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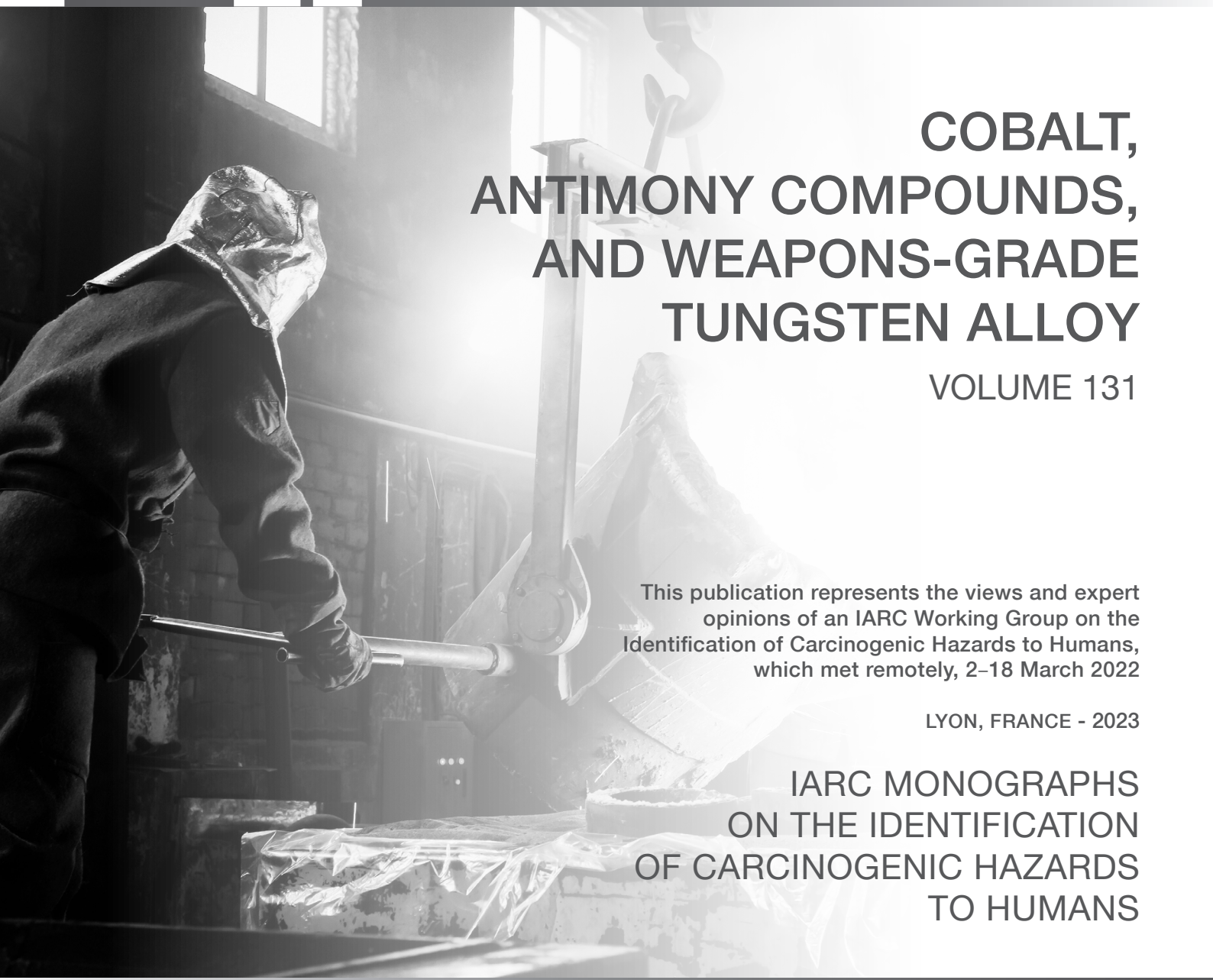

COBALT, ANTIMONY COMPOUNDS, AND WEAPONS-GRADE TUNGSTEN ALLOY

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International Agency for Research on Cancer

World Health Organization

COBALT METAL (WITHOUT TUNGSTEN CARBIDE) AND SOME COBALT COMPOUNDS

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature, synonyms, trade names, molecular formulae, and relative molecular mass

The agents considered in the present monograph include metallic cobalt (Co) (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, and the relatively insoluble compounds cobalt(II) and cobalt(II,III) oxides (CoO and Co_3O_4, respectively) and cobalt(II) sulfide (CoS). Metallic cobalt in the composite material cobalt with tungsten carbide (WC-Co), used in the hard-metal industry, has been described previously in *IARC Monographs* Volume 86 (IARC, 2006) and is not within the scope of this volume. Metallic implants containing cobalt alloys are beyond the scope of this volume and are described in *IARC Monographs* Volume 74 (IARC, 1999). Vitamin B_{12}, non-ionic organometalloid compounds containing cobalt, and radioisotopes of cobalt are also beyond the scope of this volume.

Cobalt is a naturally occurring metallic element. It is one of the first-row transition metal members of group VIII of the periodic table, members of which include iron (Fe) and nickel (Ni). The relative atomic mass of cobalt is 58.93. Only one of its isotopes, ^{59}Co, is stable and occurs naturally. There are approximately 26 known radioactive cobalt isotopes, among which only two are of commercial importance, i.e. ^{60}Co and ^{57}Co. The former provides a widely used source of radioactivity for applications in food sterilization, radiography, and radiotherapy. The latter is the most widely used isotope in γ-resonance spectroscopy (Donaldson & Beyersmann, 2011). These radioactive forms of cobalt are outside the scope of this monograph but are classified in IARC Group 1 (*carcinogenic to humans*), as described in *IARC Monographs* Volume 100D (IARC, 2012).

Pure metallic cobalt has limited uses, but it is a strategically important metal because of its use as an alloying element and as a source in chemical production (Donaldson & Beyersmann, 2011). Nomenclature, synonyms and trade names, molecular formulae, and the relative molecular masses for these cobalt compounds and salts are presented in Table 1.1 (IARC, 1991; NCBI, 2021a). The cobalt compounds and salts given in Table 1.1 are not an exhaustive list, nor are they necessarily the most commercially important cobalt-containing substances.

Table 1.1 Registry numbers, synonyms and trade names, molecular formulae, and relative molecular masses for cobalt metal and cobalt(II) salts and compounds

Chemical name	CAS No.[a]	Synonyms and trade names	Formulae[b]	Relative molecular mass[c]
Cobalt metal				
Cobalt (NCBI, 2021b)	7440-48-4 [177256-35-8; 184637-91-0; 195161-79-6; 1245817-40-6; 1262528-32-4; 132965-60-7; 335349-43-4]	CI 77320; cobalto; cobalt moncation; cobalt, elemental; cobalt-59; cobaltum; cobalt powder; cobalt metal powder; cobalt fume; cobalt foil; fine cobalt powder; cobalt nanoparticles; cobalt powder; cobalt nanofoil; cobalt nanorods; cobalt nanopowder; cobalt nanowires; cobalt rod; cobalt wire, kobalt	Co	58.93
Soluble cobalt(II) salts				
Cobalt(II) acetate (NCBI, 2021j)	71-48-7 [33327-32-1; 68279-06-1; 73005-84-2; 256431-41-1; 2649269-82-7]	Acetic acid, cobalt(2+) salt; acetic acid, cobalt salt; bis(acetato)cobalt; cobalt acetate; cobalt(2+) acetate; cobalt diacetate; cobalt(2+) diacetate; cobalt di(acetate); cobaltous acetate; cobaltous diacetate	$Co(CH_3CO_2)_2$	177.02
Cobalt(II) acetate tetrahydrate (NCBI, 2021k)	6147-53-1	Cobaltous acetate tetrahydrate; cobalt acetate tetrahydrate; cobalt diacetate tetrahydrate; cobalt(2+);diacetate;tetrahydrate; cobalt(cento) acetate tetrahydrate; bis(acetato)tetraquacobalt; acetic acid, cobalt(2+) salt, tetrahydrate; cobalt diacetate-tetrahydrate	$Co(CH_3CO_2)_2{\cdot}4H_2O$	249.08
Cobalt(II) chloride (NCBI, 2021l)	7646-79-9	Cobalt chloride ($CoCl_2$); cobalt dichloride; cobaltous chloride; cobalt chloride anhydrous; cobalt(II) chloride hydrate	$CoCl_2$	129.84
Cobalt(II) chloride hexahydrate (NCBI, 2021m)	7791-13-1	Cobalt chloride hexahydrate; cobalt dichloride hexahydrate; cobalt chloride hydrate; cobalt(II) chloride hexahydrate; cobaltous chloride, hexahydrate; cobalt chloride, hexahydrate; cobalt(2+) dichloride hexahydrate	$CoCl_2{\cdot}6H_2O$	237.93
Cobalt(II) nitrate (NCBI, 2021n)	10141-05-6 [14216-74-1]	Cobalt bis(nitrate); cobalt dinitrate; cobalt(2+) dinitrate; cobalt(2+) nitrate; cobalt nitrate salt; cobaltous nitrate; nitric acid, cobalt(2+) salt; nitric acid, cobalt salt	$Co(NO_3)_2$	182.94
Cobalt(II) nitrate hexahydrate (NCBI, 2021o)	10026-22-9	Cobaltous nitrate hexahydrate; cobalt nitrate hexahydrate; cobalt dinitrate hexahydrate; cobalt(2+) nitrate hexahydrate; cobalt(II) nitrate hexahydrate; dinitrate cobalt hexahydrate; cobalt(2+), hexaaqua-, dinitrate; cobaltous nitrate 6-hydrate	$Co(NO_3)_2{\cdot}6H_2O$	291.04
Cobalt(II) sulfate (NCBI, 2021p)	10124-43-3 [139939-65-4]	Cobalt monosulfate; cobaltous sulfate; cobalt sulfate (1:1); cobalt(2 +) sulfate; cobalt sulfate; cobalt sulfate; cobalt(II) sulfate; sulfuric acid, cobalt(2+) salt (1:1); cobalt(II) sulfate, anhydrous; cobalt brown	$CoSO_4$	155.00
Cobalt(II) sulfate heptahydrate (NCBI, 2021q)	10026-24-1	Cobalt monosulfate heptahydrate; cobalt sulfate heptahydrate; cobalt(II) sulfate (1:1), heptahydrate; cobalt(2+) sulfate heptahydrate; cobaltous sulfate heptahydrate; sulfuric acid, cobalt(2+) salt, hydrate (1:1:7); cobalt sulfate heptahydrate; sulfuric acid, cobalt(2+) salt (1:1), heptahydrate	$CoSO_4{\cdot}7H_2O$	281.11

Table 1.1 (continued)

Chemical name	CAS No.[a]	Synonyms and trade names	Formulae[b]	Relative molecular mass[c]
Cobalt(II) octanoate (NCBI, 2021r; NCBI, 2021s)	1588-79-0; 6700-85-2 [13595-74-9; 16971-12-3; 20161-50-6]	Cobalt octanoate; cobalt(2+);octanoate; cobalt dioctanoate; cobaltous octanoate; octanoic acid cobalt salt; bisoctanoic acid cobalt(II) salt	$C_{16}H_{30}CoO_4$	345.34
Cobalt(II) 2-ethylhexanoate (NCBI, 2021t)	136-52-7 [221315-92-0, 25360-65-0, 87947-13-5]	Cobalt octoate; cobaltous octoate; cobalt bis(2-ethylhexanoate); cobaltous 2-ethylhexanoate; cobalt 2-ethylhexanoate; cobalt 2-ethylhexanoate; hexanoic acid, 2-ethyl-, cobalt(2+) salt; 2-ethylhexanoic acid cobalt salt; cobalt(2+); 2-ethylhexanoate; hexanoic acid, 2-ethyl-, cobalt(2+) salt (2:1); cobalt 2-ethylcaproate; 2-ethylhexanoic acid cobalt(2+) salt; cobalt(II) bis(2-ethylhexanoate); cobalt(2+) bis(2-ethylhexanoate)	$C_{16}H_{30}CoO_4$	345.34
Insoluble cobalt(II or II,III) compounds				
Cobalt(II) oxide (NCBI, 2021u)	1307-96-6 [185461-93-2; 186373-01-3]	CI 77322; CI Pigment Black 13; cobalt black; cobalt monoxide; cobalt monooxide; cobaltous oxide; cobalt oxide (CoO); cobalt(2+) oxide; monocobalt oxide; zaffre	CoO	74.93
Cobalt(II,III) oxide (NCBI, 2021v)	1308-06-1	Cobaltic-cobaltous oxide; cobalto-cobaltic oxide; cobalto-cobaltic tetroxide; cobaltosic oxide; cobalt oxide (Co_3O_4); cobalt tetraoxide; tricobalt tetraoxide; tricobalt tetroxide (IARC, 1991)	Co_3O_4	240.80
Cobalt(II) hydroxide (NCBI, 2021w)	21041-93-0 [12672-51-4]	Cobalt(2+)hydroxide; cobalt dihydroxide; cobalt hydroxide ($Co(OH)_2$); cobalthydroxide; cefamandolenafate	CoH_2O_2	92.95
Cobalt(II) sulfide (NCBI, 2021x)	1317-42-6	Cobalt monosulfide; cobaltous sulfide; cobalt(2+) sulfide; sulfanylidenecobalt; cobalt sulphide	CoS	91.00
Organic cobalt(II) compounds				
Cobalt(II) resinate (NCBI, 2021y)	68956-82-1 (ECHA, 2021)	Cobaltous resinate; 1,4a-dimethyl-7-propan-2-yl-2,3,4,4b,5,6,10,10a-octahydrophenanthrene-1-carboxylate; cobalt(2+)	$C_{40}H_{58}CoO_4$	661.8
Cobalt(II) acetyl acetonate (NCBI, 2023)	123334-29-2	Cobalt(II) 2,4-pentanedionate; bis(2,4-pentanedionato)cobalt(II)	$C_{10}H_{14}CoO_4$	257.15
Cobalt(II) oxalate (NCBI, 2021z)	814-89-1	Cobalt; oxalic acid; ethanedioic acid	$C_2H_2CoO_4$	146.95

CAS No., Chemical Abstracts Services Registry number; CI, Colour Index.

[a] Deleted CAS Nos are shown in square brackets.

[b] Chemical formulae of cobalt alloys are shown as a list of constituent elements using square brackets.

[c] Relative molecular masses of cobalt alloys are shown as the sum of atomic masses of constituent elements calculated by the Working Group.

1.1.2 Chemical and physical properties of metallic cobalt and cobalt compounds

Cobalt is a silver-grey, shiny, hard, and ductile metal element. Cobalt commonly occurs in the 0, +2, and +3 valence states. Metallic cobalt that is commonly used in various metal alloys is in the 0 valence state, whereas cobalt in compounds occurs in two predominant oxidation states (+2 and +3 valence states). The +2 valence state (Co^{2+}) is the most common valence state in commercially available cobalt compounds and in the environment (Patnaik, 2003). Cobalt is a metal component of vitamin B_{12}, also known as cyanocobalamin, which is required for the production of erythrocytes. Other cobalt compounds are described as toxic for the environment and the human body after excessive exposure (Leyssens et al., 2017). Cobalt(II) is far more stable in normal aqueous conditions, although cobalt(III) may be stabilized by changing the ligand environment. Water-soluble cobalt compounds release cobalt(II) ions into solution that can, in turn, form various complexes with organic or inorganic anions, with equilibrium conditions depending on redox potential (E_h), pH, and the presence of anions (Krupka & Serne, 2002).

Cobalt(II) forms a wide range of simple and hydrated salts that comprise all the common anions, such as acetate, bromide, carbonate, chloride, fluoride, nitrate, perchlorate, and sulfate. Several of the hydrated salts and their solutions contain the pink octahedral $[Co(H_2O)_6]^{2+}$ ion (Donaldson & Beyersmann, 2011). When heated, cobalt is first oxidized to cobalt(II,III) oxide, then forms cobalt(III) oxide at temperatures above 900 °C. Cobalt(II) sulfide is formed by heating cobalt metal with hydrogen sulfide at 700 °C. Cobalt(II) hydroxide ($Co(OH)_2$) is a product of the hydrolysis of solutions containing Co^{2+} ions. These compounds are insoluble in water (see Table 1.2, which presents selected chemical and physical properties of metallic cobalt and cobalt compounds covered in this monograph) (Donaldson & Beyersmann, 2011).

1.1.3 Technical grade and impurities

(a) Cobalt metal

Metallic cobalt powders are widely produced in high-purity form for use in the hard-metal industry, the manufacturing of superalloys, and in other applications. Commercial metallic cobalt powders are available in purities ranging from 99% to ≥ 99.999% in many grades, particle size ranges, and forms (IARC, 2006). The agents considered in this monograph exclude WC-Co. In these hard metals, metallic cobalt powders are used as a binder, and relevant information on the hard-metal industry related to metallic cobalt powders is available in a previous monograph in *IARC Monographs* Volume 86 (IARC, 2006).

(b) Cobalt compounds

Cobalt compounds are produced in forms with purities greater than 99.9% for cobalt(II) acetate ($Co(CH_3CO_2)_2$) (Sigma-Aldrich, 2021a), cobalt(II) chloride ($CoCl_2$) (Sigma-Aldrich, 2021b), cobalt(II) hydroxide (Alfa Aesar, 2022), cobalt(II) nitrate hexahydrate ($Co(NO_3)_2{\cdot}6H_2O$) (Sigma-Aldrich, 2021c), cobalt(II) oxide (Sigma-Aldrich, 2021d), cobalt(II,III) oxide (Sigma-Aldrich, 2021e), and cobalt(II) sulfide (Sigma-Aldrich, 2022a); 99% for cobalt(II) acetate tetrahydrate ($Co(CH_3CO_2)_2{\cdot}4H_2O$) (Sigma-Aldrich, 2022b), and cobalt(II) sulfate heptahydrate ($CoSO_4{\cdot}7H_2O$) (Sigma-Aldrich, 2022c); and 98% for cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$) (Fisher Scientific, 2022). [The Working Group noted that this list is not exhaustive.]

Table 1.2 Chemical and physical properties of the pure substances containing cobalt

Chemical name	Typical physical description	Melting point (°C)	Boiling point (°C)	Density (g/cm^3)	Solubility
Cobalt metal					
Cobalt (NCBI, 2021a)	Silvery bluish-white, grey, shining, hard, magnetic, ductile, somewhat malleable metal	1495	2927	8.9 at 20 °C	Soluble in dilute acids; readily soluble in dilute nitric acid; insoluble in water
Soluble cobalt(II) salts					
Cobalt(II) acetate (NCBI, 2021j)	Light pink crystals, pink crystals	298 (dec)	NA	1.71 at 68 °F [20 °C]	Readily soluble in water; soluble in alcohol and dilute acids
Cobalt(II) acetate tetrahydrate (NCBI, 2021k)	Red crystals	140	NA	1.7	Soluble in water
Cobalt(II) chloride (NCBI, 2021l)	Pale-blue hygroscopic leaflets, colourless in very thin layers, blue hexagonal leaflets	735	1049	3.36 at 25 °C/4 °C	Soluble in water, alcohols, acetone, ether, glycerol, and pyridine
Cobalt(II) chloride hexahydrate (IARC, 1991)	Pink to red, slightly deliquescent, monoclinic, prismatic	86; loss of four H_2O at 52–56, an additional H_2O by 100, and another H_2O at 110	NA	1.92 (Nielsen et al., 2013)	Soluble in ethanol, water, acetone, diethyl ether, and glycerol
Cobalt(II) nitrate (NCBI, 2021n)	Pale red powder, red crystals	100–105 (dec)	NA	2.49	Soluble in water
Cobalt(II) nitrate hexahydrate (NCBI, 2021o)	Red crystals	55	NA	1.88	Soluble in water
Cobalt(II) sulfate (NCBI, 2021p)	Red to lavender dimorphic, orthorhombic crystals, red powder, rose-pink solid	735	NA	3.71 at 25 °C/4 °C	Dissolves slowly in boiling water; 38.3 g/100 g water at 25 °C
Cobalt(II) sulfate heptahydrate (NCBI, 2021q)	Pink to red monoclinic, prismatic crystals (O'Neil, 2001)	96.8	420	1.95	Soluble in water; slightly soluble in ethanol and methanol (O'Neil, 2001)
Cobalt(II) octanoate (NCBI, 2021r; NCBI, 2021s)	NA	NA	NA	NA	[Soluble in water]
Cobalt(II) 2-ethylhexanoate (NCBI, 2021t)	Blue liquid, blue-violet mass, purple to dark blue waxy solid at 20 °C	53–58 if heated in an aluminium crucible under nitrogen; 64–84 if heated in a glass capillary under air	Dec at approximately 90; dec before boiling if heated in an aluminium crucible under nitrogen	1.25 at 21.6 °C	Soluble in water

Table 1.2 (continued)

Chemical name	Typical physical description	Melting point (°C)	Boiling point (°C)	Density (g/cm^3)	Solubility
Insoluble cobalt(II or II,III) compounds					
Cobalt(II) oxide (NCBI, 2021u)	Powder or cubic or hexagonal crystals; colour varies from olive green to red depending on particle size, but the commercial material is usually dark grey	1935	NA	5.7–6.7	Insoluble in water and ammonium hydroxide; soluble in acids or alkalis
Cobalt(II,III) oxide (IARC, 1991)	Black or grey crystals	895; transition point to CoO is 900–950	NA	6.07 (ATSDR, 2004)	Practically insoluble in water, aqua regia, and hydrochloric or nitric acid; soluble in sulfuric acid and fused sodium hydroxide
Cobalt(II) hydroxide (Patnaik, 2003)	Rose-red powder (more stable) and bluish-green powder less stable than the red form; rhombohedral crystals	NA	NA	3.597	Insoluble in water; soluble in acids and ammonia; insoluble in dilute alkalis
Cobalt(II) sulfide (NCBI, 2021x)	Black amorphous powder	1117	NA	5.45	Insoluble in water; soluble in acid
Organic cobalt(II) compounds					
Cobalt(II) resinate (NCBI, 2021y)	NA	NA	NA	NA	NA

dec, decomposes; NA, not available.

1.2 Production and use

1.2.1 Production

(a) Cobalt metal

The main ores of cobalt are cobaltite, erythrite, glaucodot, and skutterudite. However, cobalt is produced mainly as a by-product of the mining and processing of the ores of other metals, particularly those of copper, nickel, and silver, and also gold, lead, and zinc. The first stages of the production of cobalt from its ores involve the separation of cobalt-bearing minerals – including arsenide, sulfoarsenide, sulfide, arsenic-free cobalt–copper, lateritic, and oxide ores from the gangue (the portion of the ore composed of minerals that are not of commercial value) – and from minerals that include other metals. The concentrates gained with the application of physical separation methods to ores, such as gravity separation or froth flotation, can increase the cobalt contents of cobalt-rich ores by 10–15%. Using the more common ores less rich in cobalt, these processes only increase the levels of cobalt from 0.1–0.6% to a few per cent. Subsequently, cobalt is extracted from concentrates and occasionally directly from the ore itself by hydrometallurgical, pyrometallurgical, and electrometallurgical processes (Donaldson & Beyersmann, 2011; Afolabi et al., 2021).

Global mine and refinery production figures for cobalt from 1960 to 2019 are shown in Fig. 1.1 (USGS, 2021a). Global production from cobalt mining and production of refined cobalt has increased steadily over the past two decades, reaching 144 000 and 132 000 tonnes, respectively, in 2019. Data representing global cobalt mine production and refinery production (including the production of cobalt compounds) from 2015 to 2019 are presented in Table S1.3 and Table S1.4, respectively (USGS, 2021b; Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). The largest site of cobalt mine production is in Kinshasa, the Democratic Republic of the Congo (100 000 tonnes in 2019), followed by the Russian Federation (6300 tonnes), Australia (5742 tonnes), and the Philippines (5100 tonnes). The largest producer of refined cobalt can be found in China (90 000 tonnes in 2019), followed by Finland (12 526 tonnes), Canada (6075 tonnes), and Norway (4354 tonnes).

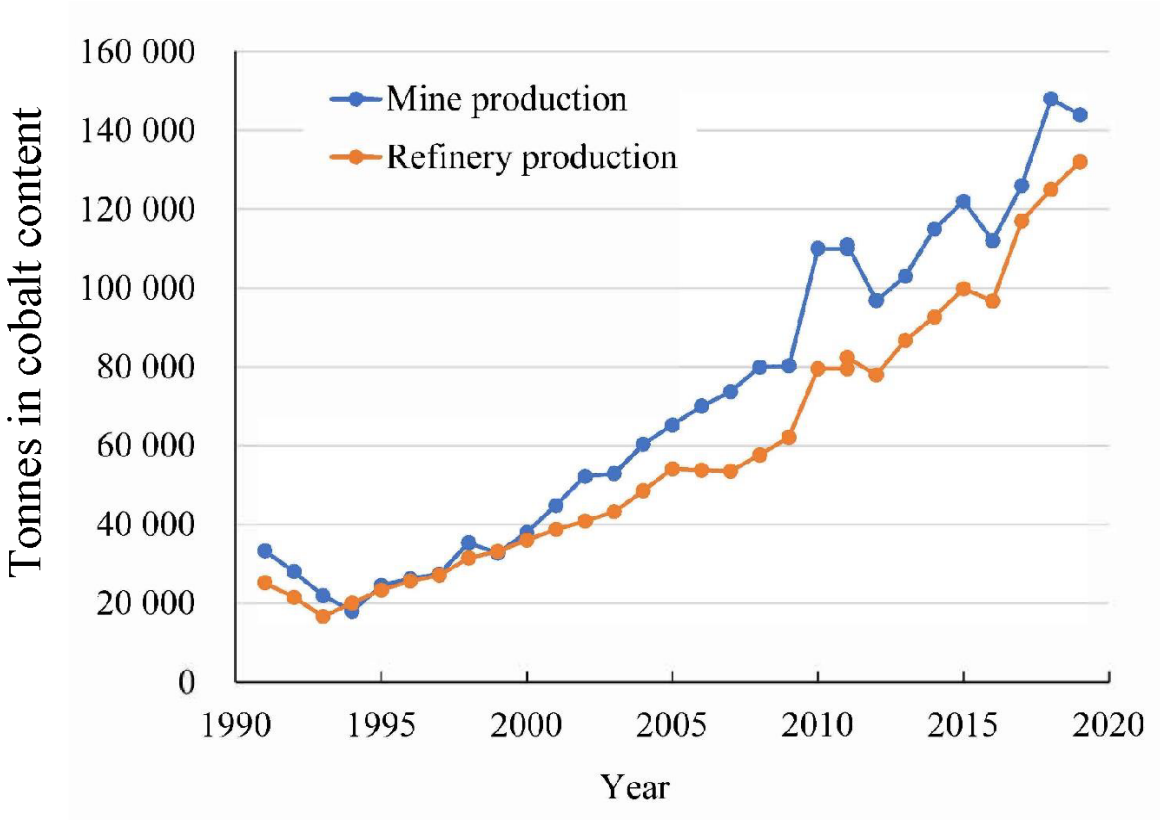
(b) Cobalt compounds

The processes used in the production of cobalt(II) salts are described in this section. Cobalt(II) acetate is prepared by dissolving cobalt(II) carbonate ($CoCO_3$) or hydroxide ($Co(OH)_2$) in dilute acetic acid, followed by crystallization. It can also be prepared by oxidation of dicobalt octacarbonyl in the presence of acetic acid (Patnaik, 2003). Cobalt(II) acetate tetrahydrate is prepared by concentrating solutions of cobalt(II) hydroxide or carbonate in acetic acid (Donaldson & Beyersmann, 2011).

Cobalt(II) chloride is prepared by the reaction of cobalt metal or its oxide, hydroxide, or carbonate with hydrochloric acid. Upon concentration and cooling, crystals of hexahydrate form from solution that, on heating with thionyl chloride, dehydrate to anhydrous cobalt(II) chloride. Alternatively, the hexahydrate may be converted to anhydrous cobalt(II) chloride by dehydration in a stream of hydrogen chloride and dried in a vacuum at 100–150 °C. The anhydrous compound also may be obtained by passing chlorine over cobalt powder (Patnaik, 2003). Cobalt(II) chloride hexahydrate is prepared by concentrating a hydrochloric acid solution of cobalt(II) oxide or carbonate (Donaldson & Beyersmann, 2011).

Cobalt(II) nitrate hexahydrate is prepared by concentrating a nitric acid solution of cobalt(II) oxide or carbonate. The hexahydrate loses water rapidly at 55 °C to yield the trihydrate; the monohydrate can also be prepared using higher temperatures (Donaldson & Beyersmann, 2011).

Fig. 1.1 Global production of cobalt in mines and refineries, 1991–2019



Data for refinery production before 1967 were not available.
Created by the Working Group with data from USGS (2021a, b).

Cobalt(II) oxide is typically prepared by the controlled oxidation of the metal at temperatures > 900 °C, followed by cooling in a protective atmosphere to prevent partial oxidation to cobalt(II,III) oxide. Cobalt(II,III) oxide can be prepared by the controlled oxidation of cobalt metal or cobalt(II) oxide, or by the thermal decomposition of cobalt(II) salts at temperatures < 900 °C (Donaldson & Beyersmann, 2011). Cobalt(II) hydroxide is obtained as a precipitate when an alkaline hydroxide is added to an aqueous solution of cobalt(II) salt (Patnaik 2003).

Cobalt(II) sulfate is prepared by dissolving cobalt(II) oxide, hydroxide, or carbonate in dilute sulfuric acid, followed by crystallization. Crystallization yields the commercial product pink heptahydrate (Patnaik, 2003).

Cobalt(II) sulfide exists in nature as the mineral sycoporite. It can also be readily prepared in the laboratory. A black precipitate of cobalt(II) sulfide is obtained by passing hydrogen sulfide through an alkaline solution of a cobalt(II) salt such as cobalt(II) chloride. Cobalt(II) sulfide can also be produced by heating cobalt metal with hydrogen sulfide at 700 °C (Patnaik, 2003).

Cobalt(II) resinate can be made by the direct reaction of cobalt powder, oxide, or hydroxide with resin acid, or by precipitation reactions involving the addition of sodium resinate to an aqueous solution of a cobalt(II) salt, such as the sulfate (Donaldson & Beyersmann, 2011).

Cobalt(II) 2-ethylhexanoate can be produced by metathesis reaction of cobalt(II) salt solutions and sodium 2-ethylhexanoate, by oxidation of cobalt metal in the presence of 2-ethylhexanoic acid, and by neutralization of 2-ethylhexanoic acid using cobalt carbonate or cobalt hydroxide (NCBI, 2021t). [Information concerning production of cobalt(II) octanoate was not available to the Working Group.]

1.2.2 Use

From a historical perspective, although very little cobalt metal was used until the 20th century, its ores have been used for thousands of years as blue colouring agents for glass and pottery. The brilliant blue pigment used for these purposes was probably produced by fusing an ore containing cobalt oxide with potash and silica to produce a vitreous material called smalt, which was powdered to produce the pigment. In the 16th century, a blue pigment called zaffre was produced from silver–cobalt–bismuth–nickel–arsenate ores found in Saxony, Germany (IARC, 2006). The next development in the use of cobalt-containing materials was the discovery, early in the 18th century, that solutions containing bismuth and cobalt could be used as sympathetic inks (Donaldson & Beyersmann, 2011). In 1923, the discovery that mixing cobalt with tungsten carbide produced hard metal initiated its use in a variety of industrial applications. Cobalt was used as a constituent in the first permanent magnetic alloy in 1933 (IARC, 2006).

Cobalt is used in many industries, including in the manufacture of cutting and grinding tools, pigments and paints, coloured glass, surgical implants, batteries, and some types of electroplating. Global consumption of cobalt has increased markedly over the past decade, as has its production. Consumption has increased at an annual rate of approximately > 5% since 2013. Much of this consumption increase is a result of the demand for cobalt in the production of lithium-ion batteries (Cobalt Institute, 2021). All lithium-ion batteries use a *p*-type semiconductor, lithium cobalt oxide ($CoLiO_2$), as the active material for the positive electrode (Tukamoto & West, 1997; Donaldson & Beyersmann, 2011). Demand for cobalt in lithium-ion batteries, used chiefly in portable electronics and electric vehicles, increased at an annual rate of 10% between 2013 and 2020. Batteries accounted for 57% of total global cobalt consumption in 2020, followed by nickel-based alloys, which accounted for 13%; other uses included tool materials, pigments, catalysts, magnets, and soaps and dryers (Cobalt Institute, 2021). In a projected growth scenario using a multiregional input–output analysis model – assuming region- and sector-specific gross domestic product growth as projected by the Organisation for Economic Co-operation and Development, constant technology, and constant background import shares – global annual cobalt demand was estimated as likely to increase from 50 000 tonnes in 2007 to 110 000 tonnes in 2030, and to 190 000 tonnes in 2050, which is an increase of roughly threefold over the given period (Tisserant & Pauliuk, 2016).

Geographically, Asia is the region that consumes the largest proportion of cobalt, accounting for around one half of total global cobalt consumption in 2020. This reflects the high production volumes of battery materials in Asia, particularly in China, Japan, and the Republic of Korea. China is the greatest cobalt consumer, accounting for 32% of global consumption in 2020. China is also a major producer of tool materials, magnets, and pigments. Europe and North America also consume large amounts of cobalt, together accounting for 40% of global consumption in 2020. In these regions, cobalt is mostly used in batteries, nickel-based alloys, and tool materials (Cobalt Institute, 2021).

(a) Cobalt metal

Pure metallic cobalt has limited applications. It is used as a source of chemical production and as an alloying element. Cobalt is not the most abundant of the elements added to steels but is an important component of high-strength steel. Maraging steels, used as ultrahigh-strength alloys, contain 5–20% cobalt, which is alloyed with nickel and molybdenum (Donaldson & Beyersmann, 2011). In electrodeposited alloys, protective coatings of electroplated nickel–cobalt alloys have been used. A 25% cobalt alloy, deposited from sulfate electrolytes, is approximately three times as hard as electroplated nickel and has almost the same hardness as pure cobalt (Donaldson & Beyersmann, 2011).

End-uses of cobalt-containing alloys include magnetic alloys for powerful permanent magnets, hard-metal alloys for cutting-tool materials, superalloys for aircraft engines, cemented carbides, corrosion-resistant alloys, wear-resistant alloys, and electrodeposited alloys to deliver wear and corrosion-resistant metal coatings (Donaldson & Beyersmann, 2011).

Several cobalt-containing alloys have special applications. For example, cobalt–chromium alloys are used as dental materials. These alloys are used for casting denture bases, complex partial dentures, and selected types of bridgework. The most widely used alloy is vitallium, which is a cobalt-based alloy that contains 64.5% cobalt, 30% chromium, 5% molybdenum, and 0.5% each of carbon and silicon. Vitallium is also used for surgical implants and bone replacement and repair (Donaldson & Beyersmann, 2011).

Metallic cobalt nanoparticles (NPs, < 10 nm in size) are used because there exists an efficient means for manipulating or modifying their optical, mechanical, magnetic, chemical, and electronic properties, which can be readily used in a wide variety of technological applications. Cobalt NPs provide excellent magnetic, electrical, and catalytic properties that are of scientific and technological interest in various fields, including recording media, magnetic sensors, magnetic memories, magnetic fluids, magnetic composites, and catalysis. Cobalt NPs are also used in biomedical-related fields, e.g. drug delivery and magnetic resonance imaging (Ansari et al., 2017). Additionally, cobalt-based NPs are produced as cobalt oxide, organometallic compounds, or biopolymers. In biomedical applications, cobalt-based NPs are used as starting materials for the formation of magnetic polymer microspheres and dextran coating (Magaye et al., 2012).

(b) Cobalt compounds

Cobalt salts are mainly used as pigments in the glass, ceramics, and paint industries, as catalysts in the petroleum industry, as paint driers, and as trace metal additives for agricultural and medical use.

Cobalt(II) acetate and cobalt(II) acetate tetrahydrate are used in the manufacturing of drying agents for lacquers and varnishes, sympathetic inks, catalysts for oxidation, pigments for oilcloth, mineral supplements, and aluminium anodizing solutions; as stabilizers for malt beverages; and in agricultural industries, for example, as treatments of soils or ruminant animals to reduce cobalt deficiencies (Patnaik, 2003; Donaldson & Beyersmann, 2011).

Anhydrous cobalt(II) chloride is blue and the change to hexahydrate red is used as a humidity indicator in silica gel desiccants. It is also used in the electroplating, ceramics, glass, malt beverage stabilizer, mineral supplement, chemical, agricultural, and pharmaceutical industries (Patnaik, 2003; Donaldson & Beyersmann, 2011). Cobalt(II) chloride is also used to prepare several other cobalt salts and in the manufacture of synthetic vitamin B_{12} (Patnaik, 2003). Cobalt(II) chloride hexahydrate is used in sympathetic inks, hydrometers, plating baths, metal refining, pigments, and catalysts (Donaldson & Beyersmann, 2011).

Cobalt(II) nitrate is an important source of high-purity cobalt for use in the electronics and related industries, and the compound also has uses in the chemical and ceramics industries. Cobalt(II) nitrate hexahydrate is used in pigments, chemicals, ceramics, feed supplements, and catalysts (Patnaik, 2003; EFSA, 2009a; Donaldson & Beyersmann, 2011).

Cobalt(II) oxide is used as a starting material for the manufacture of other chemicals and catalysts, in pigments such as colour reagents and ceramics, gas sensors, and thermistors. Cobalt(II,III) oxide is used in the production of enamels and semiconductors (Patnaik, 2003; Donaldson & Beyersmann, 2011). Cobalt(II) hydroxide is used as a drier for paints and varnishes, and is added to lithographic printing inks to enhance their drying properties. Other applications are in the preparation of cobalt salts, as a catalyst, and in storage battery electrodes (Patnaik, 2003; Donaldson & Beyersmann, 2011).

Cobalt(II) sulfate is widely used as a source of cobalt(II) in solution for the manufacturing of chemicals and the electroplating industries. The sulfates are also used in the ceramics, linoleum, and agricultural industries. Cobalt(II) sulfate heptahydrate is used for the manufacturing of other chemicals and pigments in ceramics. Cobalt(II) sulfide is used as a catalyst for the hydrogenation or hydrodesulfurization of organic compounds in petroleum refining (Patnaik, 2003; Donaldson & Beyersmann, 2011).

Cobalt(II) resinate is used as a paint and varnish drier, and as a catalyst (Patnaik, 2003; Donaldson & Beyersmann, 2011). Cobalt(II) octanoate is used in home maintenance, e.g. in oil-based polyurethane varnish for wood (NCBI, 2021w). Cobalt(II) 2-ethylhexanoate is used in paints and coatings, process regulators, surface-active agents, bonding agents, catalysts, building and construction materials (e.g. flooring, tiles, sinks, bathtubs, mirrors, wall materials/drywalls, wall-to-wall carpets, insulation, and playground surfaces), and detergent alcohol (NCBI, 2021t).

1.3 Detection and quantification

1.3.1 Air

Analytical methods used until the early 21st century to measure cobalt concentrations in airborne particulate matter have been reviewed in previous *IARC Monographs* Volume 52 and Volume 86 (IARC, 1991, 2006). Spectrometric analytical procedures, electrochemical analysis methods, and radioactivation processes continue to be employed for the analysis of cobalt and its compounds (Table 1.5). Atomic absorption spectrometry (AAS), using either flame AAS (FAAS) or electrothermal AAS (ETAAS) atomization, has been used extensively and remains the most widely employed technique for the elemental analysis of airborne particles (NIOSH, 1994; US EPA, 1999a). Other standard methods for measuring cobalt in air samples are the use of inductively coupled plasma (ICP)-atomic emission spectroscopy (ICP-AES) or ICP mass spectrometry (ICP-MS) detection systems (US EPA, 1999b; ATSDR, 2004). As shown in Table 1.5, reported sample limits of detection (LODs) for cobalt range from 0.01 ng/m^3 using ICP-MS (US EPA, 1999b) to 2.2 ng/m^3 using FAAS (US EPA, 1999a). Where available, other methods such as instrumental neutron activation analysis (INAA) and X-ray fluorescence analysis can provide quantitative data for cobalt concentrations in airborne particulate matter (Hamilton, 1994).

The advantages and shortcomings of these and other less frequently used techniques have been extensively examined (IARC, 1991, 2006; Ram et al., 2003; Pitzke et al., 2020). The more frequently reported ones are chosen on the basis of cost and efficiency. For instance, AAS instrumentation usually provides low LODs, but only one element can be analysed at a time (Schroeder

et al., 1987). Multi-element analysis techniques, such as ICP and INAA, require significant investments in equipment and are not available in all laboratories. Although more affordable, X-ray fluorescence analysis lacks sensitivity for most environmental concentrations of cobalt unless used in combination with electron microprobes (Hamilton, 1994).

1.3.2 Water

Various methodologies are currently available for the measurement of cobalt, even at low levels, in water and other environmental samples. Instrumental techniques such as electroanalytical techniques (Zhang et al., 2016), FAAS (Büyükpınar et al., 2019; Tekin et al., 2020), ETAAS (Amjadi et al., 2010), ICP-AES, ICP-MS, and ICP with optical emission spectrometry (Thomassen et al., 2004; Ndilila et al., 2014; Cheng et al., 2019; Bi et al., 2020) have been extensively reported (Table 1.5). However, low analyte concentrations and matrix effects can cause difficulties in the direct measurement of cobalt concentrations in water or other environmental samples (Aggarwal et al., 1992; Amjadi et al., 2010). Consequently, numerous preconcentration and separation techniques have been developed to extract cobalt from sample matrices. Pre-treatment procedures include ion exchange (Jiang et al., 2005), solid-phase extraction (Duran et al., 2007; Ghaedi et al., 2007; Talio et al., 2014), liquid-phase extraction (Amjadi et al., 2010; Tekin et al., 2020), liquid–liquid extraction (Todorovska et al., 2003; Reza Jamali et al., 2007), dispersive liquid–liquid microextraction (Berton et al., 2012), coprecipitation, and cloud point extraction (Ghaedi et al., 2008; Citak & Tuzen, 2010; Temel et al., 2018). The United States Environmental Protection Agency (US EPA) recommends ICP-MS for measuring the concentrations of metals in water due to its high analytical sensitivity (Table 1.5) (US EPA, 2014). ICP-MS typically requires filtration and acidification of the sample before analyses and offers LODs in the sub or low parts-per-billion range for most metals (US EPA, 2014).

New research trends include the development of chemosensors for Co^{2+} ions, characterized by high sensitivity and easy operation (Kuwar et al., 2014; Dogaheh et al., 2017). A few chemosensors using ion-selective membranes or chemical probes for fluorescence or colorimetric cobalt detection have been reported. The sensors have been applied to estimation of the concentrations of Co^{2+} ions in water, food products, and pharmaceutical formulations (Na et al., 2016; Khalil & El-Sharnouby, 2021). Colorimetric methods further allow easy monitoring of target ions with the naked eye (Na et al., 2016). [The Working Group noted that the need for cost-effective and increasingly sensitive analytical methods to quantify cobalt and cobalt speciation possibly explains the current interest in this new line of research.]

1.3.3 Other environmental samples, food and feed, consumer products, and cosmetics

Concentrations of cobalt in foodstuffs are usually very low and, despite the sensitive analytical instruments currently available (Table 1.5), pre-treatment techniques are often required (Citak & Tuzen, 2010; Tekin et al., 2020). The use of affordable and non-toxic solvents is becoming the main goal of extraction methods. Deep eutectic solvents and ionic liquids share characteristics that make them attractive as potential substitutes for traditional organic solvents. Another alternative to the traditional chemical decomposition of food samples with common acid mixtures is ultrasonic extraction (Yebra-Biurrun & Cancela-Pérez, 2007; Temel et al., 2018). For the measurement of concentrations of cobalt ions in soil samples, the US EPA (2014) recommends microwave-assisted acid digestion followed by ICP-MS analysis.

Table 1.5 Analytical methods for the measurement of cobalt in environmental samples, food and feedstuffs, consumer products, and cosmetics

Sample matrix	Sample preparation (method)	Analytical technique (method)	LOD	Reference
Environmental samples				
Air	Collection on glass fibre filters using high-volume sampler; extraction by hot acid procedure or microwave extraction; microwave extraction preferred (EPA Method IO-3.1)	AAS (EPA Method IO-3.2)	FAAS, 2.2 ng/m^3; ETAAS, 0.02 ng/m^3	US EPA (1999a)
Air	Collection on glass or quartz fibre filter; microwave or hot acid digestion (EPA Method IO-3.1)	ICP-MS (EPA Method IO-3.5)	0.01 ng/m^3	US EPA (1999b)
Air	Sample filter digested by wet acid ashing	FAAS (NIOSH Method 7027)	0.4 µg/m^3	ATSDR (2004)
Air	Sample filter digested by wet acid ashing	ICP-AES (NIOSH Method 7300)	0.5 µg/m^3	ATSDR (2004)
Inhalable aerosols	Uses ammonium citrate leachate to obtain the water-soluble fraction before the acid digestion	ICP-OES	0.1 µg/m^3	Thomassen et al. (2004)
Air: $PM_{2.5}$	High-volume sampler; microwave-assisted acid digestion	ICP-OES	NR	Bi et al. (2020)
Water	Filtration and acid digestion	ICP-MS (EPA Method 6020B)	NR	US EPA (2014)
Water	Complexation with BSOPD	UV-vis spectrophotometry	0.015 mg/L	Ahmed & Uddin (2007)
Water	Ionic liquid-based microextraction with $[C_6MIM][PF_6]$–APDC complex	ETAAS	0.04 µg/L	Amjadi et al. (2010)
Water	Online flow injection pre-concentration by ion-pair adsorption in a knotted reactor	ETAAS	5 ng/L	Benkhedda et al. (2000)
Seawater	Pre-concentration using PAR and a Dowex-1-chloride anion exchange resin	EDXRF	[1.53 µg/L][a]	Jiang et al. (2005)
Soil	Microwave-assisted acid digestion and filtration (EPA SW-846 Method 3051A)	ICP-MS (EPA Method 6020B)	NR	US EPA (2014)
Soil	Uses a photochemical vapour generation system equipped with a batch-type gas–liquid separator system to separate cobalt from the sample matrix	FAAS	8.7 µg/L	Büyükpınar et al. (2019)
Soil, house dust, and drinking-water	Metal extraction by acid digestion	ICP-AES (Method 3050B Revision 2, US EPA (1996))	NR	Ndilila et al. (2014)
Marine sediment	Slurry extraction	ETAAS	0.43 µg/g	Barciela-Alonso et al. (2003)
Wild fish	Microwave-assisted acid digestion	ICP-MS	1 µg/kg	Cheng et al. (2019)

Table 1.5 (continued)

Sample matrix	Sample preparation (method)	Analytical technique (method)	LOD	Reference
Feedstuffs, foodstuffs, consumer products, and cosmetics				
Consumer products: fashion and piercing jewellery	Sample parts were immersed in artificial sweat	ICP-OES; ICP-MS; FAAS (EN 1811:2011/AC:2012)	NR	Uter & Wolter (2018)
Cosmetics	Microwave-assisted acid digestion	ICP-OES	NR	Bruzzoniti et al. (2017)
Cosmetics (toy make-up)	Microwave-assisted acid digestion	ETAAS	0.2 µg/g	Corazza et al. (2009)
Nutrient supplements	Liquid–liquid microextraction with 1N2N–$[C_6MIM][PF_6]$ complex	ETAAS	5.4 ng/L	Berton et al. (2012)
Feed grains and forages	Extraction with 1N2N2 in glacial acetic acid	ETAAS	1 ng/g	Blanchflower et al. (1990)
Feedstuffs (grass and cereals)	Microwave partial vapour-phase acid digestion	ETAAS	NR	Araújo et al. (2000)
Foodstuffs (canned fish, black tea, green tea, tomato sauce, and honey)	Cloud point extraction with the 1-PTSC–Triton X-114 complex	FAAS	1 µg/L	Citak & Tuzen (2010)
Foodstuffs (tea leaves)	Deep eutectic solvent–liquid-phase microextraction with (*Z*)-3-bromo-5-((*p*-tolylimino)methyl)phenol	FAAS	2 µg/L	Tekin et al. (2020)

AAS, atomic absorption spectrometry; 1N2N, 1-nitroso-2-naphthol; 1-PTSC: 1-phenylthiosemicarbazide; APDC, ammonium pyrroldinedithiocarbamate; BSOPD, bis(salicylaldehyde) orthophenylenediamine; $[C_6MIM][PF_6]$, 1-hexyl-3-methylimidazolium hexafluorophosphate; EDXRF, energy-dispersive X-ray fluorescence spectrometry; EPA, Environmental Protection Agency; ETAAS, electrothermal atomic absorption spectrometry; FAAS, flame atomic absorption spectrometry; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma with optical emission spectrometry; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health; PAR, 4-(2-pyridylazo)resorcinol; $PM_{2.5}$, particulate matter with aerodynamic diameter < 2.5 µm; Triton X-114, octylphenoxypolyethoxyethanol; UV-vis, ultraviolet-visible light.

[a] Working Group conversion to International System of Units.

In recent years, increasing attention has been paid to issues concerning the exposure of skin to chemicals such as cobalt. Several studies have used methods that specifically quantify concentrations of cobalt on the skin, which has led to a better understanding of this exposure route (Lidén et al., 2008; Julander et al., 2010; Erfani et al., 2017; Kettelarij et al., 2018a; Uter & Wolter, 2018). The cobalt spot test is a simple colorimetric method that is commonly used for screening purposes (Thyssen et al., 2012; Hamann et al., 2013). Despite their reported limitations regarding the adequate quantification of exposure (Uter & Wolter, 2018), these screening tests have the advantage of helping co-allergic patients to avoid exposure to this trace metal.

1.3.4 Human biomarkers

A wide variety of techniques have been used for the determination of cobalt in human biological samples, which are roughly summarized in Table 1.6. Over the last three decades, these techniques have increased in sensitivity. Techniques such as ICP, gas chromatography–mass spectrometry, and INAA are increasingly used for multi-elemental analysis of biological samples (Goullé et al., 2005; Rocha et al., 2016; Chellini et al., 2017; Nisse et al., 2017; Capiau et al., 2020). However, ICP and INAA instruments are not available in all laboratories, and suitable alternatives are necessary for the accurate measurement of cobalt at concentrations as low as 0.5–1 µg/L. ETAAS is probably the most frequently used technique due to its sensitivity and relatively low cost of instrumentation (Todorovska et al., 2003; Berton & Wuilloud, 2010). Analytical methods recommended for determining the levels of cobalt in the blood and urine of environmentally and occupationally exposed people are ICP-MS, ICP-AES, and ETAAS (NIOSH, 1994; WHO, 1996; CDC, 2017, 2019). Since biological cobalt often occurs in complex matrices at very low levels, pre-treatment is commonly required before samples are analysed. Preconcentration techniques for cobalt quantification in biological samples are, in essence, similar to those discussed earlier (Table 1.5).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Cobalt is ubiquitous in the environment, generally occurring at low levels in rocks, soil and sediments, groundwater and surface water, and air (Hamilton, 1994; ATSDR, 2004; WHO, 2006). The upper continental crust has an average cobalt abundance of 17 mg/kg (Rudnick & Gao, 2014). Cobalt is not usually detected in drinking-water, but levels of a few micrograms per litre have been measured in samples from lakes, groundwater, and spring and well water (Hamilton, 1994). Cobalt concentrations in soil, where it usually occurs as cobalt(II), are generally within the range of 1–50 mg/kg, with an average concentration of 7 mg/kg (Hamilton, 1994; ATSDR, 2004). In ambient air, cobalt is mainly associated with the resuspension of soil particles. Atmospheric cobalt levels at unpolluted sites are generally < 2.0 ng/m^3 (Hamilton, 1994; Leyssens et al., 2017). However, anthropogenic activities such as mining, smelting and other related industrial processes, coal combustion, and vehicular traffic result in elevated levels of cobalt and cobalt compounds in the environment (Barciela-Alonso et al., 2003; Banza et al., 2009; Guéguen et al., 2012; Boev et al., 2013; Bari et al., 2015; Kamunda et al., 2016; Leyssens et al., 2017; Pan et al., 2017; Kravchenko and Lyerly, 2018; Mwaanga et al., 2019). Table 1.7 illustrates the occurrence of cobalt in environmental matrices such as ambient air, household dust, soil, and various water sources.

Because of the widespread occurrence of cobalt, the primary routes of human exposure are by inhaling ambient air (Rivas et al., 2014, Bari et al., 2015; Mohmand et al., 2015), ingesting

Table 1.6 Analytical methods for the measurement of cobalt in biological samples

Sample matrix	Sample preparation	Analytical technique (method)	LOD	Reference
Urine	Urine dilution and acidification	ICP-MS with DRC technology (CDC Method 3018.6–06)	0.023 µg/L	CDC (2019)
Urine	Urine chelation and concentration, and acidification	ETAAS	0.1–0.3 µg/L	WHO (1996)
Urine	Complexation with lithium bis(trifluoroethyl) dithiocarbamate)	GC-MS	1 µg/L	Aggarwal et al. (1992)
Urine	Extraction with HMADTC–xylene in diisopropylketone	ETAAS	6 µg/L	Bouman et al. (1986)
Urine	Urine dilution and acidification	ICP-MS with DRC technology (Method developed according to the Polish/European norm PN-EN ISO/IEC 17 025:2005)	0.004 µg/L	Brodzka et al. (2013)
Urine	Pre-concentration using 5-Br-PADAP and Amberlite XAD-7 resin	ICP-AES	25 ng/L	Farias et al. (2002)
Urine and saliva	Liquid–liquid microextraction with 1N2N–$[C_6MIM][PF_6]$ complex	ETAAS	3.8 ng/L	Berton & Wuilloud (2010)
Serum and urine	Liquid–liquid extraction with the APDC–IMBK complex	ETAAS (Method derived from the IUPAC reference method for nickel determination)	Serum, 1.93 nmol/L; urine, 1.89 nmol/L	Baruthio & Pierre (1993)
Serum	Complexation with DMG	AdSV	0.007 µg/L	Kajič et al. (2003)
Blood and urine	Ion exchange extraction	ETAAS	2–3 nmol/L	Alexandersson (1988)
Blood	Extraction with an alkaline extraction mixture	ICP-MS	NR	Capiau et al. (2020)
Whole blood	Collection in tube with anticoagulant, mixing and dilution of sample	ICP-MS (CDC Method 3030.1–03)	0.06 µg/L	CDC (2017)
Blood or tissue	Acid digestion	ICP-AES (NIOSH Method 8005)	Blood, 1 µg/100 g; tissue, 0.2 µg/g	NIOSH (1994)
Placenta and cord blood	Microwave-assisted acid digestion	ICP-MS	0.002 ng/g	Fagerstedt et al. (2015)
Hair	Microwave-assisted acid digestion	ICP-MS	0.001 µg/g	Elenge et al. (2011)

1N2N, 1-nitroso-2-naphthol; 5-Br-PADAP, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; AdSV, adsorptive stripping voltammetry; APDC, ammonium pyrroldinedithiocarbamate; $[C_6MIM][PF_6]$, 1-hexyl-3-methylimidazolium hexafluorophosphate; CDC, Centers for Disease Control and Prevention; DMG, dimethylglyoxime; DRC, dynamic reaction cell; ETAAS, electrothermal atomic absorption spectrometry; GC-MS, gas chromatography-mass spectrometry; HMADTC, *N*,*N*-hexamethyleneammonium-hexa-methylenedithiocarbamic acid; IBMK, 4-methyl-pentant-2-one (isobutyl methyl ketone); ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; ISO/IEC, International Organization for Standardization/International Electrotechnical Commission; IUPAC, International Union of Pure and Applied Chemistry; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health.

drinking-water (Amer et al., 1990; Iqbal et al., 2012; Ndilila et al., 2014; Mwesigye et al., 2016), and consuming food grown at contaminated sites (Gál et al., 2008; Mwesigye et al., 2016; Sharma et al., 2018; Cheng et al., 2019). Airborne particulate matter is a complex mixture of solids or liquids with different masses, numbers, sizes, shapes, surface areas, chemical compositions, acidities, and solubilities (Fortoul et al., 2015). The origins of the particles have critical effects on their size (or aerodynamic diameter), composition, transport in the atmosphere, and their ability to be inhaled into the respiratory system and cause health effects (Potter et al., 2021; Alghamdi et al., 2022). It is generally assumed that particulate matter with an aerodynamic diameter of 0.1 μm or less – $PM_{0.1}$ (ultrafine) particles – have highly toxic properties because they have large surface areas that can absorb a variety of toxic substances, such as cobalt (Slezakova et al., 2013). [The Working Group noted that the $PM_{0.1}$ particles described here and the NPs mentioned in Section 4 of the present monograph are generally equivalent in terms of aerodynamic diameter.] After inhalation, the ultrafine particulates can penetrate deep into the circulatory system via the respiratory tract and are translocated into various organs in the body (Kwon et al., 2020; Schraufnagel 2020; Phairuang et al., 2021; Potter et al., 2021).

Numerous studies of contaminated soils from areas where mining or smelting takes place have documented trace metal uptake by food crops, vegetables and fruits, and wild fish (Table 1.7). The results suggest that the consumption of these foodstuffs may be a significant contributor to cobalt intake (Cheyns et al., 2014; Mwesigye et al., 2016; Cheng et al., 2019). In addition, a substantial body of evidence suggests that unintentional ingestion of contaminated soil and dust, even at low cobalt concentrations, is a significant environmental exposure pathway to humans, because of the potential for long-term exposure (Cheyns et al., 2014; Ndilila et al., 2014). In soil, cobalt remains distributed between highly soluble and exchangeable fractions and relatively unreactive residual mineral phases. The distribution of cobalt in the solid phase influences the mobility and bioavailability of the element. Bioavailability based on the form of exposure (soluble versus insoluble) has been identified as an important factor influencing exposure to cobalt metal and its alloys and compounds (Behl et al., 2015). Indoor settled dust that contains cobalt may represent an important source of exposure, because people spend up to 90% of their time indoors in places such as homes, workplaces, and schools (Rivas et al., 2014). Important indoor sources of cobalt include decorative paints, cleaning products, and combustion products that result from cooking, heating, and smoking (Vilaplana et al., 1988; Bocca et al., 2014; Pinto et al., 2017). Although the process by which cobalt permeates human skin is not well documented (Larese Filon et al., 2004; Leyssens et al., 2017), skin contact with contaminated soil or water may also increase the potential for exposure (Ngole-Jeme & Fantke, 2017).

1.4.2 Occupational exposure

(a) Overview of occupational exposure scenarios

The main route of occupational exposure to cobalt is expected to be via the respiratory tract because of the inhalation of dust, fumes, or mists containing cobalt (IARC, 1991). Dermal exposure to cobalt (e.g. resulting from the deposition of particles and dust, handling of hard-metal items during production, and touching production equipment and other work material) is also a concern because of the potential for direct absorption through the skin and/or hand-to-mouth contact (Scansetti et al., 1994; Kettelarij et al., 2018a). Occupational exposure mainly occurs in industries involved in hard-metal production, processing, and use; during the production of cobalt powder; in the use of

Table 1.7 Occurrence of cobalt species in environmental matrices, foodstuffs, tobacco, and cosmetics

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Contaminated air, water, sediment, dust, and soil							
Outdoor ambient air: $PM_{2.5}$ samples	Beijing city, China, 2008–2012	$n_{suburban}$ = 59 n_{urban1} = 66 n_{urban2} = 63	Suburban: 0.38 ng/m³ (NR) Urban1: 0.36 ng/m³ (NR) Urban2: 0.35 ng/m³ (NR)	0.37 ng/m³ (0.28 ng/m³); percentiles calculated for the entire data set	ICP-MS (NR)		Wu et al. (2012b)
Indoor ambient air: PM_1 samples	Edmonton city, Canada, 2010	Winter: n = 173 Summer: n = 329	Winter: NR (0.007–1.6 ng/m³) Summer: NR (0.0–0.9 ng/m³)	Winter: 0.03 ng/m³ (NR) Summer: 0.02 ng/m³ (NR)	ICP-MS (NR)		Bari et al. (2015)
Indoor ambient air: $PM_{2.5}$ samples	Barcelona city, Spain, 2012–2013	n = 77	0.21 ng/m³ (< 0.1–0.81)	0.16 ng/m³ (NR)	ICP-MS (NR)		Rivas et al. (2014)
Household settled dust	Lahore and Sargodha cities, Pakistan, date of sample collection NR	n_{rural} = 10 n_{urban} = 10 $n_{industrial}$ = 10	Rural: [1.7 µg/g (1–2)][a] Urban: [3 µg/g (1–6)][a] Industrial: [2 µg/g (1–3)][a]	Rural: [1.6 µg/g][a] (NR) Urban: [2 µg/g][a] (NR) Industrial: [2 µg/g][a] (NR)	ICP-MS (0.001 µg/L)		Mohmand et al. (2015)
Soil and household settled dust	Copperbelt region of Zambia, date of sample collection NR	n_{soil} = 36; n_{dust} = 31	Soil: 4.63[b] mg/kg (< LOD–18.1) Dust: 1.80[b] mg/kg (< LOD–227)	NR	ICP-AES (NR)	EPA Method 3050B Revision 2, US EPA (1996)	Ndilila et al. (2014)
Household settled dust: indoor and paired outdoor samples	Katanga Copperbelt, Democratic Republic of the Congo, 2009–2011	n = 26	Indoor dust: 490 µg/g (NR) Outdoor dust: 330 µg/g (NR)	NR	ICP-OES (0.07 µg/g)		Cheyns et al. (2014)
Road dust	Xi'an city, China, 2015	n = 90	30.9 mg/kg (15.2–54.8)	29.4 mg/kg (NR)	XRF (0.5–1.0 mg/kg)		Pan et al. (2017)
Bottom ash from informal e-waste recycling	Agbogbloshie, Accra city, Ghana, 2012–2013	n = 210 (collected from 3 different sites)	Site 1: 123 mg/kg (NR) Site 2: 87 mg/kg (NR) Site 3: 96 mg/kg (NR)	NR	FAAS (NR)		Obiri et al. (2016)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Mine soil: tailings and topsoil	Witwatersrand Gold Mining Basin, South Africa, date of sample collection NR	$n = 73$	25.6 mg/kg (11.8–33.7)	NR	ICP-MS (0.001 µg/L)		Kamunda et al. (2016)
Geophagic soil samples	Democratic Republic of the Congo, Togo, South Africa, and Eswatini, date of sample collection NR	$n_{total} = 57$; $n_{DRC} = 14$; $n_{South\ Africa} = 27$; $n_{Swaziland} = 12$; $n_{Togo} = 4$	Democratic Republic of the Congo: 6.57 mg/kg (NR) South Africa: 14.30 mg/kg (NR) Eswatini: 10.86 mg/kg (NR) Togo: 4.95 mg/kg (NR)	NR	ICP-MS (NR)		Ngole-Jeme et al. (2018)
Kindergarten soil	Yerevan city, Armenia, 2012	$n = 111$	15.2 mg/kg (10.6–20.0)	15 mg/kg (NR)	XRF (5 mg/kg)	EPA Standard Method 6200	Tepanosyan et al. (2017)
Paddy field topsoil	Xiangtan city, China, 2017	$n = 63$	18.75 mg/kg (16.5–21.7)	NR	ICP-AES (NR)		Deng et al. (2019)
Lake water	Haro River, Khanpur, Pakistan, 2009	$n = 50$	0.303 mg/L (0.081–0.848)	NR	FAAS (NR)		Iqbal et al. (2012)
Groundwater	Najran city, Saudi Arabia, 2012	$n = 11$	0.02 µg/L (0.01–0.09)	NR	ICP-MS (0.002 µg/L)		Brima (2017)
Domestic water	Kilembe copper mine, Uganda, 2014	$n = 12$	20 µg/L (0.03–66)	NR	ICP-MS (NR)		Mwesigye et al. (2016)
Marine sediment	Ria of Ferrol, Spain, date of sample collection NR	$n = 35$	10 µg/g (5.0–21.0)	NR	ETAAS (0.43 µg/g)		Barciela-Alonso et al. (2003)
Mangrove sediments	Shenzhen, China, date of sample collection NR	$n = 27$	129 mg/kg (15.0–502)	NR	ICP-AES (1 mg/kg)		Xu et al. (2015)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Food, beverages, and wild fish							
Food crops: Wheat grains Mustard seeds Rice grains Maize grains	Ropar wetland, Punjab, India, 2013	n_{total} = 36 n_{wheat} = 9 $n_{mustard}$ = 9 n_{rice} = 13 n_{maize} = 5	Units, mg/kg dw: Wheat: 14.0 (12.8–14.7) Mustard: 13.5 (12.4–14.8) Rice: 15.2 (13.5–16.8) Maize: 15.1 (14.0–16.1)	NR	FAAS (NR)		Sharma et al. (2018)
Food crops: *Amaranthus tricolour* Maize Bananas	Kilembe copper mine, Uganda, 2014	31 4 5	Units, mg/kg dw: 4.20 (0.01–81) 0.16 (0.01–0.47) 0.17 (0.01–0.50)	NR	ICP-MS (NR)		Mwesigye et al. (2016)
Cassava		2	0.78 (0.15–1.41)				
Mangoes		2	0.26 (0.26–0.41)				
Rice	Gonbad county, Iran (Islamic Republic of), 2018	n = 90 (total), n = 8 (Gonbad)	NR (0–0.110 625 mg/kg)	NR	NR		Kiani et al. (2021)
Fish	Gaotang Lake, Huainan, China, date of sample collection NR	n = 28 Crucian carp: n = 5 Bighead carp: n = 5 Silver carp: n = 4 Tilapia: n = 5 Common carp: n = 5 Grass carp: n = 4	Units, mg/kg fw: Crucian carp: 0.074 Bighead carp: 0.101 Silver carp: 0.076 Tilapia: 0.089 Common carp: 0.060 Grass carp: 0.149	NR	ICP-MS (1 µg/kg)		Cheng et al. (2019)
Vegetables (excluding potatoes)	France, 2007–2009	n = 269	0.0061 mg/kg fw (NR)	NR	ICP-MS (0.002 mg/kg)	French Total Diet Study	Arnich et al. (2012)
Potatoes	Lebanon, 2008	n = 15	37.53 µg/kg fw (34.50–44.10)	NR	ICP-MS (0.0005 mg/kg)	Lebanese Total Diet Study	Nasreddine et al. (2010)

Table 1.7 (continued)

Sample type	Location and collection date	No. of samples	Mean[c] (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Beverages	France, 2006–2007	n = 143	0.007 mg/kg fw (0.001–0.032)	NR	ICP-MS (NR)	French Total Diet Study	Noël et al. (2012)
Infant formulas (RTU, CL, or P formula)	Canada, 1986–1987	n_{RTU} = 49	RTU: 1.22 ng/g (0.21–5.2)	RTU: 0.53 ng/g	GFAAS (0.04–0.21 µg/kg)		Dabeka (1989a, b)
		n_{CL} = 50	CL: 2.89 ng/g (0.25–11.8)	CL: 2.27 ng/g			
		n_{P} = 64	P: 12.7 ng/g (2.6–53)	P: 9.54 ng/g			
Tobacco and cosmetics							
Tobacco	Oporto city, Portugal, 2014	n = 40	0.84 µg/g dw (0.61–1.08)	NR	ICP-MS (0.006 µg/g)		Pinto et al. (2017)
Henna dye	Republic of Korea, date of sample collection NR	n = 15	NR (LOD–3.54 mg/kg)	NR	FAAS (1.25 mg/L)		Kang & Lee (2006)
Hair dyes	Kashan city, Iran (Islamic Republic of), 2019	n = 36	0.475 µg/g (NR)	NR	ICP-OES (0.007 µg/g)		Mostafaii et al. (2022)
Eye liner	Seoul city, Republic of Korea, date of sample collection NR	n = 13	11.80 µg/g (0.11–41.08)	NR	ICP-MS (NR)		Lim et al. (2018)

CL, concentrated liquid; DRC, Democratic Republic of the Congo; dw, dry weight; EPA, Environmental Protection Agency; ETAAS, electrothermal atomic absorption spectrometry; e-waste, electronic and/or electrical waste; FAAS, flame atomic absorption spectrometry; fw, fresh weight; GFAAS, graphite furnace atomic absorption spectrometry; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma with optical emission spectrometry; IQR, interquartile range; LOD, limit of detection; NR, not reported; P, powdered; PM_1, particulate matter with aerodynamic diameter < 1.0 µm; $PM_{2.5}$, particulate matter with aerodynamic diameter < 2.5 µm; RTU, ready to use; XRF, X-ray fluorescence spectrometry.

[a] Working Group conversion to International System of Units.

[b] Geometric mean.

[c] Mean values are expressed as the arithmetic mean unless stated otherwise.

cobalt-containing pigments and driers; battery production; and electronics recycling. The largest occupational groups exposed are likely to include welders and related machine operators; dental technologists, technicians, and laboratory assistants; and machinists and machining and tooling inspectors. Other occupations with potential for exposure to cobalt include workers involved in smelting and refining, the mining of ores containing cobalt and other metals, cobalt dye painting, cobalt chemical production, diamond polishing, glassware or porcelain work, offset printing, goldsmithing, and rockwool insulation (IARC, 1991, 2006; Donaldson & Beyersmann, 2011; CAREX Canada, 2022).

The National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), conducted between 1981 and 1983, estimated the number of workers exposed to chemical, physical, and biological agents, including cobalt compounds, on the basis of site visits to approximately 4500 workplaces, and representing roughly 1.8 million workers across the USA. Using industrial classifications, the survey estimated that metallic cobalt exposure was most common among workers in primary metal industries (19.2% of all exposed workers). Exposure to cobalt(II) compounds was most common in the chemicals and applied products industries, representing 47.0%, 40%, 55.1%, and 71.2% of all workers exposed to cobalt(II) chloride, cobalt(II) acetate, cobalt(II) oxide, and cobalt(II,III) oxide, respectively (NIOSH, 1990). From an occupational perspective, welders and cutters, chemical technicians, and clinical laboratory technologists and technicians were groups estimated to be most likely to be exposed to metallic cobalt (13.8% of exposed workers), cobalt(II) chloride (38.7% of exposed workers), and cobalt(II) acetate (15.4% of exposed workers), respectively (NIOSH, 1990).

Table 1.8 summarizes the data on occupational exposure to cobalt, as measured by analysis of concentrations in air, or via biological monitoring of cobalt concentrations in blood or urine samples among workers, in various types of industries and at different production stages. Occupational exposure to cobalt occurs predominantly during refining of cobalt, production of cobalt metals and cobalt compounds, use of diamond–cobalt tools, production of dental materials, manufacture of nickel–hydrogen batteries, and plate painting with cobalt pigments. Workers may be exposed to a mixture of various cobalt compounds and cobalt metal powders. In addition, the potential for co-exposure to nickel and other known or suspected human carcinogens has been reported in various occupational studies (see Table 1.8 and Table 1.9).

Scarselli et al. (2020) reported the ranges of exposure levels of cobalt and cobalt(II) compounds from an occupational exposure registry in Italy between 1996 and 2016. Most exposures occurred during the manufacture of fabricated metal products (50%) and among metal finishing, plating, and coating machine operators (42%). The manufacture of basic metals was the industrial sector in which exposure to cobalt, as a metal, was most frequently reported (mainly for metal smelters, casters, and rolling-mill operators), whereas exposure to cobalt nitrate was principally reported in the manufacture of fabricated metal products (mainly for metal finishing, plating, and coating machine operators). Exposure to cobalt sulfate was widespread in all sectors, except for the manufacture of basic metals. Overall, cobalt sulfate heptahydrate was the compound with the highest mean level of exposure (GM, 1.09 μg/m^3), whereas cobalt nitrate had the lowest level (GM, 0.11 μg/m^3) (Scarselli et al., 2020).

Table S1.10 summarizes the distribution of air concentrations of cobalt across the industrial sectors recorded by the Italian occupational exposure registry (see Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). The manufacture

Table 1.8 Occupational exposure to cobalt as measured among workers in various types of industries and production stages

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
General industrial settings						
Industrial settings obtained from Italian occupational exposure registry, Italy, 1996–2016	Personal or stationary air of Co and Co compounds	459	Co: 0.33 µg/m³ [a]		Over whole 8-h work shift sampling.	Scarselli et al. (2020)
		109	Co sulfate heptahydrate: Co 1.09 µg/m³ [a]			
		50	Co acetate: Co 0.81 µg/m³ [a]			
		92	Co chloride: Co 0.76 µg/m³ [a]			
		112	Co nitrate hexahydrate: Co 0.61 µg/m³ [a]			
		67	Co acetate tetrahydrate: Co 0.50 µg/m³ [a]			
		325	Co sulfate: Co 0.44 µg/m³ [a]			
		76	Co chloride hexahydrate: Co 0.42 µg/m³ [a]			
		369	Co nitrate: Co 0.11 µg/m³ [a]			
Occupational exposure in different Co industries, United Kingdom, 1988–1991	Urinary Co	780		93.0 (90th, 486) nmol/mmol creatinine	End of working week at end-of-shift sampling; workers of chemical manufacture (manufacturing and handling of Co powders, Co salts, and pigments).	White & Dyne (1994)
	Urinary Co			19.0 (90th, 107) nmol/mmol creatinine	Same sampling; workers of hard-metal manufacture (pre-sinter operations such as mixing, pressing, and furnace operation, and post-sinter grinding operations).	
	Urinary Co			17.0 (90th, 80) nmol/mmol creatinine	Same sampling; workers of hard-metal finishing (grinding and sharpening of hard-metal tools).	
	Urinary Co			< 3.0 (90th, 42) nmol/mmol creatinine	Same sampling; workers of other metal working (welding and prosthesis manufacture using Co-containing metals and alloys).	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Cobalt refinery and production of cobalt metal and salts						
Co refinery workers, Belgium, 1993	Personal air of Co dusts	82	127.5 (2–7700) μg/m³ on Monday 120.9 (1–7772) μg/m³ on Friday	84.5 μg/m³ on Monday 110.0 μg/m³ on Friday	Workers exposed to a mixture of various Co salts, oxides, and fine Co metal powders; 6 h sampling.	Swennen et al. (1993)
	Blood Co	82	1.10 (0.2–12.0) μg/dL	1.10 μg/dL	Monday end-of-shift sampling.	
	Blood Co	82	1.27 (0.2–12.0) μg/dL	1.20 μg/dL	Friday end-of-shift sampling.	
	Urinary Co	82	52.9 (2.66–2245) μg/g creatinine	44.1 μg/g creatinine	Monday end-of-shift sampling.	
	Urinary Co	82	69.8 (1.56–2038) μg/g creatinine	72.4 μg/g creatinine	Friday end-of-shift sampling.	
Co refinery workers, Belgium, 2008–2009	Personal air of Co dusts	249	NR (1–108 μg/m³) in 2007	15.0 (1.0–108.0) μg/m³ in 2007	Workers exposed to a mixture of various Co salts, oxides, and fine Co metal powders; 6 h sampling; Follow-up in engineering improvements from Swennen et al. (1993).	Lantin et al. (2011, 2013)
	Blood Co	249		0.10 (< 0.05–3.20) μg/100 mL		
	Urinary Co	249		3.9 (0.3–204.3) μg/g creatinine		
Co plant that produces fine Co metal powders, Co oxides, and Co salts, Belgium, 1988–2001	Urinary Co	122	Dry-stage area (approx. 70–250 μg/g creatinine) was the highest, followed by wet-stage area (approx. 15–55 μg/g creatinine) and mixed-exposure area (135–35 μg/g creatinine)		End of working week end-of-shift sampling; Three types of exposure: (1) production of Co metal powder, Co oxides, or salts in the dry-stage area; (2) wet-stage area, and (3) mixed exposure for maintenance workers and foremen involved at different steps of the process.	Verougstraete et al. (2004)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Production of Co metal and Co salts, Kokkola, Finland, 1967–2000	Stationary and personal air of Co dusts	110 to 93	Range, 0.05–0.25 mg/m³		Reduction and powder production.	Linna et al. (2003, 2004)
	Stationary and personal air of Co sulfates		Range, 0.02–1.0 mg/m³		Sulfatizing roasting process.	
	Stationary and personal air of Co sulfates		Range, 0.01–0.05 mg/m³		Leaching and solution purification process.	
	Stationary and personal air of mixtures of Co sulfates, carbonates, oxides, and hydroxides		Range, 0.01–0.20 mg/m³		Chemical processing department.	
	Urinary Co		Maximum, 16 000 nmol/L		Reduction and powder production.	
	Urinary Co		Range, 300–2000 nmol/L		Purification and chemical department.	
Production of Co metal and Co salts, Kokkola, Finland, 1999–2006	Urinary Co	29		240 (range, 11–4107) nmol/L	Reduction and powder production 1999–2000.	Linna et al. (2020)
	Urinary Co	113		230 (range, 6–6278) nmol/L	Reduction and powder production 2005–2006.	
Cobalt containing diamond tooling						
Diamond polisher in workshops, Belgium	Stationary air of Co dusts	8	17.9 (0.1–45.0) µg/m³		No "hard metals" have been found; 1 m sampling from the middle of the rotating disc in the worker's breathing zone.	van den Oever et al. (1990)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Diamond polisher in workshops, Belgium (date of sample collection NR)	Personal air of Co dusts	92	15.1 (0.7–42.8) µg/m³		Polishing discs showed no presence of tungsten (i.e. no "hard metal"); higher exposure group; sampling began 2 h after starting work and lasted until 1 h before the end of the working day.	Nemery et al. (1992)
	Stationary air of Co dusts	92	10.2 (3.8–19.8) µg/m³			
	Personal air of Co dusts	102	5.3 (0.2–11.2) µg/m³		Lower exposure group.	
	Stationary air of Co dusts	102	1.6 (0.5–4.3) µg/m³			
	Urinary Co	92	20.5 (2.3–75.0) µg/g creatinine		Higher exposure group.	
	Urinary Co	102	7.0 (0.7–26.5) µg/g creatinine		Lower exposure group.	
Alloys or plating containing cobalt						
Manufacturers of permanent magnets, USA, 1988	Personal air of Co dusts	100	17.5[a] (1–466) µg/m³		Whole-work-shift sampling; Ni: 4.4 (ND–368) µg/m³; Nd: 2.6 (ND–52) µg/m³. Sm: 3.9[a] (ND–528) µg/m³.	Deng et al. (1991)
Electroplaters in bright plating factory (date of sample collection NR)	Stationary air of Co dusts	42	Below LOD (< 0.12 ng/m³)		Cr: 0.0002 ± 0.0001 mg/m³.	Wultsch et al. (2017)
	Blood Co	42	0.85 ± 0.32 µg/L		Cr: 0.44 ± 0.24 (µg/L).	
Dental procedures						
Dental technicians, Sweden (date of sample collection NR)	Stationary air of Co dusts	8	Maximum value of 1.6 mg/m³		Whole-work-shift sampling; CoCrMo alloys (60–66%, 25–32%, 4–6% content, respectively); without local exhaust ventilation.	Seldén et al. (1995)

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Dental technicians of metal prostheses, Ankara, Türkiye (date of sample collection NR)	Urinary Co	23	24.8 (0.4–111.5) µg/g creatinine		End-of-shift sampling after four consecutive exposure periods; Ni: 7.7 (4.2–12.6) µg/g creatinine; Cr: 4.4 (0.5–11.9) µg/g creatinine.	Burgaz et al. (2002)
Dental technicians of metal prostheses, Indonesia (date of sample collection NR)	Blood Co	40	26.8 (95% CI, 14.8–38.7) µg/L		Morning (07:00–09:00) sampling; Ni: 36.8 (95% CI, 22.0–51.6) µg/L; Cr: 346.4 (95% CI, 303.8–388.9) µg/L.	Berniyanti et al. (2020)
Cobalt pigments						
Porcelain plate painters exposed to Co blue dye in porcelain factory, Denmark, 1981	Personal air of Co dusts	19		0.80 (0.068–8.61) Co mg/m^3	3 h sampling; Co blue underglaze colour is made by melting together a mixture of Co-Zn-silicate, Zn oxide, and silicon oxide; there was no detectable concentration of silica.	Raffn et al. (1988)
Porcelain plate painters exposed to Co blue dye in porcelain factory, Denmark, 1982	Blood Co	46	36.7 (3.40–407) nmol/L		Working 4 wk.	Raffn et al. (1988)
	Blood Co	46	8.05 (1.70–22.1) nmol/L		Off work for 6 wk.	
	Urinary Co	46	141.8 (4.04–2776) nmol/mmol creatinine		Working 4 wk.	
	Urinary Co	46	8.82 (< 1.70–65.2) nmol/mmol creatinine		Off work for 6 wk.	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Pottery plate painters, Copenhagen, Denmark, 1982 and 1984	Personal air of soluble Co-Zn-silicate	46	Range, 0.07–8.61 mg/m^3		No correlation: air/blood and air/urine.	Christensen & Mikelsen (1986)
	Personal air of insoluble Co aluminate dyes	15	Range, 0.05–0.25 mg/m^3			
	Blood Co of soluble Co-Zn-silicate	46	2.16 (0.2–24) µg/L	1.00 µg/L	Significant correlation: blood/urine (r = 0.82).	
	Blood Co of insoluble Co aluminate dyes	15	0.63 (0.37–1.58) µg/L	0.60 µg/L	Significant correlation: blood/urine (r = 0.88).	
	Urinary Co of soluble Co-Zn-silicate	46	8.35 (0.24–163) µg/mmol creatinine	2.67 µg/mmol creatinine		
	Urinary Co of insoluble Co aluminate dyes	15	0.13 (0.02–0.37) µg/mmol creatinine	0.11 µg/mmol creatinine		
Pottery plate painters, Copenhagen, Denmark, 1982–1991	Personal air of soluble Co-Zn-silicate	8–100	Mean range 1982–1991; 1356–454 nmol/m^3		Follow-up from Christensen & Mikelsen (1986).	Christensen & Poulsen (1994)
	Urinary Co of soluble Co-Zn-silicate	27–145	Mean range 1982–1991; 133.4–18.8 nmol/mmol creatinine		Follow-up from Christensen & Mikelsen (1986).	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Electrical industry						
Battery plant manufacturing Ni–hydrogen batteries, Japan (date of sample collection NR)	Personal air of Co dusts	30	0.067 (0.004–0.330) mg/m³		Over 9 h sampling; Ni: 0.481 (0.018–2.376) mg/m³; significant correlation: Co/Ni (r = 0.96).	Yokota et al. (2007)
	Urinary Co	128	38.6 (1.0–76.8) µg/L corrected for specific gravity		End of working week end-of-shift sampling. Ni: 21.5 (5.0–67.5) µg/L corrected for specific gravity. Significant correlation: Co/Ni (r = 0.70).	
Digital video cassette manufacturing plant, Japan (date of sample collection NR)	Personal air of Co oxide dusts, including both cobalt(II) oxide and cobalt(II,III) oxide	20	Range, below LOD < 1 to approx. 22 µg/m³		8 h sampling; significant correlation: air/urine (r = 0.76).	Fujio et al. (2009)
	Urinary Co	20	Range, below LOD < 1 to approx. 27.5 µg/g creatinine		End-of-shift sampling.	
E-waste recycling industry						
Recycling workers, Sweden, 2007–2009	Personal air of Co dusts	77	0.066[a] (0.0017–3.3) µg/m³		Inhalable fraction according to EN 481; 10 h work shift sampling; Hg: 0.011[a] (0.00 031–0.21) µg/m³; Pb: 7.0[a] (0.011–130) µg/m³; Cd: 0.18[a] (0.0011–11) µg/m³; Ni: 0.49[a] (0.0089–15) µg/m³; Cr: 0.45[a] (0.0050–6.9) µg/m³; As: 0.04[a] (0.001–0.730) µg/m³; Sb: 0.21[a] (0.0041–1.1) µg/m³.	Julander et al. (2014)
	Blood Co	50		0.081 (0.050–0.67) µg/L	Hg: 1.4 (0.28–18) µg/L; Pb: 32 (9.5–230) µg/L; Cr: 1.4 (0.34–5.0) µg/L.	
	Urinary Co	52		0.25 (0.12–1.3) µg/L	Hg: 1.4 (0.35–4.4) µg/L; Pb: 1.8 (0.19–17) µg/L; Cd: 0.37 (0.12–2.4) µg/L; Cr: 0.74 (0.0097–5.29) µg/L; As: 13 (2.4–410).	

Table 1.8 (continued)

Occupational group/job type, location, and time period	Monitoring method	No. of samples	Mean (range or ± SD)	Median (range or percentile)	Comments	Reference
Recycling workers, Germany, 2017–2018	Personal air of Co dusts	40	0.041[a] (0.018–0.31) μg/m^3	0.035 μg/m^3	Inhalable fraction; during disassembly work; Ni: 0.27[a] (0.058–1.9) μg/m^3; Sb: 0.091[a] (0.051–0.34) μg/m^3; Cr: 0.20[a] (0.081–1.4) μg/m^3; As: 0.033[a] (0.021–0.069) μg/m^3; Cd: 0.017[a] (0.005–0.23) μg/m^3; Hg; 0.47[a] (0.14–3.3) μg/m^3.	Gerding et al. (2021)
	Stationary air of Co dust	21	0.035[a] (0.015–0.18) μg/m^3	0.033 μg/m^3	Inhalable fraction; during disassembly work; Ni: 0.11[a] (0.052–0.69) μg/m^3; Sb: 0.067[a] (0.047–0.17) μg/m^3; Cr: 0.12[a] (0.063–0.64) μg/m^3; As: 0.031[a] (0.018–0.130) μg/m^3; Cd: 0.009[a] (0.006–0.044) μg/m^3; Hg; 0.46[a] (0.16–6.6) μg/m^3.	
	Personal air of Co dusts	4	0.021[a] (0.018–0.024) μg/m^3	0.022 μg/m^3	Respirable fraction; during disassembly work; Ni: 0.076[a] (0.064–0.088) μg/m^3; Sb: 0.076[a] (0.064–0.088) μg/m^3; Cr: 0.077[a] (0.065–0.09) μg/m^3; As: 0.025[a] (0.022–0.029) μg/m^3; Cd: 0.008[a] (0.007–0.009) μg/m^3.	
	Stationary air of Co dust	12	0.034[a] (0.018–0.062) μg/m^3	0.034 μg/m^3	Respirable fraction; during disassembly work; Ni: 0.08[a] (0.047–0.20) μg/m^3; Sb: 0.064[a] (0.047–0.14) μg/m^3; Cr: 0.11[a] (0.065–0.24) μg/m^3; As: 0.033[a] (0.021–0.077) μg/m^3; Cd: 0.007[a] (0.005–0.015) μg/m^3.	
	Urinary Co	51	0.32[a] (0.15–1.6) μg/L	0.50 μg/L	End-of-shift sampling; Ni: 0.74[a] (0.15–3.8) μg/L; Sb: 0.26[a] (0.15–2.4) μg/L; Cr: 0.10[a] (0.08–1.1;) μg/L; As: 1.96[a] (1.0–8.6) μg/L; Cd: 0.16[a] (0.08–1.8) μg/L; Hg; 0.38[a] (0.1–4.6) μg/L.	

As, arsenic; Cd, cadmium; CI, confidence interval; Co, cobalt; Cr, chromium; e-waste, electronic and/or electrical waste; Hg, mercury; LOD, limit of detection; Mo, molybdenum; Nd, neodymium; ND, not detected; Ni, nickel; NR, not reported; Pb, lead; Sb, antimony; SD, standard deviation; Sm, samarium; W, tungsten; wk, week; Zn, zinc.

[a] Geometric mean ± geometric SD.

Table 1.9 Exposures to IARC Group 1 and Group 2A agents potentially co-occurring with cobalt in epidemiological studies considered by the Working Group[a]

Agent (CAS No.)	Occupational settings[c]											IARC Group (Vol., year)	Study design and reference	
	A	B	C	D	E	F	G	H	I	J	K		Cohort	Case–control
Arsenic and inorganic arsenic compounds (7440-38-2)				✓		✓	✓	✓				1 Vols 23, Suppl. 7, 100C (2012)	Marsh et al. (2009); Moulin et al. (1993)	Grimsrud et al. (2005); Rodrigues et al. (2020)
Asbestos (all forms) and other fibres[b] (1332-21-4, 12172-73-5, 12001-29-5, 12001-28-4)			✓	✓			✓	✓		✓		1 Vols 14 Suppl. 7, 100C (2012)	Moulin et al. (1993, 1998); Tüchsen et al. (1996); Wild et al. (2000)	Grimsrud et al. (2005); Rodrigues et al. (2020)
Benzene (71-43-2)								✓				1 Vols 29, Suppl. 7, 100F, 120 (2018)		Rodrigues et al. (2020)
Beryllium and beryllium compounds (7440-41-7)								✓				1 Vols 58, 100C (2012)		Rodrigues et al. (2020)
Cadmium and cadmium compounds (7440-43-9)	✓					✓		✓				1 Vols 58, 100C (2012)	Marsh et al. (2009); Li et al. (2021a)	Rodrigues et al. (2020); Bai et al. (2019)
Chromium(VI) compounds (18540-29-9)	✓							✓			✓	1 Suppl. 7, Vols 49, 100C (2012)	Hogstedt & Alexandersson (1990); Moulin et al. (1998, 2000); Wild et al. (2000); Li et al. (2021a) (chromium species not specified)	Bai et al. (2019) (chromium species not specified); Rodrigues et al. (2020)
Crystalline silica[b] (14808-60-7)								✓			✓	1 Suppl. 7, Vols 68, 100C (2012)	Wild et al. (2000)	Rodrigues et al. (2020)
Epichlorohydrin (106-89-8)								✓				2A Vols 11, Suppl. 7, 71 (1999)		Rodrigues et al. (2020)
Formaldehyde (50-00-0)								✓				1 Suppl. 7, Vols 62, 88, 100F (2012)		Rodrigues et al. (2020)
Lead (7439-92-1)												2A Suppl. 7, Vol. 87 (2006)		Rodrigues et al. (2020)
Methylene chloride (75-09-2)								✓				2A Suppl. 7, Vols 71, 110 (2017)		Rodrigues et al. (2020)

Table 1.9 (continued)

Agent (CAS No.)	Occupational settings[c]											IARC Group (Vol., year)	Study design and reference	
	A	B	C	D	E	F	G	H	I	J	K		Cohort	Case–control
Nickel (7440-02-0)	✓	✓		✓	✓		✓	✓	✓	✓	✓	1 (Nickel compounds; and metallic) Suppl. 7, Vols 49, 100C (2012)	Cuckle et al. (1980); Hogstedt & Alexandersson (1990); Moulin et al. (1993, 1998, 2000); Sauni et al. (2017); Tüchsen et al. (1996); Wild et al. (2000); Li et al. (2021a); (nickel species not specified)	Bai et al. (2019); Grimsrud et al. (2005); Rodrigues et al. (2020); (nickel species not specified)
Perchloroethylene [tetrachloroethylene] (127-18-4)								✓				2A Suppl. 7, Vols 63, 106 (2014)		Rodrigues et al. (2020)
Sulfuric acid (7664-93-9) Included in acid mists, strong inorganic				✓				✓				1 Vols 54, 100F (2012)		Grimsrud et al. (2005); Rodrigues et al. (2020)
Trichloroethylene (79-01-6)								✓				1 Suppl. 7, Vols 63, 106 (2014)		Rodrigues et al. (2020)
Vinyl chloride (75-01-4)								✓				1 Suppl. 7, Vols 97, 100F (2012)		Rodrigues et al. (2020)

CAS No., Chemical Abstracts Service Registry number; IARC, International Agency for Research on Cancer; Suppl., supplement; Vol., volume.

