Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

22 September 2017

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Cobalt

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

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

Index Number: **027-001-00-9**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***cobalt***
EC number:	***231-158-0***
CAS number:	***7440-48-4***
Annex VI Index number:	***027-001-00-9***
Degree of purity:	***80 - 100.0%***
Impurities[a]:	***Zinc oxide*** ***Cobalt sulphate*** ***Copper*** ***Iron*** ***Oxygen-containing species (e.g. Co_3O_4)*** ***Nickel***

[a]: limited to impurities included in the publically available information on the composition of cobalt on the ECHA dissemination site.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Skin Sens. 1; H317 Resp. Sens. 1; H334 Aquatic Chronic 4; H413
Current proposal for consideration by RAC	Muta 2; H341 Carc 1B; H350, SCL 0.01% Repr 1B; H360F

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens. 1; H317 Resp. Sens. 1; H334 Muta 2; H341 Carc 1B; H350, SCL 0.01% Repr 1B; H360F Aquatic Chronic 4; H413

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Out of the scope of this proposal
2.2.	Flammable gases				Out of the scope of this proposal
2.3.	Flammable aerosols				Out of the scope of this proposal
2.4.	Oxidising gases				Out of the scope of this proposal
2.5.	Gases under pressure				Out of the scope of this proposal
2.6.	Flammable liquids				Out of the scope of this proposal
2.7.	Flammable solids				Out of the scope of this proposal
2.8.	Self-reactive substances and mixtures				Out of the scope of this proposal
2.9.	Pyrophoric liquids				Out of the scope of this proposal
2.10.	Pyrophoric solids				Out of the scope of this proposal
2.11.	Self-heating substances and mixtures				Out of the scope of this proposal
2.12.	Substances and mixtures which in contact with water emit flammable gases				Out of the scope of this proposal
2.13.	Oxidising liquids				Out of the scope of this proposal
2.14.	Oxidising solids				Out of the scope of this proposal
2.15.	Organic peroxides				Out of the scope of this proposal
2.16.	Substance and mixtures corrosive to metals				Out of the scope of this proposal
3.1.	Acute toxicity - oral				Out of the scope of this proposal
	Acute toxicity – dermal				Out of the scope of this proposal
	Acute toxicity – inhalation				Out of the scope of this proposal
3.2.	Skin corrosion / irritation				Out of the scope of this proposal
3.3.	Serious eye damage / eye irritation				Out of the scope of this proposal
3.4.	Respiratory sensitisation			Resp. Sens. 1; H334	Out of the scope of this proposal

3.4.	Skin sensitisation			Skin Sens. 1; H317	Out of the scope of this proposal
3.5.	Germ cell mutagenicity	Muta 2; H341			
3.6.	Carcinogenicity	Carc 1B; H350	0.01%		
3.7.	Reproductive toxicity	Repr. 1B; H360F			
3.8.	Specific target organ toxicity –single exposure				Out of the scope of this proposal
3.9.	Specific target organ toxicity – repeated exposure				Out of the scope of this proposal
3.10.	Aspiration hazard				Out of the scope of this proposal
4.1.	Hazardous to the aquatic environment			Aquatic Chronic 4; H413	Out of the scope of this proposal
5.1.	Hazardous to the ozone layer				Out of the scope of this proposal

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger

Pictogram: GHS08
Hazard statements: H317, H334, H341, H350, H360F, H413
Precautionary statements: not included in Annex VI of CLP

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Table 4.

Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)		
Skin Sens. 1	H317	H317		GHS08 Dgr		
Resp. Sens. 1	H334	H334				
Aquatic Chronic 4	H413	H413				

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Self-classification notifications for cobalt metal by industry are available in the C&L Inventory database. Beside classification as included in table 3.1, there are notified classifications for Acute tox 4 (H302), **Carc 1B (H350), Repr. 1B (H360)**, Repr. 2 (H361), Muta. 2 (H341), Eye irrit. 2 (H319) and classifications for aquatic acute and chronic, varying from cat 1-4. Classification was sometimes based on the presence of impurities. The registrants included classifications for Muta. 2, Carc. 1B (H350 by inhalation) and reproductive toxicity in category 1B or 2.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

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).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A substance fulfilling the criteria for classification as CMR substance shall normally be subject to harmonised classification (CLP article 36.1).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	231-158-0
EC name:	cobalt
CAS number (EC inventory):	
CAS number:	7440-48-4
CAS name:	cobalt
IUPAC name:	cobalt
CLP Annex VI Index number:	027-001-00-9
Molecular formula:	Co
Molecular weight range:	58.93

Structural formula:

Co

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Cobalt		80.0 -100%	

Current Annex VI entry:

Skin Sens. 1; H317

Resp. Sens. 1; H334

Aquatic Chronic 4; H413

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Zinc oxide			Information on concentration is confidential
Cobalt sulphate			Information on concentration is confidential
Copper			Information on concentration is confidential
Iron			Information on concentration is confidential
Oxygen-containing species (e.g. Co3O4)			Information on concentration is confidential
Nickel			Information on concentration is confidential

Current Annex VI entry:

Zinc oxide (Index number 030-013-00-7):

Aquatic Acute 1 H400

Aquatic Chronic 1 H410

Cobalt sulphate (Index number 027-005-00-0):

Tox. 4 * H302

Skin Sens. 1 H317

Resp. Sens. 1 H334

Muta. 2 H341

Carc. 1B H350i Carc. 1B; H350i: C ≥ 0,01%

Repr. 1B H360F ***

Aquatic Acute 1 H400 M=10

Aquatic Chronic 1 H410 M=10

Nickel powder (index number 028-002-01-4):

Skin Sens. 1 H317

Carc. 2 H351

STOT RE 1 H372 **

Aquatic Chronic 3 H412

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

Most studies were performed with other cobalt compounds and only some with cobalt itself. The main studies with cobalt are the carcinogenicity studies with cobalt from the NTP and the related range-finding studies. These studies were performed with cobalt with a purity of above 98% and provided by the cobalt development institute. The tested material is considered relevant for cobalt classification. For comparison, all NOAELS/LOAELS with compounds other than cobalt itself are also calculated as mg cobalt/kg bw (or /m^3). The following molecular weights are used for these calculations.

Table 9: Cobalt percentage of different cobalt compounds

	Molecular formula	Molecular weight	% cobalt/mol
Cobalt	Co	58.9	100
Cobalt sulphate heptahydrate	$CoSO_4 \cdot 7H_2O$	281.1	20.95
Cobalt chloride	$CoCl_2$	129.9	45.34
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	238.0	24.79
Cobalt acetate	$Co(C_2H_3O_2)_2$	177.0	33.28
Cobalt acetate tetrahydrate	$Co(C_2H_3O_2)_2 \cdot 4H_2O$	249.1	23.65
Cobalt nitrate	$Co(NO_3)_2$	182.9	32.34
Cobalt oxide	CoO	74.9	78.64
Cobalt (II,III) oxide	Co_3O_4	240.8	73.41
Cobalt sulfide	CoS	91.0	64.73
Cobalt(II) 4-oxopent-2-en-2-olate dihydrate	$C_{10}H_{14}CoO_4$	257.1	22.92

1.3 Physico-chemical properties

Table 10: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid, compact or particulate, metallic, odourless element	Anonymous 2005	
Melting/freezing point	1493°C 1495°C	Anonymous 2006, Anonymous 2008	Measured
Boiling point	2927 °C	Anonymous 2008	Measured
Relative density	8.92 at 20°C 8.86 at 20°C	Anonymous 2006, Anonymous 2008	measured
Vapour pressure	*ns*		
Surface tension	*ns*		
Water solubility	0.1 µg/L-12.78 mg/L at 20-22°C	Study reports 2008, 2009	Measured. Solubility depends on loading concentration and sampling time
Partition coefficient n-octanol/water	*ns*		
Flash point	*ns*		
Flammability	*ns*		
Explosive properties	*ns*		
Self-ignition temperature			
Oxidising properties	*ns*		
Granulometry	Cobalt powder (half micron) : MMAD1 = 3.00 µm and MMAD2 = 25.66 µm; GSD1 = 1.46 and GSD2 = 5.87 Cobalt fine powder: MMAD = 29.12 µm; GSD = 1.60	Study report 2010	calculated
Stability in organic solvents and identity of relevant degradation products	*ns*		
Dissociation constant	*ns*		
Viscosity	*ns*		

ns: no data in REACH registration dossier

All references are as summarised in the REACH registration dossier.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant

2.2 Identified uses

Cobalt has many uses including use as an intermediate and for the production of magnets, varistors, batteries, alloys and catalysts.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Out of scope of this proposal.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Information in this chapter is limited to information on cobalt and soluble cobalt salts as information on less soluble cobalt compounds is considered less relevant.

4.1.1 Non-human information

Oral

In vivo

No *in vivo* information is available on the absorption and bioavailability of cobalt (metal) after oral exposure.

Cobalt absorption in experimental animals is highly variable and is affected by the chemical form of the compound, age of the animal, species, and nutritional status. In rats, cobalt chloride was absorbed more efficiently from the gastrointestinal tract than insoluble cobalt oxide (Co_3O_4) (13% to 34% compared to 1% to 3%). Although no species difference was observed for absorption of cobalt oxide (Bailey *et al.*, 1989), absorption of soluble cobalt compounds was greater in rats (13% to 34%) than in cows (1% to 2%) and guinea pigs (4% to 5%). Current biokinetic models assume GI absorption of 20% to 45% for aqueous forms and 10% to 25% for solid forms. Absorption was 3- to 15-fold greater in younger animals than in adults and cobalt absorption was increased in iron-deficient rats.

Following oral administration of cobalt chloride or sulphate, 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. Fecal excretion of cobalt is the primary route of elimination in animals following oral exposure but the rate decreases as cobalt particle solubility increases (NTP 2014, 2015).

Cobalt excretion occurs rapidly with the majority of the administered dose eliminated within hours to a few days after exposure ceases.

The absorption, distribution and excretion of cobalt dichloride were determined in the rat. Intravenous injection with 4.16 mg Co^{2+}/kg bw resulted in rapid excretion within 36 hours (85.6%) of which 75.4% through the urine and 10.1% through the faces. This indicates that excretion occurs mainly via the urine. After oral gavage exposure to 33.3 mg Co^{2+}/kg bw, 27.2% was excreted via the urine and 68.6% via the faeces (total 95.8%). After correction for the partly excretion via the faeces, as observed in the intravenous study, this indicates a bioavailability of 31%. The highest concentration of Co^{2+} at 36 hours after oral gavage was detected in the liver followed by large and small intestine. Blood had the lowest concentration of all organs tested. Tmax after oral administration was approximately 3 hours with an absorption half-life of 0.9 h. Elimination

occurred in three phases with half-lives increasing from 1.3 h via 4.3 to 19 h (Ayala-Fierro *et al.*, 1999).

Cobalt naphthenate (11.9% Co^{2+}) was given to male rats orally at a dose of 3.33 mg Co(II)/kg (28 mg/kg cobalt naphthenate). The test item was prepared as an ethanol: Emulphor mixture (2:1) and administered in a final volume of approximately 0.5 mL. The test groups were allocated to be terminated from 0.5 to 36 hours (i.e. 8 time points) for necropsy and blood removal. Urine and faeces was collected separately from each animal over the 36-hour period. Blood samples were taken over a 36-hour sampling period. Those tissues found to be the target organs from previous studies were removed at the time of necropsy. The tissue and excreta samples were prepared for analysis. Cobalt was analysed by graphite furnace atomic absorption spectroscopy (GFAAS). The detection limit was 5ppb. A distribution and excretion study was performed in the same way using 0.333 mg Co(II)/kg and 33.3 mg Co(II)/kg. The blood versus time concentration curve for the low-dose group demonstrated that, although a clearly defined peak was not observed, the blood cobalt concentration was increased over control (approximately 0.025 µg Co(II)/mL) to 0.1 µg Co(II)/mL from 0.5 to 24 hours. The results from the intermediate-dose group and the high-dose group showed an elevation of 14-25-fold and 25-60-fold over the controls, respectively. The blood concentration curves for the intermediate- and high-dose groups were triphasic, and clearly demonstrated absorptive and elimination phases. Pharmacokinetic parameters were calculated for the 3.33 and 33.3 mg Co(II)/kg dose groups. The peak blood cobalt concentrations of 0.61 µg Co(II)/mL occurred at 4.3 hours for the intermediate-dose group, and 1.74 µg Co(II)/mL at 3.3 hours for the high-dose group. Only those tissues found to have significant elevated cobalt levels in the high-dose group were analysed for cobalt content in the low-dose group. A time-dependent increase in cobalt that peaked at 8 hours occurred in all tissues except the stomach and the large intestine. In the high-dose group, the stomach reached its maximal cobalt content by 2 hours post-dosing. The cobalt content in the large intestine peaked at 12 hours and then decreased rapidly over the remaining time period as cobalt was excreted. All of the organs except the heart exhibited a large increase in cobalt levels in the high-dose group as compared to the low-dose group. The maximal urinary, feacal and total excretion of cobalt for the low and high dose groups was found at approximately 12 hours. The low- and high-dose animals excreted amounts of cobalt in the urine that were not significantly different over the 36-hour period: 31.8% and 26.3%, respectively. The high-dose group excreted a larger percentage of the dose in the faeces (73.1%) as compared to the low-dose group, which excreted only 42% in the faeces by 36 hours. By 36 hours, the total percent of dose excreted was 73.8 and 99.5% in the low- and high-dose group, respectively (Firriolo *et al.*1999).

Radiolabelled (^{57}Co) tricobalt tetraoxide was given to nine male HMT rats (divided in 2 groups of 4 and 5 animals) via gavage in a single application. No information is provided regarding the dose per kg body weight. However, based on the stated specific radioactivity and volume, the estimated dose is around 1 – 8 ug/kg bw. Specific activity (if radiolabelling): Batch I: 10 GBq/g, Batch II: 80 GBq/g; ca. 0.07 Bq per particle. Suspension of particles labelled with 150 kBq ^{57}Co in 3 mL of filtered distilled water. 0.1 mL (5 kBq) per animal. Two batches with different particle sizes, 1.7 and 0.8µm were used. After administration, the animals were placed in metabolic cages for the separate collection of urine and faeces. The ^{57}Co content of the urine and the faeces was determined on day 1, 2, 5, 6 and 7 post exposure as well as the whole body burden. After the observation period of 7 days, animals were sacrificed and the ^{57}Co content of selected tissues was determined. Excretion and retention rates were calculated for both tricobalt tetraoxide batches. One week after ingestion, total urinary excretion of ^{57}Co constituted 0.49% of total ingestion for 1.7µm Co_3O_4 particles and 2.76% of total ingestion for 0.8µm Co_3O_4 particles. The tissue distribution shows very little retention in the organs. Based on the results presented in this study, the total oral absorption of cobalt into the blood (i.e. whole body burden plus urine excretion) was 0.51% for the 1.7µm

particles and 2.85% for the 0.8µm particles. Although an influence of the particle size on the oral absorption was observed, the overall difference is considered low (Collier *et al.*, 1989).

