Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

cobalt

EC Number: 231-158-0
CAS Number: 7440-48-4

CLH-O-0000001412-86-172/F

Adopted

22 September 2017

22 September 2017

CLH-O-0000001412-86-172/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **cobalt**

EC Number: **231-158-0**

CAS Number: **7440-48-4**

The proposal was submitted by **the Netherlands** and received by RAC on **14 December 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **10 January 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 February 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Tiina Santonen**

Co-Rapporteur, appointed by RAC: **Veda M. Varnai**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **22 September 2017** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	027-001-00-9	cobalt	231-158-0	7440-48-4	Resp. Sens. 1 Skin Sens. 1 Aquatic Chronic 4	H334 H317 H413	GHS08 Dgr	H334 H317 H413			
Dossier submitter's proposal	027-001-00-9	cobalt	231-158-0	7440-48-4	**Add** Carc. 1B Muta. 2 Repr. 1B	**Add** H350 H341 H360F	**Retain** GHS08 Dgr	**Add** H350 H341 H360F		**Add** Carc. 1B; H350: C ≥ 0,01 %	
RAC opinion	027-001-00-9	cobalt	231-158-0	7440-48-4	**Add** Carc. 1B Muta. 2 Repr. 1B	**Add** H350 H341 H360F	**Retain** GHS08 Dgr	**Add** H350 H341 H360F		**Add** Carc. 1B; H350: C ≥ 0,01 %	
Resulting Annex VI entry if agreed by COM	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	GHS08 Dgr	H350 H341 H360F H334 H317 H413		Carc. 1B; H350: C ≥ 0,01 %	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Toxicokinetics and bioavailability

When assessing classification of cobalt metal for carcinogenicity, mutagenicity and reproductive toxicity (CMR) properties, consideration of the toxicokinetics of the metallic cobalt is needed to evaluate the applicability of data read across to other cobalt compounds. The toxicokinetics of cobalt and its compounds has been extensively described in the CLH report by the dossier submitter (DS). There are no specific *in vivo* animal toxicokinetic studies on cobalt metal itself. However, in several inhalation studies in animals (e.g. NTP repeated dose and carcinogenicity studies) extensive bioavailability of cobalt metal after inhalation has been demonstrated. Also, in biomonitoring studies of occupationally exposed workers, similar correlations between air and urinary cobalt levels have been seen in workers exposed to soluble cobalt salts, cobalt metal. For example, in the cross-sectional study by Lison *et al.* (1994), similar regression coefficients between air and urinary cobalt levels were obtained for both cobalt metal and its salts. Although cobalt metal is poorly soluble in water and in other neutral fluids, it seems to be solubilised at low pH conditions. This has been demonstrated in *in vitro* bioaccessibility tests.

Bioelution of the substances was tested by mixing 1.0 g of the substance with 50 mL of artificial fluid (intestinal, alveolar, lysosomal, serum, synovial, gastric and interstitial) for 2, 5, 24 and/or 72 hours at 37°C (Stopford, 2003, and additional unpublished studies). The results for cobalt and the different soluble cobalt compounds in artificial fluids and testing durations are provided below.

Table: Solubility of cobalt dichloride and cobalt sulphate in artificial fluids

Substance	Bioelution (%Co release) alveolar 5 h	interstitial 5 h	lysosomal 2 or 5 h	lysosomal 24 h	lysosomal 72 h	gastric 2h	gastric 5 h	intestine 5 h
Cobalt	1.2	3.8	91.1	100	100	99	61.1	0.1-1
Cobalt dichloride	67.9	45.6	89.2	100	100	86.4	86.8	79.5
Cobalt sulphate	51.5	66.2	78.7	100	100	99.4	99.4	83.8

It seems that poorly soluble cobalt monoxide (cobalt(II)oxide) and cobalt carbonate also behave in the same way as cobalt metal in low pH fluids, see the table below.

Table: Maximum extractable cobalt levels (according to Stopford et al., 2003)

	Cobalt naphthenate (insoluble organic)	Cobalt metal, extra fine	Cobalt sulphate (soluble)	Cobalt dichloride (soluble)	Cobalt monoxide (insoluble)	Cobalt carbonate (insoluble)
Ingestion (maximum solubility %)						
Gastric fluid	>85.7	>67.3	100	>91.6	>91.8	>92
Intestinal fluid	45.4*	3.7	>83.3	>79.4	2.1	4.1
Inhalation (maximum solubility %)						
Alveolar	35.4*	4.8	>51.4	>68	2.4	2.9
Interstitial	40*	4	82.8	78.4	9.9	2.2
Serum	42.9*	11.3	>81.7	>85	19.9	10.1
Intracellular (lysosomal)	>79.1	>91.1	>83.3	>89.6	92.4	>96

*maximum extraction level at 72 hours

It has been estimated that the inhalation bioavailability of cobalt salts and lysosomal fluid soluble cobalt compounds is 20-30%. Absorbed Co^{2+} is mainly excreted to the urine.

After oral exposure, the highest tissue concentrations generally occur in the liver and kidney with lower amounts in the heart, spleen, muscle, bone, brain, pancreas, lung, and gonads. There is no animal or human data on the absorption of cobalt metal from the gastrointestinal-tract. One controlled human study shows a 10-fold lower oral absorption of insoluble tricobalt tetraoxide (cobalt(II,III)oxide) compared to the absorption of cobalt chloride (Christensen *et al.*, 1993). Cobalt(II, III)oxide may, however, be less bioaccessible than cobalt monoxide and cobalt metal, since it seems to show lower bioaccessibility in artificial body fluids (see data on the bioaccessibility from NTP, 2016). Since cobalt metal is solubilised in gastric fluid, it can be assumed that it is bioavailable also via oral exposure. The oral bioavailability of soluble cobalt substances is approximately 30%. Since the oral bioavailability of cobalt metal depends on its initial solubility in gastric fluid before entering the intestine, it can be assumed to show somewhat lower bioavailability from the gastrointestinal-tract than soluble salts. There is, however, no quantitative data on this.

There are also two human volunteer studies suggesting dermal absorption of cobalt, one with exposure of hands to hard metal powder and one with exposure to coolant solution containing cobalt (Scansetti *et al.*, 1994; Linnainmaa and Kiilunen, 1997).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Since classification of STOT RE is not part of the proposal for cobalt metal, the DS included repeated dose toxicity studies in the CLH report only as a background for the assessment of carcinogenicity and reproductive toxicity. Only a short overall summary of repeated dose toxicity is provided here as supporting evidence, but without a comparison with the criteria.

Both human and animal data are shown.

Evidence for cobalt toxicity in **humans** is briefly summarised in the CLH report, and more evidence is available in the open literature. Increases in haemoglobin and red blood cell counts, hypothyroidism and cardiotoxicity have been described in occupationally and non-occupationally exposed people (Packer, 2016). Non-occupational cobalt exposures reported to be able to induce toxicity include: treatment for anaemia (practised from the 1930s until the 1970s), heavy drinking of cobalt-fortified beer in nutritionally deficient subjects (in the mid-1960s), and total metal hip replacement or arthroplasty (includes cases reported within the last 10 years) (Packer, 2016; Bradberry *et al.*, 2014).

The toxic effects of cobalt seen in humans have been observed in experimental **animals** as well. For example, in oral studies with soluble cobalt compounds, an increase in red blood cells was observed in rats already at 2.5 mg cobalt/kg bw/d in a 3-month guideline study with cobalt chloride (CDI/CORC 2015), and cardiac damage at 12.4 mg/kg bw/dy was seen in a 3-week study in rats, also with cobalt chloride (Morvai *et al.,* 1993). In NTP inhalation studies with cobalt metal, 3-month exposure to 0.625 mg cobalt/m^3 was related to histopathological lung changes (chronic active inflammation and alveolar proteinosis) in male and female rats (NTP, 2014b), and in the lung (alveolar cellular infiltration, cytoplasmic vacuolisation of bronchiolar epithelium), nose (degeneration of olfactory epithelium) and larynx (squamous metaplasia) of male and

female mice (NTP, 2014e). At higher doses, haematological changes were also observed in these studies.

Comments received during public consultation

This hazard class was not open for comments during the public consultation.

Assessment and comparison with the classification criteria

Classification for STOT RE was not included in the proposal for cobalt metal, and a comparison with the criteria was not performed by the DS.

However, RAC points out that the toxic effects of cobalt observed in humans and animals strongly indicate classification of cobalt metal as STOT RE 1.

A more thorough analysis would be needed for such a classification (including identification of primary target organs for inclusion in the hazard statement).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify cobalt metal as Muta. 2 (H341). This is based on positive evidence on both cobalt metal and cobalt compounds from *in vitro* studies indicating DNA damage seen in the Comet assay, and chromosomal aberrations and sister chromatid exchange (SCEs), and from evidence from *in vivo* studies showing DNA damage, chromosomal aberrations and micronuclei after i.p. administration of cobalt metal and cobalt compounds. *In vivo* studies with other routes of exposure have been mainly negative. The only exception is the positive *in vivo* oral study by Palit *et al.* (1991) with cobalt chloride. In addition, the increased implantation loss in 2 dominant lethal assays was considered to point towards genotoxicity in germ cells, although the studies were considered to have significant limitations. K-ras mutations observed in the lung tumours of cobalt-exposed animals in the study by NTP was considered to provide support for the genotoxicity, at least locally.

The difference between i.p. and oral studies was considered to be due to a difference in local dose between these two routes. The DS considered that especially local mutagenicity at the port-of-entry cannot be excluded, even though the mutagenicity of cobalt may be indirect and the local concentration must reach a certain level to induce such effects. A strong increase in nuclear anomalies in the gastro-intestinal tract as shown by Kirkland *et al.* (2015) was considered as evidence for the possible local genotoxicity. It was noted that 5 cobalt salts (cobalt sulphate, cobalt nitrate, cobalt chloride, cobalt carbonate and cobalt acetate) have a harmonised classification as Muta 2. Although there were positive *in vitro* studies with cobalt metal itself no *in vivo* tests were available. Read-across from the soluble cobalt compounds to cobalt was, however, considered scientifically correct because it is shown that after inhalation exposure to cobalt, Co^{2+} is systemically available in many organs including the testes. The *in vitro* data from cobalt metal and the data that show soluble cobalt compounds can induce genotoxicity in somatic cells and possibly germ cells were considered scientifically strong enough, to fulfil the criteria for Cat. 2 and therefore, it was concluded that also cobalt metal should be considered suspected of causing genetic defects.

Comments received during public consultation

Three Member State Competent Authorities (MSCAs) supported the classification of cobalt as Muta.2.

Several Industry or trade associations and a few individuals provided comments against the classification of cobalt as a germ cell mutagen. According to these comments no classification is justified since the database for mutagenicity is considered largely negative: *in vitro* findings are considered to provide mixed results with soluble cobalt salts including many positive studies of low quality, reliability and relevance score, as well as many guideline compliant studies with negative results. It was pointed out that all GLP- and guideline compliant bacterial mutagenicity tests for cobalt metal were negative. Careful evaluation of the positive findings in *in vitro* studies were requested since according to the Industry many *in vitro* tests may have been conducted in inappropriate conditions, i.e. at concentrations above the solution limit. Regarding *in vivo* genotoxicity data, it was pointed out that positive findings were obtained only by i.p. exposure to soluble cobalt salts whereas the studies reporting positive *in vivo* findings after oral exposure were considered unreliable. These include dominant lethal assays reporting positive findings, which were considered to suffer from several deficiencies and were conducted above the maximum tolerated dose (MTD).

Reference was also made to ECHA CLP guidance (v.5.0, 2017, although the reference should in fact have been to the CLP Regulation), which states that substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens (Cat. 1A or B), shall be considered for classification as Category 2 mutagens. This condition was not considered to be fulfilled, since cobalt metal has not shown positive results *in vitro* and soluble cobalt compounds are not known germ cell mutagens (Cat. 1A or B).

It was also pointed out in the comments that mutagenicity studies need to be considered in a weight of evidence (WoE) approach, taking into account the reliability, consistency, relevance, and quality of the studies. According to Industry, a WoE assessment of the current data does not indicate germ cell mutagenicity concerns for cobalt metal, which is also consistent with the 2014 OECD conclusion that there is no evidence of genetic toxicity for cobalt salts.

Many comments addressed the fact that although cobalt compounds have shown some genotoxic responses *in vitro*, these results have not been reproduced in *in vivo* experiments with relevant routes of exposure. Therefore, the available evidence suggests that there are non-genotoxic mechanisms that exhibit thresholds playing a role in cobalt-induced carcinogenicity. The i.p. studies were not generally considered appropriate for the classification of cobalt as a germ cell mutagen, especially in the light of negative studies with more relevant routes of exposure. Use of i.p. studies were considered to be clearly not in-line with (i) the provisions laid down in the CLP regulation (ii) the test guidelines specified in Article 13(3) of the REACH regulation (iii) ECHA guidance (Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.4, v1.1, 2011).

Further information on the HPRT study by Kirkland *et al.* (2015) was provided in a comment from Covance laboratories. It was pointed out that a HPRT test was performed without particle removal hence the presence of undissolved cobalt could not be excluded. The presence of undissolved cobalt could have been indicated by the absence of such clear increases in the HPRT test with an extract of cobalt metal powder. It was also pointed out that two further cobalt compounds tested in their laboratory, cobalt sulphate and cobalt borate neodeconoate, were not mutagenic in the same test system when tested up to the limit of cytotoxicity.

The use of higher K-ras mutation frequency in the cobalt induced cancers for the supporting evidence for the classification of cobalt as a germ cell mutagen was not considered appropriate.

Assessment and comparison with the classification criteria

Bacterial mutagenicity data, cobalt metal

In bacterial mutagenicity assays conducted by the NTP (Behl and Hooth, 2014), cobalt metal induced positive results in *Salmonella typhimurium* strain TA98 without S9. Equivocal results were seen in strain TA100 in the absence of S9 metabolising enzymes. In the presence of S9, both strains yielded negative results. These results were not confirmed in TA98 in three independent tests with cobalt powder up to concentrations of 5000 µg/plate performed in three different laboratories (Kirkland *et al*., 2015). Also the *Escherichia coli* WP2 uvrA/pKM101 strain gave negative results both in the absence or presence of S9 mix after the exposure to cobalt metal (NTP, 2014). The results from the bacterial mutagenicity studies are listed in the table below.

Bacterial mutagenicity data, cobalt salts

The database of bacterial mutagenicity tests in cobalt salts is also largely negative. Some of the older positive findings have not been reproduced in more recent studies. The results from the bacterial mutagenicity studies with soluble cobalt salts are listed in the table below. There was one older positive result in strain TA98 without S9 showing positive results with cobalt chloride in *Salmonella* (Wong *et al*., 1988), but these results were not confirmed in several other studies with TA98. Positive result from the study by Pagano and Zeiger (1992) with cobalt chloride in strain TA97a were not reproduced in three independent tests with cobalt chloride performed in three different laboratories (Kirkland *et al*., 2015). Neither were positive responses in TA100 (without S9, weak positive also with S9) after exposure to cobalt sulphate reproduced in later studies in three different laboratories (Kirkland *et al*., 2015). Ogawa *et al*. (1995), reported positive findings in *E. Coli*. All the rest of the studies were negative (see table below). Overall, it can be concluded that there is a lack of mutagenic activity in bacteria. This conclusion is in accordance with the previous conclusion of RAC on cobalt salts and with the conclusion by OECD.

