ANNEX 1

in support of the Committee for Risk Assessment (RAC) for evaluation of limit values for cobalt and inorganic cobalt compounds at the workplace

ECHA/RAC/OEL-O-0000007197-68-01/F

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Table of Contents

Tables

List of abbreviations

Abbreviation	Definition
AAF	Artificial alveolar fluid
ADME	Absorption, distribution, metabolism and excretion
AGS	Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances)
AIF	Artificial interstitial fluid
ALF	Artificial lysosomal fluid
ANSES	Agence Nationale de SEcurité Sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health & Safety)
ATSDR	Agency for Toxic Substances and Disease Registry (USA)
BAL	Biological Action Levels (for occupational exposure)
BAR	Biologische Arbeitsstoff-Referenzwerte (Biological reference value; corresponds to the background level present concurrently, in a reference population of persons of working age who are not occupationally exposed to this substance).
BGV	Biological Guidance Value
BLV	Biological Limit Value
BLW	Biologischer Leit-wert (Biological guidance value at the workplace)
BME	Biomarker of exposure
CAREX	CARcinogen EXposure database
CBD	Chronic beryllium disease
CEA	Carcinoembryonic antigen
CI	Confidence Interval
CKE	Cystic keratinising epithelioma
CLP	Regulation EC No 1272/2008 on the Classification, Labelling and Packaging of substances and mixtures (CLP Regulation)
CMD / CMRD	Carcinogens and Mutagens Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. The amendment of the CMD, Directive 2022/431/EU also brought reprotoxic substances within the scope of the directive, changing the original title on the protection of workers from the risks related to exposure to carcinogens or mutagens at work to the protection of workers from the risks related to exposure to carcinogens, mutagens or reprotoxic substances at work (CMRD).
GTL	Gas to liquid
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
DLCO	Diffusing capacity for carbon monoxide
EC	European Commission
ECG	Electrocardiogram
ECHA	European Chemicals Agency
EKA	Expositionsäquivalente für krebserzeugende Arbeitsstoffe (Exposure equivalents for carcinogenic substances; an exposure equivalent, correlation between external and internal exposure.)
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EN 481	Standard: Workplace atmospheres. Size fraction definitions for measurement of airborne particles

Abbreviation	Definition
EN 482	Standard: "Workplace exposure. General requirements for the performance of procedures for the measurement of chemical agents"
EPA	Environmental Protection Agency
ERR	Exposure-risk relationship
ETAAS	Electrothermal atomic absorption spectrometry
EU	European Union
FAAS	Flame Atomic Absorption Spectrometry
FEV_1	Forced expiratory volume in one second
FEV%	Percentage of FEV_1 of the forced vital capacity (FVC)
FIOH	Finnish Institute of Occupational Health
FVC	Forced vital capacity
GC-APLI-MS	Gas chromatography (GC) coupled to atmospheric pressure laser ionization-mass spectrometry (APLI-MS)
GC-MS	Gas chromatography-mass spectrometry
GC-NICI-MS/MS	Gas chromatography/negative-ion chemical-ionization tandem mass spectrometry
GESTIS Substance Database	GEfahrSToffInformationsSystem (German information system for the safe handling of hazardous substances and other chemical substances at work) Substance Database
GLP	Good Laboratory Practice
GM	Geometric mean
GSD	Geometric standard deviation
Hb	Haemoglobin concentration
HBC-OCRV	Health-based calculated occupational cancer risk value
HIF-1α	Hypoxia-Inducible Factor-1 alpha
HP	Hypersensitivity pneumonitis
HPLC-FLD	High-performance liquid chromatography with fluorescence detection
Htc	Haematocrit
IARC	International Agency for Research on Cancer (World Health Organization)
ICP-AES	Inductively coupled argon plasma- atomic emission spectroscopy
ICP/MS	Inductively coupled plasma/mass spectrometry
IEI	integrated exposure index
IFA	Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance)
IHC	Immunohistochemistry
ILD	Interstitial lung disease
ILO	International Labour Organization, Switzerland
IPCS	The International Programme on Chemical Safety (World Health Organization)
ISO 15202-1:2020	Standard. Workplace air – Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry – Part 1: Sampling
ISO 15202-2:2020	Standard. Workplace air – Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry – Part 2: Sample preparation
ISO 15202-3:2020	Standard. Workplace air – Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry – Part 3: Analysis

Abbreviation	Definition
IUCLID	International Uniform Chemical Information Database
JEM	Job exposure matrix
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LLNA	Local lymph node assay
LMW PAH	Low molecular weight polycyclic aromatic hydrocarbon (PAH)
LOAEC	Lowest observed adverse effect concentration
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MEF_{50} MEF_{20}	Flow rates at 50% and 25% of vital capacity
M-FISH	Multicolour fluorescence in situ hybridization
MI	Mitotic index
MMAD	Median mass aerodynamic diameter
MMF	Maximum mid-expiratory flow
MNBC	Micronucleated binucleated cells
MNCL	Mononuclear Cell Leukemia
MNMC	Micronucleated mononucleated cells
MNPCEs	Micronucleated polychromatic erythrocytes
MPPD model	Multiple Path Particle Dosimetry model, a tool that can be used to estimate particle deposition in different regions of the respiratory tract
MoA	Mode of action
MPCE	mononucleated polychromatic erythrocytes
MRLs	Maximum residue levels
MTD	Maximum tolerated dose
NDI	Nuclear division index
NIOSH	National Institute for Occupational Safety and Health (USA)
NMRD	Non-malignant respiratory diseases
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOGEL	No observed genotoxic effect level
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational exposure limit
8-OH-dG	8-hydroxydeoxyguanosine
OR	Odds ratio
OSHA	Occupational Safety and Health Administration (USA)
PAH	Polycyclic aromatic hydrocarbon(s)
PBPK model	Physiologically based pharmacokinetic model
PCEs	Polychromatic erythrocytes
PCE/NCE	Polychromatic:normochromatic erythrocytes ratio
PEF	Peak expiratory flow
PMBC	Peripheral blood mononuclear cell
PRE	Protective respiratory equipment

Abbreviation	Definition
RAC	Committee for Risk Assessment
RBC	Red blood cell
REACH	Regulation (EC) No 1907/2006 of the European Union concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
RI	Replication index
ROS	Reactive Oxygen Species
RR	Relative risk
SCE	Sister-chromatid exchange
SEAC	Committee for Socio-Economic Analysis
SIR	Standardized incidence ratio
SLA	Service Level Agreement
SMR	Standardised mortality ratio
STEL	Short term exposure limit
Finland (STM)	Finland (Sosiaali- ja terveysministeriö), Ministry of Social Affairs and Health
TPA	Tissue polypeptide antigen
TWA	Time-Weighted-Average
VC	Vital capacity
VLB	Valeur limite biologique (Biological Limit Value)
WC	Tungsten carbide
WBC	White blood cell
WHO	The World Health Organization
WRA	Work-related asthma

Literature search

This report is based on international assessments such as (AGS, 2023), ANSES (2018), ANSES (2014), ATSDR (2004), DFG (2007), RAC (2020), IARC (2006), Montelius (2005). This has been complemented by a literature search of published papers from the last ten years (date of last literature search: 04/2022). Databases used were last accessed: 04/2022.

ECHA evaluation and recommendation

Cobalt and inorganic cobalt compounds are carcinogens and an exposure-risk relationship (ERR) expressing the excess risk for lung cancer in function of air concentration is therefore derived. A mode of action suggesting a key role for chronic inflammation in cobalt-induced carcinogenicity was identified.

The ERR is calculated based on available experimental data. The tables below (**Table 1** and **Table 2**) present the outcome of the scientific evaluation to derive limit values for cobalt and inorganic cobalt compounds, based on a threshold for chronic lung inflammation, and the cancer exposure-risk relationship. Below the breakpoint of 0.0005 mg/m^3 (0.5 µg/m^3), lung inflammation-related mechanisms are likely to be no longer of concern and thus the estimated cancer risk per unit exposure is lower than at exposure levels above the breakpoint.

Table 1: Outcome of the scientific evaluation

Derived Limit Values

OEL as 8-hour TWA*:	0.001 mg Co/m^3 (1 µg Co/m^3; inhalable fraction) 0.0005 mg Co/m^3 (0.5 µg Co/m^3; respirable fraction)
STEL:	Not relevant
BLV:	Not established
BGV:	Females: 0.002 mg Co/L urine Males: 0.0007 mg Co/L urine

* The proposed OEL is based on a mode of action-based threshold for the carcinogenicity of cobalt compounds

Notations

Notations:	'skin sensitisation' and 'respiratory sensitisation'

Table 2: Lung cancer exposure-risk relationship *

Air concentration of cobalt (respirable fraction; µg/m^3)	Excess life-time lung cancer risk (cases per 100 000 exposed)
0.01	0.11
0.05	0.53
0.1	1.1
0.5	5.3

Breakpoint where the slope of the dose-response changes (0.5 µg/m^3)	
1.0	58
5.0	480
10	1010
20	2070

* Assuming exposure of 8 hours per day and 5 days per week, over a 40-year working life period

1. Chemical Agent Identification and Physico-Chemical Properties

1.1 Cobalt

Cobalt has several oxidative states, as explained in Ullmann's Encyclopaedia of Industrial Chemistry 2012:" The main oxidation states of cobalt are Co^{2+} and Co^{3+}. In acid solution and in the absence of complexing agents, Co^{2+} is the stable oxidation state, with oxidation to Co^{3+} being difficult. In addition to the two most stable oxidation states, 2+ and 3+, cobalt also forms compounds in the 1-, 0, 1+, and 4+ oxidation states".[1]

Cobalt's identification and physico-chemical properties are described in **Table 3** and

Table 4:

Table 3: Substance identification

Substance name	CAS No.	EINECS/EC - No.	Description	Molecular formula
Cobalt	7440-48-4	231-158-0	Solid, compact or particulate, metallic, odourless element	Co

Table 4: Physical and chemical properties [2]

Substance name	EC/ list number	Physical state [at 20°C and 1013 hPa]	Density [g/cm³ at 20°C]	Melting point [°C]	Water solubility [mg/L at 20°C]
Cobalt	231-158-0	solid	8.86	1 495	2.94

1.2 Inorganic cobalt compounds

The inorganic cobalt compounds considered in this report are generally those for which data are available and for which use at higher tonnages is known.

The physico-chemical properties are described in tabulated summaries in **Appendix 1**.

2. EU Harmonised Classification and Labelling - CLP (EC) 1272/2008

Annex VI of the Regulation lists eight entries for the classification of cobalt and its inorganic compounds based on EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures.

Cobalt metal and several cobalt compounds may cause cancer (Carc. 1B) and may damage fertility (Repr. 1B). Furthermore, many of them have harmonised classifications as suspected of causing genetic effects (Muta. 2), and they may cause an allergic skin

[1] Ullmann's Encyclopaedia of Industrial Chemistry 2012
[2] Values obtained from registration data published on www.echa.europa.eu

reaction and may cause allergy or asthma symptoms or breathing difficulties if inhaled (Skin Sens. 1, Resp. Sens, 1).

Cobalt and key compounds that are registered are listed in the table below (**Table 5**). Full details of all compounds are given in **Appendix 1**.

Table 5: EU classification: Summary of cobalt and inorganic cobalt compounds

Index No	International chemical ID	EC No	CAS No	Annex VI of CLP hazard class and category	Hazard statement code
027-001-00-9	cobalt	231-158-0	7440-48-4	Carc. 1B Muta. 2 Repr. 1B Resp. Sens. 1 Skin Sens. 1 Aquatic Chronic 4	H350 H341 H360F H334 H317 H413
027-002-00-4	cobalt oxide	215-154-6	1307-96-6	Acute Tox. 4 * Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H400 H410
027-003-00-X	cobalt sulphide	215-273-3	1317-42-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410
027-004-00-5	cobalt dichloride	231-589-4	7646-79-9	Carc. 1B Muta. 2 Repr. 1B Acute Tox. 4 * Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H341 H360F *** H302 H334 H317 H400 H410
027-005-00-0	cobalt sulphate	233-334-2	10124-43-3	Carc. 1B Muta. 2 Repr. 1B Acute Tox. 4 * Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H341 H360F *** H302 H334 H317 H400 H410
027-009-00-2	cobalt dinitrate	233-402-1	10141-05-6	Carc. 1B Muta. 2 Repr. 1B Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H341 H360F *** H334 H317 H400 H410
027-010-00-8	cobalt carbonate	208-169-4	513-79-1	Carc. 1B Muta. 2 Repr. 1B Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H341 H360F *** H334 H317 H400 H410
028-058-00-2	cobalt lithium nickel oxide	442-750-5	-	Carc. 1A Acute Tox. 2 * STOT RE 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H350i H330 H372 ** H317 H400 H410

3. Chemical Agent and Scope of Legislation - Regulated uses of cobalt and inorganic cobalt compounds in the EU

3.1 Directive 98/24/EC and Directive 2004/37/EC

There is currently no binding or indicative occupational exposure limit value for cobalt or inorganic cobalt compounds under Directives 98/24/EC or 2004/37/EC.

3.2 REACH Registrations

There are 42 substances considered registered under REACH[3] for cobalt and inorganic cobalt compounds. For 34 of these substances, tonnage information is available as part of a REACH registration. These include 29 substances with full registrations, and five substances only registered as an intermediate. Information on the registrations is available on the ECHA website[3]. Chemical Safety Reports are only available for those with a full registration.

Table 6 provides an overview of the type of registrations with tonnage for the 18 registered cobalt substances in the highest quantities as used later in this proposal. The total tonnage reported for these 18 substances is representing about 99% of the overall tonnage reported for cobalt compounds within registrations; full details are in **Appendix 2**.

Table 6: REACH Registrations for the 18 cobalt substances in the highest quantities

Substance name	EC/List number	Intermediate registration	full registration
cobalt carbonate	208-169-4	<10 (<5 reg)	1000-10 000 (8 reg)
cobalt oxide	215-154-6	<10 (<5 reg)	1000-10 000 (26 reg)
tricobalt tetraoxide	215-157-2	10-1000 (<5 reg)	1000-10 000 (29 reg)
cobalt sulphide	215-273-3	10-1000 (12 reg)	1000-10 000 (31 reg)
cobalt	231-158-0		10 000-100 000 (96 reg)
cobalt dichloride	231-589-4	<10 (<5 reg)	1000-10 000 (6 reg)
cobalt sulphate	233-334-2	1000-10 000 (<5 reg)	>100 000 (15 reg)
cobalt dinitrate	233-402-1		1000-10 000 (10 reg)
cobalt hydroxide oxide	234-614-7	10-1000 (<5 reg)	1000-10 000 (<5 reg)

[3] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396 of 30 December 2006, p. 1; corrected by OJ L 136, 29.5.2007, p. 3)

Substance name	EC/List number	Intermediate registration	full registration
cobalt dihydroxide	244-166-4	(<5 reg)	>100 000 (19 reg)
cobalt zinc aluminate blue spinel	269-049-5		1000-10 000 (14 reg)
iron cobalt chromite black spinel	269-060-5		1000-10 000 (21 reg)
olivine, cobalt silicate blue	269-093-5		1000-10 000 (15 reg)
leach residues, zinc ore-calcine, zinc cobalt	273-769-5	1000-10 000 (6 reg)	
cobalt aluminate blue spinel	310-193-6		1000-10 000 (27 reg)
cobalt lithium manganese nickel oxide	480-390-0		10 000-100 000 (16 reg)
lithium nickel cobalt aluminium oxide	700-042-6		1000-10 000 (7 reg)
reaction mass of cobalt sulphide and nickel sulphide and trinickel disulphide	910-663-6	1000-10 000 (<5 reg)	

In addition to the above registered cobalt and its inorganic compounds, ECHA has received registrations for substances in which registrants have indicated the presence of cobalt or its inorganic compounds in concentration ranges of around 10-30% (w/w).

These substances are listed below in **Table 7**.

Table 7: Registered substances containing a significant amount of cobalt or its inorganic compounds

Substance name	EC/List number	Intermediate registration	full registration
matte, precious metal	308-506-6		1000-10 000 (<5 reg)
cement copper	266-964-1	10 000-100 000 (12 reg)	10 000-100 000 (6 reg)
leach residues, cadmium cake	293-309-7	10 000-100 000 (8 reg)	
slags, precious metal refining	308-515-5	1000-10 000 (<5 reg)	1000-10 000 (6 reg)
slimes and sludges, precious metal refining	308-516-0	1000-10 000 (<5 reg)	10 000-100 000 (5 reg)
waste solids, precious metal refining	308-526-5		1000-10 000 (5 reg)

3.3 Authorised uses under Annex XIV of REACH

Five cobalt compounds have been recommended for inclusion in Annex XIV in the Third recommendation of 20 December 2011: cobalt(II) sulphate, cobalt dichloride, cobalt(II) dinitrate, cobalt(II) carbonate and cobalt(II) diacetate. Details of the recommendations are available on ECHA's website[4].

3.4 Restricted uses under Annex XVII of REACH

The group entries 28, 29 and 30 of Annex XVII concern restrictions of substances which are, respectively, classified as carcinogen, germ cell mutagen or reproductive toxicant category 1A or 1B in Part 3 of Annex VI to Regulation (EC) No 1272/2008. Such substances:

- Shall not be placed on the market, or used, as substances, as constituents of other substances, or, in mixtures, for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than either the relevant specific or generic concentration limit in Part 3 of Annex VI to Regulation (EC) No 1272/2008
- Without prejudice to the implementation of other Community provisions relating to the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of such substances and mixtures is marked visibly, legibly and indelibly as follows: 'Restricted to professional users'.
- Certain derogations for these restrictions are listed in paragraph 2 of entries 28 to 30.

[4] Recommendations for inclusion in the Authorisation List - ECHA (europa.eu)

Such substances are also listed in Appendices 1 to 6 of Annex XVII. The following cobalt compounds are concerned:

- Cobalt, cobalt dichloride, cobalt sulphate, cobalt diacetate, cobalt dinitrate and cobalt carbonate are listed as carcinogen category 1B (**Appendix 2**) and reproductive toxicant category 1B (**Appendix 5**).
- The mixed metal compounds cobalt nickel gray periclase, cobalt nickel dioxide, cobalt nickel oxide, cobalt dimolybdenum nickel octaoxide and cobalt lithium nickel oxide are listed as carcinogen category 1A (**Appendix 1**).

In addition to the above restrictions which are already in place, ECHA submitted an Annex XV dossier, proposing restrictions of the manufacture, placing on the market or use of a substance within the EU for the following soluble cobalt salts: cobalt sulphate, cobalt dichloride, cobalt dinitrate, cobalt carbonate and cobalt di(acetate). The preparation of the restriction dossier on these five cobalt salts was initiated on the basis of Article 69(1) of the REACH Regulation, on request of the Commission[5].

The RAC and SEAC opinion was adopted in 2020 and submitted to the European Commission (RAC, 2020).

3.5 Plant Protection Products Regulation (EC) 1107/2009

There are no plant protection products authorised under Regulation (EC) No 1107/2009 which are based on or include cobalt or cobalt inorganic compounds. Cobalt and its inorganic compounds are not listed as active substances under Regulation 1107/2009/EC, as listed in Regulation (EU) No 540/2011(EU)). Also, no maximum residue levels (MRLs) have been derived for pesticides including cobalt and/or its inorganic compounds, as per Regulation (EC) No 396/2005.

3.6 Human and Veterinary Medicinal Products Directives 2001/83/EC and 2004/28/EC respectively

Cobalt chloride is used in human and veterinary medicinal products, as per Article 57 of Regulation (EC) No 726/2004. Cobalt carbonate, cobalt dichloride, cobalt oxide, cobalt sulphate and cobalt trioxide are not subject to maximum residue levels (MRLs) and are therefore included in Annex II of Council Regulation (EEC) No 2377/90, in accordance with Directive 2004/28/EC. Council Regulation (EEC) No 2377/90 has now been repealed by Regulation (EC) No 470/2009.

3.7 Biocidal Products Regulation (EU) 528/2012

There are no biocidal products authorised under Regulation (EC) No 528/2012 which are based on or include cobalt or cobalt inorganic compounds. Cobalt and its inorganic compounds are not listed as active substances under Regulation (EC) No 528/2012

3.8 Other legislations

According to Annex II of the EU Regulation (EC) No 1223/2009[6] on cosmetic products, cobalt, cobalt dichloride, cobalt sulphate, cobalt carbonate, cobalt dinitrate and cobalt (di)acetate are prohibited in cosmetic products. Cobalt (di)acetate is an organic cobalt compound. Also, cobalt nickel gray periclase, cobalt nickel dioxide, cobalt nickel oxide, cobalt dimolybdenum nickel octaoxide, cobalt lithium nickel oxide as well as the organic

[5] https://echa.europa.eu/documents/10162/13641/commissions_request_cobalt_salt_en.pdf/d21c5c69-9640-47c5-9b36-40060590c17a

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1223&from=EN

cobalt benzene sulphonate are prohibited by the same Annex. Cobalt aluminum oxide is listed in Annex IV as a colorant allowed in cosmetic products.

4. Existing Occupational Exposure Limits and Biological Limits

Several EU Member States have established OEL values for cobalt and its compounds, including inorganic ones. Some Member States have additionally established short-term limit values (STEL). **Table 8** presents these values along with those established in Norway, Switzerland, the UK and USA.

The list should not be considered as exhaustive.

Table 8: Existing Occupational Exposure Limits (OELs) indicated as 8-h Time-Weighted Average (TWA) and Short-Term Exposure Limit (STEL) (15 min) values, for cobalt and inorganic cobalt compounds

Country	TWA (8h) mg/m³	STEL (15 min) mg/m³	Remark
Austria	0.1	0.4	TRK[1] value (based on technical feasibility)
Belgium	0.02 (1)		(1) Inhalable fraction and vapour
Denmark	0.01 (1)	0.02 (1)	(1) Unspecified fraction
Finland	0.02 (1)		(1) Inhalable fraction
France (ANSES)	0.0025	0.0125	Scientific recommendation
Germany (AGS)	0.005 (1)(2) 0.0005 (1)(3)	0.04 (1)(2)(3) (5)	(1) Respirable fraction (2) Workplace exposure concentration corresponding to the proposed tolerable cancer risk. (3) Workplace exposure concentration corresponding to the proposed preliminary acceptable cancer risk. (5) 15 minutes average value
Hungary	0.1	0.4	
Ireland	0.02		
Latvia	0.5		
Netherlands	0.02(1)		(1) Inhalable fraction
Norway	0.02 (1)		(1) Unspecified fraction. Cobalt and its inorganic compounds, except Co(II)
Poland	0.02		
Romania	0.05	0.1 (1)	(1) 15 minutes average value
Spain	0.02		

Country	TWA (8h) mg/m³	STEL (15 min) mg/m³	Remark
Sweden	0.02 (1)		(1) Inhalable fraction. Cobalt and inorganic compounds
Switzerland	0.05		Inhalable aerosol
United Kingdom	0.1 (1)		(1) Inhalable fraction
USA-OSHA	0.1		

Source: GESTIS Substance Database (accessed 04/2022; searched for 'cobalt and its compounds')
Notes: [1]: Technische Richtkonzentration; Technical Guidance Concentration

Four EU Member States have established biological limit values for cobalt and cobalt compounds in the urine. These are shown in **Table 9** below.

Table 9: Biological limit values for cobalt and cobalt compounds

Country	Cobalt in urine	Specifications	Reference
France	5 µg/g creatinine (~5.7 µg/l)	(VLB): for exposure to cobalt in the form of metallic dusts, salts and oxides with the exception of cobalt in association with tungsten carbide; end of week and end of shift. Scientific recommendation.	(ANSES, 2018)
Germany	35 µg/l	(BWL at the workplace); Sampling time for long-term exposure: at the end of the shift after several shifts	(DFG, 2019)
Germany	Range of values starting from value of 3 µg/L an external concentration of 0.005 mg/m³ in air up to 300 µg/l for 0.5 mg/m³	EKA value (correlation between external and internal exposure) Sampling time: for the long-term exposure: at the end of the working shift after several shifts	(DFG, 2019)
Finland	130 nmol/l (7.7 µg/l)	(BAL): sample to be taken at the end of the work shift or work week	(STM, 2020)[7]

[7] https://www.finlex.fi/fi/laki/ajantasa/2020/20200654

Notes: VLB: "Valeur limite biologique", Biological limit value for workers; BLW: "Biologischer Leitwert", Biological guidance value at the workplace; BAL: Biological Action Levels

In addition, two EU Member States have proposed biological guidance values for cobalt and cobalt compounds in the urine and blood (**Table 10**).

Table 10: Biological guidance values for cobalt and cobalt compounds

Country	Urine/blood	Values/specifications	Reference
France	urine	(VBI): 0.6 µg/g creatinine (or 0.7 µg/l) in males and 1,5 µg/g creatinine (or 2 µg/l) in females[8]	(ANSES, 2018)
France	blood	(VBI): <0.45 µg/l in males (95th percentile) and <0.62 µg/l in females (95th percentile)[9]	(Nisse et al., 2017)
Germany	urine	(BAR): 1.5 µg/l (reference value in the non-occupationally exposed working age population)	(DFG, 2019)

Notes: VBI: "Valeur biologique d'interprétation (issue de la population générale adulte)", reference value for the non-occupationally exposed general adult population; BAR: "Biologischer Arbeitsstoff-Referenzwert", biological reference value for workplace substances of persons occupationally not exposed to cobalt

5. Occurrence, Use and Occupational Exposure

5.1 Occurrence

However, cobalt is usually found in the environment combined with other elements such as oxygen, sulphur, and arsenic. Cobalt occurs in nature in a widespread but dispersed form in many rocks and soils. The cobalt concentration in the earth's crust is about 20 mg/kg. The largest concentrations of cobalt are found in mafic (igneous rocks rich in magnesium and iron and comparatively low in silica) and ultramafic rocks; the average cobalt content in ultramafic rocks is 270 mg/kg, with a nickel:cobalt ratio of 7. Sedimentary rocks contain varying amounts of cobalt, averaging 4 mg/kg in sandstone, 6 mg/kg in carbonate rocks and 40 mg/kg in clays and shales. Concentrations of cobalt in metamorphic rock depend on the amount of the element in the original igneous or sedimentary source. Cobalt has also been found in meteorites ((Donaldson, 1986); (O'Neil, 2001); (Donaldson, 2003)). Cobalt salts occur in nature as a small percentage of other metal deposits, particularly copper; cobalt sulphides, oxides and arsenides are the largest mineral sources of cobalt (IARC, 1991, Donaldson, 2003, Schrauzer, 2004).

Small amounts of these cobalt compounds can be found in plants and animals. Cobalt is even found in water in dissolved or ionic form, typically in small amounts. A biochemically important cobalt compound is vitamin B12 ((cyano)cobalamin. Vitamin B12 is essential for good health in animals and humans (ATSDR, 2004). A dietary reference value for cobalamin (vitamin B12) (Adequate Intake (AI)) is 4 µg/day for adults based on data on different biomarkers of cobalamin status and in consideration of observed mean intakes,

[8] Based on the French Nutrition and Health Survey (ENNS) in general population (Frery et al. 2011)
[9] The study of Nisse et al. 2017 is based on an adult representative sample of the general population living in northern France (Hauts-de-France).

which range between 4.2 and 8.6 µg/day in adults in several EU countries (EFSA NDA Panel (EFSA Panel on Dietetic Products, 2015).

The cobalt concentration in the earth's crust is about 20 mg/kg. The largest concentrations of cobalt are found in mafic (igneous rocks rich in magnesium and iron and comparatively low in silica) and ultramafic rocks; the average cobalt content in ultramafic rocks is 270 mg/kg, with a nickel:cobalt ratio of 7. Sedimentary rocks contain varying amounts of cobalt, averaging 4 mg/kg in sandstone, 6 mg/kg in carbonate rocks and 40 mg/kg in clays and shales. Concentrations of cobalt in metamorphic rock depend on the amount of the element in the original igneous or sedimentary source (IARC 2006).

Cobalt salts occur in nature as a small percentage of other metal deposits, particularly copper; cobalt sulphides, oxides and arsenides are the largest mineral sources of cobalt (IARC 2006).

5.2 Production and Use Information

Cobalt is mainly mined as a by-product from copper and nickel mines. Both underground and surface mining technologies are used. The three main uses for cobalt and its inorganic compounds are battery production for electric vehicles, tablets and smartphones (57%), nickel-based alloy production (13%) and manufacturing tools (8%). A short description of the main uses for cobalt and inorganic cobalt compounds (substances listed in **Table 5**) is presented in the next sections based on information provided by the Cobalt Institute[10], the RAC and SEAC Opinion on Restriction for five cobalt salts (ECHA 2020) and data extracted from registration dossiers.

For the substances where cobalt is a significant constituent (substances in **Table 7**), the reported uses according to the registration dossiers include mainly industrial intermediate uses. Cement copper is identified to be used also in fillers, putties etc and in base metals and alloys. Slimes and sludges are reported to be used in water treatment chemicals and fillers and putties in addition to intermediate use.

5.2.1 Manufacture of batteries

Cobalt dinitrate and cobalt sulphate are used as intermediates in the manufacture of rechargeable batteries for the automotive market and for storage applications. Cobalt dinitrate and cobalt sulphate are transformed into cobalt hydroxide or tricobalt tetraoxide which are further used in the manufacture of cathodes for nickel-based batteries (NiCd and Ni-MH) and for lithium-ion batteries (LICoO2, NMC or NCA). The most popular lithium-ion technology to power portable electronic devices like phones, laptops and tablets is the lithium-cobalt oxide (LCO) battery which has a cathode composed of LiCoO2. The majority of modern electric vehicles use these battery chemistries in lithium-nickel-manganese-cobalt-oxide (NMC) batteries which have a cathode containing 10-20% cobalt.

The use of cobalt compounds in the manufacture of nickel-based batteries is expected to remain stable. However, as the transition to low-carbon transport options gains pace, more electric vehicles and plug-in hybrid vehicles are expected to be deployed and the use of cobalt compounds in the manufacture of lithium-ion batteries is expected to rise.

The battery sector is growing, and the use of cobalt compounds is crucial for the rechargeable batteries that are already powering hybrid and electric vehicles. The demand for cobalt in electric vehicles and energy storage is set to increase over the coming years[9].

[10] https://www.cobaltinstitute.org/

5.2.2 Cobalt-containing alloys

Cobalt is used as an alloying element in the production of cobalt metal alloys of different grades. Co-Ni alloys are used in jet engines, gas turbines, chemical processing, petroleum refining, marine, electronics and other industrial applications where common stainless steels may not provide adequate performance. Due to its magnetic properties, cobalt metal is also used in different Co-Ni alloys for the production of magnets and varistors. Other cobalt alloys include Co-Cr ones which are widely used as metal implants in e.g., artificial knee and hip joints due to their wear resistance characteristics. Co-Cr alloys (among other Co alloys) are also used in dental applications such as inlays, onlays, crowns and bridges where the dental restorations are produced in laboratory settings by casting. The casting of metal alloys in laboratories and in dental practices *in situ* may result in exposure to cobalt via inhalation for professionals and consumers.

5.2.3 Hard-metal production and cobalt-containing tools

Cobalt is essential for hard metal tools. Cobalt is used as a binder in the production of hard-metal (also referred to as cemented carbide and tungsten carbide). The addition of cobalt to the carbide increases resistance to wear, hardness and mechanical strength, required for cutting tools, machine tools, engine components and other industrial applications. Hard metal tools support various kinds of significant industries, such as tool manufacturing, steel- and metal processing, construction, automotive, oil & gas, aerospace, food & beverage processing, medical, hygienic, wood processing, security, packaging, energy, transport technology, bar peeling or heavy machining.

5.2.4 Manufacture of catalysts

Cobalt metal and cobalt compounds are used in the manufacture of catalysts. Cobalt-containing catalysts are used in industrial applications such as the desulphurisation of natural gas and oil.

Approximately 8% of the total tonnage of the five cobalt salts (cobalt carbonate, cobalt dinitrate, cobalt sulphate and cobalt diacetate which is an organic compound) are used in catalyst production. These substances are used as intermediates, and they are chemically transformed to produce catalyst precursors or active catalyst substances. Hence, they are not present in the final catalyst product. Cobalt compounds are used in the production of catalysts for hydrotreating/desulphurisation (oil refining) and in the production of catalysts for Gas to Liquid (GTL) (Fischer-Tropsch). The use of cobalt-containing catalysts is essential for the production of clean transport fuels which could help meet the low sulphur standards needed to avoid sulphur emissions (RAC, 2020).

5.2.5 Manufacture of pigments and dyes

Cobalt and cobalt compounds are used to produce colours (blue, purple, violet, green, turquoise etc.) for inks and pigments.

By altering the concentration of cobalt oxide and adding other metal oxides many different colours can be created which are in turn used to colour ceramics and glass. A mixture of raw materials (typically consisting of metal oxides and salts) undergoes a calcination reaction at high temperatures, forming a specific crystalline matrix. The primary raw material in the manufacture of inorganic cobalt-containing pigments is tricobalt tetraoxide. During the calcination process, raw materials are completely consumed, and the resulting substance does not contain tricobalt tetraoxide nor any other cobalt containing raw material. The resulting pigment only contains cobalt cations and other ions tightly bound in a stable crystalline structure which imitates natural coloured minerals and gemstones. The stability of their crystalline structure determines the intrinsic properties and behaviour of complex inorganic pigments, which are a specific type of chemical substances and cannot be assimilated to metal compounds. In general, complex inorganic pigments are poorly soluble and have very low reactivity and these pigments are used in high

temperature applications due to their extreme durability which offers light fastness and weather stability. These pigments are used in ceramics, metals, plastics and paints or coatings[11].

Cobalt compounds (e.g., cobalt diacetate; an organic compound, cobalt dichloride and cobalt sulphate) are used as dyes for the textile leather, wood and paper industry.

The use of the cobalt salts in the manufacture of pigments and dyes is a relatively minor use, estimated at significantly less than 100 tonnes per year representing less than 1% of the uses of the cobalt salts (RAC, 2020).

5.2.6 Surface treatment

Cobalt sulphate and cobalt dinitrate are the most commonly used cobalt salts in the surface treatment sector, with some limited use of cobalt di(acetate). It is noted that cobalt carbonate and cobalt dichloride are not widely used due to difficulties with handing and issues of corrosion, respectively.

The main uses of cobalt salts within the surface treatment sector were described in the RAC and SEAC Opinions (ECHA 2020) and are outlined below.

5.2.6.1 Passivation

Cobalt salts are used in the generation of 'conversion layers' (also called passivation), typically on zinc- or zinc alloy-coated metallic products for corrosion protection. Conversion layers delay the initial attacks on the metallic protective layer made of zinc or zinc alloy. For this reason, they are used mainly for improving the corrosion resistance of zinc plated metal, leading to longer service life and operating time of metal components, particularly in the automotive industry.

Cobalt(II) salts are added to the application solutions of Cr(III)-based conversion coatings, which are alternative surface treatments for the use of Cr(VI). In this process, the galvanized components are dipped in a treatment solution containing trivalent chromium compounds and a proportion of cobalt salts. The cobalt ions are integrated into the surface as oxides or as spinels. The addition of cobalt salts is necessary if corrosion protection is required in warm or hot environments (e.g., engine spaces, brakes, gearboxes and in electrical parts in housings, etc.).

Cobalt salts have important applications in the automotive, aerospace and defence sectors as well as for manufacture of fittings for window construction.

5.2.6.2 Plating

Cobalt salts are used in metal or metal alloy plating (mainly gold-cobalt and tin-cobalt plating) to enhance hardness and wear resistance and/or for metal colouring (RAC, 2020).

Plating is a similar process to passivation but in this case electrical current is used to form the surface. Cobalt salts are added to solutions of other metals (e.g., nickel, tungsten, iron, molybdenum, chromium, zinc, and precious metals) to form alloys in electroplating. During the plating process, the cobalt substances are transformed into cobalt metal. For example, in gold-cobalt electroplating, gold and cobalt are formed and deposited concurrently, building a surface coating of gold alloy. These alloys have improved properties (e.g., hardness, wear resistance) compared to gold on its own.

Cobalt salts are used in the watchmaking and jewellery sectors in surface treatment processes for the deposition of metal alloys (metallic coatings applied by electroplating or

[11] Information received from The Inorganic Pigments Consortium via Public Consultation

galvanic processes). Cobalt sulphate is the main cobalt salt used. It is found in some pre-gilding baths and gilding baths. The alloys deposited are composed of 94 to 98 % fine gold. These processes are either subcontracted to companies specialising in electroplating or, in the case of the largest companies, performed by the jewellery and watch-part manufacturers themselves in-house.

The amount of cobalt salts consumed in the watchmaking and jewellery sectors is extremely low and it represents a tiny fraction of their global use and even of their use in the surface treatment field.

5.2.7 Welding

Cobalt metal is used as hardener in welding processes as part of industrial and professional applications.

5.2.8 Other uses

Cobalt metal and cobalt compounds are used in the manufacture of other chemicals, including cobalt carboxylates and resonates and inorganic cobalt substances.

Cobalt salts are used as rubber adhesion promoters for bonding steel and rubber in the manufacture of tyres. However, many of these compounds e.g., cobalt carboxylates and resonates, are organic compounds. The cobalt salts are being used for more than 40 years in tyres, and their use is linked with the development and establishment of radial tyres which combine lightness with better handling, comfort, safety and durability. Cobalt compounds are also used for rubber technical products that require rubber-to-steel adhesion, such as conveyor belts.

Cobalt is used in additive manufacturing (3D printing) in powder-bed processes (Hebisch, 2021).

Cobalt metal is used as a processing aid in the production of polyamide powders for the cosmetic industry. According to the registration dossiers, formulation is the only step that takes place for this use.

The registration dossiers identify the use of cobalt dihydroxide, cobalt dichloride, cobalt carbonate and cobalt sulphate as fertilisers. This entails industrial and professional uses, including formulation. It is to be noted that, cobalt dichloride, cobalt carbonate and cobalt sulphate are not presently used as fertilisers in the EU (RAC, 2020).

Cobalt sulphate, cobalt dichloride, cobalt dinitrate and cobalt carbonate are used as oxygen scavengers in water treatment applications, helping to prevent corrosion which might lead to failures of boiler systems.

Cobalt plays an important role in renewable biogas technology. Biogas is a methane-based energy carrier and is widely used for delocalised electricity and heat production or as a renewable replacement for natural gas. The fermentation involved in biogas production can be improved by adding small amounts of cobalt sulphate, cobalt chloride, cobalt carbonate, or cobalt diacetate.

According to the registration dossiers, cobalt sulphate, cobalt dichloride and cobalt carbonate are used in animal feed as a supplementation to diets for ruminants, horses and rabbits. This is subject to Regulation (EC) no 1831/2003 on additives for use in animal nutrition, which establishes the requirement of an authorisation for this use. Cobalt salts are contained in the form of premixtures within compound feeds (defined as animal feeds containing supplements).

Cobalt dichloride is used as an indicator of humidity and moisture in the electronics industry, and in industrial and military applications. Humidity indicators can be supplied to the market in a number of formats (e.g., including plugs, cards and indicating silica gel sachets and canisters).

5.3 Occupational exposure

The Cobalt Institute provided the methodology that was applied in the occupational exposure scenarios for cobalt and 21 cobalt compounds in the registration dossiers (the sampling methodology document is available from the Cobalt Institute)[12]. Exposure data for various exposure scenarios are presented and specific monitoring data have been collected for many exposure scenarios. However, measured data were not available for all scenarios and instead available data from other cobalt compounds or published data were used to estimate exposure. Additionally, a number of exposure values are derived from modelling (MEASE (1.02.01)). In this document, only measured exposure data are considered.

The exposure data provided by the industry correspond to the inhalable fraction according to EN 481. Inhalation exposure data are from personal measurements and correspond to full-shift exposures (duration of the task is not taken into account) for cobalt metal, inorganic cobalt compounds and for cobalt carboxylates and resinates. Measurements are performed outside of any respiratory protection and the values are not corrected with the applied protection factors. An overview of the measured exposure values for different uses is extracted from the information provided by the Cobalt Institute and shown in **Table 11**. The provided occupational exposure data are presented **Appendix 3** and the sampling methodology document is available from the Cobalt Institute[13].

Table 11: Measured exposure data presented as median and 90th percentile (P90) concentration (inhalable fraction, µg Co/m3, full-shift value) for the manufacture and use of cobalt and inorganic cobalt compounds (Cobalt Institute, 2022[10])

Sector/use/Activity	Air concentration [a] µg Co/m^3 median or range of medians[b]	Air concentration [a] µg Co/m^3 P90 or range of P90s[b]
Manufacture of **cobalt metal**	1-82	5-470
-Raw material handling	9	96
-Powder production and milling	24	180
-Screening and packaging	82	470
Cobalt metal in production and use of cobalt containing alloys, steels and tools	1-221	1-1093
-Melting and casting	1	1
-Handling of powders	221	1093
Cobalt metal – service life of cobalt containing alloys, steels and tools	10-20	20-110
-Mechanical treatment of hard coated metals/alloys – low kinetic energy	10	20
-Mechanical treatment of hard coated metals/alloys – high kinetic energy	20	110
Cobalt metal – production of varistors and magnets	62	96
Inorganic cobalt compounds – manufacture	4-90	16-833
-Hot processes	90	181
-Further processing	38	239
-Packaging (moderate dustiness)	17	149
-Packaging (high dustiness)	54	833

[12] https://www.cobaltinstitute.org/about-us/contact/

Sector/use/Activity	Air concentration [a] µg Co/m³ median or range of medians[b]	Air concentration [a] µg Co/m³ P90 or range of P90s[b]
Inorganic cobalt compounds – plating processes	2-4	4-14
Inorganic cobalt compounds – fermentation processes	1	1
Inorganic cobalt compounds – humidity indicator cards	0.03-1	0.03-1
Inorganic cobalt compounds - Manufacture of pigments and dyes	5	11
Inorganic cobalt compounds - Manufacture of catalysts	2	16
Inorganic cobalt compounds - Use as catalysts	1-11	3-98
Cobalt carboxylates and resonates[c] – manufacture e.g., packaging powders	17-68	172-178
Cobalt carboxylates and resonates[c] – production and use of rubber adhesion agent	0.02-0.08	0.06-2

[a] Air monitoring measurements based on personal samplers. Full-shift values, duration of the task or the use of protective respiratory equipment (PRE) are not taken into account.
[b] In case of many sub-activities under the use, the range of medians or P90 is reported
[c] Cobalt carboxylates and resonates are organic cobalt compounds

In general terms, the highest exposure levels are measured during the packaging or handling of powders containing cobalt metal or cobalt compounds. The P90 values ranged from 149 to 1093 µg/m^3 in tasks where powders are handled. Exposure level in 'use and mechanical treatment of hard coated metals and/or alloys' depends on whether low or high kinetic energy is used in the process. The exposure level is five times higher if high kinetic energy is used, compared to low kinetic energy (P90; 20 vs 110 µg/m^3). Exposure levels (P90) in "manufacture in the catalysts industry" and "catalyst production" ranged from 3 to 98 µg/m^3, with the highest exposure measured during cleaning and maintenance tasks. Manufacture of inorganic pigments and plating processes with surface treatment created similar levels of cobalt exposure. P90 exposure values were 11 µg/m^3 and 12 µg/m^3, respectively. Exposure levels for cobalt are low when inorganic cobalt compounds are used in fermentation processes and in humidity indicator cards or in the 'production and industrial use of rubber adhesion agents', being below 6 µg/m^3 (maximum concentration).

Exposure data related to similar activities, gathered from the literature and other sources do not contradict the values presented by the registrants.

The watchmaking and jewellery sectors use cobalt salts in surface treatment processes for the deposition of metal alloys (metallic coatings applied by electroplating or galvanic processes). Cobalt sulphate is the main cobalt salt used. The levels of exposure to cobalt salts linked to these activities are very low given the low concentration in the baths (between 0.1 and 5%) and the small volume consumed, and also because of other conditions of use that reduce exposure, e.g., liquid form, application temperature below 40°C and efficient local exhaust ventilation. A measurement campaign was held during 2018. The measurements were carried out in three companies, representative of the sectors: two electroplating companies specialised in surface treatment for the watchmaking, jewellery and fashion accessory sectors and one company making watch components. At least two workers in each company were monitored. All the exposure

levels measured were lower than the limit of quantification given by the analysis laboratory, i.e., 0.1 µg Co/filter, and are therefore between lower than 0.12 µg Co/m^3 and lower than 0.23 µg Co/m^3 depending on the sampling time (4 to 7 hours) and with a flow rate of 2 l/min. [13]

Several Member States submitted data on cobalt exposure as part of the stakeholder consultation for the Restriction process (RAC, 2020). Exposure levels related to cobalt in general. The most comprehensive database was submitted by France where cobalt exposure data from 2007 to 2017 were compiled. The range of cobalt concentration from personal samples was 0.0015-1500 µg/m^3, the 90th percentile value was 24.3 µg/m^3 and the median value was 1.17 µg/m^3. The exposure was highest in sectors of dental practice (median 10.7 µg/m^3; P90 255 µg/m^3), powder metallurgies (median 30.2 µg/m^3; P90 243 µg/m^3), aeronautical and space construction (median 7.5 µg/m^3; P90 107 µg/m^3), metal processing and coating (median 2.0 µg/m^3; P90 75.5 µg/m^3) and manufacture of other tools (median 5.0 µg/m^3; P90 70.3 µg/m^3).

For feed grade material use, the median exposure level reported, based on personal sampling, was 1.0 µg/m^3, while the 90th percentile value was 32.1 µg/m^3. Slovakia reported the measurement of cobalt exposure by personal sampling in passivation to be below 4 µg/m^3. Additionally, according to the German Technical rules (TRGS 561, 2017), cobalt exposure during passivation is below 1 µg/m^3.

Hebisch et al (2021) has measured airborne cobalt exposure during 3D printing, where cobalt is used in additive manufacturing in powder-bed processes. The number of personal air samples was 14. The exposure to cobalt and its inorganic compounds (carc. 1A and 1B) measured in the respirable particle fraction varied from 0.08 to 1.1 µg/m^3 (Hebisch, 2021).

Exposures to cobalt in Italian industrial settings were evaluated by (Scarselli et al., 2020). Data on cobalt and its compounds were collected from an occupational exposure registry. Statistical analysis was carried out for some exposure-related variables (i.e., cobalt compound, activity sector, occupational group, firm size). The number of workers potentially exposed was estimated for selected industrial sectors. Overall, 1,701 measurements (personal and environmental) were analysed in the period 1996-2016. The geometric mean of cobalt airborne concentration was 0.33 µg/m^3 (GSD 8.81; 75th percentile 2.0 µg/m^3). The highest geometric mean, 3.69 µg/m^3 (N=93; GSD 2.71; 75th percentile 7.0 µg/m^3) was measured for manufacture of cutlery, tools and general hardware. Most exposures occurred in the manufacture of fabricated metal products (50%) and among metal finishing-, plating and coating-machine operators (42%). A total of 30,401 workers potentially exposed to cobalt was estimated, over 72% were male (Scarselli et al., 2020).

Hutter et al., estimated the cobalt levels in the Austrian tungsten industry at the workplace, collected from 1985 to 2012, and human biomonitoring data collected from 2008 to 2014. The median value for cobalt exposure was 20 µg/m^3 (range 1-8000 µg/m^3) and the median for cobalt in urine was 3.7 µg/l (range 1-159.7 µg/l). Both air and urine measurements exhibited an overall decreasing trend over time (Hutter et al., 2016).

[13] Information received by: The Jewellery-making, Gold Jewellery-making and Silversmiths, Gift Makers and Decorative Arts Industries Trade Association (BOCI) France Horlogerie; Time and Microtechnics Industries (FHITM); The French Union of Jewellery, Silverware, Gems and Pearls (UFBJOP), and Francéclat, the French Watch, Clock, Jewellery, Silverware and Tableware Committee

The Finnish Institute of Occupational Health (FIOH) gathered exposure concentrations of air pollutants in Finnish workplaces measured by FIOH. Samples were mainly taken from industrial environments, as well as sites of production, transportation, and disposal of commodities and/or services. The report from the years 2008-2019 and the biomonitoring measurements conducted by FIOH during 2012-2019, show that the median values for cobalt have increased slightly during the years, being 0.35 µg/m^3 for the period 2016-2019 (N=231). The 95th percentile was 155 µg/m^3 and 12% of the measurements exceeded the Finnish OEL for cobalt, 20 µg/m^3. Urinary cobalt was measured from 1511 workers during the period 2016-2019. The median value was 24.2 nmol/l and the indicative limit value, 130 nmol/l, was exceeded 185 times. Around 50% of the measurements were above the limit value for non-occupationally exposed workers (FIOH, 2019, FIOH, 2021).

Exposure levels of cobalt were regularly monitored in a Finnish cobalt plant between 1968 and 2014 (Sauni et al., 2017). After 1987, the plant produced cobalt powder, inorganic cobalt and nickel compounds using by-products of metallurgic industry as raw material. The mean exposure level of total dust, which contains 0.4% cobalt, was high (8.5 mg/m^3) in the sulphatising roasting department. Also, exposure levels in the reduction and powder production were high, with the mean value for cobalt at about 75 µg/m^3, during 2004-2014. The mean exposure level for cobalt was about 20 µg/m^3 in the leaching and solution purification and chemical department during 2004-2014.

Urinary cobalt concentrations have been high during the follow-up period from 1979 to this day (FIOH, 2019). Over 14% of urine cobalt samples exceeded the Finnish indicative limit value for urinary cobalt (130 nmol/l), in 2018. The highest urinary cobalt median concentrations were found in the '*manufacture of wire products, chain and springs'*, the *'lead, zinc and tin production'*, the '*freight transport by road'*, the '*manufacture of other inorganic basic chemicals*' and the '*electrical installation*' during the period from 1979 to 2018. In 2018, the situation was different since the highest median concentrations were in the '*manufacture of other inorganic basic chemicals'* (62 nmol/l), the '*treatment and disposal of hazardous waste*' (46 nmol/l), the '*manufacture of other fabricated metal products n.e.c.*' (37 nmol/l), the *'treatment and coating of metals*' (28 nmol/l), the '*wholesale of tools and materials'* (26 nmol/l) and the '*manufacture of tools'* (26 nmol/l). The median values were clearly under the limit value of 130 nmol/l. It appears that high individual exposures increase both the average and the 95th percentile concentrations. The most exposed groups were the mechanics, solders, miners and the chemical workers in 2018 (FIOH, 2019).

Inhalable cobalt (µg/m3) concentrations ranged from 0.16 to 19 µg/m^3, with the median value being 2.2 µg/m^3 for the hard-metal industry (Wahlqvist et al., 2020). The measurements were performed during 2017 and 2018 in two hard-metal production facilities in Sweden. The highest exposure levels occurred in the powder department (median 5.7 µg/m^3). Wahlqvist et al., examined the relationships between inhalable cobalt in air, cobalt on skin and cobalt in the blood and urine. The results showed statistically significant correlations between inhalable cobalt and cobalt concentrations in the blood and urine. Urine levels of cobalt in this study varied between 3.4–250 nmol/l, with a mean value of 34 nmol/l before shift, and 44 nmol/l after shift. The Finnish indicative limit value of 130 nmol/l was exceeded by 2% in the samples from the powder department. Workers used protective respiratory equipment and companies require such use when exposure to higher levels of dust and particles is presumed. Workers applying protective measures against exposure still had elevated urine cobalt levels, above the Finnish limit value, indicating that other factors such as exposure to skin are important. Statistically significant correlations were noted between exposure to cobalt in air with uptake of cobalt in blood and urine. Exposure to cobalt on skin showed significant correlation with uptake in blood but not urine. The data also identified urine as an indicator for cobalt uptake from exposure, though it is not correlated to exposure on skin (Wahlqvist et al., 2020).

Kettelarij et al., investigated cobalt exposure among 76 workers (58 working in the production area and 18 in offices) by monitoring cobalt exposure in the air, on skin and in urine in a hard-metal company in Sweden, in 2013. Cobalt powder used as binding agent (conc. 6-30%) for tungsten carbide to form hard-metal alloys and sintered material was handled in the company. The median values and the range of cobalt concentration in the inhalable fraction (µg Co/m^3) were 5.6 (0.82-24) in those handling raw materials, 0.13 (0.012-0.55 in those handling sintered material and 0.14 (0.026-0.45) in those working with final products. (Kettelarij et al., 2018)

The geometric mean for cobalt exposure for welders (N=8) was 0.05 µg/m^3 (GSD 1.6), for non-welding metal workers (N=8) 0.07 µg/m^3 (GSD 2.5) and for bystanders (N=16) 0.04 µg/m^3 (GSD 1.1) in small fabrication shops in the USA. The study was conducted in 2018 (Insley et al., 2019).

Occupational exposure to cobalt among recycling workers handling e-waste has been evaluated in two studies, one from Sweden and one from Germany.

Gerding et al monitored metal exposure of workers (N=51) during recycling of electronic waste in five sheltered workshops in Germany. Exposure to metals, including cobalt, was monitored with combined air monitoring and biomonitoring. Both inhalable and respirable dust fractions were sampled by personal and stationary sampling. Spot urine samples were collected at the end of the shifts. The most common work activity was electrical equipment disassembly (94%); about 50% of the workers performed the disassembly of tube displays, and 28% of flat-screen displays. The median (GM) value and range for cobalt in the respirable fraction were 0.022 (0.021) µg/m^3 and 0.018-0.024 µg/m^3, collected from personal samplers. The median (GM) and range for cobalt in the inhalable fraction were 0.035 (0.041) µg/m^3 and 0.018-0.31 µg/m^3. The range for cobalt in urine was 0.15 to 1.6 µg/l for recycling workers and 0.15 to 0.5 µg/l for controls. The measured exposure concentrations in the breathing zone and in urine were below the limit values in Germany (5 µg/m^3 from respiratory fraction and 35 µg/l BLW) (Gerling et al., 2021).

Julander et al., evaluated the exposure of recycling workers to toxic metals including cobalt, using both personal air monitoring and biomonitoring. Fifty-five workers and 10 office workers from three e-waste companies in Sweden were monitored between 2007 and 2009. The GM and range for cobalt in the inhalable fraction were 0.066 and 0.0017-3.3 µg/m^3 for recycling workers and 0.0035 and 0.0021-0.0046 µg/m^3 for office workers, respectively. Median and range for urinary Co concentrations were 0.25 and 0.12-1.3 µg/l for recycling workers and 0.24 and 0.14-0.59 µg/l for office workers (Julander et al., 2014).

5.4 Routes of exposure and uptake

5.4.1 Worker exposure

Workers may be exposed to cobalt and its inorganic compounds in the manufacture and use of the substances via inhalation, dermal and (potentially) oral routes of exposure. Prevention of dermal exposure is relevant for cobalt and cobalt compounds due to skin sensitisation.

The highest inhalable exposure is occurring during packaging or handling powders containing cobalt metal or cobalt compounds. According to the registration dossiers, cobalt powders have high dustiness, and the cobalt salts are prepared and used as solids in powder form with medium dustiness. Some of the processes (e.g., in animal feed, manufacture of catalysts, etc.) result in the transformation of the cobalt salts into dry solids (cakes, granules, pellets, etc.) with a lower potential for dust emission.

Inorganic cobalt compounds (except for cobalt carbonate) are also produced and used in liquid form, mainly as aqueous solutions. The use of aqueous solutions can lead to the

generation of mists and fumes in high energy activities such as electroplating and hot metallurgical processes.

Regarding the ratio of respirable to inhalable dust, Okamoto et al., demonstrated based on >1600 data points from different type of works, that a 20-50% respirable fraction is plausible. In his study, the highest respirable/inhalable particulates ratio was in welding (50%) and the lowest in foundries (20%). Powder handling resulted in a ratio of 40% (Okamoto et al., 1998).

In a recent study by Wippich et al (2022), cobalt exposure in respirable dust was estimated from cobalt in inhalable dust. It was concluded in the study that the ratio between inhalable and respirable dust varies according to the processes and specific exposure scenarios and therefore it is not possible to give any universal value for the ratio of inhalable and respirable fraction (Wippich et al., 2022).

5.4.2 General population

Cobalt is widely dispersed in the environment in low concentrations. General population may be exposed to small amounts of cobalt by breathing air, drinking water, and eating cobalt-containing food. Children may also be exposed to cobalt by eating dirt. People may also be exposed by skin contact with soil, water, cobalt alloys, or other substances that contain cobalt (ATSDR, 2004).

Exposure of the general population to cobalt occurs through inhalation of ambient air and ingestion of food and drinking water. In general, intake from food sources is much greater than from drinking water and air. The cobalt intake in food has been estimated to be 5.0–40.0 µg/day (ATSDR, 2004)

6. Monitoring Exposure

6.1 External exposure

Cobalt and its inorganic compounds as contained in particulates can be monitored in the workplace air using a number of validated methods.

The principle of most of the methods is trapping the sample on a suitable filter by using a particle sampler (for inhalable and/or respirable fraction). The cobalt compounds are then extracted and further analysed using a suitable technique. The limit of quantification (LOQ) is given as mass of cobalt.

The methods (included in **Table 12**) have validation data that demonstrate compliance with the requirements of the standard EN 482 *"Workplace exposure. General requirements for the performance of procedures for the measurement of chemical agents"* or the potential to meet these requirements for very low concentration of cobalt. For instance, the IFA 7808 could potentially detect 10% of the proposed OELs for both the inhalable and respirable fractions (with sampling times below 2 hours).

The table states whether the method is relevant for the sampling of inhalable fraction, the respirable or both, as reflected in the sample and analysis methods. When a specific particulate sampler (and its associated flow rate) has been recommended, the calculations of the sampling time have used the maximum flow rate recommended by the method. However, the latter does not exclude that the methods have the potential to use other sampler at different flow rates that could achieve lower LOQ or to collect a different aerosol fraction. The methods appearing under 'similar methods' follow a similar principle and analytical technique and may differ in the sample preparation or in details, such as the filter or the sampler used. These differences may induce significative difference in the analytical performance of the methods including for instance the LOQ.