[a] Not comprehensive of all possible co-exposures in the listed study populations. Cobalt metal with tungsten carbide was not included in this table as a co-exposure.

[b] A case series study was also available, Dufresne et al. (1996).

[c] Occupational settings: A, automobile manufacturing; B, manufacturing of cobalt and nickel salts; C, aluminium smelting; D, nickel refining E, metal plants; F, copper smelting; G, production of cobalt and sodium; H, semi-conductor and electronic storage-device manufacturing; I, production of cobalt powder; J, porcelain factories; K, production of stainless and alloyed steel.

of cutlery, tools, and general hardware showed the highest value (GM, 3.69 μg/m³, men; arithmetic mean, AM, 7.32 μg/m³) (Scarselli et al., 2020). Table S1.11 shows the distribution of air concentrations of cobalt across occupational groups in the industrial sectors recorded by the Italian occupational exposure registry (Scarselli et al., 2020; Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). The occupational groups with the highest measured exposures were machine-tool setters and setter operators (GM, 5.32 μg/m³, men; AM, 5.98 μg/m³), although this was based on a relatively low number of measurements (n = 50). Regarding the distributions of cobalt exposure levels according to the workforce size, microfirms (1–9 workers) had the highest value for cobalt exposure (GM, 1.06 μg/m³), whereas medium-sized firms (50–99 workers) had the lowest (GM, 0.07 μg/m³) (Scarselli et al., 2020).

The French National Institute for Research and Occupational Safety (INRS) has reported data for two periods of measurement of cobalt concentrations in air relating to industrial sectors and job titles, recorded between 1987 and 1999, and 2000 and 2020 (INRS, 2022). Table 1.12 and Table 1.13 summarize the distributions across industrial sectors and job titles, respectively, between 2000 and 2020. The manufacture of food products had the highest arithmetic mean value for personal air sampling (105 μg/m³), followed by human health and social work activities (76 μg/m³), manufacture of fabricated metal products (69 μg/m³), and mining and quarrying (49 μg/m³) (Table 1.12). Across the different job titles, clerical support workers (production clerks) had the highest arithmetic mean value (816 μg/m³), followed by farming, forestry, and fisheries advisers (616 μg/m³) (Table 1.13).

In a study in different industries in the UK in which urinary cobalt levels were collected during the period 1988–1991, it was reported that workers who manufactured and handled cobalt powders, cobalt salts, and pigments in chemical industries had the highest median concentration of cobalt in urine (93 nmol/mmol creatinine [48 μg/g creatinine]), followed by workers involved in hard-metal manufacturing (19 nmol/mmol creatinine [10 μg/g creatinine]) and hard-metal finishing (17 nmol/mmol creatinine [9 μg/g creatinine]), and workers involved in other metalworking industries (< 3.0 nmol/mmol creatinine [< 1.6 μg/g creatinine]) including welding and prosthesis manufacturing, where several cobalt-containing metals and alloys were employed (see Table 1.8) (White & Dyne, 1994).

Several scenarios or situations involving exposure to cobalt and cobalt compounds in occupational settings are summarized in the following sections.

(b) Skin exposure in occupational settings

Many cobalt alloys and platings can release significant amounts of cobalt upon contact with skin. Occupational skin exposure to cobalt has been studied in different settings (Julander et al., 2010). Skin exposure to metallic cobalt has been reported in several industrial settings as follows: in the manufacture of components for gas turbines and space propulsion using metal alloys, stainless steels, hard-metal items, and metal powders for thermal application (Julander et al., 2010); contact with raw and sintered materials and contaminated surfaces in hard-metal production (Kettelarij et al., 2018b); the handling of metal tools in the hairdressing trade, including hair clips, tweezers, sectioning clips, and straight razors (Symanzik et al., 2021); and hand-held work tools, including cutting tools, handsaws, and paint-scraping tools (Thyssen et al., 2011). Skin exposure can also occur in the construction industry. Several studies have reported hypersensitivity to cobalt chloride among construction workers (Bock et al., 2003; Uter et al., 2004; Lazzarini et al., 2012). Cases of on-the-skin exposure to cobalt have been reported for ceramics decorators (Kargar et al., 2013).

Table 1.12 Distribution of air concentrations of cobalt in industrial sectors, 2000–2020, France[a]

Industrial sector	Concentration of cobalt dusts in air (µg/m³)							
	Personal air				Stationary air			
	N	AM	Median	IQR	N	AM	Median	IQR
Mining and quarrying	11	49	12	9–29	34	23	10	5–15
Manufacture of food products	176	105	2	< LOQ–5	265	54	1	< LOQ–3
Manufacture of leather and related products	7	2	NC	NC	NC	NC	NC	NC
Manufacture of wood and of products of wood and cork, except furniture; manufacture of articles of straw and plaiting materials	45	37	11	4–42	31	17	3	< LOQ–16
Printing and reproduction of recorded media	5	< LOQ	NC	NC	NC	NC	NC	NC
Manufacture of coke and refined petroleum products	15	< LOQ	< LOQ	< LOQ to < LOQ	NC	NC	NC	NC
Manufacture of chemicals and chemical products	56	29	< LOQ	< LOQ–1	16	4	0	< LOQ–3
Manufacture of rubber and plastics products	26	8	1	0–3	7	19	NC	NC
Manufacture of other non-metallic mineral products	44	18	< LOQ	< LOQ–3	21	5	< LOQ	< LOQ to < LOQ
Manufacture of basic metals	170	20	2	< LOQ–9	100	12	4	< LOQ–8
Manufacture of fabricated metal products, except machinery and equipment	1095	69	4	< LOQ–23	371	97	2	< LOQ–9
Manufacture of computer, electronic, and optical products	35	9	< LOQ	< LOQ–4	19	1	< LOQ	< LOQ to < LOQ
Manufacture of electrical equipment	70	4	< LOQ	< LOQ–2	58	39	1	< LOQ–10
Manufacture of machinery and equipment	200	4	0	< LOQ–2	34	1	< LOQ	< LOQ to < LOQ
Manufacture of motor vehicles, trailers, and semi-trailers	46	1	< LOQ	< LOQ–0	28	1	< LOQ	< LOQ to < LOQ
Manufacture of other transport equipment	94	46	2	< LOQ–19	53	11	< LOQ	< LOQ–2
Manufacture of furniture	11	13	5	< LOQ–19	4	NC	NC	NC
Other manufacturing	334	21	2	< LOQ–10	134	6	< LOQ	< LOQ–3
Repair and installation of machinery and equipment	145	31	< LOQ	< LOQ–6	59	3	< LOQ	< LOQ to < LOQ
Electricity, gas, steam, and air-conditioning supply	9	1	NC	NC	21	< LOQ	< LOQ	< LOQ to < LOQ
Water supply; sewerage, waste management, and remediation activities	103	3	< LOQ	< LOQ–1	76	1	< LOQ	< LOQ to < LOQ
Construction	88	6	< LOQ	< LOQ–2	51	2	< LOQ	< LOQ to < LOQ
Wholesale and retail trade; repair of motor vehicles and motorcycles	105	45	1	< LOQ–12	66	17	< LOQ	< LOQ–3
Transportation and storage	24	1	< LOQ	< LOQ to < LOQ	31	< LOQ	< LOQ	< LOQ to < LOQ
Financial and insurance activities	9	3	NC	NC	58	< LOQ	< LOQ	< LOQ to < LOQ
Real estate activities	30	11	1	< LOQ–5	33	2	< LOQ	< LOQ to < LOQ

Table 1.12 (continued)

Industrial sector	Concentration of cobalt dusts in air (µg/m³)							
	Personal air				Stationary air			
	N	AM	Median	IQR	N	AM	Median	IQR
Professional, scientific, and technical activities	19	5	< LOQ	< LOQ to < LOQ	5	< LOQ	NC	NC
Administrative and support service activities	6	0	NC	NC	NC	NC	NC	NC
Public administration and defence; compulsory social security	35	< LOQ	< LOQ	< LOQ to < LOQ	5	< LOQ	NC	NC
Education	7	< LOQ	NC	NC	NC	NC	NC	NC
Human health and social work activities	25	76	2	< LOQ–25	11	< LOQ	< LOQ	< LOQ to < LOQ
Other service activities	6	1	NC	NC	29	2	< LOQ	< LOQ to< LOQ

AM, arithmetic mean; IQR, interquartile range; LOQ, limit of quantification; N, number of measurements; NC, not calculated.

[a] Data from the French National Institute for Research and Occupational Safety, INRS (2022).

Table 1.13 Distribution of air concentrations of cobalt according to job title, 2000–2020, France[a]

Job titles	Concentration of cobalt dusts in air (µg/m³)							
	Personal air				Stationary air			
	N	AM	Median	IQR	N	AM	Median	IQR
Professionals	50	358	5	< LOQ–50	46	297	12	2–59
Physical and earth science professionals	6	< LOQ	NC	NC	NC	NC	NC	NC
Farming, forestry, and fisheries advisers	29	616	34	11–94	40	341	16	5–61
Environmental protection professionals	12	2	1	< LOQ–2	6	< LOQ	NC	NC
Technicians and associate professionals	335	27	< LOQ	< LOQ–6	169	6	< LOQ	< LOQ–4
Clerical support workers	20	372	3	0–81	14	0	< LOQ	< LOQ to < LOQ
Stock clerks	8	11	NC	NC	NC	NC	NC	NC
Production clerks	9	816	NC	NC	NC	NC	NC	NC
Craft and related trades workers	1641	23	2	< LOQ–7	449	10	< LOQ	< LOQ–2
Metal, machinery, and related trades workers	1530	24	2	< LOQ–8	376	11	< LOQ	< LOQ–2
Handicraft and printing workers	48	2	< LOQ	< LOQ–2	49	1	< LOQ	< LOQ to < LOQ
Electrical and electronic trades workers	5	26	NC	NC	NC	NC	NC	NC
Food processing, wood working, garment and other craft, and related trades workers	29	26	< LOQ	< LOQ–2	16	2	< LOQ	< LOQ to < LOQ
Plant and machine operators, and assemblers	454	19	1	< LOQ–5	291	9	< LOQ	< LOQ–3
Stationary plant and machine operators	429	20	2	< LOQ–6	275	9	< LOQ	< LOQ–3
Assemblers	7	1	NC	NC	8	< LOQ	NC	NC
Drivers and mobile plant operators	18	1	< LOQ	< LOQ to < LOQ	8	< LOQ	NC	NC
Elementary occupations	122	12	< LOQ	< LOQ–2	52	1	< LOQ	< LOQ to < LOQ

AM, arithmetic mean; IQR, interquartile range; LOQ, limit of quantification; N, number of measurements.

[a] Data from the French National Institute for Research and Occupational Safety, INRS (2022).

One study reported that the excretion of cobalt in urine was elevated after skin exposure to a coolant solution containing cobalt. The amount of cobalt excreted by five people during the 24-hour period before the skin exposure averaged 18.1 nmol (range, 6.8–34.3 nmol). After the skin exposure to the coolant solution, the average amount of cobalt excreted over 24 hours increased to 38.5 nmol (range, 14.2–61.4 nmol). This increase was statistically significant (P = 0.012) (Linnainmaa & Kiilunen, 1997). The amount of cobalt on the skin has also been shown to be significantly correlated with concentration of cobalt in samples of urine (Kettelarij et al., 2018a) and blood (Klasson et al., 2017; Wahlqvist et al., 2020) obtained from workers in factories involved in hard-metal manufacturing.

Further potential for occupational exposure because of inadvertent ingestion by workers, resulting from contact between contaminated hands or objects and mouths, has been suggested (Cherrie et al., 2006). As such inadvertent ingestion exposure is anticipated to arise mainly as a result of hand-to-mouth contact, it is closely linked to dermal exposure and is potentially a significant source of occupational exposure to metals (Gorman Ng et al., 2012). Although a direct significant association between hand exposure to metals and increased metal concentrations measured by biomonitoring, such as concentrations in the urine or blood, has not been demonstrated, hand exposure to cobalt was reported to be significantly positively correlated with perioral exposure among workers in engine repair facilities (involved in painting, metal spraying, metal processing, and brazing) (Gorman Ng et al., 2017). Direct uptake of cobalt through skin has also been reported. In a simple experiment in volunteers exposed to hard-metal cobalt powder via the skin, the concentration of cobalt in urine samples increased tenfold after exposure (Scansetti et al., 1994). [The Working Group noted that a lack of data limits understanding of the role of ingestion due to hand-to-mouth contact in relation to overall occupational exposure to cobalt.]

(c) Cobalt refining and production of cobalt metal and cobalt compounds

Exposure to cobalt has been reported among workers involved in cobalt refining and the production of cobalt metal and cobalt compounds. In a cobalt refinery in Belgium that consumed a wide variety of starting raw materials (mainly cobalt metal cathodes, intermediate products, and residues), workers were exposed to a mixture of cobalt salts, oxides, and fine cobalt metal powders without being exposed to tungsten, titanium, iron or silica (or their carbides), or diamond (Swennen et al., 1993; Verougstraete et al., 2004; Lantin et al., 2011; Lantin et al., 2013). Exposure levels were high in 1993 when the median concentrations of cobalt in personal air and end-of-shift urine samples were reported to be 110.0 μg/m^3 and 72.4 μg/g creatinine, respectively (Swennen et al., 1993). Between 1992 and 2001, cobalt exposure declined sharply, as did urinary cobalt concentrations, a pattern attributed to improvements in working conditions, including the implementation of hygiene controls [details not provided in the paper] (Verougstraete et al., 2004). From 2002, all workers at the refinery were required to wear protective masks, which may have further reduced their exposure to cobalt. In 2007, the median concentration of cobalt in personal air was reported to be 15.0 μg/m^3, and mean urinary cobalt concentration was reported to be 3.9 μg/g creatinine for 2008–2009 (Lantin et al., 2011; Lantin et al., 2013). [The Working Group noted that the decrease in cobalt exposure over time reported by these studies may not be representative of trends in the industry as a whole.]

In a cobalt-producing plant in Kokkola, Finland, cobalt powder was produced from a pyrite ore concentrate between 1966 and 1987. Thereafter, cobalt powder, inorganic cobalt,

and nickel compounds were produced using by-products of the metallurgic industry as raw materials. Workers were potentially exposed to metallic cobalt and cobalt sulfates, carbonates, oxides, and hydroxides via the reduction and powder production, sulfatizing roasting, and leaching and solution purification processes, and in the chemical processing department (Linna et al., 2003, 2004, 2020). According to biological monitoring data, exposure to cobalt was highest among workers in the reduction department. The highest urinary concentration of cobalt was reported to be approximately 16 000 nmol/L (943 μg/L), compared with an average concentration of < 40 nmol/L (2.4 μg/L) among unexposed individuals (Linna et al., 2003, 2004).

(d) Cobalt-containing diamond tooling

Diamond tools are used to cut stone, marble, glass, wood, and other materials, and to grind or polish various materials (IARC, 2006). These tools do not contain hard metals and do not include tungsten carbide (van den Oever et al., 1990; Nemery et al., 1992; IARC, 2006).

Diamond polishers often use high-speed polishing discs, which have surfaces composed of micro diamonds cemented in ultrafine cobalt metal powder. During polishing activities, cobalt dust is formed and may be inhaled by the diamond polisher (Barceloux, 1999). As an example, the fashioning of a diamond involves several steps, starting with the inspection and marking of the stone, followed by cleaving, sawing, and rough-cutting processes. Finally, the stone is polished with cobalt-containing polishing discs. One study reported that the mean air concentrations of cobalt in the breathing zones of workers during such processes were 15.1 μg/m^3 and 5.3 μg/m^3 in workshops with high and low exposures to cobalt, respectively, and 0.4 μg/m^3 in a control group that was not occupationally exposed to cobalt. The same trend was reported for the mean urinary cobalt concentrations of the groups (Nemery et al., 1992).

(e) Alloys or plating containing cobalt

In a magnet-manufacturing plant located in the mid-western USA, sintered permanent magnets were produced from cobalt, nickel, and aluminium metal powders and various rare-earth metals. Exposure to cobalt, nickel, neodymium, samarium, and other metal constituents occurred during the preparation of raw materials, which included processes such as pressing, casting, break-out, blasting, and grinding (Deng et al., 1991). Exposure to cobalt was reported to be greater than to other metals (Deng et al., 1991).

In bright electroplating, materials are coated with thin layers of metal to achieve a shiny decorative surface; during the process, there is the potential for worker exposure to cobalt and chromium. Compared with hard electroplating, in which a thicker layer of metal is applied, bright electroplating has been reported to be associated with lower levels of exposure to some metals (Guillemin & Berode, 1978). In one bright-electroplating factory, the mean air concentration of cobalt in the workplace did not exceed the LOD of 0.12 ng/m^3. No significant differences ($P > 0.05$) were reported between mean concentrations of cobalt and chromium in the plasma in blood samples obtained from exposed workers (cobalt, 0.85 μg/L; chromium, 0.44 μg/L) and unexposed controls (cobalt, 0.80 μg/L; chromium, 0.41 μg/L) (Wultsch et al., 2017).

(f) Dental procedures

Dental laboratory technicians are potentially exposed to metal alloys that are used in the production of crowns, bridges, and removable partial dentures, in which alloys are frequently used for the fusion of metal frames for sectional prostheses. These alloys comprise 35–65% cobalt, 20–30% chromium, 0–30% nickel, and small amounts of molybdenum, silica, beryllium, boron, and carbon (Burgaz et al., 2002). Among

dental technicians in Türkiye and Indonesia, who worked on the production of dental prostheses, the mean cobalt levels were 24.8 µg/g creatinine in urine (Burgaz et al., 2002) and 26.8 µg/L in blood (Berniyanti et al., 2020).

(g) Cobalt pigments

Cobalt is used as a pigment in the ceramics, glass, and paint industries. Cobalt is often present in paints or inks as a siccative to facilitate the drying process, and in cobalt blue dyes for the painting of porcelain pottery. In the pigment production and paint industry, the primary exposure routes are skin contact and inhalation of paint fumes and dust (Leyssens et al., 2017). Combinations of cobalt oxides and oxides of aluminium, magnesium, zinc, and silicon are constituents of blue and green ceramic glazes and pigments (Donaldson & Beyersmann, 2011). Cobalt blue pigment is produced by calcining cobalt(II) oxide with aluminium(III) oxide (Bolt et al., 1998; Karmaoui et al., 2013). Cobalt-zinc-silicate is used in a blue underglaze paint for pieces of porcelain; the pigment was specially developed to withstand intense heat (Raffn et al., 1988).

In a porcelain factory in Denmark, porcelain plates were produced by underglazing with a blue cobalt colour. This colour is made by melting together a mixture of cobalt-zinc-silicate, zinc oxide, and silicon oxide. Among workers in the factory, mean blood cobalt concentrations approximately 4 weeks after resuming work after 6 weeks off work were 36.7 nmol/L and 4.04 nmol/L for plate painters who used this cobalt underglaze and unexposed controls, respectively. Mean urinary cobalt levels were 141.8 nmol/mmol creatinine (73.9 µg/g creatinine) for underglaze users and 1.53 nmol/mmol creatinine (0.80 µg/g creatinine) for the controls (Raffn et al., 1988).

In pottery factories producing porcelain plates in Denmark, plate painters were exposed to two different types of cobalt dyes, one type containing an insoluble cobalt aluminate compound and the other containing a soluble cobalt-zinc-silicate compound, the latter type being used most frequently. Concentrations of cobalt in personal air, blood, and urine measured in 1982 and 1984 were greater in workers exposed to cobalt-zinc-silicate compounds than in those exposed to cobalt aluminate compounds. Significant correlations between blood and urine concentrations were observed. Nevertheless, there were no significant correlations between concentrations in air and blood, or air and urine (Christensen & Mikelsen, 1986). After follow-up during the period 1982–1992, exposure of the plate painters to cobalt was reported to have decreased as a result of improvement of the working environment. From 1982 until 1991, concentrations of cobalt in personal air had decreased from 1356 to 454 nmol/m^3 in 1991 (80 to 26 µg/m^3, respectively), and in urine from 133.4 to 18.8 nmol/mmol creatinine (69.5 to 9.8 µg/g creatinine, respectively) (Christensen & Poulsen, 1994).

(h) Electrical industry

In nickel–hydrogen battery-manufacturing plants in Japan, workers were engaged in anode plate-making, which comprised mixing, filling, drying, and rolling processes, as well as board processing. A master batch for anode materials contained 200 kg by weight of nickel hydroxide, 18 kg of recycled nickel powder, 10 kg of metallic cobalt, and 10 kg of cobalt oxyhydroxide (CoO(OH)). Commercial nickel(II) hydroxide ($Ni(OH)_2$) powder of specific particle diameter (9–12 µm) included 97.2% nickel hydroxide and 2.8% cobalt hydroxide. The level of exposure to cobalt in personal air was approximately one-seventh that for nickel. A significant correlation between cobalt and nickel concentrations was reported in post-shift urine samples from workers (r = 0.833, P < 0.0001); however, the mean urinary level of cobalt was higher than that of nickel (Yokota et al., 2007).

In a digital video cassette-manufacturing plant in Japan, almost all processes were automated to avoid exposure of the workers to cobalt oxide dust. However, some of the workers involved in the cobalt vapour deposition process cleaned and removed cobalt oxides that adhered to the insides of the cobalt vapour deposition machines under dry conditions several times during their work shifts. According to personal and biomonitoring data, the air concentrations of cobalt in the personal air samples of 16 workers ranged from below the LOD to approximately 22 μg/m^3. Urinary cobalt concentrations ranged from below the LOD to approximately 27.5 μg/g creatinine in end-of-shift samples (Fujio et al., 2009).

(i) *Electronic and/or electrical waste recycling industry*

Electronic and/or electrical waste (e-waste) contains multiple metals, including cobalt. The hazardous components in e-waste include cathode ray tubes, liquid crystal display screens, light-emitting diode lights, batteries, circuit boards, mercury-containing equipment, and plastic with brominated flame retardants (Julander et al., 2014).

Julander et al. (2014) analysed exposure concentrations of 20 metals, including cobalt, among e-waste recycling workers in Sweden. The workers recycled similar types of goods, such as television sets and computers, electronic tools, toys, and small and large household appliances, and their main tasks were the dismantling, handling, inspection, and transportation of goods. The level of exposure to cobalt in personal air was approximately 19 times as high for the recycling workers as for unexposed office workers; however, no significant differences ($P = 0.05$) in blood or urine cobalt concentrations were found between the two groups.

At sheltered workshops for workers with disabilities recycling e-waste in Germany, where the workers mainly disassembled cathode ray tubes and liquid crystal displays, and performed sorting related to the recycling of small electronic devices such as consumer electronics, urinary cobalt concentrations did not differ significantly ($P < 0.05$) between recycling workers and unexposed controls (Gerding et al., 2021).

1.4.3 *Exposure of the general population*

(a) *Dietary exposure*

For the general population in non-polluted areas, dietary intake of cobalt (estimated as ranging between 3 and 40 μg per day) represents the primary source of cobalt exposure (Hokin et al., 2004; Grübl et al., 2007; Turconi et al., 2009; Nasreddine et al., 2010; Arnich et al., 2012; Domingo et al., 2012; Noël et al., 2012; Cheyns et al., 2014; Tvermoes et al., 2014; Monnot et al., 2021). Cobalt has no known nutritional function, except as a component of vitamin B_{12}. Microorganisms such as bacteria and algae synthesize vitamin B_{12} and constitute the only source of this vitamin. The vitamin B_{12} produced by microorganisms enters the human food chain through incorporation into foods of animal origin. Gastrointestinal fermentation by animals promotes the growth of these microorganisms, and vitamin B_{12} is subsequently absorbed and incorporated into animal tissues, especially the liver (WHO, 2005; González-Montaña et al., 2020). Biomagnification of cobalt up the food chain does not occur (WHO, 2006). Humans derive their required dietary vitamin B_{12} from animal tissues or products (i.e. milk, butter, cheese, eggs, meat, poultry, etc.), unless the region in which the animal is reared is geochemically deficient in cobalt. No significant amount of the vitamin B_{12} required by humans is derived from microflora (FAO & WHO, 1998). Vitamin B_{12} represents only a small fraction of total cobalt intake (Lison, 2015). The cobalt content in vitamin B_{12} varies between 4.4% and 5.8% (González-Montaña et al., 2020). Trace elements may be present in food naturally (e.g.

minerals), or may originate from either environmental contamination derived from agricultural practices (e.g. pesticide residues) or food processing and packaging (Arnich et al., 2012). In the last two decades, several countries have carried out total diet studies, which were national surveys that estimated exposure to metals (Turconi et al., 2009; Nasreddine et al., 2010; Arnich et al., 2012; Domingo et al., 2012; Noël et al., 2012). Considering data on food prepared and consumed by the populations studied, the highest mean concentrations were found in chocolate, butter, coffee, shellfish, nuts, vegetables (e.g. spinach, sweet potato leaves), and ice cream (Ghaedi et al., 2008; Noël et al., 2012; Nemery & Banza Lubaba Nkulu, 2018). In addition to traditional dietary sources, some people (e.g. athletes) may deliberately ingest cobalt in the form of cobalt-containing supplements with the aim of stimulating endogenous erythropoietin (EPO) biosynthesis, and fat and carbohydrate metabolism (Simonsen et al., 2012; Unice et al., 2012; Finley et al., 2013; Tvermoes et al., 2014; Leyssens et al., 2017). The deliberate ingestion of high concentrations of vitamin B_{12} via dietary supplements (600 µg daily for 3 months) has also been reported (Pongcharoensuk & Thaiwat, 2021). [The Working Group noted that the data reviewed for the exposure of the general population consisted of measurements of total cobalt concentrations in biological specimens, which may reflect exposure to one or several of the individual agents evaluated in this monograph.]

(b) Exposure from medical devices

Medical devices such as orthopaedic implants, prosthetics, and stents composed of cobalt-containing alloys are potential sources of cobalt exposure (van Lingen et al., 2017). Metal-on-metal implants are predominantly composed of cobalt (> 34%) and chromium (20–28%), with small amounts of other metals such as molybdenum and nickel (Leyssens et al., 2017). Metal wear or corrosion of these medical devices may lead to the dissemination of metal debris and ions throughout the body (Williams et al., 2011; Polyzois et al., 2012; Blackburn & Whitehouse, 2014; Scharf et al., 2014; Lombardi et al., 2016). Increases in concentrations of cobalt ions in serum, urine, and erythrocyte samples from patients with metal-on-metal devices have been widely reported (Dumbleton & Manley, 2005; Cobb & Schmalzreid, 2006; Witzleb et al., 2006; Imanishi et al., 2010; Friesenbichler et al., 2014), see also Table 1.14. However, it is unclear from the available studies whether increased cobalt concentrations in biological samples correlate with implant wear (Blackburn & Whitehouse, 2014; Somers et al., 2016; van Lingen et al., 2017).

(c) Cosmetics, jewellery, and other consumer products

A smaller number of studies delivered evidence for cobalt release from electronic devices, leather goods, jewellery items and coins (Hamann et al., 2013; Leyssens et al., 2017; Uter & Wolter, 2018; Alinaghi et al., 2020), household products (Basketter et al., 2003), and cosmetics, in which the trace element can be present as an impurity (Bocca et al., 2014; Bruzzoniti et al., 2017; Mostafaii et al., 2022).

Both intentional and unintentional inhalation of tobacco smoke is another potentially significant source of exposure to cobalt (Table 1.7). Although other trace elements in tobacco smoke are more commonly associated with health effects (IARC, 1987, 1990, 1993), the presence of cobalt may also contribute to the harmful effects of smoking (Pinto et al., 2017; Kaplan et al., 2019; Mansouri et al., 2020).

(d) Biomonitoring levels

Risks for toxicity in humans have been estimated mainly on the basis of measurements of cobalt content and its release from various environmental matrices (Iqbal et al., 2012; Kurt-Karakus, 2012; Obiri et al., 2016; Ngole-Jeme &

Table 1.14 Measurement of total cobalt concentrations in human biological specimens

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	95th percentile	Analytical method (LOD)	Comments	Reference
Exposure from medical devices								
Total cobalt in blood	Patients who had MoM articulations in situ for > 30 yr, United Kingdom, date of sample collection NR	MoM radiologically stable: $n = 3$ MoM radiologically loose: $n = 2$	MoM radiologically stable: 1.97 ng/g MoM radiologically loose: 35.5 ng/g (range values NR)	NR (NR)	NR	ICP-MS (0.07 ng/g)		Dunstan et al. (2005)
Total cobalt in serum	Patients with a hip replacement in place for > 10 yr, Vienna, Austria, 2003–2004	$n = 22$	NR (0.3–50.1 µg/L)	0.75 µg/L (NR)	NR	FAAS (0.3 µg/L)		Grübl et al. (2007)
Total cobalt in serum	Patients who received MoM hinged revision knee prostheses, Marburg, Germany, 2018	$n = 23$	NR [(1.0–47.5 µg/g)][a]	[10.5 µg/g][a] (NR)	NR	ICP-MS (NR)		Klasan et al. (2019)
Total cobalt in serum	Patients with MoM total hip arthroplasty Vancouver, Canada, 2004–2007	$n = 31$	NR (0.54–58.78 µg/L)	4.50 µg/L (NR)	NR	ICP-MS (NR)		Williams et al. (2011)
Environmental exposure								
Total cobalt in urine (µg/g creatinine)	Adults and children living close to the mines or smelting plants, Katanga, Democratic Republic of the Congo, 2009–2011	$n_{adults} = 79$; $n_{children} = 32$	Adults: 11.7[b] µg/g Children: 27.9[b] µg/g (range values NR)	Adults: (7.1–22.0 µg/g) Children: (13.4–62.7 µg/g) (median values NR)	NR	ICP-MS (0.018 µg/L)	Spot urine samples.	Cheyns et al. (2014)
Total cobalt in urine (µg/L × 1000)	Residents exposed to emissions from a metal recycling plant, Italy, 2011–2013	$n = 153$	0.43[b] µg/L (0.07–2.95)	0.45 µg/L (NR)	NR	ICP-MS (NR)	24 h urine samples.	Chellini et al. (2017)

Table 1.14 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	95th percentile	Analytical method (LOD)	Comments	Reference
Total cobalt in urine	People who smoked > 10 cigarettes/day for ≥ 5 yr, Iran (Islamic Republic of), 2018	$n_{\text{non-smokers}} = 35$ $n_{\text{smokers}} = 64$	NR (NR)	Non-smokers: 0.6 µg/L (0.32–0.9) Smokers: 1.22 µg/L (0.72–1.65)	NR	ETAAS (0.1 µg/L)	First morning void urine samples.	Mansouri et al. (2020)
Total cobalt in placenta and cord blood	Non-smoking mothers with anthroposophic lifestyle, Sweden, 2004–2007	$n_{\text{placenta}} = 40$ $n_{\text{cord blood}} = 40$	Placenta: (1.29–8.01 µg/kg) Cord blood: (0.04–0.51 µg/kg) (mean values NR)	Placenta: 3.13 µg/kg (NR) Cord blood: 0.08 µg/kg (NR)	NR	ICP-MS (0.002 ng/g)		Fagerstedt et al. (2015)
Total cobalt in blood	Non-occupationally exposed individuals, Canada, date of sample collection NR	$n = 100$	NR (NR)	0.25 µg/L (NR)	0.64 µg/L	ICP-MS (0.017 µg/L)		Goullé et al. (2005)
Dietary exposure								
Total cobalt in serum	Non-smoking oyster growers, British Columbia, Canada (date of sample collection NR)	$n = 39$	8.69 nmol/L[b] (6.28–106.9) [0.512 (0.370–6.30) µg/L]	7.98 nmol/L (NR) [0.470 µg/L]	12.05 nmol/L [0.710 µg/L]	ICP-MS (NR)		Clark et al. (2007)
General population, background concentrations								
Total cobalt in urine	General population, Taiwan, China, 2005–2008	$n = 780$	1.07[b] µg/L (0.05–22.43)	1.05 µg/L (0.70–1.65)	3.43 µg/L	ICP-MS (0.003 µg/L)	First morning void spot urine samples.	Liao et al. (2019)
Total cobalt in urine	Women from the general population, Japan, 2000–2005	$n = 1000$	0.68[b] µg/L (< LOD–281)	0.70 µg/L (NR)	NR	GFAAS (0.1 µg/L)		Ohashi et al. (2006)
Total cobalt in urine	The US population from the National Health and Nutrition Examination Survey, 2015–2016	$n = 3061$	0.414[b] µg/L (NR)	0.434 µg/L (NR)	1.53 µg/L	ICP-MS (0.023 µg/L)		CDC (2021)
Total cobalt in blood	General population, Canada, 2009–2011	$n = 6009$	NR (NR)	NR (NR)	0.38 µg/L	ICP-MS (0.04 µg/L)		Saravanabhavan et al. (2017)

Table 1.14 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	95th percentile	Analytical method (LOD)	Comments	Reference
Total cobalt in blood	General population, France, 2008–2010	*n* = 1992	0.30 µg/L (NR)	0.29 µg/L (0.24–0.37)	0.54 µg/L	ICP-MS (0.0016 µg/L)		Nisse et al. (2017)
Total cobalt in blood	The US population from the National Health and Nutrition Examination Survey 2015–2016	*n* = 3454	0.151[b] µg/L (NR)	0.130 µg/L (NR)	0.400 µg/L	ICP-MS (0.06 µg/L)		CDC (2021)
Total cobalt in hair	General population, Katanga province, Democratic Republic of the Congo, date of sample collection NR	*n* = 109	1.16 mg/kg (NR)	NR (NR)	4.20 mg/kg	ICP-MS (0.001 µg/g)	Hair samples were taken from the neck.	Elenge et al. (2011)

ETAAS, electrothermal atomic absorption spectrometry; FAAS, flame atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; IQR, interquartile range; LOD, limit of detection; MoM, metal-on-metal; NR, not reported; US, United States; yr, year.

[a] Working Group conversion to International System of Units.

[b] Geometric mean.

Fantke, 2017; Kiani et al., 2021; Mostafaii et al., 2022). Blood, serum, and urinary concentrations of cobalt have long been considered suitable indicators of cobalt exposure (Dunstan et al., 2005; Grübl et al., 2007; Finley et al., 2013; Chellini et al., 2017; Nisse et al., 2017; Liao et al., 2019). Of note, studies of the general population have shown wide variations in urinary cobalt concentrations (Cheyns et al., 2014; Chellini et al., 2017; Mansouri et al., 2020). This variability has been attributed to either oral or dermal absorption (Scansetti et al., 1994; Linnainmaa & Kiilunen,1997). Nevertheless, there is a general understanding that data available from the literature are sufficient for the monitoring of urinary cobalt as a biomarker of exposure to this chemical to be recommended (ANSES, 2013). Details on the absorption, distribution, metabolism, and excretion of cobalt are described in Section 4.1. Biomonitoring data obtained from a selection of studies are briefly summarized in Table 1.14. Other biological specimens have been used to estimate human exposure, either by attempting to measure cobalt concentrations at the target-organ level or because they provide information on different exposure windows. These include nails (Rogers et al., 1993; Ndilila et al., 2014; Gutiérrez-González et al., 2019), hair (Vienna et al., 1995; Elenge et al., 2011), exhaled breath condensate (Goldoni et al., 2004; Mutti & Corradi, 2006; Broding et al., 2009), saliva (Berton & Wuilloud, 2010), lacrimal fluid and sweat (Bruzzoniti et al., 2017), and placenta (Fagerstedt et al., 2015).

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

To reduce the risk of adverse health effects, several organizations have proposed occupational exposure limits for many hazardous substances. In particular, exposure limits are set for inhalable dust fractions in workplace air. The United States Occupational Safety and Health Administration permissible exposure limit for cobalt and cobalt inorganic compounds is 0.1 mg/m^3 as a time-weighted average (TWA) over an 8-hour (OSHA, 2017) or up to 10-hour working shift (NIOSH, 2007). The American Conference of Governmental Industrial Hygienists recommends a threshold limit value of 0.02 mg/m^3, to which nearly all workers may be exposed for a working lifetime without adverse health effects (ACGIH, 2019). In Europe, exposure limits vary between countries, ranging from 10 to 100 µg/m^3 (Kettelarij, 2018). Table S1.15 shows some examples of occupational exposure limits in different countries (see Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). It is of note that cobalt compounds are readily absorbed through the skin and that unintentional ingestion of cobalt can occur from mucociliary clearance after air exposure, when contaminated hands or objects come in contact with the mouth, or when cobalt is deposited around the mouth or in the oral cavity (Kettelarij, 2018). Hence, the monitoring of airborne concentrations alone may not be a reliable proxy of actual exposure (Julander et al., 2010; Klasson et al., 2016; Gorman Ng et al., 2017; Kettelarij et al., 2018a).

(b) Industrial emissions, air, water, soil, consumer products, food, and feed

In addition to occupational limit values, regulations and guidelines exist for different environmental matrices that aim to reduce exposure of the general population to cobalt. The European Union (EU) has established emission limit values for cobalt in waste gases produced by specific industrial activities (European Commission, 2010). The Environment Agency of the UK recommends a soil screening value for total cobalt of 4.2 mg/kg dry weight, based on soil ecotoxicity (Environment Agency, 2022).

British Columbia, Canada, and the USA have use-dependent soil standards and screening levels, respectively, for the protection of human health (as listed in Table S1.16, which presents a few examples of national and international guideline values for cobalt in environmental matrices, consumer products, and foodstuffs; see Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618). A safe recommended dietary allowance for cobalt intake has not been set yet. For guidance purposes only, the UK Expert Group on Vitamins and Minerals has expressed the opinion that a cobalt intake of 0.023 mg/kg body weight (bw) per day would not be expected to result in any adverse effects (FSA, 2003). Although safety data on cobalt-containing alloys, such as those used in medical devices, were specifically excluded from a European Chemicals Agency evaluation (Eichenbaum et al., 2021), European Medical Device Regulations require that medical devices containing > 0.1% w/w of cobalt are appropriately labelled with a justification for the use of the substance (European Union, 2017). The UK recommends a threshold concentration of 7 mg/L of cobalt in blood for specific medical implants, which is greater than the 3 mg/L value recommended in the USA (Matharu et al., 2017). The recommendation further indicates the need for complementary diagnostic tools for the detection of adverse reactions (AAOS, 2012). [The Working Group noted that the literature shows that guidelines for follow-up of these medical implants differ considerably between regulatory authorities worldwide, which indicates that consensus regarding acceptably safe levels has not yet been reached.] Norway has established a guidance value of 0.023 mg/kg bw per day for cobalt exposure from ceramic articles (Norwegian Scientific Committee for Food and Environment, 2007), while the EU has derived threshold values for cobalt in toys (European Commission, 2009). A few agencies have prohibited the use of specific forms of cobalt for certain purposes. For instance, the World Anti-Doping Agency has prohibited the use of cobalt at all times because it is a hypoxia-inducible factor (HIF)-activating agent (Schmidt et al., 2019). The European Food Safety Authority (EFSA, 2009b) has considered the use of cobalt(II) chloride hexahydrate for nutritional purposes as a source of cobalt in food supplements to be a safety concern. The underlying reason was the greater bioavailability of cobalt from cobalt(II) chloride than from other inorganic cobalt compounds (i.e. cobalt oxide).

1.5.2 Guidance and reference values for biological monitoring

Biological monitoring of exposure to chemicals in the workplace is used to assess exposure and reduce the risk of adverse health effects. Analysis of cobalt in biological matrices has been recommended for monitoring occupational exposure (Simonsen et al., 2012; Klasson et al., 2016). Several institutions have proposed biomonitoring guidance and reference values. As an example, for a TWA exposure threshold of 0.02 mg/m^3, the American Conference of Governmental Industrial Hygienists has defined a biological exposure index of 15 µg/L as total cobalt concentration in urine collected at the end of the shift at the end of the last day of the work week (ACGIH, 2019; see Table S1.15, Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

Biomonitoring studies to assess exposure in the general population have been conducted at the national level in Canada (Saravanabhavan et al., 2017), France (Fréry et al., 2011), and Japan (Ohashi et al., 2006). Reference values (RV95s) have been derived statistically from these studies to indicate background exposure to chemical substances in reference populations. Because these values are influenced by environmental and lifestyle factors and may differ between regions,

it has been suggested that they should be established at national/regional levels (Hoet et al., 2013). Biomonitoring guidance and reference values derived for environmental and occupational exposures are summarized in Table S1.17 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

1.6.1 *Epidemiological studies of cancer in humans*

For each study on cancer in humans, the reviews and critiques undertaken in relation to different aspects of exposure assessment are tabulated in Table S1.18 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618), and are summarized in the following sections.

(a) Exposure assessment methods

The Working Group identified 31 key studies of cancer in humans for which a critical appraisal of exposure assessment methods was undertaken. These comprised 21 industry-based studies: 18 of workers producing cobalt or other metals and products (Cuckle et al., 1980; Moulin et al., 1993; Tüchsen et al., 1996; Moulin et al., 2000; Grimsrud et al., 2005; Marsh et al., 2009; Sauni et al., 2017), including hard metals (Hogstedt & Alexandersson, 1990; Lasfargues et al., 1994; Dufresne et al., 1996; Moulin et al., 1998; Wild et al., 2000; Marsh et al., 2017a; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017), and three of other worker populations (Bai et al., 2019; Rodrigues et al., 2020; Li et al., 2021a); eight general-population studies (Rogers et al., 1993; O'Rorke et al., 2012; Kresovich et al., 2019; White et al., 2019; Duan et al., 2020; Niehoff et al., 2021; Pan et al., 2021; Mérida-Ortega et al., 2022); and two meta-analyses including both cobalt in industrial settings and populations with orthopaedic implants (Holy et al., 2022; Zhang et al., 2021a). In addition, Marsh et al. (2017b) reported a pooled analysis of hard-metal workers, as described in Marsh et al. (2017a), McElvenny et al. (2017), Morfeld et al. (2017), Svartengren et al. (2017), Wallner et al. (2017), and Westberg et al. (2017).

(i) Industry-based studies: workers producing cobalt or other products

A retrospective cohort study by Moulin et al. (1993) used company administrative records of a French electrochemical plant specializing in cobalt and sodium production to assess exposure (defined as employment for at least 1 year between 1950 and 1980) against various cancer mortality end-points. Analyses examined ever/never employed as well as a metric to apply four subgroups defined according to work areas (cobalt production, sodium production, maintenance, and administration).

Tüchsen et al. (1996) conducted a retrospective cohort study on exposure to cobalt aluminate spinel (assessed via employment history) of workers in departments responsible for plate underglazing at two porcelain factories in Copenhagen, Denmark, and assessed data against various end-points of cancer incidence. The authors noted that from 1907 to 1972 only cobalt aluminate spinel was used. Factory 1 changed from cobalt aluminate spinel to cobalt silicate in 1972. Ever/never employment categories were applied in standardized mortality ratio analyses, capturing employment periods between 1943 and 1987 at factory 1, and 1962 and 1987 at factory 2. Co-exposure to other agents, such as quartz (used in glazing until 1952 in factory 1 and for an unknown period in factory 2) and

nickel (representing less than 0.5% of cobalt dyes used) was possible.

Retrospective cohort and nested case–control studies by Moulin et al. (2000) examined workers employed for at least 1 year between 1 January 1968 and 31 December 1991 in a French factory producing stainless and alloyed steel (with employment potentially dating back to the 1920s). Company records were used to assess employment history, and a job-exposure matrix (JEM) was developed based on expert knowledge, interviews with co-workers, previous measurements in French factories, and a literature review. This information was used to assign semiquantitative estimates of exposure to metals (iron, chromium and/or nickel, and cobalt) and/or their compounds, acid mists, polycyclic aromatic hydrocarbons, silica, and asbestos. As no on-site air measurements were available for the employment period considered, an attempt was made to construct the JEM with consideration of changes in exposure over time according to periods doing different jobs on the basis of the information obtained (e.g. through workplace interviews). Categorical metrics including ever/never employed (for general-population comparison), duration of exposure, cumulative exposure, and frequency of exposure were applied in the analyses.

Grimsrud et al. (2005) conducted a nested case–control study examining lung cancer incidence among a cohort of individuals with minimum employment of 1 year at a Norwegian nickel refinery treating sulfidic nickel copper concentrate (consisting of approximately 45% nickel, 25% copper, 23% sulfur, and 2% cobalt, with < 2% iron and precious metals). Company administrative records were used to assess job histories; quantitative measurements of cobalt were used to produce cobalt:nickel ratios using a previously developed nickel JEM. Nearly 3500 personal samples, obtained and analysed for cobalt between 1982 and 1994 as part of routine sampling, were used to calculate arithmetic 8-hour TWAs for the departments in question. The cobalt:nickel ratios in air were computed for departments and periods with measured values (cobalt amounted to approximately 4–15% of total nickel except in the department where cobalt electrolysis was undertaken, where the amount of cobalt was triple that of nickel). Departments with no measurements used a ratio of 7.1% (average for all departments exclusive of cobalt electrolysis). [The Working Group highlighted the study authors' observation that cobalt would probably always be present with nickel in raw materials and intermediates at the refinery.] Exposure metrics applied in analyses included cumulative exposure, duration of employment (overall and divided between three major department groups at the refinery), and time of first employment.

A retrospective study by Sauni et al. (2017) examined incident cancer (multiple end-points) in a cohort employed for at least 1 year between 1968 and 2004 at a cobalt plant in Finland. This plant produced cobalt powder from pyrite ore concentrate between 1966 and 1987, and produced cobalt powder, inorganic cobalt, and nickel compounds using by-products of metallurgic industry as raw materials between 1987 and 1999. Company administrative records were used to assess job histories. Subcohorts categorized by exposure level were developed according to workers' first department of employment at the plant, and assessed against industrial hygiene measurements collected since 1966 (area and personal samples) and biological monitoring; sampling details are available in other publications (Linna et al., 2003, 2004, as cited in Sauni et al., 2017). Exposure metrics included categorical duration of employment as well as categorical exposure groupings based on department ("variable exposure with peaks" corresponds to factory maintenance; "low exposure" corresponds to leaching and solution purification; "moderate exposure" corresponds to chemical department, test plant; and "high

exposure" corresponds to sulfatizing, roasting, reduction, and powder production).

Studies deemed less informative to the Working Group's analysis included Cuckle et al. (1980) and Marsh et al. (2009). Marsh et al. (2009) published a retrospective cohort study of workers with employment of at least 3 years at a facility conducting copper smelter, mill, or sulfur operations at Copperhill, Tennessee, USA, between 1946 and 1996, and cancer mortality with various end-points. A JEM based on relative exposure intensities over time was used to estimate job- and time-specific exposures to six agents: lead, sulfur dioxide, arsenic, cadmium, dust, and cobalt, assessed using a modification of the "process-based projection of exposure measurements". Analyses of the cobalt exposure group (compared with an unexposed group) were based on employment. The retrospective cohort analysis of lung cancer mortality conducted by Cuckle et al. (1980) assessed historical employment (at least 12 months between 1933 and 1960) at a company manufacturing nickel and cobalt compounds in the UK. Exposure was indirectly assessed via duration of employment at the company. Various categorical metrics were applied in analyses (e.g. ever/never employed for at least 12 months, employment duration, and years since first employment). Exposure metrics were not specific to cobalt exposure for either study.

(ii) Industry-based studies: workers producing hard metals

In most studies of hard-metal workers reviewed by the Working Group, exposure to cobalt alone could not be separated from exposure to WC-Co. Two studies, Moulin et al. (1998) and Wild et al. (2000), contained additional analyses of exposure to cobalt with potential separation from WC-Co.

A retrospective cohort with nested case–control study by Moulin et al. (1998) examined lung cancer mortality in workers employed for at least 3 months in 10 French hard-metal factories, from the date the factory opened (which varied by factory) to 31 December 1991. Other production activities performed in some of the factories studied included powder metallurgy to produce equipment made of single metals (iron or nickel) or alloys (containing iron, copper, and tin), as well as foundry processes to produce cobalt superalloys and magnets (with cobalt, tungsten, nickel, chromium, and carbon). The qualitative definition of cobalt exposure was simultaneous exposure to WC-Co specific to hard-metal manufacture and other cobalt exposure resulting from other production activities. Exposure was assessed using company administrative records to assess job histories, with a JEM for exposure to cobalt and to tungsten carbide developed using expert knowledge and interviews with co-workers, which was validated with previously recorded measurements of cobalt concentrations in air (744 measurements were recorded in three factories: 382 short-duration (15–20 minutes) samples gathered between 1971 and 1983, and 362 long-duration (4–8 hours) samples (of which 264 were of personal air) gathered between 1982 and 1984). Linear regression analysis indicated significantly increasing trends between cobalt concentrations in air (excluding the cobalt powder-manufacturing workshop) and those assigned to the JEM. For simultaneous cobalt and tungsten carbide exposure, exposure metrics included ever/never employment, maximum intensity score coded according to job history, duration of exposure (years) at or above defined exposure intensity score, and estimated cumulative exposure divided into quartiles.

In their retrospective cohort study, Wild et al. (2000) examined workers employed for at least 3 months in a French factory producing hard metals as well as other metallurgical products containing cobalt (this included foundries producing magnets with cobalt and other metals; equipment made from other sintered allows of iron, nickel, copper, and tin; and production

of cobalt and tungsten carbide powders). The authors used company administrative records to categorize 14 workshop groups on the basis of the work conducted within them: powder production, hard-metal production before sintering, hard-metal production after sintering, foundries (three workshops), production of other sintered alloys (six workshops), maintenance, and other non-exposed workshops. The JEM used by Moulin et al. (1998) was used to assign exposure intensities, durations, and cumulative exposures using previous exposure measurements to validate JEM coding. Time spent at past and present workplaces was divided into up to three consecutive periods with varying exposure levels. Metrics included ever/never exposed, workshop-based categories (e.g. "ever employed in" as well as "only employed in"), highest exposure score experienced in work history, duration at or above defined exposure score, and cumulative exposure categories.

Other studies of hard-metal workers deemed uninformative by the Working Group, because of high potential for simultaneous exposure to cobalt and WC-Co, are summarized below.

Lasfargues et al. (1994) examined multiple cancer mortality end-points in a retrospective cohort study of workers employed at a plant producing hard-metal tools for at least 1 year between 1 January 1956 and 31 December 1989 in France. Categorical assignment to various "degrees of exposure" (unknown, low, medium, and high exposure) was based on job histories/work locations obtained from company administrative records, as well as previous air and biological measurements obtained from an earlier epidemiological study conducted in 1983 (Meyer-Bisch et al., 1989). Exposure metrics with categories of duration of employment (years) and years since first employment were developed for the medium- and high-exposure categories.

Dufresne et al. (1996) reported on a case series of five aluminium smelter workers, four of whom died from lung cancer and one from mesothelioma. In addition to analysis of the occupational history of each deceased worker, fibrous and non-fibrous particles in lung tissue were visualized using phase-contrast microscopy and transmission electron microscopy. The composition of particles was determined using an energy-dispersive spectrometer and spectra were compared with those in a database of known minerals. The authors reported the concentration of "metal-rich" particles (millions of particles of > 0.1 μm in lung tissue, dry weight) for each case. Cobalt in lung tissue was reported to have been observed (qualitatively) in two of the five cases (both of whom were welders).

A retrospective cohort study examined cancer mortality (lung cancer and other end-points) of workers who had been employed at three Swedish hard-metal plants for at least 1 year (Hogstedt & Alexandersson, 1990). Production at the plants had started in the late 1930s, early 1940s, and 1950s. Company administrative records and expert opinion were used to describe job roles and work locations, which were combined with air concentration data (average levels, $\mu g/m^3$) collected between 1940 and 1982 to develop five exposure categories that were ultimately collapsed into "high-exposure" and "low-exposure" categories for the analyses.

A series of companion papers examined cancer (primarily mortality) retrospectively among hard-metal production workers in five countries (Sweden, Austria, Germany, the UK, and the USA) (Marsh et al., 2017a; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017). A pooled analysis by Marsh et al. (2017b) included all these countries. In Marsh et al. (2017a, b) and McElvenny et al. (2017), exposure assessment was based on a quantitative JEM that had been constructed for cobalt, tungsten, and nickel over the period 1952 to 2014 by Kennedy et al. (2017). [The Working Group noted that none of these studies were able to separate exposure to cobalt alone from exposure to WC-Co.]

(iii) Industry-based studies: other worker populations

A nested case–control study by Rodrigues et al. (2020) examined incidence and mortality related to brain and other central nervous system (CNS) cancers of employees at three facilities engaged in the manufacture of semiconductors and electronic storage devices in the USA. Administrative records were used to assess work histories from 1965, the first year for which detailed job information was available, or the date of hire at a study facility (whichever was later). Ten primary exposure groups (PEGs) were defined on the basis of the type of production taking place, tasks performed, work environment, and the potential for chemical and physical agents to be present within that environment. Cases were assigned to PEGs on the basis of the division and department in which the individual had worked and their job title (details available in Rodrigues et al., 2019). Mean concentrations for each exposure matrix cell (chemical/PEG/manufacturing era) were linked to participants' work history.

The studies of Bai et al. (2019) and Li et al. (2021b) focused on subsets of the Dongfeng-Tongji cohort, an ongoing prospective study of 27 009 retired workers who had been employed in the automotive industry. The prospective cohort study performed by Li et al. (2021b) examined incident cancers in patients with type 2 diabetes. Fasting blood samples were collected at enrolment baseline to assess plasma levels of nine essential metals (cobalt, iron, copper, zinc, selenium, chromium, manganese, molybdenum, and nickel) and three heavy metals (arsenic, cadmium, and lead). Bai et al. (2019) conducted a nested case–control study including 440 incident lung cancer cases and 1320 controls. The authors analysed baseline plasma concentrations of 11 essential metals, including cobalt, using single- and multiple-metal models.

(iv) General-population studies

The studies of White et al. (2019) and Niehoff et al. (2021) were both based on the nationwide Sister Study (which focused on breast cancer) conducted in the USA. White et al. (2019) assessed environmental exposure to airborne cobalt and other metals by linking census tract-level concentrations obtained from the National Air Toxics Assessment (NATA) database of the US EPA for 2005 to participants' residential addresses at the time of enrolment. Niehoff et al. (2021) determined concentrations of cobalt and other metals in toenail cuttings (all toes) from each participant at the time of enrolment in their case–cohort study.

Kresovich et al. (2019) reported a population-based analysis of 696 women enrolled in the Breast Cancer Care in Chicago study who had received a breast cancer diagnosis between 2005 and 2008. The residential addresses of participants 3–6 years before diagnosis were used to assign ambient exposure to cobalt at the census tract-level using US EPA NATA data, as done in White et al. (2019).

In a population-based case–control study by Mérida-Ortega et al. (2022), concentrations of cobalt and other metals in spot urine samples (obtained during first morning void) were used to examine environmental factors associated with breast cancer among women (452 cases and 439 controls) in some states of northern Mexico between 2007 and 2011. Principal component analysis (PCA) was used to examine patterns of exposure to a variety of metals in breast cancer cases and controls.

Duan et al. (2020) analysed concentrations of urinary cobalt and other metals in urine and/or blood to assess cancer mortality in a sample of 26 056 participants drawn from the United States National Health and Nutrition Examination Survey (NHANES) 1999–2014 (a total of 82 091 participants). The authors used statistical modelling to assess whether associations

might be driven by single contaminants versus mixtures. Pan et al. (2021) described a case–control study on oesophageal precancerous lesions in study participants identified through The Early Diagnosis and Early Treatment Project of Esophageal Cancer (EDETPEC), a surveillance programme in China's Huai'an District, which is considered a high-risk area for oesophageal cancer. Exposure to cobalt was assessed with a single blood sample at enrolment and with 3-day duplicate diet samples collected on consecutive days (two work days and one weekend day); no other metals were assessed.

O'Rorke et al. (2012) assessed exposure to cobalt using toenail cuttings (big toe only) in a population-based case–control study on oesophageal adenocarcinoma and Barrett oesophagus in Ireland. Sampling took place at recruitment, and concentrations of other metals were measured alongside cobalt but were considered separately. Rogers et al. (1993) reported on a case–control study on oral cavity cancer in Washington State, USA. Exposure to cobalt was assessed in toenail cuttings at recruitment. Exposure to other metals was assessed but also considered separately.

(v) Meta-analyses

Companion meta-analyses by Zhang et al. (2021a) and Holy et al. (2022) reported a systematic review and meta-analysis of epidemiological studies to examine overall cancer risk (Zhang et al., 2021a) and multiple cancer end-points (Holy et al., 2022) in relation to exposure to cobalt in occupational cohorts, as well as in total joint-replacement patient populations.

(b) Critical review of exposure assessment

(i) Industry-based studies

An important limitation of most studies assessed was the inability to rule out the effects of other potentially carcinogenic exposures (see Table 1.9 in Section 1.4.2 for a summary of other potentially co-occurring agents classified in IARC Group 1, *carcinogenic to humans*, or Group 2A, *probably carcinogenic to humans*, reported in the epidemiological studies assessed). This was particularly apparent in studies of hard-metal workers (Hogstedt & Alexandersson, 1990; Lasfargues et al., 1994; Moulin et al., 1998; Wild et al., 2000; Marsh et al., 2017a, b; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017), which shared similar limitations with respect to simultaneous exposure to cobalt and tungsten carbide specific to hard-metal manufacture.

Two studies of hard-metal workers, by Moulin et al. (1998) and Wild et al. (2000), reported analyses of exposure to cobalt with potential for separation from exposure to WC-Co. However, in Moulin et al. (1998) it is difficult to ascertain whether cobalt was the main driver of effects observed in the "other cobalt exposure" category, which included a number of production activities besides hard-metal manufacture, since exposure to other metals may have also occurred in these settings. With their workshop-based groupings, Wild et al. (2000) attempted to separate exposure to "hard metal dust of cobalt and tungsten carbide combined" from other processes with potential for cobalt exposure. However, similarly to Moulin et al. (1998), it is not clear that cobalt exposure occurred independently via these other processes (e.g. co-exposure to tungsten carbide powders may have occurred in the powder-production workshop; the foundry grouping may have included exposure to other metals that are potentially carcinogenic, such as nickel and chromium).

In the study by Marsh et al. (2009), which was based on a cobalt semiquantitative JEM, the authors noted that exposure to lead, arsenic, cadmium, and cobalt never occurred in isolation during any of the jobs studied. Moulin et al. (2000) observed that cobalt was moderately correlated with chromium and/or nickel (r = 0.67) in their semiquantitative JEM. Moulin et al. (1993) constructed mutually exclusive subgroups (e.g. workers only employed in cobalt production) but

could not account for potential co-exposure to arsenic and nickel (Mur et al., 1987). Sauni et al. (2017) acknowledged the potential for co-exposure to nickel, while pointing out that measured levels of nickel were relatively low in the work environments studied (see Table 1.9). Tüchsen et al. (1996) identified the potential for co-exposure to other carcinogenic agents, such as quartz (used in glazing until 1952 in factory 1 and for an unknown period in factory 2) and nickel (representing less than 0.5% of cobalt dyes used), which was not accounted for in the analyses. In the case series reported by Dufresne et al. (1996), all workers had at least one type of asbestos fibre identified in their lungs and concentrations of "metal-rich" particles were reported that were not specific to cobalt.

The studies that used JEMs for exposure characterization all shared a common strength of systematic assessment but a common limitation of non-differential exposure misclassification because of broad exposure categories; these include Moulin et al. (1998, 2000), Wild et al. (2000), Grimsrud et al. (2005), Marsh et al. (2009, 2017a, b), McElvenny et al. (2017), and Morfeld et al. (2017). JEMs, typically consisting of job and exposure axes, are retrospective exposure-assessment tools that are commonly used in occupational epidemiology because they permit the translation of job histories into exposures in a systematic and unbiased way (Peters, 2020). However, the informativeness and quality of JEMs vary depending on the specificities of job titles, the extent of quantitative exposure information available, and whether the exposure data cover the entire study period. Because a JEM assigns the same exposure estimates to all workers with the same job title (or other grouping), it may not reflect variability between workers in the same group (Kromhout et al., 1993). Any misclassification introduced by a JEM is expected to be non-differential with respect to the outcome, attenuating the risk estimates.

The JEM described by Kennedy et al. (2017) was used in the international pooled analysis reported by Marsh et al. (2017b), as well as in the UK- and USA-based studies by Marsh et al. (2017a, b) and McElvenny et al. (2017). The quantitative nature of this JEM and the extensive nature of the underlying data (site visits, industrial hygiene record reviews, and quantitative measurements) were strengths compared with other studies. However, a key limitation of these studies was that independent exposure estimates of cobalt versus WC-Co could not be generated due to their co-occurrence at some level for all job classes within the JEM. Furthermore, measurements were not available for all facilities and countries studied or for earlier periods, which challenges the validity of extrapolation across facilities and time periods.

Several studies reported the use of statistical modelling (linear models with the outcome being the natural logarithm of exposure measurements because of the usually skewed distribution of environmental exposures) of available exposure measurements, arguably the most efficient way to extract information from exposure databases while accounting for the combined influence of several exposure determinants (Burstyn & Teschke, 2010). In the study by Wallner et al. (2017) concerning an Austrian plant, the models were based on a relatively small number of measurements (~150 measurements of cobalt in air) although trends were reported to correlate well with ~250 urinary cobalt measurements. High correlation was observed between cobalt dust and tungsten. Westberg et al. (2017) used statistical models, based on ~2700 measurements collected from the early 1970s to 2012, to estimate airborne cobalt exposure across three plants. Sensitivity analyses were run to assess different back-extrapolation methods before 1970. Exposure to nickel and tungsten was dichotomized into two intensity groups because of the low number of measurements. A similar approach was reported by Svartengren et al. (2017). A study of hard-metal

workers employed at three production sites in Germany (Morfeld et al., 2017) also reported using statistical modelling based on ~1500 measurements, using two exposure groups (low versus high), production site, and calendar time as the main predictors.

A limitation of multiple studies reviewed was the use of the qualitative exposure metric of "employed versus never employed" for external comparison in retrospective occupational cohort studies conducted on workers exposed to cobalt (Cuckle et al., 1980; Moulin et al., 1993; Tüchsen et al., 1996; Moulin et al., 1998; Moulin et al., 2000; Wild et al., 2000; Marsh et al., 2009; Marsh et al., 2017a; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017). This metric suggests that exposure occurred in all employed individuals, with high potential for non-differential exposure misclassification. The use of "no" versus "low" versus "high" exposure in another study (Hogstedt & Alexandersson, 1990) has a similar likelihood of exposure misclassification because of broadly defined exposure groups.

In a similar vein, the subcohort analyses of some studies exclusively applied time-dependent exposure metrics (e.g. duration of employment, years since first employment) to serve as a proxy of exposure. A key limitation of these metrics was that they are not specific to a particular contaminant and do not account for differences in exposure according to which tasks were performed and where. Therefore, the studies relying on employment duration or related metrics had similar limitations and are likely to have high potential for non-differential exposure misclassification due to the grouping of workers across exposure conditions that may have been very different. Moulin et al. (1993) attempted to address some of these concerns by defining exposure groupings where workers were employed exclusively in one department (e.g. cobalt production or sodium production) for some analyses. Similar classification was performed by Sauni et al. (2017) and Wild et al. (2000).

(ii) General-population studies

A common limitation in the population-based cohort study of adults participating in NHANES by Duan et al. (2020) and the assessment of urinary cobalt among women in northern Mexico by Mérida-Ortega et al. (2022) is the measurement of metal concentrations in spot urine samples. Urinary levels of cobalt have relatively short half-lives; hence, the measured concentrations may have reflected recent rather than long-term exposure. In addition, Mérida-Ortega et al. (2022) assessed exposure after participants had received a cancer diagnosis, which is a limitation because the relevant exposure window may not have been reflected in the exposure estimates. In the study by Duan et al. (2020), metals were measured in NHANES 1999–2002 and mortality between 1999 and 2015 was assessed, potentially resulting in a short time period between any measured exposure to cobalt and the outcome under study. A strength of both studies was their use of statistical approaches that accounted for co-exposure to multiple metals.

Three studies conducted among women in the USA (Kresovich et al., 2019; White et al., 2019; Niehoff et al., 2021) assessed metals singly and as mixtures; White et al. (2019) and Niehoff et al. (2021) both reported on analyses from the Sister Study cohort. The exposure metrics in the studies by White et al. (2019) and Kresovich et al. (2019) are similar; modelled census tract-level estimates of outdoor air levels of cobalt and antimony were linked to residential addresses. In White et al. (2019), this was done for a single year and for participants' addresses at enrolment; in Kresovich et al. (2019), participants' addresses 3–6 years before diagnosis were used for exposure assessment. In both cases, the exposure assessment did not capture temporal trends in outdoor air concentrations of metals, nor did it account for variation in levels within census

tracts. Furthermore, there was no consideration of residential mobility. In White et al. (2019), a sensitivity analysis was conducted that was restricted to women who did not move during the follow-up period. Another investigation of the Sister Study cohort used toenail cuttings (all toes) to assess exposures to cobalt and antimony (together with 15 other metals) (Niehoff et al., 2021). Two additional case–control studies also used toenail cuttings (big toes) (Rogers et al., 1993; O'Rorke et al., 2012). As a biomarker of exposure, toenail cuttings are advantageous because collection is simple and non-invasive. As with other biomarkers, toenails reflect exposure from all routes. A strength of the study by Niehoff et al. (2021) was that toenail cuttings were collected at enrolment in the cohort study and before the outcome diagnosis, whereas toenail cuttings were collected at enrolment, but after the outcome had been diagnosed among cases, in the case–control studies by Rogers et al. (1993) and O'Rorke et al. (2012), which is a limitation and probably contributed to non-differential exposure misclassification. An important consideration, and potential limitation, is the period of exposure reflected in samples of toenail cuttings and whether it is aligned with the relevant at-risk period for the outcome under study. Metal concentrations in toenails have been shown in a recent review to represent exposures 3–12 months before sampling (Gutiérrez-González et al., 2019). In occupational studies of metals, the correlation between metal concentrations in toenails and exposure is stronger for the 7–12-month period before sampling (Laohaudomchok et al., 2011; Grashow et al., 2014). In a general-population study by Garland et al. (1993) and a breast cancer case–control study by O'Brien et al. (2019), correlations between repeated measures of metals in toenail clippings were reported for participants with no identified occupational exposures to metals. Garland et al. (1993) found that correlations between metals in toenails collected ~6 years apart ranged from $r = 0.26$ for copper to $r = 0.58$ for zinc; the correlation for cobalt was $r = 0.35$. O'Brien et al. (2019) reported correlations between metal concentrations in two toenail samples collected ~8 years apart in the Sister Study cohort in the USA that ranged from $r = 0.18$ for antimony to $r = 0.71$ for mercury, among cases and controls; the correlation for cobalt was $r = 0.34$ overall ($r = 0.46$ for controls and $r = 0.24$ for cases).

The serum samples used in the case–control study by Pan et al. (2021) integrate exposure from all routes of exposure. The exposure measures are limited by the fact that they rely on one blood sample collected after the diagnosis of the outcome, and thus may not have captured the relevant exposure window, leading to potential non-differential misclassification of exposure. Pan et al. (2021) also collected dietary samples, representing ingestion (intentional) exposure. Although dietary samples were collected in triplicate, they were collected on consecutive days and may not reflect variability in dietary patterns.

(iii) Meta-analyses

A major limitation of the meta-analyses by Zhang et al. (2021a) and Holy et al. (2022) is the lack of comparability between study populations with respect to exposure contexts, routes, and metallic speciation for occupationally exposed workers compared with patients implanted with artificial joints. In occupational analyses, meta-estimates included studies on both hard and non-hard metals, resulting in a lack of interpretability for exposure to cobalt without tungsten carbide. In addition, only broad exposure metrics were extracted from the occupational cohort studies (e.g. employment in cobalt-related work for at least 1 year), which further limits their informativeness.

1.6.2 Mechanistic studies in humans

The Working Group identified 27 mechanistic studies in which a critical appraisal of exposure assessment methods was undertaken. The critiques undertaken for each study in relation to different aspects of exposure assessment are tabulated in Table S1.19 (Annex 1, Supplementary material for Section 1, Exposure Characterization, web only, available from: https://publications.iarc.fr/618).

(a) Exposure assessment methods

The studies identified included seven observational studies of the general population (Arslan et al., 2011; Calderón-Garcidueñas et al., 2013; Johnstone et al., 2014; Bibi et al., 2016; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021), 16 industry-based studies (Bencko et al., 1983, 1986a; Nemery et al., 1990; Gennart et al., 1993; Swennen et al., 1993; Rizzato et al., 1994; Shirakawa & Morimoto, 1997; De Boeck et al., 2000; Hengstler et al., 2003; Krakowiak et al., 2005; Mateuca et al., 2005; Walters et al., 2012; Tilakaratne & Sidhu, 2015; Princivalle et al., 2017; Wultsch et al., 2017; Andersson et al., 2021), and three experimental studies (L'vova et al., 1990; Katsarou et al., 1997; Amirtharaj et al., 2008).

(i) General-population studies

Most of the general-population studies were cross-sectional (Calderón-Garcidueñas et al., 2013; Bibi et al., 2016; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021) with two being case–control studies (Arslan et al., 2011; Johnstone et al., 2014). Five studies used biological samples to quantitatively assess exposure. Xue et al. (2021) and Li et al. (2021b) assessed concentrations of cobalt and other metals in blood samples from individuals living near e-waste–recycling sites and individuals living elsewhere. Calderón-Garcidueñas et al. (2013) assessed cobalt quantitatively in the frontal cortex tissues of 59 decedents living in cities with differing levels of air pollution. Howe et al. (2021) used spot urine samples from women in the first trimester of pregnancy to quantitatively assess concentrations of cobalt and eight other metals (cross-sectional analysis within a prospective cohort study). Bibi et al. (2016) assessed concentrations of cobalt in the urine, blood, and nails, although the analysis of mechanistic end-points only used data relating to the blood samples.

In the case–control studies, Arslan et al. (2011) used biological samples (blood) in their study of oxidative stress in those with and without malignant glial tumours, while Johnstone et al. (2014) assessed urinary concentrations of cobalt (along with several other metals and trace elements) among 495 women participating in the Endometriosis Natural History, Diagnosis and Outcomes study.

(ii) Experimental studies

Three experimental studies were also identified for review (L'vova et al., 1990; Katsarou et al., 1997; Amirtharaj et al., 2008). Amirtharaj et al. (2008) examined the role of oxidative stress and fatty acids in modulating cobalt binding to albumin in patients with fatty liver disease by applying a known amount of cobalt chloride (0.232 mmol/L) to serum samples (ex vivo) to assess cobalt binding. In a larger study of 180 cement workers who participated in repeated patch testing, Katsarou et al. (1997) examined the T-cell responses of 20 people: 10 who had consistently positive patch-test results and 10 who had previously had a positive patch test and now had a negative patch test. In this study, cobalt chloride was applied to primary T-cells isolated from the individuals, and responses were assessed (ex vivo), but neither the amount nor the concentration of cobalt applied was reported. L'vova et al. (1990) applied cobalt chloride to human primary cell cultures (ex vivo) to assess mutagenicity.

(iii) Industry-based studies

Four publications reported on case studies or a case series. Tilakaratne & Sidhu (2015) described the work histories and patch test results for two workers with histories of work in home renovations, one with a diagnosis of cutaneous T-cell lymphoma (the other was a suspected case). Nemery et al. (1990) measured the concentration of cobalt (as well as iron, nickel, and chromium) in postmortem lung tissue collected from a 52-year-old man who had worked as a diamond polisher using polishing discs containing cobalt, and who died from interstitial fibrosis (Nemery et al., 1990). Rizzato et al. (1994) assessed cobalt concentrations in various biological specimens (e.g. blood, urine, pubic hair, nails, and sperm) from three individuals with sarcoidosis who all had an occupational history of exposure to cobalt. Krakowiak et al. (2005) described the occupational history of a diamond polishing-disc former with asthma. The patient's response to controlled exposure to cobalt chloride was assessed using patch testing and nasal-provocation testing.

There were 12 cross-sectional studies focused on different occupational groups, many of which relied on biological samples for the exposure assessment. Cobalt exposure was prospectively assessed in urine and blood samples collected at multiple time points from 34 workers in a hard-metal manufacturing plant (Principalle et al., 2017). Although there was potential exposure to WC-Co, no data on WC-Co exposure were reported. Hengstler et al. (2003) assessed cobalt exposure using air and urine samples (spot samples given the same day as air sampling) from 78 workers at 10 facilities engaged in either the production of cadmium-containing pigments or batteries, or the recycling of electric tools; exposures to lead and cadmium were additionally assessed. Walters et al. (2012) reported on 62 workers employed at an aerospace manufacturing company. Urinary cobalt was assessed using spot samples (as was urinary chromium). In addition, semiquantitative exposure groups were constructed on the basis of exposure to metal working fluids. Wultsch et al. (2017) measured cobalt concentrations in blood samples collected from 42 workers at a bright electroplating factory (comparison group, 43 jail wardens). In addition to the concentration of cobalt in blood, duration of exposure (years) was considered as a semiquantitative (categorical) exposure metric. De Boeck et al. (2000) and Mateuca et al. (2005) reported on the same study population, which included workers exposed to cobalt, workers exposed to WC-Co, and workers with neither exposure. Although urinary cobalt concentration was assessed with a single end-of-week sample collection, qualitative groups were used in the analysis (cobalt-exposed, hard-metal (WC-Co) exposed, and unexposed controls). Gennart et al. (1993) assessed cobalt concentrations in urine samples from 24 men employed at a metal-powder production factory and compared them with those of 23 clerical workers. Results from the biological samples suggested higher levels of exposure among the production workers, but mechanistic outcomes were limited to comparisons between the two groups (exposed/unexposed) and by duration of exposure categories (0 years, less than 5 years, and at least 5 years). Andersson et al. (2021) assessed cobalt exposure among 72 Swedish hard-metal workers via quantitative measures of inhalation exposure, using personal and stationary samples, that described multiple size fractions for mass concentrations (total, inhalable, and respirable) as well as particle surface areas and numbers. Andersson et al. (2021) also used biological monitoring (blood and urine) to assess internal cobalt concentrations.

In the other studies, exposure was defined in a qualitative manner (Swennen et al., 1993; Shirakawa & Morimoto, 1997). Swennen et al. (1993) compared 82 workers at a cobalt plant with 82 unexposed workers. Employment at the plant (yes/no) was used as the exposure measure

in the analysis of mechanistic end-points; air and biological samples demonstrated low exposure among the comparison group (Swennen et al., 1993). Shirakawa & Morimoto (1997) qualitatively assessed hard-metal exposure (yes/no) among workers at hard-metal plants. Workers included in the analysis were engaged in the production of hard metals, a process that included other metals (e.g. tungsten, nickel, and molybdenum), as described by Kusaka et al. (1986). Two studies did not provide information on the occupational settings in which the exposed workers were employed (Bencko et al., 1983, 1986a), only describing the exposure group as "occupationally exposed" to cobalt.

(b) Critical review of exposure assessment

Many studies used biological measures to assess exposure to cobalt (Nemery et al., 1990; Gennart et al., 1993; Hengstler et al., 2003; Arslan et al., 2011; Walters et al., 2012; Calderón-Garcidueñas et al., 2013; Johnstone et al., 2014; Wultsch et al., 2017; Andersson et al., 2021; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021). The timing of biological samples is important because it determines the period of exposure reflected in sample results. Different types of biological sample (e.g. urine, blood, hair) are likely to represent different time periods of exposure. The exposure-only study from Princivalle et al. (2017) reported that cobalt in urine has a shorter half-life (5.3 days) than that of cobalt in blood (12.3 days), and the authors concluded that measurement of cobalt concentrations in blood is preferable to measurement in urine because the results are more reliable; this would apply to studies of the general population and to groups of workers. Two studies used data from deceased individuals (Nemery et al., 1990; Calderón-Garcidueñas et al., 2013) and thus were limited to available samples. Many studies relied on a single biological sample to assess exposure (Gennart et al., 1993; Hengstler et al., 2003; Arslan et al., 2011; Walters et al., 2012; Johnstone et al., 2014; Wultsch et al., 2017; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021), which was a limitation because single samples do not capture exposure variability and introduce non-differential exposure misclassification. A strength of the study by Andersson et al. (2021) was the collection of two biological samples in the same week, which allowed for comparison between the two time points.

A strength of several studies was the inclusion of air monitoring data that quantitatively described the external exposure conditions in the workplaces. Results from air sampling were reported in five studies, including those concerning workers involved in cobalt refining (Swennen et al., 1993), hard-metal production (Shirakawa & Morimoto, 1997), cadmium pigment/battery production and electric tool recycling (Hengstler et al., 2003), bright electroplating (Wultsch et al., 2017), and metal-powder production (Gennart et al., 1993). Only in the cases of Shirakawa & Morimoto (1997) and Hengstler et al. (2003) were these external exposure measures considered in the statistical analyses reported.

Several studies examined working populations for which co-exposure to other metals was probable, including those involved in hard-metal production (Shirakawa & Morimoto, 1997; Princivalle et al., 2017; Andersson et al., 2021), the production of cadmium-containing pigments (Hengstler et al., 2003), battery manufacture (Hengstler et al., 2003), recycling of electric tools (Hengstler et al., 2003), bright electroplating (Wultsch et al., 2017), aerospace manufacturing (Walters et al., 2012), and metal-powder production (Gennart et al., 1993). As noted in Section 1.6.1(b)(i), a limitation of these studies was the inability to rule out the effects of co-exposures to other metals, some of which are known carcinogens (e.g. chromium(VI), nickel, and cadmium). A summary of other potentially co-occurring IARC Group 1 and Group 2A agents in selected occupational settings is presented

in Table 1.9 A key limitation of the studies of hard-metal workers was the probable co-exposure to WC-Co (Shirakawa & Morimoto, 1997; Princivalle et al., 2017; Andersson et al., 2021). The study by Shirakawa & Morimoto (1997) was limited because the authors did not assess exposure to cobalt specifically, instead using exposure to hard metal (yes/no) as the exposure metric. Princivalle et al. (2017) prospectively assessed cobalt concentrations in urine and blood samples collected from hard-metal workers, and while the potential for co-exposure to WC-Co was a limitation, the mechanistic outcomes under study were specific to cobalt. Andersson et al. (2021) characterized cobalt exposure and particulate matter exposure in detail among a population of Swedish hard-metal workers (and the cobalt exposures were relatively low, as noted by the authors), but the reporting of tungsten exposure in all job groups under study was a limitation.

A strength of the studies by Hengstler et al. (2003), Princivalle et al. (2017), and Wultsch et al. (2017) was the use of biological samples (urine or blood) to quantify exposure to cobalt. Gennart et al. (1993) used biological samples to assess exposure among production and clerical workers, but analysis of mechanistic end-points was limited to qualitative and semiquantitative exposure metrics (e.g. exposed/unexposed, duration of exposure) that were not specific to cobalt exposure. Shirakawa & Morimoto (1997) and Walters et al. (2012) both employed qualitative exposure definitions that were not specific to cobalt (e.g. exposed/unexposed, work areas). Exposure assessment was probably more specific to cobalt in Swennen et al. (1993), where cobalt production workers were under study and a qualitative metric of exposure was used (exposed/unexposed). A strength of De Boeck et al. (2000) and Mateuca et al. (2005), two studies based on the same population, was the identification of a group of workers with only cobalt exposure, which was compared with a group of workers who had WC-Co exposure.

A strength of many population-based studies and studies of workers was the inclusion of exposure assessment for co-exposure to metals other than cobalt (Arslan et al., 2011; Walters et al., 2012; Calderón-Garcidueñas et al., 2013; Johnstone et al., 2014; Bibi et al., 2016; Wultsch et al., 2017; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021). One exception was the study of hard-metal workers by Shirakawa & Morimoto (1997) that only considered cobalt exposure (qualitatively), which was a limitation of this study. Despite the large number of studies that measured exposure to other metals, only Hengstler et al. (2003) accounted for exposure to other metals (cadmium, nickel, and lead) in their analysis. All case reports and case series (Nemery et al., 1990; Rizzato et al., 1994; Krakowiak et al., 2005; Tilakaratne & Sidhu, 2015) noted other potential exposures. Nemery et al. (1990) measured concentrations of cobalt, iron, nickel, and chromium in lung tissue. Tilakaratne & Sidhu (2015) discussed nickel and chromium as potential co-exposures, but only qualitatively, and did not confirm exposure to cobalt. There was potential exposure to WC-Co and other metals in the case reported by Krakowiak et al. (2005), but challenge testing was completed only for cobalt. Rizzato et al. (1994) quantified cobalt, tungsten, and tantalum in all biological samples.

By definition, cross-sectional studies are limited because exposure and outcome are assessed at the same point in time and thus cannot provide evidence of temporality (Bencko et al., 1983, 1986a; Gennart et al., 1993; Swennen et al., 1993; Shirakawa & Morimoto, 1997; De Boeck et al., 2000; Hengstler et al., 2003; Mateuca et al., 2005; Walters et al., 2012; Calderón-Garcidueñas et al., 2013; Bibi et al., 2016; Wultsch et al., 2017; Andersson et al., 2021; Howe et al., 2021; Li et al., 2021b; Xue et al., 2021). Two studies reporting on the same population did not provide sufficient information for the exposure assessment to be assessed, which was a major limitation because no information on the industry or occupational

exposure of workers, or the potential for co-exposure, could be ascertained (Bencko et al., 1983, 1986a).

A strength of the experimental studies was that the exposure is a known, and controlled, element of the experiment (L'vova et al., 1990; Katsarou et al., 1997; Amirtharaj et al., 2008) and thus the exposure is well characterized. The potential limitations of these studies pertained to the relevance of the experimental exposure level to real-world exposure levels and the lack of variability in the experimental exposure levels, as would be expected among exposed humans.

2. Cancer in Humans

In this section, a review of the evidence from studies of cancer in humans exposed to cobalt metal (without tungsten carbide) and cobalt compounds is presented. Cobalt metal without tungsten carbide was evaluated previously in 2003 and is described in *IARC Monographs* Volume 86 (IARC, 2006). In that volume, the evidence relating to cancer in humans consisted of a series of studies of the French and Swedish hard-metal industries (e.g. Hogstedt & Alexandersson 1990; Moulin et al., 1998; Wild et al., 2000), the French cobalt-production industry (Moulin et al., 1993), and a Danish study of porcelain painters (Tüchsen et al., 1996). Cobalt metal without tungsten carbide was evaluated as IARC Group 2B, *possibly carcinogenic to humans*, with a determination that the evidence in humans was *inadequate*. *IARC Monographs* Volume 86 also found that the evidence regarding cancer in humans was *inadequate* for other forms of cobalt besides WC-Co. Since then, several additional studies of occupational cohorts and the general population have been published. In considering occupational cohort studies with multiple publications, only those with the most recent cancer follow-up were reviewed in detail.

The Working Group identified 12 occupational studies on the risk of lung and other cancers at hard-metal production sites (see Table 2.1), but the majority were considered uninformative because there were no specific analyses related to cobalt without simultaneous co-exposure to tungsten carbide. Most workers in the hard-metal industry are exposed to a composite of WC-Co, unsintered or sintered. The production of WC-Co hard metals involves the mixing and granulation of tungsten carbide and cobalt powders. After mixing, the material is pressed and shaped by cutting and drilling. These steps give rise to unsintered hard-metal dust that results in simultaneous exposure to cobalt and tungsten carbide. The pieces are then sintered at a high temperature in an oxygen-free furnace and are finally finished by grinding and drilling, giving rise to simultaneous exposure to sintered WC-Co hard-metal dust. Exposure to WC-Co was evaluated in *IARC Monographs* Volume 86 as having *limited evidence* for cancer in humans on the basis of positive associations with lung cancer in cohorts of workers involved in hard-metal production. Because of the nature of the hard-metal production process, it is difficult to evaluate risk of exposure to cobalt alone (i.e. in the absence of the WC-Co composite material) in most studies of hard-metal workers. The Working Group reviewed the 12 hard-metal production studies to determine whether any presented analyses for cobalt exposure might be relevant for this evaluation. Two such studies were identified (Moulin et al., 1998; Wild et al., 2000).

Five occupational studies of other industries that reported results for lung cancer in relation to cobalt exposure were reviewed (Moulin et al., 1993; Tüchsen et al., 1996; Moulin et al., 2000; Grimsrud et al., 2005; Sauni et al., 2017). Two additional studies on lung cancer among workers at a nickel refinery and copper smelter were not considered informative for this evaluation (Cuckle et al., 1980; Marsh et al., 2009). One other

Table 2.1 Epidemiological studies of cancer of the lung in workers producing hard metals

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1998) France 1942–1991/ 1968–1991 Cohort	7459 workers (5777 men, 1682 women) with ≥ 3 mo experience in hard-metal production (10 facilities); for 61 lung cancer cases with job histories, exposure–response analyses used a nested case–control design in which 3 controls were sampled from each case's risk set, matching on sex and date of birth (± 3 mo) (180 controls) Exposure assessment method: Cohort: exposure via all routes (indirectly) assessed qualitatively using company administrative records; exposure metrics: ever employment (≥ 3 mo) Nested case–control: JEM used to assign cumulative exposure based on maximum intensity score and duration (unweighted or additionally weighted by frequency)	Lung, mortality	SMR:			Age, sex, and calendar period	*Exposure assessment critique*: Key strengths include: JEM validated with atmospheric cobalt measurements. Key limitations include: Non-differential exposure misclassification likely (broad exposure categories for cohort and JEM for case–control study). Cobalt exposure not assessed independently (all analyses included co-exposure to tungsten carbide or other agents). *Other strengths:* A large study involving nearly 7500 workers, 684 deaths (any cause), 63 from lung cancer. In the nested case–control study, smoking status was known for about 80% of the cases and controls, and there was a suggestion of slight negative confounding. *Other limitations*: No adjustment for occupational exposures other than for WC-Co and other cobalt exposure. *General comments*: Exposures lagged 10 yr.
			Whole cohort (men and women, any exposure)	63	1.30 (1.00–1.66)		
		Lung, mortality	Level of WC-Co exposure, smoking habits known (OR):			Age, sex, year of birth	
			0–1	NR	1		
			2–9	NR	2.29 (1.08–4.88)		

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1998) France 1942–1991/ 1968–1991 Cohort (cont.)		Lung, mortality	Level of WC-Co exposure, smoking habits known (OR):			Age, sex, year of birth, smoking	
			0–1	NR	1		
			2–9	NR	2.60 (1.16–5.82)		
		Lung, mortality	Level of WC-Co exposure, and other cobalt exposure (ever/never) (OR):			Age, sex, year of birth, exposure to WC-Co, and "other cobalt exposure" mutually adjusted	
			0–1	26	1		
			2–9	35	1.93 (1.03–3.62)		
			Other cobalt exposure	15	2.21 (0.99–4.90)		
		Lung, mortality	Level of WC-Co exposure, and other cobalt exposure (ever/never) (OR):				
			0–1	26	1		
			2–3	8	3.37 (1.19–9.56)		
			4–5	19	1.54 (0.76–3.12)		
			6–9	8	2.79 (0.96–8.10)		
			Other cobalt exposure	15	2.05 (0.94–4.45)		
			Trend-test *P*-value, 0.08 (for WC-Co)				
		Lung, mortality	Duration of WC-Co exposure at level ≥ 2, and other cobalt exposure (ever/never) (OR):				
			Non-exposed	26	1		
			≤ 10 yr	19	1.61 (0.78–3.34)		
			10–20 yr	12	2.77 (1.12–6.82)		
			> 20 yr	4	2.03 (0.49–8.51)		
			Other cobalt exposure	15	2.20 (0.99–4.87)		
			Trend-test *P*-value, 0.03 (for WC-Co)				

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1998) France 1942–1991/ 1968–1991 Cohort (cont.)		Lung, mortality	Unweighted cumulative dose of WC-Co (months × levels), and other cobalt exposure (ever/ never) (OR):			Age, sex, year of birth, exposure to WC-Co, and "other cobalt exposure" mutually adjusted	
			< 32	6	1		
			32–142	16	2.64 (0.93–7.47)		
			143–299	16	2.59 (0.88–7.60)		
			> 299	23	4.13 (1.49–11.47)		
			Other cobalt exposure	15	1.83 (0.86–3.91)		
			Trend-test *P*-value, 0.01 (for WC-Co)				

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wild et al. (2000) France 1950–1992/ 1968–1992 Cohort	2860 workers (2216 men, 644 women); ≥ 3 mo experience in the largest hard-metal facility in France Exposure assessment method: exposure to cobalt via all routes (indirectly) assessed qualitatively using company administrative records; exposure metric: ever employment, workshop-based categories; JEM used to assign semiquantitative exposure intensity, duration, and cumulative exposure (1, sum of score by duration; 2, score weighted by frequency code)	Lung, mortality	Employed ≥ 3 mo (SMR):			Age, calendar period	*Exposure assessment critique*: Key strengths include: low job turnover noted at this site (most with same job for entire work history) and large exposure gradient noted for hard-metal dust across work areas. Key limitations include: non-differential exposure misclassification likely (broad exposure categories). High likelihood of simultaneous exposures, particularly in maintenance (where asbestos exposure estimated in JEM). *Other strengths*: Prospective follow-up. Nearly full work history (at the plant). *Other limitations*: Smoking habits collected retrospectively from former workers. Simultaneous exposure to powders containing known or potentially carcinogenic metals or metal compounds. *General comments*: Follow-up ended at age 85 yr, death, or end of 1992. Exposures lagged 10 yr.
			Men	46	1.70 (1.24–2.26)		
			Women	1	1.19 (0.02–6.62)		
		Lung, mortality	Men only employed in powder production (SMR):	2	1.39 (0.17–5.02)		
		Lung, mortality	Men ever employed in powder production (SMR):	5	1.92 (0.62–4.49)		
		Lung, mortality	Men ever exposed to cobalt except in combination with tungsten carbide (SMR):	15	1.95 (1.09–3.22)		

Table 2.1 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wild et al. (2000) France 1950–1992/ 1968–1992 Cohort (cont.)		Lung, mortality	Exposed to IARC carcinogens, men (SMR):			Age, calendar period	
			Asbestos	13	1.95 (1.04–3.33)		
			PAH	13	1.99 (1.09–3.40)		
			Silica	9	1.73 (0.79–3.29)		
			Nickel compounds	15	1.76 (0.99–2.91)		
			Chromium compounds	2	2.08 (0.23–7.52)		
			Any of the above	26	2.05 (1.34–3.00)		
		Lung, mortality	Exposed to any IARC carcinogen (asbestos, PAH, silica, nickel, chromium compounds), men (RR):			Age, calendar period, smoking, exposure to dust from sintered hard metal (yes/ no), duration of exposure to dust from unsintered hard metal (unexposed, 3 levels of duration)	
			Never	NR	1		
			Ever	NR	1.48 (0.81–2.68)		

CI, confidence interval; IARC, International Agency for Research on Cancer; JEM, job-exposure matrix; mo, month; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; SIR, standardized incidence ratio; SMR, standardized mortality ratio; RR, relative risk; WC-Co, cobalt with tungsten carbide; yr, year.

study examined the incidence of lung cancer in relation to plasma levels of metals, including cobalt, in a population of retired workers who had been employed in the automotive industry (Bai et al., 2019). Only one occupational study was identified that did not report results for lung cancer. Rodrigues et al. (2020) analysed the risk of malignant CNS cancers in workers employed in the semiconductor and electronic storage device-manufacturing industry.