Table: Bacterial mutagenicity data with cobalt metal and cobalt salts

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
Ames test (TA 98, TA 100, E. coli WP2)	cobalt	–S9: 500 µg/plate (TA 100), 100 µg/plate (TA 98), 450 µg/plate (E. coli) +S9: 7500 µg/plate	-S9: positive (TA 98) +S9: negative	OECD TG 471	NTP, 2014
Ames test (TA 98)	cobalt powder	5000 µg/plate	-S9: negative +S9: negative	OECD TG 471 3 test labs	Kirkland et al., 2015
Ames test (TA 98, TA 102, TA 1535, TA 1537)	cobalt chloride	40 µg/mL	-S9: positive (TA 98) -S9:negative (TA102, TA 1535, TA 1537)	No guideline	Wong, 1988

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
			+S9: negative		
Ames test (TA 97)	cobalt chloride	13 µg/mL	-S9:positive	No guideline; methodical and reporting deficiencies	Pagano and Zeiger, 1992
Ames test (TA 98, TA 100, TA 1537, TA 2637)	cobalt(II)chloride	130000 µg/plate	-S9:negative	No guideline	Ogawa et al., 1986
Ames test (E. coli SY1032/pKY241)	cobalt chloride	2.6 µg/mL	S9: positive		Ogawa et al., 1999
Ames test (TA 100)	cobalt chloride hexahydrate	23800 µg/mL	-S9:negative		Tso and Fung, 1981
Ames test (TA 98, TA 100, TA 1535, TA 1537, TA 1538, E. coli WP2)	cobalt chloride hexahydrate	?	-S9:negative		Arlauskas et al., 1985
Ames test (TA 98, TA 1538)	cobalt chloride hexahydrate	20 µg/mL	-S9:negative		Mochizuki and Kada, 1982
Ames test (E. coli WP2)	cobalt chloride hexahydrate	20 µg/mL	-S9:negative		Kada and Kanematsu, 1978
Ames test (E. coli WP2)	cobalt chloride hexahydrate	50 µg/mL	-S9:negative		Leitao et al., 1993
Ames test (TA 97a)	cobalt chloride	5000 µg/plate	-S9: negative +S9: negative	OECD TG 471 3 test labs	Kirkland et al., 2015
Ames test (TA 98, TA 100, TA 1535)	cobalt sulphate heptahydrate	-S9: 3 µg /mL (TA 100) 10.000µg/mL (TA98, 1535) + S9: 10.000 µg/mL	-S9: positive (TA 100) -S9:negative (TA98, TA 1535) +S9: negative	OECD TG 471	Publication NTP, 1998
Ames test (TA 98, TA 100, TA 1535)	cobalt(II)sulphate heptahydrate	100 µg/plate (TA 100) 10000 µg/plate (TA 98, 1535)	-S9: negative +S9: negative	Comparable to guideline	Zeiger, et al., 1992
Ames test (TA 100)	cobalt sulphate	5000 µg/plate	-S9: negative +S9: negative	OECD TG 471 3 test labs	Kirkland et al., 2015

Gene mutations in mammalian cells - cobalt metal and cobalt salts

Mutagenicity of cobalt metal in mammalian cells has been studied in an HPRT assay in L5178Y cells. The study with cobalt metal resulted in a weakly positive response in the presence of S9. As described in Kirkland *et al.* (2015), test item precipitation and thus the presence of particulate

matter may have contributed to the results. In order to avoid the presence of particulate matter, the experiment was replicated with the extract of the cobalt metal powder. Relative cell survival was reduced to <20% under all treatment conditions, indicating that divalent Co cations were liberated during the extraction process and induced toxic effects in the cells. The repetition of the HPRT assay using the extract of cobalt metal powder resulted in a negative result for mutagenicity (Kirkland *et al*., 2015).

An HPRT assay in L5178Y cells was also performed with cobalt monoxide, cobalt sulphate and cobalt sulphide. None of these studies resulted in conclusively positive responses (Kirkland *et al*., 2015). Thus, these new studies could not reproduce the positive responses in two older, non-guideline HPRT studies with cobalt dichloride. In other non-guideline gene mutation tests cobalt chloride or sulphide did not induce mutations in tk locus, V79-8AG locus or in the Gpt locus, whereas positive responses were seen in one study with cobalt chloride and sulphide in the Gpt locus in transgenic G12 cells, which are sensitive mutations. These data on gene mutations in mammalian cells are summarised in the table below.

Overall, it is concluded that cobalt metal does not cause gene mutations in mammalian cells.

Table: Mutagenicity of cobalt metal and cobalt salts in mammalian cells

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
Mammalian cell gene mutation test (hprt locus)	cobalt metal powder	30 µg/mL	-S9: negative +S9: positive	OECD TG 476	Kirkland et al., 2015
Mammalian cell gene mutation test (tk locus)	cobalt dichloride hexahydrate	57.11 µg/mL	negative	No guideline. Treatment was only 3 hours	Amacher, and Paillet, 1980
Mammalian cell gene mutation test (hprt locus)	cobalt dichloride hexahydrate	13 µg//mL	positive	No positive control. No data on cytotoxicity. No confirmatory experiment.	Hartwig et al., 1990 and 1991
Mammalian cell gene mutation test (hprt locus)	cobalt dichloride (nature salt unknown). Purity >99%	26 µg/mL	positive	No guideline. Only 1 concentration tested	Miyaki et al., 1979
Mammalian cell gene mutation test (Gpt locus and transgenic G12, Gpt locus)	cobalt chloride	13 µg/mL 6.5 µg/mL	Negative (Gpt locus) Positive (transgenic G12)		Kitahara et al., 1996
Mammalian cell gene mutation test (V79-8AG locus negative)	cobalt chloride hexahydrate	2 µg/mL	negative		Yokoiyama et al., 1990
Mammalian cell gene mutation test (hprt locus)	cobalt sulphate	100 µg/mL	-S9: negative +S9: negative	OECD TG 476	Kirkland et al., 2015
Mammalian cell gene mutation test (hprt locus)	cobalt oxide	120 µg/mL	-S9: negative +S9: negative	OECD TG 476	Kirkland et al., 2015

Mammalian cell gene mutation test (hprt locus)	cobalt sulfide	922 µg/mL	-S9: negative +S9: negative	OECD TG 476	Kirkland et al., 2015
Mammalian cell gene mutation test (Gpt locus and transgenic G12, Gpt locus)	cobalt sulfide (CoS2 and CO3S4) particles	1 µg/mL 0.5 µg/mL	Negative (Gpt locus) Positive (transgenic G12)		Kitahara et al., 1996

Genotoxicity in vitro in mammalian cells - cobalt metal and cobalt salts

Cobalt metal and soluble cobalt compounds have shown consistently positive responses in Comet assay and other tests measuring DNA strand breaks *in vitro* (see table below). In chromosomal aberration and micronucleus tests *in vitro*, cobalt metal, cobalt oxide and soluble cobalt salts have caused positive responses in the majority of the tests (table below). These data indicate that even though cobalt does not have mutagenic activity in bacterial and mammalian tests, cobalt can cause genotoxicity by inducing DNA and chromosomal breaks *in vitro*.

Table: DNA strand breaks and chromosomal damage caused by cobalt metal and cobalt salts in mammalian cells

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
Alkaline elution in murine 3T3 fibroblasts	cobalt (metal)	1 µg/mL	positive for DNA strand breaks		Anard *et al.*, 1997
Alkaline sucrose gradient in CHO cells	cobalt chloride	260 µg/mL	positive for DNA strand breaks		Hamilton-Koch *et al.*, 1986
Nucleoid sedimentation in CHO cells	cobalt chloride	1,300 µg/mL	negative for DNA strand breaks		Hamilton-Koch *et al.*, 1986
DNA damage in BALB/3T3 cells	cobalt chloride	1 µM	positive		Ponti *et al.*, 2009
DNA damage in rat neuronal PC12 cell	cobalt chloride	100 µM	positive in mitochondrial DNA, not in nuclear DNA		Wang *et al.*, 2000
Sucrose gradient in CHO cells	cobalt sulfides (CoS2 and CO3S4) particles	10 µg/mL	positive: strand breaks in		Robison *et al.*, 1982
Comet assay (human leukocytes)	cobalt metal	0.6 µg/mL	positive		Van Goethem et al., 1997
Comet assay (Alkaline elution assay) (human lymphocytes)	cobalt metal	4.5 µg/mL	positive		Anard et al., 1997
Comet assay (human lymphocytes)	cobalt metal	0.3 µg/mL	positive		De Boeck et al., 1998
Comet assay (human PBMC)	Cobalt metal	0.6 µg/mL	positive		De Boeck et al., 2003
Comet assay (human lymphocytes)	cobalt chloride	0.3 µg/mL	positive		De Boeck et al., 1998
Comet assay (human HepG2 cells)	cobalt chloride	10 µg/mL	positive		Alarifi et al., 2013

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
Comet assay (human peripheral blood leukocytes)	cobalt chloride	100 µM	negative		Colognato et al., 2008
Comet assay (human lung epithelial cells)	cobalt chloride	150 µM	positive		Patel et al., 2012
Comet assay (human fibroblasts)	cobalt chloride	0.84 µM	positive		Davies et al., 2005
Comet assay (human T-cells)	cobalt chloride	30 µM	negative		Jiang et al., 2012
Comet assay (human T-cells)	cobalt chloride	5 mM	positive		Caicedo et al., 2004
SCE (mouse macrophage-like cells)	cobalt chloride	13 µg/mL	positive		Andersen, 1983
SCE (human lymphocytes)	cobalt chloride	1.3 µg/mL	positive		Andersen, 1983
Chromosome aberration (human lymphocytes)	cobalt sulphate	4.5 µg/mL	negative	Experimental deficiencies (inappropriate dose stepping, no positive control, no duplicate cultures, short cytoB exposure)	Olivero et al., 1995
Chromosome aberration (human fibroblasts)	cobalt chloride hexahydrate	1.3 ppb	positive		Fairhall et al., 1949
Chromosome aberration (human fibroblasts)	cobalt chloride hexahydrate	50 µM	positive		Smith et al., 2014
Chromosome aberration (human fibroblasts)	cobalt chloride hexahydrate	25 µM	weakly positive	Numerical aberrations	Figgitt et al., 2010
Chromosome aberration (human fibroblasts and mononuclear leukocytes)	cobalt nitrate	0.15 µg/mL	negative	No guideline	Paton and Allison, 1972
Chromosome aberration (human lymphocytes)	cobalt acetate tetrahydrate	0.6 µg/mL	negative		Voroshilin et al., 1978
Chromosome aberration (human lymphocytes)	cobalt oxide	0.6 µg/mL	negative		Voroshilin et al., 1978
Chromosome aberration (human fibroblasts)	cobalt oxide	0.5 µg/mL	positive		Smith et al., 2014
Mammalian cell micronucleus test (human cells)	cobalt metal; purity 99.87%, median particle size 4 µm	0.6 µg/mL	positive	No guideline	van Goethem et al. 1997
Mammalian cell micronucleus test (human cells)	cobalt; purity 99.5%; median particle size 1-4 µm	0.75 µg/mL	positive	No guideline, poorly described	Miller et al. 2001
Mammalian cell micronucleus test (human cells)	cobalt metal	3 µg/mL	positive		De Boeck et al., 2003b

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
Mammalian cell micronucleus test (BALB/c bone marrow)	cobalt(II) dichloride hexahydrate	50 µg/mL	negative		Suzuki et al. 1993
Mammalian cell micronucleus test (BALB/3T3)	cobalt chloride	10 µM	negative		Ponti et al., 2009
Mammalian cell micronucleus test (human peripheral blood leukocytes)	cobalt chloride	40 µM	positive	High variability in response of donors	Colognato et al., 2008
Mammalian cell micronucleus test (Syrian hamster embryo cells)	cobalt sulphate heptahydrate	? 1-4 µg/mL	positive	No guideline	Gibson et al., 1997
Mammalian cell micronucleus test (human lymphocytes)	cobalt sulphate	4.5 µg/mL	negative	Experimental deficiencies (inappropriate dose stepping, no positive control, no duplicate cultures, short cytoB exposure)	Olivero et al., 1995

In vivo genotoxicity

There is only one *in vivo* study available on the genotoxicity of cobalt metal. This is the inhalation micronucleus study by NTP (2014) using a single dose (10 mg/m3) performed in conjunction with a 3 month repeated dose inhalation study. It did not result in any increases in the peripheral blood erythrocyte micronucleus levels, even though this dose was able to induce significant decreases in sperm counts and motility as well as degeneration of testes and epididymis.

Soluble cobalt salts have been tested for *in vivo* genotoxicity in several studies. Four of these studies have used i.p. administration of cobalt salts. All these i.p. studies have resulted in positive responses, i.e. increased incidences of micronuclei in bone marrow or in peripheral blood lymphocytes, oxidative DNA damage in different tissues or aneuploidy in bone marrow and in germ cells (see table below). Although there were shortcoming in these studies, including poor reporting, high doses, uncertainty in the biological relevance of the results (Farah, 1983), and in the case of the Rasgele *et al.*, (2013) and Suzuki (1993) studies a potential role of increased erythropoiesis behind the increase in micronuclei level, these data suggested that cobalt salts are genotoxic *in vivo* after i.p. administration.

On the other hand, the data from the oral route showed mostly negative results. In the most recent study (Kirkland *et al*., 2015) chromosome aberrations caused by cobalt compounds in bone marrow of rats (Hsd:SD) was evaluated. The study included a preliminary single dose study with the doses up to 320 mg/kg bw of cobalt sulphate and up to 1000 mg/kg bw of cobalt monoxide. No treatment related effects were seen in chromosome abberation frequencies in cobalt-exposed animals. No apparent effect on mitotic indexes were seen, either. In the multi-dose study, animals were treated with cobalt sulphate (100, 300 and 1000 mg/kg bw/d equivalent to 21, 63 and 210 mg Co/kg bw/d), tricobalt tetraoxide (200, 600 and 2000 mg/kg bw/day equivalent to 47, 141 and 470 mg Co/kg bw/d) and cobalt oxide (200, 600 and 2000 mg/kg bw/d equivalent to 157, 472 and 1573 mg Co/kg bw/d) by gavage using 1% methyl cellulose in water as the vehicle for 5 consecutive days. General toxicity was observed after

exposure to cobalt sulphate and cobalt oxide, resulting in mortalities and a reduction in the number of exposure days for the remaining animals. Therefore, no chromosome abberation frequency could be determined for some groups. There was evidence of bone marrow toxicity with both cobalt sulphate and cobalt monoxide (which has similar solubility in body fluids as cobalt metal) based on decreases in the mitotic index. Some increased chromosome abberation frequencies were seen in the top dose groups treated twice with cobalt sulphate and cobalt monoxide. Cobalt monoxide resulted in the death of all females, therefore the chromosome abberation frequency could be determined only in males. Because chromosome abberation frequencies in vehicle control animals were low (historical control data in the range of 0-2%), the finding of 1.8% cells with chromosome abberation (although statistically significant) in the high dose sulphate and monoxide groups of males and the presence of a dose-effect relation for cobalt oxide could be only indicative of a clastogenic response.

There is another OECD guideline compatible bone marrow chromosomal aberration tests available with cobalt chloride showing no increased incidences of chromosomal aberrations after oral administration (table below, Gudi *et al.*, 1998). Also, a micronucleus test performed by the same group (Gudi *et al.*, 1998) remained negative. The highest doses used by Gudi *et al.* (1998) were very high and probably above the MTD. Some reduction in mitotic index and the percent of the polychromatic erythrocytes were reported. The only positive *in vivo* cytogenetic assay with cobalt compounds is the study by Palit *et al*. (1991). In this study, male Swiss mice were treated with a single dose of cobalt chloride (0, 20, 40 or 80 mg/kg bw, equivalent to 4.96-19.8 mg cobalt/mg bw) by gavage. After post exposure periods of 6, 12, 18 and 24 hours, dose- and time-related increases in chromosome abberation frequency were seen in all treated groups. The reliability of the study by Palit *et al*. (1991) has been questioned as it has been considered unusual for genotoxins to produce dose-related responses at all sampling times tested (OECD, 2014, and Kirkland *et al*., 2015) meaning that they would cause effects at all stages of the cell cycle. In addition, the number of polyploid cells was statistically significantly increased at all time points (including 6 h), although the increase was not as high as at 18 and 24 h. The increase at shorter time points is questionable since polyploid cells can normally only be generated after a full cell cycle, which takes 24h.

Regarding germ cells, Kirkland *et al.* (2015) reported a spermatogonial chromosomal aberration assay performed in Sprague Dawley CD rats with 0, 3, 10 and 30 mg/kg bw/d of cobalt chloride (equivalent to 0, 0.7, 2.5 and 7.4 mg cobalt/kg bw by gavage, for 28 days). Also this remained negative; in all treated groups mean structural chromosome abberation frequencies fell below the control levels. No polyploid cells were found from 1000 metaphases scored in each of the groups (Kirkland *et al.,* 2015).

Overall, there is one negative *in vivo* inhalation micronucleus study with cobalt metal, negative single and multidose chromosome abberation studies with cobalt monoxide and sulphate, as well as negative single dose micronucleus and chromosome abberation studies with cobalt chloride and one negative 1-month spermatogonial chromosome abberation study with cobalt chloride; there is a single positive oral *in vivo* cytogenetic study with cobalt chloride. As there are doubts about the validity of the study by Palit *et al.* (1991), the overall evidence suggests that systemic genotoxic effects caused by physiological routes of exposure are minor. The difference between the i.p. and oral/inhalation studies is likely to be dose-related; i.p. injection is likely to result in significantly higher systemic doses in internal organs like in bone marrow.

Kirkland *et al.* (2015) also reported an increase in nuclear anomalies in intestinal cells after exposure to cobalt sulphate. These nuclear anomalies are produced as a result of apoptosis. Although analysis of nuclear anomalies/apoptotic cells is not a standard method for the assessment of genotoxicity and it represents a mechanism for the tissue gets rid of damaged

cells, apoptosis is generally seen in tissues following exposure to DNA damaging agents. Therefore, this increase in nuclear anomalies/apoptotic cells in intestinal cells may indicate a potential for local genotoxicity.