Comparable low oral absorption (0.3% and 0.39%) was found for Cobalt oxides in the F344 rat in a comparable study by Patrick et al (1989) and in the baboon (1.9% and 2.6%) by Andre *et al.* (1989).

Pregnant female Sprague Dawley rats (3-18/dose) were given 0, 25, 50 or 100 mg/kg bw of cobalt sulphate heptahydrate by gavage daily during GD1-20. Cobalt concentration in maternal blood, fetal blood and amniotic fluid (24 hours after the last exposure on day 20) increased in a dose dependent matter. The cobalt concentration in fetal blood was higher than in maternal blood showing placental transfer. A single gavage administration of 100 mg/kg bw of cobalt chloride or cobalt sulphate resulted in a Tmax of the blood of 2 hours. The cobalt sulphate administration resulted in an almost two fold higher blood concentration compared to cobalt chloride. This difference cannot be explained by differences in cobalt content. Maternal blood concentration decreased approximately 3 fold from 2 hours to 24 hours after exposure to cobalt sulphate. Comparing the cobalt concentration in maternal blood at 24 hours after a single administration and repeated administration (20 days) does not show a clear difference indicating limited accumulation Szakmary *et al* (2001).

Inhalation

In vivo

Multiple studies of cobalt metal, cobalt oxides, or soluble cobalt salts show that cobalt is absorbed rapidly following inhalation exposure in animals and distributed to various tissues similar to that observed for other routes with the exception of greater retention in the lung for both soluble and insoluble cobalt.

Lung clearance kinetics of cobalt particles include both mechanical transport (by mucociliary action) and translocation. Lung clearance of inhaled cobalt metal particles in rats and mice showed a well-defined two-phase elimination profile following 3-month or 2-year studies (NTP 2014). The majority (> 95% in rats and > 82% in mice) of the deposited cobalt was cleared rapidly (half-life of 1 to 5 days) while the remainder was cleared more slowly (half-lives of ~20 to > 400 days) depending on the concentration and study duration. Lung steady-state burdens were reached after approximately 6 months and were similar in rats and mice. Lung cobalt burdens were well below the levels that would cause lung overload. Initial mechanical clearance rates were typically 10- to 20-fold greater in rodents than in other species, decreased monotonically with time, and were similar for different particle sizes. In contrast, interspecies differences in translocation rates varied by 3- to 10-fold, remained constant or increased and then decreased with time, and were affected by particle size.

Soluble cobalt compounds are cleared from the lungs at a faster rate than less soluble compounds. The rate of urinary excretion correlates with the rate of translocation of cobalt from the lungs to the blood while fecal excretion rates correlate with the rate of mechanical clearance of cobalt particles from the lung (summarized by NTP 2016a).

Following inhalation exposure of rats to 0.0004 to 0.2 ppm (0.001 to 0.5 mg/m^3) pure cobalt for 24 hours per day for 3 months, a dose-dependent distribution and accumulation of cobalt was reported in the thyroid gland, spleen, liver, kidney, and lung. In SD-Jcl rats exposed to 0.880 ppm (2.12 mg/m^3) cobalt aerosol for 5 hours/day for 4 days, the average cobalt content of the lung and blood 2 hours after the last exposure was 6.42 µg/g and 28.94 µg/L, respectively. The values 28 days after exposure were 0.09 µg/g (1.5 nmol/g) and 0.40 µg/L (6.8 nM), respectively, for lung and blood. The clearance of cobalt in both blood and lung was biphasic with half-lives in the lung of 52.8 and 156 hours and in the blood of 52.8 and 172.8 hours, for the first and second phases, respectively. In

miniature swine following inhalation exposure to 0.04 to 0.41 ppm (0.1 to 1.0 mg/m^3) pure cobalt powder for 6 hours/day, 5 days/week for 3 months, cobalt was excreted mostly by the kidney (summarized by NTP 2014).

Cobalt levels in rat urine 24 hours following intratracheal instillation of a tungsten carbide-cobalt mixture were approximately 3-fold higher compared to instillation of cobalt powder at the same dose. It was later confirmed that this was not due to higher bioavailability but due to rapid urinary excretion following exposure to the tungsten carbide-cobalt mixture. The mean lung cobalt concentration of rats given cobalt was two times more than that of rats given a tungsten carbide-cobalt mixture at 48 hours following exposure; by day 7, mean levels had decreased significantly to almost the same level in all exposed rats (summarized by NTP 2014).

Toxicokinetic study results on cobalt as summarised by NTP (2014) (Basic study descriptions: see repeated dose toxicity)

In the 16/17 day study:

"Urine was collected from core study rats for 16 hours beginning day 12; volume and creatinine and cobalt metal concentrations were determined. Blood was collected from the retro orbital sinus of core study rats and mice and two female tissue burden study rats and mice per group on the last day of exposure and from three female tissue burden study rats and mice per group 3 weeks post exposure; blood and serum were analysed for cobalt metal concentration. Following blood collection, the right femur, heart, right kidney, liver (right lateral and caudate lobes), right lung lobe, and right testis were collected from core study animals and weighed. In addition, whole liver, whole lung, and left lung plus mainstem bronchi were removed and weighed and the right and left lung lobes were collected and weighed individually. Tissues were analysed for cobalt metal concentration.

Results rat
Tissue weights and concentrations were determined in male and female rats at terminal kill and in additional female rats held for 3 weeks post exposure. Data were generated on male rats exposed to 10 mg/m^3 or less due to mortality at 20 mg/m^3. In females, data were generated on all exposure groups; however, a relatively small number of samples (n=1 to 3) was available in 20 mg/m^3 females due to decreased survival.
Male and female rat lung weights increased with increasing exposure concentration at terminal kill and in females held for the 3-week recovery period; these increases were significant at higher exposure concentrations in females. In general, kidney, liver, heart, and femur weights decreased with increasing exposure concentration in males and females; some of these decreases were significant at higher exposure concentrations. In males exposed to 10 mg/m^3, testis weights were decreased in comparison to chamber controls. Because of the significant changes in female lung weights, lung burdens rather than concentrations were evaluated for toxicokinetic parameters.
At terminal kill, cobalt concentrations and burdens increased with increasing exposure concentration in all tissues examined. In general, normalized burdens did not increase with increasing exposure concentration, with the exception of the liver in males and females. Cobalt concentrations in tissues decreased in the order of lung>liver>kidney>femur>heart>serum>blood ~ testes (males). Cobalt burdens in the tissues of male and female rats decreased in the order of liver>lung>kidney>heart>femur ~ testes (males). These data indicate that the tissues examined tended to accumulate cobalt at concentrations greater than could be found in blood and serum, that cobalt was distributed to extra-pulmonary tissues, and that more cobalt accumulated in the liver than in the lung, particularly at the higher concentrations. At 3 weeks post exposure in female rats, cobalt concentrations were markedly reduced in blood, serum, and lung.

Kinetic analysis of data from female rats exposed to 20 mg/m^3 or less indicated elimination half-lives of 9.2 to 11.1 days (blood), 2.8 to 3.4 days (serum; 10 and 20 mg/m^3 only, due to undetectable serum concentrations of cobalt at lower exposure concentrations at 3 weeks post exposure) and 4.2 to 5.6 days (lung). Lung cobalt deposition rates and predicted steady-state lung cobalt burdens generally increased less than proportionally across exposure concentrations except when comparing 10 and 20 mg/m^3.
In general, the volume of urine collected from male and female rats during the 16-hour collection period after exposure on day 12 decreased with increasing exposure concentration. Increased creatinine concentrations were observed in both sexes in the higher exposure concentration groups. Urinary cobalt concentration increased with increasing exposure concentration in both sexes. When normalized to creatinine, cobalt concentrations increased approximately in proportion to exposure concentration. Total cobalt excreted increased with exposure at lower concentrations before decreasing at higher concentrations.

Results mice
Tissue weights and concentrations were determined in male and female mice at terminal kill and in additional female mice held for 3 weeks after the exposure. Data were generated on male and female mice in all exposure groups; however, relatively small numbers of samples (n=1 to 2) were available in 40 mg/m^3 females due to decreased survival.
Male and female mouse lung weights increased with increasing exposure concentration, reaching weights that were up to 1.5- to 2-fold greater than those of the chamber controls at terminal kill. In female mice that were held for the 3-week recovery period, lung weights of exposed groups recovered such that they were similar to those of the chamber controls at the end of the recovery period. In both males and females, treatment-related decreases in the weights of all other tissues occurred. Because of the significant changes in lung weight, lung cobalt burdens rather than lung concentrations were evaluated for toxicokinetic parameters.
At terminal kill, cobalt concentrations and burdens increased with exposure concentration in all tissues examined. Cobalt concentrations in tissues decreased in the order of lung>liver>kidney>serum>heart approximately equal to femur>blood>testes (males). Tissue cobalt burdens in male mouse tissues decreased in the order of lung>liver>kidney>heart>femur>testes. With the exception of testes, all tissues examined represented sites where cobalt could accumulate at concentrations greater than observed in the blood or serum. Mice of both sexes accumulated large amounts of cobalt in the liver. While lung cobalt burdens were generally higher than liver cobalt burdens at exposures of 20 mg/m^3, liver and lung burdens were similar in females exposed to 20 mg/m^3 or less, and liver burdens were greater than lung burdens in 40 mg/m^3 males and females. Normalized tissue burdens generally remained the same or decreased with increasing exposure concentration.
Kinetic analysis of data from female mice exposed to 20 mg/m^3 or less indicated elimination half-lives of 4.1 to 7.3 days (blood), 2.9 to 3.7 days (serum), or 5.5 to 6.6 days (lung); in general, half-lives decreased with increasing exposure concentration. Lung cobalt deposition rates and predicted steady-state lung cobalt burdens increased in proportion to exposure concentrations of 2.5 and 5 mg/m^3, but the increases were less than proportional at greater exposure concentrations."

In the 90-day study:

"Lungs and blood (retro orbital sinus) were collected from three special study female rats and mice per exposure group on days 5, 12, 26, 40, 61, and 89 and on days 7, 14, 28, and 42 post exposure. Liver (right lateral and caudate lobes) was also collected on days 26 and 40. Liver and lungs were weighed; blood, liver, and lungs were analysed for cobalt metal concentration.

Results rat

Lung and liver weights and lung, blood, and liver cobalt concentrations were determined in female rats. Lung weights were increased in all exposed groups starting on day 40 (5 mg/m^3) or day 61 (2.5 mg/m^3 or less) and remained greater than those in the chamber controls throughout the exposure and post exposure periods. Because of the significant changes in lung weights with exposure concentration, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Liver weights of exposed groups of females were either decreased or similar to chamber controls at each time point.

Lung cobalt concentrations and burdens increased with increasing exposure concentration and were significantly increased over chamber controls with all exposure concentrations at all time points. By day 26, the concentrations and burdens of cobalt in the lung of all exposed groups appeared to reach steady state and did not change significantly through the end of exposure (day 89) before decreasing rapidly during the first week of the post exposure period and then more slowly until the end of the post exposure period. Lung cobalt concentrations in chamber control animals were at or below the limit of detection (LOD) at all time points. Lung cobalt burden data normalized to exposure concentration indicated increases in burden that were proportional to exposure concentration.

During the 3-month exposure, blood cobalt concentrations in chamber control animals were at or below the LOD at all time points and concentrations in the exposed groups generally increased in proportion to exposure concentration at all time points. Within each exposure concentration, blood cobalt concentrations appeared to be at or near steady state starting from the earliest time point and continuing throughout the exposure period. However, during the recovery period, blood cobalt concentrations fell very rapidly; the largest declines occurred during the first week post exposure. Accordingly, because of the extensive elimination of cobalt from the blood, it was not possible to demonstrate dose proportionality from blood concentration data collected during the recovery period. In addition, it was not possible to fit the blood data to a two-compartment model due to the lack of early sampling times; however, it appears that there were both rapid and slow clearance phases from the blood.

Liver cobalt concentrations in the chamber control group were at or below the LOD and concentrations and burdens in the exposed groups increased with increasing exposure concentration at both time points (days 26 and 40). Cobalt concentrations and burdens in the liver of exposed animals were generally lower on day 26 compared to day 40. The normalized liver cobalt burdens were similar across the exposed groups at both time points. At both time points liver cobalt burdens were similar to and in some cases greater than the corresponding lung cobalt burdens.

Pulmonary clearance of cobalt during the recovery period showed a well-defined two-phase elimination profile. The rapid phase exhibited half-lives ranging from 1.8 to 2.6 days and was followed by a slower lung clearance phase with half-lives of 19 to 23 days. A two-compartment clearance model could not be fit to the lung cobalt burden data collected during the 3-month study due to the lack of data collected prior to 5 days of exposure, but a one-compartment model provided an adequate fit to these data. The results indicated that half-lives ranged from 4.7 to 9.0 days.

Results mice:

Lung and liver weights and lung, blood, and liver cobalt concentrations were determined in female mice. During the exposure period, lung weights of the 5 and 10 mg/m^3 groups were significantly greater than those of the chamber controls starting on study day 12 and generally remained elevated compared to the chamber controls until the end of the post exposure period. Increased lung weights

were occasionally observed at 2.5 mg/m^3. Because of the significant changes in lung weights with exposure concentration, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Lung cobalt concentrations and burdens increased with increasing exposure concentration and were increased over chamber controls. Lung cobalt concentrations in chamber control animals were near or below the LOD at all time points. By day 40, lung cobalt concentrations in all exposed groups appeared to be approaching steady state and did not change significantly through the end of exposure (day 89) before steadily decreasing during the recovery period. Lung cobalt burdens increased rapidly within the first 5 to 26 days, but by days 12 to 40, the rate of increase slowed as lung burdens asymptotically approached steady state with the higher concentrations taking longer to approach steady state. During the recovery period, lung cobalt burdens decreased very rapidly during the first week, after which lung clearance of cobalt slowed significantly. Normalized lung cobalt burdens tended to increase with exposure concentration up to 5 mg/m^3 but were lower in animals exposed to 10 mg/m^3 than in animals exposed to 5 mg/m^3, indicating a lack of a nonproportional accumulation at 10 mg/m^3.