There were also several studies that examined associations between general-population exposures to cobalt and various cancer types. White et al. (2019) examined the relationship between estimated air concentrations of cobalt in the participants' census tract of residence at the time of enrolment and breast cancer incidence. Studies have also examined associations of cobalt with various cancers using biomarkers of exposure, including studies investigating cobalt concentrations measured in toenail cuttings in relation to breast, larynx, oral cavity, and oesophageal cancers (including oesophageal precursor lesions) (Rogers et al., 1993; O'Rorke et al., 2012; Niehoff et al., 2021), as well as studies of cobalt concentrations measured in plasma, serum, or urine (Bai et al., 2019; Duan et al., 2020; Li et al., 2021a; Pan et al., 2021; Mérida-Ortega et al., 2022). The types and routes of cobalt exposure assessed via biological samples represented all routes of exposures, including ingestion through the diet as well as inhalation of ambient air.

Workers in smelting processes and other industries studied were potentially exposed to cobalt metal and cobalt compounds. Co-exposure to substances evaluated by IARC as *carcinogenic to humans (Group 1)* with evidence for lung cancer was present in most occupational studies and was a key consideration of the Working Group in evaluating studies.

The outcomes examined in most occupational studies were based on cancer mortality rather than incidence. For lung cancer, which tends to have a shorter survival time, this is a reasonable approximation of lung cancer incidence. However, this is not the case for many other types of cancers and is particularly problematic when evaluating all other types of cancers combined, which comprises a heterogeneous group of outcomes. Furthermore, the case definitions for the incidence studies tended to be more valid and based on histological confirmation.

The Working Group noted that the meta-analysis studies of Zhang et al. (2021a) and Holy et al. (2022) included studies of hard-metal production workers. While the meta-analyses also included some studies that were considered to be informative, the reported meta-estimates could not be adequately interpreted for exposure specific to cobalt and were therefore considered uninformative for this evaluation.

2.1 Lung cancer

2.1.1 Studies of workers producing hard metals

See Table 2.1.

Particles of metal carbides cemented in a metal binder constitute the most common composite hard metals used for cutting tools and other industrial purposes. The carcinogenic potential of exposure to WC-Co was evaluated by the *IARC Monographs* programme in 2003 (Volume 86; IARC, 2006) and is not within the scope of this monograph, in which exposure to cobalt metal alone or with co-exposure to substances other than WC-Co are evaluated. Nonetheless, the Working Group reviewed studies of hard-metal workers to identify analyses that may contribute to the present evaluation, for example, where exposure to cobalt could potentially be assessed independently of exposure to WC-Co. Studies without data relevant for this purpose are only briefly mentioned and are not shown in Table 2.1.

There were four studies of lung cancer mortality among hard-metal workers from the 20th century: three conducted in France and

one in Sweden. Two of them covered only exposure to WC-Co (Hogstedt & Alexandersson, 1990; Lasfargues et al., 1994). They were evaluated by *IARC Monographs* Volume 86 (IARC, 2006) and are not considered here. Moulin et al. (1998) assessed lung cancer mortality among 7459 workers at 10 hard-metal production facilities in France, who were employed for at least 3 months, with follow-up from 1968 to 1991. The study included the largest production site that was investigated separately by Wild et al. (2000), described below. The study by Moulin et al. (1998) reported that, across the 10 cohorts assessed, the overall risk of lung cancer mortality was elevated compared with national mortality rates, with a standardized mortality ratio (SMR) of 1.30 (95% confidence interval, CI, 1.00–1.66; 63 deaths). Exposures during the 10 years before death were disregarded in nested case–control analyses. In the case–control analyses, risk was estimated in models including two types of cobalt exposure: cobalt in dust from hard metal (WC-Co) with different exposure metrics (categories of duration, intensity, and cumulative measures) and ever exposure to cobalt in other situations with no WC-Co, denoted "other cobalt exposure" (dichotomous variable). For workers exposed to WC-Co, there were signs of increasing risk with duration of exposure and with increasing cumulative exposure, adjusted for "other cobalt exposure". The risk of lung cancer mortality in workers ever exposed to "other cobalt exposure" ranged between odds ratios (ORs) of 1.83 and 2.21 in different models (15 exposed cases), with lower bounds of the 95% confidence intervals varying between 0.86 and 0.99. However, these models were not adjusted for exposure to other agents classified by IARC in Group 1, *carcinogenic to humans*, with *sufficient* evidence in the lung, such as asbestos, arsenic, and chromium, nickel, and cadmium compounds, which were known to be present at the facilities under study. Analyses that included smoking habits, which involved 80% of the nested case–control sample, suggested a slight negative confounding effect of smoking on exposure to WC-Co. [The Working Group considered subanalyses of "other cobalt exposure" potentially informative because the models controlled for exposure to WC-Co. However, there were no exposure–response analyses presented for other cobalt exposures. Another concern is that there may be residual confounding from exposure to WC-Co, the predominant form of cobalt exposure at the facilities, which may not have been fully captured by the models. Importantly, this study did not account for exposures to other lung carcinogens present at the plants and had no information about the extent or level of exposure to other lung carcinogens, which makes it difficult to rule out their contribution to the observed excess of lung cancer.]

Wild et al. (2000) analysed lung cancer mortality data from 1968 to 1992 among 2860 workers employed at the largest of the production sites studied by Moulin et al. (1998) (see above). Complete work histories were available for most workers, and the workers were followed for 1 year longer than in the study by Moulin et al. (1998). The overall risk of lung cancer in the cohort was higher among men than among the local population (SMR, 1.70; 95% CI, 1.24–2.26). A subgroup of men was engaged in the production of cobalt powder by reducing cobalt hydroxide. Powder production also included the manufacture of tungsten carbide powder from wolframite ore, but risk estimates were not reported specifically for workers exposed only to cobalt metal powders. In analyses in which exposure groups were categorized by production department (workshop), men ever employed in departments of powder production (i.e. exposed to cobalt powder only, or separately to tungsten carbide powder and to cobalt powder) had a lung cancer standardized mortality ratio of 1.92 (95% CI, 0.62–4.49; 5 deaths). For men only employed in powder production, the standardized mortality ratio for lung cancer was 1.39 (95% CI, 0.17–5.02; 2 deaths). When exposure

was defined according to a JEM, men exposed to cobalt excluding WC-Co had a standardized mortality ratio for lung cancer of 1.95 (95% CI, 1.09–3.22; 15 deaths). In further analyses of exposures to specific agents classified by IARC as "lung carcinogens", including asbestos and metal compounds, standardized mortality ratios were in the range of 1.73–2.08. [The Working Group noted that the authors selected five Group 1 agents with *sufficient* evidence for lung cancer in humans, although there is no *IARC Monographs* evaluation of "PAHs" as a class of compounds.] The standardized mortality ratio for men with exposure to any "IARC carcinogen" was 2.05. In an internal analysis with Poisson regression, the relative risk associated with exposure to agents classified by IARC as lung carcinogens among men was 1.48 (95% CI, 0.81–2.68), adjusted for age, calendar period, smoking, and exposure to dust from WC-Co. [The Working Group considered the subanalyses of "powder production workers" and workers exposed to "cobalt except hard metal" as potentially informative. However, it was not possible to estimate the risk from cobalt exposure adjusted for occupational exposures other than for those exposed to dust from WC-Co. According to the JEM, exposure to IARC-classified lung carcinogens was common at the plant and positively associated with an increased risk of lung cancer among the workers.]

Additional cohort studies published in 2017 reported lung cancer mortality or incidence among hard-metal workers in Sweden, Germany, Austria, the UK, and the USA, together with a pooled analysis (Marsh et al., 2017a, b; McElvenny et al., 2017; Morfeld et al., 2017; Svartengren et al., 2017; Wallner et al., 2017; Westberg et al., 2017). None of these studies reported risk in workers exposed to cobalt without concomitant exposure to WC-Co. [The Working Group considered these studies uninformative for the purpose of the present monograph.]

2.1.2 *Studies of workers with other occupational exposure to cobalt*

See Table 2.2.

Seven unique study populations were identified that constituted non-hard-metal cobalt production workers.

A cohort of 1143 workers with a minimum of 1 year of employment at a factory producing cobalt and sodium in south-eastern France was followed up for mortality from 1950 to 1980 by Mur et al. (1987). Some analyses were conducted only among workers born in France because of high loss to follow-up among workers born outside of France. The cohort ($n = 1148$) mortality and job histories were updated by Moulin et al. (1993) until 1988. In the second follow-up (Moulin et al., 1993), a small excess of lung cancer was reported (Moulin et al., 1993), with a standardized mortality ratio of 1.16 (95% CI, 0.24–3.40) compared with French national rates (SMR, 1.16; 95% CI, 0.24–3.40), based on three lung cancer deaths among French-born workers employed exclusively in cobalt production. [The Working Group noted that interpretation of this study was limited given the very small number of expected cases in the cobalt production workshop, resulting in statistical imprecision, and the possible confounding with asbestos exposure.]

A population of Danish women working in two porcelain factories in Copenhagen, comprising 874 workers exposed to a dye containing cobalt and 520 who were not exposed, was followed up for mortality (from 1968 until the end of 1992) and cancer incidence (from 1943 or 1962 to 1992) by Tüchsen et al. (1996). The dye used was reported to contain 25% cobalt and < 0.5% nickel. Cobalt aluminate spinel dye was replaced by cobalt silicate dye in factory 1 in 1972 and in factory 2 in 1989. Industrial hygiene measurements of dust (possibly quartz) and ambient air cobalt silicate concentrations were measured on an annual basis between 1982 and 1988 in factory 1 and between 1981 and 1988

Table 2.2 Epidemiological studies of cancer of the lung in workers with other occupational exposure to cobalt

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1993) France Enrolment, 1950–1980/ follow-up, 1988 Cohort	1148 men employed at an electrochemical plant producing cobalt and sodium for ≥ 12 mo between 1950 and 1980 (same population as in Mur et al., 1987) Exposure assessment method: exposure to cobalt via all routes (indirectly) was assessed qualitatively based on production area using company job history records	Lung, mortality	Employed in cobalt production, French-born workers (SMR): Exclusively in cobalt production Ever in cobalt production	 3 4	 1.16 (0.24–3.40) 1.18 (0.32–3.03)	Age, calendar period	*Exposure assessment critique*: Key strengths include: clearly defined exposure groups. Key limitations include: non-differential exposure misclassification likely (broad exposure categories). Possible co-exposures identified could not be fully accounted for in analyses. *Other strengths*: Analyses in subgroup without loss to follow-up. *Other limitations*: causes of death before 1968 assessed by physicians. Incomplete follow-up among non-French-born. No smoking data. *General comments*: results from several analyses could not be adequately interpreted for exposure to cobalt alone.

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Tüchsen et al. (1996) Denmark Enrolment, factory 1, 1943–1987; enrolment, factory 2, 1962–1987/ follow-up, 1992 Cohort	874 exposed/520 unexposed; all women working in one of two porcelain factories employed in the plate underglazing departments (exposed to cobalt) and a referent population (unexposed) working in cobalt-free departments in the same factories Exposure assessment method: exposure (indirectly) to cobalt aluminate spinel via all routes was assessed qualitatively using company administrative records; exposure metrics: ever/never employed	Lung, incidence	Exposure group (SIR):			Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely. Possible co-exposure to dusts (including quartz) and nickel at "insignificant" levels not accounted for in analyses. *Other strengths*: a long follow-up period and few lost to follow-up. Cancer incidence obtained through the national registers. *Other limitations*: a limited population with corresponding low expected numbers of cancers.
			All exposed	8	2.35 (1.01–4.62)		
			Factory 1, exposed	3	[1.60 (0.41–4.37)]		
			Factory 2, exposed	5	[3.25 (1.19–7.20)]		
			Referents	7	1.99 (0.80–4.11)		
		Lung, incidence	Exposure group (RR):				
			Referents	7	1		
			All exposed	8	1.2 (0.4–3.8)		

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (2000) France Enrolment, 1968–1991/ follow-up, 1992 Nested case–control	Cases: 54; death from lung cancer in a cohort of workers in a stainless steel-producing factory, employed for ≥ 1 yr between 1968 and 1991 Controls: 162; 3 workers per case sampled from the case's risk set and matched on sex and date of birth (± 6 mo) Exposure assessment method: Cohort: employment of ≥ 1 yr in steel-producing factory assessed qualitatively using company administrative records; exposure metric: ever employment of ≥ 1 yr between 1968 and 1991 Case–control: semiquantitative JEM used to assign exposures examining duration, intensity, frequency of exposure by job period with 10 yr lag period applied; employment by work area also examined	Lung, mortality	Cobalt exposure, 10 yr lag, smoking history known (OR):			Age, sex, year of birth, PAH, silica, smoking status	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely (broad exposure categories for cohort and JEM for case–control). Co-exposure to chromium and/or nickel likely. *Other strengths*: a relatively large cohort with complete administrative records of job histories. *Other limitations*: incomplete smoking histories (only known for 67% of cases and 73% of controls). No exposure measurements. Co-exposure to other metals not accounted for in analyses.
			None	NR	1		
			Ever	12	0.44 (0.17–1.16)		
		Lung, mortality	Exposure level (OR):			Age, sex, year of birth, smoking status	
			Per 1 unit increase in level (0, none; 1, low; 2, medium; 3, high)	12	0.54 (NR)		
			Trend-test *P*-value, 0.09				
		Lung, mortality	Duration of exposure (OR):				
			Per one-category increase (1, < 10 yr; 2, 10–19 yr; 3, 20–29 yr; 4, ≥ 30 yr)	12	0.56 (NR)		
			Trend-test *P*-value, 0.04				
		Lung, mortality	Frequency unweighted cumulative dose quartile (OR):				
			Per one-quartile increase (quartiles among controls, coded 1–4)	12	0.55 (NR)		
			Trend-test *P*-value, 0.02				
		Lung, mortality	Frequency-weighted cumulative dose quartile (OR):				
			Per one-quartile increase (quartiles among controls, coded 1–4)	12	0.54 (NR)		
			Trend-test *P*-value, 0.02				

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Grimsrud et al. (2005) Norway 1952–1995 Nested case–control	Cases: 213; lung cancer cases among employees with employment ≥ 1 yr in a nickel refinery Controls: 525; controls matched to cases by sex and year of birth, free from lung cancer at the time of diagnosis Exposure assessment method: exposure to cobalt (all routes) assessed qualitatively and quantitatively in a cohort of nickel refinery workers based on company records, using job categories and quantitative exposure ratios with nickel	Lung, incidence	Cumulative cobalt exposure (OR):			Age, sex, year of birth, smoking in 5 categories (never, former, current: 1–10, 11–20, > 20 g/day)	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely. Exposure to nickel and cobalt highly correlated. *Other strengths*: available historical personnel files. Smoking information obtained from subjects or next of kin. A large study with corresponding large power. *Other limitations*: inability to evaluate cobalt independently from nickel.
			Unexposed	9	1		
			Low: 0.31–29.5 μg/m³-years	49	1.5 (0.6–3.8)		
			Medium 29.7–142 μg/m³-years	73	2.4 (1.0–5.6)		
			High 144–3100 μg/m³-years	82	2.9 (1.2–6.8)		
		Lung, incidence	Cumulative cobalt exposure (OR):				
			Per 1 unit increase [(mg/m³) × yr]	303	1.3 (0.9–1.8)		
		Lung, incidence	Cumulative cobalt exposure (OR):			Age, sex, year of birth, smoking in 5 categories (never, former, current: 1–10, 11–20, > 20 g/day); cumulative exposure to nickel, arsenic, asbestos, sulfuric acid, carcinogenic work outside the refinery (year)	
			Per 1 unit increase [(mg/m³) × yr]	303	0.7 (0.3–1.4)		

Table 2.2 (continued)

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sauni et al. (2017) Finland Enrolment, 1968–2004/ follow-up, 2013 Cohort	995; men employed for ≥ 1 yr at a Finnish cobalt plant between 1968 and 2004 Exposure assessment method: exposure to cobalt via all routes (indirectly) assessed semiquantitatively using company administrative records; exposure metrics: duration and departmental exposure groupings	Lung, incidence Lung, incidence Lung, incidence	Incidence (SIR): Total Exposure group (SIR): Variable exposure Low Moderate High Duration of employment (SIR): > 1 yr > 5 yr	 6 0 2 0 4 6 5	 0.50 (0.18–1.08) 0 (0–6.68) 0.41 (0.05–1.47) 0 (0–5.56) 0.67 (0.18–1.72) 0.50 (0.18–1.08) 0.52 (0.17–1.22)	Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential misclassification likely. Possible co-exposure to nickel. *Other strengths*: complete work histories from personnel records. Precise exposure characterization. Cancer incidence rather than mortality. Some smoking data suggested that the low lung cancer risk was not due to lower smoking rates. *Other limitations*: relatively small number of expected cases. Possible co-exposure to nickel not accounted for in analyses.

CI, confidence interval; JEM, job-exposure matrix; mo, month; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; SIR, standardized incidence ratio; SMR, standardized mortality ratio; RR, relative risk; yr, year.

in factory 2. Cobalt concentrations in air in these factories were occasionally noted to be high (sometimes > 1000 μg/m^3) (Christensen & Poulsen, 1994). Both exposed women (standardized incidence ratio, SIR, 2.35; 95% CI, 1.01–4.62; n = 8) and the reference group (SIR, 1.99; 95% CI, 0.80–4.11; n = 7) had greater risk of lung cancer than the national reference rate. However, the exposed group had a relative risk ratio of 1.2 (95% CI, 0.4–3.8) when compared with the reference group. [The Working Group noted that the low number of lung cancer cases limited the statistical precision of the results. Inclusion of an internal unexposed referent group is an important strength of the study, and elevated standardized incidence ratios in both the exposed and referent groups suggest that the increased risk of lung cancer was not related to cobalt exposure.]

Workers at a factory producing stainless and alloyed steel (n = 4897) were followed up for mortality between 1968 and 1992 (Moulin et al., 2000). A JEM was developed to assign semiquantitative estimates of exposure to cobalt, as well as other metals and agents. Cobalt exposure was correlated with nickel and chromium (r = 0.67) as classified by the JEM. Within this factory, a metal powder production process for cobalt, nickel, and iron was initiated in 1972. No information was available regarding the number of workers in this workshop or expected numbers of lung cancer cases, and no lung cancer cases were observed. In a nested case–control study including 54 lung cancer cases and 162 controls, only 1 control and no cases had been involved in metal powder production, indicating that the expected number of cases was very small in this workshop. Based on 12 exposed cases, the odds ratio for ever versus never cobalt exposure, lagged by 10 years and adjusted for smoking and exposure to polycyclic aromatic hydrocarbons and silica, was equal to 0.44 (95% CI, 0.17–1.16). Inverse trends were observed for increasing exposure-level categories, duration of exposure, and cumulative exposure indices (based on exposure level and duration), adjusted for smoking. [The Working Group noted that this study provides no information on lung cancer risk associated with cobalt given the very small number of expected cases in the cobalt production workshop, and the likelihood of confounding by nickel or chromium in the stainless-steel production process.]

Grimsrud et al. (2005) conducted a nested case–control study within a Norwegian cohort of refinery workers in which there were 213 cases of lung cancer and 525 controls matched on age, sex, and year of birth. A positive trend in smoking-adjusted odds ratios was observed for cobalt exposure assessed using a JEM based on quantitative exposure measurements. However, associations with cobalt exposure were confounded by nickel exposure, as all individuals who were exposed to cobalt were also exposed to nickel, generally at much higher levels than to cobalt. The positive trend with cobalt was reversed after further adjusting for other occupational exposures (nickel, arsenic, asbestos, sulfuric acid mist, and carcinogenic exposure in work outside the refinery). [The Working Group noted that no dose-related positive trend was seen for cobalt exposure after adjustment for exposure to known lung carcinogens.]

A cohort of 995 workers at a metal refinery – who were potentially exposed to metallic cobalt powder, cobalt salts, oxides, sulfides, and nickel compounds – in Kokkola, Finland, was followed up for cancer incidence through 2013 (Sauni et al., 2017). The cohort was further divided into subcohorts by exposure levels, according to the departments in which employees had first worked at the plant. The exposures in different departments were based on regular industrial hygiene measurements taken between 1986 and 2014. The mean concentrations of cobalt exposure varied between < 0.02 mg/m^3 and 0.10 mg/m^3 depending on when the measurement was taken and the workshop. The standardized incidence ratio for lung cancer among men employed for more than 1 year was 0.50 (95% CI, 0.18–1.08;

6 cases) (the rates used for comparison were local rates from Central Ostrobothnia, Finland). An exposure subgroup analysis (variable, low, moderate, or high) showed no statistically significant excesses in any subgroup nor any positive trends. Smoking rates among workers towards the end of the follow-up period were higher in the study population than in the region from which comparative lung cancer rates were available. Thus, the lower-than-expected lung cancer incidence cannot be explained by lower smoking rates. The cohort was relatively young, with person-years above age 60 years accounting only for 15% of the total person-years. [The Working Group noted that the small number of cases and the relatively young age of the cohort limited the potential for a meaningful exposure–response analysis to be conducted.]

[Two additional occupational studies of lung cancer in industries with potential cobalt exposures were considered uninformative by the Working Group. A cohort study of workers at a company manufacturing nickel and cobalt salts in Clydach, south Wales, UK (Cuckle et al., 1980), was considered uninformative because of co-exposure to nickel, an IARC Group 1 lung carcinogen, and a lack of analyses specifically related to cobalt. A study by Marsh et al. (2009) of workers employed at a facility conducting smelter, mill, or sulfur operations between 1946 and 1996 in Copperhill, Tennessee, USA, was considered uninformative because the prevalence of cobalt exposure in the study population was unclear and exposure to other metals was common, making it difficult to interpret findings for the evaluation of cobalt.]

2.1.3 Studies of other populations

See Table 2.3.

One nested case–control study from China (Bai et al., 2019) examined the association between plasma concentrations of selenium and other metals [most of them essential elements] and the risk of lung cancer among a cohort of retired workers who had been employed at a car production facility. [The Working Group noted that the occupational cohort was not selected on the basis of high cobalt exposure, and it was unclear whether the study participants had any specific exposure to cobalt in their work.] The study was based on the Dongfeng-Tongji cohort and included 27 009 retired workers from an automotive manufacturing company who were recruited to the study between 2008 and 2010. Between April and October 2013, an additional 14 120 new retirees were also enrolled. A total of 452 lung cancer cases were identified with follow-up to the end of 2016 (440 who had available fasting peripheral venous blood samples were included). The self-reported new lung cancer cases were confirmed from medical records or death certificates. The diagnosis of lung cancer for surgery patients was based on histopathological analysis of surgical pathology archives from the Tongji Hospital. Healthy controls (n = 1320) were frequency matched on age and sex. Baseline plasma concentrations of 11 elements including cobalt were measured. The main focus of the study was the potential protective effect of zinc exposure on lung cancer risk. A one-unit increase in the natural log-transformed concentration of cobalt in plasma (μg/L) was weakly associated with increased risk of incident lung cancer in all participants (OR, 1.07; 95% CI, 0.86–1.32), men only (OR, 1.10; 95% CI, 0.84–1.45), and women only (OR, 1.07; 95% CI, 0.76–1.51) in single-metal models, which were adjusted for potentially confounding factors, but the associations were imprecise. [The Working Group noted that the plasma concentration of cobalt was measured only once, at the start of the study, and may not reflect long-term exposures that are likely to be associated with lung cancer. The time period between the measurement of metal concentrations and outcome was small (≤ 8 years).]

Table 2.3 Epidemiological studies of cancer of the lung in other populations

Reference Location Enrolment/follow-up period Study design	Population size, description Exposure assessment method	Organ site, incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bai et al. (2019) China Enrolment, 2008–2010 and 2013/ follow-up, 2016 Nested case–control	Cases: 440 incident cases of lung cancer in the Dongfeng-Tongji cohort; cohort consisted of retired (n = 27 009, 87% of invited participants agreeing) and newly retired (n = 14 120) employees of an automotive-manufacturing company; participants provided baseline blood samples and questionnaire information between September 2008 and June 2010; the newly retired workers were recruited between April and October 2013. Controls: 1320 healthy controls frequency-matched (1:3) to cases by age (± 3 yr) and sex – who were free of cancer, diabetes, and cardiovascular disease at baseline and during the follow-up period – were selected from the Dongfeng-Tongji cohort. Exposure assessment method: exposure to cobalt through all routes was assessed quantitatively using blood samples (focus on "essential metals" in an occupational cohort)	Lung, incidence	Plasma cobalt level (μg/L) (OR): Median, 0.14 μg/L; IQR, 0.09–0.27 μg/L; per 1 unit increase (natural log-transformed) Plasma cobalt level (μg/L), men (OR): Per 1 unit increase (natural log-transformed) Plasma cobalt level (μg/L), women (OR): Per 1 unit increase (natural log-transformed)	 440 275 165	 1.07 (0.86–1.32) 1.10 (0.84–1.45) 1.07 (0.76–1.51)	Age, sex, BMI, education level, smoking status, alcohol-drinking status, regular physical activity status, pack-year smoking, family history of cancer	*Exposure assessment critique*: Key limitations include: differential misclassification possible, non-differential likely. Possible co-exposure to other carcinogens in workplace not discussed. Time period between metal measurement and outcome potentially very small. *Other strengths*: Large cohort. Results adjusted for covariates. *Other limitations*: Plasma concentrations of cobalt measured only once at baseline, which does not consider the possible variability over time.

BMI, body mass index; CI, confidence interval; IQR, interquartile range; OR, odds ratio; yr, year.

2.2 Breast cancer

See Table 2.4.

Three cohort studies, one case series, and one case–control study investigated the association between cobalt exposure and breast cancer.

The study by Tüchsen et al. (1996), described in Section 2.1.2 above, estimated the risk of breast cancer among women occupationally exposed to cobalt aluminate and cobalt silicate spinel dye at two porcelain factories in Copenhagen, Denmark. The cohort consisted of 874 women exposed occupationally to cobalt and 520 women employed at the factories but not exposed to cobalt (referents). Cancer cases were identified from the Danish Cancer Registry, and there was a long follow-up period (1943–1992). When compared with national reference rates, standardized incidence ratios for breast cancer were not notably increased among exposed workers overall (SIR, 0.93; 95% CI, 0.53–1.52), among workers in factory 1 (SIR, 0.76; 95% CI, 0.31–1.57), or among workers in factory 2 (SIR, 1.12; 95% CI, 0.52–2.13). The standardized incidence ratio among the unexposed workers was 0.81 (95% CI, 0.44–1.38). [The Working Group noted that the results were not adjusted for reproductive risk factors like parity and age at first birth. The number of cancer cases was also rather low and risk estimates were imprecise.]

The study population analysed in the studies by White et al. (2019) and Niehoff et al. (2021) was based on the nationwide Sister Study in the USA, a cohort of 50 884 women (age, 35–74 years) who had a sister who had received a diagnosis of breast cancer. However, in the latter study, the researchers selected a race-stratified case–cohort consisting of non-Hispanic Black and non-Hispanic White participants.

The study by White et al. (2019) identified a total of 2587 breast cancer cases during follow-up (mean, 7.4 years). Exposure to cobalt was estimated on the basis of the 2005 US EPA's NATA census tract estimates of metal concentrations in outdoor air at participants' places of residence at the time of enrolment. The results were reported according to the quintiles of air concentrations of cobalt. The adjusted hazard ratios for quintiles 2 and 3 were 1.2 (95% CI, 1.0–1.3) and 1.2 (95% CI, 1.0–1.3), respectively. Quintiles 4 and 5 did not indicate an increased risk of breast cancer. The adjusted hazard ratios for postmenopausal breast cancer in quintiles 2 and 3 were 1.2 (95% CI, 1.0–1.3) and 1.2 (95% CI, 1.0–1.4), respectively. In the premenopausal group, there was no evidence of an association between exposure to cobalt and breast cancer. In analyses stratified by estrogen receptor (ER) status, positive associations with no clear exposure gradient were noted among the ER-positive cases versus non-cases, but not among ER-negative cases. [The Working Group noted that census tract-level concentrations used in the study are very broad proxies for personal exposures. In addition, the evaluation of cobalt concentration in air represented only one time point and the baseline cobalt air concentration in the Sister Study was very low. Exposure may vary locally, and people may move during the follow-up period. However, the hazard ratio for breast cancer according to cobalt exposure was not increased among those who never moved during follow-up.]

In the other case–cohort study nested within the Sister Study cohort (Niehoff et al., 2021), exposure to metals, including cobalt, was based on analyses of toenail cuttings. The toenail cuttings were collected at baseline before breast cancer diagnosis. The cohort consisted of 1499 patients with breast cancer and a subcohort of 1607 women in the USA and Puerto Rico who were selected at random. The period of follow-up was 7.5 years on average. The study did not identify increased hazard ratios for breast cancer in any of the cobalt concentration tertiles. Relative to tertile 1, the hazard ratio for tertile 2 was 1.00 (95% CI, 0.82–1.21) and for tertile 3 was 1.01 (95% CI, 0.83–1.22). The results were further stratified by race/ethnicity, ER status (ER-positive

Table 2.4 Epidemiological studies of cancer of the breast and exposure to cobalt

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Tüchsen et al. (1996) Denmark Enrolment, factory 1: 1943–1987; factory 2: 1962–1987/ follow-up, 1992 Cohort	874 exposed/520 unexposed; all women working in one of two porcelain factories employed in the plate underglazing departments (exposed to Co) and a referent population (unexposed) working in Co-free departments in the same factories Exposure assessment method: exposure to Co aluminate spinel via all routes (indirectly) was assessed qualitatively using company administrative records	Breast, incidence	Exposure group (SIR): All exposed Factory 1, exposed Factory 2, exposed Referents	 14 6 8 12	 [0.93 (0.53–1.52)] [0.76 (0.31–1.57)] [1.12 (0.52–2.13)] [0.81 (0.44–1.38)]	Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential exposure misclassification likely. Possible co-exposure to dusts (e.g. quartz) and Ni at "insignificant" levels. *Other strengths*: Long follow-up period. Exposure measurements from the two factories from several years. Linkage of the cohort with national cancer register. *Other limitations*: The results were not adjusted for confounders including co-exposures. High number of emigrated workers. Information bias possible.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2015 Cohort	2587 breast cancer cases; prospective cohort study of 50 884 women (age 35–74 yr) who had a sister diagnosed with breast cancer but no prior breast cancer at enrolment (Sister Study), followed through July 2015 Exposure assessment method: assessment of Co metal exposure was made for a single year in time quantitatively based on address at enrolment before the development of the outcome; annual census-tract estimates of metal concentrations in air (μg/m³) for Co – along with As, Cd, Cr, Pb, Mn, Hg, Ni, Sb, and Se from the US EPA 2005 NATA – were linked to participants' geocoded residences at baseline and categorized into quintiles for analysis	Breast, incidence	Quintiles of residential airborne Co concentration (HR):			Age, race, education, annual household income, marital status, parity, census-tract median income, geographical region	*Exposure assessment critique*: Key strengths include: weighted quantile sum regression was used to assess metal mixtures. Key limitations include: non-differential exposure misclassification likely, as neither temporal trends in outdoor metal levels nor residential mobility was accounted for in the exposure assessment, and potential for within census-tract variability in outdoor air levels of metals also probably introduced error in the exposure assessment. *Other strengths*: Large prospective cohort study. Address ascertained at baseline. Extensive covariate information.
			Quintile 1	472	1		
			Quintile 2	549	1.2 (1.0–1.3)		
			Quintile 3	552	1.2 (1.0–1.3)		
			Quintile 4	508	1.1 (0.94–1.2)		
			Quintile 5	487	1.0 (0.91–1.2)		
			Trend-test *P*-value, 0.9				
		Breast, incidence	Quintiles of residential airborne Co concentration, premenopausal women (HR):				
			Quintile 1	88	1		
			Quintile 2	114	1.1 (0.86–1.5)		
			Quintile 3	109	1.1 (0.79–1.4)		
			Quintile 4	104	0.97 (0.72–1.3)		
			Quintile 5	119	1.1 (0.83–1.5)		
			Trend-test *P*-value, 0.9				
		Breast, incidence	Quintiles of residential airborne Co concentration, postmenopausal women (HR):				
			Quintile 1	383	1		
			Quintile 2	435	1.2 (1.0–1.3)		
			Quintile 3	442	1.2 (1.0–1.4)		
			Quintile 4	403	1.1 (0.95–1.3)		
			Quintile 5	367	1.0 (0.87–1.2)		
			Trend-test *P*-value, 0.9				
		Breast, ER+ vs non-cases, incidence	Quintiles of residential airborne Co concentration (HR):				
			Quintile 1	269	1		
			Quintile 2	333	1.26 (1.07–1.48)		
			Quintile 3	313	1.19 (1.01–1.40)		
			Quintile 4	298	1.14 (0.96–1.36)		
			Quintile 5	274	1.07 (0.89–1.27)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2015 Cohort (cont.)		Breast, ER– vs non-cases, incidence	Quintiles of residential airborne Co concentration (HR):			Age, race (non-Hispanic White, other), education, annual household income, marital status, parity (continuous), census-tract median income, geographical region	An independent validation study in California found good agreement between monitored data and certain air toxics in the 2005 NATA data release. *Other limitations*: For exposure analysis, only the exposure levels at the enrolment residence were considered.
			Quintile 1	61	1		
			Quintile 2	48	0.79 (0.54–1.15)		
			Quintile 3	58	0.92 (0.63–1.34)		
			Quintile 4	47	0.72 (0.48–1.08)		
			Quintile 5	47	0.72 (0.48–1.08)		
		Breast: ER+ vs ER– cases, incidence	Quintiles of residential airborne Co concentration (HR):			Age, race, education, annual household income, marital status, parity, census-tract median income, geographical region	
			Quintile 1	NR	1		
			Quintile 2	NR	1.59 (1.05–2.41)		
			Quintile 3	NR	1.29 (0.85–1.95)		
			Quintile 4	NR	1.58 (1.02–2.45)		
			Quintile 5	NR	1.49 (0.95–2.32)		
		Breast, incidence	Quintiles of residential airborne Co concentration, BMI < 25 (HR):				
			Quintile 1	158	1		
			Quintile 2	204	1.25 (1.01–1.54)		
			Quintile 3	216	1.35 (1.10–1.67)		
			Quintile 4	178	1.08 (0.87–1.35)		
			Quintile 5	173	1.11 (0.88–1.39)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
White et al. (2019) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2015 Cohort (cont.)		Breast, incidence	Quintiles of residential airborne Co concentration, BMI ≥ 25 (HR):			Age, race, education, annual household income, marital status, parity, census-tract median income, geographical region	
			Quintile 1	314	1		
			Quintile 2	345	1.10 (0.94–1.28)		
			Quintile 3	336	1.04 (0.89–1.22)		
			Quintile 4	330	1.06 (0.91–1.25)		
			Quintile 5	314	0.99 (0.84–1.17)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Niehoff et al. (2021) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2017 Cohort	50 884 women (age 35–74 yr), who had a sister diagnosed with breast cancer but no prior breast cancer at enrolment (Sister Study); case–cohort study design evaluated 1495 incident breast cancers (all non-Hispanic Black cases and a random sample of non-Hispanic White cases) and 1605 women randomly sampled from the cohort, stratified by race/ethnicity Exposure assessment method: concentrations of 15 metals, including Co, were measured in toenail clippings collected at baseline and categorized into tertiles for analysis.	Breast, incidence	Co level (HR):			Age, education, race/ethnicity, BMI, smoking status, parity/ breastfeeding	*Exposure assessment critique*: Key strengths include: exposures were assessed before the development of the outcome and analyses considered the metal mixture. Key limitations include: non-differential exposure misclassification was likely, and metals in toenails typically represent exposures 3–12 mo before sampling (Gutiérrez-González et al., 2019); hence, a single toenail specimen may not have represented average exposure during the follow-up period. *Other strengths*: Cases and controls were drawn from a large, national prospective study population.
			Tertile 1 (< 5.2 ng/g)	524	1		
			Tertile 2 (5.2–10.5 ng/g)	488	1.00 (0.82–1.21)		
			Tertile 3 (> 10.5 ng/g)	483	1.01 (0.83–1.22)		
			Trend-test *P*-value, 1.0				
		Breast, ER+	Co level (HR):				
			Tertile 1 (< 5.2 ng/g)	366	1		
			Tertile 2 (5.2–10.5 ng/g)	355	1.01 (0.82–1.24)		
			Tertile 3 (> 10.5 ng/g)	372	1.07 (0.87–1.32)		
		Breast, ER–	Co level (HR):				
			Tertile 1 (< 5.2 ng/g	67	1		
			Tertile 2 (5.2–10.5 ng/g)	64	1.07 (0.72–1.58)		
			Tertile 3 (> 10.5 ng/g)	55	0.92 (0.61–1.37)		
		Breast	Co level, non-Hispanic White (HR):				
			Tertile 1 (< 5.2 ng/g)	402	1		
			Tertile 2 (5.2–10.5 ng/g)	418	1.03 (0.84–1.27)		
			Tertile 3 (> 10.5 ng/g)	432	1.02 (0.84–1.26)		
		Breast	Co level, non-Hispanic Black (HR):				
			Tertile 1 (< 5.2 ng/g)	122	1		
			Tertile 2 (5.2–10.5 ng/g)	70	0.71 (0.49–1.01)		
			Tertile 3 (> 10.5 ng/g)	51	0.78 (0.52–1.18)		
		Breast (ductal carcinoma in situ)	Co level (HR):				
			Tertile 1 (< 5.2 ng/g)	121	1		
			Tertile 2 (5.2–10.5 ng/g)	116	1.06 (0.78–1.43)		
			Tertile 3 (> 10.5 ng/g)	104	0.95 (0.70–1.29)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Niehoff et al. (2021) USA and Puerto Rico Enrolment, 2003–2009/ follow-up, 2017 Cohort (cont.)		Breast (invasive breast cancer)	Co level (HR):			Age, education, race/ethnicity, BMI, smoking status, parity/ breastfeeding	The case–control study had a large sample size and extensive covariate information *Other limitations*: Unclear how informative the exposure contrast in the study was, as there are no agreed toxic or harmful levels of the metals measured in the toenails.
			Tertile 1 (< 5.2 ng/g)	403	1		
			Tertile 2 (5.2–10.5 ng/g)	372	0.98 (0.80–1.20)		
			Tertile 3 (> 10.5 ng/g)	379	1.02 (0.83–1.25)		
		Breast	Co level, postmenopausal women (HR):				
			Tertile 1 (< 5.2 ng/g)	449	1		
			Tertile 2 (5.2–10.5 ng/g)	396	0.92 (0.75–1.13)		
			Tertile 3 (> 10.5 ng/g)	398	0.95 (0.77–1.16)		
		Breast	Co level, premenopausal women (HR):				
			Tertile 1 (< 5.2 ng/g)	75	1		
			Tertile 2 (5.2–10.5 ng/g)	92	1.04 (0.67–1.62)		
			Tertile 3 (> 10.5 ng/g)	85	0.90 (0.91–1.10)		

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kresovich et al. (2019) USA Enrolment, 2005–2008 Case series [case–case comparison study]	Cases: 696; incident breast cancers among women (age 30–79 yr) diagnosed with a first primary in situ or invasive breast cancer, self-identified as non-Latina White, non-Latina Black, or Latina living in the metropolitan Chicago area at time of diagnosis, enrolled in the Breast Cancer Care in Chicago study and evaluated for ER/PR status (ER/PR-negative if both ER-negative and PR-negative (n = 147); ER/PR-positive, otherwise (n = 549)) Exposure assessment method: total ambient inhalation exposure to Co was quantitatively estimated at the census-tract level using the US EPA NATA data that account for mobile and stationary sources of exposure, but do not include indoor sources or other occupational exposures	Breast, incidence	Quintiles of residential airborne cobalt in ER/PR-negative vs ER/PR-positive cases (OR): Quintile 1 (< 0.010 ng/m^3) Quintile 2 (0.010–0.014 ng/m^3) Quintile 3 (0.014–0.017 ng/m^3) Quintile 4 (0.017–0.024 ng/m^3) Quintile 5 (> 0.024 ng/m^3) Trend-test P-value, 0.04	 NR NR NR NR	 1 1.4 (0.7–2.8) 2.2 (1.2–4.2) 1.8 (0.9–3.4) 2.0 (0.9–4.4)	Age, race/ethnicity, education, BMI, income, census-tract affluence and disadvantage, reproductive factors	*Exposure assessment critique*: Key strengths include: evaluation of co-exposure to other metals in ambient air. Key limitations include: non-differential misclassification likely. Timing of exposure measurement may be outside the relevant time window of exposure for cancer outcome under study. Census-tract level concentrations are broad proxies for personal exposures. The reliance on residential address at a single point in time may have introduced non-differential misclassification.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kresovich et al. (2019) USA Enrolment, 2005–2008 Case series [case–case comparison study] (cont.)							*Other strengths*: High proportion of ER/PR-negative cases (21%) giving enough power to detect etiological heterogeneity. Co-exposures considered in analyses. *Other limitations*: 11% of participants were excluded due to missing residential history and 20% were missing information on tumour ER/PR status.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mérida-Ortega et al. (2022) Northern Mexico Enrolment, 2007–2011 Case–control	Cases: 452 histopathologically confirmed breast cancer cases from main public and academic hospitals (age ≥ 18 yr), no personal history of other type of cancer, ≥ 1 year residence in study area, creatinine concentration in normal range (20–300 mg/dL), and available information for urinary metal concentrations Controls: 439 women with ≥ 1 yr residence in study area with no personal history of cancer, creatinine concentration in normal range (20–300 mg/dL), and available information for urinary metal concentrations, matched by age to cases (± 5 yr) Exposure assessment method: this study quantitatively assessed Co exposure (all routes) in urine samples collected at a single point in time; in addition, exposure to other metals and trace elements was assessed	Breast	Co quartile (μg/g creatinine): Quartile 1 Quartile 2 Quartile 3 Quartile 4	 NR NR NR Trend-test *P*-value, 0.000	 1 1.20 (0.82–1.75) 0.79 (0.54–1.18) 0.45 (0.28–0.70)	Age, schooling, estrogenic index, alcohol consumption, BMI	*Exposure assessment critique*: Key strengths include: consideration of co-exposure to other metals and trace elements. Key limitations include: reliance on a single spot urine sample. The use of a single sample may not reflect the relevant exposure window, particularly as the sample was collected after the outcome. *Other strengths*: Population-based case–control study design; area studied has natural contamination by metals in water and the largest non-ferrous metal processing site worldwide. Exposure to other metals accounted for in the statistical analysis.

Table 2.4 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mérida-Ortega et al. (2022) Northern Mexico Enrolment, 2007–2011 Case–control (cont.)							*Other limitations*: Urine samples collected after diagnosis, leading to potential for reverse causality. Spot urine sample may not reflect exposures during biologically relevant time period.

As, arsenic; BMI, body mass index; Cd, cadmium; CI, confidence interval; Co, cobalt; Cr, chromium; ER, estrogen receptor; Hg, mercury; HR, hazard ratio; LOD, limit of detection; Mn, manganese; Mo, molybdenum; mo, month; NATA, National Air Toxics Assessment; ND, not detected; Ni, nickel; OR, odds ratio; Pb, lead; PR, progesterone receptor; Sb, antimony; SD, standard deviation; Se, selenium; US EPA, United States Environmental Protection Agency; W, tungsten; wk, week; yr, year; Zn, zinc.

versus ER-negative), menopausal status, and invasiveness. Cobalt concentration was not associated with risk of breast cancer in any of these additional analyses. [The Working Group noted the strengths of the study with respect to design and sample size. Assessment of exposure at a single point in time was a limitation, and there was only a twofold difference in cut-off points used to define the lowest (< 5.2 ng/g) and highest (> 10.5 ng/g) exposure groups. Sample sizes were small for some subgroup analyses, resulting in wide confidence intervals for ER-negative breast cancers and cancers among non-Hispanic Black women.]

The association between the ER and progesterone receptor (PR) status of breast cancer and cobalt exposure was also examined by Kresovich et al. (2019) in a case series [case–case comparison] study. Altogether, 989 participants with diagnoses of incident cases of breast cancer were enrolled into the Breast Cancer Care in Chicago study between 2005 and 2008; ER/PR status was known for 696 cases. The 2002 US EPA's NATA census tract estimates of metal concentrations including cobalt were matched to participants' places of residence in the same year. When the highest and lowest quintiles were compared, elevated odds of ER/PR-negative tumours were identified for cobalt (OR, 2.0; 95% CI, 0.9–4.4; *P* for trend = 0.04). [The Working Group noted that while the authors describe this as a case series study, it might more accurately be described as a case–case comparison study. This design is of limited usefulness because it does not directly estimate the risk of breast cancer associated with cobalt exposure. It is included here because the associations between cobalt exposure and ER/PR-negative and ER/PR-positive breast cancer in women can be compared with the findings by White et al. (2019).]

A population-based case–control study in Mexico examined associations of certain metals or metalloids with incident breast cancer in a region that has naturally high levels of metals in water, and which houses the world's fourth largest non-ferrous metal processing facility (Mérida-Ortega et al., 2022). Women with histopathologically confirmed breast cancer (*n* = 499) were identified from public hospitals in several states in northern Mexico and were age-matched (± 5 years) with controls. [The Working Group noted that the authors did not mention how the case group was selected from the larger case pool of 1045 histopathologically confirmed cases.] Interviews were conducted to obtain covariate information, and height and weight were measured. Metal concentrations were measured in urine samples (first morning void) near the time of interview and for the women with breast cancer before any treatment had begun (on average, 2 months after diagnosis). After excluding cases and controls with exceptionally low or high creatinine concentrations, 452 cases and 439 controls were analysed. Odds ratios were calculated for creatinine-adjusted metal concentrations, both individually in models and grouped together using PCA to assess mixture patterns. [The Working Group noted the somewhat imprecise age matching as a limitation and the high response rates (> 90%) of both cases and controls, as well as good control for confounding factors – such as body mass index (BMI), endogenous estrogen exposure, and alcohol consumption – as strengths.] The median cobalt concentration in urine samples from women with breast cancer was significantly lower than that of controls. An inverse association was noted between cobalt urine concentration and breast cancer overall (highest quartile OR, 0.45; 95% CI, 0.28–0.70). A positive interaction was reported between cobalt and molybdenum regarding their association with breast cancer. [The Working Group noted that the interaction results were not shown and that the reported interaction was hard to interpret, because both cobalt and molybdenum were strongly inversely associated with breast cancer. Case exposure was measured near the time of diagnosis. The study was conducted in

a population with suspected exposure to metals that is greater than that of the general population because of its geographical location.]

2.3 Cancer of the oral cavity, pharynx, larynx, and oesophagus

See Table 2.5.

In total, five epidemiological studies reported on the association between cobalt exposure and the risk of cancer of the oral cavity, larynx, and oesophagus. Data on Barrett oesophagus and oesophageal precancerous lesions were included because these lesions were considered precursors of oesophageal cancer.

Moulin et al. (1993) followed a cohort of 1148 men employed at a cobalt- and sodium-producing plant for at least 1 year between 1950 and 1980 in France. The study is an update of a previous study by Mur et al. (1987), which is not described here. Exposure was assessed by records of work areas: cobalt production, sodium production, production of other chemicals, maintenance, and general service. One quarter of the workforce was born outside of France, and the vital status of these workers could not be assessed completely. Causes of death were ascertained from 1950 to 1967 based on medical records, and from 1968 to 1988 based on death certificates retrieved from the national register at INSERM (the French National Institute of Health and Medical Research). Results were reported as standardized mortality ratios using French national death rates as standards. In the cohort as a whole, 12 workers died from cancer of the buccal cavity and pharynx (SMR, 1.47; 95% CI, 0.76–2.57); of these 9 were born in France (SMR, 1.56; 95% CI, 0.71–2.96). Four workers died from oesophageal cancer (SMR, 0.51; 95% CI, 0.14–1.31); of these three were born in France (SMR, 0.55; 95% CI, 0.11–1.61). Six workers died from laryngeal cancer (SMR, 0.91; 95% CI, 0.34–1.99); all of these were born in France (SMR, 1.31; 95% CI, 0.48–2.85). Data were not reported by work area for these cancer sites. [The Working Group noted that workers were identified from company records only. For these uncommon cancers, results were not presented separately for exposure subgroups.]

Rogers et al. (1993) undertook a case–control study in three counties in Washington State, USA, in which 960 patients who had received diagnoses of cancer of the larynx, oesophagus, or oral cavity, in 1983–1987 were identified. Controls were identified via random-digit dialling. For 3798 households that were willing to participate, 625 eligible controls (frequency-matched to cases on sex and 5-year age intervals) were identified. Toenail cuttings collected at recruitment to the study (available for 354 cases and 434 controls) were used to assess exposure to cobalt. Concentrations of iron, calcium, zinc, chromium, and cobalt were measured in the toenail cuttings and used to stratify the participants into three exposure groups. Unconditional logistic regression was used to assess the association between cobalt exposure groups and risk of cancer of the larynx, oesophagus, or oral cavity. For cancer of the oral cavity, odds ratios were 1.5 (95% CI, 0.9–2.6) and 1.9 (95% CI, 1.0–3.6) in individuals with cobalt concentrations of 0.05–0.17 ppm and > 0.17 ppm, respectively, using individuals with concentrations of < 0.05 ppm as the referent. For oesophageal cancer, the odds ratio for individuals with cobalt concentrations of 0.05–0.17 ppm was 2.4 (95% CI, 0.8–7.2), and there was an increased odds ratio of 9.0 (95% CI, 2.7–30.0) for those with concentrations of > 0.17 ppm, using individuals with concentrations of < 0.05 ppm as the referent.No association was found between cobalt concentration and laryngeal cancer. [The Working Group noted that toenail cuttings were collected some length of time after diagnosis, and some eligible cases died between the time of diagnosis and the request for toenail cuttings (Rogers et al., 1991). Therefore,

Table 2.5 Epidemiological studies of cancer of the oral cavity, larynx, pharynx, and oesophagus and exposure to cobalt

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Moulin et al. (1993) France Enrolment, 1950–1980/ follow-up, 1988 Cohort	1148 men employed for ≥ 1 yr at a plant producing cobalt and sodium Exposure assessment method: exposure to cobalt via all routes (indirectly) was assessed qualitatively and semiquantitatively using company job history records; exposure metrics: employed ≥ 12 mo between 1950 and 1980, occupational categories, time since first employment (man-years), and duration of employment	Buccal cavity and pharynx, mortality	Employed in cobalt production (SMR):			Age, calendar period	*Exposure assessment critique*: Non-differential exposure misclassification likely (broad exposure categories). Possible co-exposures identified could not be fully accounted for in analyses. *Strengths*: Cohort study. *Limitations*: Identification of cohort based on company records only. Incomplete vital status assessment for foreigner workers. Cause of death 1950–1967 based on medical records only.
			All workers	12	1.47 (0.76–2.57)		
			French-born workers	9	1.56 (0.71–2.96)		
		Oesophagus, mortality	Employed in cobalt production (SMR)				
			All workers	4	0.51 (0.14–1.31)		
			French-born workers	3	0.55 (0.11–1.61)		
		Larynx, mortality	Employed in cobalt production (SMR)				
			All workers	6	0.91 (0.34–1.99)		
			French-born workers	6	1.31 (0.48–2.85)		

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rogers et al. (1993) Washington State, USA, 3 counties 1983–1987 Case–control	Cases: 960 individuals aged 20–70 yr diagnosed with cancers of the oral cavity (516), oesophagus (203), or larynx (241) through the local SEER cancer registry; toenail samples available for only 354 cases (oral cavity, 281; oesophagus, 73; larynx, 153) Controls: 625 controls identified using random-digit dialling, frequency-matched to oral cancer cases by sex and 5 yr age intervals, but toenail samples available for only 434 Exposure assessment method: exposure to cobalt through all routes was assessed quantitatively using toenail samples	Oral cavity, incidence	Cobalt level in nail tissue (OR):			Age, sex, pack-year of cigarette use, drink-years of alcohol, energy intake (kcal/day), β-carotene intake (mg/day), ascorbic acid intake (mg/day)	*Exposure assessment critique*: Key limitations include: the exposure assessment may not have captured the relevant exposure window for the development of the cancer outcomes under study (oral, oesophageal, and laryngeal) potentially leading to non-differential misclassification of exposure. *Other strengths*: Population-based case–control study. Measurement of metal in toenail samples. *Other limitations*: Toenail samples available for only some of eligible cases and controls. [sum of case numbers reported by level of exposure does not match total number].
			< 0.05 ppm	NR	1		
			0.05–0.17 ppm	NR	1.5 (0.9–2.6)		
			> 0.17 ppm	NR	1.9 (1.0–3.6)		
		Oesophagus, incidence	Cobalt level in nail tissue (OR):				
			< 0.05 ppm	NR	1		
			0.05–0.17 ppm	NR	2.4 (0.8–7.2)		
			> 0.17 ppm	NR	9.0 (2.7–30.0)		
		Larynx, incidence	Cobalt level in nail tissue (OR):				
			< 0.05 ppm	NR	1		
			0.05–0.17 ppm	NR	2.0 (1.0–3.8)		
			> 0.17 ppm	NR	1.0 (0.4–2.6)		

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
O'Rorke et al. (2012) Ireland Enrolment, 2002–2004 Case–control	Cases: 451 participants aged ≤ 85 yr diagnosed with adenocarcinoma of the oesophagus (227) or Barrett oesophagus (224); toenail clippings available for 137 and 182 cases, respectively Controls: 260 population controls aged 35–84 yr with no prior history of oesophageal/ gastrointestinal malignancy selected at random from general practitioner lists in Ireland, frequency-matched to cases on sex and age (5 yr bands); toenail clippings available for 221 controls Exposure assessment method: exposure to cobalt through all routes was assessed quantitatively using toenail samples	Oesophagus (adenocarcinoma), incidence	Natural log-transformed cobalt level in nail tissue (μg/g) (OR):			Age at interview, sex, smoking status, gastro-oesophageal reflux symptoms, education, location, *H. pylori* infection	*Exposure assessment critique*: Key limitations include: the exposure assessment may not have captured the relevant exposure window for the development of oesophageal cancer or Barrett oesophagus, potentially leading to non-differential misclassification of exposure. *Other strengths*: Population-based measurements in toenail clippings. *Other limitations*: Toenail clippings only available for part of eligible cases and controls.
			< −5.4824	34	1		
			−5.4824 to < −4.4705	39	1.06 (0.57–1.98)		
			≥ −4.4705	52	1.54 (0.84–2.85)		
			Trend-test *P*-value, 0.16				
		Oesophagus (Barrett oesophagus), incidence	Natural log-transformed cobalt level in nail tissue (μg/g) (OR):				
			< −5.4824	55	1		
			−5.4824 to < −4.4705	54	1.08 (0.55–2.10)		
			≥ −4.4705	64	1.97 (1.01–3.85)		
			Trend-test *P*-value, 0.05				

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sauni et al. (2017) Finland Enrolment, 1968–2004/ follow-up, 2013 Cohort	995 men employed for ≥ 1 yr at a Finnish cobalt plant Exposure assessment method: exposure to cobalt via all routes (indirectly) assessed semiquantitatively using company administrative records Exposure metrics: duration and departmental exposure groupings	Tongue, incidence	Exposure group (SIR):			Age, calendar period	*Exposure assessment critique*: Key limitations include: non-differential misclassification likely. Possible co-exposure to nickel not accounted for in analyses. *Other strengths*: Identification of cohort members and follow-up for deaths and emigration were complete. *Other limitations*: Identification of cobalt from company records only. Concomitant exposure to iron, zinc, and nickel.
			Variable exposure	1	26.4 (0.67–14.0)		
			Low	1	6.48 (0.16–36.1)		
			Moderate	0	0 (NR)		
			High	1	6.12 (0.15–34.1)		
		Tongue, incidence	Duration of employment (SIR):				
			> 1 yr	3	7.39 (1.52–21.6)		
			> 5 yr	3	10.0 (2.06–29.2)		
		Oesophagus, incidence	Duration of employment (SIR):				
			> 1 yr	2	1.74 (0.21–6.28)		
			> 5 yr	2	2.24 (0.27–8.08)		
		Larynx, incidence	Duration of employment (SIR):				
			> 1 yr	2	2.45 (0.30–8.86)		
			> 5 yr	2	3.09 (0.37–11.2)		

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pan et al. (2021) Huai'en district, China Enrolment, 2015–2017 Case–control	Cases: 100 participants identified as having mild/moderate OPL from a cohort of 1731 residents, aged 35–75 yr, who underwent endoscopic examination; 144 mild/moderate OPL identified; 100 randomly selected for study Controls: 100 healthy controls, matched to cases on sex, age (± 2 yr), and village Exposure assessment method: exposure to cobalt was assessed quantitatively in two ways: (1) repeated dietary samples (triplicate) to assess ingestion; (2) single blood sample (plasma) to assess exposure through all routes (indirectly)	Oesophagus (OPL), incidence	Dietary cobalt intake (μg/day) (OR):			Sex, age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	*Exposure assessment critique*: Key strengths include: measurements in serum and duplicate diet portions. Key limitations include: the exposure assessment may not have captured the relevant exposure window for the development of OPL, potentially leading to non-differential misclassification of exposure. Results for dietary cobalt do not consider other routes of exposure that may be significant for some participants (e.g. inhalation). *Other limitations*: Many comparisons and inconsistency between pattern in men and women.
			Quartile 1 (4.67–20.92)	32	1		
			Quartile 2 (20.92–32.14)	28	0.84 (0.35–2.04)		
			Quartile 3 (32.14–58.05)	24	0.37 (0.15–0.93)		
			Quartile 4 (58.05–1915.77)	16	0.34 (0.12–0.96)		
			Trend-test *P*-value, 0.034				
		Oesophagus (OPL), incidence	Dietary cobalt intake (μg/day), men (OR):			Age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	
			Quartile 1 (4.67–20.92)	12	1		
			Quartile 2 (20.92–32.14)	16	1.43 (0.39–5.22)		
			Quartile 3 (32.14–58.05)	10	1.20 (0.29–5.04)		
			Quartile 4 (58.05–1915.77)	14	0.88 (0.25–3.03)		
			Trend-test *P*-value, 0.537				
		Oesophagus (OPL), incidence	Dietary cobalt intake (μg/day), women (OR):				
			Quartile 1 (4.67–20.92)	20	1		
			Quartile 2 (20.92–32.14)	12	0.58 (0.16–2.09)		
			Quartile 3 (32.14–58.05)	14	0.40 (0.12–1.37)		
			Quartile 4 (58.05–1915.77)	2	0.12 (0.02–0.80)		
			Trend-test *P*-value, 0.025				

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pan et al. (2021) Huai'en district, China Enrolment, 2015–2017 Case–control (cont.)		Oesophagus (OPL), incidence	Plasma cobalt (μg/L) (OR):			Sex, age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	
			Quartile 1 (0.00–0.58)	26	1		
			Quartile 2 (0.58–1.19)	25	0.53 (0.21–1.32)		
			Quartile 3 (1.19–1.78)	26	0.65 (0.24–1.75)		
			Quartile 4 (1.78–20.82)	23	0.49 (0.17–1.45)		
			Trend-test *P*-value, 0.253				
		Oesophagus (OPL), incidence	Plasma cobalt (μg/L), men (OR):			Age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	
			Quartile 1 (0.00–0.58)	15	1		
			Quartile 2 (0.58–1.19)	11	0.62 (0.18–2.16)		
			Quartile 3 (1.19–1.78)	10	0.27 (0.07–1.04)		
			Quartile 4 (1.78–20.82)	11	0.70 (0.20–2.46)		
			Trend-test *P*-value, 0.611				
		Oesophagus (OPL), incidence	Plasma cobalt (μg/L), women (OR):				
			Quartile 1 (0.00–0.58)	11	1		
			Quartile 2 (0.58–1.19)	14	1.31 (0.31–5.49)		
			Quartile 3 (1.19–1.78)	16	1.35 (0.34–5.41)		
			Quartile 4 (1.78–20.82)	7	0.14 (0.02–0.80)		
			Trend-test *P*-value, 0.020				

Table 2.5 (continued)

Reference Location Enrolment/ follow-up period Study design	Population size, description Exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pan et al. (2021) Huai'en district, China Enrolment, 2015–2017 Case–control (cont.)		Oesophagus (OPL), incidence	Serum Tc II level (ng/L) (OR): Quartile 1 (49.36–73.21) Quartile 2 (73.21–86.30) Quartile 3 (86.30–116.24) Quartile 4 (116.24–675.39) Trend-test *P*-value, < 0.001	 37 33 17 13	 1 0.85 (0.29–2.49) 0.22 (0.08–0.64) 0.19 (0.07–0.51)	Sex, age, village, BMI, smoking status, education, income, alcohol intake, fruit and vegetable consumption, meat intake	

BMI, body mass index; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; mo, month; NR, not reported; OPL, oesophageal precancerous lesion; OR, odds ratio; SEER, Surveillance, Epidemiology, and End Results; SIR, standardized incidence ratio; SMR, standardized mortality ratio; Tc II, transcobalamin II; yr, year.

the survivors may not be representative of the recruited cases. The numbers of cases included in the analyses with cobalt concentrations in toenail cuttings were not clearly reported.]

O'Rorke et al. (2012) undertook an all-Ireland case–control study to assess the association between toenail concentrations of cobalt and incidence of oesophageal adenocarcinoma and Barrett oesophagus. In total, 227 patients with oesophageal adenocarcinoma and 224 with Barrett oesophagus who received diagnoses between 2002 and 2004 were recruited along with 260 population-based controls. Toenail cuttings were obtained from participants (oesophageal adenocarcinoma, n = 137; Barrett oesophagus, n = 182; controls, n = 221) and analysed for iron, selenium, zinc, cobalt, chromium, cerium, and mercury concentrations. The mean concentration of cobalt in toenail cuttings was 0.02 µg/g for oesophageal adenocarcinoma, Barrett oesophagus, and controls. Exposure levels were stratified into tertiles on the basis of levels in controls. Using the lowest tertile as baseline, the adjusted odds ratios for oesophageal adenocarcinoma with medium and high cobalt exposure were 1.06 (95% CI, 0.57–1.98) and 1.54 (95% CI, 0.84–2.85), respectively (P for trend = 0.16). For Barrett oesophagus, the adjusted odds ratios for medium and high exposure were 1.08 (95% CI, 0.55–2.10) and 1.97 (95% CI, 1.01–3.85), respectively (P for trend = 0.05). [The Working Group noted the low exposure level in the general population.]

Sauni et al. (2017) identified and followed 995 men, employed for at least 1 year during the period 1968–2004, at the Kokkola cobalt plant, Finland. From 1966 to 1987, cobalt was produced from pyrite ore concentrate, and after 1987 from by-products of the metallurgical industry. Cohort members were followed up for cancer incidence by linkage to the Finnish Cancer Register for the period 1968–2013, and standardized incidence ratios were calculated using cancer incidence rates from the local area around the Kokkola plant. The study reported a substantially increased risk of cancer of the tongue across the entire cohort (SIR, 7.39; 95% CI, 1.52–21.6; 3 cases) and for workers employed > 5 years (SIR, 10.0; 95% CI, 2.06–29.2; 3 cases), although there were few cases. There were 2 cases of cancer of the larynx (SIR, 2.45; 95% CI, 0.30–8.86), 2 cases of oesophageal cancer (SIR, 1.74; 95% CI, 0.21–6.28), and fewer than 2 cases of cancer of the pharynx with no results reported for this cancer. [The Working Group noted the small size of the cohort as a limitation of this study. Overall smoking prevalence in the cohort was fairly similar to that in the local area.]

Pan et al. (2021) undertook a case–control study nested in a screening programme for detection of oesophageal precancerous lesions in Huai'an District, China, a region that has high rates of oesophageal squamous cell carcinoma. In the period 2015–2017, 1731 residents aged 35–75 years underwent endoscopic examination. Of these, 144 received diagnoses of mild/moderate oesophageal precancerous lesion; 100 were randomly selected as cases, and controls were 100 sex-, age-, and village-matched screen-negative individuals. Among other exposures, plasma cobalt concentration and dietary cobalt intake were measured using fasting blood samples and 3-day duplicate dietary samples. Levels of cobalt were divided into quartiles, and associations between exposure and risk of oesophageal precancerous lesions were assessed with conditional logistic regression. For dietary cobalt intake there was a decreasing risk of oesophageal precancerous lesions with increasing exposure level (P = 0.034; adjusted OR for highest versus lowest, 0.34; 95% CI, 0.12–0.96). There was no association between plasma cobalt concentration and risk of oesophageal precancerous lesion (P = 0.253). [The Working Group noted that the results were not consistent between men and women, and that data were not presented for oesophageal cancer.]

2.4 Other cancers and all cancers combined

See Table S2.6 (Annex 2, Supplementary material for Section 2, Cancer in Humans, web only, available from: https://publications.iarc.fr/618).

Five cohort studies (three industrial cohorts and two other cohorts) and one nested case–control study were available for the Working Group to review.

The study of Moulin et al. (1993) was an extension of a previous study published by the same research group (Mur et al., 1987). Both studies were based on a cohort of workers in an electrochemical factory that produced cobalt and sodium in France. The mortality data reported in the first study were based on medical records from the years 1950 to 1980, but Moulin et al. (1993) extended the study to 1981–1988 and used data from death certificates. In the study by Moulin et al. (1993), the analyses were performed in two separate cohorts: one overall cohort including all workers and the other including only workers born in France, since many foreign-born workers were lost to follow-up. The overall cohort comprised 1148 people. Neither of the studies identified increased mortality for all cancers combined. In the study by Moulin et al. (1993), the standardized mortality ratio was 0.83 (95% CI, 0.66–1.03) in the overall cohort and 1.00 (95% CI, 0.78–1.26) for workers born in France. In total, there were 5 deaths from brain cancer (SMR, 3.57; 95% CI, 1.16–8.32), of which 4 were among French-born workers (SMR, 3.98; 95% CI, 1.08–10.19). The workers who died had been involved in maintenance and administration. The study did not find increased mortality from cancers of the stomach, intestine, rectum, pancreas, urinary bladder, or prostate, or from lymphoma, leukaemia, or bone sarcoma. [The Working Group noted that non-differential exposure misclassification in the study was probable, as broad exposure categories were used. Possible co-exposure to other carcinogens (e.g. asbestos) was also identified. Follow-up was incomplete among those born outside of France.]

In the cohort study by Tüchsen et al. (1996), which concerned two Danish porcelain factories, the cancer risk of several organ sites was examined. The cohort consisted of 874 women exposed occupationally to cobalt and 520 women who were employed at the same factories but who had not been exposed to cobalt. Standardized incidence ratios using national reference rates were reported separately for all exposed workers, those exposed in factory 1, those exposed in factory 2, and the unexposed workers. Standardized incidence ratios were increased for cancer of the uterine cervix among all exposed workers (SIR, 2.31; 95% CI, 1.19–4.03) and for cancer of the uterine corpus among the unexposed workers (SIR, 3.02; 95% CI, 1.38–5.73). There were 4 cases of brain cancer: 1 case in a cobalt-exposed worker (SIR, 0.50 [95% CI, 0.03–2.48]) and 3 in non-exposed workers (SIR, 1.68 [95% CI, 0.43–4.59]). The standardized incidence ratio for all cancers combined was 1.20 (95% CI, 0.94–1.52) for all exposed workers, 1.12 [95% CI, 0.79–1.55] for workers exposed in factory 1, 1.29 [95% CI, 0.90–1.79] for those exposed in factory 2, and 0.99 [95% CI, 0.76–1.27] for unexposed workers. [The Working Group noted that the number of observed and expected cases for less common types of cancer was quite low, resulting in imprecise risk estimates. Inclusion of an internal unexposed referent group was an important strength of the study.]

A Finnish cohort study of 995 men, all cobalt-production workers, investigated risk of cancers of the stomach, colon, rectum, pancreas, prostate, kidney, and urinary bladder, and of melanoma, non-melanoma skin cancer, basal cell carcinoma, non-Hodgkin lymphoma, and leukaemia (Sauni et al., 2017). The cohort was further divided into subcohorts by exposure levels, according to the departments in which employees had first worked at the plant.

Exposures in different departments were based on regular industrial hygiene measurements recorded between 1986 and 2014. There were no notable increases in risk for any of the specific cancer types assessed. The standardized incidence ratios of all cancers combined were 1.00 (95% CI, 0.81–1.22) for those employed for at least 1 year and 1.08 (95% CI, 0.85–1.34) for those employed for at least 5 years. [The Working Group noted that the number of observed and expected cases for less common types of cancer was quite low, resulting in imprecise risk estimates.]

Rodrigues et al. (2020) undertook a case–control study nested in a cohort of workers from three facilities engaged in the manufacture of semiconductor and electronic storage devices, located in New York, Vermont, and California, USA. In total, 126 836 workers had been included in a 1965–1999 mortality study, and 89 054 of them in a 1976–1999 cancer incidence study. Fatal cases of malignant CNS neoplasms were identified from the National Death Index. Incident cases were identified from linkage with state cancer registries. For each case, 10 cohort members were selected as controls using incidence density sampling and further matched on year of birth, facility, sex, and race. On the basis of information about the workers' roles in production, 10 PEGs were constructed. For each case and control, work history was mapped on the basis of combinations of division, department, and job title, and these combinations were classified by PEG. Manufacturing periods were divided into eras, and a chemical/PEG/manufacturing era matrix was constructed for 31 chemicals. Odds ratios were calculated for the associations between tertiles of cumulative exposure to cobalt and risk of CNS cancer, stratified by facility. In total, 120 cases and 1028 controls were identified. There was little evidence of positive associations between cumulative exposure to cobalt and CNS cancer in any of the three facilities, and estimates were statistically imprecise. [The Working Group noted that workers could have been exposed to more than one chemical in a given PEG, and that half of the cases and controls had worked in more than one PEG. Numerous exposures were assessed in this study, and positive associations were found for the highest tertile of estimated cumulative exposure for several chemicals, including 2-butoxyethanol, cyclohexanone, *ortho*-dichlorobenzene, cadmium, molybdenum, trichloroethylene, and vinyl chloride.]

The study by Duan et al. (2020) was based on a sample (n = 26 056) drawn from the NHANES 1999–2014 cohort, and mortality was followed until the end of 2015. The study investigated the association between heavy metal concentrations in urine (barium, cadmium, cobalt, caesium, molybdenum, lead, antimony, titanium, tungsten, and uranium) at the time of the enrolment and cancer mortality. Urinary cobalt concentration (median, 0.35 µg/L) was not associated with increased overall cancer mortality (relative risk, 1.05; 95% CI, 0.85–1.30) when cobalt was analysed with covariate adjustment for other metals in the multiple-metal analysis. When the association between single urinary metals and cancer mortality was estimated, cobalt was associated with all cancers combined in a model adjusted for sex, age, age^2, ethnicity, and urine creatinine concentration (relative risk, 1.23; 95% CI, 1.03–1.46). The association remained similar but was slightly attenuated in two other models adjusted for additional medical and socioeconomic risk factors. [The Working Group noted that the metals were measured in NHANES 1999–2014, and mortality was assessed between 1999 and 2015; therefore, there was a short time period between exposure and outcome.]

Li et al. (2021a) studied the associations between plasma concentrations of 12 metals (iron, copper, zinc, selenium, chromium, manganese, molybdenum, cobalt, nickel, arsenic, cadmium, and lead) at baseline and cancer risk in 4573 patients with type 2 diabetes. The participants were from the Dongfeng-Tongji cohort,

which comprises 27 009 retired workers who had been employed by an automotive manufacturing corporation. [The Working Group noted that the occupational cohort was not selected on the basis of high cobalt exposure, and that it was unclear whether the study participants had any specific exposure to cobalt in their work.] Enrolment took place between 2008 and 2010 and follow-up through 2018. The results were reported according to the quartiles of plasma concentrations of cobalt. The hazard ratios for all types of cancer combined were not associated with the plasma concentrations of cobalt in any of the quartiles. When compared with the lowest exposure quartile, the hazard ratios were 0.96 (95% CI, 0.76–1.20), 0.79 (95% CI, 0.62–1.00), and 0.80 (95% CI, 0.63–1.02) for quartiles 2, 3, and 4, respectively. [The Working Group noted that the plasma level of cobalt, natural log-transformed for skewness, was lower in cancer cases (median [presumed], 0.24 μg/L; interquartile range, 0.19–0.31) than in non-cases (median [presumed], 0.26 μg/L; interquartile range, 0.20–0.32). The timing of exposure measurement may be outside the relevant time window of exposure for the cancer outcome under study.]

2.5 Evidence synthesis for cancer in humans

The studies considered by the Working Group to be most relevant to the evaluation of cancer in humans resulting from exposure to cobalt metal (without tungsten carbide or other metal alloys) and cobalt compounds included occupational studies of cobalt-exposed workers, one study in an occupational group not specifically exposed to cobalt, and several studies in the general population. Among the occupational studies considered relevant for this evaluation, two concerned hard-metal industries and six investigated other industries. All these studies analysed lung cancer, and some analysed cancers at other organ sites. Among the non-occupational studies, one analysed risk of breast cancer in relation to baseline residential air pollution levels, and several studies of other cancer types used biomarkers from one-time samples of toenail cuttings, blood, or urine to estimate individual exposures. No informative studies were found that permitted the separation of the effects of soluble cobalt(II) salts, the insoluble compounds cobalt(II) oxide or cobalt(II,III) oxide, cobalt(II) sulfide, or other forms of cobalt from those of cobalt metal.

2.5.1 Quality of exposure assessment for cobalt and co-exposures

Quality of the exposure assessment was an important factor in evaluating the informativeness of studies by the Working Group. Detailed reports on the strengths and limitations of exposure evaluations in cohort and case–control studies are provided in Section 1.6.1.

The most informative occupational studies assessed potential associations between cobalt exposure and lung cancer. Weaknesses in the exposure assessments in many of these studies included use of qualitative rather than quantitative exposure metrics for cobalt, lack of control for co-exposures to the WC-Co composite (an agent excluded from this evaluation) in studies of the hard-metal industry, and lack of control for carcinogenic co-exposures that may have been present in many of the facilities studied, including recognized lung carcinogens such as asbestos, arsenic, and chromium, nickel, and cadmium compounds. The study by Moulin et al. (1998) included an analysis of lung cancer mortality in relation to "other cobalt exposures", which included exposure to cobalt alone or simultaneously with agents other than WC-Co. Because of the potential for confounding by WC-Co in other production areas and co-exposure to other lung carcinogens, which could not be fully adjusted for, the exposure metric

assessed in this study was considered uninformative for this evaluation. Similarly, although the study by Wild et al. (2000) used a detailed JEM to assess exposure–response in relation to exposure to WC-Co, the exposure group considered most relevant for the current evaluation was workers "ever employed" or "only employed" in the production of cobalt powder, who may have also been exposed separately to tungsten carbide powders. This exposure subgroup analysis was of limited informativeness because individuals in the "ever employed" category had potential exposure to other lung carcinogens during work in other production areas of the plant (there were only two lung cancer deaths in the more informative category of "only exposed").

Reviews and critiques of the exposure assessment methodology for studies of cobalt exposure and lung cancer in other industries (Moulin et al., 1993, 2000; Tüchsen et al., 1996; Grimsrud et al., 2005; Sauni et al., 2017) are described by the Working Group in Section 1.6.1. These studies varied in exposure assessment quality, and most had potential misclassification of cobalt exposure and confounding by co-exposure to other lung carcinogens. None of these studies found positive associations with cobalt exposure for which confounding by co-exposure to other lung carcinogens could be ruled out.

Among the studies reflecting exposures of the general population (as opposed to occupational exposures), the study by White et al. (2019) linked modelled census tract-level estimates of cobalt concentrations in outdoor air for a single year to each woman's address at enrolment. This failed to account for temporal trends in outdoor air concentrations of metals within census tracts.

One study within the prospective Sister Study cohort used cobalt concentrations in toenail cuttings as biological markers of exposure (Niehoff et al., 2021). In this study, toenail cuttings were obtained at baseline, thus enabling assessment of exposure before the onset of disease. Although one-time measurement of metal concentrations in toenail cuttings may not represent cumulative exposures to cobalt during biologically relevant time periods of exposure, they appear to be moderately correlated within individuals over a period of several years (see Section 1.6.1). A strength of the exposure assessments in the general-population studies was that much more extensive information was provided on individual-level covariates and quantitative estimates of exposure to other environmental contaminants. Although not a reflection of low-quality exposure assessment, the small range of exposures in the general-population studies may limit the informativeness of exposure–response analyses.

2.5.2 Lung cancer

In two high-quality studies of hard-metal manufacturing facilities, where there was some overlap in populations, elevated standardized mortality ratios or odds ratios for lung cancer were observed in workers "ever exposed" to cobalt with no exposure to tungsten carbide (Moulin et al.,1998; Wild et al., 2000). The study by Moulin et al. (1998) included an analysis of lung cancer mortality in relation to "other cobalt exposures", which included cobalt alone or simultaneous exposure to cobalt and agents other than WC-Co. Because exposures were defined as "ever" working in the departments where there was no tungsten carbide exposure, rather than "only" working in those departments, there is potential for confounding by exposure to WC-Co and co-exposure to other lung carcinogens. This study did not present exposure–response analyses for "other cobalt exposures", although it did control for categorical metrics of WC-Co in analyses of "other cobalt exposures". In addition, the study did not control for exposure to other carcinogens, and there was no information provided about the extent or intensity of exposure to them. Therefore, after careful consideration, the Working Group viewed this

study as uninformative for the present evaluation. Similarly, although the study by Wild et al. (2000) observed elevated lung cancer risks among workers ever employed in the production of cobalt and tungsten carbide powders, workers in this subgroup may have worked in other production areas that had potential for exposure to WC-Co and co-exposure to other lung carcinogens. There were only two deaths in the more informative category of "only exposed" in powder production. The Working Group considered this study to be uninformative for the evaluation because of the strong potential for exposure to WC-Co and other confounding exposures. In the occupational studies of other industries involving cobalt exposures, some of which had confounding or limited statistical precision, no consistent elevated risks of lung cancer were observed (Moulin et al., 1993, 2000; Tüchsen et al., 1996; Grimsrud et al., 2005; Sauni et al., 2017).

2.5.3 Breast cancer

One occupational study and three population-based studies examined breast cancer incidence after exposure to cobalt. The occupational study did not find an excess of breast cancer incidence among women employed in two Danish porcelain factories (Tüchsen et al., 1996). Limitations of this study included low statistical power and an inability to control for important breast cancer risk factors, including parity and age at first birth. In the study on the relation between residential exposure to cobalt and breast cancer within the Sister Study population, there was no clear gradient of increasing risk with increasing exposure (White et al., 2019). Limitations of this study included the low levels and small range of cobalt exposures and the use of broad proxies in the exposure assessment. The study on cobalt concentrations in toenail cuttings and breast cancer within the Sister Study population did not find associations with increasing levels of cobalt exposure (Niehoff et al., 2021). This study was also limited by low cobalt concentrations and a small range of cobalt exposures, as well as the considerations regarding the use of measurements of metal concentrations in toenail cuttings discussed in Section 2.5.1. One case–control study in an area of Mexico where metal exposures were thought to be high did not find a positive association between urinary cobalt concentrations and breast cancer (Mérida-Ortega et al., 2022).

2.5.4 Other cancers and all cancers combined

In total, five epidemiological studies reported on the association between cobalt exposure and the risk of cancer of the oral cavity, larynx, oesophagus, and oesophageal precursor lesions (O'Rorke et al., 2012; Moulin et al., 1993; Rogers et al., 1993; Sauni et al., 2017; Pan et al., 2021). Although suggestive evidence of positive associations for some cancer sites or subsites was reported in some studies, interpretation of evidence from these studies was limited by inconsistent findings and small numbers of cases.

Other cancer types (digestive organs, pancreas, kidney, urinary bladder, prostate, uterus, ovary, and brain) and melanoma, non-melanoma skin cancer, lymphoma, osteosarcoma, and leukaemia were assessed in three cohort studies (Moulin et al., 1993; Tüchsen et al., 1996; Sauni et al., 2017) and one nested case–control study (Rodrigues at al., 2020). Mostly, the risk estimates were imprecise and not substantially elevated. Tüchsen et al. (1996) reported an increased standardized incidence ratio for cancer of the uterine cervix among all exposed workers and for cancer of the uterine corpus among the unexposed workers. In the study by Moulin et al. (1993), the standardized mortality ratio for brain cancer was increased both among all workers and among a subpopulation of workers born in France. Overall, there were possible co-exposures in all three studies

that were not adjusted for in the analyses, therefore these studies are of moderate quality and informativeness.

Of the five studies assessing the risk of all cancers combined (Moulin et al., 1993; Tüchsen et al., 1996; Sauni et al., 2017; Duan et al., 2020; Li et al., 2021a), only one population-based study found a significant increase related to cobalt exposure in one of three adjusted models of analysis (Duan et al., 2020). A limitation of this study was the relatively short follow-up period, which yielded a small number of death outcomes. The exposure assessment was based on one-time urine analyses, which may not have reflected cobalt exposures over a biologically relevant time period.

Although several studies examined risks of other cancers, these studies did not provide an adequate basis for evaluation because of low statistical power and the small number of studies for each cancer type. Results of analyses of all cancers combined are generally less informative for carcinogenicity evaluations because this is a heterogeneous outcome.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Cobalt metal

3.1.1 Mouse

Inhalation

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were exposed by inhalation (whole-body) to cobalt metal-particle aerosol concentrations of 0, 1.25, 2.5, or 5 mg/m^3 (concentrations were based on the findings from a 13-week study) for untreated controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, 98.2% ± 0.6%; mass median aerodynamic diameter, MMAD, 1.5–2.0 μm; geometric standard deviation, GSD, 1.6–1.9 μm) for 6 hours plus T_{90} (12 minutes; the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation) per day, 5 days per week for 105 weeks (NTP, 2014). Surviving mice were killed at age 109–111 weeks. At study termination, survival of male mice was 39/50, 31/50, 29/50, and 25/50, and for female mice was 36/50, 36/50, 27/50, and 26/50, for the controls and groups at the lowest, intermediate, and highest concentration, respectively. These survival rates included one male in the group at the intermediate concentration, three males at the highest concentration, and two females at the lowest concentration that died during the last week of the study. Survival of males in groups at the intermediate or highest concentration was significantly less than that of controls. The mean body weights of the males and females at the highest concentration were significantly decreased, and at least 10% less than those of the control groups after weeks 85 and 21, respectively. Abnormal breathing and thinness were noted in exposed male and female mice. Tissue burden studies were conducted only in females; lung cobalt concentrations and burdens increased with increasing exposure concentrations and were greater than those of controls. The values of maximum cobalt lung burdens observed in the 2-year studies indicated that lung overload was not reached in these studies. All mice underwent complete necropsy with histopathological evaluation.