Table: In vivo genotoxicity, cytogenetic studies

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
Cobalt metal, inhalation					
micronucleus assay mice, inhalation	cobalt	10 mg/m3	Negative	OECD TG 474	NTP, 2014
Cobalt salts, i.p. administration					
micronucleus assay mice, i.p.	cobalt(II)chloride hexahydrate	50 mg/kg bw (12.4 mg cobalt/kg bw)	Positive	No guideline	Suzuki, 1993
micronucleus assay mice, i.p.	cobalt chloride hexahydrate	11.25 mg/kg bw (2.8 mg cobalt/kg bw)	Positive		Rasgele et al., 2013
DNA damage rat, i.p.	cobalt acetate	50 µmol /kg bw (12.5 mg cobalt acetate/kg bw or 2.9 mg cobalt/kg bw)	Positive (kidney, liver, lung)		Kasprzak et al., 1994
mammalian germ cell cytogenetic assay hamster, i.p.	cobalt chloride	400 mg/kg bw (99 mg cobalt/kg bw)	Positive (bone marrow and testes)	No guideline; experimental and reporting deficiencies	Farah, 1983
Cobalt salts and cobalt monoxide, oral					
chromosome aberration mice, oral	cobalt chloride hexahydrate	4.96 mg/kg (1.2 mg cobalt/kg bw)	Positive	No guideline	Palit et al., 1991
chromosome aberration rat, oral, single dose	cobalt oxide	1000 mg/kg bw (786 mg cobalt/kg bw)	Negative	OECD TG 475, minor deviations	Study report, Legault et al., 2009 (also Kirkland et al., 2015)
chromosome aberration rat, oral, 5 doses	cobalt oxide	2000 mg/kg bw (1573 mg cobalt/kg bw)	Negative		Kirkland et al., 2015
micronucleus assay rat, oral	cobalt chloride hexahydrate	600 mg/kg bw (149 mg cobalt/kg bw)	Negative	OECD TG 474	Study report, Gudi et al., 1998
chromosome aberration rat, oral	cobalt chloride hexahydrate	600 mg/kg bw (149 mg cobalt/kg bw)	Negative	OECD TG 475	Study report, Gudi et al., 1998

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
chromosome aberration rat, oral, single dose	cobalt sulphate heptahydrate	320 mg/kg bw (67 mg cobalt/kg bw)	Negative	OECD TG 475, minor deviations	Study report, Legault et al., 2009 (also reported by Kirkland et al., 2015)
chromosome aberration rat, oral, 5 doses	cobalt sulphate heptahydrate	1000 mg/kg bw (210 mg cobalt/kg bw)	Negative		Kirkland et al., 2015
spermatogonial chromosome aberration test	cobalt chloride hexahydrate	30 mg/kg bw/day (7.4 mg cobalt/kg bw)	Negative		Kirkland et al., 2015

In addition, there are two dominant lethal assays (DLA) available on cobalt chloride. In the first one (Pedigo *et al*., 1993), ten male B6C3F1 mice were treated with cobalt chloride hexahydrate (400 ppm Co, estimated as 67 mg Co/kg bw/d) in drinking water for 10 weeks. After completion of the 10-week exposure period, 10 control and 10 cobalt-treated males were mated with untreated females over a period of 2 weeks.

Fertility of the males was maintained during the 10-week cobalt treatment period, decreased during the DLA (1.8% vs 82.4% in controls) after 12 weeks of treatment, and recovered over the next 6 weeks (for tables, see 'RAC evaluation of reproductive toxicity'). There was a decrease in testes weight. Sperm parameters at the end of the DLA and the recovery period showed that cobalt decreased all parameters measured at 12 weeks, but these parameters, except sperm concentration, recovered to control levels by 18 weeks.

There was a decrease in total implantations, an increase in average pre-implantation losses and a decrease in total and live births, but no change in post-implantation losses (which would indicate a dominant lethal effect) in litters at day 19 of gestation.

Tissue concentrations of cobalt measured by atomic absorption analysis were increased in liver, kidney, testis, and epididymis after 12 weeks of cobalt treatment. General toxicity or other effects were not determined in this study. It should be noted that the number of animals used for the test was low compared to the recommended number of animals in the DLA. In addition, only one dose level was used. Pre-implantation losses were interpreted as an effect of the cobalt on fertility. Since doses not causing effects on fertility should be used in the DLA, the dose was inappropriate for the DLA.

In the other, non-guideline, DLA (Elbetieha *et al*., 2008), animals were treated with cobalt chloride mixed in their drinking water. Three doses were used, corresponding to an intake of up to 23.1 mg Co/kg bw/d. Increased numbers of resorptions were observed already from the lowest dose (corresponding to about 6 mg Co/kg bw/d) indicating a possible dominant lethal effect. However, also the number of pregnant females was decreased at mid- and high dose and the number of implantation sites/female was reduced from the lowest dose but the effect did not show a dose response relationship. The number of viable foetuses was also decreased in all groups, but the effect did not follow a dose response pattern. Both the body weights and fluid intake were decreased in all treated groups and decreased sperm counts, changes in weight and morphology of male reproductive oragans were observed at 11.6 and 23.1 mg Co/kg bw/d (testes weight and epididymal sperm numbers were affected already at 6 mg Co/kg bw/d) indicating

adverse effects of cobalt chloride hexahydrate on fertility. Since the DLA should be conducted at doses not causing effects on fertility, at least the mid- and high doses were clearly inappropriate doses.

The study included several deficiencies (e.g. significantly lower number of animals and implantations than recommended by the OECD guideline, evidence of mating, e.g. number of sperm-positive females, was not stated, positive controls and data on historical controls were not included). Two animals at the highest dose and one at the mid-dose died during the treatment, but no further information was given regarding pathological changes in these animals (namely, no clinical signs of toxicity were observed in surviving animals in groups in which mortality occurred). Because of these deficiencies and the high doses affecting fertility, the study is not considered reliable for showing a dominant lethal effect.

Human data

In a study by De Boeck *et al.* (2000), 35 workers were exposed to cobalt dust from three refineries. To determine chromosomal damage, 8-OHdG was measured in the urine, and results of the comet assay and micronuclei in their peripheral blood lymphocytes were evaluated and compared to those of the 35 matched control subjects. No significant increases of genotoxic effects were detected in workers exposed to cobalt-containing dust at a mean level of 20 µg Co per gram of creatinine in urine, equivalent to a time weighted average (TWA) exposure of 20 µg/m³ Co.

Mechanisms of cobalt causing genotoxicity

According to the available evidence presented above, the cobalt ion is not directly mutagenic, although it can cause clastogenic chromosal damage. There are studies on the mechanisms of the DNA damage caused by cobalt, which have shown that induction of reactive oxygen species (ROS) and oxidative stress may play a significant role in the genotoxicity of cobalt. The cobalt(II) ions are able to induce the formation of ROS both *in vitro* and *in vivo*, and they catalyse the generation of hydroxyl radicals from hydrogen peroxide in a Fenton type reaction. In the i.p. study by Kasprzak *et al*. (1994), cobalt resulted in the formation of oxidative DNA base damage in kidneys, liver and lungs (ECHA, 2016). In the NTP carcinogenicity study of cobalt sulphate heptahydrate in B6C3F1 mice (NTP, 1998) K-ras mutation frequency and spectra in lung tumours were evaluated. A higher frequency (5/9; 55%) of G to T transversions was detected in codon 12 of K-ras compared with chamber controls (0/1) or historical controls (1/24). G to T transversions are common DNA changes associated with reactive oxygen species. Since these mutations are indicative of oxidative damage, this supports the conclusion that cobalt indirectly damages DNA by oxidative stress.

In addition, as also discussed in the RAC reference dose response document on cobalt salts (ECHA, 2016), impairment of DNA repair by cobalt is also likely to contribute to the chromosomal damage observed with cobalt *in vitro*. There is *in vitro* evidence on the ability of cobalt to inhibit DNA repair. Cobalt(II) ions have been shown to substitute for zinc in the zinc-finger domain of some important proteins, including those controlling cell cycle and DNA repair. This substitution results in proteins with modified catalytic activity. Also substitution of cobalt for magnesium in DNA polymerases or topoisomerases and modulation of the DNA binding capacity of p53 protein by cobalt(II) ions has been proposed as potential mechanisms of cobalt-caused indirect DNA damage.

Comparison to the classification criteria

The classification for Germ cell mutagenicity, Category 1A, is based on positive evidence from human epidemiological studies. Since there is no such evidence for cobalt, Category 1A is not applicable.

The classification in Category 1B is based on: 1) positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or 2) positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells; or 3) positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny.

In the case of cobalt, there is no data from heritable germ cell mutagenicity tests in mammals. For soluble cobalt salts, there is one oral study suggesting a positive dominant lethal effect but since the study had several deficiencies (including the fact that the doses used already induced fertility effects) and the results are not supported by the other studies, this result is not considered relevant for classification for germ cell mutagenicity.

Regarding *in vivo* somatic cell genotoxicity data, one inhalation micronucleus study with cobalt powder did not show an increase in micronuclei in peripheral blood erythrocytes. In the case of cobalt salts*, in vivo* studies using the i.p. route has shown positive results whereas oral studies have mainly remained negative. Only one non-guideline study has shown positive results after oral administration of cobalt salt. The reliability of this study has been questioned by the OECD (2014) and Kirkland *et al.*, (2015). The difference between the results in i.p. and oral/inhalation studies is likely to be dose-related; i.p. injection is likely to result in significantly higher systemic doses in internal organs such as in the bone marrow. Taking into account the mechanisms of action of cobalt-induced DNA damage, which are likely to be related to the oxidative damage and impairment of DNA repair, the genotoxicity of cobalt may exert a threshold. There is no experimental data showing that cobalt can reach germ cells and result in DNA damage in germ cells. Recent spermatogonial chromosomal aberration test with cobalt chloride hexahydrate (Kirkland *et al.*, 2015) remained negative. Therefore, classification in Category 1B is not appropriate. However, classification in Category 2 should be considered.

According to the CLP Regulation, classification in Category 2 is based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:
    - Somatic cell mutagenicity tests *in vivo*, in mammals; or
    - Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

It is also noted in the CLP Regulation that substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens. The moiety causing systemic genotoxic effects is the cobalt ion in all cases and according to the toxicokinetic data, cobalt metal can be absorbed in the body at similar levels as cobalt salts after inhalation, it is scientifically justified to use data on cobalt salts in the classification of cobalt metal.

As described above, cobalt metal and cobalt salts can cause DNA damage measured by Comet assay and chromosomal aberrations and micronuclei *in vitro*, although they do not cause direct mutagenic effects. These effects may be threshold-based. Existence of a threshold could explain the fact that *in vivo* genotoxic effects are seen mainly in i.p. studies which are likely to result in higher systemic doses than inhalation or oral studies. The lack of clear evidence of systemic genotoxic effects after oral or inhalation exposure at the doses causing other toxicity indicates that the systemic genotoxic effects of cobalt might be relatively weak and below the detection limit of the oral, dermal, and inhalative test assays. However, this may not be enough to justify non-classification (see CLP Guidance 2017, Section 3.5.2.4).

In the case of cobalt, there is mechanistic evidence suggesting a possible threshold for its genotoxic effects. However, identification of such a threshold is not possible. The only studies

supporting systemic genotoxic effects via relevant routes are the dominant lethal test by Elbetieha *et al.* (2008), which has severe deficiencies and the *in vivo* oral micronucleus test by Palit *et al.* (1991), the validity of which has been also questioned. Local genotoxicity of cobalt is also possible when taking into account the data by Kirkland *et al.* (2015) reporting an increase in nuclear anomalies (apoptotic cells) in intestinal cells after exposure to cobalt sulphate. This increase in nuclear anomalies/apoptotic cells in intestinal cells may indicate potential for local genotoxicity. However, it should be noted that apoptosis may be caused also by other kinds of cell injury and these findings cannot be regarded as proof of local genotoxicity. The NTP study showing K-Ras mutations in cobalt-induced cancers can be regarded as supportive of local oxidative DNA damage in tumour cells but it is not considered to be sufficient proof of local genotoxicity.

Overall, the critical issue is whether the available *in vivo* data gathered via physiological exposure routes can provide enough evidence to conclude that genotoxicity *in vivo* is not relevant via these routes. If not, classification as Muta. 2 is warranted based on i.p. data and *in vitro* data. At present, although the recent studies using oral or inhalation routes suggest that genotoxicity may be below the detection limit of these test assays, it is difficult to exclude relevant systemic genotoxicity, especially when there are additionally some indications from earlier – although less reliable - studies on the genotoxic effects via physiological routes.

Therefore, the criteria for Muta. 2 are considered to be fulfilled, and RAC agrees with the DS's proposed **classification as Muta. 2; H341**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify cobalt metal as carcinogenic, Category 1B, on the basis of two inhalation carcinogenicity studies, which are available for the substance, one in rats and one in mice. Due to co-exposure to other carcinogens, epidemiological studies in humans were not considered to provide sufficient evidence for the carcinogenicity of cobalt in humans.

Two inhalation carcinogenicity studies on cobalt sulphate heptahydrate (in rats and mice) and one intratracheal study in rats with cobalt oxide were used as supporting evidence. Although there are no carcinogenicity studies using other routes of exposure, according to the CLP Regulation the route of exposure should be stated only if it is conclusively proven that no other routes of exposure cause the hazard. Since there was evidence showing that cobalt is distributed through the body and in inhalation studies also an increase in adrenal tumours were seen, the DS considered that tumours after exposure via e.g. the oral route cannot to be excluded and therefore, the criteria for specifying the route of exposure were not met.

The DS also proposed a specific concentration limit (SCL) of 0.01% for cobalt. This is based on the guidance given in EC 1999. According to this guidance, an SCL for non-threshold carcinogens can be derived by calculating the T25[1] (Dybing *et al.*, 1997, and comparing it to guidance levels given in the document. Using the highest net tumour incidence observed in the inhalation study

[1] The T25 estimate of potency and it is defined as the daily dose (in mg/kg bw) inducing a tumour incidence of 25% upon lifetime exposure.

with cobalt metal in male mice, a T25 of 0.1 mg/kg bw/d was obtained. Since this was below the limit of 1 mg/kg bw/d for high potency carcinogens, an SCL of 0.01% was proposed.

Comments received during public consultation

Three MSCAs supported the proposed classification as carcinogen Category 1B (H350) without specifying the route of exposure and with an SCL of 0.01%. Several Industry or Trade Associations and a few individuals provided comments arguing against the classification of cobalt as carcinogenic via all routes of exposure and against the SCL of 0.01%. The majority of the comments were related to the implications of this classification to alloys containing cobalt, and the use of bioelution tests for the classification of metal alloys and slags. However, since this proposal is limited to the classification of the substance cobalt based on information on cobalt and read-across from other cobalt compounds, the use of bioelution tests for the classification of alloys and slags is beyond the scope of this proposal.

According to Industry, the weight of evidence does not support the carcinogenicity of cobalt in sites other than lungs and therefore carcinogenicity classification via all routes of exposure is not warranted. Detailed comments on the relevance and possible mechanisms of systemic tumours observed in cobalt inhalation studies were provided. These included:

- the possible lung damage and hypoxia as a mechanism of carcinogenicity for pheochromocytomas observed in rats;
- non-relevance of the (non-exposure related) increase in MN occurrence in female rats;
- the lack of statistical significance for kidney adenomas and carcinomas;
- the inability to assess the relevance of the pancreatic islet cancers because of the lack of historical control data in this strain/colony of rats, which use was discontinued soon after these studies with cobalt.

According to Industry, there is insufficient evidence to conclude that these systemic neoplasms are caused by the cobalt ion and none of the systemic neoplastic findings fulfil the criteria for Category 1B. Reference to the EFSA (2012) evaluation on cobalt in animal feed and NTP (2016) monograph on cobalt and cobalt compounds that release cobalt ions *in vivo* was made. EFSA (2012) concluded the following:

"Cobalt(II) cations are considered genotoxic under *in vitro* and *in vivo* conditions. Cobalt(II) cations have CMR (carcinogen, mutagen and reproduction toxicant) properties. No data are available on the potential carcinogenicity of cobalt(II) following oral exposure either in humans or in experimental animals. However, oral exposure may potentially entail a number of adverse effects in humans (cardiac effects, effects on erythropoiesis, effects on thyroid, developmental effects and allergic dermatitis). For these threshold effects, the FEEDAP Panel developed a health-based guidance value of 0.0016 mg/kg bw and day (see Appendix B)."

Considering the toxicological profile of cobalt(II)-containing compounds, the FEEDAP Panel also recommended to minimise the exposure of users to cobalt(II) compounds at several levels of feed formulation and animal nutrition. According to Industry's comments, the NTP evaluation of the hip implant data showed that there is no conclusive finding indicating that cobalt is carcinogenic by this route of exposure. It seems that NTP considered the available data on the hip implants uninformative since many of the available studies were case studies and the available cohort studies (mainly record linkage studies) did not provide information on the types of implants (whether they contained cobalt and how much) or the exposure to cobalt.