Blood cobalt concentrations in the chamber control animals were at or below the LOD at all time points. During the 3-month exposure, blood cobalt concentrations generally increased in proportion to exposure concentration at all time points and were increased over chamber controls in all groups and all exposure time points and remained elevated through the later post exposure time points as exposure concentration increased. Within each exposure concentration, blood cobalt concentrations appeared to be at or near steady state by study day 12. However during the recovery period, blood cobalt concentrations fell very rapidly to concentrations that were near or below the LOD in an exposure concentration-related manner. Accordingly, because of the rapid and extensive elimination of cobalt from the blood, it was not possible to demonstrate dose proportionality from blood concentration data collected during the recovery period.

Liver weights of the 5 and 10 mg/m^3 groups were significantly less than that of the chamber control group on day 26; similar, although not statistically significant decreased liver weights in these exposed groups were observed on day 40. Liver cobalt concentrations in chamber control animals were at or below the LOD at both time points. During the 3-month exposure, liver cobalt concentrations and burdens generally increased with exposure concentration and were increased compared to the chamber controls at both time points. Liver cobalt concentrations and total liver cobalt burdens for exposed animals were higher at all exposure concentrations on day 26 compared to day 40 (except for cobalt concentration in animals exposed to 10 mg/m^3).

Pulmonary clearance of cobalt during the recovery period showed a well-defined two-phase elimination profile. The rapid phase exhibited half-lives ranging from 1.4 to 3.2 days and was followed by a slower lung clearance phase with half-lives of 27 to 39 days; there was no clear relationship to exposure concentration in either phase. A two-compartment clearance model could not be fit to the lung cobalt burden data collected during the 3-month study due to the lack of data collected prior to 5 days of exposure, however a one-compartment model provided an adequate fit to these data. The results indicated that half-lives ranged from 2.4 to 17 days (increased with increasing exposure concentration) for animals exposed to 5 mg/m^3 or less. The half-life in animals exposed to 10 mg/m^3 was 122 days, but the standard errors for the clearance rate constant and subsequently the calculated half-life were high (>80%) making these data unreliable."

In the 2 year study:

"On days 1, 2, 3, 4, 184, 366, and 548, lungs were removed from five female lung burden study rats and mice per group, weighed, and analysed for cobalt metal concentration.

Results rat

Lung weights and lung cobalt burdens were determined in female rats. Lung weights increased in all exposed groups; however, increases in lung weights occurred earlier in the study (day 184) in the 2.5 and 5 mg/m^3 groups than in the 1.25 mg/m^3 group (day 366). Because of the significant changes in lung weights with increasing exposure concentration, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Cobalt concentrations and burdens in the lung increased with increasing exposure concentration and were significantly increased in all exposed groups of female rats at all time points compared to those in the chamber control group. Cobalt concentrations in the chamber control group were at or below the LOD at all time points except day 548 (one animal had a lung cobalt concentration exceeding the LOD but less than the experimental limit of quantitation (ELOQ). By day 184, lung cobalt concentrations for all exposed groups appeared to reach steady state and did not change significantly through day 548; lung cobalt burdens increased rapidly by day 4, but by day 184 the rate of increase slowed as lung burdens asymptotically approached steady state. Analysis of normalized lung cobalt burdens revealed no tendency toward disproportionate changes and no biologically significant differences in normalized burdens with increasing exposure concentration.

The lung cobalt burden data from the exposure phases of the 3-month and 2-year studies were modeled using a two-compartment model; these data show that steady state was clearly reached at 2.5 and 5 mg/m^3 but not at 1.25 mg/m^3. Rapid clearance phase half-lives were between 1.53 days and 2.37 days, while slow clearance phase half-lives were 789 days, 167 days, and 83 days for 1.25 mg/m^3, 2.5 mg/m^3, and 5 mg/m^3, respectively. The apparent lack of achievement of steady state and long half-life at 1.25 mg/m^3 are likely spurious findings due to uncertainty in the model. Cobalt deposition rates were 1.4, 2.1, and 5.6 μg cobalt/day during the rapid clearance phase and 0.018, 0.078, and 0.29 μg cobalt/day during the slow clearance phase at 1.25, 2.5, and 5 mg/m^3, respectively. Steady-state lung cobalt burdens including both the rapid and slow clearance phases (LSSa + LSSb) were approximately 25.4, 27.8, and 46.8 μg cobalt/lung in animals exposed to 1.25, 2.5, and 5 mg/m3, respectively. The fractions of deposition in the slow clearance phase (FB) for the exposed groups were quite low, increasing from 0.012 to 0.049 as exposure concentrations increased, corresponding to total slow phase lung cobalt clearances of 1.2% to 4.9%; clearances of total deposited cobalt during the rapid clearance phase ranged from 98.8% to 95.1% [(1-FB) × 100] with increasing exposure concentration.

Results mice

Lung weights of female mice were significantly increased starting on day 4 in groups exposed to 2.5 or 5 mg/m^3 and continuing until day 548. At 1.25 mg/m^3, lung weights were increased on days 366 and 548; because of these increases in lung weights, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Cobalt concentrations and burdens in the lung increased with increasing exposure concentration and were significantly increased in all exposed groups of female mice at all time points compared to those in the chamber control group. Cobalt concentrations in the chamber control group were at or below the LOD at all time points. By day 184, lung cobalt concentrations for all exposed groups appeared to reach steady state and did not change significantly through day 548. Lung cobalt burdens increased rapidly by day 4, but by day 184, the rate of increase slowed as lung burdens asymptotically approached steady state. Analysis of lung cobalt burdens normalized to exposure concentration indicated that there were proportional increases between the 1.25 and 2.5 mg/m^3

groups, but nonproportional increases were observed between the 2.5 and 5 mg/m^3 groups. At the earlier time points, normalized lung cobalt burdens were lower in animals exposed to 5 mg/m^3 than in those exposed to 2.5 mg/m^3; however the opposite was true at the longer exposure durations, where normalized cobalt burdens were greater than proportional relative to the 2.5 mg/m^3 group.

The lung cobalt burden data from the exposure phases of the 3-month and 2-year studies were modelled using a two-compartment model. Rapid clearance phase half-lives were 1.2, 1.1, and 5.2 days, respectively, for the 1.25, 2.5, and 5 mg/m^3 groups, indicating a slightly longer half-life in animals exposed to 5 mg/m^3. Cobalt deposition rates for the rapid clearance phase were 0.87, 1.84, and 1.18 µg cobalt/day at 1.25, 2.5, and 5 mg/m^3, respectively. Slow clearance phase half-lives revealed the opposite trend, with half-lives of 409, 172, and 118 days with increasing exposure concentration. Cobalt deposition rates for the slow clearance phase were 0.027, 0.075, and 0.25 µg cobalt/day. The overall theoretical steady-state lung cobalt burdens, including both the rapid and slow clearance phases (LSSa + LSSb), were approximately 17.8, 21.4, and 51.8 µg cobalt/lung in the 1.25, 2.5, and 5 mg/m^3 groups, respectively; these data support the achievement of steady state in the 2.5 and 5 mg/m^3 groups but not in the 1.25 mg/m^3 group. The fractions of deposition in the slow clearance phase (FB) for the exposed groups were quite low, increasing from 0.031 to 0.176 as exposure concentration increased, corresponding to total slow phase lung cobalt clearances of 3.1% to 17.6%; clearances of total deposited cobalt during the rapid clearance phase ranged from 96.9% to 82.4% [(1–FB) × 100] with increasing exposure concentration."

NTP overall conclusion

"Multiple lines of evidence, including the rapid clearance of cobalt from the lung and blood, the low lung cobalt burdens, the absence of particle overload, the systemic distribution and elimination of cobalt, and the observed toxicity/carcinogenicity to extrapulmonary sites are consistent with relatively soluble cobalt particles rather than insoluble particles (Kreyling *et al.*, 1986; Collier *et al.*, 1989; Kyono *et al.*, 1992). Cobalt has been reported to be insoluble in aqueous environments but able to be solubilized by strong mineral acids (Takahashi and Koshi, 1981; Kyono *et al.*, 1992). In vivo studies by Rae (1975) show that macrophages were able to dissolve a significant amount of cobalt, despite toxicity to the cell. Based on this evidence, alveolar macrophages likely contributed to the solubilizaton and systemic absorption of cobalt via the lung in the current studies. Furthermore, studies by Stopford *et al.* (2003) using artificial fluids to mimic ingestion and inhalation indicate that lysosomes are likely responsible for dissolving cobalt taken up by macrophages and that any cobalt ingested via grooming or mucocilliary clearance would be solubilized by gastric juices. Because dissolution of cobalt results in toxicity to the macrophages, it is likely that the clearance of cobalt is due primarily to the dissolution and absorption of cobalt, rather than alveolar macrophage mediated clearance of intact particles via mucocilliary clearance. However, gastrointestinal absorption and systemic distribution following grooming or mucocilliary clearance may have also contributed to the tissue distribution of cobalt.

(Overload was originally studied in F344 rats and assumes a density of one; however, for the current studies, the ratio of mouse to rat lung weight at 18 months of the chronic study and the use of the density of the cobalt test article (approximately 8.81 g/cm^3) allowed for evaluation of overload specific to rats and mice exposed to cobalt metal. Based on these assumptions, 13.2 mg (rats) or 2.1 mg (mice) would be required to cause overload. These values are 264 (rats) or 42 (mice) times the maximum lung burdens observed in the 2-year studies, indicating that overload was not approached in these studies.)"

As inhalation exposure to cobalt powder in rats and mice resulted in comparable effects (increase in red blood parameters) as exposure to soluble Co^{2+} compounds and seen the high level of urinary excretion of cobalt (speciation not stated) after inhalation cobalt powder exposure, it is considered likely that cobalt powder is at least partly oxidised to Co^{2+}. Cobalt in the trivalent state, on dissolution, the Co^{3+} is expected to undergo protonation forming an unstable hydrated species giving rise to the divalent Co^{2+} ion (Cotton & Wilkinson, 1988). Co^{2+} is expected to be stable in biological fluids.

The information on the toxicokinetics in the carcinogenicity studies with $CoSO_4.7H_2O$ and the preceding range-finding studies is limited to information on urine excretion in the 90-day rat study shown in the table below. Based on these values the estimated urine excretion as percentage of the exposure was estimated at 22% (30 mg/m^3, 6 hours/day, inhalation volume rat 0.288 m^3/kg for 6 hours, 0.38 fraction cobalt in cobalt sulphate, 300 g male rat, factor 1.5 for 16 to 24 hour extrapolation and factor 7/5 for 5 days a week exposure). This shows that a substantial percentage of the inhaled substance is systemically available.

Table 11: Cobalt content in urine of rats in the thirteen-week inhalation studies of cobalt sulphate heptahydrate.

	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
Male	0.22 ±0.03	2.51 ±0.23	5.21 ± 0.34	33.4 5.15	42.6 ± 7.6	105 ± 11.8
Female	0.17 ± 0.05	1.99 ±0.47	2.36 ± 0.28	18.1 ± 1.23	21.4 ± 1.64	66.9 ±4.0

µg Co excreted per 16 hours; mean ± standard error for groups of 10 animals.

Rats were exposed for 6 hours per day. Inhalation doses are expressed for the anhydrous $CoSO_4$.

Dermal

In vivo

Dermal absorption of cobalt (applied as cobalt chloride) has been investigated in mice, guinea pigs, and hamsters. Dermal absorption of cobalt applied to intact or acid-burned skin of mice was about 0.1% after one hour but increased to 25% to 50% when applied to skin damaged by incision, abrasion, or punctures. In a similar study in guinea pigs, absorption of cobalt through intact skin was less than 1% while absorption through abraded skin was about 80% 3 hours after exposure. It was reported that small amounts of cobalt were detected in urine 24 to 48 hours after application to the intact skin of hamsters and that much of the metal was retained in the skin after 48 hours. In this study it was also reported that uptake of cobalt by keratinocytes exposed in vitro was about 5% of the dose (NTP 2016a).

In vitro

In an *in vitro* dermal absorption study according to OECD TG 428 (GLP compliant), cobalt dichloride hexahydrate showed an absorption (including strips 6-20) of 0.38% and 1.08% through human skin with 8 hour exposure and 64 hour post-exposure monitoring period at nominal dose levels of 100 and 1000 ug/cm^2, respectively (Cobalt registration Exp Key Dermal absorption.001).

4.1.2 Human information

Absorption

Cobalt absorption after oral exposure varies considerably (5-97% of the dose) and depends on type and dose. In addition, the sex and nutritional status of the subject play a role. GI uptake in women is higher than in men, probably due to a higher iron deficiency in women. The primary route of elimination in humans following oral exposure is through feces.

Workers exposed to cobalt dust and fumes had cobalt levels in blood and urine that generally increased in proportion to inhalation exposure levels especially when exposed to soluble cobalt-containing particles. Exposure to less soluble cobalt oxide particles results in a lower absorption rate and longer retention time in the lungs.

Recent *in vitro* studies with human lung cells show that insoluble cobalt oxide particles (CoO or Co_3O_4) are readily taken up through endocytosis and are partially solubilized at the low pH within lysosomes while soluble cobalt salts utilize cellular transporters such as calcium channels or the divalent metal ion transporter to enter cells.

Dermal absorption of cobalt was demonstrated in two studies that measured increased cobalt concentrations in the urine of volunteers who immersed their hands in hard metal dust containing 5 to 15% cobalt (85-95% tungsten carbide) for 90 minutes or in a used coolant solution containing 1,600 mg/L cobalt for one hour. In volunteers who placed their fingers in a cobalt salt solution 10 minutes per day for 2 weeks (10-50 mg/l in the first week, 100-200 mg/l in the second week), cobalt accumulated in the fingernails. (ATSDR 2004, NTP 2014, 2015).

Distribution

In humans, inorganic cobalt is distributed to liver, kidney, heart, and spleen with lower concentrations found in bone, hair, lymph, brain, and pancreas. Inorganic cobalt administered intravenously (i.v.) or orally to human volunteers was distributed primarily to the liver (10-30%). Due to a rapid distribution to tissues, plasma cobalt levels decline rapidly. However, about 9-16% of the administered dose was retained with a half-life of about 800 days. Most of the cobalt in plasma is bound to leukocytes or plasma proteins with a maximum free fraction of 12%. Free cobalt is also taken up by red blood cells via a membrane transport pathway shared with calcium (Simonsen *et al.* 2012, Simonsen *et al.* 2011). Uptake of cobalt by red blood cells is practically irreversible because the ions bind to haemoglobin and are not extruded by the calcium pump. Cobalt can also transfer to human milk and across the placenta (NTP 2016a).