Table 12: Overview of sampling and analytical methods for monitoring cobalt and cobalt compounds (as cobalt) in workplace air, based on digestion of the loaded filter

Method/ Fraction	Analytical technique	LOQ and sampling volume and time	Similar methods/ comments
IFA 7808 Inhalable and respirable (IFA, 2021)	ICP-MS	0,029 µg/m^3 (for a 1.2 m^3 air sample (2 hours at 10 l/min)	
MTA/MA – 065/A16 (INSHT, 2016) Inhalable and respirable	ICP-AES	0.29 µg/m^3 (for a 480 l sample) less than 1 hour at a flow rate: 10 l/min/ 4 hours for a flow rate of 2l/min)[1]	
NIOSH 7300 and 7301 ((NIOSH, 2003a) and (NIOSH, 2003b)) Inhalable fraction (sampler not completely fitting the standard)	ICP-AES	0,000083 mg/m^3 for a 480 l sample (less than 1 hour at a flow rate: 10 l/min/ 4 hours for a flow rate of 2l/min)[1] 0.000003 mg/m^3 for a 1200 l sample (for 2 hours for a flow rate of 10l/min)	The sampler is not an inhalable sampler. (A sampler fitting the EN 481 could be used instead) A sampler for the respirable fraction could be used if required reaching same LOQs
ISO 15202- parts 1,2, and 3 (ISO, 2020a), (ISO, 2020b, ISO, 2004) (Inhalable or respirable fraction)	ICP-AES	0.0004 mg/m^3 for a 480 l sample (less than 1 hour at a flow rate: 10 l/min/ 4 hours for a flow rate of 2l/min)[1]	Métropol 003, NIOSH 7300, NIOSH 7301, NIOSH 7303, OSHA ID-125G
BGI 505–15E (DFG, 2012b) (Inhalable fraction)	ETAAS (electrothermal atomic absorption spectrometry)	0.0018 mg/m^3 for a 1.2 m^3 air sample (2 hours at 10 l/min)	A sampler for the respirable fraction could be used if required reaching same LOQs

[1] Sampling time calculated for the maximum flow of 10 l/min (maximum flow rate for common inhalable and respirable fraction samplers) and for a flow rate of 2 l/min (common flow rate for inhalable samplers)

6.2 Biomonitoring of exposure (internal exposure)

As for other metals, the determination of the metal in blood or urine has been used as indicator of the internal exposure. Both determinations are in principle suitable as indicators.

The fact that sampling is non-invasive and that the cobalt concentration in urine corresponds more closely to external cobalt exposure, argues in favour of determining cobalt in urine. The cobalt concentrations in urine are about ten times higher than those in blood. Internal cobalt exposures are therefore both diagnostically as well as analytically more reliable to detect, using the urine as biological material (DFG, 2016).

6.2.1 Background levels

Exposure of the general population to cobalt occurs through inhalation of ambient air and ingestion of food and drinking water. In general, intake from food sources is much greater than from drinking water and air (ATSDR, 2004).

Several publications in and outside of Europe have looked at background concentrations in the non-occupationally exposed populations. **Table 13**, below, adapted from DFG (2019), summarises the most relevant studies.

Table 13: Studies on background concentration of cobalt in the general (non-occupationally exposed) population

Country, collective	Cobalt in urine		References
	[µg/L]	[µg/g creatinine]	
Germany n = 87 Age: 18–65 years	95th percentile: 1.53 Range (0.02–3.3)		(Heitland and Köster, 2006)
Italy n = 34 (14 male, 20 female) Age: 61.4 ± 12.4 (28–80) years	95th percentile: 1.16 Range (0.08–1.21)		(De Palma et al., 2010)
Spain n = 50 Age: 21–83 years		1.2 ± 0.4 (1.1–1.5) [AM ± SD (range)] 95th percentile< 1.3	(Torra et al., 2005)
France n=1991 Age 18 to 74 of which 1235 females 756 males	Total 0.24 (0.23–0.25)) [GM (95% CI] 1.400 (1.250–1.660)] [95th percentile (95% CI)] Female 0.26 (0.24–0.28)] [GM (95% CI] 1.951 (1.73–2.17)] [95th percentile (95% CI)] Male 0.22 (0.21–0.0.23)] [GM (95% CI] 0.697 (0.57–0.83)] [95th percentile (95% CI)]	Total 0.210 (0.200–0.220)) [[GM (95% CI] 1.130 (1.040–1.240)] [95th percentile (95% CI)] Female 0.27 (0.25–0.29)] [GM (95% CI] 1.454 (1.31–1.60)] [95th percentile (95% CI)] Male 0.15 (0.16–0.17)] [GM (95% CI] 0.569 (0.44–0.70)] [95th percentile (95% CI)]	(Fréry et al., 2011)
Finland n = 118 Age: working age	95th percentile: 1.4 (23.7 nmol/L) AM: 0.55 (9.4 nmol/L)		(FIOH, 2012)
Pakistan n = 75 (male) Age: 25–55 years	**1.5 ± 0.4 (1.05–1.96)** [AM ± SD (range)] 95% CI: 1.35–1.57		(Afridi et al., 2009)

Country, collective	Cobalt in urine		References
	[µg/L]	[µg/g creatinine]	
Japan n= 13000 urine samples from 1000 (female) Age: 47.5 ± 10.4 years (20–81) [AM ± SD (range)]	0.68 ± 3.04 (< 0.1 (LOD)–281) [GM ± SD (range)]	0.6 ± 2.75 (< 0.1 (LOD)–77) [GM ± SD (range)]	(Ohashi et al., 2006)
n = 25 (male) Age: 45.0 ± 2.9 years [AM ± SD]		0.5 ± 0.4 (AM ± SD)	(Fujio et al., 2009)
United States n = 1811 > 20 years	95th percentile: 1.23 (95% CI: 1.17–1.34)		(CDC, 2017)
Taiwan (data 2005-2008) Young n=103 Age: 7-17 Adults n=677 Age >18	 1.59 (1.77) [GM (GSD] 4.78 (3.38–8.39)] [95th percentile (95% CI)] 1.01 (2.04) [GM (GSD)] 3.31 (2.82–3.85) [95th percentile (95% CI)]		(Liao et al., 2019)

The studies above show that for the studies in the European general population, the 95th percentile value for the cobalt is similar cross Europe. Studies in the European general population indicated that urinary cobalt levels were significantly higher in females than males.
Since all studies are reporting similar findings, the data of Fréry et al. (2011) (more comprehensive study) are considered as representative for the European general population. Consequently, a background levels of 2 µg/L and 0.7 µg/L are identified for females and males, respectively, and can be used for biological guidance values (BGV).

6.2.2 Occupational exposure

Many studies have followed the correlation between air concentration of cobalt and internal concentration (as cobalt in blood or urine). This section focuses on the studies that correlate air concentration and urine concentration, as it is considered the more suitable biomarker (see introduction to this section).

6.2.2.1 Correlations used to derive the DFG values

DFG derived a first EKA value (correlation between external and internal exposure) in 1994 (DFG, 2012a).

The EKA correlation was based on the correlations proposed by the four studies, shown in **Table 14**.

Table 14: Correlations used by DFG (2012a) to derive EKA correlations

Regression equation (Co_{Urine} in µg/l, Co_{Air} in (µg/m3)	Correlation coefficient	Reference
Co_{Urine} = 0.34 Co_{Air} + 19.9	0.606	
Co_{Urine} = 0.67 Co_{Air} + 0.9	0.99	(Ichikawa et al., 1985)
Co_{Urine} = 0.29 Co_{Air} + 0.8	0.83	(Morgan, 1983)
Co_{Urine} = 0.70 Co_{Air} + 0.7	0.81	(Scansetti et al., 1985)

The EKA correlations were re-evaluated in 2006 and 2017 (DFG, 2016, DFG, 2019). More recent studies were evaluated and those were in good accordance with the correlation developed. The correlation was also extended to lower concentrations.

Table 36 in **Appendix 4**, summarizes the studies that had sufficient data to be used to re-evaluate the correlation. The studies covered a wide range of cobalt species, such as metallic cobalt, cobalt oxides, cobalt salts, cobalt sulphides etc., and there was no indication that these different exposures are reflected by different concentrations of cobalt in urine.

The EKA correlations derived on the basis of the available data are presented in **Table 15**, below (DFG, 2019).

Table 15: EKA correlations derived by DFG (DFG, 2019)

Cobalt in air (mg/m³)	Cobalt in urine (µg/L)
0.005	3
0.010	6
0.025	15
0.050	30
0.100	60
0.500	300

6.2.2.2 Correlations used to derive the ANSES value

ANSES (ANSES, 2015) proposed an OEL and a corresponding biomarker of exposure (BME) in 2015. The BME uses cobalt in urine as a biomarker and its value represents the expected internal concentration when the worker is exposed to a concentration of 2,5 µg/m³. The BME proposed was 5 µg/g creatinine (corresponding to circa 7 µg/L using a correction of 1.4 µg/L of creatinine). The assessment carried out by the Committee evaluated data of biological monitoring of cobalt and its compounds with the exception of cobalt associated with tungsten carbide.

The ANSES documentation considered most of the publications reported by DFG (see **Appendix 4**).

The BME value proposed by the ANSES Committee is based on the publications by (Nemery et al., 1992) and (Lison et al., 1994) as those were the only publications where no association of cobalt with hard-metals was reported.

6.2.2.3 Correlations used to derive the Finnish BLV

In the derivation of the Finnish limit value for cobalt in urine, the correlations reported in (Alexandersson, 1988, Alexandesson and Lidums, 1979, Ichikawa et al., 1985, Linnainmaa and Kiilunen, 1997, Scansetti et al., 1985) were taken into consideration. It was concluded

that a BLV of 130 nmol/L (7.7 µg/L) is expected to correspond to 8 hours of cobalt inhalation exposure at 0.01 mg/m^3 (STM, 2016).

6.2.2.4 Conclusion

A **Biological limit value is not proposed** because the air levels corresponding the proposed OELs are likely to result in urinary levels which are very close to the 95th percentiles of the general population.

6.2.3 Biomonitoring analytical methods

There are analytical methods available to measure cobalt in urine that are able to reach concentrations well below the proposed BLV. Some suitable methods are detailed in the table below (**Table 16**).

Table 16: Analytical methods for cobalt in urine

METHOD Analytical technique LOQ /Range	METHOD Analytical technique LOQ /Range	METHOD Analytical technique LOQ /Range
(Goullé et al., 2005)	Inductively coupled plasma mass spectrometry (ICP/MS)	0.06 µg/L
(DFG, 1985)	Flame Atomic Absorption Spectrometry (FAAS)	0.1 µg/L
(Heinrich and Angerer, 1984)	Voltammetry and Electrothermal Atomic Absorption Spectrometry	0.2 µg/L

7. Health Effects

The human epidemiological data concerning the hazardous properties of cobalt and inorganic cobalt compounds are based on two main occupational exposure settings:

- Firstly, those related to production and use of hard-metal and those related to diamond polishing, where exposure to cobalt occurs not alone but always together with exposure to metal carbides or diamond dust, respectively. Tungsten carbide (WC) is the most common hard-metal, formed by binding or cementing metallic carbides with a metal binder, usually cobalt or nickel.
- Secondly, those related to production of cobalt and use of cobalt compounds, where exposure is without tungsten or other metal carbides or diamond dust but may involve exposure to both metallic cobalt and cobalt salts with varying solubility.

For the hazard endpoints discussed in this section, when applicable, the human studies are grouped accordingly, to separately describe effects observed in these two main exposure settings, as there is indication for some adverse effects that effect levels in humans are different when exposure is to cobalt alone compared to cobalt exposure in hard-metal work or those related to diamond polishing.

In addition to these main occupational exposure settings, there are abundant human data on systemic health effects from non-occupational oral exposure to cobalt salts and systemic exposure resulting from metal-to-metal (hip) joint implants. As exposure via such routes is not directly relevant for quantitative setting of an occupational limit value, those non-occupational studies are only summarized based on reviews and, when available, complemented with a description of those human studies that have assessed the risk of related systemic endpoints specifically following (inhalation) exposure in the

occupational environment. Human data relevant for understanding mode of action of cobalt compounds are, when relevant, described in chapter 8.

7.1 Toxicokinetics (Absorption, distribution, metabolism and excretion - ADME)

7.1.1 Human data

7.1.1.1 Absorption

Exposure to cobalt particles via inhalation results in considerable deposition in the upper and lower respiratory tract, depending on the size and other properties of the particles. According to Foster et al. (1989), the proportion deposited was around 50% for cobalt oxide particles with a diameter of 0.8 µm and 75% for particles with a diameter of 1.7 µm. Six months after inhalation exposure of radiolabelled cobalt particles, almost half of the originally detected lung burden persisted and it seems like the half-time for elimination increases during time.

Cobalt may be absorbed directly in the blood stream from the airways, if deposited cobalt particles ions are dissolved, or after phagocytosis by macrophages in the lower respiratory tract. Mechanical transfer by mucociliary action from the upper airways to the gastrointestinal tract may also occur. The gastrointestinal absorption of cobalt has been reported to vary markedly, between 18 and 97% of the orally administered dose (ATSDR, 2004, WHO, 2006).

There are some studies showing data that indicate dermal absorption of cobalt. Scansetti et al. (1994) reported increased urinary levels of cobalt in persons who had placed their hands in a box with hard-metal dust (containing 5-15% cobalt metal) for 90 minutes. In the study by Nielsen et al. (2000), three volunteers placed their fingers in cobalt solution for 10 minutes per day for 10 days, after which fingernail analyses detected the presence of cobalt.

In the study by Klasson et al. (2017), the aim was to examine the correlation between amounts of cobalt on workers' skin (n=62), air concentrations of cobalt, and blood cobalt levels of workers exposed in hard metal manufacture. Significant correlations between blood and skin, blood and inhalable air, and skin and inhalable air cobalt concentrations were reported. The results indicated that a twofold increase in cobalt concentrations at the skin caused a 3-14% increase in blood levels at a given air concentration. The authors noted that unintentional ingestion as a result of hand-to-mouth contact may partly explain the measured blood cobalt concentrations.

7.1.1.2 Distribution

Cobalt is part of vitamin B_{12} and is thus an essential element and can be detected in a large part of the body tissues. Highest amounts of systemically distributed cobalt have been found in the liver and kidneys. Inhalation exposure results in accumulation of cobalt particles in the lungs and consequently increased lung tissue concentrations of cobalt (ATSDR, 2004, WHO, 2006).

7.1.1.1 Metabolism

Cobalt is a metal and is not metabolised per se.

7.1.1.2 Excretion

Absorbed cobalt is excreted in the urine whereas foecal excretion is expected to be the main route for insoluble / non-absorbed cobalt. Following dermal exposure, urinary excretion is the main route (ATSDR, 2004, Scansetti et al., 1994).

7.1.2 Animal data

7.1.2.1 Absorption

Inhalation absorption depends on cobalt particles size; larger particles (>2 µm) tend to deposit on the higher tract (ATSDR, 2004) and subsequently either adsorbed directly via dissolution or transported mechanically to the gastro-intestinal tract by mucociliary action, while the smaller particles reach the lower respiratory tract where they tend to remain until dissolved, phagocytised and then adsorbed. After inhalation exposure, the elimination rate depends on solubility, with soluble salts eliminated faster from the lungs, mainly via adsorption into the blood, followed by excretion in the urine and, to a lesser extent, via the faeces. The elimination via the faeces seems to derive from the fraction that is mechanically moved up the respiratory tract and subsequently swallowed.

In rats, inhaled radiolabelled cobalt oxide particles were nearly completely removed from the lungs after 6 months (ATSDR, 2004). Ultrafine cobalt particles (20 nm) injected intraperitoneally in rats were solubilised in the lungs within a few hours, while larger particles exhibited a half-life of a few days (3-4) (DFG, 2007).

In rats, oral absorption of cobalt dichloride is between 13 to 34% of the administered dose, while the absorption rate of the physiologically insoluble cobalt oxide particles is between 1 and 3%, with no significant effects of the particle size on the absorption rate. Several studies demonstrated the influence of counter ion, cobalt speciation, vehicle, dose, iron status, age of the animal on the absorption rate. Generally, higher absorption was found for more water soluble compounds (ATSDR, 2004).

Dermal absorption has been studied in guinea pigs and Syrian hamsters and was found to be very small through unabraded skin. However, exposure of a cobalt dichloride solution on abraded skin of guinea pigs resulted to almost 80% absorption (ATSDR, 2004).

In another report, Sprague Dawley rats (n=10/sex/dose) were administered cobalt dichloride intravenously (0.0248 mg cobalt/kg bw) and cobalt dichloride (2.48 mg cobalt/kg bw), tricobalt tetraoxide (220 mg cobalt/kg bw), cobalt lithium oxide (180 mg cobalt/kg bw), and cobalt sulphide (194 mg cobalt/kg bw), orally. The bioavailability (%) for each substance was determined based on the plasma concentrations achieved, when compared to intravenous delivery (100%), and was reported to be 7-12% for cobalt dichloride in male and female, 0.3 and 0.25% in male and female, respectively, for cobalt lithium oxide, and finally, less than 0.01% for both tricobalt tetraoxide and cobalt sulphide (water solubility < 0.004 g/L for both). Differences in other toxicokinetic parameters were observed among the substances along with C_{max} variations between males and females (Danzeisen et al., 2020).

7.1.2.2 Distribution

Cobalt is an essential element as it is a constituent of vitamin B12, and therefore was found in all tissues even in non-exposed animals. In animals exposed via inhalation, an increased concentration was found in the lungs. High concentrations have also been recorded in the liver and kidneys.

After oral exposure, the highest concentration was found in the liver, and increased concentrations were measured in the heart, kidneys and the gastro-intestinal tract (ATSDR, 2004). In a dominant lethal assay in male mice, cobalt dichloride was administrated for 10 weeks via drinking water and increased concentration were found in the testes (2.5 times), followed by kidneys (2.2), liver (1.9) and epididymides (1.7) (Pedigo and Vernon, 1993). In male rats, following cobalt dichloride administration through the diet for 14 weeks, increased concentrations were found in the blood, brain, intestine, kidneys, liver and testes, at levels up to 100 times the control value (Nation et al., 1983). Szakmary et al. demonstrated that cobalt sulphate can cross the rat placenta

barrier since a dose-dependent increase in cobalt was measured in foetal blood and amniotic fluid (Szakmáry et al., 2001).

After intravenous injection (i.v.) of cobalt dichloride or dinitrate in rats, increased concentrations were observed in the liver, kidneys and intestines. Following a repeated i.v. exposure of cobalt dichloride for 100 or 132 days, the highest concentrations were measured in the spleen, heart and bone. In the same experiment, the concentrations in liver and kidney decreased from the highest levels at the beginning of the exposure to being significantly lower than in the above-mentioned organs by the end of the treatment period (ATSDR, 2004). After 24h from a single i.v. injection of radioactive cobalt, a small fraction was detected in the testis (0.056%) and epididymis (~0.036%), but none in the germinal cells (Edel et al., 1994).

A slightly different distribution was found after injection of cobalt chelated within porphyrin rings, e.g., mesoporphyrin, protoporphyrin compounds, or lysosomes encapsulated cobalt (ATSDR, 2004).

7.1.2.3 Metabolism

Cobalt is a metal and is not metabolised per se.

7.1.2.4 Excretion

After a single exposure, an initial high rate of elimination via the faeces was observed followed by the majority of the dose being eliminated via the urine. Similar results were noted after 3 months of repeated exposure. In several studies, the insoluble tricobalt tetraoxide (Co_3O_4) was cleared from the lungs almost completely in about 6 months, in several animal species, with clearance rates decreasing from mouse, rat, hamster, guinea pig, baboon and human, to beagle dog (ATSDR, 2004).

After oral exposure, cobalt is excreted mainly via the faeces with inverse proportionality to the solubility of the cobalt compound. For example, the majority of insoluble tricobalt tetraoxide (Co_3O_4) (>96%) was eliminated via the faeces in several animal species, while 70-83% of cobalt dichloride was excreted via the faeces, with the rest of the administered dose being eliminated primarily via the urine in rats. A small percentage of cobalt dichloride (~0.012%) was found in cow milk (ATSDR, 2004). Generally, a decreased iron status leads to a lower cobalt excretion, while co-administration of cobalt and iron enhances cobalt elimination (ATSDR, 2004).

After a single dermal exposure to cobalt dichloride, Syrian hamsters eliminated the majority of the adsorbed dose via the urine within 48 hours (Lacy et al., 1996, ATSDR, 2004).

For i.v. or intramuscular (i.m.) injections, the main elimination route was the urine accounting for ~70 to ~90% of the administered dose within 21 days after exposure, with slight differences amongst species.

After injection, cobalt bound to porphyrins was retained longer in the body than soluble salts and an increased fraction was eliminated via the faeces. Consequently, it was postulated that elimination is faster and primarily via the urine for soluble salts, and becomes slower and increasingly via the faeces for less soluble compounds (ATSDR, 2004).

Male and female Sprague Dawley rats were administered by gavage a single dose of cobalt dichloride (2.48 mg cobalt/kg bw) or tricobalt tetraoxide (220 mg cobalt/kg bw). Excretion was practically complete after 72 hours, with the majority of the cobalt substances eliminated within the first 24 hours. Cobalt was mainly excreted via the faeces; >82% and 95% in rats dosed with cobalt dichloride and tricobalt tetraoxide, respectively. Urinary excretion was above 12% and about 0.1% for the two compounds, respectively (Danzeisen et al., 2020).

7.1.3 *In vitro* data

Tricobalt tetraoxide was found to be poorly dissolved (<2%) in a study using simulated lung fluids. However, other studies in the presence of lung cells showed a more significant release of cobalt ion. In the presence of canine (mongrel) alveolar macrophages, after two week of culture, 0.3 µm-sized particles of tricobalt tetraoxide were solubilised up to 50%, while larger particles were solubilised between 2 to 5%. The authors demonstrated that the soluble fraction in the presence of macrophages increased over time, while it remained constant in culture medium. They speculated that the difference could be due to the macrophages uptake. In the same study, in the presence of human alveolar macrophages, the dissolution rates were comparable to those observed in *in vivo* translocation from human lungs clearance (Kreyling et al., 1990, NTP, 2016).

Ortega et al. studied the toxicity of tricobalt tetraoxide particles (100-400 nm range) towards BEAS-2B human lung cells. Their study confirmed the very low metal release (up to about 0.4%) of the cobalt particles in culture medium, after 7 days, however when the lung cells were included, the particles entered the cells via endocytosis and therein released cobalt ions thereby increasing the dissolution rate to about 2% and 5% after 3 and 7 days, respectively. This suggests that tricobalt tetraoxide releases cobalt ions *in vivo* to a higher extent to what is measured *in vitro* (Ortega et al., 2014).

In another study, CoO nanoparticles (0.27 to 3.56 µm) were tested in the human lung fibroblast cell line, WTHBF-6. The study demonstrated a concentration-dependent increase of the intracellular cobalt concentration (Corzilius et al., 2014).

Fifteen cobalt salts were used to assess their metal release in artificial biological fluids in order to estimate their bioaccessibility. A fixed amount of each salt (0.1 g) was placed in 50 ml of simulated gastric (pH 1.5) or intestinal (pH 7.4) fluid. The authors measured a significant variability in solubility among the tested salts, up to 100 and 1000-fold in simulated gastric or intestinal fluids, respectively (Danzeisen et al., 2020). Cobalt solubility in simulated gastric was measured at 98.5%, 99.7%, 0.58%, 0.2%, 0.85% and 5.9% for cobalt dichloride, cobalt sulphate, cobalt sulphide, tricobalt tetraoxide, cobalt hydroxide oxide or cobalt lithium oxide, respectively, and at 79%, 64%, 0.33%, 0.05%, 0.06% or 0.02% in simulated intestinal fluid (Danzeisen et al., 2020).

The release of cobalt from 12 cobalt salts (cobalt dichloride, dinitrate, sulphate, diacetate, sulphide, carbonate, oxide, cobalt metal, cobalt dihydroxide, hydroxyide oxide, tricobalt tetraoxide, and lithium cobalt dioxide) was tested in three different artificial lung fluids, namely artificial interstitial fluid (AIF), artificial alveolar fluid (AAF) and artificial lysosomal fluid (ALF). These fluids are designed to mimic the different conditions the cobalt particles are exposed to, in the lungs. AIF is representative of the extracellular environment in the deep lungs with a pH around 7.4; AAF of the alveolar space, with a neutral pH (7.3-7.4) and includes surfactants; ALF, of the environment after phagocytosis by alveolar or the microphages, with a lower pH (4.5-5.0) containing enzymes and a high concentration of citric acid. Due to the chelating properties of citric acid and the lower pH, the solubility is expected to be higher than in the other fluids. Each compound was tested at a concentration of 2 g/l and metal release in the fluids measured after 2 or 5 and 24 hours. Based on their results after the 2 or 5-hour extractions, the authors divided the salts in three groups: those exhibiting high release in all fluids (cobalt dichloride, dinitrate, sulphate, diacetate), those with low release in AIF and AAF but high in ALF (cobalt carbonate, oxide, cobalt metal, cobalt dihydroxide), and the last group which showed poor release in all fluids (cobalt hydroxyide oxide, sulphide, tricobalt tetraoxide, and lithium cobalt dioxide) (Verougstraete et al., 2022).

The percutaneous penetration of cobalt metal powder was studied using Franz diffusion cells and comparing intact and damaged human skin. The results indicated that cobalt can permeate through damaged skin more easily than through intact skin (Filon et al., 2009). Later studies demonstrated the skin penetration of cobalt nanoparticles, reaching the

dermis and epidermis. Permeation through the skin was detected in a model using damaged skin (Larese Filon et al., 2013, Mauro et al., 2015).

Penetration of cobalt ions of aqueous solutions of cobalt (II) chloride heptahydrate salt into the epidermis of human skin in an ex vivo setting was detected in the study by Hagvall et al. (2021).

Midander et al. (2020) studied the penetration of cobalt metal (alone or together with nickel or chromium) in artificial sweat through intact piglet skin. Cobalt was detected in the receptor solution at concentrations increasing over time.

7.1.4 Toxicokinetic modelling

Application of a physiologically based pharmacokinetic (PBPK) model for human respiratory tract, developed by ICRP, estimated that the absorption of cobalt chloride and nitrate is fast (100% absorption within 10 minutes), and that of cobalt oxides, cobalt metal and cobalt alloys is fast or medium (70-100% absorption within 10 minutes) (WHO, 2006).

In an unpublished study by the Cobalt Institute (2016), a range of 0.4% to 3.4% alveolar deposition was estimated for workers being exposed to cobalt dust. The estimations were conducted using the MPPD-model, which is a tool that can be used to estimate particle deposition in different regions of the respiratory tract. Particle size distribution data of samples collected in actual workplace measurements were used as input parameters. Furthermore, by taking into consideration the estimated alveolar deposition in workers and data on alveolar deposition in rats, an approach to estimate human equivalent concentrations for rat doses was presented.[14]

7.1.5 Biological monitoring

For human biomonitoring, excreted cobalt concentrations can be measured in urine using different methods. Samples are normally recommended to be collected at the end of the last work shift of a week, to reflect exposure during that period of work. There is a good correlation between blood levels and occupational exposure to cobalt, but biomonitoring of cobalt in blood is not common (WHO, 1996).

Further information on this topic, including analytical methods, background levels, and relationships found between airborne and urine concentrations, is presented in section 6.2.

7.1.6 Summary

Several parameters affect cobalt absorption such as particle size, type of cobalt compound, and iron status of the animal (or human). Inhaled cobalt is absorbed after dissolution in the lung fluid or via phagocytosis by macrophages. Upon oral exposure, absorption can occur after mechanical transfer of the particles deposited in the upper part of the respiratory tract. Oral adsorption is higher for water soluble salts, however other factors can influence the total adsorption. Cobalt tends to accumulate in the liver and kidney, but experimental studies have also indicated the heart, gastrointestinal tract or muscle, brain and testes as organs with possible accumulation of cobalt. After inhalation, elimination is faster for the more soluble compounds and happens via transfer to the blood and excretion via the urine and the faeces. Elimination via the faeces seems to correlate with the mechanical clearance from the lungs and is therefore more important for insoluble compounds than for those with higher solubility. After oral exposure, the primary elimination route is via the faeces.

[14] https://www.cobaltinstitute.org/about-us/contact/

7.2 Acute toxicity

7.2.1 Human data

7.2.1.1 Acute oral toxicity

DFG (2007) cites a case report of a mineral oil refinery worker who inadvertently received in his mouth a considerable quantity of cobalt phthalocyanine in powder form (Merox catalyst) when opening a bag containing it (Schulz, 1978). Five months later, a giant cell tumour developed in his mouth.

7.2.1.2 Acute dermal toxicity

There are no human data on acute toxicity via the dermal route.

7.2.1.3 Acute inhalation toxicity

Exposure to high concentrations of fumes of several metals can lead to acute pulmonary manifestations like metal fume fever or pneumonitis. Inhalation of dusts containing cobalt has also been linked to such effects (Nemery 1990). However, information on exposure levels is not available. The lung parenchymal hard-metal disease (further described in section 7.3.1.1) can also manifest as a relatively acute disease with sometimes a fatal outcome (Lison 1996, Nemery 1990).

7.2.2 Animal data

7.2.2.1 Acute oral toxicity

Acute toxicity of cobalt compounds was studied in Wistar rats; the lowest LD_{50} value was 42.4 mg/kg bw for cobalt dichloride and the highest was 3672 mg/kg bw for tricobalt tetraoxide, an insoluble salt. Cobalt carbonate LD_{50} was determined to be 317 mg/kg bw. Oral LD_{50} values of 89.3 and 123 mg/kg bw were estimated in male Swiss mice for cobalt dichloride and sulphate respectively, which correspond to 40 and 46 mg/kg bw of cobalt (ATSDR, 2004) (**Table 17**).

Table 17: Oral LD_{50} values

Species	Oral LD_{50} (mg/kg bw)	Oral LD_{50} (mg Co/kg bw)[#]	References
Wistar rats	42.4 $CoCl_2$	19.2	(ATSDR, 2004)
Wistar rats	3672 Co_3O_4	899	(ATSDR, 2004)
Wistar rats	317 $CoCO_3$	157	(ATSDR, 2004)
Swiss mouse	123 $CoSO_4$	46	(ATSDR, 2004)
Swiss mouse	89.3 $CoCl_2$	40	(ATSDR, 2004)

[#] values calculated by ECHA

7.2.2.2 Acute dermal toxicity

No mortality was reported in an acute dermal toxicity study with tricobalt tetraoxide (LD50 ≥ 2500 mg/kg bw (ECHA, 2022).

7.2.2.3 Acute inhalation toxicity

Sprague Dawley rats (3 sex/dose) were exposed via inhalation for 4h to 5000 mg/m^3 aerosol of eight cobalt compounds (cobalt metal powder, dihydroxide, monoxide, sulphate, carbonate, sulphide, tricobalt tetraoxide and cobalt lithium dioxide). If mortality was observed, five animals/sex were exposed to decreasing concentrations (1000, 500, 100 or 50 mg/m^3 MMAD for all study 1-4 µm) in a stepwise manner, until the LD_{50} or the lowest concentration was reached (Viegas et al., 2022). Exposure to cobalt sulphate was aimed to assess a time and concentration dependency of the irritation response, thus five Fischer F344 rats were exposed to different concentrations (0.1, 0.3, 1, 10 and 30 mg/m^3, MMAD for all study 1-4 µm), and based on the lack of any effects, a NOAEL of 1 mg/m^3 was determined. The LD_{50} values were <50 mg/m^3 for cobalt metal powder and cobalt dihydroxide, 50 mg/m^3 for cobalt monoxide, and above 5000 mg/m^3 for cobalt carbonate, sulphide, tricobalt tetraoxide and cobalt lithium dioxide. In addition, the authors scored the effects observed based on the inflammatory response (lung inflammation (inflammatory oedema (perivascular), alveolar pulmonary oedema and pneumonia) and upper respiratory tract reactivity in the larynx (squamous cell meta- or hyperplasia)) and followed it for 14 days post exposure. They concluded that there is no correlation between LD_{50} values and modelled lung deposition or cobalt content of the compounds. Based on their scoring, the group of substances considered as 'poorly reactive' (cobalt hydroxide oxide, cobalt sulphide, tricobalt tetraoxide, and lithium cobalt dioxide; see tiered inhalation approach in section 8.1.1 (Viegas et al., 2022)) do not induce pulmonary oedema or larynx squamous metaplasia but induce a low-grade perivascular oedema (Viegas et al., 2022).

7.2.3 *In vitro* data

No relevant data were found.

7.2.4 Summary

The human data on acute toxicity are limited and consist of occasional case reports in which exposure levels were not quantified.

In animals, the LC_{50} values after oral exposure ranged from 42.4 mg/kg bw for cobalt dichloride to 3672 mg/kg bw for the insoluble salt tricobalt tetraoxide, probably due to lower absorption. In rats, the LC_{50} via inhalation was measured between <50 to above 5000 mg/m^3, depending on the tested substance.

7.3 Specific target organ toxicity/Repeated dose toxicity

7.3.1 Human data

7.3.1.1 Parenchymal lung disease (from exposure to hard-metal or diamond polishing dust)

Hard-metal lung disease occurs in workers exposed to cobalt in association with tungsten carbide (hard-metal) (Lison (1996), Nemery (1990)). It also occurs in some other processes, such as diamond polishing where diamond discs cemented with cobalt are used, resulting in exposure to mixed particles including cobalt (Demedts et al. (1984), Nemery et al. (1992), Nemery et al. (2001)). Therefore, the term cobalt lung disease has also been proposed, although also that term is not ideal either as not all cobalt exposures are capable to produce the disease (Nemery et al., 2001). No cases have been documented in exposure settings other than those somehow related to hard-metal or diamond polishing. The disease is an interstitial pneumonitis characterised by radiographic infiltrates, reduced diffusing capacity and lung volume (Fischbein et al. (1992)) and a histology of fibrosing alveolitis with unusual multinucleated giant cells (Nemery et al., 1990, Lison, 1996). Fatal cases have been reported (Nemery et al. (1990), Ruokonen et al. (1996)) while the predictors of fatality are not clear. The clinical picture is variable, and the interstitial disease related to hard-metal exposure presents in at least two forms, an acute or

subacute alveolitis and a more latent form, characterized by progressive interstitial fibrosis (Lison, 1996).

As reviewed by Lison (1996) and Nemery (1990), while cobalt alone may lead to the development of occupational asthma, the exact role of cobalt in the pathogenesis of the lung parenchymal cobalt lung disease has long been debated. Involvement of the lung parenchyma has only been reported in clinical and epidemiological studies among hard-metal and diamond polishing workers, i.e., when cobalt is inhaled in association with other components, such as tungsten carbide or diamond dust. Parenchymal toxicity seems to be absent when exposure is to cobalt alone, apart from rare potential cases in exposure setting before and around second world war. Whilst animal models have proved useful for the demonstration of the toxic synergy between cobalt and carbides (e.g., tungsten carbide), most animal models have remained descriptive and have not provided information on the mechanism for this synergy. In particular, the bizarre multinucleated giant cells which are an important hallmark of the human disease, have not been reproduced consistently in experimental animals. Since cobalt is a known sensitiser, the possible involvement of immunological mechanisms in the pathogenesis of the parenchymal (interstitial) disease has been speculated yet remains unclear. Hard-metal disease has characteristics similar to chronic beryllium disease (CBD) and hypersensitivity pneumonitis (HP) where adaptive immune responses play a role (Adams et al. (2017), Nemery et al., (2001), (Nemery, 1990, Lison, 1996). Like CBD or HP, hard-metal disease occurs in only a small percentage of exposed workers and lacks a linear dose-response relationship with some indication that smokers would be less often affected than non- or ex-smokers (Lison, 1996, Nemery, 1990) Posgay et al. (1993), Nemery et al., (2001)).

In contrast to numerous case reports or clinical surveys, there is only a limited number of specifically designed epidemiological studies assessing the prevalence or incidence of interstitial lung disorders among hard-metal workers and the comparison of their results is hampered by the use of different criteria for the detection of interstitial lung disease (radiological and/or functional), by previous exposure to other agents causing lung fibrosis (silica or coal dust) and in some cases, by the lack of an unexposed control group. It is to be noted that both radiographic lung profusion scores according to the ILO pneumoconiosis classification (ILO, 2011) and lung function measurements typical of lung parenchymal disease are not specific to any given causative agent of the lung parenchymal disease (Lison, 1996).

Sprince et al. (1984) studied cross-sectionally 290 workers from two US hard-metal production plants. The study subjects represented a 19% sample of all workers of the plants. The selection was non-random and focused on those with the greatest potential exposures to cobalt. There was no control group unexposed to hard-metal dust. In plant A, 9/150 (6%) of workers showed radiological and/or lung function findings indicative of a lung parenchymal disease. The exposure duration was 21-35 years and all nine had worked in operations with peak exposure levels exceeding 500 µg/m^3. However, four of the nine subjects had also past employment in coal mines or foundries, indicating potential exposure also to other causative agents of lung parenchymal disease. In plant B, 2/140 (1.4%) of workers had interstitial lung infiltrates. Their duration of employment in hard-metal production was 23 and 35 years, respectively, and both had worked in operations with peak exposure levels exceeding 500 µg/m^3.

Sprince et al. (1988) later performed a cross-sectional study among 1039 hard-metal workers from 11 companies. The mean exposure duration was 7 years with a mean current airborne cobalt level of 48 µg/m^3 and a mean cumulative exposure of 370 µg/m^3 x year. The exposure assessment was based on measurements performed for each major process step at each plant (194 personal cassette samples for TWA calculation and 273 short-term samples with high volume samplers). Interstitial lung disease (ILD) was defined as a combination of (1): a chest X-ray ILO profusion score of $\geq$1/1, (2): forced vital capacity (FVC) or diffusing capacity for carbon monoxide (DLCO) of $\leq$70% of reference value and

(3): forced vital capacity in one second percentage (FEV1/FVC %) of at least 75%. Based on seven observed cases, the prevalence of ILD was 0.7%. The risk of an ILO profusion score of $\geq$ 1/0 was increased among those with an average life-time exposure of at least 100 µg/m^3 (OR 5.1, p = 0.029), compared to those with less than 100 µg/m^3. When comparing those with an average life-time exposure of at least 50 µg/m^3 with those with less than 50 µg/m^3, the risk was not significantly increased (OR 2.0, p = 0.29). Of the seven cases with ILD, three were estimated to never had been exposed to short-term cobalt levels above 50 µg/m^3 and their life-time average cobalt exposure levels were 3, 4 and 7 µg/m^3, respectively. The rest of the ILD cases had highest short-term exposure levels exceeding 300 µg/m^3 and life-time average exposure levels of 36 – 180 µg/m^3. No lung biopsies were available to assess the causal factors of the ILD and there was no control group unexposed to hard-metal dust.

Kusaka et al. (1986b) studied respiratory health in a cross-sectional study of 319 Japanese hard-metal production workers (282 still exposed and 37 with only prior exposure). Airborne cobalt concentrations in the different departments of the factory ranged from 3 to 1290 µg/m^3. Chest X-rays of 3 workers showed parenchymal small opacities with an ILO score of 1/1 or more. However, none of the cases was compatible with the clinical picture of hard-metal disease. In two cases, this was confirmed with lung biopsy and these subjects also had been exposed to other lung fibrosis causing dusts before employment in hard-metal production. The third case refused lung biopsy and the cause of the radiological parenchymal changes could not be confirmed. However, as there were no respiratory symptoms noted, rales were not heard in auscultation and pulmonary function tests were normal, a diagnosis of hard-metal disease was not suspected.

Kusaka et al. (1990) surveyed between 1981 and1990, all 700 Japanese hard-metal production company workers with or without exposure to hard-metal. The number of exposed workers and the level of exposure were unspecified. The authors identified four cases of radiographic interstitial fibrosis of ILO profusion score of 1/1 or more. Histological examination revealed the presence of cobalt and other metals in the lung tissue of the two cases who underwent a transbronchial biopsy. No giant multinucleated cells were found in the lung specimens. A lymphocyte transformation test was performed in one of the cases with a positive response to cobalt. No work-related respiratory symptoms or restrictive pulmonary function impairment was seen.

Meyer-Bisch et al. (1989) conducted a cross-sectional survey among 433 workers from three French hard-metal production factories. Comparison was made to a group of 88 workers not exposed to hard-metal dust. The exposed workers had an average duration of exposure of 14 years. Current mean airborne cobalt concentrations (4-hour samples) varied between 30 and 272 µg/m^3 and mean urinary cobalt levels between about 10 and 100 $\mu g_{Co}/g_{creatinine}$ in the various departments of the factories. Slight abnormalities of chest radiographs suggestive of parenchymal disease (ILO scores of 0/1 and 1/1) were more prevalent in exposed subjects than in controls (12.8 vs. 1.9%). This difference was maintained following correction for smoking habits. No dose-response analyses of prevalence of radiographic abnormalities by level of cobalt exposure were reported. The prevalence of ILD defined as a combination of abnormal radiographic profusion score and restrictive impairment in the pulmonary function tests was not reported.

Nemery et al. (1992) conducted a cross-sectional study among 194 diamond polishing workers, from 10 workshops, exposed to cobalt when using polishing disks made of microdiamonds cemented with cobalt and 59 control subjects from three other diamond industry workshops without cobalt exposure (diamond sawing or cleaving or jewellery drawing). The airborne cobalt concentrations were assessed with area samples. Personal samples were also collected from a few workers in each workshop. Urinary cobalt concentrations were measured. Based on airborne cobalt concentrations in the workshops, the exposed workers were further divided into low exposure (n= 102) and high exposure (n=92) groups. The mean airborne cobalt concentrations in area samples were 0.4, 1.6

and 10.2 µg/m^3 in control, low and high exposure groups, respectively and mean personal sample concentrations were 0.4, 5.3, 15.1 µg/m^3, respectively. Mean urinary cobalt concentrations were 2.1, 7.0, 20.5 $\mu g_{Co}/g_{creatinine}$, respectively. Chest X-rays to assess for any radiological abnormalities, indicative of an interstitial lung disease, were not taken. However, the study showed respiratory tract symptoms and small, but statistically significant effects on lung function in the high exposure group. These are further described in section 7.3.1.2.

Marsh et al. (2017b) conducted a pooled mortality follow-up among 32 354 hard-metal production workers from 17 manufacturing sites from five countries (see section 7.7.1 for further details). Special emphasis was on lung cancer risk, but some data on mortality from non-malignant respiratory diseases (NMRD) were also reported. There was a statistically significant excess in NMRD overall, when comparing to regional rates (SMR = 1.20; 95% CI 1.10 – 1.31), which was primarily due to an excess of emphysema (SMR = 1.44; 95% CI 1.10 – 1.86) affecting mainly workers with less than a year of employment in hard-metal production. Among workers with at least 1 year of employment, the mortality of NMRD was not increased (SMR = 0.93; 95% CI 0.82 – 1.05). Among these workers, there was no evidence of an occupationally-related risk for the NMRD subcategory 'other NMRD', in which had hard-metal disease and pneumoconiosis are contained (SMR = 0.99; 95% CI 0.76 – 1.26). The analyses were not presented separately by estimates of average or cumulative exposure to Co, W or overall hard-metal dust. However, exposure in the pooled cohorts ranged was from 1-300 µg Co/m^3, 3-300 µg W/m^3 and 1-30 µg Ni/m^3. The median values were 6 µg Co/m^3, 30 µg W/m^3 and 3 µg Ni/m^3. The ratio mean was 13 µg Co/m^3, 89 µg W/m^3 and 5 µg Ni/m^3. The ratio mean was calculated as the sum of cumulative exposure divided by the sum of exposure durations across all workers with known work history.

Hard-metal disease or cobalt lung disease (i.e., involvement of the lung parenchyma), have only been reported in clinical and epidemiological studies among hard-metal and diamond polishing workers, i.e., when cobalt is inhaled in association with other components, such as tungsten carbide or diamond dust. With regard to exposure to cobalt without such co-exposures, Linna et al. (2003) more recently performed a cross-sectional survey on the respiratory health of 110 cobalt production plant workers and 140 unexposed controls. Among the cobalt production workers, the mean duration of exposure was 22.1 years, and the mean cumulative exposure was 1.0 mg/m^3 x years. The average exposure intensity was thus 45 µg/m^3. Based on a respiratory questionnaire, lung function tests, chest X-ray and, when necessary, high resolution computed tomography, no one in the study group showed findings of hard-metal disease.

7.3.1.2 Irritation of the respiratory tract

Exposure to cobalt compounds is an established cause of occupational asthma and exposure to cobalt-containing hard-metal or diamond polishing dust is an established cause of parenchymal lung disease (hard-metal disease or cobalt lung disease). The epidemiological studies that have investigated such clinical entities are described in the sections of respiratory sensitisation (Section 7.5.1) and parenchymal lung disease (Section 7.3.1.1), respectively. While these diseases cause respiratory tract symptoms and may affect lung function (spirometry) parameters, several studies have also assessed the prevalence of respiratory tract symptoms and effects on lung function parameters, in populations with long-term exposure. The changes observed may represent either nonspecific irritating effects of cobalt-containing dust or an immunologically-mediated reaction and are both described in this section. It is noted that the study of Kusaka et al. (1986a) also includes a short-term exposure of 6 hours of healthy volunteers which is also described in this section together with the rest of the Kusaka et al. (1986a) results.

Hard-metal or diamond polishing exposure

Alexandersson and Bergman (1978) studied exposure levels, based on personal samples from the breathing zone, in four Swedish hard-metal production plants and Alexandersson (1979) subsequently performed a cross-sectional study on the prevalence of respiratory symptoms, self-reported in a physician's interview (details of the interview protocol were not disclosed). The prevalence of symptoms in six different occupational groups was compared to non-exposed office worker control groups from the same industries, matched for gender, age, height and smoking. The number of exposed-control pairs in the occupational groups varied from 29 to 63 and the mean cobalt exposure levels amongst the exposed in the six groups were 2, 3, 5-10, 8, 12 and 60 µg/m^3. The mean duration of employment in hard-metal industry was 4-11 years. The prevalence of irritative effects of the respiratory tract was higher among exposed persons than among their controls, although there was no clear dose-response for any of the symptoms studied. In the group with the lowest average exposure (2 µg/m^3) irritation of eyes, nose or throat ($p=0.008$) and cough with phlegm ($p=0.004$) were more common than in controls. The same irritation effects were also more prevalent in the group with a mean exposure of 3 µg/m^3 compared to their controls ($p=0.03$ and 0,04, respectively). The prevalence of irritation of eyes, nose or throat was significantly higher in each occupational group, while the prevalence of cough with phlegm was not increased among those with mean exposure level of 5-10 and 12 µg/m^3. Chronic bronchitis was significantly more prevalent in the highest (60 µg Co/m^3), but not in the lower exposure groups. Ventilatory function measurements were performed before shift on Mondays and Fridays and after shift on a Friday. The highest exposure group (60 µg Co/m^3) revealed significant impairment in pre-working week FEV_1 (forced expiratory volume in one second), FEV% (percentage of FEV_1 of the forced vital capacity, FVC), and MMF (maximum mid-expiratory flow) compared to their paired controls and a reduction of FVC, FEV_1, and MMF over the working week. In the group with a mean exposure of 12 µg Co/m^3, a tendency for impairment of the FVC compared to controls was seen and in those with a mean exposure to 8 µg Co/m^3, a decreasing trend of FEV_1 and MMF over the working week. No significant impairment of lung function parameters was found in the other exposure groups. It should be noted that exposure measurements were the most recent ones, performed within a couple of years (no further details given). As exposures were markedly higher in the past (Alexandersson and Bergman (1978) chronic symptoms may have been caused by earlier, higher exposures. A 5-year follow-up of 27 workers showed additional FEV_1 impairment in smokers. The mean exposure of these workers decreased from 80 to 30 µg Co/m^3 during this follow-up period (Alexandersson and Randma, 1986).

Kusaka et al. (1986a) studied respiratory irritation and ventilatory function in 15 healthy male volunteers without previous exposure to hard-metal dust. The men were exposed to hard-metal dust for 6 hours, seated at rest, in the shaping room of a hard-metal factory. Symptoms were recorded and lung auscultation and function tests were performed at the beginning and end of the exposure period. Control measurements to adjust for diurnal variation in lung function were also performed during a 6-hour period without exposure. The mean exposure during the exposure period was 38 (range 14 – 76) µg/m^3 cobalt and 800 (400 – 1600) µg/m^3 hard-metal dust. All study subjects complained of coughing, expectoration, or a sore throat during and after the exposure, but neither rales nor wheezing were observed. The FVC decreased slightly (- 100 ml) but significantly ($p<0.05$) after exposure to hard-metal, compared with the physiological diurnal change (+ 50 ml). There were no significant correlations between the decrease in FVC and the hard-metal concentration or the cobalt concentration. No changes were observed in other lung function parameters (e.g., airway obstruction parameters like FEV_1). The authors considered that the mechanism of the ventilatory change was probably the result of an irritant effect on the large bronchi. There was no exposure group that would have been exposed to cobalt alone, without the other components of hard-metal dust.

Kusaka et al. (1986a) also studied the same effects pre-shift and post-shift in 42 shapers and in a control group of 84 unexposed workers, matched for sex, age, height, and smoking habits. The mean duration of exposure to hard-metal for the 42 shapers was 10 (range 2-20) years. Four of them previously worked in departments other than shaping. Three of the shapers had occupational asthma related to hard-metal. None had interstitial pneumonitis. None of the shapers wore respirators on the day the respiratory function was measured. The mean exposure during the working day was 85 (range 17-610) $\mu g/m^3$ cobalt and 1400 (200-2400) $\mu g/m^3$ hard-metal dust. There were no significant changes in any lung function parameter over the working day in the shapers. However, compared to the controls, lung function parameters were lower in the shapers and the difference was statistically significant ($p<0.05$) for FEV1% (i.e., FEV_1/FVC). Qualitatively, it was reported that irritant symptoms, similar to those seen in the above study among 15 healthy volunteers, were also observed among the hard-metal workers during the years preceding the study and that several workers developed not asthma but irritation of the airways between a couple of days and a few weeks after they were first employed in the factory or were transferred to more dusty work. They recovered fully when they wore respirators or were transferred to non-dusty work. In some cases, respiratory symptoms disappeared spontaneously.

Nemery et al. (1992) conducted a cross-sectional study among 194 diamond polishing workers, from 10 workshops, exposed to cobalt when using polishing disks made of microdiamonds cemented with cobalt and 59 control subjects from three other diamond industry workshops without cobalt exposure (diamond sawing (N=15) or cleaving (N=10) or jewellery drawing (N=37), three subjects were excluded). Due to contamination with cobalt exposure in the originally planned control group, the final control group had to be recruited only after completion of the main study and was examined more than a year after the other groups. Consequently, in the jewellery drawing subgroup of the controls (63 % of all controls), no questionnaire was administered, and no workplace measurements were performed. In the other control and diamond polisher workshops the airborne cobalt concentrations were assessed with area samples. Also, personal samples were collected from a few workers in each workshop. Urinary cobalt concentrations were measured. Based on airborne cobalt concentrations in the workshops, exposed workers were further divided into low exposure (n= 102) and high exposure (n=92) groups. The mean airborne cobalt concentrations in area samples were 0.4, 1.6 and 10.2 $\mu g/m^3$ in control, low and high exposure groups, respectively and mean personal sample concentrations were 0.4, 5.3, 15.1 $\mu g/m^3$, respectively. Mean urinary cobalt concentrations were 2.1, 7.0, 20.5 $\mu g_{Co}/g_{creatinine}$, respectively. There were no statistically significant differences in prevalence or respiratory symptoms between the low exposure and control groups, while the high exposure group had statistically ($p < 0.05$) higher prevalence of the following symptoms: upper respiratory tract symptoms in general, work-related upper respiratory tract symptoms, cough in general and work-related cough. As regards phlegm, dyspnoea or wheezing, there was no difference in the prevalence in the high exposure and control groups. All groups had mean FVC and FEV_1 values at or above the values predicted from population reference values and there was no difference between the groups in the prevalence of abnormal values. However, both mean FVC, FEV_1 and MMEF (maximal mid-expiratory flow, or mean forced expiratory flow between 25 and 75% of the FVC) were slightly but statistically significantly lower in the high exposure group than in controls. In a two-way analysis of variance by cobalt exposure category (no, low, high) and smoking status (non-smoker, ex-smoker, smoker) there was a strong and consistent effect of smoking on lung function, but this did not cancel out the effect of exposure category, resulting in p values of < 0.01, < 0.001 and < 0.01 for smoking controlled for exposure category and < 0.02, < 0.02 and 0.06 for exposure category controlled for smoking, for FVC, FEV1 and MMEF, respectively. However, it is noted that an association between the intensity of Cobalt exposure and lung function was found only at the group level (i.e. not by correlating each individuals pulmonary function to his/her

cobalt exposure level and smoking) and there was no control for potential confounding by overall dust concentration or any specific component of it (e.g. diamond dust). Also, a covariance analysis of lung function against smoking status was performed, taking urinary cobalt concentration as a covariate by giving each subject the mean urinary cobalt excretion value of his or her workshop. The expected differences in lung function between smoking categories were found, but there were also significant correlations between FEV_1 or FVC and urinary cobalt. The study indicates small, but statistically significant effects on lung function at cobalt exposure levels of 15 µg/m^3, but not at 5 µg/m^3 (personal samples) that occurred in combination with diamond dust, when the comparison was made to workers with diamond dust exposure only. However, the exposure assessment and lung function measurements were cross-sectional and for example, the effect of past exposures (cobalt or other) could not be assessed. In addition, the covariance analysis described above, did not use urine cobalt values of the individual, but the mean of his or her workshop. It is also noted that although the control group had exposure to diamond dust (but no cobalt exposure), the intensity of diamond dust or total dust in the three groups was not characterized and consequently not adjusted for, in the analyses concerning lung function. Given that the observed effects on lung function were quite small (mean values in each group were within the general population reference values) and that after the redesign of the control group, the control group's exposure to diamond dust resulted from relatively different process activities such a control for potential confounding of workplace exposures other than cobalt would seem important. The only workplace exposures that were quantified were cobalt and iron and only cobalt was included in analyses. Due to the cross-sectional nature of the study the long-term predictive value of the small lung function changes observed at group level remains uncertain.

Kennedy et al. (1995) conducted a cross-sectional study among 118 saw filers in eight volunteer lumber mills in coastal British Columbia. The saw filers were exposed to cobalt containing hard-metal dust during maintenance operations involving e.g., operating machinery to solder or weld new tips to the saw body, grinding and sharpening tips, and occasionally soldering and welding tips manually. The objective was to determine whether or not these workers were exposed to excessive concentrations of cobalt or chromium, and if so, to evaluate the determinants of exposure. Consequently, no unexposed control group was originally included in the study protocol. However, the respiratory health survey results were afterwards compared to those obtained separately from bus mechanics that had been studied with the identical respiratory symptom questionnaire and pulmonary function testing procedures and equipment, but in a separate, earlier study. It is also to be noted that due to the voluntary participation of the saw filers, selection bias towards the more symptomatic or those more frequently exposed is possible. Nevertheless, the participation was quite complete as from the total of 131 filing room personnel eligible to participate, 118 took part (90%). The industrial hygiene measurements (personal sampling) showed detectable cobalt concentrations in 62 of the 278 air samples collected. Of the samples above the detection limit, the mean (SD, maximum) cobalt concentration was 9 (20, 106) µg/m^3 while it was reported that average level of cobalt exposure was about 5 µg/m^3 (presumably refers to arithmetic average of all samples, but not specified) Chromium was detected in 105 of the air samples with a mean (SD, maximum) of 4 (9, 55) µg/m^3. The analytical method used in this study did not distinguish the valency of chromium present. In comparison with the bus mechanics, the saw filers had more respiratory symptoms (phlegm, cough, wheeze, $p < 0.05$ for all). However, the analyses were not performed by level of exposure, nor adjusted for potential confounding by exposure to other workplace dust. There were no significant differences in FEV_1 or FVC between saw filers and bus mechanics after controlling for age, height, race, smoking and amount of smoking. In the internal comparison within the saw filer group, reduction of FEV_1 correlated with the level of estimated Co exposure and reduction of FVC with the duration of tungsten carbide exposure ($p< 0.05$, both), after controlling for age, height, race, smoking and amount of smoking and Co/tungsten carbide exposure. Again, potential

confounding by other workplace dust exposure was not adjusted for. The reduction in lung function was seen particularly among those performing wet grinding with a coolant.

Rehfisch et al. (2012) studied 582 Swedish hard-metal production workers with several spirometries available since 1982 and performed a follow-up spirometry in 2008-2009. On the basis of measurements performed in eight production departments and one administrative department, a job exposure matrix was constructed. Due to production changes and industrial hygiene improvements, measurements and work histories before and after 2000 were considered separately. The exposure in the matrix was defined in four different exposures: category 0, unexposed; category 1, mean cobalt concentration of less than 0.99 µg/m^3; category 2, mean cobalt concentration 1–49 µg/m^3; and category 3, mean cobalt concentration greater than 50 µg/m^3. A qualitative cumulative exposure index was then calculated summing up the products of exposure category (0,1,2,3) and years for each worker. The relationship between the estimated exposure and the change in FEV_1 over time was analysed using a linear mixed model. This regression model takes into account that individuals have repeated measurements over time for both exposure and outcome, and it allows for modelling of individual effects that are allowed to vary between individuals. In addition to cumulative exposure, the final model included gender, age, height, and smoking. In both smokers and non-smokers without asthma, a statistically nonsignificant, dose–response effect was seen between increasing cobalt exposure and decline in FEV_1. In all exposure categories, the FEV_1 in smokers declined 10 ml more per year than for non-smokers. The authors concluded that the amount of the annual decrease was low in relation to the effect of aging.

Andersson et al. (2020) studied the respiratory symptoms and pulmonary function among 72 Swedish hard-metal production plant workers. Inhalable cobalt and total dust exposures were measured as 8-hour TWA based on personal samples and correcting (when necessary) for the use of respiratory protection to estimate true exposure. Using existing historical data, cumulative exposure was also estimated. Respiratory symptoms were assessed with a questionnaire. In internal comparisons by tertiles, when those with a cumulative exposure to inhalable cobalt of >70 µg/m^3 x year and 20 – 60 µg/m^3 x year were compared to those with $\leq$20 µg/m^3 x year there was no significant dose-response in prevalence of either bronchitis symptoms or cough with phlegm when adjusted for age, smoking, gender and for the use of respirators. Similarly, when those with full-shift inhalable Co 8-hour TWA of 0.44–1.7 and $\geq$1.7 µg/m^3 were compared to those with <0.44 µg/m^3, there was no significant dose-response in the prevalence of these symptoms in the adjusted analysis. Additionally, the pulmonary function tests indicated a better lung function among those with higher cumulative cobalt exposure, especially for FVC and to lesser extent for FEV%, but the differences were not statistically significant. Of the early markers of general lung inflammation, there was no significant dose-response by cumulative cobalt exposure for the fraction of exhaled nitric oxide while the serum club cell secretory protein 16 (CC16) was significantly higher in the highest tertile of cumulative cobalt exposure but not in the middle one, when compared to the lowest tertile.

Other cobalt exposures

Raffn et al. (1988) performed a cross-sectional study among 46 plate painters, heavily exposed to cobalt blue dye in a porcelain factory and 51 unexposed top-glaze painters from the same factory. They also studied the plate painters twice, first after a 6-week exposure free period and second time a few weeks after they resumed work. The study comprised among others a questionnaire, a health examination and a lung function test. The results of the questionnaire showed that the plate painters complained significantly more than the top-glaze painters of itching skin, irritation of the nose, mouth and throat, and of cough and expectoration (no quantitative data or significance value reported). Furthermore, the plate painters reported more dyspnoea during work and on exertion. The mucous membrane symptoms increased after the plate painters resumed work. There was no difference between the two groups concerning symptoms of bronchitis. The pattern of

lung function changes among the non-smoking and smoking plate painters indicated obstruction for both groups, but the changes seemed more pronounced for the non-smokers, especially for flow rates at 50% and 25% of vital capacity (MEF_{50}, and MEF_{25}). However, the differences between the two groups were significant only for MEF_{25}. When comparing the lung function tests of the plate painters in the two examinations, the peak expiratory flow PEF and FEV_1 increased significantly from the first to the second examination, and the MEF_{50}, and MEF_{25} (flow rates at 25% and 50% of vital capacity) decreased significantly. The mean duration of employment was 11 years (range 2-25), for the plate painters. The results were not correlated to airborne cobalt levels, but it was reported that seven months before the examinations, the median concentration of airborne cobalt in the breathing zones of the plate painters was 16 times the Danish occupational exposure limit for cobalt of 50 µg/m^3, while industrial hygiene improvements were made thereafter, and measurements conducted one month after the end of the study showed airborne cobalt levels just around 50 µg/m^3. No adjustment for potential confounding by any other workplace exposure was made.