In addition, one recently published additional epidemiological study from Finland was provided (Sauni *et al*., 2014). In this study, occupational exposure to cobalt was not associated with an increased overall cancer risk or lung cancer risk among workers exposed to cobalt between 1968

and 2004 in Finnish cobalt plants. However, because of the small number of cancer cases (92) the results must be interpreted with caution. In addition, information on the large international occupational epidemiologic investigation of hardmetal workers were provided. The results of this study are expected to be published in the autumn 2017, but manuscripts of the research papers were provided to RAC for review (Marsh *et al.*, 2017).

Assessment and comparison with the classification criteria

Human carcinogenicity data

There are limited data available on the carcinogenicity of cobalt in humans. The majority of the available data came from the hard metal Industry where the workers are exposed to cobalt and tungsten carbide. There was experimental evidence showing that the mixture of cobalt and tungsten carbide causes effects that are more severe than those observed with cobalt metal alone (IARC, 2006). Therefore, it is difficult to draw conclusions on the carcinogenicity of cobalt on the basis of exposures in the hard metal Industry. These studies have been summarised in the table below.

Table: Published epidemiological studies on the cancer risk caused by cobalt (with or without tungsten carbide)

Study	Cohort population	Result	Remarks
Hardmetal production (co-exposure to cobalt and tungsten carbine)			
Lasfargues *et al*., 1994	709	SMR = 2.13, 95% CI = 1.02–3.93 for the cancer of the trachea, bronchus and lung	an effect of smoking not entirely ruled out
Moulin *et al.*, 1998	7459 workers, 10 plants	OR = 2.21, 95% CI = 0.99 to 4.90 for lung cancer when higher exposure groups (2-9) were compared to no or mildly exposed (0-1), non-statistically significant exposure-response relationship across the levels, duration and cumulative level of exposure	cohort study with internal nested case-control analysis
Wild *et al.* 2000	2860	SMR = 1.95, 95% CI = 1.09 to 3.22 for lung cancer, no information on exposure-response relationship	subcohort of Moulin *et al.* 1998 from the largest plant
Cobalt manufacturing (exposure to cobalt)			
Moulin *et al.*, 1993		SMR = 0.85, 95% CI = 0.18-2.50 (whole cohort) SMR = 1.16, 95% CI 0.24-3.40 (French sub-cohort)	Extension of Mur *et al.* (1987) study
Sauni *et al.*, 2017	995	SIR = 1.00, CI = 0.81-1.22, all cancers) SIR = 0.50, CI = 0.18-1.08, lung cancer) SIR = 7.39, CI 1.52–21.6, tongue cancer	also exposure to nickel may have occurred

SMR: standardized mortality ratio, CI: confidence interval, OR: odds ratio, SIR: standardized incidence ratio.

Moulin *et al.* (2000) also reported a nested case-control study of stainless and alloyed steel workers conducted in one factory in France (N = 4897), in which no association between cobalt exposure and lung cancer was found.

There are only two of studies available on the exposure to cobalt (without tungsten carbide) in the cobalt manufacturing sector. The first one is the study by Moulin *et al.* (1993), which is actually the extension of the study published earlier by Mur *et al.* (1987). In Mur *et al.* (1987), slightly but not significantly higher overall death rate was observed among cobalt workers when compared to the national rate: the standardized mortality ratio (SMR) for cobalt production workers was 1.29. Mortality from malignant tumours was reported to be increased (SMR = 1.65), especially from lung cancer (SMR 4.66; $p < 0.05$; 4 cases). In the follow-up study (Moulin *et al.*,

1993) the SMR for all causes of death was 0.85 (95%, CI 0.76-0.95) for the whole cohort, and 0.95 (95%, CI 0.83-1.08) for the sub-cohort of workers born in France. For lung cancer mortality among cobalt production workers, the SMRs were 0.85 (95%, CI 0.18-2.50, 3 cases) for the whole cohort and 1.16 (95%, CI 0.24-3.40, 3 cases) for the sub-cohort. In maintenance workers, an elevated risk for lung cancer (1.80, 95%, CI 0.78-3.55) was observed but this might be related to asbestos exposure.

The second one is a recent study from Finland (Sauni *et al.*, 2017), which evaluated the cancer incidence among workers employed in a Finnish cobalt plant since the beginning of production in 1968. The study cohort consisted of 995 males employed by the Finnish cobalt plant for at least a year during 1968–2004. The cohort was divided into subcohorts by exposure levels. During the follow-up period, 92 cases of cancer were diagnosed (SIR 1.00, 95%, CI 0.81–1.22), six of which were lung cancer cases (SIR 0.50; 95%, CI 0.18–1.08). The only cancer type with increased incidence was tongue cancer (three cases, all smokers, SIR 7.39; 95% CI 1.52–21.6). The cohort was divided into subcohorts by exposure levels assessed on the basis of industrial hygienic measurements and biomonitoring, according to the department in which they had started working during their employment at the plant. No dose response relationship was observed across the different exposure levels and the incidence of any cancer type. During the first years of cobalt production, the cobalt levels in some department of the plant may have exceeded 1 mg/m^3, and some co-exposure to nickel may also have occurred. Because of the small size of the study the results must be interpreted with caution.

Two case-control studies found an association between toenail levels of cobalt and elevated risk of oesophageal cancer (O'Rorke *et al.*, 2012; Rogers *et al.*, 1993). In the Rogers *et al.* (1993) study, iron and calcium levels were also higher in cancer patients and it was speculated that there may be differences in mineral intake or metabolism between individuals who develop some carcinomas of the upper aerodigestive tract and those who do not. In the case of O'Rorke *et al.* (2012), an association with toenail zinc levels was also seen. Since the association of the toenail cobalt levels and the cumulative/long term cobalt exposure is not clear, no conclusions on the carcinogenic potency of cobalt can be made on the basis of these studies.

During the opinion development process manuscripts of a large International cancer study were provided by the Industry. The main results were described in Marsh *et al.* (2017), "Mortality among hardmetal production workers: pooled analysis of cohort data from an international investigation" (accepted for publication in Journal of Occupational and Environmental Medicine). Additionally, several associated manuscripts related to the exposure assessment and different sub-cohorts were provided. The study combined 5 individual country-cohorts from Austria, Germany, Sweden, UK and USA and altogether involved 32354 workers from three companies and 17 manufacturing sites. Exposure assessment was based on air measurements and in some cases on biomonitoring (in Germany and Austria). For 13 job classes exposure exceeded the current American Conference of Governmental Industrial Hygienists Threshold Limit Value (ACGIH TLV) of 0.02 mg/m^3 for Co. Two job classes, scrap recycling and milling and drying had exposures between 0.05-0.1 mg/m^3. SMRs were calculated for all causes of death, all cancers and lung cancer and confounding factors, such as smoking, were taken into account. In the pooled analysis, the lung cancer SMR was 1.26 (95% CI 1.15-1.38) or 1.20 (1.09-1.31) compared to national or regional rates, respectively. However, further analysis showed that the risk was mainly observed in short-term workers whereas in long term workers no statistically increased lung cancer mortality was seen (SMR 1.02 and 1.10 when using 5- and 1-year cut-points, respectively). Thus, no evidence of any exposure-response relationship was seen. For all cancers, the SMR was 1.07 (1.02-1.11) and 1.06 (1.01-1.11) when compared to national or regional rates, respectively. In the Swedish sub-cohort, elevated risks for several causes of death, including lung cancer were seen, however, detailed assessment revealed that elevated risk was

present only in short term workers (employment between 1 day and 1 year), but not in long-term workers. In the German sub-cohort, elevated SMRs were found for all-cause heart disease, and non-malignant respiratory diseases mortality, but not for lung cancer and in the Austrian sub-cohort a dose response relationship for three observed cases of chronic obstructive pulmonary disease (COPD) were observed but no excesses of lung cancer were seen. In the US sub-cohort overall deficits in deaths for mortality, all cancers and lung cancer were seen. Also in the UK sub-cohort no increased mortality from any cause, including lung cancer, was observed.

Thus, this large study showed no consistent evidence of elevated lung cancer mortality risk among cobalt-exposed hardmetal workers.

Animal carcinogenicity data, local effects

Cobalt metal caused clear increases in the alveolar adenomas and carcinomas in the NTP 2-year inhalation carcinogenicity studies, both in F344/NTac rats and B6C3F1/N mice in both sexes. Increases in cancer incidences were evident at all doses; 1.25, 2.5 and 5 mg/m^3 (see tables below).

In rats, survival of females exposed to 2.5 mg/m^3 was significantly less than that of the chamber control group. This may, however, be related to tumour development. Mean body weights of ≥ 2.5 and 5 mg/m^3 males were at least 10% less than those of the chamber control group after weeks 99 and 12, respectively, and those of ≥ 2.5 and 5 mg/m^3 females were at least 10% less after weeks 57 and 21, respectively. Exposure-related clinical findings included abnormal breathing and thinness in male and female rats, however, e.g. abnormal breathing began relatively late and was observed only in a small fraction of the animals. Non-neoplastic lesions in the lung at these doses included increased incidences of alveolar epithelium hyperplasia, alveolar proteinosis, chronic active inflammation, and bronchiole epithelium hyperplasia in all exposed groups. Chronic active inflammation and proteinosis in the lungs was evident already in a 90-day study at the lowest dose of 0.625 mg/m^3 (NTP, 2014) The finding was evident in almost all exposed animals. Also, a spectrum of non-neoplastic lesions occurred in the nose of both sexes, including chronic active and suppurative inflammation, hyperplasia, metaplasia, and necrosis of the respiratory epithelium and atrophy of the turbinate. These effects were seen already in a 90-day study starting from the 1.25 mg/m^3 dose group.

In mice, the survival of males exposed to 2.5 or 5 mg/m^3 was significantly less than that of the chamber control group. Like in female rats, this may have been related to tumour development. Mean body weights of males and females in the 5 mg/m^3 group were at least 10% less than those of controls after weeks 85 and 21, respectively. Abnormal breathing and thinness were noted in exposed male and female mice at all doses, however, abnormal breathing began relatively late during the course of the study. Non-neoplastic findings in the lungs included alveolar/bronchiolar epithelium hyperplasia and cytoplasmic vacuolisation, alveolar epithelium hyperplasia, proteinosis, and alveolus cellular infiltration with histiocytes which were significantly increased in all exposed groups. In addition, for example erosion of the bronchiolar epithelium and suppurative inflammation of the airways was increased in males at 2.5 mg/m^3 and higher doses. Also nasal epithelium showed inflammatory and atrophic changes as well as hyperplasia and metaplasia at all doses. Like in the case of rats, these effects were observed also already in a 90-day study at the same dose levels.

*Table: Respiratory tract tumours in male and female **rats** after two year exposure to cobalt metal*

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Alveolar/bronchiolar Adenoma, Multiple	1	3	2	6
Alveolar/bronchiolar Adenoma (includes multiple)[c]				
Overall rate[d]	2/50 (4%)	10/50 (20%)	10/50 (20%)	14/50 (28%)
Adjusted rate[e]	5.0%	24.1%	23.3%	32.5%
Terminal rate[f]	1/17 (6%)	6/20 (30%)	2/16 (13%)	4/16 (25%)
First incidence (days)	611	577	535	478
Poly-3 test[g]	P=0.011	P=0.015	P=0.018	P<0.001
Alveolar/bronchiolar Carcinoma, Multiple	0	6*	14**	30**
Alveolar/bronchiolar Carcinoma (includes multiple)[h]				
Overall rate	0/50 (0%)	16/50 (32%)	34/50 (68%)	36/50 (72%)
Adjusted rate	0.0%	38.2%	76.8%	80.6%
Terminal rate	0/17 (0%)	7/20 (35%)	16/16 (100%)	14/16 (88%)
First incidence (days)	—[i]	580	472	552
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma[j]				
Overall rate	2/50 (4%)	25/50 (50%)	39/50 (78%)	44/50 (88%)
Adjusted rate	5.0%	58.0%	84.6%	93.6%
Terminal rate	1/17 (6%)	13/20 (65%)	16/16 (100%)	16/16 (100%)
First incidence (days)	611	577	472	478
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Cystic Keratinizing Epithelioma[h]	0	1	0	1
Female				
Alveolar/bronchiolar Adenoma, Multiple	0	1	3	4
Alveolar/bronchiolar Adenoma (includes multiple)[k]				
Overall rate	2/50 (4%)	7/50 (14%)	9/50 (18%)	13/50 (26%)
Adjusted rate	4.5%	16.2%	22.1%	30.9%
Terminal rate	1/35 (3%)	5/26 (19%)	6/24 (25%)	8/25 (32%)
First incidence (days)	698	590	587	579
Poly-3 test	P=0.002	P=0.072	P=0.016	P<0.001
Alveolar/bronchiolar Carcinoma, Multiple	0	4	3	18**
Alveolar/bronchiolar Carcinoma (includes multiple)[h]				
Overall rate	0/50 (0%)	9/50 (18%)	17/50 (34%)	30/50 (60%)
Adjusted rate	0.0%	21.3%	42.0%	69.2%
Terminal rate	0/35 (0%)	9/26 (35%)	14/24 (58%)	20/25 (80%)
First incidence (days)	—	730 (T)	690	471
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma (combined)[l]				
Overall rate	2/50 (4%)	15/50 (30%)	20/50 (40%)	38/50 (76%)
Adjusted rate	4.5%	34.7%	48.5%	86.2%
Terminal rate	1/35 (3%)	13/26 (50%)	14/24 (58%)	25/25 (100%)
First incidence (days)	698	590	587	471
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Cystic Keratinizing Epithelioma[h]	0	4	1	2
Squamous Cell Carcinoma	0	0	0	1

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical control incidence for 2-year studies (all routes) (mean ± standard deviation): 5/100 (5.0% ± 1.4%), range 4%-6%
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h] Historical control incidence: 0/100
[i] Not applicable; no neoplasms in animal group
[j] Historical control incidence: 5/100 (5.0% ± 1.4%), range 4%-6%
[k] Historical control incidence: 2/100 (2.0% ± 2.8%), range 0%-4%
[l] Historical control incidence: 2/100 (2.0% ± 2.8%), range 0%-4%

*Table: Respiratory tract tumours in male and female **mice** after two year-exposure to cobalt metal*

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Alveolar/bronchiolar Adenoma, Multiple	0	1	1	0
Alveolar/bronchiolar Adenoma (includes multiple)[d]				
Overall rate[e]	7/50 (14%)	11/49 (22%)	15/50 (30%)	3/50 (6%)
Adjusted rate[f]	14.7%	24.5%	35.9%	7.3%
Terminal rate[g]	5/39 (13%)	7/31 (23%)	14/29 (48%)	2/25 (8%)
First incidence (days)	684	571	660	571
Poly-3 test[h]	P=0.254N	P=0.176	P=0.016	P=0.226N
Alveolar/bronchiolar Carcinoma, Multiple	3	18**	24**	36**
Alveolar/bronchiolar Carcinoma (includes multiple)[i]				
Overall rate	11/50 (22%)	38/49 (78%)	42/50 (84%)	46/50 (92%)
Adjusted rate	22.8%	79.4%	87.6%	93.8%
Terminal rate	8/39 (21%)	24/31 (77%)	25/29 (86%)	22/25 (88%)
First incidence (days)	561	551	382	425
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma[j]				
Overall rate	16/50 (32%)	41/49 (84%)	43/50 (86%)	47/50 (94%)
Adjusted rate	33.0%	85.0%	89.7%	95.9%
Terminal rate	11/39 (28%)	26/31 (84%)	26/29 (90%)	23/25 (92%)
First incidence (days)	561	551	382	425
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

Female

Alveolar/bronchiolar Adenoma, Multiple	0	1	0	1
Alveolar/bronchiolar Adenoma (includes multiple)[k]				
Overall rate	3/49 (6%)	9/50 (18%)	8/50 (16%)	10/50 (20%)
Adjusted rate	6.9%	19.9%	18.9%	24.5%
Terminal rate	3/36 (8%)	7/35 (20%)	6/27 (22%)	6/26 (23%)
First incidence (days)	731 (T)	505	626	593
Poly-3 test	P=0.037	P=0.067	P=0.087	P=0.024
Alveolar/bronchiolar Carcinoma, Multiple	1	7*	20**	24**
Alveolar/bronchiolar Carcinoma (includes multiple)[l]				
Overall rate	5/49 (10%)	25/50 (50%)	38/50 (76%)	43/50 (86%)
Adjusted rate	11.3%	53.8%	78.9%	87.7%
Terminal rate	3/36 (8%)	18/35 (51%)	19/27 (70%)	21/26 (81%)
First incidence (days)	583	537	457	478
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma[m]				
Overall rate	8/49 (16%)	30/50 (60%)	41/50 (82%)	45/50 (90%)
Adjusted rate	18.0%	63.7%	84.6%	91.6%
Terminal rate	6/36 (17%)	22/35 (63%)	21/27 (78%)	22/26 (85%)
First incidence (days)	583	505	457	478
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 39/300 (13.0% ± 4.2%), range 8%-20%; (all routes): 145/950 (15.3% ± 6.2%), range 2%-26%
[e] Number of animals with neoplasm per number of animals with lung examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by N.
[i] Historical incidence for inhalation studies: 59/300 (19.7% ± 3.4%), range 16%-24%; (all routes): 132/950 (13.9% ± 7.1%), range 4%-24%
[j] Historical incidence for inhalation studies: 90/300 (30.0% ± 5.5%), range 26%-40%; (all routes): 263/950 (27.7% ± 5.7%), range 16%-40%
[k] Historical incidence for inhalation studies: 16/299 (5.4% ± 3.7%), range 2%-12%; (all routes): 54/949 (5.7% ± 3.6%), range 0%-12%
[l] Historical incidence for inhalation studies: 13/299 (4.4% ± 4.3%), range 0%-10%; (all routes): 38/949 (4.0% ± 3.6%), range 0%-14%
[m] Historical incidence for inhalation studies: 28/299 (9.4% ± 4.8%), range 2%-16%; (all routes): 90/949 (9.5% ± 4.8%), range 2%-22%

In addition, these carcinogenic findings in the lungs were supported by two-year inhalation studies with soluble cobalt sulphate heptahydrate in Fischer 344 rats and in B6C3F1 mice at doses of 0, 0.3, 1.0, or 3.0 mg/m^3. In rats, the combined incidence of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) was significantly increased in 3.0 mg/m^3 males and exceeded the historical control range. In females exposed to ≥ 1.0 mg/m^3, the incidences of alveolar/bronchiolar adenomas, carcinomas and adenomas/carcinomas combined were significantly increased and exceeded the historical control ranges. There were no effects on survival or body weight but irregular breathing in females was observed at 3.0 mg/m^3 and inflammatory changes, proteinosis, metaplasia and fibrosis was observed at all doses. These doses of cobalt sulphate heptahydrate corresponded to doses of 0.06, 0.2 and 0.6 mg/m^3 of cobalt metal.