In male mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) [P = 0.011, Cochran–Armitage test]. The incidence of bronchioloalveolar adenoma (includes multiples) in the groups at 0 (control), 1.25, 2.5, and 5 mg/m^3 was 7/50 (14%), 11/49 (22%), 15/50 (30%), and 3/50 (6%), respectively, and was significantly

Table 3.1 Studies of carcinogenicity in experimental animals exposed to different forms of cobalt

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Cobalt metal				
Full carcinogenicity Mouse, $B6C3F_1$/N (M) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 39, 31, 29, 25	*Lung* Bronchioloalveolar adenoma (includes multiple) 7/50 (14%), 1 1/49 (22%), 15/50 (30%)*, 3/50 (6%) Bronchioloalveolar carcinoma (includes multiple) 11/50 (22%), 38/49 (78%)*, 42/50 (84%)*, 46/50 (92%)* Bronchioloalveolar carcinoma, multiple 3/50, 18/49*, 24/50*, 36/50* Bronchioloalveolar adenoma or carcinoma (combined) 16/50 (32%), 41/49 (84%)*, 43/50 (86%)*, 47/50 (94%)*	[$P = 0.011$, Cochran–Armitage trend test] *$P = 0.016$, poly-3 test [$P = 0.0448$, Fisher exact test] $P < 0.001$, poly-3 trend test; [Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test] [$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.01$, poly-3 test [$P \leq 0.002$, Fisher exact test] $P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, adequate number of mice, adequate duration of exposure and observation, and multiple doses based on a 3-month study were used. Other comments: MMAD, 1.5–2.0 µm; GSD, 1.6–1.9 µm. Survival rates in the groups exposed to 2.5 and 5 mg/m^3 significantly less than that of control. Historical controls: Bronchioloalveolar adenoma (includes multiple): inhalation studies 39/300 (13.0 ± 4.2%), range, 8–20%; all routes 145/950 (15.3 ± 6.2%), range, 2–26%. Bronchioloalveolar carcinoma (includes multiple): inhalation studies 59/300 (19.7 ± 3.4%), range, 16–24%; all routes 132/950 (13.9 ± 7.1%), range, 4–24%. Bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 90/300 (30.0 ± 5.5%), range, 26–40%; all routes 263/950 (27.7 ± 5.7%), range, 16–40%.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, $B6C3F_1$/N (F) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 36, 36, 27, 26	*Lung* Bronchioloalveolar adenoma (includes multiple) 3/49 (6%), 9/50 (18%), 8/50 (16%), 10/50 (20%)* Bronchioloalveolar carcinoma (includes multiple) 5/49 (10%), 25/50 (50%)*, 38/50 (76%)*, 43/50 (86%)* Bronchioloalveolar carcinoma, multiple 1/49, 7/50*, 20/50**, 24/50** Bronchioloalveolar adenoma or carcinoma (combined) 8/49 (16%), 30/50 (60%)*, 41/50 (82%)*, 45/50 (90%)*	$P = 0.037$, poly-3 trend test *$P = 0.024$, poly-3 test [$P = 0.0387$, Fisher exact test] $P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test] [$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P = 0.0317$, Fisher exact test] **$P \leq 0.01$, poly-3 test [$P < 0.001$, Fisher exact test] $P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, adequate number of mice, adequate duration of exposure and observation, and multiple doses based on a 3 mo study were used. Other comments: MMAD, 1.5–2.0 µm; GSD, 1.6–1.9 µm. Historical controls: Bronchioloalveolar adenoma (includes multiple): inhalation studies 16/299 (5.4 ± 3.7%), range, 2–12%; all routes 54/949 (5.7 ± 3.6%), range, 0–12%. Bronchioloalveolar carcinoma (includes multiple): inhalation studies 13/299 (4.4 ± 4.3%), range, 0–10%; all routes –38/949 (4.0 ± 3.6%), range, 0–14%. Bronchioloalveolar adenoma or carcinoma (combined): inhalation studies 28/299 (9.4 ± 4.8%), range, 2–16%; all routes – 90/949 (9.5 ± 4.8%), range, 2–22%.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (M) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 17, 20, 16, 16	*Lung* Bronchioloalveolar adenoma (includes multiple) 2/50 (4%), 10/50 (20%)*, 10/50 (20%)*, 14/50 (28%)**	$P = 0.011$, poly-3 trend test [$P = 0.015$, Cochran–Armitage trend test] *$P \leq 0.018$, poly-3 test [$P = 0.0139$, Fisher exact test] **$P < 0.001$, poly-3 test [$P = 0.0009$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan, male and female rats used, adequate number of rats; adequate duration of exposure and observation, and multiple doses based on a 3-mo study were used. Other comments: MMAD, 1.4–2.0 μm; GSD, 1.6–1.9 μm. Body weights of rats treated with 2.5 and 5 mg/m^3 were ≥ 10% less than those of controls after weeks 99 and 12, respectively. Historical controls: Bronchioloalveolar adenoma (includes multiple): 5/100 (5.0 ± 1.4%), range, 4–6%. Bronchioloalveolar carcinoma (includes multiple): 0/100. Bronchioloalveolar adenoma or carcinoma (combined): 5/100 (5.0 ± 1.4%), range, 4–6%. Cystic keratinizing epithelioma: 0/100. Adrenal medulla, benign pheochromocytoma: 25/100 (25 ± 7.1%), range, 20–30%. Adrenal medulla, malignant pheochromocytoma: 2/100 (2.0% ± 2.8%), range, 0–4%. Adrenal medulla, benign or malignant pheochromocytoma: 27/100 (27 ± 9.9%), range, 20–34%. Pancreatic islets, adenoma: all routes, 0/100. Pancreatic islets, carcinoma: all routes, 2/100 (2 ± 2.8%), range, 0–4%. Pancreatic islets, adenoma or carcinoma (combined): 2/100 (2.0 ± 2.8%), range, 0–4%.
		Bronchioloalveolar carcinoma (includes multiple) 0/50, 16/50 (32%)*, 34/50 (68%)*, 36/50 (72%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	
		Bronchioloalveolar carcinoma, multiple 0/50, 6/50*, 14/50**, 30/50**	[$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P = 0.0133$, Fisher exact test] **$P \leq 0.01$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Bronchioloalveolar adenoma or carcinoma (combined) 2/50 (4%), 25/50 (50%)*, 39/50 (78%)*, 44/50 (88%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (M) 5–6 wk 105 wk NTP (2014) (cont.)		Cystic keratinizing epithelioma 0/50, 1/50 (2%), 0/50, 1/50 (2%)	NS	
		Adrenal medulla Benign pheochromocytoma (includes bilateral) 15/50 (30%), 23/50 (46%), 37/50 (74%)*, 34/50 (68%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P < 0.001$, Fisher exact test]	
		Benign pheochromocytoma, bilateral 4/50 (8%), 13/50 (26%)*, 22/50 (44%)**, 21/50 (42%)**	[$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P < 0.01$, Fisher exact test] **$P \leq 0.01$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Malignant pheochromocytoma (includes bilateral) 2/50 (4%), 2/50 (4%), 9/50 (18%)*, 16/50 (32%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P = 0.03$, poly-3 test) [$P = 0.0256$, Fisher exact test] **$P < 0.001$, poly-3 test [$P = 0.0002$, Fisher exact test]	
		Malignant pheochromocytoma, bilateral 0/50, 0/50, 0/50, 7/50 (14%)*	*$P \leq 0.01$, poly-3 test [$P = 0.0062$, Fisher exact test]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (M) 5–6 wk 105 wk NTP (2014) (cont.)		Benign or malignant pheochromocytoma (combined) 17/50 (34%), 23/50 (46%), 38/50 (76%)*, 41/50 (81%)*	*P* < 0.001, poly-3 trend test [*P* < 0.001, Cochran–Armitage trend test] **P* < 0.001, poly-3 test [*P* < 0.0001, Fisher exact test]	
		Pancreatic islets Adenoma 0/50, 1/50 (2%), 6/48 (13%)*, 3/49 (6%)	**P* = 0.015, poly-3 test [*P* = 0.0117, Fisher exact test]	
		Carcinoma 2/50 (4%), 1/50 (2%), 5/48 (10%), 6/49 (12%)	*P* = 0.021, poly-3 trend test NS, poly-3 test; [Fisher exact test]	
		Adenoma or carcinoma (combined) 2/50 (4%), 2/50 (4%), 10/48 (20%)*, 9/49 (18%)**	*P* = 0.002, poly-3 trend test [*P* = 0.007, Cochran–Armitage trend test] **P* = 0.013, poly-3 test [*P* = 0.0113, Fisher exact test] ***P* = 0.022, poly-3 test [*P* = 0.0235, Fisher exact test]	
		Kidney Renal tubule adenoma or carcinoma (standard and extended evaluation, combined) 3/50, 1/50, 1/50, 7/50	*P* = 0.023, poly-3 trend test [*P* = 0.039, Cochran–Armitage trend test]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (F) 5–6 wk 105 wk NTP (2014)	Inhalation (whole-body exposure) Co metal, 98.2% Clean air 0, 1.25, 2.5, 5 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 35, 26, 24, 25	*Lung* Bronchioloalveolar adenoma (includes multiple) 2/50 (4%), 7/50 (14%), 9/50 (18%)*, 13/50 (26%)**	$P = 0.002$, poly-3 trend test [$P = 0.022$, Cochran–Armitage trend test] *$P = 0.016$, poly-3 test [$P = 0.0256$, Fisher exact test] **$P < 0.001$, poly-3 test [$P = 0.0019$, Fisher exact test]	Principal strengths: GLP study, study covered most of lifespan; male and female rats used, adequate number of rats, adequate duration of exposure and observation, and multiple doses based on a 3-mostudy were used. Other comments: MMAD, 1.4–2.0 µm; GSD, 1.6–1.9 µm. Survival rate in the group exposed to 2.5 mg/m^3 was significantly less than that of control, body weights of rats exposed to 2.5 or 5 mg/m^3 were ≥ 10% less than those of controls after weeks 57 and 21, respectively. Historical controls: Bronchioloalveolar adenoma: 2/100 (2.0 ± 2.8%), range, 0–4%. Bronchioloalveolar carcinoma (includes multiple): 0/100. Bronchioloalveolar adenoma or carcinoma (combined): 2/100 (2.0 ± 2.8%), range, 0–4%. Cystic keratinizing epithelioma: 0/100. Adrenal medulla, benign pheochromocytoma: 7/100 (7 ± 7.1%), range, 2–12%. Adrenal medulla, malignant pheochromocytoma: 1/100 (1 ± 1.4%), range, 0–2%. Adrenal medulla, benign or malignant pheochromocytoma (combined): 8/100 (8 ± 5.7%), range, 4–12%. All organs, mononuclear cell leukaemia: 35/100 (35 ± 4.2%), range, 32–38%. Pancreatic islets, adenoma or carcinoma (combined): 2/100 (2.0 ± 0.0%), range, 2%.
		Bronchioloalveolar carcinoma (includes multiple) 0/50 (0%), 9/50 (18%)*, 17/50 (34%)**, 30/50 (60%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0013$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Bronchioloalveolar adenoma or carcinoma (combined) 2/50 (4%), 15/50 (30%)*, 20/50 (40%)**, 38/50 (76%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0005$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Cystic keratinizing epithelioma 0/50, 4/50 (8%), 1/50 (2%), 2/50 (4%)	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (F) 5–6 wk 105 wk NTP (2014) (cont.)		*Adrenal medulla* Benign pheochromocytoma (includes bilateral) 6/50 (12%), 12/50 (24%), 22/50 (44%)*, 36/50 (72%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0003$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Benign pheochromocytoma, bilateral 2/50 (4%), 4/50 (8%), 8/50 (16%)*, 19/50 (38%)**	[$P < 0.001$, Cochran–Armitage trend test] *$P \leq 0.05$, poly-3 test [$P = 0.0458$, Fisher exact test] **$P < 0.01$, poly-3 test [$P < 0.0001$, Fisher exact test]	
		Malignant pheochromocytoma (includes bilateral) 0/50, 2/50 (4%), 3/50 (6%), 11/50 (22%)*	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0073$, Fisher exact test]	
		Malignant pheochromocytoma, bilateral 0/50, 1/50, 1/50, 4/50 (8%)*	*$P \leq 0.05$, poly-3 test	
		Benign or malignant pheochromocytoma (combined) 6/50 (12%), 13/50 (26%), 23/50 (46%)*, 40/50 (80%)**	$P < 0.001$, poly-3 trend test [$P < 0.001$, Cochran–Armitage trend test] *$P < 0.001$, poly-3 test [$P = 0.0002$, Fisher exact test] **$P < 0.001$, poly-3 test [$P < 0.0001$, Fisher exact test]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/NTac (F) 5–6 wk 105 wk NTP (2014) (cont.)		*Pancreatic islets* Adenoma or carcinoma (combined) 1/50, 0/50, 0/50, 3/50 *All organs* Mononuclear cell leukaemia 16/50 (32%), 29/50 (58%)***, 28/50 (56%)**, 27/50 (54%)*	NS NS, poly-3 trend test [$P = 0.036$, Cochran–Armitage trend test] *$P = 0.019$, poly-3 trend test [$P = 0.0214$, Fisher exact test] **$P = 0.013$, poly-3 test [$P = 0.0131$, Fisher exact test] ***$P = 0.007$, poly-3 test [$P = 0.0077$, Fisher exact test]	
Full carcinogenicity Rat, Hooded (M) 2–3 mo 119 wk Heath (1956)	Intramuscular injection Co metal powder, spectroscopically pure [no further details provided] Fowl serum 0, 28 mg once 10, 10 NR, average survival: 71 wk	*Injection site* Rhabdomyofibrosarcoma or sarcoma (NOS) (combined) 0/10, 4/10* Rhabdomyofibrosarcoma 0/10, 3/10 Sarcoma (NOS) 0/10, 1/10	*[$P < 0.05$, Fisher exact test] [NS] [NS]	Principal strengths: the duration of observation was adequate, the rats were randomly allocated in groups. Principal limitations: small number of rats per group, use of single dose, limited reporting of study details, vehicle used was serum not from the same species, and statistical analysis not performed. Other comments: Co metal powder in 0.4 mL fowl serum was injected once into the left thigh of the rat; control rats received fowl serum alone. Particle size, ranging from 3.5 µm × 3.5 µm to 17 µm × 12 µm, with several long narrow particles of 10 µm × 4 µm; clumps of 100 µm × 100 µm were present.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hooded (F) 2–3 mo 122 wk Heath (1956)	Intramuscular injection Co metal powder, spectroscopically pure [no further details provided] Fowl serum 0, 28 mg once 10, 10 NR, average survival: 71 wk	*Injection site* Fibrosarcoma, rhabdomyosarcoma, or rhabdomyofibrosarcoma (combined) 0/10, 5/10* Fibrosarcoma 0/10, 3/10 Rhabdomyosarcoma 0/10, 1/10 Rhabdomyofibrosarcoma 0/10, 1/10	 *[$P < 0.02$, Fisher exact test] [NS] [NS] [NS]	Principal strengths: rats randomly allocated in groups, the duration of observation was adequate. Principal limitations: small number of rats per group, use of single dose, limited reporting of study details, vehicle used was serum not from the same species, and statistical analysis not performed. Other comments: Co metal powder in 0.4 mL fowl serum was injected once into the left thigh of the rat; control rats received fowl serum alone. Particle size, ranging from 3.5 µm × 3.5 µm to 17 µm × 12 µm, with several long narrow particles of 10 µm × 4 µm; clumps of 100 µm × 100 µm were present.
Full carcinogenicity Rat, Hooded (F) 2–3 mo 105 wk Heath (1956)	Intramuscular injection Co metal powder, spectroscopically pure [no further details provided] Fowl serum 0 (control, Zn powder), 0 (control, W powder), 28 mg once 5, 5, 10 NR, NR, 1 (average survival: 43 wk)	*Injection site* Rhabdomyofibrosarcoma, fibrosarcoma, or sarcoma (NOS) (combined) 0/5, 0/5, 8/10* Rhabdomyofibrosarcoma 0/5, 0/5, 5/10 Sarcoma (NOS) 0/5, 0/5, 2/10 Fibrosarcoma 0/5, 0/5, 1/10	 *[$P = 0.007$, Fisher exact test] [NS] [NS] [NS]	Principal strengths: the duration of observation was adequate. Principal limitations: small number of rats per group, only one sex, use of single dose, lack of untreated control group, limited reporting of study details, vehicle used was serum not from the same species, and statistical analysis not performed. Other comments: two groups served as controls, one group of 5 rats received Zn powder and another group of 5 rats received W powder in the same manner as the exposed group. Particle size, ranging from 3.5 µm × 3.5 µm to 17 µm × 12 µm, with several long narrow particles of 10 µm × 4 µm; clumps of 100 µm × 100 µm were present.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) ~44 days 3 mo Wen et al. (2020)	Intramuscular implantation Co metal pellet, > 99.99% Implantation Ta (control): diameter 1 mm, length 2 mm; Co: diameter 1 mm, length 2 mm 1× for 3 mo 8, 8 NR, NR	*Limb gastrocnemius muscle* Neoplasms or inflammation (combined) 0/8, 4/8*	*[P = 0.0385, Fisher exact test]	Principal limitations: statistics not provided, only one sex, small number of rats per group, no untreated controls. Other comments: the control group received Ta implants in the same manner as the Co-exposed group to serve as surgery sham controls.
Full carcinogenicity Rat, Sprague-Dawley (M) ~44 days 6 mo Wen et al. (2020)	Intramuscular implantation Co metal pellet, > 99.99% Implantation Ta (control): diameter 1 mm, length 2 mm; Co: diameter 1 mm, length 2 mm 1× for 6 mo 8, 8 NR, NR	*Limb gastrocnemius muscle* Neoplasms or inflammation (combined) 0/8, 6/8*	*[P = 0.0035, Fisher exact test]	Principal limitations: statistics not provided, only one sex, small number of rats per group, no untreated controls. Other comments: the control group received Ta implants in the same manner as the Co-exposed group to serve as surgery sham controls.
Full carcinogenicity Rat, Sprague-Dawley (M) ~44 days 12 mo Wen et al. (2020)	Intramuscular implantation Cobalt metal pellet, > 99.99% Implantation Ta (control): diameter 1 mm, length 2 mm; Co: diameter 1 mm, length 2 mm 1× for 12 mo 8, 8 NR, NR	*Limb gastrocnemius muscle* Rhabdomyosarcoma or spindle cell tumours (NOS) (combined) 0/8, 7/8* Spindle cell tumours (NOS) 0/8, 5/8* Rhabdomyosarcoma 0/8, 2/8	*[P = 0.0007, Fisher exact test] *[P = 0.0128, Fisher exact test] [NS]	Principal strengths: the duration of exposure and observation was adequate. Principal limitations: statistics not provided, only one sex, small number of rats per group, no untreated controls. Other comments: the control group received Ta implants in the same manner as the cobalt-exposed group to serve as surgery sham controls.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Age not specified 8 mo Hansen et al. (2006)	Subcutaneous implantation Co metal as bulk, NR None Diameter 6.5 mm, height 1 mm 1× for 6 or 8 mo 10 NR	*Implantation site* Sarcoma 0/10	NA	Principal strengths: the duration of exposure and observation was considered adequate. Principal limitations: statistics not provided, no controls, only one sex, small number of rats per group, each rat was exposed to the two test agents concurrently. Other comments: this group of 10 male rats was subcutaneously implanted with Co as bulk material on the right side of the vertebral column and implanted as nanoparticulate material on the left side in the paravertebral muscle (see study below).
Full carcinogenicity Rat, Sprague-Dawley (M) Age not specified 8 mo Hansen et al. (2006)	Intramuscular implantation Co metal as nanoparticulate material: average size 120 nm (range, 50–200 nm), NR (Sigma Chemicals, Deisenhofen, Germany) None 60–100 mg 1× for 6 or 8 mo 10 NR	*Implantation site* Sarcoma (NOS) (at 8 mo) 5/6	NA	Principal strengths: the duration of exposure and observation was considered adequate. Principal limitations: statistics not provided, no controls, only one sex, small number of rats per group, each rat was exposed to the two test agents concurrently. Other comments: this group of 10 male rats was implanted with Co as nanoparticulate material on the left side of the vertebral column in the paravertebral muscle and subcutaneously implanted with as bulk material on the right side (see study above).
Full carcinogenicity Rat, Sprague-Dawley (F) NR [bw, 120–140 g] 12 mo Jasmin & Riopelle (1976)	Intrarenal injection Co metal, NR (reagent grade) Glycerin (0.05 mL) 0, 5 mg Single injection into each pole of the right kidney 16, 18 NR	*Kidney* Tumours 0/16, 0/18	NA	Principal limitations: only one dose and sex used, number of rats at start unclear, short duration, step section method not used for histopathological examination, small number of rats per group.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Soluble cobalt(II) salts				
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1, 3 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 22, 31, 24, 20	*Lung* Bronchioloalveolar adenoma 9/50 (18%), 12/50 (24%), 13/50 (26%), 18/50 (36%)* Bronchioloalveolar carcinoma 4/50 (8%), 5/50 (10%), 7/50 (14%), 11/50 (22%)* Bronchioloalveolar adenoma or carcinoma (combined) 11/50 (22%), 14/50 (28%), 19/50 (38%), 28/50 (56%)*	 *P* = 0.005, life-table trend test *P* = 0.018, logistic regression trend test *P* = 0.029, Cochran–Armitage trend test **P* = 0.024, life-table test *P* = 0.027, logistic regression test *P* = 0.035, Fisher exact test *P* = 0.004, life-table trend test *P* = 0.006, logistic regression trend test *P* = 0.021, Cochran–Armitage trend test **P* = 0.031, life-table test *P* = 0.033, logistic regression test *P* = 0.045, Fisher exact test *P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.001, life-table test; logistic regression test; Fisher exact test	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, multiple-dose study, adequate number of rats, adequate duration of exposure and observation. Other comments: MMAD, 1.5–1.6 µm; GSD, 2.3 µm. Mean body weights of 3 mg/m^3 mice (M) were less than those of controls from week 96 until the end of the study. Historical controls: Bronchioloalveolar adenoma: 141/947 (14.9 ± 7.0%), range, 6–36%. Bronchioloalveolar carcinoma: 75/947 (7.9 ± 5.7%), range, 0–16%. Bronchioloalveolar adenoma or carcinoma (combined): 205/947 (21.7 ± 8.0%), range, 10–42%.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1.0, 3.0 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 34, 37, 32, 28	*Lung* Bronchioloalveolar adenoma 3/50 (6%), 6/50 (12%), 9/50 (18%), 10/50 (20%)* Bronchioloalveolar carcinoma 1/50 (2%), 1/50 (2%), 4/50 (8%), 9/50 (18%)* Bronchioloalveolar adenoma or carcinoma (combined) 4/50 (8%), 7/50 (14%), 13/50 (26%)*, 18/50 (36%)**	*P* = 0.014, life-table trend test *P* = 0.024, logistic regression trend test *P* = 0.045, Cochran–Armitage trend test **P* = 0.016, life-table test *P* = 0.024, logistic regression test *P* = 0.036, Fisher exact test *P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test) **P* < 0.01, life-table test; logistic regression test; Fisher exact test *P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* = 0.016, life-table test, logistic regression test, Fisher exact test ***P* < 0.001, logistic regression test; Fisher exact test	Principal strengths: GLP study, study covered most of lifespan, male and female mice used, multiple-dose study, adequate number of mice, adequate duration of exposure and observation. Other comments: MMAD, 1.5–1.6 µm; GSD, 2.3 µm. For all exposed groups body weights were significantly greater than those of controls. Historical controls: Bronchioloalveolar adenoma: 61/939 (6.5 ± 3.2%), range, 0–14%. Bronchioloalveolar carcinoma: 38/939 (4.1 ± 3.2%), range, 0–12%. Bronchioloalveolar adenoma or carcinoma (combined): 97/939 (10.3 ± 3.7%), range, 0–16%.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1.0, 3.0 mg/m^3 6 h+ T90 (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 17, 15, 21, 15	*Lung* Bronchioloalveolar adenoma 1/50 (2%), 4/50 (8%), 1/48 (2%), 6/50 (12%)	$P = 0.042$, life-table trend test	Principal strengths: GLP study, study covered most of lifespan, male and female rats used, multiple-dose study, adequate number of rats, adequate duration of exposure and observation. Other comments: MMAD, 1.4–1.6 µm; GSD, 2.1–2.2 µm. Historical controls: Bronchioloalveolar adenoma: 17/654 (2.6 ± 3.6%), range, 0–10%. Bronchioloalveolar carcinoma: 6/654 (0.9% ± 1.0%), range 0–2%. Bronchioloalveolar adenoma or carcinoma (combined): 23/654 (3.5 ± 3.7%), range, 0–10%. Adrenal medulla, benign pheochromocytoma: 163/623 (26.2 ± 13.2%), range 0–50%. Adrenal medulla, benign, complex, or malignant pheochromocytoma (combined): 176/623 (28.3 ± 12.0%), range, 8–50%.
		Bronchioloalveolar carcinoma 0/50, 0/50, 3/48 (6%), 1/50 (2%)	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 1/50 (2%), 4/50 (8%), 4/48 (8%), 7/50 (14%)*	$P = 0.027$, life-table trend test $P = 0.032$, logistic regression trend test $P = 0.038$, Cochran–Armitage trend test *$P = 0.030$, life-table test $P = 0.029$, logistic regression test $P = 0.030$, Fisher exact test	
		Adrenal medulla Benign pheochromocytoma 14/50 (28%), 19/50 (38%), 23/49 (47%)*, 20/50 (40%)	NS, life-table trend test; logistic regression trend test; Cochran–Armitage trend test *$P = 0.041$, Fisher exact test	
		Benign, complex, or malignant pheochromocytoma (combined) 15/50 (30%), 19/50 (38%), 25/49 (51%), 20/50 (40%)	NS, life-table trend test; logistic regression trend test; Cochran–Armitage trend test *$P < 0.05$, logistic regression test; Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$), approximately 99% Clean air 0, 0.3, 1.0, 3.0 mg/m^3 6 h + T_{90} (12 min) per day, 5 days/wk for 105 wk 50, 50, 50, 50 28, 25, 26, 30	*Lung* Bronchioloalveolar adenoma 0/50, 1/49 (2%), 10/50 (20%)**, 9/50 (18%)*	*P* = 0.003, life-table trend test *P* = 0.001, logistic regression trend test *P* = 0.002, Cochran–Armitage trend test) **P* < 0.003, life-table test; logistic regression test *P* = 0.001, Fisher exact test ***P* < 0.001, life-table test; logistic regression test; Fisher exact test	Principal strengths: GLP study, study covered most of lifespan; male and female rats used, multiple-dose study, adequate number of rats, adequate duration of exposure and observation. Other comments: MMAD, 1.4–1.6 µm; GSD, 2.1–2.2 µm. Historical controls: Bronchioloalveolar adenoma: 7/650 (1.1 ± 1.6%), range, 0–4%. Bronchioloalveolar carcinoma: 0/650. Bronchioloalveolar adenoma or carcinoma (combined): 7/650 (1.1 ± 1.6%), range, 0–4%. Adrenal medulla, benign pheochromocytoma: 35/608 (5.8 ± 4.9%), range, 0–14%. Adrenal medulla, benign, complex, or malignant pheochromocytoma (combined): 39/608 (6.4 ± 4.4%), range, 2–14%.
		Bronchioloalveolar carcinoma 0/50, 2/49 (4%), 6/50 (12%)*, 6/50 (12%)*	*P* < 0.05, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.05, life-table test; logistic regression test; Fisher exact test	
		Bronchioloalveolar adenoma or carcinoma (combined) 0/50, 3/49 (6%), 15/50 (30%)*, 15/50 (30%)*	*P* < 0.001, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.001, life-table test; logistic regression test; Fisher exact test	
		Adrenal medulla Benign pheochromocytoma 2/48 (4%), 1/49 (2%), 3/50 (6%), 8/48 (17%)*	*P* < 0.01, life-table trend test; logistic regression trend test; Cochran–Armitage trend test **P* < 0.05, logistic regression test; Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6 wk 105 wk NTP (1998) (cont.)		Benign, complex, or malignant pheochromocytoma (combined) 2/48 (4%), 1/49 (2%), 4/50 (8%), 10/48 (21%)*	$P < 0.001$, logistic regression trend test; Cochran–Armitage trend test $P = 0.001$, life-table trend test *$P < 0.02$, life-table test; logistic regression test; Fisher exact test	
Full carcinogenicity Rat, Wistar albino (M) About 4 wk 12 mo Shabaan et al. (1977)	Subcutaneous injection Cobalt(II) chloride ($CoCl_2$); purity, NR Physiological saline 0, 4 mg/100 g bw 1× in 2 courses of 5 days separated by a 9 day interval 20, 20 19, 11	*Subcutaneous tissue* Fibrosarcoma 0/20, 8/20*	*[$P = 0.0016$, Fisher exact test]	Principal strengths: adequate duration and schedule of exposure. Principal limitations: statistics not provided, only one sex, small number of rats per group, the control group was also used in the experiment below, use of single dose, necropsy was limited to macroscopic lesions only. Other comments: none of the 9 treated rats who died during the study (almost 50%) were examined. Most tumours induced have been described as developing at the injection site; however, this study also showed occurrence of tumours remote from the site of injection.
Full carcinogenicity Rat, Wistar albino (M) About 4 wk 8 mo Shabaan et al. (1977)	Subcutaneous injection Cobalt(II) chloride ($CoCl_2$); purity, NR Physiological saline 0, 4 mg/100 g bw 1× in 2 courses of 5 days separated by a 9 day interval 20, 20 20, 16	*Subcutaneous tissue* Fibrosarcoma 0/20, 6/20*	*[$P = 0.010$, Fisher exact test]	Principal strengths: adequate duration and schedule of exposure. Principal limitations: statistics not provided, only one sex, small number of rats per group, the control group was also used in the experiment above, use of single dose, necropsy was limited to macroscopic lesions only. Other comments: the 4 rats that died throughout the study were not examined. Most tumours induced have been described as developing at the injection site; however, this study also showed occurrence of tumours remote from the site of injection.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Co-carcinogenicity Rat, Wistar (M + F combined) 3 mo Lifetime Zeller (1975)	Subcutaneous injection Cobalt(II) chloride hexahydrate ($CoCl_2·6H_2O$), NR NR 5 (DEN), 5 (DEN) + 0.5 ($CoCl_2·6H_2O$) mg/kg bw 1×/wk for 43 wk 24, 24 NR	*All sites* Total tumours 19/24, 18/24	[NS]	Principal strengths: male and female rats used. Principal limitations: only one dose, no untreated control group was available, data combined for both sexes, small number of rats per group, limited reporting of the study, no statistics reported. Other comments: DEN (5 mg/kg bw) injected subcutaneously 1×/wk for 43 wk, no tumours were observed in another control group of 12 rats [sex distribution unspecified] treated with $CoCl_2·6H_2O$ only.
Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide				
Full carcinogenicity Mouse, Swiss (F) 2–3 mo 751 days Gilman & Ruckerbauer (1962)	Intramuscular injection Cobalt(II) oxide (CoO), NR 10% suspension in aqueous penicillin G procaine (60 000 IU) 0, 10 mg/site once 51, 50 NR, 12	*Injection site* Tumour 0/48, 0/46 *Lung* Adenoma 0/48, 2/46 (4%)	NA NS	Principal strengths: the duration of observation was adequate, adequate number of mice. Principal limitations: one sex and only one dose were used, neither microphotographs nor histopathology description provided. Other comments: particle size, < 5 µm.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime Steinhoff & Mohr (1991)	Intratracheal instillation Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0 (untreated), 0 (vehicle), 2, 10 mg/kg bw 1×/2 wk for 18 treatments and then 1×/4 wk from the 19th to the 39th treatment, over 2 yr 50, 50, 50, 50 NR	*Lung* Total tumours 0/50, 0/50, 1/50, 5/50* Bronchioloalveolar adenoma 0/50, 0/50, 0/50, 2/50 Bronchioloalveolar adenocarcinoma 0/50, 0/50, 0/50, 1/50 Adenocarcinoma 0/50, 0/50, 0/50, 2/50 Benign squamous epithelial tumour 0/50, 0/50, 1/50, 0/50	[P = 0.047, Cochran–Armitage trend test] *[P = 0.0281, Fisher exact test] [NS] [NS] [NS] [NS]	Principal strengths: multiple-dose study, male and female rats used, adequate number of rats used, randomly allocated in groups, adequate duration of exposure and observation. Principal limitations: no statistical analysis was performed, the particle size was potentially not appropriate for this route of exposure. Other comments: CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range. Total tumours were bronchioloalveolar adenoma, bronchioloalveolar adenocarcinoma, adenocarcinoma, and benign squamous epithelial tumours.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 10 wk Lifetime Steinhoff & Mohr (1991)	Intratracheal instillation Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0 (untreated), 0 (vehicle), 2, 10 mg/kg bw 1×/2 wk for 18 treatments and then 1×/4 wk from the 19th to the 39th treatment, over 2 yr 50, 50, 50, 50 NR	*Lung* Bronchioloalveolar adenoma 0/50, 0/50, 1/50, 0/50 Bronchioloalveolar carcinoma 0/50, 0/50, 0/50, 1/50	[NS] [NS]	Principal strengths: multiple-dose study, male and female rats used, adequate number of rats used, randomly allocated in groups, adequate duration of exposure and observation. Principal limitations: no statistical analysis was performed, the particle size was potentially not appropriate for this route of exposure. Other comments: CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime Steinhoff & Mohr (1991)	Subcutaneous injection Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0, 2 mg/kg bw 5×/wk for 2 yr 10, 10 NR, NR	*Injection site* Malignant histiocytoma or sarcoma (NOS) (combined) 0/10, 5/10*	 *[P = 0.0163, Fisher exact test]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: statistics not provided, only one sex, small number of rats per group, survival and body weight data not reported, only one dose. Other comments: CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range.
Full carcinogenicity Rat, Sprague-Dawley (M) 10 wk Lifetime Steinhoff & Mohr (1991)	Subcutaneous injection Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0, 10 mg/kg bw 1×/wk for 2 yr 10, 10 NR, NR	*Injection site* Malignant histiocytoma or sarcoma (NOS) (combined) 0/10, 4/10	 [NS]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: statistics not provided, only one sex, small number of rats per group, survival and body weight data not reported, only one dose. Other comments: CoO, 76.7% Co; ~80% of particles are in the 5–40 µm range.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Wistar (M + F combined) 2–3 mo Up to 519 days Gilman & Ruckerbauer (1962)	Intramuscular injection Cobalt(II) oxide (CoO) powder, spectroscopically pure Aqueous penicillin G procaine (90 000 IU) 0, 30 mg/site (thigh) once 10, 10 NR, 260 days (average survival)	*Injection site* Rhabdomyosarcoma 0/10, 5/10*	*[P = 0.0163, Fisher exact test]	Principal strengths: rats randomly allocated in groups, the duration of observation was adequate. Principal limitations: statistics not provided, data combined for both sexes, small number of rats per group, use of single dose, limited reporting of study details. Other comments: particle size, ≤ 5 µm.
Full carcinogenicity Rat, Wistar (M + F combined) 2–3 mo ≤ 342 days Gilman (1962)	Intramuscular injection Cobalt(II) oxide (CoO), spectroscopically pure Aqueous penicillin G procaine 20 mg/site (thigh) once 32 5	*Injection site* Sarcoma, mostly rhabdomyofibrosarcoma 12/24	NA	Principal strengths: adequate number of rats used, randomly allocated in groups, the duration of observation was adequate. Principal limitations: lack of control group, use of single dose, data combined for both sexes, histopathological confirmation was not consistently performed. Other comments: particle size, ≤ 5 µm. Among the effective number (24) of rats (those surviving ≥ 90 days), 5 were treated in both thighs and 19 in one thigh.
Full carcinogenicity Rat, Sprague-Dawley (M + F combined) 10 wk Lifetime Steinhoff & Mohr (1991)	Intraperitoneal injection Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 0, 200 mg/kg bw 3× at intervals of 2 mo 20, 20 NR, NR	*Injection site* Total tumours 1/20, 14/20* Malignant histiocytoma 1/20, 10/20* Sarcoma (NOS) 0/20, 3/20 Malignant mesothelioma 0/20, 1/20	*[P < 0.0001, Fisher exact test] *[P = 0.0017, Fisher exact test] [NS] [NS]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: statistics not provided, data for both sexes combined, small number of rats per group, survival and body weight data not reported. Other comments: the control and treated groups of 20 rats included 10 male and 10 female. CoO, 76.7% Co; ~80% of particles were in the 5–40 µm range.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Co-carcinogenicity Rat, Sprague-Dawley (F) 10 wk Lifetime Steinhoff & Mohr (1991)	Intratracheal instillation Cobalt(II) oxide (CoO), reported as "pure" Physiological saline 20 (benzo[*a*]pyrene), 20 (benzo[*a*]pyrene) + CoO 10 or 20 mg/kg bw CoO: 10 mg/kg bw, 1×/wk for a total of 7 treatments; then 20 mg/kg bw every 14 days for a total of 20 treatments (total dose, 470 mg/kg bw). Benzo[*a*]pyrene: 20 mg/kg bw, 1×/wk; starting from the 8th treatment dose given every 14 days for a total of 10 treatments (total dose, 200 mg/kg bw) 20, 20 NR	*Lung* Carcinomas (all) 1/20, 9/20* Squamous cell carcinoma 1/20, 8/20* Adenocarcinoma 0/20, 1/20	 *[$P = 0.0042$, Fisher exact test] *[$P = 0.0098$, Fisher exact test] [NS]	Principal strengths: the duration of exposure and observation was adequate, the schedule of exposure was adequate. Principal limitations: small number of rats, only one dose and sex, survival and body weight data not reported, no statistics reported, the particle size was potentially not appropriate for this route of exposure. Other comments: the alternating period between treatment with CoO and benzo[*a*]-pyrene was 4 days, the control group was treated with benzo[*a*]pyrene alone in physiological saline. CoO, 76.7% Co; ~80% of particles in the 5–40 µm range.
Full carcinogenicity Hamster, Syrian golden (M) 2 mo Lifetime Wehner et al. (1977)	Inhalation (whole-body exposure) Cobalt(II) oxide (CoO), reported as "purified" Clean air 0, 10 mg/m^3 7 h/day, 5 days/wk for life 51, 51 NR	*All sites combined* Total tumours (malignant) 1/51, 2/51 Reticulum cell sarcoma 0/51, 1/51 Carcinoma 0/51, 1/51	 [NS] [NS] [NS]	Principal limitations: only one sex, poor survival, limited reporting of the study, only one dose, no statistics reported. Other comments: median survival, 16.6 mo in cobalt-exposed hamsters vs 15.3 mo in controls.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Co-carcinogenicity Hamster, Syrian golden(M) 2 mo Lifetime Wehner et al. (1977)	Inhalation (whole-body exposure) Cobalt(II) oxide (CoO), reported as "purified" Clean air Cigarette smoke + 0 (CoO), cigarette smoke + 10 (CoO) mg/m^3 7 h/day, 5 days/wk for life 51, 51 NR	*All sites combined* Total tumours (benign) 5/51, 8/51 Polyps 0/51, 1/51 Squamous cell papilloma 0/51, 1/51 *Adrenal cortex*: adenoma 4/51, 5/51 *Vascular system*: haemangioma 0/51, 1/51	 [NS] [NS] [NS] [NS] [NS]	Principal limitations: only one sex, limited reporting of the study, only one dose, no statistics reported. Other comments: cigarette smoke exposure (3×/day for 10 min, nose-only) in modified Hamburg II smoking machine; twice before and once after the 7 h CoO exposure or sham dust exposure. Median survival, 21.6 mo in CoO + cigarette smoke-exposed hamsters vs 19.3 mo in cigarette smoke only-exposed controls.
Full carcinogenicity Rat, Wistar (M + F combined) 2–3 mo ≤ 365 days Gilman (1962)	Intramuscular injection Cobalt(II) sulfide (CoS), spectroscopically pure Aqueous penicillin G procaine 20 mg/site (thigh) once 30 1	*Injection site* Sarcoma, mostly rhabdomyofibrosarcoma 28/29	 NA	Principal strengths: adequate number of rats used, randomly allocated in groups, the duration of observation was adequate. Principal limitations: lack of control group, data combined for sexes, use of single dose, histopathological confirmation was not consistently performed. Other comments: particle size, ≤ 5 µm. Among the effective number of rats (rats surviving ≥ 90 days), nearly all (27) were treated in both thighs and 2 in one thigh.
Full carcinogenicity Rat, Sprague-Dawley (F) NR [body weight, 120–140 g] 12 mo Jasmin & Riopelle (1976)	Intrarenal injection Cobalt(II) sulfide (CoS), NR (reagent grade) Glycerin 0, 5 mg Single injection into each pole of the right kidney 16, 20 NR	*Kidney* Tumours 0/16, 0/20	 NA	Principal limitations: only one dose and sex, number of rats at start unclear, short duration, step section method not used for histopathological examination, small number of rats per group. Other comments: glycerin, 0.5 mL.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Hamster, NR (M + F combined) 9 wk ≤ 110 wk Farrell & Davis (1974)	Intratracheal instillation Cobalt(II,III) oxide (Co_3O_4), NR Gelatin in saline 0, 4 mg 1×/wk [assumed] for 30 wk 50, 50 (25 M and 25 F/group) 43, 43	*Respiratory tract* Total tumours 5/44, 2/48 *Alveoli* Tumours 0/44, 2/48	 [NS] [NS]	Principal limitations: data combined for both sexes, histopathological examination was limited to the respiratory tract, only one dose, no statistical analysis reported. Other comments: particle size, 0.5–1.0 µm. The week after the last injection, the intratracheal instillations were started (1×/wk [assumed] for 30 wk); Co_3O_4 was suspended in 0.2 mL of 0.5% gelatin in saline; controls were instilled with 0.2 mL of 0.5% gelatin in saline only; the hamsters were observed for an additional 43–68 wk.
Initiation promotion (tested as promoter) Hamster, NR (M + F combined) 9 wk ≤ 110 wk Farrell & Davis (1974)	Intratracheal instillation Cobalt(II,III) oxide (Co_3O_4), NR Gelatin in saline DEN + vehicle, DEN + 4 mg Co_3O_4 1×/wk [assumed] for 30 wk 50, 50 (25 M and 25 F/group) 33, 39	*Respiratory tract* Total tumours 30/48, 37/48 *Nasal cavity* Tumours 1/48, 2/48 Carcinoma 0/48, 1/48 Papilloma 7/48, 12/48 *Trachea:* papilloma 27/48, 33/48 *Bronchi*: tumours 0/48, 1/48 *Alveoli*: tumours 7/48, 5/48	 [NS] [NS] [NS] [NS] [NS] [NS]	Principal limitations: data combined for both sexes, histopathological examination was limited to the respiratory tract, only one dose, no statistical analysis reported. Other comments: particle size, 0.5–1.0 µm. DEN treatment (0.5 mg/0.25 mL saline) by subcutaneous injection 1×/wk for 12 wk; the week after the last injection, the intratracheal instillations were started (1×/wk [assumed] for 30 wk); Co_3O_4 was suspended in 0.2 mL of 0.5% gelatin in saline. Controls were instilled with 0.2 mL of 0.5% gelatin in saline only. The hamsters were observed for an additional 43–68 wk.

bw, body weight(s); Co, cobalt; DEN, diethylnitrosamine; F, female; GLP, Good Laboratory Practice; GSD, geometric standard deviation; IU, International Unit; M, male; min, minute; MMAD, mass median aerodynamic diameter; mo, month; NA, not applicable; No., number; NOS, not otherwise specified; NR, not reported; NS, not significant; T_{90}, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation; Ta, tantalum; vs, versus; W, tungsten; wk, week; yr, year; Zn, zinc.

increased (P = 0.016, poly-3 test) [P = 0.0448, Fisher exact test] in the group at the intermediate concentration, exceeding the upper bound of the range observed in historical controls from this laboratory (inhalation studies, 39/300; 13.0 ± 4.2%; range, 8–20%; and all routes: 145/950; 15.3 ± 6.2%; range, 2–26%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test]. The incidence of bronchioloalveolar carcinoma (includes multiples) was significantly increased (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test] in all exposed groups (control, 11/50; lowest concentration, 38/49; intermediate concentration, 42/50; highest concentration, 46/50). In addition, there was a significant positive trend in the incidence of multiple bronchioloalveolar carcinoma [P < 0.001, Cochran–Armitage test], with the incidence (control, 3/50; lowest concentration, 18/49; intermediate concentration, 24/50; highest concentration, 36/50) being significantly increased ($P \leq 0.01$, poly-3 test) [$P \leq 0.002$, Fisher exact test] in all exposed groups. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test], with the increase in incidence (control, 16/50; lowest concentration, 41/49; intermediate concentration, 43/50; highest concentration, 47/50) being significant (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test] at all concentrations.

In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) (P = 0.037, poly-3 test). The incidence of bronchioloalveolar adenoma (includes multiples) in the groups at 0 (control), 1.25, 2.5, and 5 mg/m^3 was 3/49 (6%), 9/50 (18%), 8/50 (16%), and 10/50 (20%), respectively, and significantly increased (P = 0.024, poly-3 test) [P = 0.0387, Fisher exact test] in the group at the highest concentration. The incidence of bronchioloalveolar adenoma (includes multiples) in all exposed groups exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 16/299; 5.4 ± 3.7%; range, 2–12%; all routes: 54/949; 5.7 ± 3.6%; range, 0–12%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test] with the increase in incidence (control, 5/49; lowest concentration, 25/50; intermediate concentration, 38/50; highest concentration, 43/50) being significant (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test] at all concentrations. In addition, there was a significant positive trend in the incidence of multiple bronchioloalveolar carcinoma [P < 0.001, Cochran–Armitage test], with the incidence (control, 1/49; lowest concentration, 7/50; intermediate concentration, 20/50; highest concentration, 24/50) being significantly increased in all exposed groups (lowest concentration, $P \leq 0.05$, poly-3 test [P = 0.0317, Fisher exact test]; intermediate and highest concentration, $P \leq 0.01$, poly-3 test [P < 0.001, Fisher exact test]). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test] with the increase in the incidence (control, 8/49; lowest concentration, 30/50; intermediate concentration, 41/50; highest concentration, 45/50) being significant at all concentrations (P < 0.001, poly-3 test) [P < 0.001, Fisher exact test].

Regarding non-neoplastic lesions in treated males and females, bronchioloalveolar epithelial hyperplasia and cytoplasmic vacuolization of the bronchioloalveolar epithelium occurred in the epithelium of the peri-acinar region of the lung, which encompassed the terminal bronchioles, associated alveolar ducts, and immediately adjacent alveoli (NTP, 2014). Moreover, instances of alveolar epithelial hyperplasia, alveolar proteinosis, alveolar histiocytic infiltration, bronchiolar epithelial erosion, and suppurative inflammation occurred. In general, the severity

of these non-neoplastic lesions increased with increasing exposure concentration. In the nasal cavity, larynx, and trachea, non-neoplastic changes also occurred in both treated males and females. [The Working Group noted that this was a well-conducted study that complied with GLP, both sexes were used, the duration of exposure and observation was adequate, there was a sufficient number of animals per group, and multiple concentrations based on a 3-month study were tested.]

3.1.2 Rat

(a) Inhalation

In a well-conducted study that complied with GLP, groups of 50 male and 50 female Fischer 344/NTac rats (age, 5–6 weeks) were exposed by inhalation (whole-body) to cobalt metal-particle aerosol concentrations of 0, 1.25, 2.5, or 5 mg/m^3 (concentrations were based on the findings from a 13-week study) for untreated controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, 98.2% ± 0.6%; MMAD, 1.4–2.0 μm; GSD, 1.6–1.9 μm) for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks (NTP, 2014). Surviving rats were killed at age 109–110 weeks. At study termination, survival of male rats was 17/50, 20/50, 16/50, and 16/50, and for female rats was 35/50, 26/50, 24/50, and 25/50 (including one rat that died during the last week of the study), for the controls and groups at the lowest, intermediate, and highest concentration, respectively. Survival of females at the intermediate concentration was significantly less than that of controls. The mean body weights of rats in the groups at the intermediate and highest concentration were at least 10% less than those of controls after weeks 99 and 12, respectively, for male rats, and weeks 57 and 21, respectively, for female rats. Abnormal breathing and thinness were noted in exposed male and female rats. Tissue burden studies were conducted only in females; lung cobalt concentrations and burdens increased with increasing exposure concentrations and were significantly greater than in controls. The values of maximum cobalt lung burdens observed in the 2-year studies indicated that lung overload was not reached in these studies. All rats underwent complete necropsy with histopathological evaluation.

In male rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) (P = 0.011, poly-3 test) [P = 0.015, Cochran–Armitage test]. The incidence of bronchioloalveolar adenoma (includes multiples) in the groups at 0 (control), 1.25, 2.5, and 5 mg/m^3 was 2/50, 10/50, 10/50, and 14/50, respectively, and significantly increased (lowest and intermediate concentration, $P \leq 0.018$, poly-3 test [P = 0.0139, Fisher exact test]; highest concentration, $P < 0.001$, poly-3 test [P = 0.0009, Fisher exact test]) in all exposed groups. There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence (control, 0/50; lowest concentration, 16/50; intermediate concentration, 34/50; highest concentration, 36/50) being significantly increased ($P < 0.001$, poly-3 test) [$P < 0.001$, Fisher exact test] in all exposed groups. In addition, there was a significant positive trend in the incidence of multiple bronchioloalveolar carcinoma [$P < 0.001$, Cochran–Armitage test], with the incidence (control, 0/50; lowest concentration, 6/50; intermediate concentration, 14/50; highest concentration, 30/50) being significantly increased (lowest concentration, $P \leq 0.05$, poly-3 test [P = 0.0133, Fisher exact test]; intermediate and highest concentration, $P \leq 0.01$, poly-3 test, [$P < 0.0001$, Fisher exact test]) in all exposed groups. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the increase in incidence (control, 2/50; lowest concentration, 25/50; intermediate concentra-

tion, 39/50; highest concentration, 44/50) being significant ($P < 0.001$, poly-3 test) [$P < 0.0001$, Fisher exact test] at all concentrations. Cystic keratinizing epithelioma was observed only in the groups at the lowest (1/50, 2%) and highest (1/50, 2%) concentration. Incidence of cystic keratinizing epithelioma exceeded the incidence observed in historical controls from this laboratory (all routes including current study: 0/100). There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test]. Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 15/50 (30%), 23/50 (46%), 37/50 (74%), and 34/50 (68%), respectively, and significantly increased ($P < 0.001$, poly-3 test) [$P < 0.001$, Fisher exact test] at the intermediate and highest concentration. The incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) in all treated groups exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 25/100; 25 ± 7.1%; range, 20–30%). In addition, there was a significant positive trend in the incidence of bilateral benign pheochromocytoma of the adrenal medulla [$P < 0.001$, Cochran–Armitage test]. Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 4/50 (8%), 13/50 (26%), 22/50 (44%), and 21/50 (42%), respectively, and significantly increased (lowest concentration, $P \leq 0.05$, poly-3 test [$P < 0.01$, Fisher exact test]; intermediate and highest concentration; $P \leq 0.01$, poly-3 test, [$P < 0.0001$, Fisher exact test]) in all exposed groups, and exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 25/100; 25.0% ± 7.1%; range, 20–30%) at the intermediate and highest concentration. There was a significant positive trend in the incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test]. Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 2/50 (4%), 9/50 (18%), and 16/50 (32%), respectively, and significantly increased at the intermediate ($P < 0.001$, poly-3 test) [$P = 0.0256$, Fisher exact test] and highest concentration ($P < 0.001$, poly-3 test) [$P = 0.0002$, Fisher exact test]. [The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only (see Patterson et al., 1995; Thompson, 2002).] The incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) in the groups at the intermediate and highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 2/100; 2.0% ± 2.8%; range, 0–4%). In addition, the incidence of bilateral malignant pheochromocytoma of the adrenal medulla at the highest concentration, which was 7/50 (14%), was significantly increased ($P \leq 0.01$, poly-3 test) [$P = 0.0062$, Fisher exact test], exceeding the upper bound of the range observed in historical controls from this laboratory (all routes: 2/100; 2.0% ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 17/50 (34%), 23/50 (46%), 38/50 (76%), and 41/50 (81%), respectively, and significantly increased ($P < 0.001$, poly-3 test) [$P < 0.0001$, Fisher exact test] at the intermediate and highest concentration. The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in all treated groups exceeded the upper bound of the range observed in historical controls (all routes: 27/100; 27 ± 9.9%; range, 20–34%). There was a significant increase ($P = 0.015$, poly-3 test) [$P = 0.0117$, Fisher exact test] in the incidence of pancreatic islets adenoma (control, 0/50;

lowest concentration, 1/50; intermediate concentration, 6/48; highest concentration, 3/49) in the group at the intermediate concentration, with incidence at all concentrations exceeding the incidence observed in historical controls from this laboratory (all routes: 0/100). There was a significant positive trend in the incidence of pancreatic islets carcinoma (P = 0.021, poly-3 test). Incidence in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 1/50 (2%), 5/48 (10%), and 6/49 (12%), respectively, and the groups at the intermediate and highest concentration exceeded the upper bound of the range observed in historical controls (all routes: 2/100; 2 ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of pancreatic islets adenoma or carcinoma (combined) (P = 0.002, poly-3 test) [P = 0.007, Cochran–Armitage test]. The incidence of pancreatic islets adenoma or carcinoma (combined) in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 2/50 (4%), 10/48 (20%), and 9/49 (18%), respectively, and significantly increased at the intermediate (P = 0.013, poly-3 test) [P = 0.0113, Fisher exact test] and highest concentration (P = 0.022, poly-3 test) [P = 0.0235, Fisher exact test]. At the intermediate and highest concentration, incidence exceeded the upper bound of the range observed in historical controls (all routes: 2/100; 2.0 ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of renal tubular adenoma or carcinoma (combined) (P = 0.023, poly-3 test) [P = 0.039, Cochran–Armitage test] for standard evaluations (single-sections) and extended evaluations (step sections) (combined), with the incidence being control, 3/50; lowest concentration, 1/50; intermediate concentration, 1/50; and highest concentration, 7/50. The incidence of renal tubular adenoma or carcinoma (combined) for standard evaluations (single-sections) of controls and groups at the lowest, intermediate, and highest concentration was 0/50, 1/50 (2%), 0/50, and 4/50 (8%), respectively. The incidence of renal tubular adenoma or carcinoma (combined) for standard evaluations observed in historical controls was only 1/100 (range, 0–2%).

In female rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (includes multiples) (P = 0.002, poly-3 test) [P = 0.022, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 2/50 (4%), 7/50 (14%), 9/50 (18%), and 13/50 (26%), respectively, and significantly increased at the intermediate (P = 0.016, poly-3 test) [P = 0.0256, Fisher exact test] and highest concentration (P < 0.001, poly-3 test) [P = 0.0019, Fisher exact test]. The incidence of bronchioloalveolar adenoma (includes multiples) at all concentrations exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 2/100; included current study, 2.0 ± 2.8%; range, 0–4%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (includes multiples) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test], with the incidence (control, 0/50; lowest concentration, 9/50; intermediate concentration, 17/50; highest concentration, 30/50) being significantly increased in all exposed groups (lowest concentration, P < 0.001, poly-3 test [P = 0.0013, Fisher exact test]; intermediate and highest concentration, P < 0.001, poly-3 test [P < 0.0001, Fisher exact test]). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001, poly-3 test) [P < 0.001, Cochran–Armitage test], with the increase in the incidence (control, 2/50; lowest concentration, 15/50; intermediate concentration, 20/50; highest concentration, 38/50) being significant (lowest concentration, P < 0.001, poly-3 test [P = 0.0005, Fisher exact test]; intermediate and highest concentration, P < 0.001, poly-3 test [P < 0.0001, Fisher exact test]) at all concentrations. Cystic keratinizing epithelioma was observed in all exposed groups, with incidence of

controls and groups at the lowest, intermediate, and highest concentration being 0/50, 4/50 (8%), 1/50 (2%), and 2/50 (4%), respectively. The incidence of cystic keratinizing epithelioma in all treated groups exceeded the incidence observed in historical controls from this laboratory (all routes, included current study: 0/100). There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test] with the incidence of controls and groups at the lowest, intermediate, and highest concentration being 6/50 (12%), 12/50 (24%), 22/50 (44%), and 36/50 (72%), respectively, and significantly increased at the intermediate ($P < 0.001$ poly-3 test [$P = 0.0003$, Fisher exact test] and highest concentration ($P < 0.001$ poly-3 test) [$P < 0.0001$, Fisher exact test]. In addition, the incidence of bilateral benign pheochromocytoma of the adrenal medulla in the controls and groups at the lowest, intermediate, and highest concentration was 2/50 (4%), 4/50 (8%), 8/50 (16%), and 19/50 (38%), respectively, and significantly increased at the intermediate ($P \leq 0.05$, poly-3 test) [$P = 0.0458$, Fisher exact test] and highest concentration ($P \leq 0.01$ poly-3 test) [$P < 0.0001$, Fisher exact test]. The incidence of benign pheochromocytoma of the adrenal medulla (includes bilateral) in all exposed groups, and of bilateral benign pheochromocytoma of the adrenal medulla at the intermediate and highest concentration, exceeded the range observed in historical controls from this laboratory (all routes: 7/100; 7 ± 7.1%; range, 2–12%). There was a significant positive trend in the incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 0/50, 2/50 (4%), 3/50 (6%), and 11/50 (22%), respectively, and significantly increased ($P < 0.001$, poly-3 test) [$P = 0.0073$, Fisher exact test] at the highest concentration. [The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only.] The incidence of malignant pheochromocytoma of the adrenal medulla (includes bilateral) in the groups at the intermediate and highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (all routes: 1/100; 1 ± 1.4%; range, 0–2%). In addition, the incidence of bilateral malignant pheochromocytoma of the adrenal medulla in the controls and groups at the lowest, intermediate, and highest concentration was 0/50, 1/50 (2%), 1/50 (2%), and 4/50 (8%), and significantly increased in the group at the highest concentration ($P \leq 0.05$, poly-3 test). There was a significant positive trend in the incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla ($P < 0.001$, poly-3 test) [$P < 0.001$, Cochran–Armitage test], with the incidence in the controls and groups at the lowest, intermediate, and highest concentration being 6/50 (12%), 13/50 (26%), 23/50 (46%), and 40/50 (80%), respectively, and significantly increased at the intermediate ($P < 0.001$, poly-3 test) [$P = 0.0002$, Fisher exact test] and highest concentration ($P < 0.001$, poly-3 test) [$P < 0.0001$, Fisher exact test]. The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in all exposed groups exceeded the range observed in historical controls from this laboratory (all routes: 8/100; 8 ± 5.7%; range, 4–12%). The incidence of pancreatic islets adenoma or carcinoma (combined) in the controls and groups at the lowest, intermediate, and highest concentration was 1/50 (2%), 0/50, 0/50, and 3/50 (6%), respectively, and at the highest concentration exceeded the range observed in historical controls from this laboratory (all routes: 2/100; 2.0 ± 0.0%; range, 0–2%). There was a significant positive trend in the incidence of mononuclear cell leukaemia (all organs) [$P = 0.036$, Cochran–Armitage test]. The incidence of mononuclear cell leukaemia (all

organs) in the controls and groups at the lowest, intermediate, and highest concentration was 16/50 (32%), 29/50 (58%), 28/50 (56%), and 27/50 (54%), respectively, and significantly increased at the lowest ($P = 0.007$, poly-3 test) [$P = 0.0077$, Fisher exact test], intermediate ($P = 0.013$, poly-3 test) [$P = 0.0131$, Fisher exact test], and highest concentration ($P = 0.019$, poly-3 test) [$P = 0.0214$, Fisher exact test], exceeding the range observed in historical controls for all concentrations from this laboratory (all routes: 35/100; 35 ± 4.2%; range, 32–38%).

Regarding non-neoplastic lesions, the incidence of alveolar epithelial hyperplasia, alveolar proteinosis, chronic active inflammation in the lung, and bronchiolar epithelial hyperplasia in all exposed groups of male and female rats was significantly higher than that in the control groups. The incidence of medullary hyperplasia in the adrenal gland was significantly increased in female rats in the groups at the lowest and intermediate concentration; the incidence of this lesion was significantly decreased in male rats in groups at the intermediate and highest concentration. [The Working Group noted that this was a well-conducted study that complied with GLP, males and females were used, the duration of exposure and observation was adequate, there was a sufficient number of animals per group, and multiple concentrations based on a 3-month study were tested.]

(b) Intramuscular injection

Groups of 10 male and 10 female Hooded rats (age, 2–3 months) were treated with cobalt metal microparticles (spectroscopically pure [no further details provided], 400 mesh; ranging from 3.5 × 3.5 μm to 17 × 12 μm, with several long narrow particles of 10 × 4 μm; clumps of 100 × 100 μm were present as well; suspended in 0.4 mL fowl serum) at a dose of 0 (controls, injected with 0.4 mL fowl serum alone at the same site) or 28 mg by single intramuscular injection into the left thigh muscle (Heath, 1956), and were then observed for up to 119 weeks for males and 122 weeks for females. Tumour-bearing rats were killed a few weeks after tumour appearance. [The Working Group determined the average survival time in treated males (71 weeks) and treated females (61 weeks); survival of the controls was not reported.] The first tumours were observed macroscopically at 5 months and the last ones by 12 months. All the tumours occurred at the injection site.

In male rats, there was a significant increase [$P < 0.05$, Fisher exact test] in the incidence of rhabdomyofibrosarcoma or sarcoma (not otherwise specified, NOS) (combined) at the injection site in the treated group (control, 0/10; treated, 4/10).

In female rats, there was a significant increase [$P < 0.02$, Fisher exact test] in the incidence of fibrosarcoma, rhabdomyosarcoma, or rhabdomyofibrosarcoma (combined) at the injection site in the treated group (control, 0/10; treated, 5/10) (Heath, 1956). [The Working Group noted that the duration of observation was adequate, and the animals were randomly allocated in groups. However, the number of rats per group was small, a single dose was tested, the reporting of study details was limited, the vehicle used was serum that was not from the same species, and statistical analysis was not performed. The Working Group suggested that rhabdomyofibrosarcoma could be, in this case, a myofibroblastic reaction in association with rhabdomyosarcoma other than a dual differentiation of a sarcoma after the evaluation of the microphotographs.]

Groups of 10 female Hooded rats (age, 2–3 months) were treated with cobalt metal micro-particles (spectroscopically pure [no further details provided], 400 mesh; ranging from 3.5 × 3.5 μm to 17 × 12 μm, with large numbers of long narrow particles of the order of 10 × 4 μm; suspended in 0.4 mL fowl serum) at a dose of 28 mg by single intramuscular injection into the right thigh muscle (Heath, 1956), and then observed for up to 105 weeks.

Tumour-bearing rats were killed a few weeks after tumour appearance. Five control rats were treated with zinc powder (spherical particles ranging from 1.5 to 44 μm diameter, with most of the particles having diameters between 4 and 20 μm, and no clumps were observed; suspended in 0.4 mL fowl serum) at a dose of 28 mg by intramuscular injection, and another 5 control rats were treated with tungsten powder (rectangular particles, with sizes ranging from 5 × 5 μm diameter to 50 × 50 μm diameter, with most of them having diameters between 8 × 12 μm and 10 × 30 μm, and some clumps up to 170 × 170 μm were observed; suspended in 0.4 mL fowl serum) at a dose of 28 mg by intramuscular injection. [The Working Group determined the average survival time for cobalt-treated rats (43 weeks); the survival of the controls was not reported.]

The first tumours were observed macroscopically at 5 months and the last ones by 12 months. All the tumours occurred at the injection site. There was a significant increase [P = 0.007, Fisher exact test] in the incidence of rhabdomyofibrosarcoma, fibrosarcoma, or sarcoma (NOS) (combined) at the injection site in the treated group (zinc powder controls, 0/5; tungsten powder controls, 0/5; treated, 8/10) (Heath, 1956). [The Working Group noted that the duration of observation was adequate. However, the number of animals per group was small, only one sex and dose was used, there was no untreated control group, the reporting of study details was limited, the vehicle used was serum that was not from the same species, and statistical analysis was not performed. The Working Group suggested that rhabdomyofibrosarcoma could be, in this case, a myofibroblastic reaction in association with rhabdomyosarcoma other than a dual differentiation of a sarcoma.]

A group of 30 male Hooded rats (age, 2–3 months) were treated with cobalt metal powder (spectroscopically pure [no further details provided]; suspended in 0.4 mL fowl serum) at a dose of 28 mg by single intramuscular injection into the right thigh muscle. A control group of 15 male rats received a single intramuscular injection of 0.4 mL fowl serum alone (Heath, 1960). A number of rats (not reported) were killed at daily intervals 1–28 days after injection, or at 2-week intervals up to 20 weeks after injection [no further details provided]. [The Working Group noted that the survival of controls and treated rats was not reported but that the number of treated animals was adequate. However, the duration of exposure and observation was short since some rats were killed on a daily basis during the first month of the study, only one sex and dose was used, the number of controls was small, no data on tumour incidence were given for controls, the vehicle used was serum that was not from the same species, the reporting of study details was limited, and statistical analysis was not conducted. Therefore, the Working Group considered the study inadequate for the evaluation of the carcinogenicity of cobalt metal in experimental animals.]

(c) Intramuscular or subcutaneous implantation

In a study using embedded metals (modelling "shrapnel wounds"), eight groups of 8 male Sprague-Dawley rats (age, ≥ 44 days) were treated with a single intramuscular implantation of four cobalt metal pellets (cylinders, 1 mm in diameter and 2 mm in length) (purity, > 99.99%) or tantalum (surgical-sham controls), with two pellets inserted into the gastrocnemius muscle of each limb, and were analysed for changes in the gastrocnemius muscle at 1, 3, 6, or 12 months. (Wen et al., 2020). No untreated controls were used.

No tumours or inflammation of the gastrocnemius muscle were observed in any of the tantalum-implanted rats or in rats in the cobalt-implanted group analysed after 1 month (incidence, 0/8). Tumours or inflammation (combined) of the gastrocnemius muscle were observed starting 3 months post-implantation in

the group implanted with cobalt (incidence, 4/8) [$P = 0.0385$, Fisher exact test], and the incidence of tumours or inflammation (combined) significantly increased over time: 6/8 in the group implanted with cobalt analysed at 6 months and 7/8 [all lesions were tumours] in the group analysed at 12 months [$P = 0.0035$ and $P = 0.0007$, Fisher exact test, respectively]. Tumours identified in the group implanted with cobalt analysed at 12 months were described as 5 spindle cell tumours [NOS] and 2 rhabdomyosarcomas; tumour types found at earlier time points were not identified (Wen et al., 2020). [The Working Group noted that the duration of exposure and observation was adequate. However, only one sex was used, there was a small number of animals per group and no untreated controls, and statistical analysis was not provided.]

One group of 10 male Sprague-Dawley rats [age at start, not specified] were treated with a single subcutaneous implantation of cobalt metal as bulk material (diameter, 6.5 mm; height, 1 mm) on the right side of the vertebral column, and a single intramuscular implantation of nanoparticulate cobalt metal (60–100 mg; average size, 120 nm; range, 50–200 nm; the particles covered an area of approximately 2 cm^2) on the left side in the paravertebral muscle of each rat, and followed for 6 or 8 months (Hansen et al., 2006). A 12-month observation period had been planned; however, one rat of this group died 8 months post-implantation, thus all remaining rats were killed at 8 months. There was no control group. Histological examination of the implantation sites showed a high incidence of malignant mesenchymal tumours (sarcomas, NOS) around nanoparticulate material implantation sites (tumour incidence, 5/6 at 8 months). Most tumours were localized intramuscularly (i.e. at the original implantation site). Malignant mesenchymal tumours (sarcomas) were not observed around the bulk material implantation sites (tumour incidence, 0/10). [The Working Group noted that the duration of exposure and observation was considered adequate. However, each rat was exposed to the two test agents concurrently, the number of animals was small, statistical analysis was not provided, only one sex was used, and the study did not include a control group.]

(d) Intrarenal injection

A group of 18 female Sprague-Dawley rats [number of rats at start unclear], weighing 120–140 g [age, not reported], were treated with a single intrarenal injection of 5 mg cobalt metal [powder] (reagent grade) [purity, not reported] suspended in 0.05 mL glycerin into each pole of the right kidney (Jasmin & Riopelle, 1976). In addition, a group of 16 female rats were treated with injections of 0.05 mL glycerin only into each pole of the kidney (controls). After 12 months, all rats were necropsied; no tumours were observed in the kidneys of cobalt-exposed or control rats. [The Working Group noted the use of only one dose and sex, the short duration of exposure and observation, and the small number of animals per group. Moreover, the Working Group noted that the authors did not mention the use of step sections in evaluating small kidney tumours.]

(e) Intrathoracic injection

Two groups of 10 female Hooded rats (age, 2–3 months) were treated with cobalt metal microparticles (spectrographically pure [no further details provided]; particle size: 400 mesh; 3.5 × 3.5 µm to 17 µm × 12 µm, with many long narrow particles of the order of 10 × 4 µm; suspended in serum [species, not reported]) at a dose of 28 mg by single intrathoracic injection through the right dome of the diaphragm (first group) or through the fourth left intercostal space (second group), and then observed for up to 28 months (Heath & Daniel, 1962). Within 3 days post-injection, 6/10 rats injected through the diaphragm and 2/10 rats injected through the intercostal space had died. The remaining rats in the group injected through the diaphragm

survived for between 11 and 28 months, and the group injected through the intercostal space survived for between 7.5 and 17.5 months.

Of the 12 rats that survived the injection, four rats (two in each group) developed intrathoracic sarcomas (three of mixed origin, including rhabdomyosarcomatous elements, and one rhabdomyosarcoma arising in the intercostal muscles). Three of these tumours appeared to have originated in the cardiac muscle, which was thoroughly intermingled with tumour cells. These three tumours also had certain histological features in common: all had myosarcomatous histopathology with occasional neoplastic giant cell transformation and haemangiosarcomatous changes (Heath & Daniel, 1962). [The Working Group noted the appropriate duration of the experiment and the malignant features of the tumours. However, the Working Group noted the lack of controls, the small number of animals per group, the low number of rats surviving the treatment, and the use of only one dose and sex. The study was considered inadequate for the evaluation of the carcinogenicity of cobalt metal in experimental animals.]

3.2 Soluble cobalt(II) salts

3.2.1 Cobalt(II) sulfate heptahydrate

(a) Mouse

Inhalation

In a well-conducted study that complied with GLP undertaken by the National Toxicology Program (NTP, 1998) and also reported by Bucher et al. (1999), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were exposed by inhalation (whole-body) to aqueous aerosols at concentrations of 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt(II) sulfate heptahydrate for controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, approximately 99%; MMAD, 1.5–1.6 μm; GSD, 2.3 μm) for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. No effects on survival were observed in exposed males or females compared with controls. At study termination, survival of male mice was 22/50, 31/50, 24/50, and 20/50, and for female mice was 34/50, 37/50, 32/50, and 28/50 for the controls and groups at the lowest, intermediate, and highest concentration, respectively. The mean body weights were significantly higher in all groups of females exposed to cobalt(II) sulfate heptahydrate from week 20 to 105 and lower in males at the highest concentration from week 96 to 105 compared with controls, but changes were within 10% of controls for both males and females. All mice underwent complete necropsy with histopathological evaluation.

In male mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (P = 0.005, life-table test; P = 0.018, logistic regression test; P = 0.029, Cochran–Armitage test). The incidence of bronchioloalveolar adenoma for controls and groups at the lowest, intermediate, and highest concentration was 9/50 (18%), 12/50 (24%), 13/50 (26%), and 18/50 (36%), respectively, and significantly increased at the highest concentration (P = 0.024, life-table test; P = 0.027, logistic regression test; P = 0.035, Fisher exact test). The incidence of bronchioloalveolar adenoma in the group at the highest concentration was at the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 141/947; 14.9 ± 7.0%; range, 6–36%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (P = 0.004, life-table test; P = 0.006, logistic regression test; P = 0.021, Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 4/50 (8%), 5/50 (10%), 7/50 (14%), and 11/50 (22%), respectively, and significantly increased at the highest concentration (P = 0.031, life-table test; P = 0.033, logistic regression test; P = 0.045, Fisher exact test), exceeding the upper bound of the range

observed in historical controls from this laboratory (inhalation studies: 75/947; 7.9 ± 5.7%; range, 0–16%). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$, life-table test, logistic regression test, and Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 11/50 (22%), 14/50 (28%), 19/50 (38%), and 28/50 (56%), respectively, and significantly increased at the highest concentration ($P < 0.001$, life-table test, logistic regression test, and Fisher exact test), exceeding the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 205/947; 21.7 ± 8.0%; range, 10–42%).

In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma ($P = 0.014$, life-table test; $P = 0.024$, logistic regression test; $P = 0.045$, Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 3/50 (6%), 6/50 (12%), 9/50 (18%), and 10/50 (20%), respectively, and significantly increased ($P = 0.016$, life-table test; $P = 0.024$, logistic regression test; $P = 0.036$, Fisher exact test) at the highest concentration. The incidence in the groups at the intermediate and highest concentrations also exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 61/939; 6.5 ± 3.2%; range, 0–14%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma ($P < 0.001$, life-table test, logistic regression test, and Cochran–Armitage test), with the incidence for controls and groups at the lowest, intermediate, and highest concentration being 1/50 (2%), 1/50 (2%), 4/50 (8%), and 9/50 (18%), respectively, and significantly increased ($P = 0.007$, life-table test; $P = 0.009$, logistic regression test; $P = 0.008$, Fisher exact test) at the highest concentration, which exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 38/939; 4.1 ± 3.2%; range, 0–12%). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.001$; life-table test, logistic regression test, and Cochran–Armitage test), with the increase in the incidence for controls and groups at the lowest, intermediate, and highest concentration being 4/50 (8%), 7/50 (14%), 13/50 (26%), and 18/50 (36%), respectively, and significantly increased at the highest ($P < 0.001$; life-table test, logistic regression test, and Fisher exact test) and intermediate concentrations ($P = 0.016$; life-table test, logistic regression test, and Fisher exact test). The incidence in the groups at the intermediate and highest concentrations also exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 97/939; 10.3 ± 3.7%; range, 0–16%).

Regarding non-neoplastic lesions, in all exposed groups of males and females, the incidence of cytoplasmic vacuolization of the bronchi was significantly higher than that in the control groups. The incidence of diffuse histiocytic cell infiltration in males and of focal histiocytic cell infiltration in females at the highest concentration was significantly greater than that in the controls. In addition, there was a significant increase in the incidence of non-neoplastic lesions of the nasal cavity and larynx in both treated males and females (NTP, 1998; Bucher et al., 1999). [The Working Group noted that this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, males and females were used, there was a sufficient number of animals per group, and multiple concentrations were tested.]

(b) Rat

Inhalation

In a well-conducted study that complied with GLP undertaken by the NTP (1998) and also reported by Bucher et al. (1999), groups of 50 male and 50 female Fischer 344/N rats (age,

6 weeks) were exposed by inhalation (whole-body) to aqueous aerosols of cobalt(II) sulfate heptahydrate at concentrations of 0, 0.3, 1.0, or 3.0 mg/m^3 for controls and groups at the lowest, intermediate, and highest concentration, respectively (purity, approximately 99%; MMAD, 1.4–1.6 μm; GSD, 2.1–2.2 μm) for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. No effects on survival or mean body weight were observed in exposed males or females compared with controls. At study termination, survival of male mice was 17/50, 15/50, 21/50, and 15/50, and for female mice was 28/50, 25/49, 26/50, and 30/50 for the controls and groups at the lowest, intermediate, and highest concentration, respectively. All rats underwent complete necropsy with histopathological evaluation.

In male rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (P = 0.042, life-table test). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 1/50 (2%), 4/50 (8%), 1/48 (2%), and 6/50 (12%), respectively. Incidence in the group at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 17/654; 2.6 ± 3.6%; range, 0–10%). The incidence of bronchioloalveolar carcinoma for the controls and groups at the lowest, intermediate, and highest concentration was 0/50, 0/50, 3/48 (6%), and 1/50 (2%), respectively. No statistically significant increase in the incidence of bronchioloalveolar carcinoma was observed; however, the incidence at the intermediate concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 6/654; 0.9 ± 1.0%; range, 0–2%). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P = 0.027, life-table test; P = 0.032, logistic regression test; P = 0.038, Cochran–Armitage test). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 1/50 (2%), 4/50 (8%), 4/48 (8%), and 7/50 (14%), respectively, and significantly increased (P = 0.030, life-table test; P = 0.029, logistic regression test; P = 0.030, Fisher exact test) at the highest concentration. The incidence of bronchioloalveolar adenoma or carcinoma (combined) at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 23/654; 3.5 ± 3.7%; range, 0–10%). The incidence of benign pheochromocytoma of the adrenal medulla for the controls and groups at the lowest, intermediate, and highest concentration was 14/50 (28%), 19/50 (38%), 23/49 (47%), and 20/50 (40%), respectively, and significantly increased (P = 0.041, Fisher exact test) at the intermediate concentration. The incidence of benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla for the controls and groups at the lowest, intermediate, and highest concentration was 15/50 (30%), 19/50 (38%), 25/49 (51%), and 20/50 (40%), respectively, and significantly increased (P < 0.05, logistic regression test, Fisher exact test) at the intermediate concentration. The incidence for the group at the intermediate concentration was also slightly above the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 176/623; 28.3 ± 12.0%; range, 8–50%). [The Working Group noted that complex pheochromocytoma is an adrenal medullary tumour, characterized histologically by a pheochromocytoma component and a coexisting, variably differentiated neural component composed of ganglioneuroma and/or neuroblastoma, nerve fibres, and Schwann cells (Martinez & Mog, 2001). The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only (see Patterson et al., 1995; Thompson, 2002). The Working Group also noted the absence of a concentration–response relationship, the significant increase only at the intermediate concentration, and the high background of these tumours,

and considered that this increase may or may not have been treatment-related.]

In female rats, there was a significant positive trend in the incidence of bronchioloalveolar adenoma (P = 0.003, life-table test; P = 0.001, logistic regression test; P = 0.002, Cochran–Armitage test), with the incidence for the controls and groups at the lowest, intermediate, and highest concentration being 0/50, 1/49 (2%), 10/50 (20%), and 9/50 (18%), respectively, and significantly increased at the intermediate (P < 0.001, life-table test, logistic regression test, Fisher exact test) and highest concentration (P = 0.003, life-table test, logistic regression test; P = 0.001, Fisher exact test). The incidence of bronchioloalveolar adenoma at the intermediate and highest concentrations exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 7/650; 1.1 ± 1.6%; range, 0–4%). There was a significant positive trend in the incidence of bronchioloalveolar carcinoma (P = 0.033, life-table test; P = 0.023, logistic regression test; P = 0.022, Cochran–Armitage test), with the incidence for the controls and groups at the lowest, intermediate, and highest concentration being 0/50, 2/49 (4%), 6/50 (12%), and 6/50 (12%), respectively, and significantly increased in the groups at the intermediate and highest concentrations (P < 0.05, life-table test, logistic regression test, Fisher exact test). The incidence of bronchioloalveolar carcinoma at all concentrations exceeded the incidence observed in historical controls from this laboratory (inhalation studies: 0/650). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) (P < 0.001; life-table test, logistic regression test, Cochran–Armitage test). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 0/50, 3/49 (6%), 15/50 (30%), and 15/50 (30%), respectively, with the increase in incidence being significant at the intermediate and highest concentrations (P < 0.001, life-table test, logistic regression test, Fisher exact test). The incidence of bronchioloalveolar adenoma or carcinoma (combined) at all concentrations exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 7/650; 1.1 ± 1.6%; range, 0–4%). There was a significant positive trend in the incidence of benign pheochromocytoma of the adrenal medulla (P = 0.006, life-table test; P = 0.004, logistic regression test; P = 0.003, Cochran–Armitage test), with the incidence for the controls and groups at the lowest, intermediate, and highest concentration being 2/48 (4%), 1/49 (2%), 3/50 (6%), and 8/48 (17%), respectively, and significantly increased (P < 0.05; logistic regression test, Fisher exact test) at the highest concentration. The incidence of benign pheochromocytoma of the adrenal medulla at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 35/608; 5.8 ± 4.9%; range, 0–14%). There was a significant positive trend (P ≤ 0.001, life-table test, logistic regression test, Cochran–Armitage test) in the incidence of benign, complex, or malignant pheochromocytoma of the adrenal medulla (combined). The incidence for the controls and groups at the lowest, intermediate, and highest concentration was 2/48 (4%), 1/49 (2%), 4/50 (8%), and 10/48 (21%), respectively, with the incidence being significantly increased (P < 0.02; life-table test, logistic regression test, Fisher exact test) at the highest concentration. The incidence of benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla at the highest concentration exceeded the upper bound of the range observed in historical controls from this laboratory (inhalation studies: 39/608; 6.4 ± 4.4%; range, 2–14%). [The Working Group noted that complex pheochromocytoma is an adrenal medullary tumour, characterized histologically by a pheochromocytoma component and a coexisting, variably differentiated neural component composed of ganglioneuroma and/or neuroblastoma, nerve fibres, and Schwann cells

(Martinez & Mog, 2001). The Working Group indicated that the differential diagnosis of malignant pheochromocytoma is difficult to assess on the basis of histomorphology only (see Patterson et al., 1995; Thompson, 2002).]

Regarding non-neoplastic lesions, in all exposed groups of male and female rats, the incidence of alveolar proteinosis, alveolar epithelial metaplasia, granulomatous alveolar changes, and interstitial fibrosis in the lung was significantly higher than in the controls. The incidence of hyperplasia of the adrenal medulla was not significantly increased in exposed males or females (NTP, 1998; also reported by Bucher et al., 1999). [The Working Group noted that this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, males and females were used, there was an adequate number of animals per group, and multiple concentrations were tested.]

3.2.2 Cobalt(II) chloride

(a) Rat

(i) Subcutaneous injection

In one study, in a first experiment, two groups of 20 male Wistar albino rats (age, about 4 weeks) were treated with cobalt(II) chloride [purity not given] at a dose of 0 (control) or 4 mg/100 g bw suspended in physiological saline by daily subcutaneous injection into the central abdominal wall, in two courses of 5 days separated by a 9-day interval, and then observed for about 12 months (Shabaan et al., 1977). Survival rates at the end of the 12th month were 19/20 and 11/20 for controls and treated rats, respectively. Surviving rats were killed after 12 months and only tumours that were visible macroscopically were assessed microscopically; however, rats that died during the experiment were not examined.

There was a significant increase [$P = 0.0016$, Fisher exact test] in the incidence (control, 0/20; treated, 8/20) of fibrosarcomas of the subcutaneous tissue. Some fibrosarcomas were pleomorphic in appearance with numerous mitoses, and one had the appearance of a fibromyxosarcoma. Four rats developed fibrosarcomas at a distance from the sites of injection; these tumours were more pleomorphic than those developing in the vicinity of the injection sites. There was no evidence of metastases in any of the rats. [The Working Group noted that the duration and schedule of exposure were considered adequate. However, the number of animals per group was small, the control group was also used for another experiment (see experiment below), only one sex and dose was used, the necropsy was limited to macroscopic lesions only, and statistical analysis was not reported.]

In the same study by Shabaan et al. (1977), in a second and concurrent experiment, two groups of 20 male Wistar albino rats (age, about 4 weeks) were treated with cobalt(II) chloride [purity not given] at doses of 0 (control) [same control group as the first experiment] or 4 mg/100 g bw suspended in physiological saline by daily subcutaneous injection into the central abdominal wall, in 2 courses of 5 days separated by a 9-day interval, and then observed for about 8 months. Survival rates at the end of the 8-month experiment were 20/20 and 16/20 for controls and treated rats, respectively. Surviving treated rats were killed after 8 months and only tumours that were visible macroscopically were assessed microscopically; however, rats that died during the experiment were not examined. There was a significant increase [$P = 0.010$, Fisher exact test] in the incidence (control, 0/20; treated, 6/20) of tumours [reported to be of the same nature as those described in the first experiment, i.e. fibrosarcomas of the subcutaneous tissue]. [The Working Group noted that the duration and the schedule of exposure were considered adequate. However, the number of animals per group was small, the control group was also used for another experiment (see experiment above), only one sex and dose was used, the necropsy was limited to

macroscopic lesions only, and statistical analysis was not reported.]

(ii) Co-carcinogenicity

In a study by Zeller (1975), Wistar rats (age, 3 months) were divided into groups, each containing 12 males and 12 females. Two groups received diethylnitrosamine (DEN) at a dose of 5 mg/kg bw injected subcutaneously under the dorsal skin once per week for 43 weeks. One of these two groups also received cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) [no further details reported] at a dose of 0.5 mg/kg bw. The total dose of DEN was 215 mg/kg bw in all groups, and that of cobalt chloride was 21.5 mg/kg bw. A control group of 12 rats [sex distribution, not reported] was treated with cobalt chloride alone at the same dose by subcutaneous injection. All rats were observed until their natural death [but no surviIal information between groups was reported]. No tumours developed in the group treated with cobalt chloride alone (control). No local tumours were observed in either DEN-treated group. The incidence of tumours of the respiratory tract (nasal cavity) was approximately twice the incidence of tumours of the liver in both DEN-treated groups. There was no significant difference in the incidence of respiratory tract tumours or in the incidence of liver tumours between DEN-treated groups. [The Working Group noted that only one dose was tested, no statistical analysis was performed, data were combined for males and females, the small number of animals per group, no untreated control group was used, and limited reporting.]

Ivankovic et al. (1972) investigated whether the carcinogenicity of ethylnitrosourea (ENU) in rats could be significantly increased by metal ions, including Co^{2+} (from cobalt(II) chloride). In a first experiment, adult BD strain rats [age at start and sex not reported] were treated with ENU at a dose of 25–90 mg/kg bw with copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) or cobalt(II) chloride hexahydrate at doses of 5–10 mg/kg bw [purity, not reported; group size unclear; no further details provided] by single intraperitoneal injection. After a mean induction time of 190 days, local sarcomas were observed in the abdominal cavity of 6/20 rats. In the control groups (exposed to the same dose of metal salt only or of ENU only) [no further details on these controls provided], no such lesions were reported. In a second experiment, rats were treated with ENU at a dose of 10 mg/kg bw per week with cobalt chloride at 1 mg/kg bw or copper sulfate at 1 mg/kg bw [age at start and sex, not reported; purity, not reported; group size unclear] by single subcutaneous injection. [The Working Group noted the small number of animals, the very limited reporting of the study including the unspecified number of control animals, the absence of details on group size and dosing regimen, and the lack of survival and body-weight data. The study was considered inadequate for the evaluation of the carcinogenicity of cobalt chloride in experimental animals.]

Three groups of 70–72 female CBA × C57BL mice were treated with 0.5% 20-methylcholanthrene [a carcinogen] in a volume of 0.02 mL of benzene by application to a shaved area of the skin (in the interscapular region) once per week until the end of the study [presumably for life] (Finogenova, 1973). Mice were treated with cobalt(II) chloride hexahydrate at a dose of 0, 10, or 100 µg (in 0.1 mL saline) for the controls and groups at the lower and higher dose, respectively, by intraperitoneal injection, twice per week for 8 weeks. The first injection was administered on the same day as the 20-methylcholanthrene application, subsequent injections were administered 3 days later. [The Working Group noted that an adequate number of animals per group and multiple doses were used. However, this study used benzene (classified by IARC as *carcinogenic to humans*, Group 1) as a vehicle, had a short duration, used only one sex, and did not report on tumour incidence or multiplicity, but only on tumour latency. The study

was considered inadequate for the evaluation of the carcinogenicity of cobalt chloride in experimental animals.]

3.2.3 Cobalt(II) nitrate

Rabbit

Subcutaneous injection

Two female rabbits [not further specified] (age, at least 9 months) were treated with cobalt(II) nitrate (equivalent to 1 mg of cobalt metal) in 10 mL of saline (9 g/L) or cobalt(II) nitrate (equivalent to 1 mg cobalt metal) in 10 mL of purified deproteinized amniotic fluid by subcutaneous injection [injection site, not reported] once per day for 5 days (Thomas & Thiery, 1953). From the start of the injections, there was a dramatic decrease in body weight. The rabbits were killed after 37 days (cobalt nitrate in saline) and 22 days (cobalt nitrate in amniotic fluid). [The Working Group noted the limited reporting, the very small number of animals, the use of a single dose, and the lack of controls. The study was considered inadequate for the evaluation of the carcinogenicity of cobalt nitrate in experimental animals.]

3.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

3.3.1 Cobalt(II) oxide

(a) Mouse

Intramuscular injection

A group of 50 female Swiss mice (age, 2–3 months) was treated with cobalt(II) oxide [purity not reported] at a dose of 10 mg per site, as a 10% suspension in aqueous penicillin G procaine, by single intramuscular injection into each thigh muscle using a 22-gauge needle (Gilman & Ruckerbauer, 1962; Gilman, 1962). The particles of cobalt oxide used were milled to a size of 5 μm or less by means of a laboratory pebble mill followed by air elutriation. A vehicle control group of 51 female Swiss mice (age, 2–3 months) was treated with aqueous penicillin G procaine (60 000 IU) alone by single intramuscular injection into both thigh muscles. The experiment was terminated 751 days post-injection and after 772 days for vehicle controls. Of the treated mice, 12 survived (12/50) until day-751, and no tumours were observed at the sites of injection in any of the 46 effective mice (those surviving 90 days post-injection) in the cobalt oxide-exposed group, but two pulmonary adenomas were found. [Neither microphotographs nor histopathology descriptions were provided.] No tumours were observed at the injection sites in 48 effective vehicle controls [survival not reported], 772 days post-injection. [The Working Group noted the duration of observation and the number of animals used per group were adequate, but only one sex and dose was used.]

(b) Rat

(i) Intratracheal instillation

Four groups each of 50 male and 50 female Sprague-Dawley rats (age, 10 weeks) were treated with cobalt(II) oxide microparticles (76.7% cobalt; purity, "chemically pure"; ~80% of particles in the 5–40 μm range) in physiological saline (sodium chloride) at doses of 0, 0, 2, and 10 mg/kg bw for untreated controls, vehicle controls (physiological saline only), and groups at the lower and higher dose, respectively, by intratracheal instillation once every 2 weeks for the first 18 treatments, and then every 4 weeks from the 19th to 39th treatments over 2 years (Steinhoff & Mohr, 1991). The rats were allowed to live until natural death or were killed when moribund. They underwent full autopsy and organs and tissues were histologically examined. Regarding body weight and survival, there were no appreciable differences between rats in the groups treated with cobalt oxide and the controls.

In male rats, there was a significant positive trend in the incidence (untreated, 0/50; vehicle, 0/50; lower dose, 1/50; higher dose, 5/50) of all tumours of the lung combined [P = 0.047, Cochran–Armitage test]. The incidence of all tumours of the lung combined at the higher dose (5/50: bronchioloalveolar adenoma, 2/50; bronchioloalveolar adenocarcinoma, 1/50; adenocarcinoma, 2/50) [P = 0.0281, Fisher exact test] was significantly higher than that in the controls (1/50, benign squamous epithelial tumour at the lower dose). In the treated groups, the incidence of bronchioloalveolar adenoma, of bronchioloalveolar adenocarcinoma, of adenocarcinoma of the lung, or of benign squamous epithelial tumours of the lung was not significantly higher than that in the control groups.

In female rats, the incidence of bronchioloalveolar adenoma and of bronchioloalveolar carcinoma was not significantly higher than that in the controls. [The Working Group noted that an adequate number of rats per group was used, rats were randomly allocated in groups, the duration of exposure and observation was adequate, the schedule of exposure was adequate, multiple doses were tested, and males and females were used. The Working Group also noted that the particle size was potentially not appropriate for this route of exposure, and that no statistical analysis was performed.]

(ii) Subcutaneous injection

In two concurrent studies using groups of 10 male Sprague-Dawley rats (age, 10 weeks), two groups treated with cobalt(II) oxide microparticles (76.7% cobalt; purity, "chemically pure"; ~80% of particles in the 5–40 μm range) in physiological saline (sodium chloride, 1 mL/kg bw per treatment) at doses of 0 (control) or 2 mg/kg bw by subcutaneous injection, 5 days/week for 2 years; two other groups were given the same type of cobalt(II) oxide in physiological saline at doses of 0 (control) or 10 mg/kg bw by subcutaneous injection, once per week for 2 years (Steinhoff & Mohr, 1991). The rats were allowed to live until natural death or were killed when moribund.

In the first study, the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site was significantly higher [P = 0.0163, Fisher exact test] in the group of rats treated with 2 mg/kg bw compared with controls (5/10 and 0/10, respectively). In the second study, there was no significant difference between the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site between the group of rats treated with 10 mg/kg bw and controls (4/10 and 0/10, respectively) (Steinhoff & Mohr, 1991). [The Working Group noted that the duration of exposure and observation was adequate, and the schedule of exposure was adequate. However, the number of animals per group was small, only one sex and dose was used, survival and body-weight data were not included, and no statistical analysis was performed.]

(iii) Intramuscular injection

A group of 10 male or female Wistar rats [sex distribution, not reported] (age, 2–3 months) was treated with cobalt(II) oxide powder (particle size up to 5 μm) [purity, not reported] at a dose of 30 mg as a 10% suspension in an aqueous suspension of penicillin G procaine (90 000 IU) by single intramuscular injection per thigh (Gilman & Ruckerbauer, 1962). A control group of 10 male or female rats [sex distribution, not reported] received single injections of aqueous penicillin G procaine alone. [Survival of both the control and treated rats was not specified.] Control and treated rats were observed for up to 519 and 489 days, respectively.

Cobalt(II) oxide caused a significant [P = 0.0163, Fisher exact test] increase in the incidence of rhabdomyosarcoma (control, 0/10; treated, 5/10) in male and female (combined) Wistar rats compared with controls. All five tumours occurring at the site of cobalt oxide injection appeared to be of muscle cell origin. Metastases involving the lymph nodes and lung

occurred in four of these five cases. No tumours were observed at the sites of injection in any of the controls (Gilman & Ruckerbauer, 1962). [The Working Group noted that the duration of exposure was adequate. However, the number of animals per group was small, data for males and females were combined, a single dose was tested, there was limited reporting of the study, and statistical analysis was not performed.]