Swennen et al. (1993) studied respiratory symptoms and lung function among 82 workers exposed to cobalt oxides, cobalt salts, and cobalt metal in a cobalt production plant and compared them with 82 age matched control workers not exposed to lung irritants, recruited in the mechanical workshops of a nearby plant belonging to the same company. The exposed group had been exposed for 8 years on average (range 0.3-39) and the geometric mean current 8-hour TWA cobalt exposure assessed with personal samplers was about 125 (range 1-7800) µg/m^3 and 25% of the values were higher than 500 µg/m^3. The respiratory symptoms studied were mostly those more closely linked with asthma than with irritation as such. Cobalt exposed workers complained more often of daily wheezing (6.1% vs. 0%, $p<0.05$) and dyspnoea (e.g., dyspnoea during normal exercise 12.1% vs 2.4%, $p<0.05$). Wheezing was also more commonly observed in the clinical examination (16% vs 6%, $p<0.05$). No statistically significant differences were observed in lung function measurements between the exposed and controls. However, within the exposed group, a dose-effect relation was found between the reduction of the forced expiratory volume in one second divided by vital (FEV_1/VC) capacity and the intensity of current exposure to cobalt assessed by the measurement of cobalt in the air or in urine ($p<0.01$ for both). It is noted that the control group was "not exposed to lung irritants". However, it is not further explained how the lack of such exposure could be excluded in a population was recruited from a mechanical workshop. A logistic regression model was used to assess the probability of abnormal results as a function of the exposure variables. Only dyspnoea during exercise was found to be related to current concentrations of Co in air. Based on a graphical presentation of this association a 10% probability of dyspnoea at exercise is predicted at about 60 µg/m^3 of Co.

Verougstraete et al. (2004) performed a longitudinal analysis in the same plant. Male workers (n= 122) subjected to at least three lung function measurements between 1988 and 2000, as part of their heath surveillance programme were studied. An additional spirometry was performed in 2001. In conjunction with each lung function test, smoking habits had also been recorded and an end of week urine cobalt measurement had been performed. Changes in height and weight adjusted FEV_1 and FVC between each lung function measurement were analysed, according to smoking status. In the best-fit model, age at baseline and time elapsed since the first lung function test, significantly and negatively influenced FEV_1 and FVC values over the years. Cobalturia contributed significantly to the deterioration of FEV_1, but only in association with smoking. No influence of cobalturia on FVC was observed. The interaction between cobalturia and time was not a significant determinant either of FEV_1 or FVC. Data in the cross-sectional 2001 measurements did not reveal differences in FEV_1 or FVC between the three exposure patterns in the plant, despite marked differences in air borne cobalt exposure levels (dry-stage area; mixed exposure; wet-stage area). However, these results were reported only qualitatively and there was also no comparison to an unexposed reference group. As

regards the statistically significant association between urine Co level and FEV_1 decline in smokers observed in the fitted statistical model the authors reported that "a cobalturia of 10, 20, or 40 µg/g creatinine, corresponding roughly to a time-weighted average exposure at 10, 20 or 40 µg/m^3 would cause, for a 30-year-old smoking worker, an additional decrement of 64, 84 or 103 ml of FEV1 after 10 years of work. The amplitude of this additional decrement is, however, relatively small compared with the expected decline, 518 ml, in a smoking subject over the same period in the absence of occupational exposure."

Linna et al. (2003) performed a cross-sectional respiratory health survey among 110 cobalt production plant workers and 140 unexposed controls, both having an employment duration of at least 10 years. The prevalence of chronic bronchitis was higher among the exposed, but the difference was not statistically significant. Of the respiratory tract symptoms, a statistically significant increase in prevalence was reported for phlegm at least 3 months a year, cough with wheezing, dyspnoea with wheezing and breathlessness on exertion, but not for cough for 3 months a year. The results remained the same also after the multivariate analysis in which the effect of age and smoking was taken into account. The risks were not explored by level of exposure. However, among the cobalt production workers, the mean duration of exposure was 22.1 years and mean cumulative exposure 1.0 mg/m^3 years. The average exposure intensity for cobalt was thus 45 µg/m^3. The exposed workers also had a cumulative exposure of 400 µg/m^3 years of nickel compounds, 7.5 ppm-years of H_2S, 3.7 ppm-years of SO_2, 27 ppm-years of NH_3 and 19400 µg/m^3 years total dust. Further multivariate analyses were performed in the exposed group and exposure to any of the specific chemicals (including cobalt) did not have a significant effect on increasing the risk of any of the studied respiratory symptoms. As regards ventilatory function analyses, smokers had lower lung functions in both the exposed and the control groups. The FEV_1, MEF_{50}, and MEF_{25} values of the smokers in the exposed group were lower than those of the smokers in the control group. In the analysis of variance, the difference in the MEF_{50} and MEF_{25} values remained when smoking (packyears) was included as a covariate in the model. Among the non-smokers, none of the values of the measured lung function parameters differed between the groups. In the analyses of variance, smoking was the only significant factor explaining lower lung functions; no above-mentioned occupational chemical exposure alone (including cobalt), or in a combination, had any significant effect on lung function.

7.3.1.3 Cardiac effects

As reviewed by Leyssens et al. (2017) the cardiotoxic potential of cobalt was first discovered in the 1960s when heavy beer drinkers presented with symptoms of cardiomyopathy, which was attributed to the use of cobalt chloride or cobalt sulphate as foam stabilisers in beer. However, it is likely that the poor nutritional status of these subjects and the alcoholism itself were contributing factors for the cardiac effects, rendering the persons more susceptible to systemic cobalt toxicity. According to Packer (2016), the clinical emergence of cobalt cardiomyopathy requires the coexistence of one or more cofactors, particularly a low-protein diet, thiamine deficiency, alcoholism, and hypothyroidism. Cobalt cardiomyopathy has also been described among individuals with metal-on-metal hip implants.

There have also been rare cases of cardiomyopathy among hard-metal or other cobalt exposed workers. Although it remains somewhat uncertain whether these isolated reports represent occurrence of an idiopathic congestive cardiomyopathy in workers exposed to cobalt or whether occupational exposure was a real contributing factor (Lison 1996). Kennedy et al. (1981) described a fatal case in a man who had worked for 4 years in a company making hard-metal cutting tips for drills, bits, and dies. Industrial hygiene measurements from the plant indicated exposures "well in excess" the limit value of 100 µg/m^3 and urinary cobalt values in the colleagues of the deceased worker indicated values between 60 and 300 $\mu g_{Co}/g_{creatinine}$. Jarvis et al. (1992) described two cases who had

worked in sample preparation of mineral assay laboratories where the work included, among others, crushing of cobalt ores. No workplace air cobalt concentration measurements or biological monitoring results were reported. In a group of 30 hard-metal processing plant workers, a weak but still significant inverse correlation was found between exposure time and the left ventricular ejection fraction as measured by radionuclide ventriculography (Horowitz et al., 1988). The level of cobalt exposure was not reported but the mean duration of employment in hard-metal work was 10 years.

Sjogren et al. (2020) assessed the occupational chemical exposures and cardiovascular disease. The epidemiological studies regarding cobalt exposure and cardiac effects mainly consisted of hard metal worker cohorts. Sjogren et al. (2020) concluded that "*The majority of cohorts comprising cobalt-exposed hard metal workers did not exhibit an increased risk of circulatory diseases or ischaemic heart disease. Cardiomyopathy has been described after intake of cobalt-containing beer and after inhalation of high cobalt dust concentrations. However, despite a large number of exposed industrial workers, such reports are exceptionally rare. Signs of cor pulmonale have been observed as a result of fibrotic lung disease".* Overall Sjogren et al. (2020) concluded that "*There is insufficient evidence for an association between exposure to cobalt and cardiovascular disease"*.

Some individual studies are further described below.

Studies in workers with exposure to hard-metal

Alexandersson and Atterhög (1980) and Alexandersson and Atterhög (1983) compared 42 dry grinders, 43 wet grinders and 61 powder handlers with 126 controls. The mean Co exposures of the groups were 10 µg Co/m^3 for both the dry and the wet grinders, 60 µg Co/m^3 for the powder handlers and no exposure for the control group. Only the wet grinders had ST- and T-depressions in the electrocardiogram (ECG) with an over-frequency of ectopic beats but they had no pulmonary dysfunction. In the other groups, no effects on the heart function were found. The small ECG changes in the wet grinders had disappeared after 4 weeks of vacation (Alexandersson and Atterhög, 1983).

D'Adda et al. (1994) studied cardiac function of a group of 31 hard metal workers with or without pulmonary disease and with mean exposure duration of 10.4 years and mean exposure levels of 9 to 13 600 µg Co/m^3. The most evident finding concerned abnormality of the right ventricular function observed in the patients could be a manifestation of initial pulmonary artery hypertension or of early occult cor pulmonale due to fibrotic lung disease.

Studies in workers with other cobalt exposure

Linna et al. (2004) compared in 1999-2000, echocardiographic parameters in a cross-sectional study of cobalt powder production plant workers and unexposed referents. There were 55 workers with high cumulative exposure (> 470 µg/m^3-years), 54 with low exposure (< 470 µg/m^3-years) and 57 unexposed controls. The potential explanatory factors included cumulative exposure, age, blood pressure, smoking status, overuse of alcohol, and physical activity. In the adjusted analysis two of the 16 echocardiography parameters measured were associated with cobalt exposure. In the high exposure group, the left ventricular isovolumic relaxation time (mean 53.3, 49.1, and 49.7 ms in the high exposure, low exposure, and control groups, respectively, p=0.01) and the deceleration time of the velocity of the early rapid filling wave (mean 194.3, 180.5, and 171.7 ms for those in the high exposure, low exposure, and control groups respectively, p=0.001) were prolonged, indicating altered left ventricular relaxation and early filling. There were no significant differences in the rest of the echocardiographic parameters or ECG findings or conduction parameters between the exposed and unexposed.

Linna et al. (2020) re-examined 93 of the exposed and 49 of unexposed after a 6-year follow-up in 2006. Blood pressure was measured, and ECG, laboratory tests, 24-hour

Holter registration of ECG, and echocardiography were conducted for all participants. No differences were found between the exposed and unexposed groups for any of the echocardiographic parameters in 2006. There were no differences in the laboratory test values, the ECG parameters, or the results of the Holter registration of the exposed and unexposed workers. Unlike in 1999-2000, in the 2006 follow-up echocardiographs, the left ventricular isovolumic relaxation times (mean 66.9, 68.5, and 69.6 ms were similar in the high exposure, low exposure, and control groups, respectively, p = 0.602) and so were the deceleration times of the velocity of the early rapid filling wave (mean 187.4, 183.4, and 193.9 ms for those in the high exposure, low exposure, and control groups, respectively, p=0.117). After the 6-year follow-up, the high cumulative exposure consisted of >550 µg/m^3-years and low of exposure <550 µg/m^3-years. Four employees from the earlier exposed group and one from the unexposed group had died. No deaths from cardiomyopathy had occurred. The cause of death of one of the exposed workers was heart infarction. The deaths of the other deceased workers were not related to cardiovascular diseases. The 6-year follow-up examinations did not confirm the associations observed earlier.

Raffn et al. (1988) performed a cross-sectional study among 46 plate painters, heavily exposed to cobalt blue dye in a porcelain factory and 51 unexposed top-glaze painters from the same factory. The study comprised a questionnaire, a health examination, a lung function test, and the determination of the blood and urinary cobalt levels. Regarding cardiac health, ECGs were analysed, blood pressure was measured, and heart auscultation performed. There were no significant differences in the ECG parameters or blood pressure between the exposed and unexposed. The pulse rate was higher among the exposed but was not associated with the cobalt levels in the blood or urine. The mean duration of employment was 11 (range 2-25) years for the plate painters. It was reported that seven months before the examinations, the median concentration of airborne cobalt in the breathing zones of the plate painters was 16 times the Danish occupational exposure limit for cobalt of 50 µg/m^3 while industrial hygiene improvements were made thereafter, and measurements made one month after the end of the study showed airborne cobalt levels just around 50 µg/m^3.

7.3.1.4 Haematological effects

As reviewed by Leyssens et al. (2017) and ATSDR (2004), cobalt has a known stimulant effect on the red blood cell (RBD) production and it has also been used as treatment for anaemia. Exposure to cobalt compounds may thus increase the RBC count (polycythemia), the haematocrit and haemoglobin levels which normalise within few weeks after cessation of exposure. There are also anecdotal case reports of polycythemia in metal-to-metal hip implant patients. A few studies have also investigated the occurrence of haematological effects in the occupational setting, after inhalation exposure and are described below.

Lantin et al. (2011) conducted in 2008, a cross-sectional survey among 249 male workers from a Belgian cobalt production department. Cobalt in blood and cobalt in urine were used as measures of current exposure. Samples were taken in the afternoon of the fourth or fifth day of the work week. In addition, an integrated exposure index based on an individual's all urine samples was calculated to reflect cumulative exposure and expressed as $\mu g_{Co}/g_{creatinine}$ x years. As outcome variables, white blood cell count (WBC), haemoglobin concentration (Hb), haematocrit (Htc) and red blood cell count (RBC) were measured on whole blood. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the measured values. In the multiple regression analyses, the following confounding factors were included: age, physical exercise, smoking and alcohol intake. In the multivariate model adjusted for potential confounders, urine or blood cobalt did not correlate with any of the outcome variables, while a negative effect of integrated exposure index (IEI) was observed on MCHC. However, in neither of the two component measures used to calculate this parameter (Hb and Htc), an effect correlation with IEI was seen. The airborne cobalt

concentrations in 2007 ranged from 1 to 108 µg/m^3 (no mean or median reported), while earlier measurements were not directly reported but had been higher (see earlier study below). The median duration of employment was 12 (range 1 – 36) years. The urinary cobalt concentration $\mu g_{Co}/g_{creatinine}$ showed a median of 3.9, mean 4.4 and range 0.3 – 204.

In an earlier study from the same plant, Swennen et al. (1993) reported cross-sectional results from a health survey of 82 male workers and a control group of 82 age-matched unexposed workers. Between 1992 and 2001, cobalt exposure in the plant had sharply declined and urine cobalt values followed the same pattern (Verougstraete et al., 2004). In the Swennen et al. (1993) study, the geometric mean of the end of week post-shift urinary cobalt levels among the exposed group was 70 $\mu g_{Co}/g_{creatinine}$. It was reported that the exposed group had been exposed for 8 years on average (range 0.3 - 39) and that the geometric mean current 8-hour TWA cobalt exposure assessed with personal samplers was about 125 µg/m^3 and 25% of the values were higher than 500 µg/m^3. The following outcome variables were analysed: RBC, WBC, Hb, Htc, MCV, MCH, MCHC and platelet count. RBC, Hb, Htc were statistically significantly lower in the cobalt exposed group whereas the WBC count was significantly higher ($p < 0.05$ for all). It is noted that the lower RBC, Hb, Htc values in the exposed is at odds to the known haematopoietic effects of cobalt.

Raffn et al. (1988) performed a cross-sectional study among 46 plate painters, heavily exposed to cobalt blue dye in a porcelain factory and 51 unexposed top-glaze painters from the same factory. They also studied the plate painters twice, first after a 6-week exposure-free period, and a second time a few weeks after they resumed work. The study comprised a questionnaire, a health examination, a lung function test, and the determination of the blood and urinary cobalt levels. As regards haematological outcomes, RCB, Hb, Hct, MCHC and MCV were analysed. There were no statistically significant differences between the plate painters in the first examination and the referents. In the second examination of the plate painters, the Hct decreased from 0.41 to 0.40 and the mean MCV decreased from 93.2 to 92.0 fl. These small changes, however, were statistically significant ($p < 0.01$). The Hb and RBC decrease, and the MCHC increase were of borderline significance ($0.07 < p < 0.12$), but these changes were also very small. These changes did not correlate with those in the concentration of cobalt in the blood or urine. The mean urine concentration of cobalt in controls, plate painters at the end of a work-free period and after resuming work, were 0.80, 4.6 and 74 $\mu g_{Co}/g_{creatinine}$, respectively and the blood cobalt concentration means 4.0, 8.1 and 37 mml/l, respectively. The mean duration of employment was 11 (range 2-25) years for the plate painters. It was reported that seven months before the examinations, the median concentration of airborne cobalt in the breathing zones of the plate painters was 16 times the Danish occupational exposure limit for cobalt of 50 µg/m^3, while industrial hygiene improvements were made thereafter, and measurements made one month after the end of the study showed airborne cobalt levels just around 50 µg/m^3.

7.3.1.5 Thyroid gland effects

As reviewed by Leyssens et al. (2017), thyroid-related changes from cobalt have been observed in the non-occupational setting both after oral exposure and exposure from hip implants. Autopsy of the previously mentioned beer drinkers with cardiomyopathy often revealed thyroid changes. Furthermore, numerous human studies have reported endocrine effects (e.g., goitre development and reduced iodide uptake) in subjects treated orally with cobalt for anaemia in the 1950s and 1960s. The average daily doses (25 to 300 mg $CoCl_2$/day) were considerably high and often taken for several months. Additionally, chronic thyroiditis, disturbance of the thyroid hormone metabolism (mostly hypothyroidism) and a reduced thyroid volume have been described in individuals exposed to cobalt from metal-on-metal hip implants. A few studies have also investigated the occurrence of thyroid-related effects in the occupational setting, after inhalation exposure and are described below.

Lantin et al. (2011) conducted in 2008 a cross-sectional survey among 249 male workers from a Belgian cobalt production department. Cobalt in blood and cobalt in urine were used as measures of current exposure. Samples were taken in the afternoon of the fourth or fifth day of the workweek. In addition, an integrated exposure index based on an individual's all urine samples was calculated to reflect cumulative exposure and expressed as $\mu g_{Co}/g_{creatinine}$ x years. As outcome variables, thyrotropin (TSH), free thyroxin (FT4), free triiodothyronine (FT3), total thyroxin (T4) and total triiodothyronine (T3) were measured in serum. In the multiple regression analyses, the following confounding factors were included: age, physical exercise, smoking, alcohol intake and ethnicity. No exposure variable correlated significantly with any of the outcome variables either in the unadjusted model or in the model adjusted for potential confounders. The airborne cobalt concentrations in 2007 ranged from 1 to 108 $\mu g/m^3$ (no mean or median reported), while earlier measurements were not directly reported but had been higher (see earlier study below). The median duration of employment was 12 (range 1 – 36) years. The urinary cobalt concentration $\mu g_{Co}/g_{creatinine}$ showed a median of 3.9, mean 4.4 and range 0.3 – 204.

In an earlier study from the same plant, Swennen et al. (1993) reported cross-sectional results from a health survey of 82 male workers and a control group of 82 age-matched unexposed workers. Between 1992 and 2001, cobalt exposure in the plant had sharply declined and urine cobalt values followed the same pattern (Verougstraete et al. 2004). In the Swennen et al. (1993) study, the geometric mean of the end of week post-shift urinary cobalt levels among the exposed group was 70 $\mu g_{Co}/g_{creatinine}$. It was reported that the exposed group had been exposed for 8 years on average (range 0.3 - 39) and that the geometric mean current 8-hour TWA cobalt exposure assessed with personal samplers was about 125 $\mu g/m^3$ and 25% of the values were higher than 500 $\mu g/m^3$. As outcomes, TSH, T3, T4 and T3 uptake were measured. Mean T3 level was statistically significantly lower in the exposed group ($p<0.05$), while there were no significant differences in the mean of the other thyroid parameters. However, the percentage of abnormal values was significantly higher among those exposed for TSH, T4 and T3 uptake ($p<0.05$). For T3 the abnormal values were also more frequent among the exposed (7.3% vs. 1.2%) but did not reach statistical significance (only one abnormal value among the non-exposed).

Prescott et al. (1992) conducted in 1988 a cross-sectional study on 61 female plate painters exposed to cobalt blue dyes in two Danish porcelain factories (36 and 25 exposed participants) and 48 unexposed referents. The first factory used cobalt aluminate, an insoluble form of cobalt, and the second used cobalt-zinc silicate, a semi-soluble form of cobalt. Urinary cobalt was used as the exposure metric and measured from samples taken in the afternoon of the 4th day of the working week (reported as μg_{Co}/mmol $_{creatinine}$, but here converted to $\mu g_{Co}/g_{creatinine}$·for the ease of comparison). As outcome variables, TSH, FT4, FT3, T4 and T3 were measured in serum. Also, the volume of the thyroid gland was determined by ultrasonography. In factory 1 using an insoluble cobalt compound, the mean urinary cobalt concentration was only slightly higher than in controls (1.77 vs. 1.15 $\mu g/g_{creatinine}$) and there was no significant difference in any thyroid parameter when compared to the controls. In factory 2 using a semi-soluble cobalt compound, the urinary cobalt concentration was significantly higher than in controls (10.4 vs. 1.15 $\mu g/g_{creatinine}$, $p<0.0001$). However, these subjects had increased not decreased levels of serum T4 and FT4 ($p=0.0001$ and 0.0029, respectively), unaltered serum TSH and T3, and marginally reduced FT3 ($p=0.08$), whereas thyroid volume tended to be lower ($p=0.14$). The authors concluded that the study did not demonstrate any inhibitory effect of cobalt on thyroid function, and it did not suggest cobalt to be a goitrogen at the measured level of urinary concentration. Industrial hygiene measurements in 1987-1988 showed cobalt concentrations around 50 $\mu g/m^3$ in the two factories.

7.3.1.6 Nervous and sensory effects

As reviewed by Leyssens et al. (2017) cobalt-related neurotoxicity may cause peripheral as well as central nervous system deficits. A variety of symptoms have been described,

related to hearing and balance, cognitive function, and sensory and motor performance. Moreover, these symptoms often coincide with polyneuropathy. The evidence is mainly based on exposure from metal-on-metal hip implants. Only few small studies have investigated occurrence of neurological effects in the occupational setting after inhalation exposure and are described below.

Botelho et al. (2009) studied steel workers (aged 18-50 years), exposed to noise (group I, 81 workers), and exposed to noise and chemical products (group II, 71 workers) for a period that varied from 3 to 20 years. The chemicals considered for group II were acetone, styrene, resins, and cobalt and "others of less relevance" which were not further specified. The prevalence of impaired hearing in left, right, or either ear, were all higher in group II. However, the data were not presented separately for the chemicals considered, neither was the fraction of workers that had been specifically exposed to cobalt reported.

Meecham and Humphrey (1991) presented a case report of a 48-year-old man with total bilateral neuronal deafness after 20 months of work with exposure to cobalt powder. Despite wearing a mask, inhalation exposure had occurred. No air concentration measurements were reported, while the blood cobalt was elevated (234 µg/l, normal value reported as < 2 µg/l) and the urine 24-hour cobalt was also increased (119 µg/24h, normal value reported as < 51 µg/24h). The patient also had optic atrophy. The man stopped working with cobalt and his hearing started to improve. The audiograph two years later indicated normal values for frequencies 500-2000 Hz while for higher frequencies both air and bone conductive hearing was still decreased.

Jordan et al. (1990) examined memory functioning on the Wechsler Memory Scale-Revised in a group of 12 adult, former hard-metal workers who were exposed to hard-metals consisting primarily of tungsten carbide and cobalt, in both a dust powder form and in a mist form with an organic solvent as the vehicle. It is noted that bias seems to have resulted from the fact that from among the hard-metal disease patients, only those who had memory complaints were selected for the study, while there was no similar selection among the controls. Therefore, the study is not described further.

7.3.2 Animal data

Several repeated dose studies have been performed in animals and demonstrate that cobalt is toxic and can be lethal depending on the administered doses.

7.3.2.1 Lethality

All five F344/N rats and five B6C3F1 mice exposed to cobalt sulphate (19 mg cobalt/m^3) as aerosol for 16 days, 6 h/d, 5 d/week died. The same NTP group exposed groups of 10 animal/sex (F344/N rats and B6C3F1 mice) for 13 weeks (6 h/d, 5 d/week, MMAD 1.61-2.00 µm); at the high dose of 30 mg/m^3 only two male mice died (Bucher, 1991, ATSDR, 2004).

In an NTP repeated dose study, F344/N rats were exposed to cobalt metal particles at doses of 0, 2.5, 5, 10, 20 or 40 mg/m^3 for 6 h/d 5 d/week, for 16 days (MMAD 1.79-1.94 µm). All rats exposed to the highest dose, and all male and three females exposed to 20 mg/m^3 died before the end of the study (NTP, 2014). In the correspondent study in B6C3F1/N mice, five animals/sex/dose were exposed to cobalt metal particles at doses of 0, 2.5, 5, 10, 20 or 40 mg/m^3 for 6 h/d 5 d/week, for 17 days. Three males and three females died at the high dose (NTP, 2014).

Oral administration of cobalt sulphate (20 mg cobalt/kg bw/d) by gavage for 5 weeks resulted in the death of 20-25% of exposed guinea pigs, while co-exposure to alcohol did not increase the lethality rate (Mohiuddin et al., 1970, ATSDR, 2004).

In a combined repeated dose toxicity study with reproduction/developmental toxicity screening test according to the OECD guideline 422, Sprague Dawley rats (10/sex/dose)

received cobalt powder (0, 30, 100, 300 or 1000 mg/kg bw/day) by gavage from 2 weeks before mating for 4 weeks (males) or until PND3 (females). All females and 9 out of 10 males died at the highest dose, 8 and 5 females also died at 300 and 100 mg/kg bw/day (ECHA, 2022, RAC, 2017).

7.3.2.2 Respiratory tract effects

A large number of the guinea pigs exposed to cobalt sulphate by gavage for 5 weeks developed tachypnea (Mohiuddin et al., 1970, ATSDR, 2004).

Lesions in the alveolar region, interstitial inflammation and fibrosis were observed in the lungs of rats and rabbits after 3-4 months of exposure to mixed cobalt oxides in concentrations between 0.4 to 9 mg cobalt/m^3 (ATSDR, 2004). Sensitised and non-sensitised guinea pigs were exposed to cobalt dichloride (2.4 mg cobalt/m^3), with the inflammatory changes observed in the lungs were different between the two groups (Camner et al., 1993). Hamsters exposed to cobalt monoxide aerosol (7.9 mg/m^3, 7h/d 5/d/week) developed emphysema (Wehner et al., 1977). No information on the MMADs is available.

Cobalt dichloride was administered for 3 months via drinking water to 20 Sprague-Dawley male rats (30.2 mg cobalt/kg bw/d) and resulted in a significant increase of lung weight in the absence of histological or morphological changes (Domingo et al., 1984, ATSDR, 2004).

In a 16-day NTP experiment, rats and mice exposed to aerosols of cobalt sulphate (19 and 1.9 mg cobalt/m^3, respectively, MMAD 1.79-1.94 µm) developed necrosis and inflammation of the nasal cavities, larynx, trachea and bronchioles. In rats and mice exposed to cobalt sulphate aerosols for 13 weeks at concentrations of 0, 0.3. 1, 3, 10 or 30 mg/m^3 corresponding to approximately 0, 0.11, 0.38, 1.14, 3.8 or 11.4 mg cobalt/m^3, degeneration of the olfactory epithelium, inflammation and necrosis on the respiratory tract in all parts were the main findings. Metaplasia in the larynx and histiocytic infiltration were detected in all exposed groups. In rats, it was the chronic inflammation of the larynx to be first observed at 0.38 mg cobalt/m^3, while in mice it was the nose inflammation at 1.14 mg cobalt/m^3. The effects extended to other parts of the respiratory tract and increased in severity with increasing doses in both species (Bucher, 1991).

In the chronic inhalation NTP study (see also section 7.7.2), F344/N rats and B6C3F1 mice were exposed to cobalt sulphate aerosol at doses of 0, 0.3, 1.0, or 3.0 mg/m^3 for 6 h/d 5 d/week for 104 weeks (MMAD 1.2-1.8 µm). Non-neoplastic lesions occurred in all exposed groups of mice (≥0.3 mg/m^3; unless stated otherwise) and included inflammation of the lungs, nose and larynx, atrophy of the olfactory epithelium (≥1 mg/m^3), hyperplasia (3mg/m^3) in the nose, metaplasia (squamous) in the larynx (, fibrosis, several types of alveolar lesions, and histiocytic infiltration (3 mg/m^3) (Bucher et al., 1999, NTP, 1998).

In an NTP repeated dose inhalation study, F344/N rats (5/sex/dose) were exposed to cobalt metal particles at doses of 0, 2.5, 5, 10, 20 or 40 mg/m^3 for 6 h/d 5 d/week for 16 days (MMAD 1.79-1.94 µm). Dark lungs were observed at necropsy in all early-death rats of both sexes exposed to 40 mg/m^3 and most rats exposed to 20 mg/m^3. Pale lungs were noted in two females (20 mg/m^3), four males (10 mg/m^3), and one male at 5 mg/m^3. Absolute and relative lung weights were significantly increased, and increased incidences of lesions of the lung occurred in exposed male and female rats and included haemorrhage, acute inflammation, alveolar epithelium hyperplasia, histiocytic cellular infiltration of the alveolus, cytoplasmic vacuolization of bronchiolar epithelium, necrosis of the bronchiolar epithelium, and interstitial fibrosis of the alveolar epithelium were detected. The authors reported increased incidences of nasal lesions including olfactory epithelium necrosis, olfactory epithelium atrophy, respiratory epithelium necrosis, and respiratory epithelium squamous metaplasia (NTP, 2014). In the correspondent study in B6C3F1/N mice, 5 animals per sex per dose were exposed to cobalt metal particles at doses of 0, 2.5, 5, 10,

20 or 40 mg/m^3 for 6 h/d 5 d/week for 17 days (MMAD 1.79-1.94 µm). Tan lungs were observed in most animal treated at or above 20 mg/m^3. One of the males that died before the end of the study had dark lung lobes. Significant increase of lung weights was recorded in both sexes exposed at or above 10 mg/m^3. In all exposed animals an increased incidence of non-neoplastic lung lesions were reported, and those included alveolar histiocytic cellular infiltration, cytoplasmic vacuolization of the bronchiolar epithelium, alveolar/bronchiolar epithelium karyomegaly, interstitial fibrosis, and acute inflammation. Similarly, an increased incidence of non-neoplastic nasal lesions was reported, and included acute inflammation, olfactory epithelium atrophy, olfactory epithelium necrosis, cytoplasmic vacuolization of the respiratory epithelium, and squamous metaplasia of the respiratory epithelium (NTP, 2014).

In a longer NTP repeated dose toxicity study, F344/N rats and B6C3F1/N mice (10/sex/dose) were exposed to cobalt metal particles at doses 0, 0.625, 1.25, 2.5, or 5 mg/m^3 (or 10 mg/m^3 mice only) for 6 h/d 5 d/week for 13 weeks (MMAD 1.61-2.00 µm). In all exposed rats, lung weights were significantly greater than controls. In the lung, chronic active inflammation and alveolar proteinosis occurred in all animals at all doses, and bronchiole epithelium hyperplasia occurred in all animals from 1.25 mg/m^3. In the nose, incidences of olfactory epithelium degeneration and respiratory epithelium hyperplasia were significantly increased in all animals exposed to and above 2.5 mg/m^3, while olfactory epithelium hyperplasia was significantly increased at and above 2.5 or 5 mg/m^3 in males or females, respectively. Increased incidences of turbinate atrophy were detected from 2.5 mg/m^3 in all rats, statistically significant at 5 or 2.5 mg/m^3 in males or females, respectively. In exposed mice, lung weights were significantly exposed from ≥ 2.5 or 5 mg/m^3 in male or females, respectively. In the lung, alveolar histiocytic cellular infiltration and bronchiole epithelium cytoplasmic vacuolization occurred in all exposed animals. Other statistically significant effects at higher doses included bronchiole epithelium hyperplasia (≥ 2.5 mg/m^3), alveolar proteinosis and alveolar/bronchiolar epithelium karyomegaly (≥ 5 mg/m^3), and incidences of haemorrhage (≥ 5 mg/m^3). In the nose, the incidences of olfactory epithelium degeneration were significantly increased in mice exposed at or above ≥ 1.25 mg/m^3. Incidences of respiratory epithelium degeneration were significantly increased from ≥ 1.25 or 2.5 mg/m^3 in male or females, respectively, while incidences of respiratory epithelium squamous metaplasia were significantly increased in animals from 2.5 mg/m^3. At and above 5 mg/m^3, the incidences of turbinate atrophy and chronic active inflammation were significantly increased in males and females. The incidences of squamous metaplasia were significantly increased in the larynx of all exposed mice (NTP, 2014).

In a carcinogenicity NTP study, F344/NTac rats (50/sex/dose) were exposed to cobalt metal particles at doses 0, 1.25, 2.5, or 5 mg/m^3 for 6 h/d 5 d/week for 105 weeks (MMAD 1.4-2.0 µm). The incidences of alveolar epithelium hyperplasia, alveolar proteinosis, chronic active inflammation, and bronchiole epithelium hyperplasia were significantly higher in all exposed rats. Nasal non-neoplastic lesions included chronic active and suppurative inflammation, respiratory metaplasia, atrophy, hyperplasia, basal cell hyperplasia, and necrosis of the olfactory epithelium; hyperplasia, squamous metaplasia, and necrosis of the respiratory epithelium and atrophy of the turbinate, observed in all exposed animals (NTP, 2014).

In a carcinogenicity NTP study, B6C3F1/N mice (50/sex/dose) were exposed to cobalt metal particles at doses 0, 1.25, 2.5, or 5 mg/m^3 for 6 h/d 5 d/week for 105 weeks (MMAD 1.4-2.0 µm). In all exposed animals, the incidences of alveolar/bronchiolar epithelium hyperplasia and cytoplasmic vacuolization, alveolar epithelium hyperplasia, proteinosis, and alveolus infiltration cellular histiocyte were significantly increased. The incidences of bronchiole epithelium hyperplasia were significantly increased in the high dosed males and in the mid dosed group in females. The incidence of bronchiole epithelium erosion was significantly increased in the mid and high dosed males. The incidences of suppurative

inflammation were significantly increased in exposed males (mid dose) and females (high dose). In the nose, the incidences of suppurative inflammation; olfactory epithelium atrophy, hyperplasia, and respiratory metaplasia; cytoplasmic vacuolization and squamous metaplasia of the respiratory epithelium; and atrophy of the turbinate were significantly increased in all exposed animals. The incidences of atypical respiratory metaplasia of the olfactory epithelium and hyaline droplet accumulation of the respiratory epithelium were significantly increased in the low and mid dosed animals. In all exposed animals, the incidences of respiratory epithelium squamous metaplasia and cytoplasmic vacuolization of the larynx were statistically increased, while the incidence of squamous epithelium hyperplasia was significantly increased at the high dose. In the trachea, the incidences of epithelium cytoplasmic vacuolization were significantly increased in all exposed animals. In the lung, cobalt concentrations increased dose-dependently (NTP, 2014).

Wistar rats (10/sex/group) were exposed for 6h/d for 28 days to tricobalt tetraoxide aerosol (5, 20, or 80 mg/m^3, MMAD 1.41-1.88 µm). Half of the animal in each dose were allowed to recover for 90 days. Inflammatory responses were observed from the mid dose and was detected also at the end of the recovery period. The histopathology revealed effects in the lung, larynx, nasal cavity, trachea and lung lymph nodes, while a slight inflammatory response in the alveoli and slight bronchio-alveolar hyperplasia were also detected. The main study was preceded by a 14-day dose range finding one, with rats exposed to 12, 48 or 192 mg/m^3 tricobalt tetraoxide. Dose-dependent accumulation of particle in the lungs and in macrophages was observed and an increase of inflammatory response in all animals from the mid dose, while HIF1-α increase was statistically significant only in the top dose (Burzlaff et al., 2022).

7.3.2.3 Cardiovascular effects

Wistar male rats were administered a single dose of cobalt sulphate (100 mg cobalt/kg bw) dissolved in water followed by daily oral doses (26 mg cobalt/kg bw/d) for eight weeks. Histological changes in the myocardium, accumulation of fat droplets, decrease of myofibrils, and minimal inflammation were reported (Grice et al., 1969).

Mohiuddin et al. exposed guinea pigs to cobalt sulphate by gavage in the presence or absence of alcohol to mimic human toxicity after ingestion of cobalt in beer for 5 weeks. After exposure to 20 mg cobalt/kg bw/d, cardiomyopathy, increase in heart weight, lesions in the pericardium, myocardium, and endocardium were observed (Mohiuddin et al., 1970, ATSDR, 2004), while concomitant exposure to alcohol did not affect the severity or incidence of the findings.

Multifocal myocytolysis, with degeneration of myofibrilles was recorded in CFY male rats after exposure to cobalt dichloride (50 mg/kg bw/d or 12.4 mg cobalt/kg bw/d) for 3 months in drinking water in the presence of alcohol and sugar (10 and 5% respectively). The authors concluded that alcohol did not cause significant direct damage to the heart but enhanced the hypoxia created by cobalt exposure (Morvai et al., 1993)

Cobalt dichloride was administered for 3 months via drinking water to twenty Sprague-Dawley male rats (30.2 mg cobalt/kg bw/d) and resulted in a significant increase of heart weight in the absence of histological or morphological changes (Domingo et al., 1984, ATSDR, 2004).

Rats exposed to cobalt sulphate (40 mg/bw/d or 8.4 mg cobalt/kg bw/d for 16 or 24 weeks in diet) had significantly increased myocardial cobalt concentration at both time points. After 24 weeks, a significant decrease in left ventricular systolic and diastolic functions, along with left ventricular hypertrophy were noted (Haga et al., 1996).

A slight increase in severity of cardiomyopathy was measured in rats after 13 weeks exposure to cobalt sulphate (11.4 mg cobalt/m^3, MMAD 1.79-1.94 µm) (Bucher, 1991).

However, it was not detected in the carcinogenicity study in mice or rats (Bucher et al., 1999, NTP, 1998).

To characterise the severe cardiac insufficiency after cobalt exposure, rats were exposed to cobalt sulphate (8.4 mg cobalt/kg bw/d) in the diet for 24 weeks. The exposure resulted in decreased manganese-superoxide dismutase activity (pronounced), mitochondrial ATP production rate (moderate) and capacity of the respiratory chain (Clyne et al., 2001).

The NTP conducted a 2-phase retrospective study on male B6C3F1 mice exposed by inhalation to 9 particulate compounds for 2-year period to understand the potential relationship between particulate matter induced inflammation and vascular disease (Moyer et al., 2002). The 9 particulate compounds were indium phosphide, cobalt sulphate heptahydrate, vanadium pentoxide, gallium arsenide, nickel oxide, nickel sulphide, nickel sulphate hexahydrate, talc, and molybdenum trioxide. On the first phase, they evaluated selected tissues (heart, kidney, lung) from all control and high dose male, and successively tissues (heart, lungs, kidney, and mesentery) from a 90-day studies for control and high dose male and female mice for the substances for which and effects was observed on phase 1. In the 2 years study, indium phosphide, cobalt sulphate heptahydrate, vanadium pentoxide, gallium arsenide (MMAD 1.1-1.3, 1.5-1.8, 1.0, 1.0, respectively) caused increased incidence of arteritis (statistically significant for indium phosphide, cobalt sulphate heptahydrate, and marginally increased for the other 2 substances) while was only occasionally found in the other substances. Furthermore, the authors observed that arteritis lesions within the heart was distinctly different from the degenerative changes associated with age-related cardiomyopathy in the B6C3F1 mouse. The authors speculated an (in)direct toxic effect possibly via disruption in vascular tone. No arteritis was observed in the 90d studies, suggesting the need for chronic exposure for these effects to manifest (Moyer et al., 2002).

7.3.2.4 Haematological effects

Cobalt dichloride was administered for 3 months via drinking water to 20 Sprague-Dawley male rats (30.2 mg cobalt/kg bw/d) and resulted in a significant increase of haematocrit and haemoglobin (Domingo et al., 1984, ATSDR, 2004).

Sprague Dawley rats were maintained on a diet containing 265 ppm cobalt dichloride (20 mg cobalt/kg bw/day) for up to 98 days. Mean erythrocyte counts, packed cell volume, and haemoglobin concentrations were significantly higher than the controls on days 84 and 98 (Corrier et al., 1985).

Pregnant Sprague-Dawley rats were dosed with cobalt dichloride by gavage (0, 25, 50, 100 mg/kg bw/day) on GD6–15. A significant increase in haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, and reticulocytes was observed at the high dose (Paternain et al., 1988).

Cobalt dichloride was administrated to Crl:CD(SD) rats (10/sex/dose) for 3 months by gavage at doses of 0, 3, 10 or 30 mg/kg bw/day (0, 0.74, 2.5, or 7.4 mg cobalt/kg bw/day). Several statistically significant haematological changes were observed at the mid and high dose, e.g., haemoglobin (in males +11% at 10 mg/kg bw/d; in males +25% and in females 14% 30 mg/kg bw/d), number of red blood cells (in males +10% at 10 mg/kg bw/d; in males 19%**/+11%** m/f at 10 or 30 mg/kg bw/d, ** statistically significant, $p \leq 0.01$) (ECHA, 2022).

In the NTP study, after a 13-week exposure to 30 mg/m^3 cobalt sulphate (11.4 mg cobalt/m^3, MMAD 1.79-1.94 µm), an increased number of red blood cells (polycythaemia) was noted in rats, but not mice (Bucher et al., 1999, NTP, 1998, Bucher, 1991).

In several animal studies, oral administration of cobalt (from 0.5 mg cobalt/kg bw/d, 3 to 8 weeks) resulted in a noticeable rise in concentration of erythrocytes (polycythaemia), haematocrit, and haemoglobin levels (ATSDR, 2004).

In a longer NTP repeated dose toxicity study, F344/N rats and B6C3F1/N mice (10/sex/dose) were exposed to cobalt metal particles at doses 0, 0.625, 1.25, 2.5, or 5 mg/m^3 (or 10 mg/m^3 mice only) for 6 h/d 5 d/week for 13 weeks (MMAD 1.4-2.0 µm). Dose-dependent increase in haemoglobin concentration, erythrocyte count, haematocrit value, and manual packed cell volume were reported for all rats at the end of the exposure period. In mice, small (<5%) but statistically significant increases were recorded at the end of the study, in haemoglobin concentration (m) and erythrocyte count (m/f) in animals exposed to 10 mg/m^3 (NTP, 2014).

In a study conducted according to OECD 408, 10 Sprague Dawley rats/sex/dose were administered by gavage cobalt dichloride (0, 0.74, 2.48 or 7.44 mg cobalt/kg bw) or tricobalt tetraoxide (0, 73.4, 220 or 734 mg cobalt/kg bw) for 90 days, with an additional 10 animals/sex used for the recovery study control and high dose groups. After administration of cobalt dichloride, statistically significant changes in haematological parameters (increase in haematocrit, haemoglobin and red blood cells), were observed in males from the mid dose and in females receiving the high dose. In males, a decrease in reticulocytes and platelets was also measured at the same doses. Erythroid hyperplasia in the bone marrow was dose-dependent and statistically significant from the mid dose. All effects were reversible after the 4-week recovery period. Haematological parameters were also affected after the administration of tricobalt tetraoxide including increases in haematocrit, haemoglobin and red blood cells below 10% and above 20%, in the mid and high dose, respectively in male rats. In the mid dose females, a 5% increase in the same haematological parameters was observed, statistically significant only for red blood cells. In the high dose group in females, all increases were statistically significant and above 10% (Danzeisen et al., 2020).

7.3.2.5 Effects on other organs

Cobalt dichloride was administered for 3 months via drinking water to twenty Sprague-Dawley male rats (30.2 mg cobalt/kg bw/d) and resulted in a hypertrophy of the spleen (Domingo et al., 1984, ATSDR, 2004).

In the rats and mice that died after exposure to 2 mg/m^3 cobalt sulphate (19 mg cobalt/ m^3, MMAD not specified) for 16 days, liver congestion and necrosis, thymus necrosis and congestion of the brain vessels were observed (Bucher, 1991). Hyperplasia of the mediastinal lymph nodes was measured in mice after 13 weeks exposure to 11.4 mg cobalt/m^3 as cobalt sulphate (Bucher, 1991). No histological effects were observed in the kidneys after exposure for 16 days, 13 weeks or 2 years (Bucher, 1991, Bucher et al., 1999).

Oral exposure to cobalt dichloride for 5 months resulted in increased liver weight in rats (10 mg cobalt/kg bw/d), however no morphological or enzymatic liver changes were observed in 3 studies in rats with doses from 2.5 to 30.2 mg cobalt/kg bw/d with exposures duration between 3 to 7 months (ATSDR, 2004).

Renal tubular degeneration was detected in rats exposed orally to cobalt dichloride (10-18 mg cobalt/kg bw/d) for 4 or 5 months (ATSDR, 2004).

Necrosis, inflammation and histopathological changes of the thyroid were detected after exposure to cobalt dichloride (26 mg cobalt/kg bw/d in drinking water) for 45 days in female Parker mice (ATSDR, 2004). In Sprague-Dawley rats fed a diet containing cobalt dichloride (3.79 mg/cobalt kg bw/d) for 28 days, thymus atrophy was detected. Lower immunological reactivity was observed in rats dosed with cobalt dichloride (from 0.5 mg cobalt/kg bw/d) for 6 to 7 months (ATSDR, 2004).

Shrivastava et al. (1996) reported histopathological changes in the thyroid gland of female mice exposed to 49.4 mg Co/kg-d (HED of 6.9 mg Co/kg-d) in drinking water for 15 to 45 d (LOEL of 2100 µg/L) (Finley et al., 2012).

In an NTP repeated dose study, F344/N rats and B6C3F1/N mice (5/sex/dose) were exposed to cobalt metal particles at doses of 0, 2.5, 5, 10, 20 or 40 mg/m^3 for 6 h/d 5 d/week for 16 days or 17 days, respectively (MMAD not specified). Decreased body weight body weight gain and liver weight was reported in all animals at the high dose. In addition, in rats, thymus and kidneys weight were also decreased (Bucher, 1991).

In a 14-week NTP repeated dose toxicity study, F344/N rats and B6C3F1/N mice (10/sex/dose) were exposed to cobalt metal particles at doses 0, 0.625, 1.25, 2.5, or 5 mg/m^3 (or 10 mg/m^3 mice only) for 6 h/d 5 d/week for 14 weeks (MMAD 1.4-2.0 µm). In rats, statistically significant decreases in body weight (m/f) and body weight (m) gain were reported from 5 mg/m^3. In mice, statistically significant decreases in body weight and body weight gain were reported from 10 mg/m^3. In addition, statistically significant increases of liver weights (10, ≥ 2.5 mg/m^3 males or females, respectively) and kidney weights (≥ 5 mg/m^3) was recorded (NTP, 2014).

In a carcinogenicity NTP study, F344/N rats (50/sex/dose) were exposed to cobalt metal particles at doses 0, 1.25, 2.5, or 5 mg/m^3 for 6 h/d 5 d/week for 105 weeks (MMAD 1.4-2.0 µm). Decreased mean body weights (>10%) in all animals exposed at and above 2.5 mg/m^3 were measured after weeks 99 and 57 for males and females, respectively. Incidences of hyperplasia of the adrenal medulla were significantly increased in female rats exposed to 1.25 or 2.5 mg/m^3 (NTP, 2014).

In a carcinogenicity NTP study, B6C3F1/N mice (50/sex/dose) were exposed to cobalt metal particles at doses 0, 1.25, 2.5, or 5 mg/m^3 for 6 h/d 5 d/week for 105 weeks (MMAD 1.4-2.0 µm). Statistically significant lower mean body weights (>10%) were observed in animals exposed at the high dose after weeks 85 and 21 for males and females, respectively (NTP, 2014).

Cobalt dichloride was administrated to Crl:CD(SD) rats (10/sex/dose) for 3 months by gavage at doses of 0, 3, 10 or 30 mg/kg bw/day (0, 0.74, 2.5, or 7.4 mg cobalt/kg bw/day). A significant increase and dose-dependent in severity of erythroid hyperplasia in the bone marrow of the male and female animals at the mid and high dose groups, was reported in 4/7 and 7/7 m/f vs 0/0 in the control (ECHA, 2022).

Neurological effects have been observed in rat studies, manifesting as increased sensitivity, behaviour reactivity to stress, and latent reflex period (ATSDR, 2004).

7.3.3 *In vitro* data

No relevant data were found.

7.3.4 Summary

In human studies among workers exposed to hard-metal or diamond polishing, i.e., cobalt exposure in combination with either tungsten carbide or diamond dust, an increased incidence of lung parenchymal disease (hard-metal disease or cobalt lung disease) has been observed. In such exposure settings an increased incidences of respiratory irritation, thyroid effects and haematological effects have also been observed. Of these effects, the lowest adverse effect concentration was observed for respiratory irritation and/or small but observable effects in lung function parameters. In a study of Swedish hard-metal workers in the late 1970s, indications of respiratory irritation symptoms were seen at exposure levels of 2-3 µg/m^3 and statistically significant effects on lung function at 60 µg/m^3, with some statistically non-significant indications of effects around 10 µg/m^3. In a recent study in Swedish hard-metal workers, respiratory irritation effects and lung function measurements did not correlate with exposure levels when groups of <0.44, 0.44-1.7 and >1.7 µg/m^3 were compared. In a study of Belgian diamond polishers, small (all groups had mean values within the population reference values) but statistically significant effects on lung function were observed at cobalt exposure levels of 15 µg/m^3 - but not at 5 µg/m^3

(personal samples) - that occurred in combination with diamond dust, when the comparison was made to workers with diamond dust exposure only. However, the exposure assessment and lung function measurements were cross-sectional and e.g., the level of past exposures and their potential contribution in the effects observed could not be assessed. It is also noted that although the control group had exposure to diamond dust (but no cobalt exposure), the intensity of diamond dust or total dust in the control, low and high exposure groups was not measured and consequently not adjusted for, in the analyses concerning lung function. The control group was also compiled after the main study and not all measurements of the main study were performed in all controls. The other studies on hard-metal or diamond polishing workers have concerned much higher exposure levels.

In human studies among workers exposed in cobalt production or use of cobalt compounds in the absence of tungsten or diamond dust, no cases of cobalt lung parenchymal disease have been observed. Increased incidences of respiratory irritation, thyroid effects and haematological effects were observed similar to those in hard-metal workers, but at higher exposure levels. Such effects were observed in studies with exposure levels of tens or hundreds of $\mu g/m^3$. For respiratory tract effects LOAECs of about 50 $\mu g/m^3$ or more were reported in four studies covering various populations of cobalt production workers.

Human studies in populations with non-occupational oral exposure from medical treatment or diet and systematic exposure from hip implants have also indicated neurological effects and cardiomyopathy. However, such effects have either not been observed in occupationally exposed populations or are limited to few case reports concerning exposures much higher than those described above for irritation effects, or concern studies with possibility of confounding.

In animals, dose-dependent lethality has been observed in rats, mice and guinea pigs. Cobalt exerted toxic effects on several organs or systems. After inhalation, the respiratory tract toxicity depends on the size of the particles: larger particles (>2 µm) deposit on the upper respiratory tract (ATSDR, 2004) and cause necrosis and inflammation in nasal cavities, larynx, trachea as well as atrophy of the olfactory epithelium, hyperplastic lesions in the nose, and fibrosis. Smaller particles reach the alveoli where they cause several types of lesions, inflammation, necrosis and fibrosis. Effects on the respiratory tract were observed also in rats after exposure via drinking water; tachypnea was described after gavage administration in guinea pigs. Several cardiovascular effects were observed in the animal studies: cardiomyopathy, lesions in the myocardium and pericardium, degeneration of myofibrillas were those most commonly observed. Increases of erythrocytes, haematocrit and haemoglobin were recurrent, after administration of soluble and less soluble compound such as tricobalt tetraoxide. Less frequently reported findings include renal tubular degeneration, inflammation and necrosis of the thymus and thyroid, hyperplasia on the lymph nodes, and some neurological effects. Dermal application resulted in reversible scrabs.

7.4 Irritancy and corrosivity

7.4.1 Human data

7.4.1.1 Skin irritation

The differentiation of an irritant reaction from skin sensitisation may be difficult and both types of responses can coexist (Lison, 1996). However, there are no human data on skin irritation as such, following exposure to cobalt compounds. Swennen et al. (1993) for example, studied various health outcomes among 82 workers exposed to cobalt oxides, cobalt salts, and cobalt metal in a cobalt production plant and compared them with 82 unexposed workers. The clinical examinations detected more subjects with skin problems (eczema, erythema) (51% vs 25%, $p<0.001$) in the exposed than in the control group but did not characterise the nature of those skin lesions, further.

7.4.1.2 Respiratory irritation

Exposure to cobalt compounds is an established cause of occupational asthma and exposure to cobalt containing hard-metal or diamond polishing dust is an established cause of parenchymal lung disease (hard-metal disease or cobalt lung disease). The epidemiological studies that have investigated such clinical entities are described in the sections of respiratory sensitization (Section 7.5.1) and repeated dose toxicity (Section 7.3.1), respectively. Several studies have also assessed the prevalence of respiratory tract (and eye) symptoms and the effects on lung function parameters in exposed populations. The changes observed may represent either nonspecific irritating effects of cobalt-containing dust or an immunologically mediated reaction. As such studies deal with working populations with long term exposure, they are described in the repeated dose toxicity section 7.3.1.2., although one of them includes an embedded human voluntary study (Kusaka et a. 1986a) with a 6-hour exposure to hard-metal dust which indicated respiratory irritation symptoms and a small but statistically significant effect on forced ventilatory capacity at a mean exposure level of 38 µg/m^3 cobalt, combined with a mean level of 800 µg/m^3 of total hard-metal dust. No similar short-term exposure studies were identified for populations with exposure to cobalt or cobalt compounds alone.

7.4.2 Animal data

Cobalt dinitrate was applied to the skin of Vienna white rabbits according to OECD 404 test guideline, an average score of 1 was observed for 2/3 animals for erythema, which was fully reversible within 72 h (ECHA, 2022).

Cobalt dinitrate was applied to the eyes of New Zealand White rabbits according to OECD 405 test guideline; an average score of 1 was observed for 2/3 animals for cornea opacity and iritis, both fully reversible within 72h. Conjunctivae scores (3, 2, 1.3) and chemosis scores (3, 3, 0.7) were not reversible within 21 days (ECHA, 2022).

7.4.3 *In vitro* data

No relevant data were found.

7.4.4 Summary

The human data on skin effects are limited and concern only non-specific skin effects without separation of irritation and other effects.

The human data on respiratory irritation concern mostly working populations with long term exposure. They are described in the repeated dose toxicity section 7.3.1.2. One human volunteer study with a 6-hour exposure to hard-metal dust indicated respiratory irritation symptoms and a small but statistically significant effect on forced ventilatory capacity at a mean exposure level of 38 µg/m^3 cobalt, combined with a mean level of 800 µg/m^3 of total hard-metal dust. No similar short-term exposure studies were identified for populations with exposure to cobalt or cobalt compounds alone.

Skin irritation scabs were observed in guinea pigs after repeated exposure, no irritation was detected on rabbits after a single exposure. Cobalt dinitrate was corrosive to the eyes of New Zealand White rabbits. Respiratory tract effects are described in section 7.3.2.2.

7.5 Sensitisation

7.5.1 Human data

7.5.1.1 Respiratory sensitisation

7.5.1.1.1 Asthma

Exposure to cobalt is an established cause of occupational asthma and the evidence points also to the involvement of non-immunological mechanisms (Baur, 2013). However, in

some cases of cobalt asthma, a type I allergic reaction has been suspected, because specific IgE antibodies against a complex of cobalt with albumin could be identified, while for the remaining patients, the mechanism of cobalt-induced asthmatic reaction wass not elucidated (Lison 1996). Immunological mechanisms similar to those in hard-metal disease may also be involved (Cirla, 1994).

In the context of the RAC opinion on the restriction proposal of soluble cobalt salts, information from the stakeholder consultation indicated that under the current exposure conditions, cobalt-induced occupational asthma is rare (RAC, 2020). More specifically information from three Member States suggested an incidence of 0 to 1 asthma cases per year, related to exposure to cobalt compounds. However, RAC noted, that the information was too scarce to draw any firm conclusions on the occurrence of occupational asthma related to cobalt exposure in the EU. Incidences from data sources other than the above stakeholder consultation were not described.

A more extensive literature search indicates that large epidemiological studies on cobalt asthma are lacking, and the knowledge is largely based on case reports or small series often with limited analysis of risk by level of exposure. This is a typical situation in occupational asthma epidemiology.

Studies in populations with exposure to hard-metal

Kusaka et al. (1986b) studied asthma prevalence in a cross-sectional study of 319 hard-metal production workers (282 current and 37 with earlier exposure). It is noteworthy that the asthma definition included not only new onset of asthma but also asthma that had started before employment in hard-metal production, if it was aggravated during employment in hard-metal production. There were 18 (5.6%) prevalent cases of asthma. The latency time between the start of exposure and the onset of asthma ranged from 3 months to 10 years, while the exposure measurement data covered only the survey period (1981-84). Nine of the asthma cases underwent a bronchial provocation test with cobalt while nine refused the test. The nine tests were positive comprising two dual reactions, two immediate and five late reactions. Only two of the nine individuals had a positive skin patch test to cobalt. The working departments of the asthma cases were reported and also the mean and range of the cobalt concentrations of all personal air measurements (at the time of the survey) in those departments were reported: 8 cases occurred in the powder department (mean (range) 690 (6 – 6400) µg/m^3), 6 cases in grinding (dry grinding 1300 (1100 – 1500) µg/m^3, wet grinding 53 (11 – 1500) µg/m^3), 3 cases in shaping (130 (6 - 1200) µg/m^3) and 1 case in sintering (26 (2 – 150) µg/m^3). In addition, for eight of the asthma cases, air measurements (at the time of the survey) based on their personal samples were also reported (mean (range if reported), µg/m^3): >1200, 890 (600-1200), 330, 160 (31-440), >31, 24 (8-48), >18, 18 (5-29).