Excretion

Renal excretion of absorbed cobalt is rapid over the first days but is followed by a second, slower phase that lasts several weeks. Controlled experimental studies in humans indicate that 3% to 99% of an orally administered dose of cobalt is excreted in the feces and primarily represents unabsorbed cobalt. Following i.v. administration of cobalt chloride to 6 volunteers, fecal elimination accounted for about 2% to 12% of the administered dose while about 28% to 56% was eliminated in the urine after 8 days.

Following inhalation exposure to insoluble cobalt compounds such as cobalt metal and cobalt oxide, three-phase elimination kinetics were observed in humans. The half-life for the first phase, likely representing mucociliary clearance in the tracheobronchial region, was approximately 2 to 44 hours. The second phase with a half-life of approximately 10 to 78 days may represent macrophage-mediated clearance of cobalt particles from the lung. The third phase clearance with a half-life on the order of years may represent long-term clearance from the lung. Controlled aerosol studies in human volunteers show that about 40% the initial lung burden of inhaled cobalt oxide (Co3O4) particles was retained in

the respiratory tract after six months. About 33% of the initial lung burden was found in the urine with 28% in feces 6 months after exposure (NTP 2014, 2015).

4.1.3 Other information

In vitro

In vivo information on the bioavailability of cobalt and its compounds is limited. The same applies to the toxicological information. Therefore, the registrants determined the dissolution of many cobalt compounds in several artificial body fluids and used them to support read-across between tested and untested cobalt compounds.

The bio-elution of the substances was tested by mixing 1.0 g of the substance with 50 ml (20 g/L) 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). Some studies used other initial loading concentrations (100 mg/L). 5% CO_2 in nitrogen was bubbled through the intestinal, interstitial and alveolar fluids to maintain the required pH. Extracts were separated from the solids by filtration or centrifugation. Cobalt was determined in the extracts using flame atomic absorption spectrophotometry. If the 2 hour extract contained more than 5 ppm cobalt, another extraction was performed using 100 mg per 50 ml (2 g/L) fluid to avoid erroneous low values caused by mass ion effect. Once 50% had solubilised, no additional extractions were performed and 100% solubilisation was assumed for longer incubation periods.

The results for cobalt and the different soluble cobalt compounds for which toxicological data are used in this proposal in artificial fluids and testing durations are provided below.

Table 12: 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

Cobalt metal and several water-soluble compounds (cobalt sulphate heptahydrate and chloride) but also some water-insoluble cobalt compounds were found to be soluble in gastric and lysosomal fluids. These two fluids are both acidic. In contrast to the soluble cobalt salts, cobalt was only limitedly soluble in alveolar, interstitial and intestine fluid. These fluids are more neutral in pH.

After oral exposure, cobalt and cobalt compounds may dissolve in the stomach due to the low pH depending on their solubility and solubility rate. The dissolved Co^{2+} is moved to the intestine where the pH is raised to normal using carbonate. Absorption is expected to occur in the intestine. This could potentially result in the formation of a precipitate of cobalt carbonate as cobalt carbonate has a limited solubility in intestinal fluid (4%). However, the same argument would apply for soluble cobalt compounds such as cobalt chloride for which a bioavailability is estimated of 30%. This indicates that dissolved Co^{2+} at low pH does not precipitate when the pH is raised to normal. This is confirmed in a study conducted by Firriolo (1992) which showed that when Cobalt naphtenate is dissolved in ethanol is added to PBS with pH = 7.3 there is no strong precipitation. This is also confirmed by the comparable *in vivo* bioavailability of cobalt chloride and cobalt naphtenate. Therefore, it is likely that substances dissolved in gastric fluid will remain dissolved when coming

into contact with intestinal fluid. The dissolution in gastric fluid is considered determinative for the oral bioavailability.

The same argumentation is applicable to the inhalation route. Large cobalt particles will deposit in the respiratory airways and be transported upwards by mucocilliary action. After swallowing, this fraction will follow the oral route described above. Small cobalt particles will be transported into the alveoli where they cannot be removed by mucocilliary action. When they do not dissolve in the alveolar fluid they will be taken up by cells and transported into the lysosomes. Table 12 shows that cobalt and soluble cobalt compounds dissolve in lysosomal fluid. Co2+ ions diffuse from the lysosomes into the cell and outside the cell and become locally and systemically available. The solubility in the other fluids is less relevant and may only increase the bioavailability. Therefore, read-across from tested soluble cobalt compounds to cobalt compounds with a comparable solubility in gastric and/or lysosomal fluid is justified.

The dissolution of a substance (percentage of the loading dissolved) in these *in vitro* systems can be determined by 3 factors namely the dissolution rate (how fast the substance dissolves), the solubility (the maximum amount that can dissolve in the tested amount of fluid) or the applied amount (some substances dissolve quickly and completely in the fluid after which no additional substance can dissolve but the maximum solubility is not reached). When the dissolution rate is determinative, as indicated by an increase of the percentage dissolved over time, this depends amongst others on the amount (loading) and on particle size of the substance. Therefore, a higher dose level in mg/kg bw or air concentration will result in a higher dissolved concentration. When the solubility is determinative, the substance cannot dissolve completely in the test system indicated by no increase in dissolved percentage over time. Therefore, a higher *in vivo* external dose level (mg/kg bw/day or air concentration) will not result in a higher dissolved concentration. When the test system is determinative, the substance is completely dissolved (close to 100%) often already at the first time point. In such cases no correct dissolution rate can be determined from the data. It is also formally not known whether a higher dose will result in an increased concentration. However, it is considered likely because it is very unlikely that the substance was tested exactly at the maximum solvability. Therefore, it is likely that a higher external dose will result in a higher dissolved concentration, in a higher internal concentration and increased toxicity.

Information is available on the solubility after 2, 5, 24 and/or 72 hours. For oral exposure prediction via gastric fluid, the shortest period is considered the most relevant as gastric content in the rat is removed within 2 hours. Bio-elution was tested at 100 mg per 50 ml fluid meaning a concentration of 2 mg/ml and for some substances at 100 mg/L meaning a concentration of 0.1 mg/ml. Several substances showed solubility in gastric fluid at these concentrations close to 100% already after 5 hours showing that the results were limited by the amount of substances added to the gastric fluid and not by the solution rate or the solubility of the substance. The relevance of the tested concentration compared to the potential concentration in the stomach and intestine is not stated in the registrations.

The gastric fluid volume in the rat (average of fasted and fed) is 3.2 g/kg bw (McConnell *et al.*, 2008) and the gastric fluid production is approximately 10 ml/hour kg bw as estimated from the measurements by Brodie (1966) and 2.2 ml/hour kg according to Areche (2008). The average is approximately 5 ml/hour per kg. A rat eats approximately 40 g / kg bw per day and drinks 40 ml / kg bw per day (ECHA guidance R8). Over a day for a diet study this means that 40 g of food is mixed with 40 ml (water intake) plus 24 hours/day * 5 ml/kg bw. hour = 120 g gastric fluid resulting in 200 ml per kg bw. Testing the maximum dose of 1000 mg/kg bw per day for an oral study results in a concentration of 5 mg/ml (1000 mg in 200 ml). This is somewhat above the concentration used in most bioelution tests. A gavage study using 2 ml/kg bw for 1000 mg/kg bw would result in a stomach concentration of 46 mg/ml (1000 mg/kg bw in 21.8 ml/kg bw consisting

of 2 ml/kg bw for gavage application, 3.2 g/kg bw gastric volume, 5 ml/kg bw/h * 2 h for gastric fluid production, 3.3 g/kg bw for food uptake in two hours and 3.3 ml/kg bw for water uptake. This is clearly above the concentration used in most bioelution tests. This calculation also indicates that the concentration in the stomach is much higher after gavage exposure compared to a diet exposure. This higher stomach concentration may limit the dissolution and therefore limit bioavailability.

Table 13: Relation between external oral dose and internal concentration in the stomach

External dose (mg/kg bw/day)	Diet stomach concentration (mg/ml)	Gavage stomach concentration (mg/ml)
1000	5.0	46
100	0.50	4.6
10	0.05	0.46
1	0.005	0.046

For the inhalation route no release of particles from the lysosomes is expected. Therefore, the longest period in a bioelution test is considered the most relevant for bioavailability after inhalation exposure.

Read-across

The systemic effects of cobalt and cobalt compounds are determined by the concentration of Co^{2+} systemically available. It is assumed that transport of Co^{2+} ions across the intestinal wall and in the alveoli only depends on the concentration of dissolved Co^{2+} ions in the intestine and the alveoli. The toxicity of Co compounds is also dependant on the toxicity of the counter ion or the combined toxicity of cobalt and its counter ion as this may determine the highest administered dose (i.e. external exposure level) in a study. The intestinal and alveoli concentration is also dependant on the solubility in biological fluids. For cobalt and cobalt compounds with a different speciation (Co^0 and Co^{3+}), oxidation or reduction to Co^{2+} is also relevant.

The available bioelution data on cobalt indicate that it is dissolved after oral exposure in the stomach and therefore will be taken up in the intestine. Therefore, a good bioavailability of cobalt is expected after oral exposure. The bioavailability is expected to be higher in a diet study compared to a gavage study. After inhalation exposure, cobalt particles need to be taken up by cells such as macrophages and transported to the lysozymes where they can dissolve. The available lysosomal bioelution data show that cobalt dissolves readily under the tested conditions. The Co^{2+} ions can than diffuse to other parts of the cell, the lung and the body. Therefore, a good bioavailability of cobalt is also expected after inhalation exposure. The bioavailability of cobalt after inhalation exposure is also shown in the NTP inhalation studies.

4.1.4 Summary and discussion on toxicokinetics

The oral bioavailability of cobalt and cobalt compounds varies depending on substance, species, age and dose. Studies with dissolved cobalt compounds show a bioavailability of approximately 30%. For Co^{2+} substances which do not dissolve in water, the dissolution rate in gastric fluid is expected

to be determinative for the bioavailability. Indeed for substances with a low water and gastric fluid solubility like Co_3O_4, the bioavailability is lower. For Co^0 and Co^{3+} compounds, in addition to solution also oxidation or reduction to Co^{2+} is required. This is not expected to be the rate limiting step.

Uptake in cells involves at least partly active transport.

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. A study with pregnant rats (day 20) show that cobalt is transferred over the placenta resulting in higher foetal blood concentrations compared to the maternal concentration.

Absorbed Co^{2+} is mainly excreted via the urine (88%) whereas unabsorbed cobalt and cobalt compounds are excreted via the faeces. Elimination is fast and occurs in three phases with half-lives increasing from 1.3 h via 4.3 to 19 h (blood).

For cobalt powder, no *in vivo* kinetic studies are available. *In vitro* studies show a high bioaccesability in gastric and lysosomal fluid. Therefore, a good bioavailability is expected after oral exposure. However, this good bioavailability is not confirmed in the combined repeated dose toxicity and reproductive screening study with gavage exposure of cobalt particles suspended in water, as indicated by the absence of or presence of very limited haematological effects (typical for systemic Co^{2+}) (CoRC/CDI, 2015). The bioavailability of cobalt is expected to be higher after diet exposure compared to gavage exposure. This is expected because diet exposure results in a lower amount of cobalt present in the stomach at one time resulting in a higher percentage of dissolution. In addition, uptake of cobalt ions may be an active process with a limited capacity as shown for other metal ions. A short but high intraluminal concentration after gavage exposure may result in a lower active transport compared to a continuous low level intraluminal concentration after diet exposure. Therefore, read-across from oral studies with water soluble cobalt compounds to cobalt is considered justified.

The bioavailability of cobalt and cobalt compounds after inhalation exposure also varies depending on substance, particle size and dose. Large particles are mainly cleared by mucociliary clearance in the tracheobronchial region. This results in exposure via the gastric/intestinal route. Smaller particles in the alveoli are cleared by macrophages at a much lower rate. Cobalt compounds which are soluble in lung fluids can be cleared by diffusion or active transport from the lung. Cobalt and cobalt substances which dissolve in lysosomal fluid can also become systemically available. The bioavailability of water soluble and lysosomal fluid soluble cobalt and cobalt compounds is estimated at 20 – 30%.

Distribution is comparable to the oral route. However, there is additional retention in the lungs.

Inhalation studies with cobalt in rats and mice indicate a rapid first phase with half-lives of 1 to 5 days followed by a much slower second phase (20 - >400 days).

For cobalt, the bioavailability after inhalation exposure is shown by the measurements of cobalt in several tissues and the observation of systemic effects typical for Co^{2+}. This is supported by the lysosomal bioelution data on cobalt. Therefore, read-across from inhalation studies with water soluble cobalt compounds to cobalt is considered justified.

The available data indicate that the dermal bioavailability of cobalt and cobalt compounds on the undamaged skin occurs but is low.

4.2 Acute toxicity

Out of scope of this proposal

4.3 Specific target organ toxicity – single exposure (STOT SE)

Out of scope of this proposal.

4.4 Irritation

Out of scope of this proposal.

4.5 Corrosivity

Out of scope of this proposal.

4.6 Sensitisation

4.6.1 Skin sensititsation

Out of scope of this proposal.

4.7 Repeated dose toxicity

Classification of STOT-RE is not part of this proposal for cobalt metal. Repeated dose toxicity studies are included as background for the assessment of carcinogenicity and reproduction toxicity. Only the study summaries are therefore included and a short overall summary of repeated dose toxicity but no comparison with the criteria.

The summarised studies are based on available summaries and are limited to cobalt compounds with reasonable solubility as these are most relevant for read-across. Repeated dose toxicity tests with low solubility or with toxicity of the counter ion are not included.