A group of 32 male or female Wistar rats (age, 2–3 months) [sex distribution, not reported] were treated with cobalt(II) oxide powder [purity not reported] (particle size up to 5 μm) at a dose of 20 mg per thigh by intramuscular injection, and then observed for up to 342 days (Gilman, 1962). No information was given on control groups. The effective number of rats (surviving at least 90 days after the start of the treatment) was 24 in the treated group; 5 rats in the treated group were injected in both thighs, and 19 in one thigh only, because of toxicity [no further details provided]. Five treated rats survived to the end of the experiment.

Cobalt oxide-induced sarcomas (mostly rhabdomyofibrosarcomas) were observed at the injection site, with an incidence of 12/24 for treated males and females combined. Most tumours in the cobalt oxide-treated group were pleomorphic, highly cellular sarcomas of striated muscle origin, showing frequent metastasis to the lung and lymph nodes. Both regional and distant metastases occurred in 25% of the rats in the treated group. In all cases, the primary tumours, once established, tended to grow rather rapidly and often to a very large size around the site of injection (Gilman, 1962). [The Working Group noted that the duration of exposure was adequate, a sufficient number of animals was used and randomly allocated in groups, and males and females were used. However, not all animals were necropsied, histopathological confirmation was not consistently performed, data for males and females were combined, a single dose was tested, and there was no control group.]

(iv) Intraperitoneal injection

Two groups each of 10 male and 10 female Sprague-Dawley rats (age, 10 weeks) were treated with cobalt(II) oxide microparticles (76.7% cobalt; purity, "chemically pure"; ~80% of particles in the 5–40 μm range) at doses of 0 (controls, received saline only) or 200 mg/kg bw suspended in physiological saline by intraperitoneal injection, three times at intervals of 2 months, and then followed by lifetime observation (Steinhoff & Mohr, 1991).

The incidence of malignant histiocytoma at the injection sites of treated males and females combined was significantly higher [$P = 0.0017$, Fisher exact test] than that in the controls (control, 1/20; treated, 10/20); however, the incidence of sarcoma was not significantly higher than that in the controls (control, 0/20; treated, 3/20), nor was the incidence of malignant mesothelioma (control, 0/20; treated, 1/20). Total tumour incidence at the injection site (control, 1/20; treated, 14/20) was significantly increased [$P < 0.0001$, Fisher exact test] (Steinhoff & Mohr, 1991). [The Working Group noted that the duration of exposure and observation was adequate as was the exposure schedule. However, the number of animals per group was small, survival and body-weight data were not included, data for males and females were combined, and statistical analysis was not performed.]

(v) Co-carcinogenicity

A group of 20 female Sprague-Dawley rats (age, 10 weeks) were treated with alternating treatments of: (a) cobalt(II) oxide microparticles (76.7% cobalt; "chemically pure" [purity, not reported]; ~80% of particles in the 5–40 μm range) in physiological saline at an initial dose of 10 mg/kg bw once per week and starting from the 8th treatment at a dose of 20 mg/kg bw every 14 days (total of 27 treatments, total dose of 470 mg/kg bw); and (b) benzo[*a*]pyrene in physiological saline at a dose of 20 mg/kg bw once per week and starting from the 8th treatment

at the same dose every 14 days (total of 10 treatments, total dose of 200 mg/kg bw) by intratracheal instillation (Steinhoff & Mohr, 1991). The alternating period between treatments with the two compounds was 4 days. A control group of 20 female rats was given similar treatment with benzo[*a*]pyrene and physiological saline. The rats were allowed to live until natural death or were killed when moribund.

The incidence of pulmonary carcinoma in the group treated with cobalt oxide plus benzo[*a*]-pyrene was significantly higher [$P = 0.0042$, Fisher exact test] than the control group treated with benzo[*a*]pyrene only (control, 1/20; treated, 9/20). The incidence of squamous cell carcinoma of the lung (control, 1/20; treated, 8/20) was significantly higher [$P = 0.0098$, Fisher exact test] in the treated group than in the controls. One adenocarcinoma of the lung was observed in the treated group, but none were observed in controls (0/20, 1/20) (Steinhoff & Mohr, 1991). [The Working Group noted that the duration of exposure and observation was adequate as was the exposure schedule. The Working Group also noted that only one sex and dose was used, a small number of animals per group, survival and body-weight data were not included, and that the particle size was potentially not appropriate for this route of exposure]

(c) Hamster

Inhalation and co-carcinogenicity

In a study investigating full carcinogenicity, groups of 51 male Syrian golden hamsters (ENG:ELA strain) (age, 2 months) were exposed by inhalation (whole-body) to cobalt(II) oxide respirable aerosol (with a MMAD of 0.45 μm) [purity, unspecified, reported as "purified"] concentrations of 0 (control) or 10 mg/m^3 for 7 hours per day, 5 days per week, for life (Wehner et al., 1977). Median survival of treated hamsters was 16.6 months compared with 15.3 months for controls. No significant difference in the incidence of any tumour type was observed between the treated hamsters and controls.

In a concomitant co-carcinogenicity experiment using a similar study design, two groups of 51 male Syrian golden hamsters (age, 2 months) were exposed by inhalation to cigarette smoke (3 times per day for 10 minutes, nose-only exposure) (controls) or to cigarette smoke plus cobalt oxide aerosol (10 mg/m^3) for life. There was no significant difference in the incidence of any tumours, including lung tumours, between the group of hamsters exposed to cigarette smoke plus cobalt oxide and the controls. [The Working Group noted the limited reporting of the study, the poor survival of the groups of cobalt- exposed hamsters and controls, that only one dose was tested, and the use of one sex only.]

3.3.2 Cobalt(II) sulfide

Rat

(i) Intramuscular injection

A group of 30 male and female Wistar rats [sex distribution, not reported] (age, 2–3 months) was treated with cobalt(II) sulfide [purity, not given] (particle size up to 5 μm) at a dose of 20 mg per thigh by single intramuscular injection, and then observed for 365 days (Gilman, 1962). No information was given on control groups. The effective number of rats (those surviving at least 90 days after the start of the treatment) was 29; nearly all rats ($n = 27$) were treated in both the left and right thigh, but two rats received one dose only. Only one treated rat survived until the end of the experiment.

Sarcomas (mostly rhabdomyofibrosarcomas) were induced at the injection site in treated rats; tumour incidence was 28/29 for treated males and females combined. Several of the tumours examined were highly anaplastic and difficult to categorize; however, the predominant histological type was a striated muscle tumour. Both regional and distant metastases occurred in

55% of rats in the treated group. In all cases, the primary tumours, once established, tended to grow rather rapidly and often to a very large size around the site of injection (Gilman, 1962). [The Working Group noted that the duration of exposure was adequate, a sufficient number of animals was used and randomly allocated in groups, and both males and females were used. However, not all rats were necropsied, histopathological confirmation was not consistently performed, data for males and females were combined, a single dose was tested, and there was no control group.]

(ii) Intrarenal injection

A group of 20 female Sprague-Dawley rats [number of rats at start unclear], weighing 120–140 g [age, not reported] treated with cobalt(II) sulfide (reagent grade) [purity and particle size, not reported] at a dose of 5 mg suspended in 0.05 mL glycerin by single intrarenal injection into each pole of the right kidney. A group of 16 female rats receiving injections of 0.05 mL of glycerin alone into each pole of the kidney served as controls. After 12 months, all rats were necropsied; no tumours were observed in the kidneys of treated or control rats (Jasmin & Riopelle, 1976). [The Working Group noted the use of only one dose and sex, and the small number of animals per group. Moreover, the Working Group noted that the authors did not mention the use of step sections in evaluating small kidney tumours.]

3.3.3 Cobalt(II,III) oxide

Hamster

Intratracheal instillation and initiation–promotion

In an experiment to study the effects of particulates on carcinogenesis in the respiratory tract induced by DEN, two groups of 50 (25 males and 25 females) hamsters [strain, not reported] (age, 7 weeks), were treated with DEN at a dose of 0 (controls) or 0.5 mg in 0.25 mL of saline by subcutaneous injection once per week for 12 weeks (total DEN dose, 6 mg) (Farrell & Davis, 1974). One week after the last injection of DEN, both groups were treated with cobalt(II,III) oxide powder (particle size, 0.5–1.0 μm [purity, not reported]) at a dose of 4 mg suspended in 0.2 mL of 0.5% gelatin in saline by intratracheal instillation, once per week [assumed] for 30 weeks. Two other groups of 50 (25 males and 25 females) hamsters, treated with either DEN or saline by subcutaneous injection, plus gelatin-saline by intratracheal instillation, served as controls. At the end of treatment (at week 42), there were 39, 33, 43, and 43 hamsters [male and female combined, sex distribution not reported] still alive in the groups exposed to DEN plus cobalt oxide, DEN plus gelatin-saline, saline plus cobalt oxide, or saline plus gelatin-saline, respectively. The hamsters were then observed for an additional 43–68 weeks (total study duration, up to 110 weeks) after the last intratracheal instillation.

In the initiation–promotion study, the incidence of respiratory tract tumours at various sites in hamsters treated with DEN plus cobalt oxide was not significantly different from that in hamsters receiving DEN plus gelatin-saline. In the group treated by subcutaneous injections of saline plus intratracheal instillations of cobalt oxide, 2/48 hamsters [sex distribution not reported] developed pulmonary alveolar tumours, although this increase was not statistically significant. No such tumours were observed in the 44 control hamsters in the group given subcutaneous injections of saline plus intratracheal instillations of gelatin-saline (Farrell & Davis, 1974). [The Working Group noted that data were combined for males and females, only one dose was tested, statistical analysis was not performed, and that histopathological examination was limited to the respiratory tract.]

3.4 Other cobalt(II) compounds

No data were available to the Working Group.

3.5 Evidence synthesis for cancer in experimental animals

3.5.1 Cobalt metal

In a well-conducted study on inhalation (whole-body exposure) of cobalt metal microparticles in male and female B6C3F$_1$/N mice that complied with GLP, there was a significant increase in the incidence, with a significant positive trend, of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined) in males and females (NTP, 2014).

In a well-conducted study on inhalation (whole-body exposure) of cobalt metal microparticles in male and female Fischer 344/NTac rats that complied with GLP, there was a significant increase in the incidence, with a significant positive trend, of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), benign pheochromocytoma of the adrenal medulla, malignant pheochromocytoma of the adrenal medulla, and benign or malignant pheochromocytoma (combined) of the adrenal medulla in males and females. In males, there was a significant increase in the incidence, with a significant positive trend, of pancreatic islet adenoma or carcinoma (combined), a significant increase in the incidence of pancreatic islet adenoma, and a significant positive trend in the incidence of pancreatic islet carcinoma and renal tubule adenoma or carcinoma (combined). In females, there was a significant increase in the incidence of mononuclear cell leukaemia (NTP, 2014).

In one study in male and female Hooded rats treated with cobalt metal microparticles by intramuscular injection, there was a significant increase in the incidence of rhabdomyofibrosarcoma or sarcoma (NOS) (combined) at the injection site in males, and of fibrosarcoma and rhabdomyosarcoma or rhabdomyofibrosarcoma (combined) at the injection site in females (Heath, 1956). In another study in female Hooded rats treated with cobalt metal microparticles by intramuscular injection, there was a significant increase in the incidence of rhabdomyofibrosarcoma and fibrosarcoma or sarcoma (NOS) (combined) at the injection site (Heath, 1956).

In one study in male Sprague-Dawley rats treated with cobalt metal microparticles by subcutaneous implantation, there was no occurrence of tumours at the implantation site (Hansen et al., 2006).

In a study in male Sprague-Dawley rats treated with cobalt metal nanoparticles by intramuscular implantation, there was a high incidence of sarcomas (NOS) at the implantation site [but there was a lack of concurrent controls] (Hansen et al., 2006). In a study in male Sprague-Dawley rats treated with cobalt metal pellets by intramuscular implantation, there was a significant increase in the incidence of rhabdomyosarcoma or spindle cell tumours (NOS) (combined) of the limb gastrocnemius muscle (Wen et al., 2020).

In one study in female Sprague-Dawley rats treated with cobalt metal by intrarenal injection (Jasmin & Riopelle, 1976), there was no significant increase in the incidence of tumours.

One study in Hooded rats treated with cobalt metal microparticles by intrathoracic injection (Heath & Daniel, 1962) and one study where cobalt metal microparticles were administered by intramuscular injection (Heath, 1960) were judged to be inadequate for the evaluation of the carcinogenicity of cobalt metal in experimental animals.

3.5.2 Soluble cobalt(II) salts

(a) Cobalt(II) sulfate heptahydrate

In a well-conducted study on inhalation (whole-body exposure) of cobalt(II) sulfate heptahydrate aerosols in male and female B6C3F$_1$ mice that complied with GLP, there was a significant increase in the incidence, with a significant positive trend, of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined) in males and females (NTP, 1998).

In a well-conducted study on inhalation (whole-body exposure) of cobalt(II) sulfate heptahydrate aerosols in male and female Fischer 344/NTac rats that complied with GLP, there was a significant positive trend in the incidence of bronchioloalveolar adenoma, a significant increase in the incidence – with a significant positive trend – of bronchioloalveolar adenoma or carcinoma (combined), and a significant increase in the incidence of benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla in males. In females, there was a significant increase in the incidence – with a significant positive trend – of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), benign pheochromocytoma of the adrenal medulla, and benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla (NTP, 1998).

(b) Cobalt(II) chloride

In a study that included two experiments involving male Wistar rats treated with cobalt(II) chloride by subcutaneous injection, there was a significant increase in the incidence of fibrosarcoma of the subcutaneous tissue in both experiments (Shabaan et al., 1977).

In a study of co-carcinogenicity of cobalt(II) chloride (administered by subcutaneous injection) in male and female Wistar rats (Zeller, 1975), there was no significant increase in the incidence of tumours in males and females combined.

Two studies of co-carcinogenicity, in which cobalt(II) chloride was administered to BD strain rats (Ivankovic et al., 1972) or to female CBA × C57BL mice (Finogenova, 1973) by intraperitoneal injection, were judged to be inadequate for the evaluation of the carcinogenicity of cobalt(II) chloride in experimental animals.

(c) Cobalt(II) nitrate

One study in rabbits treated with cobalt(II) nitrate by subcutaneous injection (Thomas & Thiery, 1953) was judged to be inadequate for the evaluation of the carcinogenicity of cobalt(II) nitrate in experimental animals.

3.5.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

(a) Cobalt(II) oxide

In a study in male and female Sprague-Dawley rats treated with cobalt(II) oxide microparticles by intratracheal instillation, there was a significant increase in the incidence of bronchioloalveolar adenoma, bronchioloalveolar adenocarcinoma, or adenocarcinoma (combined) of the lung in males, with a significant positive trend (Steinhoff & Mohr, 1991).

In two concurrent studies in male Sprague-Dawley rats treated with cobalt(II) oxide microparticles by subcutaneous injection, there was a significant increase in the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site in one study (Steinhoff & Mohr, 1991).

In two studies in male and female Wistar rats treated with cobalt(II) oxide microparticles by intramuscular injection, in one study there was a significant increase in the incidence of rhabdomyosarcoma at the injection site in males and females combined (Gilman & Ruckerbauer, 1962). In the other study there was a high incidence of sarcoma (mostly rhabdomyofibrosarcoma) at the

injection site in males and females combined [but there was a lack of concurrent controls] (Gilman, 1962).

In a study in male and female Sprague-Dawley rats treated with cobalt(II) oxide microparticles by intraperitoneal injection, there was a significant increase in the incidence of malignant histiocytoma, sarcoma (NOS), or malignant mesothelioma (combined), and of malignant histiocytoma (both at the injection site) in males and females combined (Steinhoff & Mohr, 1991).

In a study in Swiss mice treated with cobalt(II) oxide microparticles by intramuscular injection (Gilman, 1962; Gilman & Ruckerbauer, 1962), there was no significant increase in the incidence of tumours.

In a study of co-carcinogenicity in female Sprague-Dawley rats treated with cobalt(II) oxide microparticles with benzo[*a*]pyrene by intratracheal instillation (Steinhoff & Mohr, 1991), there was a significant increase in the incidence of malignant lung tumours (total) and squamous cell carcinoma of the lung compared with benzo[*a*]pyrene treatment only. In a study of full carcinogenicity and another concomitant study of co-carcinogenicity involving cobalt(II) oxide inhalation (whole-body exposure) in male Syrian golden hamsters, there was no significant increase in the incidence of tumours (Wehner et al., 1977).

(b) Cobalt(II) sulfide

In a study in male and female Wistar rats (combined) treated with cobalt(II) sulfide microparticles by intramuscular injection, there was a high incidence of sarcomas (mostly rhabdomyofibrosarcoma) at the injection site in males and females combined [but there was a lack of concurrent controls] (Gilman, 1962).

In a study in female Sprague-Dawley rats treated with cobalt(II) sulfide by intrarenal injection (Jasmin & Riopelle, 1976), there was no significant increase in the incidence of tumours.

(c) Cobalt(II,III) oxide

In studies on intratracheal instillation and initiation–promotion in male and female hamsters treated with cobalt(II,III) oxide, there was no significant increase in the incidence of tumours (Farrell & Davis, 1974).

3.5.4 Other cobalt(II) compounds

No data were available to the Working Group.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

[The Working Group noted that there is a paucity of information available on the pharmacokinetics of the individual forms of cobalt considered in the present monograph.]

(a) Absorption

In a study of four volunteers by Foster et al. (1989), the average fractional depositions of inhaled radiolabelled (^{57}Co) cobalt(II,III) oxide particles with geometric mean diameters of 0.8 and 1.7 μm in the lung were 52% and 78%, respectively. [The Working Group noted that these data were also reported in Bailey et al. (1989).]

Cobalt and tungsten concentrations measured in exhaled breath condensates from workers involved in the production of diamond tools or hard-metal inserts, collected post-shift, were higher than those in condensate samples collected before the beginning of the work shift (Goldoni et al., 2004). Cobalt concentration measured in samples of urine collected from workers is also a surrogate indicator of cobalt lung absorption. Workers exposed to cobalt metal or cobalt oxide have increased urine cobalt concentrations after

conclusion of their work shifts, suggesting that lung absorption and systemic delivery occurred (Apostoli et al., 1994; Fujio et al., 2009; Martin et al., 2010; Princivalle et al., 2017). [The Working Group noted that this finding could also result from non-inhalation occupational exposure.] Other studies suggest that absorption of cobalt from the lungs is dependent on the solubility of the cobalt compound. Soluble forms of cobalt are more readily absorbed than insoluble forms (Christensen & Poulsen, 1994; Lison et al., 1994).

Large interindividual variability for absorption rates in humans has been reported after ingestion. Studies using radiolabelled cobalt(II) chloride indicate that gastrointestinal absorption of cobalt in humans varies from 5% to 45% (Paley & Sussman, 1963; Valberg et al., 1969; Sorbie et al., 1971; Smith et al., 1972; Holstein et al., 2015; Tvermoes et al., 2015). The fraction of ingested cobalt that is absorbed from the gastrointestinal tract depends on an individual's nutritional status, and the dose and form of cobalt to which they are exposed. Overnight fasting and iron deficiency increase cobalt absorption (Valberg et al., 1969; Sorbie et al., 1971; Smith et al., 1972). Serum ferritin levels were inversely correlated with blood cobalt concentrations in some studies (Bárány et al., 2005; Meltzer et al., 2010). Gastrointestinal uptake of soluble cobalt(II) chloride was higher than that seen after ingestion of cobalt oxide, a less soluble form of cobalt (Christensen et al., 1993). Gastrointestinal uptake of cobalt is higher in women than men (Christensen et al., 1993; Tvermoes et al., 2014). Uptake of carrier-loaded inorganic cobalt (cobalt(II) chloride) is lower than for carrier-free cobalt (Holstein et al., 2015).

Dermal absorption of cobalt was assessed experimentally in four participants who exposed one hand to either freshly mixed powder (5–15% cobalt) or waste dry powder from a hard-metal production facility for 90 minutes. Increased urinary cobalt concentrations were seen post-exposure with both cobalt sources (Scansetti et al., 1994). A cohort of five volunteers placed their hands in a used coolant that contained cobalt at 1600 mg/L for 1 hour. Urinary excretion of cobalt averaged 18.1 nmol (range, 6.8–34.3 nmol) during the 24-hour period before exposure and increased to 38.5 nmol (range, 14.2–61.4 nmol) in the 24-hour period after exposure. One participant had no apparent increase in urinary cobalt concentration after exposure (Linnainmaa & Kiilunen, 1997). [The Working Group noted that both studies provided limited information concerning the participants and forms of cobalt used.]

Occupational studies examining dermal absorption have focused on workers in hard-metal production facilities. [The Working Group noted that these studies can be informative regarding the distribution of cobalt after absorption from the skin.] These studies show associations between exposure to cobalt on the skin and concentrations in blood or urine (Scansetti et al., 1994; Klasson et al., 2017; Kettelarij et al., 2018a; Wahlqvist et al., 2020). Exposure to cobalt on the skin was statistically associated with levels of cobalt in the blood but not in the urine (Wahlqvist et al., 2020). Linear regression analysis of blood concentrations in an occupational cohort demonstrated significant positive relationships with cobalt skin concentrations and with cobalt air concentrations (Klasson et al., 2017). Increased cobalt skin concentrations were associated with increased cobalt concentrations in the blood or urine, suggesting that systemic absorption of cobalt occurred (Klasson et al., 2017; Kettelarij et al., 2018a).

(b) Distribution

Cobalt is broadly distributed throughout the human body. Cobalt is distributed to the serum, whole blood, liver, lung, kidney, heart, and spleen, with lower amounts reported in the skeleton, hair, lymphatic circulation, and pancreas (Gerhardsson et al., 1984; Collecchi et al., 1986; Ishihara et al., 1987; Hewitt, 1988; Muramatsu &

Parr, 1988; Takemoto et al., 1991). [The Working Group noted that autopsy specimens collected from workers or individuals with cancer or other disease states have been used to assess the distribution of cobalt in humans.] Experimental studies involving intravenous administration of radiolabelled cobalt(II) chloride to volunteers have shown that the liver accumulates approximately 10–50% of the body burden of this metal (Smith et al., 1972; Jansen et al., 1996).

(c) Metabolism

Metabolism of cobalt consists of the formation of complexes with a variety of protein and non-protein ligands (see Section 4.2.1). Cobalt is not subject to direct metabolism by enzymatic pathways but gets distributed or excreted as the parent compound.

(d) Excretion

Studies in humans given cobalt chloride by intravenous injection have shown that most excreted cobalt is found in urine and faeces (Kent & McCance, 1941; Smith et al., 1972; Curtis et al., 1976). Similar excretion patterns are found after ingestion. For example, Sorbie et al. (1971) reported that 9–23% of an orally administered dose of radiolabelled cobalt(II) chloride was excreted in the urine within 24 hours of administration. Increased urinary cobalt excretion (23%–42%) was seen in individuals with iron deficiency (Sorbie et al., 1971). Aerosol exposure of human volunteers to radiolabelled cobalt oxide revealed that approximately 40% of the initial lung burden of inhaled cobalt was retained for 6 months after exposure. Cumulative elimination of 33% of the initial lung burden was found in the urine and 28% was found in the faeces (Foster et al., 1989).

Half-lives of elimination have also been estimated in several other studies. Finley et al. (2013) studied cobalt pharmacokinetics in volunteers who ingested cobalt at approximately 1000 µg per day (10–19 µg/kg per day) as cobalt(II) chloride for 31 days. Steady-state whole-blood and erythrocyte cobalt concentrations were achieved within 14–24 days. Clearance of cobalt from blood and serum followed a two-phase exponential decay curve, with an initial rapid phase followed by a slower second phase. The fast-phase half-life was 3 days. For the slower phase, the half-life was 16 days for serum and 39 days for whole blood, with 23% of the cobalt found in serum and 39% found in whole blood (Finley et al., 2013). Tvermoes et al. (2014) also reported that cobalt elimination from whole blood and serum also followed a two-phase exponential decay curve in volunteers ingesting 1 mg per day for 90 days. Elimination from erythrocytes correlated with their lifespan of 120 days.

(e) In vitro studies

Several relevant in vitro studies using human tissues were also identified. Absorption of powdered cobalt, suspended in artificial sweat, by human abdominal skin has been assessed in vitro (Filon et al., 2004, 2009).

Several studies have examined the dissolution of cobalt in vitro. Percutaneous permeation through intact skin was approximately 0.01 µg/cm^2 per hour with an average lag time of 1.6 hours, and was increased in abraded skin (Filon et al., 2009). Studies suggest that lung clearance depends on dissolution of cobalt particles in alveolar macrophages, resulting in cobalt delivery from the lung to the systemic circulation, with smaller particles having faster dissolution rates than larger particles (Kreyling et al., 1990).

Several studies have examined dissolution of cobalt in simulated body fluids. [The Working Group noted that these studies used cell-free artificial fluids that mimic those found in humans.] Dissolution of ultrafine cobalt powder in artificial lung fluid appears to be six times as high as that of standard cobalt powder (Kyono et al., 1992). Dissolution rates are also influenced by the chemical form of cobalt. Water-soluble forms of cobalt (cobalt(II) chloride and cobalt(II) sulfate)

have higher dissolution rates in artificial lung fluids than less soluble forms of cobalt, including cobalt(II,II) oxide and cobalt hydroxide oxide. In contrast, increased surface area of particles was not associated with enhanced cobalt release (Verougstraete et al., 2022). Dissolution rates in simulated gastric and intestinal fluids also reflect water solubility, with higher rates occurring with cobalt(II) chloride and cobalt(II) sulfate than cobalt(II,III) oxide or cobalt oxyhydroxide. Cobalt metal powder was very soluble in gastric fluid and poorly soluble in intestinal fluid (Danzeisen et al., 2020).

4.1.2 Experimental systems

(a) Absorption

(i) Oral administration

Gastrointestinal absorption varies depending on animal species and age. [The Working Group focused on monogastric species, including rodents, dogs, and swine.] Naylor & Harrison (1995) reported that gastrointestinal absorption of cobalt(II) citrate in Harwell Mouth Tumour (HMT) rats was about 25% and was lower in Dunkin-Hartley guinea-pigs after single-dose oral administration (10–100 µL: 1–10 kBq ^{57}Co, depending on animal age, from age 1 to 200 days). Younger animals showed higher intestinal absorption and retention than adult animals. Cobalt absorption was 3%–15% greater in young rats and guinea-pigs than in adult animals (Naylor & Harrison, 1995). Oral administration of a low dose of cobalt(II) chloride (≤ 229 µg/kg per day) to juvenile swine for 14 or 21 days did not affect average blood cobalt concentration (Suh et al., 2019).

(ii) Inhalation

The pulmonary absorption of inhaled cobalt metal is rapid in animals, and the predominant fraction of body burden is in the lung. Kyono et al. (1992) reported that in rats exposed to ultrafine cobalt powder aerosols (consisting of loose aggregates of primary particles; diameter, 20 nm; MMAD, 0.76 µm for secondary particles), cobalt concentrations in lung and blood were 6.42 µg/g (wet) and 28.94 µg/L, respectively, 2 hours after exposure to 2.12 ± 0.55 mg/m^3 for 4 days at 5 hours per day, suggesting that cobalt accumulated in the lung after inhalation and was transferred rapidly to the blood (Kyono et al., 1992). Studies have reported that the rate of translocation of cobalt(II,III) oxide from lung to blood varies depending on the size, density, and surface area of materials, but there were no significant species-related differences (Bailey et al., 1989; Kreyling et al., 1991). The initial translocation of cobalt particles of diameter 0.8 µm ranged from 0.4% per day in humans and baboons to 1.6% per day in HMT rats. Translocation of 1.7 µm particles was lower in all species and ranged from 0.2% per day in baboons to 0.6% per day in HMT rats (Bailey et al., 1989).

(iii) Other routes of administration

Lacy et al. (1996) reported that dermal application of 100 µL of 2% cobalt(II) chloride to Syrian hamsters resulted in low concentrations of cobalt in the urine 24 or 48 hours after application, suggesting some systemic distribution [The Working Group noted that limited details were available to determine if oral exposure may have occurred.] In addition, cobalt was retained at the site of dermal application 48 hours after it was applied on the skin (Lacy et al., 1996). A study of cobalt metal embedded in the muscle of Sprague-Dawley rats showed that the implanted metals rapidly solubilized; a considerable concentration of cobalt was found in the urine, and cobalt was also detected in the brain (Hoffman et al., 2021a, b). Intranasal administration of radioactive $^{57}Co^{2+}$ (0.8 ng cobalt(II) chloride, 15 µCi) in Sprague-Dawley rats showed that cobalt was absorbed by the olfactory mucosa and was transported to the olfactory bulb of the brain and retained at high concentrations in this tissue (Persson et al., 2003).

(b) Distribution

(i) Oral administration

In general, oral exposure of cobalt to mice or rats caused increased cobalt concentrations in the liver and kidney; however, increased cobalt concentrations were also found in the spleen, pancreas, heart, blood, bone, brain, testis, and intestine (Nation et al., 1983; Domingo et al., 1984; Bourg et al., 1985; Edel et al., 1994; Kirchgessner et al., 1994; Zheng et al., 2019). For example, after oral administration of cobalt(II) chloride at 500 ppm in drinking-water given to male Sprague-Dawley rats for 3 months, a significant increase of cobalt was found in the liver, kidney, heart, and spleen (Domingo et al., 1984). In male Sprague-Dawley rats given feed supplemented with cobalt(II) sulfate (20 mg and 40 mg/kg bw per day for 8 weeks), increased concentrations of cobalt were found in the three tissues examined: myocardium, followed by the skeletal muscle and blood (Clyne et al., 1990a, b). In male Sprague-Dawley rats whose feed was supplemented with cobalt(II) chloride, leading to exposures of 5 mg/kg bw or 20 mg/kg bw per day for 69 days, atomic absorption spectrophotometric analyses after 69 days revealed a dose-dependent tissue accumulation of cobalt in blood, bone, brain, hair, small intestine, kidney, liver, and testis (Nation et al., 1983).

In another study, groups of three pregnant OFA-Sprague-Dawley rats were treated by gavage with cobalt(II) sulfate at a dose of 25, 50, or 100 mg/kg bw on days 1–20 of gestation, and cobalt concentrations were measured in maternal and fetal blood. Cobalt concentrations increased dose-dependently in the maternal blood, and the compound was found to cross the placenta and appear in the fetal blood. The concentration of cobalt was higher in fetal than in maternal blood. For example, cobalt concentrations were about 0.8 mg/L and 2 mg/L in maternal blood and fetal blood, respectively, 24 hours after the last administration of cobalt(II) sulfate at 100 mg/kg bw (Szakmáry et al., 2001).

(ii) Inhalation

Generally, after inhalation exposure, cobalt accumulates and is retained in the lung, translocates to the blood, and distributes to extrapulmonary tissues (Rhoads & Sanders, 1985; Bailey et al., 1989; Kyono et al., 1992; NTP, 2014). Lung retention varies depending on the species and also on the nature of the particle (size, density, and surface area); it is generally greater for larger particles than for smaller particles (Bailey et al., 1989; Kreyling et al., 1991; NTP, 2014).

In a study by Kyono et al. (1992), rats were exposed to ultrafine cobalt aerosol (consisting of loose aggregates of primary particles; diameter, 20 nm; MMAD, 0.76 µm for secondary particles) at 2.12 mg/m^3, 5 hours per day for 4 days. They found that the cobalt concentration in the lung reached 6.42 µg/g (wet) 2 hours after the last exposure; the control level was 0.006 µg/g (wet). The cobalt concentration in the lung had decreased to 0.09 µg/g (wet) by 28 days post-exposure.

In an inhalation study by Bailey et al. (1989), humans and various species of experimental animals – including baboon, dog, guinea-pig, rat, hamster, and mouse – were exposed to cobalt(II,III) oxide with different particle sizes (0.8 and 1.7 µm). Generally, lung retention at 90 days and at 180 days was less for the smaller (0.8 µm) particles than for the larger (1.7 µm) particles in most species. However, retention varied between species, with humans having higher values than experimental animals. For instance, 6 months after inhalation of 0.8 µm cobalt particles, lung retention ranged from 1% of the initial lung deposit in mice and rats to 45% in humans and ranged from 8% of the initial lung deposit in rats to 56% in humans for the 1.7 µm cobalt particles (Bailey et al., 1989). Kreyling et al. (1991) reported that particle density influenced lung retention. Administration of cobalt(II,III) oxide by inhalation to baboons, dogs, and rats

showed that there was increased lung retention when they were exposed to 0.9 µm solid cobalt particles compared with 0.8 µm porous cobalt particles (Kreyling et al., 1991).

Patrick et al. (1994) measured the retention of radioactive cobalt isotope (^{57}Co) deposited in the lung by intratracheal instillation of cobalt(II) chloride or cobalt(II) nitrate at various doses, ranging from 0.006 to 5.4 µg depending on the species used. Species included baboon, guinea-pig, rat (all three species exposed for 100 days), hamster (exposed for 121 days), and dog (exposed for > 1000 days); dogs were treated with cobalt nitrate. Cobalt was deposited directly in the lung, but no significant accumulation of the compound was observed in other organs. In the same study, it was observed that lung retention and whole-body retention differed significantly between species. After 100 days, the fraction of radioactive cobalt remaining in the lung ranged from 0.13% (hamster) and 0.58% (rat) to 1.2% (estimated for dog) of the amount administered, while the proportion of cobalt remaining in the body ranged from 0.35% (hamster) to 3.2% (dog) of the amount administered.

In the inhalation studies conducted by the NTP (2014) using cobalt metal particulate aerosols, cobalt concentrations and burdens increased with increasing cobalt concentration in the 2-week, 3-month, and 2-year studies. In the 2-week study, concentrations increased in the liver, lung, kidney, bone, heart, serum, blood, and testis. Cobalt was distributed to extrapulmonary tissues, and a large amount of cobalt was accumulated in the liver. Cobalt tissue burdens in the liver were similar to or greater than those in lung (NTP, 2014).

(iii) Intraperitoneal and intravenous injection

Numerous studies using injections of Co^{2+} in rats and mice have demonstrated that cobalt distribution to multiple different tissues depends on the dosage and route of administration. Liver, lung, kidney, heart, bone, pancreas, the gastrointestinal tract, and muscle have been reported to be the target tissues (Stenberg, 1983; Llobet et al., 1988; Roshchin et al., 1989; Szebeni et al., 1989; Edel et al., 1994; Bingham et al., 1997; Horiguchi et al., 2004).

In other studies, Wistar rats treated with cobalt(II) chloride at a dose of 0.75 mg/kg bw by daily intraperitoneal injection for 3 weeks showed an accumulation of cobalt in the liver, kidney, brain, gastrointestinal tract, spleen, heart, and lung, with a predominance in the liver (14.4 µg/g) (Houeto et al., 2018). Llobet et al. (1988) also reported that in Sprague-Dawley rats treated with cobalt(II) chloride at a dose of 0.06 mmol/kg bw per day, 3 days/week for 4 weeks by intraperitoneal injection, cobalt was detected in the kidney, liver, heart, spleen, brain, and blood (Llobet et al., 1988). Szebeni et al. (1989) administered 2 mL of a solution containing 0.09–0.11 µCi ^{57}Co (cobalt(II) chloride) [final cobalt concentration was not specified] intravenously to Sprague-Dawley rats and detected cobalt in the liver, bone, heart, lung, kidney, gastrointestinal tract, and blood 2 hours after exposure. In a study by Edel et al. (1994), 24 hours after male Sprague-Dawley rats were treated with cobalt(II) chloride (10 ng/rat) by single intravenous injection, high concentrations of cobalt were detected in the lung, kidney, liver, and spleen, and about 0.06% of the administered dose was detected in the testis. In another group of rats treated with cobalt(II) chloride (10 ng/rat) by single intraperitoneal injection, high concentrations of cobalt were detected in the pancreas, kidney, small intestine, and liver at day 3 after injection, and cobalt was retained at high concentrations in the kidney, liver, and spleen at day 7 after injection. A third group was assessed 100 days after exposure by single intraperitoneal injection (lowest dose, 5 µg/rat; highest dose, 1 mg/rat); tissue distribution was affected by the dose, with the lowest dose leading to high cobalt concentrations in the spleen, pancreas, and bone, and the higher dose leading

to highest concentrations in the bone samples, indicating that the compound accumulates in this tissue. Bingham et al. (1997) gave ^{57}Co [not further specified] to male HMT rats (0.2 mL of 50 kBq/mL ^{57}Co) by intravenous administration and found that the highest concentrations of the compound were found in the kidney and liver, with transient accumulation in the prostate, but no accumulation in the testis.

(c) Metabolism

Cobalt is not subject to direct metabolism by enzymatic pathways.

(d) Excretion

(i) Oral administration

After oral administration of cobalt, faecal excretion is the major route of elimination (Reuber et al., 1994; Ayala-Fierro et al., 1999). In particular, Ayala-Fierro et al. (1999) found that 36 hours after oral administration of cobalt(II) chloride at a dose of 33.3 mg/kg bw by gavage to male Fischer 344 rats, about 74.5% of the substance was eliminated in the faeces.

(ii) Inhalation

Cobalt compounds are cleared rapidly from the lung. As shown through a two-compartment model of the relevant data, clearance of cobalt from the lung is biphasic, with a first rapid phase and a second slow phase. Elimination of cobalt from blood is also indicated to have a rapid phase and a slow phase. A study by Kreyling et al. (1986) exposed beagle dogs to radioactive particles of cobalt oxides of different sizes (0.3–2.7 µm) by inhalation and found that smaller particles were cleared more rapidly from the lung (Kreyling et al., 1986). In a study by Sisler et al. (2016a), mice were exposed to cobalt(II) oxide nanoparticles (NPs) at 10 or 30 mg/m^3 (72 ± 16 nm) for 6 hours per day for 4 days by inhalation. The lung burden after 1 hour of exposure was found to be 7.7 µg/lung and 18.7 µg/lung for exposure at the lowest and highest dose, respectively; less than 1% of the cobalt(II) oxide NPs remained in the lungs at 56 days post-exposure. Kyono et al. (1992) reported that in rats exposed to an ultrafine cobalt aerosol (consisting of loose aggregates of primary particles; diameter, 20 nm; MMAD, 0.76 µm for secondary particles), the clearance of cobalt was biphasic: in the first phase about 75% of the cobalt was cleared from the lungs within 3 days with a half-life of 53 hours, while in the second phase (occurring within 3–28 days) a slower clearance rate was evident with a half-life of 156 hours (Kyono et al., 1992). When Syrian golden hamsters were exposed to cobalt(II) oxide by inhalation for 60 days, it was found that cobalt was eliminated 6 days after exposure, and only about 27% remained in the carcass, lung, liver, and kidney (of which 23.3% was in the carcass), 24 hours after inhalation of cobalt oxide (Wehner & Craig, 1972).

To provide data on pulmonary retention, clearance, and systemic distribution of cobalt metal, toxicokinetic studies were performed in Fischer 334/N rats and B6C3F$_1$/N mice by the NTP (2014). Animals were exposed to cobalt metal particulate aerosol in an inhalation chamber. The details of the 2-week, 3-month, and 2-year exposure studies are as follows.

In the 2-week exposure studies, rats or mice were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days/week for 16–17 days. Cobalt concentrations were found to increase with increasing exposure levels of cobalt in all tissues examined, including the liver, lung, kidney, bone, heart, serum, blood, and testis. Cobalt was distributed to extrapulmonary tissues, and a large amount of cobalt was accumulated in the liver. Liver burdens were similar to or greater than those in the lung. In general, normalized tissue burdens did not increase or decrease with increasing exposure concentration. At 3 weeks post-exposure, cobalt concentrations were markedly reduced in the blood, serum, and lung.

Urinary cobalt concentrations increased with increasing cobalt exposure concentration. The clearance half-lives were 9.2–11.1 days (blood), 2.8–3.4 days (serum), and 4.2–5.6 days (lung) in rats. In mice, the half-lives were 4.1–7.3 days (blood), 2.9–3.7 days (serum), and 5.5–6.6 days (lung).

In the 3-month exposure studies, female rats were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 0.625, 1.25, 2.5, or 5 mg/m^3, and female mice were exposed by inhalation to cobalt metal particulate aerosol at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. It was observed that lung cobalt concentrations and burdens increased with increasing exposure concentration. The lung cobalt burden data from the exposure phases of these studies were analysed using a two-compartment model. Lung cobalt burdens increased rapidly within the first few days, and steady states were reached in all groups by day 26 (rat) or day 40 (mouse). During the 42-day recovery period, lung cobalt burdens decreased rapidly during the first week, after which lung clearance of cobalt slowed significantly. Liver cobalt concentrations increased with exposure concentration at both time points (day 26 and 40). Pulmonary clearance of cobalt during the recovery period showed a well-defined two-phase profile. In the rapid phase, half-lives were 1.8–2.6 and 1.4–3.2 days for rats and mice, respectively, and were 19–23 and 27–39 days for rats and mice, respectively, in the slower lung clearance phase that followed. The lung cobalt burden data were analysed using a one-compartment model, and the resulting half-lives were 4.7–9.0 and 2.4–17 days, respectively, for rats and mice.

In the 2-year studies, female rats and mice were exposed by inhalation to cobalt metal particulate aerosol at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, for 6 hours plus T_{90} (12 minutes) per day, 5 days/week for 105 weeks. Cobalt concentrations and burdens in the lung increased with increasing exposure concentration. The lung burden data from the exposure phases were analysed using a two-compartment model. In rats, lung burdens increased rapidly by day 4; in mice, lung burdens increased rapidly within the first 5–26 days, and all exposed groups of rats and mice reached steady states by day 184. The majority of the deposited cobalt (more than 95% in rats and more than 82% in mice) was cleared quickly. The half-lives in the rapid clearance phase were between 1.5–2.9 days (rat) and 1.1–5.2 days (mouse). In the slow clearance phase, half-lives for rats and mice were 789 and 409, 167 and 172, and 83 and 118 days, respectively, for the exposures at 1.25, 2.5, and 5 mg/m^3. Cobalt deposition rates for rats and mice were 1.4 and 1.2, 2.1 and 1.1, and 5.6 and 5.2 µg/day during the rapid clearance phase, respectively, and 0.018 and 0.027, 0.078 and 0.075, and 0.29 and 0.25 µg/day during the slow clearance phase, for the exposures at 1.25, 2.5, and 5 mg/m^3.

(iii) Intravenous injection

Intravenous injection studies in mice and rats have indicated rapid elimination of cobalt. The excretion was predominantly via the urine, but a small proportion (2–15%) was also eliminated via bile and faeces (Cikrt & Tichý, 1981; Gregus & Klaassen, 1986; Llobet et al., 1986; Ayala-Fierro et al., 1999; Weber et al., 2012).

Weber et al. (2012) intravenously administered a single dose of radioactive cobalt(II) chloride (11.2 kBq of $^{60}CoCl_2$) to Fischer 344 rats and found that the liver, gastrointestinal tract, and muscle were the tissues with the greatest burdens of cobalt. These tissues cleared quickly, and less than 3% of the recovered dose remained in the body 28 days after administration (67.6% was removed with a half-life of 1.9 hours, and the remaining amount was eliminated with a half-life of 45 hours).

After rats were exposed to cobalt(II) chloride by a single intravenous injection, 70% and

7% of the dose given was excreted via the urine and faeces, respectively, during the first 3 days (Onkelinx, 1976). Another study showed that after exposure of rats to cobalt(II) chloride by intravenous injection, 73% and 15% were excreted via the urine and faeces, respectively, during the 4 days after dosing (Gregus & Klaassen, 1986). After various species of experimental animals were exposed to a radioactive cobalt nitrate (^{57}Co-labelled cobalt(II) nitrate) solution by injection, over 60% of the ^{57}Co was excreted during the first day, and over 90% was excreted by 3 weeks (Bailey et al., 1989). Thirty days after various species of experimental animals were exposed to ^{60}Co-labelled cobalt(II) chloride by intravenous administration, a significant amount (70–85%) was excreted in the urine during the first day; however, the long-term whole-body retention times were significant, being 495, 309, 183, and 180 days in mouse, rat, monkey, and dog, respectively (Thomas et al., 1976).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether cobalt metal (without tungsten carbide), including cobalt metal nanoparticles, and soluble cobalt(II) salts are electrophilic or can be metabolically activated to an electrophile; are genotoxic; induce oxidative stress; induce chronic inflammation; modulate receptor-mediated effects; induce immortalization; and alter cell proliferation, cell death, or nutrient supply. Evidence is also reported as to whether soluble cobalt(II) salts alter DNA repair or cause genomic instability; induce epigenetic alterations; or are immunosuppressive. Evidence is also summarized as to whether cobalt(II) oxide and cobalt(II,III) oxide (including cobalt oxide nanoparticles) are genotoxic; induce oxidative stress; or modulate receptor-mediated effects. Few data are described for the other key characteristics for cobalt metal, soluble cobalt salts, and cobalt oxides. Sparse data for the key characteristics of carcinogens are reported for cobalt sulfide and other cobalt(II) compounds.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

See Table 4.1.

No studies on cobalt–DNA adducts were available to the Working Group; however, there was one study on cobalt–protein adduct formation in an exposed population.

In an occupational study, Princivalle et al. (2017) assessed cobalt–haemoglobin adducts in the blood of a subset of Italian hard-metal workers employed at several plants where they were exposed to both cobalt metal powder and cobalt oxide in various mixing and sintering operations. They reported positive correlations between geometric mean cobalt concentrations in blood and cobalt–haemoglobin adducts ($r = 0.9650$; $P < 0.001$) in 22 workers. This correlation between cobalt–haemoglobin adducts and exposure to cobalt was also seen in a larger population ($n = 66$) from the same group of plants ($r = 0.8440$; $P < 0.001$). [The Working Group noted that there was little potential for exposure misclassification. This study was focused on cobalt exposure and excretion over short time periods (days to weeks). Co-exposure to WC-Co was probable in this group of hard-metal workers; however, there was a substantial subset of workers whose work tasks primarily involved the handling of cobalt powder before the synthesis of hard-metal composites.]

Shirakawa & Morimoto (1997) examined a population exposed only to hard metal (WC-Co). [The Working Group evaluated the study and deemed it uninformative because the population

was exposed only to WC-Co), which was inseparable from exposure to cobalt alone. There is potential for non-differential misclassification in the exposure groups. A key limitation was the lack of information on the exposure groups and how they were constructed, particularly the timing of when these groups were defined in relation to the outcome measures. There are exposure metrics reported in the multiple logistic regression that are not described in the methods. Co-exposure to tungsten carbide is probable.]

[The Working Group also evaluated but excluded a study by Amirtharaj et al. (2008) as uninformative because there was no in vivo exposure to cobalt.]

(ii) Human cells in vitro

No data on direct cobalt–DNA binding in human cells in vitro were available to the Working Group.

Primary erythrocytes from healthy volunteers were treated with ^{57}Co as a tracer. Chromatographic analysis showed that ^{57}Co co-migrated with haemoglobin (Simonsen et al., 2011). [The Working Group noted that this might indicate cobalt binding to haemoglobin protein.]

When cells were depleted of zinc, the extracted wildtype human recombinant p53 proteins (produced via a baculovirus expression system) lost their DNA-binding capacity. In human MCF7 cells in vitro, addition of extracellular Zn^{2+} (5 μM) renatured and reactivated the p53. The addition of Co^{2+} (125 μM cobalt(II) chloride) had a similar effect to that of Zn^{2+} (i.e. renaturation, reactivation, and restoration of the DNA-binding capacity of p53), suggesting that Co^{2+} can substitute for Zn^{2+} binding in p53 protein (Méplan et al., 2000). [The Working Group noted that this "substitution" might be considered protein binding.]

(iii) Acellular systems

Cobalt binding to human albumin is relevant to the clinical use of the albumin-cobalt-binding (ACB) assay to detect the presence of myocardial ischaemia. The ACB assay was developed based on decreased cobalt binding to "ischaemia-modified albumin (IMA)" compared with normal human albumin and has great specific negative predictive value to reliably exclude myocardial ischaemia when the assay readout is low. However, increased assay readout is of low specificity because IMA is seen in many diseases or conditions other than ischaemia (Lu et al., 2012).

Human serum albumin has four known metal binding sites: A and B, an N-terminal binding site (NTS), and cysteine 34 (Coverdale et al., 2018). Cobalt binds to the first three sites, with a preference for sites A and B over the NTS; the affinity of Co^{2+} binding was strongest at site B, followed by site A, and weakest at the NTS (Sokołowska et al., 2009). Within the NTS, the 3-histidine and α-amino group are essential moieties that interact with Co^{2+} (Cho et al., 2022).

Using serum albumin from patients with fatty liver and healthy controls, Amirtharaj et al. (2008) showed that cobalt binding was lower in patients with fatty liver. In both purified albumin and normal serum, hydroxyl radicals [produced by hydrogen peroxide (H_2O_2) and copper (Cu)] (representative of oxidative stress) decreased cobalt binding to human albumin in vitro, and this decrease was reversed by catalase (CAT). These data were confirmed by the use of various combinations of xanthine/xanthine oxidase (to generate superoxide radicals) with CAT or superoxidase dismutase (SOD) (to scavenge superoxide), demonstrating that superoxide radicals, did not affect cobalt–albumin binding. The binding is affected by production of hydroxyl radicals as shown by the incubation with copper sulfate ($CuSO_4$) and H_2O_2, or $CuSO_4$ and H_2O_2 and thiourea and mannitol (Amirtharaj et al.,

Table 4.1 Cobalt-derived adducts in exposed humans

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Co–Hb adducts	Blood, urine	Italy, serial biomonitoring, longitudinal (pre/post-work shift Co measurements)	Hard-metal manufacturing workers in several plants, exposed to cobalt(II) metal and cobalt(III) oxide powders and sintering operations (n = 66)	Positive correlation between geometric mean Co concentrations in urine, in blood, and Co–Hb adducts in 22 of the workers; r = 0.965; P < 0.001); n = 22 workers Additional similar finding in total cohort: positive correlation log Co in blood (µg/L) and Hb (ng/g globin): log Co concentration in blood, −0.2894 + 0.6758 (log Co concentration in globin); n = 66; r = 0.8440, P < 0.001)	NR	There was little potential for misclassification. This study was focused on Co exposure and excretion over short time periods (days to weeks). Co-exposure to WC-Co was likely.	Principalle et al. (2017)

Co, cobalt; Hb, haemoglobin; NR, not reported; WC-Co, cobalt with tungsten carbide; wk, week.

2008). [The Working Group noted that albumin has at least seven fatty acid-binding sites, including a high-affinity site (FA2) near site A (Simard et al., 2005), and removing fatty acids from purified albumin also decreased cobalt–albumin binding, but fatty acid removal did not exacerbate the hydroxyl radical-induced reduction in cobalt–albumin binding (Amirtharaj et al., 2008).]

Cobalt(II,III) oxide NPs (diameter, 50 nm) with a defined crystalline phase caused minor changes in the tertiary structure of human serum albumin, as measured by fluorescence spectroscopy (Arsalan et al., 2020), but did not alter the secondary structure of albumin (amount of α-helix, β-sheet, and random coil structures), as measured by circular dichroism. When clusters of cobalt(II,III) oxide NPs were set to diameters of 0.5, 1, 1.5, and 2 nm, a computer docking program (HEX 6.3 software) calculated that the highest binding energy with human serum albumin was from the 1.5-nm diameter cluster.

(b) Experimental systems

(i) Non-human mammals in vivo

No data on cobalt–DNA or cobalt–protein binding were available to the Working Group.

(ii) Non-human mammalian cells in vitro

BALB/3T3 mouse fibroblasts, or isolated cytosolic and nuclear extracts, were acutely exposed to different forms of cobalt (at similar cobalt concentrations) (Sabbioni et al., 2014b). The amount of cobalt binding to DNA was consistently greater for cobalt microparticles (^{60}Co-labelled; mean diameter, 2.2 µm; form aggregates up to 15 µm in size [which is close to the diameter of fibroblast cells]; specific surface area, 0.75 m^2/g) than for cobalt NPs (^{60}Co-labelled; mean diameter, 3.4 nm; mean aggregate size, 200 nm; range, 100–400 nm, Zeta potential, 14 ± 4.2 mV; specific surface area, 14.4 m^2/g), which in turn was much greater than the DNA binding of Co^{2+} ions (from Co^{57}-labelled cobalt(II) chloride hexahydrate; metal purity, 99.998%) (Sabbioni et al., 2014b). [The Working Group noted that the greater amount of cobalt–DNA binding in cells treated with cobalt NPs, compared with Co^{2+}, was partially explained by the greater cellular uptake of cobalt NPs and the higher ratio of cobalt in the nuclei/whole cell compared with equivalent concentrations of Co^{2+}.]

When rat Novikoff ascites hepatoma cells were exposed to 1 mM of cobalt(II) chloride in vitro for 8 hours, the cell viability was greater than 85% and DNA–protein cross-links were seen in electrophoresis results (Wedrychowski et al., 1986). The majority of cross-linked proteins were between 94 and 200 kDa. [The Working Group noted that no digital image analysis or statistical analysis was performed.]

(iii) Acellular systems

In an acellular system, different forms of crystallized synthetic DNA were soaked in a cobalt(II) chloride solution, and Co^{2+} exhibited binding exclusively to the N^7 position of guanine (Gao et al., 1993). The binding affinity was influenced by the microenvironment of the guanine bases, as Co^{2+} in the presence of magnesium bound to different sites in the synthetic sequences CGCGCG and CGCGTG. The binding of Co^{2+} to the N^7 position of guanine probably changes the conformation of either B-DNA or A-DNA (Gao et al., 1993).

When synthetic DNA was crystallized in the presence of Co^{2+} from cobalt(II) chloride, it was observed that Co^{2+} bound to only the N^7 positions of terminal guanines (not non-terminal positions) in the resulting B-DNA crystals (Labiuk et al., 2003). Furthermore, it was shown that cobalt does not bind CGC nor B-DNA in a stable manner (Labiuk et al., 2003).

Lu et al. (2012) demonstrated that myristate, which has albumin-binding characteristics similar to those of the physiological fatty acids stearate and oleate, decreased the binding capacity of Co^{2+} (from cobalt(II) chloride

Table 4.2 Genetic and related effects of cobalt in exposed humans

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)[a]	Covariates controlled	Comments	Reference
DNA single-strand breaks (alkaline elution method)	PBMC	Germany, 10 facilities where high air concentrations of Cd were expected, cross-sectional	Workers with mixed metal exposures (Co, Cd, and Pb) ($n = 78$)	Positive correlation between Co air concentrations and DNA-SSB ($P < 0.001$, $r = 0.401$); similarly, Co in urine correlated with DNA-SSB ($P < 0.001$; $r = 0.381$)	Age, sex, alcohol, smoking status	The potential for differential exposure misclassification is low with the measurements collected. The consideration of co-exposures to Pb and Cd (both also quantitatively assessed) is a strength. The air samples (inhalation exposure) and biological samples (all routes of exposure) represent different time periods of exposure. Limitation of the study: reliance on a single biological measure of exposure.	Hengstler et al. (2003)
DNA single-strand breaks (comet assay, alkaline elution)	PBMC	European, multiple factories, cross-sectional study of genotoxic outcomes by exposure and DNA repair capacity	Co-exposed ($n = 21$) WC-Co-exposed ($n = 26$) Non-exposed controls ($n = 26$)	Comet assay: *XRCC3* × smoking status, $P = 0.037$ *XRCC1*, $P = 0.053$	Genotypes, age, exposure, type of plant, smoking, interaction terms	The potential for differential exposure misclassification is low. There was likely exposure to other metals that was not considered. Estimated Co exposure: 20 µg cobalt/m^3. This finding is for mixed (Co and WC-Co) exposure.	Mateuca et al. (2005)
Micronucleus formation (CBMN assay)	Peripheral blood lymphocytes			↑ MNMC for WC-Co-exposed workers, $P = 0.011$ ↑ MNCB for Co only-exposed workers, $P = 0.022$		Attributed to WC-Co exposure. Attributed to Co-only exposure.	Mateuca et al. (2005)

Table 4.2 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)[a]	Covariates controlled	Comments	Reference
DNA strand breaks (comet assay)	Peripheral blood lymphocytes	Belgium, Norway, Finland, Sweden, and England, workers from several refinery factories exposed to Co-containing dust, cross-sectional	Co-exposed (n = 35/24*) WC-Co-exposed (n = 29) Non-exposed controls (n = 35/27*)	Difference NSS between exposed and controls	Creatinine, urine Vitamin E (α-tocopherol), serum Selenium, serum Independent variables: Exposure, plant type, Co-urine, age, smoking, vitamin E-serum, interaction between smoking and hard-metal exposures	Key limitation of the study: reliance on a single spot urine sample. The single sample may not reflect the relevant exposure window for all outcomes. Co exposure concentration approximates current TLV-TWA (20 μg/m^3). Multiple regression for influence of independent variables on outcomes including plant type (WC-Co or Co-alone exposure). Reduced number of participants for Co-exposed workers and non-exposed controls after one plant dropped from study because of age differences.	De Boeck et al. (2000)
Micronucleus formation (CBMN assay)	Peripheral blood lymphocytes			MNMC: interaction term (smoking status by plant type) P = 0.0145, but for WC-Co plant. ↑ MNCB: influenced by Co-only plant exposure and smoking as an interaction term (P = 0.0011).			
DNA damage (comet assay)	Sperm DNA	China, partners of fertility clinic patients, cross-sectional	n = 516	No associations between urinary metal levels and sperm DNA integrity parameters after adjustment for multiple testing (FDR-adjusted P for trend, > 0.10)	Age, BMI, abstinence time, smoking status, daily cigarette consumption, and urinary creatinine	Urine samples collected at two points close in time on the same day as semen sample limits the findings. Only outcome for Co-only exposure in the interaction.	Wang et al. (2016a)

↑, increased; BMI, body mass index; CBMN, cytokinesis-block micronucleus; Cd, cadmium; Co, cobalt; DNA-SSB, DNA single-strand break; FDR, false discovery rate; MNCB, micronucleated binucleated cell; MNMC, micronucleated mononucleated cell; NSS, not statistically significant; Pb, lead; PBMC, peripheral blood mononuclear cell; TLV-TWA, threshold limit value/time-weighted average; WC-Co, cobalt with tungsten carbide.

[a] +, positive; –, negative.

hexahydrate) to mature bovine serum albumin (with metal- and fatty acid-binding properties similar to those of human serum albumin). Because ischaemia also increases plasma fatty acid levels, ACB assay results might not be attributable to albumin changes. Other conditions that increase fatty acid levels would also decrease measured cobalt binding in the ACB assay.

Cobalt has been shown to bind to the globin moieties of bovine haemoglobin (Hoffman & Petering 1970) and sperm whale myoglobin (Sato et al., 1990).

4.2.2 Is genotoxic

The genotoxicity of cobalt and cobalt compounds was previously evaluated in *IARC Monographs* Volumes 52 (IARC, 1991) and 86 (IARC, 2006). The following is a summary of the studies relevant to key characteristic 2 – "is genotoxic" – and reported in Tables 4.2 to 4.5 and Table S4.6 (see Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618), with a particular emphasis on the studies in exposed humans, human cells in vitro, and experimental (non-human mammalian) systems.

(a) Humans

(i) Exposed humans

See Table 4.2.

Evidence for genotoxic effects in human populations exposed to cobalt included a cross-sectional study of German workers exposed to cobalt, cadmium, and lead (Hengstler et al., 2003). A positive correlation was observed between cobalt air concentrations and DNA single-strand breaks in blood mononuclear cells (r = 0.401; P < 0.001). Similarly, cobalt concentrations in urine positively correlated with DNA single-strand breaks (r = 0.381; P < 0.001). [The Working Group noted that the authors indicated that cobalt exposure was comparatively low (range, 0–10 μg/m³ air) compared with the German TRK permissible exposure limit value of 100 μg/m³. The Working Group also noted that DNA damage in the exposed individuals might be a secondary event due to DNA repair inhibition. DNA repair inhibition might also explain interactions between heavy metals since a decreased repair capacity will increase susceptibility to direct genotoxic effects. The Working Group noted that the potential for differential exposure misclassification is low with the measurements collected. The consideration of co-exposures to lead and cadmium, both also quantitatively assessed, was a strength. However, the Working Group noted that non-standard analytical approaches were used to assess confounding (e.g. Greenland, 1989). The air (inhalation exposure) and biological samples (all routes of exposure) represent different time periods of exposure.]

In another cross-sectional study of European factory workers across several plants, some with exposure to cobalt alone and others to WC-Co, and including non-exposed controls, Mateuca et al. (2005) examined several end-points of genotoxic damage, including DNA single-strand breaks measured in peripheral blood mononuclear cells (PBMCs) using the comet assay, and mono- and binucleated cells in whole blood using the cytokinesis-block micronucleus assay. Only the amount of micronucleated binucleated cells in the micronucleus assay was found to be significantly increased in the workers exposed to cobalt alone (P = 0.022). [The Working Group noted that the potential for differential exposure misclassification was low. There was probable exposure to other metals that was not considered.]

In a similar cohort to that described above by Mateuca et al. (2005), DeBoeck et al. (2000) assessed genotoxic end-points in a group of European factory workers from several sites, with exposure to either metallic tungsten carbide particles or cobalt metal alone, and compared them with non-exposed controls from the same plants, adjusting for age and smoking status. No statistically significant differences

Table 4.3 Genetic and related effects of cobalt in human cells in vitro

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Cobalt metal					
DNA single-strand breaks and alkali-labile sites (alkaline comet assay)	Lymphocytes	+[b]	0.3 µg/mL (d_{50}, 4 µm)	Difficult to infer LEC from the graphic representation.	De Boeck et al. (1998)
Oxidative DNA damage (comet assay with Fpg enzyme)	Mononuclear leukocytes	–[b]	6 µg/mL (d_{50}, 4 µm)		De Boeck et al. (1998)
DNA strand breaks (alkaline elution)	Lymphocytes	+[b]	3 µg/mL (d_{50}, 4 µm)		Anard et al. (1997)
DNA single-strand breaks and alkali-labile sites (alkaline comet assay)	Lymphocytes	+[b]	4.5 µg/mL (d_{50}, 4 µm)		Anard et al. (1997)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Mononuclear leukocytes	+[b]	0.6 µg/mL (d_{50}, 4 µm)		Van Goethem et al. (1997)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Leukocytes	–	6 µg/mL (d_{50}, 4 µm)		De Boeck et al. (2003)
DNA strand breaks (alkaline elution assay)	Human osteoblast-like cells, HOS Osteoblast-like cells (immortalized, non-tumourigenic), TE85 (clone F-5)	–	6 µg/mL	Purity, 99.5%; d_{50} = 1–4 µm, A heavy metal W alloy [pure mixture of W (92%), Ni (5%), and Co (3%)] was also tested with positive (synergistic) results.	Miller et al. (2001)
Micronucleus formation (CBMN assay)	Lymphocytes	+[b]	0.6 µg/mL (d_{50}, 4 µm)		VanGoethem et al. (1997)
Micronucleus formation (CBMN assay)	Lymphocytes	(+)	3 µg/mL (d_{50}, 4 µm)	Difficult to infer LEC from the data presented.	De Boeck et al. (2003)
Micronucleus formation (CBMN assay)	Human osteoblast-like cells, HOS osteoblast-like cells (immortalized, non-tumourigenic), TE85 (clone F-5)	(+)	0.75 µg/mL	Purity, 99.5%; d_{50}, 1–4 µm. Statistically significant increase without concentration–response relationship. A heavy metal W alloy [pure mixture of W (92%), Ni (5%), and Co (3%)] was also tested with positive results.	Miller et al. (2001)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Cobalt metal NPs					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Mononuclear leukocytes	+	50 µM [3 µg/mL], 2 h	d_{50} = 246 nm (100–500 nm) [given that the authors designated them as NPs, it is presumed that ≥ 50% of the particles have one dimension < 100 nm].	Colognato et al. (2008)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Blood T-cells	+	3 µM [0.53 µg/mL], 4 h	d_{50}, 50 nm (30–70 nm). Concurrent assessment of Co dissolution in culture medium, cytotoxicity.	Jiang et al. (2012)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) DNA double-strand breaks (γH2AX assay)	Primary CD34+ haematopoietic stem cells and haematopoietic progenitor cells (isolated from cord blood)	(+) (+)	200 µM [11.8 µg/mL]	Size, 50–200 nm. Single concentration of Co metal NPs tested; the aim was to test protective effect of selenomethionine against Co metal NPs. Concomitant assessment of cytotoxicity, apoptosis, oxidative stress and modulation of DNA repair response	Zhu et al. (2021b)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	+	5 µg/mL	Mean diameter, 20 nm Only 2 concentrations tested; no positive control described	Wan et al. (2012)
DNA double-strand breaks (γH2AX assay)		+	10–15 µg/mL	Concomitant assessment of NP uptake by cells, cytotoxicity, oxidative stress, and gene expression (genes involved in DNA damage response). DNA damage was significantly attenuated by pre-treatment with antioxidants (NAC or catalase); dose- and time-dependent increased expression of RAD51, phosphorylated p53, and ATM.	Wan et al. (2012)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549; human bronchial epithelial cells, HBEC3-kt (immortalized bronchial epithelial cells) Human lung alveolar cell line, A549	(+) + (Fpg)	40 µg/mL, 3 h and 24 h 20 µg/mL, 24 h	Co metal NP purity, 99.8%; different dimensions and shapes (from spheres to parallelepipeds); average size, 25 ± 8.8 nm. Only 2 concentrations tested; only positive at the highest concentration tested; no concentration–response. Concurrent assessment of cytotoxicity, ROS formation.	Cappellini et al. (2018)
Micronucleus formation (CBMN assay)	Lymphocytes	+	20 µM [1.18 µg/mL]	d_{50}, 246 nm.	Colognato et al. (2008)
Soluble cobalt(II) salts					
Cobalt(II) chloride ($CoCl_2$)					
DNA strand breaks (fluorescence analysis of DNA unwinding)	Mononuclear leukocytes	(+)	50 µM [6.5 µg/mL]	One concentration tested. No cytotoxicity assessment.	McLean et al. (1982)
DNA strand breaks (alkaline sucrose gradient)	Human diploid fibroblasts, HSBP	(+)	5 mM [650 µg/mL]	One concentration tested.	Hamilton-Koch et al. (1986)
DNA strand breaks (nick translation)	Human diploid fibroblasts, HSBP	(+)	10 mM [1300 µg/mL]	One concentration tested.	Hamilton-Koch et al. (1986)
DNA strand breaks (nucleoid sedimentation)	Human diploid fibroblasts, HSBP	(–)	10 mM [1300 µg/mL]	One concentration tested.	Hamilton-Koch et al. (1986)
DNA single strand breaks and alkali-labile sites (alkaline comet assay)	Lymphocytes	+		Not possible to infer LEC from the graphic representation.	De Boeck et al. (1998)
DNA cleavage (topoisomerase II-mediated DNA cleavage)	Human breast cancer cell line, MCF7	(+)	200 µM [26 µg/mL], 24 h	A single concentration tested.	Baldwin et al. (2004)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Primary human fibroblasts	(+)	0.84 µM [0.11 µg/mL]	Very limited data presented for cobalt(II) chloride. Artificial salt solution (synthesized fluids) containing cobalt(II) chloride alone at 0.84 µm induced DNA damage but at a lower level than that produced by a Co–Cr alloy solution.	Davies et al. (2005)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Hepatocellular carcinoma-derived cell line, HepG2	(+)	10 µg/mL [77 µM], 48 h	Two concentrations (10 and 15 µg/mL).	Alarifi et al. (2013)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Blood T-cells	–	30 µM [3.9 µg/mL]	Concurrent assessment of Co dissolution in culture medium, cytotoxicity.	Jiang et al. (2012)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human keratinocyte cell line, HaCaT	+	40 µM [5.2 µg/mL], 6 h and 24 h	Concomitant intracellular Co determination and cytotoxicity assessment.	Gault et al. (2010)
DNA double-strand breaks (neutral comet assay)	Human lymphoma cell line, Jurkat (CD4+ T-cells obtained from a T-helper lymphoma)	(+)	5 mM [650 µg/mL], 48 h	Only positive at the highest concentration tested; no concentration–response [the use of $CoCl_2$ or $CoCl_2{\cdot}7H_2O$ is not specified].	Caicedo et al. (2008)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Mononuclear leukocytes	–	100 µM [13 µg/mL], 2 h		Colognato et al. (2008)
DNA breaks Single-cell array-based halo assay (alkaline conditions)	Fetal fibroblast cells	(+)	10 µM [1.3 µg/mL], 1 h	Method developed by the authors [source of Co^{2+} not mentioned, no positive control, number of independent experiments/ replicates not specified, primary or immortalized cells not specified].	Qiao & Ma (2013)
DNA double-strand breaks (γH2AX assay)	Human lung carcinoma cell line, H460	+	300 µM [39 µg/mL]	Concurrent cell viability, apoptosis, and ROS assessment.	Patel et al. (2012)
DNA double-strand breaks (γH2AX assay)	Human colorectal cancer cell lines, HCT116, SW620, and LOVO cells	(+)	100 µM [13 µg/mL], 24 h	A single concentration of $CoCl_2$ tested.	Zhong et al. (2020)
Micronucleus formation (CBMN)	Lymphocytes	+	40 µM [5.2 µg/mL]		Colognato et al. (2008)
Sister-chromatid exchange	Lymphocytes	+	10 µM [1.3 µg/mL]		Andersen (1983)
Aneuploidy	Lymphocytes	+	3.7 µg/mL [28.5 µM]		Resende de Souza Nazareth (1976)
Cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$)					
DNA strand breaks (nucleoid sedimentation)	Human cervical cancer cell line, HeLa	+	50 µM [11.9 µg/mL]		Hartwig et al. (1990)
DNA strand breaks (alkaline elution)	Lymphocytes	(–)	25 µg/mL [105 µM]	A single concentration tested.	Anard et al. (1997)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme or hOGG1 enzyme)	Human bronchial epithelial cell line, BEAS-2B	+ + (Fpg) + (hOGG1)	2.5 µg/mL [10.5 µM], 2 h 1.25 µg/mL [5.25 µM], 2 h, Fpg 5 µg/mL [21 µM], 24 h, hOGG1	[No data for ROS production or oxidative stress markers.]	Uboldi et al. (2016)
DNA double-strand breaks (γH2AX)		+	2.5 µg/mL [10.5 µM]		Uboldi et al. (2016)
Chromosomal aberration, including translocations and aneuploidy (M-FISH)	Primary fibroblasts	+ (structural + numerical chromosomal aberration) + (aneuploidy)	1.3 ppb (metal ion species) [0.005 µM] 25 ppb [0.105 µM]	Co tested at physiological doses (found in peripheral blood of exposed humans). Numerical chromosomal aberrations were predominantly present. Structural aberrations such as translocations and dicentrics were not observed.	Figgitt et al. (2010)
Chromosomal aberration	Primary normal human bronchial epithelial cells, NHBE	+	175 µM [41.7 µg/mL], 24 h	Concomitant cytotoxicity and intracellular Co ion concentration measured. Comparison with Co oxide NPs: at similar intracellular Co ion levels, $CoCl_2$ induces more chromosome damage than Co oxide NPs. The most common aberration observed was chromatid lesions.	Xie et al. (2016)
Chromosomal aberration	Human primary bronchial fibroblast-derived cell line, WTHBF-6 (hTERT immortalized clonal cell line)	+	50 µM [11.9 µg/mL], 24 h	Concomitant cytotoxicity and intracellular Co ion concentration measured. Based on intracellular Co levels, exposure to soluble or particulate Co induced relatively similar levels of genotoxicity. The most common aberrations observed were chromatid lesions.	Smith et al. (2014)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Chromosomal aberration	Human urothelial cells, hTU1–38 (hTERT immortalized subclone of human urothelial hTU1cell line)	+	175 µM [41.7 µg/mL], 24 h	Co concentrations were chosen based on physiologically relevant exposure levels in patients who received hip implants (mean cobalt ion urine concentration, 75.40 µg/L). The most common aberration observed was chromatid lesions. Concomitant cytotoxicity and intracellular Co concentration assessment. Comparison with Co oxide NPs (see below).	Speer et al. (2017)
Micronucleus formation (CBMN assay)	Human bronchial epithelial cell line, BEAS-2B	+	1.25 µg/mL [5.25 µM]	Cytotoxicity/apoptosis reported.	Uboldi et al. (2016)
Cobalt(II) acetate tetrahydrate ($Co(CH_3CO_2)_2 \cdot 4H_2O$)					
Chromosomal aberration	Leukocytes	–	0.6 µg/mL [2.4 µM]		Voroshilin et al. (1978)
Cobalt(II) nitrate ($Co(NO_3)_2$)					
Chromosomal aberration	Diploid fibroblasts, WI_{38} and MRC_5	–	0.08 µM [0.015 µg/mL]		Paton & Allison (1972)
Chromosomal aberration	Mononuclear leukocytes	–	0.8 µM [0.15 µg/mL]		Paton & Allison (1972)
Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$)					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	(+)	800 µg/mL, 4 h (soluble and unfiltered fractions)	Cobalt content, 21%. Only one concentration tested. Marked induction of DNA strand breaks seemed to coincide with significant reduction in cell number and thus cytotoxic activity of the cobalt compound.	Kirkland et al. (2015)
Oxidative DNA damage (comet assay with hOGG1 enzyme)		(-)			
Cobalt(II) octoate					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	+	800 µg/mL (soluble fraction); 200 µg/mL (total fraction), 4 h	Cobalt content, 17%.	Kirkland et al. (2015)
Oxidative DNA damage (comet assay with hOGG1 enzyme)		+	800 µg/mL, 4 h		Kirkland et al. (2015)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Insoluble cobalt (II or II,III) compounds					
Cobalt(II) oxide (CoO)					
Chromosome aberrations	Human primary normal bronchial epithelial cells, NHBE	+	0.1 µg/cm², 24 h	Size, ≤ 10 µm. Same comments as above for soluble $CoCl_2 \cdot 6H_2O$.	Xie et al. (2016)
Chromosome aberrations	Human bronchial fibroblasts, WTHBF-6 (hTERT immortalized clonal cell line derived from primary human bronchial fibroblasts)	+	0.5 µg/cm², 24 h	Size (average), 1 µm (0.27–3.56 µm). Same comments as above for soluble $CoCl_2 \cdot 6H_2O$.	Smith et al. (2014)
Chromosome aberrations	Human urothelial cells, hTU1–38 (hTERT immortalized subclone of human urothelial hTU1cell line)	+	1 µg/cm², 24 h	Particle size, ≤ 10 µm. Same comments as above for soluble $CoCl_2 \cdot 6H_2O$.	Speer et al. (2017)
Cobalt(II,III) oxide (Co_3O_4)					
DNA strand breaks, and alkali-labile sites (comet assay) Oxidative DNA damage (comet assay with Fpg and hOGG11enzymes)	Human bronchial epithelial cell line, BEAS-2B	+ + (with Fpg or hOGG1)	10 µg/mL, 2 h 2.5 µg/mL, 24 h 2.5 µg/mL, 2 h and 24 h, Fpg 1.25 µg/mL, 2 h, and 24 h, hOGG1	Purity, 98.4%. Co_3O_4 particles were mainly aggregated and exhibited a polyhedral structure with heterogeneous sizes: 100–400 nm. Concomitant assessment of secondary physicochemical properties in cell culture medium (DLS) and cytotoxicity/apoptosis. Comparison with $CoCl_2 \cdot 6H_20$. [Very comprehensive study.] Oxidative DNA damage was observed after Co particles (10–1000 nm) (10–20 µg/mL) and $CoCl_2$ (1.25–10 µg/mL) treatment. No data for ROS production or oxidative stress markers.	Uboldi et al. (2016)
DNA double-strand breaks (γH2AX assay)		+	2.5 µg/mL		Uboldi et al. (2016)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549	(+)	20 µg/cm²	Size, < 10 µm. Only 2 concentrations tested, positive at the lower concentration only, Good physicochemical characterization of particles. Concomitant evaluation of ROS and formation of 8-oxo-dG adducts.	Kain et al. (2012)
Oxidative DNA damage (comet assay with Fpg enzyme)		–	40 µg/cm²		
DNA strand breaks and alkali labile sites (comet assay)	Human bronchial epithelial cell line, BEAS-2B	(+)	40 µg/cm²	Size, < 10 µm. Only 2 concentrations tested; positive at the higher concentration only. Good physicochemical characterization of particles; concomitant evaluation of ROS and formation of 8-oxo-dG adducts.	Kain et al. (2012)
Oxidative DNA damage (comet assay with Fpg enzyme)		+	20 µg/cm²		
Micronucleus formation (CBMN assay)	Human bronchial epithelial cell line, BEAS-2B	+	1.25 µg/mL	Purity, 98.4%. Same comment as for DNA strand breaks results above.	Uboldi et al. (2016)
Cobalt(II) oxide (CoO) NPs					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human bronchial epithelial cells, HBEC3-kt (immortalized bronchial epithelial cells)	(+)	60 µg/mL, 3 h	Metal purity, 99.99%; mainly spherical and fused into large agglomerates with a pristine dimension of 43 ± 9 nm. Only 2 concentrations tested; only positive at the higher concentration tested; no concentration–response. Concurrent assessment of cytotoxicity, ROS formation, [Quite comprehensive study.]	Cappellini et al. (2018)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549	(+) (+) (Fpg)	60 µg/mL, 24 h 60 µg/mL, 24 h	Only positive at the highest concentration tested; no concentration–response relationship.	Cappellini et al. (2018)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Cobalt(II,III) oxide (Co_3O_4) NPs					
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human lung alveolar cell line, A549; human bronchial epithelial cells	–	60 µg/mL, 3 h and 24 h	Size, 51 ± 11 nm. Concurrent assessment of cytotoxicity, ROS formation.	Cappellini et al. (2018)
Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549	– (Fpg)	60 µg/mL, 3 h and 24 h		Cappellini et al. (2018)
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Human hepatocellular carcinoma-derived cell line, HepG2; human colon cancer cell line, Caco-2 cells; human neuroblastoma cell line, SH-SY5Y	–	100 µg/mL, 24 h	Size, 47.0 ± 20.3 nm. Concomitant assessment of secondary physicochemical properties in cell culture medium (TEM and DLS); uptake analysis; cytotoxicity/apoptosis and oxidative damage assessment.	Abudayyak et al. (2017)
	Human lung alveolar cell line, A549	+	0.1 µg/mL, 24 h	Concentration–response relationship but low level of induction of DNA breaks.	
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Lymphocytes	(+)	100 µg/mL, 24 h	Diameter, 96.4 ± 0.57 nm. Characterization of secondary physicochemical properties. Concurrent assessment of cytotoxicity and oxidative stress. A single concentration tested.	Rajiv et al. (2016)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human bronchial epithelial cell line, BEAS-2B	+ + (Fpg sites)	40 µg/mL, 2 h and 24 h 5 µg/mL, 2 h and 24 h	Purity, ≤ 99.5%; elongated hexagonal shape; d_{50}, 22.1 ± 7.2 nm. Direct DNA damage: dose-dependent trend, statistically significant only at the higher concentration tested. Concurrent evaluation of cytotoxicity and immunotoxicity.	Cavallo et al. (2015)
DNA single-strand breaks and alkali-labile site (alkaline comet assay) Oxidative DNA damage (comet assay with Fpg enzyme)	Human lung alveolar cell line, A549	+ + (Fpg sites)	40 µg/mL, 2 h 20 µg/mL, 24 h 20 µg/mL, 2 h and 24 h	Purity, ≤ 99.5%; elongated hexagonal shape; d_{50}, 22.1 ± 7.2 nm.	Cavallo et al. (2015)

Table 4.3 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA single-strand breaks and alkali-labile site (alkaline comet assay)	Hepatocellular carcinoma-derived cell line, HepG2	+	10 µg/mL, 24 h 5 µg/mL, 48 h	Polygonal shape; size, ~21 nm.	Alarifi et al. (2013)
Chromosomal aberration	Lymphocytes	(+)	100 µg/mL, 1 h	Diameter: 96.4 ± 0.57 nm. Characterization of secondary physicochemical properties. Concurrent assessment of cytotoxicity and oxidative stress. A single concentration tested.	Rajiv et al. (2016)
Organic cobalt(II) compounds					
Cobalt(II) resinate					
Chromosomal aberration	Lymphocytes	(–) with S9 (+) with S9	300 µg/mL, (–S9) 37.5 or 75 µg/mL, (+S9), 3 h	Purity, 83.7%. Data from 3 (–S9) and 4 (+S9) experiments. Cytotoxicity evaluated by mitotic index. Results difficult to interpret due to inconsistencies; the impact of cytotoxicity is unclear because of the confounding factor of precipitation.	Kirkland et al. (2015)

8-oxodG, 8-oxo-2′-deoxyguanosine; CBMN, cytokinesis-block micronucleus; Co, cobalt; Cr, chromium; d_{50}, median diameter; DLS, dynamic light scattering; Fpg, formamidopyrimidine DNA glycosylase; γH2AX, gamma-H2A histone family member X; HIC, highest ineffective concentration; hOGG1, human 8-oxoguanine DNA *N*-glycosylase-1; hTERT, human telomerase reverse transcriptase; LEC, lowest effective concentration; M-FISH, multicolour fluorescence in situ hybridization; NAC, *N*-acetyl cysteine; Ni, nickel; NP, nanoparticle; ppb, part per billion; ROS, reactive oxygen species; TEM, transmission electron microscopy; W, tungsten.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

[b] Refers to the same experiment in which Co and Co with tungsten carbide were compared.

for measurements of DNA single-strand breaks or micronuclei formation in peripheral blood lymphocytes were found between exposed groups and controls. The frequency of micronuclei in binucleated cells (MNCB) was influenced by cobalt-only plant exposure and smoking as an interaction term (P = 0.0011). [The Working Group noted that a key limitation of this study was the reliance on a single spot urine sample. The single sample may not reflect the relevant exposure window for all outcomes.]

The association between urinary metal levels and sperm DNA damage, as measured by comet assay, was assessed in partners of Chinese fertility clinic patients in a cross-sectional study by Wang et al. (2016a). No significant (or suggestive) associations were found between urinary cobalt levels and sperm DNA integrity parameters after adjustment for age, BMI, abstinence time, smoking status, daily cigarette consumption, and concentrations of urinary creatinine and other metals, nor for multiple testing. [The Working Group noted that collection of urine samples at two points close in time on the same day as semen sample collection limits the findings.]

[The Working Group noted that three of four studies reported some evidence of cobalt-associated genotoxicity, including DNA strand breaks and micronucleus formation in binucleated cells.]

Three other studies examined the effects of cobalt exposure in humans: L'vova et al. (1990), Gennart et al. (1993), and Wultsch et al. (2017). [After evaluation, the Working Group excluded these studies. In L'vova et al. (1990), the study description was considered inadequate for the evaluation of the exposure assessment and the findings. The study by Gennart et al. (1993) was deemed uninformative because of the population's mixed exposure to other carcinogens, including chromium and nickel, without any regression analysis to isolate the effects of cobalt. Similarly, the study by Wultsch et al. (2017) was excluded because the selected population was co-exposed to chromium and probably to other metals, including Ni, and no regression analysis was performed to isolate correlations with cobalt. The potential for differential exposure misclassification is low. In addition, the authors relied on a single biological measure of exposure.]

(ii) Human cells in vitro

The results of the genotoxic effects of exposure to cobalt metal or cobalt(II) salts, including cobalt-based NPs, in human cells are summarized in Table 4.3.

Cobalt metal

Cobalt metal exposure was able to induce DNA strand breaks in human primary lymphocytes (Anard et al., 1997; De Boeck et al., 1998) and in mononuclear leukocytes (Van Goethem et al., 1997), although a negative result was also reported in leukocytes (De Boeck et al., 2003). Cobalt exposure did not induce formamidopyrimidine DNA glycosylase (Fpg)-sensitive sites in mononuclear leukocytes (De Boeck et al., 1998). Cobalt metal did not induce DNA breaks in non-tumourigenic osteoblast-like cells (Miller et al., 2001). However, it induced a significant and concentration-dependent increase in micronucleus formation frequencies in lymphocytes (Van Goethem et al., 1997; De Boeck et al., 2003) and in human osteoblast-like cells (Miller et al., 2001).

Soluble cobalt(II) salts

Cobalt(II) chloride induced DNA strand breaks in human primary leukocytes and lymphocytes (McLean et al., 1982; De Boeck et al., 1998). In addition, an artificial salt solution containing Co^{2+} at a concentration of 0.84 µM caused a low but significantly increased level of DNA damage, as assessed by alkaline comet assay, in human primary fibroblasts (Davies et al., 2005). However, more recent studies using the same assay reported no induction of DNA lesions in primary blood leukocytes (Colognato et al., 2008) and primary T-cells exposed to cobalt(II) chloride (Jiang et al., 2012).

The induction of DNA strand breaks after exposure to cobalt(II) chloride was shown in HSBP cells (normal human foreskin fibroblasts, diploid) through an alkaline sucrose gradient or nick translation (but not by nucleoid sedimentation assay) (Hamilton-Koch et al., 1986), and in fetal fibroblast cells (Qiao & Ma, 2013), as assessed by the single cell array-based halo assay developed by the authors. In line with these reports, Gault et al. (2010) showed the induction of DNA damage in a human keratinocyte cell line, as measured by comet assay. Weakly positive results were detected in the human hepatoma-derived HepG2 (Alarifi et al., 2013) and Jurkat CD4+ T-cell (Caicedo et al., 2008) lines, the latter using the neutral version of the comet assay that enables measurement of DNA double-strand breaks.

Cobalt(II) chloride induced sister-chromatid exchange (Andersen, 1983) and aneuploidy (Resende de Souza-Nazareth, 1976) in human primary lymphocytes. Using the cytokinesis-block micronucleus assay, a significant and concentration-dependent increase in micronuclei frequency and a parallel decrease in the cytokinesis-block proliferation index (a measure of cytostasis) were reported in human primary lymphocytes exposed to cobalt(II) chloride, suggesting clastogenic/aneugenic potential (Colognato et al., 2008).

More recently, Patel et al. (2012) investigated the effects of cobalt(II) chloride alone, nickel chloride ($NiCl_2$), or a mixture of the two on a human lung carcinoma cell line (H460) using the phosphorylation of histone H2AX (γH2AX foci formation) as a marker of double-strand breaks. A significant induction of γH2AX foci formation was reported for cobalt(II) chloride, and a concomitant induction of reactive oxygen species (ROS) was found. The measured reduction of double-strand breaks after pre-treatment with an antioxidant agent (*N*-acetyl cysteine) suggested that ROS formation may underlie the genotoxicity of cobalt(II) chloride (Patel et al., 2012) (see also Section 4.2.5 and Table 4.13). The capacity of a single concentration of cobalt(II) chloride (100 μM) to induce the formation of γH2AX foci was additionally explored in several *KRAS*-mutant intestinal cancer cell lines (HCT 116, SW620, and LoVo cells), resulting in positive responses (Zhong et al., 2020). Furthermore, the same cobalt(II) chloride concentration (with treatment referred to as mimicking a hypoxic environment in cancer cells) markedly downregulated expression levels of *RAD51* and the percentage of *RAD51* focus-positive cells, indicating compromised capacity for homologous recombination repair (see Section 4.2.3). Cobalt(II) chloride tested at a single concentration (200 μM) was also observed to have the capacity to impair human topoisomerase IIα activity in the MCF7 cell line (Baldwin et al., 2004), as assessed by topoisomerase II-mediated DNA cleavage assay. [The Working Group noted that the results of parallel acellular assays further supported the idea that this compound may act as a topoisomerase II poison, suggesting that this mechanism contributes to its genotoxicity.]

Cobalt(II) chloride hexahydrate did not induce DNA strand breaks in human primary lymphocytes (Anard et al., 1997). In contrast, this cobalt(II) salt generated DNA strand breaks in the HeLa cell line, as assessed by nucleoid sedimentation assay (Hartwig et al., 1990), and in the human bronchial epithelial (BEAS-2B) cell line, as assessed by comet assay (Uboldi et al., 2016). Furthermore, using a modified version of the comet assay with the formamidopyrimidine glycosylase (Fpg) and human 8-oxoguanine DNA *N*-glycosylase-1 (hOGG1) enzymes to detect oxidized bases, positive results were also reported, indicating induction of oxidative DNA damage (Uboldi et al., 2016) (see also Section 4.2.5 and Table 4.13).

The ability of cobalt(II) chloride hexahydrate to induce numerical and/or structural chromosomal alterations has been assessed in human primary fibroblasts (Figgitt et al., 2010) and

human primary normal bronchial epithelial cells (Xie et al., 2016), and in other cell lines representative of the respiratory (WTHBF-6 cells) and urothelial (hTU1-38 cells) tracts (Smith et al., 2014; Speer et al., 2017). Of note, in two studies, the concentration ranges for cell treatment were selected on the basis of physiologically relevant exposure levels measured in samples of peripheral blood (Figgitt et al., 2010) or urine (Speer et al., 2017) collected from patients who had received cobalt-containing implants. All studies that analysed unstable chromosomal damage showed significant induction of chromosomal aberrations, with chromatid breaks being the aberration most frequently observed (Smith et al., 2014; Xie et al., 2016; Speer et al., 2017). By contrast, Figgitt et al. (2010), using full multicolour fluorescence in situ hydridization to analyse translocation and aneuploidy, found predominantly numerical chromosomal aberrations in primary fibroblasts, suggesting that this cobalt(II) salt displays aneugenic potential. The positive results described by Uboldi et al. (2016) with an in vitro cytokinesis-block micronucleus assay and a γH2AX assay in the BEAS-2B cell line support the clastogenic and/or aneugenic effects of this soluble cobalt(II) salt in immortalized bronchial cells.

Cobalt(II) acetate tetrahydrate did not induce chromosomal aberrations in human primary lymphocytes (Voroshilin et al., 1978).

Cobalt(II) nitrate did not induce chromosomal aberrations in human primary mononuclear leukocytes (Paton & Allison, 1972) or diploid fibroblasts (WI-38 or MRC-5 cells).