Shirakawa et al. (1989) studied the immunological mechanisms involved in cobalt-induced occupational asthma. Eight cases were non-randomly selected from the hard-metal production plant, already studied by Kusaka et al. (1986b). It seems that the two studies overlap as the case collection for the Shirakawa et al. (1989) study was said to have started in 1981 and four out of the eight cases selected had their asthma onset before that and had changed department before the immunological tests by Shirakawa et al. (1989) were performed. The cases were chosen based on (1) good response to bronchodilator which was compatible with asthma and; (2) baseline lung function volumes more than 70% of reference values. The latent period from onset of employment to diagnosis ranged from 2 months to 20 years and the mean exposure level in the current job (personal sampling) was from 7 to 230 µg/m^3. Only the mean-no range-was reported. As mentioned, 4/8 of the cases had changed department and for them the air concentration measurements did not represent their exposure level at the time of the onset of asthma. The absence of a co-existing hard-metal disease was confirmed with computer tomography, broncho-alveolar lavage and transbronchial lung biopsy. The cases

were compared to 8 controls not exposed to hard-metal dust (three with atopic asthma, three with non-atopic asthma and two without asthma). A positive patch test for cobalt chloride was seen in two cases and in none of the controls. In the radioallergosorbent test (RAST) with cobalt-conjugated human serum albumin, elevated scores were identified in four of the cases, while the four remaining cases had scores similar to the control subjects. A specific bronchial challenge test with cobalt chloride was positive in all cases but in none of the controls. There were two immediate reactions, four late reactions and two dual reactions.

Andersson et al. (2020) studied the respiratory symptoms and pulmonary function among 72 Swedish hard-metal production plant workers. Inhalable cobalt and total dust exposures were measured as 8-hour TWA based on personal samples and correcting (when necessary) for the use of respiratory protection to estimate true exposure. Using existing historical data, cumulative exposure was also estimated. Respiratory symptoms were assessed with a questionnaire. Asthma prevalence was not compared to an external unexposed population. In internal comparisons by tertiles, when those with cumulative exposure to inhalable cobalt of >70 µg/m^3 x year and 20 – 60 µg/m^3 x year were compared to those with $\leq$20 µg/m^3 x year, there was no significant dose-response in asthma symptom prevalence when adjusted for age, smoking, gender and for the use of respirators. Similarly, when those with full-shift inhalable Co 8-hour TWA of 0.44 – 1.7 and $\geq$1.7 µg/m^3 were compared to those with <0.44 µg/m^3, there was no significant dose-response in asthma symptom prevalence in the adjusted analysis. As further described in the section of respiratory irritation, the pulmonary function tests did not indicate an effect from cumulative cobalt exposure, either.

Al-Abcha et al. (2021) described the incidence of work-related asthma in cobalt-exposed workers in 1988-2017, in the State of Michigan, US. The study was based on the obligatory reporting by physicians and hospitals of work-related asthma (WRA). A standardized telephone interview of each reported case was conducted. An industrial hygienist evaluated the reported cases' workplace, and a physician reviewed the results to confirm the diagnosis. The case definition included both new onset of work-related asthma and an aggravation at work of pre-existing asthma. However, all the cobalt-related cases were cases of new onset of asthma. There were 35 cases, i.e., 1.2 per year. The incidence rate in the entire working population was not calculated in the report. However, it is noted that in 2011-2017, the average employed population in Michigan was about 4.5 million and thus the incidence rate per the entire working population would be of the order of 0.25/million per year. All cases were related to hard-metal exposure, 65% were workers involved in manufacturing tungsten carbide tools and 35% used those tools in manufacturing processes of other products. The cases emerged from 26 facilities and industrial hygiene measurements indicated cobalt exposure levels above 50 µg/m^3 8-h TWA in 25% of those 24 facilities, where measurements were performed. The mean or other summary exposure levels in the rest of the facilities were not further characterised.

Studies in populations with other cobalt exposure

Morgan (1983) did not find any cases of asthma in a cross-sectional study among 49 workers exposed to cobalt metal and cobalt oxide. Compared to an unexposed control group of 46 workers, there was also no difference in the ventilatory function parameters. The air measurements from personal samples of the exposed men indicated cobalt concentrations from 100 to 3000 µg/m^3 (mean 520, median 200).

Swennen et al. (1993) studied respiratory symptoms and lung function among 82 workers exposed to cobalt oxides, cobalt salts, and cobalt metal in a cobalt production plant and compared them with 82 age matched control workers, not exposed to lung irritants, who were recruited in the mechanical workshops of a nearby plant belonging to the same company. The exposed group had been exposed for 8 years on average (range 0.3-39) and the geometric mean current 8-hour TWA cobalt exposure assessed with personal

samplers was about 125 (range 1-7800) µg/m^3 and 25% of the values were higher than 500 µg/m^3. The urine and blood cobalt measurements in control group workers and a few air measurements at their workplace indicated no cobalt exposure among them. Asthma prevalence was not analysed. However, the cobalt-exposed workers complained more often of daily wheezing (6.1% vs. 0%, $p< 0.05$) and dyspnoea (e.g., dyspnoea during normal exercise 12.1% vs 2.4%, $p<0.05$). Wheezing was also more commonly observed in the clinical examination (16% vs 6%, $p<0.05$). No statistically significant differences were observed in lung function measurements between the exposed and control groups. However, within the exposed group, a dose-effect relation was found between the reduction of the forced expiratory volume in one second divided by vital (FEV_1/VC) capacity and the intensity of current exposure to cobalt assessed by the measurement of cobalt in the air or in urine ($p<0.01$ for both).

The most extensive case series with exposure information is by Sauni et al. (2010) and concerns all incident occupational asthma cases during 1967-2003, in a Finnish cobalt production plant which had started operation in 1966. During 1967–2003, about 700 workers worked at the cobalt plant, including workers hired for 6 months or longer. The case ascertainment of a cobalt-induced asthma included a specific bronchial provocation test in all cases. All cases occurred in the three departments with concomitant exposure to irritant gases (SO_2, NH_3 or H_2S, median exposures from 1 to 3.5 ppm), while in the chemical department where such irritant gas exposure did not occur, there were no asthma cases. In 1987 the process was modified so that exposure to SO_2 would no longer occur in the plant and in 1987-2003, only one case of asthma occurred. Therefore, the incidence density in different departments was calculated only for 1967-87, based on 22 incident cases. Regular occupational hygiene measurements had been performed in the cobalt plant since 1966, both personal and stationary sampling, and were used to describe the exposure levels in different departments. The incidence density per person-years was 0.02 in reduction and powder production (median level of exposure 150, range 100 - 400 µg/m^3), 0.006 in sulphatising roasting (median level of exposure 100, range 6 - 1000 µg/m^3) and 0.005 in leaching and solution purification (median level of exposure 30, range 10 - 100 µg/m^3) and 0 in the chemical department (median level exposure 120, range 20 - 300 µg/m^3). The latter department had 102 person-years accrued by 34 workers. Yet, it is noted that during the last 16 years of follow-up (1987-2003), more person-years without new cases of asthma were obviously accrued by these workers but were not reported as there were no longer any asthma cases in any of the departments (see above) and person-year data were not reported for this period. The only asthma case that occurred after 1987 was a maintenance worker who repaired machinery from various departments and moved around the plant, and neither the level of exposure to cobalt was estimated, nor exposure to other harmful agents was reported. It is noteworthy that the 22 cases of cobalt-induced asthma in the plant corresponded to about half of all cobalt-induced asthma cases reported in Finland during the study period. None of the asthma patients had positive reactions against cobalt in skin prick test, indicating a non-immunologic mechanism. The reactions in the bronchial challenge test were mostly late or dual, rather suggesting a non-immunologic mechanism of asthma. The mean age at diagnosis of asthma was 46 years and the median working time before onset of symptoms was 0.5 years in sulphatising roasting, 3.0 years in reduction and powder production and 7.5 years in leaching and solution purification. It is noted that the asthma cases occurred among workers exposed concomitantly to cobalt and irritant gases. However, specific bronchial challenge tests with cobalt chloride or cobalt sulphate were performed in 17 cases to verify a cobalt related reaction. Nevertheless, in five cases the specific bronchial challenge test used a less specific substance, i.e. cobalt powder or dust from sulphatising roasting.

In an earlier case-control study from the same plant, Roto (1980) reported an increased risk of asthma overall (OR 4.8; 95% CI 2.0 – 11.7) and that 6 out of the 15 cobalt exposed cases had a positive bronchial provocation test. However, no risk estimates were reported by level of exposure or by exposure to other (irritant) workplace exposures. Linna et al.

(2003) also performed a cross-sectional survey in the same plant. Respiratory health was assessed in 110 cobalt workers and 140 unexposed controls, both having an employment duration of at least 10 years. There was an increased prevalence of asthma and of suspected work-related asthma among the exposed and the results remained the same even after the multivariate analysis, in which the effect of age and smoking was taken into account. Among the cobalt production workers, the mean duration of exposure was 22.1 years and mean cumulative exposure 1000 µg/m^3 years. The average exposure intensity for cobalt was thus 45 µg/m^3. The exposed workers also had a cumulative exposure of 400 µg/m^3 years of nickel compounds, 7.5 ppm-years of H_2S, 3.7 ppm-years of SO_2, 27 ppm-years of NH_3 and 19400 µg/m^3 years total dust. Further multivariate analyses were performed in the exposed group, and exposure to any of the specific chemicals (including cobalt) did not have a significant effect on increasing the risk of asthma.

Walters et al. (2014) described a series of 14 occupational asthma cases identified among UK Mid Westland manufacturer of automotive engine valves, diagnosed between 1996 and 2005. Valve seats and tips were welded with stellite, an alloy containing 30–65% cobalt, and finished by grinding and polishing with metalworking fluid lubrication. Case ascertainment included, among others, serial peak expiratory flow (PEF) measurements. Sensitization to cobalt chloride was demonstrated in nine workers, either by skin prick test or specific inhalation challenge. There was no information on cobalt air measurement levels in the plant.

7.5.1.1.2 Rhinitis

Rhinitis and related nasal symptoms are described in this section. However, it is acknowledged that the studies cited usually focused on rhinitis in general, without differentiating irritation or an immunological reaction.

Hard-metal exposure

In the study by Kennedy et al. (1995) among Canadian saw filers (see section 7.3.1.2), there was no difference in the prevalence of nasal symptoms between the saw filers and bus mechanics.

Andersson et al. (2020) studied respiratory symptoms among 72 Swedish hard-metal production plant workers. Inhalable cobalt and total dust exposures were measured as 8-hour TWA based on personal samples and correcting (when necessary) for the use of respiratory protection to estimate true exposure. Using existing historical data, cumulative exposure was also estimated. Respiratory symptoms were assessed with a questionnaire. The prevalence of nasal symptoms was not compared to an external unexposed population. In an internal analysis by tertiles, when those with full-shift inhalable Co 8-hour TWA of 0.44 – 1.7 and $\geq$1.7 µg/m^3 were compared to those with < 0.44 µg/m^3, there was no statistically significant trend in the prevalence of dripping of blocked nose, for at least 1 month, or for dripping, blocked or itching nose during the last 2 weeks, while the prevalence was somewhat higher in those with higher exposure. There were also no significant trends by tertiles of cumulative exposure to inhalable cobalt (>70 µg/m^3 x year, 20-60 µg/m^3 x year, $\leq$20 µg/m^3 x year).

Other cobalt exposure

Swennen et al. (1993) studied respiratory symptoms among 82 workers exposed to cobalt oxides, cobalt salts, and cobalt metal in a cobalt production plant and compared them with 82 unexposed workers. The prevalence of self-reported daily rhinitis for at least 3 months a year was not significantly different between the exposed (13%) and the unexposed (10%) groups. No analyses by level of cobalt exposure were reported. It was disclosed that the exposed group had been exposed for 8 years on average (range 0.3 - 39) and that the geometric mean current 8-hour TWA cobalt exposure assessed with personal

samplers was about 125 (range 1-7800) µg/m^3, with 25% of the values being higher than 500 µg/m^3.

Linna et al. (2003) studied the occurrence of respiratory diseases and symptoms among 110 workers with at least 10 years of exposure to cobalt, in a Finnish cobalt production plant (the same plant as Sauni et al. (2017) above for asthma) and 140 unexposed controls having worked at least 10 years on the same plant or as municipal workers in the same city. Workers having worked also in other metallurgical plants or having regularly performed welding for at least 6 months were excluded from both groups. The prevalence of physician diagnosed allergic rhinitis was similar between the exposed (2.8%) and unexposed (2.9%) subjects. Self-reported symptoms of allergic rhinitis were less common among the exposed (8.2%) than the unexposed (15.2%), but the difference was not statistically significant. Among the cobalt production workers, the mean duration of exposure was 22.1 years and mean cumulative exposure was 1000 µg/m^3 years. The average exposure intensity was thus 45 µg/m^3. The exposed workers also had a cumulative exposure of 400 µg/m^3 years of nickel compounds, 7.5 ppm-years of H_2S, 3.7 ppm-years of SO_2, 27 ppm-years of NH_3 and 19400 µg/m^3 total dust. Further multivariate analyses were performed in the exposed group, and exposure to any of the specific chemicals (including cobalt) did not have a significant effect on increasing the risk of diagnosed or self-reported allergic rhinitis.

7.5.1.2 Skin sensitisation

Based on general population studies, cobalt is one of the major contact allergens. A large European survey from 2002-2010 reported patch test results from 10 617 patients with occupational contact dermatitis and 24 177 patients with non-occupational contact dermatitis (Pesonen et al., 2015). Positive patch test reactions for cobalt(II)chloride were detected in 9.3% of the occupational dermatitis and 6.8% of the non-occupational dermatitis patients. Studies have shown that patch test positivity for cobalt has been prevalent among workers (with or without dermatitis) in some occupations, including cement workers (7%), dental technicians (12%), and hard-metal workers (5%) (Fischer and Rystedt (1983),Wang et al. (2011) (Lee et al., 2001). Solitary cobalt allergy, without simultaneous contact allergy to nickel or chromate is seen mainly among hard-metal workers and in glass and pottery industry and it is considered that the simultaneous allergy is not a result of cross-reactivity but rather stems from combined exposure (Montelius, 2005). Significant dermal doses may result from short-term contact (Kettelarij et al., 2018). In a hard-metal production plant, the highest dermal doses were among raw material handlers, but also production workers showed significant doses while many occupational groups had low, but measurable doses (Midander et al., 2014).

Data are sparse as regards incidence or prevalence of (clinical) occupational contact dermatitis due to cobalt.

In the context of the RAC opinion on restriction of cobalt salts, information from the stakeholder consultation indicated that under the current exposure conditions, cobalt-induced occupational skin diseases are rare (RAC, 2020). More specifically information from three Member States suggested an incidence of 1 to 3 cases of occupational skin disease per year related to exposure to cobalt compounds. However, RAC noted, that the information was too scarce to draw any firm conclusions on the occurrence of occupational skin diseases related to cobalt exposure in the EU. Incidence from data sources other than the above stakeholder consultation were not described by RAC.

Large epidemiological studies on cobalt-induced occupational dermatitis in industry-based cohorts are lacking. Some national health surveillance data are reported below.

Kanerva et al. (2000) reported the incidence rate of occupational allergic contact dermatitis caused by metals, over a 7-year period (1991-1997) in the Finnish working

population, averaging 2.13 million per year during that period. There were 41 cobalt induced cases, i.e., about 6 per year or 2.7/million employed per year.

Athavale et al. (2007) reported the incident of occupational skin diseases in 1993-2004 from a voluntary reporting scheme of UK dermatologists. There were 823 cases of cobalt-related skin disease, i.e., 68 per year. For employed men the annual incidence rates were 2.0/million among those aged 16-29 and 4.7/million among those aged > 60 years and for employed women in the same age categories 4.9/million and 0.63/million, respectively.

Swennen et al. (1993) studied various health outcomes among 82 workers exposed to cobalt oxides, cobalt salts, and cobalt metal in a cobalt production plant and compared them with 82 unexposed workers. The clinical examinations detected more subjects with skin problems (eczema, erythema) (51% vs 25%, $p<0.001$) in the exposed than in the control group. It was reported that the exposed group had been exposed for 8 years on average (range 0.3 - 39) and that the geometric mean current 8-hour TWA cobalt exposure assessed with personal samplers was about 125 (range 1-7800) µg/m^3 and 25% of the values were higher than 500 µg/m^3. Dermal exposure was not characterised.

7.5.2 Animal data

7.5.2.1 Respiratory sensitisation

No studies are available.

7.5.2.2 Skin sensitisation

A local lymph node assay (LLNA) was conducted using cobalt dichloride in dimethylsulfoxide or aqueous ethanol solution on BALB/c mice. With both solvents, a dose-dependent increase in proliferation was recorded (Ikarashi et al., 1992a). The same group performed LLNA on CBA/N mice, F344 rats and Hartley guinea pigs; in all three species the stimulation index was equal or above 3, at slightly different concentrations of cobalt dichloride: in mice at 1, 2.5 or 5% (10.8, 27, 64.1 mg cobalt/kg bw/d), in rats at 2.5 and 5% (9.6, 19.2 mg cobalt/kg bw/d), and in guinea pigs at 5% (14.7 mg cobalt/kg bw/d) in dimethyl sulfoxide (Ikarashi et al., 1992b).

Positive reactions for skin sensitisation were obtained in all test animals in a guinea pig maximisation test conducted with cobalt dichloride (challenge dose concentration 1%) (Wahlberg and Boman, 1978).

Cobalt sulphate applied at a concentration of 0.03% caused skin sensitisation in all test animals in a Guinea pig sensitisation test (Yanagi et al., 2001).

LLNA studies conducted with cobalt zinc silicate blue phenacite and dipotassium hexacyanocobalt(II)-ferrate(II) showed positive skin sensitisation reactions, whereas the results of LLNAs with iron cobalt chromite black spinel or cobalt zinc aluminate blue spinel did not indicate sensitisation (ECHA, 2022).

7.5.3 *In vitro* data

Cobalt dichloride was tested on skin from healthy humans in the reconstructed human epidermis (RhE) IL-18 assay. It gave positive results in all 3 repetitions and was classified as strong sensitiser, with an EC_{50} value for the RhE assay of 4.69%. The authors compared their values with existing data: cobalt dichloride and sulphate were categorised as strong sensitisers based on LLNA results (EC3; the EC3 is the estimated concentration in % that causes a 3-fold increase in draining lymph node cell proliferative activity) between 0.1 and 1 (Gibbs et al., 2018).

7.5.4 Summary

Human data indicate that exposure to cobalt compounds is an established cause of occupational asthma. The evidence points also to the involvement of non-immunological

mechanisms. However, in some cases of cobalt asthma a type I allergic reaction has been suspected, because specific IgE antibodies against a complex of cobalt with albumin could be identified. Studies among hard-metal workers exposed to cobalt in combination with tungsten have observed multiple cases of occupational asthma at exposure levels of several tens to several hundreds of µg/m^3 cobalt and one reported case showing an exposure level of 7 µg/m^3 at the time of diagnosis, but with unknown levels of previous exposure. A recent study among Swedish hard-metal workers did not find a correlation between asthma symptom prevalence and exposure level when comparing those with cobalt exposure of <0.44, 0.44-1.7 and >1.7 µg/m^3.

In workers exposed in circumstances other than hard-metal-related, cases have also been reported at exposure levels of tens (lowest plant department level median 30 µg/m^3 cobalt) to hundreds of µg/m^3 cobalt but usually in combination with exposure to irritant gases. In the absence of such irritant exposure, no cases of asthma were seen in a cobalt plant chemical department with median cobalt exposure of 120 µg/m^3. However, only 102 person-years of follow-up were accrued by the 34 workers of the department. Yet the person-years without asthma cases seem to be actually higher, as the authors of the study did not report the person-year data of the chemical department for the more recent 16 years, when no asthma cases were observed in any of the departments of the plant.

Recently conducted human studies as well as the data from national surveillance systems indicate that while heavy exposure to cobalt and cobalt compounds increases the risk of asthma, in current exposure settings (with exposure levels in the order of 10 µg/m^3 or below), the risk is low and it is difficult to identify it, let alone firmly quantify it in an epidemiological setting, given the size of existing, currently exposed worker populations.

The human studies for rhinitis have observed increased incidences of physician-diagnosed rhinitis or of nasal symptoms in heavily exposed populations. However, these studies focused on rhinitis in general without differentiating irritation or an immunological reaction.

According to human data, cobalt is one of the major contact allergens. Positive patch test reactions for cobalt(II) chloride are often detected both in occupational and non-occupational dermatitis patients. In the occupational setting, significant dermal doses may result even from short-term contact. However, data are sparse as regards incidence or prevalence of clinical occupational contact dermatitis due to cobalt. Data from a few national surveillance schemes indicate an annual incidence rate of the order of magnitude of 1/million working population. However, this relates to the entire working population and no incidence rate data are available for the sub-population exposed to cobalt compounds.

Cobalt dichloride was tested *in vitro* in an RhE assay, and *in vivo* in LLNA studies performed on mice, rats and guinea pigs. In all assays, cobalt dichloride showed skin sensitisation properties.

7.6 Genotoxicity

7.6.1 Human data

De Boeck et al. (2000) performed a cross-sectional study among 35 male workers exposed to cobalt dust, recruited from three refineries in Belgium, Norway, and Finland and 29 male workers exposed to hard-metal dust recruited from two plants in Sweden and England. An attempt was made to match both groups for age, smoking habits, and cobalt exposure as assessed by cobalt in urine (Friday end of shift sampling). A urinary concentration of 20 µg cobalt per gram of creatinine was targeted, which is equivalent to an airborne exposure level of 20 µg/m^3 (the basis of this equation was not further described). A third group of matched control subjects (n=35) comprised workers with similar socioeconomic status, not exposed to cobalt or hard-metal, who were recruited from the five respective plants (warehouse, general services, or administration). The study

design integrated complementary methodologies to assess biomarkers of effects that represent both initial DNA damage (8-hydroxydeoxyguanosine [8-OHdG] in urine and comet assay on lymphocytes) and definitive chromosome breakage/loss (micronuclei in lymphocytes). Micronuclei were scored both as binucleates (MNCB) and as mononucleates (MNMC). There was no statistically significant difference in the group means of urinary 8-OHdG when control and exposed, or cobalt and hard-metal workers were compared. Results from the alkaline comet assay did not show any statistically significant differences, neither between exposed and control workers nor between cobalt and hard-metal workers, for any of the three parameters measuring DNA damage (tail length, tail DNA, tail moment). There was no statistically significant increase of DNA migration in any of the worker groups when the comet assay was combined with the Fpg enzyme (formamidopyrimidine) to detect oxidative DNA damage. The frequency of MNCB was not statistically different between control and exposed workers. This parameter was however significantly higher in cobalt- compared to hard-metal workers. It was found that the smoking status affected the levels of MNCB. in lymphocytes, the frequency of MNMC did not vary among the different worker groups.

Hengstler et al. (2003) studied DNA-damage in mononuclear blood cells from 78 metal workers from ten different facilities, selected because of the high air concentrations of cadmium expected. These included various types of facilities specialised in the production of cadmium-containing pigments, production of cadmium-containing batteries, galvanization and recycling of electric tools, especially television sets. Exposures assessed included cobalt, lead and cadmium based on urine (Cd and Co), blood (Cd and Pb) and air samples (Cd, Co, Pb, 6-hour personal sampling immediately before urine and blood sampling). A control group without heavy metal exposure (n=22) was used to dichotomize DNA single strand breaks (DNA SSB) into 'normal' and 'increased' levels but was not included into the regression analysis itself. The concentrations in the air samples ranged from 0.05 to 138.00 µg/m^3 for cadmium, from 0 to 10 µg/m^3 for cobalt and from 0 to 125 µg/m^3 for lead. Cobalt was not detectable in the air samples of 33 (42%) of the workers. In univariate analysis, cadmium in air, cadmium in blood, cobalt in air and cobalt in urine correlated with DNA single strand breaks. In a multivariate analysis including age, gender, smoking, alcohol consumption, iron in serum and the various heavy metal exposure metrics, cadmium in air, cadmium in blood and cobalt in air correlated significantly with DNA single strand breaks. The authors concluded that cobalt was the strongest determinant for DNA damage but acknowledged that interpretation of the observed correlations between cadmium as well as cobalt concentrations with DNA damage was difficult, since there were correlations between cobalt air concentration with cadmium concentrations in air. It is noted that DNA SSB was the only parameter analysed and represents reversible lesions. Later letters to the editor debated some potential methodological and result interpretation problems in the study (Kirsch-Volders and Lison (2003), Hengstler (2003)).

DFG (2007) referred to a study by Oesch et al. (1999), in which increased numbers of DNA single strand breaks and reduced repair capacity for oxidative DNA damage in lymphocytes were found in a subgroup of 11 workers from a group of 78 metal workers, who were exposed to >4 µg/m^3 cobalt (species not indicated) at the work site. Although the workers were also exposed to considerably higher cadmium levels, statistical analysis revealed that the cobalt exposure had the dominant impact on the occurrence of strand breaks. Similar cobalt concentrations were linked to the inhibition of 8-oxoguanine repair as for induction of DNA single strand breaks supporting the hypothesis that inhibition of DNA repair processes could have caused the DNA damage observed. It is noted that this study seems to concern the same group of 78 metal workers and similar analyses as the study by Hengstler et al. (2003).

Gennart et al. (1993) studied 26 male workers exposed at least for two years to iron, chromium, cobalt and nickel dust in a metal powder producing factory and 25 controls

matched for age, smoking habits and alcohol consumption. Sister-chromatid exchange (SCE) in blood lymphocytes, serum tumours markers, carcinoembryonic antigen (CEA) and tissue polypeptide antigen (TPA), and urinary excretion of chromium, cobalt and nickel were determined. An analysis of variance on the SCE rank values revealed that both exposure status overall (workers exposed to metal dust overall vs controls) and smoking habits (smokers and former smokers vs never smokers) had a statistically significant effect. For the tumour markers, the analysis of variance did not reveal a statistically significant difference between exposed persons and controls. However, CEA serum levels were significantly correlated not only with smoking habits but also with the duration of exposure. Exposure occurred simultaneously to several metal dusts and there was no multivariate analysis on the roles of individual metal dusts.

7.6.2 Animal data (*in vivo*)

Relevant *in vivo* genotoxicity studies are described in detail and tabulated in **Appendix 5**. A brief overview of the main findings is provided below.

A limited number of micronucleus *in vivo* studies have been conducted with cobalt metal (3-month inhalation study in mice (NTP, 2014) and cobalt chloride hexahydrate (delivered i.p in mice (Suzuki et al., 1993, Goc Rasgele et al., 2013) and orally in rats (Gudi and Ritter, 1998)). The oral and inhalation studies yielded negative results, while positive responses were observed in the i.p. studies in mice. In the one of the two i.p. studies, cobalt chloride was injected as pre-treatment to induce erythropoietin followed by treatment with different mutagens. A micronucleus test with WC-Co in rats, was also positive (**Table 37**).

Treatment of rats (i.v.) with cobalt diacetate resulted in a dose-dependent increase of DNA base products, characteristic of promutagenic oxidative DNA damage in kidney, liver and lung samples (Kasprzak et al., 1994). In a more recent study, no increase in 8-OH-dG lesions, was detected in lung tissues of rats treated with 80 mg/m^3 tricobalt tetraoxide in a 28-day inhalation exposure study, indicating an absence of oxidative DNA damage (Burzlaff et al., 2022).

Following oral administration of cobalt dichloride to Swiss male mice, a dose-dependent increase of chromosomal aberrations, including clastogenic effects (chromatid breaks and gaps) were reported (Palit et al., 1991b), however this non-GLP, non-guideline compliant study presents biologically implausible observations and is therefore of limited value. In contrast, high oral doses of cobalt chloride hexahydrate produced negative responses in a study in rats (Gudi and Ritter, 1998).

Cobalt sulphate, cobalt monoxide and tricobalt tetraoxide were evaluated for *in vivo* induction of bone marrow chromosomal aberrations in rats. No increased chromosomal aberration frequencies were seen in animals treated with tricobalt tetraoxide, while only marginal increases (up to 1.8%) compared to vehicle controls were observed in the highest dose groups in males treated with cobalt sulphate and cobalt monoxide, consequently the studies were considered negative (Kirkland et al., 2015).

No spermatogonial chromosomal aberrations were detected after exposing Sprague-Dawley rats orally to cobalt dichloride hexahydrate, for 28 days (Kirkland et al., 2015).

Chromosomal aberrations in bone marrow cells and DNA damage in bone marrow and brain tissue samples were also reported after 4 injections peri-articularly, in female mice of CoCr micron-sized particles (Brown et al., 2013).

Dose-dependent increases in the frequencies of *Kras* mutations, the majority of which being G→T transversions, in the alveolar/bronchiolar carcinomas of exposed animals compared to concurrent/historical or sourced from other NTP studies controls, were reported in the 2-year inhalation studies with cobalt sulphate in mice and with cobalt metal in both rats and mice (NTP, 1998, NTP, 2014). Additionally the occurrence of *Egfr* and

Tp53 mutations were also noted in the lung neoplasms of rats and mice exposed to cobalt metal (NTP, 2014). All the above mutations were not detected in spontaneous carcinomas of concurrent control animals, suggesting that they were related to chemical exposure. These genetic alterations, indicative of indirect DNA damage by oxidative stress, are reflective of secondary genotoxic events and could be associated to the production of ROS and the modulation of inflammatory responses by the cobalt species (see also section 7.7.2).

Relevant *in vitro* genotoxicity studies are described and tabulated in **Appendix 6**. A brief overview of main findings is provided below.

7.6.3 *In vitro* genotoxicity

7.6.3.1 Bacterial test systems

Published studies on the genotoxicity of cobalt metal and soluble cobalt compounds (such as cobalt chloride and cobalt sulphate) have been inconsistent, reporting conflicting findings.

Cobalt metal

The mutagenic potential of cobalt metal against *Salmonella typhimurium* (TA98, TA100) and *Escherichia coli* (WP2 *uvrA*/pKM101) tester strains, with or without S9 activation, has been evaluated in an NTP study (NTP, 2014) (**Appendix 6**; **Table 38**.). Cobalt metal produced a weaky positive response in strain TA98, in the absence of exogenous activation. No mutagenic activity was detected in *E. coli*. When probed again in GLP tests, cobalt metal did not produce a mutagenic response in TA98, regardless of S9 activation (Kirkland et al., 2015).

Cobalt salts

Cobalt salts yielded largely negative results with only isolated positive responses in specific strains (**Appendix 6**, **Table 38**). Cobalt chloride was reported in early studies to be mutagenic in strain TA98 and to a lesser extent in TA1537, in the absence of S9 activation (Wong, 1988). It was also shown to be readily mutagenic in the TA97 strain (Pagano and Zeiger, 1992). Cobalt sulphate heptahydrate was weakly mutagenic in the TA100 strain, in the absence of S9 metabolic activation, and in the presence of 5% hamster or rat liver S9 (Zeiger et al., 1992, NTP, 1998). The two cobalt salts were tested again in GLP compliant studies, in the relevant strains, at multiple concentrations, yet neither substance produced a mutagenic response, regardless of S9 activation, failing to reproduce the previously positive findings at similar concentrations.

Collectively, bacterial mutation assays were all (except one) negative, in the presence of S9 activation. In the absence of S9, sporadic indications of mutagenic activity of cobalt and soluble cobalt compounds in specific *Salmonella typhimurium* tester strains were not confirmed in follow-up GLP studies, pointing to an overall lack of mutagenic activity in bacteria.

7.6.3.2 *In vitro* genotoxicity in mammalian cells

Cobalt metal powder has been tested for its potential to induce *Hprt* mutations in mouse lymphoma L5178Y (Kirkland et al., 2015) (**Appendix 6**; **Table 39**). Although it did not induce statistically or biologically significant increases in mutant frequency, when tested up to highly toxic concentrations (50 mg/ml), in the absence of S9, cobalt metal produced weak, reproducible mutagenic effects upon exogenous activation. This finding was not however corroborated when the experiment was repeated with extract of cobalt metal powder (Kirkland et al., 2015).

Cobalt chloride was initially reported to be mutagenic, increasing the *Hprt* mutation frequency in V79 cells (Hartwig et al., 1990) (Hartwig et al., 1991), but cobalt chloride

hextahydrate was negative in the mouse lymphoma *Tk* mutation assay (Amacher and Paillet, 1980).

Comprehensive *Hprt* mutation studies were later undertaken for a number of cobalt substances (Kirkland et al., 2015). When tested for 3 hours (and in some cases for 24 hours), up to toxic or precipitating concentrations, cobalt dihydroxide, lithium cobalt dioxide, cobalt oxalate, cobalt hydroxide oxide, tricobalt tetraoxide, cobalt sulphate and cobalt sulphide did not induce statistically and/or biologically significant increases in *Hprt* mutation frequencies compared to vehicle controls, regardless of S9 activation. Cobalt monoxide also gave negative results up to precipitating concentrations, under the same experimental conditions (Kirkland et al., 2015). In other non-guideline gene mutation tests, cobalt chloride exhibited measurable mutagenic activity only in one (G12) of two *gpt*$^+$ transgenic V79 cell lines (Yokoiyama et al., 1990, Kitahara et al., 1996). Similar results in the same testing system were obtained for cobalt sulphide (Kitahara et al., 1996).

Collectively, positive results in rodent cells occurred mainly for the *hprt* locus, however OECD guidelines compliant *HPRT* assays failed to yield any positive effects, suggesting that cobalt metal and compounds do not elicit any mutagenic activity in mammalian cells.

An overview of other relevant genotoxicity studies is presented in **Table 40** (**Appendix 6**). The *in vitro* genotoxic activity of cobalt metal and cobalt compounds has been extensively reviewed by (Beyersmann and Hartwig, 1992) (Lison et al., 2001), (Kirkland et al., 2015) and more recently by (Lison et al., 2018). **Table 40** additionally features findings with cobalt containing alloys and micro/nano-sized particles. Although, as discussed earlier, considered not mutagenic in bacterial and mammalian test systems, different cobalt species have consistently been shown to induce DNA strand breaks, as detected by the alkaline comet (often modified to include lesion-specific endonucleases), alkaline elution, nuclear sedimentation, and phosphorylation of H2Ax assays, in a range of hamster, rodent and human cell lines. The *in vitro* genotoxic activity stemming from the DNA damaging potential of cobalt metal and compounds has been confirmed in cytogenetic assays. Micronucleus tests (cytokinesis-blocked) and sister chromatid exchange studies were mostly positive in human and rodent cells. Chromosomal aberrations have also been documented in human and hamster fibroblasts by cobalt alloy particles in the micro and nano size range, and cobalt chloride and cobalt oxide particles (**Table 40**). The genotoxic activity of cobalt and compounds appears to derive from the capacity of bioavailable cobalt ions to generate ROS and oxidative DNA damage. Other indirect mechanisms involve the impairment of critical DNA repair processes through the inhibition of key proteins, or via the enhancement of Topoisomerase-mediated DNA cleavage, contributing to cobalt's clastogenic activity. Overall, *in vitro* studies indicate a genotoxic potential for cobalt and cobalt compounds.

7.6.4 Summary

Only very limited and non-conclusive human data are available with respect to the assessment of genotoxic effects from cobalt compound exposure. The study populations are small and either no statistically significant differences between exposed and unexposed were found or when differences were found, correlations between exposure to cobalt and other exposures complicated the interpretation of these differences within the small study samples.

In bacterial mutagenicity assays conducted by the NTP ((NTP, 2014), cobalt metal yielded (weakly) positive results in the *Salmonella typhimurium* strain TA98 and equivocal results in strain TA100 in the absence of S9 metabolising enzymes. No mutagenic activity was detected in *E. coli*. Later GLP studies, failed to corroborate the positive result in the TA98 strain, regardless of S9 activation(Kirkland et al., 2015)). Cobalt salts produced largely negative results with only isolated positive responses in specific strains. Similar to cobalt

metal, early positive findings with cobalt chloride and cobalt sulphate heptahydrate in the T97 and T100 strains, were not reproduced in later GLP studies. Overall, the data point to the lack of mutagenic activity in bacteria. The same applies for mammalian cells. With the exception of a positive response in the *gpt* locus in transgenic G12 cells, most studies focusing on the *hprt* locus, were negative for cobalt metal and cobalt compounds (e.g., cobalt monoxide, sulphate and sulphide) as they failed to induce statistically and/or biologically significant increases in *Hprt* mutation frequencies compared to vehicle controls, regardless of S9 activation, in OECD compliant tests. With regard to the *in vitro* genotoxicity in mammalian cells, cobalt metal and soluble cobalt compounds have consistently produced positive responses in the majority of the comet assays, chromosomal aberrations, micronucleus, and H2Ax phosphorylation tests *in vitro*. These data indicate that even though cobalt and soluble cobalt salts do not exhibit mutagenic activity in bacterial and mammalian tests systems, they can cause genotoxicity by inducing DNA strand and chromosomal breaks *in vitro*.

Early *in vivo* micronucleus and chromosomal aberration studies conducted with cobalt dichloride delivered both i.p. and orally produced in some cases positive results, in mice and hamsters. However, more recent NTP and other studies designed according to OECD test guidelines did not confirm either these early findings in animals or the genotoxic activity observed *in vitro*. No increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood of male or female mice exposed to cobalt metal for 3 months by inhalation (NTP, 2014). Similarly, no increases in chromosomal aberrations were noted in bone marrow analyses of rats treated orally with cobalt sulphate, cobalt monoxide and tricobalt tetraoxide. The lack of clastogenic activity was also extended in spermatogonial cells of rats exposed to cobalt dichloride hexahydrate (Kirkland et al., 2015). Increased frequencies of *Kras* and to a lesser extent *Egfr* and *Tp53* mutations were however noted, exclusively in lung carcinomas of animals exposed to cobalt sulphate or cobalt metal, in the respective NTP studies (NTP, 1998, NTP, 2014).

In a NTP review, the authors stated that "*protein binding is important in the consideration of genotoxicity assay results for cobalt compounds because cobalt binding in vivo, e.g., to serum proteins, could render it less effective than when tested in vitro for the same endpoint. The available data suggests that cobalt compounds are clastogenic and can induce DNA and chromosomal damage, i.e., micronucleus formation, chromosomal aberrations, and aneuploidy, as shown by results reported for in vitro assays (NTP, 2016).*" The absence of chromosome damage *in vivo*, in the more reliable studies, could also be attributed to more effective protective processes in whole mammals, preventing ROS-mediated DNA damage as suggested by (Kirkland et al., 2015). The overall negative oral and inhalation studies provide no evidence of genetic toxicity of cobalt metal and the tested cobalt substances with relevance to humans.

7.7 Carcinogenicity

Cobalt metal and several cobalt compounds may cause cancer and have a harmonised classification under CLP as Carc 1B.

Furthermore, IARC (2006) assessed the carcinogenicity of metallic cobalt particles (with or without tungsten carbide) and classified cobalt metal with tungsten carbide as "probably carcinogenic to humans" (Group 2A). Cobalt metal without tungsten carbide, cobalt sulphate and other soluble cobalt(II) salts were considered as "possibly carcinogenic to humans (Group 2B)". Very recently, IARC re-assessed the evidence for cobalt metal (without tungsten carbide or other metal alloys), soluble cobalt(II) salts, cobalt(II) oxide, cobalt(II,III) oxide, cobalt(II) sulphide, and other cobalt(II) compounds. Only a concise news 'online first' article is available at the time of drafting this report (Karagas et al., 2022). Cobalt metal and soluble cobalt(II) salts were classified as "probably carcinogenic to humans" (Group 2A) based on "sufficient" evidence for cancer in experimental animals

and "strong" mechanistic evidence in human primary cells". Cobalt(II) oxide was classified as "possibly carcinogenic to humans" (Group 2B) based on "sufficient" evidence in experimental animals. Regarding cobalt(II,III) oxide, cobalt(II) sulphide, other cobalt(II) compounds, the conclusion was "not classifiable as to its carcinogenicity to humans" (Group 3).(Karagas et al., 2022)

7.7.1 Human data

As stated at the beginning of section 7, the human epidemiological data concerning the hazardous properties of cobalt and inorganic cobalt compounds is based on two main occupational exposure settings: those related to production and use of hard-metal and those related to production of cobalt and use of cobalt compounds. Tungsten carbide (WC) is the most common hard-metal, formed by binding or cementing metallic carbides with a metal binder, usually cobalt (Co) or nickel (Ni).

In the evaluation by IARC (2006) it was concluded for human data that:

- There is *limited evidence* in humans for the carcinogenicity of cobalt metal with tungsten carbide.
- There is *inadequate evidence* in humans for the carcinogenicity of cobalt metal without tungsten carbide.

The IARC *limited evidence* for cobalt metal with tungsten carbide was based on early Swedish and French studies assessing lung cancer risk in hard-metal production further described below.

Ward et al. (2010) then set the remaining research priorities for some substances evaluated by IARC. For epidemiology of metallic cobalt (with or without tungsten carbide), these research recommendations included updating the French and Swedish studies and studying additional cohorts of hard-metal manufacturing workers; including assessment of molecular biomarkers of early cellular effects and genetic polymorphisms associated with cellular protective systems and doing further research into the toxicity of exposure to cobalt with tungsten carbide in the nanoparticle size range.

Since the IARC assessment and Ward et al. (2010), some more human data on cancer and cobalt exposure have been published. These are summarised together with the most important studies already available in past assessments, structured to describe separately; (1) those related to hard-metal exposure , (2) the cobalt production related and, (3) those related to the use of cobalt or cobalt compounds.

In the recent re-evaluation by IARC (Karagas et al., 2022), it was concluded there was "inadequate" evidence from human data, regarding carcinogenicity in humans for cobalt metal (without tungsten carbide or other metal alloys) and for cobalt(II) and (II,III) compounds. It was explained that "the available studies did not permit the separation of cobalt's effects from those of the cobalt–tungsten carbide composite or other confounding exposures, or did not show positive associations".

7.7.1.1 Workers exposed in hard-metal production

Hogstedt and Alexandersson (1990) followed 3163 male workers employed in three Swedish hard-metal factories, for cancer mortality in 1951-82. Based on 17 deaths from lung cancer, the SMR was increased but without statistical significance (1.34; 95% CI 0.77–2.13). The SMRs were similar between those with low exposure (1.31; 95% CI 0.65–2.34) and high exposure (1.39; 95% CI 0.51–3.04). However, there was a higher and statistically significantly increased risk among those who had at least 10 years of exposure and at least 20 years from the first exposure (2.78; 95% CI 1.11–5.72). Smoking habits were not reported, and the risk estimates were not adjusted for potential confounding by smoking. The risk estimates were not calculated by mean exposure intensity or cumulative exposure to cobalt.

Lasfargues et al. (1994) followed for cancer mortality in 1956-1989, those 709 men who had been employed in a French plant producing lhard-metals using cobalt as a binder. The mortality from lung cancer was increased (SMR 2.13; 95% CI 1.02 – 3.93, 10 deaths), mainly due to an excess in the highest exposure group (SMR 5.03; 95% CI 1.85 – 10.9, 6 deaths). However, the risk did not increase with duration of employment or time since the start of employment. Smoking data were available for 81% of the workers and 69% of the deceased and showed that smoking alone was unlikely to explain the observed excess in lung cancer mortality. Potential confounding by other occupational carcinogens was not analysed. The risk estimates were not calculated by mean exposure intensity or cumulative exposure to cobalt.

Moulin et al. (1998) studied the association between lung cancer and exposure to cobalt and tungsten carbide among 5777 men and 1682 women who had worked for at least 3 months in one of the 10 French hard-metal production plants. The cohort was followed for cancer mortality in 1968-1991 and a nested case-control study (61 cases and 180 controls) was also performed. Exposure to cobalt and to tungsten carbide was assessed based on a job exposure matrix (JEM) of 320 job periods and was graded semi quantitatively on a scale from 0 (no exposure) to 9 (highest exposure). Smoking habits were known for 82% of the cases and 79% of the controls.

As regards atmospheric cobalt concentrations, there were 382 short duration (15-20 minutes) area samples from 1971-1983 and 362 long duration (4-8 hours) samples from 1984-94. Of the latter, 264 were personal samples. The cobalt measurements were not used for the JEM development but were later used for validation purposes. A linear regression between the cobalt levels that the experts assigned in the job-exposure matrix and the log-transformed measurements, showed significantly increasing trends in the short-duration area samples ($p<0.0001$), long-duration area samples ($p=0.015$), and long-duration personal samples ($p=0.015$). The personal sample arithmetic and geometric means (μg Co/m^3) ranged from about 40 and 20 in JEM group 2 to about 100-170 and 90-170 in JEM groups 6-7, respectively. For JEM groups 0-1 and 8-9, there were no measurements available.

Compared to the general population, mortality from lung cancer was increased (SMR 1.30; 95% CI 1.00-1.66, 63 deaths). In the case-control analysis, simultaneous exposure to cobalt and tungsten carbide (JEM groups $\geq$ 2 vs 0-1) increased the risk of lung cancer (OR 1.96; 95% CI 1.03 – 3.62). The risk increased significantly with exposure duration at JEM score $\geq$ 2 (p for trend=0.03) and cumulative exposure as expressed in months x JEM score $\geq$2 ($p=0.01$). Exposure to cobalt and tungsten carbide before and after sintering was considered simultaneously in a multiple regression analysis. Before-sintering exposure was associated with an elevated risk (OR 1.69; 95% CI 0.88 - 3.27), which increased significantly with frequency-weighted cumulative exposure. The risk was lower for after-sintering exposure (OR 1.26; 95% CI 0.66 - 2.40), while no significant trend was observed for cumulative exposure.

Control for confounding in the subset of cases and controls with known smoking habits did not indicate important confounding by smoking. There was also no indication of important confounding by exposure to the other occupational carcinogens considered (polycyclic aromatic hydrocarbons (PAHs), asbestos, silica, certain chromium compounds, certain nickel compounds, arsenic compounds, cadmium compounds, nitrosamines, and benzene). Except the above-mentioned semi-quantitative JEM-based intensity or cumulative exposure to cobalt, there were no analyses by more quantitative exposure metrics.

Wild et al. (2000) extended for one additional year (1968-1992) the follow-up of the largest factory among the French cohorts of the Moulin et al. (1998) study. The follow-up was concluded for 2860 subjects (2216 men). Compared to the general population, there was a statistically significant lung cancer excess (SMR 1.70; 95% CI 1.24 – 2.26, 46

deaths). The risk was higher among those having worked in hard-metal production steps before sintering (SMR 2.42; 95% CI 1.10 – 8.63, 9 deaths), than in the workshops after sintering (SMR 1.28; 95% CI 0.41 – 5.9, 5 deaths). According to the job-exposure matrix score of $\geq$ 2, the risk of all hard-metal exposure was similar as in the Moulin et al. (1998) study (SMR 2.02; 95% CI 1.32 – 2.96, 26 deaths) and increased with duration and semi-quantitative cumulative exposure.

Later studies performed in Austrian, German, Swedish, UK and US hard-metal worker manufacturing cohorts, with most recent follow-ups published in 2017 (Wallner et al. (2017), Morfeld et al. (2017), Marsh et al. (2017a) (Svartengren et al., 2017) (McElvenny et al., 2017)). These cohorts were then pooled by Marsh et al. (2017b) in the so far largest epidemiological study related to cancer among hard-metal production workers. By combining data from national cohorts, they studied total and cause-specific mortality among 32 354 hard-metal production workers from 17 manufacturing sites in these five countries. Special emphasis was on lung cancer risk in relation to exposure to cobalt, nickel and tungsten. Mortality data were collected for the years 1952-2014. About 38% of the workers were born after 1960, 53% were hired after 1979 and 73% were men.

Based on quantitative job-exposure matrices, exposure estimates were generated for Co, Ni and W for the period of 1952-2014 (jobs held before 1952 were assigned 1952 exposures). The job-specific exposure data used, covered the set of industrial hygiene measurements available for all the manufacturing sites involved (Kennedy et al., 2017). Personal sampler data were preferred. Although there was variation as regards if total aerosol or inhalable particles had been measured, the available particle size distribution data indicated that any correction for that would only have a minimal effect and was therefore not applied. Respirable fraction estimates were not generated as there was limited availability of such measurement data and also lacking process/engineering data that would allow generating modelled estimates. The exposure estimates were not corrected for any possible time trends in use of personal protective equipment e.g., in the highest exposure operation that may have influenced the ratio between true inhalation exposure and the measured airborne concentrations.

The exposure range was from 1 to 300 µg Co/m^3, from 3 to 300 µg W/m^3 and 1 to 30 µg Ni/m^3. The median values were 6 µg Co/m^3, 30 µg W/m^3 and 3 µg Ni/m^3. The ratio mean was 13 µg Co/m^3, 89 µg W/m^3 and 5 µg Ni/m^3. The ratio mean was calculated as the sum of cumulative exposure divided by the sum of duration of exposure across all workers with known work history. Due to process improvements and tightening national occupational exposure limits, the Co exposures indicated a decline over time. There were too few data to determine whether W or Ni exposures decreased over time.

About 30% of the cohort members had worked less than 1 year in hard-metal production. The detailed risk analyses were focused on those cohort members who had worked at least 1 year in hard-metal production (22 506 persons, 544 845 person-years of follow-up). According to the authors the rationale for this choice was that in general, short-term workers are known to have a less favourable mortality pattern due to unhealthy behavioural and lifestyle characteristics associated with short-term workers, compared with longer-term workers. Risk estimates were calculated using both internal (relative risk (RR) compared to the lowest exposure category) and external (standardised mortality ratios (SMR) compared to regional rates) references.

Overall, the lung cancer mortality was not statistically significantly increased among workers with at least 1 year of employment (SMR 1.10 (95% CI 0.97 – 1.23). The risk was higher among those workers who had worked only less than 1 year in hard-metal production (SMR 1.42 (95% CI 1.21 – 1.65) and thus the SMR of lung cancer in the entire cohort was increased (SMR 1.20, 95% CI 1.09 – 1.31). There was no significant trend in SMR of lung cancer by duration of employment (p=0.12) or time since first exposure (p=0.11). Analyses by exposure to cobalt, nickel or tungsten and lung cancer risk did not

reveal significant trends in relative risks with the internal comparison group or SMRs compared to the general population by average intensity or cumulative exposure to either Co, Ni or W (**Table 18**). There was also no trend by exposure years, for any of these metals. The Swedish cohort contributed about one half of the pooled cohort and showed a higher lung cancer risk than the rest of the national cohorts. According to a Poisson regression analysis of the lung cancer risk, there was heterogeneity between the five national cohorts ($p < 0.001$) but not between the four cohorts, other than the Swedish one ($p=0.503$).

Table 18: Relative risk (RR) and standardized mortality ratio (SMR) of lung cancer by mean intensity of exposure and cumulative exposure to Cobalt, Tungsten and Nickel (pooled hard-metal worker cohorts, at least 1 year of employment)-data from Marsh et al. (2017b)

Exposure	No of deaths	RR* (95% CI)	p for trend	SMR (95% CI)
Cobalt				
Mean intensity (µg Co/m^3)			0.065	
< 1.9	35	1.00 ref		1.04 (0.73 – 1.45)
2.0 – 4.9	73	0.84 (0.56 – 1.27)		0.93 (0.73 – 1.16)
5.0 – 10.9	95	1.22 (0.82 – 1.82)		1.30 (1.05 – 1.58)
≥ 11.0 -	82	1.18 (0.79 – 1.77)		1.15 (0.92 – 1.43)
Cumulative (µg Co/m^3 -yrs)			0.302	
< 9.1	29	1.00 ref		0.83 (0.56 – 1.19)
9.1 – 39.3	74	1.31 (0.85 – 2.03)		1.15 (0.90 – 1.44)
39.4 – 127	91	1.51 (0.99 – 2.31)		1.31 (1.06 – 1.61)
≥ 128	91	1.31 (0.85 – 2.01)		1.03 (0.83 – 1.27)
Tungsten				
Mean intensity (µg W/m^3)			0.081	
< 6.9	62	1.00 ref		0.95 (0.73 – 1.22)
7.0 – 39.9	92	1.32 (0.95 – 1.83)		1.18 (0.95 – 1.45)
40.0 – 289	69	1.34 (0.94 – 1.90)		1.09 (0.85 – 1.38)
≥ 289 -	62	1.39 (0.96 – 2.01)		1.23 (0.95 – 1.58)
Cumulative (µg W/m^3 -yrs)			0.158	
< 12.3	15	1.00 ref		0.97 (0.55 – 1.61)
12.3 – 54.8	35	0.94 (0.51 – 1.73)		1.00 (0.70 – 1.39)
54.9 – 250	59	1.11 (0.62 – 1.97)		1.04 (0.79 – 1.34)
250 - 869	85	1.38 (0.79 – 2.41)		1.32 (1.06 – 1.64)
≥ 869	91	1.19 (0.68 – 2.08)		1.07 (0.86 – 1.31)
Nickel				
Mean intensity (µg Ni/m^3)			0.227	
0	28	1.00 ref		1.38 (0.92 – 2.00)
0.001 – 3.19	133	0.78 (0.49 – 1.23)		1.00 (0.84 – 1.19)
≥ 3.2	124	1.01 (0.64 – 1.58)		1.19 (0.99 – 1.42)
Cumulative (µg Ni/m^3 -yrs)			0.305	
< 1.9	35	1.00 ref		1.30 (0.91 – 1.81)
1.9 – 19.6	66	0.84 (0.54 – 1.31)		1.01 (0.78 – 1.28)
19.7 – 68.6	92	0.95 (0.62 – 1.46)		1.13 (0.91 – 1.39)
≥ 68.7	92	1.09 (0.70 – 1.68)		1.11 (0.90 – 1.36)

* Adjusted for age, calendar time, gender and country

The estimates of each metal are not adjusted for exposure level of the two other metals. Cobalt exposure related risks for example, are not adjusted for mean intensity or cumulative exposure to tungsten or nickel.

The risk estimates were not adjusted for smoking. However, separate analyses using the indirect adjustment method of Richardson (2010) and Richardson et al. (2014) were performed to assess confounding by smoking but only at dichotomous level of exposure to Co (exposed/unexposed). The smoking unadjusted relative risk from cobalt exposure of 1.07 (95% CI 0.74 – 1.53) was reduced to 0.91 (95% CI 0.53 – 1.30) after adjustment for smoking. Assuming that adjustment for smoking would influence the dose-specific RRs of **Table 18** similarly, those risk estimates above 1 would be reduced towards 1. However, such analyses were not performed.

The two highest exposure intensity categories in **Table 18** represent exposures either slightly below or above 10 µg Co/m^3.

Marsh et al. (2017b) also analysed the risk by pre- and post-sintering operations. The risks were quite similar: SMR (95% CI) for those with pre-sintering jobs was 1.15 (0.91–1.44), for those with post-sintering jobs only 1.10 (0.84–1.42), for those with mixed pre/post-sintering jobs 1.42 (0.97–2.00) and for those with no pre/post-sintering jobs 1.00 (0.83–1.20). There was no trend of risk by increasing employment duration, either for pre- or for post-sintering operations.

Of the cancer sites other than lung, the SMR compared to regional rates was statistically significantly increased for buccal cavity and pharynx (1.69; 95% CI 1.30 – 2.16) and all digestive organs and peritoneum combined (1.10; 95% CI 1.01 – 1.19). However, when focusing on workers with at least 1 year of employment, there was no significant excess for either of these cancer sites, SMRs 1.40 (95% CI 0.96 – 1.96) and 1.08 (95% CI 0.97 – 1.19), respectively. No analyses by duration of employment, time since first exposure or exposure metrics of Co, Ni or W were performed for cancer sites other than lung cancer. There was no obvious indication of a healthy worker bias (e.g. SMRs for all causes, all cancer and cardiovascular diseases were 1.12, 1.07, and 1.07, respectively).

To conclude, the pooled analysis in a population with the highest analysed exposure categories representing mean intensities around 10 µg Co/m^3, identified no clear relationship between the exposure to Co (concomitantly with tungsten carbide) and mortality from lung cancer. The authors also noted that although all risk estimates were standardized for age, the study populations in most countries were young, contributing a relatively small percentage of total deaths before the end of the observation period (e.g., in Austrian, German, UK, and US cohorts, only 9.0, 11.0, 11.9, and 14.9%, respectively, of workers had died by the end of the follow-up). In the entire cohort, about 40% were born after 1960, making them 55 years old or younger at the end of study, and nearly one-third of the cohort was hired at age 35 or older. More than half the cohort was hired after 1979. At the end of the study period, 24,674 study members (76.3%) were assumed alive and 7187 (22.2%) were known to be dead. Consequently, many members did not reach the older age groups for which mortality rates for many causes of death categories increase dramatically in the general population. It is also noted that the exposure estimates were not corrected for any possible time trends in use of personal protective equipment e.g., in the highest exposure operation that may have influenced the ratio between true inhalation exposure and the measured airborne concentrations.

7.7.1.2 Workers exposed in cobalt production

Moulin et al. (1993) extended the earlier (cancer) mortality study of Mur et al. (1987) and followed until the end of 1988 those 1148 men who had worked at least one year in a French cobalt production plant between 1950 and 1980. In the plant, cobalt was obtained by etching (HCl) of the roasted ore, then by neutralization (NaOH, $Ca(OH)_2$), multiple filtrations, and finally by electrolysis of the cobalt dichloride solution. The cobalt metal

manufacturing process also included oxides and cobalt salts production. No exposure measurement data were reported.

Compared to the general population, the overall cancer mortality was not increased in the entire cohort (SMR 0.83; 95% CI 0.66 – 1.03) or in those workers who were born in France (SMR 1.00; 95% CI 0.78 – 1.26). Lung cancer mortality was not increased in either cohort; SMRs 0.89 (95% CI 0.53 – 1.38. 19 deaths) and 1.12 (95% CI 0.65 – 1.80, 17 deaths), respectively. The lung cancer mortality was not increased in any of the worker groups investigated (cobalt workers, sodium workers, maintenance workers and administrative workers). The SMR for lung cancer was the highest in maintenance workers but did not show any trend by duration of exposure or time since first exposure. No analyses were reported by intensity of cobalt exposure for any of the groups studied. The only cancer site with statistically significantly increased mortality was brain with SMRs of 3.57 (95% CI 1.16-8.32, 5 deaths) and 3.98 (95% CI 1.08-10.2, 4 deaths) in the entire and French-born cohorts, respectively. The increase in brain cancer mortality was due to an increase among the maintenance workers and administrative workers, while cobalt or sodium production workers did not show an increase. It thus seems unlikely that this increase was causally related to cobalt exposure.

Sauni et al. (2017) followed until the end of 2013, the cancer incidence among those 995 men who had worked in a Finnish cobalt production plant for at least one year in 1968-2004. For their exposure, it was reported that in sulphatising roasting, the dust in the ambient air was found to contain 15–20% iron, 1% zinc, 0.4% cobalt, and 0.2% nickel, whereas in leaching building, the dust consisted of metal sulphides and sulphates. In the reduction plant and powder production facility, cobalt was mainly in the form of cobalt powder and fine powder. In the chemical department, the cobalt and nickel compounds were mainly sulphates, carbonates, oxides, and hydroxides. During 1968-2014, the mean cobalt exposure levels ranged from about 20 µg/m^3 in the chemical department and in the leaching and purification department, to about 80-100 µg/m^3 in the sulphatising roasting and in reduction and powder production.