Similarly, mice showed increased incidences of alveolar/bronchiolar adenoma and/or carcinoma at 3.0 mg/m^3 in males and at 1.0 mg/m^3 and higher in females, which generally exceeded the historical control ranges for inhalation studies. No effects on survival or body weight were observed but irregular breathing in females was observed at 1.0 mg/m^3 and inflammatory and atrophic changes at 1.0-3.0 mg/m^3.

Intratracheal instillation of 0, 2 and 10 mg cobalt(II)oxide/kg bw (1 dose/2 week × 18 doses, then 1 dose/4 weeks × 11 doses, then 1 dose/2 weeks × 9 doses; total 39 doses) resulted in significant increases in lung neoplasms (alveolar/bronchiolar adenoma, benign squamous epithelial neoplasm, or alveolar/bronchiolar carcinoma combined) in male rats. Non-significant increases in lung neoplasms were seen in females. There were significant increases in alveolar/bronchiolar proliferation (types of lesions not described) in both sexes combined (Steinhoff and Mohr, 1991).

These data on the lung carcinogenicity of cobalt metal, supported by the data on soluble cobalt sulphate and poorly soluble cobalt(II)oxide, are sufficient to conclude that the criteria for Cat. 1B (H350) for carcinogenicity are fulfilled. However, to conclude if cobalt can be considered carcinogenic only via the inhalation route, anevaluation of the data on systemic cancer findings in animals as well as a consideration of the toxicokinetics and mechanisms of the carcinogenicity of cobalt are needed.

Animal carcinogenicity data, systemic effects and carcinogenicity via other routes of exposure

There are no animal studies on the carcinogenicity of cobalt metal or cobalt compounds via routes of exposure other than inhalation.

In the inhalation carcinogenicity study with cobalt metal in mice, no systemic tumours were observed. However, in rats, statistically significantly increased incidences of cancers in different organs were observed. These included pheochromocytomas in both male and female rats, pancreatic islet tumours in male rats, mononuclear cell leukaemias in females and non-statistically significant increases in renal tubule tumours in male rats. Incidences of pheochromocytomas are presented in the table below. Statistically significant increases compared to the concurrent controls were seen at doses of 2.5 and 5 mg/m^3. At these levels also historical control incidences were exceeded. However, it should be noted that the historical control database in this strain of rats is limited to only 100 rats since the strain was used only in few cancer studies due to some non-cancer problems (chylothorax, seizures, declining fertility) observed and their use was discontinued soon after the cobalt study.

Table: Incidences of adrenal tumours in male and female rats

	Chamber Control	1.25 mg/m³	2.5 mg/m³	5 mg/m³
Male				
Benign Pheochromocytoma, Bilateral	4	13*	22**	21**
Benign Pheochromocytoma (includes bilateral)[c]				
Overall rate[d]	15/50 (30%)	23/50 (46%)	37/50 (74%)	34/50 (68%)
Adjusted rate[e]	35.8%	54.3%	81.2%	76.4%
Terminal rate[f]	3/17 (18%)	12/20 (60%)	15/16 (94%)	14/16 (88%)
First incidence (days)	519	583	582	572
Poly-3 test[g]	P<0.001	P=0.059	P<0.001	P<0.001
Malignant Pheochromocytoma, Bilateral	0	0	0	7**
Malignant Pheochromocytoma (includes bilateral)[h]				
Overall rate	2/50 (4%)	2/50 (4%)	9/50 (18%)	16/50 (32%)
Adjusted rate	5.0%	5.0%	21.4%	39.1%
Terminal rate	0/17 (0%)	2/20 (10%)	3/16 (19%)	9/16 (56%)
First incidence (days)	668	729 (T)	628	646
Poly-3 test	P<0.001	P=0.693N	P=0.030	P<0.001
Benign or Malignant Pheochromocytoma[i]				
Overall rate	17/50 (34%)	23/50 (46%)	38/50 (76%)	41/50 (82%)
Adjusted rate	40.2%	54.3%	82.7%	90.7%
Terminal rate	3/17 (18%)	12/20 (60%)	15/16 (94%)	16/16 (100%)
First incidence (days)	519	583	582	572
Poly-3 test	P<0.001	P=0.130	P<0.001	P<0.001
Female				
Benign Pheochromocytoma, Bilateral	2	4	8*	19**
Benign Pheochromocytoma (includes bilateral)[j]				
Overall rate[d]	6/50 (12%)	12/50 (24%)	22/50 (44%)	36/50 (72%)
Adjusted rate[e]	13.6%	27.2%	52.1%	80.6%
Terminal rate[f]	6/35 (17%)	5/26 (19%)	13/24 (54%)	21/25 (84%)
First incidence (days)	730 (T)	598	590	579
Poly-3 test[g]	P<0.001	P=0.091	P<0.001	P<0.001
Malignant Pheochromocytoma, Bilateral	0	1	1	4*
Malignant Pheochromocytoma (includes bilateral)[k]				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	11/50 (22%)
Adjusted rate	0.0%	4.7%	7.5%	27.0%
Terminal rate	0/35 (0%)	2/26 (8%)	2/24 (8%)	9/25 (36%)
First incidence (days)	—[l]	730 (T)	715	712
Poly-3 test	P<0.001	P=0.228	P=0.102	P<0.001
Benign or Malignant Pheochromocytoma[m]				
Overall rate	6/50 (12%)	13/50 (26%)	23/50 (46%)	40/50 (80%)
Adjusted rate	13.6%	29.4%	54.5%	89.4%
Terminal rate	6/35 (17%)	6/26 (23%)	14/24 (58%)	24/25 (96%)
First incidence (days)	730 (T)	598	590	579
Poly-3 test	P<0.001	P=0.058	P<0.001	P<0.001

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical control incidence for 2-year studies (all routes) (mean ± standard deviation): 25/100 (25.0% ± 7.1%), range 20%-30%
[d] Number of animals with neoplasm per number of animals with adrenal medulla examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.
[h] Historical control incidence: 2/100 (2.0% ± 2.8%), range 0%-4%
[i] Historical control incidence: 27/100 (27.0% ± 9.9%), range 20%-34%
[j] Historical control incidence: 7/100 (7.0% ± 7.1%), range 2%-12%
[k] Historical control incidence: 1/100 (1.0% ± 1.4%), range 0%-2%
[l] Not applicable; no neoplasms in animal group
[m] Historical control incidence: 8/100 (8.0% ± 5.7%), 4%-12%

Pheochromocytomas in rats originate from chromaffin cells of the adrenal medulla and they occur with relatively higher frequency in male rats. Their occurrence has been shown to be linked to hypoxia, uncoupling of oxidative phosphorylation, disturbance in calcium homeostasis, and disturbance of the hypothalamic endocrine axis (Greim *et al.*, 2009). Substances that interfere with these biochemical endpoints may produce pheochromocytomas in animal carcinogenicity studies. It has been proposed that in the case of cobalt, lung damage resulting in reduced oxygen concentration may have contributed to the increased incidences of pheochromocytomas at high doses. At these doses, cobalt inhalation causes chronic active inflammation in the lungs, which was seen already in a 90-day study (NTP, 2014). At the doses of 2.5 and 5 mg/m^3 mean body weights of exposed animals were significantly lower compared to the controls (16 and 30% in females and 11 and 29% in males, respectively) at the end of the study, but at the dose of 2.5 mg/m^3 a significantly lower body weight was observed only at the late stages of the study. Although high dose animals showed thin and abnormal breathing, this was also observed only at late stages of the study. There was no measured data on the hypoxia caused by the local lung effects of cobalt. If lung damage is considered as the main mechanism for pheochromocytomas in rats, the relevance of these tumours to humans and to the exposure to cobalt via other routes of exposure could be considered minimal. However, at present, it is unsure whether lung damage may have contributed to adrenal tumorigenesis. Cobalt has also been shown to promote a hypoxia-like state even with normal molecular oxygen pressure, by stabilising hypoxia-inducible factor (HIF-1α), which is a major regulator of the adaptation of cancer cells to hypoxia. This occurs via the ability of cobalt(II) to compete with the iron binding site of HIF-1α (prolyl) hydroxylase, preventing its hydroxylation and the degradation of HIF-1α. As noted by Greim *et al.* (2009), the mechanisms of action identified in rats are to be expected in humans as well and can, particularly after exposure conditions similar to those used in animal studies, induce pheochromocytomas. Since this specific mechanism of action is not related to the lung damage caused by the cobalt, it is difficult to definitely conclude that pheochromocytomas would occur only via inhalation, although the lung damage caused by the inhalation has very likely contributed to the tumours response.

Pheochromocytomas were also observed in the study with cobalt sulphate heptahydrate. Statistically significantly increased incidences of benign and total (benign, complex or malignant) pheochromocytomas were observed in females at 3 mg/m^3 (incidences were 4, 2, 6 and 17% for benign tumours at the doses of 0, 0.3, 1 and 3 mg/m^3, respectively, and 4, 2, 8, 21% for total tumours). In males, statistically significant increase in total tumours was seen only at the second highest dose, the overall rates being 30, 38, 51 and 40% for 0, 0.3, 1 and 3 mg/m^3, respectively. Also, cobalt sulphate heptahydrate showed lung toxicity (inflammation, proteinosis, fibrosis) at these dose levels. These doses of cobalt sulphate heptahydrate corresponded to doses of 0.06, 0.2 and 0.6 mg/m^3 of cobalt metal.

In addition to pheochromocytomas, a statistically significantly increased incidence of pancreatic islet tumours (combined adenoma and carcinoma) was observed in male rats at 2.5 and 5 mg/m^3, whereas in females no statistically significant increases were seen. At the highest dose group, the female incidence exceeded the historical control range, but as explained above, the historical control database in this strain of rats is very limited and it should be noted that this dose exceeded the MTD (body weight of these rats were >10% lower compared to the controls already from week 12 of the study). For comparison, also nickel metal has caused similar increases in the incidence of pheochromocytomas and adrenal tumours at high doses (above the MTD). The mechanism for both of these tumours has been considered to be related to hypoxia caused by the lung damage, especially since oral exposure to soluble nickel salts, resulting in several times higher blood nickel levels, did not induce an increase in these tumours (Oller *et al.*, 2008). According to Greim *et al.* (2009), also nickel(II) is able to stabilise HIF-1α.

Incidences of pancreatic tumours in rats after exposure to cobalt metal are presented in the table below. Statistically significantly increased incidences of combined adenomas and carcinomas were seen at the highest dose in male rats. The small size of the historical control database limited the comparison with historical control data. Pancreatic tumours were not observed in corresponding rat cancer study with cobalt sulphate heptahydrate. The mechanisms of these cancer types remains unclear. Although hypoxia generally inhibits cell growth, it has also been suggested that hypoxia-mediated oxidative stress may facilitate the growth of neoplasm by the degradation of oncogene MUC4 (Joshi, 2016).

Table: Pancreatic tumours observed in rats after inhalation exposure to cobalt metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Adenoma[a]				
Overall rate[b]	0/50 (0%)	1/50 (2%)	6/48 (13%)	3/49 (6%)
Adjusted rate[c]	0.0%	2.5%	15.1%	7.7%
Terminal rate[d]	0/17 (0%)	0/20 (0%)	1/16 (6%)	3/16 (19%)
First incidence (days)	—[f]	684	618	729 (T)
Poly-3 test[e]	P=0.052	P=0.504	P=0.015	P=0.116
Carcinoma[g]				
Overall rate	2/50 (4%)	1/50 (2%)	5/48 (10%)	6/49 (12%)
Adjusted rate	5.0%	2.5%	12.6%	15.1%
Terminal rate	0/17 (0%)	0/20 (0%)	3/16 (19%)	2/16 (13%)
First incidence (days)	675	675	618	679
Poly-3 test	P=0.021	P=0.496N	P=0.213	P=0.129
Adenoma or Carcinoma (combined)[h]				
Overall rate	2/50 (4%)	2/50 (4%)	10/48 (21%)	9/49 (18%)
Adjusted rate	5.0%	4.9%	24.7%	22.6%
Terminal rate	0/17 (0%)	0/20 (0%)	3/16 (19%)	5/16 (31%)
First incidence (days)	675	675	618	679
Poly-3 test	P=0.002	P=0.689N	P=0.013	P=0.022
Female				
Adenoma[i]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	0.0%	2.5%
Terminal rate	0/35 (0%)	0/26 (0%)	0/24 (0%)	1/25 (4%)
First incidence (days)	—	—	—	730 (T)
Poly-3 test	—[j]	—	—	—
Carcinoma[k]				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	7.2%
Terminal rate	0/35 (0%)	0/26 (0%)	0/24 (0%)	1/25 (4%)
First incidence (days)	234	—	—	506
Poly-3 test	P=0.060	P=0.512N	P=0.523N	P=0.279
Adenoma or Carcinoma[l]				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	7.2%
Terminal rate	0/35 (0%)	0/26 (0%)	0/24 (0%)	1/25 (4%)
First incidence (days)	234	—	—	506
Poly-3 test	P=0.060	P=0.512N	P=0.523N	P=0.279

(T) Terminal kill

[a] Historical control incidence for 2-year studies (all routes) (mean ± standard deviation): 0/100

[b] Number of animals with neoplasm per number of animals with pancreatic islets examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.

[f] Not applicable; no neoplasms in animal group

[g] Historical control incidence for all routes: 2/100 (2.0% ± 2.8%), range 0%-4%

[h] Historical control incidence for all routes: 2/100 (2.0% ± 2.8%), range 0%-4%

[i] Historical control incidence for all routes: 1/100 (1.0% ± 1.4%), range 0%-2%

[j] Value of statistic not computed because all exposure groups have fewer than two neoplasms.

[k] Historical control incidence for all routes: 1/100 (1.0% ± 1.4%), range 0%-2%

[l] Historical control incidence for all routes: 2/100 (2.0% ± 0.0%), range 2%

In female rats, mononuclear cell leukaemias were increased at all doses, exceeding the available historical control range (35/100, 35 ± 4.2%, range 32-38%). The incidences were: 16/50 (32%) for the controls, 29/50 (58%), 28/50 (56%), 27/50 (54%) for 1.25, 2.5 and 5 mg/m^3, respectively. There was no clear dose response relationship. In addition, these types of tumours are very common in aging Fischer rats, and in males the background incidences in cancer bioassays have even exceeded 50%. There is no corresponding tumour type for MNCL in humans. Cobalt may have contributed to the occurrence of this tumour type in female rats by its ability to stimulate erythropoietin and thereby modulate haematopoiesis. Tumours of this type were not observed in corresponding cancer studies with cobalt sulphate.

In kidneys, a non-statistically significantly increased incidence in kidney tumours was seen in male rats at the highest dose level. Although the historical control incidence was exceeded, it should be noted that the historical control database for this strain of rats is limited and the effect was observed only at the highest dose, which resulted in a mean body weight which was 29% lower in males and 30% lower in females when compared to the controls. Thus, at this high dose level MTD was exceeded.