Table 14: Summary table of relevant repeated dose toxicity studies

Method	Test substance	Results	Remarks	Reference
oral				
Combined repeated dose toxicity and reproduction screening study in rats (10/sex/dose) 0, 30, 100, 300 or 1000 mg/kg bw (gavage: undissolved) 2 weeks before mating – 2 weeks after mating (males) or ppd 3 (females)	Cobalt powder	≥ 100 mg/kg bw: Mortality, clinical effects, macroscopic intestinal changes NOAEL: 30 mg/kg bw/day	OECD 422	CDI/CORC 2015
Oral developmental study in female rabbits (8-25/dose) (gavage) 0, 20, 100 or 200 mg cobalt sulphate/kg bw (GD6-20)	cobalt sulphate heptahydrate	≥ 20 mg/kg bw: mortality, circulatory failure, reduced bw gain LOAEL: ≤ 20 mg/kg bw (4.2 mg Co/kg bw)		Szakmáry, E. *et al.* 2001
8 weeks oral study in male rats (20/group) 100 mg cobalt sulphate/kg bw, followed 26 mg/kg bw/day	cobalt sulphate	degenerative heart lesions LOAEL: ≤ 26 mg/kg bw (5.46 mg Co/kg bw)	Initial dose (given once) 100 mg/kg bw	Grice, H.C. *et al.*, 1969
5 weeks oral study in guinea pigs (gavage) 0 or 20 mg cobalt/kg bw	cobalt sulphate	cardiomyopathy LOAEL ≤ 20 Co mg/kg bw		Mohiuddin *et al.* 1970
24 week diet study in rats 0, 4.2 or 8.4 mg cobalt/kg bw/day (diet)	cobalt sulphate	4.2 mg cobalt/kg bw: decreased bw gain (33%). 8.4 mg cobalt/kg bw: significant reductions in a number of enzymes in cardiac tissues LOAEL ≤ 4.2 mg cobalt/kg bw		Clyne *et al.*, 2001
3 months oral toxicity study in male rats (40/dose) (drinking water) 0 or 30 mg cobalt/kg bw/day	Cobalt dichloride	Increased hematocrit and Hb, increased urea, decreased GPT, increased lung and heart weight, decreased testicle weight. LOAEL: ≤ 500 ppm (30 mg cobalt/kg/day)		Domingo, J.L. *et al.* 1984
3 months oral toxicity study in male rats (diet) 0 or 20 mg cobalt/kg bw/day	Cobalt dichloride hexahydrate	Increased erythrocyte count, packed cell volume, and haemoglobin concentration LOAEL: ≤ 265 ppm 20 mg Co/kg bw/day		Corrier, D.E. *et al.*, 1985
3 months oral (gavage)	Cobalt dichloride	≥ 10 mg/kg bw:	OECD 408	CDI/CORC

toxicity study in rats (10/sex/dose) 0, 3, 10 or 30 mg cobalt chloride/kg bw/day	hexahydrate	decreased body weight gain, changed hematological parameters 30 mg/kg bw: erythroid hyperplasia of the femur LOAEL: 10 mg/kg bw (2.5 mg cobalt/kg bw) NOAEL: 3 mg/kg bw (0.7 mg cobalt/kg bw)		2015
Oral developmental study in female rats (20/dose) (gavage) 0, 25, 50 or 100 mg cobalt dichloride/kg bw/day (GD6-15)	Cobalt dichloride hexahydrate	≥ 25 mg/kg bw: decreased body weight gain ≥ 50 mg/kg bw: decreased GOT and creatinine ≥ 100 mg/kg bw: increased Hb, Ht, MCV, MCH and reticulocytes; increased cholesterol LOAEL: ≤ 25 mg/kg bw (6.2 mg cobalt/kg bw)		Paternain, J.L. *et al.*, 1988
3 week study in male rats 0 or 12.4 mg cobalt/kg bw/day	Cobalt chloride	12.4 mg cobalt/kg bw: cardiac damage LOAEL: ≤ 12.4 mg cobalt/kg bw		Morvai *et al.*, 1993.
8 week study in rats 0, 0.6 or 2.5 mg cobalt/kg bw/day	Cobalt chloride	2.5 mg cobalt/kg bw: polycythemia NOAEL: 0.6 mg cobalt/kg bw LOAEL: 2.5 mg cobalt/kg bw		Stanley *et al.* 1947
4 month gavage study in rats 0 or 18 mg cobalt/kg bw/day	Cobalt chloride	18 mg cobalt/kg bw: renal injury LOAEL: ≤ 18 mg cobalt/kg bw		Holly, 1955
5 month study in rats 0 or 10 mg cobalt/kg bw/day	Cobalt chloride	10 mg cobalt/kg bw: increased liver weight (17%). LOAEL: ≤ 10 mg cobalt/kg bw		Murdock 1959
28 days oral toxicity study in rats (5/sex/dose) (gavage) 0, 15, 50 or 150 mg cobalt(II) 4-oxopent-2-en-2-olate dehydrate/kg bw/day	cobalt(II) 4-oxopent-2-en-2-olate dihydrate; purity 98.9%	≥ 50 mg/kg bw: reduced bw gain, increased Hb ≥ 150 mg: increased red blood cells, increased hematocrit LOAEL 50 mg/kg bw (11.5 mg cobalt/kg bw) NOAEL: 15 mg/kg bw (3.4 mg cobalt/kg bw)	EU Method B.7	Study report, 2007
inhalation				

16 days inhalation toxicity study in rats (5/sex/dose) 0, 2.5, 5, 10, 20 or 40 mg/m^3	Cobalt Purity >98% MMAD: 1.8-1.9 µm GSD: 1.7-1.8	≥ 2.5 mg/m^3: decreased liver weight, atrophy and necrosis olfactory epithelium, cytoplasmic vacuolization bronchioli ≥ 5 mg/m^3: pale lungs, lung infiltration ≥ 10 mg/m^3:decreased body weight, decreased kidney and thymus weight, increased lung weight, fibrosis and necrosis in the lung ≥ 20 mg/m^3: mortality LOAEC: ≤ 2.5 mg/m^3		NTP 2014
17 days inhalation toxicity study in mice (5/sex/dose) 0, 2.5, 5, 10, 20 or 40 mg/m^3	Cobalt Purity >98% MMAD: 1.8-1.9 µm GSD: 1.7-1.8	≥ 2.5 mg/m^3: decreased liver weight, vacuolization lung and resp epithelium, atrophy olfactory epithelium ≥ 5 mg/m^3: increased lung weight, infiltration and karyomegaly in the lung, inflammation resp epithelium, necrosis olfactory epithelium ≥ 10 mg/m^3: squamous metaplasia resp. epithelium ≥ 20 mg/m^3: decreased body weight 40 mg/m^3: mortality LOAEC: ≤ 2.5 mg/m^3		NTP 2014
90 days inhalation toxicity study in rats (10/sex/dose) 0, 0.625, 1.25, 2.5 or 5 mg/m^3	Cobalt; Purity >98% MMAD: 1.6-2.0 µm GSD: 1.7-2.0	≥ 0.625 mg/m^3: increased lung weight, decreased sperm motility, inflammation lung, proteinosis alveoli ≥ 1.25 mg/m^3: hyperplasia bronchioli, degeneration olfactory epithelium ≥ 2.5 mg/m^3: hyperplasia olfactory and resp. epithelium, turbinate atrophy ≥ 5 mg/m^3: decreased body weight LOAEC: ≤ 0.625 mg/m^3	OECD 413	NTP, 2014
90 days inhalation toxicity study in mice (10/sex/dose) 0, 0.625, 1.25, 2.5, 5 or 10 mg/m^3	Cobalt; Purity >98% MMAD: 1.6-2.0 µm GSD: 1.7-2.0	≥ 0.625 mg/m^3: infiltration lung, vacuolization bronchiole, squamous metaplasia larynx ≥ 1.25 mg/m^3:	OECD 413	NTP, 2014

		degeneration olfactory and resp. epithelium ≥ 2.5 mg/m^3: decreased liver weight, increased lung weight, decreased sperm motility, hyperplasia bronchiole and resp. epithelium, squamous metaplasia resp. epithelium ≥ 5 mg/m^3: tan lungs, decreased kidney and testis weight, decreased sperm activity, proteinosi and karyomegaly alveoli, tubinate atrophy, lung hemorrhage, inflammation lung and nose ≥ 10 mg/m^3: decreased body weight, degeneration testes, atrophy and cytopl. vacuolization epididymis, hypospermia, exfoliated germ cells. LOAEC: ≤ 0.625 mg/m^3		
combined repeated dose and carcinogenicity inhalation study in rats (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	Cobalt Purity >98% MMAD: 1.4-2.0 µm GSD: 1.6-1.9	≥ 2.5 mg/m^3: decreased survival, decreased body weight, necrosis olfactory epithelium ≥ 1.25 mg/m^3: hyperplasia, proteinosis, inflammation, atrophy, squamous metaplasia in nose and lung LOAEC: ≤ 1.25 mg/m^3		NTP, 2014
combined repeated dose and carcinogenicity inhalation study in mice (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	Cobalt Purity >98% MMAD: 1.5-2.1 µm GSD: 1.6-1.9	5 mg/m^3: decreased body weight ≥ 2.5 mg/m^3: decreased survival, inflammation and erosion lung ≥ 1.25 mg/m^3: hyperplasia, cytoplasmic vacuolization, proteinosis, infiltration, atrophy, metaplasia in lung, nose, larynx and trachea LOAEC: ≤ 1.25 mg/m^3		NTP, 2014
3 month study in pigs (5/dose) 0, 0.1 or 1 mg/m^3, 6h/day, 5 days/week	Cobalt metal	At 0.1 mg/m3: Decreased lung compliance, ECG abnormalities that may reflect ventricular impairment		Kerfoot 1975

		LOAEC: ≤ 0.1 mg/m^3		
16 days inhalation toxicity study in rats (5/sex/dose) 0, 0.1, 0.5, 5, 50 or 200 mg cobalt sulphate heptahydrate/m^3, 6h/day, 12 exposures over 16 days	cobalt sulphate heptahydrate; purity 99%; MMAD: 0.8-1.1 µm	≥ 50 mg/m^3 mortality, decreased body weight, inflammation and necrosis of respiratory epithelium, necrosis of thymus, testis atrophy. 200 mg/m^3: necrosis in liver LOAEC: 50 mg/m^3 (10.5 mg cobalt/m^3) NOAEC: 25 mg/m^3 (1.1 mg cobalt/m^3)		NTP, 1991
16 days inhalation toxicity study in mice (5/sex/dose) 0, 0.1, 0.5, 5, 50 or 200 mg cobalt sulphate heptahydrate/m^3, 6h/day, 12 exposures over 16 days	cobalt sulphate heptahydrate; purity 99%; MMAD: 0.8-1.1 µm	≥ 5 mg/m^3 , inflammation and necrosis of the respiratory epithelium. ≥ 50 mg/m^3 mortality LOAEC: 5 mg/m^3 (1.1 mg cobalt/m^3) NOAEC: 0.5 mg/m^3 (0.1 mg cobalt/m^3)		NTP, 1991
90 days inhalation toxicity study in rats (10/sex/dose) 0, 0.3, 1, 3, 10 or 30 mg cobalt sulphate heptahydrate/m^3, 6h/day, 5 days/week	cobalt sulphate heptahydrate; purity 99%; MMAD: 0.8-1.1 µm	≥ 0.3 mg/m^3: respiratory metaplasia. At higher doses, also inflammation, hyperplasia, necrosis and fibrosis are observed LOAEC: ≤ 0.3 mg/m^3 (0.06 mg cobalt/m^3)	Comparable to OECD 413	NTP, 1991
90 days inhalation toxicity study in mice (10/sex/dose) 0, 0.3, 1, 3, 10 or 30 mg cobalt sulphate heptahydrate/m^3, 6h/day, 5 days/week	cobalt sulphate heptahydrate; purity 99%; MMAD: 0.8-1.1 µm	≥ 0.3 mg/m^3: respiratory respiratory metaplasia. At higher doses, also inflammation, hyperplasia, necrosis and fibrosis are observed LOAEC: ≤ 0.3 mg/m^3 (0.06 mg cobalt/m^3)	Comparable to OECD 413	NTP, 1991
combined repeated dose and carcinogenicity inhalation study in rats (50/sex/dose) 0, 0.3, 1.0, or 3.0 mg cobalt sulphate heptahydrate /m³ 6 hours per day, 5 days per week, for 105 weeks)	cobalt sulphate heptahydrate; purity 99%; MMAD: 1.1-1.8 µm GSD: 1.9-2.6	≥ 0.3 mg/m^3 respiratory hyperplasia, inflammation, metaplasia and fibrosis LOAEC: ≤ 0.3 mg/m^3 (0.06 mg cobalt/m^3)		NTP, 1998
combined repeated dose and carcinogenicity inhalation study in mice (50/sex/dose) 0, 0.3, 1.0, or 3.0 mg cobalt sulphate heptahydrate /m³ 6 hours per day, 5 days per	cobalt sulphate heptahydrate; purity 99%; MMAD: 1.1-2.0 µm GSD: 2.1-3.0	≥ 0.3 mg/m^3 respiratory hyperplasia, inflammation, metaplasia and fibrosis Liver inflammation, karyomegaly, oval cell hyperplasia, and regeneration		NTP, 1998

week, for 105 weeks)		LOAEC: ≤ 0.3 mg/m^3 (0.06 mg cobalt/m^3)		
3-4 month study in rats and rabbits 0.4 – 9 mg Co/m^3	Cobalt oxides (mixed)	0.4–9 mg cobalt/m^3 : lesions in the alveolar region of the respiratory tract LOAEC: ≤ 0.4 mg cobalt/m^3		Johansson *et al.* 1984, 1987, 1991, 1992; Kyono *et al.* 1992; Palmes *et al.* 1959
Carcinogenicity inhalation study in hamster (51/group) 0 or 10 g/L 7h/day, 5 days/week, for 17-21 months	Cobalt oxide	7.9 mg cobalt/m^3: emphysema LOAEC: ≤ 7.9 mg cobalt/m^3		Wehner, 1977

4.7.1 Non-human information

Besides repeated dose toxicity studies, also several fertility or developmental studies provide some information on repeated dose toxicity. Effects due to repeated dose toxicity are discussed below, for effects on fertility/development, see 4.11: Reproductive toxicity.

4.7.1.1 Repeated dose toxicity: oral

Studies with cobalt metal

In a combined repeated dose toxicity study with reproduction/developmental toxicity screening test according to OECD guideline 422, rats (SD) (n=10 / dose / sex) were treated by gavage with powdered cobalt (0, 30, 100, 300 or 1000 mg/kg bw/day, purity >99.8%) (vehicle 0.5% hydroxypropyl methylcellulose gel) (particle size: D50=12.8 μm) from 2 weeks before mating until approximately 2 weeks after mating (males) or 3 days post-partum (females). Testing at 30 mg/kg bw was performed after the mortality at the higher dose levels became evident. The limit for statistical significance was set at $p<0.01$ for some effects and for others at $p<0.05$. All females and 9 out of 10 males died at 1000 mg/kg bw/day (see table 15). No mortality occurred in males at lower dose levels. Eight out of 10 females treated with 300 mg/kg bw/day and five out of 10 females treated with 100 mg/kg bw/day died during the mating, gestation or lactation period. No mortality was observed in females treated with 30 mg/kg bw/day. Specific effects on fertility and development can be found in paragraph 4.11 Toxicity for reproduction.