Cobalt(II) sulfate heptahydrate caused a marked induction of DNA breaks (as assessed by comet assay) after exposure of the A549 cell line (lung alveolar epithelial-like cells) to a single concentration of the compound (800 μg/mL, 4 hours), but no oxidative DNA damage was detected after treatment with the hOGG1 enzyme (Kirkland et al., 2015). [The Working Group noted that induction of DNA damage seemed to coincide with cytotoxic activity of the compound, and therefore the results should be interpreted with caution.]

Cobalt(II) octanoate, at a concentration of 800 (soluble fraction) or 200 (total fraction) μg/mL, induced a significant increase in DNA strand breaks and oxidative DNA damage in A549 cells, as assessed by comet assay without and with the hOGG1 enzyme (Kirkland et al., 2015).

Insoluble cobalt(II or II,III) compounds

Cobalt(II) oxide particles have consistently been observed to have the capacity to induce chromosomal aberrations in different types of human cells. Speer et al. (2017) showed that cobalt(II) oxide particles (size, up to 10 μm), tested at physiologically relevant exposure levels, are more genotoxic than soluble cobalt (cobalt(II) chloride hexahydrate) in human immortalized urothelial (hTU1-38) cells. However, at similar intracellular cobalt ion concentrations, particulate and soluble cobalt induced similar extents of chromosomal damage, the most common being chromatid lesions. The results were in line with those previously reported in human immortalized bronchial fibroblasts exposed to cobalt oxide particles (d_{50}, 1 μm) when compared with soluble cobalt (Smith et al., 2014). Of note, the induction of chromosomal damage by soluble and insoluble cobalt was also observed by Xie et al. (2016) using human primary bronchial epithelial cells; however, at similar intracellular cobalt levels, the frequency of chromosomal aberrations induced by cobalt(II) oxide particles was lower than that induced by soluble cobalt(II) salt.

Cobalt(II,III) oxide particles differing in their physicochemical properties (e.g. size) have been observed to have slightly different genotoxicity outcomes. Cobalt(II,III) oxide particles with a high degree of purity, that were poorly soluble and had a polyhedral structure and constituted sizes ranging from 100 to 400 nm, induced micronuclei and the formation of γH2AX foci (a marker of DNA double-strand breaks) in the BEAS-2B

cell line (Uboldi et al., 2016). In addition, the authors reported the induction of primary DNA lesions and oxidative DNA damage (see also Section 4.2.5 and Table 4.13), as assessed by modified comet assay with Fpg and hOGG1 enzymatic cleavage. Comparison with the results obtained in parallel for a soluble cobalt(II) salt showed that the observed genotoxicity of cobalt(II,III) oxide particles is induced by the particles themselves and cannot be solely attributed to the release of Co^{2+} ions (Uboldi et al., 2016). In previous work, larger cobalt(II,III) oxide particles (size, < 10 µm) induced DNA breaks in the BEAS-2B and A549 cell lines, but only at a single concentration (Kain et al., 2012). However, oxidative DNA damage was solely detected in the BEAS-2B cells and at a single concentration of particles, as assessed by Fpg-modified comet assay, while some interference of particles with the Fpg enzyme was noted (see also Section 4.2.5).

Cobalt-based nanoparticles

Cobalt metal NPs have been assessed for genotoxicity in vitro using mainly conventional assays, such as the comet assay, the γH2AX assay, and micronuclei formation in human primary cells and lung cell lines. Exposure of human primary blood mononuclear cells to cobalt metal NPs (d_{50}, 246 nm, or d_{50}, 50 nm) has resulted in consistently increased levels of DNA strand breaks and alkali-labile sites, as assessed by comet assay (Colognato et al., 2008; Jiang et al., 2012), and (cobalt metal NPs: d_{50}, 246 nm) have induced micronuclei formation in cytokinesis-blocked lymphocytes (Colognato et al., 2008). Zhu et al. (2021b) reported the induction of DNA single- and double-strand breaks by a single concentration of cobalt metal NPs (size, 50–200 nm) in human primary haematopoietic stem cells and haematopoietic progenitor cells (isolated from cord blood). Positive findings have been also reported in cells representative of the respiratory tract. Cappellini et al. (2018) reported induction of Fpg-sensitive sites (assessed by Fpg-modified comet assay) after exposure of the A549 cell line and induction of DNA strand breaks in immortalized human bronchial epithelial cells to the highest tested concentration (40 µg/mL, for 3 and 24 hours) of cobalt metal NPs with different dimensions and shapes (average size, 25 ± 8.8 nm). Cobalt metal NPs were taken up by both cell lines, and parallel analyses showed that they induced ROS formation, which may mediate the observed genotoxicity (see also Section 4.2.5). In agreement with these findings, another study also showed that cobalt metal NPs (mean diameter, 20 nm) caused an increase in DNA single- and double-strand breaks, as assessed by comet and γH2AX assays, respectively (Wan et al., 2012). The observed genotoxicity was significantly attenuated by pre-treatment of the cells with ROS scavengers, which also abolished ROS induction (see also Section 4.2.5).

Cobalt(II) oxide NPs (size, 43 ± 9 nm) (Cappellini et al., 2018) were tested for genotoxicity in immortalized human bronchial epithelial cells and in the alveolar A549 cell line with positive results, as assessed by standard (tested in both cells) and Fpg-modified (tested in A549 cells only) comet assays for the highest concentration tested (Cappellini et al., 2018). Other end-points were simultaneously analysed, such as cytotoxicity and ROS formation (see also Section 4.2.5).

Cobalt(II,III) oxide NPs were assessed for genotoxicity in various human cell lines, mainly by comet assay (and modified versions of it with DNA repair enzymes), generating mixed results (Alarifi et al., 2013; Cavallo et al., 2015; Rajiv et al., 2016; Abudayyak et al., 2017; Cappellini et al., 2018). A study using human primary lymphocytes reported the induction of DNA strand breaks and chromosomal aberrations by cobalt(II,III) oxide NPs (diameter, 96.4 ± 0.57 nm), at a concentration of 100 µg/mL (Rajiv et al., 2016). In a study by Abudayyak et al. (2017), human hepatoma (HepG2), intestine (Caco-2), and nervous system (SH SY5Y) cell lines were exposed to dose ranges (up to 100 µg/mL) of

Table 4.4 Genetic and related effects of cobalt in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Cobalt metal (Co)							
Gene mutation (K-*Ras*, *Egfr*, *Tp53*)	Mouse, $B6C3F_1$/N (M/F)	Alveolar/ bronchiolar carcinomas	+	All exposed groups combined (1.25, 2.5, and 5 mg/m^3)	Inhalation, 2 yr	Evaluation of mutation spectra in cobalt-caused cancers. The spectra were compatible with oxidative damage.	NTP (2014)
	Rat, F344/ NTac (M/F)		+	All exposed groups combined (1.25, 2.5, and 5 mg/m^3)		Evaluation of mutation spectra in cobalt-caused cancers. The spectra were compatible with oxidative damage.	
Micronucleus formation	Mouse, $B6C3F_1$ (M/F)	Peripheral blood NCEs	–	10 mg/m^3	Inhalation, 90 days, 3 dose levels	Guideline-based study, no decrease in PCE/NCE ratio. No bone marrow toxicity observed but general toxicity observed at the highest dose.	NTP (2014)
8-Oxo-dG adduct formation (immunohistochemistry)	Mouse, $B6C3F_1$/N (M/F)	Lung	+	10 mg/m^3	Inhalation, 90 days		Ton et al. (2021)
Cobalt metal (Co) NPs							
Gene mutation (*Gpt* locus)	Mouse, G*pt* delta transgenic (M/F)	Lung	+	50 µg/mouse	Intratracheal installation	Only one dose level.	Wan et al. (2017)
Oxidative DNA damage (8-OHdG, ELISA)	Mouse, *Gpt* delta transgenic (M/F)	Lung	+	50 µg/mouse	Intratracheal installation	Only one dose level.	Wan et al. (2017)
Soluble cobalt(II) salts							
Cobalt(II) chloride ($CoCl_2$)							
Aneuploidy, pseudodiploidy, and hyperploidy	Hamster (M)	Bone marrow and testes	(+)	400 mg/kg bw	Intraperitoneal injection, total dose given to each hamster over 9 days	Only one dose level, limited reporting, not sure if $CoCl_2$ anhydrous or hexahydrate. In testes, significant effect seen only in metaphase I, not in metaphase II.	Farah (1983)

Table 4.4 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Cobalt(II) acetate ($Co(CH_3CO_2)_2$)							
Oxidative DNA base damage (GC-MS)	Rat, F344/NCr (M/F)	Kidney, liver, and lung	+	50 µmol/kg bw [8.85 mg/kg]	Single intraperitoneal injection, evaluation 2 or 10 days after dosing, 2 dose levels	The bases determined were typical products of hydroxyl radical attack, mainly in liver and kidney.	Kasprzak et al. (1994)
Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)							
DNA damage (comet assay)	Rat, Sprague-Dawley (M)	Liver	(+)	10.37 ± 0.38 mg/day per rat	Oral via drinking-water, 4 wk exposure	Only one dose resulting in other liver effects (enzyme induction).	Khalil et al. (2020)
Micronucleus formation	Mouse, BALB/c AnNCj (M)	Bone marrow	+	50 mg/kg bw	Single intraperitoneal injection, 3 dose levels	Dose–response relationship observed, PCE/NCE ratio was decreased at the highest dose.	Suzuki et al. (1993)
Micronucleus formation	Mouse, Swiss albino (M)	Bone marrow	+	11.25 mg/kg bw	single intraperitoneal injection, 24 or 48 h after dosing, 3 dose levels	Dose-dependent increase, more significant effects 48 h after dosing, PCE/NCE ratio was unchanged or rather increased (highest dose, 48 h after the dosing).	Rasgele et al. (2013)
Chromosomal aberration	Mouse, Swiss albino (M)	Bone marrow	(+)	20 mg/kg bw	Oral, evaluation 6, 12, 18, and 24 h after dosing, 3 dose levels, 1×	Low number of cells analysed. Increases in chromosomal aberration frequencies and polyploid cells at all treatment times (including 6 h) raises questions.	Palit et al. (1991a)
Chromosomal aberration	Rat, Sprague-Dawley CD (M)	Testis (spermatogonia)	–	30 mg/kg bw	Gavage, 28 days, 3 dose levels	100 and 300 mg/kg resulted in overt toxicity. No concurrent positive control, but the laboratory had history of positive responses with mitomycin C.	Kirkland et al. (2015)

Table 4.4 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Rat, Wistar albino (M)	Bone marrow	(+)	300 mg/L in drinking-water [~36 mg/kg, estimated by the Working Group]	Oral via drinking-water, 7 days, 3 dose levels	The study had limited reporting (e.g. no information on the number of cells analysed or PCE/NCE ratios). No positive control, micronucleus induction several times that in other studies.	Awoyemi et al. (2017)
Micronucleus formation	Rat, Sprague-Dawley (M)	Bone marrow	–	600 mg/kg bw	Gavage, 3 dose levels, 1×	Non-published guideline-based study report, cited in ECHA (2016, 2017).	Study report, Gudi et al. (1998) in ECHA, (2016, 2017)
Chromosomal aberration	Rat, Sprague-Dawley (M)	Bone marrow	–	600 mg/kg bw	Gavage, 3 dose levels, 1×	Non-published guideline-based study report, cited in ECHA (2016, 2017).	Study report, Gudi et al. (1998) in ECHA, (2016, 2017)
Oxidative DNA damage (8-OHdG, ELISA)	Rat, Sprague-Dawley (M)	Kidney	(+)	300 mg/L in drinking-water	Oral via drinking-water, 4 wk	Only one dose, water consumption not reported, general toxicity seen as decreased weight gain, weights not reported.	Abdel-Daim et al., 2020
Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$)							
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	(–)	1000 mg/kg bw per day	Gavage, 2–5 days, 3 dose levels	Design complied with OECD guidelines, but deaths and early kills at two highest dose levels compromised the compliance. Two highest doses exceeded MTD, all groups were not evaluated and exposure duration of remaining rats was reduced.	Kirkland et al. (2015)

Table 4.4 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Insoluble cobalt (II or II,III) compounds							
Cobalt(II) oxide (CoO)							
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	–	2000 mg/kg bw per day	Gavage, 5 days, 3 dose levels	OECD guideline-based study, no major limitations.	Kirkland et al. (2015)
Cobalt tetraoxide (Co_3O_4)							
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	–	2000 mg/kg bw per day	Gavage, 5 days, 3 dose levels	OECD guideline-based study, no major limitations.	Kirkland et al. (2015)
Chromosomal aberration	Rat, Sprague-Dawley (Hsd:SD) albino (M/F)	Bone marrow	–	2000 mg/kg bw per day	Gavage, 2–4 days, 3 dose levels	Design complied with OECD guidelines, but deaths and early kills compromised the compliance. Two highest doses exceeded MTD, all study groups were not evaluated, and exposure duration of remaining rats was reduced.	Kirkland et al. (2015)
Organic cobalt(II) compounds							
Micronucleus formation	Mouse, Swiss Ico: OF1 (IOPS Caw) (M/F)	Bone marrow	–	500 mg/kg cobalt(II) acetyl acetonate	Gavage, dosing after 24 h, 3 dose levels	Highest dose resulted in deaths.	Kirkland et al. (2015)
Micronucleus formation	Mouse, Swiss Ico: OF1 (IOPS Caw) (M/F)	Bone marrow	–	1500 mg/kg cobalt(II) resinate	Gavage, dosing after 24 h, 3 dose levels	Highest dose resulted in decrease in PCE/NCE ratio.	Kirkland et al. (2015)

8-OHdG, 8-hydroxy-2′-deoxyguanosine; bw, body weight; ELISA, enzyme-linked immunosorbent assay; F, female; GC-MS, gas chromatography-mass spectrometry; *Gpt*, guanine phosphoribosyltransferase; HID, highest ineffective dose; LED, lowest effective dose; M, male; MTD, maximum tolerated dose; NCE, normochromatic erythrocyte; OECD, Organisation for Economic Co-operation and Development; PCE, polychromatic erythrocyte; wk, week; yr, year.

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality.

well-characterized cobalt(II,III) oxide NPs (size, 47.0 ± 20.3 nm), with no induction of DNA strand breaks observed. In contrast, a concentration-dependent increase in DNA breaks was observed in the A549 cell line, although within the low dose range of 0.1–100 µg/mL. In agreement with this finding, a previous study using the A549 and BEAS-2B cell lines showed that exposure to elongated hexagonal cobalt(II,III) oxide NPs (d_{50}, 22.1 ± 7.2 nm) resulted in a dose-dependent positive trend with a significant increase in DNA damage solely observed at the highest concentration tested (Cavallo et al., 2015). Alarifi et al. (2013) showed that low concentrations of polygonal NPs (size, approximately 21 nm) induced DNA damage in the HepG2 cell line. Cappellini et al. (2018) reported that cobalt(II,III) oxide NPs (size, 51 ± 11 nm) failed to induce DNA strand breaks and oxidative DNA damage in the A549 cell line and immortalized human bronchial epithelial cells, suggesting that cobalt(II,III) oxide NPs have lower genotoxic potential than do cobalt metal NPs and cobalt(II) oxide NPs (Cappellini et al., 2018).

Organic cobalt(II) compounds

Cobalt(II) resinate did not induce chromosomal aberrations in human primary lymphocytes. However, the presence of the liver S9 metabolic activation system significantly increased the frequency of chromosomal aberrations compared with the negative control (Kirkland et al., 2015). [The Working Group noted that the results are difficult to interpret due to inconsistencies among experiments, the impact of cytotoxicity, and the occurrence of precipitation.]

(b) Experimental systems

The genotoxicities of cobalt metal and cobalt(II) salts have been extensively assessed in experimental systems both in vivo and in vitro. In vivo data have been summarized in Table 4.4 according to the form of cobalt studied, including cobalt metal, soluble cobalt(II) salts, insoluble cobalt(II or II,III) compounds, and cobalt metal NPs. Table 4.5, Table S4.6, and Table S4.7 include in vitro genotoxicity studies in non-human mammalian cells, non-mammalian cells, and acellular systems, respectively (for Tables S4.6 and S4.7, see Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). Most of the data come from the cobalt(II) salts, most notably soluble cobalt(II) chloride. The data on cobalt metal are more limited when compared with the cobalt(II) salts. Although this evaluation is focused on cobalt metal and divalent cobalt salts, relevant data relating to some other cobalt compounds (like trivalent cobalt compounds) are briefly reviewed but not included in the tables.

(i) Non-human mammals in vivo

See Table 4.4.

Cobalt metal

There is only one in vivo animal study available on the genotoxicity of cobalt metal. NTP (2014) assessed micronucleus formation in peripheral blood normochromatic erythrocytes of male and female mice after 3 months of whole-body inhalation exposure to metallic cobalt at concentrations of 0.625–10 mg/m^3. No increases in the frequencies of micronucleus formation were seen. Although exposure to the highest dose resulted in decreased body weight and lung toxicity, no bone marrow toxicity (measured as a change in the percentage of polychromatic erythrocytes, PCEs) was observed. Immunohistochemical analysis of 8-oxo-2′-deoxyguanosine (8-oxo-dG) in the lungs of both male and female mice at the highest dose (10 mg/m^3) revealed oxidative DNA damage to be significantly increased compared with non-exposed controls (see also Section 4.2.5) (Ton et al., 2021).

Analysis of the mutational spectra of lung tumours induced by cobalt metal in the NTP (2014) studies in mice and rats showed mutations

(especially G→T transversions) in K-*Ras*, *Tp53*, and *Egfr* genes compatible with oxidative DNA damage (Hong et al., 2015). [The Working Group noted that cobalt was one of just three carcinogens that showed specific carcinogen-induced genomic signatures when lung tumours caused by 20 different chemicals were analysed (Riva et al., 2020).]

Soluble cobalt(II) salts

Few in vivo genotoxicity studies have been reported that have investigated soluble cobalt(II) salts. Four studies performed using intraperitoneal administration of cobalt(II) salts have resulted in consistently positive genotoxic responses. These include an early study by Farah (1983), which reported increased numbers of hyperploid and pseudodiploid cells in the bone marrow of Syrian golden hamsters after repeated intraperitoneal administration of cobalt(II) chloride. In testis, an increased number of bivalents during meiotic metaphase I (but not during metaphase II) was reported. Suzuki et al. (1993) reported increased frequency of micronuclei in PCEs of BALB/c mice after single intraperitoneal administration of cobalt(II) chloride. In this study, cobalt chloride was shown also to enhance the genotoxicity of the known genotoxic agents 1,1-dimethylhydrazine and benzo[*a*]-pyrene, which was partly considered to be due to an acceleration of erythropoiesis (Suzuki et al., 1993). An increase in bone marrow PCE micronuclei frequencies in Swiss albino mice after intraperitoneal administration of cobalt(II) chloride has also been reported by Rasgele et al. (2013). The most significant increases were seen 48 hours after treatment.

Dose-dependent increases in oxidative DNA damage were seen in kidney, liver, and lung tissues in rats treated by intraperitoneal administration of cobalt(II) acetate 2 and 10 days before tissue collection. The most significant increases were seen in kidney and liver, which is in accordance with the distribution of soluble cobalt(II) salts after intraperitoneal administration (Kasprzak et al., 1994) (see also Table 4.4 and Section 4.2.5).

The data available relating to genotoxicity after oral administration are less clear. One study has reported DNA damage, as assessed by comet assay, in liver after 4-week exposure to cobalt(II) chloride in drinking-water (reported cobalt dose, 10.37 ± 0.38 mg/day per rat) (Khalil et al., 2020). [The Working Group noted that the study included only one dose level, which appeared to cause liver toxicity indicated by significant elevations in liver enzyme and bilirubin levels.] Palit et al. (1991a) reported dose- and time-dependent increases in chromosomal aberration frequencies in bone marrow in mice 6–24 hours after oral administration by gavage of a single dose of cobalt(II) chloride (0, 20, 40 or 80 mg/kg bw). Similar dose responses were reported at all sampling times tested. [The Working Group noted that this finding, suggesting similar effects at all stages of the cell cycle, is a rather uncommon finding for genotoxicants.] Also, the frequency of several polyploid cells was increased at all time points. [The Working Group noted that the increases in the number of polyploid cells starting at the 6-hour time point is hard to explain, which raises questions about the reliability of this study.] The same group has also published related studies evaluating the modifying roles of calcium and chlorophyllin on the clastogenicity of cobalt(II) chloride (Ghosh et al., 1991; Palit et al., 1991b). Since these studies report the same cobalt(II) chloride results as Palit et al. (1991a), they have not been included in Table 4.4. Kirkland et al. (2015), on the other hand, reported negative results from a set of guideline-based chromosomal aberration studies in rats after oral administration, by gavage, of different (soluble and insoluble) cobalt(II) salts: cobalt(II) sulfate heptahydrate (100, 300, or 1000 mg/kg bw per day), tricobalt tetraoxide (Co_3O_4, synonym of cobalt tetraoxide or cobalt(II,III) oxide) (200, 600, or 2000 mg/kg bw per day), or cobalt monoxide (CoO, synonym

Table 4.5 Genetic and related effects of cobalt in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Cobalt metal						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	+	30 µg/mL	OECD guideline 476, high level of cobalt powder precipitation was observed.	Kirkland et al. (2015)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	350 µg/mL	OECD guideline 476, extracts of cobalt metal powder were tested.	Kirkland et al. (2015)
Cobalt metal NPs						
DNA strand breaks (comet assay)	Mouse (BALB/3T3), fibroblasts	+	NT	1 µM	No dose–response, but high cytotoxicity at highest doses.	Ponti et al. (2009)
Micronucleus formation	Mouse (BALB/3T3), fibroblasts	+	NT	1 µM		Ponti et al. (2009)
DNA strand breaks, comet assay	Rat, kidney, NRK cells	(+)	NT	100 µM	Diameter, 20–50 nm. Single dose only.	Liu et al. (2017)
DNA strand breaks (comet assay)	Rat, liver, BRL-1A cells	(+)	NT	10 µM	Median size, 30–70 nm. The study does not provide quantitative results from the comet assay.	Liu et al. (2016)
DNA damage (comet assay without Fpg enzyme)	Mouse, embryo, fibroblasts (wildtype *Ogg*$^{+/+}$ and MEF *Ogg*$^{-/-}$ cells)	–	NT	1 µg/mL	Size, < 50 nm. No change in % tail DNA	Annangi et al. (2015)
Oxidative DNA damage (comet assay with Fpg enzyme)		+ (*Ogg*$^{-/-}$) – (*Ogg*$^{+/+}$)	NT NT	0.1 µg/mL 1 µg/mL	Dose-dependent effect observed only in Ogg1 knockout (mouse embryonic fibroblast *Ogg1*$^{-/-}$) cells but not in wildtype cells.	Annangi et al. (2015)
DNA strand breaks (comet assay)	Rat, CD34+ haematopoietic stem cells and haematopoietic progenitor cells	(+)	NT	200 µM [11.8 µg/mL]	Size, 50–200 nm. One dose only.	Zhu et al. (2021b)
Oxidative DNA damage (8-OHdG, ELISA)		(+)	NT	200 µM	One dose only	Zhu et al. (2021b)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Activation of γH2AX		(+)	NT	200 µM	One dose only.	Zhu et al. (2021b)
Rtkn-GFP and Srxn1-GFP reporter activity	Mouse embryonic stem cells (mES)	↑ Rtkn-GFP ↑ Srxn1-GFP	NR	Co (0–10 µg/mL), CoO and Co_3O_4 (0–100 µg/mL), 24 h	Assessment of (geno)toxicity including oxidative stress using ToxTracker assay.	Cappellini et al. (2018)
Soluble cobalt(II) salts						
Cobalt(II) chloride (hydrate not specified)						
DNA strand breaks (alkaline sucrose gradient)	Chinese hamster, ovary	+	NT	5 mM [650 µg/mL]		Hamilton-Koch et al. (1986)
DNA strand breaks (nucleoid sedimentation assay)	Chinese hamster, ovary	–	NT	10 mM [1300 µg/mL]		Hamilton-Koch et al. (1986)
DNA–protein cross links	Rat, Novikoff ascites hepatoma cells	+	NT	1 mM [130 µg/mL]		Wedrychowski et al. (1986)
DNA damage, integrity of nuclear genome (qPCR assay)	Rat, neuronal PC-12 cells	–	NT	100 µM [13 µg/mL]	Negative response in nuclear genome, but positive mitochondrial DNA (which might be related to hypoxia).	Wang et al. (2000)
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cells	(+)	NT	200 µMM [26 µg/mL]	Weak response and only one dose with high cytotoxicity.	Miyaki et al. (1979)
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cell	–	NT	100 µM [13 µg/mL]		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, V79 *Gpt*⁺ transgenic cell line G10	–	NT	100 µM [13 µg/mL]		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, *Gpt*⁺ transgenic cell line G12	+	NT	50 µM [6.5 µg/mL]		Kitahara et al. (1996)
Sister-chromatid exchange	Mouse, macrophage-like cells, $P388D_1$	+	NT	100 µM [13 µg/mL]		Andersen (1983)
Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_20$)						
DNA strand breaks (comet assay)	Mouse (BALB/3T3), fibroblasts, mouse	+	NT	1 µM [0.24 µg/mL]		Ponti et al. (2009)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA degradation (flow cytometry)	Mouse, heart, HL-1	(+)	NT	500 µM [120 µg/mL]	One dose only. Glucocorticoids protected from the cobalt-induced DNA damage.	Cruz-Topete et al. (2016)
Gene mutation (*Tk* locus)	Mouse, lymphoma L5178Y cells	–	NT	57.11 µg/mL		Amacher & Paillet (1980)
Gene mutation (*Hprt* locus, *8AG*-resistant mutation)	Chinese hamster, V79 cells	–	NT	9 µM [2 µg/mL]	Low dose compared with in V79 cells Hartwig et al. (1990).	Yokoiyama et al. (1990)
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cells	+	NT	100 µM [24 µg/mL]		Hartwig et al. (1990)
Gene mutation (*Hprt* locus)	Mouse, mammary carcinoma, FM3A cells	(+)	NT	200 µM [48 µg/mL]	Modified HPRT assay, only weak response.	Morita et al. (1991)
Sister-chromatid exchange	Chinese hamster, V79 cells	+	NT	10 µM [2.4 µg/mL]		Hartwig et al. (1991)
Micronucleus formation	Mouse (BALB/c), bone marrow cells, PCE	–	–	50 µg/mL		Suzuki et al. (1993)
Micronucleus formation	Mouse (BALB/3T3), fibroblasts	–	NT	10 µM [2.4 µg/mL]		Ponti et al. (2009)
Cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$)						
Micronucleus formation	Syrian hamster, embryo	+	NT	1 µg/mL		Gibson et al. (1997)
Cobalt(II) sulfate ($CoSO_4$)						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	100 µg/mL	OECD guideline test, including extended 24 h treatment time.	Kirkland et al. (2015)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	120 µg/mL	Cobalt(II) 2-ethylhexanoate ($C_8H_{15}CoO_2$+).	Kirkland et al. (2015)
Insoluble cobalt(II or II,III) compounds						
Cobalt(II) sulfide (CoS)						
DNA strand breaks (alkaline sucrose gradient)	Chinese hamster, ovary, CHO cells	+	NT	10 µg/mL		Robison et al. (1982)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation (*Hprt* locus)	Chinese hamster, V79 cells	–	NT	1 µg/cm²		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, transgenic cell line G10	–	NT	1 µg/cm²		Kitahara et al. (1996)
Gene mutation (*Gpt* locus)	Chinese hamster, transgenic cell line G12	+	NT	0.5 µg/cm²		Kitahara et al. (1996)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	922 µg/mL	OECD guideline test, including extended 24 h treatment time.	Kirkland et al. (2015)
Cobalt(II) oxide (CoO)						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	60 µg/mL	OECD guideline test, including extended 24 h treatment time.	Kirkland et al. (2015)
Cobalt tetraoxide						
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	2000 µg/mL without S9 750 µg/mL with S9	OECD guideline test.	Kirkland et al. (2015)
Cobalt dihydroxide						
Gene mutation, *Hprt* locus	Mouse, lymphoma L5178Y cells	–	+/–	30 µg/mL	Positive responses in 2 out of 3 experiments at the highest dose showing high cytotoxicity.	Kirkland et al. (2015)
Cobalt(II,III) oxide NPs						
DNA strand breaks (comet assay)	Rat, cardiomyocytes	+	NT	5 µg/mL	Co_3O_4 NPs; non-spherical; mean diameter, 17 nm; forming agglomerates of tens of NPs.	Savi et al. (2021)
Organic cobalt(II) compounds						
Gene mutation (*Tk* locus)	Mouse, lymphoma L5178Y *Tk*± cells	–	–	100 µg/mL	Cobalt resinate ($C_{40}H_{58}CoO_4$): top two doses resulted in precipitation.	Kirkland et al. (2015)

Table 4.5 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation (*Tk* locus)	Mouse, lymphoma L5178Y *Tk*$^{\pm}$ cells	+/–	–	91.625 µg/mL	Cobalt acetyl acetonate ($C_{10}H_{14}CoO_4$): positive responses mainly at the highest dose in the presence of significant cytotoxicity.	Kirkland et al. (2015)
Gene mutation (*Hprt* locus)	Mouse, lymphoma L5178Y cells	–	–	70 µg/mL	Cobalt oxalate (CoC_2O_4): highest dose resulted in precipitation, cytotoxicity noted at lower concentrations as well.	Kirkland et al. (2015)

8AG, 8-azaguanine; FPG, formamidopyrimidine DNA glycosylase; *Gpt*, guanine phophoribosyltransferase; γH2AX, phosphorylated form H2A histone family member X; HID, highest ineffective dose; *Hprt*, hypoxanthine-guanine phosphoribosyltransferase; LED, lowest effective dose; NT, not tested; OECD, Organisation for Economic Co-operation and Development; Ogg, 8-oxoguanine DNA glycosylase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCE, polychromatic erythrocyte; qPCR, quantitative polymerase chain reaction; *Tk*, thymidine kinase.

[a] ↑ increased; +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

of cobalt(II) oxide) (200, 600, or 2000 mg/kg bw per day) for 2–5 consecutive days that did not result in treatment-related increases in chromosomal aberration frequencies. However, exposure to cobalt(II) sulfate or cobalt oxide resulted in mortalities, and thus the exposure duration was reduced from the original 5 days for the remaining animals (Kirkland et al., 2015). [The Working Group noted that due to high mortality, no chromosomal aberration frequency could be determined for some groups and that in other groups the number of cells examined was reduced.] No increase in spermatogonial chromosomal aberrations was observed in rats treated with cobalt(II) chloride hexahydrate by gavage for 28 days at doses of 3, 10, and 30 mg/kg bw per day (Kirkland et al., 2015). One additional study reported a frequency of bone marrow micronuclei in rats treated with drinking-water containing cobalt(II) chloride for 7 days that was many times that of controls (Awoyemi et al., 2017). [The Working Group noted that the frequency of micronucleus formation in PCEs of exposed rats was several times higher when compared with observations in other in vivo studies. In addition, no positive control was included, and no information on, for example, the numbers of cells counted or PCE/normochromatic erythrocyte ratios was provided.]

Existing cobalt assessment reports (OECD, 2014; ECHA, 2016, 2017) include OECD guideline-compatible bone marrow chromosomal aberration and micronucleus tests, with cobalt(II) chloride showing no increased incidence of chromosomal aberrations after oral doses of up to 600 mg/kg bw (cited as "Study report, Gudi et al., 1998"). The highest doses were very high and probably above the maximum tolerated dose. Some reductions in the mitotic index and the percentage of PCEs were reported. [The Working Group noted that since the original study report is not publicly available, full evaluation of this study was not possible.] One study showed increased 8-OHdG levels, a marker of oxidative DNA damage, in rat kidney after administration of cobalt(II) chloride. However, only one dose was tested (see also Section 4.2.5) (Abdel-Daim et al., 2020).

Cobalt metal nanoparticless

In mice, intratracheal administration of cobalt metal NPs (mean diameter, 20 nm) (single dose, 50 μg/mouse) resulted in increased frequencies of guanine phosphoribosyltransferase gene (*Gpt*) mutations in lung genomic DNA and increased 8-OHdG levels (see also Section 4.2.5) (Wan et al., 2017). Analysis of the mutational spectra revealed that exposure to cobalt metal NPs caused mainly G→C to T→A transversions, which is in agreement with oxidative DNA damage.

Organic cobalt(II) compounds

Kirkland et al. (2015) reported results of studies of bone marrow micronucleus formation after mice were treated with the organic cobalt compounds cobalt(II) resinate and cobalt(II) acetyl acetonate, orally administered twice at three different dose levels. No increase in the frequency of micronucleus formation was observed when the compounds were tested up to toxic levels.

(ii) Non-human mammalian cells in vitro

See Table 4.5.

Cobalt metal

In a guideline-based study, exposure to cobalt metal induced increased incidence of hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) mutations when tested with metabolic activation in mouse L5178Y lymphoma cells (Kirkland et al., 2015). Instead results were largely negative without metabolic activation. However, the cobalt metal powder clearly precipitated in culture medium, at multiple concentrations. Extracts (non-precipitated fractions) of cobalt metal powder, prepared from culture media buffered solutions, were also tested up to cytotoxic levels. The weak responses seen with cobalt metal

powder were not reproduced with extracts of the powder.

Soluble cobalt(II) salts

Cobalt(II) chloride (either anhydrous or hexahydrate) has been tested in several experimental systems for its ability to cause DNA strand breaks, gene mutations, or chromosomal damage. It has been shown to induce DNA strand breaks via an alkaline sucrose gradient (but not nucleoid sedimentation assay) and a reduction of cloning efficiency in Chinese hamster ovary cells (Hamilton-Koch et al., 1986). Fibroblasts from BALB/3T3 mice, as assessed by comet assay, showed increased levels of DNA damage after 2-hour exposure to subtoxic concentrations of cobalt(II) chloride (Ponti et al., 2009). Wang et al. (2000) reported increased DNA damage in mitochondrial, but not nuclear, DNA of the rat neuronal PC-12 cell line. Cobalt(II) chloride has been also reported to cause protein–DNA cross-links in Novikoff ascites hepatoma cells (Wedrychowski et al., 1986) and DNA degradation in the mouse cardiomyocyte cell line HL-1 (Cruz-Topete et al., 2016).

Studies concerning gene mutations caused by cobalt(II) chloride have shown inconsistent results. Cobalt(II) chloride hexahydrate induced gene mutations in the *Hprt* locus in the Chinese hamster V79 cell line in one study (Hartwig et al., 1990), whereas another study using cobalt(II) chloride showed only weakly positive results (Miyaki et al., 1979) and a third study reported negative results (Kitahara et al., 1996). Kitahara et al. (1996) studied induction of gene mutations in the *Gpt* locus in Chinese hamster *Gpt*$^+$ transgenic G10 and G12 cell lines: in G12 cells, clear induction of mutations was observed, whereas no induction was seen in G10 cells (Kitahara et al., 1996). Weak induction of mutations was observed in the mouse mammary carcinoma FM3A cell line, as assessed by modified *Hprt* assay, in a study by Morita et al. (1991). No increased gene mutation frequencies after exposure to cobalt(II) chloride hexahydrate were observed at the *Tk* locus in the mouse lymphoma L5178Y cell line (Amacher & Paillet, 1980) or at the *Hprt* locus (8-azaguanine-resistant) in the Chinese hamster V79 cell line (Yokoiyama et al., 1990). [However, the Working Group noted that the doses in Yokoiyama et al. (1990) were low compared with other gene mutation studies using cobalt(II) chloride hexahydrate.]

Cobalt(II) chloride has been reported to induce sister-chromatid exchange in the mouse P388D1 (Andersen, 1983) and Chinese hamster V79 cell lines (Hartwig et al., 1991). However, tests of micronucleus formation in BALB/3T3 mouse fibroblasts and in BALB/c mouse bone marrow cell suspensions have been negative (Suzuki et al., 1993; Ponti et al., 2009).

In addition to cobalt chloride, in vitro genotoxicity studies have been performed with other cobalt(II) compounds. Kirkland et al. (2015) reported a series of *Hprt* locus gene mutation studies in the mouse lymphoma L5178Y cell line using different (soluble and insoluble) cobalt(II) compounds, including cobalt sulfate, cobalt 2-ethyl hexanoate, cobalt sulfide, cobalt tetraoxide, cobalt(II) oxide, and cobalt dihydroxide; (organic) cobalt(II) oxalate; and some other cobalt compounds not considered relevant for this assessment (lithium cobalt dioxide, cobalt oxide hydroxide, and cobalt borate neodecanoate). Although sporadic and statistically significant increases in mutation frequencies were seen with some of these compounds, there were no dose–response relationships. The resulting increases in mutation frequencies were below biological significance, which was considered to be an increase of threefold over the mean level for historical controls.

Cobalt(II) sulfate heptahydrate has been reported to induce a dose-dependent increase in micronucleus formation in Syrian hamster embryo (SHE) cells (Gibson et al., 1997).

Insoluble cobalt(II or II,III) compounds

In earlier studies with cobalt(II) sulfide, Kitahara et al. (1996) reported induction of gene mutations at the *Gpt* locus in Chinese hamster *Gpt*$^{+}$ transgenic G12 cells, but not in G10 cells. Negative results were obtained in the *Hprt* locus of Chinese hamster V79 cells (Kitahara et al., 1996). Co-culture with H_2O_2 did not result in increased mutation frequencies. Cobalt(II) sulfide particles have also been reported to induce DNA strand breaks in Chinese hamster ovary cells (Robison et al., 1982).

Cobalt-based nanoparticless

Cobalt metal NPs (size range, 20–500 nm; peak, 80 nm) induced DNA damage (as assessed by comet assay) and micronucleus formation in BALB/3T3 mouse fibroblasts, which were not dose-dependent. The reported lack of dose-dependency may have been due to the cytotoxicity at higher doses. [The Working Group noted that genotoxic effects were associated with significant uptake of cobalt metal NPs into the cells, possibly via a Trojan horse-type mechanism (Ponti et al., 2009).] Exposure of the rat kidney NRK cell line to cobalt NPs (diameter, 20–50 nm) at a concentration of 100 µM for 4–48 hours resulted in DNA damage, as assessed by comet assay, and cytotoxicity in a study by Liu et al. (2017). The same group also reported time- and dose-dependent genotoxicity, as assessed by comet assay, in normal rat liver cells cultured in vitro; however, no quantitative results were provided (Liu et al., 2016). No increase in DNA damage, as assessed by comet assay, was observed in wildtype mouse embryonic fibroblasts (MEF *Ogg1*$^{+/+}$) and derived 8-oxoguanine DNA glycosylase (*Ogg1*) knockout (MEF *Ogg1*$^{-/-}$) cells after exposure to cobalt NPs (Annangi et al., 2015). However, when the comet assay was performed with Fpg enzyme to detect 8-OHdG, a dose-dependent increase in oxidative DNA damage was observed in knockout (MEF *Ogg1*$^{-/-}$) cells but not in wildtype cells (Annangi et al., 2015) (see also Section 4.2.5). In a study by Zhu et al. (2021b), exposure of rat CD34+ haematopoietic stem cells/haematopoietic progenitor cells to cobalt NPs (size, 50–200 nm) resulted in DNA damage (as assessed by comet assay), activation of DNA damage-responsive γH2AX, and increased 8-OHdG levels (see also Section 4.2.5).

Cobalt(II,III) oxide NPs (non-spherical; mean diameter, 17 nm; forming small agglomerates of tens of NPs) have been reported to induce dose-dependent DNA strand breaks in rat cardiomyocytes cultured in vitro, as assessed by comet assay (Savi et al., 2021).

Organic cobalt(II) compounds

Two organic cobalt(II) compounds, cobalt resinate and cobalt acetyl acetonate, were tested for their ability to induce mutations in the *Tk* locus, as assessed by mouse lymphoma L5178Y Tk$^{+/-}$ assay (Kirkland et al., 2015). Cobalt resinate yielded negative results, whereas cobalt acetyl acetonate gave equivocal results with some positive responses at the highest doses tested mainly without metabolic activation. Relatively high toxicity was also observed at these doses.

(iii) Non-mammalian experimental systems

See Table S4.6 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt metal

Cobalt metal has been tested in *Salmonella typhimurium* strains TA98 and TA100 (NTP, 2014; Kirkland et al., 2015) and in *Escherichia coli* strain WP2 (NTP, 2014) in reverse mutation assays. A weakly positive response was observed in the first study in strain TA98 without metabolic activation (NTP, 2014). In strain TA100, the result was equivocal in the absence of metabolic activation and negative in its presence. In *E. coli*, results remained negative both with and without metabolic activation (NTP, 2014). Kirkland et al. (2015) reported Ames test results, using *S.*

typhimurium strain TA98, from three individual laboratories that complied with GLP. All these tests gave negative results both with and without metabolic activation.

Soluble cobalt(II) salts

Numerous studies have tested cobalt(II) chloride (either anhydrous or hexahydrate) using non-mammalian experimental systems including bacteria (*S. typhimurium*, *E. coli*, and *Bacillus subtilis*), yeast (*Saccharomyces cerevisiae*), zebrafish (*Danio rerio*), earthworms (*Eisenia hortensis*), and fruit flies (*Drosophila melanogaster*). Reinardy et al. (2013) measured the induction of DNA damage in male zebrafish sperm by exposure to cobalt(II) chloride, as assessed by comet assay. Increased incidence of DNA strand breaks was observed immediately after exposure, but induction of DNA damage did not differ from controls when measured after a 6-day recovery period. Although there were also statistically significant changes in the expression of DNA repair genes, these changes were not dose-dependent (Reinardy et al., 2013). Induction of micronucleus formation and DNA strand breaks in coelomocytes of earthworms has been also reported after exposure to cobalt(II) chloride at dose levels close to the LD_{50} (Ciğerci et al., 2016).

Cobalt(II) chloride has been shown to induce mutations in *D. melanogaster mwh/flr* strain in multiple studies (Ogawa et al., 1994; Kaya et al., 2002; Demir et al., 2009, Vales et al., 2013, Ertuğrul et al., 2020). In yeast, cobalt(II) chloride (and its hexahydrate) has been reported to induce respiratory deficiency "petite" mutations and *Trp* conversions, but mostly negative results have been obtained for the induction of *Ilv* reverse mutations (Prazmo et al., 1975, Putrament et al., 1977, Egilsson et al., 1979; Fukunaga et al., 1982; Singh, 1983; Kharab & Singh, 1985; Kharab & Singh, 1987) (see Table S4.6 in Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). Cobalt(II) chloride (anhydrous or hexahydrate) has been reported to be negative in multiple reverse mutation assays in several strains of *S. typhimurium* (TA98, TA100, TA102, TA1535, TA1538, and TA2637) (Tso & Fung, 1981; Mochizuki & Kada,1982; Arlauskas et al., 1985; Ogawa et al., 1986) (see Table S4.6 in Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). Only positive findings were reported for the studies by Wong (1988) using strains TA98 and 1537 (without S9 mix), and by Pagano & Zeiger (1992) using strain TA97 in a preincubation assay. One study reported cobalt(II) chloride-induced mutagenesis in the *supF* transfer RNA gene of *E. coli* (Ogawa et al., 1999), whereas other available *E. coli* studies reported negative results (Kada & Kanematsu, 1978; Rossman et al., 1984; Arlauskas et al., 1985). Positive results were reported for one out of three mutation assays employing *B. subtilis* (Kanematsu & Shibata, 1980; Kanematsu et al., 1980); negative results were reported for the two other assays (Nishioka, 1975; Inoue et al., 1981). Chromosomal aberration test and comet assay results reported for a study of onion bulbs (*Allium cepa* L.) showed positive responses in one study at concentrations that also caused cytotoxicity (decreased mitotic index) (Yıldız et al., 2009).

Fewer data were available for other cobalt(II) salts. Cobalt(II) nitrate hexahydrate induced gene mutation and chromosomal deletion, non-disjunction, or mitotic recombination in *D. melanogaster* (Yeşilada, 2001). Cobalt(II) nitrate hexahydrate induced micronucleus formation and chromosomal aberration in onion bulbs (*A. cepa* L.) (Macar et al., 2020; Kalefetoğlu Macar et al., 2021). The concentration used in these studies resulted also in a significant reduction in mitotic index. Erturk et al. (2013) reported induction of DNA damage in maize (*Zea mays* L.) after exposure to cobalt(II) nitrate hexahydrate. In an older study, nitrate salt of cobalt – either cobalt(II) or cobalt(III) nitrate – induced

mutations in the chlorophyll genes of *Pisum abyssinicum* (Von Rosen, 1964).

Cobalt(II) sulfate heptahydrate induced reverse mutations (without metabolic activation) in *S. typhimurium* strain TA100, but not in TA98 or TA1535 (Zeiger et al., 1992). However, this positive finding in TA100 was not repeated in subsequent studies performed independently by three different laboratories (Kirkland et al., 2015). A slightly positive response was observed by Rec assay in *B. subtilis* (strain H17 Rec+) exposed to cobalt(II) sulfate (Kanematsu & Shibata, 1980; Kanematsu et al., 1980). Cobalt(II) sulfate induced DNA double-strand breaks in *E. coli* (Kumar et al., 2017). These strand breaks were not associated with the induction of oxidative stress; however, cobalt(II) sulfate was observed to effectively inhibit the ROS-induced DNA repair pathways (Kumar et al., 2017). Cobalt(II) sulfate has been also reported to induce chromosomal aberrations and aneuploidy in plants (*A. cepa*) (Gori & Zucconi, 1957).

Cobalt(II) acetate tetrahydrate caused a positive response in a reverse mutation assay in *E. coli* strain WP2 without metabolic activation (Maeda et al., 2021).

Cobalt-based nanoparticles

Cobalt metal NPs (diameter, < 50 nm) have been reported to induce mutations in *D. melanogaster* mainly via somatic recombination mechanisms (Vales et al., 2013; Ertuğrul et al., 2020). Cobalt metal NPs also caused DNA damage in *D. melanogaster* haemocytes, as assessed by comet assay (Ertuğrul et al., 2020).

Cobalt(II,III) oxide NPs induced DNA strand breaks, as assessed by comet assay, in eggplant (*Solanum melongena* L. cv. Violetta lunga 2) (Faisal et al., 2016). The effects occurred concurrently with an increase in ROS levels. [The Working Group noted that significant induction of apoptosis was also observed at all concentrations tested.] Negative results were obtained in *S. typhimurium* strain TA98, as assessed by reverse mutation assay, using two different sizes of cobalt(II,III) oxide NPs (average size, 10–30 and 80–150 nm) (Kong et al., 2020).

Organic cobalt(II) compounds

Two organic cobalt(II) compounds, cobalt resinate and cobalt acetyl acetonate, did not increase mutation frequencies in any of the five different *S. typhimurium* strains tested (Kirkland et al., 2015).

(iv) Acellular systems

See Table S4.7 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt metal or cobalt metal nanoparticles

Cobalt metal induced DNA strand breaks in a study using purified DNA from mouse 3T3 cells (Anard et al., 1997). Ultrafine cobalt particles induced free-radical (oxidative) damage to supercoiled (bacteriophage) plasmid DNA (Zhang et al., 1998). [The Working Group noted that the mean diameter of the particles was 20 nm, being in the nanosized range.]

Soluble cobalt(II) salts

In early experiments conducted using calf thymus DNA, cobalt(II) chloride displaced acridine orange from DNA when assessed by fluorescence polarization, indicating an interaction of cobalt with DNA (Richardson et al., 1981). However, the effect was weaker than with, for example, iron or copper. The effect correlated with that reported by Sirover & Loeb (1976) on the fidelity of DNA synthesis. In their study, Sirover & Loeb (1976) reported that Co^{2+} substitutes Mg^{2+} in DNA polymerase (derived from *E. coli*, sea urchin, and avian myeloblastosis virus), resulting in an increase in the error frequency during DNA replication. Cobalt(II) chloride has induced DNA cleavage of the human c-Ha-*RAS*-1 proto-oncogene, but only in the presence of H_2O_2 (Kawanishi et al., 1989a, b; Yamamoto et al., 1989).

Table 4.8 Altered DNA repair and genomic instability in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
8-oxoG repair activity	Blood mononuclear cells	German, cross-sectional	78 workers with mixed metal exposures (cobalt, cadmium, and lead)	↓ 8-oxoG repair with increasing cobalt exposure; inversely correlated with the level of DNA single-strand breaks (R = –0.427, Pearson test; P = 0.001)	Age, sex, alcohol, smoking status	The potential for differential exposure misclassification is low with the measurements collected. The consideration of co-exposures to lead and cadmium, both also quantitatively assessed, is a strength. The air samples (inhalation exposure) and biological samples (all routes of exposure) represent different time periods of exposure. A limitation of this study is the reliance on a single biological measure of exposure. Detailed description of the 8-oxoG repair data not shown.	Hengstler et al. (2003)
DNA repair	Autopsy samples of brain and lung tissue	Mexico, urban air pollution exposure vs 2 less-exposed locations	47 exposed and 12 controls from less polluted cities (adults and children)	↓ OGG1 expression in olfactory bulb with ↑ frontal concentrations of cobalt (prob. t = 0.0161) ↓ LIG1 expression in olfactory bulb with ↑ frontal concentrations of cobalt (prob. t = 0.0306)	Age	Cobalt and other metals were quantitatively assessed using accepted methods. The potential for misclassification of cobalt exposure is low. A limitation of this study is the reliance on a single time point for exposure information, and whether this time point captures the relevant window of exposure for the mechanistic end-point of interest. Age, sex, place of residency, cause of death, and time between death and autopsy noted. Cause of death was considered for all subjects to rule out infection, inflammatory events, drug exposure, brain ischaemia, and hypoxia. Cobalt measured quantitatively as predictor of outcomes.	Calderón-Garcidueñas et al. (2013)

↑, increase; ↓, decrease; 8-oxoG, 8-oxoguanine; LIG1, DNA ligase I; OGG1, 8-oxoguanine DNA glycosylase; prob., probability; vs, versus.

Chemical changes were observed in chromatin isolated from the human K562 cell line and isolated calf thymus DNA after exposure to cobalt(II) sulfate, but only in the presence of H_2O_2 (Nackerdien et al., 1991). Kumar et al. (2017) showed that exposure to cobalt(II) sulfate changes the ellipticity of the plasmid DNA and inhibits DNA synthesis (Kumar et al., 2017). Exposure to cobalt(II) sulfate induced production of reactive hydroxyl species and resulted in deoxyribose degradation, which was only observed with co-treatment with H_2O_2 (Moorhouse et al., 1985).

4.2.3 Alters DNA repair or causes genomic instability

With the exception of direct reversal repair, all major DNA repair pathways progress in the same order: recognition of erroneous DNA, recruitment of repair components, removal of the erroneous part, reconstruction of the DNA, and reinstatement of the repaired part into the rest of the DNA structure (Kelley & Fishel, 2016). Specific players for each step vary by the types of DNA damage and the repair pathways deployed. Among major repair pathways – single-strand break repair includes base excision repair (BER), nucleotide excision repair (NER, including global genome NER and transcription-coupled NER), and mismatch repair; and double-strand break repair includes homologous recombination and non-homologous end joining (NHEJ). The effects of exposure to cobalt(II) salts in various steps involved in NER and in the reinstatement step of homologous recombination were studied in human cells in vitro and acellular systems, whereas the effects on BER were studied only assessed using a bacterial enzyme. Studies on other DNA repair pathways were not available to the Working Group. Cobalt(II) salts also decreased the mRNA (but not protein) expression of retrotransposons in two human cancer cell lines, which could result in genomic instability.

(a) Humans

(i) Exposed humans

See Table 4.8.

Evidence for perturbations in DNA repair in human populations exposed to cobalt included a cross-sectional study of German workers exposed to cobalt, cadmium, and lead (Hengstler et al., 2003). 8-Oxoguanine repair activity in blood mononuclear cells decreased with increasing cobalt exposure and was inversely correlated with the levels of DNA single-strand breaks ($r = -0.427$; $P = 0.001$). A similar dose–response relationship for cobalt (5–10 μg/m³) was observed for inhibition of 8-oxoguanine repair as for induction of DNA single-strand breaks; that is, repair activity for 8-oxoguanine decreased at concentrations of cobalt > 4 μg/m³. [The Working Group noted that the exposure to cobalt was low (range, 0–10 μg/m³ air) compared with the German TRK permissible exposure limit value of 100 μg/m³. In addition, while co-exposure to several metals was noted, regression analyses were performed to isolate the effects of cobalt alone.]

Another study examined brain and lung tissue samples, obtained via autopsies, to assess urban air pollution effects, including from exposure of people from urban versus less-exposed (control) locations in Mexico to metals (Calderón-Garcidueñas et al., 2013). While the cobalt concentrations in control versus urban-exposed frontal lobes were not significantly different (controls, 15 ± 2 μg/g dry weight tissue; exposed, 17 ± 2 μg/g dry weight tissue), regression analyses revealed that increased frontal lobe concentrations of cobalt correlated with decreases in 8-oxoguanine DNA glycosylase (*OGG1*) expression, which encodes the enzyme responsible for the excision of 8-oxoguanine in the olfactory bulb (probability, $t = 0.0161$). Cobalt also correlated inversely with the LIG1 gene (encodes for DNA ligase I), which functions in DNA replication and BER processes, in

Table 4.9 Altered DNA repair and genomic instability in human cells in vitro, non-human mammalian cells in vitro, and acellular systems exposed to cobalt

Pathway	Steps in the DNA repair pathway					Overall repair and notes
	Recognition→	Recruitment→	Removal→	Reconstruction→	Reinstatement	
Single-strand break repair	Co^{2+} did not affect polynucleotide kinase phosphatase function (Whiteside et al., 2010)[a]	NR	NR	NR	NR	NR
Nucleotide excision repair[b]	$CoCl_2$ ↑ XPA (Cycs4) protein level (Liu et al., 2012a)[c] $CoCl_2$ ↓ mouse XPA activity (Asmuss et al., 2000a, b)[e] $Co(NO_3)_2 \cdot 6H_2O$ substitute zinc in XPA zinc finger (Kopera et al., 2004)[a]		$CoCl_2$ ↓ incision (Kasten et al., 1997)[d]	$CoCl_2$ ↓ polymerization (Kasten et al., 1997)[d]	$CoCl_2$ did not affect ligation (Kasten et al., 1997)[d]	$CoCl_2$ ↓ overall repair (Hartwig et al., 1991)[c] $CoCl_2$ ↓ 8-oxo-dGTPase activity of human MTH1 protein (Porter et al., 1997)[a]
Base excision repair	$CoCl_2$ ↓ 8-oxo-dGTPase activity of bacterial MutT protein (Porter et al., 1997)[f]	NR	NR	NR	NR	NR
Homologous recombination repair	NR	NR	NR	NR	$CoCl_2$ ↓ *RAD51* mRNA level (Zhong et al., 2020)[c] $CoCl_2$ ↓ RAD51-positive foci (indicating repair) (Zhong et al., 2020)[c]	NR

↑, increased or upregulated; ↓, decreased or downregulated; 8-oxo-dGTPase, 8-oxo-2′-deoxyguanosine-5′-triphosphatase; mRNA, messenger RNA; MTH1, 8-oxo-dGTPase activity of recombinant human MutT homologue 1; NR, not reported (no study found); XPA, xeroderma pigmentosum complementation group A.

[a] Acellular system with human cell components.

[b] XPA is involved in both recognition and recruitment.

[c] Human cell line(s).

[d] Human primary cells.

[e] Used an acellular experimental system with mammalian cell components.

[f] Used an acellular experimental system with bacterial components.

the olfactory bulb (probability, $t = 0.0306$). [The Working Group noted that while co-exposure to several metals was noted, regression analyses were performed for individual metals and did not account for co-exposures. Cobalt and other metals were quantitatively assessed using accepted methods. The potential for misclassification of cobalt exposure is low. A limitation of this study was the reliance on a single time point for exposure information, and whether this time point captures the relevant window of exposure for the mechanistic end-point of interest.]

[The Working Group noted that there were two studies of cobalt-exposed populations with some evidence of alterations in DNA repair.]

(ii) Human cells in vitro

See Table 4.9.

Non-cytotoxic concentrations of cobalt(II) chloride inhibited the incision and polymerization steps of NER of ultraviolet (UV)-induced DNA damage in primary human skin fibroblast cells, which were derived from a normal donor (Kasten et al., 1997). The incision of UV-induced DNA damage was inhibited by 2-hour exposure to cobalt(II) chloride at 50 µM, while incision at cyclobutane pyrimidine dimer sites was inhibited only at concentrations of 150 or 200 µM, suggesting that cobalt might affect the removal of (6–4) photoproducts more than the removal of pyrimidine dimers (Kasten et al., 1997). Co-induced inhibition of polymerization was reversed by bivalent magnesium, suggesting metal substitution. The ligation step was not affected by cobalt (Kasten et al., 1997).

The removal of UV-induced thymine dimers in the HeLa cell line was decreased by cobalt(II) chloride hexahydrate at non-cytotoxic concentrations without increasing UV-induced thymine dimers or strand breaks (Hartwig et al., 1991).

When the human lung cancer cell lines Calu-6 and SK-LU-1 were exposed to cobalt(II) chloride [source and purity not reported] at a concentration of 500 µmol/L for 24 or 48 hours, protein levels of HIF-1α and xeroderma pigmentosum complementation group A (XPA) were increased (Liu et al., 2012a). Cobalt(II) chloride-induced increases in HIF-1α and XPA protein levels were not seen in the H358 cell line, which has "high levels of XPA protein concomitant with enrichment of the HIF-1α protein at the XPA promoter", suggesting that the increase of XPA was via HIF-1α binding to the promoter of XPA (Liu et al., 2012a).

Exposure to cobalt(II) chloride at a concentration of 100 µM for 24 or 72 hours decreased *RAD51* messenger RNA (mRNA) levels and RAD51 foci-positive cells, respectively, suggesting impaired homologous recombination repair, while γH2AX foci-positive cells increased, suggesting an increase in double-strand breaks (see Section 4.2.2) (Zhong et al., 2020). All these effects were in human colorectal cancer cell lines with *K-RAS* mutation – HCT 116, SW620, and LoVo – with the exception that *RAD51* mRNA was not measured in the LoVo cell line. [The Working Group noted that cobalt(II) chloride might decrease DNA repair, because double-strand breaks would increase *RAD51* mRNA, which plays an important role in homologous recombination, rather than the observed mRNA decrease.] [The Working Group noted that hypoxia might be how cobalt(II) chloride decreased *RAD51* mRNA, because bevacizumab (humanized anti-VEGF monoclonal antibody) also induced hypoxia and decreased *RAD51* gene expression (Zhong et al., 2020).]

The human genome rearranges itself via mobile elements, which include transposons and retrotransposons. Transposons operate in a "cut-and-paste" fashion, while retrotransposons operate in a "copy-and-paste" fashion and consequently increase their copy numbers in the genome faster than transposons (Kim et al., 2012). The only autonomous and active retrotransposons are the long interspersed elements (LINEs), including LINE-1, LINE-2, and LINE-3. LINE-1 can contribute to carcinogenesis via

insertion into oncogenes and tumour suppressor genes, creating DNA damage, increasing genomic instability, and affecting immune function (Zhang et al., 2020). While subtoxic concentrations of cobalt(II) chloride increased *LINE-1* mRNA levels in human neuroblastoma BE(2)-M17 and HeLa cell lines, but not in primary human fibroblasts derived from ATM patients (denoted "HF D") or healthy individuals ("HF WT"), it did not significantly increase LINE-1 promoter activity or protein levels in any of the four cell types tested (Habibi et al., 2014). Furthermore, retrotransposition of LINE-1 was not increased in HeLa cells (El-Sawy et al., 2005; Habibi et al., 2014) or neuroblastoma cells (not tested in HF D or HF WT cells) (Habibi et al., 2014).

(iii) Acellular systems

See Table 4.9.

The 8-oxo-2′-deoxyguanosine-5′-triphosphatase (8-oxo-dGTPase) activity of recombinant human MutT homologue 1 (MTH1) protein was inhibited by 2.5-minute exposure to 6 mM Co^{2+} (cobalt(II) chloride) in the presence of 8 mM Mg^{2+} (magnesium acetate) (Porter et al., 1997). In the absence of Mg^{2+}, the natural activator of 8-oxo-dGTPase, Co^{2+} could restore roughly one third of the activity of MTH1, while other bivalent metals were much less effective or had no effect (Porter et al., 1997).

After preincubation with bivalent Co^{2+} [cobalt compound form, source, and purity were not reported] at a concentration of 200 μM for 10 minutes, whole-cell extracts from the human lung fibroblast MRC-5 cell line showed the same levels of phosphatase activity, kinase activity, and nick ligation of recombinant polynucleotide kinase phosphatase in repairing single-strand breaks on synthetic substrates as untreated whole-cell extracts (Whiteside et al., 2010). The substrates were double-stranded oligonucleotides with a nick in one strand, a single-stranded oligonucleotide, and a double-stranded oligonucleotide with nearly one half of its length being only one-stranded. With the placement of two different fluorophores and the addition of phosphate, the substrates enabled the investigation of different functions of polynucleotide kinase phosphatase.

p53 family members (p53, p63, p73, and their isoforms) are facilitators of DNA repair. p53 causes cell cycle arrest to allow time for repair, and p53 family members also control the transcription of many genes involved in DNA repair. Cobalt(II) chloride impaired the DNA-binding capacity of p53 protein in a study using purified protein and synthetic DNA. Cobalt(II) chloride decreased purified human p53 protein binding to supercoiled plasmid DNA that did not contain the p53 consensus binding site sequence (p53CON) as well as to p53CON in a linear DNA fragment (Paleček et al., 1999). Compared with $ZnCl_2$, cobalt(II) chloride had a weaker inhibitory effect on DNA–protein interaction (Paleček et al., 1999). DNA binding of p63 and p73 proteins was also inhibited by cobalt(II) chloride (Adámik et al., 2015). More specifically, the binding of p53 DNA binding domain, p63 protein, and p73 protein to the 474-base pair-long DNA fragment of p53CON was inhibited in each case by preincubating the proteins with cobalt(II) chloride (Adámik et al., 2015).

XPA proteins recognize DNA damage in global genome NER and recruit other DNA repair proteins in both global genome NER and transcription-coupled NER. Using a synthetic peptide representing the human XPA zinc finger sequence (XPAzf), Kopera et al. (2004) showed that Co^{2+} (from cobalt(II) nitrate hexahydrate) can substitute Zn^{2+} (from zinc(II) nitrate hexahydrate, $Zn(NO_3)_2{\cdot}6H_2O$) in XPAzf while keeping the XPAzf structure equivalent to the native zinc finger, although the interaction between Co^{2+} and XPAzf was 100 times weaker than Zn^{2+}. [The Working Group noted that the effects of Co^{2+} substitution on XPAzf function were not investigated and remain unclear.] Low oxidative activity

Table 4.10 Epigenetic alterations in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Extracellular vesicle miRNA	Maternal plasma	Multiple samples analysis within a prospective cohort study of pregnant women, USA	Subset (n = 184) of larger cohort of pregnant women with early and late pregnancy samples available	Inverse association between cobalt and miR-150-5p after multiple testing corrections; stronger effect estimate when evaluating late pregnancy miRNA levels (β, −0.20; 95% CI, −0.31 to −0.09 compared with β, −0.16; 95% CI, −0.24 to −0.07 for early pregnancy)	None	The potential for differential exposure misclassification is low. Exposure to other metals was considered. A limitation of this study is the reliance on a single biological measure of exposure.	Howe et al. (2021)

CI, confidence interval; miRNA, microRNA.

Table 4.11 Epigenetic alterations in human cells in vitro exposed to cobalt

End-point	Cell line	Results	Concentration	Comments	Reference
Cobalt(II) salts					
Histone post-translational modifications	Human embryonic kidney cells (HEK-293)	H3-Ser10 dephosphorylation and ↓ H3 pan-acetylation	50 µg/mL and 1000 µM for 24 h	Used two doses that are very similar	Verma et al. (2011)
	Human neuroepithelioma cells (SK-N-MC)	↑ H3K4me3, H3K9me2, H3K9me3, H3K27me3, H3K36me3, uH2A, and uH2B; ↓ H4ac	50 µg/mL and 1000 µM for 24 h		
Histone post-translational modifications	Human lung alveolar carcinoma cells (A549)	↑ H3K4me3, H3K9me2, H3K9me3, H3K27me3 and H3K36me3 as well as uH2A and uH2B at >200 mM; ↑ at 500 mM histone ubiquitination of di-uH2A; ↓ acetylation at histone H4 (AcH4)	200 µM for 24 h		Li et al. (2009)
	Human bronchial epithelial cells (BEAS-2B)	↑ H3K4me3, H3K9me2 and H3K27me3, ↓ AcH4 at 25, 50, 100 and 200 mM, whereas ↑ H3K9me3 only at 200 mM, H3K36me3 (50–200 mM), uH2A (150 mM) and di-uH2A (200 mM)			
Histone post-translational modifications	Human neuroblastoma cells (SHSY5Y)	↓ H3ac and H4ac	100~400 µM for 24 h		Guo et al. (2021)
m^{6}A modification	Human neuroblastoma cells (H4)	↓ expression of m^{6}A modification enzymes and inactivate demethylase to alter the m^{6}A methylation levels of genes	400 µM for 24 h	Did not measure the cytotoxic effects of $CoCl_2$ on H4 cells	Tang et al. (2020)
Histone post-translational modifications	Human renal proximal tubular cells (HK-2)	↑ H3K27ac	300 µM for 7 h	300 µM caused cytotoxic effects	Ha et al. (2018)
miRNA expression	Human umbilical vein endothelial cells (HUVECs)	↑ miR-21 expression	150 µM for 24 h	Only one dose	Xu et al. (2017)
miRNA expression	Human colorectal adenocarcinoma cells (HT-29)	↑ miR-210 expression	300 µM for 24 h	Only one dose	Nersisyan et al. (2021)
	Human colon adenocarcinoma cells (Caco-2)	↑ miR-210 expression	300 µM for 24 h	Only one dose	
miRNA expression	Human colon adenocarcinoma cells (Caco-2)	↓ miR-148a expression	300 µM for 24 h	Only one dose	Nersisyan et al. (2021)

↑, increased or upregulated; ↓, decreased or downregulated; ac, acetylation; H, histone; K, lysine; LEC, lowest effective concentration; m^{6}A, methylation of the adenosine base at the N^6 position of mRNA; me2, demethylation; me3, trimethylation; miRNA, microRNA; uH, ubiquitinated histone.

in the Co^{2+}–XPAzf complex was observed. [The Working Group noted that the functional effects of this are unclear.]

(b) Experimental systems

(i) Non-human mammals in vivo and non-human mammalian cells in vitro

No studies were available to the Working Group.

(ii) Acellular systems

See Table 4.9.

After 15-minute preincubation with Co^{2+} [cobalt form, source, and purity were not reported] (lowest effect concentration 100 μM), the DNA–protein binding activity of recombinant mouse XPA protein to UVC-damaged oligonucleotide was inhibited, as assessed by gel mobility shift assay (Asmuss et al., 2000a, b). [The Working Group considered that because of the presence of excess Zn^{2+} (simultaneous incubation with Zn^{2+} at 5 or 10 times the Co^{2+} concentration) largely prevented cobalt-induced XPA inactivation, the Co^{2+} effects might be via displacement of zinc in the zinc finger structure of the protein (Asmuss et al., 2000a).] In contrast, 15-minute preincubation of Co^{2+} at concentrations up to 1000 μM did not affect bacterial Fpg activity on oxidatively damaged supercoiled DNA derived from bacteriophage PM2 (Asmuss et al., 2000a, b).

In BER, the 8-oxo-dGTPase activity of recombinant bacterial MutT protein was inhibited by 5-minute exposure to 12 mM Co^{2+} (cobalt(II) chloride) in the presence of 8 mM Mg^{2+} (magnesium acetate) (Porter et al., 1997). In the absence of Mg^{2+}, the natural activator of 8-oxo-dGTPase, Co^{2+} could restore roughly three quarters the activity of MutT, while other bivalent metals (concentration range, 2.4–0.1 mM) were much less effective or had no effect (Porter et al., 1997).

4.2.4 Induces epigenetic alterations

(a) Human

(i) Exposed humans

See Table 4.10.

In a multiple-sample analysis as part of a prospective cohort study in pregnant women in the USA, Howe et al. (2021) examined maternal plasma for external vesicle microRNA, which contributes to maternal–fetal communication and is dysregulated during pregnancy complications. Within a subset (n = 184) of the larger cohort, an inverse association between cobalt levels (early pregnancy) and miR-150-5p (in late pregnancy) was reported after multiple testing corrections. The findings showed a stronger effect estimate when evaluating late pregnancy microRNA levels (β, −0.20; 95% CI, −0.31 to −0.09 compared with β, −0.16; 95% CI, −0.24 to −0.07 for early pregnancy).

(ii) Human cells in vitro

See Table 4.11.

Epigenetic modifications are stable and heritable alterations that are mainly driven by three tightly regulated and interconnected processes: DNA methylation, modification of histones, and regulation of non-coding RNAs that alter DNA accessibility and chromatin structure, thereby modulating gene expression patterns (Strahl & Allis, 2000; Robertson, 2005; Portela & Esteller, 2010; Chervona & Costa, 2012). [The Working Group noted that exposure to cobalt metal or cobalt(II) salts appears to induce epigenetic alterations; however, most of the results were in cancer cell lines.]

Exposure of human embryonic kidney (HEK-293) and neuroepithelioma (SK-N-MC) cell lines to cobalt(II) chloride at 50 μg/mL and 1000 μM for 24 hours caused dramatic dephosphorylation of serine 10 on histone H3 and decreased pan-acetylation of histone H3 (Verma et al., 2011).

Exposure to cobalt(II) chloride (at concentrations ≥ 200 μM) for 24 hours caused alterations in histone post-translational modifications (increased trimethylation of lysine 4, lysine 9, lysine 27, and lysine 36, and increased dimethylation of lysine 9 on histone H3; increased ubiquitination of histones H2A and 2HB; and decreased acetylation of histone H4) in human lung carcinoma (A549) or human bronchial epithelial (BEAS-2B) cell lines (Li et al., 2009). Co^{2+} may compete with iron (Fe^{2+}) for binding to JMJD2A, thus directly inhibiting JMJD2A demethylase activity and resulting in increased histone methylation. In addition, Co^{2+} may prevent de-ubiquitination, leading to increased ubiquitination of histone H2 (Li et al., 2009). GeneChip microarray results showed that exposure of A549 cells to cobalt(II) chloride at 200 μM for 24 hours caused alterations in the expression of multiple genes, including increased expression of genes in different functional classes, such as transcriptional activation (i.e. JMJD1A), cell defence (i.e. HMOX1 and BN1P3L), and DNA repair and cell cycle checkpoint control (i.e. GADD45A), and decreased expression of genes involved in tumour suppression (i.e. NBL1 and MTUS1) (Li et al., 2009).

Exposure of the human neuroblastoma SH-SY5Y cell line to cobalt(II) chloride (100–400 μM) for 24 hours caused decreased acetylation of histone H3 and H4 in a time- and dose-dependent manner. In addition, cobalt(II) chloride selectively decreased HAT activity and protein expression but had no effect on HDAC in the SH-SY5Y cell line (Guo et al., 2021). Exposure of the human renal proximal tubular epithelial cell line HK-2 to cobalt(II) chloride (300 μM) for 7 hours increased the level of acetylated lysine 27 residues on histone H3 (Ha et al., 2018). In addition, exposure to cobalt(II) chloride (400 μM for 24 hours) caused significant differences in the pattern of methylation of the adenosine base at the N^6 position of mRNA (m^6A) in the human brain neuroblastoma cell line H4 (Tang et al., 2020, 2022). Cobalt(II) chloride-induced ROS affected the m^6A modification of apoptosis-related genes by decreasing the expression of FTO, resulting in the activation of apoptosis (Tang et al., 2022). In addition, cobalt(II) chloride exposure induced changes in gene expression related to the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signalling pathway, AMP-activated protein kinase, insulin signalling, MAPK, and the axon guidance signalling pathway (Tang et al., 2020).

Exposure to cobalt(II) chloride also caused microRNA dysregulation. Exposure of primary human umbilical vein endothelial cells (HUVECs) to cobalt(II) chloride (150 μM) for 24 hours caused upregulation of miR-21, downregulation of programmed cell death 4 (PDCD4) protein expression, and attenuation of apoptosis (Xu et al., 2017). [The Working Group noted that miR-21 is one of the earliest identified cancer-promoting "oncomiRs", targeting numerous tumour suppressor genes associated with proliferation, apoptosis, and invasion (Si et al., 2007).] Exposure of the human colorectal cancer cell lines HT-29 and Caco-2 to cobalt(II) chloride (300 μM) for 24 hours caused consistent upregulation of miR-210; however, cobalt(II) chloride exposure caused downregulation of miR-148a in Caco-2 cells (Nersisyan et al., 2021). [The Working Group noted that miR-210 not only targets numerous prosurvival proteins and angiostatic factors, but also directly silences proteins essential for mitochondrial respiration and DNA repair (Devlin et al., 2011). In addition, miR-210 has been extensively studied in cancer progression (Bavelloni et al., 2017). It is known that miR-210 generally exhibits oncogenic properties, because it is frequently elevated in several cancers including breast, lung, head and neck, and pancreatic cancer, and glioblastoma (Bavelloni et al., 2017; Devlin et al., 2011; Qin et al., 2014). miR-148a is aberrantly expressed in a variety of tumours, which has been linked to tumour size, stage of development, metastasis,

Table 4.12 Oxidative stress in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Antioxidant enzyme activity (GSH, GPX, RG, CAT, and SOD)	Blood, urine, nails	Pakistan, Lahore district, cross-sectional	48 people from 4 different industrially contaminated areas with environmental exposure to chromium, cadmium, nickel, cobalt, lead, and zinc; 48 controls (not well defined); metals measured in urine, blood, and nails	↓ GPX with ↑ blood [cobalt] ($R = -0.358$; $P = 0.016$)	Age	Correlation for cobalt and effect; questionnaire used but statistics did not include covariates, except age.	Bibi et al. (2016)
8-OHdG adducts	Urine	European, multiple factories, cross-sectional study of genotoxic outcomes by exposure and DNA repair capacity	21 cobalt-exposed; 26 WC-Co-exposed; 26 non-exposed controls	For the total population, including controls ($n = 72$): ↑ 8-OHdG adducts for interaction term: exposure (cobalt alone or WC-Co) × smoking ($R^2 = 0.155$; $P = 0.003$) For the exposed only group ($n = 47$): ↑ 8-OHdG adducts for interaction term: type of plant (cobalt alone or WC-Co) × smoking ($R^2 = 0.173$; $P = 0.004$)	Genotypes, age, exposure, type of plant, smoking, interaction terms	The potential for differential exposure misclassification is low. There was likely exposure to other metals that was not considered. Cobalt exposure: 20 μg/m^3. Raw data not shown for adduct measures. No effect of repair gene polymorphisms.	Mateuca et al. (2005)

Table 4.12 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
MDA, 8-I	Blood	China, two sites (one former e-waste site, one control site)	Individuals (n = 62) exposed to lead, nickel, cobalt, mercury, copper, zinc, tin, and cadmium Non-exposed controls (n = 47)	Positive correlation between cobalt and 8-I in multilinear regression; B = 2176.57 (244.31–4108.83); P = 0.03 No significant association between cobalt and MDA	[Unclear as text states "different variables" considered in regression] [No statistical differences in age, height, weight, and BMI between exposed and controls]	There is potential for non-differential misclassification as the biological samples may not be reflective of historically relevant exposure. This study did assess and explicitly investigate other metals as co-exposures. The key limitation of this study is the reliance on a single biological sample. It is mentioned that the exposed group did not have drug or alcohol exposure, but it is unclear for the controls. Question about exposure measurement being reflective of historical exposure.	Xue et al. (2021)
MDA, 8-I	Blood	China, two sites (one former e-waste site, one control site)	Exposed (n = 69); non-exposed controls (n = 53) Significant ↑ blood concentrations of lead, nickel, cobalt, and mercury in exposed vs controls	In regression analyses, cobalt was positively correlated with 8-I (R = 0.456; P = 0.00) and MDA (R = 0.171; P = 0.161).	None	The potential for differential exposure misclassification is low with the measurements collected. Though other metals were measured and assessed independently against the oxidative stress and blood–brain barrier disturbance markers, they were not accounted for within the cobalt analyses. A key limitation of this study is the reliance on a single biological measurement for exposure assessment. Although other metals were measured and assessed independently against the oxidative stress and blood–brain barrier disturbance markers, they were not accounted for within the cobalt analyses.	Li et al. (2021b)

Table 4.12 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
8-OHdG adducts	Urine	Refinery workers from several factories in Belgium, Norway, Finland, Sweden, and England	Cobalt-exposed (n = 35/24*) WC-Co-exposed (n = 29) Non-exposed controls (n = 34/27)	NSS between exposed and controls Significant interaction effect for the type of plant (cobalt alone or WC-Co) and age (P = 0.0294)	Creatinine, urine Vitamin E (α-tocopherol), serum Selenium, serum Independent variables: exposure, plant type, cobalt in urine, age, smoking, vitamin E in serum, interaction between smoking and hard-metal exposures	A key limitation of this study is the reliance on a single spot urine sample. The single sample may not reflect the relevant exposure window for all outcomes. Target cobalt: current TLV-TWA (20 mg/g creatinine). Multiple regression for influence of independent variables on outcomes including plant type (WC-Co or cobalt-alone exposure). *Reduced number of cobalt-exposed workers and non-exposed controls after one plant was dropped from study due to worker age differences.	De Boeck et al. (2000)

↓, decreased; ↑, increased; +, positive(ly); 8-I, 8-isoprostane; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BMI, body mass index; CAT, catalase; e-waste, electronic and/or electrical waste; GPX, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; NSS, not statistically significant; RG, reduced glutathione; SOD, superoxide dismutase; TLV-TWA, threshold limit value/time-weighted average; vs, versus; WC-Co, cobalt with tungsten carbide.

and prognosis (Li et al., 2016). miR-148a has also been reported to inhibit the migration, invasion, and proliferation of the colorectal cancer cell lines LoVo and SW480 in vitro (Zhao et al., 2019).]

(b) Experimental systems

(i) Non-human mammals in vivo

In the study by Tang et al. (2020), male C57BL/6 mice were intraperitoneally injected with 0, 4, 8, or 16 mg/kg bw of cobalt(II) chloride, once per day for 30 days. The results showed that the m^6A proportion of total RNA in the cortex of the cobalt(II) chloride-exposed mice was significantly decreased. Cobalt(II) chloride exposure caused alterations in m^6A methylation by dysregulating m^6A methyltransferases (METTLL3, METTL14, and WTAP) and demethylases (FTO and ALKBH5).

(ii) Non-human mammalian cells in vitro

Exposure of the mouse myoblast cell line C2C12 to 50 µg/mL of cobalt(II) chloride for 24 hours or hippocampal primary neurons (derived from P2 mouse pups) for 1 week caused dramatic dephosphorylation of serine 10 on histone H3 and decreased histone H3 pan-acetylation (Verma et al., 2011). Exposure of the rat pheochromocytoma PC-12 or mouse neuroblastoma Neuro-2a (N2a) cell lines to a single dose (300 µM) of cobalt(II) chloride for 24 hours caused decreased acetylation of histones H3 and H4 (Guo et al., 2021). Furthermore, exposure of the monkey kidney COS-7 cell line to cobalt(II) chloride also decreased global histone H3 and H4 acetylation levels in a dose-dependent manner. In addition, exposure to cobalt(II) chloride decreased acetylated histone enrichment within the proximal promoter region of the enzyme extracellular superoxide dismutase 3 (SOD3), leading to its decreased expression (Hattori et al., 2016).

4.2.5 Induces oxidative stress

(a) Humans

(i) Exposed humans

See Table 4.12.

In a study of people living in areas of the Islamic Republic of Pakistan that were industrially contaminated by multiple metals, including cobalt, Bibi et al. (2016) assessed a group of oxidative markers in blood, including glutathione (GSH), glutathione peroxidase (GPX), reduced GSH, CAT, and superoxide dismutase (SOD). Only GPX was associated with cobalt blood levels in correlation analyses, revealing an inverse correlation with cobalt exposure ($r = -0.358$; $P = 0.016$). [The Working Group noted that although other co-exposures were assessed, they were only considered individually in the analyses reported.]

In a cross-sectional study of European factory workers across several plants, some with exposure to cobalt alone and others to tungsten carbide particles (WC-Co), and including non-exposed controls, Mateuca et al. (2005) examined urinary 8-OHdG concentrations as a measure of systemic oxidative DNA damage. At the plants with cobalt-only exposure, no correlation was observed between urinary cobalt and urinary 8-OHdG concentrations. In multiple regression analyses of the total population, including the controls, a positive correlation was observed for 8-OHdG and the interaction term: any cobalt exposure (cobalt alone or WC-Co) × smoking ($R^2 = 0.155$; $P = 0.003$). When the exposed only group (either Co alone or WC-Co) was considered, regression models showed a positive correlation for 8-OHdG and the interaction term: type of plant (Co alone or WC-Co) × smoking ($R^2 = 0.173$; $P = 0.004$). [The Working Group noted that both findings were reported by the authors as having been observed only in the plants with WC-Co exposure and were thus uninformative.]

DeBoeck et al. (2000) assessed a similar cohort to that reported by Mateuca et al. of European factory workers from several plants, who were exposed to either WC-Co or cobalt metal alone (at different facilities) and compared oxidative stress markers in urine with non-exposed controls from the same plants, matching groups for age and smoking. No significant differences were seen between 8-OHdG concentrations in cobalt-exposed workers compared with controls. 8-OhdG was reported to be elevated in smokers who were exposed to hard-metal dust but not in those exposed to cobalt alone. [The Working Group noted that a significant interaction effect for the type of plant (exposure to cobalt alone or WC-Co) and age (P = 0.0294) was reported but attributed the findings to WC-Co exposure.]

In a Chinese study of e-waste-exposed populations living in close proximity to electronics recycling facilities, Xue et al. (2021) measured concentrations of multiple metals including cobalt and various end-points of interest, including levels of 8-isoprostane (8-I) and malondialdehyde (MDA) as indicators of an oxidative stress effect, in blood samples. A positive association for cobalt and 8-I in multilinear regression was reported (B, 2176.57; 95% CI, 244.31–4108.83; P = 0.03), but no significant association between cobalt and MDA was found. [The Working Group noted there is potential for non-differential misclassification, as the biological samples may not be reflective of historically relevant exposure. This study used multilinear models to adjust for co-exposures to other metals. The key limitation of this study was the reliance on single biological samples.]

In another similar study of e-waste-exposed populations in China, Li et al. (2021b) also measured multiple metals and 8-I and MDA in blood to identify evidence of oxidative stress. In regression analyses, cobalt exposure was positively correlated with 8-I (r = 0.456; P = 0.000) and MDA (r = 0.171; P = 0.161). [The Working Group noted that the potential for differential exposure misclassification is low with the measurements collected. Though other metals were measured and assessed independently against the oxidative stress and blood–brain barrier disturbance markers, they were not accounted for within the cobalt analyses. The key limitation of this study was the reliance on a single biological measurement for exposure assessment.]

[The Working Group noted that three out of five studies of cobalt-exposed populations showed some evidence of oxidative stress.]

Scharf et al. (2014) examined the effects of cobalt and chromium in patients who had received metal hip replacement. [The Working Group deemed the study as uninformative because it was related to the effects of metal-on-metal implants, which were outside the scope of this evaluation.]

Walters et al. (2012) examined workers exposed to metalworking fluids. [The Working Group reviewed and excluded the study because 90% of the exposed workers also had elevated urinary levels of chromium, and effects attributable to cobalt exposure alone were not isolated using any statistical analyses. A key limitation of this study was the reliance on a single spot urine sample. Another limitation was the potential for exposure to other metals, including chromium and tungsten.] In addition, Arslan et al. (2011) measured oxidative stress in patients with malignant glioma compared with controls. [The Working Group reviewed and excluded the study because, although levels of multiple metals were measured, these metal levels were not used as predictor variables for the outcomes, and no differences in cobalt levels were observed between exposed individuals and controls. A key limitation of this study was the reliance on a single spot urine sample. The use of a single sample may not reflect the relevant exposure window, particularly as the sample was collected after the outcome.]