Compared to the general population, the overall cancer incidence was not increased (SIR 1.00, 95% CI 0.81 – 1.22). The only cancer site with a statistically increased incidence was cancer of the tongue based on only 3 observed cases (SIR 7.39; 95% CI 1.52 – 21.6). The incidence of lung cancer (SIR 0.50; 95% CI 0.18 – 1.08, 6 observed cases) was not increased. Using an exposure grading (low, moderate, high) based on working department, there was no indication of a dose-response for all cancer, lung cancer, tongue cancer or bladder cancer. Two of the lung cancer cases occurred in the low exposure category (SIR 0.41: 95% CI 0.05 – 1.47) and four in the high exposure category (SIR 0.67, 95% CI 0.18- 1.72). The cobalt exposure levels in these categories were reported as 16-20 and 65-100 µg/m^3, respectively. As regards the three workers with tongue cancer, it was reported that all three were smokers while their alcohol consumption habits were unknown. No adjustment for potential confounding was possible as the comparison was made to the general population with unknown life-style habits at individual level.

Sauni et al. (2017) considered that the healthy worker effect did not play a marked role in their study. In the cobalt plant, there was no selection of workers because of possible cancer risk. No markers or tests were used in pre-employment health examinations of the plant to exclude individuals that could be at risk of cancer. They could also follow the workers after the end of employment. Thus, if they had to leave work because of health reasons, they were still included in the cohort follow-up. It is further noted that the overall risk of cancer (SIR = 1.00) does not indicate a healthy worker bias. The study was a follow-up for cancer incidence via the national cancer registry and thus no risk estimates for non-cancer or overall mortality/incidence were reported.

7.7.1.3 Workers exposed in use of cobalt compounds

Tüchsen et al. (1996) followed 875 women exposed to cobalt-aluminate spinel in plate under-glazing departments of two Danish porcelain factories and compared them with 520 women from cobalt-free departments of one the two plants. The exposed women had worked in the factories in the periods 1943-87 (factory 1) and 1962-87 (factory 2), and the non-exposed women in 1943-87 (factory 1) and were followed for cancer incidence until 1992.

All exposed workers performed the same tasks, but the number of plates produced per day varied. The workers sprayed cobalt blue dye on bare plates and later removed superfluous dye from parts of the plate, in order to form a picture in white and various blue colours. In both factories, an exhaust cupboard was built into the workplace of each worker. Until 1972, cobalt-aluminate spinel (based on cobalt oxide) was used and thereafter cobalt silicate dye in factory 1 (and in factory 2 workers since 1989, when these workers moved to factory 1). Both dyes had a cobalt content of about 25%. No worker worked only with the latter dye and for most workers included in the study, the work with the latter dye covered only a short period of the entire exposure period. Until 1981, only measurements for total dust were performed. In 1981, cobalt measurements were performed in 19 workers and the industrial hygiene standard was exceeded in all of them, in the range of 1.3 to 172 times. The national standard is not reported, but it was supposedly higher than the current national OELs (8-hour TWA limit value of 10 µg/m^3 and STEL of 20 µg/m^3). In a separate paper (Raffn et al. 1988) from the same factory, reference is made to a national limit value of 50 µg/m^3. This indicates exposures up to higher than 1000 µg/m^3 for the time period relevant for this cancer follow up.

Compared to the general population, there was a statistically significantly increased incidence of lung cancer among the cobalt exposed women (SIR 2.35; 95% CI 1.01 – 4.62, 8 cases). However, the risk was nearly as high among the cobalt non-exposed women (SIR 1.99, 95% CI 0.80 – 4.11, 7 cases) and in the internal comparison of the cobalt exposed to the non-exposed, no significant increase in the relative risk ratio was observed (RR 1.2; 95% CI 0.4 - 3.8). This may indicate an effect from exposure to a factor other than cobalt that affected both groups of women in the factory. Information on smoking habits was only sporadically available and potential confounding by smoking could not be adjusted for. The only other cancer sites showing significantly elevated risks were cervical cancer among the exposed women (SIR 2.31; 95% CI 1.19 - 4.03, 12 cases) and cancer of corpus uteri among the non-exposed women (SIR 3.02; 95% CI 1.38-5.73, 9 cases). No analyses by intensity of cobalt exposure were reported for any of the comparisons above.

Moulin et al. (2000) performed a nested lung cancer case-control study in a cohort of 4897 workers that had worked at least one year between 1968 and 1991, in a French factory that produced stainless steel and metallic alloys. There were 54 cases and 162 gender and age matched controls. Smoking habits were available from medical records and job histories from administrative records. A job exposure matrix (JEM) was developed to estimate exposure to metals (iron, chromium and/or nickel, cobalt) and/or their compounds, acid mists, PAHs, silica and asbestos. The crude odds ratio (OR) of lung cancer was not increased among those exposed to cobalt (OR = 0.62; 95% CI 0.26 - 1.46) nor was the OR increased when adjusted for smoking and exposure to PAHs and silica (OR = 0.44; 95% CI 0.17 - 1.16).

7.7.1.4 Other work-related exposure

Grimsrud et al. (2005) performed a nested case-control study among 213 lung cancer cases and 525 age-matched controls from a Norwegian nickel refinery, having also experienced exposure to cobalt. When adjusted for smoking, there was a non-significant, slightly increased risk of lung cancer by cumulative exposure to cobalt as a continuous

variable (OR = 1.3; 95% CI 0.9 – 1.8) per mg/m^3-years). However, when adjusted for exposure to nickel, asbestos and other carcinogens, the risk per cumulative cobalt exposure was no longer increased (OR = 0.7: 95% 0.3 – 1.4) per mg/m^3-years). In a categorical analysis (low, moderate, high cumulative cobalt exposure), the smoking-adjusted risk of lung cancer increased by exposure. However, that analysis could not be adjusted for nickel exposure due to collinearity of the two exposures.

A case report of an oral cavity cancer following acute oral exposure is described in section 7.2.1.

7.7.1.5 Non-occupational exposure

Further to the occupational studies via the inhalation route, there have been some concerns regarding an increased risk for cancer in patients with metal-on-metal implants. Numerous epidemiological studies were conducted to evaluate the total and specific cancer rates in implanted patients, but none revealed any indication of an increased cancer risk (see Leyssens et al. (2017) for review). No human studies in non-occupational populations exposed via the inhalation route are available.

7.7.2 Animal data

In the most recent evaluation by IARC (Karagas et al., 2022) regarding cancer data in experimental animals, it was concluded that "The evidence for cancer in experimental animals was "sufficient" for cobalt metal, for soluble cobalt(II) salts, and for cobalt(II) oxide". However, for the other cobalt compounds included in the evaluation, the conclusion was that the evidence regarding cancer in experimental animals was "limited" for cobalt(II) sulphide, and "inadequate" for cobalt(II,III) oxide and for other cobalt(II) compounds. This evaluation was largely based on earlier studies in rodents (i.e., NTP 1998 and NTP 2014 and a subset of studies in **Table 19** and **Table 24**), described below. A previous evaluation by IARC (2006), had reached the same conclusion, with regard to cobalt metal powder and cobalt sulphate carcinogenicity in experimental animals. Evidence was deemed limited for metal alloys containing cobalt and cobalt–aluminum–chromium spinel.

A number of carcinogenicity studies with cobalt metal powder, cobalt compounds and cobalt containing alloys, involving different animal species and routes of administration, were first reviewed by IARC in 1991 (IARC, 1991). Inhalation exposure of male Syrian golden hamsters (n=51) to 0 or 10 mg/m^3 cobalt(II) oxide dust, for 7 hours per day, five days per week, for life, did not result in an increased incidence of any tumour type, compared to controls, in a study limited by poor survival (Wehner et al., 1977). Chronic exposure of Sprague-Dawley rats (n=50/sex/dose) to 2 or 10 mg/kg bw cobalt(II) oxide powder via intratracheal instillation, in a dose regimen as that shown in Table 24, resulted in two benign lung tumours, in one male and one female, in the low dose group. In the high dose group, two bronchoalveolar adenomas occurred in males, and one bronchoalveolar carcinoma and three adenocarcinomas in females and males, respectively (total of 6/100 alveolar/bronchiolar adenomas/carcinomas combined) (Steinhoff and Mohr, 1991). In a smaller experiment by the same group, intratracheal instillation of cobalt(II) oxide potentiated the effect of benzo(a)pyrene when used in combination, in female Sprague-Dawley rats (n=20) with eight rats presenting with squamous-cell carcinomas and one with an adenocarcinoma of the lung (Steinhoff and Mohr, 1991). Cobalt metal and compounds exhibited carcinogenic potential in connective and striated muscle tissue, when delivered by injection, consistently producing mostly localised sarcomas at the injection site, in a subset of studies summarised in **Table 19**. When delivered by alternative routes such as intratracheal instillation, subcutaneous, intramuscular and intra-osseous implantation and intrarenal administration, cobalt and compounds were largely inactive (IARC, 1991). In its evaluation, IARC concluded that there was sufficient evidence for the carcinogenicity of cobalt metal powder and cobalt (II) oxide in experimental animals, limited evidence for the carcinogenicity of cobalt (II)

sulphide, cobalt(II)chloride and metal alloys containing cobalt, chromium and molybdenum, while evidence was deemed inadequate for cobalt-aluminium-chromium spinel, cobalt(II, III) oxide, cobalt naphthenate and cobalt diacetate.

Table 19: Summary of studies of cobalt, cobalt compounds and cobalt containing alloys delivered by injection (data extracted from (IARC, 1991))

Test substance	Species, strain, sex, No	Dose/ schedule/ duration	Route of administration	Findings/incidences in survivors/Remarks	Reference
Cobalt metal powder	Hooded, Rats, M+F, n=10	28 mg	i.m.	6 months observation (at time of publication); malignant tumours (rhabdoomyofibro-sarcomas) at site of injection in 2/10 in M (one tumour developed a lymph node metastasis) and 1/10 in F rats	(Heath, 1954)
Cobalt metal powder	Hooded rats, M+F, n=10	28 mg	i.m.	observation/autopsy (21-122 weeks); injection site tumours (mostly rhabdomyofibro-sarcomas) in 4/10 M (one lymph node metastasis) and in 5/10 F; 0/10 in controls	(Heath, 1956)
	Hooded rats, F, n=10	28 mg	i.m.	autopsy (10-105 weeks); injection site tumours (mostly rhabdomyofibro-sarcomas) in 8/10 F	
Cobalt Metal powder	Hooded rats, F, n=10	28 mg	intra-thoracic (diaphragm)	observation up to 28 months; intrathoracic tumours in 2/4 (one tumour being a heart muscle rhabdomyo-sarcoma	(Heath and Daniel, 1962)
	Hooded rats, F, n=10	28 mg	intra-thoracic (intercostal)	observation up to 17,5 months; intrathoracic tumours in 2/8 (one rhabdomyosarcoma of both cardiac and intercostal muscle and one skeletal muscle rhabdomyosarcoma), 3/4 tumours involved the cardiac muscle	
Cobalt (II) oxide unwashed/ washed	Swiss mice, F, n=50	1x10 mg/site	i.m.	observed up to 110 weeks; failure to induce tumours: 0/46	(Gilman and Ruckerbau er, 1962)
Cobalt(II) oxide	Wistar rat, M+F, n=10	1x30 mg/site (10	i.m.	Observed for 74 weeks: injection site rhabdomyosarcomas in	

Test substance	Species, strain, sex, No	Dose/ schedule/ duration	Route of administration	Findings/incidences in survivors/Remarks	Reference
		effectively exposed sites)		5/10 (metastases to the lung and lymph nodes in 4 cases); (0/10 in controls)	
Cobalt(II) oxide	Swiss Mice, M+F, n=46	1x10 mg/site (both thighs) (total sites injected= 92)	i.m.	2-year observation; failure to induce tumours; 0/46	
Cobalt(II) oxide	Wistar rat, n=32	1x20 mg in one (n=19) or both thighs (n=5) (total injection sites=29)	i.m.	20 months following injection; sarcomas of striated muscle origin in 12/24 (13/29 sites); 25% (3/12) showing metastases to the lung and lymph nodes)	(Gilman, 1962)
Cobalt(II) oxide	Sprague-Dawley rats, M, n=10	2x5 and 10x1 mg/kg bw/week, for 2 years	s.c.	local malignant tumours in 5/10 and 4/10 treated rats; (controls 0/20)	(Steinhoff and Mohr, 1991)
	Sprague-Dawley rats, M/F, n=20	3x200 mg/kg bw at 2-month intervals	i.p.	malignant i.p. tumours in 14/20 rats; (controls 1/20)	
Cobalt sulphide	Wistar rat, n=30	20 mg/site (total injection sites=58)	i.m.	observation up to 1 year; predominantly striated muscle tumours in 28/29 rats (35/58 sites), metastases noted in 55% (16/29); sulphide markedly more active than oxide (in number and average progression time of tumours)	(Gilman and Ruckerbauer, 1962)
Cobalt (II) dichloride	Wistar rats, M, n=20	40 mg/kg bw/ 2x5 d, at 9-d interval	s.c.	observation period: 12 months; s.c sarcomas in 8/11 treated rats; 4/8 tumours distant from injection site; 0/19 in controls	(Shabaan et al., 1977)
Cobalt(II) dichloride	Wistar rats, M, n=20	as above	s.c.	observation period: 8 months; s.c sarcomas in 6/16 treated rats; 1/6 distant from injection site	

Test substance	Species, strain, sex, No	Dose/ schedule/ duration	Route of administration	Findings/incidences in survivors/Remarks	Reference
Cobalt alloys Co/Cr/Mo (66.5% cobalt in wear particles)	Hooded rats, F	28 mg of alloy detritus/wear particles (samples A, B and C)	i.m.	Total of 14 tumours (A: 3/11; B: 4/14 and C:7/50); 7/14 microscopically examined; mostly fibrosarcomas; 4/7 produced metastases	(Heath et al., 1971)
Co/Cr/Mo	Hooded rats,	As above	i.m.	observation up to 29 months after injection; tumours at injection site (mostly rhabdomyo-sarcomas) in 22/80 rats, metastases in the lymph node noted for 9/22	(Swanson et al., 1973)
Co/Al/Cr spinel	Sprague-Dawley rats, M/F, n=20	3x200 mg/kg at 2-month intervals	i.p.	malignant i.p tumours in 2/20; (controls 1/20); no statistically significant carcinogenic effect	(Steinhoff and Mohr, 1991)

Cobalt sulphate heptahydrate was administered to F344/N rats and $B6C3F_1$ mice (n=5-10/sex/group) in subchronic inhalation studies of 16-day and 13-week exposures to 0.1-200 mg/m^3 and 0.3-30 mg/m^3 cobalt compound, respectively. A spectrum of nonneoplastic lesions and neoplasms in the respiratory tract of exposed animals, was reported. In the 13-week study, lesions included olfactory epithelium, squamous metaplasia of the respiratory epithelium, and inflammation in the nose; inflammation, necrosis, squamous metaplasia, ulcers (rats), and inflammatory polyps (rats) of the larynx; squamous metaplasia of the trachea (mice); and histiocytic infiltrates, bronchiolar regeneration, peribronchiolar and septal fibrosis, and epithelial hyperplasia in the alveoli of the lung. The respiratory tract was thus identified as the major target of toxicity and larynx as the most sensitive tissue. Although a no-observed-adverse effect level was not identified since squamous metaplasia occurred in rats and mice at the lowest exposure concentration of 0.3 mg/m^3, these studies informed the dose selection for subsequent chronic inhalation ones (Bucher, 1991, Bucher et al., 1990).

In the 2-year inhalation studies conducted by the NTP in 1998, male and female F344/N rats and $B6C3F_1$ mice (n=50/sex/group) were exposed to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt sulphate heptahydrate (corresponding to 0.114, 0.32 or 1.14 mg cobalt/m^3), 6 hours per day, 5 days per week, for 105 weeks (NTP, 1998, Bucher et al., 1999). In rats, no exposure-related effects on survival or mean body weights were noted. The combined incidences of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) in the high dose 3.0 mg/m^3 exposure group in males, were significantly greater than in chamber controls, providing *'some evidence'* of carcinogenic activity. In female rats exposed to 1.0 or 3.0 mg/m^3 cobalt sulphate, the incidences of alveolar/bronchiolar neoplasms were significantly greater than those in the control group and exceeded the NTP historical control ranges, providing *'clear evidence'* of carcinogenicity (**Table 20**). Additionally, there were two occurrences of squamous cell carcinoma in female rats; one in the 1 mg/m^3 and one in the 3 mg/m^3 dose groups.

Table 20: Incidences of neoplastic effects in the lungs and the adrenal medulla in male and female F344/N rats (50/sex/group), in a 2-year inhalation study of cobalt sulphate heptahydrate ((adapted from (National Toxicology, 1998))

Doses (mg/m³)	0		0.3		1.0		3.0	
Lung	M	F	M	F	M	F	M	F
Alveolar/ bronchiolar adenoma	1/50[a]	0/50	4/50	1/49	1/48	10/50**	6/50	9/50***
Alveolar/ bronchiolar carcinoma	0/50	0/50	0/50	2/49	3/48	6/50*	1/50	6/50*
Alveolar/ bronchiolar adenoma or carcinoma	1/50 (2%)	0/50 (0%)	4/50 (8%)	3/49 (6%)	4/48 (8%)	15/50** (30%)	7/50* (14%)	15/50** (30%)
Adrenal medulla								
Benign, complex, or malignant pheochromocytoma	15/50[a] (30%)	2/48 (4%)	19/50 (38%)	1/49 (2%)	25/49* (51%)	4/50 (8%)	20/50 (40%)	10/48* (21%)

[a] Overall rate; number of neoplasm-bearing animals/number of animals examined micro-scopically; significantly different (*p<0.05, **p<0.001, ***p=0.003) to chamber controls by the logistic regression test

The combined incidences of benign, complex, or malignant adrenal pheochromocytoma in the 1.0 mg/m³ group of males and in 3.0 mg/m³ exposed females were significantly greater than those in chamber controls, exceeding the historical control ranges, and rendering the finding in females, exposure-related thus providing *'clear evidence'* of carcinogenicity (**Table 20**).

In the concurrent studies in mice (n=50/sex/group), under the same experimental conditions, survival of the exposed animals was similar to that of controls. A decrease in mean body weights in the 3.0 mg/m³ male group was noted from week 96. In contrast, the mean body weights of all exposed groups of female mice were generally greater than those of chamber controls from week 20 until the end of the study. The main finding in mice, as was the case in rats, was the induction of alveolar/bronchiolar neoplasms (adenomas/carcinomas), which in the 1.0 mg/m³ exposure group in females and in the 3.0 mg/m³ groups in both sexes, was significantly greater than controls, exceeding historical control ranges for inhalation studies, and thus providing 'clear evidence' of cobalt sulphate heptahydrate carcinogenic activity in mice (**Table 21**). Increased incidences of hemangiosarcoma in all exposed groups of male mice, reaching statistical significance in the 1.0 mg/m³ group, and in the same dose group in female mice, exceeded the range observed in historical controls for inhalation studies. Interpretation of this observation was however confounded as the study was later presumed to have been impacted by a *Helicobacter hepaticus* infection, manifesting with the presence of the typical liver lesions and silver-staining helical organisms. *H. hepaticus*-associated hepatitis has been associated with increases in the incidences of hepatocellular neoplasms in male mice, in NTP studies (NTP, 1998).

Having established the carcinogenicity of soluble cobalt sulphate, NTP later conducted a number of subacute/subchronic (i.e., 2 weeks and 3 months) and chronic inhalation studies to assess the toxicity and carcinogenic potential of insoluble cobalt metal in rats

and mice, and compare and contrast them to the cobalt salt findings (Behl et al., 2015, NTP, 2014).

Table 21: Incidences of neoplastic effects in the lungs in male and female B6C3F$_1$ mice (50/sex/group), in a 2-year inhalation study of cobalt sulphate heptahydrate (adapted from (National Toxicology, 1998) and (Bucher et al., 1999)

Doses (mg/m^3)	0		0.3		1.0		3.0	
Lung	M	F	M	F	M	F	M	F
Alveolar/bronchiolar adenoma	9/50[a]	3/50	12/50	6/50	13/50	9/50	18/50*	10/50*
Alveolar/bronchiolar carcinoma	4/50	1/50	5/50	1/50	7/50	4/50	11/50*	9/50**
Alveolar/bronchiolar adenoma or carcinoma	11/50 (22%)	4/50 (8%)	14/50 (28%)	7/50 (14%)	19/50 (38%)	13/50* (26%)	28/50** (56%)	18/50** (36%)

[a] Overall rate; number of neoplasm-bearing animals/number of animals examined micro-scopically; significantly different (*p<0.05, **p<0.001) from chamber controls by the logistic regression test

In concurrent 2-year GLP studies, F344/NTac rats and B6C3F1/N mice (n=50/sex/group) were exposed to cobalt metal by inhalation of particulate aerosol at concentrations of 0, 1.25, 2.5, or 5.0 mg/m^3, for 6 hours per day, 5 days per week, for up to 105 weeks. Survival was significantly decreased in female rats exposed to 2.5 mg/m^3 and in the 2.5 and 5 mg/m^3 male mice groups, compared to controls. Decreased mean body weights by at least 10% were noted in the 2.5 and 5 mg/m^3 groups in male rats (after weeks 99 and 12, respectively), in the 2.5 and 5.0 mg/m^3 females (after week 57 and 21, respectively), and in 5 mg/m^3 male and female mice (after week 85 and 21, respectively). Common sites of chronic exposure-related carcinogenicity between cobalt sulphate and cobalt metal, included the lung and the adrenal gland. In the lung, significantly increased incidences of multiple alveolar/bronchiolar adenomas occurred in all exposed groups of male rats and in females exposed to 5 mg/m^3 cobalt metal (**Table 22**). Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) increased with increasing cobalt concentrations and were significantly greater than chamber controls, at all dose levels, in both sexes. These findings, providing 'clear evidence' of carcinogenic activity of cobalt metal were generally higher (40/100 male and female rats affected) and were dominated by carcinomas compared to the previously recorded cobalt sulphate responses (22/100 male and female rats affected), at the only comparable concentrations of the two cobalt species between the two studies (i.e., 3.0 mg/m^3 cobalt sulphate equivalent to 1.14 mg Co/m^3 in the NTP 1998 study and 1.25 mg Co/m^3 group in the NTP 2014 study). Additionally, occurrences of cystic keratinising epithelioma were noted in all exposed groups of female rats which were considered cobalt exposure-related based on the rarity of these neoplasms in NTP inhalation studies and the increase over the historical control range for all routes of administration. Two males in the 1.25 and 5 mg/m^3 exposure groups also presented with cystic keratinising epithelioma (**Table 22**). One female rat in the high dose exposure group developed squamous cell carcinoma.

Another major target organ for carcinogenicity in rats was the adrenal medulla. Incidences of benign, complex or malignant pheochromocytoma (combined) increased with increasing cobalt metal exposure concentrations, and were significantly greater than chamber controls, in male and female rats exposed to 2.5 and 5.0 mg/m^3, providing 'clear evidence' of the carcinogenic activity of cobalt metal (**Table 22**). These increases were of similar extent in animals exposed to cobalt sulphate (10/50 animals affected at 1.14 mg Co/m^3) and cobalt metal (13/50 affected at 1.25 mg Co/m^3), between the two NTP chronic studies.

Distinct sites of carcinogenicity, specific to cobalt metal exposure included the pancreas, the blood and the kidney. The incidences of pancreatic islet adenoma or carcinoma (combined) in male rats (2/50, 2/50, 10/48, 9/49), were significantly increased in the 2.5 and 5.0 mg/m^3 groups, compared to controls, exceeding the historical control range for all routes of administration and considered therefore exposure-related. This finding thus provided 'some evidence' of carcinogenic activity of cobalt metal in male rats. In female rats, a slight, non-significant increase in the highest dose group only (3/50), exceeded the historical controls for all routes of administration and was deemed equivocal evidence. The incidence of mononuclear cell leukemia (MNCL) was significantly increased in female rats, at all exposure levels (16/50, 29/50, 28/50, 27/50) compared to controls, exceeding the historical control incidence, but without exhibiting any clear exposure-concentration relationship. This finding in females was considered by the authors related to cobalt exposure, however it should be noted that elevated incidences of spontaneous MNCL are common in Fisher rats which are generally prone to developing this type of tumour as they age.

Table 22: Incidences of neoplastic effects in the lungs and the adrenal medulla in male and female F344/NTac rats (50/sex/group), in a 2-year inhalation study of cobalt metal (adapted from NTP, 2014 and Behl et al, 2014)

Doses (mg/m^3)	0		1.25		2.5		5	
Lung	M	F	M	F	M	F	M	F
Alveolar/bronchiolar adenoma	2/50[a]	2/50	10/50*	7/50	10/50*	9/50*	14/50**	13/50**
Alveolar/bronchiolar carcinoma	0/50	0/50	16/50**	9/50**	34/50**	17/50**	36/50**	30/50**
Alveolar/bronchiolar adenoma or carcinoma	2/50 (4%)	2/50 (4%)	25/50** (50%)	15/50** (30%)	39/50** (78%)	20/50** (40%)	44/50** (88%)	38/50** (76%)
CKE[b]	0/50	0/50	1/50	4/50	0/50	1/50	1/50	2/50
Adrenal medulla								
Benign, complex, or malignant pheochromocytoma	17/50 (34%)	6/50 (12%)	23/50 (46%)	13/50 (26%)	38/50** (76%)	23/50** (46%)	41/50** (82%)	40/50** (80%)

[a] Overall rate; number of neoplasm-bearing animals/number of animals with lungs examined microscopically; [b] CKE = Cystic keratinising epithelioma; significantly different (*p≤0.05, **p<0.001) from chamber controls by the poly-3 test

In the kidney, slightly increased incidences of renal tubule adenoma or carcinoma in male rats exposed to 5 mg/m^3 (overall rate 7/50 (14%) compared to controls (overall rate 3/50 (6%), were noted, albeit without reaching statistical significance, yet exceeding the historical control incidences for all routes of administrations. Following an extended microscopic evaluation after step-sectioning the kidney to obtain additional sections, this finding was regarded as equivocal, since despite the additional renal tubule adenomas and hyperplasias observed, no additional renal tubule carcinomas were identified.

In mice (n=49-50/sex/group), the incidences of alveolar/bronchiolar carcinoma and the combined incidences of alveolar adenoma or carcinoma were significantly greater in all exposed groups of both sexes, than in chamber controls, exceeding the historical controls for inhalation studies and all routes of administration (**Table 23**). The combined response (71/99 male and female mice affected at 1.25 mg/m^3 cobalt) was greater compared to that observed for the similar concentration of cobalt sulphate (46/100 male and female mice affected at 3 mg/m^3=1.14 mg Co/m^3), mirroring the superior cobalt metal effect exerted in rats.

Table 23: Incidences of neoplastic effects in the lungs in a 2-year inhalation study of cobalt metal in B6C3F1/N mice (49-50/sex/group) (adapted from NTP, 2014 and Behl et al, 2014)

Doses (mg/m³)	0		1.25		2.5		5	
Lung	M	F	M	F	M	F	M	F
Alveolar/bronchiolar adenoma	7/50[a]	3/49	11/49	9/50	15/50*	8/50	3/50	10/50*
Alveolar/bronchiolar carcinoma	11/50	5/49	38/49**	25/50**	42/50**	38/50**	46/50**	43/50**
Alveolar/bronchiolar adenoma or carcinoma	16/50 (32%)	8/49 (16%)	41/49** (84%)	30/50** (60%)	43/50** (86%)	41/50** (82%)	47/50** (94%)	45/50** (90%)

[a] Overall rate; number of neoplasm-bearing animals/number of animals with lungs examined microscopically; significantly different (*p≤0.05, **p<0.001) from chamber controls by the poly-3 test

Collectively, these 2-year inhalation NTP studies provided clear evidence of carcinogenic activity of cobalt metal in male and female F344/Ntac rats and B6C3F$_1$/N mice, based predominantly on the incidences of alveolar/bronchiolar adenoma and carcinoma in the lung and increased incidences of malignant pheochromocytoma of the adrenal medulla in male rats and this is reflected in IARC's latest evaluation of cobalt metal carcinogenicity in experimental animals ((Karagas et al., 2022). Along with establishing the carcinogenic activity of both cobalt species in the NTP chronic inhalation studies in rodents, mutation analysis of homologues of human cancer genes often acquiring 'driver mutations' associated with lung carcinogenesis, were also carried out to elucidate potential mechanisms and pathways implicated in the observed carcinogenesis. *Kras* mutations were the most frequently detected mutations in cobalt metal induced carcinomas in exposed rodents (31% in rats and 67% in mice), with exon 1, codon 12 G→T transversions being the predominant point mutations (57% in rats and 80% in mice), similar to the observations made in cobalt sulphate-exposed mice (55% codon 12 G→T transversions). *Egfr* mutations were detected in 17% cobalt metal-induced lung tumours in rats and mice, while *Tp53* mutations were detected in 23% of rats and in 19% of mice occurring alveolar/bronchiolar carcinomas. The *Kras* G→T transversions, readily detected in rats and mice exposed to both cobalt species, are the most common *Kras* mutations in human lung cancer and are commonly associated with the production of reactive oxygen species (ROS) during oxidative damage to DNA ((Janssen et al., 1993) (Shigenaga and Ames, 1991, Tchou et al., 1991). These DNA base changes in *Kras* appear to correlate with the presence of (8-OHG) adducts that typically result from oxidative stress (Shigenaga and Ames, 1991, Tchou et al., 1991). Cobalt has been shown to induce hypoxia and upregulate HIF-1a signalling (see section 8.1), thereby modulating inflammatory responses and inducing oxidative stress, while cobalt sulphate has been shown to catalyse the production of oxygen-based free radicals, supporting the notion of indirect DNA damage which would also explain why these mutations were not detected in control samples in either mice (concurrent) or rats (other NTP chronic bioassays' controls) (Simonsen et al., 2012). Both EFGR and KRAS are upstream effectors in cancer-promoting/sustaining signalling pathways, including the MAPK signalling cascades and mutations can lead to aberrant activation of these pathways. The positive results in the NTP bacterial mutagenicity studies, only in the *S. typhimurium* strain TA98 among the tester strains, further highlight the G:C sequence specificity of the mutational events. Cobalt metal-induced mutagenicity was not evident upon S9 activation in any of the bacterial strains tested and it has been proposed that this could be attributable to the presence of antioxidant, radical scavenging enzymes such as glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase, and superoxide dismutase in the S9 mix, or to the direct binding of

cobalt to S9 proteins. Although mutations within *Egfr* and *Kras* are considered mutually exclusive in human lung and colon cancers, in the cobalt metal NTP study, 25% of mice and 38% of rats harbouring *Egfr* mutations, also had *Kras* mutations, with isolated cases of one mouse and one rat possessing mutations in all three genes. Cobalt may affect *Tp53* transcription and expression through DNA damage, DNA binding, the inhibition of DNA repair, and induction of ROS which in turn can affect signal transduction (including MAPK cascades) and activate downstream factors such as NFkB, AP-1, and HIF-1 (reviewed in (Hong et al., 2015)).

Finally, numerous *in vivo* studies entailing exposures to particulate cobalt (typically micronsized) and CoCr alloys exposures via different routes of administration, including implantation, and across different species, have been reviewed by (Christian et al., 2014). A subset of studies with implanted CoCr alloys reported the occurrence of tumours at the implant site, however none of the studies reported a statistically significant increase in primary systemic tumours.

7.7.3 Summary

Early studies in hard-metal production workers provided indications of an increased risk of lung cancer. However, potential confounding by smoking or by occupational factors other than cobalt was not assessed comprehensively in those studies. More importantly, risk estimates were not provided by intensity of hard-metal or cobalt exposure. Descriptive information indicates that mean exposures were of the order of tens or more µg Co/m^3. A more recent pooled analysis among about 22500 hard-metal production workers with at least 1 year of employment provided risk estimates by exposure intensity with highest analysed exposure categories representing mean intensities around 10 µg Co/m^3 and identified no clear relationship between exposure to cobalt (concomitantly with tungsten carbide) and mortality from lung cancer. None of the available studies with hard-metal exposure allows to distinguish potential cobalt effects from those of the cobalt–tungsten carbide.

In the cohorts exposed in cobalt manufacturing or in use of cobalt compounds (other than hard-metal) no consistent evidence of an increased cancer risk has been observed. For some of these cohorts no information on exposure levels was available. For cohorts with information, exposure levels were tens of µg/m^3 or more.

A number of early animal carcinogenicity studies with cobalt metal powder, cobalt compounds and cobalt containing alloys entailing i.m and i.p administration, produced mostly localised sarcomas at the injection site (**Table 19**). More relevant inhalation studies on cobalt sulphate heptahydrate in rats and mice, initially sub-chronic studies, identified the lower respiratory tract/lungs as the major target of toxicity. Prior to this, cobalt(II) oxide had exhibited no carcinogenic activity in a chronic inhalation study in hamsters, while intratracheal instillation in rats produced a limited number of lung neoplasms (NTP, 1998, Steinhoff and Mohr, 1991) (**Table 24**). In order to investigate the carcinogenic potential of both soluble and insoluble cobalt compounds, the NTP conducted two chronic carcinogenicity studies of cobalt sulphate heptahydrate and cobalt metal, in rats and mice (NTP, 1998, NTP, 2014) (main findings summarised in **Table 24**). Inhalation exposure of cobalt metal resulted in significantly increased, dose-related incidences of alveolar/bronchiolar adenomas and carcinomas (combined) in the lung, compared to chamber controls, in all exposed groups (i.e., ≥1.25 mg/m^3), in both species (n=50/sex/dose in each species). In addition, the incidences of mononuclear cell leukemia were significantly increased in all exposed groups of female rats. Cobalt exposure-related occurrences of cystic keratinising epithelioma were also noted in all dose groups of female rats (total of 7 animals affected), with single incidences also occurring in two exposure groups in male rats. One female rat in the high dose exposure group (5 mg/m^3) developed squamous cell carcinoma. Another major target organ for carcinogenicity in rats was the adrenal medulla, with significant, dose-related increases in the incidences of benign,

complex or malignant pheochromocytoma (combined) compared to chamber controls, in animals of both sexes, exposed to ≥2.5 mg/m^3 cobalt. Finally, the combined incidences of carcinoma/adenoma of the pancreatic islets were significantly increased in male rats exposed to ≥ 2.5 mg/m3 cobalt, while a slight increase was noted in females in the highest exposure group. No systemic tumours were observed in mice exposed to cobalt under the same experimental conditions as the rats.

Table 24: Summary of key animal carcinogenicity studies

Method	Test substance	Results	Remarks*	Reference
Repeated dose/ carcinogenicity study in F344/NTac rats; n=50/sex/dose; 0, 1.25, 2.5, or 5 mg/m^3, 6 h plus T90 (12 minutes) per day, 5 days per week for up to 105 weeks **inhalation**	cobalt metal >98% purity	**≥1.25 mg/m^3:** significantly increased incidences of alveolar/bronchiolar neoplasms in the lung (m/f) Significantly increased incidence of mononuclear cell leukemia (f); occurrences of cystic keratinising epitheliomas (m/f); 1 female rat exposed to 5 mg/m^3 had a squamous cell carcinoma. **≥2.5 mg/m^3:** significantly increased incidences of benign, complex or malignant pheochromocytomas of the adrenal medulla (m/f) Significantly increased neoplasms (adenomas/carcinomas (combined) of the pancreatic islets (m) **5 mg/m^3:** Non-significant, non dose-related Renal tubule adenomas/carcinomas (m) Increase in neoplasms (adenomas/carcinomas (combined) of the pancreatic islets (f)	*"Clear evidence of carcinogenic activity"* *"exposure related"* *"(possibly) exposure related" (f)* *"clear evidence"* *"Some evidence; exposure related"* *"possibly exposure related"* *"equivocal"*	(NTP, 2014)
Repeated dose/ carcinogenicity	cobalt metal >98% purity	**≥1.25 mg/m^3:** significantly increased	*"Clear evidence"*	(NTP, 2014)

Method	Test substance	Results	Remarks*	Reference
study in B6C3F$_1$/N mice; n=50/sex/dose; 0, 1.25, 2.5, or 5 mg/m^3, 6 h plus T90 (12 minutes) per day, 5 days per week for up to 105 weeks **inhalation**		incidences of alveolar/bronchiolar carcinomas in the lung (m/f)		
Repeated dose/ carcinogenicity study in F344/NT rats; n=50/sex/dose; 0, 0.3, 1.0, or 3.0 mg/m^3, 6 h per day, 5 days per week for 105 weeks **inhalation**	cobalt sulphate heptahydrate (approximately 99% pure)	**≥1.0 mg/m^3** (=0.38 mg Co/m^3): statistically increased incidence of alveolar/bronchiolar neoplasms in the lung in (f) and at **3 mg/m^3** (=1.14 mg Co/m^3) in (m); 1.0 mg/m^3: statistically increased, yet marginal incidence of benign, complex, or malignant pheochromocytoma of the adrenal medulla in (m) and at **3.0 mg/m^3** in (f); 2 occurrences of squamous cell carcinoma in (f)	*"Clear evidence"(f)* "some evidence"(m) *"equivocal; possibly exposure related" (m)* *"Clear evidence"(f)*	(NTP, 1998)
Repeated dose/carcinogenicity study in B6C3F$_1$/N mice; n=50/sex/dose; 0, 0.3, 1.0, or 3.0 mg/m^3, 6 h per day, 5 days per week for 105 weeks **inhalation**	cobalt sulphate heptahydrate (approximately 99% pure)	**≥1.0 mg/m^3**: statistically increased incidence of alveolar/bronchiolar neoplasms in the lung (f) and at 3 mg/m^3 in (m);	*"Clear evidence"*	(NTP, 1998)
Chronic carcinogenicity inhalation study in Syrian golden hamsters; 10 g/l, 7 h/day, 5 days/week (median survival in treated animals 16.6 months)	cobalt(II) oxide	No carcinogenic effects		(Wehner et al., 1977)

Method	Test substance	Results	Remarks*	Reference
inhalation				
Sprague-Dawley rats; 2 or 10 mg/kg bw, 1 dose/2 weeks, 1 dose/4 weeks (19-30th treatment), then 1 dose/ 2 weeks x 9 doses; total 39 doses) for 2 years (total dose 78 and 390 mg/kg bw); n=50/sex **intra-tracheal implantation**	cobalt(II) oxide	2 mg/kg bw: 2/100 benign pulmonary tumours 1/100 alveolar/bronchiolar adenoma/carcinomas (combined) 10 mg/kg bw: 2/100 alveolar/bronchiolar adenomas 6/100 alveolar/bronchiolar adenoma/carcinomas (combined)		(Steinhoff and Mohr, 1991)

*the statements in brackets reflect the respective study authors' assessment

Similar to cobalt metal, inhalation exposure to cobalt sulphate heptahydrate, resulted in significant increase in the incidence of lung neoplasms (alveolar/bronchiolar adenomas and carcinomas (combined), in ≥ 1.0 mg/m^3 (=0.38 mg Co/m^3) groups in females and the 3.0 mg/m^3 (=1.14 mg Co/m^3) exposure group in males, in both rats and mice. The other common site of exposure-related carcinogenicity was the adrenal medulla. The incidences of benign, complex, or malignant adrenal pheochromocytoma was significantly increased in male and female rats, exposed to 1.0 and 3.0 mg/m^3 cobalt sulphate heptahydrate, respectively (**Table 24**).

A higher frequency of *Kras* mutations in the alveolar/bronchiolar adenomas/carcinomas of exposed animals, compared to spontaneous lung tumours in controls, was noted in both NTP studies. *Kras* mutations were predominantly G→T transversions, commonly associated with 8-Oxoguanine lesions resulting from oxidative stress, suggesting that oxidative DNA damage may be a contributing factor to the observed carcinogenesis.

Collectively, the NTP study of cobalt metal, provided clear evidence of carcinogenic activity in F344/NTac rats and B6C3F1/N mice of both sexes, on the basis of the increased incidences of alveolar/bronchiolar adenomas and carcinomas in the lung, and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla in female rats. Systemic tumours considered related or possibly related to exposure, occurring in the pancreas, the liver and blood were additionally observed in rats.

7.8 Reproductive toxicity

7.8.1 Human data

There are no human data on fertility effects of cobalt or its inorganic compounds.

No developmental effects were observed in the children of 78 women given cobalt chloride orally during pregnancy for the treatment of anaemia (Holly, 1955). Doses up to 0.6 mg cobalt/kg/day for 90 days were given, either together with an iron supplement or alone. Examination of the foetuses, however, was limited to the reporting of obvious birth defects, and for most women exposure only occurred in the final trimester. Details of the developmental endpoints examined, were not reported.

There are no human data on developmental effects via other routes of exposure.

7.8.2 Animal data

In the 1991 NTP study, subchronic inhalation of 50 mg/m^3 cobalt sulphate heptahydrate for 16 days resulted in atrophy of the testis, characterised by a decreased number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts, in F344/N rats, and coincided with marked weight loss and reduced survival (Bucher, 1991). A longer exposure of 13-weeks, at the highest concentration of 30 mg/m^3 produced limited, non-significant reductions in sperm motility and sperm counts, an increase in the abnormal sperm incidence and a longer oestrous cycle in females, compared to those of controls (Bucher, 1991). Under the same experimental conditions (30 mg/m^3, 13 weeks), significant effects in exposed B6C3F1 male mice comprised decreased testis and epididymal weight and an increased incidence of abnormal sperm, compared to controls (**Table 25**). At the same dose, atrophy of the testis, consisting of a loss of germinal epithelium in the seminiferous tubules, was observed in 9/10 mice examined. Additionally, sperm motility was significantly reduced in the ≥3 mg/m^3/day exposure groups, while in females exposed to 30 mg/m^3/day, the oestrous cycle was significantly longer than in controls. The magnitude of these effects pointed to a direct toxic effect of cobalt in the reproductive system with the rat appearing less susceptible to cobalt than the mouse (Bucher, 1991).

Table 25: Summary of significant effects of cobalt sulphate heptahydrate in reproductive tissues/oestrous cycle in male and female B6C3F1 mice in a 13-week inhalation study (adapted from (Bucher, 1991))

Doses (mg/m^3)	0	3	10	30
Mice (n=10)				
Testis weight (absolute; mg)	120	120	121	57**
Right epididymal weight (mg)	0.042	0.043	0.045	0.034**
Abnormal sperm (%)	1.29(9)	1.38	0.98	3.80**(8)
Sperm motility (%)	87.0	78.6**	75.6**	46.6**(8)
Testis atrophy/mineralisation	0/0	0/0	0/0	9**/4*
Oestrous cycle length	4.20	4.11	4.20	5.00**

() Number of animals assessed, if not n=10; *p<0.05; **p<0.01

In a subsequent 2-year cobalt sulphate heptahydrate inhalation NTP study (Bucher et al., 1999, NTP, 1998), no statistically significant or biologically relevant exposure-related non-neoplastic findings of the genital system, in either rats or mice, of either sex, were reported up to doses of 3 mg/m^3/day, equivalent to 0.6 mg cobalt/m^3/day.

Cobalt metal was evaluated in a set of NTP subchronic (2 weeks, 3 months) and chronic (2 years) inhalation studies in rats and mice, against a number of reproductive toxicity endpoints. The absolute testis weight of rats exposed to 10 mg/m^3, the highest dose with survivors at the end of a preliminary 2-week study, was significantly less than that of chamber controls (no histopathologic assessment was performed). In a 3-month study, there were no marked changes in testis weight and the only significant effect was a decrease of sperm motility in rats exposed to 1.25, 2.5, or 5 mg/m^3 cobalt metal, by up to 8% in the high dose group, suggesting a potential for cobalt metal to be a reproductive toxicant in male rats (**Table 26**). In the 2-year study, the incidence of testes infarct was significantly increased in male rats exposed to 5 mg/m^3, from 1/50 in controls to 12/50 in

the exposed group. Infarcts were mostly unilateral, with signs of necrosis and the presence, in a subset of affected testes, of multifocal intratubular mineralisation. Male mice again appeared more susceptible to cobalt-related reproductive toxicity compared to rats, under the same experimental conditions. In the 2-week exposure study, absolute testis weight in the 40 mg/m^3 group was significantly less than that of the chamber controls. At this dose, there was a significant body weight loss and only 2/5 mice survived at 17 days. In the 3-month study, males exposed to 10 mg/m^3 exhibited significant decreases in the weights of the cauda epididymis, epididymis, and testis; testis weight was also significantly decreased in the 5 mg/m^3 group (**Table 26**).

Other significant decreases occurred in the number of spermatid heads per testis (10^6/testis), and total sperm per epididymis and per gram epididymis in 5 and 10 mg/m^3 exposed males. Sperm motility was significantly less at all exposure levels dropping from 86% in controls to 2.6% at the highest dose of 10 mg/m^3, while total sperm count per epididymis and per gram epididymis was significantly decreased in both the 5 and 10 mg/m^3 exposure groups. Findings in the 10 mg/m^3 exposure group were associated with significant histopathologic changes; these included a marked degeneration of the germinal epithelium in the testes, with affected testes having a reduced cross-sectional diameter compared to chamber controls and all or most seminiferous tubules presenting irregular outlines with markedly decreased or completely absent germinal epithelium, vacuolated Sertoli cells, aspermia, intraluminal clumps of sloughed germinal cells and amorphous mineralized debris. In the epididymis, significant, albeit mild/moderate or even subtle, effects at 10 mg/m^3 exposure included an increase in the incidences of exfoliated germ cells, hypospermia, cytoplasmic vacuolization, and atrophy.

In female mice, the oestrous cycle was significantly longer in the 10 mg/m^3 group, while tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated no significant differences in oestrous cyclicity of the exposed and chamber control groups.

In the 2-year study, the incidence of minimal to mild germinal epithelium degeneration in male mice exposed to 5 mg/m^3 was significantly greater than in the chamber controls (chamber control, 9/50; 5 mg/m^3, 21/50).

Collectively, cobalt metal exposure for 3 months had significant effects on the male reproductive system, especially in mice, including concentration-related decreases in reproductive tissue weights, spermatid and epididymal spermatozoa counts, and sperm motility in combination with histopathologic findings in both the testis and epididymis, indicating that cobalt metal is likely to be a reproductive toxicant in male mice. Localised hypoxia was proposed as a possible mechanism for these cobalt-exerted effects (see section 8.1). The effects in females' oestrous cycle were of unclear toxicological significance (NTP, 2014).

Table 26: Summary of significant effects of cobalt metal in reproductive tissues/oestrous cycle in male and female B6C3F1 mice and F344/N rats in a 3-month inhalation study (adapted from (NTP, 2014))

Doses (mg/m^3)	0	1.25	2.5	5.0	10
Rats (n=10)					
Sperm motility (%)	88.8	86.0*	83.8**	81.9**	-
Mice (n=10)					
Testis weight (g)	0.1185	-	0.1132	0.1027**	0.0316**

Doses (mg/m^3)	0	1.25	2.5	5.0	10
Epididymis weight (g)	0.0603	-	0.0578	0.0614	0.0429*
Cauda epididymis (g)	0.0217	-	0.0210	0.0231	0.0168**
Spermatid heads (10^6/testis)	22.34	-	22.22	18.90*	0.53**
Sperm motility (%)	86.0	-	82.0*	82.2*	2.6**
Sperm (10^6/cauda epididymis)	11.55	-	10.53	9.62**	0.71**
Germinal epithelium degeneration (testes)[a]	2	-	0	1 (1.0)	10**(4.0)[b]
Exfoliated Germ cell	0	-	0	0	10**(2.7)
Hypospermia	0	-	0	0	10**(2.9)
Cytopasmic vacuolisation	0	-	0	0	9**(1.0)
Atrophy	0	-	0	0	10**(1.0)
Estrous cycle length (days)	4.1	-	4.0	4.1	4.9*

*p≤0.05; **p≤0.01; [a] number of animals with lesion/number of animals with tissue examined microscopically; [b] average severity of grade of lesions (1=minimal, 2=mild, 3=moderate, 4=marked)

Sprague Dawley rats (10/sex/dose) were treated by gavage with powdered cobalt (0, 30, 100, 300 or 1000 mg/kg bw/day) from 2 weeks before mating until approximately 2 weeks after mating (males) or 3 days post-partum (females). All females and 9/10 males died in the 1000 mg/kg bw/day dose group. Eight out of 10 females treated with 300 mg/kg bw/day and 5/10 females treated with 100 mg/kg bw/day died during the mating, gestation or lactation period. Lower body weight was recorded during the gestation period (300 mg /kg bw/day) and at end of the study, the decrease became statistically significantly lower than control on lactation day 1. No effects on fertility of females or sperm parameters were observed. A significant increase of post-implantation loss (30.9%, control 12.6%) and decrease in the live birth index (75.9%, control: 100 %) (both $p \leq 0.01$) was recorded at 300 mg/kg bw/day, however at this dose 8/0 dams died. A slight decrease in the viability index (95.4% and 95.5% at 100 and 300 mg/kg bw/day vs 100% in control) was recorded. A slightly lower pups' weight at 30 and 100 mg/kg bw/day on lactation days 1 and 4, which increased for pups at 300 mg/kg bw/d was recorded. No external abnormalities were observed in any of the pups examined (ECHA, 2022).

Forty Swiss mice were exposed to cobalt dichloride in drinking water (0, 6.4, 11.6, 23.1, mg cobalt/kg bw/d) for 12 weeks. Effects were observed in the sexual organs; statistically significant decreased epididymal absolute weight at the high dose, testes weights at all doses, and absolute weight of seminal vesicles at the mid and high dose. In addition, epididymal sperm counts were decreased at all exposure doses, statistically significantly from the mid dose, while testicular sperm count was significantly decreased from the mid dose. Histological examination of the testes revealed necrosis of the seminiferous tubules and interstitial tissues, congested blood vessels, hypertrophy of the interstitial Leydig cells and degeneration of the spermatogonial cells. After the exposure period, each male was caged with 2 females not exposed to cobalt. A decrease in the number of pregnant females was observed (95%, 15%, 12%, 7%), statistically significant from the mid dose. The

number of implantation and viable foetuses were also reduced (7.89, 5.67, 5.42, 6.43 and 7.74, 5.00, 4.67, 5.83 respectively), while the number of resorption and the total number of females with resorption was increased at all concentrations (Elbetieha et al., 2008).

Male Sprague Dawley rats (6/dose) received cobalt dichloride in chow (0, 5 or 20 mg cobalt/kg bw/day) for 69 days. No information on general toxicity was provided. Significantly decreased testis weight and testicular atrophy was observed at 20 mg/kg bw/day, but not at 5 mg/kg bw/day. Testicular weights decreased to 42% of control values (Nation et al., 1983).

Male CD1 mice exposed via drinking water to cobalt dichloride (5 per dose, 23, 42 or 72.1 mg/kg bw/day for 12 weeks) showed decrease fertility from 2 weeks of exposure: dose-dependent decreases in testicular weight, epididymal sperm concentration and sperm motility were recorded (**Table 27**). At the high dose, a decrease in fertility was recorded. Serum testosterone was significantly higher than in control in all groups while FSH and LH levels were not affected. The authors concluded that cobalt is directly or indirectly interfering with spermatogenesis and with local regulatory mechanisms in testosterone synthesis (Pedigo et al., 1988).

Table 27: Effects on testicular weight and sperm concentration in CD1 male mice oral administration of cobalt chloride 5 per dose (Pedigo et al., 1988)

Dose (mg/kg bw/d)	0	23	42	72.1
Testicular weight (% control value)	100	71	52	30
Epididymal sperm concentration (% control value)	100	66	29	8
Fertility	82.3			7.8

In a dominant lethal assay, cobalt dichloride was administrated for 10 weeks via drinking water to 10 B6C3F1 male mice, which were subsequently mated. After 10 weeks of dosing, all males were fertile, however, mating resulted in a lower number of pregnancies (58% vs 91% in control), a lower number of total implantations per pregnant female (6.5 vs 8.3 in control) and an increase of the average pre-implantation losses (2.4 vs 0.43 in control), with all three observations being statistically significant. Testicular function at 12 weeks was reduced with respect to control and was almost completely restored at control level after 6 weeks of recover (**Table 28**) (Pedigo and Vernon, 1993).

Table 28: Testicular function in B6C3F1 mice after oral administration (n=10/dose) (Pedigo and Vernon, 1993)

Testicular function in mice	12 weeks (% of control)	18 weeks (6 weeks recovery, % of control)
Sperm concentration	15.3	63.8
Sperm motility	18.3	Control level
Path velocity	30.8	Control level
Progressive velocity	22.2	Control level
Linear index	75.7	Control level
Progressive motility	17.2	Control level
Track speed	43.7	Control level
Testicular weight	41	60

Effects on mice testes were observed in animals exposed to 400 ppm cobalt dichloride (24 mg cobalt/kg bw/day) in drinking water for 13 weeks. Seminiferous tubule degeneration, vacuolation of Sertoli cells, and shrinkage of tubules were among the effects observed (Anderson et al., 1992).

Sprague Dawley rats maintained on a diet containing 265 ppm cobalt dichloride (20 mg cobalt/kg bw/day) for up to 98 days, exhibited progressive degenerative changes in testicular morphology (e.g., formation of "giant" cells, alteration of sperm tail filaments, degeneration of sperm mitochondria and a decrease in testicular volumes), which were attributed to tissue hypoxia (see section 8.1), mediated by the thickening of the basal lamina and the increased packing density of red blood cells (Mollenhauer et al., 1985). Under the same experimental conditions, cobalt-induced polycythaemia produced necrotic lesions in the germinal epithelium and seminiferous tubules of rats (Corrier et al., 1985).

Pregnant Wistar rats, 15 per group, were dosed with cobalt dichloride 0, 12, 14 and 48 mg/kg bw/day (0, 3, 6, 12 mg cobalt/kg bw/day) from gestation day 14 (GD14) until post-natal day 21 (PND21). A dose-dependent decrease in number of litters, increase in dead pups per litter, decrease foetal weight and general growth delay, as well as an increase in pups mortality were reported (Domingo et al., 1985).

The potential developmental toxicity of cobalt dichloride was evaluated in 20 pregnant Sprague-Dawley rats administered by gavage, a daily dose of 0, 25, 50, 100 mg/kg bw/day on GD6–15. Maternal effects were evident in the highest dose group and included significant reductions in weight gain and food consumption, and changes in blood chemistry. However, no significant treatment-related adverse changes were reported in the number of total implants, resorptions, the number of live and dead foetuses, foetal size parameters and sex distribution, or foetal gross external abnormalities. The authors therefore suggested that cobalt does not exert significant teratogenic or foetotoxic effects in the rat, at doses as high as 100 mg/kg bw/d (Paternain et al., 1988).

Szakmáry et al. administered cobalt sulphate heptahydrate by gavage to pregnant CD1 mice, Wistar rats and New Zealand White rabbits (Szakmáry et al., 2001). Pregnant female C57BL mice (25 or 20/dose) were given 0 or 50 mg/kg bw of cobalt sulphate heptahydrate by gavage daily during GD6-15. Maternal weight gain was non-significantly decreased (49.4% vs 61.3% in control). An increased frequency of foetuses with retarded body weight gain (24.7% vs 4.7% in control) and statistically significant skeletal retardation (58 vs 27 in control) was observed. In addition, a statistical increase of malformed foetuses (major anomalies of eyelids, kidneys, cranium and spine) was noted, although the increase of each singular malformation was not statistically significant (Szakmáry et al., 2001).

Pregnant female Sprague Dawley rats (3-18/dose) were given 0, 25, 50 or 100 mg/kg bw/day of cobalt sulphate heptahydrate by gavage during GD1-20 and were sacrificed on GD 21. In a second study, rats were treated until GD 21 (only 0 and 25 mg/kg bw/day) and were allowed to give birth. In this study, the development of the pups was followed up until PND 21. Cobalt concentration in maternal blood, foetal blood and amniotic fluid (24 hours after the last exposure on day 20) increased in a dose-dependent matter. The cobalt concentration in foetal blood was higher than in maternal blood showing placental transfer. Maternal body weight gain was not significantly affected. The relative liver, adrenal and spleen weight were increased at the highest dose level. Several clinical chemical parameters were significantly changed compared to the controls at the highest dose. RBC and Hb were increased at the highest dose but not significantly. There were no effects on litter size, resorptions or post-implantation loss. The frequency of foetuses with retarded body weights and skeletal and visceral retardation significantly increased with the dose of cobalt sulphate. The two higher doses increased the frequency of malformations of the skeleton (0, 0, 4, 3, control, low, mid and hight dose respectively) and the urogenital system (dilated ureter, 0, 1, 1, 4 in control, low, mid and hight dose, respectively). No statistically significant increase in a particular type of malformation was

observed. The number of dams that died during delivery increased dose-dependently (0, 1, 5, 12 in the control, low, mid and hight dose, respectively). However, it is unclear how these dams died during delivery, as the protocol states that these dams were processed (meaning opening of the uterus) on day 21 of gestation. In the group followed until PND21, the perinatal index decreased statistically significantly (**Table 29**). The presence of post-natal maternal toxicity is not stated. Survival index was not affected. Foetal body weight was significantly reduced on PND 1 and 7, but not on PND 14 and 21 (**Table 29**). Some effects on the maturation of the nervous system in the pups was observed at 25 mg/kg bw/day, but this may be related to the lower body weights (Szakmáry et al., 2001).

Table 29: Effects of cobalt sulphate on postnatal development in rats (adapted from Szakmáry et al., 2001)

Effect	Control	25 mg/kg bw/d
Perinatal index (%)	92.0 ± 7.0	73.3* ± 6.6
Survival index (%)	85.1 ± 8.5	87.5 ± 4.2
Offspring bw		
PND 1	6.6 ± 0.09	5.7* ± 0.09
PND 7	14.5 ± 0.34	12.5 ± 0.47
PND 14	29.2 ± 0.95	28.2 ± 1.13
PND 21	51.7 ± 1.33	48.2 ± 1.87

p≤0.05; perinatal index: 100(number of live pups on PND5)/(number of live newborns); survival index: 100*(number of live pups on PND21)/(number of live pups on PND5)

Pregnant New Zealand White rabbits (8-25/dose) were treated daily with cobalt sulphate (0, 20, 100, or 200 mg/kg bw/day) by gavage during GD6-20. All doses resulted in dams' mortality (5/25, 4/13 and 7/8), due to circulatory failure. Total resorption was found in the only surviving high dose dam, in all the 9 survivors mid dose dams, and in 6/20 surviving low dose dams. Skeletal retardation was the only statistically significant effect observed at the low dose (sternum hypoplasia, double vertebral ossification centres, shortened rib 13, dilated cranial sutures, in 22 pups vs 14 in control). Cobalt sulphate at 20 mg/kg bw/day proved to be embryotoxic for the surviving foetuses with inhibition of skeletal development and did not induce malformations in rabbits (Szakmáry et al., 2001).

Cobalt dichloride was administered by gavage (0, 25, 50, or 100 mg/kg bw/day in tap water) to 25 pregnant Crl:CD(SD) rats on GD6–19. Maternal toxicity included piloerection reduced motility and salivation from the mid dose, while in the high dose a haemorrhagic nose/snout was recorded for 3/20 dams on GD19 or 20. Additionally, corrected bw gain was decreased dose-dependently. A statistically significant reduction on mean foetal weights in the mid and high dose groups (8% both doses) was the only developmental effect recorded (ECHA, 2022).