Table 15. Effects on mortality in rats after repeated exposure to cobalt metal

	0	30	100	300	1000
Males	0/10	0/10	0/10	0/10	9/10
females	0/10	0/10	5/10	8/10	10/10

Piloerection was observed at ≥ 100 mg/kg bw. Reduced motility and soft faeces were observed in males at 1000 mg/kg and in females at ≥ 100 mg/kg bw (depending on the period in the study). Pre

lethal symptoms also included hunched posture, increased or decreased respiratory rate, reduced body temperature (animal cold at touch), dyspnoea, tremor, miosis, ptosis, a haemorrhagic nose, haemorrhagic urine or a pale skin in some animals.

A neurological screening was performed between weeks 4-9, in animals dosed with 0, 30, 100 and 300 mg/kg bw. In females (300 mg/kg bw), several changes were noted in the observational screening, but these were related to the moribund condition of 2 of 4 of the animals. Furthermore, reduced forelimb grip strength was observed in both sexes at ≥ 300 mg/kg bw and in males also at 100 mg/kg bw.

Body weight of the male rats was reduced from test week 2 onwards, being 13% or 16% (300 and 1000 mg/kg bw) below the control value in test week 3 (mating period). Body weight at autopsy was reduced as well in the 300 mg/kg bw group (12% below the control value). Body weight of the female rats (300 mg/kg bw) was below the control on gestation day 20 (by 11%) and on lactation day 1 (by 21%). The body weight of the two surviving females was still reduced on lactation day 4 and at autopsy (20% or 19% below the control value). Food intake was not changed in males. In females, relative food intake of the animals treated with 100 or 300 mg /kg bw was below that of the control group by minus 37% or minus 68% on lactation day 1.

No test item-related changes in haematological or biochemical parameters were observed on day 15, although some small increases were observed in HGB (males, 4-8%). No biologically relevant effects on organ weights were reported with the exception of effects on the spleen. In females the relative weight was increased at 30 mg/kg bw and showed a dose related increase up to 165% (except at 1000 mg/kg bw due to 100% mortality). In males there was also an increase although not statistically significant at $p<0.01$ at all dose levels up to 134%. All these parameters were only statistically tested for $p<0.01$.

At 1000 mg/kg bw/day, a reddened stomach was noted in the only survivor of ten males of the high dose level. Macroscopic inspection of the prematurely deceased nine males revealed pathological changes of the adrenals (enlarged and / or reddened) and the gastro-intestinal region (reddened intestines, caecum or stomach) in nearly all animals. In addition, lesions of the lungs (oedematous) were noted in some animals, changes of thymus (reddened) were seen in two of nine animals. In females, at ≥ 100 mg/kg bw, changes of the gastro-intestinal tract (reddened, haemorrhagic foci, filled with fluid) were noted - in relation to the dose - in a few to several animals. In addition, a reddened thymus was noted in a few females treated with 100 mg/kg bw. Further changes were noted at 1000 mg/kg bw in the form of enlarged and / or reddened adrenals in nearly all animals and oedematous lungs in some animals.

The histological examination of rat organs did not reveal any morphological lesions which are considered to be related to the test item. For the macroscopic lesions noted at necropsy no histological correlate could be found. However, adrenal congestion was observed in 4 out of 5 females at 300 mg/kg bw compared to 0 out of 5 females in the controls. At 100 mg/kg bw, only one female was examined also showing adrenal congestion. No animals at 30 mg/kg bw were examined. Also an increase in placenta with mild or moderate congestion was observed at 300 mg/kg bw. The NOAEL was 30 mg/kg bw/day (CDI/CORC 2015).

Studies with soluble cobalt compounds

Cobalt sulphate (heptahydrate)

fertility/development, see 4.11: Reproductive toxicity.

Pregnant New Zealand White rabbits (8-25/dose) were treated daily with cobalt sulphate (0, 20, 100, or 200 mg/kg bw) by gavage during GD6-20. All doses resulted in mortality (5/25, 4/13 and 7/8 dams died), due to circulatory failure. Maternal body weight gain was reduced at ≥ 20 mg/kg bw (Szakmáry, E. *et al.* 2001).

Three groups of 20 male Wistar rats were given different diets each for eight weeks (differences in thiamine, potassium phosphate and /or calcium carbonate content). After eight weeks, half the rats of each diet group were given an initial oral dose of 100 mg/kg of cobalt sulphate dissolved in water, followed by daily oral doses of 26 mg/kg of cobalt sulphate for eight weeks. Five rats of each diet group died before the experiment was terminated. Histological changes, involving both myocardial cells and interstitium, were seen in 26 of the 30 rats given cobalt. The initial changes involved oedematous separation of cells, some fragmentation and vacuolization of myocardial cells, and minimal inflammatory cellular response. There was a slight swelling of the myocardial cells and an apparent increase in ground substance, along with a decrease in the number of myofibrils so that only a few myofibrils remained in some cells. Electron microscopy indicated that mitochondria in areas of greatest damage tended to be slender and smaller than normal, averaging 0.12 µ in diameter. Prominent myofibrillar degeneration was evidenced by the focal and segmental occurrence of brightly acidophilic contraction bands in some myocardial cells.
In certain cells the thinning, disappearance, and separation of myofibrils was associated with an accumulation of small vacuoles appearing in rows between the myofibrils. Electron microscopical observations revealed focal areas of degeneration, in which some muscle cells contained fat droplets, 0.4 - 0.8µ in diameter. The focal fragmentation of muscle fibers was pronounced in some areas, and fragmented fibers became rounded, decreased in number and size, and replaced by loose fibrous tissue. Histological changes involving slight separation of muscle fibers and small focal areas of fibrous tissue replacement of myocardial cells were seen in two rats given diet 2 without added cobalt and in four rats given diet 3 without added cobalt. No abnormalities were seen in the hearts of animals given diet 1 alone. (Grice, H.C. *et al.*, 1969).

In an experiment designed to simulate conditions leading to beer-cobalt cardiomyopathy in humans, guinea pigs were given 20 mg cobalt/kg/day as cobalt sulphate by gavage either alone or in combination with ethanol (as part of a liquid diet) for 5 weeks (Mohiuddin *et al.* 1970). The experiment resulted in cardiomyopathy, which was characterized by abnormal EKGs; increased heart weights; lesions involving the pericardium, myocardium, and endocardium; and disfigured mitochondria. Alcohol did not intensify the cardiac effects (as summarised by ATSDR, 2004).

Clyne et al. (2001) reported that exposure of rats to 8.4 mg cobalt/kg/day, as cobalt sulphate, in the diet for 24 weeks resulted in significant reductions in a number of enzymes in cardiac tissues, including manganese-superoxide dismutase, succinate-cytochrome c oxidase, NADH-cytochrome c reductase, and cytochrome c oxidase, as well as reducing the mitochondrial ATP production rate. In addition, a significant decrease (33%) in body weight gain was observed following 8 weeks of exposure of rats to 4.2 mg cobalt/kg/day as cobalt sulphate (as summarised by ATSDR 2004).

Cobalt chloride (hexahydrate)

Male Sprague-Dawley rats (40/dose) were given cobalt chloride in drinking water for three months at a concentration of 0 or 500 ppm (30 mg Co/kg bw/day). Body weight gain was reduced only in the first 1.5 month of treatment. A significant increase in the haematocrit and haemoglobin was observed. A significant increase was observed for urea and a significant decrease was observed for GPT. Lung and heart weight were significantly increased. Testicle weight was significantly decreased. Hypertrophy was observed in the spleen. No morphological changes or atypical intracellular deposits were noted (Domingo 1984).

Male Sprague Dawley rats were given a daily diet containing 0 or 265 ppm cobalt chloride hexahydrate (20 mg cobalt/kg bw/day) during a 98 day study period. Cardiac blood was collected from rats sacrificed on day 84 and 98 (3 rats/dose/time point). No effects on body weight were observed. Mean erythrocyte counts, packed cell volume, and haemoglobin concentrations of the cobalt-fed rats were significantly higher than the controls on days 84 and 98 (Corrier, 1985).

In a 90 day repeated dose toxicity study according to OECD GL 408, Crl:CD(SD) rats (10/sex/dose) were given 0, 3, 10 or 30 mg cobalt chloride/kg bw/day by gavage (vehicle: tap water). Recovery animals (5/sex/dose extra) were included in the control and high dose group. No justification for the test dose levels was provided. In addition to the standard requirements testosterone, progesterone and 17β-estradiol concentrations were determined in blood at 0, 6 and 13 weeks. Additional sampling for cobalt determination was performed. However, these results were reported separately (not yet available).

No substance-related mortality was observed. No relevant neurological changes were noted. Body weight was reduced at the end of the study (6 and 11% for males in the 10 and 30 mg/kg bw group and 9% for females in the 30 mg/kg bw group, only statistically significant in high dose males). No effects were observed on the oestrous cycle. Several haematological changes were observed in the mid and high dose groups (see table 16 below). In addition, plasma levels of bilirubin were increased by 14% for the male animals treated with 10 mg Cobalt dichloride hexahydrate/kg bw/day and by 29% to 34% for the male and 16% for the female animals treated with 30 mg/kg bw. No test item-related effects were observed on urinalysis, organ weight (with the possible exception of a small increase in relative spleen weights) and macroscopic post mortem findings. Some slight alterations were observed in the hormone levels of Testosterone, and 17β-Estradiol, however, they were not considered treatment-related (see table 17). In addition, the control values varied over time making assessment difficult. Microscopic evaluation revealed test item-related changes in the bone marrow (erythroid hyperplasia) of the femur. There was a significant and test item-related increase for erythroid hyperplasia in the bone marrow of the male and female animals treated with 30 mg Cobalt dichloride hexahydrate/kg b.w./day compared to the controls: 7 of 10 animals for both sexes in the high dose group versus 0 of 10 in controls. Bone marrow of animals treated with 10 mg Cobalt dichloride hexahydrate/kg b.w./day displayed significant erythroid hyperplasia, although of marginal to slight severity when compared to controls (see table 18). No effects were observed in the bone marrow of low dose animals. Histopathological examination of testes and epididymis did not show test-item related effects.

Recovery: body weight of the male and female animals previously treated with 30 mg Cobalt dichloride hexahydrate/kg b.w./day was still statistically significant reduced by 17% or by 13%, respectively, on test day 118 compared to the control group. However, all changes previously observed in haematological and biochemical parameters and at histological examination had subsided after 4 weeks of recovery (CDI/CORC 2015).

Table 16: Changes in haematological parameters in rats after 13 weeks exposure to cobalt chloride

Maximum changes in haematological parameters compared to control group 1 (vehicle) [%] (test day 91 and 92 combined)				
Parameter	**Group 3 10 mg/kg**		**Group 4 30 mg/kg**	
	males	**females**	**males**	**females**
	Test day 91/92			
HGB	+11**	none	+25**	+14**

RBC	+10**	none	+19**	+11**
Reti	-33*	none	-24	none
PLT	-13	none	-26**	-12
HCT	+12**	none	+23**	+14**
TPT	none	none	+7**	none
aPTT	none	none	+8*	none
MCV	none	+4*	+4*	+3
MCH	none	none	+5**	none

** = statistically significant at $p \leq 0.01$

* = statistically significant at $p \leq 0.05$

Table 17 Hormone levels in rats after 13 weeks exposure to cobalt chloride

Parameter	controls	30 mg/kg bw	Sex	Test day	Statistical significance
Testosterone (ng/ml serum)	10.109±4.410	6.307±3.110	m	predose	$p \leq 0.05$
	0.483±0.041	0.652±0.095	f	predose	$p \leq 0.01$
	6.304±3.024	3.940±2.104	m	42	$p \leq 0.05$
	0.603±0.117	0.751±0.244	f	42	$p \leq 0.05$
	3.459±1.541	1.477±0.730	m	91/92	$p \leq 0.01$
	1.167±0.333	0.713±0.125	f	91/92	$p \leq 0.01$
	2.587±0.734	1.119±0.394	m	119	$p \leq 0.01$
	1.338±0.268	0.728±0.216	f	119	$p \leq 0.01$
17β-Estradiol (pg/ml serum)	6.68±5.60	14.06±9.80	f	predose	$p \leq 0.05$
	7.30±6.94	29.53±18.17	f	91/92	$p \leq 0.01$

Table 18 Histopathology of bone marrow in rats after 13 weeks exposure to cobalt chloride

Removal Reason: ALL	Male				Female			
Group:	Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 1	Gr. 2	Gr. 3	Gr. 4
Number of Animals:	10	10	10	10	10	10	10	10
Number of Completed Animals:	10	10	10	10	10	10	10	10
bone marrow (os femoris)								
Examined	10	10	10	10	10	10	10	10
No abnormalities detected	10	10	6	3	10	10	3	3
erythroid hyperplasia	0	0	4	7 ¹	0	0	7 ¹	7 ¹
.... marginal	0	0	3	3	0	0	5 *+	1
.... slight	0	0	1	4	0	0	2	5 *+
.... moderate	0	0	0	0	0	0	0	1

+ [Footnote is displayed in the Comments and Markers page] - General Footnote: [Fisher's Two-Tailed Exact Test Performed: = 5% Sign*

*1 [** - Test: Fisher's Exact 2 Sided p < 0.01]*

Pregnant Sprague-Dawley rats (20/dose) were given a daily dose of 0, 25, 50, and 100 mg/kg cobalt chloride by gavage on days 6 - 15 of gestation. A dose related decrease in body weight gain was observed in all treated groups. A significant increase in haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, and reticulocytes was observed at 100 mg/kg bw. GOT and creatinine levels were significantly reduced at ≥ 50 mg/kg bw, whereas total protein concentration was significantly increased. Cholesterol level was significantly increased at 100 mg/kg bw. Quantitative data were not available. No effects were observed on organ weight (Paternain, 1988).

Three weeks of exposure to 12.4 mg cobalt/kg/day as cobalt chloride in male rats resulted in cardiac damage, presenting as incipient, multifocal myocytolysis, with degeneration of myofibrilles (Morvai *et al.* 1993 as summarised by ATSDR 2004).

No morphological changes in the liver, lungs or gastrointestinal system of rats were observed following exposure for 4 months to 18 mg cobalt/kg/day as cobalt chloride by gavage. However, renal injury, evidenced by histologic alteration of the proximal tubules (necrosis) was observed (Holly 1955 as summarised by ATSDR 2004).

Increased liver weight (17%) was found in rats exposed to 10 mg cobalt/kg/day (as cobalt chloride) for 5 months. No effects on body weight were observed. Renal injury, evidenced by histologic alteration of the proximal tubules was observed (Murdock 1959 as summarised by ATSDR 2004).

8-week study in rats (Stanley *et al.* 1947), which reported dose- and time-related increases in erythrocyte number following oral administration of cobalt chloride, with an apparent NOAEL of 0.6 mg cobalt/kg/day and a LOAEL of 2.5 mg cobalt/kg/day (as summarised by ATSDR 2004).