Table 4.13 Oxidative stress in human cells in vitro exposed to cobalt

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
Primary cells						
Cobalt(II) chloride ($CoCl_2$)						
Expression of HMOX1	Human primary PBMCs	–	No changes	24 µg/mL	Also tested Co metal (6 µg/mL) with same outcome.	Lombaert et al. (2013)
Mitochondrial superoxide	Human primary PBMCs	+	↑	20 µM	Only one end-point for oxidative stress measurement.	Chamaon et al. (2019)
ROS and GSH levels	Human dermal microvascular endothelial cells isolated from juvenile foreskin	+	↑ ROS level after 4 h and ↓ GSH after 24 h	0.5 mM	No. of samples was not specified.	Peters et al. (2007)
ROS level	Primary human MDMs; primary human pulmonary alveolar cells; human monocytic cell line U937	+	↑	100 µM		Nyga et al. (2015)
Oxidative DNA damage; 8-OHdG detection	Human diploid fibroblasts	–	No changes	250 µM		Ivancsits et al. (2002)
ROS level	Primary human dermal fibrobalsts	+	↑	100–300 µM		Xu et al. (2018a)
Cobalt(II) sulfate ($CoSO_4$)						
Superoxide radical (H_2O_2) formation	Human polymorphonuclear leukocytes obtained from healthy subjects	–	No changes	1 µM/2.5 × 10^5 cells for 30 min	$CoSO_4$ No. of samples was not specified. Only one concentration was tested.	Zhong et al. (1990)
Cobalt metal NPs						
Activities of SOD, GPX, and CAT	Human primary T-cells isolated from peripheral blood collected from healthy donors	+	↓	6 µM for 4 h	NP size, 30–70 nm Donor number was not specified. ↓ SOD, ↓ GPX, and ↓ CAT.	Jiang et al. (2012)
ROS level; GSH	Human dermal microvascular endothelial cells isolated from juvenile foreskin	+	↑ ROS level after 4 h and ↓ GSH after 24 h	25 µg/mL	NP size, 28 nm. No. of samples was not specified.	Peters et al. (2007)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level, 8-OHdG, GSH, and GPX activity	Human CD34+ haematopoietic stem cells/ haematopoietic progenitor cells were isolated from human cord blood vessels	+	↑ ROS level; ↓ GSH; ↓ GPX; ↑ 8-OHdG	200 µM	NP size, 50–200 nm. Decreased cell viability Selenomethionine partially attenuated NP-induced increase in ROS production, and restored the total antioxidant capacity and GSH level. No. of samples was not specified. IC_{50} for cell viability was 200 µM; 200 µM was the only concentration tested for oxidative stress.	Zhu et al. (2021a)
ROS level; GSH level; lipid peroxidation; HO-1 expression	Endothelial cells derived from human aorta (HAECs) and human umbilical vein (HUVECs)	+	↑ ROS level; ↓ GSH; ↑ lipid peroxidation; ↑ HO expression	20 µg/mL	NP size, 17 nm.	Alinovi et al. (2015)
ROS level	Human vascular endothelial HUVEC and HMEC-1 cells	+	↑	800 µM for 24 h	NP size, < 50 nm.	Zhu et al. (2021b)
Cobalt(II) oxide (CoO) NPs						
ROS level	Human primary lymphocytes from health subjects	+	↑	5 µg/mL	NP size, 62 ± 4 nm. Concentration-dependent increase, NAC has significant effects on preventing NP-induced cytotoxicity.	Chattopadhyay et al. (2015a)
Cobalt(II,III) oxide (Co_3O_4) NPs						
ROS level; lipid peroxides; CAT, SOD, and GSH activity	Human peripheral lymphocytes from healthy volunteers	+	↑ ROS level; ↑ lipid peroxides; ↓ CAT; ↓ SOD; and ↓ GSH	50–100 µg/mL for 24 h	NP size, 35.8 + 0.8 nm. Increased cytotoxicity ($n = 3$).	Rajiv et al. (2016)
ROS level	Primary human MDMs; primary human pulmonary alveolar cells; human monocytic cell line U937	+	↑	5 µg/mL	NP size, 2–60 nm. A comparable concentration of Co^{2+} ions did not exhibit such effects.	Nyga et al. (2015)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
Immortalized cell lines						
Cobalt(II) chloride ($CoCl_2$)						
Protein oxidation and nitration; HO-1, GPX, and CAT expression	MG-63 osteoblast-like cells	+	↑ Protein oxidation; ↑ nitration; ↓ HO-1 expression; ↑ GPX; no change to CAT	2.5–10 ppm		Fleury et al. (2006)
ROS level; HO-1 expression	MG-63 osteoblast-like cells	+	↑ ROS levels; ↑ HO-1 expression	200 µM		Li et al. (2017)
Protein oxidation	Macrophage cell line U937	+	↑	10 ppm	Statistical analysis was not clear.	Petit et al. (2005)
Protein nitration	Macrophage cell line U937	+	↑	10 ppm	Statistical analysis was not clear.	Petit et al. (2006)
ROS level	Human prostate cancer cell line PC-3M	+	↑	200 µM		Lu et al. (2007)
Cell viability; clonogenic survival; NAC addition	Human lung cancer cell line H460	+/–	H460 cells treated with cobalt(II) chloride at 300 and 400 µM for 48 h significantly decreased cell viability, at 100–300 µM for 48 h clonogenic survival significantly decreased; an antioxidant scavenger, was able to preserve cell viability and clonogenic survival	LEC was 300 µM for cell viability. LEC was 100 µM for clonogenic survival end-point.	This study did not directly analyse the effect on ROS production or oxidative stress markers.	Ma et al. (2011)
ROS level	Prostate cancer cell line PC-3	+	↑	21.91 mg/L	Only one end-point measured for oxidative stress.	Mahey et al. (2016)
GSH level	Human neuroblastoma cell line SHSY5Y	+	↓	300 µM		Olivieri et al. (2001)
GSH level	Human neuroblastoma cell line SHSY5Y	+	↓	300 µM		Olivieri et al. (2002)
GSH level	H460 human lung epithelial cells	+	↓	100, 200, 300 µM	NAC protective effects of cytoxicity and pro-oxidant Co effects	Luczak and Zhitkovich (2013)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level (indirect)	Human embryonic kidney cell line HECK293T	+	↓	$CoCl_2$ (0, 50, 100, 150, and 200 mM) and/or ascorbic acid (0, 50, 100, 150, and 200 mM) for 3 h	Indirect measurements: whereas ascorbic acid repressed Co(II)-induced OCT4 expression	Yao et al. (2014)
ROS level	Human lung epithelial cells H460	+	↑	150 μM		Patel et al. (2012)
GSH level; MDA formation	Human vascular endothelial cell line EA.hy926	+	↓ GSH; ↑ MDA	100–250 μM		Qiao et al. (2009)
HO-1, Mn-SOD, Cu/Zn-SOD, CAT, and GPX	Human macrophage-like cells U937	+	↑ HO-1; no change to Mn-SOD, Cu/Zn-SOD, CAT, and GPX	7.5 ppm	HO-1 was the only oxidative stress-related marker changed.	Tkaczyk et al. (2010)
ROS level	Human keratinocyte cell line HaCaT	+	↑	400 μM		Yang et al. (2011a)
ROS level	Human transfected trophoblast HTR-8/SVneo cells	+	↑			Wang et al. (2021)
Cobalt(II) sulfate ($CoSO_4$)						
ROS level	Human lung alveolar cancer cell line A549, and immortalized human bronchial epithelial cell line BEAS-2B	+	↑	2.5 mM		Ton et al. (2021)
Cobalt(II,III) oxide (Co_3O_4)						
Oxidative DNA damage measured by alkaline comet assay modified with the enzymes Fpg and hOGG1	Human bronchial epithelial cell line BEAS-2B	+ (with Fpg or hOGG1)	↑	2.5 μg/mL, 2 h and 24 h, Fpg 1.25 μg/mL, 2 h and 24 h, hOGG1	Oxidative DNA damage was observed after cobalt particles (10–1000 nm) (10–20 μg/mL) and $CoCl_2$ (1.25–10 μg/mL) treatment. No data for ROS production or oxidative stress markers.	Uboldi et al. (2016)
Cobalt(II,III) oxide (Co_3O_4) NPs						
Level of MDA; 8-OHdG, GSH	HepG2, A549, and SHSY5Y	+	↑ MDA; ↑ 8-OHdG; ↓ GSH	10 μg/mL		Abudayyak et al. (2017)
Level of MDA; 8-OHdG, GSH	Caco-2 cells	–	↑ MDA; ↑ 8-OHdG; ↓ GSH	10 μg/mL		Abudayyak et al. (2017)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
GSH, lipid hydroperoxide, ROS level, SOD, and CAT activity	Hepatocellular carcinoma-derived cell line HepG2	+	↑ ROS levels; ↑ lipid hydroperoxide; ↑ SOD; ↑ CAT; ↓ GSH	5–15 µg/mL	NP size, 21 nm.	Alarifi et al. (2013)
Intracellular and mitochondrial ROS levels	Human fetal hepatic L02 cells	+	↑ ROS; ↑ mitochondrial ROS	2.5 µg/mL	NP size, 20 nm.	Feng et al. (2020)
ROS level; lipid peroxidation, protein oxidation; HO-1 expression	Human lung alveolar cancer cell line A549	+	↑	50 µg/mL	NP size, < 50 nm.	Alinovi et al. (2017)
ROS level	Human endothelial EVC-304 cells and hepatocellular carcinoma-derived cell line HepG2	+	↑	LEC was 14.7 µg/mL for Co_3O_4 NPs; HIC was 99.2 µg/mL for $CoCl_2$	NP size, 45 nm. Under non-cytotoxic concentrations, Co_3O_4 NPs (14.7–88.2 µg/mL) induced a concentration-dependent increase in ROS generation in both cell lines, whereas $CoCl_2$ (12.4–99.2 µg/mL, the same amount of cobalt in an ionic form) showed no effect on ROS generation.	Papis et al. (2009)
Oxidative DNA damage (ROS level)	Human normal bronchial epithelial cell line BEAS-2B	+	↑	20 µg/cm^2	NP size, 9–62 nm.	Kain et al. (2012)
ROS level	Human lung alveolar cancer cell line A549	+	↑	40 µg/cm^2	NP size, 9–62 nm.	Kain et al. (2012)
ROS level	Human lung alveolar cancer cell line A549	+	↑	30 ppm	NP size, 20–75 nm. Only one end-point for oxidative stress.	Limbach et al. (2007)
ROS level, 8-OHdG level	Human lung alveolar cancer cell line A549	+	↑	5 µg/mL	NP size, 20 nm. NPs composed of 85–90% cobalt metal and 10–15% Co_3O_4.	Wan et al. (2012)
Oxidative DNA damage	Human lung alveolar cancer cell line A549 and human normal bronchial epithelial cell line BEAS-2B	+	↑	20 µg/mL in A549 cells; 5 µg/mL in BEAS-2B cells	NP size, 22.1 + 7.2 nm. Only comet assay, no other end-points for oxidative stress measurement.	Cavallo et al. (2015)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level; GSH; lipid peroxidation	Human breast cancer cell line MCF-7	+	↑	50 µM	NP size, 30 ± 20 nm. NAC, a precursor of GSH, restored GSH depletion and (BSO, an inhibitor of GSH synthesis pathway) aggravated the depletion of GSH.	Akhtar et al. (2017)
ROS level	Human leukaemia cell line K562	+	↑	142 µg/mL	NP size, 50 nm. Only one concentration and one end-point for oxidative stress.	Arsalan et al. (2020)
ROS level	Human leukaemia cell line K562, Jurkat cells, KG1-A cells	+	↑	1 µg/mL	NP size, 74 ± 8 nm. Dose-dependent effect (1–50 µg/µL).	Chattopadhyay et al. (2015b)
ROS level	Human monocytic cell line U937	+	↑	100 µM	NPs were composed of 90% cobalt metal and 10% Co_3O_4. NP size, 2–60 nm.	Xu et al. (2018b)
Cobalt(II) oxide (CoO) NPs						
ROS level; HIF-1α protein expression; MT3, NOS2, PTGS2(Cox2), and SOD3 gene expression	Human small airway epithelial cells, HSAEc	+	↑	25 µg/mL	NP size, 53.55 nm.	Sisler et al. (2016b)
Hypoxia-related studies: primary cells						
Cobalt(II) chloride ($CoCl_2$)						
HO-1	Human trophoblasts isolated from normal-term placentas	+	↑	250 µM	Also increased in 500 µM.	Ma et al. (2011)
Mitochondria-derived ROS	Human periodontal ligament stem cells	+	↑	200 µM, 72 h	Induced apoptosis.	He et al. (2018)
Hypoxia-related studies: immortalized cell lines						
Cobalt(II) chloride ($CoCl_2$)						
ROS level; COX-2	Human skin keratinocyte cell line HaCaT	+	↑	400 µM, 24 h		Yang et al. (2011b)
Cellular ATP	Human prostate carcinoma cell line DU145	+	↓	25 µM		Lee et al. (2006)
ROS level	Human colorectal carcinoma epithelium cell line HCT 116	+	↑	100 µM, 30 min		Seo et al. (2016)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
PLD activity; COX-2	Human astroglioma cell line U-87 MG	+	↑	200 µM	Results occurred in a dose- and time-dependent manner.	Ahn et al. (2007)
ROS level	Human glioma cells with oxidative phosphorylation-dependent (U251-MG) and glycolytic-dependent (D54-MG) phenotypes	+	↑	100 µM		Griguer et al. (2006)
Intracellular OH radical levels	Hepatocellular carcinoma-derived cell line HepG2	+	↓	100 µM, 24 h		Porwol et al. (1998)
ROS level	Human colorectal adenocarcinoma cell line Caco-2	+	↑	100 µM		Liu et al. (2012b)
ROS level	Hep3B wildtype and Hep3B depleted of mitochondrial DNA	+	↑	100 µM		Chandel et al. (1998)
ROS level; COX-2	Pulmonary artery smooth muscle cells	–	No changes	25 µM, 24 h	Induced cell proliferation.	Li et al. (2014)
HO-1, ROS level	Human microvascular endothelial cell line HMEC-1	+	↑	250 µM		Loboda et al. (2005)
ROS level	Human retinal ganglion cells	+	↑	100 µM, 24 h		Tulsawani et al. (2010)
ROS level	Trophoblast cell line HRT-8/SVneo	+	↑	500 µM		Zhao et al. (2014)
ROS level	Human skin keratinocyte cell line HaCaT	+	↑	750 µM, 6 h	Resulted in cytotoxicity of HaCaT cells.	Yang et al. (2018)
ROS level	Endothelial cell line EA.hy926	+	↑	300 µM	Induces excessive apoptotic cell death.	Tan et al. (2009)
ROS level	Trophoblast cell line HTR-8	+	↑	500 µM	Proliferation of H8 cells gradually decreased as the $CoCl_2$ treatment concentration increased.	Zheng et al. (2016)
ROS level	Hepatocellular carcinoma-derived cell line HepG2	–	No changes	200 µM	Increased mRNA levels of pro-fibrotic cytokines TGF-β1, α-SMA.	Hernández et al. (2020)
ROS level; mitochondrial ROS; lipid 8-Is; 4-HNE protein adducts; NF-κB activation	Human retinal epithelium cell line hRPE	+	↑	200 µM	Decrease cell viability after 12 h.	Cervellati et al. (2014)

Table 4.13 (continued)

End-point	Tissue, cell line	Results[a]	Direction of response	Concentration	Comments	Reference
ROS level	Human retinal pigment epithelial cells (ARPE-19 cell line)	+	↑	600 µM, 24 h	Decreased cell viability.	Li et al. (2013)
ROS level; COX-2; phosphorylation of NF-κB p65 subunit	Human skin keratinocyte cell line HaCaT	+	↑	500 µM	Reduced cell viability and oversecretion of IL-6 and IL-8.	Yang et al. (2011a)
ROS level	Human cervical cancer cell line HeLa	+	↑	150 µM	Decreased cell proliferation.	Triantafyllou et al. (2006)
SOD; MDA	Spiral arterial smooth muscle cells	+	↑	50 µM, 24 h	Decreased cell viability, increased apoptosis.	Xiao et al. (2020)
LDH; ROS level	Human colorectal adenocarcinoma cell line Caco-2	+	↑	100 mM		Basavaraju et al. (2021)

↑, increased or upregulated; ↓, decreased or downregulated; 4-HNE, 4-hydroxynonenal; 8-I, isoprostane; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ATP, adenosine triphosphate; BSO, buthionine-(S,R)-sulfoximine; CAT, catalase; COX-2, cyclooxygenase-2; Cu/Zn-SOD, copper/zinc superoxide dismutase; Fpg, formamidopyrimidine DNA glycosylase; GPx, glutathione peroxidase; GSH, glutathione; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; HIF-1α, hypoxia-inducible factor-1α; HMOX1, haem oxygenase 1; HO, haem oxygenase; HO-1, haem oxygenase isoenzyme 1; hOGG1, human 8-oxoguanine DNA *N*-glycosylase-1; IC_{50}, half maximal inhibitory concentration; IL-6/8, interleukin-6/8; LDH, lactate dehydrogenase; LEC, lowest effective concentration; MDA, malondialdehyde; MDM, monocyte-derived macrophages; min, minute; Mn-SOD, manganese superoxide dismutase; MT3, metallothionein 3; NAC, *N*-acetyl cysteine; NF-κB, nuclear factor-kappa B; NOS2, nitric oxide synthase 2; PBMC, peripheral blood mononuclear cell; PLD, phospholipase D; ppm, parts per million; PTGS2(Cox2), prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2); ROS, reactive oxygen species; SOD, superoxide dismutase.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

(ii) Human cells in vitro

See Table 4.13.

Primary cells

Cobalt metal or soluble cobalt(II) salts

A study by Lombaert et al. (2013) reported that in human PBMCs isolated from healthy donors (n = 3), exposure to cobalt metal (6.0 μg/mL) or 6.0 μg/mL of cobalt-equivalent cobalt(II) chloride (24 μg/mL) did not result in significant induction of the antioxidant enzyme haem oxygenase 1, which is encoded by the HMOX1 gene (Lombaert et al., 2013). Cobalt(II) sulfate at 1 μM per 2.5×10^5 cells did not stimulate superoxide radical (O_2^-) formation in human polymorphonuclear leukocytes obtained from healthy volunteers (Zhong et al., 1990) [The Working Group noted that the number of volunteers was not specified]; however, Chamaon et al. (2019) reported that in PBMCs isolated from healthy donors (n = 2), exposure to cobalt(II) chloride at concentrations of 20, 200, and 300 μM for 24 hours significantly increased mitochondrial superoxide radical (O_2^-) formation. An increase in ROS formation, using 5-(and 6-)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) was observed in human dermal fibroblasts after a 6-hour exposure to cobalt(II) chloride at 100–300 μM (Xu et al., 2018a).

Cobalt-based nanoparticles

Chattopadhyay et al. (2015a) reported that intracellular production of ROS increased by 1.25–4.18-fold in human lymphocytes isolated from healthy donors (n = 6) treated with cobalt(II) oxide NPs (size, 62 ± 4 nm) at 1–50 μg/mL. Pre-treatment with the antioxidant *N*-acetylene cystine attenuated cobalt(II) oxide NP-induced cytotoxicity, indicating that ROS play a role in the cytotoxicity induced by cobalt(II) oxide NPs (Chattopadhyay et al., 2015a). Similarly, Rajiv et al. (2016) showed that cobalt(II,III) oxide NPs (size, 35.8 ± 0.8 nm; 50–100 μg/mL) caused concentration-dependent cytotoxicity and oxidative stress in human lymphocytes isolated from healthy volunteers (n = 3). The evidence for oxidative stress included increases in ROS and lipid peroxidation levels, and decreases in CAT, SOD, and GSH levels (Rajiv et al., 2016).

Nyga et al. (2015) performed a comparison study of cobalt(II,III) oxide NPs (2–60 nm) and cobalt(II) chloride in primary human monocyte-derived macrophages from healthy donors (n = 6), in primary human pulmonary alveolar macrophages from tissues obtained after resection for lung carcinoma (n = 6), and in the human monocytic U937 cell line. Cobalt(II,III) oxide NPs (5–20 μg/mL) induced cytotoxicity and increased ROS levels after exposure for 3 and 6 hours in all three types of monocytic cell models, whereas cytotoxic effects were not observed after treatment with an equivalent concentration of cobalt(II) chloride. Exposure to cobalt(II,III) oxide NPs increased the level of the HIF-1α transcription factor, leading to the upregulation of HIF target genes, which include BNIP3, COX2, GLUT1, and HO1. The antioxidants ascorbic acid and GSH both prevented ROS generation; however, only ascorbic acid reduced HIF-1α levels and prevented cell death (GSH had no effect in preventing cell death) (Nyga et al., 2015). [The Working Group noted that an ROS-independent pathway could be also involved in cytotoxicity induced by cobalt-based NPs.]

In a study conducted in human dermal microvascular endothelial cells isolated from juvenile foreskin [The Working Group noted that the number of samples was not specified], both cobalt metal NPs (size, 28 nm; 25 and 50 μg/mL) and cobalt(II) chloride (0.5 and 1 mM) increased ROS generation after 4-hour exposure (Peters et al., 2007). Another study reported that treatment of human CD34+ haematopoietic stem cells/haematopoietic progenitor cells that were isolated from human cord blood vessels in the placentas of full-term infants with cobalt metal

NPs (size, 50–200 nm) at a concentration of 200 μM (IC_{50}) increased ROS and 8-OHdG levels and decreased GPX activity. [The Working Group noted that the number of samples was not specified.] The addition of selenomethionine partially attenuated cobalt metal NP-induced ROS overproduction and restored total antioxidant capacity and the level of GSH (Zhu et al., 2021a).

Induction of oxidative stress by cobalt metal NPs was also detected in two primary endothelial cells derived from human aorta (HAECs) and human umbilical vein (HUVECs) (Alinovi et al., 2015; Zhu et al., 2021b) as well as from the HMEC-1 cell line (Zhu et al., 2021b), as demonstrated by increased levels of ROS production and lipid and protein peroxidation, and decreased GSH levels (Alinovi et al., 2015; Zhu et al., 2021b). Similarly, Jiang et al. (2012) observed reduction in the activities of superoxide dismutase, glutathione peroxidase and CAT enzymes after a 4-hour exposure to cobalt metal NPs at 6 μM in human primary T-cells isolated from peripheral blood collected from healthy donors (Jiang et al., 2012).

Immortalized cell lines

Soluble cobalt(II) salts

Increases in ROS levels were detected in the following human cell lines after exposure to soluble cobalt(II) salts: prostate cancer PC-3M cells (Lu et al., 2007; Mahey et al., 2016), lung epithelial H460 cells (Patel et al., 2012), osteoblast-like MG-63 cells (Li et al., 2017), keratinocyte 926 cells (Yang et al., 2011a), lung cancer A549 and immortalized bronchial epithelial BEAS-2B cells (Ton et al., 2021), vascular endothelial EA.hy926 cells (Qiao et al., 2009), and transfected trophoblasts (HTR-8/SVneo) (Wang et al., 2021).

In human MG-63 (osteoblast-like) and U937 monocytic cells, soluble cobalt(II) salts increased protein oxidation and nitration, which are two markers of oxidative stress (Petit et al., 2005, 2006; Fleury et al., 2006).

Soluble cobalt(II) salts elevated the expression of antioxidant enzymes such as haem oxygenase 1 (HO-1) and GPX in human MG-63 (osteoblast-like) cells (Fleury et al., 2006; Li et al., 2017) and in U937 (macrophage) cells (Tkaczyk et al., 2010). Studies also showed that the oxidative stress effect induced by soluble cobalt(II) salts is cell type-dependent (Ivancsits et al., 2002; Chamaon et al., 2019).

Studies also showed some evidence that soluble cobalt(II) salts induce oxidative stress because antioxidants (*N*-acetyl cysteine, ascorbic acid, and melatonin) showed protective effects against cobalt(II)-induced cytotoxicity (Olivieri et al., 2001, 2002; Luczak & Zhitkovich, 2013; Yao et al., 2014; Uboldi et al., 2016).

Cobalt-based nanoparticles

Several studies found that in human alveolar carcinoma (A549) and human normal bronchial epithelial (BEAS-2B) cells cultured in vitro, cobalt(II,III) oxide NPs caused increased levels of intracellular ROS, gene expression of haem oxygenase 1, lipid peroxidation, and oxidative stress-associated DNA damage (Limbach et al., 2007; Kain et al., 2012; Wan et al., 2012; Cavallo et al., 2015; Alinovi et al., 2017; Cappellini et al., 2018). (The NPs tested in the Wan et al. study consisted of 85–90% cobalt metal NPs and 10–15% cobalt(II,III) oxide NPs.) [The Working Group noted that some studies reported that the addition of antioxidants attenuated the adverse effects induced by the NPs.]

In two hepatic cell lines, human hepatocarcinoma (HepG2) and human fetal hepatic (L02) cells, cobalt(II,III) oxide NPs increased levels of ROS and lipid hydroperoxide, SOD, and CAT activity; and decreased the concentration of GSH. Soluble cobalt(II) salts also induced cytotoxicity and oxidative stress, but the effects were less significant compared with the NPs (Papis

et al., 2009; Alarifi et al., 2013; Abudayyak et al., 2017; Feng et al., 2020).

The induction of oxidative stress by cobalt(II,III) oxide NPs has also been reported in the following cell lines: human breast cancer (MCF7) cells (Akhtar et al., 2017), as demonstrated by increased ROS production and reduced GSH; human leukaemia (K562) cells (Arsalan et al., 2020; Chattopadhyay et al., 2015b); and by cobalt(II) oxide NPs in human small airway epithelial cells (HSAEs) (Sisler et al., 2016b), as demonstrated by increased ROS levels. Xu et al. also observed an increase of ROS level in human monocytic cells (U937 cell line) treated with 100 μM cobalt nanoparticles composed of 90% cobalt metal and 10% Co_3O_4 (Xu et al., 2018b).

Cobalt(II) chloride-induced hypoxia

Oxidative stress has been assessed in studies that used cobalt(II) chloride as a means of chemically inducing hypoxia, mostly after exposure to concentrations ≥ 100 μM (e.g. Porwol et al., 1998), with little evidence of hypoxia induction reported at lower concentrations (Lee et al., 2006; Xiao et al., 2020). The expression of HIF-1α, a key regulator of hypoxia, was frequently assessed in these studies. In two studies conducted with human primary cells, cobalt(II) chloride increased HO-1 expression, a sensor of cellular oxidative stress (Ma et al., 2011), and levels of ROS within the mitochondria (He et al., 2018) in trophoblasts isolated from normal-term placentas and human periodontal ligament stem cells, respectively. Numerous studies conducted in a variety of human immortalized cell lines (e.g. Yang et al., 2011a; Liu et al., 2012b; Seo et al., 2016) demonstrated an ability of cobalt(II) chloride to stimulate an intracellular hypoxia-like condition by regulating the stability of HIF-1α, thus rapidly increasing levels of intracellular ROS or other markers of oxidative stress (see Table 4.13).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table S4.14 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt metal or cobalt-based nanoparticles

After 12 months of follow-up, Sprague-Dawley rats with cobalt metal cylinders (diameter, 1 mm; length, 2 mm) surgically implanted into the gastrocnemius muscle showed no significant alterations in thiobarbituric acid-reacting substances (TBARS) and GSH in serum (Kalinich et al., 2022). Ultrafine cobalt metallic particles administrated by intratracheal instillation (1 mg/rat) increased the levels of lipid peroxides in bronchoalveolar lavage fluid (BALF) from male Wistar rats (Zhang et al., 1998). Elevated MDA and 8-OHdG levels were shown in the temporal lobe and hippocampus of rats after exposure to cobalt metal NPs (intraperitoneal administration at 2–8 mg/kg bw per day for 20 days) (Zheng et al., 2019), and in the lungs of mice exposed to cobalt-based NPs (85–90% cobalt metal NPs and 10–15% Co_3O_4 NPs) at 50 μg/mouse by intratracheal instillation for up to 28 days (Wan et al., 2017). In addition, Nrf2 and HO-1 levels showed dose-dependent increases in rat hippocampus (Zheng et al., 2019). In line with these findings, increased HO-1 levels were found in the lungs of mice exposed to a single dose of cobalt(II) oxide NPs (20 μg/mouse) by oropharyngeal aspiration (Zhang et al., 2012). Ton et al. (2021) also reported on the levels of 8-OHdG in lungs of mice exposed via inhalation to cobalt metal for 90 days in a study conducted by the NTP (2014). Ultrafine tricobalt tetraoxide [cobalt(II,III) oxide] enhanced hydroxyl radical generation in rat BALF (Dick et al., 2003). [The Working Group noted that this study tested only a single dose, showing a clear limitation of the study.]

Soluble cobalt(II) salts

Cobalt(II) chloride has been shown to induce alterations in multiple oxidative stress-related markers – including MDA, superoxide, H_2O_2, nitric oxide, and AOPP (advanced oxidation protein products) – in a wide range of tissues and organs (brain, blood, liver, heart, and kidney) of rats, rabbits, and pigs (Kuno et al., 1980; Morita et al., 1982; Johansson et al., 1986; Wang et al., 1993; Daido & Aniya, 1994; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Sumbayev, 2001; Gonzales et al., 2005; Kalpana et al., 2008; Garoui et al., 2011, 2013; Ajibade et al., 2017; Awoyemi et al., 2017; Akinrinde & Adebiyi 2019; Oyagbemi et al., 2019). In addition, oxidative stress-related enzymes were also reported to respond substantially to cobalt(II) chloride exposure. For example, HO-1 activity was found to be induced significantly in the liver of mice, rats, and pigs (Numazawa et al., 1989a; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005). In contrast, GPX activity displayed significant changes with varied patterns in a large number of studies (Hatori et al., 1993; Daido & Aniya, 1994; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Ajibade et al., 2017; Awoyemi et al., 2017; Akinrinde & Adebiyi, 2019; Oyagbemi et al., 2019). In agreement with the trend reported for GPX caused by cobalt(II) chloride exposure, the activities/levels of a group of antioxidant enzymes had distinct responses to exposure. While several studies found that glutathione *S*-transferase (GST) activity (Daido & Aniya, 1994), GSH levels (Nordström et al., 1990; Christova et al., 2001), CAT activity (Christova et al., 2002), glutathione reductase activity (Christova et al., 2001, 2002), and PSH (protein thiol) levels (Oyagbemi et al., 2019) were increased, many studies reported that cobalt(II) chloride exposure decreased GST activity (Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Ajibade et al., 2017; Abdel-Rahman Mohamed et al., 2019; Akinrinde & Adebiyi, 2019; Oyagbemi et al., 2019), SOD activity (Hatori et al., 1993; Llesuy & Tomaro, 1994; Christova et al., 2001, 2002, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Awoyemi et al., 2017; Abdel-Rahman Mohamed et al., 2019; Akinrinde & Adebiyi, 2019), CAT activity (Llesuy & Tomaro, 1994; Christova et al., 2001, 2003; Gonzales et al., 2005; Garoui et al., 2011, 2013; Awoyemi et al., 2017; Abdel-Rahman Mohamed et al., 2019; Oyagbemi et al., 2019), and PSH (protein thiol) and NPSH (non-protein thiol) levels (Garoui et al., 2013; Ajibade et al., 2017). Furthermore, gene and/or protein expression responses related to oxidative stress have also been investigated by several studies. Increased mRNA and/or protein expression of HO-1/2, copper/zinc-SOD, and NOX2 were found in multiple organs of rats exposed to cobalt(II) chloride (Bauer et al., 1998; Gonzales et al., 2005; Guan et al., 2015; Nordquist et al., 2015; Abdel-Rahman Mohamed et al., 2019). In contrast, decreased Nrf2 levels were reported in the brain of cobalt(II) chloride-treated rats (Guan et al., 2015). [The Working Group noted that, with regard to study quality, most of the studies tested only a single dose, which was a clear limitation. However, the large number of reports counterbalanced the limitations. In addition, several studies showed dose- and time-dependent effects or presented multiple end-points related to oxidative stress, supporting the accuracy and reliability of the studies.]

Studies on cobalt(II) chloride hexahydrate also demonstrated that multiple oxidative stress markers were significantly altered in a wide range of tissues and organs in rat models. Namely, one or more alterations in MDA, H_2O_2, nitric oxide, ROS, protein oxidation, 8-OHdG, total antioxidant capacity (TAC), and protein carbonyl levels have been reported in a variety of studies (Shrivastava et al., 2008; Saxena et al., 2010; Akinrinde et al., 2016; Zheng et al.,

2019; Abdel-Daim et al., 2020; Oyagbemi et al., 2020). Additionally, cobalt(II) chloride hexahydrate exposure induced alterations in oxidative stress-related enzymes. For example, augmented activities of haem oxygenase (Stelzer & Klaassen, 1985), GST (Akinrinde et al., 2016), CAT, and MPO (Akinrinde et al., 2016; Abdel-Daim et al., 2020; Oyagbemi et al., 2020), as well as increased GSH (glutathione) levels with declining GSSG (glutathione disulfide) levels accompanied by an increased ratio of GSH/GSSG (Stelzer & Klaassen, 1985; Shrivastava et al., 2008; Saxena et al., 2010), were found in animals exposed to cobalt(II) chloride hexahydrate. In addition, increased Nrf2 and HO-1 protein levels were observed in the hippocampus and/or gastrocnemius muscle of rats after administration of cobalt(II) chloride hexahydrate by injection (Saxena et al., 2010; Zheng et al., 2019). There are also several studies that used other cobalt forms, such as cobalt(II) sulfate and cobalt(II) acetate. One study demonstrated that cobalt(II) sulfate exposure caused suppression of manganese-SOD activity in the myocardium of rats (Clyne et al., 2001). [The Working Group noted as a clear limitation that both studies tested only a single dose.] Cobalt(II) acetate exposure in rats resulted in a dose-dependent increase in oxidative DNA damage and higher levels of products of hydroxyl radical attack in the kidney, liver, and lung (Kasprzak et al., 1994).

[The Working Group noted that, with regard to the exposure routes of the studies summarized above, most experiments investigating soluble cobalt(II) salt-induced alterations in end-points related to the key characteristic oxidative stress were carried out by acute or subacute intraperitoneal and subcutaneous injection using a dose range of up to 80 mg/kg bw. There were also several experiments performed by oral administration (100–650 mg/L) and, on occasion, gavage or inhalation exposure were used.]

(ii) Non-human mammalian cells in vitro

Cobalt metal nanoparticles

Cobalt metal NPs increased levels of multiple oxidative stress markers and various oxidative stress-related enzymes. Increased ROS levels, the most common oxidative stress markers in most studies, were found in the rat RAW 264.7 cell line and Wistar rat LV myocytes (Zhang et al., 2012; Savi et al., 2021).

Soluble cobalt(II) salts

Soluble cobalt(II) salts, mainly cobalt(II) chloride, increased multiple oxidative stress markers (e.g. ROS and MDA), oxidative stress-related enzymes (e.g. SOD, CAT, and GSH), and gene/protein expression (e.g. HO-1 and NOX) in various non-human mammalian cells in vitro. [The Working Group noted that an increase in ROS levels was the most common oxidative stress marker assessed in cells exposed to cobalt(II) chloride (Karovic et al., 2007; Chen et al., 2010b, 2018b, 2019; Kamiya et al., 2008, 2010; Tong et al., 2012; Lan et al., 2013; Wang et al., 2013c; Wenker et al., 2013; Guan et al., 2015; Shweta et al., 2015; Lee et al., 2016; Zimmerman et al., 2018; Manu et al., 2019; Yin et al., 2021; Luo et al., 2022).] In addition to increased ROS, cobalt(II) chloride increased MDA levels in various types of cells, including rat primary hepatocytes, cardiomyocyte H9c2 cells, and PC-12 cells (Wang et al., 2013c; Chen et al., 2019; Manu et al., 2019; Yin et al., 2021). In line with the above findings, the levels or activities of oxidative stress-related enzymes – including haem oxidase (Maines & Sinclair, 1977), GSH and GSSG (Maines & Sinclair, 1977; Chen et al., 2010b; Guan et al., 2015; Wang et al., 2016a), SOD (Yang et al., 2011c; Tong et al., 2012, Wang et al., 2013c, 2016a; Yin et al., 2021), CAT (Tong et al., 2012; Wang et al., 2016a; Yin et al., 2021), and NOX2 (NADPH oxidase 2) (Chen et al., 2019) – were also significantly altered by cobalt(II) chloride exposure. In addition, cobalt(II) chloride induced changes in

Table 4.15 Chronic inflammation in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
COX-2; IL-1β	Autopsy samples of brain and lung tissue	Mexico, urban air pollution exposure vs 2 less-exposed locations	47 exposed and 12 controls (adults and children)	↓ COX-2 with ↑ frontal lobe Co ($R = -1.96 \times 10^4$; probability $t = 0.0024$) Effect of Co on IL-1β in brain NSS	Age	Co and other metals were quantitatively assessed using accepted methods. The potential for misclassification of Co exposure is low. A limitation of this study is the reliance on a single time point for exposure information, and whether this time point captures the relevant window of exposure for the mechanistic end-point of interest. Age, sex, place of residency, cause of death, and time between death and autopsy noted. Cause of death was considered for all subjects to rule out infection, inflammatory events, drug exposure, brain ischaemia, and hypoxia impact on mRNA levels of the inflammatory markers measured in the study. Co measured quantitatively as predictor of outcomes. While specific metal effects were explored through regression analysis for some brain findings (Table 3), other outcome comparisons between exposed individuals and controls for lung and brain sites found significant correlations between any lung metal concentrations and COX-2 or IL-1β lung expression.	Calderón-Garcidueñas et al. (2013)

Table 4.15 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Inflammatory infiltrate with fibrosis	Postmortem lung tissue and blood	Belgium, worker case report	Diamond polisher using Co-containing discs for 35 yr	+ Lung histology for active inflammation with multinucleated giant cells and a mononuclear inflammatory infiltrate in the alveolar septa with prominent fibrosis. ↑ Blood sedimentation rate (28 mm/h) and ↑ leukocyte count of 10 500/mm^3 (with 71% neutrophils)		The case report provides detailed qualitative narrative exposure history and quantitative measurements of Co in lung tissue for 1 patient. The key limitation is the nature of a case report with a sample size of 1.	Nemery et al. (1990)
Inflammatory markers	Serum	Belgium, Co refinery workers	82 Co-exposed males 82 non-exposed controls (age-matched)	↑ Sedimentation rate in exposed vs controls but NSS difference ↑ leukocyte count ($P < 0.05$) in Co-exposed vs controls	Controls were age, education, and SES matched	There is limited potential for non-differential misclassification in the exposure groups. This study focuses on comparisons between exposed and unexposed groups working at different companies. The key limitation of this study is the reliance on the qualitative exposure assessment approach (exposed/unexposed) based on location of employment. These findings might not be clinically relevant nor indicative of chronic inflammation.	Swennen et al. (1993)

Table 4.15 (continued)

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Inflammatory and fibrotic markers (TGF-β and α-SMA)	Blood	China, 2 sites (1 former e-waste site, 1 control site)	Individuals (n = 62) exposed to Pb, Ni, Co, Hg, Cu, Zn, Sn, and Cd Non-exposed controls (n = 47)	In multiple linear regression analyses, Co was positively correlated with α-SMA (β = 323.18; P = 0.03) and TGF-β (β = 4814.69; P < 0.01) in blood from the exposed group	[Unclear as text states "different variables" considered in regression.] [No statistical differences in age, height, weight, and BMI between exposed individuals and controls]	There is potential for non-differential misclassification as the biological samples may not be reflective of historically relevant exposure. This study did assess and explicitly investigate other metals as co-exposures. The key limitation of this study is the reliance on a single biological sample. Study mentions that the exposed group did not have drug or alcohol exposure, but unclear about the controls. End-points measured as indicators of inflammation and fibrosis. Question about exposure measurement being reflective of historical exposure.	Xue et al. (2021)
Serum proteins	Serum	Czechia production workers, cross-sectional	38 Ni-exposed 35 Co-exposed Non-exposed controls	↑ A_1AT, A_2M, CPL, and LYS in Co-exposed compared with controls (P < 0.001 to P < 0.005)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1983)
Serum proteins	Serum	Czechia production workers, cross-sectional	38 Ni-exposed 35 Co-exposed Non-exposed controls	↑ A_2M, TRF, A_1AT, CPL, LYS, and A_1GP in Co-exposed compared with controls (P < 0.05 for TRF; P < 0.001 for all others)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1986b)

+, positive(ly); ↓, decreased; ↑, increased; A_1AT, alpha$_1$-anti-trypsin; A_2M, alpha$_2$-macroglobulin; A_1GP, alpha$_1$-glycoprotein; BMI, body mass index; Cd, cadmium; Co, cobalt; COX-2, cyclooxygenase-2; CPL, ceruloplasmin; Cu, copper; e-waste, electronic and/or electrical waste; IL-1β, interleukin 1 beta; Hg, mercury; LYS, lysozyme; mRNA, messenger RNA; Ni, nickel; NSS, not statistically significant; Pb, lead; α-SMA, α-smooth muscle actin; Sn, tin; TGF-β, transforming growth factor-β; TRF, transferrin; vs, versus; yr, year; Zn, zinc.

protein levels and/or mRNA expression of many antioxidants and ROS-generating enzymes, including GST, SOD, CAT, HO-1, NOX1/2, and iNOS (inducible nitric oxide synthase). Cobalt(II) chloride hexahydrate also induced oxidative stress markers. For instance, ROS and protein carbonyl levels were increased in rat RAW 264.7 cells (Zhu et al., 2017; Salloum et al., 2018). [The Working Group noted that most studies were relevant, because they presented more than one end-point related to oxidative stress.]

[The Working Group noted that cobalt(II) chloride and cobalt(II) chloride hexahydrate were commonly used as hypoxia mimics in many studies in which oxidative stress-related markers were assessed. Most of the studies had limitations related to the concentration used and end-point selected; however, the major findings of the studies conducted in non-human mammalian cells in vitro supported the conclusion that soluble cobalt(II) salts are oxidative stressors.]

4.2.6 Induces chronic inflammation

(a) Humans

(i) Exposed humans

See Table 4.15.

A Mexican autopsy study in brain and lung tissue samples investigated air pollution effects, including from metal exposure in people from urban versus less-exposed (control) locations (Calderón-Garcidueñas et al., 2013). There was a difference (not statistically significant) in cobalt concentrations in frontal lobe or lung tissue samples from urban-exposed versus control individuals. However, regression analyses revealed that increased frontal lobe concentrations of cobalt correlated inversely with gene expression of the cyclooxygenase-2 (COX2) enzyme, a pro-inflammatory marker (probability, $t = 0.0024$). A cobalt-dependent effect on the pro-inflammatory cytokine interleukin-1β (IL-1β) in brain tissue was not observed. COX-2 expression in the lung was also measured and found to be significantly elevated in the urban-exposed group versus controls ($P = 0.01$), but no significant correlations were found for any metal-specific effects. [The Working Group considered this study of air pollution mixtures to be minimally informative for the evaluation of cobalt with respect to this end-point.]

Nemery et al. (1990) described the autopsy findings of a Belgian diamond polisher who had worked using cobalt-containing polishing discs for 35 years. They reported mostly normal blood haematology and chemistry results, except for an elevated sedimentation rate of 28 mm/hour and a leukocyte count of 10 500/mm^3 with 71% neutrophils. The diamond polisher's lung histology showed active inflammation with multinucleated giant cells and a mononuclear inflammatory infiltrate in the alveolar septa upon necropsy. Prominent fibrosis was also observed. [The Working Group noted that this case report provided detailed qualitative narrative exposure history and quantitative measurements of cobalt in lung tissue for one patient. The key limitation was the nature of a case report with a sample size of one.]

In a study conducted in Belgium, Swennen et al. (1993) assessed health effects in 82 cobalt refinery workers employed for an average duration of 8 years, with a geometric mean TWA exposure to cobalt dust of 125 μg/m^3. This included 25% of the worker population with exposure > 500 μg/m^3. All study participants were grouped into the exposed or unexposed group on the basis of their location of employment. Within the exposed group, exposure was further assessed through inhalation (air samples) and all routes (biological measurements). Results were compared with an age-matched group of non-cobalt dust-exposed controls. Leukocyte counts were elevated in the cobalt-exposed group compared with controls (8.03 versus 6.73 × 10^9/L; $P < 0.05$). The sedimentation rate was also higher, but not statistically significant, in the

cobalt-exposed group compared with controls (5.00 versus 3.00 mm/hour). [The Working Group noted that these findings might not be clinically relevant nor indicative of chronic inflammation. There is limited potential for non-differential misclassification in the exposure groups. This study focused on comparisons between exposed and unexposed groups working at different companies. A potential limitation of this study was the reliance on the qualitative exposure assessment approach, which was based on the location of employment, but air sampling and biological monitoring confirmed good exposure contrast between the groups.]

In a Chinese study of e-waste-exposed populations living near former electronics recycling plants, Xue et al. (2021) measured concentrations of metals and assessed markers of inflammation and fibrosis in blood, including transforming growth factor-β and α-smooth muscle actin. In multiple linear regression analyses, cobalt was positively correlated with α-SMA (α-smooth muscle actin) (β = 323.18; P = 0.03) and TGF-β (transforming growth factor-β) (β = 4814.69; $P < 0.01$) in blood from the exposed group. [The Working Group noted that this study did assess other metals as co-exposures and that these were accounted for in the results for cobalt. However, a key limitation of this study was the reliance on a single biological sample and whether this was reflective of historical exposure.]

Other evidence of an altered inflammatory response in cobalt-exposed human populations was reported in two cross-sectional studies of cobalt production workers in Czechia compared with non-exposed, age-matched controls (Bencko et al., 1983, 1986b). In the earlier study (1983), average values of acute-phase reactant proteins including α1-anti-trypsin, α_2-macroglobulin, ceruloplasmin, and lysozyme were elevated in the cobalt-exposed individuals ($P < 0.001$ to $P < 0.005$). In the later study (1986), acute-phase reactant proteins were also assessed, of which, α1-anti-trypsin, α_2-macroglobulin, transferrin, ceruloplasmin, lysozyme, and α1-glycoprotein were all significantly elevated in the cobalt-exposed individuals compared with controls ($P < 0.05$ for transferrin; $P < 0.001$ for all others). [The Working Group noted that the key limitation of this study was the limited information on the exposure definition, which provided no information on the industry or occupation of the exposed workers, or the potential for co-exposure.]

[The Working Group noted that four out of six studies of cobalt-exposed populations showed some evidence of being possibly associated with chronic inflammation.]

Katsarou et al. (1997) examined Greek cement workers with co-exposure to chromium. [The Working Group evaluated and excluded the study as uninformative because no statistical analyses were performed to isolate the effects of cobalt alone. Furthermore, the study design makes the evaluation of exposure assessment challenging. The exposure was a controlled aspect of the experimental design; there is no potential for exposure misclassification.]

The study by Krakowiak et al. (2005) is a case report of a diamond-polishing disc former with 9 years of exposure to hard metal (WC-Co). [The Working Group reviewed but excluded the study as uninformative because the effects of cobalt alone were not examined.]

Walters et al. (2012) investigated metal-working fluid-exposed workers. [The Working Group reviewed but excluded the study because 90% of workers also had elevated urinary levels of chromium and effects attributable to cobalt exposure alone were not isolated using any statistical analyses.]

The study by Rizzato et al. (1994) is a case report of four workers exposed to hard metal. [The Working Group deemed the study uninformative and excluded it.]

Shirakawa & Morimoto (1997) assessed a population exposed to hard metal. [The Working Group evaluated but excluded the study as uninformative because the population

was exposed only to hard metal (WC-Co), which was inseparable from exposure to cobalt alone. Furthermore, there is potential for non-differential misclassification in the exposure groups. A key limitation was the lack of information on the exposure groups and how they were constructed, particularly the timing of when these groups were defined in relation to the outcome measures. There were exposure metrics reported in the multiple logistic regression that were not described in the methods.]

(ii) Human cells in vitro

Numerous studies have shown that cobalt compounds – including cobalt metal, soluble cobalt(II) salts (mostly cobalt(II) chloride), and cobalt-based NPs – have pro-inflammatory effects on human cells, which include the induction of cytokines/chemokines and cell surface activation markers and adhesion molecules (see Table S4.16, Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618). [The Working Group noted that the studies using human cells in vitro present acute (short-term) end-points but may be relevant to chronic pro-inflammatory effects induced by various forms of cobalt in the context of potential repeated exposure over time.]

(b) Experimental systems

(i) Non-human mammals in vivo

[The Working Group noted that only those studies that include end-points ≥ 7 days are reported.]

Cobalt metal or cobalt-based nanoparticles

In a subchronic study conducted by the NTP, male and female Fischer 344/NTac rats and $B6C3F_1$ mice were exposed to cobalt metal by inhalation for 14 weeks (6 hours per day, 5 days per week) at concentrations of 0.625, 1.25, 2.5, 5, and (mice only) 10 mg/m^3. The incidence of chronic active inflammation was significantly increased in the lungs of male and female rats at all exposure levels, as was the incidence of alveolar histiocytic cell infiltration in male and female mice. The incidence of chronic active inflammation in the nose in male and female mice was significantly increased at concentrations of 5 and 10 mg/m^3.

In a chronic study conducted by the NTP, male and female Fischer 344/NTac rats and $B6C3F_1$ mice were exposed to cobalt metal by inhalation for 2 years (6 hours per day, 5 days per week) at concentrations of 1.25, 2.5, or 5 mg/m^3. The incidence of chronic active inflammation was significantly increased in the lungs of male and female rats at all exposure concentrations. The incidence of histiocytic cell infiltration (in alveoli) was significantly increased in the lungs of male and female mice at all exposure concentrations, as was the incidence of suppurative inflammation in male (2.5 and 5 mg/m^3) and female (5 mg/m^3) mice. In the nose, the incidence of chronic active inflammation in male and female rats, and of suppurative inflammation in male and female rats and mice, was significantly increased at all exposure concentrations (NTP, 2014). Transcriptomic profiling of bronchioloalveolar carcinomas from the cobalt-exposed mice showed significant alterations in molecular pathways related to immune response signalling including "IL-8 signalling", "melatonin signalling", "B cell receptor signalling", and "regulation of IL-2 expression in activated and anergic T lymphocytes", with pro-inflammatory IL-8 signalling being one of the most significantly altered (Ton et al., 2021).

In a study by McNamara & Williams (1981), cobalt metal discs (diameter, 5 mm; thickness, 2 mm) were surgically implanted intramuscularly in male Lister rats, and histological effects were assessed for up to 52 weeks. Breakdown of muscle fibres was observed near the site of implantation. Many inflammatory cells had infiltrated between fibres and there were several "lymphoid clumps" that were mainly composed of plasma

cells. In some areas, bloodborne lymphocytes were attached to the blood vessels.

In another implantation study, cobalt metal pellets were surgically implanted into the skeletal muscle of male Sprague-Dawley rats, which induced spindle-cell tumours as early as 3 months. The Gene Ontology (GO) of differentially expressed genes (DEGs) with increased expression 3 months after implantation were "leukocyte cell-cell adhesion", "leukocyte proliferation", "lymphocyte proliferation", "mononuclear cell proliferation", and "adaptive immune response". The transcriptomic profile and GO of DEGs with increased expression in muscle tissue closer to the implant were "cell activation", "leukocyte activation", "lymphocyte activation", "T cell activation", and "positive regulation of immune system process" at 1 month, and "immune system process", "immune response", "regulation of immune system process", "cell activation", and "leukocyte activation" at 3 months (Wen et al., 2020).

In a study by Hansen et al. (2006), male Sprague-Dawley rats were implanted intramuscularly with cobalt metal NPs (average size, 120 nm) or implanted subcutaneously with bulk cobalt metal (4.73 mm^{-1}) and assessed at 6, 8, or 12 months. For the bulk cobalt metal, but not the cobalt metal NPs, inflammatory infiltrates (composed of mononuclear cells and lymphocytes) and granulomas were reported in muscle tissue after exposure for 6 months.

In a study by Kalinich et al. (2022), male Sprague-Dawley rats were implanted intramuscularly with cobalt metal pellets (cylinders of 1 mm in diameter, 2 mm in length). Interferon-gamma (IFN-γ), IL-4, IL-5, IL-6, IL-10, and IL-13 protein levels were significantly increased in serum samples 3–12 months after exposure. Keratinocyte chemoattractant/human growth-regulated oncogene chemokine (KC/GRO) and tumour necrosis factor alpha (TNFα) protein levels did not change. Administration of ultrafine cobalt metal [the Working Group noted that this was probably cobalt metal NPs] to female Sprague-Dawley rats by single intratracheal instillation at 0.06, 0.3, 0.6 mg/100 g bw significantly increased the number of total cells, macrophages, neutrophils, and lymphocytes in BALF on day 1. However, no significant effects were observed in BALF at day 28 or 1 month after 4 instillations (administered once per month) of 0.06 mg/100 g bw (Lasfargues et al., 1995). In a study by Zhang et al. (1998), male Wistar rats were treated with ultrafine cobalt (consisting of cobalt metal and cobalt(II,III) oxide) [The Working Group noted that these were cobalt-based NPs with a diameter of 20 nm] administered by intratracheal instillation (1 mg/mL). There were significant increases in the number of total cells and neutrophils (and a decrease in macrophages) in BALF on days 1, 3, 7, 15, and 30, together with a significant increase in lymphocytes on days 15 and 30.

In *gpt* delta transgenic mice treated with cobalt metal NPs at a dose of 50 µg (size, 20 nm) by intratracheal instillation, lung inflammation and injury were induced. Neutrophils were significantly increased in BALF on days 1, 3, 7, and 28 (although the numbers recorded on days 7 and 28 were lower than on days 1 and 3). The chemokine (C-X-C motif) ligand 1 (CXCL1)/keratinocyte chemoattractant (KC) was significantly increased in BALF on days 1, 3, and 7. Infiltration of macrophages and neutrophils into lung tissue (alveolar space/septa and interstitium) was identified on day 7 by histological assessment, and inflammatory cells were still present 4 months post-exposure (Wan et al., 2017).

In a study by Viegas et al. (2022), male and female Sprague-Dawley rats were exposed for 4 hours to aerosols of cobalt metal and various other (soluble and insoluble) cobalt(II) compounds (including cobalt dihydroxide, cobalt monoxide, cobalt(II) sulfate heptahydrate (in Fischer 344 rats), tricobalt tetraoxide [cobalt(II,III) oxide], cobalt(II) sulfide, and cobalt(II) sulfate). Histopathological assessment

showed increased lung perivascular (inflammatory) oedema at day 1 and up to days 3, 5, 14, or 16 post-exposure, except after exposure to cobalt dihydroxide and cobalt(II) sulfate.

In a study by Burzlaff et al. (2022), Wistar rats were exposed by inhalation to tricobalt tetraoxide [cobalt(II,III) oxide] particles at a concentration of 5, 20, or 80 mg/m^3 for 6 hours per day for 28 days. Neutrophil numbers were significantly increased in BALF at exposure concentrations of 20 and 80 mg mg/m^3 at day 1 and at day 91 after the end of the exposure period in both males and females.

In a study by Sisler et al. (2016a), male C57BL/6 mice were exposed to cobalt(II) oxide NPs (72 ± 16 nm) administered by inhalation (whole-body) for 4 days at concentrations of 10 or 30 mg/m^3. Neutrophils, eosinophils, and lymphocytes were significantly increased in BALF of mice 1 day (together with IL-1β and IL-6, but not TNFα and KC/GRO) and 7 (but not 56) days after the last exposure at a concentration of 30 mg/m^3, whereas alveolar macrophages were significantly increased in BALF at all three time points (including day 56).

Soluble cobalt(II) salts

Male Sprague-Dawley rats were exposed to cobalt(II) chloride in drinking-water (300 mg/L per day) for 4 weeks, which induced kidney injury. There were significant increases in pro-inflammatory response protein biomarkers – nitric oxide, TNFα (tumour necrosis factor-α), MPO (myeloperoxidase), and CRP (C-reactive protein) – and mRNAs (NF-κB and IL-6) in kidney tissue (Abdel-Daim et al., 2020). In a study by Oyagbemi et al. (2019), peritubular and periglomerular inflammation were increased in the kidney of male Wistar rats exposed to cobalt(II) chloride in drinking-water (150 or 300 ppm) for 7 days, along with focal areas of glomerulonecrosis at the highest exposure concentration. In male Wistar rats exposed by gavage to cobalt(II) chloride hexahydrate (150 mg/kg bw) for 8 days, infiltration of kidney by inflammatory cells and increased levels of NF-κB in the heart and kidney were reported (Oyagbemi et al., 2020).

In a study by Awoyemi et al. (2017), mild infiltration of hepatic interstitium by inflammatory cells was observed in male Wistar rats exposed to cobalt(II) chloride in drinking-water (600 mg/L) for 7 days.

In one study, male Sprague-Dawley rats were treated with feed containing cobalt(II) chloride hexahydrate (12.5 mg/kg bw) for 7 days. There were no significant treatment-related changes in the levels of inflammatory cytokines IFNα, IFN-γ, or MCP-1 in BALF (Shukla et al., 2009).

In a study using transgenic mice with a conditional deletion of lung epithelium-derived HIF-1α (Saini et al., 2010), non-transgenic control mice (on a mixed C57BL/6 and FVB/N background) were exposed to cobalt(II) chloride (30 or 60 μg) via oropharyngeal aspiration, 5 days per week for 2 weeks. The mice exhibited lung injury with histopathological evidence of mild-to-moderate chronic bronchopneumonia (marked inflammation), which consisted of infiltrates of mixed inflammatory cells (including monocytes/macrophages, neutrophils, eosinophils, lymphocytes, and occasionally plasma cells) 72 hours after the last exposure. Total cell counts and absolute numbers of macrophages, lymphocytes, neutrophils, and eosinophils, as well as protein levels of some pro-inflammatory cytokines/chemokines (for example, RANTES) were increased in BALF, but the changes were not statistically significant compared with saline-treated mice. In another study, exposure of the same non-transgenic control male mice to cobalt(II) chloride (60 μg) by oropharyngeal aspiration induced a significant increase in total cell counts and number of macrophages in BALF after 5 or 10 doses, and of neutrophils, eosinophils, and lymphocytes after 5 (but not 10) doses (Proper et al., 2014).

In a metal sensitization study by Bonefeld et al. (2015), mice were sensitized dermally with

10% cobalt(II) chloride and then challenged dermally twice with 10% cobalt(II) chloride 3 weeks later. CD4+ (BrdU+) T-cells, CD8+ (BrdU+) T-cells, and CD19+ (BrdU+) B-cells were significantly increased in the draining lymph nodes after challenge. In a study by Tsui et al. (2020), male BALBalb/c mice were sensitized dermally with 5% cobalt(II) chloride hexahydrate on days 1 and 8, and then challenged by inhalation of 0.05% cobalt(II) chloride hexahydrate on days 15, 17, 19, 22, and 24. Neutrophils and eosinophils, as well as the chemokines MCP-1, MIP-2, and KC, were significantly increased (and macrophages decreased) in BALF 24 hours after the last challenge.

In a metal sensitization study, guinea-pigs were sensitized dermally with 5% cobalt(II) chloride on days 0, 2, 7, and 9 and then challenged by cobalt(II) chloride inhalation at 2.4 mg/m^3 for 6 hours per day for 2 weeks, starting on day 42. Neutrophils and eosinophils were significantly increased in BALF after the last challenge (Camner et al., 1993).

In a study by Johansson et al. (1984), male rabbits were exposed by inhalation to cobalt(II) chloride at concentrations of 0.4–0.6 mg/m^3 for 4–6 weeks (6 hours per day, 5 days per week), but no inflammation was observed. However, in another study by Johansson et al. (1983), there was a significant increase in alveolar macrophages (with increased metabolic activity) in BALF after 1 month of exposure. Chronic exposure of male rabbits to cobalt(II) chloride at a concentration of 2 mg/m^3 for 14–16 weeks (6 hours per day, 5 days per week) by inhalation also resulted in a significant increase in alveolar macrophages (with increased metabolic activity) in BALF (Johansson et al., 1986), and an increase in interstitial and intra-alveolar leukocytic and/or lymphocytic inflammation in the lung (Johansson et al., 1987).

Male and female Fischer 344/N rats and B6C3F$_1$ mice were subchronically exposed to cobalt(II) sulfate heptahydrate at concentrations of 0.3, 1, 3, 10, or 30 mg/m^3 by inhalation for 13 weeks (6 hours per day, 5 days per week). In male and female rats, incidence of inflammation (≥ 1 mg/m^3) and inflammatory polyps (≥ 10 mg/m^3) in the larynx, and of inflammation (≥ 3 mg/m^3) and histiocytic infiltrates (≥ 1 mg/m^3) in the lung, were significantly increased. In male and female mice, incidence of inflammation in the nose (≥ 3 mg/m^3) and larynx (≥ 10 mg/m^3), and of chronic inflammation (≥ 10 mg/m^3) and histiocytic infiltrates (≥ 0.3 mg/m^3) in the lung, were significantly increased (Bucher et al., 1990).

In a chronic study by the NTP, male and female Fischer 344/N rats and B6C3F$_1$ mice were exposed by inhalation to cobalt(II) sulfate heptahydrate at concentrations of 0.3, 1, or 3 mg/m^3 for 2 years (6 hours per day, 5 days per week). Incidence of granulomatous alveolar inflammation was significantly increased in the lung of male and female rats at all exposure concentrations (NTP, 1998; Bucher et al., 1999). Histological re-evaluation of the lungs of male rats reported a significant increase in the incidence of chronic active inflammation and histiocytosis (Ozaki et al., 2002). The incidence of diffuse and focal histiocytic cell infiltration was significantly increased in the lungs of male and female mice, respectively, at the highest exposure concentration. The incidence of suppurative inflammation in the nose was also significantly increased in male (3 mg/m^3) and female (1 mg/m^3) mice (NTP, 1998; Bucher et al., 1999).

(ii) Non-human mammalian cells in vitro

[The Working Group noted that in vitro studies in experimental systems with acute end-points were not included in the evaluation since they were not considered to be very relevant to chronic pro-inflammatory effects in humans.]

Table 4.17 Immunosuppression in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Ig subclasses	Serum	Czechia production workers, cross-sectional	38 nickel-exposed 35 cobalt-exposed Non-exposed controls	↑ IgA in cobalt-exposed group compared with controls ($P < 0.05$)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1983)
Ig subclasses	Serum	Czechia production workers, cross-sectional	38 nickel-exposed; 35 cobalt-exposed Non-exposed controls	↑ IgA in cobalt-exposed group compared with controls ($P < 0.05$) ↓ IgE in cobalt-exposed compared with controls (NSS)	Age-matched controls	The key limitation of this study is the limited information on the exposure definition, which makes it challenging to evaluate the exposure assessment.	Bencko et al. (1986b)

↓, decreased; ↑, increased; Ig, immunoglobulin; NSS, not statistically significant.

4.2.7 Is immunosuppressive

(a) Humans

(i) Exposed humans

See Table 4.17.

Evidence for immunomodulation in human populations exposed to cobalt has been reported in two cross-sectional studies in Czechia that compared cobalt production workers with non-exposed, age-matched controls (Bencko et al., 1983, 1986b). In the earlier study (1983), significantly increased average values were obtained for immunoglobulin A (IgA) in serum samples collected from cobalt-exposed workers compared with controls ($P < 0.05$).

In the later study (1986), levels of IgG, IgA, IgM, and IgE were measured; levels of IgA in cobalt-exposed workers were significantly higher than those of controls ($P < 0.05$). However, levels of IgE in cobalt-exposed individuals were lower than those of controls, although the difference was not statistically significant.

[The Working Group evaluated the study by Tilakaratne & Sidhu (2015) but excluded it as uninformative because it was a case report of T-cell populations from two cement workers with mixed exposure to chromium and nickel, without any regression analyses to isolate the effects of cobalt alone. The Working Group noted that the case report provides a narrative work history. Both cases reported probably also had exposure to chromate. The key limitations of this study were the lack of quantitative exposure data and the nature of a case report with a sample size of two.]

(ii) Human cells in vitro

[The Working Group noted that decreased cell proliferation and increased apoptosis/cytotoxicity of human leukocyte cell populations (including lymphocytes) in vitro induced by cobalt could be relevant to immunosuppression. All but one of the studies of human cells in vitro (described below) used primary cells.]

Wang et al. (1996) investigated cytotoxicity, T- and B-cell proliferation, and cytokine release by human peripheral blood mononuclear cells (PBMC) from whole blood after exposure to cobalt(III) chloride ($CoCl_3$). PBMC were exposed to cobalt(III) chloride (0.01, 0.1, 1.0, 10, 100 ng/mL) which was not cytotoxic at any concentration tested after a 72-hour exposure. However, phytohaemagglutinin-induced T-cell proliferation and lipopolysaccharide-induced B-cell proliferation were significantly inhibited by 48-hour exposure to cobalt(III) chloride at all tested concentrations and at ≥ 0.1 ng/mL, respectively. Furthermore, the release of the cytokines IL-2, IL-6, and IFN-γ by phytohaemagglutinin-stimulated PBMCs was significantly decreased after a 48-hour exposure to cobalt(III) chloride at all tested concentrations for IL-6 and IFN-γ, and at ≥ 0.1 ng/mL for IL-2. [The Working Group noted that the cobalt(III) chloride form has a valence state of +3 and, as such, is unstable/elusive. It is more likely that the cobalt(II) chloride form is responsible for observed effects.]

Akbar et al. (2011) studied the effects of cobalt(II) chloride on primary human lymphocytes in terms of cell viability, apoptosis, proliferation, and cytokine release. Lymphocytes were exposed to Co^{2+} (as cobalt(II) chloride) at concentrations of 0.1, 1, 10, or 100 µM for 24 or 48 hours. Exposure of resting lymphocytes to Co^{2+} at 100 µM resulted in a significant decrease in cell viability. This cytotoxic response was enhanced in lymphocytes stimulated with anti-CD3 antibody and observed after exposure to Co^{2+} at 10 µM for 24 and 48 hours, suggesting that activated cells are more sensitive to Co^{2+}-induced reductions in viability than resting lymphocytes. There was also a slight (but significant) increase in apoptosis of resting and CD3-activated lymphocytes after exposure to Co^{2+} at 100 µM for 24 and 48 hours. After 48-hour exposure, lymphocyte proliferation and cytokine (IFN-γ and IL-2) release by CD3-activated lymphocytes, with and without anti-CD28 antibody stimulation,

were significantly decreased after exposure to Co^{2+} at concentrations of 100 (proliferation), 0.1 (IL-2), or 10 (IFN-γ) μM.

Hagmann et al. (2013) investigated the effect of cobalt(II) chloride on primary human CD4+ T-lymphocytes with the aim of assessing lymphocyte viability, apoptosis/necrosis, and cell surface lymphocyte activation markers (CD25, CD38, CD69, and CD95). Co^{2+} (as cobalt(II) chloride) at a concentration of 1000 μg/L induced significant decreases in cell viability (with increases in the rates of apoptosis and necrosis) and the expression of CD25, CD38, and CD95 after exposure for 120 hours.

Bruzzese et al. (2016) treated peripheral blood lymphocytes isolated from whole blood and a human lymphoma CD4+ T-(CEM) cell line expressing $A_{2A}R$ with cobalt(II) chloride at concentrations of 50–800 μM to induce hypoxia. Resting peripheral blood lymphocytes and CEM cells exposed to cobalt(II) chloride for 24 hours showed a significant dose-dependent decrease in cell viability. Cells activated with phorbol 12-myristate 13-acetate plus phytohaemagglutinin and then treated with cobalt(II) chloride showed a greater dose-dependent decrease in cell viability than did resting cells.

In the study by Paladini et al. (2011), primary PBMCs extracted from whole-blood samples from 18 healthy donors were exposed to cobalt(II) chloride hexahydrate at concentrations of 42–336 μM for up to 6 days. Treatment with cobalt(II) chloride caused a significant reduction in T-lymphocyte numbers (84 μM cobalt(II) chloride reduced T-lymphocyte viability and increased apoptosis), and the expression of p53 in CD3+ T-lymphocytes was significantly increased 16 hours after exposure. Monocytes exposed to the same concentrations of cobalt(II) chloride did not display decreased cell viability (increased apoptosis), but p53 expression was significantly increased. Monocytes exposed to cobalt(II) chloride showed an increase in cytoplasmic $p21^{Cip1/WAF1}$, an inhibitor of pro-caspase-3 (an anti-apoptotic effect), which supports the results reported regarding the survival of monocytes treated with cobalt(II) chloride. Other experiments using monocytes showed that cobalt(II) chloride exposure increased NF-κB activity, as well as the expression and secretion of pro-inflammatory cytokines (TNFα and IL-1β), suggesting a role for cobalt(II) chloride in the activation of monocytes. The expression of human leukocyte antigen (HLA) class II molecules and the ability of the cells to capture and present antigens decreased over time with exposure, suggesting that cobalt(II) chloride maintains monocytes in a partially activated (but not fully differentiated) pro-inflammatory state. [The Working Group noted that decreased antigen presentation would dampen immune activation.]

Posada et al. (2015) studied the effects of Co^{2+} ions on primary human lymphocytes in terms of cell viability, apoptosis, proliferation, and cytokine production. Cell viability was measured 24, 48, and 120 hours after exposure to Co^{2+} at 0.1 μM (source of Co^{2+} not specified). [The Working Group noted that this concentration was chosen to mimic the metallosis that can occur in the local environment surrounding an implant.] The results showed that there were no significant changes in the numbers of lymphocytes or metabolic activity after exposure to Co^{2+} at 0.1 μM for 24 or 120 hours, but lymphocyte proliferation was significantly decreased after 48-hour (but not 120-hour) exposure. IL-2 secretion by lymphocytes after 24- and 48-hour exposure to Co^{2+} at 0.1 μM was significantly decreased, but significant alterations in the secretion of IL-6, IFN-γ, and TNFα were not reported.

Primary human PBMCs isolated from whole blood were exposed to cobalt metal NPs (size, < 50 nm), particles, or cobalt(II) chloride at a concentration of 10^{-5}, 10^{-6}, and 10^{-7} M to assess the resulting effects on the production of several cytokines. Exposure to cobalt particles induced a significant decrease in the release of IFN-γ,

TNFα, IL-10, IL-4, and IL-2 at all concentrations tested, and of IL-6 at 10^{-7} M. Exposure to cobalt metal NPs significantly decreased the production of IL-2 and IL-10 at all concentrations tested, and stimulated the release of TNFα at 10^{-6} and 10^{-7} M, and IFN-γ at 10^{-7} M. Exposure to cobalt(II) chloride significantly decreased the production of IL-10, IL-2, and TNFα at 10^{-5} M (Petrarca et al., 2006).

Verstraelen et al. (2014) assessed expression profiles of genes involved in immune signalling in human alveolar (A549) and bronchial (BEAS-2B) epithelial cells after exposure to cobalt(II) oxide NPs (size, 7.04 ± 0.81 nm) at concentrations of 9.1×10^{13}/mL [60.91 μg/mL] and 9.1×10^{12}/mL [6.091 μg/mL], respectively, for 3, 6, 10, 24 hours. Exposure to cobalt(II) oxide NPs at a concentration of 60.91 μg/mL was cytotoxic to BEAS-2B, but not A549, cells after exposure for 24 hours. BEAS-2B cells were more sensitive to the effects of cobalt(II) oxide NPs and had higher numbers of differentially expressed transcripts than A549 cells, including differentially expressed transcripts of immune markers, especially after 24-hour exposure. Cobalt(II) oxide NPs predominantly induced downregulation of the expression of genes involved in immune signalling over time after exposure. The downregulated genes included TLR6, HLA-DRB3, TIRAP, and HLA-A. The mRNA transcript that had the largest decrease was HLA-DRB3, which plays an important role in presenting peptides derived from extracellular proteins. [The Working Group noted that the decreased antigen presentation would dampen immune activation.] The TLR6 gene was suppressed in both cell types. In contrast, the ITGB2 and PAG1 genes, which encode a protein involved in the adhesion and migration of leukocytes and a protein thought to be involved in the regulation of T-cell activation, respectively, were upregulated.

(b) Experimental systems

(i) Non-human mammals in vivo

In a study by Chetty et al. (1979), Sprague-Dawley rats were treated with feed containing cobalt(II) chloride (0–300 ppm) with sufficient or deficient levels of iron. Rats that received cobalt(II) chloride at concentrations of ≥ 100 ppm (iron-sufficient and -deficient groups) had reduced body and thymus weights; however, non-lymphoid tissues such as liver, kidney, and heart were not affected. In exposed rats, there was also a significant decrease in sheep haemagglutinins (200 ppm, iron-sufficient group; ≥ 50 ppm, iron-deficient group) and antibody-producing cells as assessed by plaque-forming cell assays using sheep erythrocytes (≥ 100 ppm, iron-sufficient group; ≥ 50 ppm, iron-deficient group).

Nagai et al. (1989) examined the effects of cobalt(II) chloride on the production of specific IgM and polyclonal IgG antibodies as assessed by direct plaque-forming cell and reverse plaque-forming assays, respectively. Mice given cobalt(II) chloride hexahydrate (9.0, 0.9, or 0.45 mg/kg bw) by intraperitoneal injection every other day (for a total of three injections) and then immunized with sheep erythrocytes on the last injection day showed an increase in the number of IgM plaque-forming cells in spleen (9.0 and 0.9 mg/kg bw). However, numbers of polyclonal IgG plaque-forming cells (9.0 or 0.9 mg/kg bw) in the spleen did not differ from those in the control group.

(ii) Non-human mammalian cells in vitro

In a study by Shweta et al. (2015) in peritoneal macrophages and splenocytes from BALB/c mice exposed to cobalt(II) chloride (0.05 mM) for 24 hours, there was a significant decrease in the expression of TLR4 by macrophages and the cell surface activation markers CD25, CD40, and CD69 (but not CD95) by splenocytes; however, cobalt(II) chloride induced a significant increase in the secretion of pro-inflammatory TNFα and

Table 4.18 Modulation of receptor-mediated effects in humans exposed to cobalt

End-point	Biosample type	Location, setting, study design	Study population	Response (significance)	Covariates controlled	Comments	Reference
Thyroid hormone levels	Serum	Belgium, highly exposed cobalt refinery workers	82 cobalt-exposed men 82 non-exposed controls (age-matched)	Negative association for T3 with cobalt Mean 150.00 ng/dL in controls vs 140.18 ng/dL in exposed ($P < 0.05$) Effect of cobalt on FT4 and TSH	Controls were age-, education-, and SES-matched	There is limited potential for non-differential misclassification in the exposure groups. This study focuses on comparisons between exposed and unexposed groups working at different companies. A key limitation of this study is the reliance on the qualitative exposure assessment approach (exposed/unexposed) based on location of employment.	Swennen et al. (1993)
Reproductive hormones	Serum	China, partners of fertility clinic patients, cross-sectional	$n = 511$	No associations between urinary cobalt levels and serum reproductive hormones after adjustment for multiple testing (FDR-adjusted P for trend for each hormone > 0.50)	Age, BMI, abstinence time, smoking status, daily cigarette consumption, and urinary creatinine	Urine samples collected at two close points in time on the same day as semen sample limits the findings.	Wang et al. (2016a)
Thyroid hormone levels	Blood	China, Hangzhou Birth Cohort Study, cross-sectional	$n = 915$ pregnant women	While the middle tertile of urinary cobalt was associated with a decrease in FT3 ($P < 0.05$), no associations between urinary cobalt levels and serum reproductive hormones in single-metal models were observed after adjustment for multiple testing (FDR-adjusted for each hormone, P for trend ranged from > 0.26 to 0.88)	Maternal age, education, household income, working in pregnancy, second-hand smoke in pregnancy, drinking in pregnancy, gestational age at measurement of thyroid hormone levels, parity, history of hyperglycaemia in pregnancy, and pre-pregnancy BMI	Single blood samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Co-exposures to other metals (arsenic, cobalt, selenium, manganese, and nickel) were evaluated in the statistical analyses if they produced statistically significant results ($P < 0.05$) when evaluated one at a time. Logistic regression results presented for manganese, nickel, and antimony with FT4.	Guo et al. (2018)

BMI, body mass index; FDR, false discovery rate; FT3, free triiodothyronine; FT4, free thyroxine; SES, socioeconomic status; T3, triiodothyronine; TSH, thyroid stimulating hormone; vs, versus.

IL-6 (but not IFN-γ). The expression of NF-κB by macrophages was also increased.

4.2.8 Modulates receptor-mediated effects

(a) Humans

(i) Exposed humans

See Table 4.18.

Swennen et al. (1993) assessed health effects in 82 workers at a cobalt refinery in Belgium who had an average duration of exposure of 8 years and a geometric mean TWA exposure to cobalt dust of 125 μg/m³. This included 25% of the worker population with exposure > 500 μg/m³. Results were compared with an age-matched group of non-cobalt dust-exposed controls. Mean triiodothyronine (FT3) concentrations were lower in the cobalt dust-exposed population (140.18 ng/dL) than in non-exposed controls (150.00 ng/dL) ($P < 0.05$). However, other thyroid hormone measurements, including free thyroxine (FT4) and thyroid stimulating hormone (TSH), did not differ between the groups.

In the Chinese Hangzhou Birth Cohort Study, Guo et al. (2018) examined thyroid hormone levels in 915 pregnant women grouped into tertiles of six metals: arsenic, cobalt, manganese, nickel, antimony, and selenium. While the middle tertile of urinary cobalt was associated with a decrease in free triiodothyronine (FT3) ($P < 0.05$), no associations between urinary cobalt levels and levels of reproductive hormones in serum in single-metal models were observed after adjustment for multiple testing (FDR-adjusted model; P for trend ranged from 0.26 to 0.88) across the hormones assessed. Covariates considered included maternal age, education, household income, working during pregnancy, exposure to second-hand smoke during pregnancy, drinking during pregnancy, gestational age at measurement of thyroid hormones, parity, history of hyperglycaemia during pregnancy, and pre-pregnancy BMI. [The Working Group noted that single blood samples were used to assess exposures to metals concurrently with the assessment of the end-point of interest. Co-exposures to other metals (arsenic, cobalt, selenium, manganese, and nickel) were assessed in the statistical analyses if they produced statistically significant results ($P < 0.05$) when considered one at a time. Logistic regression results were presented for manganese, nickel, and antimony with free thyroxine.]

The association of urinary metal levels with reproductive hormones was assessed in partners of fertility clinic patients in a cross-sectional study in China by Wang et al. (2016a). No significant (or suggestive) associations were found between urinary cobalt levels and any hormones assessed after adjustment for age, BMI, smoking status, daily cigarette consumption, abstinence time, and concentrations of urinary creatinine and other metals, nor for multiple testing.

[The Working Group noted that there was only one of three studies of cobalt-exposed populations with some evidence related to receptor-mediated effects. The Working Group also noted that the occupational exposure levels to metals in Swennen et al. (1993) were probably much higher than the environmental exposure levels occurring in the two cohort studies.]

Johnstone et al. (2014) investigated exposure to trace metals as a predictor of benign uterine fibroid tumour development. [The Working Group evaluated this study but excluded it as uninformative because exposure to trace metals is an uncertain indicator, and while the authors reported higher cobalt concentrations in patients with fibroids compared with non-fibroid controls, it was not a predictor of a fibroid diagnosis.]

The study by Tilakaratne & Sidhu (2015) was a case report of T-cell populations from samples collected from two cement workers with mixed exposure to chromium and nickel. [The Working Group evaluated the study but excluded it as uninformative because it referred

to co-exposure and was without any regression analyses to isolate the effects of cobalt alone.]

(ii) Human cells in vitro

Cobalt(II) salts

The effects of cobalt(II) chloride on steroid hormone receptor signalling pathways, specifically ERα and PR, were studied in the ERα-positive human breast cancer cell line MCF7 (Martin et al., 2003; Cho et al., 2005). Similar to estradiol at 1 nm (24-hour exposure), exposure to cobalt(II) chloride at 1 μM (24-hour exposure) decreased ER mRNA and protein levels, increased pS2 (an estrogen-responsive gene) mRNA, increased PR mRNA and protein levels via interacting with the ER, and increased MCF7 cell proliferation (Martin et al., 2003). [The Working Group noted that cobalt(II) chloride, presumably in ion form, binds to ERα non-competitively and activates ERα through the hormone-binding (not DNA-binding) domain (Martin et al., 2003).] Investigating the mechanisms of hypoxia-induced ERα downregulation, Cho et al. (2005) used cobalt(II) chloride to induce cellular hypoxia in MCF7 cells and found that inhibition of HIF-α protein synthesis could partially prevent the cobalt(II) chloride (hypoxia)-induced ERα protein decrease. Protein kinase inhibitors of MAPK, P13K, and p38 did not prevent cobalt(II) chloride-induced downregulation of ERα (Cho et al., 2005).

Using a recombinant ER DNA-binding domain polypeptide, Predki & Sarkar (1992) restored the nonspecific DNA-binding property of the apo-polypeptide, which was previously inhibited by treatment with metal chelators, by dialysis with buffer containing zinc, cobalt, or cadmium (but not copper or nickel). [The Working Group noted that the authors did not specify the species on which the recombinant polypeptide was based. It was likely to be human recombinant ER.]

Retinoic acid receptor-related orphan receptor α (RORα) and peroxisome proliferator-activated receptor α (PPARα), both of which can have potential pro- and anticancer effects, were assayed in various human cells exposed to cobalt(II) chloride. After the human hepatoma cell line HepG2 was treated with cobalt(II) chloride, the increase in the level of *RORA* gene expression (encoding RORα) and overall protein production was statistically significant, although the increase in RORα transcriptional activity was not statistically significant (Chauvet et al., 2002). While the study by Chauvet et al. did not investigate downstream effects of RORα, cobalt(II) chloride was reported by Daoud et al. (2005) to result in decreased PPARα mRNA levels in isolated human placental trophoblast cells, where decreased PPARβ and PPARγ mRNA levels were also observed. [The Working Group noted that PPARα has several potential anticancer effects, including the induction of decreased pro-inflammatory transcription factors via interaction with the ER.]

Cobalt(II) chloride treatment or transfection of HIF-1α decreased constitutive androstane receptor (CAR) protein expression as well as *CAR* gene promoter activity in the human cancer cell lines AGS (gastric), SW480 (colon), and PC-3 (prostate) (HIF-1α was tested in AGS cells only) (Küster et al., 2010). [The Working Group noted that CAR, a tumour suppressor, also has pro-cancer activities (e.g. increases cell proliferation and metastasis), so the effects of decreased CAR expression remain unclear.]

Aryl hydrocarbon receptor nuclear translocator (ARNT) regulates many genes involved in tumour growth and angiogenesis. Exposure to either cobalt(II) chloride at 50 μM (chemically induced hypoxia) or 1% oxygen (1% oxygen hypoxia) for 24 hours increased ARNT protein levels in the human cell lines MCF7 (breast ductal carcinoma), Hep3B (hepatocellular carcinoma), and REPC (primary human kidney), while only cobalt(II) chloride (not 1% oxygen hypoxia) increased ARNT protein levels in HepG2 (hepatocellular carcinoma). In HeLa

(cervical carcinoma) and Kelly (human neuroblastoma) cells, neither cobalt(II) chloride nor 1% oxygen hypoxia altered ARNT protein levels (mRNA levels were not measured). The mRNA levels of ARNT did not always change in the same direction as the protein. ARNT mRNA levels were decreased by cobalt(II) chloride (but not 1% oxygen hypoxia) in MCF7 cells, decreased by both cobalt(II) chloride and 1% oxygen hypoxia in Hep3B cells, and unchanged in both HeLa and REPC cells (Wolff et al., 2013).

Cobalt has been reported to affect the receptors of two peptides that influence vascular tone: adrenomedullin (ADM), a vasodilator, and endothelin (ET), a vasoconstrictor. Cobalt(II) chloride increased mRNA levels of ADM but decreased the mRNA levels and mRNA stability of an ADM receptor component, receptor activity modifying protein 2 (RAMP2), in two human neuroblastoma cell lines (IMR-32 and NB-69) (Kitamuro et al., 2001). [The Working Group noted that in studies not using cobalt, ADM was induced by HIF-1α and displayed several pro-cancer effects, including anti-apoptosis signalling and stimulation of cell growth, and indirectly decreased immune responsiveness (Zudaire et al., 2003).] Like estradiol, cobalt(II) chloride increased mRNA and protein levels of endothelin-A receptor (ETAR) in several human breast cancer cell lines (Wülfing et al., 2005). The functions of endothelin and its receptors, including ETAR, include mitogenesis, anti-apoptosis, angiogenesis, synergism with growth factors, and promoting tumour cell growth, in addition to vasocontraction (Grant et al., 2003; Jovanović, 2018). [The Working Group noted that the effects of cobalt(II) chloride on receptors of ADM and ET are likely to contribute to carcinogenesis.]

(b) Experimental systems

(i) Non-human mammals in vivo

Male Sprague-Dawley rats given drinking-water containing cobalt(II) chloride at 2 mM for 10 days showed decreased mRNA of PPARα- and PPARα-regulated genes, such as pyruvate dehydrogenase kinase 4 (PDK4), muscle carnitine palmitoyltransferase I (mCPT I), and malonyl-CoA decarboxylase (MCD) in heart cells (Razeghi et al., 2001). [The Working Group noted the high dose of cobalt(II) chloride used in this study and that no water intake information was provided.]

PPARα and PPARγ (but not PPARδ) mRNA levels were increased in adipose tissue of male Slc:ICR mice fed a high-fat diet for 14 days. When cobalt(II) chloride was administered at 1, 3, 5, or 7.5 mg/kg bw per day for 10 days via subcutaneous injection after the high-fat diet began, both PPARα and PPARγ mRNA levels were restored to the same levels as in the group given standard diet (Kawakami et al., 2012). [The Working Group noted that the effective dose of cobalt(II) chloride was not specified in the results.]

Investigating the link between CAR and HIF-1α, Shizu et al. (2013) used cobalt(II) chloride to activate HIF-1α. In male C3H/HeN mice, administration of cobalt(II) chloride (40 mg/kg bw) by a single intraperitoneal injection increased CAR protein expression, nuclear CAR accumulation, CAR-mediated phenobarbital-responsive enhancer module transactivation, and gene expression of CAR target genes in the liver (Shizu et al., 2013).

(ii) Non-human mammalian cells in vitro

Cobalt(II) chloride treatment of ovarian granuloma cells collected from pigs caused decreased estradiol synthesis but had no effects on progesterone production (Grasselli et al., 2005). In contrast, treatment with cobalt(II) sulfate heptahydrate had no effect on 17β estradiol release by rat ovarian fragments (Roychoudhury et al.,

Table 4.19 Immortalization in mammalian cells in vitro exposed to cobalt

End-point	Species, tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Soluble cobalt(II) salts					
Cobalt(II) chloride ($CoCl_2$)					
Morphological transformation assay (type III foci)	Mouse, embryonic fibroblasts, BALB/3T3	(–)	50 µM [~6 µg/mL]	$CoCl_2 \cdot 6H_20$; 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Data in Figure 1, subfigure titles, and legend do not match text. Negative and several positive controls included.	Sabbioni et al. (2014a)
Morphological transformation assay (type III foci)	Mouse, embryonic fibroblasts, BALB/3T3	–	70 µM [~9.1 µg/mL]	$CoCl_2$; 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Negative and positive controls included.	Ponti et al. (2009)
Morphological transformation assay (type II foci; type III foci were not observed)	Mouse, embryonic fibroblasts, C3H10T1/2	(+)	5 µg/mL [~40 µM]	$CoCl_2$; 48 h exposure, 42-day growth period. Effective dose range induced ≥ 95% cytotoxicity. Dose-dependent response, negative and positive controls included.	Doran et al. (1998)
Cobalt(II) sulfate ($CoSO_4$)					
Reduced pH morphological transformation assay	Syrian hamster, embryo, SHE	(+)	0.125 µg/mL [~0.75 µM]	$CoSO_4$ hydrate; 24 h exposure. Source and purity of cobalt not reported. Similar magnitude of effect at all doses, effective dose range induced ≤ 50% cytotoxicity.	Kerckaert et al. (1996)
Cobalt(II) acetate ($Co(CH_3CO_2)_2$					
Enhancement of simian adenovirus SA7-induced transformation	Syrian hamster, embryo, SHE	(+)	200 µM [~35 µg/mL]	$(Co(CH_3CO_2)_2$; 18 h exposure, 25–30-day growth period. Source and purity of cobalt not reported. Only reported effects at a single dose. Only reported ratio of enhancement.	Casto et al. (1979)
Cobalt metal (Co) or cobalt metal NPs					
Morphological transformation assay (type II and III foci), clonal growth in soft agar, Tumour growth in female athymic nude mice	Human, osteosarcoma, HOS TE85 [*p53* mutation at codon 156]	–	3 µg/mL cobalt powder	Cobalt metal particle size: d_{50}, 1–4 µm); 24 h exposure, 35-day growth period. Dose range based on minimal to 60% cytotoxicity. Dose-dependent response in cytotoxicity. Negative and positive control included.	Miller et al. (2001)
Anchorage-independent colony growth on soft agar	Human, cervical adenocarcinoma, HeLa	(+)	0.05 µg/mL	NP size: d_{50}, 30 nm; hd_{50}, 505 nm; 5–12 wk exposure, 21-day growth period on soft agar. Indirect exposure of HeLa cells fed media conditioned by *Ogg*$^{-/-}$ mouse embryonic fibroblasts exposed to cobalt nanoparticles. Cobalt source specified, but not purity. No discussion of cytotoxicity following 5–10 wk of exposure. Dose-dependent response, negative control included.	Annangi et al. (2015)
	Mouse, embryonic fibroblasts, MEF (WT)	+	0.05 µg/mL		
	Mouse embryonic fibroblasts, MEF (*Ogg*$^{-/-}$)	+	0.05 µg/mL		

Table 4.19 (continued)

End-point	Species, tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Morphological transformation assay (type II and III foci)	Mouse, embryonic fibroblasts, C3H10T1/2	(–)	500 µg/mL	Cobalt metal particle size: d_{50}, < 5 µm; 42-day exposure and growth period. Particle size was determined visually by light microscopy, exposures continued beyond 48 h throughout 42-day growth period. Dose range based on minimal to > 95% cytotoxicity, negative and positive controls included.	Doran et al. (1998)
Morphological transformation (type III foci), following exposure as initiator or promoter in two-stage carcinogenesis bioassay	Mouse embryonic fibroblasts, BALB/3T3	+ –	10 µM [2.0 µg/mL] (initiator) 2.5 µM [0.5 µg/mL] (promoter)	NP size: d_{50}, 28 nm; hd_{50}, 141 nm; 72 h exposure as initiator, 9-day exposure as promoter, 42-day growth period. Cobalt source specified, but not purity. Dose range based on 50–80% cytotoxicity, negative and positive controls included.	Sighinolfi et al. (2016)
Morphological transformation assay (type III foci)	Mouse embryonic fibroblasts, BALB/3T3	(+) (+)	1 µM [~0.13 µg/mL] cobalt metal 5 µM [~0.65 µg/mL] cobalt metal NPs	Cobalt metal particles (hd_{50}, 2.2 µm), cobalt metal NPs (hd_{50}, 200 nm); 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Dose-dependent response, negative and several positive controls included. Data in Figure 1, subfigure titles, and legend do not match text.	Sabbioni et al. (2014a)
Morphological transformation assay (type III foci)	Mouse embryonic fibroblasts, BALB/3T3	+	7 µM [~1.4 µg/mL]	Cobalt metal NPs (d_{50}, 80 nm); 72 h exposure, 35-day growth period. Dose range based on 20–80% cytotoxicity. Dose-dependent response, negative and positive controls included.	Ponti et al. (2009)
Cobalt(II) sulfide or disulfide (CoS or CoS_2)					
Morphological transformation assay (disordered colony morphology)	Syrian hamster, embryo, SHE	+ +/–	1 µg/mL CoS_2 particles 5 µg/mL CoS particles	CoS_2 crystalline particles (d_{50}, 1.25 µm), CoS amorphous particles (d_{50}, 2.0 µm); 48 h exposure, 20-day growth period. Cobalt source specified, but not purity. Dose range based on minimal to > 50% cytotoxicity. Dose-dependent response, negative control included, morphology scored by 3 blinded observers.	Abbracchio et al. (1982); Costa et al. (1982)

d_{50}, mean particle diameter; hd_{50}, hydrated mean particle diameter; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; wk, week; WT, wildtype.
[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality.

2014), but did decrease progesterone release by both rat ovarian fragments (Roychoudhury et al., 2014) and porcine primary ovarian granulosa cells (Kolesarova et al., 2010). Progesterone production by primary cultures of canine corpus luteum cells was also decreased after exposure to cobalt(II) chloride (Sousa et al., 2016).

Steroid production by male reproductive Leydig cells was decreased by cobalt(II) chloride. In Leydig cells collected from adult rats, cobalt(II) chloride decreased the maximum rate of luteinizing hormone (LH)-stimulated androgen production (Moger, 1983). By using inhibitors, Moger (1983) determined that the inhibition occurred between cAMP formation and the 3β-hydrogenase step. When the mouse Leydig tumour-derived MA-10 cell line was exposed to cobalt(II) chloride, progesterone production was decreased via increased ROS production, increased HIF-1α activity, and decreased basal mRNA levels of the specific enzyme cytochrome P450 side-chain cleavage ($P450_{scc}$) (Kumar et al., 2014). [The Working Group noted that in MA-10 cells, the steroidogenesis product is progesterone due to the lack of 17α hydroxylase.]

Exposure of Cos7 cells (SV40-transformed African green monkey renal cells) to either cobalt(II) chloride (50 μM, 24 hours) or hypoxia (1% oxygen, 24 hours) increased ARNT protein levels (Wolff et al., 2013).

Cobalt(II) chloride (100 μM) decreased mRNA levels of macrophage scavenger receptor 1 (MSR1) (via increasing HIF-1α expression) in a mouse cell line (RAW 264) (24-hour exposure) and in primary macrophages (8-hour exposure) collected from mouse peritoneal exudate (Shirato et al., 2009). [The Working Group noted that while decreased levels of MSR1 were associated with worse recurrence-free survival of patients with prostate cancer (Cao et al., 2017), the role of macrophage scavenger receptor 1 in carcinogenesis remains to be determined.]

4.2.9 Causes immortalization

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Cobalt metal or cobalt metal nanoparticles

See Table 4.19.

Direct evidence is limited to a single study that was previously reported in *IARC Monographs* Volume 86 (IARC, 2006). Exposure of the human osteosarcoma (HOS) cells (TE85, cone F-5) to cobalt metal at subcytotoxic concentrations for 24 hours (3 μg/mL; mean particle diameter, 1–4 μm) did not increase transformation after a 35-day period of clonal growth in soft agar or tumourigenesis when cells were implanted in athymic nude mice (Miller et al., 2001). [The Working Group noted that simulated W/Ni/Co (a mixture of metals including cobalt) increased transformation frequency, anchorage-independent growth of transformed clones in soft agar, and formation of injection-site tumours in athymic nude mice (Miller et al., 2001).]

Indirect evidence is also available from the observation of a concentration- and duration-dependent increase in the anchorage-independent growth of HeLa cells in soft agar, when fed with media conditioned by MEF *OGG1*$^{-/-}$ cells exposed to cobalt metal NPs at concentrations of 0.05 and 0.1 μg/mL for up to 10 weeks (Annangi et al., 2015). [The Working Group noted that *hOGG1* encodes a protein responsible for the BER of oxidative 8-OHdG lesions. The Working Group also noted that the effects of conditioned media cannot be attributed to the presence of cobalt metal NPs specifically, as the potential confounding effects of cell-derived mediators such as matrix metalloproteinases 2 and 9, which were also increased, cannot be ascertained.]