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422), 10 Sprague Dawley rats/sex/dose were administered by gavage cobalt sulphide (0, 64.8, 194, or 648 mg Co/kg bw/day) or tricobalt tetraoxide (0, 73.4, 220 or 734 mg cobalt/kg bw). For both males and females dosing started 2 weeks before mating and it ended 2 weeks after mating for males (at least 28 days exposure), and on day 3 post-partum for females. The viability index for the high dose rats administered with tricobalt tetraoxide was decreased (87.6%) due to the total loss of a

litter, and statistically significant lower body weight was recorded for these pups. No findings were observed in the rats dosed with cobalt sulphide (Danzeisen et al., 2020).

A summary of the main findings on reproductive effects from relevant repeated dose and reproductive toxicity studies is presented in **Table 30**.

Table 30: Summary table of relevant repeated dose and reproductive toxicity studies

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt powder Combined repeated dose toxicity and reproduction screening study in rats (10/sex/dose) 0, 30, 100, 300 or 1000 mg/kg bw 2 weeks before mating – 2 weeks after mating (males) or PND3 (females)	≥ 100 mg/kg bw: mortality, clinical effects, macroscopic intestinal changes	≥ 100 mg/kg bw **development**: decreased viability index ≥ 300 mg/kg bw **fertility**: decreased implantation sites and life birth index **development**: increased pre- and post-implantation loss, decreased live birth index, decreased pup weight **remarks**: a, b, c	(ECHA, 2022)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt Purity >98% MMAD: 1.8-1.9 µm GSD: 1.7-1.8 16 days inhalation in rats (5/sex/dose) 0, 2.5, 5, 10, 20 or 40 mg/m^3	≥ 2.5 mg/m^3: decreased liver weight, atrophy and necrosis olfactory epithelium, cytoplasmic vacuolization bronchioles ≥ 5 mg/m^3: pale lungs, lung infiltration ≥ 10 mg/m^3: decreased body weight, decreased kidney and thymus weight, increased lung weight, fibrosis and necrosis in the lung ≥ 20 mg/m^3: mortality	≥ 10 mg/m^3 **fertility**: decreased testis weight **remarks**: a	(NTP, 2014)
Cobalt Purity >98% MMAD: 1.8-1.9 µm GSD: 1.7-1.8 17 days inhalation in mice (5/sex/dose) 0, 2.5, 5, 10, 20 or 40 mg/m^3	≥ 2.5 mg/m^3: decreased liver weight, vacuolization lung and respiratory epithelium, atrophy olfactory epithelium ≥ 5 mg/m^3: increased lung weight, infiltration and karyomegaly in the lung, inflammation respiratory epithelium, necrosis olfactory epithelium ≥ 10 mg/m^3: squamous metaplasia resp. epithelium ≥ 20 mg/m^3: decreased body weight 40 mg/m^3: mortality	LOEAL: 40 mg/m^3 **fertility**: decreased testis weight **remarks**: a	(NTP, 2014)
Cobalt Purity >98% MMAD: 1.6-2.0 µm GSD: 1.7-2.0 14 weeks inhalation in rat (10/sex/dose) 0, 0.625, 1.25, 2.5 or 5 mg/m^3	≥ 0.625 mg/m^3: increased lung weight, inflammation lung, proteinosis alveoli ≥ 1.25 mg/m^3: hyperplasia bronchioles, degeneration olfactory epithelium ≥ 2.5 mg/m^3: hyperplasia olfactory and resp. epithelium, turbinate atrophy ≥ 5 mg/m^3: decreased body weight	≥ 1.25 mg/m^3 **fertility**: decreased sperm motility **remarks**: a, b, c	(NTP, 2014)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt Purity >98% MMAD: 1.6-2.0 µm GSD: 1.7-2.0 14 weeks inhalation in mice (10/sex/dose) 0, 0.625, 1.25, 2.5, 5 or 10 mg/m3	≥ 0.625 mg/m^3: infiltration lung, vacuolization bronchiole, squamous metaplasia larynx ≥ 1.25 mg/m^3: degeneration olfactory and resp. epithelium ≥ 2.5 mg/m^3: decreased liver weight, increased lung weight, hyperplasia bronchiole and resp. epithelium, squamous metaplasia resp. epithelium ≥ 5 mg/m^3: tan lungs, decreased kidney weight, proteinosis and karyomegaly alveoli, turbinate atrophy, lung haemorrhage, inflammation lung and nose ≥ 10 mg/m^3: decreased body weight	≥ 2.5 mg/m^3 **fertility**: decreased sperm motility ≥ 5 mg/m^3 **fertility**: reduced sperm activity and count, decreased testis weight 10 mg/m^3 **fertility**: degeneration testes epithelium, exfoliated germ cells, hypospermia, vacuolization and atrophy epididymis. Increase oestrus length **remarks**: a, b, c	(NTP, 2014)
Cobalt Purity >98% MMAD: 1.4-2.0 µm GSD: 1.6-1.9 combined repeated dose and carcinogenicity inhalation study in rats (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	≥ 2.5 mg/m^3: decreased survival, decreased body weight, necrosis olfactory epithelium ≥ 1.25 mg/m^3: hyperplasia, proteinosis, inflammation, atrophy, squamous metaplasia in nose and lung	5 mg/m^3 **fertility**: testes infarct **remarks**: a, b	(NTP, 2014)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt Purity >98% MMAD: 1.5-2.1 µm GSD: 1.6-1.9 combined repeated dose and carcinogenicity inhalation study in mice (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	5 mg/m³: decreased body weight ≥ 2.5 mg/m³: decreased survival, inflammation and erosion lung ≥ 1.25 mg/m³: hyperplasia, cytoplasmic vacuolization, proteinosis, infiltration, atrophy, metaplasia in lung, nose, larynx and trachea	LOAEL: 5 mg/m³ **fertility**: degeneration germinal epithelium testes **remarks**: a, b	(NTP, 2014)
Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm 16 days inhalation in rats (5/sex/dose) 0, 0.1, 0.5, 5, 50 or 200 mg/m³, 6h/day, 12 exposures over 16 days (0, 0.042, 0.1, 1.05, 10.5, or 42 mg cobalt/m³)	≥ 50 mg/m³ (≥ 10.5 mg Co/m³) mortality, decreased body weight, inflammation and necrosis of respiratory epithelium, necrosis of thymus 200 mg/m³: necrosis in liver	≥ 50 mg/m³ (≥ 10.5 mg Co/m³) **fertility**: testes atrophy **remarks**: a, b	(Bucher, 1991)
Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm 16 days inhalation in mice (5/sex/dose) 0, 0.1, 0.5, 5, 50 or 200 mg /m³, 6h/day, 12 exposures over 16 days (0, 0.042, 0.1, 1.05, 10.5, or 42 mg cobalt/m³)	≥ 5 mg/m³ (≥ 1.05 mg Co/m³), inflammation and necrosis of the respiratory epithelium ≥ 50 mg/m³ mortality	NOAEL: ≥ 50 mg/m³ (≥ 10.5 mg Co/m³) **remarks**: a, b	(Bucher, 1991)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm 13 weeks inhalation in rats (10/sex/dose) 0, 0.3, 1, 3, 10 or 30 mg /m^3, 6h/day, 5 days/week (0, 0.06, 0.21, 0.6, 2.1, or 6.3 mg cobalt/m^3)	≥ 0.3 mg/m^3 (≥ 0.06 mg Co/m^3): respiratory metaplasia. At higher doses, also inflammation, hyperplasia, necrosis and fibrosis were observed	NOAEL: 30 mg/m^3 (6.3 mg Co/m^3) **remarks**: a, b	(Bucher, 1991)
Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm 13 weeks inhalation in mice (10/sex/dose) 0, 0.3, 1, 3, 10 or 30 mg/m^3, 6h/day, 5 days/week (0, 0.06, 0.21, 0.6, 2.1, or 6.3 mg cobalt/m^3)	≥ 0.3 mg/m^3 (≥ 0.06 mg Co/m^3): respiratory metaplasia. At higher doses, also inflammation, hyperplasia, necrosis and fibrosis were observed	≥ 3 mg/m^3 (≥ 0.6 mg Co/m^3) **fertility**: decreased sperm motility 30 mg/m^3 (≥6.3 mg Co/m^3) **fertility**: decreased testes and epididymal weight, increased abnormal sperm count, testes atrophy and mineralisation, significant increase oestrous cycle **remarks**: a, b, c	(Bucher, 1991)
Cobalt sulphate heptahydrate purity 99%; MMAD: 1.1-2.0 GSD: 1.9-3.0 combined repeated dose and carcinogenicity inhalation study in rats and mice (50/sex/dose) 0, 0.3, 1.0, or 3.0 mg/m^3 (0, 0.06, 0.21, 0.6 mg cobalt/m^3) 6 hours per day, 5 days per week, for 105 weeks	Rats: ≥ 0.3 mg/m^3 (≥ 0.06 mg Co/m^3) respiratory hyperplasia, inflammation, metaplasia and fibrosis Mice: ≥ 0.3 mg/m^3 (≥ 0.06 mg Co/m^3) respiratory hyperplasia, inflammation, metaplasia and fibrosis Liver inflammation, karyomegaly, oval cell hyperplasia, and regeneration	NOAEL: ≥ 3 mg/m^3 (≥ 0.6 mg Co/m^3) **remarks**: a, b	(Bucher et al., 1999, NTP, 1998)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt dichloride hexahydrate Dominant lethal assay in male mice (10/dose), oral administration in drinking water for 12 weeks 0 or 67 mg cobalt/kg bw/day	General toxicity or other effects were not determined in this study	400 ppm (approximately 67 mg Co/kg bw): **fertility**: reduced fertility, increased preimplantation loss, reduced sperm parameters. remarks: a, c	(Pedigo and Vernon, 1993)
Cobalt dichloride hexahydrate Dominant lethal assay in male mice (10/dose), 12 weeks oral administration in drinking water 0, 25.7, 46.9 or 93 mg/kg bw/day (0, 6.4, 11.6, or 23.1, mg cobalt/kg bw/d)	≥ 25.7 mg /kg bw/day (≥ 6.4 mg Co/kg bw/day): decreased body weight ≥ 46.9 mg /kg bw/day (≥ 11.6 mg Co/kg bw/day): mortality	≥ 25.7 mg /kg bw/day (≥ 6.4 mg Co/kg bw/day) **fertility**: decreased sperm count **development**: increased resorptions, decreased number of viable pups ≥ 46.9 mg /kg bw/day (≥ 11.6 mg Co/kg bw/day) **fertility**: reduced testes weight, histopathological changes in the testes, reduced pregnancies, reduced implantation sites 93 mg/kg bw/day (≥ 23.1 mg Co/kg bw/day) **fertility**: reduced epididymal weight **remarks**: a, b, c	(Elbetieha et al., 2008)
Cobalt dichloride 69 days study in male rats, oral diet administration 0, 5 or 20 mg cobalt/kg bw/day	no information on general toxicity provided	20 mg cobalt/kg bw/day **fertility**: decreased testis weight, testicular atrophy **remarks**: a, b	(Nation et al., 1983)
Cobalt dichloride hexahydrate 3 months oral in male mice (5/dose) 0, 23, 42 or 72 mg/kg bw/day (~5.8, 10.5 or 18 mg Co/kg bw/day) (drinking water)	72 mg/kg bw/day (18 mg Co/kg bw/day): reduced body weight, reduced fluid intake	≥ 23 mg/kg bw/day (≥ 11.6 mg Co/kg bw/day) **fertility**: reduced testicular weight, reduced sperm concentration 72 mg/kg bw (18 mg Co/kg bw/day) **fertility**: reduced fertility remarks: a, c	(Pedigo et al., 1988)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt dichloride hexahydrate 13 weeks study in male mice (10/dose), oral administration (drinking water) 0 or 400 ppm/day, i.e. 92 mg/kg bw/day (23 mg Co/kg bw/day)	no information on general toxicity provided	92 mg/kg bw/day (24 mg Co/kg bw/day) **fertility**: reduced testicular weight, degeneration seminiferous tubules, altered testicular vessel epithelium **remarks**: a, b	(Anderson et al., 1992)
Cobalt dichloride 98 days oral (diet) administration in rats 0 or 265 ppm/day (0 or 20 mg cobalt/kg bw/day)	no information on general toxicity provided	265 ppm/day (20 mg cobalt/kg bw/day) **fertility**: degenerative, changes in the testes remarks: b, c	(Mollenhauer et al., 1985)
Cobalt dichloride hexahydrate 3 months oral (diet) toxicity study in male rats 0 or 265 ppm/day (0 or 20 mg cobalt/kg bw/day)	265 ppm/day (20 mg cobalt/kg bw/day): Increased erythrocyte count, packed cell volume, and haemoglobin concentration	265 ppm/day (20 mg cobalt/kg bw/day) **fertility**: degenerative, non-necrotic and necrotic lesions were present in the seminiferous tubules remarks: b, c	(Corrier et al., 1985)
Cobalt dichloride hexahydrate 3 months oral (gavage) toxicity study in rats (10/sex/dose) 0, 3, 10 or 30 mg/kg bw/day (0, 0.74, 2.5, or 7.4 mg cobalt/kg bw/day)	≥ 10 mg/kg bw/day (2.5 mg cobalt/kg bw/day): decreased body weight gain, changed haematological parameters 30 mg/kg bw/day (7.4 mg cobalt/kg bw/day): erythroid hyperplasia of the femur	NOAEL: 30 mg/kg bw/day (7.4 mg cobalt/kg bw/day)	(ECHA, 2022)
Cobalt dichloride Oral developmental study in female rats (15/dose) 0, 12, 14 or 48 mg/kg bw/day (0, 3, 3.5, 12 mg cobalt/kg bw/day) (GD14-PND21)	no general toxicity reported	≥ 12 mg/kg bw/day (3/5.4 mg cobalt/kg bw/day) **development**: decreased number of litters, increased dead pups/litter, decreased foetal weight	(Domingo et al., 1985)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt dichloride hexahydrate Oral developmental study in female rats (20/dose) (gavage) 0, 25, 50 or 100 mg/kg bw/day (~6.2, 12.4 or 24.8 mg cobalt/kg bw/day) (GD6-15)	≥ 25 mg/kg bw/day (6.2 mg cobalt/kg bw/day): decreased body weight gain ≥ 50 mg/kg bw/day (12.4 mg cobalt/kg bw/day): decreased GOT and creatinine ≥ 100 mg/kg bw/day (24.8 mg cobalt/kg bw/day): increased Hb, Ht, MCV, MCH and reticulocytes; increased cholesterol	NOAEL: ≥ 100 mg/kg bw/day (24.8 mg cobalt/kg bw/day	(Paternain et al., 1988)
Cobalt dichloride hexahydrate Oral developmental study in female rats (25/dose) (gavage) 0, 25, 50 or 100 mg/kg bw/day (~6.2, 12.4 or 24.8 mg cobalt/kg bw/day) (GD6-19)	≥ 100 mg/kg bw/day (24.8 mg cobalt/kg bw/day: reduced bw gain ≥ 50 mg/kg bw/day (12.4 mg cobalt/kg bw/day): gastro-intestinal lesions	≥ 50 mg/kg bw/day (12.4 mg Co/kg bw/day) **development**: slightly lower pups bw	(ECHA, 2022)
Cobalt sulphate heptahydrate Oral developmental study in female mice (20 or 25/dose) (gavage) 0 or 50 mg/kg bw/day (10.5 mg cobalt/kg bw/day) (GD6-15)	no relevant maternal toxicity	≥ 50 mg /kg bw/day (10.5 mg Co/kg bw/day) **development**: retarded bw gain, increased skeletal retardation, increased malformations	(Szakmáry et al., 2001)
Cobalt sulphate heptahydrate Oral developmental study in female rats (3-18/dose) 0, 25, 50 or 100 mg/kg bw/day (5.2, 10.5 or 21 mg cobalt/kg bw) (GD1-20/21) (gavage)	no relevant maternal toxicity	≥ 25 mg/kg bw/day (5.2 mg cobalt/kg bw/day) **development** skeletal retardation ≥ 50 mg /kg bw/day (10.5 mg cobalt/kg bw/day) **development**: retarded bw gain, visceral retardation, increased malformations	(Szakmáry et al., 2001)

Test substance and method	General toxicity	Reproductive effects	Reference
Cobalt sulphate heptahydrate Oral developmental study in female rabbits (8-25/dose) 0, 20, 100 or 200 mg /kg bw (4.2, 21 or 42 mg cobalt/kg bw/day) (GD6-20) (gavage)	≥ 20 mg/kg bw/day (4.2 mg cobalt/kg bw/day): mortality, circulatory failure, reduced bw gain	≥ 20 mg/kg bw/day (4.2 mg cobalt/kg bw/day) **development**: increased resorptions, skeletal retardation	(Szakmáry et al., 2001)
Cobalt sulphide (0, 64.8, 194, or 648 mg Co/kg bw/day) Tricobalt tetraoxide (0, 73.4, 220 or 734 mg cobalt/kg bw). Oral (gavage) combined repeated dose toxicity and reproduction screening study in SD rats (10/dose) 2 weeks before mating until 2 weeks after mating for males and PND3 for females		tricobalt tetraoxide 734 mg cobalt/kg bw **development**: decreased viability index and pup weight cobalt sulphide: no effects	(Danzeisen et al., 2020)

Remarks: a: organ weight (testes and/or epididymis) analysed; b: histopathology reproductive organs (testis and epididymis) performed; c: sperm analysis performed

7.8.3 Summary

There are no human data on fertility effects of cobalt or its inorganic compounds and the data on developmental effects are limited to an old study which did not report any adverse effects, without specifying which developmental endpoints were examined.

A large number of studies have demonstrated that cobalt affects male fertility in mice and rats. Decreased sperm motility has been recorded in a 14-week cobalt metal inhalation study in rats starting from doses of 1.25 mg cobalt/m^3 in rats. Other severe effects, such as testis infarct (5 mg cobalt/m^3) were observed in chronic studies. Overall, inhalation studies show evidence of an increase in effects with increasing dose and time. Effects on male testes were also recorded in oral studies, with a decrease in fertility becoming visible after more than a month of continuous exposure. For example, in a dominant lethal assay, male exposed to cobalt dichloride for 12 weeks were mated with non-exposed females. A dose-dependent decrease of pregnancies was observed (95%, 15%, 12%, 7%, for control, 6.4, 11.6, or 23.1 mg cobalt/kg bw/d), statistically significant from the mid dose (Elbetieha et al., 2008). Effects on female fertility have been recorded in only two NTP studies, where a statistically significant increase in oestrous cycle length was reported in mice after 13 weeks inhalation exposure to cobalt sulphate at 30 mg/m^3/day (6.3 mg cobalt/m^3/day) (Bucher, 1991).

Several oral developmental studies demonstrated reduced pup weight and skeletal retardation as the main effect. Other effects among the less frequently reported were decreases in the number of litters, viability index, and perinatal index as recorded in a rats and mice developmental study. In one series of studies, several type of malformations

(skeletal and visceral), non-statistically significant, were observed in both mice and rats (Szakmáry et al., 2001).

8. Other considerations

8.1 Mode of action (MoA) considerations

8.1.1 Considerations on human data

No consistent indication of an increase in cancer risk has been found in data on occupationally exposed workers with exposure to cobalt compounds only. Early studies in hard-metal production workers (cobalt together with tungsten) provided indications of an increased risk of lung cancer. However, potential confounding by smoking or by occupational factors other than cobalt was not assessed comprehensively in those studies. In hard-metal production worker cohorts reflecting the more recent exposure levels, no indication of an increased risk of cancer was observed. It is however not possible to use the available epidemiological data to draw conclusions on no/low cancer potential or threshold/non-threshold mechanism. As animal data show significant induction of tumours, such data are relevant for the identification of MoA.

8.1.2 Considerations on *in vivo* and *in vitro* data

The carcinogenicity of cobalt metal and cobalt compounds is considered not to be the result of direct genotoxicity. Instead, several experimental studies indicate secondary genotoxicity, involving chronic inflammation, increased ROS production and oxidative damage as the main reasons behind the observed carcinogenic effects predominantly in the lung (AGS, 2023, RAC, 2016, RAC, 2020). Evidence in some studies points toward cobalt genotoxicity (mainly clastogenicity) being the result of two mechanisms: a direct increase of oxidative DNA damage via a Fenton mechanism, and an indirect effect via the inhibition of DNA repair mechanisms (NTP, 2016).

8.1.3 Recent additional data

Recently a series of articles proposed a tiered approach to explore cobalt inhalation toxicity and obtain more information on parameters that may have an impact on the MoA for lung tumours in rodents. Briefly, the first paper introduces the methodologies, while the following articles provide study results to support the approach described in the first publication (Danzeisen et al., 2022a).

In their tiered approach, the authors do not specifically aim to characterise the MoA, but to describe a testing procedure to assess and group cobalt compounds without the need to test all of them for all endpoints. The first step consisted of the estimation of cobalt ion release in artificial lung fluid which according to a previous study by the same group is considered a reliable marker for cobalt toxicity (Danzeisen et al., 2020). Thus the cobalt release in artificial lung fluids was measured for 12 cobalt salts, see section 7.1.3 (Verougstraete et al., 2022). The second tier entails the characterisation of local inflammation caused by oxidative stress due to the increase in reactive oxygen species (ROS) and the characterisation of cobalt induced hypoxic cellular responses, (see **Appendix 6**; *in vitro* genotoxicity in mammalian cells). In the same tier, the potential primary genotoxicity has been evaluated; the authors found negative results on all their *in vitro* tests (Derr et al., 2022b). These *in vitro* tiers are then followed by *in vivo* tiers. Firstly, an acute toxicity test with extended histopathology was conducted (see 7.2.2, followed by 28 days repeated dose inhalation toxicity study with tricobalt tetraoxide (see section 7.3.2) (Burzlaff et al., 2022)), which is considered by the authors as the representative substance for the 'poorly reactive/poorly bioavailable' cobalt compounds group. The last tier in their approach is a planned 90-day repeated inhalation study. The tiered approach has been constructed to decrease the number of substances to be tested on each tier: based on the results of the low tiers, cobalt compounds are divided in groups

so that only one or few substances representative for each group are tested in the high *in vivo* tiers (Danzeisen et al., 2022b) .

The results from the above tiered approach were incorporated in a MoA approach based on the "International Programme on Chemical Safety Conceptual Framework for Evaluating a MOA for Chemical Carcinogenesis" (Sonich-Mullin et al., 2001) (Danzeisen et al., 2022a). Following inhalation, the cobalt substance is deposited in the lungs, and successively cobalt ion is released into tissues (initiating event, IE) where it accumulates (key event 1, KE1). The authors estimated a threshold between the IE and the KE1 of 34 mg cobalt/g sample and an inverse limit of 230 mg cobalt/g sample, above which all key events are triggered ((Danzeisen et al., 2022a) based on the sum of Co ion release in three artificial lung fluids and the presence/absence of data in different tiers, as reported by (Verougstraete et al., 2022, Derr et al., 2022b, Viegas et al., 2022, Burzlaff et al., 2022)) . Once in the lung tissue (KE2), cobalt is responsible for i) the generation of ROS directly and indirectly by acting as pro-inflammatory agent, inducing oxidative stress and for ii) mimicking hypoxic conditions by preventing the degradation of HIF-1α (Hypoxia-Inducible Factor-1 alpha), either by binding directly to HIF-1α and thus inhibiting the interaction between HIF-1α and the von Hippel-Lindau protein (pVHL) or by blocking the iron binding site of the prolyl hydroxylase. As a result HIF-1α which accumulates in the cells. The cytotoxicity (iii) observed after exposure to highly soluble and bioaccessible cobalt substances is considered part of the KE2, occurring in parallel to ROS and hypoxia and not a distinct key event. Inflammatory responses comprise KE3 and are observed when cobalt accumulates at levels above the threshold triggering KE1. Chronic inflammation leads to non-neoplastic lesions, such as oedema, epithelial hyperplasia and necrosis. These effects are considered by the authors as possibly early events of KE4 which is hyperplasia followed by tumour formation (Danzeisen et al., 2022a)The tiered approach showed that the first group of cobalt compounds, i.e., the highly soluble cobalt salts (cobalt dichloride, dinitrate, sulphate, diacetate), triggered all effects included under KE2 (ROS formation, hypoxia and cytotoxicity), while only hypoxia was observed for group 2 substances (cobalt carbonate, oxide, cobalt metal, cobalt dihydroxide). None of the effects in KE2 was observed for group 3 substances (cobalt hydroxide oxide, sulphide, tricobalt tetraoxide, and lithium cobalt dioxide), which, according to the authors, supported the hypothesis of a threshold (Burzlaff et al., 2022) , see section 7.1.3 for the groups definition (Verougstraete et al., 2022).

8.1.4 Conclusions

Chronic inflammation, increased ROS production and oxidative damage are considered as the main events behind the genotoxic and carcinogenic effects of cobalt. Based on the involvement of secondary genotoxicity, the mechanisms responsible for the carcinogenicity of cobalt could be regarded as threshold events. However, as already identified by RAC (RAC, 2020), genotoxic effects at exposure levels below the threshold cannot be fully excluded. In such a situation, a non-threshold approach would normally be the default approach. It is however important to note that the dose-response curve is likely to be less steep at lower than at higher concentrations. For the purpose of identifying a MoA for the OEL setting, the scope is on low exposure levels, and therefore, following a linear dose-response approach is likely to be very conservative.

The sublinear approach described by RAC (RAC, 2020; See section 9.1.1.3) is considered relevant and appropriate. Identification of a dose level at which lung inflammation is not induced anymore can be considered as a breakpoint in the dose-response curve. At doses below that breakpoint, chronic lung inflammation is unlikely to occur, and consequently the risks of oxidative stress and secondary genotoxicity are significantly reduced. Remaining cancer risks can, however, not be fully excluded at exposures below the breakpoint. At exposure concentrations below the breakpoint, the cancer risk is anyhow considered to be significantly lower than the risk levels that could be derived from a non-threshold based linear extrapolation curve.

Regarding those cobalt compounds which have been demonstrated as poorly soluble in artificial body fluids (See section 7.1.3), it is noted that there is some uncertainty on the MoA, particularly due to the lack of sub-chronic or chronic experimental data which could confirm whether those substances have a different toxicity profile than cobalt metal or other cobalt compounds. Therefore, there is currently no data available to confirm whether the sublinear approach with same breakpoint and similar dose-response slopes apply for these substances, or whether a threshold would apply.

8.2 Lack of specific scientific information

Repeated dose/chronic toxicity inhalation studies on compounds with very low solubility in biological fluids are lacking.

8.3 Groups at Extra Risk

No specific groups at extra risk were identified.

9. Evaluation and recommendations

9.1 Cancer risk assessment

9.1.1 Published approaches for cancer risk assessment

9.1.1.1 ANSES

ANSES (2014) assessed available data on cobalt and its compounds (excluding cobalt in association with tungsten carbide) and considered the existing human and animal data too uncertain to derive an OEL and consequently derived a pragmatic OEL based on respiratory impairment effects (see details in 9.2.1.1).

9.1.1.2 AGS

A recent assessment by AGS done in 2022 (AGS, 2023) focused on cobalt metal and seven cobalt compounds, which are all classified as carcinogens (Carc. 1B). The assessment was based on in vivo inhalation carcinogenicity study data on cobalt sulphate in female rats (NTP, 1998), from which a breakpoint in the dose-response was identified, being equivalent to a human exposure concentration of 2.2 µg Co/m^3 (respirable). For the purpose of exposure-response relationship (ERR) extrapolations regarding excess life-time risks of developing cancer from cobalt inhalation exposure over working life, benchmark-dose modelling was conducted. A BMD10 of 0.051 mg Co/m^3 was derived and used as point-of-departure for the ERR. Based on this, exposure risk relationships (ERR) for cancer caused by cobalt exposure were derived. Additional nominal risks of developing cancer from inhalation exposure over working life are shown in **Table 31** below.

Table 31: Exposure risk relationship (ERR) for cobalt metal, inorganic cobalt compounds and cobalt-containing metal carbides (AGS, 2023)

Risk estimate	Cobalt concentration (µg/m^3, respirable fraction, long-term mean value, 40 years of workplace exposure)
Risk 4:1000	4
Risk 4:10 000	2
Risk 4:100 000	0.2

9.1.1.3 RAC (2020)

In its Opinion on restrictions of five cobalt salts (RAC, 2020), RAC took into consideration various approaches for the assessment of excess cancer risk among workers. It is noted that an earlier linear dose-response relationship for lung cancer (RAC, 2016) was not used, because RAC agreed that the use of a linear dose-response approach would be very conservative, resulting in overestimation of risks at lower exposure levels. Also, RAC concluded that the available data were insufficient to derive a mode of action-based (health-based) threshold. Instead, RAC acknowledged the role of chronic inflammation in the mode of action of genotoxicity and cancer, upon exposure to cobalt.

RAC derived an estimated threshold level of 0.5 µg Co/m^3 (respirable fraction; estimated to correspond to 1 µg inhalable Co/m^3), from animal data, for chronic pulmonary inflammation. This was considered to present a breakpoint in the dose-response of cobalt carcinogenicity, but it was stressed by RAC that "this cannot be identified as a health-based (fully safe) threshold below which cancer risks can be considered negligible". It was, however, concluded that "below this level, the cancer risk is likely to be reduced significantly compared to the risk estimated on the basis of linear extrapolation".

The starting point for the derivation of the breakpoint value was information from chronic inhalation studies with cobalt sulphate hexahydrate in rats and mice, which showed inflammatory effects in the lungs at all tested dose levels (0.3, 1, 3 mg/m^3) (see sections 7.3.2.2 and 7.7.2). The lowest dose (0.3 mg cobalt sulphate hexahydrate/m^3, corresponding to 0.067 mg Co/m^3) was thus the LOAEC and can be used as point of departure for the calculation of a concentration which would represent the breakpoint in the dose-response curve.

As the point of departure 0.067 mg Co/m^3 relates to animal exposure, it was converted to a worker equivalent dose, considering breath volumes (moderate physical activity) and exposure duration (8 h working day):

$$0.067\ mg/m^3 * 6h/8h * 6.7\ m^3/10\ m^3 = 0.034\ mg/m^3.$$

In addition, the following default assessment factors were applied to cover uncertainties: a factor of 5 for severity (LOAEC versus NOAEC), a factor of 2.5 for interspecies differences, and a factor of 5 for worker intraspecies differences, resulting in a total assessment factor of 62.5.

By applying the assessment factors, a limit value of 0.0005 mg/m^3 (0.5 µg/m^3) was derived by RAC. This value corresponds to the respirable fraction. RAC considered that the respirable fraction can be expected to represent ≤50% of inhalable dust. Following a worst-case approach assuming 50% respirable dust, a limit value of 0.001 mg Co/m^3 (1 µg/m^3) was calculated for the inhalable fraction. It is relevant to note that RAC stressed that "this cannot be identified as a health-based (fully safe) threshold below which cancer risks can be considered negligible". It was, however, concluded that "below this level, the cancer risk is likely to be reduced significantly, compared to the risk estimated on the basis of linear extrapolation".

Furthermore, RAC (2020) noted that no dose-response for effects caused by inhalable cobalt particles can be derived, as was already concluded earlier by RAC (RAC, 2016). The risk of upper respiratory tract cancers was considered to be more than one order of magnitude lower than that of lung cancer, on the basis of animal study data with absence of tumours in the upper respiratory tract at the highest tested dose of 3.0 mg/m^3 cobalt sulphate hexahydrate, compared to the increased incidence of lung tumours at 0.3 mg/m^3. The mechanism of cancers observed in the upper respiratory tract of test animals was considered as potentially related to the high doses applied and may exert a threshold. As there seems to be a clear potency difference between lung effects and effects in the upper respiratory tract, the considerations on effect levels of respirable particles were identified to cover also possible cancer risks that exposure to non-respirable cobalt particles may

induce. RAC (RAC, 2020) considered that it would not be appropriate to apply the earlier lung cancer dose-response (RAC, 2016) in the characterisation of the risk for other types of cancer caused by non-respirable cobalt dust.

Regarding information on human cobalt exposure, RAC (2020) concluded that "[…] human data have not shown any clearly increased cancer risk in occupationally exposed workers. This cannot, however, be used to exclude the cancer risk seen in animal studies. Neither does it provide additional information on a potential threshold for carcinogenicity. Thus, the quantification of cancer risk needs in the case of cobalt to be based on animal data".

In the Opinion on restrictions of five cobalt salts, RAC (2020) considered that the excess lifetime cancer risk at doses above the breakpoint level (0.5 µg Co/m^3; respirable fraction) can be calculated using the formula:

1.0576 x exposure concentration (as mg Co/m^3, respirable fraction) - 0.0004763

Below the breakpoint (0.5 µg Co/m^3; respirable fraction) the dose-response is expected to be less steep, and the excess lifetime cancer risk can be estimated by the formula:

0.105 x exposure concentration (as mg Co/m^3).

Based on this, exposure at concentrations ≤ 0.5 µg Co/m^3 (respirable fraction; 1 µg Co/m^3 as inhalable fraction), can be calculated as corresponding to an excess lifetime cancer risk below 5.25 x 10^{-5}.

The starting point for the dose-response modelling was the $BMDL_{10}$ of 0.414 mg/m^3 as cobalt sulphate hexa/heptahydrate (0.093 mg/m^3 as cobalt) based on lung tumours (adenoma or carcinoma) in a rat inhalation carcinogenicity study (NTP, 1998). This BMDL refers to an exposure scheme of 6h/d, 5d/week, and for 105 weeks life-time.

For the workplace, a $BMDL_{10(worker)}$ of 0.095 mg/m^3 can be derived: (0.093 mg Co/m^3 x 6/8 hours x 6.7/10 m^3 x 52/48 weeks/year x 75/40 years).

9.1.2 Cancer risk assessment

Since the human data on the carcinogenicity of cobalt is limited, the cancer risk assessment is based on experimental data from animals. However, some comparisons of the animal data based ERR and the epidemiological observational evidence are made in Appendix 7. For the cancer risk assessment, lung cancer findings in experimental animals are considered as the critical effects. There was no clear evidence of systemic cancers in carcinogenicity studies with soluble cobalt salts. Some indications of systemic carcinogenicity have been raised from studies with cobalt metal, in which somewhat increased incidences of pheochromocytomas, pancreatic cancers and mononuclear cell leukaemia were reported in rats (but not in mice). These, however, are not considered key effects for the cancer risk assessment.

As no significant new studies supporting other approaches have been published since the assessment of RAC (RAC, 2020), the identification of a breakpoint for lung cancer as an estimated threshold level for chronic lung inflammation is still considered a relevant and appropriate approach for the cancer risk assessment (see detailed description in section 9.1.1.4 above). Although the RAC Opinion on the restriction (RAC, 2020) focused on five cobalt salts only, RAC noted that "cobalt metal and other cobalt compounds can cause similar risks to the five cobalt salts covered by the restriction". Cobalt metal and several other poorly water-soluble cobalt compounds have been found to be soluble in biological fluids and can thus release cobalt(II) ions *in vivo*. Differences in solubility of different cobalt salts have been observed in *in vitro* studies with artificial body fluids (See section 7.1.3). Due to the lack of, for example, subchronic or chronic inhalation studies with poorly soluble cobalt salts, it is proposed that no separation is made between different types of inorganic cobalt compounds, and a conservative approach based on existing data on

soluble compounds is followed. Thus, the previous approach of RAC (RAC, 2020) is followed for the calculation of excess lung cancer risk. Excess lung cancer risks at different exposure concentrations of respirable cobalt were calculated using the formulas presented by RAC (see section 9.1.1.3) and are summarised in the table below (**Table 32**). As already concluded by RAC (2020), no dose-response for effects caused by inhalable cobalt particles can be derived.

Table 32: Lung cancer exposure-risk relationship (40 year working life exposure to a given 8-hour air concentration for five working days a week)

Air concentration of cobalt (respirable fraction; µg/m³)	Excess life-time lung cancer risk (cases per 100 000 exposed)
0.01	0.11
0.05	0.53
0.1	1.1
0.5	5.3
Breakpoint where the slope of the dose-response changes (0.5 µg/m³)[a]	
1.0	58
5.0	480
10	1010
20	2070

[a] Below the breakpoint of 0.5 µg/m³, lung inflammation-related mechanisms are likely to be no longer of concern and thus the estimated cancer risk per unit exposure is lower than at exposure levels above the breakpoint (See section 9.1.1.3)

9.2 Derived Occupational Exposure Limit (OEL) Values

9.2.1 Published approaches to establishing OELs

9.2.1.1 ANSES

OEL, STEL and notations

ANSES (2014) conducted the scientific expert evaluation for setting occupational exposure limit values for cobalt and its compounds (excluding cobalt in association with tungsten carbide). ANSES concluded that there have been only a limited number of studies investigating the carcinogenicity of cobalt in humans. Furthermore, ANSES noted that in the animal carcinogenicity assays with cobalt sulphate heptahydrate by inhalation, although a broad spectrum of inflammatory and proliferative symptoms as well as pulmonary lesions were observed, many of the cellular tumours were morphologically similar to those occurring spontaneously. ANSES stated that: 1) there is only limited evidence of the carcinogenicity of cobalt and its compounds, 2) that the dose-response relationships for this effect are uncertain and therefore it was decided to establish a pragmatic 8h-OEL for an effect other than cancer. However, ANSES acknowledged that cancer is a stochastic effect, and it is therefore possible that as such it may occur at lower doses than non-carcinogenic effects. The purpose of the recommended OEL is therefore

not to avoid possible carcinogenic effects but rather to serve as a means for reducing exposure.

Impairment of the respiratory system was selected as the critical effect for establishing a pragmatic 8h-OEL. The two-year NTP inhalation study in rats was selected as the key study, the proteinosis observed in male rats as the critical effect and a BMDL at 10% of 70 µg Co/m^3 was established as point of departure. An interspecies assessment factor of 10 was applied (the selection of the assessment factor was justified "It should be remembered that the OEL proposed concerns all cobalt compounds, irrespective of their solubility. Studies show a species-dependent gradient of elimination for insoluble compounds; it was observed that rats eliminate cobalt from the lungs much more rapidly than humans, indicating greater sensitivity in the human species and fully justifying this safety factor"). As regards intraspecies assessment factor, ANSES concluded that for a population of workers (healthy adults) a degree of homogeneity is assumed, so an assessment factor of 3 was recommended. An 8-hour TWA OEL of 2.5 µg of cobalt/m^3 was derived (70/(10*3)=2.33).

ANSES (2014) noted that there was no dose-response relationship on which to establish a STEL. In the absence of such substance-specific data, in accordance with the national methodology adopted, ANSES recommended that workers not be exposed for 15 min at values higher than 5 x 8h-OEL, or 12.5 µg cobalt/m^3.

ANSES (2014) further noted that the following *in vitro* application of a 0.085 M solution of cobalt dichloride for 4 hours on the skin of a human abdomen (autopsy material, washed with soap and water and frozen before use) (Wahlberg, 1965), a significant dermal absorption was observed. Consequently, ANSES (2014) recommended a skin notation to be applied to soluble (but not to insoluble) cobalt compounds.

BLV and BGV

ANSES (2018) reviewed the literature to explore; (1): if the body of scientific evidence is sufficient to quantify a dose-response relationship with certainty, biological limit values (BLVs) on the basis of health data and, (2): if not, BLVs could be calculated on the basis of the expected concentration of the biomarker of exposure (BME) when the worker is exposed to the 8-hour OEL. ANSES (2018) noted that the health effects observed can be very different depending on the type of exposure. For this reason, it was decided to consider cobalt in soluble and/or insoluble forms separately from cobalt in association with tungsten carbide.

ANSES (2018) firstly concluded that the reported findings concerning (systemic) health effects, like haematotoxicity and thyroid effects, related to exposure to cobalt compounds are too heterogeneous to recommend or identify a biomarker of effect, suitable for biomonitoring.

ANSES (2018) secondly concluded that the (diamond polisher) study of Nemery et al. (1992) allows to determine from a graph the urinary concentration of cobalt at the end of week and end of shift at about 5 µg/g creatinine, corresponding to metallic cobalt exposure at the 8-hour OELV of 2.5 µg/m^3, proposed by ANSES (2014). This result is confirmed by the work of Lison et al. (1994), estimating the urinary concentrations of cobalt using the regression equation derived for exposure to a range of cobalt compounds (salts, metals and hard-metals).

ANSES (2018) also noted the urine cobalt concentration corresponding to the 95th percentile of the distribution of values from the French ENNS study of the general population (reported by Fréry et al. (2011)), should be used as reference values indicating an overexposure when compared to the general population exposure. These biological reference values were 1.95 µg/L (1.45 µg/g creatinine) in females and 0.70 µg/L (0.57 µg/g creatinine) in males.

Urine cobalt at end of week and end of shift

- BLV based on exposure to the 8h-OEL (2.5 µg/m^3): 5 µg/g creatinine. This value applies to cobalt in the form of metallic powders, salts and oxides. It does not apply to exposure to cobalt associated with tungsten carbide.
- BGV:
    - 1.5 µg/g creatinine or 2 µg/l (women)
    - 0.6 µg/g creatinine or 0.7 µg/l (men)

9.2.1.2 RAC (2020)

In the RAC Opinion on the restriction of five cobalt salts (RAC, 2020), the need for a BOELV for cobalt and its compounds was raised: "RAC considers it necessary, and proposes to the European Commission, to derive a binding occupational exposure limit value (BOELV) for cobalt and its compounds according to Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD). RAC recommends that this value should be identical to the limit values given in this restriction". The 8-h TWA limit values identified by RAC were 0.5 µg Co/m^3 for the respirable fraction (corresponding to 1 µg Co/m^3 for the inhalable fraction). The approach for the derivation of an estimated threshold level (breakpoint for the dose-response) which could be used as limit values for carcinogenicity is presented in section 9.1.1.3.

Regarding non-cancer effects, including respiratory sensitisation and non-cancer lung effects, it was noted that only limited dose-response data are available. RAC noted that according to the report by Nemery et al. (1992), no effect on lung function is expected in exposed workers at levels below 5 µg Co/m^3. By applying an assessment factor of 5 for inter-individual differences, a limit value of 1 µg Co/m^3 (inhalable fraction) was identified. Furthermore, RAC pointed out that based on recent information, asthma caused by cobalt exposure seems to be uncommon nowadays, and therefore an 8-h limit value of 1 µg Co/m^3 (inhalable fraction) is expected to reduce the risk of respiratory sensitisation as well.

Although the restriction was limited to five specific cobalt salts, RAC concluded that "The limit values proposed here by RAC can be considered applicable also for cobalt metal and other cobalt compounds releasing cobalt ions in contact with body fluids". RAC also noted that attention should be paid to the prevention of exposure via the skin, to protect workers from skin sensitisation.

9.2.1.3 Finland (STM)

The current Finnish 8-h OEL of 20 µg Co/m^3 (inhalable) was set to decrease the risk of hazardous effects due to cobalt exposure. Irritation of the nose, eyes and throat as well as effects on pulmonary function were identified as critical effects (STM, 2011).

In addition to the air value, a biological limit value was set; 130 nmol Co/L urine, corresponding to 8-h of exposure at 10 µg Co/m^3. (STM, 2016)

9.2.1.4 AGS

In the recent assessment by AGS, which focused on cobalt metal and seven cobalt compounds classified as carcinogens (Carc. 1B), ERR calculations for lung cancer were presented (see section 9.1.1.2) (AGS, 2023).

In addition, OELs were derived for non-cancer effects. The LOAEC of 67 µg Co/m^3 for local pulmonary effects with cobalt sulphate heptahydrate (NTP, 1998) was used to derive an OEL (8h TWA, respirable). This concentration was extrapolated to occupational exposure situations (high physical activity, 8 h/day; factor of 2), and assessment factors were applied to cover uncertainties related to exposure duration and LOAEC-NOAEC

extrapolation (total factor of 5), and inter- and intraspecies differences (total factor of 3). The outcome of those calculations was an OEL of 2 µg Co/m^3 (respirable).

In addition, an OEL of 20 µg Co/m^3 (inhalable) was derived. This was based on an average of the LOAECs identified in four epidemiological studies on workers exposed in cobalt production. The LOAECs were identified for respiratory sensitisation (30 µg Co/m^3, Sauni et al., 2010), dyspnoea during exercise (60 µg Co/m^3, Swennen et al., 1983), clinical respiratory findings including dyspnoea, cough, phlegm and wheezing (45 µg Co/m^3, Linna et al., 2003), and FEV1 decline observed only in smokers (40 µg Co/m^3, Verougstraete et al., 2004). By application of an assessment factor of 2 for LOAEC-NOAEC extrapolation, a limit value of ≈ 20 µg Co/m^3 (inhalable) was calculated. (AGS, 2023)

9.2.2 Occupational Exposure Limits (OELs) - 8h TWA

Carcinogenicity is considered the critical toxicological effect of cobalt. No threshold could be identified for the carcinogenicity of cobalt and its inorganic compounds, but it is considered that a "breakpoint" can be identified. There are no human data that could be used for setting the limit value or deriving dose-responses, but on the basis of the arguments and approach presented earlier by RAC (RAC, 2020) (explained in sections 8.1, 9.1.1.3 and 9.1.2), and considering that no crucial new data have been presented lately, a sublinear approach, deriving dose-responses from animal data, is considered relevant for estimating lung cancer risks of cobalt.

An exposure level of 0.5 µg cobalt/m^3 (respirable fractions) was identified as the breakpoint for the sublinear dose-response (see section 9.1.1.3). Below the breakpoint of 0.5 µg/m^3, lung inflammation-related mechanisms are likely to be no longer of concern and thus the estimated cancer risk per unit exposure is lower than at exposure levels above the breakpoint. Estimated excess cancer risks at different exposure concentrations were calculated and are shown in section 9.1.2.

The identified breakpoint can be used to set an **OEL (8h TWA) of 0.0005 mg Co/m^3** (0.5 µg/m^3), for the **respirable fraction.** This is derived from animal data (NTP, 1998): 0.067 mg/m^3 (LOAEC) * 6/8 hours * 6.7/10 m^3 / 2.5 (interspecies differences) / 5 (intraspecies differences) / 5 (severity LOAEC-NOAEC) (for details on the calculations see section 9.1.1.3).

In addition, for the **inhalable fraction, an OEL (8h TWA) of 0.001 mg Co/m^3** (1 µg/m^3), can be derived from human data on non-cancer effects (Nemery et al., 1992): 0.0051 mg/m^3 (NOAEC) / 5 (intraspecies differences) = 0.001 mg/m^3. According to the report by Nemery et al. (1992), no effects on lung function are expected in exposed diamond polishing workers at levels below 0.0051 mg/m^3 (5 µg Co/m^3) (see discussion in the RAC Opinion and in section 9.2.1.2 of this Annex).

Effects on reproductive toxicity, specifically male fertility, have consistently been reported in several experimental studies in rats and mice. A NOAEC for such effects was identified as 0.625 mg Co/m^3 in a sub-chronic inhalation study with respirable cobalt metal in rats; at 1.25 mg Co/m^3 (LOAEC), slightly decreased sperm motility was reported (NTP, 2014, see section 7.8.2). Cobalt sulphate hexa/heptahydrate was tested earlier by NTP (Bucher 1991) with a LOAEC identified in mice at 0.67 mg Co/m^3 (3 mg/m^3 for the sulphate hexa/heptahydrate) for reduced sperm motility as most sensitive effect. No NOAEC was identified. From the LOAEC of 0.67 mg Co/m^3 a hypothetical (conservative) OEL could be calculated as follows:

- Correction of the starting point to consider exposure duration (6 h per day for rats versus 8 h for workers) and inhalation volumes of rat versus humans:
  0.67 mg/m^3 *x* (6 h/ 8 h) *x* (6.7 m^3/10 m^3) = 0.337 mg/m^3
- Application of an assessment factor of 3 for LOAEC-to-NOAEC extrapolation, a factor of 2 to cover extrapolation from sub-chronic to chronic exposure, a factor of

5 for intraspecies differences, and a factor of 2.5 for interspecies differences (workers):
0.337 mg/m^3 / (3 *x* 2 *x* 5 *x* 2.5) = 0.004 mg/m^3 (4 µg/m^3).

No human data on developmental effects were found. In animals, several developmental toxicity studies have been conducted, including developmental toxicity studies in rats, mice, and rabbits, with different design and duration (e.g., only organogenesis period, or until end of pregnancy, or late pregnancy including lactation period), dominant lethal tests, sub-chronic exposure of males with mating untreated females, and reproductive toxicity screening studies. Most of these studies were conducted with cobalt dichloride and cobalt sulphate heptahydrate and were published in the literature. All had deficiencies and limitations. However, the effects reported included reduced foetal weight, growth and skeletal retardation, resorptions, and pup mortality. The effect levels varied, but in general developmental effects were seen at higher doses than those causing fertility effects (see above). LOAELs reported were as low as 5.4 mg Co/kg bw/d (Domingo et al., 1985, cobalt dichloride) and NOAELs as high as 24.8 mg/kg bw/d (Paternain 1988, cobalt dichloride). Thus, the DNELs for developmental effects would be higher than the DNEL calculated for fertility.

In summary, the lowest reproductive DNEL of 4 µg/m^3 is derived for male fertility and this DNEL exceeds the proposed OELs for the respirable fraction and inhalable factions. Therefore, the OELs proposed based on lung effects are also protective for toxic effects on reproduction and fertility.

Cobalt and several cobalt compounds are classified as respiratory sensitisers. From the available data it is not possible to identify a dose-response or derive a limit value for respiratory sensitisation caused by cobalt. However, based on the available data described in section 7.5.1 and summarised in 7.5.4, it can be anticipated that cases of asthma would be unlikely at low exposure levels around the breakpoint identified for the cancer ERR.

9.2.3 Short-term Exposure Limits (STELs)

No STEL is proposed. The critical effects caused by cobalt exposure are related to long-term exposure.

9.2.4 Biological Limit Value (BLV)

Correlations between air concentrations and urine cobalt levels are presented in section 6.2.2. No BLV is proposed because exposure at air levels corresponding the proposed OELs are likely to result in urinary levels which are very close to the 95th percentiles of the general population.

9.2.5 Biological Guidance Value (BGV)

Studies in the European General population indicate that the 95th percentile levels for cobalt in urine, for males and females (combined), are usually between 1.0 µg/L and 2.24 µg/L, but when analysed separately females show higher levels than males.

Based on the data from the most comprehensive European study by Fréry et al. (2011), a value of 0.7 µg Co/L urine for males and 2 µg Co/L urine for females is proposed as a BGV unless there is specific national data supporting the use of other values.

9.3 Notations

Cobalt metal and several cobalt compounds have a harmonised classification as skin sensitisers and respiratory sensitisers. Therefore, 'skin sensitisation' and 'respiratory sensitisation' notations are recommended.

There are only limited data on systemic uptake via the skin and thus no 'skin' notation is proposed.