Table: Kidney tumours observed in rats after inhalation exposure to cobalt metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Single Sections (Standard Evaluation)				
Renal Tubule, Adenoma, Multiple	0	0	0	1
Renal Tubule, Adenoma (includes multiple)[b]	0	1	0	3
Renal Tubule, Carcinoma[c]	0	0	0	2
Renal Tubule, Adenoma or Carcinoma[d]	0	1	0	4
Step Sections (Extended Evaluation)				
Renal Tubule, Adenoma	3	1	1	3
Renal Tubule, Carcinoma	0	0	0	2
Renal Tubule, Adenoma or Carcinoma	3	1	1	5
Renal Tubule, Oncocytoma	0	0	1	0

Single Sections and Step Sections (Combined)				
Renal Tubule, Adenoma (includes multiple)	3	1	1	6
Renal Tubule, Carcinoma	0	0	0	2
Renal Tubule, Adenoma or Carcinoma				
Overall rate[e]	3/50 (6%)	1/50 (2%)	1/50 (2%)	7/50 (14%)
Adjusted rate[f]	7.5%	2.5%	2.4%	17.4%
Terminal rate[g]	0/17 (0%)	1/20 (5%)	1/16 (6%)	4/16 (25%)
First incidence (days)	678	729 (T)	729 (T)	691
Poly-3 test[h]	P=0.023	P=0.302N	P=0.294N	P=0.158

(T) Terminal kill

[a] Number of animals with lesion

[b] Historical control incidence for 2-year studies (all routes) (mean ± standard deviation): 1/100 (1.0% ± 1.41%), range 0%-2%

[c] Historical control incidence: 1/100 (1.0% ± 1.41%), range 0%-2%

[d] Historical control incidence: 1/100 (1.0% ± 1.41%), range 0%-2%

[e] Number of animals with neoplasm per number of animals with kidney examined microscopically

[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[g] Observed incidence at terminal kill

[h] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose/an exposure group is indicated by N.

Overall, there are some concerns also on the systemic tumours, mainly pheochromocytomas and pancreatic cancers, induced by the inhalation exposure to cobalt metal. Cobalt has been shown to be absorbed from the lungs and as discussed in the toxicokinetics chapter, its absorption from the gastrointestinal tract is also likely, although it may be more limited. There are no oral carcinogenicity studies available on cobalt or its compounds, which could provide evidence on the lack of cancer via other routes of exposure. Therefore, it is difficult to definitely exclude the possibility of induction of cancers via other routes of exposure. However, when taking into account the potential mechanisms of action of cobalt and the fact that these systemic cancers occurred mainly at the highest dose level, which is considered to exceed the MTD, they are likely to exert a threshold. At the second highest dose level, only the incidence of pheochromocytomas was increased. The mechanism for these pheochromocytomas is unclear but may be related to local lung effects and HIF-1 activation as has been postulated also in the case of nickel metal that has caused similar effects. Therefore, it is very likely that high doses are needed to induce systemic cancers by the oral route of exposure. On the other hand, local carcinogenicity in the gastrointestinal tract after oral exposure cannot be excluded, especially when taking into account that repeated dose toxicity studies with cobalt and cobalt chloride affect the gastro-intestinal tract and Kirkland *et al.* (2015) demonstrated nuclear anomalies (apoptotic changes) in the gastrointestinal-tract after single dose oral exposure (see 'RAC evaluation of germ cell mutagenicity').

Potency and mechanism of action

The DS calculated the T25 for cobalt according to the guidelines given in EC (1999). The lowest dose with increased tumour incidence of 1.25 mg cobalt/m^3 and the highest net tumour increase at this dose observed in male mice, for alveolar/bronchiolar carcinomas (78 and 22% in 1.25 and 0 mg/m^3 group, respectively, resulting in a net dose of 56%) was used for the T25 calculation. Correction factors were applied for dosing for 5 days/week instead of 7 (d*5/7) and for mg/m3 to mg/kg bw (d*1/3.9, default value as provided in the guidance). This results in a T25 of 1.25*5/7*1/3.9*25/56 = 0.10 mg cobalt/kg bw/d, which falls in the category of high potency carcinogen according to EC (1999), which gives a T25 limit of 1 mg/kg bw for high potency.

These rules for potency evaluation, however, assume a linear dose response. Therefore, there is a need to consider further potential mechanisms of action of cobalt and the dose response relationship. As also discussed in the RAC reference dose response document for the soluble cobalt salts (ECHA, 2016), the mode of action of lung carcinogenicity of cobalt is likely to involve

mechanisms which exert a threshold. The cobalt ion is not directly mutagenic although it can cause clastogenic chromosal damage. The main mechanisms of the DNA damage caused by cobalt are 1) induction of ROS and oxidative stress, 2) impairment of DNA repair and, 3) stabilisation of hypoxia-inducible factor HIF-1α. The cobalt(II) ions are able to induce the formation of reactive oxygen species (ROS) both *in vitro* and *in vivo*, and furthermore they catalyse the generation of hydroxyl radicals from hydrogen peroxide in a Fenton type reaction. NTP (1998) evaluated oncogene alterations in tumours induced by cobalt metal and found tumours with K-ras alterations in 67% of mouse pulmonary neoplasms and 31% of rat pulmonary neoplasms. Exon 1 codon 12 G to T transversions were the most common mutation observed (80% of mouse K-ras alterations and 57% of K-ras alterations) in the rat. These types of mutations are known to be related to reactive oxygen species and support the role of ROS in the carcinogenicity of cobalt.

Inhibition of repair of DNA damage by cobalt may include substitution of cobalt ions for zinc ions resulting in proteins with modified catalytic activity or substitution of cobalt for magnesium in DNA polymerases or topoisomerases or modulation of the DNA binding capacity of p53 protein by cobalt(II) ions. As discussed in the case of pheochromocytomas, there is experimental support for the involvement of HIF-1 activation in cobalt-induced carcinogenesis. HIF-1α is a major regulator of the adaptation of cancer cells to hypoxia and may contribute to tumour development and progression by decreasing both repair and removal of mutated cells, selecting for cells with genetic instability, reducing p53 transcriptional activity, evading growth arrest checkpoints, and inducing apoptosis resistance.

All of these three possible modes of action proposed for the carcinogenic effects of cobalt ion may involve a threshold, although there are some uncertainties pertaining to whether the initial event of a catalytic effect of the cobalt(II) ions leading to oxidative DNA damages through a Fenton-like mechanism can be considered a threshold or a non-threshold effect (ECHA, 2016). In the case of lung carcinogenesis caused by cobalt dust, a particle effect and local tissue damage are also likely to play a role. It should be noted that at the doses resulting in increased cancer levels, chronic inflammation and proteinosis were observed together with hyperplasia. It cannot be concluded whether the induction of alveolar proteinosis, chronic inflammation and hyperplasia (threshold events) are prerequisites for the development of a carcinogenic response of Co(II) in lungs, but it is likely that they are contributing the lung carcinogenicity of cobalt.

The fact that there is no clear evidence on the carcinogenicity in humans regardless of long term use of cobalt may be related to the low exposure levels.

Overall evaluation and comparison with the criteria

A substance should be classified in Category 1A if it is known to have a carcinogenic potential in humans. Category 1A is largely based on human evidence. Category 1A requires that human studies establish a causal relationship between human exposure to a substance and the development of cancer. There are few epidemiological studies suggesting a correlation between cobalt exposure and lung cancer. However, in all these studies there is co-exposure to other carcinogens, limiting the suitability of these studies for classification purposes. In addition, the recent large international study from cobalt-exposed hardmetal workers provided no consistent evidence on the association between cobalt exposure and lung cancer. Therefore, Cat. 1A is not applicable for cobalt.

Category 1B is indicated in the case of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in at least two species or in two independent studies in one species. In the case of cobalt, these criteria are fulfilled since increased incidence of lung cancers were observed in both rats and mice in both sexes after inhalation exposure. Supporting

information is available from studies with cobalt sulphate heptahydrate. There is no data available on the carcinogenicity via other routes of exposure.

According to the CLP regulation the route of exposure should be stated if it is conclusively proven that no other routes of exposure cause the hazard. For soluble cobalt salts, inhalation has been specified as the relevant route of exposure, but the classification of these compounds was made before the CLP regulation came into force. There are no oral and dermal carcinogenicity studies available on cobalt or its compounds which could provide evidence on the lack of cancer via other routes of exposure. Cobalt has been shown to be absorbed from the lungs and, as it is discussed in the toxicokinetics chapter, absorption from the gastrointestinal tract is also likely, although it may be more limited than for soluble salts. In addition, there are some concerns on the systemic carcinogenicity of cobalt since pheochromocytomas and pancreatic cancers were observed after the inhalation exposure to cobalt metal. Since these systemic cancers were occurring only close to or above the MTD and are likely to exert a threshold, it is very likely that high doses are needed to induce systemic cancers by oral route of exposure (if they are induced at all). Nevertheless, this reasoning cannot to be used to exclude the possibility of cancer via other routes of exposure and to justify the classification of cobalt as a carcinogen via the inhalation route only. Also, local carcinogenicity in the gastrointestinal tract after oral exposure cannot be excluded especially when taking into account that repeated dose studies with cobalt and cobalt chloride affect the gastro-intestinal tract and Kirkland *et al.* (2015) demonstrated nuclear anomalies (apoptotic changes) in the gastrointestinal-tract after single dose oral exposure (see 'RAC evaluation of germ cell mutagenicity'). Therefore, RAC proposes to classify cobalt as **Carc. Cat. 1B (H350) without specifying the route of exposure.**

Specific Concentration Limit

CLP Article 10.1 allows the use of specific concentration limits (SCL) based on the potency of the carcinogen(s). Calculation of T25 for carcinogenicity can be used to assist in establishing SCLs for carcinogens together with considerations of the cancer mechanisms, toxicokinetic factors and e.g. the shape of the dose response relationship. A T25 below 1 mg/kg bw as an oral dose is considered as a limit for high potency carcinogens, for which a specific concentration limit of 0.01% could be applied unless there are additional elements which decrease the concern. These include non-linear dose response, non-genotoxic mechanisms and lower sensitivity of humans to the mechanisms behind the cancers or e.g. toxicokinetic differences.

For cobalt, the DS calculated a T25 of 0.1 mg/kg bw, which falls in the category of high potency carcinogens according to EC (1999). The starting assumption for this potency grouping is an assumption of a linear dose response relationship. However, the three main modes of action proposed for the carcinogenic effects of cobalt ion (ROS and oxidative stress, inhibition of DNA repair and upregulation of HIF-1α) are mechanisms, which are likely to have a threshold, although there are some uncertainties related to the threshold for oxidative damage. A possible threshold mode of action (and therefore lower potency at low exposure levels) could partly explain the lack of clear evidence from epidemiological studies on the carcinogenicity of cobalt regardless of its long term use.

In the case of lung carcinogenesis caused by cobalt dust, a particle effect and local tissue damage are also likely to play a role. It should be noted that at the doses resulting in increased cancer levels, chronic inflammation and proteinosis were observed together with hyperplasia. Whether the induction of alveolar proteinosis, chronic inflammation and hyperplasia are prerequisites for the development of a carcinogenic response of Co(II) in lungs cannot, however, be concluded but it is very likely that they have contributed the lung carcinogenicity of cobalt.

According to the guidelines (EC, 1999), a non-linear (sublinear) dose response can be used to justify the move of substances near the potency borders into a lower potency group. The guideline does not define what is "near the potency borders" but in the case of cobalt, the T25 is an order of magnitude lower than the potency border and is therefore not considered to be "near the potency border". It can be argued on the basis of the epidemiological data that the potency of cobalt in humans is far lower than in animals and that this should be taken into account when considering the SCL. Indeed, in humans, no consistent evidence of increased cancer mortality (including lung cancer mortality) was seen in a recent study in hardmetal workers even though exposures in the highest exposure categories were up to the level of T25 observed in animals. Although this decreases the concern for carcinogenicity in humans, the current SCL criteria are based on animal data and the lack of human epidemiological evidence is not given as an element which could be used to move the substance into a lower potency group and no guidance for these cases is given. **Thus, based on the calculated T25, a specific concentration limit of 0.01% is proposed for cobalt.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Twenty-seven studies evaluating reproductive toxicity of cobalt were presented in the CLH report. Sexual function and fertility was assessed in 7 oral and 12 inhalation studies, developmental toxicity in 5 oral studies, and both endpoints in 3 oral studies.

Only one oral study with cobalt metal (cobalt powder, CDI/CORC 2015a) was available. In other oral studies cobalt chloride (hexahydrate) or cobalt sulphate (heptahydrate) were used.

Six inhalation studies with cobalt metal were described, while in the other 6 inhalation studies cobalt sulphate heptahydrate was applied.

Out of these 27 studies, only three were guideline compliant studies: CDI/CORC 2015a, Combined repeated dose toxicity and reproduction screening study in rats (according to OECD TG 422), CDI/CORC 2015b, a 3-month oral (gavage) study in rats with cobalt chloride hexahydrate (in a 90-day repeated dose toxicity study, according to OECD TG 408), and CDI/CORC 2015c, Prenatal developmental toxicity (PNDT) study with cobalt chloride hexahydrate in pregnant rats (according to OECD TG 414).

A. Sexual function and fertility

Studies evaluating adverse effects of cobalt on sexual function and fertility that are presented in the CLH report are listed below.

1. <u>Studies with cobalt metal</u>
   1.1. Oral exposure
   CDI/CORC 2015a - Combined repeated dose toxicity and reproduction screening study in rats with cobalt metal
   1.2. Inhalation exposure - six NTP inhalation studies with cobalt metal in male and female F344/N or F344/NTac rats and B6C3F1/N mice:
      1.2.1 NTP 2014a: 16-day inhalation study in rats with cobalt metal
      1.2.2. NTP 2014b: 14-week inhalation study in rats with cobalt metal
      1.2.3. NTP 2014c: combined repeated dose and carcinogenicity study in rats with cobalt metal
      1.2.4. NTP 2014d: 17-day inhalation study in mice with cobalt metal

1.2.5. NTP 2014e: 14-week inhalation study in mice with cobalt metal
1.2.6. NTP 2014f: combined repeated dose and carcinogenicity study in mice with cobalt metal

2. Studies with cobalt compounds

2.1. Oral expsoure

Seven non-guideline studies published in peer-reviewed journals and one guideline study (CDI/CORC 2015b; confidential report) were available with cobalt chloride hexahydrate:

2.1.1. Pedigo and Vernon, 1993: Dominant lethal assay in male mice
2.1.2. Elbetieha et al., 2008: Dominant lethal assay in male mice
2.1.3. Nation et al., 1983: 69-day diet study in male rats
2.1.4. Pedigo et al., 1988: 3-month oral (drinking water) study in male mice
2.1.5. Anderson et al., 1992: 13-week oral (drinking water) study in male mice
2.1.6. Mollenhauer et al., 1985: 98-day diet study in rats
2.1.7. Corrier et al., 1985: 3-month diet study in male rats
2.1.8. CDI/CORC 2015b: 3-month oral (gavage) study in rats.

2.2. Inhalation exposure - six NTP inhalation studies with cobalt sulphate heptahydrate in F344/N rats and B6C3F1 mice:

2.2.1. NTP 1991a: 16-day inhalation studies with cobalt sulphate heptahydrate in rats and mice
2.2.2. NTP 1991b: 13-week inhalation studies with cobalt sulphate heptahydrate in rats and mice
2.2.3. NTP 1998: Toxicology and carcinogenesis inhalation studies of cobalt sulphate heptahydrate in rats and mice.

Conclusion of the Dossier Submitter proposal

The DS concluded that although actual effects on fertility are only tested and shown in one species, namely mice, the effects on sperm parameters are consistently observed in both mice and rats, and with several cobalt compounds, including cobalt metal. The absence of such effects in the two recent regulatory studies (CDI/CORC, 2015a and c) could be explained by the low dose level in the oral 90-day study with cobalt chloride and by the short exposure duration and possibly low bioavailability of cobalt after gavage exposure.

Adverse effects on the reproductive system were observed in multiple studies and in two species. Therefore, the DS considered that there is clear evidence for an effect on (male) fertility, and proposed to classify cobalt metal as Repr. 1B.

A specific concentration limit for Repr. 1B, H360F was not proposed, since severe effects on male reproductive organs and on fertility were only seen at dose levels above 4 mg/kg bw/d.