Significantly increased erythrocyte (polycythemia), hematocrit, and hemoglobin levels were found in animals treated orally with cobalt chloride as a single dose of 161 mg cobalt/kg (Domingo and Llobet 1984) or with longer-term exposure (3 weeks to 2 months) to ≥0.5 mg/kg/day (Brewer 1940; Davis 1937; Domingo *et al.* 1984; Holly 1955; Krasovskii and Fridlyand 1971; Murdock 1959; Stanley *et al.* 1947) (as summarised by ATSDR 2004).

Cobalt(II) 4-oxopent-2-en-2-olate dihydrate

Sprague Dawley (5/sex/dose) were exposed by gavage to cobalt(II) 4-oxopent-2-en-2-olate dihydrate at concentrations of 0, 15, 50 or 150 mg/kg bw for **28 days** (vehicle 0.5% methylcellulose). No mortality occurred during the study. Hypersalivation was noted in 4/5 females (but not in males) given 150 mg/kg/day (starting in week 2 or 3 and lasting for between 9 and 16 days). Mean body weight gain in the top dose group was lower than in controls from day 1-8 (-38%, $p<0.01$ in males and -34% in females). In males but not females, body weight gain was also statistically significant reduced in the second half of the study (overall bw gain -22%, $p<0.01$). At 50 mg/kg bw, bw gain was also reduced in males (-18%, $p<0.01$). At 15 mg/kg bw a similar trend was observed, but this was not statistically significant.

Red blood cell count was statistically significant increased when compared to controls at 150 mg/kg/day (males: +15%, $p<0.01$ and females: +20%, $p<0.01$). Hemoglobin concentration was significantly increased at 150 mg/kg/day (both sexes) and 50 mg/kg/day (males only). A slight, but statistically significant increase in PCV (hematocrit) was noted in males and females treated at 150 mg/kg/day. A statistically significant low plasma cholesterol level (1.1 versus 1.9 mmol/L, -42%, $p<0.01$) was noted in males treated at 150 mg/kg/day.

No toxicologically relevant effects on organ weights were observed. No treatment-related necropsy findings or microscopic changes were noted. The NOAEL was 15 mg/kg bw, based on reduced body weight in males at 50 mg/kg bw (Study report 2007).

4.7.1.2 Repeated dose toxicity: inhalation

Studies with cobalt metal

F344/N rats (5/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **16 days**. No hematology was performed in this study. All rats exposed to 40 mg/m^3 and all male and three female rats exposed to 20 mg/m^3 died before the end of the study; the majority of deaths occurred by study day 7. Mean body weight and body weight gain were significantly decreased in male and female rats exposed to ≥ 10 mg/m^3 (see table 19). Exposure-related clinical findings included abnormal breathing, lethargy, and thinness in male rats exposed to 20 or 40 mg/m^3, and in females exposed to 40 mg/m^3. Dark lungs were observed at necropsy in all early-death rats of both sexes exposed to 40 mg/m^3 and most rats exposed to 20 mg/m^3. Pale lungs were noted in two females exposed to 20 mg/m^3, four males exposed to 10 mg/m^3, and one male exposed to 5 mg/m^3. Absolute and relative lung weights were significantly increased. Overall, absolute and relative liver and thymus weights and absolute kidney and testis weights were significantly decreased (for details, see table 20). Increased incidences of lesions of the lung occurred in exposed male and female rats and included hemorrhage, acute inflammation, alveolar epithelium hyperplasia, histiocytic cellular infiltration of the alveolus, cytoplasmic vacuolization of bronchiolar epithelium, necrosis of the bronchiolar epithelium, and interstitial fibrosis of the alveolar epithelium. Increased incidences of lesions of the nose occurred in exposed male and female rats and included olfactory epithelium necrosis, olfactory epithelium atrophy, respiratory epithelium necrosis, and respiratory epithelium squamous metaplasia (for details, see table 21). Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined. The LOAEC that can be derived from this study is 2.5 mg/m^3 (lowest dose administered) (NTP 2014).

Table 19: Survival and body weight of rats in the 2 week inhalation study of cobalt metal

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	102 ± 2	144 ± 3	41 ± 2	
2.5	5/5	102 ± 2	144 ± 2	42 ± 2	100
5	5/5	102 ± 3	140 ± 4	38 ± 2	97
10	5/5	100 ± 3	115 ± 6**	14 ± 4**	80
20	0/5[c]	103 ± 3	—	—	—
40	0/5[d]	101 ± 3	—	—	—
Female					
0	5/5	88 ± 4	112 ± 4	24 ± 2	
2.5	5/5	88 ± 2	112 ± 2	24 ± 1	100
5	5/5	86 ± 3	107 ± 3	21 ± 1	96
10	5/5	87 ± 4	98 ± 4**	11 ± 1**	88
20	2/5[e]	86 ± 3	61 ± 5**	–23 ± 0**	55
40	0/5[f]	86 ± 3	—	—	—

** Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 2 weeks/number initially in group

[c] Days of deaths: 5, 5, 5, 9, 13

[d] Days of deaths: 5, 6, 6, 7, 7

[e] Days of deaths: 5, 7, 13

[f] Days of deaths: 5, 6, 6, 6, 7

Table 20: Selected organ weights and organ weight to body weight ratios for rats in the 2 week inhalation study of cobalt metal

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Male						
n	5	5	5	5	0	0
Necropsy body wt	144 ± 3	144 ± 2	140 ± 4	115 ± 6**		
L. Kidney						
Absolute	0.61 ± 0.02	0.61 ± 0.01	0.58 ± 0.01	0.52 ± 0.02**		
Relative	4.25 ± 0.06	4.26 ± 0.08	4.12 ± 0.09	4.57 ± 0.10*		
Liver						
Absolute	5.84 ± 0.16	5.10 ± 0.09**	5.08 ± 0.15**	4.29 ± 0.24**		
Relative	40.61 ± 0.46	35.40 ± 0.28**	36.35 ± 0.63**	37.43 ± 0.86**		
Lung						
Absolute	1.14 ± 0.10	1.16 ± 0.08	1.19 ± 0.04	1.28 ± 0.12		
Relative	7.91 ± 0.61	8.07 ± 0.55	8.49 ± 0.30	11.13 ± 0.50**		
L. Testis						
Absolute	0.886 ± 0.040	0.928 ± 0.017	0.852 ± 0.035	0.590 ± 0.088**		
Relative	6.165 ± 0.246	6.446 ± 0.155	6.103 ± 0.248	5.053 ± 0.502		
Thymus						
Absolute	0.374 ± 0.013	0.358 ± 0.025	0.358 ± 0.007	0.284 ± 0.008**		
Relative	2.605 ± 0.054	2.485 ± 0.161	2.560 ± 0.023	2.498 ± 0.112		
Female						
n	5	5	5	5	2	0
Necropsy body wt	112 ± 4	112 ± 2	107 ± 3	98 ± 4**	61 ± 5**	
L. Kidney						
Absolute	0.52 ± 0.02	0.50 ± 0.01	0.50 ± 0.02	0.46 ± 0.01*	0.35 ± 0.00**	
Relative	4.66 ± 0.11	4.46 ± 0.05	4.63 ± 0.08	4.74 ± 0.12	5.75 ± 0.42**	
Liver						
Absolute	4.07 ± 0.16	3.77 ± 0.05	3.61 ± 0.13**	3.44 ± 0.05**	2.57 ± 0.06**	
Relative	36.37 ± 0.49	33.59 ± 0.16	33.78 ± 1.08	35.17 ± 1.00	42.15 ± 2.12**	
Lung						
Absolute	0.86 ± 0.04	0.83 ± 0.01	0.91 ± 0.04	1.03 ± 0.06*	1.01 ± 0.04*	
Relative	7.71 ± 0.36	7.44 ± 0.07	8.49 ± 0.34	10.54 ± 0.69**	16.54 ± 0.56**	
Thymus						
Absolute	0.317 ± 0.016	0.324 ± 0.011	0.352 ± 0.022	0.289 ± 0.011	0.064 ± 0.016**	
Relative	2.842 ± 0.167	2.895 ± 0.126	3.289 ± 0.201	2.948 ± 0.092	1.024 ± 0.178**	

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test

** P≤0.01

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data are available for 20 mg/m^3 males or 40 mg/m^3 males or females due to 100% mortality.

Table 21: Incidences of selected nonneoplastic lesions of the respiratory system in rats in the 2 week inhalation study of cobalt metal

	Chamber Control	2.5 mg/m³	5 mg/m³	10 mg/m³	20 mg/m³	40 mg/m³
Male						
Lung[a]	5	5	5	5	5	5
Hemorrhage[b]	0	0	0	1 (1.0)[c]	5** (1.2)	5** (3.0)
Inflammation, Acute	0	0	0	0	4* (1.3)	5** (2.0)
Alevolar Epithelium, Hyperplasia	0	0	0	0	3 (1.7)	5** (1.4)
Alveolus, Infiltration Cellular, Histiocyte	0	0	4* (1.0)	3 (1.3)	5** (2.0)	5** (1.2)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	5** (1.2)	5** (1.6)	5** (2.0)	1 (2.0)	0
Bronchiole, Epithelium, Necrosis	0	0	0	0	2 (1.0)	3 (1.0)
Interstitium, Fibrosis	0	0	0	5** (1.2)	2 (3.0)	0
Nose	5	5	5	5	5	5
Olfactory Epithelium, Necrosis	0	3 (1.0)	4* (1.3)	4* (1.0)	4* (2.8)	5** (3.0)
Olfactory Epithelium, Atrophy	0	5** (1.6)	5** (1.8)	5** (2.4)	3 (1.7)	3 (1.7)
Respiratory Epithelium, Necrosis	0	0	0	1 (1.0)	3 (1.3)	5** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	2 (1.0)	1 (1.0)
Female						
Lung	5	5	5	5	5	5
Hemorrhage	0	0	0	0	3 (2.0)	5** (2.8)
Inflammation, Acute	0	0	0	0	2 (1.0)	5** (1.4)
Alevolar Epithelium, Hyperplasia	0	0	0	0	2 (1.0)	2 (1.0)
Alveolus, Infiltration Cellular, Histiocyte	0	0	0	0	5** (2.0)	5** (1.8)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	4* (1.0)	5** (1.0)	5** (1.8)	3 (1.7)	0
Bronchiole, Epithelium, Necrosis	0	1 (1.0)	1 (1.0)	4* (1.0)	3 (1.0)	3 (1.0)
Interstitium, Fibrosis	0	0	0	4* (1.0)	3 (3.0)	0
Nose	5	5	5	5	5	5
Olfactory Epithelium, Necrosis	0	5** (1.0)	3 (1.0)	5** (1.0)	5** (2.0)	5** (3.0)
Olfactory Epithelium, Atrophy	0	5** (1.8)	5** (2.0)	5** (2.0)	4* (2.8)	1 (2.0)
Respiratory Epithelium, Necrosis	0	0	0	0	5** (1.4)	5** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	1 (1.0)	0

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[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

B6C3F1/N mice (5/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **17 days**. No hematology was performed in this study. Three male and three female mice exposed to 40 mg/m^3 died before the end of the study. Mean body weight and body weight gain were significantly decreased in male and female mice exposed to ≥ 20 mg/m^3 as well as body weight gain of the lower dose females (see table 22). Exposure-related clinical findings included

abnormal breathing, lethargy, and thinness in male rats exposed to ≥ 20 mg/m^3 and females exposed to 10 mg/m^3 or greater. At necropsy, tan lungs were observed in most males and females exposed to ≥ 20 mg/m^3. Dark lung lobes were observed in one early-death male.

Lung weights of both sexes exposed to ≥ 10 mg/m^{3}or greater were significantly increased. Liver weights of exposed male and female mice were significantly decreased (except relative weight at 40 mg/m^3) (see table 23). Increased incidences of nonneoplastic lesions of the lung occurred in exposed male and female mice and included alveolar histiocytic cellular infiltration, cytoplasmic vacuolization of the bronchiolar epithelium, alveolar/bronchiolar epithelium karyomegaly, interstitial fibrosis, and acute inflammation. Increased incidences of nonneoplastic lesions of the nose occurred in exposed groups of male and female mice and included acute inflammation, olfactory epithelium atrophy, olfactory epithelium necrosis, cytoplasmic vacuolization of the respiratory epithelium, and squamous metaplasia of the respiratory epithelium (see table 24). Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined. The NOAEC of this study is 2.5 mg/m^3 (lowest dose (NTP 2014).