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Appendix 1. Summary of the physico-chemical properties of inorganic cobalt compounds

Table 33: Physical and chemical properties for inorganic cobalt compounds[15]

EC/List number	Substance name	Density [g/cm³ at 20°C]	Water Solubility	Melting point [°C]	BoilingPoint [°C]
208-169-4	Cobalt carbonate	4.17	12.98 µg/L 11430.0 µg/L		
215-153-0	Cobalt trihydroxide	1.41	1.29 mg/L		
215-154-6	Cobalt oxide	6.656	4.88 mg/L	1883.0°	
215-157-2	Tricobalt tetraoxide	6.11	1.62 mg/L		
215-273-3	Cobalt sulphide	5.45	1240.3 mg/L	1117.0°C	
231-158-0	Cobalt	8.89	2.94 mg/L	1494.0°C	2927.0°C
231-589-4	Cobalt dichloride	3.36	585.8 g/L	736.0°C	1049.0°C
233-254-8	Cobalt wolframate				
233-334-2	Cobalt sulphate	3.71	376.7 g/L	700.0°C	
233-402-1	Cobalt dinitrate	2.49	669.6 g/L		
233-514-0	Octacarbonyldicobalt				
234-614-7	Cobalt hydroxide oxide	3.72	746.0 µg/L		
235-362-0	Cobalt lithium dioxide	4.82	304.0 µg/L		
235-762-5	Aluminum cobalt oxide	4.57			
237-358-4	Cobalt molybdate	4.32	508.0 mg/L		
237-742-1	Tripotassium hexacyanocobaltate	1.9			
244-166-4	Cobalt dihydroxide	3.6	2300.0 µg/L		

[15] Values obtained from registration data published on www.echa.europa.eu

EC/List number	Substance name	Density [g/cm³ at 20°C]	Water Solubility	Melting point [°C]	BoilingPoint [°C]
269-047-4	Cobalt titanite green spinel	4.95	0.5 mg/L		
269-049-5	Cobalt zinc aluminate blue spinel	4.49			
269-060-5	Iron cobalt chromite black spinel	5.15			
269-072-0	Cobalt chromite blue green spinel	4.96			
269-093-5	Olivine, cobalt silicate blue	3.74			
269-101-7	Cobalt chromite green spinel	5.31			
269-102-2	Iron cobalt black spinel	4.67			
270-208-6	Cobalt zinc silicate blue phenacite	4.06			
273-769-5	Leach residues, zinc ore-calcine, zinc cobalt	3.39		71.0°C -	
310-193-6	Cobalt aluminate blue spinel	4.26			
480-390-0	Cobalt Lithium Manganese Nickel Oxide	4.63	0.143 mg/L	360.0°C	
603-073-2	Dipotassium hexacyanocobalt(II)-ferrate(II)	2.21	0.89 mg/L	450.0°C	
700-042-6	Aluminum cobalt lithium nickel oxide	1.2	1.0 mg/L		
839-353-8	Nickel Cobalt Manganese Hydroxide	3.5709	0.786 mg/L		
931-895-4	Reaction product of soluble nickel salt, cobalt salt, manganese salt with alkalines	2.0			
942-358-9	Cobaltate (3-), hexakis (cyano-.kappa.C)-, zinc, hydrate (2:3:12). (OC-6-11) OR 'Trizinc bis(hexacyanidocobaltate(III)) dodecahydrate	1.692	182.3 mg/L		
951-904-5	Cobalt Nickel Manganese Oxide	5.3485	0.0311 mg/L		

Appendix 2. REACH Registrations

Table 34: REACH Registrations

EC Number	Substance name	Intermediate registration	Full registration
208-169-4	cobalt carbonate	<10 (<5 reg)	1000-10 000 (8 reg)
215-153-0	Cobalt trihydroxide		
215-154-6	cobalt oxide	<10 (<5 reg)	1000-10 000 (26 reg)
215-157-2	tricobalt tetraoxide	10-1000 (<5 reg)	1000-10 000 (29 reg)
215-273-3	cobalt sulphide	10-1000 (12 reg)	1000-10 000 (31 reg)
231-158-0	cobalt		10 000-100 000 (96 reg)
231-589-4	cobalt dichloride	<10 (<5 reg)	1000-10 000 (6 reg)
233-254-8	Cobalt wolframate		
233-334-2	cobalt sulphate	1000-10 000 (<5 reg)	>100 000 (15 reg)
233-402-1	cobalt dinitrate		1000-10 000 (10 reg)
233-514-0	octacarbonyldicobalt	<10 (<5 reg)	
234-614-7	cobalt hydroxide oxide	10-1000 (<5 reg)	1000-10 000 (<5 reg)
235-362-0	cobalt lithium dioxide		10-1000 (7 reg)
235-762-5	Aluminum cobalt oxide	<10 (<5 reg)	
237-358-4	cobalt molybdate	<10 (<5 reg)	10-1000 (<5 reg)
237-742-1	tripotassium hexacyanocobaltate		10-1000 (<5 reg)
244-166-4	cobalt dihydroxide	(<5 reg)	>100 000 (19 reg)
269-047-4	cobalt titanite green spinel		10-1000 (7 reg)
269-049-5	cobalt zinc aluminate blue spinel		1000-10 000 (14 reg)
269-060-5	iron cobalt chromite black spinel		1000-10 000 (21 reg)
269-072-0	cobalt chromite blue green spinel		10-1000 (19 reg)
269-093-5	Olivine, cobalt silicate blue		1000-10 000 (15 reg)
269-101-7	cobalt chromite green spinel		10-1000 (12 reg)
269-102-2	iron cobalt black spinel		<10 (<5 reg)
270-208-6	cobalt zinc silicate blue phenacite		<10 (<5 reg)
273-769-5	Leach residues, zinc ore-calcine, zinc cobalt	1000-10 000 (6 reg)	
310-193-6	Cobalt aluminate blue spinel		1000-10 000 (27 reg)
442-750-5	N/A		
480-390-0	Cobalt Lithium Manganese Nickel Oxide		10 000-100 000 (16 reg)
485-210-4	N/A		
603-073-2	Dipotassium hexacyanocobalt(II)-ferrate(II)		<10 (<5 reg)

EC Number	Substance name	Intermediate registration	Full registration
696-062-7	Cobaltate(1-), tetracarbonyl-, sodium (1:1), (T-4)-		
700-042-6	Lithium Nickel Cobalt Aluminium Oxide		1000-10 000 (7 reg)
839-353-8	cobalt manganese nickel dihydroxide		<10 (<5 reg)
910-663-6	Reaction mass of cobalt sulphide and nickel sulphide and trinickel disulphide	1000-10 000 (<5 reg)	
912-664-7	Reaction mass of cobalt and copper and iron	<10 (<5 reg)	
931-895-4	Reaction product of soluble nickel salt, cobalt salt, manganese salt with alkalines		
939-184-0	N/A		
942-358-9	Trizinc bis[hexacyanidocobaltate] dodecahydrate		<10 (<5 reg)
945-045-5	alumina doped with cobalt		<10 (<5 reg)
951-904-5	(nickel cobalt manganese) monoxide and tri(nickel cobalt manganese) tetraoxide		<10 (<5 reg)

Appendix 3. Occupational exposure data (Cobalt Institute)

Table 35: Statistical summary of personal inhalable air monitoring measurements (µg Co/m3) used for exposure estimation (Methodology applied in the occupational exposure scenarios for cobalt and cobalt compounds (IUCLID Section 13). Summary report. November 2021[16]

Code for Dataset [1]	Counts	Median	GM	GSD	P75	P90	P95	Max	Statistic [2]
CM-Manufacture of cobalt-Raw material handling	49	9.00	10.18	4.43	19	96	148	537	**P90**
CM-Manufacture of cobalt-Leaching unit	133	3	3	2.56	6	10	24	51	**P75**
CM-Manufacture of cobalt-Solvent extraction unit	27	1	2	2.40	2	5	7	40	**P90**
CM-Manufacture of cobalt-Tankhouse (electrowinning)	41	19	19	2.24	32	52	65	126	**P90**
CM-Manufacture of cobalt-Shearhouse (cutting)	9	11	12	1.81	19	24	27	30	**P90**
CM-Manufacture of cobalt-Powder production and milling	141	24	27	3.99	72	180	310	702	**P90**
CM-Manufacture of cobalt-Screening and packaging	191	82	72	4.75	235	470	680	5200	**P90**
CM-Manufacture of cobalt-Packaging of metal chips	10	2	2	2.64	6	8	9	9	**P95**
CM-Manufacture of cobalt-Supervision/control room	53	7	7	1.91	10	14	16	27	**P75**
CM-Production and industrial use of cobalt containing alloys, steels and tools-Melting and Casting	7	1	1	1.32	1	1	2	2	**P90**
CM-Production and industrial use of cobalt containing alloys, steels and tools-Handling of powders	19	221	192	3.77	489	1093	1221	1419	**P90**
CM-Service life of cobalt containing alloys, steels and tools in industrial settings-Use and mechanical treatment of hard coated metals and/or alloys-Mechanical treatment of hard coated metals and/or alloys – low kinetic energy	22	10	11	1.31	10	20	20	20	**P75**

[16] The summary is available from the Cobalt Institute https://www.cobaltinstitute.org/about-us/contact/

Code for Dataset [1]	Counts	Median	GM	GSD	P75	P90	P95	Max	Statistic [2]
CM-Service life of cobalt containing alloys, steels and tools in industrial settings-Use and mechanical treatment of hard coated metals and/or alloys-Use and mechanical treatment of hard coated metals and/or alloys – high kinetic energy	16	20	20	3.26	30	110	208	290	**P90**
CM-Industrial use of cobalt in the production of varistors and magnets (calcination/sintering processes)-Preparation of pre-sintered materials	11	62	34	2.78	66	96	110	123	**P95**
CM-Surface treatment-Finishing of surface treated objects	6	2	2	1.62	2	3	3	4	**P90**
CI-Manufacture of the substance-Raw material handling	65	22	20	6.19	45	172	326	680	**P90**
CI-Manufacture of the substance-Preparation of raw material	163	7	7	3.09	12	25	33	730	**P75**
CI-Manufacture of the substance-Wet process	77	4	4	3.76	8	21	49	146	**P90**
CI-Manufacture of the substance-Hot process	25	90	60	3.67	137	181	231	300	**P90**
CI-Manufacture of the substance-Further processing	75	38	30	5.43	79	239	301	670	**P90**
CI-Manufacture of the substance-Packaging of substances with moderate dustiness potential	147	17	20	4.55	59	149	252	797	**P90**
CI-Manufacture of the substance-Packaging of substances with high dustiness potential	91	54	72	5.96	262	833	1247	2823	**P90**
CI-Manufacture of the substance-Supervision	45	4	4	2.83	11	16	19	30	**P90**
CI-Manufacture of the substance-Cleaning & Maintenance	187	15	17	4.44	44	109	246	1482	**P90**
CI-Formulation-Surface Treatment-Raw material handling (solids)	4	2	3	2.37	4	8	10	11	**Max**
CI-Formulation-Surface Treatment-Filling of solutions containing <25 %	1	1	1	n.a.	n.a.	n.a.	n.a.	1	**Max*2**

Code for Dataset [1]	Counts	Median	GM	GSD	P75	P90	P95	Max	Statistic [2]
CI-Formulation-Surface Treatment-Raw material handling of low dusty solids	1	0.1	0.1	n.a.	n.a.	n.a.	n.a.	0.1	**Max*2**
CI-Plating processes in surface treatment-Cleaning & Maintenance	9	4	4	2.66	6	14	17	20	**P95**
CI-Plating processes in surface treatment-Plating	60	3	3	2.82	6	12	14	40	**P75**
CI-Plating processes in surface treatment-Raw material handling (solutions)	9	2	2	1.90	3	4	6	8	**P90**
CI-Use in fermentation processes, in scientific research, standard analysis and biogas production-Handling at laboratory scale	6	1	1	1.00	1	1	1	1	**P90**
CI-Use in fermentation processes, in scientific research, standard analysis and biogas production-Raw material handling	6	1	1	1.00	1	1	1	1	**P90**
CI-Use in humidity indicator cards, plugs and/or bags with printed spots-Handling of humidity indicator cards or spotted bags	6	0.03	0.03	1.06	0.03	0.03	0.03	0.03	**P90**
CI-Use in humidity indicator cards, plugs and/or bags with printed spots-Handling of liquid raw material	2	1	1	1.46	1	1	1	1	**P95**
CI-Use in humidity indicator cards, plugs and/or bags with printed spots-Further processing	10	0.3	0.3	2.46	0.4	1	1	2	**P95**
CI-Manufacture of inorganic pigments, frits, ceramic ware, glass -Raw material handling	13	5	4	3.12	9	11	15	20	**P90**
CI-Manufacture in the catalyst industry-All workplaces	141	2	2	4.81	7	16	22	110	**P90**
CC-Manufacture of the substance-Raw material handling	28	28	22	5.17	80	172	302	330	**P90**
CC-Manufacture of the substance-Packaging of powders	12	17	21	5.61	39	375	469	541	**P95**

Code for Dataset [1]	Counts	Median	GM	GSD	P75	P90	P95	Max	Statistic [2]
CC-Manufacture of the substance-Packaging of low and/or medium dusty materials	27	68	55	3.58	125	178	234	360	**P90**
CC-Production and industrial use of rubber adhesion agent using cobalt carboxylates- Raw material handling	33	0.080	0.118	11.10	1	2	5	6	**P90**
CC-Production and industrial use of rubber adhesion agent using cobalt carboxylates- Kneading (mixing)	36	0.057	0.080	7.11	0.239	2.345	4.175	4.200	**P90**
CC-Production and industrial use of rubber adhesion agent using cobalt carboxylates- Shaping	38	0.021	0.018	4.51	0.037	0.081	0.360	0.360	**P90**
CC-Production and industrial use of rubber adhesion agent using cobalt carboxylates- Finishing and shipping	4	0.051	0.050	1.14	0.053	0.056	0.056	0.057	**P95**
CC-Use of cobalt di(acetate) as catalyst-Use of catalyst	6	0.76	0.50	5.45	1.87	2.65	2.88	3.1	**Max**
CI-Catalysts-Delivery, transfer, storage	27	1.1	1.2	2.18	1.8	2.9	4.5	10.4	**P90**
CI-Catalysts-Addition of reagents, dissolution, sampling	6	0.6	1.1	2.96	1.9	4.5	5.7	6.8	**P95**
CI-Catalysts-Addition of reagents, impregnation, transfer to dryer, drying	4	9.7	6.6	2.23	10.4	10.4	10.4	10.4	**Max**
CI-Catalysts-Transfer to calciner, calcination	2	2.4	1.9	2.57	3.1	3.5	3.7	3.8	**Max**
CI-Catalysts-Screening to adjust particle size distribution	8	11.7	9.3	2.41	17.0	20.9	21.3	21.7	**P95**
CI-Catalysts-Cleaning and maintenance	26	11.1	11.0	4.07	21.5	97.9	131.7	150.8	**P90**

[1] Key: A combination of substance group (or substance name), original exposure scenario title and original workplace title is provided in this column. Substance groups are abbreviated as CM (cobalt metal), CI (inorganic cobalt substances) and CC (cobalt carboxylates and resinates)

[2] Statistic: Statistic selected as reasonable worst-case estimate (RWC), according to Vetter et al. (2016); the maximum value was selected in cases in which the required number of observations does not comply with R.14 (ECHA 2012) of the REACH (Table 14-2) guidance for precautionary reasons

Appendix 4. Summary table of studies on exposure correlations used by (DFG, 2016) and (DFG, 2016)

Table 36: Studies on correlations between air cobalt and cobalt in urine at workplaces; (DFG, 2016) and (DFG, 2016)

Workplace, workers	Cobalt in air [µg/m³]	Cobalt in urine [µg/L]	Cobalt in urine [µg/g creatinine]	References
Hard-metal grinders and -beaters; n = 50; no other details	68 ± 39 (AM ± SD; n = 13)	2.6–38[1] (range; n = 13)	–	(Stebbins et al., 1992)
Nickel refinery: cobalt metal and cobalt salts; n = 82 male; age 19–56 years	121; 110 (1–7 772) [GM; median (range); n = 82]		69.8; 72.4 (1.56–2 038) [GM; median (range); n = 82]	(Swennen et al., 1993)
Metal powder production (Co, Cr, Ni); n = 23 male; no further details	40; 5.0 (GM; GSD)		23.6 (6.4–173.1) [GM; (range); n = 26]	(Gennart et al., 1993)
Grinding with hard-metal tools;16 workers in 6 firms; with exhaustion without exhaustion	4.43 ± 2.70 (GM ± GSD; n = 17) 47.75 ± 3.53 (GM ± GSD; n = 18)	2.66 ± 1.69 (GM ± GSD; n = 8) 28.5 ± 3.97 (GM ± GSD; n = 6)		(Cereda et al., 1994)
Ceramics industry; welding and polishing of cobalt alloy moulds; welding	161.2; 1.5 (GM; GSD; n = 7) 37.1; 1.3	142.4 ± 95.6 (AM ± SD; n = 7) 29.9 ± 16.9 (AM ± SD; n = 6)		(Ferri et al., 1994)

Workplace, workers	Cobalt in air [µg/m³]	Cobalt in urine [µg/L]	Cobalt in urine [µg/g creatinine]	References
polishing	(GM; GSD; n = 6)			
Hard-metal tool production; 20 female	690; (4–1 100) [GM; (range); n = 11] 115; (2–510) [GM; (range); n = 22]	550 (median; no other data) 85 (6–505) [median; (range); n = 15]	– –	(Ferdenzi et al., 1994)
Hard-metal production; n = 8 male + 18 female: age 34.2 ± 8.3 years	83 (2–115) [median (range); n = 26]	–	33.8 ± 5.3 (GM ± GSD; n = 26; Friday start of shift)	(Franchini et al., 1994)
Hard-metal production (**cobalt powder**); n = 74	100 (AM; n = 12; value from graph equation)	80 (AM; n = 12; value from graph equation)[1]	–	(Scansetti et al., 1994)
Hard-metal production (**cobalt powder**); Factory A n = 112; age 15–65 years	288–1 600 (range; n = 112)	68; 303.6 ± 837.5 (0.75–5 500) [median ; AM ± SD; (range); n = 88]	-	(Sabbioni et al., 1994)
Factory B 1; n = 33	64 ± 20(19–90) [AM ± SD (range); n = 33]	42.1 ± 12.3 (16.1–70.5) [AM ± SD (range); n = 33]	-	
Factory B 2; n = 29	240 ± 120 (105–480) [AM ± SD (range); n = 29]	79.6 ± 28.0 (38.9–142.7) [AM ± SD (range); n = 29]	-	
Factory B 3; n = 10	840 ± 300 (520–1 320) [AM ± SD (range); n = 10]	141.7 ± 32.8 (99.8–210.4) [AM ± SD (range); n = 10]		

Workplace, workers	Cobalt in air [µg/m³]	Cobalt in urine [µg/L]	Cobalt in urine [µg/g creatinine]	References
Flame spraying, plasma spraying; n = 34; age 20–62 years	23 (1–390) [AM (range); n = 54] 27 (1–80) [AM (range); n = 29]		0.52; 5.2 (< 0.15–133) [median ; AM; (range); n = 89] 8.9;11.3 (0.7–33) [median; AM (range); n = 27]	(Chadwick et al., 1997)
Ceramics industry; **coating with cobalt blue paints**				(Christensen and Poulsen, 1994)
1982	80 (AM; n = 19)		22; 69 (median; AM; n = 46)	
1984	22 (AM; n = 39)		16; 22 (median; AM; n = 49)[2)]	
1985	30 (AM; n = 8)		12; 14 (median; AM; n = 27)	
1986	26 (AM; n = 35)		12; 17 (median ; AM; n = 66)	
1989	26 (AM; n = 60)		5; 12 (median; AM; n = 145)	
1990	22 (AM; n = 100)		2; 6 (median; AM; n = 94)	
1991	27 (AM; n = 57)		5; 10 (median; AM; n = 107)	
Cobalt metal; n = 35	383 (17–10 767) [GM (range); n = 35]		161.6 (13.1–1 534.0) [GM (range); n = 35]	(Lison et al., 1994)
Cobalt salts;	89 (1–4 690)		45.6 (1.6–666.1)	

Workplace, workers	Cobalt in air [µg/m³]	Cobalt in urine [µg/L]	Cobalt in urine [µg/g creatinine]	References
n = 72 **Cobalt oxides**; n = 15 **Hard-metal**; n = 10	[GM (range); n = 72] 467 (23–7 772) [GM (range); n = 15] 19 (1–203) [GM (range); n = 10]		[GM (range); n = 72] 70.0 (13,5–2 037.5) [GM (range); n = 15] 17.6 (3.0–85.6) [GM (range); n = 10]	
Wet grinding of hard-metal; n = 131; 16 workplaces	graphic presentation (n = 75)	8.9; 14.2 (0.5–159.4)[1] [median; AM (range); n = 131]	–	(Linnainmaa and Kiilunen, 1997)
Hard-metal production (**cobalt powder**); n = 30 male + 20 female; age 38 ± 5 years	21.85 ± 24.25 (5–92) [AM ± SD (range); n = 6]	22.28 ± 21.46 (1.15–88.6) [AM ± SD (range); n = 45]	–	(Scansetti et al., 1998)
Hard-metal production	100 ± 25 (79–130) [AM ± SD (range)]		46 ± 17 (11–110) [AM ± SD (range)]	(Torra et al., 2005)
Manufacture of tungsten carbide cutting tools n = 16 male	4.9–144.2 (GM range) 108 samples	2.3–15.5 (GM range) 507 samples	1.6–16.4 (GM range) 507 samples	(Martin et al., 2010)
Manufacture of tungsten carbide cutting tools n = 55 (37 male, 18 female) Age: 38.5 ± 10.4 years (21–61) Pre-sintering: Pouder weighing	 1.7	 10.28 ± 2.09		(De Palma et al., 2010)

Workplace, workers	Cobalt in air [µg/m³]	Cobalt in urine [µg/L]	Cobalt in urine [µg/g creatinine]	References
Pouder pressing	2.5	n = 13		
Sintering:	0.45	3.38 ± 2.24 n = 6		
Wet grinding:	1.50	4.47 ± 3.44 n = 13 (GM ± GSD		
Manufacture of nickel-hydrogen accumulators; Co-metal, $CoO(OH)$, $Ni(OH)_2$ N = 16 male; Mean age: 39 years dust masks	67 (4–330) [AM (range)] TWA	38.6 ± 47.4 (1.0–176.8)1) 28.2 ± 34.0 (1.0–127.8)1) [AM ± SD (range)]		(Yokota et al., 2007)
Manufacture of digital video cassettes (cobalt oxide) n = 16 male Age: 46.3 ± 4.8 years (AM ± SD) no dust respirators	<50 60 samples		(diagram)	(Fujio et al., 2009)
Metallic powders (Low exposure) (High exposure)	5 [0.2-11] (AM [range]) 15	9.0 ± 7.2 (0.1–34.8) [AM ± SD (range); n = 73][1] 25.2 ± 23.8 (2.0–137)	7 [0.7-27] (AM [range]) n = 60 21	(Nemery et al., 1992)

Workplace, workers	Cobalt in air [µg/m³]	Cobalt in urine [µg/L]	Cobalt in urine [µg/g creatinine]	References
	[0.7-43] (AM [range])	[AM ± SD (range); n = 86][1]	[2.3-75] (AM [range])	

(1) correlation between Co (air) and Co (urine)

Appendix 5. Genotoxicity, Animal data (*in vivo*)

The *in vivo* genotoxicity of cobalt and cobalt compounds has been assessed in a number of studies, as outlined in Table 37.

In an *in vivo* micronucleus study in mice, performed according to OECD TG474, no increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood of male or female mice exposed to cobalt metal (0.625 to 10 mg/m^3) for 3 months by inhalation. No significant alterations in the percentages of reticulocytes (polychromatic erythrocytes; PCEs) were seen in male or female mice either, suggesting that under these conditions, cobalt metal did not cause bone marrow toxicity (NTP, 2014).

In an *in vivo* micronucleus study, cobalt dichloride was administered via i.p. to male mice (n=5) (25-90 mg/kg bw) as pre-treatment to induce erythropoietin followed by treatment with different mutagens. After the treatment with cobalt dichloride, an increase in the frequency of micronucleated PCEs (MNPCEs) was observed (Suzuki et al., 1993). In contrast, high oral doses (probably above the maximum tolerated dose) of cobalt dichloride produced negative results at two different sampling times when tested for induction of chromosomal aberrations and micronuclei in rat bone marrow rats (Gudi and Ritter, 1998) as reported in (Kirkland et al., 2015)).

Dose and time-dependent increases in chromosomal aberrations, including clastogenic effects (chromatid breaks and gaps) were reported in bone marrow cells of Swiss male mice, after oral administration of different fractions of the lethal toxic dose of cobalt chloride hexahydrate ((Palit et al., 1991a), however this study is of limited value due to biologically implausible findings.

Swiss albino mice (n=5 males) administered cobalt chloride (0, 11.25, 22.5 or 45 mg/kg bw), intraperitoneally, for 24 or 48 hours, exhibited a significant increase in the frequency of MNPCEs in the bone marrow at the mid and high dose, after 24 hours, and at all doses after 48 hours exposure. The polychromatic:normochromatic erythrocytes (PCE/NCE) ratio was not significantly reduced in any concentration or time point (Goc Rasgele et al., 2013).

Aneuploidy was observed in the bone marrow and testes of male Syrian hamsters dosed intraperitoneally on five consecutive days with cobalt chloride (Farah, 1983). However, limitations of this study were recognised and the results were deemed difficult to interpret "since control levels of hyperdiploid cells were high, the definition of pseudodiploid cells is unclear, and there was no measure of bone marrow toxicity," (Kirkland et al., 2015).

Cobalt dichloride was additionally assessed for its potential to induce spermatogonial chromosomal aberrations *in vivo*, in Sprague-Dawley rats, dosed orally, once daily, for 28 days. No increases in the frequency of chromosomal aberrations compared to vehicle controls, and no bone marrow toxicity as measured by mitotic index, were observed in any of the three dose levels included in the analysis (0, 3, 10 or 30, mg/kg bw/day), with the highest dose identified as the maximum tolerated dose (MTD). Additionally, no polyploid cells were detected in the metaphases examined in each of the groups (Kirkland et al., 2015).

DNA strand breaks were detected by the alkaline comet assay in the sperm of male zebrafish, exposed to sub-lethal concentrations of cobalt chloride, up to 25 mg/l, for 13 days. Induction of the expression levels of selected analysed DNA repair genes (*rad51*, *xrcc5* and *xrcc6*) in the testes was also reported (Reinardy et al., 2013)

An intravenous dose of 50 or 100 µmol cobalt diacetate/kg bw (0, ~2.9 or 5.9 mg cobalt/kg bw) was administered to F344/NCr rats (n=12/sex/dose). Chromatin analysis revealed DNA base products, characteristic of promutagenic oxidative DNA damage (e.g. 8-oxo-Gua and 5-OHMe-Ura), occurring in a dose dependent manner in kidney, liver and lung

samples (Kasprzak et al., 1994). In a more recent study, no increase in 8-OH-dG lesions as detected by immunohistochemistry, was observed in lung tissues of rats treated with 80 mg/m^3 tricobalt tetraoxide, in a 28-day inhalation exposure study, indicating an absence of oxidative DNA damage (Burzlaff et al., 2022).

Cobalt sulphate, cobalt monoxide and tricobalt tetraoxide were evaluated for induction of bone marrow chromosomal aberrations *in vivo*, in non-GLP studies including a single dose phase (n=2/sex/dose) which informed the dose selection for the subsequent multi-dose phase study. The latter comprised 5 oral administrations in Sprague-Dawley rats (n=5/sex/dose) (doses: cobalt sulphate 21, 63 or 210 mg cobalt/kg bw/day; cobalt monoxide: 157, 472 or 1573 mg cobalt/kg bw/day; tricobalt tetraoxide: 47, 141 or 470 mg cobalt/kg bw/day). These studies were not fully compliant with OECD guidelines due to the severity of clinical signs, early sacrifice and mortalities occurring during the multi-dose phase, for cobalt sulphate and cobalt oxide, at dose levels that proved to be above the maximum tolerable dose, resulting in number of groups/animals sampled to be reduced below the normally recommended levels. No increased chromosomal aberration frequencies were seen in animals treated with tricobalt tetraoxide, while only marginal increases (up to 1.8%) compared to vehicle controls were seen in the highest dose groups in males treated with cobalt sulphate and cobalt monoxide. These were deemed not biologically significant, as they were within the range of normal variance for controls. It was therefore concluded that none of the cobalt salts induced chromosomal aberrations in rat bone marrow, at high doses (at or above the MTD) in the multi-dose phase (Kirkland et al., 2015).

Brown et al. (2013) reported chromosomal aberrations and DNA damage in femur bone marrow of mice dosed four times periarticularly (knee joint) for up to 18 weeks with nano- and micron-sized CoCr particles (Brown et al., 2013). The same group found no increase of g-H2AX foci and increased levels of total chromosome aberrations, simple aneuploidy, complex aneuploidy and chromosome fragments. However only the increases in incidences of total aberrations and chromosome fragments, 4 weeks after injection of micron sized particles were significant (Brown et al., 2013).

A 2-year inhalation study provided 'clear evidence' of carcinogenic activity of cobalt sulphate heptahydrate in female rats, and B6C3F$_1$ mice of both sexes, based on increased incidences of alveolar/bronchiolar neoplasms (NTP, 1998). DNA isolated from tissue sections from both cobalt sulphate-induced lung neoplasms in B6C3F$_1$ mice (3 different dose groups) and spontaneous neoplasms from controls, was used for PCR amplification, restriction fragment length polymorphic identification, single-strand conformation polymorphism analysis or direct sequencing for molecular analysis for genetic alterations. Of the *Kras* mutations detected at the second base of codon 12 (35%, 9/26), a higher frequency of G→T transversions (5/9, 55%) was detected in the neoplasms of exposed mice, compared to those of concurrent (0/1, 0%) and historical controls (1/24, 4%). No *Kras* codon 61 CTA or CGA mutations were detected. The frequency of *Kras* mutations exhibited a dose-response relationship trend and it was suggested that the higher incidence of G→T transversions at codon 12 produced by cobalt sulphate heptahydrate may be associated with DNA damage by oxidative stress, since these point mutations are typically associated with active oxygen species and are consistent with the generation of 8-hydroxydeoxyguanine (8-OH-G) adducts, implicated in tumorigenesis (Tchou et al., 1991) (Shigenaga and Ames, 1991, Janssen et al., 1993, Grollman and Moriya, 1993). Consistent with this, is the report by (Shi et al., 1993) that cobalt sulphate heptahydrate catalyses the production of oxygen-based free radicals. Thus, cobalt salt exposure in B6C3F$_1$ mice may have resulted in the generation of hydroxyl radicals, indirectly damaging DNA, thereby promoting G→T transversions.

Clear evidence of carcinogenic activity was also provided for cobalt metal by a set of 2-year inhalation NTP studies in F344/NTac rats and in B6C3F$_1$/N mice of both sexes, based on the increased incidences of alveolar/bronchiolar adenomas and carcinomas in the lung

(NTP, 2014). Mutation analysis of the rodent homologues of the most commonly altered genes in human lung cancer (i.e., *KRAS*, *EGFR* and *TP53*) was conducted in samples from lung carcinoma tissues derived from all cobalt metal-exposed groups and from spontaneous alveolar/bronchiolar carcinomas occurring in control animals (i.e., concurrent controls in mice and controls from other NTP bioassays in rats). Overall, there was a significantly higher incidence of *Kras* mutations, and a lower occurrence of *Egfr* and *Tp53* mutations in the lung carcinomas of chronically exposed rats and mice. None of these mutations were detected in control tissues suggesting that these genetic alterations are related to chemical exposure (NTP, 2014, Hong et al., 2015). *Kras* mutations were the most frequently detected mutations in the carcinomas of exposed rodents (31% in rats and 67% in mice), with exon 1, codon 12 G→T transversions being the predominant point mutations (80% in mice and 57% in rats), similar to the observations made in mice exposed to cobalt sulphate. *Egfr* mutations were detected in 17% cobalt metal-induced lung tumours in rats and mice, dominated by exon 20 G→A transitions (50% in rats and 42% in mice). *Tp53* mutations were detected in 23% (rats) and in 19% (mice), dominated by exon 6 transition in rats and exon 5 transversions in mice. As discussed above, the G→T transversions are commonly associated with the production of reactive oxygen species (ROS) during oxidative damage to DNA. Cobalt metal has been shown to induce hypoxia and upregulate HIF-1a signalling, thereby modulating inflammatory responses and inducing oxidative stress (Simonsen et al., 2012). The prominence of G→T transversions in the *Kras* mutations occurring almost exclusively in the alveolar/bronchiolar carcinomas of cobalt metal and cobalt sulphate exposed animals, suggests a common mechanisms of mutation induction with a G:C sequence specificity. In the NTP bacterial mutagenicity assays the cobalt substances produced positive results only in *S. typhimurium* strains able to detect mutational events at G:C base pairs (section 7.6 and **Appendix 6**). In view of the weak, if any, activity of cobalt metal in gene mutation assays *in vitro* (section 7.6 and **Appendix 6**), it appears likely that the *Kras* mutations reflect secondary genotoxic events associated with inflammatory and oxidative stress induced in the lung upon cobalt metal particles exposure (NTP, 2014).

The early positive findings of *in vivo* genotoxicity (micronuclei and chromosome aberrations in bone marrow and germ cells) of cobalt chloride administered orally or i.p. in rats, mice and hamsters were not reproduced in the more recent studies designed to comply with OECD protocols, with various cobalt compounds administered orally, up to the maximum tolerated dose in rats. In the latter studies no significant or biologically meaningful genotoxic response was recorded (Kirkland et al., 2015). Similarly, no increase in peripheral erythrocyte micronuclei was noted in mice from the 3-month NTP study cobalt metal study (NTP, 2014).

Table 37: Summary of *in vivo* genotoxicity studies

Method	Test substance	Lowest effective* or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
Micronucleus test in peripheral blood erythrocytes in male and female mice (n=10/sex)	Cobalt metal (>98% purity)	10 mg/m^3	negative	OECD TG471, 3-month study, inhalation	(NTP, 2014)
Micronucleus test in bone marrow cells, BALB/c AnNCrj male mice (n=5)	Cobalt dichloride hexahydrate	50 mg/kg (=12.4 mg cobalt/kg bw)/(90 mg/kg)	**positive** Dose-dependent increase in MPCEs ($CoCl_2$ single treatment) Significant enhancement of MPCEs induced by mutagenic substances following pretreatment with Cobalt dichloride	Combination studies of Co with mutagens, Cobalt dichloride administered i.p, mice sacrificed 30 h after mutagen treatment (12-72 h post Co injection)	(Suzuki et al., 1993)
Micronucleus assay in bone marrow cells, rat	Cobalt dichloride hexahydrate	600 mg/kg bw/ (149 mg cobalt/kg bw)	negative	Compatible with OECD TG 474, oral administration	(Gudi and Ritter, 1998), as reported in (Kirkland et al., 2015)
Micronucleus test in Wistar rat type II pneumocytes (AT-II) and PBMCs	WC-Co (6.3% cobalt, 84% tungsten and 5.4% carbon), 2 mm	AT-II 1.8 mg WC-Co/kg bw/ (49.8 mg WC-Co/kg body w)) PBMC	**positive** ‰ of micronucleated AT-II cells was maximal at 72 h after i.t. instillation Dose-related increase at doses 1.8-16.6 mg WC-Co/kg/ bw negative	The frequency of micronuclei was assessed in isolated type II pneumocytes (ex vivo) and in PBMC (ex vivo/*in vitro*, cytokinesis-block method); time course (72 h selected) and dose-effect experiments (rats exposed to 4 doses of WC-Co)	(De Boeck et al., 2003a)

Method	Test substance	Lowest effective* or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
Micronucleus test in bone marrow cells, Swiss albino mice (n=5)	Cobalt dichloride	24 h 22.5 mg/kg bw/ (45 mg/kg bw) 48 h: 11.25 mg/kg bw/ (45 mg/kg bw)	**positive** significant increase in the number of MPCEs at 24 h and 48 h. No significant reduction of the PCE/NCE	i.p. administration, 24 and 48 h exposures	(Goc Rasgele et al., 2013)
Chromatin analysis by gas chromatography and mass spectrometry, male and female F344/NCr rats (n=12/sex/dose)	Cobalt diacetate	50 and 100 µmol/kg bw (= 12.5. 25 mg cobalt acetate/kg bw or 2.9, 4.9 mg cobalt/kg bw	**positive** DNA base products, typical of oxidative DNA damage, increased 30%-≥200% over control levels, with increasing Co dose	DNA base damage, in renal, hepatic, and pulmonary chromatin, single i.p. administration, sacrifice 2-10 days	(Kasprzak et al., 1994)
Mammalian bone marrow and germ cell cytogenetic assay, hamster	Cobalt dichloride	400 mg/kg bw (99 mg cobalt/kg bw)	**positive** Bone marrow and testes	No guideline; experimental and reporting deficiencies, 7 days, i.p.	(Farah, 1983)
Chromosome aberrations in bone marrow cells, Swiss Albino male mice (n=5/dose)	Cobalt dichloride	Different dilutions of the lethal toxic dose, 80 (1/10), 40 (1/20), and 20 1/40) mg/ kg bw per time point	**positive** Significant, dose-dependent increase in chromosomal aberration (chromatid gaps/breaks, polyploids) at all time points, peaking at 24 h	Oral administration, observations made after 6, 12, 18 and 24 h (n=20/set including controls)	(Palit et al., 1991c)
Chromosome aberrations (M-FISH), bone marrow cells (n=6)	CoCr nano and micron-sized particles	$4.8x10^6$ mm^3 micron/nanometre sized particles	**positive** levels of total chromosome aberrations, simple aneuploidy, complex aneuploidy and chromosome fragments were higher in the mice exposed to both the nm and mm sized particles than controls, however		(Brown et al., 2013)

Method	Test substance	Lowest effective* or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
			only the increases in incidences of total aberrations and chromosome fragments, 4 weeks after injection of micron sized particles were significant		
Chromosome aberrations, rat	Cobalt dichloride hexahydrate	600 mg/kg bw (149 mg cobalt/kg bw)	negative	Compatible with OECD TG 475, oral administration	(Gudi and Ritter, 1998), as reported in (Kirkland et al., 2015)
Chromosome aberrations in bone marrow cells, Sprague-Dawley albino rats (n=5/sex/dose)	Cobalt sulphate Cobalt monoxide Tricobalt tetraoxide	1000 mg/kg bw/day (=210 cobalt mg/kg bw/day) 2000 mg/kg bw/day (=1573 cobalt mg/kg bw/day) 2000 mg/kg bw/day (=470 cobalt mg/kg/day)	negative Evidence of bone marrow toxicity with both cobalt sulphate and cobalt monoxide, but mitotic index (MI) increased at the low and mid-doses of cobalt tetraoxide	Administered orally, sampled 16 h after the last (or only) treatment	(Kirkland et al., 2015)
Spermatogonial chromosomal aberration, Sprague-Dawley CD rats	Cobalt dichloride hexahydrate	30 mg/kg bw/day	negative No bone marrow toxicity as measured by mitotic index	Oral administration, 3 dose levels, for 28 days	(Kirkland et al., 2015)
Alkaline comet assay, Wistar rat type II pneumocytes (AT-II), cells obtained after broncho-alveolar lavage (BAL) and PBMCs	WC-Co (6.3% cobalt, 84% tungsten and 5.4% carbon), 2 mm	16.6 mg WC-Co/kg body wt (=1.0 mg Co/kg body wt)	**positive** Statistically significant increase of DNA damage in AT-II cells at 12 h Negative in PBMCs and BAL	Single intra-tracheal (i.t.) instillation of WC-Co, dose producing mild pulmonary toxicity	(De Boeck et al., 2003a)

Method	Test substance	Lowest effective* or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
Alkaline comet assay, DNA damage in sperm, Zebrafish, (n=2-3)	Cobalt dichloride Cobalt sulphate	15 mg/l (25 mg/l)	**positive** DNA damage in sperm increased with Co concentration after exposure but returned to control levels after a 6-day recovery	A day after the end of exposure (day 13), 3 males were sampled, followed by a 6-day recovery period	(Reinardy et al., 2013)
Alkaline comet assay in cells from the bone marrow of the right femur and dissociated cells from frontal cortex of C3H female mice (n=6)	CoCr nano and micron-sized particles	$4.8x10^6$ mm^3 micron/nanometre sized particles	**positive** Bone marrow cells: significantly increased DNA damage at 1 and 40 weeks after final injection of nm sized particles, non significant trend for mm sized frontal cortex cells: significant increase in DNA damage, 40 weeks post-injection (for nm sized only)	4 injections peri-articularly, in the right knee joint at 0, 6, 12 and 18 weeks, doses representative of low/high wear, n=10 were sacrificed at weeks 1, 4 and 40 following the final injection, n=6 of high dose mice at each time point were assessed	(Brown et al., 2013)
g-H2AX phosphorylation, immunofluorescence of cerebral cortex tissues from mice (n=4)	CoCr nano and micron-sized particles	$4.8x10^6$ mm^3 micron/nanometre sized particles	negative no significant increase in g-H2AX foci in mice exposed to either nm or mm sized particles		(Brown et al., 2013)
8-OH-dG lesions as markers for oxidative DNA damage in lung tissue, detected by IHC, 28-day inhalation toxicity study in male/female Wistar rats (n=10/sex/group)	tricobalt tetraoxide cobalt sulphate	80 mg/m^3	negative	In vivo 28-day inhalation study was conducted according to OECD TG 412, nose-only inhalation, 6 h/day, 8-OH-dG IHC performed on day 1 and day 90 post exposure, high dose group	(Burzlaff et al., 2022)
carcinogenicity study F344/N rats and B6C3F1	cobalt sulphate	0.3 mg/m^3/day/((3.0 mg/m^3/day)	**positive in mice** increased frequency of *Kras*		(NTP, 1998)

Method	Test substance	Lowest effective* or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
mice			mutations and increased G to T transversions in codon 12) in mice		
carcinogenicity study F344/N rats and B6C3F1 mice	cobalt metal	1.25 mg/m^3/day	**positive in mice and rats** increased frequency of *Kras* mutations (and G to T transversions in codon 12), lower occurrence of *Egfr* and *Tp53* mutations		(NTP, 2014)

* the lowest effective dose corresponds to the dose of a perceived increase in the amplitude of a response and does not always coincide with the dose which reaches statistical significance; WC: Tungsten-carbide; MPCEs: mononucleated polychromatic erythrocytes; PMBC: peripheral blood mononuclear cell; M-FISH: Multicolour in situ hybridization; IHC: immunohistochemistry; i.p: intraperitoneally, i.t: intra-tracheally; MI: mitotic index; 8-OH-dG: 8-hydroxydeoxyguanosine

Appendix 6. *In vitro* genotoxicity

Published studies on the genotoxicity of cobalt metal and soluble cobalt compounds (such as cobalt chloride and cobalt sulphate) have been inconsistent, reporting conflicting findings.

Bacterial test systems

Cobalt metal

Cobalt metal has been assessed for bacterial mutagenicity in *Salmonella typhimurium* (TA98, TA100) and *Escherichia coli* (WP2 *uvrA*/pKM101) tester strains, either in buffer or S9 mix, as part of a toxicity/carcinogenicity NTP study (NTP, 2014) (**Table 38**). Cobalt metal (100 to 5000 µg/plate) produced an overall equivocal response in the TA100 strain, in the absence of S9 activation, by inducing only small, non dose-related increases in revertant colonies compared to solvent controls in 2/3 occasions. No mutagenic activity was seen in the presence of 10% rat liver S9, at doses up to 7,500 µg/plate. In strain TA98, cobalt metal (100 to 3500 µg/plate) was mutagenic, yielding two positive and one equivocal response, in the absence of exogenous activation. These responses were overall weak and did not correlate with the dose level. In contrast, no activity in that strain was evident upon S9 activation, at doses up to 7500 mg/plate. No mutagenic activity was detected in the *E. coli* strain WP2 *uvrA*/pKM101, at doses of up to 450 µg/plate, with or without S9 activation (NTP, 2014). In light of the positive results in strain TA98, cobalt metal was probed again in the same strain, in GLP, single strain Ames tests, conducted in three different laboratories. Cobalt metal did not produce a mutagenic response, regardless of S9 activation, failing to corroborate the previous finding, even at the higher concentration of 5000 mg/plate (Kirkland et al., 2015).

Cobalt salts

Cobalt salts evaluated in bacterial mutagenicity tests yielded largely negative results (**Table 38**) with only isolated positive responses in specific strains.

Evidence of cobalt chloride mutagenicity in strain TA98 and to a lesser extent in TA1537, in the absence of S9 activation was first reported by Wong et al (Wong, 1988). Dose-response curves were derived, employing a range of (unspecified) cobalt concentrations between the 50% and 90% inhibitory doses (40 and 120 ppm, respectively), as determined by growth inhibition experiments in *Salmonella typhimurium* strains TA98, TA102, TA1535 and TA1537. Based on the number of revertants/mg (normalised to controls), at doses corresponding to the linear portion of these curves, cobalt chloride was approximately 4-fold more mutagenic in TA98 than TA1537, without S9 activation (Wong, 1988). Cobalt chloride was shown to be readily mutagenic in the *S. typhimurium* strain TA97, under different preincubation buffers and agar components (salts), producing a higher level of response compared to the other metals tested (Pagano and Zeiger, 1992). TA97 revertants increased with increasing cobalt chloride concentrations, reaching 5-6-fold the control levels, at 800 mM.

Cobalt chloride was tested for its modulatory effect on the mutagenic potential of several chemical and physical agents, in a number of combination studies in bacteria, without exhibiting any marked mutagenic activity in single exposures ((Ogawa et al., 1986, Ogawa et al., 1988, Kada, 1978, Mochizuki and Kada, 1982, Clarke and Shankel, 1988, Leitao et al., 1993, Kada and Shimoi, 1987).

Cobalt sulphate heptahydrate (10 to 10000 µg/ml) was weakly mutagenic in the TA100 strain, in the absence of S9 metabolic activation, using the pre-incubation method. Peak responses of approximately 2-fold increases in T100 revertants compared to controls, were detected at 333 mg/plate cobalt sulphate, in three separate experiments and were regarded as weakly positive/positive. Weakly positive responses resulting in <2-fold

increased mutations compared to controls, were also seen in the presence of 5% hamster or rat liver S9. Higher percentages of rat/hamster S9 generated either equivocal or negative results. No increased induction of mutations was reported in the other two strains examined, TA98 or TA1535, with or without S9 (Zeiger et al., 1992, NTP, 1998).

Strain T98 detects a -1 frameshift that disrupts a dinucleotide run of $(CG)_4$ residues; strain TA100 detects reverse mutations at a codon for proline (GGG) in *hisG46*, and the *E. coli* WP2 *uvrA*/pKM101 strain detects reverse mutations at the *trpE* ochre (TAA) codon. There is therefore a correlation between the mutagenic responses elicited by cobalt sulphate and cobalt metal in the bacterial strains and the ability of these test systems to detect mutational events at G:C base pairs. This finding is complementary to a cobalt chloride-related observation, where sequencing of the *supF* tRNA mutational reporter gene in exposed bacteria revealed that all mutational events (base substitutions and frameshifts) occurred exclusively at G:C base pairs (Ogawa et al., 1999).

Cobalt chloride and cobalt sulphate were tested again in GLP compliant studies, in the TA97a and TA100 strains, respectively, at multiple concentrations of up to 5000 mg/plate. Neither substance produced a mutagenic response, regardless of S9 activation, failing to reproduce the previously positive findings at similar concentrations (Kirkland et al., 2015).

Table 38: Selected bacterial mutagenicity studies with cobalt metal and cobalt salts

Assay/ species strains	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
Bacterial reverse mutation assay/*S. typhimurium* TA98, TA100; *E. coli* WP2 *uvrA*/pKM101	cobalt metal (>98% purity)	-S9: TA100: 500 mg/plate/ (5000 mg/plate) TA98: 100 mg/plate/ (3500 mg/plate) + S9: TA100, TA98: 7500 mg/plate *E.coli:* +/- S9: 450 mg/plate	-S9: TA100: 2/3 equivocal, 1/3 negative TA98: 2/3 **positive,** 1/3 equivocal + S9 (10% rat): negative *E. coli* +/- S9: negative	OECD TG471 2 independent experiments, 5 concentrations tested in triplicate, concurrent positive and negative controls, preincubation method	(NTP, 2014)
Bacterial reverse mutation assay/*S. typhimurium* TA98	cobalt metal (ultrafine powder, 99.94% pure)	5000 mg/plate	-/+ S9: negative	OECD TG471, GLP compliant, plate incorporation and pre-incubation methods, 3 test laboratories, each tested at least 6 concentrations/chemical, at least in triplicate, small differences in methodologies/data evaluation; precipitation/toxic	(Kirkland et al., 2015)

Assay/ species strains	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
				ity observed at the highest concentration	
Bacterial reverse mutation assay/*S. typhimurium* TA98, TA102, TA1535, TA1527	cobalt chloride	+/- S9: Doses (unspecified) within the linear portion of the dose-response curves, between the 50% and 90% toxic doses (40-120 ppm, respectively), obtained from growth inhibition experiments	-S9: TA98: **positive** -S9: TA1527: **positive** -S9: TA102, TA1535: negative +S9: negative	Plate incorporation assay, performed as per (Maron and Ames, 1983), range of concentrations induced 50-90% toxicity	(Wong, 1988)
Bacterial reverse mutation assay/ *S. typhimurium* TA97	cobalt chloride	13 mg/ml/(800 mM (=104 mg/ml corresponding to 72.8 mg/plate)	-S9: Readily detected as **positive**	Preincubation mutagenesis tests in triplicate	(Pagano and Zeiger, 1992)
Bacterial reverse mutation assay/ *S. typhimurium* TA98, TA100, TA1537, TA2637	cobalt chloride	(130000 µg/plate) (=1000 mmoles/plate)	-S9: negative ($CoCl_2$ alone)	Combined mutagenicity studies of heteroaromatic compounds with $CoCl_2$, as per (Ames et al., 1975)	(Ogawa et al., 1986)
Bacterial reverse mutation assay/ *S. typhimurium* TA98, TA97a, TA100, TA1537, TA2637	cobalt chloride	(300 mmoles/plate) (TA1537, TA2637)	-S9: negative ($CoCl_2$ alone)	Combined mutagenicity studies with 4-substituted pyridines	(Ogawa et al., 1988)
Mutations in the *E.coli supF* gene/ pUB3 vector, *E.coli* SY1032/pKY241 host cells	cobalt chloride	2.6 µg/mL/(20 mM)	-S9: **positive** deletions/ frameshifts (61%); base substitutions (29%); 18/19 base	2 h treatment of pUB3 with 20 mM $CoCl_2$	(Ogawa et al., 1999)

Assay/ species strains	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
			substitutions and 8/10 frameshifts occurred at G:C sites		
Reverse Mutation assay/ *E. coli* WP2	cobalt chloride hexahydrate	(20 µg/ml)	-S9: negative ($CoCl_2$ alone)	Effects of $CoCl_2$ on cellular viability and mutation induction of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced mutations in *E.coli* WP2 Try^-	(Kada, 1978)
Bacterial reverse mutation assay/ *S. typhimurium* TA100	cobalt chloride hexahydrate	(23800 µg/ml) (= 0.1 M)	-S9: negative	As per (Ames et al., 1975)	(Tso and Fung, 1981)
Bacterial reverse mutation assay/ *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 *uvrA*/pKM101	cobalt chloride hexahydrate (97.5% purity)	Non-toxic concentrations determined by preliminary toxicity assays in TA100 (unspecified)	-S9: negative	Standard and modified medium, plate incorporation/fluctuation assays	(Arlauskas et al., 1985)
Bacterial reverse mutation assay/ *S. typhimurium* TA98, TA1538	cobalt chloride hexahydrate	(20 µg/mL)	-S9: negative	Probing the antimutagenic effects of $CoCl_2$ in 'top agar' and 'liquid phase' experiments with regard to Trp-P-l-induced mutagenesis in strains TA98 and TA1538	(Mochizuki and Kada, 1982)
Reverse mutation assay/ *E. coli* strain ND-160	cobalt chloride	(15 mg/ml)	Negative cobalt ions did not reduce spontaneous reversions to		(Clarke and Shankel, 1988)

Assay/ species strains	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
			Lac^+, partially decreased the numbers of caffeine induced revertants when used in combination		
Inhibition-induction of SOS responses / *E.coli* WP2s	cobalt chloride hexahydrate	(200 mg/ml)	-S9: Negative ($CoCl_2$ alone)	Probing the effects of $CoCl_2$ on UV-induced mutagenesis, lysogenic induction and phage reactivation	(Leitao et al., 1993)
Bacterial reverse mutation assay/ *S. typhimurium* TA97a	cobalt chloride (>99% pure)	-/+S9: (5000 mg/plate)	-/+ S9: negative	OECD TG471, GLP compliant, plate incorporation and pre-incubation methods, 3 test laboratories, each tested at least 6 concentrations/chemical, at least in triplicate, small differences in methodologies/data evaluation; toxicity at the highest concentrations (3600 and 5000 mg/plate)	(Kirkland et al., 2015)
Bacterial reverse mutation assay/ *S. typhimurium* TA98, TA100, TA1535	cobalt sulphate heptahydrate	-S9: TA100: 10 µg/plate/ (10000 mg/plate) -S9: TA98: (10000 µg/plate) TA1535: (1000 mg/plate) +S9: TA100: 3 mg/plate/ (10.000 µg/plate)	-S9: TA100: 2/3 weakly **positive**; 1/3: positive -S9: TA98, TA 1535 negative +S9-5% hamster/rat: TA100: 2/2 weakly **positive**	Testing was performed as per (Zeiger et al., 1992), each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of cobalt sulphate heptahydrate.	(NTP, 1998, Zeiger et al., 1992)

Assay/ species strains	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
		+S9: TA1535: (1000 mg/plate) TA98: (10000 mg/plate)	+S9-10% and 30% hamster TA100: 2/3 equivocal, 1/3 negative +S9-10% and 30% rat TA100: 2/3 negative, 1/3 equivocal +S9-5% hamster/rat: TA1535: 2/2 negative +S9-5-30% hamster: TA98: 3/4 negative, 1/4 equivocal +S9-5-30% rat: TA98: negative		
Bacterial reverse mutation assay/ *S. typhimurium* TA100	cobalt sulphate	-/+ S9: (5000 mg/plate)	-/+S9: negative	OECD TG471, GLP compliant, plate incorporation and pre-incubation methods, 3 test laboratories, each tested at least 6 concentrations/chemical, at least in triplicate, small differences in methodologies/data evaluation; toxicity at the highest concentration (5000 mg/plate)	(Kirkland et al., 2015)

Collectively, bacterial mutation assays were all (except one) negative, in the presence of S9 activation. In the absence of S9, sporadic indications of mutagenic activity of cobalt and soluble cobalt compounds in specific *Salmonella typhimurium* tester strains were not confirmed in follow-up GLP studies, therefore it can be concluded that there is a lack of mutagenic activity in bacteria.

Gene mutations in mammalian cells

Cobalt metal powder was tested for its potential to induce *Hprt* mutations in mouse lymphoma L5178Y cells, in the absence or presence of S9, after 3-hour treatments (Kirkland et al., 2015) (**Table 39**). Cobalt precipitated in culture medium and undissolved metal was present at many lower concentrations, potentially exerting direct toxic effects on cells due to its physical presence. It was concluded that cobalt metal powder did not induce statistically or biologically significant increases in mutant frequency, when tested up to highly toxic concentrations (50 mg/ml), in the absence of S9. However, it produced weak, reproducible, and at single intermediate concentrations significant mutagenic effects in the presence of S9, starting at 30 mg/ml. In order to avoid any potential effects from undissolved cobalt, the experiment was repeated with extracts of cobalt metal powder. At higher concentrations, relative survival was reduced to <20% of control. Extract was used up to highly toxic concentrations of 290 and 350 mg cobalt (used for extraction)/ml, in the absence or presence of S9, respectively, for 3-hour treatments. The extract was also tested in the absence of S9 for a longer 24-hour treatment. Under all conditions, the extract of cobalt metal powder elicited only sporadic positive responses of no biological relevance, regardless of S9 activation or treatment time, therefore failing to corroborate the earlier positive findings of cobalt metal (Kirkland et al., 2015).

Cobalt chloride was initially reported to be mutagenic inducing an increase in the *Hprt* mutation frequency by 4.2-fold in V79 cells, after 24 hours of treatment at 100 mM (Hartwig et al., 1990). Cobalt chloride mutagenicity albeit weak, was confirmed by the same group, as evidenced by a 2.9-fold increase in the spontaneous mutation frequency at 250 mM, after a 23-hour treatment (Hartwig et al., 1991). In contrast, Amacher and Pailat had previously reported that $CoCl_2$ at a concentration range between 5.69 to 57.11 mg/ml, did not increase trifluorothymidine resistant colonies in the mouse lymphoma *Tk* mutation assay, compared to solvent controls, in cultures exposed to the cobalt salt for 3 hours (Amacher and Paillet, 1980).

Comprehensive *Hprt* mutation studies were later undertaken for a number of cobalt substances (Kirkland et al., 2015). From those tested for 3 hours, up to toxic or precipitating concentrations, cobalt dihydroxide, lithium cobalt dioxide, cobalt oxalate, cobalt hydroxide oxide and tricobalt tetraoxide did not induce statistically and/or biologically significant increases in *Hprt* mutation frequencies compared to vehicle controls, regardless of S9 activation. From the substances tested for 3 hours in the absence and presence of S9, and for 24 hours in the absence of S9, neither cobalt sulphate nor cobalt sulphide induced any statistically and/or biologically significant increases in mutation frequencies compared to controls, when tested up to toxic concentrations. Cobalt chloride was not reinvestigated for gene mutation induction in this set of studies, however the negative *Hprt* mutation results with cobalt sulphate (which would equally expose the cells to the cobalt cation) indicate that cobalt chloride would not be mutagenic, either. Cobalt monoxide also gave negative results up to precipitating concentrations, under the same experimental conditions (Kirkland et al., 2015).

Table 39: Summary of gene mutation studies in mammalian cells

Assay/ test system	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
Mammalian cell gene mutation	cobalt metal powder (99.98%	-S9: (50 mg/ml)	-S9: negative	OECD TG476, 3 h incubations	(Kirkland et al., 2015)

Assay/ test system	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
test (*Hprt* locus)/ mouse lymphoma (L5178Y) cells	Pure)	+S9: 30 mg/ml/(250 mg/ml	+S9: **positive**		
Mammalian cell gene mutation test (*Hprt* locus)/ mouse lymphoma (L5178Y) cells	extracts of cobalt metal powder	-S9: 3 h: 290 mg of cobalt used for extraction/ml) 24h: 150 mg of cobalt used for extraction/ml) +S9: 3h: 350 mg/ml	-/+ S9 (3/24h): negative	OECD TG476, 3 or 24 h incubations	(Kirkland et al., 2015)
Mammalian cell gene mutation test (*Tk* locus)/ mouse lymphoma cells	cobalt chloride hexahydrate	57.11 mg/ml	negative	Assay performed as per (Clive et al., 1972); 3 h treatments, no metabolic activation	(Amacher and Paillet, 1980)
Mammalian cell gene mutation test (*Hprt* locus)/ chinese hamster (V79) cells	cobalt dichloride hexahydrate	100 mM	**positive** 4.2-fold enhancement of mutation frequency		(Hartwig et al., 1990)
Mammalian cell gene mutation test (*Hprt* locus)/ chinese hamster (V79) cells	cobalt chloride hexahydrate	250 mM/(250 mM)	**positive** 2.9-fold increase of spontaneous mutation frequency, only at the highest concentration	Total incubation time of 25 h, single treatments and in combination with UV; 250 mM is toxic reducing the colony forming ability of the treated cells to 44% of control	(Hartwig et al., 1991)
Mutation assay (*Hprt* locus)/	cobalt chloride hexahydrate	$1x10^{-4}$ M/ ($2.5x10^{-4}$ M)	**positive** produced (2-12)-fold increases of	Modification of the method (Nakamura and	(Morita et al., 1991)

Assay/ test system	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
mouse mammary carcinoma (FM3A) cells			control number of mutant colonies	Okada, 1983), metabolic activation, 48 h treatments, dose-dependent increase in cytotoxicity, mutagenic only in a narrow range of concentrations, diminished at the highest dose	
Mammalian cell gene mutation test (*Hprt* locus)/ chinese hamster (V79) cells	cobalt dichloride (nature salt unknown). Purity >99%	26 µg/ml/ (26 µg/ml)	**positive**	No guideline. Only 1 concentration tested	(Miyaki et al., 1979)
Mammalian cell gene mutation test (*gpt*$^+$ transgenic) / chinese hamster (G12), (G10) and parental (V79) cells	cobalt chloride cobalt sulphide	50 mM (=6.5 mg/ml)/ (100 mM) 0.25 µg/cm^2/ (1.0 mg/cm^2)	**positive** Significant increase of 6TG resistant colonies at the toxic doses of 50-100 mM in G12 cells (surviving fractions at these doses <40% control) negative in G10 and parental V79 cells **positive** non dose-related, significant increase in mutation frequency in G12 cells (0.25 µg/cm^2, surviving fraction ≈ 80% control) negative in G10 and parental V79 cells	24 h treatments	(Kitahara et al., 1996)
Mammalian cell gene	cobalt chloride	<9x10^{-5} M	negative ($CoCl_2$ alone)	Effect of $CoCl_2$ on frequency of	(Yokoiyama et al.,

Assay/ test system	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
mutation test/ chinese hamster (V79) cells-8AG locus negative	hexahydrate			8AG-resistant mutations alone or in combination with UV and g-rays	1990)
Mammalian cell gene mutation test (*Hprt* locus)/ mouse lymphoma L5178Y cells	cobalt dihydroxide lithium cobalt dioxide cobalt oxalate cobalt oxide hydroxide tricobalt tetraoxide	-S9: (26 mg/ml) +S9: (35 mg/ml) -S9: (60[P] mg/m) +S9: (50/600[P] mg/ml) -S9: (55 mg/ml) +S9: (70[P] mg/ml) -S9: (15[P] mg/ml) +S9: (15[P] mg/ml) -S9: (2408[P] mg/ml) +S9: (750 mg/ml)	-/+ S9: negative	OECD TG476, 3 h incubations	(Kirkland et al., 2015)
Mammalian cell gene mutation test (*Hprt* locus)/ mouse lymphoma (L5178Y) cells	cobalt sulphate	-S9: 3 hr: (60 mg/ml) 24 hr: (35 mg/ml) +S9: 3 hr: (100 mg/ml)	-/+ S9 3 hr or – S9 24 h: negative	OECD TG476, 3 and 24 h incubations	(Kirkland et al., 2015)
Mammalian cell gene mutation test (*Hprt* locus)/ mouse lymphoma (L5178Y) cells	cobalt sulphide	-S9: 3 hr: (922 mg/ml) 24 hr: (800 mg/ml) +S9: 3 hr: (922 mg/ml)	-/+ S9 3 hr or – S9 24 h: negative	OECD TG476, 3 and 24 h incubations	(Kirkland et al., 2015)
Mammalian	cobalt	-S9:	-/+ S9 3 hr or	OECD TG476, 3	(Kirkland et

Assay/ test system	Test substance	Lowest effective or highest ineffective dose/(top concentration tested)	Findings	Remarks	Reference
cell gene mutation test (*Hprt* locus)/ mouse lymphoma (L5178Y) cells	monoxide	3 hr: (80[P] mg/ml) 24 hr: (60 mg/ml) +S9: 3 hr: (60[P] mg/ml)	– S9 24 h: negative	and 24 h incubations	al., 2015)

6TG: 6-thioguanine; 8AG: 8-azaguanine, [P] = precipitate persisted until end of treatment

In other non-guideline gene mutation tests, cobalt chloride did not induce any 8AG resistant mutations in V79 cells in single treatments and exhibited measurable mutagenic activity only in one (G12) of two *gpt*$^{+}$ transgenic V79 cell lines (Yokoiyama et al., 1990, Kitahara et al., 1996). Similar results in the same testing system were obtained for cobalt sulfide (Kitahara et al., 1996).

Collectively, positive results in rodent cells occurred mainly for the *hprt* locus, however OECD guidelines compliant *HPRT* assays failed to yield any positive effects, concluding therefore that cobalt metal and compounds do not elicit any mutagenic activity in mammalian cells.

In vitro genotoxicity in mammalian cells

The *in vitro* genotoxic activity of cobalt metal and cobalt compounds has been extensively reviewed by (Beyersmann and Hartwig, 1992) (Lison et al., 2001), (Kirkland et al., 2015) and more recently by (Lison et al., 2018). **Table 40** provides an overview of the relevant assays along with the main findings, covering cobalt metal, cobalt compounds, and cobalt containing alloys and micro/nano-sized particles. The genotoxic activity is mediated either by the intra/extra cellular solubilisation of Co(II) ions from e.g., inorganic salts and oxides, which can in turn inhibit enzymes and DNA repair processes and produce ROS through a Fenton-like reaction in the presence of hydrogen peroxide, or by the surface corrosion of metallic materials and ensuing release of Co(II) ions which coupled with the reduction of oxygen, can also produce ROS (Lison et al., 2018). These mechanisms render therefore the bioavailability of the solubilised Co(II) content a key determinant of the genotoxic activity. An intracellular solubilised Co(II) ions-independent mechanism of genotoxicity has also been suggested for the documented activity of poorly soluble mixed Co (II,III) oxide particles at non cytotoxic concentrations in human bronchial epithelial cells (Uboldi et al., 2016).

In early studies in mammalian cells in culture, cobalt(II) was shown to induce DNA strand breaks, DNA-protein crosslinks, sister-chromatid exchanges as well as micronuclei (as reviewed in (Beyersmann and Hartwig, 1992).

Cobalt metal, cobalt containing alloys and cobalt salts can affect DNA integrity by readily producing DNA damage, as evidenced by the induction of alkali labile sites and single and double DNA strand breaks, often in a time and dose-dependent fashion, as consistently detected by the alkaline comet assay (including the modified version which employs lesion-specific endonucleases), alkaline elution, nuclear sedimentation, and phosphorylation of H2Ax in different hamster, rodent and human cells (**Table 40**). A more recent study however employing a mouse embryonic stem cells, GFP-based reporter assay (ToxTracker), showed no activation of the interrogated DNA damage biomarkers, by any

of the nine cobalt substances tested (including cobalt metal, cobalt dichloride, cobalt sulphide etc), under the experimental conditions, while five substances induced oxidative stress reporters (Derr et al., 2022a). Similarly, the preferential induction of oxidative stress reporters by the pro-oxidant DEM, with no concurrent activation of the DNA damage reporters, in an earlier report of the Toxtracker assay, however, suggests that oxidative stress is a poor inducer of the DNA damage response (Hendriks et al., 2016).