B. Development

In the CLH report, developmental toxicity was evaluated in the following studies:

1. CDI/CORC, 2015a: Combined repeated dose toxicity and reproduction screening study in rats with cobalt metal
2. Szakmáry *et al.,* 2001: study with cobalt sulphate heptahydrate in pregnant mice, rats and rabbits
3. Domingo *et al.,* 1985: study with cobalt chloride hexahydrate in pregnant rats
4. Paternain *et al.,* 1988: study with cobalt chloride hexahydrate in pregnant rats
5. Elbetieha *et al.,* 2008: Dominant lethal assay in male mice (cobalt chloride)
6. Pedigo and Vernon, 1993: Dominant lethal assay in male mice (cobalt chloride)

7. CDI/CORC, 2015c: PNDT study with cobalt chloride hexahydrate in pregnant rats

Conclusion of the Dossier Submitter's proposal

A limited number of developmental studies are available, and some of them showed effects on development in rats and mice (including reduced body weight and body length, reduced viability and malformations of skeleton and urogenital system), at doses that were not toxic to the dams. However, no specific type of malformation was statistically significant and the effects were not observed in a PNDT guideline study (OECD TG 414) in rats (CDI/CORC, 2015d), at comparable level of exposure to cobalt.
In a study with soluble cobalt salt death of dams during delivery was observed in rats, but there were limitations in reporting, it is unclear whether this effect is a reproductive effect or maternal toxicity, and the effects were not observed in the screening study with cobalt powder (CDI/CORC, 2015b). More precisely, dam mortality around GD 20 and 21 was observed in the study with cobalt metal, but also at other time points (mating and lactation). In a study with soluble cobalt compounds, postnatal mortality (on PND 5) was increased at a dose level without maternal toxicity, as well as in the Domingo *et al*. study (1985), at dose levels with unknown maternal toxicity. The DS highlighted limitations of both studies, and did not consider these effects reliable enough for classification purposes.
To summarise, the DS considered that classification for developmental toxicity is not warranted.

Comments received during public consultation

Three MSCAs supported classification as Repr. 1B; H360F, and two supported no classification for developmental effects. One MSCA stated that "although there is no strong evidence of a developmental effect, this endpoint should be evaluated carefully".
Other comments were provided by Industry, trade associations or individuals. These comments argued against classification as Repr. 1B; H360F, and in support of self-classification as Repr. 2. The majority of the comments were related to socio-economic implications of this classification, and anticipated changes in the manufacturing process, and some of them were related to classification of cobalt-containing alloys.

The main comments that were related to scientific aspects of classification for reproductive effects (primarily from CDI/CORC), included:

- in the NTP Co metal inhalation studies, all effects on testes were observed in the presence of severe lung toxicity, sometimes with haematological effects, and, therefore, fertility effects were considered to be secondary to hypoxia;
- in the studies with cobalt metal, no fertility effects were observed at below the MTD;
- the three GLP-compliant CDI/CORC studies on cobalt metal powder and cobalt chloride, are the key studies for the endpoint reproductive toxicity that should be considered as primary evidence, showed no effects on reproductive endpoints;
- fertility effects in the 3-month NTP inhalation study with cobalt metal in mice were observed at dose levels at which respiratory toxicity was also present;
- no fertility effect was observed in the 2-year NTP studies with cobalt sulphate (in rats and mice), although severe lung toxicity was observed;
- limitations of non-guideline studies published in peer-review journals were stressed (primarily regarding lack of reporting of general toxicity and data on feed/water consumption);
- "in accordance with the ECHA Guidance on the preparation of CLH dossiers (Version 2.0, August 2014), a decision on the classification proposal for fertility impairment of cobalt metal should be postponed, in the light of the fact that a testing proposal for a EOGRTS (extended one generation reproductive toxicity study) has been submitted by CoRC for

the soluble cobalt substances group, in which cobalt metal is included"; Industry, therefore, proposed "to remove the entire section on reproductive toxicity and to re-open the CLH procedure for the endpoint reproductive toxicity, after the proposed experimental testing is final".

Assessment and comparison with the classification criteria

Fertility

Males

Fertility effects in male animals exposed to cobalt metal or soluble cobalt compounds (cobalt chloride, cobalt sulphate), at dose levels that did not induce marked general (systemic) toxicity, were observed in inhalation studies in rats and mice, and oral studies in mice (see tables below).

Inhalation studies in rats with cobalt metal

In NTP inhalation studies with cobalt metal in rats, reduced testis weight and sperm motility, and increased incidence of testicular infarction were observed.

A decrease in absolute (by 33% compared to control) and relative (by 18%) **testis weight** was observed in males administered 10 mg Co/m^3 in a 16-day inhalation study in rats (NTP, 2014a). At this dose level, a 20% reduction of body weight was noted in males, with 15-23% reduced absolute weights of liver, kidney and thymus, as well as increased lung absolute (12%) and relative (41%) weight. However, no mortality or clinical signs of toxicity were present at this dose level, and lung changes (cytoplasmic vacuolisation and necrosis of bronchiolar epithelium, interstitial fibrosis) were described to be of minimal to mild grade of severity. Histopathology of testes was not performed at this dose level (only for 0, 20 and 40 mg Co/m^3 groups), and sperm analysis was not performed in the study. Nevertheless, absolute testis weight is considered as a precise indicator of gonadal injury and a significant increase (or decrease) is indicative of an adverse effect (US EPA, 1996). According to ECHA CLP Guidance (2017), "Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes". Regarding interpretation of severity of a 20% reduction in body weight in terms of systemic toxicity, RAC is not aware of any quantitative limit or guidelines regarding the magnitude of the effect. It has been recognised that severe reduction in food intake and body weight can damage spermatogenesis (Haschek *et al.*, 2009). Reduced testosterone, epididymal sperm and testicular spermatids were found in CD mice maintained for 90 days at 70% of control body weight. However, no effect on testicular or sperm parameters were observed either in rats at this level of body weight reduction, or in mice maintained at 80% or 90% of control body weight (Haschek *et al.*, 2009). These results can serve only as a general example, since body weight was reduced by a diet restriction *per se*, without exposure to a toxicant.

In conclusion, NTP study results described above indicate that the observed reduction in testis absolute weight is not a secondary consequence of marked systemic toxicity, and that direct effect of cobalt on male fertility cannot be ruled out. The CLP Guidance points out that "There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity."

In a 14-week inhalation study in rats (NTP, 2014b) **sperm motility** was slightly (by 8%), but statistically significantly reduced at the top dose, 5 mg/m^3, and it was part of a dose response trend (according to OECD Draft guidance document on reproductive toxicity testing and

assessment, 2004, there is a 95% probability of detecting a change of 6% in a sperm motion parameter with a group size of 10 rats, if the study methodology is adequate). No effects on weight and morphology of male reproductive organs were observed. At this dose level (5 mg/m^3) a reduction in body weight (by 7%), pathological changes in respiratory system (lung changes graded as minimal to mild, except for mild to moderate alveolar proteinosis and degeneration of olfactory epithelium), and changes in haematological (e.g. red blood cell count (RBC) increase by 29%) and clinical biochemistry parameters (decrease in cholesterol and blood glucose) were noted in males. Mortality or clinical signs of cobalt toxicity were not observed. Nevertheless, since the effect on sperm motility in this study was very small in magnitude it is considered only as supportive evidence for cobalt-related effects on male fertility.

It is pointed out in the CLH report that it was suggested that "the effects on the testes can be caused by the increase in RBC causing a slow blood flow resulting in a reduction in oxygen supply to the testes". This hypothesis is not supported by the inhalation study with cobalt metal in mice (14-week study, NTP 2014e), in which, in the presence of only subtle increase in red blood cells count (<5%), a 97% decrease in sperm motility (compared to controls), as well as other fertility effects, were observed in males.

Increased incidence of **testis infarction** was noted in rats exposed to 5 mg Co/m^3 in combined repeated dose and carcinogenicity study in rats (NTP, 2014c). Although at this dose level the survival rate was not affected, significant (29%) reduction of body weight at the end of the study was noted, as well as clinical signs of toxicity (abnormal breathing and thinness) and increased number of non-neoplastic and neoplastic changes (non-neoplastic lung changes were graded as moderate to marked). These findings, in RAC's opinion, limit the relevance of testis infarction as a fertility effect for classification purposes.

Inhalation studies in rats with cobalt sulphate

In 16-day, 13-week and 2-year NTP (1991a,b, 1998) inhalation studies with cobalt sulphate in rats, fertility effects were not observed, except for **testis atrophy** (with decreased number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts) in animals dosed at 19 mg Co/m^3 in the 16-day study. However, at this dose level, 40% mortality was present in males, as well as a 47% reduction in body weight. Beside the lesions in respiratory tract typical for inhalation exposure to cobalt, thymus and liver necrosis were found, and congestion of vessels in the brain and meninges. Although in this study pronounced general toxicity limits the relevance of testis atrophy as a fertility effect for classification purposes, this change fits in the pattern of cobalt-related fertility effects observed in the studies without marked systemic toxicity.

Table: Overview of inhalation studies in rats

Dose (mg Co/m^3)	2 weeks *#		3 months		2 years *#	
	Co metal	$CoSO_4 \times 7H_2O$	Co metal	$CoSO_4 \times 7H_2O$ **	Co metal	$CoSO_4 \times 7H_2O$
76		**100% mortality**				
40	**100% mortality**					
19 - 20	**100% mortality**	**40% mortality,** clinical signs, 47%↓ body wt, ↓thymus wt, ↑ lung wt, resp. organs lesions, thymus and liver necrosis, brain vessels congestion, testis atrophy				
10 - 11.4	20%↓body wt, ↓liver and kidney wt, ↑ lung wt, resp. organs lesions (min.-mild), 33% ↓ testis a. wt			clinical signs, 14%↓body wt, ↑ lung wt, resp. organs lesions, 32%↑ RBC		
3.8 - 5	↓liver wt, resp. organs lesions (min.-mild)		29%↑ RBC, ↑ lung wt, resp. organs lesions (min.-mod.), 8%↓ sperm motility	↑ lung wt, resp. organs lesions, 17%↑ RBC	29%↓ body wt, resp. organs lesions (mod.-marked), infarct testes	
1.9 - 2.5	↓liver wt, resp. organs lesions (min.-mild)	red discoloration and increased firmness in the lungs (no histologic examination)	28%↑ RBC, ↑ lung wt, resp. organs lesions (min.-mild), 6%↓ sperm motility		11%↓ body wt, resp. organs lesions (min.-mod.)	
1 - 1.3			22%↑ RBC, ↑ lung wt, resp. organs lesions (min.-mild), 3%↓ sperm motility	↑ lung wt, resp. organs lesions, 4%↑ RBC	resp. organs lesions (min.-mod.)	resp. organs lesions
0.3 - 0.6			5%↑ RBC, ↑ lung wt, resp. organs lesions (min.-mild)	↑ lung wt, resp. organs lesions		resp. organs lesions
0.1 - 0.2		(no histologic examination)		↑ lung wt, resp. organs lesions		resp. organs lesions

*No sperm analysis performed; # No haematology performed; ** Sperm analysis not performed at 0.1 mg Co/m3
RBC - red blood cells count; wt - weight; a. - absolute; resp. - respiratory; min. - minimal; mod. - moderate

Inhalation studies in mice with cobalt metal

Absolute **testis weight** was significantly lower (by 29% compared to controls) in mice dosed at 40 mg/m^3 (highest dose tested) in the 17-day inhalation study in mice (NTP, 2014d). However, at this dose level mortality occurred (2 out of 5 males died), body weight was decreased (by 27% in males), and pulmonary toxicity was observed (clinical signs, increased lung weight, increased incidence of non-neoplastic lesions of the lung). Although severe general toxicity observed at a dose level at which fertility effect occurred limits its relevance for classification purposes, decreased testis weight was also observed in a mice study of a longer duration (14 weeks, NTP, 2014e), in which systemic cobalt toxicity was not pronounced.

Number of fertility indices of affected male were observed in the 14-week inhalation study in mice (NTP, 2014e). Decreased testis weight was observed in males dosed at 5 mg/m^3 (by 13% in absolute weight and 10% in relative weight) and 10 mg/m^3 (by 73% in absolute weight and 68% in relative weight). Decreased epididymis weight was noted at 10 mg/m^3 (by 23% in relative weight of cauda epididymis, and by 29% in epididymis absolute weight). The number of spermatids (per testis) was decreased at 5 and 10 mg/m^3 (by 15% and 98%, respectively), and already at 2.5 mg/m^3 a decrease in sperm motility (by 5%) and sperm number (by 8-9%) was noted. The severity of these effects increased in a dose-related manner (e.g. sperm motility at the top dose decreased by 97% compared to controls, and sperm number per cauda epididymis by 94%). At the highest dose tested (10 mg/m^3), histopathologic findings in the testis (marked degeneration of germinal epithelium) and epididymis (exfoliated germ cell and hypospermia with average severity of moderate grade; minimal cytoplasmic vacuolisation and atrophy) were observed. In this study, exposure to cobalt did not induce mortality even at the highest dose tested, and only at the highest dose reduced body weight (by 14%) and clinical sign of toxicity (abnormal breathing) were observed in males. At this dose level, lung weight increased by 50% and histopathological lung changes were predominately moderate. Although already at lower doses increased lung weight was observed (by 15% at 2.5 mg/m^3 and 35% at 5 mg/m^3), histopathological lung changes at these dose levels were minimal to mild, except for cytoplasmic vacuolisation of bronchiolar epithelium which was mild to moderate at 5 mg/m^3. Haematological

changes were observed only at the highest dose tested, and were minimal (< 5% increase compared to control).

RAC considers that the effects on male fertility in the study were observed in the absence of marked systemic cobalt toxicity, and are therefore relevant for classification purposes.

In the combined repeated dose and carcinogenicity study in mice (NTP, 2014f) an increased incidence of minimal to mild germinal epithelium degeneration was noted at 5 mg/m^3 (highest dose tested). At the same dose survival of males was significantly lower compared to controls (44% *vs.* 78% in controls) and final body weight was 23% lower. At this dose also increased number and severity of non-neoplastic and neoplastic lung lesions was noted. RAC considers that marked general toxicity observed at a dose level at which fertility effect occurred limits its relevance for classification purposes, but points out that the effect observed (germinal epithelium degeneration) fits in the spectrum of cobalt-related fertility changes described in the studies without marked systemic toxicity.

Inhalation studies in mice with cobalt sulphate

Fertility effects were observed in 13-week inhalation study in mice (NTP 1991b). At the highest dose, 11.4 mg Co/m^3, decreased testis absolute weight (by 52% compared to control), decreased epididymal absolute weight (by 19%), atrophy of the testis with a loss of germinal epithelium in the seminiferous tubules, 3-fold increased abnormal sperm count, and 46% decreased sperm motility were found. At this dose level marked general toxicity was observed, with 20% mortality and 77% increase in lung absolute weight. Nevertheless, 13% and 10% decrease in sperm motility was observed at lower doses as well (3.8 and 1.1 mg Co/m^3, respectively; sperm parameters were not analysed at lower doses), at which general toxicity was not pronounced. Namely, at doses below 11.4 mg Co/m^3, there was no mortality or significant changes in body weights (compared to controls). There was an 18% increase in lung absolute weight at 3.8 mg Co/m^3, and no increase at 1.1 mg Co/m^3. No consistent or dose-related haematological effects were observed at any dose level. RAC considers that the effects on sperm motility observed at dose levels without marked systemic cobalt toxicity are relevant for classification purposes.

Table: Overview of inhalation studies in mice

Dose (mg Co/m^3)	2 weeks*#		3 months		2 years*#	
	Co metal	$CoSO_4 \times 7H_2O$ $	Co metal	$CoSO_4 \times 7H_2O$ *	Co metal	$CoSO_4 \times 7H_2O$
76		**100% mortality**				
40	**60% mortality**, 27%↓body wt, clinical signs, ↓liver wt, ↑ lung wt, resp. organs lesions (min.-mod.), 29%↓ testis a. wt					
19 - 20	9%↓body wt, clinical signs, ↓liver wt, ↑ lung wt, resp. organs lesions (min.-mild)	**80% mortality**, 33%↓body wt, clinical signs, ↓thymus wt, ↑ lung wt, resp. organs lesions				
10 - 11.4	↓liver wt, ↑ lung wt, resp. organs lesions (min.-mild)		14%↓ body wt, clinical signs, 5%↑ RBC, ↓liver and kidney wt, ↑ lung wt, resp. organs lesions (min.-marked), 73%↓ testis a. wt, 29%↓ epididymis a. wt, 98%↓ spermatid heads count, 94%↓ sperm count, 97%↓ sperm motility	**20% mortality**, 14%↓body wt, ↑ lung wt, resp. organs lesions, testis atrophy, 19%↓ epididymis wt, 3x↑ abnormal sperm count, 46%↓ sperm motility		
3.8 - 5	↓liver wt, ↑ lung wt, resp. organs lesions (min.-mild)		↓kidney wt, ↑ lung wt, resp. organs lesions (min.-mild), 13%↓ testis a. wt, 15%↓ spermatid heads count, 17-20%↓ sperm count, 4%↓ sperm motility	↑ lung wt, resp. organs lesions, 13%↓ sperm motility	**44%↓ survival**, 23% body wt, resp. organs lesions (min.-marked), ↑ germinal epithelium degeneration (testis)	
1.9 - 2.5	↓liver wt, ↑ lung wt, resp. organs lesions (min.-mild)	resp. organs lesions	↑ lung wt, resp. organs lesions (min.-mild), 5%↓ sperm motility		**28%↓ survival**, 8%↓ body wt, resp. organs lesions (min.-mod.)	
1 - 1.3			resp. organs lesions (min.-mild) (no sperm analysis)	resp. organs lesions, 10% sperm motility	resp. organs lesions (min.-mild)	10%↓ body wt, resp. organs lesions
0.3 - 0.6			resp. organs lesions (min.-mild) (no sperm analysis)	resp. organs lesions (no sperm analysis)		resp. organs lesions
0.1 - 0.2		(no histologic examination)		resp. organs lesions (no sperm analysis)		resp. organs lesions

*No sperm analysis performed; # No haematology performed; *No significant effect on RBC count; RBC - red blood cells count
wt - weight; a. - absolute; resp. - respiratory; min. - minimal; mod. - moderate

Oral studies in mice

In all four oral studies in mice (non-guideline studies from open literature), performed with cobalt chloride hexahydrate in drinking water, fertility effects were observed (decreased male reproductive organs weight, sperm count, fertilisation rate, histopathologic changes in testis).