Cobalt compounds

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

See Table 4.19.

Cobalt metal or cobalt metal nanoparticles

Exposure of MEF BALB/3T3 cells to cobalt metal at (non-cytotoxic and cytotoxic) concentrations of ≥ 0.13 µg/mL for 72 hours significantly increased transformation frequency as determined by a concentration-dependent increase in type III foci, which was inhibited by co-treatment with ascorbic acid at 50 µM (Sabbioni et al., 2014a). [The Working Group noted concerns, as described in Table 4.19, namely, apparent inconsistencies in the reporting of results that decrease confidence in the interpretation of these findings.] However, cobalt metal particles < 5 µm in diameter did not increase the incidence of type II or III foci formation in C3H10T1/2 cells after exposure at concentrations ≤ 500 µg/mL, despite significantly increased cytotoxicity (Doran et al., 1998). [The Working Group noted that it was impossible to remove particulates from cell monolayers, so cells were exposed throughout the 42-day growth period, greatly exceeding the intended 48-hour exposure period.]

Cobalt metal NPs (mean diameter, 0.08 µm) induced morphological transformation as determined by increased incidence of type III foci in MEF BALB/3T3 cells after 72-hour exposures at concentrations ≥ 1.4 µg/mL (Ponti et al., 2009); larger cobalt metal particles (diameter, 0.2 µm) significantly increased transformation after 72-hour exposures to concentrations ≥ 0.65 µg/mL, including non-cytotoxic and cytotoxic exposures, which was inhibited by co-treatment with 50 µM ascorbic acid (Sabbioni et al., 2014a). [The Working Group noted concerns with the study by Sabbioni et al. (2014a) as described in Table 4.19 and discussed above.] When used as the initiating agent in a two-stage transformation bioassay, cobalt metal NPs (mean diameter, 0.028 µm; hydrated mean diameter, 0.14 µm) at a concentration of 2.0 µg/mL increased transformation of BALB/3T3 cells after a 3-day exposure, in the absence of any promoter. When cobalt metal NP exposure was followed by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion, the number of transformed foci increased ninefold. Cobalt metal NPs were not effective as a promoter after 9-day exposure at the lower concentration of 0.5 µg/mL (Sighinolfi et al., 2016). [The Working Group noted that activity in the initiation phase might indicate that cobalt metal NPs can induce transformation in non-neoplastic cells. The Working Group also noted that the number of foci observed with 3-methylchloanthrene initiation followed by cobalt metal NP promotion was reduced, suggesting that cobalt metal NP treatment suppressed 3-methylchloanthrene-initiated colony formation, possibly due to increased cytotoxicity over the extended exposure duration.]

After 12 weeks of exposure at concentrations that were non-cytotoxic in the short-term, exposure of MEF cells deficient in Ogg1 (*Ogg1*$^{-/-}$) to cobalt metal NPs at a concentration of 0.05 µg/mL (mean diameter, 0.03 µm; hydrated mean diameter, 0.5 µm) induced morphological changes including cell elongation, but not in isogenic wildtype MEFs. After 10 weeks of exposure, there was a concentration-dependent increase in anchorage-independent soft agar colony formation in both wildtype and *Ogg1*$^{-/-}$ MEFs exposed to 0.05 or 0.1 µg/mL cobalt metal NPs; colony formation was greater in the *Ogg1*$^{-/-}$ versus wildtype MEFs, and colony formation increased after exposure to 0.1 µg/mL of cobalt metal NPs for only 5 weeks in *Ogg1*$^{-/-}$ but not wildtype MEFs (Annangi et al., 2015).

Soluble cobalt(II) salts

Cobalt(II) chloride did not increase morphological transformation (by evaluation of type III foci) in the MEF BALB/3T3 cell line after 72-hour

Table 4.20 Alterations in cell proliferation, cell death, or nutrient supply in human primary cells exposed to cobalt

End-point	Tissue, cell line	Results[a]	Concentration	Comments	Reference
Angiogenesis					
VEGF expression	Primary human fetal striatal neuroblasts	+	400 µM for 48 h		Ambrosini et al. (2015)
VEGF expression	Bone marrow mesenchymal stem cells	+	100 µM for 24 h	4 donors.	Nguyen et al. (2020)
VEGF expression	Fibroblasts and HUVECs	+	100 µM for 18 h	Statistical analysis was not performed.	Minchenko et al. (1994)
VEGF expression	Dental pulp-derived cells	+	300 µM, for 24 h	3 donors. Non-cytotoxic concentration.	Müller et al. (2012)
VEGF expression Decreased SDF-1 expression	Human endometrial stromal cells	+ +	10–100 µM for 48 h	22 donors.	Tsuzuki et al. (2012)
VEGF mRNA and protein expression	Retinal pigment epithelial cells	(+)	600 µM for 12 and 24 h		Gu et al. (2021)
VEGF expression	Retinal pigment epithelial cells	+	50–200 µM for 24 h	1 donor. Non-cytotoxic concentration.	Rosen et al. (2015)
VEGF expression	Nasal polyp fibroblasts	+	500 µM for 8 and 12 h	8 donors. VEGF was not increased after 24 h.	Lin et al. (2008)
VEGF expression	Periosteum-derived mesenchymal stem cells	+	50–200 µM for 7 day	$CoCl_2 \cdot 6H_20$. Cells obtained from periosteal biopsies from the proximal tibia of adolescent and adult patients during total knee replacement procedure or distraction osteogenesis.	Chai et al. (2018)
VEGF expression	HUVECs	+	100 µM for 24 h	Statistical analysis was not performed.	Namiki et al. (1995)
VEGF expression	HUVECs	+	50 µM for 4 and 24 h		Qiu et al. (2012)
VEGF expression Capillary-like tube structure formation	HUVECs	(+) +	100 µM for 24 h	Figure legend describing VEGF expression seems inverted.	Liu et al. (2009)
VEGF expression PECAM-1 expression	HUVECs	+ +	0.5–50 µM for 48 h 25 µM for 48 h	Non-cytotoxic concentrations.	Bosch-Rué et al. (2021)
VEGF expression	HUVECs	+	100 µM for 24 h		Wei et al. (2019)
VEGF expression	CD133+ cells from umbilical cord blood samples	+	100–200 µM		Zan et al. (2012)

Table 4.20 (continued)

End-point	Tissue, cell line	Results[a]	Concentration	Comments	Reference
Glycolysis					
ATP production through glycolysis	Skin fibroblasts	+	70 µM for 24 h	Non-cytotoxic concentration.	Vincent et al. (2008)
Cell proliferation					
Cell growth	Cholesteatoma keratinocytes	+	50–200 µM for 24 h		Zhang et al. (2021b)
Cell viability	Primary human fetal striatal neuroblasts	(+)	300–400 µM for 48 h	Cell viability used as an indirect marker of cell proliferation.	Ambrosini et al. (2015)
Cell viability	CD133+ cells from umbilical cord blood samples	(+)	50–200 µM for 72 h	Cell viability used as an indirect marker of cell proliferation.	Zan et al. (2012)
Cell viability increased CDK4 and cyclin D1 expression	HUVECs	+	20–200 µM for 24 h	Cell viability used as an indirect marker of cell proliferation.	Wei et al. (2019)
		+	100 µM for 24 h		
Cell number	Bone marrow mesenchymal stem cells	–	100 µM for 24 h	4 donors.	Nguyen et al. (2020)
Cell death					
Decreased apoptosis	Primary human umbilical vein endothelial cells (HUVECs)	+	100 µM for 24 h		Wei et al. (2019)
Increased Bcl-2 expression		+			
Decreased Bax and caspase-3 expression		+			
Decreased apoptosis	Primary human umbilical vein endothelial cells (HUVECs)	+	150 µM for 24 h	This effect of $CoCl_2$ is dependent on increased miR-21 expression.	Xu et al. (2017)
Decreased PDCD4 expression		+			

ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma-2; CDK4, cyclin-dependent kinase 4; HUVEC, human umbilical vein endothelial cell; mRNA, messenger RNA; PDCD4, programmed cell death protein 4; PECAM-1, platelet endothelial cell adhesion molecule-1; SDF-1, stromal cell-derived factor-1; VEGF, vascular endothelial growth factor.
[a] +, positive; –, negative; (+), positive in a study of limited quality.

exposures at concentrations ≤ 9.1 μg/mL, despite concentration-dependent increases in cytotoxicity as assessed by decreased relative plating efficiency (Ponti et al., 2009; Sabbioni et al., 2014a). [The Working Group noted that these negative studies were inconsistent with the positive results reported in earlier studies and summarized in *IARC Monographs* Volume 86 (IARC, 2006), although several quality and reporting concerns exist, as noted in Table 4.19, including a lack of concentration dependence in responses or effects only observed at concentrations associated with more than 90% cytotoxicity.] Briefly, concentration-dependent increases in the number of type II foci were observed in the MEF C3H10T1/2 cell line after 48-hour exposure to 5–20 μg/mL cobalt(II) chloride, concentrations that induced reductions of more than 95% in relative plating efficiency (Doran et al., 1998). An increased rate of morphological transformation was also observed in SHE cells, cultured at pH 6.7, after 24-hour exposure to ≥ 0.125 μg/mL cobalt(II) sulfate hydrate, including both subcytotoxic and cytotoxic concentrations, although the magnitude of effect was not concentration-related (Kerckaert et al., 1996). Similarly, 18-hour treatment with cobalt(II) acetate at 35 μg/mL enhanced simian adenovirus SA7-induced transformation in SHE cells (Casto et al., 1979).

Cobalt(II) sulfide

As reported previously in *IARC Monographs* Volume 86 (IARC, 2006), particles of crystalline CoS_2 and amorphous CoS (mean diameters, 1.25 μm and 2.0 μm, respectively) induced morphological transformation in a concentration-dependent manner in SHE cells after 48 hours of exposure to relatively noncytotoxic levels of 1 and 5 μg/mL, respectively, with greater transformation reported with crystalline versus amorphous forms, and at cytotoxic concentrations of 10 μg/mL (Abbracchio et al., 1982; Costa et al., 1982).

4.2.10 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Primary cells

See Table 4.20.

Cobalt(II) chloride has been frequently used to model the effects of chemically induced hypoxia because both cobalt(II) chloride and cobalt(II) sulfate induce responses similar to physiological hypoxia in cells (i.e. exposure to 1% oxygen), with the reported IC_{50} for induction of hypoxia-response element (HRE)-regulated genes around 10–30 μM for cobalt(II) sulfate, and VEGF induction occurring in a HIF-1α-dependent manner at concentrations ≤ 100 μM (Vengellur et al., 2003; Xia et al., 2009). In fact, the main hypoxia biomarker and signal transducer activated by cobalt(II) chloride is HIF-1α. [The Working Group noted that the mechanisms for HIF-1α activation are generally thought to involve replacing Fe^{2+} ions in the prolyl hydroxylase active centre leading to stabilization of HIF-1α protein, and possibly other activities.] Cobalt(II) chloride has been shown to induce hypoxia in 19 studies conducted using human primary cells (Table 4.20). In these studies, hypoxia effects induced by cobalt(II) chloride were associated with end-points related to key characteristic 10: "alters cell proliferation, cell death, or nutrient supply".

Regarding angiogenesis, increased VEGF mRNA and/or protein expression was shown in cells – such as bone marrow mesenchymal stem cells (Nguyen et al., 2020), dental pulp-derived cells (Müller et al., 2012), endometrial stromal cells (Tsuzuki et al., 2012), nasal polyp fibroblasts (Lin et al., 2008), and periosteum-derived mesenchymal stem cells (Chai et al., 2018)

– treated with cobalt(II) chloride at various concentrations (50–500 μM). Cobalt(II) chloride treatment (600 μM) of human retinal pigment epithelial cells (ARPE-19 cell line) significantly increased HIF-1α and VEGF mRNA and protein levels at 12 and 24 hours, respectively (Gu et al., 2021). Concentration-dependent increases in VEGF expression were observed in human retinal pigment epithelial cells (Rosen et al., 2015), human endometrial stromal cells (Tsuzuki et al., 2012), and CD133+ cells derived from human umbilical cord blood (Zan et al., 2012). In addition, different concentrations of cobalt(II) chloride (20–200 μM) were shown to induce VEGF expression in human umbilical vein endothelial cells (HUVECs) (Namiki et al., 1995; Liu et al., 2009; Qiu et al., 2012). In the study by Liu et al. (2009), cobalt(II) chloride also increased the formation of capillary-like tube structures, a marker of angiogenesis. In terms of nutrient supply, cobalt(II) chloride (70 μM) increased ATP production via glycolysis in human skin fibroblasts (Vincent et al., 2008) (Table 4.20). [The Working Group noted that for the most informative studies, with statistical significance, showing VEGF expression induced by cobalt(II) salts in human primary cells, cytotoxicity was controlled and/or concentration-dependent effects were observed (Müller et al., 2012; Vincent et al., 2008 ; Tsuzuki et al., 2012 ; Qiu et al., 2012 ; Zan et al., 2012 ; Rosen et al., 2015).]

Fewer studies have assessed the effects of cobalt(II) chloride on cell proliferation in human primary cells. Treatment of human cholesteatoma keratinocytes with cobalt(II) chloride induced cell proliferation, which was dependent on p-PDK1 and p-Akt signalling (Zhang et al., 2021b). Concentration-dependent increases in cell proliferation were observed in primary human fetal striatal neuroblasts (Ambrosini et al., 2015) and umbilical cord blood-derived CD133+ cells (Zan et al., 2012). [The Working Group noted that cell viability was assessed in both studies; this represents an indirect marker of cell proliferation.] Increased cell proliferation induced by cobalt(II) chloride in HUVECs involved increased CDK4 and CCND1 expression (Wei et al., 2019). On the other hand, no effects on cell proliferation were observed in bone marrow mesenchymal stem cells treated with cobalt(II) chloride (Nguyen et al., 2020). Only two studies showed inhibition of apoptosis by cobalt(II) chloride in HUVECs (Xu et al., 2017; Wei et al., 2019). In these studies, inhibition of apoptosis was associated with increased BCL2 and decreased BAX and caspase-3 expression and was dependent on increased miR-21 expression. All references are shown in Table 4.20.

Immortalized cells

See Table S4.21 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Cobalt(II) chloride has been extensively shown in 49 studies to be a hypoxia inducer in human immortalized cell lines. In these studies, increased HIF-1α expression was associated with hypoxia induced by cobalt(II) chloride. The hypoxic effects induced by cobalt(II) chloride were associated with end-points related to key characteristic 10: "alters cell proliferation, cell death, or nutrient supply".

Regarding angiogenesis, increased VEGF mRNA and/or protein expression was shown in studies with cobalt(II) chloride (50–200 μM) in several cell lines, such as choroidal vascular endothelial cells (Balaiya et al., 2013), human microvascular endothelial cells (Loboda et al., 2005), human Müller cells (Sears & Hoppe, 2005), and osteoblast-like cell lines (Kim et al., 2002) obtained from healthy tissue. In addition, several studies conducted in human retinal pigment epithelial cells (ARPE-19 cell line), have shown increased VEGF expression induced by different concentrations (100–300 μM) of cobalt(II) chloride (Oh et al., 2013; Park et al., 2015; Zhao et al., 2015; Wang et al., 2016b; Chang et al., 2017).

In this same cell line, cobalt(II) chloride (100 µM) induced downregulation of SIRT1 and acetylation of the pro-angiogenic factor HMGB1 (Chang et al., 2017).

Furthermore, cobalt(II) chloride (50–200 µM) increased VEGF mRNA and/or protein expression in several immortalized cell lines, such as endometrial carcinoma (Molitoris et al., 2009), cervical carcinoma (Osera et al., 2015), mesothelioma (Sato et al., 2014), colon adenocarcinoma (Law et al., 2012), pancreatic carcinoma (Wen et al., 2016), prostate carcinoma (Lee et al., 2006), and oral squamous carcinoma cells (Stewart et al., 2003). Such effects were also reported after treatment with cobalt(II) sulfate (100 µM) (Xia et al., 2009). Studies demonstrated that the effects of cobalt(II) chloride on VEGF expression were dependent on several factors including: HuR (human antigen R) (Osera et al., 2015); Yes, a member of the Src family of kinases (Sato et al., 2014); STAT, which binds the VEGF promoter (Gray et al., 2005); AMP-activated protein kinase (Lee et al., 2006); or PI3K signalling (Stewart et al., 2003). In the study by Maurage et al. (2009), cobalt(II) chloride (100 µM) also increased secretion of endocan (also known as endothelial cell-specific molecule 1), a pro-angiogenic factor, by a glioblastoma cell line. In terms of nutrient supply, cobalt(II) chloride (150 µM) induced the expression of GLUT-1 and/or hexokinase II in human cervical cancer (Cheng et al., 2013) and mammary cancer (Chen et al., 2010a) cells (see Table S4.21, Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Fewer studies have assessed the effects of cobalt chloride on cell proliferation in normal human cell lines. Concentration-dependent increases in cell proliferation were observed in ARPE-19 cells (Wang et al., 2016b). [The Working Group noted that cell viability was assessed, which represents an indirect marker of cell proliferation.] Increased proliferation of pulmonary artery smooth muscle cells, induced by cobalt(II) chloride, was dependent on PGI_2 downregulation and low levels of hydrogen sulfide (Li et al., 2014). In addition, studies demonstrated induction of cell proliferation using cobalt(II) chloride at different concentrations, and different neoplastic cell lines, such as gastric adenocarcinoma (Bi et al., 2010), cervical cancer cells (Cheng et al., 2013), pancreatic cancer cells (Chen et al., 2018a), and renal cell carcinoma (Zhang et al., 2017). Induction of cell proliferation involved increased ERK-MAPK signalling (Bi et al., 2010) and inhibition of p53 (Lee et al., 2001). On the other hand, cobalt(II) chloride induced concentration-dependent decreases in cell proliferation (cell viability) in colon cancer (Yang et al., 2016) and breast cancer (Barrak et al., 2020) cells. In addition, no alterations in cell proliferation were observed in studies conducted with cobalt(II) chloride in fetal mesencephalic neural progenitor cells (Milosevic et al., 2009), Hodgkin lymphoma (Kewitz et al., 2016), and ovarian cancer cell lines and clear cell carcinoma cells (Nunes et al., 2018). No effects (Xu et al., 2014) or increases (Cheng et al., 2013) in apoptosis were observed after treatment of hepatoma and cervical cancer cells with cobalt(II) chloride, respectively. All references are shown in Table S4.21 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

[The Working Group noted that the most informative data refer to the increased expression of VEGF in human primary cells and human immortalized cells induced by cobalt(II) chloride. Few of these available studies controlled for cobalt(II) chloride cytotoxicity. However, cytotoxic effects are normally seen with concentrations > 300 µM, while increased expression of VEGF was observed at lower concentrations.]

(b) Experimental systems

(i) Non-human mammals in vivo

Cobalt metal or cobalt metal nanoparticles

Cobalt metal microparticles induced an array of epithelial cell damage and proliferative responses in the respiratory tracts of male and female Fischer 344/N or Fischer 344/Ntac rats exposed to aerosols (MMAD, 1.4–2.0 μm) at 1.25 mg/m^3 for 2, 14, or 105 weeks (NTP, 2014), like that reported after exposure to cobalt(II) salts. Dose- and duration-dependent increases in hyperplastic, metaplastic, and fibrotic responses were observed in the alveolar and bronchiolar epithelium, along with olfactory epithelial degeneration and hyperplasia, nasal respiratory epithelium necrosis and squamous metaplasia, and turbinate atrophy. Lung was the tissue most sensitive to injury after cobalt metal microparticle exposure, as significant effects in the trachea were not reported, unlike those observed after exposure to cobalt(II) salts (NTP, 2014). While injury was not observed after 1 day of exposure to cobalt metal NPs in male JcL:SD rats, 4 days of exposure to 2.12 ± 0.55 mg/m^3 aerosols (d_{50}, 20 nm; MMAD, 760 nm) appeared to induce a variety of similar lung pathologies evident by transmission electron microscopy, including bronchiolar hypertrophy and proliferation, bronchiolization of alveolar ducts, type II pneumocyte proliferation, and mitosis of fibroblasts (Kyono et al., 1992). In male Sprague-Dawley rats, bulk cobalt metal and cobalt metal NPs (d_{50}, 120 nm) were implanted on contralateral sides of the vertebral column (subcutaneously and intramuscularly, respectively), and subdermal lesions were assessed after 6 months due to early deaths or 8 months due to unacceptable lesion morbidity. In 4/10 rats, capsules with fibroblastic proliferations were described, which were described as having preneoplastic characteristics, along with increased proliferating cell nuclear antigen (PCNA) expression (Hansen et al., 2006). [The Working Group noted that several conduct and reporting issues were identified in this study, including lack of reporting on comprehensive histopathological lesion incidence, severity, or magnitude, incidence reported only in terms of numbers of rats with fibroblastic proliferation, and no description of PCNA evaluation or comparison method.]

Similar to the effects observed in rats, cobalt metal aerosols induced an array of epithelial cell damage and proliferative responses in the respiratory tracts of male and female B6C3F$_1$/N mice exposed at 1.25 mg/m^3 (MMAD, 1.4–2.0 μm) for 2, 14, or 105 weeks, with damage also evident in the larynx and lung after exposures at 0.625 mg/m^3 for 14 weeks (NTP, 2014). Dose- and duration-dependent increases in necrotic, hyperplastic, metaplastic, and fibrotic responses were observed in the alveolar and bronchiolar epithelium, along with olfactory epithelial necrosis, atrophy, and hyperplasia, nasal respiratory epithelium degeneration and squamous metaplasia, and turbinate atrophy. Unlike in similarly exposed rats, but comparable with the results reported in B6C3F$_1$/N mice after exposure to cobalt(II) salts, larynx was the tissue most sensitive to injury, because squamous metaplasia was induced at the lowest concentrations after subchronic or longer exposure durations (NTP, 2014). Intratracheal instillations of cobalt metal NPs (d_{50}, 20 nm; hd_{50}, 260 nm) induced proliferative responses in the lung epithelial cells of male and female transgenic C57Bl/6J mice (*gpt*, lambda phage EG10 DNA), elevating Ki-67 and PCNA expression 1 and 16 weeks after exposure to 50 μg/mouse [~2 mg/kg bw] (Wan et al., 2017).

[The Working Group noted that Viegas et al. (2022) exposed male and female Crl:CD(SD) rats to a variety of (soluble and insoluble) cobalt compounds via nose-only inhalation for 4 hours (cobalt metal powder, cobalt(II) hydroxide, cobalt(II) sulfate, cobalt carbonate, tricobalt tetraoxide [cobalt(II,III) oxide], lithium cobalt oxide, or cobalt(II) sulfide). However, the authors

Table 4.22 Alterations in cell proliferation, cell death, or nutrient supply in non-human mammals in vivo exposed to cobalt

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Soluble cobalt(II) salts					
Cell proliferation					
Cobalt(II) chloride ($CoCl_2$)					
Epithelial cell damage and proliferative response (nodular aggregation of alveolar type II epithelial cells, and concentrations of type II cells in other areas of the lung, with some AAH characteristics)	Rabbit, lung	+ (4–6 wk) + (15 wk) + (16 wk)	0.4–0.6 mg Co^{2+}/m^3 0.4 ± 0.2 mg Co^{2+}/m^3 0.6 ± 0.5 mg Co^{2+}/m^3	$CoCl_2$ aerosol: MMAD, 1 µm. Cobalt source and purity not reported, evaluations were blinded to exposure condition, dose-dependent response, $n = 8$, rabbits housed individually, males evaluated, negative control included.	Johansson et al. (1992, 1987, 1984)
Epithelial cell proliferation (PCNA expression and BrdU incorporation)	Rat (Wistar), kidney (in ablation/infarction model of chronic kidney disease)	+ (8 day)	10 mg/kg per day (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, $n = 8$, males evaluated, positive and negative controls included.	Deng et al. (2010)
Epithelial cell proliferation (PCNA staining)	Wistar rats, kidney (in right kidney subtotal nephrectomy model of ischaemia)	+ (2–9 wk)	5 mg/kg (subcutaneous injection, 3×/wk)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, $n = 4–8$, females evaluated, negative control included.	Tanaka et al. (2005a)
Epithelial cell proliferative capacity (ODC and HO-1 activity by bioassay)	Rat (Wistar), liver	+ (6–36 h)	125 µmol/kg [92.6 mg/kg] (subcutaneous injection; single or multiple)	Cobalt source and purity not reported, subcutaneous injection route $n = 3–4$, dose- and duration-dependent responses, males evaluated, negative controls included.	Numazawa et al. (1989b)
Epithelial cell proliferation (BrdU staining)	Rat (SD), bladder	+ (6 days)	200 µM every 2 days, for 30 min (intravesical infusion)	Cobalt purity not reported, single dose evaluated, intravesical exposure route, $n = 6$, males evaluated, vehicle control included.	Buttyan et al. (2003)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Cobalt(II) sulfate ($CoSO_4$)					
Epithelial cell damage and proliferative response Lung: alveolar epithelial hyperplasia, AAH, metaplasia, interstitial fibrosis; bronchiolar epithelium regeneration Nose: olfactory epithelial atrophy, degeneration, metaplasia; respiratory epithelium hyperplasia, squamous metaplasia Larynx: ulcer, necrosis, squamous metaplasia	Rat (F344/N), lung, nose, and larynx	+ (13 wk) + (13 wk) + (13 wk) + (104 wk)	0.3 mg/m³ (larynx); 1.0 mg/m³ (lung); 10 mg/m³ (nose); 0.3 mg/m³ (lung, nose, and larynx)	$CoSO_4 \cdot 7H_2O$ aerosol: MMAD, 1–3 µm. Gold-standard for design, methodology, and reporting. Dose-dependent responses, negative controls included, 13 wk (n = 10), 104 wk (n = 50), males and females evaluated, evaluations were blinded to exposure condition.	Bucher et al. (1990, 1999)
Epithelial cell damage and proliferative response Lung: cytoplasmic vacuolization of the bronchiolar epithelium, regeneration; alveolar epithelial hyperplasia Nose: olfactory epithelial atrophy, degeneration, hyperplasia; respiratory epithelium squamous metaplasia Larynx: necrosis, squamous metaplasia Trachea: squamous metaplasia	Mouse ($B6C3F_1$/N), lung, nose, larynx, and trachea Mouse ($B6C3F_1$/N), lung, nose, larynx	+ (13 wk) + (13 wk) +/– (13 wk) + (104 wk) + (104 wk)	0.3 mg/m³ (lung and larynx); 3.0 mg/m³ (nose); 30 mg/m³ (trachea); 0.3 mg/m³ (lung and larynx); 1.0 mg/m³ (nose)	$CoSO_4 \cdot 7H_2O$: MMAD, 1–3 µm. Gold-standard for design, methodology, and reporting in 2 yr inhalation bioassay. Dose-dependent responses, 13 wk (n = 10), 104 wk (n = 50), males and females evaluated, negative controls included, evaluations were blinded to exposure condition.	Bucher et al. (1990, 1999)
Angiogenesis					
Cobalt(II) chloride ($CoCl_2$)					
VEGF protein expression, EPO mRNA by qRT-PCR	Rats (Wistar), kidney (in ablation/infarction model of chronic kidney disease)	+ (8 days)	10 mg/kg per day (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, n = 8, males evaluated, positive and negative controls included.	Deng et al. (2010)
VEGF and EPO mRNA levels by qRT-PCR, and VEGF protein by IHC	Rat (Wistar), kidney (in right kidney subtotal nephrectomy model of ischaemia)	+ (2–9 wk)	5 mg/kg (subcutaneous injection, 3×/wk)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, n = 4–8, females evaluated, negative control included.	Tanaka et al. (2005b)
VEGF mRNA expression by northern blot	Rat (Wistar), liver, heart, kidney, muscle Rat (Wistar), brain	+ (6–8 h) – (6–8 h)	60 mg/kg (subcutaneous injection)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, n = 5, males and females evaluated, negative control included.	Minchenko et al. (1994)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
VEGF mRNA expression by northern blot	Rat (Wistar), heart Rat (Wistar), brain and kidney	(+/–) (4 h) (–) (4 h)	1 mL of 50 or 75 mM solution [32 or 48 mg/kg] (subcutaneous injection)	Cobalt source and purity not reported, treatment dosage unclear [1 mL of 50 or 75 mM], single dose evaluated, subcutaneous injection route, (*n* = unclear), negative control included.	Ladoux & Frelin (1994)
Factor XIII by IHC and microvessel enumeration, HIF-1α and VEGF protein expression by western blot	Rat (SD), bladder	+ (6 days)	200 µM every 2 days, for 30 min (intravesical infusion)	Cobalt purity not reported, single dose evaluated, intravesical exposure route, *n* = 6, males evaluated, vehicle control included.	Buttyan et al. (2003)
VEGF, EPO, and HO-1 mRNA levels by qRT-PCR, VEGF protein by western blot	Rat (SD), kidney (unephrectomized Thy1 nephritis model)	+ (3 wk)	2.7 mg/kg every 3 days (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, *n* = 2–9, males evaluated, negative control included.	Tanaka et al. (2005b)
HIF-1α and VEGF mRNA by qRT-PCR and protein by western blot	Rat (SD), tibial bone	+ (unclear duration)	15 mg/kg per day (intraperitoneal injection)	Cobalt source and purity not reported, uncertain treatment duration, single dose evaluated, *n* = 8, females evaluated, negative control included.	Huang et al. (2015)
HIF-1α, VEGF, and EPO mRNA expression by RT-PCR and protein expression by western blot, and HIF-1α DNA-binding	Rat (SD), heart	+ (7 days)	12.5 mg/kg per day (oral gavage)	Co source and purity not reported, single dose evaluated, *n* = 3–10, negative control included.	Singh et al. (2010)
VEGF and EPO mRNA by qRT-PCR	Rat (SD), kidney (ischaemic renal injury model)	+ (days –10 to 3 post-injury)	2 mM in drinking-water [440 mg/kg per day]	Cobalt source and purity not reported, single dose evaluated, *n* = 2–3, males evaluated, vehicle control included.	Matsumoto et al. (2003)
Microvessel density by morphometric evaluation of new, immature arterioles	Rat (SD), heart	+ (5 wk)	75 mg/kg, 3×/wk (intraperitoneal injection)	Cobalt source and purity not reported, single dose evaluated, polycythemia observed, *n* = 8–10, males evaluated, vehicle control included.	Rakusan et al. (2001)
VEGF and GLUT1 protein expression in cerebrum microvessels by western blot, point counting of capillary density	Rat (SD), brain	+ (12 days) – (12 days)	2 mM in drinking-water [260 mg/kg per day]	Cobalt purity not reported, single dose evaluated, *n* = 12, males evaluated, vehicle control included, evaluations were blinded to exposure condition.	Badr et al. (1999)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Flt-1 and EPO mRNA by RNase protection assay, VEGF, and flt-1 mRNA expression	Rat (SD), liver Rat (SD), lung	+ (6 h) – (6 h)	60 mg/kg (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, $n = 6$, males evaluated, negative control included.	Sandner et al. (1997)
EPO mRNA by RNse protection assay, serum EPO concentrations, VEGF mRNA expression	Rat (SD), liver and kidneys Rat (SD), lung, liver, kidneys, and heart	+/– (6 h) – (6 h)	60 mg/kg (subcutaneous injection)	Cobalt source and purity not reported, limited method reporting, $n = 6$, males evaluated, negative control included.	Sandner et al. (1996)
HIF-1α and HIF-2α protein levels by western blot and/or IHC, VEGF and EPO mRNA levels by qRT-PCR	Rat (SHR/NDmcr-cp), kidney (diabetic nephropathy model)	+ (26 wk)	200 µM in drinking-water (1 mg/rat per day) [2.1 mg/kg per day]	Cobalt source and purity not reported, single dose evaluated, $n = 10$, males evaluated, negative control included.	Ohtomo et al. (2008)
HIF-1α protein level by western blot, VEGF and EPO mRNA expression by qRT-PCR	Mouse (BALB/c), brain and hippocampus	(+)	60 mg/kg (unspecified injection)	Cobalt source and purity not reported, uncertain treatment route, single dose evaluated, duration-independent, $n = 3–6$, males evaluated, negative control included.	Zhang et al. (2014)
HIF-1α and VEGF protein levels by IHC and western blot, and mRNA expression by qRT-PCR	Mouse (BALB/c), nose, nasal mucosa (in ovalbumin-induced allergic rhinitis model)	+ (29 days)	9.8 mg/kg per day (intraperitoneal injection)	Cobalt purity not reported, single dose evaluated, $n = 4–6$, negative control included.	Zhou et al. (2012)
Enumerating blood vessels and vessel density; no effects with $CoCl_2$ in absence of MatLyLu cells	Chicken, chorioallantoic membrane in whole egg	+ (5 days)	100 µM (implanted ring containing rat prostate tumour cells [MatLyLu] cells)	Cobalt purity not reported, single dose evaluated, $n = 5$, vehicle and negative control included.	Van Lieshout et al. (2003)
Skin flap necrosis by visual evaluation of margins, resulting from improved blood flow	Rat (Wistar), skin flap	(+) (13 wk pre-conditioning)	10% gel (skin application)	$CoSO_4 \cdot 7H_2O$. Cobalt source and purity not reported, observations were not blinded, single dose evaluated, macroscopic necrosis margins estimated visually, $n = 8$, "no treatment" control included but no gel vehicle control, males and females evaluated.	Bobek et al. (2005)
Apoptosis					
Cobalt(II) chloride ($CoCl_2$)					
TUNEL staining	Rat (Wistar), kidney (in right kidney subtotal nephrectomy model of ischaemia)	+ ↓ (2–9 wk)	5 mg/kg (subcutaneous injection, 3×/wk)	Cobalt purity not reported, single dose evaluated, subcutaneous injection route, $n = 4–8$, females evaluated, negative control included.	Tanaka et al. (2005a)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Apoptosis: TUNEL staining in a partially VEGF-dependent manner	Rat (SD), kidney (unnephrectomized Thy1 nephritis model)	+ ↓ (3 wk)	2.7 mg/kg every 3 days (subcutaneous injection)	Cobalt source and purity not reported, single dose evaluated, subcutaneous injection route, n = 2–9, males evaluated, negative control included.	Tanaka et al. (2005b)
Cobalt metal or cobalt metal NPs					
Cell proliferation					
Epithelial cell damage and proliferative response Lung: alveolar epithelial hyperplasia, cytoplasmic vacuolization, interstitial fibrosis; bronchial epithelial hyperplasia, cytoplasmic vacuolization, erosion, necrosis Nose: olfactory epithelial atrophy, necrosis, degeneration, hyperplasia; respiratory epithelium cytoplasmic vacuolization, degeneration, squamous metaplasia; turbinate atrophy Larynx and trachea: squamous metaplasia, cytoplasmic vacuolization	Mouse, ($B6C3F_1$/N), lung and nose Mouse ($B6C3F_1$/N), lung, nose, and larynx Mouse ($B6C3F_1$/N), lung, nose, larynx, and trachea	+ (2 wk) + (14 wk) + (14 wk) + (105 wk)	2.5 mg/m³ (lung and nose); 0.625 mg/m³ (lung and larynx); 1.25 mg/m³ (nose); 1.25 mg/m³ (lung, nose, larynx, and trachea)	Aerosol particle size: MMAD, 1.4–2.0 µm. Gold-standard for design, methodology, and reporting in 2 yr inhalation bioassay, dose-dependent responses, survival-adjusted analyses: 2 wk (n = 5), 14 wk (n = 10), and 104 wk (n = 50), males and females evaluated, negative control included, evaluations were blinded to exposure condition.	NTP (2014)
Epithelial cell damage and proliferative response Lung: alveolar epithelial hyperplasia, interstitial fibrosis; bronchial epithelial hyperplasia, necrosis Nose: olfactory epithelial atrophy, necrosis, degeneration, hyperplasia, metaplasia; respiratory epithelium necrosis, hyperplasia, metaplasia; turbinate atrophy	Rat (F344/N), lung and nose Rat (F344/N), lung and nose Rat (F344/NTac), lung and nose	+ (2 wk) + (14 wk) + (14 wk) + (105 wk)	2.5 mg/m³ (lung and nose); 1.25 mg/m³ (lung); 2.5 mg/m³ (nose); 1.25 mg/m³ (lung and nose)	Aerosol particle size: MMAD, 1.4–2.0 µm. Gold-standard for design, methodology, and reporting in 2 yr inhalation bioassay, dose-dependent responses, survival-adjusted analyses: 2 wk (n = 5), 14 wk (n = 10), 104 wk (n = 50), males and females evaluated, negative control included, evaluations were blinded to exposure condition.	NTP (2014)

Table 4.22 (continued)

End-point	Species, tissue	Results[a]	Dose	Comments	Reference
Epithelial cell damage and proliferative response: bronchiolar hypertrophy and proliferation, damage to cilia and bronchioalveolar duct junctions, bronchiolization of alveolar ducts; type II pneumocyte proliferation, interstitial oedema, fibroblast mitosis	Rat (SD, Jcl:SD), lung	(–) (1 days) (+) (4 days)	2.72 ± 0.44 mg/m^3 2.12 ± 0.55 mg/m^3	Aerosol NP size: d_{50}, 20 nm; MMAD, 760 nm. Cobalt purity not reported, no negative controls, procedures for pathological evaluation not described, *n* = 2–5, males evaluated, duration-dependent responses.	Kyono et al. (1992)
Fibroblast proliferation: encapsulated fibroblastic proliferation with preneoplastic characteristics, PCNA expression by IHC	Rat (SD), dermis	(+) (26–35 wk)	"Bulk" cobalt metal pellets subcutaneously; 60–100 mg cobalt metal NPs intramuscularly; agglomerate of particles of 10 µM size.	"Bulk" cobalt metal pellets (4.73 mm^1) and cobalt metal NPs (d_{50}, 120 nm) implanted on contralateral sides of vertebral column. Cobalt purity not reported, detailed lesion incidence and PCNA expression not clearly reported, *n* = 10, males evaluated, positive and negative controls included.	Hansen et al. (2006)
Altered cell proliferation and/or cell death: percentage of CD34$^+$ HSC/HPC by fluorescence-activated cell sorting	Rat (SD), bone marrow	(+ ↓) (6 wk)	1 mg/kg (3×/wk injections into right hip)	NP size: diameter, 50–200 nm. Cobalt purity and rat source not specified, hip injection as route of exposure, experimental allocation unclear (may be *n* = 6), males evaluated, negative control included.	Zhu et al. (2021a)
Epithelial cell damage and proliferative response: focal alveolar epithelial cell hyperplasia, LDH in BALF, proliferation rate by Ki-67 and PCNA IHC 1 wk; interstitial fibrosis, bronchiolization of the alveola, proliferation rate by Ki-67 and PCNA IHC 16 wk	Mouse (C57Bl/6J, *Gpt* transgenic, lambda phage EG10 DNA), lung	+ (1 wk) + (16 wk)	50 µg/mouse [~2 mg/kg] (intratracheal instillation) 50 µg/mouse [~2 mg/kg] (intratracheal instillation)	NP size: d_{50}, 20 nm; hd_{50}, 260 nm. Cobalt purity not specified, intratracheal route of exposure, *n* = 4–8, males and females evaluated, negative control included.	Wan et al. (2017)

AAH, atypical adenomatous hyperplasia; BALF, bronchoalveolar lavage fluid; BrdU, bromodeoxyuridine; CD34, cluster of differentiation 34; d_{50}, mean particle diameter; EPO, erythropoietin; flt, Fms related receptor tyrosine kinase 1; Gpt, guanine phosphoribosyltransferase; hd_{50}, hydrated mean particle diameter; HIC, highest ineffective concentration; HO-1, haem oxygenase isoenzyme 1; HPC, haematopoietic progenitor cell; HSC, haematopoietic stem cell; IHC, immunohistochemistry; LDH, lactate dehydrogenase; LEC, lowest effective concentration; min, minute; MMAD, mass median aerodynamic diameter; mRNA, messenger RNA; PCNA, proliferating cell nuclear antigen; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RNase, ribonuclease; RT-PCR, reverse transcription polymerase chain reaction; SD, Sprague-Dawley; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; VEGF, vascular endothelial growth factor; wk, week; yr, year.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative in a study of limited quality; ↓, decreased.

presented only a summary severity score based upon the aggregation of four histopathological end-points from the upper respiratory tract. Due to the lack of primary data reporting and other quality concerns, this study was not considered for further evaluation.]

Soluble cobalt(II) salts

Cobalt(II) chloride disrupted the organization of type II pneumocytes in the lungs of male rabbits exposed by inhalation to Co^{2+} aerosol at 0.4–0.6 mg/m^3 (MMAD, 1 μm) for 4–16 weeks. The effect manifested by the nodular aggregation of alveolar type II epithelial cells in some regions of the distal lung, coinciding with decreasing proportions of these cells in adjacent alveoli, consistent with epithelial cell proliferation in response to alveolar injury (see Table 4.22) (Johansson et al., 1984, 1987, 1992). The authors observed that treatment-related effects in type I pneumocytes were less frequent, less severe, and appeared later in time compared with the aggregation observed in type II cells. [The Working Group noted that some aspects of the nodular aggregation reported after longer durations appear consistent with atypical adenomatous hyperplasia.] A greater degree and variety of epithelial cell damage and proliferative responses were observed in the respiratory tracts of male and female Fischer 344/N rats exposed by inhalation to cobalt(II) sulfate aerosols (MMAD, 1–3 μm) at 0.3 mg/m^3 for 13 weeks and 2 years, in inhalation bioassays. While concentration- and duration-dependent increases in hyperplastic, metaplastic, and fibrotic responses were observed in the alveolar epithelium, along with olfactory epithelial degeneration and squamous metaplasia of the nasal respiratory epithelium, necrosis and squamous metaplasia of the larynx appeared earliest and at the lowest exposure concentrations assessed, in response to significant tissue injury (Bucher et al., 1990, 1999). Similarly, although generally less severe, pathology was also observed in the lungs, nasal passages, and larynx of male and female B6C3F$_1$/N mice that were similarly exposed, with the addition of squamous metaplasia appearing in a few animals after subchronic exposure to the highest concentrations (Bucher et al., 1990, 1999).

Increased epithelial cell proliferation and/or decreased apoptosis have been reported in rat kidney after cobalt(II) chloride administration via subcutaneous or intraperitoneal injection (Tanaka et al., 2005a, b; Deng et al., 2010), as well as in the urinary bladder after intravesical infusion (Buttyan et al., 2003).

Increased HIF-1α mRNA and protein expression have been widely reported after exposure to cobalt(II) chloride (single doses to multiple weeks) in several strains of rats and BALB/c mice by injection, gavage, and drinking-water (e.g. Ohtomo et al., 2008; Deng et al., 2010; Zhang et al., 2014; Huang et al., 2015). Because of this chemically induced hypoxia, cobalt(II) chloride has been used to investigate the effects of hypoxia-mediated protection in various rodent models of human diseases and tissue injury, including allergic rhinitis (Zhou et al., 2012), chronic kidney disease (Tanaka et al., 2005a, b; Deng et al., 2010), ischaemia (Rakusan et al., 2001; Matsumoto et al., 2003; Singh et al., 2010; Zhang et al., 2014), and bone fracture (Huang et al., 2015). Increased mRNA and/or protein levels of various angiogenic mediators induced by HIF-1α activation have also been reported, including VEGF and EPO (e.g. Minchenko et al., 1994; Matsumoto et al., 2003; Tanaka et al., 2005a, b; Ohtomo et al., 2008; Deng et al., 2010; Singh et al., 2010; Zhou et al., 2012; Zhang et al., 2014; Huang et al., 2015). However, in several studies evaluating effects in rat tissues 4–6 hours after treatment with 30–60 mg/kg cobalt(II) chloride administered via subcutaneous injection, mRNA expression of VEGF and the VEGF receptor Flt-1 increased marginally in the heart but not in the brain, kidney, liver, or lung (Ladoux & Frelin, 1994; Sandner et al., 1996, 1997).

Few studies reporting direct measures of angiogenesis after cobalt(II) chloride exposures were identified; increased microvessel formation coinciding with increased VEGF protein expression was observed in the bladders of Sprague-Dawley rats (Buttyan et al., 2003), increased formation of new, immature arterioles in the heart coincided with decreases in some measures of existing capillary supply (Rakusan et al., 2001), and no change in microvessel density in the cerebrum was reported despite increased VEGF protein expression (Badr et al., 1999). Interestingly, while exposure to cobalt(II) chloride alone had no effect on angiogenesis in a chick chorioallantoic membrane (CAM) model, the inclusion of cobalt(II) chloride-exposed MatLyLu cells (Dunning rat prostate tumour cells) increased both the number and density of blood vessels (Van Lieshout et al., 2003). [The Working Group noted that while cobalt(II) chloride appeared to widely stimulate the activities of HIF-1α and EPO, increased expression of downstream mediators of angiogenesis such as VEGF, and the ultimate stimulation of new vessel formation, may occur in a tissue-specific manner.] While studies using cobalt(II) chloride to induce hypoxia typically only assessed a single dose, multiple subcutaneous cobalt(II) chloride injections every 6–12 hours increased activity of the downstream HIF-1α target ornithine decarboxylase (ODC) in a greater-than-additive manner in male Wistar rat liver, compared with a single injection of 125 µmol/kg [92.6 mg/kg bw] (Numazawa et al., 1989b).

[The Working Group noted that Burzlaff et al. (2022), in experiments described only in the supplementary materials associated with their manuscript, exposed male and female Wistar (Han) rats via nose-only inhalation to cobalt(II) sulfate aerosols (MMAD, 1.6 µm) at 2 mg/m^3 for 28 days, and reported increased focal squamous metaplasia at the base of the epiglottis in most exposed rats. Owing to quality concerns regarding study reporting, this study was not considered for further evaluation.]

(ii) Non-human mammalian cells in vitro

Soluble cobalt(II) salts

While increased cyclin B1 content suggestive of increased cellular proliferation was reported in porcine ovarian granulosa cells exposed to high concentrations of cobalt(II) sulfate (90 µg/mL) (Kolesarova et al., 2010), no increases in cellular proliferation, or decreases in apoptosis inferred by alterations in relative plating efficiency (RPE), were reported by several studies in mouse embryonic fibroblasts (MEF) or immortalized RAW 264.7 macrophages exposed to cobalt(II) chloride at concentrations ≥ 2 µM (for examples see: Kerckaert et al., 1996; Doran et al., 1998; Ponti et al., 2009; Sabbioni et al., 2014a; Salloum et al., 2018; Zhang et al., 2021b). In other cell types, cobalt(II) chloride concentrations ≥ 100 µM induced cellular proliferation (Wenker et al., 2013), had no appreciable effects (Yang et al., 2004; Lu et al., 2009; Wu et al., 2012a), or failed to attenuate caspase-3 activation and apoptotic cell death (Yang et al., 2011d; Mo et al., 2016).

Cobalt(II) chloride has been extensively used in numerous studies as a means of chemically inducing hypoxia in cell lines from various mammalian species and many tissue types after exposures to concentrations ≥ 100 µM (e.g. Xia et al., 2009), with little evidence of any consistent concentration–response relationship at lower concentrations (Kuo et al., 2019). While HIF-1α protein expression or DNA-binding activity is commonly increased, along with increased expression and activity of downstream HIF-1α targets such as VEGF associated with increased measures of angiogenesis (e.g. Zhang et al., 2021b), cobalt(II) chloride exposure does not consistently increase HIF-1α mRNA expression levels (e.g. Kumar et al., 2012; Wang et al., 2013b). Cobalt(II) chloride exposure increased VEGF expression in primary cells isolated from

bovine and porcine ovaries (Grasselli et al., 2005; Zhang et al., 2011), rabbit retinal pigment epithelial cells (Wang et al., 2003), rat kidney cortical cells (Deng et al., 2010), and rat heart myocyte cultures (Ladoux & Frelin, 1994). In primary pancreatic islets isolated from C57Bl/6 mice, cobalt(II) chloride increased VEGF mRNA and protein expression, which was associated with an expanded number and network of endothelial cells (Sankar et al., 2019), with similar results also reported in bone marrow populations isolated from 129S1/SvlmJ mice (García-Román et al., 2010). Exosomes derived from RAW 264.7 macrophages that were stimulated with lipopolysaccharide and exposed to 200 μM cobalt(II) chloride induced neoangiogenesis when administered to a human endothelial cell line (EA.hy926) installed in Matrigel plugs in male Sprague-Dawley rats (Zhang et al., 2021b).

Cobalt metal nanoparticles

At concentrations of ≥ 100 μM, cobalt metal NPs (diameter, 50–500 nm) did not increase cellular proliferation, viability, or attenuate cell death in Sprague-Dawley rat primary bone marrow CD34+ haematopoietic stem and progenitor cells (Zhu et al., 2021a). Similar results were reported in several studies in MEFs (e.g. Miller et al., 2001; Ponti et al., 2009; Sabbioni et al., 2014a; Annangi et al., 2015; Sighinolfi et al., 2016).

Other relevant information

Swennen et al. (1993) assessed health effects in 82 cobalt refinery workers in Belgium with an on average 8-year duration of exposure and a geometric mean TWA exposure to cobalt dust of 125 μg/m^3. This included 25% of the refinery workers who had been exposed to > 500 μg/m^3. Results were compared with an age-matched group of non-cobalt dust-exposed controls. Of interest, three erythrocyte measurements were all significantly lower in the exposed population than controls: the total erythrocyte count (4.85 versus 5.48 × 10^{12}/L), haemoglobin (15.05 versus 15.59 g/100 mL), and haematocrit (44.03% versus 45.54%). [The Working Group noted that these minor differences in erythrocyte parameters were unlikely to be clinically relevant, but that cobalt often causes the opposite effects in erythrocytes.]

4.2.11 Multiple characteristics identified by transcriptomics (or other experimental approaches)

(a) Humans

(i) Human cells in vitro

See Table S4.23 (Annex 3, Supplementary material for Section 4, Mechanistic Evidence, web only, available from: https://publications.iarc.fr/618).

Soluble cobalt(II) salts

Several studies have assessed the transcriptional responses of various human cell lines at the mRNA expression and/or proteomics level, typically after exposures to high levels (e.g. 100–300 μM) of cobalt(II) chloride for up to 24 hours. In a study that assessed the extent of similarity in gene expression between cells after cobalt or hypoxia treatment, cobalt(II) chloride treatment of RKO colon carcinoma cells induced differential gene expression in 54 out of 150 "hypoxia upregulated" and 12 out of 76 "hypoxia downregulated" genes from a repository of hypoxia-related gene sets that were published on the MSigDB database (Sheffer et al., 2011). Genes that were upregulated impacted the carbohydrate metabolism and glycolysis, oxidoreductase activity, drug transport, cell survival, and angiogenesis pathways. Co-treatment with zinc(II) chloride was found to at least partially reverse expression in roughly one half of the hypoxia-associated genes and appeared to globally reverse the differential expression induced by cobalt(II) chloride exposure, when assessed by principal component analysis (PCA) (Sheffer et al., 2011).

[The Working Group noted that zinc supplementation may downregulate HIF-1/2 activity, and that the reverse of gene expression changes induced by cobalt(II) chloride may reflect zinc-mediated antagonism of HIF-1α activity.] In studies examining the effects of hypoxia induced by 24 hours of exposure to cobalt(II) chloride at 300 µM in differentiated enterocyte-like Caco-2 cells, activation of hallmark gene sets (described in Liberzon et al., 2015) associated with hypoxia induction via HIF signalling was reported – for example, protein tyrosine phosphatase, receptor type, f polypeptide, interacting protein, α 4 (*PPFIA4*) and B-cell CLL/lymphoma 2 apoptosis regulator (*BCL2*) – in addition to the TNFα-mediated NF-κB inflammation pathway, mTORC1 proliferation, and p53 cell cycle regulation (Knyazev et al., 2021). In a similar study, HIF-1α mRNA levels were not increased in Caco-2 cells but did increase in another colorectal adenocarcinoma cell line (HT-29), consistent with previous observations that HIF-1 may be regulated primarily by post-translational stabilization in a cell type-specific manner (Nersisyan et al., 2021).

Compared with another chemical method of hypoxia induction (oxyquinoline derivative), which stabilizes HIF-1α via inhibition of HIF prolyl hydroxylases, cobalt(II) chloride uniquely and independently stimulated dozens of genes in Caco-2 or HT-29 cells including elements involved in the oxidative stress and pro-inflammatory response, upregulation of anaerobic glycolysis (Knyazev et al., 2021), and the major histocompatibility complex class I antigen presentation pathway (Nersisyan et al., 2021). While more than 230 proteins were also differentially regulated, few changes in protein levels coincided with differential mRNA expression levels (Knyazev et al., 2021). [The Working Group noted this lack of concordance between mRNA and protein expression levels is not uncommon in such studies that typically assess a single high concentration of exposure at a single time point, and could result from several factors including the extent of protein regulation by post-translational modification, which underscores the difficulty of attempting to apply data from hypothesis-generating studies to specific hypothesis-testing applications.] Of the 17 genes previously reported by Benita et al. (2009) to be widely associated with hypoxia across various tissues, only 5 were found to be associated with cobalt(II) chloride exposure at both the mRNA and protein levels: aldolase, fructose-bisphosphate C (*ALDOC*), pyruvate dehydrogenase kinase 1 (*PDK1*), N-myc downstream regulated 1 (*NDRG1*), BCL2 interacting protein 3 (*BNIP3*), and prolyl 4-hydroxylase subunit α 1 (*P4HA1*) (Knyazev et al., 2021).

Concentrations of cobalt(II) chloride inducing 50% cytotoxicity in placental trophoblast cells (HTR-8/SVneo) broadly decreased intracellular levels of metabolite profiles including saturated and unsaturated fatty acids, GSH, amino acids, and tricarboxylic acid cycle intermediates such as malic and citric acid; effects on secreted metabolites were limited to decreased methionine, citramalic acid, and the two unsaturated fatty acids gamma-linolenic acid and conjugated linoleic acid, which also decreased internally (Chen et al., 2020). When the effect of a non-cytotoxic concentration of cobalt(II) chloride (20 µg/mL or approximately 150 µM) on the proteins secreted by BEAS-2B lung bronchial epithelial cells was investigated, 23 of the predicted 66 proteins in the extracellular "secretome" were found to be suppressed, with the most dramatic decreases reported in fibrillins 1 and 2, carboxypeptidase A4, biglycan, complement factor B, and cysteine rich transmembrane BMP regulator 1 (Malard et al., 2012). Only endoplasmic reticulum aminopeptidase 1 was induced, providing evidence that cobalt exposure generally reduces protein secretion in lung epithelial cells (Malard et al., 2012). Earlier gene expression studies evaluating supraphysiological LD_{50} concentrations (2 mM) in A549 cells reported differential expression of

genes involved in cobalt transport, transcription factors, the stress response, and cellular metabolism, including the induction of several HIF-1α target genes: aldolase, fructose-bisphosphate A (*ALDOA*), solute carrier family 2 member 1 (*SLC2A1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPD*), lactate dehydrogenase A (*LDHA*), BCL2 interacting protein 3 like (*BNIP3L*), phosphoglycerate kinase 1 (*PGK1*), and transferrin receptor (*TFRC*) (Malard et al., 2007). While this study assessed nine candidate genes coding for secreted proteins as potential biomarkers of cobalt(II) chloride exposure and confirmed that TIMP2 was significantly decreased in A549 cells, none of these candidates were differentially regulated after subcytotoxic exposures in BEAS-2B cells (Malard et al., 2012).

In neoplastic U266 multiple myeloma cells, treatment with cobalt(II) chloride at 100 μM induced approximately 30% cytotoxicity and upregulated the expression of genes associated with cellular development and death, as well as the immune response and B-cell activation, while genes involved in the biological processes regulating cell cycle, transcription, and kinase activity experienced both up- and downregulation (Bae et al., 2012). One of the most highly induced was oxidative stress induced growth inhibitor 1 (*OKL38*), a tumour suppressor gene that can inhibit neoplastic proliferation by inducing apoptosis. In U937 acute promonocytic leukaemia cells, cobalt(II) chloride at 50 μM induced accumulation of HIF-1α protein, coinciding with decreased content of proteins associated with physiological hypoxia and regulating cellular metabolism, cell proliferation, and differentiation; increases were only observed in the *NDRG1* gene (Han et al., 2006). In human HaCaT keratinocytes, non-cytotoxic cobalt(II) chloride concentrations (3 μg/mL or approximately 23 μM) also induced genes associated with HIF-1 activation such as *BNIP3* and *ALDOC*, as well as gene sets implicated in glycolysis and carbohydrate metabolism, and focal adhesion (Busch et al., 2010). While similar changes were also observed in liver Hep3B carcinoma cells exposed to cobalt(II) chloride at 100 μM, the authors noted that differences in gene expression were evident when compared with physiological hypoxia and cautioned against considering them equivalent (Vengellur et al., 2005).

Insoluble cobalt(II or II,III) oxide compounds

The effects of exposure to cobalt(II) oxide NPs in BEAS-2B lung bronchial epithelial cells and A549 alveolar adenocarcinoma cells at non-cytotoxic levels (6.09 μg/mL and 60.9 μg/mL, respectively) were assessed at the transcriptomic level (Verstraelen et al., 2014). Despite the lower concentration of cobalt(II) oxide NPs, there were approximately 100-fold more differentially expressed, and primarily downregulated, transcripts in BEAS-2B compared with A549 cells. In A549 cells, cobalt(II) oxide NPs primarily induced expression of seven genes associated with cellular metabolism: aryl hydrocarbon receptor nuclear translocator like 2 (*ARNTL2*), excision repair 4, endonuclease catalytic subunit (*ERCC4*), folylpolyglutamate synthase (*FPGS*), G protein nucleolar 3 like (*GNL3L*), hook microtubule tethering protein 3 (*HOOK3*), protein kinase cAMP-dependent type II regulatory subunit α (*PRKAR2A*), and zinc finger protein 721 (*ZNF721*). A similar induction in cellular metabolism was also observed in BEAS-2B cells, but the different genes were upregulated in a cell-specific manner: angiopoietin-like 4 (*ANGPTL4*), basic helix-loop-helix family member e40 (*BHLHE40*), endothelin 2 (*EDN2*), hexokinase 2 (*HK2*), pyruvate dehydrogenase kinase 1 (*PDK1*), protein phosphatase 1 regulatory subunit 3C (*PPP1R3C*), ribosomal modification protein rimK like family member A (*RIMKLA*), very low-density lipoprotein receptor (*VLDLR*) (Verstraelen et al., 2014), and both solute carrier family 2 member 1 (*SLC2A1*) and transferrin receptor (*TFRC*) which were reported by Malard et al. (2007) to be elevated in A549 cells after exposure to cobalt(II)

chloride. In addition, downregulation of several transcripts associated with immune system signalling were observed only in BEAS-2B cells, including toll-like receptor 6 (*TLR6*) and MHC (major histocompatibility complex) class I, A (*HLA-A*), with the most significant decrease noted in MHC class II, DR β 3 (*HLA-DRB3*) (Verstraelen et al., 2014).

(b) Experimental systems

(i) Non-human mammals in vivo

In Sprague-Dawley rats given drinking-water containing cobalt(II) chloride at a dose of 12.5 mg/kg bw per day for 7 days, proteomic analysis of plasma proteins indicated changes in proteins involved in lipid and mineral metabolism, as well as increased levels of albumin, apolipoproteins ApoA1 and ApoA4, and complement C3 (Ahmad et al., 2016).

Transcriptomic analysis was performed on lung bronchioloalveolar carcinomas induced in male and female $B6C3F_1$/N mice exposed via inhalation to cobalt metal aerosols (MMAD, 1.4–2.0 μm) at 5 mg/m^3 for 105 weeks, as reported by the NTP (2014), compared with spontaneous bronchioloalveolar carcinomas from unexposed mice, as well as normal, unaffected lung tissue from control mice (Ton et al., 2021). One significant difference in cobalt metal-induced bronchioloalveolar carcinomas compared with spontaneous bronchioloalveolar carcinomas or normal lung tissue was the identification of Kras as a primary regulator of downstream pathway activation, consistent with *Kras* mutations being present in 67% of cobalt metal-induced bronchioloalveolar carcinomas in mice compared with 27% of spontaneous lung tumours (Hong et al., 2015; Ton et al., 2021). [The Working Group noted that Kras mutations stimulate activation of downstream effectors via numerous pathways, including the Raf-MEK-MAPK signalling cascade.] Canonical pathways (as assessed by Ingenuity Pathway Analysis) uniquely altered in cobalt metal-induced, but not spontaneous, lung tumours included those related to MAPK signalling: integrin-linked kinase (ILK), p21-activated kinase (PAK), phosphoinositide 3-kinase/Akt (PI3K/AKT), ERBB, melatonin, and IL-8. Interestingly, amphiregulin (AREG) and epiregulin (EREG), ERBB family receptor ligands capable of stimulating both MAPK and PI3K/AKT pathways, were included among the most highly induced genes in cobalt metal-induced bronchioloalveolar carcinomas. Other pathways were overrepresented in both tumour types, including Rho family GTPases, signal transducer and activator of transcription 3 (STAT3), B-cell receptor, retinoic acid receptor, and xenobiotic metabolism. When expression profiles of cobalt metal-induced mouse bronchioloalveolar carcinomas were compared with the published transcriptomic data sets from human stage I lung adenocarcinomas, the most concordant canonical signalling pathways included Nop56p-associated pre-rRNA complex, 60S ribosomal subunit, dilated cardiomyopathy, focal adhesion, and hypertrophic cardiomyopathy (Ton et al., 2021).

(ii) Non-human mammalian cells in vitro

Cobalt(II) salts induced a variety of changes in gene expression when differentiated rat PC-12 adrenal pheochromocytoma cells were exposed to cobalt(II) acetate concentrations associated with 20% cytotoxicity (at < 20 μM), primarily involving an oxidative stress response with a marked increase in the HIF-1α downstream target haem oxygenase 1 (HMOX1) (Adams et al., 2015). When exposed to cobalt(II) chloride at concentrations associated with similar low cytotoxicity, changes in RNA and protein expression in the rat liver-derived cell lines H4-II-E-C3 and MH1C1 were generally consistent with those observed in human cell lines and in rodent studies in vivo, with modulation of pathways associated with responses to NRF2-mediated oxidative stress, acute-phase stress, and hypoxia-like stress.

Differential expression of genes downstream of HIF-1α signalling was reported and associated with changes in carbohydrate metabolism and other energy metabolism-related pathways (Permenter et al., 2013). Modulation of extracellular proteins and/or transcripts was also assessed, and while there were some thematic similarities with the "secretome" of human bronchial epithelial cells reported by Malard et al. (2012), no identical matches were described in rat liver cells (Permenter et al., 2013). Cobalt(II) salts were demonstrated to induce a transcriptomic response highly similar to physiological hypoxia in a HIF-1α-dependent manner in MEFs (wildtype and HIF-1α$^{-/-}$) exposed to 100 μM cobalt(II) chloride (Vengellur et al., 2003), while in BALB/3T3 fibroblasts exposed to cobalt(II) chloride at 1 μM, cobalt metal, or cobalt metal NPs (mean aggregate diameter, 450 nm or 500 nm, respectively), cobalt metal NPs induced the largest magnitude of differential RNA expression, involving the cell stress response and repair pathways (Papis et al., 2007). In another murine fibroblast cell line (PW), HIF-1α was induced at non-cytotoxic concentrations of cobalt metal NPs as low as 19 μM (mean diameter, 20 nm; hydrated mean diameter, 260 nm), while growth arrest and DNA damage inducible α (GADD45α) mRNA and protein expression increased at ≥ 75 μM in a HIF-1α-dependent manner (Feng et al., 2015).

4.2.12 Evaluation of high-throughput in vitro toxicity screening data

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 131 was informed by data from high-throughput screening assays generated by the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Cobalt(II) sulfate heptahydrate was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2022a).

The ToxCast/Tox21 high-throughput screening results are presented based on the assays that have been mapped to the key characteristics (Reisfeld et al., 2022). The detailed results are available in supplementary information for this volume (Annex 4, Supplementary material for Section 4, Evaluation of high-throughput in vitro toxicity screening data, available from: https://publications.iarc.fr/618). Here, for brevity, assays for which there is a positive "hit call" are referred to as "active" assays. A summary of these results is given below as the number of active assays (without any caution flags)/total number of key characteristic-related assays for the chemical.

Cobalt(II) sulfate heptahydrate was active in two assays mapped for key characteristic 5, "induces oxidative stress". These results include the significantly increased activity of HIF-1α in the cervical cell line ME-180 at AC_{50} of 31.4 μM, and increased activity of nuclear factor erythroid 2-related factor 2 (NRF2) in the liver cell line HepG2 at AC_{50} of 36.7 μM. In addition, cobalt(II) sulfate heptahydrate was active in one assay mapped for key characteristic 8, "modulates receptor-mediated effects". The result showed increased activity of nuclear receptor subfamily 3, group C, member 1 (*NR3C1*, glucocorticoid receptor) in the cervical adenocarcinoma cell line HeLa at AC_{50} of 23.28 μM. [The Working Group noted that the purity of the compound evaluated could not be determined because the quality control grade is listed as "unknown/inconclusive" (NCATS, 2022).]

5. Summary of Data Reported

5.1 Exposure characterization

The agents evaluated in the present monograph include: metallic cobalt (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, and the relatively insoluble compounds cobalt(II) or cobalt(II,III) oxide and cobalt(II) sulfide. Cobalt is ubiquitous in the environment, generally occurring at low levels in rocks, soil and sediments, groundwater and surface water, and air as oxides, sulfides, arsenides, and sulfoarsenides. Anthropogenic activities such as mining, smelting and other related industrial processes, coal combustion, and vehicular traffic have resulted in elevated levels of cobalt and cobalt compounds in the environment. Cobalt is produced mainly as a by-product of the mining and processing of ores of other metals. The global production of cobalt from mines and refineries has increased steadily over the past two decades. Cobalt is used in many industries, including in the manufacture of cutting and grinding tools, in pigments and paints, coloured glass, medical implants, batteries, and electroplating. Its use has increased at an annual rate of approximately 5% since 2013, particularly driven by the production of lithium-ion batteries. Occupational exposure to cobalt is expected to occur predominantly during the refining of cobalt, in the production of cobalt metals and cobalt compounds, during the use of diamond–cobalt tools, during the production of dental materials, in plate painting with cobalt pigments, and during the manufacture of nickel–hydrogen batteries. The main route of occupational exposure to cobalt is expected to be the respiratory tract; however, skin exposure and inadvertent ingestion may also occur. Workers may be exposed to various cobalt compounds and cobalt metal powders together with other agents. For the general population, food is usually the primary source of cobalt exposure; exposure may also occur via medical implants. Blood, serum, and urinary concentrations of cobalt are commonly used as indicators of exposure. Cobalt levels in other biological matrices such as nails or exhaled breath condensate have also been used to estimate human exposure. Occupational exposure to cobalt is regulated in many countries, particularly the inhalable dust fraction in workplace air; monitoring via analysis of cobalt in biological matrices such as blood and urine has also been recommended by several regulatory agencies. Some environmental guidelines exist for cobalt in natural water sources, foodstuffs, and dietary supplements.

5.2 Cancer in humans

Several studies on cobalt exposure in humans were available. Two high-quality occupational cohort studies in the hard-metal industry were considered potentially informative and found positive associations with lung cancer. However, in these studies it was difficult to separate a cobalt-specific effect from the effect of co-exposure to WC-Co or other lung carcinogens present at the work sites. Five studies in other industries did not show a consistent association between cobalt exposure and risk of lung cancer. Several studies in the general population considered cobalt exposure in relation to many other cancer sites, including breast, and none of the studies found strong or consistent evidence for a positive association. Most of the studies in the general population were limited by the use of one-time exposure assessments, which may or may not have reflected biologically important windows of exposure and temporal variability. The studies in the general population also had a relatively small range of exposures to evaluate exposure–response relations. Overall, the available studies did not permit a conclusion to be drawn about the presence of a causal association between cobalt exposure and lung cancer or other cancers in humans. No informative studies were found that permitted the separation of the

effects of soluble cobalt(II) salts, the insoluble compounds cobalt(II) or cobalt(II,III) oxide, cobalt(II) sulfide, or other forms of cobalt from those of cobalt metal.

5.3 Cancer in experimental animals

5.3.1 Cobalt metal

Treatment with cobalt metal caused an increase in the incidence of tumours in both sexes of two species in well-conducted studies that complied with Good Laboratory Practice (GLP).

Cobalt metal microparticles administered by inhalation (whole-body exposure) in one well-conducted GLP study in male and female $B6C3F_1$/N mice caused an increase in the incidence of bronchioloalveolar carcinoma in males and females.

Cobalt metal microparticles were administered by inhalation (whole-body exposure) in one well-conducted GLP study in male and female F344/NTac rats. Cobalt metal microparticles caused an increase in the incidence of bronchioloalveolar carcinoma and malignant pheochromocytoma of the adrenal medulla in males and females; of pancreatic islet adenoma or carcinoma (combined), and a positive trend in the incidence of pancreatic islet carcinoma and renal tubule adenoma or carcinoma (combined) in males; and of mononuclear cell leukaemia in females.

Cobalt metal microparticles administered by intramuscular injection in one study in female Hooded rats caused an increase in the incidence of rhabdomyofibrosarcoma, fibrosarcoma or sarcoma (not otherwise specified, NOS) (combined). Cobalt metal microparticles administered by intramuscular injection in one study in male and female Hooded rats caused an increase in the incidence of rhabdomyofibrosarcoma or sarcoma (NOS) (combined) in males, and of fibrosarcoma and rhabdomyosarcoma or rhabdomyofibrosarcoma (combined) in females.

Cobalt metal pellets administered by intramuscular implantation in one study in male Sprague-Dawley rats caused an increase in the incidence of rhabdomyosarcoma or spindle cell tumours (NOS) (combined) of the limb gastrocnemius muscle. Cobalt metal nanoparticles administered by intramuscular implantation in one study in male Sprague-Dawley rats caused a high incidence of sarcomas (NOS) at the implantation site (but there was a lack of concurrent controls).

5.3.2 Soluble cobalt(II) salts

(a) Cobalt(II)sulfate

Treatment with cobalt(II)sulfate caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of two species in well-conducted studies that complied with GLP.

Cobalt(II) sulfate administered by inhalation (whole-body exposure) in one well-conducted study that complied with GLP in male and female $B6C3F_1$ mice caused an increase in the incidence of bronchioloalveolar carcinoma in males and females.

Cobalt(II) sulfate was administered by inhalation (whole-body exposure) in one well-conducted study that complied with GLP in male and female Fischer 344/NTac rats. Cobalt(II) sulfate caused an increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined) in males; and an increase in the incidence of bronchioloalveolar carcinoma, and benign, complex, or malignant pheochromocytoma (combined) of the adrenal medulla in females.

(b) Cobalt(II) chloride

Treatment with cobalt(II) chloride caused an increase in the incidence of malignant neoplasms in a single experiment in one species (the rat).

Cobalt(II) chloride administered by subcutaneous injection in one study in male Wistar rats (including two experiments) caused an increase in the incidence of fibrosarcoma of the subcutaneous tissue in both experiments.

5.3.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

(a) Cobalt(II) oxide

Treatment with cobalt(II) oxide caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in more than one study in one species (the rat), carried out at different times or in different laboratories and/or under different protocols.

Cobalt(II) oxide microparticles administered by intratracheal instillation in one study in male and female Sprague-Dawley rats caused an increase in the incidence of bronchioloalveolar adenoma, bronchioloalveolar adenocarcinoma, or adenocarcinoma of the lung (combined) in males.

Cobalt(II) oxide microparticles administered by subcutaneous injection in one study in male Sprague-Dawley rats caused an increase in the incidence of malignant histiocytoma or sarcoma (NOS) (combined) at the injection site.

Cobalt(II) oxide microparticles administered by intramuscular injection in one study in male and female Wistar rats caused an increase in the incidence of rhabdomyosarcoma at the injection site in males and females combined. Cobalt(II) oxide microparticles were administered by intramuscular injection in one study in male and female Wistar rats. Cobalt(II) oxide microparticles caused a high incidence of sarcomas (mostly rhabdomyofibrosarcoma) in males and females combined (but there was a lack of concurrent controls).

Cobalt(II) oxide microparticles administered by intraperitoneal injection in one study in male and female Sprague-Dawley rats caused an increase in the incidence of malignant histiocytoma, sarcoma (NOS), or malignant mesothelioma (combined), and of malignant histiocytoma, both at the injection site, in males and females combined.

(b) Cobalt(II) sulfide

Treatment with cobalt(II) sulfide caused a high incidence of malignant neoplasms in a single experiment in one species (the rat).

Cobalt(II) sulfide microparticles were administered by intramuscular injection in one study in male and female Wistar rats. Cobalt(II) sulfide microparticles caused a high incidence of sarcomas (mostly rhabdomyofibrosarcoma) in males and females combined (but there was a lack of concurrent controls).

(c) Cobalt(II,III) oxide

Treatment with cobalt(II,III) oxide did not cause a carcinogenic effect in a single study.

Cobalt(II,III) oxide microparticles administered by intratracheal instillation in male and female hamsters did not cause a significant increase in the incidence of tumours in males and females combined.

5.3.4 Other cobalt(II) compounds

No data on other cobalt(II) compounds were available to the Working Group.

5.4 Mechanistic evidence

The Working Group considered multiple forms of cobalt in evaluating the mechanistic evidence of carcinogenicity. Appropriate particles of all sizes were grouped together on the basis of similar chemical forms of cobalt, since observed differences regarding the effects of particles of micron size and smaller on mechanistic end-points were deemed minor on the basis of evidence available to the Working Group.

Data were available on the absorption and distribution of cobalt in humans and experimental systems. Inhaled cobalt can be deposited and absorbed in the respiratory tract or cleared by mucociliary clearance and swallowed. Deposition, absorption, and clearance of cobalt in the respiratory tract is influenced by particle size and surface area, and solubility of the compound. More soluble forms of cobalt have a higher rate of absorption from the lungs and gastrointestinal tract. Dermal absorption of cobalt from intact skin is low (less than 1%) but is higher through abraded skin. Cobalt is primarily distributed to the serum, whole blood, liver, kidneys, heart, and spleen, with lower amounts reported in the skeleton, hair, lymphatic circulation, pancreas, and other organs. Cobalt crosses the placenta and appears in fetal blood. Cobalt is not subject to metabolism by enzymatic pathways and is excreted primarily in the urine and faeces, with elimination half-lives of several hours to 1 week in humans.

Several studies in humans with either occupational or environmental exposure to cobalt were available to the Working Group. These studies provided some evidence of cobalt-induced effects on key characteristics of carcinogens for genotoxicity, induction of oxidative stress, and modulation of receptor-mediated effects. However, these studies did not identify the form of cobalt to which exposure occurred, therefore they have not been included in the summary. One occupational study with possible mixed exposure to cobalt metal and cobalt oxides was retained, and appears in the summary of cobalt metal, cobalt(II) oxide, and cobalt(II,III) oxide.

5.4.1 Cobalt metal

There is consistent and coherent evidence that cobalt metal exhibits key characteristics of carcinogens.

Cobalt metal is genotoxic. In exposed humans, evidence for genotoxicity is suggestive, as positive correlations were observed between urinary cobalt concentrations and DNA single-strand breaks and the frequency of micronuclei in blood binucleated cells in one occupational study. Consistent and coherent evidence for genotoxicity comes from multiple studies in human primary cells showing that cobalt metal induces DNA strand breaks and increases the frequency of micronucleus formation. Consistent and coherent evidence for genotoxicity also comes from experimental systems, including DNA and chromosomal damage in multiple studies in human cell lines, and in non-human mammalian experimental systems in vivo and in vitro.

Cobalt metal induces oxidative stress. No data were available in humans identified as exposed specifically to cobalt metal. There is consistent and coherent evidence of increased ROS levels or other oxidative stress biomarkers in multiple studies in human primary cells. One study showed oxidative DNA damage. Additional consistent and coherent evidence comes from in vivo rodent studies, in vitro studies showing oxidative stress and oxidative DNA damage in human cell lines, and several in vitro studies in non-human mammalian cell lines.

Cobalt metal induces chronic inflammation. No data were available in humans identified as exposed specifically to cobalt metal. There is consistent and coherent evidence of chronic inflammation from chronic rodent inhalation studies that demonstrated increased inflammation in the nose and larynx, and chronic active inflammation (and increased histiocytic infiltrates) in the lung. Shorter-term inhalation and implant studies in rodents provide further evidence of chronic inflammation after cobalt metal exposure.

Cobalt metal alters cell proliferation, cell death, or nutrient supply. No data were available in exposed humans. Cobalt metal induced progressive, proliferative lesions, including hyperplasia and metaplasia, in a dose-responsive manner in the upper and lower respiratory tract

of rats and mice after acute to chronic inhalation exposures. There was no evidence of increased cell proliferation or cell viability, or attenuated apoptosis, in several studies in non-human mammalian cell lines.

There is suggestive evidence that cobalt metal modulates receptor-mediated effects. Exposure to cobalt metal and/or oxides was associated with decreased levels of thyroid hormone (T3) in an occupational cohort.

There is suggestive evidence that cobalt metal causes immortalization. Mixed evidence from two studies showed an increase in anchorage-independent growth in neoplastic human cell lines. Additional evidence comes from several in vitro studies in murine embryonic fibroblast cell lines showing cobalt metal-induced morphological transformation. Cobalt metal also acted as an initiator in a single two-stage (initiator–promoter) in vitro assay.

For other key characteristics of carcinogens, there is a paucity of available data.

5.4.2 Soluble cobalt(II) salts

There is consistent and coherent evidence that soluble cobalt(II) salts exhibit key characteristics of carcinogens.

Soluble cobalt(II) salts are genotoxic. No data were available in exposed humans. There was consistent and coherent evidence based on multiple studies using human primary cells showing that cobalt(II) salts induce chromosomal aberrations, DNA strand breaks, and, in one study, sister-chromatid exchange. Consistent and coherent evidence for genotoxicity is also available from experimental systems, including studies in human cell lines. Several rodent studies using intraperitoneal administration of cobalt(II) salts consistently reported genotoxic effects. Oral exposure of rodents to cobalt salts, and studies using in vitro non-human mammalian and non-mammalian systems, provide mixed results.

Soluble cobalt(II) salts induce oxidative stress. No data were available in exposed humans. There is consistent and coherent evidence from numerous studies using immortalized human cell lines in vitro, rats and mice in vivo, and non-human mammalian cell lines in vitro of increased levels of oxidative stress biomarkers, including oxidative DNA damage. There is suggestive evidence in a few studies in human primary cells that yielded mixed results.

Soluble cobalt(II) salts induce chronic inflammation. No data were available in exposed humans. Consistent and coherent evidence of increased nasal and laryngeal inflammation, as well as chronic active inflammation (and histiocytic infiltrates) in the lung, is provided by subchronic and chronic inhalation studies in rodents. Increased levels of macrophages in bronchoalveolar lavage and markers of interstitial or intra-alveolar lung inflammation were also reported after subchronic inhalation exposures in rabbits, and acute exposures in mice and rats. In dermal sensitization studies, leukocyte accumulation was induced in both mice and guinea-pigs.

Soluble cobalt(II) salts are immunosuppressive. No data were available in exposed humans. There is consistent and coherent evidence of immunosuppression based on studies in experimental systems and suggestive evidence in human primary cells. Decreased thymus weight and antibody-producing cells in response to sheep erythrocyte stimulation were reported after oral exposure to cobalt salts in a single study in rats, while increased production of IgM but not IgG antibodies was reported in a single study in mice. Decreased toll-like receptor 4 expression was reported in a single study in primary mouse macrophages. In addition, cobalt(II) salts induced decreased lymphocyte viability, activation, proliferation, and/or cytokine expression in human primary cells in several studies, and in a single study in primary mouse splenocytes. Decreased expression of human leukocyte

antigen HLA class II molecules was reported in one study in human primary cells.

Soluble cobalt(II) salts alter cell proliferation, cell death, or nutrient supply. No data were available in exposed humans. Consistent and coherent evidence of increased vascular endothelial growth factor expression was reported in numerous studies in human primary cells, immortalized human cell lines, and rodent studies in vivo. Increased formation of capillary-like tube structures was reported in one study in primary human umbilical vein endothelial cells and two studies in mouse endothelial cell cultures. Stimulated cell proliferation, or increased cell viability, was seen in several studies in human primary cells or human cell lines, but the evidence for attenuated cell death was mixed. Cobalt(II) salts also induced progressive, proliferative lesions, including hyperplasia and metaplasia, in a dose-responsive manner in the upper and lower respiratory tract of rabbits, rats, and mice after acute to chronic inhalation exposures. Apoptosis was decreased in two in vivo rat studies.

There is suggestive evidence that soluble cobalt(II) salts at non-cytotoxic concentrations alter DNA repair, based on one study in human primary cells and on studies in experimental systems using human cell lines and acellular systems.

There is suggestive evidence that soluble cobalt(II) salts induce epigenetic alterations, based on a single study in human umbilical vein endothelial cells. Suggestive evidence is also available from several studies in human cell lines showing changes in histone acetylation, mRNA methylation, and altered expression of miRNAs after cobalt(II) salt treatment. Altered methylation of RNA was reported after repeated intraperitoneal administration in one mouse study. Several studies in non-human mammalian cells in vitro also revealed consistent evidence of decreased histone acetylation after exposure to cobalt(II) salts.

There is suggestive evidence that soluble cobalt(II) salts modulate receptor-mediated effects based on measurements of protein levels of (largely) individual receptors in human cell lines. Consistent with the observations in human cell lines, decreased peroxisome proliferator-activated receptor PPAR mRNA levels were reported in two in vivo rat studies.

For other key characteristics of carcinogens, there is a paucity of available data.

5.4.3 Cobalt(II) oxide

For cobalt(II) oxide, the mechanistic evidence is suggestive. While consistent and coherent evidence was reported for oxidative stress in experimental systems (described below), this was not supported by such evidence for other key characteristics of carcinogens.

There is suggestive evidence that cobalt(II) oxide is genotoxic based on one study in human primary cells and in multiple studies using human cell lines. In these studies, cobalt(II) oxide induced chromosome aberrations and DNA strand breaks. One in vivo study in rats showed negative results for chromosomal aberrations. Findings in non-human mammalian cells in vitro and in non-mammalian systems typically yielded mixed results in a small number of studies.

There is consistent and coherent evidence from two experimental systems that cobalt(II) oxide induces oxidative stress, including one in human cell lines and one in vivo study in rodents. There is suggestive evidence that cobalt(II) oxide induces oxidative stress in a single study in human primary cells.

There is suggestive evidence that cobalt(II) oxide modulates receptor-mediated effects. Exposure to cobalt metal and/or oxides was associated with decreased levels of thyroid hormone (T3) in an occupational cohort.

For other key characteristics of carcinogens, there is a paucity of available data.

5.4.4 Cobalt(II,III) oxide

For cobalt(II,III) oxide, the mechanistic evidence is suggestive. While consistent and coherent evidence was reported for oxidative stress in experimental systems (described below), this was not supported by such evidence for other key characteristics of carcinogens.

There is suggestive evidence that cobalt(II,III) oxide is genotoxic, based upon induction of chromosome aberrations and DNA strand breaks in one study in human primary cells. There is suggestive evidence in human cell lines for DNA strand breaks and micronuclei formation. One oral study in rodents and one study in non-human mammalian cells reported negative findings.

There is consistent and coherent evidence that cobalt(II,III) oxide induces oxidative stress based on increased levels of reactive oxygen species in two studies in human primary cells, and increased measurements of oxidative stress, including oxidative DNA damage, in multiple studies in human cell lines. Similar effects were also reported in one in vivo rodent study.

There is suggestive evidence that cobalt(II,III) oxide modulates receptor-mediated effects. Exposure to cobalt metal and/or oxides was associated with decreased levels of thyroid hormone (T3) in an occupational cohort.

There are either no data or sparse data for cobalt(II,III) oxide for all other key characteristics.

5.4.5 Cobalt sulfide

There are either no data or sparse data for all key characteristics.

5.4.6 Other cobalt(II) compounds

There are either no data or sparse data for all key characteristics.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of cobalt metal (without tungsten carbide or other metal alloys) and of soluble and insoluble cobalt(II) and cobalt(II,III) compounds.

6.2 Cancer in experimental animals

6.2.1 Cobalt metal

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt metal.

6.2.2 Soluble cobalt(II) salts

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt(II) sulfate.

There is *limited evidence* in experimental animals for the carcinogenicity of cobalt(II) chloride.

6.2.3 Insoluble cobalt(II) oxide, cobalt(II,III) oxide, and cobalt(II) sulfide

There is *sufficient evidence* in experimental animals for the carcinogenicity of cobalt(II) oxide.

There is *inadequate evidence* in experimental animals regarding the carcinogenicity of cobalt(II,III) oxide.

There is *limited evidence* in experimental animals for the carcinogenicity of cobalt(II) sulfide.

6.2.4 Other cobalt(II) compounds

There is *inadequate evidence* in experimental animals regarding the carcinogenicity of other cobalt(II) compounds.

6.3 Mechanistic evidence

There is *strong evidence* in human primary cells and in experimental systems that cobalt metal (including particles of all sizes) exhibits key characteristics of carcinogens.

There is *strong evidence* in human primary cells and in experimental systems that soluble cobalt(II) salts exhibit key characteristics of carcinogens.

For cobalt(II) and cobalt(II,III) oxides (including particles of all sizes), there is *limited* mechanistic evidence.

For cobalt(II) sulfide, there is *inadequate* mechanistic evidence.

For other cobalt(II) compounds, there is *inadequate* mechanistic evidence.

6.4 Overall evaluation

Cobalt metal (without tungsten carbide or other metal alloys) is *probably carcinogenic to humans (Group 2A)*.

Soluble cobalt(II) salts are *probably carcinogenic to humans (Group 2A)*.

Cobalt(II) oxide is *possibly carcinogenic to humans (Group 2B)*.

Cobalt(II,III) oxide is *not classifiable as to its carcinogenicity to humans* (*Group 3*).

Cobalt(II) sulfide is *not classifiable as to its carcinogenicity to humans* (*Group 3*).

Other cobalt(II) compounds are *not classifiable as to their carcinogenicity to humans* (*Group 3*).

6.5 Rationale

The Group 2A evaluation for cobalt metal (without tungsten carbide or other metal alloys) is based on *sufficient evidence* for cancer in experimental animals and *strong* mechanistic evidence in human primary cells. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of two species in well-conducted studies that complied with Good Laboratory Practice. There is *strong evidence* in human primary cells and in experimental systems that cobalt metal (including particles of all sizes) is genotoxic and induces oxidative stress. There is *strong evidence* in experimental systems that cobalt metal (including particles of all sizes) induces chronic inflammation and alters cell proliferation, cell death, or nutrient supply. There is *inadequate evidence* regarding cancer in humans.

The Group 2A evaluation for soluble cobalt(II) salts is based on *sufficient evidence* for cancer in experimental animals and *strong* mechanistic evidence in human primary cells. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms caused by soluble cobalt(II) sulfate in both sexes of two species in well-conducted studies that complied with Good Laboratory Practice, and on an increase in the incidence of malignant neoplasms caused by soluble cobalt(II) chloride in a single experiment in one species (the rat). There is *strong evidence* in human primary cells and in experimental systems that soluble cobalt(II) salts are genotoxic, and alter cell proliferation, cell death, or nutrient supply. There is *strong evidence* in experimental systems that soluble cobalt(II) salts induce oxidative stress and chronic inflammation, and that they are immunosuppressive. There is *inadequate evidence* regarding cancer in humans.

The Group 2B evaluation for cobalt(II) oxide is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in more than one study in one species (the rat), carried out at

different times or in different laboratories and/or under different protocols. There is *limited* mechanistic evidence in exposed humans, in human primary cells, and in experimental systems for insoluble cobalt(II) oxide. While there is consistent and coherent evidence that insoluble cobalt(II) oxide (including particles of all sizes) induces oxidative stress in experimental systems, there is not for other key characteristics of carcinogens. There is *inadequate evidence* regarding cancer in humans.

The Group 3 evaluation for cobalt(II,III) oxide is based on *inadequate evidence* regarding cancer in humans and in experimental animals and on *limited* mechanistic evidence. There is *limited* mechanistic evidence in exposed humans, in human primary cells, and in experimental systems for cobalt(II,III) oxide. While there is consistent and coherent evidence that cobalt(II,III) oxide (including particles of all sizes) induces oxidative stress, there is not for other key characteristics of carcinogens.

The Group 3 evaluation for cobalt(II) sulfide is based on *inadequate evidence* regarding cancer in humans, on *limited evidence* for cancer in experimental animals, and on *inadequate* mechanistic evidence. The *limited evidence* for cancer in experimental animals is based on a high incidence of malignant neoplasms observed in a single experiment in one species (the rat).

The Group 3 evaluation for other cobalt(II) compounds is based on *inadequate evidence* regarding cancer in humans and in experimental animals, and on *inadequate* mechanistic evidence. No studies were available in experimental animals, and the few mechanistic studies were largely negative.

All classifications above should be presumed to apply to all size classes for the listed agents.

The evidence in humans is *inadequate* regarding the carcinogenicity of cobalt metal (without tungsten carbide or other metal alloys) and non-metallic forms of cobalt. Among the available human cancer studies, the studies of exposures to cobalt in the hard-metal industry did not permit separation of cobalt's effects from those of the cobalt and tungsten carbide composite, or other known or suspected lung carcinogens, in examining lung cancer risk, and other occupational studies were either confounded by other known lung carcinogens or did not show associations of cobalt with lung cancer. None of the four available studies in workers or the general population found convincing associations of cobalt with breast cancer risk. Five studies examining other cancer sites either sporadically found positive associations or were considered of low quality or uninformative. No informative studies were found that permitted the separation of the effects of soluble cobalt(II) salts, the insoluble compounds cobalt(II or II,III) oxide, cobalt(II) sulfide, or other forms of cobalt from those of cobalt metal.

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