The *in vitro* genotoxic activity stemming from the DNA damaging potential of cobalt metal and compounds including cobalt/cobalt oxide nanoparticles, has been confirmed in cytogenetic assays. Micronucleus tests (cytokinesis-blocked) and sister chromatid exchange studies were mostly positive in human and rodent cells (**Table 40**). (Van Goethem et al., 1997) reported significant, dose-dependent induction of micronuclei in human lymphocytes treated for 15 minutes with cobalt metal powder. These results were later corroborated in studies employing the same range of cobalt concentrations by (De Boeck et al., 2003c) and (Miller et al., 2001). Positive results were also reported for cobalt chloride, cobalt sulphate and cobalt alloy particles (Colognato et al., 2008, Ponti et al., 2009, Moche et al., 2015, Tsaousi et al., 2010). Chromosomal aberrations were less evident, especially in earlier studies with negative results in human lymphocytes with cobalt sulphate, nitrate and oxide. However cobalt alloy particles in the micro and nano size range, and cobalt chloride and cobalt oxide particles did produce aberrations in human and hamster fibroblasts (**Table 40**).

The above *in vitro* genotoxic effects manifesting mainly as DNA strand breaks and chromosomal aberrations, are consistent with a reactive oxygen species-mediated DNA damaging mechanism, as discussed above and proposed by a number of studies (De Boeck et al., 2003c, Kirkland et al., 2015, Paget et al., 2015, Lison et al., 2001). Oxidative DNA damage was confirmed by employing the hOGG1/Fpg modified alkaline comet assay which detects DNA lesions which are substrates for the two repair enzymes and manifests as aggravated DNA strand breaks in the detected comets. This effect was shown for cobalt chloride, cobalt sulphate, cobalt oxide and tungsten carbide-cobalt nanoparticles, while the use of free oxygen radical scavenger inhibitors in certain cases attenuated the ROS-mediated DNA damage ((Cavallo et al., 2015, Moche et al., 2015, Uboldi et al., 2016, Kirkland et al., 2015, Wan et al., 2012, Lugun et al., 2022, Patel et al., 2012). Dose-dependent increases in the fluorescence output (e.g., DCF or DHE fluorescence) in relevant assay (e.g., the 6-carboxy-2,70-dichlorodihydro-fluorescein diacetate (DCFH-DA) assay), indicative of intracellular ROS production, have also been directly measured in cells treated with cobalt sulphate, cobalt chloride and cobalt nanoparticles ((Kirkland et al., 2015, Lugun et al., 2022, Patel et al., 2012, Annangi et al., 2015). Consistent with these observations, exposure of mouse embryonic stem reporter cell lines to cobalt monoxide, cobalt metal powder, cobalt carbonate, cobalt dihydroxide and cobalt dichloride activated the oxidative damage reporters in the ToxTracker assay (Derr et al., 2022a). The magnitude of the oxidative stress response correlated with the concentration of soluble cobalt ion, with the reactive substances also inducing the expression of HIF1a target genes.

Cobalt and cobalt compounds can also exert indirect effects on DNA integrity through the inhibition of proteins involved in key DNA repair processes. Ionic cobalt(II) (75 mM $CoCl_2$) for example, has been shown to be comutagenic with UV in V79 cells by inhibiting the excision of UV-induced pyrimidine dimers after UV irradiation, by interfering either with the DNA polymerases' activity or the ligation step of the excision repair (as shown in HeLa cells) (Hartwig et al., 1991). Similarly, low, non-cytotoxic concentrations of $CoCl_2$ inhibited the incision and polymerisation step of the nucleotide excision repair of UV-induced DNA damage in human fibroblasts, by competing with essential magnesium ions (Kasten et al., 1997). $CoCl_2$ did not affect the base excision repair (BER) bacterial formamidopyrimidine-DNA glycosylase (Fpg), but it reduced the DNA binding of another zinc finger protein, the mammalian XPA protein (involved in nucleotide excision repair) while also modulating – albeit at high concentrations - the binding of p53 to DNA ((Asmuss et al., 2000, Palecek

et al., 1999, Hartwig et al., 2002). Interference of cobalt particles with the repair of mutagen induced DNA damage has been demonstrated in combination studies, where cobalt metal inhibited the repair of methyl methanesulphonate (MMS)-induced DNA lesions in human lymphocytes treated ex vivo, by interfering either at the incision or the polymerisation step (De Boeck et al., 1998). The H_2O_2-induced activation of the poly(ADP-ribose)polymerase (PARP), containing two zinc fingers and being involved in DNA strand break detection and apoptosis was greatly reduced by Co(II) (Hartwig et al., 2002). Cobalt (II) also enhances the error frequencies of DNA polymerases from different organisms *in vitro* (Sirover and Loeb, 1976a; Zakour et al., 1981).

Another mechanism contributing to the genotoxic potential of cobalt, including its clastogenic activity is cobalt's effect on Topoisomerase IIa. Cobalt(II) ($CoCl_2$) was shown to enhance enzyme-mediated DNA cleavage, stimulating scission and inhibiting the rate of religation, acting like a Topoisomerase II poison in cultured human MCF-7 cells (Baldwin et al., 2004).

The overall positive results across a range of different *in vitro* testing systems, indicate a genotoxic potential for cobalt metal and cobalt compounds.

Table 40: Summary of *in vitro* genotoxicity studies in mammalian cells

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
DNA strand breaks (molecular weight DNA)/ chinese Hamster Ovary (CHO) cells	CoS	10 mg/ml	**positive**	Treatment for 24 h	(Robison et al., 1982)
Alkaline sucrose sedimentation/ human diploid fibroblasts (HSBP)	$CoCl_2$	3h: 10 mM/(10 mM) 16h: 2 mM/(10 mM)	**positive** Concentration-dependent induction of DNA strand breaks after 3h and 16h treatments		(Hamilton-Koch et al., 1986)
Alkaline sucrose sedimentation/ chinese hamster ovary (CHO) cells	$CoCl_2$	3h: (10 mM) 16h: 2 mM/(10 mM)	negative No DNA strand breaks at this concentration and exposure time **positive** Concentration-dependent induction of DNA strand breaks	CHO cells more sensitive than human fibroblasts	(Hamilton-Koch et al., 1986)
Nucleoid sedimentation/ chinese hamster ovary (CHO) cells and human diploid fibroblasts (HSBP)	$CoCl_2$	(10 mM)	negative	1 h treatment	(Hamilton-Koch et al., 1986)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
Nucleoid sedimentation/ HeLa cells	$CoCl_2$	(100 mM)	negative in single Co single treatments. Increased number of DNA single strand breaks when in combination with UV, presumably by inhibiting the polymerisation/ligation step of the excision repair	Single and combination treatments with UV	(Hartwig et al., 1991)
Alkaline elution/ human lymphocytes	Cobalt WC-Co $CoCl_2$	3 mg/ml/(15 mg/ml) 25 mg/ml; 1.5 mg Co/ml/(250 mg/ml; 15 mg Co/ml) (102 mM)	**positive** Dose-dependent increase in DNA single strand breaks by Co (and rWC-Co particles) negative no increase compared to controls, alone or in combination with WC	15 mins exposure, <15% reduction of viability	(Anard et al., 1997)
Alkaline elution/ human osteosarcoma (TE85) cells	Cobalt metal (extra fine powder, 99.5% purity) Heavy metal pure mixture of tungsten metal (W) (92%), Ni (5%) and Co (3%), without extensive milling (rWNiCo)	6 mg/ml/(6 mg/ml) 25 mg/ml/(200 mg/ml)	**weakly positive** Marginal induction of DNA strand breaks **positive** Marked increase of DNA strand breaks by rWNiCo, reaching 850-900% untreated controls' levels at the highest concentration; synergistic effect of composite metals in the mixture, rWNiCo>Co	Assay based on the method reported by (Kohn et al., 1976), 1 h exposures	(Miller et al., 2001)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
Alkaline comet assay/ human leukocytes	Cobalt metal powder (99.87% purity) Cobalt-tungsten carbide (WC-Co) (6% Co + 94% WC particles)	0.6 mg/ml/(12 mg/ml) 10 mg/ml; 0.6 mg/ml Co-equivalent)/(100 mg; 6.0 mg/ml Co-equivalent)	**positive** Dose-dependent induction of DNA strand breaks by Co and WC-Co with WC-Co > Co; mixture produced a higher level of DNA damage than Co alone (synergistic effect), WC alone was inert	Assay performed as per (Singh et al., 1988), 15 min treatments, 1 donor	(Van Goethem et al., 1997)
Alkaline comet assay/ human lymphocytes)	Cobalt metal WC-Co	0.6 mg/ml/(6 mg/ml) 50-75 mg/ml; 3-4.5 mg Co/ml)/(100 mg/ml; 6 mg Co/ml)	**positive** Dose and time-dependent increase in DNA strand breaks, WC-Co>Co	Assay performed as per (Singh et al., 1988) with modifications, 15 mins treatments, 1 donor	(Anard et al., 1997)
Alkaline comet assay (-/+ Fpg)/ human lymphocytes	Cobalt metal (98.87% purity), WC-Co (Co 6%, WC 94%), Cobalt chloride hexahydrate	0.3 mg/ml Co-equivalent/(6 mg/ml Co-equivalent) (values for 15 min treatments)	**positive** Dose-dependent increase in DNA damage (DNA strand breaks, alkali labile sites), peak of response at 6 h treatment, damage not substrate for 8-oxoguanine DNA glycosylase (Fpg), enhanced DNA damage after post-incubation or co-treatment of MMS-treated cells with Co particles suggesting inhibition of excision repair, Co and $CoCl_2$ produced comparable responses	Assay as per (Singh et al., 1988) Exposure time ranging from 15 mins – 72 hours, 3 donors, inter-donor/inter-experimental variation	(De Boeck et al., 1998)
Alkaline Comet assay/ human PMBC	Cobalt and Co with powder mixtures of Cr_3C_2, Mo_2C and NbC, and	(6 mg/ml Co-equivalent)	negative Co alone did not increase Tail DNA or Tail length (15 mins treatment) Carbides alone were also inert **positive** interaction of NbC, Cr_3C_2 (and WC) with Co particles	Assay performed as per (Singh et al., 1988), with PBMCs from 2 donors inter-experimental and inter-donor differences	(De Boeck et al., 2003b)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	WC (Co 6% and carbides 94%)		Mixtures produced a higher mutagenic effect than the individual metal particles, in the order of: WC–Co> Cr_3C_2–Co > NbC–Co. Carbides alone inert (except WC). negative No interaction of Co with Mo_2C particles	Treatments for 15 min, 24 h after the onset of PHA stimulation	
Alkaline comet assay/ human fibroblasts	Synovial fluid from CoCr alloy prostheses-bearing patients; solution of corroded P21 CoCr alloy, $CoCl_2$ derived artificial solution of Co (II) mimicking corrosion of CoCr alloy	metal-on-metal implants: 0.92 µM – 2.64 µM Co metal-on-polyethylene implants: 0.01 µM – 0.62 µM Co corroded P21 Co-Cr alloy/artificial solution: 0.84 mM Co	**positive** Significant increases in DNA strand breaks induced by CoCr metal-on-metal implants (in 6/6 samples), CoCr metal-on-polyethylene implants (in 4/6 samples), Co (II) containing artificial fluid and corroded CoCr alloy pellets.	48 h treatments probing level of DNA damage induced by culturing human fibroblasts in synovial fluid retrieved at revision arthroplasty (controls samples were synovial fluid from primary arthroplasty)	(Davies et al., 2005)
Alkaline comet assay/ human fibroblasts	Cobalt chrome alloy (CoCr) particles (62.243% Co, 28.7% Cr, 6.3% Mo, 0.87% Si,	24 h: 5 mm^3/cell/(5000 mm^3/cell)	**positive** 24 h: Dose-dependent increase of DNA strand breaks 5 days: DNA breaks were reduced at the higher doses and increased at the lower doses. Greater DNA damage was observed by particles (5000:1 m^3/cell) without incubation in medium	Assay performed as per (Singh et al., 1988), 1 to 5 days exposure	(Papageorgiou et al., 2007b)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	0.71% Ni, 0.59% Mn, 0.53% Fe, 0.057% C); 2.9 mm		as opposed to particles exposed to medium for 24 h, with and without the same culture medium) Metal particles and cobalt released from free corrosion of pellets of orthopaedic CoCr alloy after 1 month, were also positive in inducing DNA damage. Only in the latter case, DNA damage was abolished by the addition of NAC		
Alkaline comet assay/ human fibroblasts	Cobalt chrome alloy (CoCr) particles (62.243% Co, 28.7% Cr, 6.3% Mo, 0.87% Si, 0.71% Ni, 0.59% Mn, 0.53% Fe, 0.057% C); 2.9 mm	5000:1 and 10000:1 mm^3/cell	**positive** DNA damage increased in both young (PD10) and older (PD35) cells, after exposure to cobalt chrome particles for 6 and 24 hours; no significant difference between the two species and treatments	*In vitro* model of cellular ageing comprising human fibroblasts of different population doublings (PD10 and PD35)	(Papageorgiou et al., 2007b)
Alkaline comet assay/ human dermal fibroblasts	CoCr alloy nano and micron-sized (62.2% Co, 28.7% Cr, 6.3% Mo, 0.87% Si, 0.71% Ni, 0.59% Mn,	0.0005 mm^3/cell/ (5000 mm^3/cell)	**positive** 24 h: both types of particles caused DNA damage in a dose-dependent manner, nano- more potent than micron-sized particles at higher doses 3 days: mean level of DNA damage decreased, micron-more potent than nano-sized particles 5 days: not enough nanoparticles available for testing	Assay performed as per (Singh et al., 1988), 24h, 3 and 5 day treatments	(Papageorgiou et al., 2007a)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	0.53% Fe, C 0.057%)				
Neutral comet assay/ Jurkat T-lymphocytes	$CoCl_2$	5 mM/(5 mM)	**positive** Significant DNA damage only at the highest concentration tested	48 h treatments	(Caicedo et al., 2008)
Alkaline comet assay/ Balb/3T3 mouse fibroblasts	$CoCl_2$ Cobalt nanoparticles (Co-NPs, <500 nm)	1 mM/(5 mM) 1 mM/(5 mM)	**positive** Comparable, significant induction of DNA damage for both Co-NPs (non dose-dependent) and Co^{2+} (dose-dependent), at subtoxic concentrations.	Assay performed as per (Singh et al., 1988) with some modifications, 2h treatments	(Ponti et al., 2009)
Alkaline comet assay/ human HaCaT keratinocyte cells	Cobalt chloride hexahydrate	40 mM/(1000 mM)	**positive** Significant increase in DNA strand breaks at all concentrations tested (for $CoCl_2$ single treatments), effects more pronounced at 24 h compared to 6 h	Assay performed as per (Singh et al., 1988) with modifications, sub-toxic concentrations used, 6 and 24 h treatments, cobalt used alone or in combination with irradiation	(Gault et al., 2010)
Alkaline comet assay/ human T-cells	$CoCl_2$ Cobalt nanoparticles (Co-NPs)	(30 mM) 3 mM/(6 mM)	negative **positive** Significant, concentration-dependent increase of DNA damage at 3 and 6 µM	Assay performed as per (Singh et al., 1988), 4 h treatments	(Jiang et al., 2012)
Alkaline comet assay/ human peripheral blood leukocytes	$CoCl_2$ Metal Co-NPs	(10^{-5}, $5x10^{-5}$ and 10^{-4} M) $5x10^{-5}$ M/(10^{-4} M)	negative No statistically significant increase in DNA strand breaks over control values **positive**	Assay performed as per (Collins et al., 1996), 3 donors, 2h incubation	(Colognato et al., 2008)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	(100–500 nm)		Statistically significant, dose-related increase in DNA strand breaks		
Alkaline comet assay/ human fibroblasts	$CoCl_2$ CoCr alloy (nano and micrometre sized particles; 29.5 nm and 2.9 mm, respectively)	0.4 mM 0.036 mg/cm^2/(0.36 mg/cm^2)	**positive** Significant increases of DNA damage after direct, indirect or 'insert' exposure; applying blockers of gap junctions/hemichannels above or below the BeWo barrier (indirect exposure to CoCr-NP) protected from DNA damage, while a peptide inducing connexin expression and ATP release enhanced DNA damage	Assay performed as per (Papageorgiou et al., 2007a), confluent layer of BeWo cells used as a cellular barrier (indirect exposure) and compared to direct (onto fibroblasts) and transwell 'insert' (without the BeWo barrier) treatments for 24 h. For single $CoCl_2$ treatments, 'insert' and indirect treatments were applied	(Bhabra et al., 2009)
Alkaline comet assay/ human lung carcinoma (A549) cells	Nanosized Cobalt (nano-Co), 20 nm	5 mg/ml/ (15 mg/ml)	**positive** dose-dependent and time-dependent, significant increase in DNA damage, the effect was ROS-mediated as it was significantly attenuated after pretreatment with the ROS inhibitors NAC or catalase	12-48 h exposures	(Wan et al., 2012)
Alkaline comet assay/ human hepato-carcinoma (HepG2) cells)	Co^{2+} Cobalt oxide nanoparticles (Co_3O_4 NPs)	10 mg/ml/(15 mg/ml) 5 mg/ml/(15 mg/ml)	**positive** Statistically significant dose and time-dependent increase in DNA damage Co_2^+< Co_3O_4 NPs	Method as per (Singh et al., 1988) with modifications, 24 and 48h treatments, 3 independent experiments	(Alarifi et al., 2013)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
Alkaline comet assay +/- Fpg/ Wild-type mouse embryonic fibroblasts (MEF *Ogg1*$^{+/+}$) and (MEF Ogg1$^{-/-}$)	CoNPs (< 50nm)	(1 mg/ml) 0.1 mg/ml/(1 mg/ml)	negative No significant increase in genotoxic (- Fpg) DNA damage in either MEF *Ogg1*$^{+/+}$ or MEF Ogg1$^{-/-}$ cells; no increase in oxidative (+Fpg) DNA damage in MEF Ogg1$^{+/+}$ cells **positive** significant dose-dependent increase in oxidative DNA damage (+Fpg) in MEF Ogg1$^{-/-}$ cells	Assay performed as per (Bach et al., 2014), 24 h exposures at sub-toxic concentrations	(Annangi et al., 2015)
Alkaline comet assay, hOGG1 modified/ human lung carcinoma (A549) cells	Cobalt sulphate heptahydrate $CoSO_4$	800 mg/ml (one concentration tested)	**positive** Significant increase in DNA strand breaks induced by both unfiltered and filtered fraction at comparable levels, further enhanced by the presence of hOGG1	Assay performed as per (Singh et al., 1988) and (Smith et al., 2006), 4 h treatments with unfiltered or soluble fractions of the extracts in AAF, 3 independent experiments, unfiltered fraction more cytotoxic than filtered	(Kirkland et al., 2015)
Alkaline comet assay (+/- Fpg modification)/ human lung carcinoma (A549) cells	Co_3O_4 NPs (22 nm)	(-Fpg) 2 h, 24h: 1 mg/ml/(40 mg/ml) (+ Fpg) 2 h, 24h: 1 mg/ml/(40 mg/ml)	**positive** Dose-dependent increase in direct DNA damage at both 2 h (significant at 40 mg/ml) and 24 h (significant at 20 mg/ml) **positive** Dose-dependent increase in oxidative DNA damage at both 2 h and 24 h (significant at 20 mg/ml, in both instances)	Assay performed as per (Collins et al., 1993), +/- Fpg, 2 and 24 h treatments	(Cavallo et al., 2015)
Alkaline comet assay (+/- Fpg	Co_3O_4 NPs (22 nm)	(-Fpg) 2 h, 24h:	**positive** Dose-dependent increase in direct DNA damage	Assay performed as per (Collins et al., 1993),	(Cavallo et al., 2015)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
modification)/ human bronchial epithelial (BEAS-2B) cells		1 mg/ml/(40 mg/ml) (+ Fpg) 2 h, 24h: 1 mg/ml/(40 mg/ml)	at both 2 h (significant at 40 mg/ml, in both instances) **positive** Non dose-dependent increase in oxidative DNA damage at both 2 h and 24 h (significant at 5 mg/ml, in both instances)	+/- Fpg, 2 and 24 h treatments	
Alkaline comet assay modified with Fpg/ mouse lymphoma (L5178Y $tk^{+/-}$) cells	WC–Co NP (8% w/w Co); 60–250 nm	(100 mg/ml) 80-100 mg/ml	negative -Fpg **positive** +Fpg, slight increase in Fpg-substrate damage sites, at the highest doses	Assay performed as per (Singh et al., 1988), 4 h treatments, 45 ± 5% cytotoxicity at highest concentration	(Moche et al., 2015)
Alkaline comet assay modified with Fpg/ human lymphocytes	WC–Co NP (8% w/w Co); 60–250 nm	60 mg/ml/(120 mg/ml) (120 mg/ml)	**positive** -Fpg, significant (at most doses) increase of direct DNA damage negative +Fpg, no increase in Fpg-sensitive sites	As above, marked variability between the two donors	(Moche et al., 2015)
Alkaline comet assay/ human lung epithelial (BEAS-2B) cells	$CoCl_2$ poorly soluble, submicronic cobalt oxide Co_3O_4	2 h: 1.25 mg/ml (significant at 2.5 mg/ml)/(10 mg/ml) 2 h: 1.25 µg/ml; 24 h: 1.25 µg/ml/(20 mg/ml)	**positive** 2 h: Non dose-related, significant increase in DNA strand breaks at ≥2.5 mg/ml negative 24 h: only marginal increase at 24 h exposure **positive** 2 h: dose-related increase in DNA strand breaks, significant at 10 and 20 mg/ml 24 h: non dose-dependent increase in DNA strand breaks, significant at ≥2.5 mg/ml	2 and 24 h treatments	(Uboldi et al., 2016)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	particles		Co_2O_4P > $CoCl_2$ at 24 h		
Alkaline comet assay modified with Fpg/hOGG1/ human lung epithelial (BEAS-2B) cells	$CoCl_2$ poorly soluble, submicronic cobalt oxide (Co_3O_4) particles	2 h: Fpg: 1.25 mg/ml/(10 mg/ml) hOGG1: 1.25 mg/ml/(10 mg/ml) 24h Fpg: (10 mg/ml) hOGG1: 1.25 mg/ml/(10 mg/ml) 2 h: Fpg: 2.5 mg/ml/(20 mg/ml) hOGG1: 1.25 mg/ml/(20 mg/ml) 24 h: Fpg: 2.5 mg/ml/(20 mg/ml) hOGG1: 1.25 mg/ml/(20 mg/ml)	**positive** 2 h: Modification with both Fpg and hOGG1 enzymes detected significant oxidative DNA damage, at all concentrations tested negative 24 h: no increase in DNA strand breaks with Fpg modification **positive** 24 h: increased hOGG1-detected oxidative DNA damage, significant at ≥ 5 mg/ml **positive** at both 2 h and 24 h, Fpg and hOGG1 detected significant oxidative DNA damage at most concentrations	Modified alkaline comet assay (+Fpg or hOGG1), 2 h and 24 h exposures	(Uboldi et al., 2016)
Alkaline comet assay/ chinese hamster lung	cobalt oxide nanoparticles (Co_3O_4 NPs),	20 mg/ml/(60 mg/ml)	**positive** Significant, concentration-dependent increase in DNA strand breaks, ROS-mediated potent and	Assay performed as in (Singh et al., 1988), treatment for 24 h	(Lugun et al., 2022)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
fibroblast (V79) cells	<50 nm		dose-dependent induction of DNA lesions by non-cytotoxic concentrations of Co_3O_4 NPs		
SCE/ $P388D_1$ macrophage cell line, human lymphocytes	$CoCl_2$	$P388D_1$:10 mM (=13 mg/ml)/(100 mM) human lymphocytes: 1 mM (=1.3 mg/ml)/(10 mM)	**positive** Elevated SCE in both $P388D_1$ and human lymphocytes, significantly at the highest doses		(Andersen, 1983)
SCE/ chinese hamster (V79) cells	Cobalt chloride hexahydrate	10 mg/ml/(100 mg/ml)	**positive** Significant increase in SCEs/cell induction, compared to controls, in single $CoCl_2$ treatments; synergistic enhancement of UV-induced SCEs	Single and combined treatments with UV	(Hartwig et al., 1991)
Chromosome condensation (DAPI staining)/ human hepato-carcinoma (HepG2) cells	Co_3O_4 NPs	5-10-15 mg/ml	**positive**	Method as per (Dhar-Mascareno et al., 2005), 24 and 48 h treatments	(Alarifi et al., 2013)
Chromosome aberration/ diploid human fibroblasts (WI.38 and MRC5), human leucocytes	Cobalt nitrate	Not specified	negative	Leukocytes cultures: 24, 48 and 72h treatments Fibroblasts: treated with sub-toxic concentrations for 24 h or cultured for several weeks in metal-treated serum	(Paton and Allison, 1972)
Chromosome aberration	Cobalt oxide	(0.6 mg/ml)	negative		(Voroshilin et al., 1978)
Chromosome	Cobalt	(4.5 mg/m)	negative	Experimental	(Olivero et

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
aberration/ human lymphocytes	sulphate			deficiencies (inappropriate dose stepping, no positive control, no duplicate cultures, short cytoB exposure)	al., 1995)
Chromosome aberration M-FISH / primary human skin fibroblasts	Cobalt chrome alloy (CoCr) particles (62.243% Co, 28.7% Cr, 6.3% Mo, 0.87% Si, 0.71% Ni, 0.59% Mn, 0.53% Fe, 0.057% C); 2.9 mm	5000:1 mm^3/cell	**positive** In younger cell populations (PD10), marked increase of deletions and fragments after 1 day of exposure, effect greatly reduced after 2 days of exposure and unchanged after 15 days. 15 days: PD10=PD35 in overall levels of structural and numerical aberrations, but different distributions in the patterns of chromosome loss/gain	*In vitro* model of cellular ageing comprising human fibroblasts at different population doublings (PD10 and PD35), 1, 2 and 15 days exposure	(Papageorgiou et al., 2007b)
Chromosome aberration FISH / Primary BJ human fibroblasts	CoCr alloy (nano-particles); 29.5 nm	Low dose NP exposure 0.036 mg/cm^2/(0.36 mg/cm^2)	**positive** Increased tetraploidy, no increase of dicentric chromosomes or chromosome translocations, and no statistically significant increase of aneuploidy or chromosome breaks after 'indirect' exposure to CoCr nanoparticles; treatment with a gap junction blocker (connexin mimetic peptide) abolished the effect	Confluent layer of BeWo cells used as a cellular barrier between CoCr NPs NPs and the human fibroblasts ('indirect' exposure)	(Bhabra et al., 2009)
Chromosome aberration, FISH/ human	CoCr alloy particles (in the	Chromosome loss: 1 mg/T-75 flask/(5 mg/T-75 flask)	**positive** Substantial number of metaphases with both clastogenic (chromosome	Treatments for 24 h	(Tsaousi et al., 2010)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
fibroblasts	micrometre range in size)	Gain: 2 mg/T-75 Polyploidy: 1 mg/T-75/(5 mg/T-75) Sister-chromatic separation: 2 mg/T-75/(5 mg/T-75)	fragmentation) and aneugenic lesions (mainly chromosome loss and tetraploidy), chromosome gain and premature sister-chromatid separation also detected		
Chromosome aberrations (M-FISH)/ human primary fibroblasts	$CoCl_2$ hexahydrate (alone or in combination with Cr(VI) or Cr(III)	25 ppb (=0.105 mM) for simple aneuploidy; 50 ppb (=0.210 M) for complex aneuploidy/(50 ppb)	**positive** weak, dose-dependent, significant increase in incidence of (numerical) chromosome aberrations, Co alone least effective, most effective in combination with Cr(VI) causing prolonged aneuploidy negative Only marginal increase in chromosome fragments, only at the highest dose of 50 ppb	Treatments for 24 h	(Figgitt et al., 2010)
Chromosome aberrations (M-FISH)/ human BJ fibroblasts	CoCr particles prepared by both thermal plasma and pin-on-plate methods (20-80 nm)	0.005 mm^3/cell to 50 mm^3/cell	**positive** high incidence (40-50%) of metaphases with significant numerical chromosomal aberrations (simple/complex aneuploidy); no structural aberrations, presence of nucleoplasmic bridges	24 h exposure	(Raghunathan et al., 2013)
Chromosome aberration assay/ human lung fibroblast	Cobalt oxide	0.1 mg/cm^2/(5 mg/cm^2)	**positive** Concentration-dependent increase in % metaphases with chromosome damage and total aberrations/100 metaphases.	Assay performed as per (Wise et al., 2002), 24 h exposures, concentration-	(Smith et al., 2014)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
(WTHBF-6) cell line	$CoCl_2$ hexahydrate	50 mM/(250 mM)	**positive** $CoCl_2$ = Cobalt oxide in terms of genotoxicity, based on intracellular cobalt concentrations, cell cycle arrest effect: $CoCl_2$>Cobalt oxide	dependent increase in cytotoxicity, soluble $CoCl_2$ more cytotoxic than particulate cobalt oxide based on intracellular cobalt ion levels, chromatid lesions predominant aberrations	
Chromosome aberration/ human lymphocytes	WC–Co NP (8% w/w Co); 60–250 nm	30 mg/ml/(60 mg/ml)	**positive** Significant increase in the number of chromosome and chromatid breaks, increase in the number of polyploid cells, at the two highest concentrations (40 and 60 mg/ml).	Assay performed according to the OECD guideline TG473, no metabolic activation, 24 h treatment, relative mitotic index (53%) at the highest concentration	(Moche et al., 2015)
Chromosome aberration assay/ chinese hamster lung fibroblast (V79) cell line	Cobalt oxyhydroxide	(300^{P} mg/ml) 1500^{P} mg/ml/(1500^{P} mg/ml) 200^{P} mg/ml/(500^{P} mg/ml)	negative 4 h, -S9 **positive** 4h, + S9 **positive** 20 h, -S9	OECD recommended protocol; each trial in duplicates, 2 independent experiments, 4 h treatments (+/- S9) and 20 h treatments (– S9), higher concentrations associated with a mitotic index reduction of >50%, precipitation present at all concentrations tested, positive response	(Kirkland et al., 2015)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
				possible indirect consequence of physiological stress, V79 cells particularly susceptible to 'false positives'	
Chromosome aberration assay/ human lung epithelial cells	Cobalt oxide $CoCl_2$ hexahydrate	0.1 mg/cm^2/(1 mg/cm^2) 100 mM/(250 mM)	**positive** concentration dependent increase in % metaphase with chromosome damage and total aberrations in 100 metaphases **positive** $CoCl_2$ > Cobalt oxide as per intracellular Cobalt ion measurements	Assay performed as per (Wise et al., 2002), 24 h exposure, concentration-dependent increase in cytotoxicity, comparable cytotoxicity between $CoCl_2$ and cobalt oxide based on intracellular cobalt concentrations, chromatid lesions predominant aberrations	(Xie et al., 2016)
Chromosome aberration assay/ human urothelial (hTU1-38) cell line	Cobalt oxide (particle sizes ≤10 µm) $CoCl_2$	1 mg/cm^2/(10 mg/cm^2) 100 mM/(250 mM)	**positive** Concentration-dependent increase in damage in % metaphases and total aberrations/100 metaphases **positive** Equally potent o cobalt oxide with regard to genotoxicity, when normalised to intracellular cobalt concentrations, highest concentration induced cell cycle arrest	Assay performed as per (Wise et al., 2010), 24 h treatments, soluble $CoCl_2$ more cytotoxic than particulate cobalt oxide based on intracellular cobalt ions concentration, chromatid lesions (breaks and gaps) predominant aberrations	(Speer et al., 2017)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
Chromosome aberration assay/ chinese hamster lung fibroblast (V79) cell line	Cobalt oxide nanoparticles (Co_3O_4 NPs, <50 nm)	20 mg/ml/(30 mg/ml)	**positive** Induction of structural aberrations (fragments and chromatid breaks)	Treatments for 6 h	(Lugun et al., 2022)
Mammalian cell micronucleus test/ cultured bone marrow cells from BALB/c mice	Cobalt chloride hexahydrate	(50 mg/ml)	negative	30 min treatment, +/- S9	(Suzuki et al., 1993)
Mammalian cell micronucleus test/ human lymphocytes	Cobalt sulphate	(4.5 mg/ml)	negative	Experimental deficiencies (inappropriate dose stepping, no positive control, no duplicate cultures, short cyt-B exposure)	(Olivero et al., 1995)
Mammalian cell micronucleus test/ human lymphocytes	Cobalt metal powder (99.87% purity) Cobalt-tungsten carbide (WC-Co) (6% Co + 94% WC	Cobalt: 0.6 mg/ml/(6.0 mg/ml) WC-Co: 10 mg/ml (0.6 mg/ml Co-equivalent)/(100 mg; 6.0 mg/ml Co-equivalent)	**positive** Co alone and in alloy with WC, induced a dose-dependent, highly statistically significant increase of ‰ MNCB (WC-Co>Co); a decrease of the % CB was found at all concentrations studied	1 donor, 15 min treatments, 24 h after stimulation, cytokinesis-blocked (cyt-B) lymphocytes	(Van Goethem et al., 1997)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	particles)				
Mammalian cell micronucleus test/ syrian hamster embryo (SHE) cells	Cobalt sulphate hydrate	1 mg/ml/(4 mg/ml)	**positive** Significant increases in MNBC	24 h treatment	(Gibson et al., 1997)
Mammalian cell micronucleus test/ human osteo-sarcoma (TE85) cells	Cobalt metal (extra fine powder, 99.5% purity) Pure mixture of W (92%), Ni (5%) and Co (3%) (rWNiCo)	0.75 mg/ml/(6 mg/ml) 25 mg/ml/(200 mg/ml)	**positive** Significant, dose-dependent increase in % MNCB induced by both Co and reconstituted mixture rWNiCo	Assay performed as per (Perry and Wolff, 1974), 1 h exposure, cyt-B blocked TE85 cells	(Miller et al., 2001)
Mammalian cell micronucleus assay/ human PBMCs	Cobalt metal Cr_3C_2–Co, Mo_2C–Co, NbC–Co and WC–Co alloys	0.6 mg/ml/(6 mg/ml) 0.6 mg/ml/(6 mg/ml)	**positive** Statistically significant, dose-dependent increase in ‰ MNBN by Co alone **positive** Marked (not significant) increase in in ‰ MNBN by carbide-Co treatments over Co/controls; Cr_3C_2–Co>WC–Co>NbC–Co>Co>Mo_2C–Co	Assay performed as per (Van Goethem et al., 1997) and (Fenech, 2000), 2 donors, 15 mins of exposure, cyt-B blocked cells, inter-experimental and inter-donor variability	(De Boeck et al., 2003c)
Mammalian micronucleus assay, + pancentro-meric	CoCr alloy nano and micron-sized (62.2% Co,	5-50 mm^3/cell/ (500 mm^3/cell)	**positive** Both nano- and microparticles caused a dose-dependent increase of micronuclei (and nucleoplasmic bridges) with no significant	Assay performed as per (Fenech and Morley, 1985), 12 h exposure, cytoB treatment	(Papageorgiou et al., 2007a)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
probe/ human fibroblasts	28.7% Cr, 6.3% Mo, 0.87% Si, 0.71% Ni, 0.59% Mn, 0.53% Fe, C 0.057%)		difference between them; Nanoparticles increased nuclear blebs more than microparticles at the highest dose, significantly more centromere-positive micronuclei in cells exposed to nanoparticles than in cells exposed to microparticles		
Mammalian cell micronucleus test/ human peripheral blood leukocytes	$CoCl_2$ metal cobalt nanoparticles (CoNP 100–500 nm)	10^{-5} M (significant at $4x10^{-5}$ M/($8x10^{-5}$ M) 10^{-5} M (significant at $4x10^{-5}$ M/($8x10^{-5}$ M)	**positive** dose-dependent significant increase in the frequency of BNMN ‰ **positive** significant increase in the frequency of BNMN ‰	Assay performed as per (Migliore et al., 2002), 72 h treatment, cyt-B blocked, high variability among donors in the induction of micronuclei,	(Colognato et al., 2008)
Mammalian cell micronucleus test/ mouse Balb/3T3 fibroblasts	$CoCl_2$ Cobalt nanoparticles (<500 nm)	(10 mM) 1 mM/(10 mM)	negative **positive** statistically significant induction of chromosomal aberrations (‰ BNMN), at all the concentrations tested (decreasing with increasing concentration)	24 h treatments, cyt-B blocked, 3 doses tested, the highest corresponding to the IC_{50}	(Ponti et al., 2009)
Mammalian cell micronucleus assay/ primary human fibroblasts	CoCr alloy particles (mm range size)	0.5 mg/T-75 flask)/(5 mg/T-75 flask)	**positive** 24 h: dose-dependent increase in MNBNCs	Assay performed as per (Fenech and Morley, 1985, Fenech, 2000), cyt-B blocked, 24 h exposure, significant dose-dependent reductions in NDI after 24 h exposure to >0.5	(Tsaousi et al., 2010)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
				mg/T-75 CoCr particles	
Mammalian cell micronucleus test/ human hepato-carcinoma (HepG2) cells	$CoCl_2$ (large) tungsten-based nanoparticles (WC_L-Co) (10% (w/w) cobalt)	50 mM/(200 mM) 33 mg/ml	**positive** number of micronuclei was slightly increased, only reaching significance at the highest concentration tested (200 mM) negative no significant increase in the rate of micronuclei was observed in the concentration range tested	Assay performed according to ISO 21427-2, 3 h treatments	(Kuhnel et al., 2012)
Mammalian cell micronucleus test/ human BJ fibroblasts	CoCr particles prepared by both thermal plasma and pin-on-plate Methods (20-80 nm)	unspecified	**positive** Significant increase of micronuclei, multinucleated cells and cells with nucleoplasmic bridges at all NP exposures; greatest incidence after exposures to 80 nm Thermal plasma particles and 30 nm Pin-on-plate NPs; decreased incidence of micronuclei in 30 nm and 80 nm NPs treated cells when mitochondrial ROS was inhibited by MitoQ but not decylTPP	Treatments for 24 h, 48 h, 3 days, no cyt-B treatment, 5 days, +/- MitoQ and decylTPP bromide	(Raghunathan et al., 2013)
Mammalian cell micronucleus test - FISH + pancentro-meric probe/ mouse lymphoma (L5178Y) cells	WC–Co NP (8% w/w Co); 60–250 nm	4 h: 100 mg/ml/(120 mg/ml) 24 h: 40 mg/ml/(100 mg/ml)	**positive** 4 h: significant increase in the total number of MN cells and centromere negative MN at concentrations of 100 and 120 mg/mL 24 h: significant increases in total number of MN in all concentrations tested and in centromere negative and positive cells at ≥60 mg/ml	4 and 24 h treatments, RPD was decreased to ≈50% at the two highest concentrations	(Moche et al., 2015)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
			overall responses 24 h > 4 h		
Mammalian cell micronucleus test – FISH + pancentro-meric probe/ human lymphocytes	WC–Co NP (8% w/w Co); 60–250 nm	4 h: 60 mg/ml/(90 mg/ml) 24 h: 20 mg/ml/(80 mg/ml)	**positive** 4 h: Significant increase in MNBC and MNMC only at the lowest concentration (60 mg/ml) **positive** 24 h: significant increase in MNM cells and centromere positive MN at ≥ 20 mg/ml, significant increase in MNBN cells at 60 and 80 mg/ml overall responses 24 h > 4 h	As above + cyt-B block, RI was decreased to <80% at the two highest concentrations	(Moche et al., 2015)
Mammalian cell micronucleus test/ human bronchial epithelial (BEAS-2B) cells	$CoCl_2$ poorly soluble Co_3O_4 Particles	1.25 mg/ml/(100 mg/ml)	**positive** both Co_3O_4P and $CoCl_2$ exerted a highly statistically significant dose-dependent micronuclei formation in BN cells, $CoCl_2$ > Co_3O_4P	24 h exposure, cyt-B blocked	(Uboldi et al., 2016)
H2AX phospho-rylation, immuno-fluorescence/ human fibroblasts	$CoCl_2$ CoCr alloy (nano and micrometre sized particles; 29.5 nm and 2.9 mm, respectively)	0.4 mM 0.036 mg/cm^2/(0.36 mg/cm^2)	**positive** Significant (for the CoCr particles only) increases in mean number of cells with ≥ 4 g-H2AX foci, in all treatments; applying blockers of gap junctions/hemichannels above or below the BeWo barrier ('indirect' exposure to CoCr NPs) protected from DNA damage	Confluent layer of BeWo cells used as a cellular barrier ('indirect' exposure) and compared to direct (onto fibroblasts) and transwell 'insert' (without the BeWo barrier) treatments for 24 h. For $CoCl_2$ treatments only 'insert' and indirect treatments	(Bhabra et al., 2009)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
				applied	
H2AX phospho-rylation, immuno-fluorescence/ human fibroblasts	CoCr alloy particles (size in the micrometre range)	2-5 mg/T-75/(5 mg/T-75 flask)	**positive** Significantly decreased number of cells with very low levels of DSBs and significant increase of cells with low, medium and high levels of DSBs at 2 and 5 mg/T-75 flask, compared to controls	g-H2AX foci/cell, 24 h treatment	(Tsaousi et al., 2010)
H2AX phospho-rylation, immuno-blotting/ human lung epithelial (H460) cells	$CoCl_2$	200 mM/(300 mM)	**positive** increase in DNA DSBs as evidenced by a corresponding increase in g-H2Ax expression levels at the highest concentrations (200-300 mM)	g-H2Ax detected by immunoblotting; cells exposed for 24 h, pretreatment with NAC abolished the production of DNA DSBs	(Patel et al., 2012)
H2AX phospho-rylation, immune-fluorescence and immuno-blotting/ human lung epithelial (A549) cells	Nanosized Cobalt (nano-Co), 20 nm	5 mg/ml/(15 mg/ml)	**positive** Dose and time-dependent increase in g-H2AX foci formation and g-H2AX expression levels	1-24 h treatment	(Wan et al., 2012)
H2AX phospho-rylation, Immuno-fluorescence/ human liver (Hep3B) and kidney (Caki-1) cell lines	Wc-Co NPs (8% wt/wt Co); 60 nm	25 mg/ml/(75 mg/ml)	**positive** dose-dependent increase in g-H2AX foci/nucleus in both cell lines	Assay performed as per (Paget et al., 2014), 24 h exposures, g-H2AX foci/nucleus detected by confocal microscopy	(Paget et al., 2015)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
H2AX phospho-rylation, Immuno-fluorescence/human lung epithelial (BEAS-2B) cells	$CoCl_2$ poorly soluble Co_3O_4 Particles	2.5 mg/ml/(20 mg/ml) 2.5 mg/ml/(20 mg/ml)	**positive** dose-dependent, statistical increases in g-H2Ax foci Co_2O_4P < $CoCl_2$	DNA DSBs detected as g-H2Ax foci/cell by immunofluorescence, 24 h exposures, pretreatment with NAC induced a highly statistically significant decrease in the number of cells with g-H2Ax foci in cells exposed to both $CoCl_2$ and Co_3O_4 particles (at 10 and 20 mg/ml treatments)	(Uboldi et al., 2016)
DNA damage markers (QPCR)/ rat neuronal (PC12) cells (pheochromocytoma)	$CoCl_2$	100 mM (4-24h)/(200 mM)	**positive** mitochondrial DNA damage, time-dependent increase of MYH glycosylase levels, transient, concentration-dependent modulation of hypoxia/DNA damage response factors (HIF-1a, p53, p21, PCNA) negative Nuclear DNA damage: only marginal, at the highest concentration (200 mM) after 24 h treatment	QPCR assay for mitochondrial DNA damage performed as per (Yakes and Van Houten, 1997), protein levels detected by immunoblotting, 4-24 h treatments	(Wang et al., 2000)
Induction of biomarkers; (ToxTracker), Flow Cytometry (Mouse	Cobalt metal powder Cobalt carbonate Cobalt	20 mg/ml 26 mg/ml 23 mg/ml	negative DNA damage No GFP reporter induction of at least 2-fold compared to vehicle control treatment for any of the substances, -/+ S9	ToxTracker comprises a panel of GFP-based mouse embryonic stem (mES) reporter cell lines: DNA damage-	(Derr et al., 2022a)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
embryonic stem cells with stable integration of GFP-tagged biomarkers)	dichloride Cobalt dihydroxide Cobalt monoxide Cobalt oxyhydroxide Cobalt Sulphide Lithium cobalt dioxide Tricobalt tetraoxide	56 mg/ml 14 mg/ml 1.4 mg/ml 0.89 mg/ml 0.041 mg/ml 0.50 mg/ml	(>2-fold Btg2-GFP induction denoting p53 activation by cobalt dihydroxide and cobalt carbonate, -S9)	specific: Bscl2-GFP and Rtkn-GFP reporters, -/+ S9, a single stock solution, containing the maximum soluble cobalt concentration in culture medium was prepared and used, 24 h treatment with 5 concentrations of each tested compound, derived from dose range finding studies	
Induction of biomarkers; (ToxTracker), Flow Cytometry (Mouse embryonic stem cells with stable integration of GFP-tagged biomarkers)	Cobalt metal powder Cobalt monoxide Cobalt carbonate Cobalt dihydroxide Cobalt dichloride Tricobalt tetraoxide Cobalt Sulphide Lithium cobalt	10 mg/ml /(20 mg/ml) 7.0 mg/ml /(14 mg/ml) 13 mg/ml /(26 mg/ml) 14 mg/ml /(56 mg/ml) 11.5 mg/ml /(23 mg/ml) (0.50 mg/ml) (0.89 mg/ml) 0.041 mg/ml 1.4 mg/ml	**positive** Induction of both oxidative stress reporters (associated with cytotoxicity) by at least >2-fold compared to controls, Srxn1-GFP reporter> Blvrb-GFP reporter negative no activation of any of the oxidative stress ToxTracker reporters, no cytotoxicity	ToxTracker, Oxidative stress-specific mES reporter cell lines incorporating Srxn1-GFP and Blvrb-GFP	(Derr et al., 2022a)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	dioxide Cobalt oxyhydroxide				
Hypoxia qPCR/(Mouse embryonic stem cells)	Cobalt dichloride Cobalt carbonate Cobalt monoxide Cobalt dihydroxide Cobalt metal powder Cobalt Sulphide Lithium cobalt dioxide Tricobalt tetraoxide Cobalt oxyhydroxide	≈20 mg/ml (high dose,) <30 mg/ml (high dose) ≈15 mg/ml (high dose) < 30 mg/ml (high dose) < 15 mg/ml (high dose)	**positive** increased expression of the HIF1a target genes: Hmox1, Slc2A1, Bnip3 and Ddit4 (>2-fold for Cobalt dichloride) **positive** Only for Hmox1, Slc2A1 and Ddit4 negative	Parental mES cells were exposed to two concentrations (4-fold dilution) of the filtered medium of the test substances for 8 h in triplicate, -S9 Activation of HIF1a target genes increased with increasing cobalt concentrations	(Derr et al., 2022a)
ELISA-HIF-1a quantification/(A 549)	Co hydroxide oxide, LCO, CoS and Co_3O_4	1000 mM (concentration in µM Co equivalents) 250 mM/(1000 mM)	Negative No effect on intracellular HIF-1a content **positive**	ELISA performed with commercially available kit, 24 h treatments,	(van den Brule et al., 2022)

Assay/ test system	Test substance	Lowest effective* or highest ineffective dose/ (top concentration tested)	Findings	Remarks	Reference
	$CoCl_2$, $CoSO_4$, Co dinitrate, Co dihydroxide, Co diacetate, and Co metal 2 Co metal 1, CoO, and Co carbonate	(concentration in µM Co equivalents)	HIF-1a stabilisation at low doses dose-dependently stabilised HIF-1α up to the highest concentration tested		

* the lowest effective dose corresponds to the dose of a perceived increase in the amplitude of a response and does not always coincide with the dose which reaches statistical significance; WC: Tungsten-carbide; Fpg: formamidopyrimidine DNA glycosylase; PMBC: peripheral blood mononuclear cell; NAC: N-acetyl cysteine; hOGG1: 8-oxyguanine DNA N-glycosylase 1; AAF: artificial alveolar fluid; SCE: sister chromatid exchange; M-FISH: Multicolour in situ hybridization; MNBC: micronucleated binucleated cells; MNMC: micronucleated mononucleated cells cyt-B: cytochalasin B; NDI: nuclear division index; RPD: relative population doubling; RI: replication index; DSBs: Double strand breaks; MEF: mouse embryonic fibroblasts, ^{P} =precipitate observed

Appendix 7. Comparison of the animal data based ERR with the observational epidemiological evidence from Marsh et al. (2017) and Sauni et al. (2017)

ECHA (2020) previously compared the relative risk estimates of Marsh et al. (2017) and absolute excess risks provided by the dose-response derived from animal data (see section B.4.4.2 of the Background document of the RAC opinion).

ECHA noted that *the two highest exposure intensity categories in Marsh et al. (2017) represent exposures either slightly below or above 10 µg Co/m^3. For 10 µg Co/m^3 the dose-response derived from lung tumour incidence in animal data predicts an excess lifetime risk of lung cancer of 1 x 10^{-2} assuming an exposure duration of 40 years at work. The cumulative lung cancer incidence in the European population until the age of 75 years is 5.5 x 10^{-2} for men (i.e. 5.5%), 2.2 x 10^{-2} for women and 3.8 x 10^{-2} both sexes combined (IARC Globocan database), i.e. observing the excess lifetime risk of 1 x 10^{-2} above the background incidence in the general population would roughly correspond to a relative risk in the exposed population of 1.2 (= (1 + 5.5)/5.5) for men or 1.3 (= (1 + 3.8)/3.8) both sexes combined.* ECHA (2020) noted that while no clear correlation was observed between lung cancer mortality and exposures occurring in hard-metal production, it is to be noted that detecting or excluding with confidence such levels of relative risk in an epidemiological study is challenging. ECHA (2020) further noted that actually, the smoking-unadjusted relative risks observed for lung cancer (albeit without statistical significance) for these groups around average intensity of 10 µg Co/m^3 are not clearly deviating from the order of magnitude of risk predicted by the animal data.

It is noted that the study of Marsh et al. (2017) is large (see section 7.7.1) and that the overall lung cancer mortality was not statistically significantly increased and the upper limit of the 95% CI is about 1.2 (SMR 1.10 (95% CI 0.97 – 1.23). However, as indicated by ECHA (2020) the animal data based ERR predicts quite low relative risks even for the highest exposure categories reported by Marsh et al. (2017) when assuming life-time follow-up.

Nevertheless, it is also noted that when comparing ERR predictions and the human experience from the cohort studies it is challenging to fully account for three main differences. Firstly, the ERR presents the risk by air concentration of respirable particles, while the Marsh et al. (2017) risk estimates are presenting the relative risk by concentration of inhalable particles. Secondly the ERR assumes an exposure duration of 40 years while in the hard-metal worker cohort follow-up the average exposure time is shorter. Marsh et al. (2017) reported that after exclusion of those with less than 1 year of employment, roughly one third had an exposure duration of 1-4 years, one third 5-19 and one third at least 20 years, although such distributions are not reported for the subcategory with highest mean intensity of exposure. Thirdly, the ERR calculates the excess risk for the entire life-time while the observational evidence (RR in the exposed vs unexposed or highest exposure vs lowest exposure or the expected and observed cancer cases in SMR) relate only to the person-years in each age category experienced so far in the cohort. There is no straightforward way to divide the life-time excess risk predicted by the ERR to certain (age) periods of life-time. Thus, in order to compare the ERR with human data one has to estimate life-time risks also for human data. Thus, crude comparisons using life-time lung cancer (absolute) risk in the general population and life-time (absolute) excess risk predicted by the ERR seem the best way for estimation of relative risks that would have been observed at given exposure levels had the exposure in the exposed cohort stayed at a given level for 40 years and had the cohort been followed for life-time (e.g. until 75 years of age as a proxy of life-time) as assumed by the ERR.

For the Marsh et al. (2017) study these sensitivity calculations are done below using both male and male+female background rates as the Marsh et al. (2017) study included male and female workers in the ratio of about 70:30 both in terms of numbers and person-years but does not report the gender distribution for the highest exposure category used below. The population followed by Sauni et al. (2017) was entirely male. The most recent cumulative incidence of lung cancer until age 75 in Europe is 5.4% in men and 3.7% combining both genders (IARC Globocan database, accessed 13 June 2022, https://gco.iarc.fr/)

It is noted that RAC (ECHA 2020) assumed as a worst-case approach that 50% of inhalable particles concern respirable dust ECHA (2020). Table 41 and Table 42 present similar calculations as above, but assuming 50% of respirable particles and focusing on the highest exposure category reported by Marsh et al. (2017) where an excess risk should most likely have been observed, i.e. > 11 µg Co/m^3 (range 11 – 300 µg Co/m^3). Marsh et al. (2017) does not report the mean or median exposure in this category. Calculations are therefore made for exposure intensities (1) at lower limit of this category, (2) lower limit + 10% of range and (3) lower limit + 50% of range. It is noted that Marsh et al. (2017) report that in the entire pooled cohort, mean exposure calculated as cumulative exposure divided by duration of exposure was 13 µg Co/m^3. Thus, in the highest exposure category, the mean exposure must have been quite clearly above the lower limit of that exposure category (11 µg Co/m^3). Table 41 presents estimations based on EU male background cumulative lung cancer incidence and Table 42 those based on background cumulative incidence combining both genders. It is noted that in addition to European cohorts Marsh et al. (2017) included a US cohort, but European rates are assumed to be a reasonable comparison. For this exposure category Marsh reported a RR of 1.18 (95% 0.79 – 1.77) based on internal comparison to the lowest exposure category and a SMR of 1.15 (95% 0.92 – 1.43) based on external comparison to national rates. The estimations in Table 41 and Table 42 result in slightly lower risk when exposure intensity is assumed to have been at the lower limit of the exposure range covered by that category, which seems, however a clear underestimation of exposure in that category given the overall mean exposure of 13 µg Co/m^3 reported for the entire cohort. Assuming an exposure at lower limit + 10% of range results in the ERR overestimating the risk but still being within the 95% confidence interval of Marsh et al. (2017) RR an SMR. Assuming an exposure level at lower limit + 50% of range results in RR estimates that are higher than the upper 95% CI of lung cancer RR and SMR. As explained in section 7.7.1 the exposure category specific risks were not adjusted for smoking. However, the authors found indication of confounding by smoking which, when adjusted for, reduced the risk estimate in the analyses concerning the overall cobalt related RR. Finally, the exposure estimates were not corrected for any possible time trends in use of personal protective equipment. It is also noteworthy that about 40% of the cohort members were aged 55 or younger at the end of the follow-up and 76% were still alive. The mean follow-up time per worker was 24 years, thus the RR and SMR estimates are based already on quite a long observation period.

Overall the comparison of the empirical epidemiological with the animal data based ERR indicates that no extra interspecies assessment factor seems warranted to adjust the ERR for higher risk of humans.

Table 41: Relative risk estimated from ERR excess risk and EU background rate of lung cancer (male). Risks calculated for levels that correspond to the lower limit, lower limit + 10% of range and lower limit + 50% of range of the highest exposure category (11 – 300 µg/m³) of Marsh et al. (2017).

Exposure intensity (µg Co/m³) inhalable	Exposure intensity (µg Co/m3) respirable	Predicted life-time excess per 100	Background life-time risk per 100	Estimated RR
11	5.5	0.5341	5.4	1.10
40	20	2.676	5.4	1.38
156	78	8.2017	5.4	2.52

* Respirable fraction estimated as 50% of inhalable fraction. Estimated RR = (Excess predicted by ERR + background risk)/background risk.

Table 42: Relative risk estimated from ERR excess risk and EU background rate of lung cancer (male + female). Risks calculated for levels that correspond to the lower limit, lower limit + 10% of range and lower limit + 50% of range of the highest exposure category (11 – 300 µg/m³) of Marsh et al. (2017).

Exposure intensity (µg Co/m³) inhalable	Exposure intensity (µg Co/m3) respirable	Predicted life-time excess per 100	Background life-time risk per 100	Estimated RR
11	5.5	0.5341	3.8	1.14
40	20	2.676	3.8	1.56
156	78	8.2017	3.8	3.22

* Respirable fraction estimated as 50% of inhalable fraction. Estimated RR = (Excess predicted by ERR + background risk)/background risk.

Sauni et al. (2017) studied cancer incidence in a cobalt production plant, where exposure included several cobalt compounds, not only metallic. The average exposure levels in the departments varied between 20 and 80-100 µg/m^3 (inhalable). Assuming an overall average of 50 µg/m^3 and 50% of respirable particles would correspond to a mean exposure of 25 µg/m^3 of respirable cobalt and applying the ERR would indicate an excess risk of 2.5 x 10^{-2}. Observing the excess lifetime risk of 2.5 x 10^{-2} above the male background incidence in the general population would roughly correspond to a relative risk in the exposed population of 1.5 (= (2.5 + 5.4)/5.4). Sauni et al. (2017) observed no increase in lung cancer risk and an upper 95% CI below this risk (SIR 0.50; 95% CI 0.18 – 1.08, only 6 observed cases). Sauni et al. (2017) did not report the average duration of exposure in the cohort, nor estimated cumulative exposures, and therefore it is not possible to assess how the cumulative exposure corresponds to the assumption of a 40-year career long exposure used in calculating the ERR from animal data. Sauni et al. (2017) did not adjust for smoking. The mean follow-up time per worker was 26 years, thus the RR and SMR estimates are based already on quite a long observation period, while the mean age at hire or end of follow-up was not reported, nor was the proportion of cohort that was still alive at end of follow-up.

The above rough estimations indicate that the risk estimates by the ERR may result in overestimation. However, the effects of assumptions concerning the ratio respirable/inhalable particles, duration of exposure in the cohorts vs the assumed 40 career used in the ERR and effect of potential confounding, especially by smoking, as well as the effect of use of personal respiratory protective equipment in the actual exposure levels experienced by the cohorts cannot be fully assessed assumption by assumption, while they might operate to different directions. It is, however, also important to underline

the overall lack a significantly increased risk in the cohorts, i.e., the point estimate in the Marsh et al. (2017) highest exposure category was not statistically significantly deviating from unity and no significant trend of increasing risk by increasing mean or cumulative exposure was observed and Sauni et al. (2017) did not observe an increased risk of lung cancer with an upper 95% confidence limit quite close to unity. To be noted that there is no obvious indication that healthy worker effect would have biased the lung cancer risk estimates of Marsh et al. (2017) or Sauni et al. (2017), see section 7.7.1. Given the above, it seems robust to conclude that at exposure levels experienced by the workers of the Marsh et al. (2017) and Sauni et al. (2017) studies, humans are not more sensitive to carcinogenic risks than the predictions made by the animal data derived ERR indicate. There is some indication that at such exposure levels the animal data derived ERR may somewhat overestimate the risk. However, as pointed out by ECHA (2020) it is to be noted that detecting or excluding with confidence low levels of relative risk in an epidemiological study is challenging.