However, in two of these studies general toxicity data are not presented (Pedigo and Vernon, 1993, Anderson *et al.*, 1992).

In one study (Elbetieha *et al.*, 2008, DLA in male mice), general toxicity data are given (mortality, body weight, clinical signs of toxicity), but there are various deficiencies in the methodology and reporting. Namely, lower number of animals was used than recommended by the OECD test guideline, and evidence of mating (e.g. number of sperm-positive females), positive control, and historical control data are not stated. Also, no further information is provided regarding pathological changes in animals that died during the exposure period (no clinical signs of toxicity were observed in surviving animals in groups in which mortality occurred). In this study, a dose-dependent decrease in testis absolute weight and sperm count, as well as changes in fertility indices (ratio between pregnant and mated females, number of resorptions) were observed, including doses with 10-20% mortality. Nevertheless, 10% decreased testis absolute weight, 13% decreased epididymal sperm count (per mg tissue), and increased number of resorptions were noted at the lowest dose level, 6.4 mg Co/kg bw/d, at which no mortality occurred and body weight was decreased by only 5% compared to controls. In the opinion of RAC, however, the study presents only supportive evidence for cobalt effects on male fertility, bearing in mind methodological and reporting deficiencies.

In the well-reported Pedigo *et al.* (1988) 3-month oral study in mice (*Dose response study*), pronounced, dose-related effects on male fertility were observed at dose levels without marked systemic toxicity (no mortality, approximately 10% decrease in body weight at the top dose, no statistically significant effect on haematocrit in any dose group). These effects included up to 70% decreased testicular relative weight and up to 92% decrease in epididymal sperm concentration. At the top dose, 72.1 mg Co/kg bw/d, sperm motility was also decreased (by 58% compared to controls), and fertility (expressed as % ova fertilised) was decreased by 90%. The effects observed at the top dose were reproduced (with similar magnitude) in the second part of the study (*Time course study*).

Although the study is a non-guideline study, and a relatively small number of animals (5 per dose) was used, RAC is of the opinion that the study's methodology and reporting is adequate enough to consider fertility effects observed in this study as relevant for classification purposes.

Table: Overview of oral studies in mice

Dose (mg Co/kg bw/day)	Pedigo & Vernon 1993	Elbetieha et al. 2008	Pedigo et al. 1988*	Anderson et al. 1992
67 - 72	98%↓ fertilisation rate, 59%↓ testis wt, 85%↓ sperm count, general tox?		10%↓ body wt, 90%↓ fertility, 70%↓ testis r. wt, 92%↓ sperm count	↓ testis size, testicular congestion, degeneration, germinal epitel damage, general tox?
42			48%↓ testis wt, 71%↓ sperm count	
23		**20% mortality**, 7%↓ body wt, ↓fertility, 29%↓ testis a. wt, 8%↓ epididymis a. wt, 77%↑ seminal vesicles, 22%↓ epid. sperm count	29%↓ testis wt, 34%↓ sperm count	
11.6		**10% mortality**, 6%↓ body wt, ↓fertility, 14%↓ testis a. wt, 54%↑ seminal vesicles, 14%↓ epid. sperm count		
6		5%↓ body wt, ↓ fertility, 10%↓ testis a. wt, 13%↓ epid. sperm count		
	12 -13 weeks exposure (cobalt chloride hexahydrate in drinking water)			

wt - weight; a. - absolute; r. - relative; general tox - general toxicity; epid. - epididymal; *No significant effect on haematocrit

Oral studies in rats

RAC considers that fertility effects observed in oral studies in rats could not be adequately evaluated. In two out of five of these studies fertility effects were observed but general toxicity was not reported (Nation *et al.*, 1983; Mollenhauer *et al.*, 1985), and in two studies (Corrier *et al.*, 1985; Mollenhauer *et al.*, 1985) fertility effects (testicular degeneration, degenerative and necrotic changes in the germinal epithelium) were observed in the congested testes. In the Corrier *et al.*, 1985, study moderate to marked testicular congestion and 41% increase in red blood cells count was observed (compared to control), and the abdominal viscera, blood and testes of the exposed rats were dark-red and cyanotic. Testicular changes could therefore be a secondary effect of cobalt-induced polycythaemia that may have produced a prolonged state of tissue hypoxia.

In two out of five oral studies in rats (CDI/CORC 2015a,b; studies with cobalt metal and cobalt chloride hexahydrate), fertility effects were not observed. In the oral CDI/CORC (2015b) rat study in which cobalt chloride was given (by gavage), polycythaemia was also observed, but the doses applied (up to 7.4 mg cobalt/kg bw/d) were below the dose shown to produce testicular effects in the other three oral rat studies (20 mg cobalt/kg bw/d; Corrier *et al.,* 1985, Nation *et al.*, 1983 and Mollenhauer *et al.*, 1985). It should be stressed that RAC does not consider that the negative CDI/CORC oral study with cobalt chloride in rats contradicts the positive oral studies in mice. Rats seem to be, in general, less sensitive to cobalt-related fertility effects, compared to mice. For example, comparing the 3-month inhalation study with cobalt metal in rats and mice, it could be observed that at the same dose level (5 mg Co/m3), 8% decreased sperm motility was observed in rats, while in mice in addition to decreased sperm motility, decreased testis absolute weight (13%), spermatid count (15%) and sperm count (17-20%) was found. In rats, more pronounced effects on testis (testis atrophy, degeneration, infarction and necrosis) were present at doses at which also marked general toxicity was observed (e.g. mortality, marked decrease in body weight), or at which marked increase in RBC was found (e.g. 41%). Since possible secondary effect of hypoxia and blood congestion cannot be excluded in these cases, these effects were not considered relevant for classification purposes, and were taken into account as a supportive evidence only. Relatively lower doses applied in the CDI/CORC 3-month study in rats with cobalt chloride are, therefore, not expected to produce marked effects on rat testes. At the similar increase in RBC (22%) in the 3-month NTP inhalation study with cobalt metal in rats, only a slight decrease in sperm motility was observed (3%).

Contrary to the above-mentioned studies and other studies in rats (oral studies with cobalt chloride and inhalation studies with cobalt metal or cobalt sulphate), in the CDI/CORC (2015a) study in which cobalt metal was given as a powder (via gavage), polycythaemia was not observed (only small increase in haemoglobin values was observed in males, 4-8%), in spite of high doses applied (up to 1000 mg/kg bw/d) and 90% mortality at the top dose (1000 mg/kg bw/d). The findings indicate that systemic availability of orally given cobalt metal is lower compared to cobalt chloride, and that local effects in the gastrointestinal tract could contribute to morbidity (including lethality at the top doses in males and females), as proposed by the DS. Indeed, changes in the gastro-intestinal tract were observed in males dosed at 1000 mg/kg bw/d (reddened stomach, intestines, caecum or stomach) and in females dosed at ≥ 100 mg/kg bw/d (dose-related reddened, haemorrhagic foci, filled with fluid in the gastrointestinal tract). Gavage, as a method of application of cobalt, could contribute to the effects observed, i.e. reduced systemic availability (concentration of cobalt in the stomach is expected to be much higher after gavage exposure compared to a diet exposure, which may limit the dissolution of cobalt and therefore limit bioavailability; p. 29 in the CLH report) and local effects in the gastrointestinal tract, as pointed out by the DS. In addition, this study was of shorter duration compared to other oral studies in which fertility effects were observed (5-6 weeks *vs.* 10-14 weeks). For example, in the Pedigo *et*

al. (1988) oral study in mice with cobalt chloride, testis weight started to be significantly decreased at week 9, and fertilisation rate (% of fertilised ova) decreased after the 11th exposure week (at the top dose of 58.9 mg Co/kg bw/d, at which 10% body weight reduction was noted). Uncertainty, therefore, remains whether a diet study of longer duration (e.g. 3 months) with cobalt metal would show fertility effects similar to those observed in inhalation studies with cobalt metal in rats.

Table: Overview of oral studies in rats

Dose (mg Co/kg bw/day)	5 - 6 wk	10 wk	13 wk	14 wk	14 wk
	Cobalt metal	Cobalt chloride hexahydrate			
	CDI/CORC 2015 (gavage)	Nation et al. 1983 (diet)	CDI/CORC 2015 (gavage)	Mollenhauer et al. 1985 (diet)	Corrier et al. 1985 (diet)
1000	**90% mortality**, 16%↓ body wt, piloerection, ↑ spleen r. wt				
300	13%↓body wt, piloerection, ↓grip strength, ↑ spleen r. wt				
100	piloerection, ↓ grip strength, ↑ spleen r. wt				
30	↑ spleen r. wt				
20		42%↓ testis wt, testis atrophy general tox?		testicular congestion and degeneration general tox?	41%↑ RBC, testicular congestion, degeneration, necrosis
7.4			11%↓ body wt, 20%↑ RBC, bone marrow erythroid hyperplasia		
5		general tox?			
2.5			10%↑ RBC, bone marrow erythroid hyperplasia		
0.7			NS		

wt - weight; r. - relative; RBC - red blood cells count; NS - no significant findings; general tox - general toxicity

Changes in fertility parameters in females were not observed, except for a 19-20% longer oestrous cycle in top dose mice in the 14-week inhalation study with cobalt metal (NTP, 2014e) and the 13-week inhalation studies with cobalt sulphate heptahydrate (NTP, 1991b). Although the increases were statistically significant, the oestrous cycle length in both cases was within normal range for laboratory mice (4-6 days, according to the literature data; Byers *et al.*, 2012), i.e. average length at the top dose was 4.9 ± 0.36 days in a study with cobalt metal, and 5.00 ± 0.24 days in a study with cobalt sulphate.

Conclusion on fertility

To summarise, fertility effects in male animals exposed to cobalt metal or soluble cobalt compounds, at dose levels that did not induce marked general (systemic) toxicity, were observed in inhalation studies in rats and mice, and oral studies in mice.

The NTP (1991, 1998 and 2014) inhalation studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations, and in compliance with NTP chemical health and safety requirements (which must meet or exceed all applicable Federal, state, and local health and safety regulations), as well as audited by an independent quality assessment contractor.

RAC considers the studies to be of high reliability, and the effects of inhaled cobalt metal and cobalt sulphate heptahydrate on male fertility observed in these studies relevant for classification. These effects primarily include:

- decreased testis weight (in the 16-day inhalation study in rats with cobalt metal, and in the 14-week inhalation study in mice with cobalt metal);
- decreased epididymis weight (in the 14-week inhalation study in mice with cobalt metal);
- decreased number of spermatids and sperm number (in the 14-week inhalation study in mice with cobalt metal);
- testis atrophy and histopathologic changes in testis and epididymis (in the 14-week inhalation study in mice with cobalt metal);
- decreased sperm motility (in the 13-week inhalation study in mice with cobalt sulphate heptahydrate).

The following effects on male fertility were also observed in inhalation studies, but they occurred at dose levels at which marked general toxicity, including mortality, was present:

- testis infarction (in the combined repeated dose and carcinogenicity inhalation study in rats with cobalt metal);
- testis atrophy (in the 16-day inhalation study in rats, and in the 13-week inhalation study in mice with cobalt sulphate heptahydrate);
- decreased testis weight (in the 17-day inhalation study in mice with cobalt metal, and in the 13-week with cobalt sulphate heptahydrate inhalation study in mice);
- increased incidence of germinal epithelium degeneration (in the combined repeated dose and carcinogenicity inhalation study in mice with cobalt metal);
- increased abnormal sperm count (in the 13-week inhalation study in mice with cobalt sulphate heptahydrate).

The effects observed in the 3-month oral study in mice with cobalt chloride hexahydrate (Pedigo *et al.*, 1988), decreased testicular weight, epididymal sperm concentration, sperm motility and fertilisation rate, were noted at dose levels without marked systemic toxicity and are considered relevant for classification.

Fertility effects observed in male cobalt-exposed rats and mice in other oral studies are considered as a supportive evidence only, since in these studies either general toxicity was not reported (Nation *et al.*, 1983; Mollenhauer *et al.*, 1985; Pedigo and Vernon, 1993; Anderson *et al.*, 1992), or methodological and reporting deficiencies limit study reliability (Elbetieha *et al.*, 2008). In the Corrier *et al.*, 1985, study there was a possibility that the effect (degenerative and necrotic changes in the germinal epithelium) was a secondary consequence of cobalt-induced polycythaemia, and the same applies to the Mollenhauer *et al.*, 1985, study.

Comparison with the criteria

Effects on (male) fertility, observed primarily as dose-related testis toxicity, based on clear evidence in two animal species (mice and rats) at dose levels at which marked systemic cobalt toxicity was not observed, and which are not considered to be a secondary non-specific consequence of other toxic cobalt-related effects. RAC agrees with the DS that **cobalt metal should be classified as Repr. 1B; H360F**.

It is not proposed to specify the exposure route (fertility effects were observed both in inhalation and oral studies in rodents, and no dermal studies are available hence effects via this route cannot be excluded).

Specific Concentration Limits

Using linear extrapolation for epididymal sperm concentration data from the 3-month oral study with cobalt chloride hexahydrate in male mice study (Pedigo *et al.*, 1988), as the only oral study available in which toxic effect on fertility fulfilled the criteria for classification for reproductive toxicity, an ED_{10} of 11 mg Co/kg bw/d is derived (STATA SE 14.2; linear regression analysis). Since this value is above the limit value of 4 mg/kg bw/d for high potency, see CLP guidance (2017), and since route-to-route extrapolation (in this case inhalation-to-oral route) is associated with a high degree of uncertainty (ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8), **no SCL is proposed**.

Development

Although certain developmental effects were observed at dose levels without significant maternal toxicity, these effects were seen in non-guideline studies (research articles available in open literature; Szakmary *et al.*, 2001; Domingo *et al*., 1985; Elbetieha *et al.*, 2008) with limitations (e.g. deficient reporting, no maternal toxicity data presented), and they were contradicted by other studies with similar design (Paternain *et al.*, 1988; Pedigo and Vernon, 1993) and by two guideline studies (CDI/CORC, 2015a,c; studies with cobalt metal and cobalt chloride) which did not show developmental effects at doses without significant maternal toxicity.

In a study with cobalt sulphate in mice, rats and rabbits (Szakmary *et al*., 2001), retarded skeletal growth and increased incidence of skeletal malformations in mice, retarded skeletal and visceral growth, increased incidence of skeletal and urogenital malformations, and decreased perinatal survival and body weight gain in rats, were observed at dose levels without significant maternal toxicity. Due to the high mortality, RAC considers that the effects seen in rabbits are not relevant for classification.

In RAC's opinion, this study has several serious limitations, including lack of historical control data and deficient reporting (e.g. as noticed by the DS, it is stated in the article that the number of rat dams that died during delivery dose-dependently increased, but, according to study protocol these dams were processed, by opening of the uterus, on GD 21; for mice it is stated that increased frequency of foetuses with retarded body weight was found, but "retarded body weight" is not defined, i.e. quantified).

Postnatal survival, body weight gain and body length were decreased in the Domingo *et al.* (1985) study with cobalt chloride in pregnant rats, however maternal toxicity data are lacking, and there are some other deficiencies in reporting, as described previously.

Limitations of the Elbetieha *et al.*, (2008) study (Dominant lethal assay in male mice), in which reduced foetal survival was noted, were described in the fertility section. In addition, although decreased number of viable mice foetuses was observed in this study, there was no clear dose response pattern. Similarly, no dose response was observed in the number of females with resorptions, and the ratio between number of resorptions and number of implantations was markedly increased (5 times compared to control group) already at the lowest dose tested (6.4 mg Co/kg bw/d), although no effect on post-implantation loss and *in vitro* embryo development was observed at a 10 times higher level of exposure in mice (67 mg Co/kg bw/d in the Pedigo and Vernon (1993) study, with comparable study design). Taking also into account the previously mentioned study deficiencies, RAC considers these effects not robust enough to trigger classification for developmental effects.

Therefore, RAC agrees with the DS's proposal not to classify cobalt metal for developmental effects.

Overall, RAC agrees with the DS, and proposes to classify cobalt metal as **Repr. 1B; H360F** for effects on (male) fertility, without setting an SCL.

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).