Table 22: Survival and body weight of mice in the 2 week inhalation study of cobalt metal[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	23.4 ± 0.3	25.7 ± 0.5	2.3 ± 0.3	
2.5	5/5	23.5 ± 0.3	25.0 ± 0.5	1.5 ± 0.2	97
5	5/5	23.6 ± 0.3	25.9 ± 0.3	2.2 ± 0.4	101
10	5/5	23.8 ± 0.3	25.3 ± 0.5	1.5 ± 0.2	98
20	5/5	23.1 ± 0.4	23.4 ± 0.4**	0.2 ± 0.4**	91
40	2/5[c]	23.0 ± 0.4	18.9 ± 1.1**	–4.7 ± 1.7**	73
Female					
0	5/5	19.1 ± 0.3	20.8 ± 0.1	1.7 ± 0.3	
2.5	5/5	19.8 ± 0.5	20.3 ± 0.5	0.5 ± 0.2*	98
5	5/5	19.8 ± 0.5	20.1 ± 0.5	0.3 ± 0.4*	97
10	5/5	19.4 ± 0.4	20.0 ± 0.6	0.6 ± 0.4*	96
20	5/5	19.0 ± 0.3	17.4 ± 0.4**	–1.6 ± 0.2**	84
40	2/5[d]	18.9 ± 0.2	13.0 ± 1.6**	–6.1 ± 1.1**	62

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** P≤0.01
[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 2 weeks/number initially in group
[c] Days of deaths: 5, 5, 8
[d] Days of deaths: 6, 7, 9

Table 23: Selected organ weights and organ weight to body weight ratios for mice in the 2 week inhalation study of cobalt metal

	Chamber Control	2.5 mg/m³	5 mg/m³	10 mg/m³	20 mg/m³	40 mg/m³
n	5	5	5	5	5	2
Male						
Necropsy body wt	25.7 ± 0.5	25.0 ± 0.5	25.9 ± 0.3	25.3 ± 0.5	23.4 ± 0.4**	18.9 ± 1.1**
Liver						
Absolute	1.13 ± 0.04	0.98 ± 0.04**	0.98 ± 0.04**	0.99 ± 0.02**	0.89 ± 0.02**	0.83 ± 0.01**
Relative	43.88 ± 0.80	39.18 ± 1.35*	37.67 ± 1.09**	39.32 ± 0.91*	37.93 ± 0.51**	44.20 ± 2.99
Lung						
Absolute	0.18 ± 0.01	0.21 ± 0.01	0.23 ± 0.01*	0.24 ± 0.01**	0.29 ± 0.01**	0.36 ± 0.05**
Relative	7.08 ± 0.15	8.33 ± 0.32	8.73 ± 0.33	9.61 ± 0.62*	12.62 ± 0.51**	19.31 ± 3.73**
L. Testis						
Absolute	0.098 ± 0.002	0.104 ± 0.001	0.099 ± 0.004	0.084 ± 0.009	0.089 ± 0.003	0.070 ± 0.002**
Relative	3.834 ± 0.074	4.180 ± 0.114	3.812 ± 0.149	3.322 ± 0.311	3.807 ± 0.088	3.731 ± 0.314
Female						
Necropsy body wt	20.8 ± 0.1	20.3 ± 0.5	20.1 ± 0.5	20.0 ± 0.6	17.4 ± 0.4**	13.0 ± 1.6**
Liver						
Absolute	0.93 ± 0.03	0.81 ± 0.02**	0.80 ± 0.03**	0.75 ± 0.03**	0.69 ± 0.03**	0.61 ± 0.06**
Relative	44.56 ± 1.13	40.09 ± 0.31**	39.75 ± 0.82**	37.40 ± 1.12**	39.73 ± 0.70**	46.88 ± 1.36
Lung						
Absolute	0.19 ± 0.01	0.19 ± 0.00	0.22 ± 0.01*	0.23 ± 0.01**	0.29 ± 0.01**	0.33 ± 0.02**
Relative	9.34 ± 0.38	9.49 ± 0.37	11.14 ± 0.24*	11.77 ± 0.59**	16.80 ± 0.58**	25.67 ± 1.53**

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test

** $P \leq 0.01$

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table 24: Incidences of selected nonneoplastic lesions of the respiratory system in mice in the 2 week inhalation study of cobalt metal

	Chamber Control	2.5 mg/m³	5 mg/m³	10 mg/m³	20 mg/m³	40 mg/m³
Male						
Lung[a]	5	5	5	5	5	5
Alveolus, Infiltration Cellular, Histiocyte[b]	0	2 (1.0)[c]	5** (1.0)	5** (1.4)	5** (2.4)	5** (2.0)
Bronchiole, Epithelium, Vacuolization, Cytoplasmic	0	4* (1.0)	3 (1.0)	5** (1.6)	3 (1.0)	3 (1.3)
Alveolar/bronchiolar Epithelium, Karyomegaly	0	0	4* (1.0)	5** (1.0)	5** (1.8)	4* (1.5)
Interstitium, Fibrosis	0	0	0	3 (1.0)	5** (2.2)	3 (2.7)
Inflammation, Acute	0	0	0	0	0	3 (1.7)
Nose	5	5	5	5	5	5
Inflammation, Acute	0	0	1 (1.0)	5** (2.4)	5** (1.6)	5** (1.8)
Olfactory Epithelium, Atrophy	0	5** (1.0)	5** (1.0)	5** (1.8)	5** (1.8)	4** (2.0)
Olfactory Epithelium, Necrosis	0	2 (1.0)	3 (1.0)	0	5** (1.2)	5** (1.4)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	4* (1.0)	5** (1.0)	4* (1.0)	5** (1.2)	5** (1.2)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	4* (1.0)	4* (1.0)	2 (1.0)
Female						
Lung	5	5	5	5	5	5
Alveolus, Infiltration Cellular, Histiocyte	0	2 (1.0)	5** (1.4)	5** (1.6)	5** (2.6)	5** (2.4)
Bronchiole, Epithelium, Vacuolization, Cytoplasmic	0	2 (1.0)	4* (1.0)	3 (1.7)	2 (1.0)	1 (1.0)
Alveolar/bronchiolar Epithelium, Karyomegaly	0	3 (1.0)	4* (1.0)	5** (1.2)	4* (1.3)	4* (1.0)
Interstitium, Fibrosis	0	0	0	2 (1.0)	5** (2.8)	2 (3.5)
Inflammation, Acute	0	0	2 (1.0)	1 (1.0)	3 (1.0)	2 (1.5)
Nose	5	5	5	5	5	5
Acute Inflammation	0	0	5** (2.0)	5** (2.6)	5** (2.4)	5** (2.2)
Olfactory Epithelium, Atrophy	0	5** (1.4)	5** (1.6)	5** (1.8)	5** (2.2)	3 (2.0)
Olfactory Epithelium, Necrosis	0	3 (1.0)	5** (1.0)	2 (1.5)	4* (1.5)	3 (1.7)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	5** (1.0)	5** (1.0)	5** (1.2)	4* (1.0)	4* (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	3 (1.0)	1 (1.0)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[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

Groups of F344/N rats (10/sex/dose) were to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **14 weeks**. All male and female rats survived to the end of the study. Mean body weights of males and females exposed to 5 mg/m^3 were significantly less than those of the chamber controls, and the mean body weight gain of 5 mg/m^3 males was significantly less than that of the chamber controls (table 25). There were no clinical signs related to cobalt metal exposure. At necropsy, pale foci were noted in the lungs of most exposed male and female rats.

In male rats, exposure concentration-related increases in the hemoglobin concentration, erythrocyte count, hematocrit value, and manual packed cell volume occurred at $\geq$ 2.5 mg/m^3 on days 3 and 23

and in all exposed groups by week 14; at week 14, female rats also had increases in these parameters. Exposure concentration-related decreases in cholesterol concentrations were observed at all three time points in male and female rats. While this change was not always observed in the lower exposure groups, decreases were consistently observed at ≥ 2.5 mg/m^3 in both sexes on day 23 and at week 14. In addition, glucose concentration was decreased in males exposed to ≥ 1.25 mg/m^3 at week 14. Lung weights of all exposed groups of males and females were significantly greater than those of the chamber controls. Sperm motility was significantly decreased in all males exposed to cobalt (2.8-7.9% lower than control), suggesting a potential for cobalt metal to be a reproductive toxicant in male rats.

In the lung, chronic active inflammation and alveolar proteinosis occurred in all exposed males and females, and bronchiole epithelium hyperplasia occurred in all males and females exposed to ≥ 1.25 mg/m^3. In the nose, incidences of olfactory epithelium degeneration and respiratory epithelium hyperplasia were significantly increased in males and females exposed to ≥ 2.5 mg/m^3. The incidences of olfactory epithelium hyperplasia were significantly increased in ≥ 2.5 /m^3 males and in 5 mg/m^3 females. Significantly increased incidences of turbinate atrophy occurred in 2.5 mg/m^3 females and 5 mg/m^3 males and females (table 26). Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined. LOAEC of this study is 0.625 mg/m^3 (lowest dose administered) (NTP 2014).

Table 25: Survival and body weight of rats in the 3 month inhalation study of cobalt metal

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	107 ± 2	319 ± 5	212 ± 4	
0.625	10/10	107 ± 3	336 ± 6	229 ± 4	105
1.25	10/10	107 ± 2	327 ± 7	220 ± 6	102
2.5	10/10	107 ± 3	326 ± 6	220 ± 5	102
5	10/10	107 ± 3	297 ± 5*	190 ± 4**	93
Female					
0	10/10	88 ± 3	201 ± 3	113 ± 4	
0.625	10/10	88 ± 3	205 ± 4	117 ± 5	102
1.25	10/10	89 ± 3	198 ± 4	109 ± 2	98
2.5	10/10	88 ± 2	199 ± 4	111 ± 3	99
5	10/10	87 ± 2	187 ± 3*	100 ± 4	93

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
** P≤0.01
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 3 months/number initially in group

Table 26: Incidences of selected nonneoplastic lesions of the respiratory system in rats in the 3 month inhalation study of cobalt metal

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male					
Lung[a]	10	10	10	10	10
Inflammation, Chronic Active[b]	0	10** (1.9)[c]	10** (1.9)	10** (1.5)	10** (2.4)
Alveolus, Proteinosis	0	10** (1.8)	10** (2.2)	10** (2.2)	10** (2.7)
Bronchiole, Epithelium, Hyperplasia	0	0	10** (1.0)	10** (1.4)	10** (2.2)
Alveolar Epithelium, Hyperplasia	0	0	0	0	2 (1.5)
Nose	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	2 (1.0)	9** (1.0)	10** (2.5)
Olfactory Epithelium, Hyperplasia	0	0	2 (1.0)	6** (1.2)	10** (1.7)
Respiratory Epithelium, Hyperplasia	0	0	3 (1.0)	9** (1.0)	10** (1.8)
Turbinate, Atrophy	0	0	0	3 (1.0)	9** (1.0)
Female					
Lung	10	10	10	10	10
Inflammation, Chronic Active	2 (1.0)	10** (1.9)	10** (1.5)	10** (1.6)	10** (2.4)
Alveolus, Proteinosis	0	10** (1.8)	10** (1.9)	10** (1.9)	10** (2.1)
Bronchiole, Epithelium, Hyperplasia	0	0	10** (1.0)	10** (1.3)	10** (2.0)
Alveolar Epithelium, Hyperplasia	0	0	0	0	1 (1.0)
Nose	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	5* (1.0)	10** (1.0)	10** (2.5)
Olfactory Epithelium, Hyperplasia	0	0	0	3 (1.0)	10** (2.2)
Respiratory Epithelium, Hyperplasia	0	1 (1.0)	0	9** (1.0)	10** (1.8)
Turbinate, Atrophy	0	0	0	4* (1.0)	6** (1.0)

* Significantly different (P≤0.05) from the chamber control group by the Fisher exact test
** P≤0.01
[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

Groups of 10 male and 10 female B6C3F1/N mice were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **14 weeks.** One 2.5 mg/m^3 female mouse was accidentally killed during the first week of the study; all other mice survived to the end of the study. Mean body weight and body weight gain of males and females exposed to 10 mg/m^3 were significantly less than those of the chamber controls (table 27). Abnormal breathing was noted in approximately 50% of males and females exposed to 10 mg/m^3. At necropsy, tan lungs were noted in mice exposed to 5 or 10 mg/m^3. Statistically significant, but minimal (<5%) increases were observed in hemoglobin concentration and erythrocyte count of 10 mg/m^3 males and in the erythrocyte count of 10 mg/m^{3}females at 14 weeks.

Lung weights of males exposed to ≥ 2.5 mg/m^3 or greater and females exposed to ≥ 5 mg/m^3 were significantly increased. Liver weights of males exposed to 10 mg/m^3 and females exposed to ≥ 2.5 mg/m^3, kidney weights of males and females exposed to ≥ 5 mg/m^3 and testes weights of males exposed to ≥ 5 mg/m^3 were significantly decreased (table 28). Exposure concentration-related decreases in reproductive tissue weights, spermatid and epididymal spermatozoa counts, and sperm motility in combination with histopathologic findings in both the testis and epididymis indicate that cobalt metal is likely to be a reproductive toxicant in male mice (table 29).
In the lung, alveolar histiocytic cellular infiltration and bronchiole epithelium cytoplasmic vacuolization occurred in the lung of all exposed male and female mice. Bronchiole epithelium hyperplasia occurred in all mice exposed to ≥ 2.5 mg/m^3. Alveolar proteinosis and alveolar/bronchiolar epithelium karyomegaly occurred in all males and females exposed to ≥ 5 mg/m^3. The incidences of hemorrhage were significantly increased in 5 mg/m^3 females and in ≥ 5 mg/m^{3}males. In the nose, the incidences of olfactory epithelium degeneration were significantly increased in males and females exposed to ≥ 1.25 mg/m^3. Incidences of respiratory epithelium degeneration were significantly increased in males exposed to ≥ 1.25 mg/m3 and females exposed to ≥ 2.5 mg/m^3. Incidences of respiratory epithelium squamous metaplasia were significantly increased in males and females exposed to ≥ 2.5 mg/m^3, and incidences of turbinate atrophy and chronic active inflammation were significantly increased at ≥ 5 mg/m^3 in males and females. The incidences of squamous metaplasia were significantly increased in the larynx of all exposed groups of males and females (table 30). Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined. LOAEC of this study is 0.625 mg/m^3 (lowest dose administered) (NTP 2014).

Table 27: Survival and body weight of mice in the 3 month inhalation study of cobalt metal

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	23.7 ± 0.2	37.7 ± 0.8	14.0 ± 0.7	
0.625	10/10	23.7 ± 0.3	38.2 ± 0.6	14.5 ± 0.4	101
1.25	10/10	23.7 ± 0.2	37.9 ± 0.8	14.2 ± 0.8	101
2.5	10/10	23.8 ± 0.2	37.0 ± 0.5	13.3 ± 0.4	98
5	10/10	23.7 ± 0.2	37.0 ± 0.9	13.4 ± 0.8	98
10	10/10	23.8 ± 0.2	32.5 ± 0.5**	8.7 ± 0.5**	86
Female					
0	10/10	20.5 ± 0.3	30.9 ± 1.0	10.4 ± 1.1	
0.625	10/10	20.0 ± 0.3	31.6 ± 1.1	11.6 ± 1.3	102
1.25	10/10	20.2 ± 0.4	31.4 ± 0.9	11.2 ± 0.7	102
2.5	9/10[c]	19.8 ± 0.2	30.1 ± 0.7	10.1 ± 0.7	97
5	10/10	20.1 ± 0.3	29.0 ± 1.1	8.9 ± 1.0	94
10	10/10	19.8 ± 0.2	26.8 ± 1.0**	7.0 ± 1.0*	87

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** P≤0.01
[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 3 months/number initially in group
[c] Week of death: 1

Table 28: Selected organ weights and organ weight to body weight ratios for mice in the 3 month inhalation study of cobalt metal[a]

	Chamber Control	0.625 mg/m³	1.25 mg/m³	2.5 mg/m³	5 mg/m³	10 mg/m³
Male						
n	10	10	10	10	10	10
Necropsy body wt	37.7 ± 0.8	38.2 ± 0.6	37.9 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
R. Kidney						
Absolute	0.31 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.00	0.29 ± 0.01**	0.23 ± 0.01**
Relative	8.360 ± 0.192	8.441 ± 0.122	8.333 ± 0.237	8.507 ± 0.047	7.714 ± 0.145**	7.176 ± 0.131**
Liver						
Absolute	1.48 ± 0.04	1.53 ± 0.04	1.51 ± 0.07	1.49 ± 0.04	1.42 ± 0.05	1.15 ± 0.03**
Relative	39.217 ± 0.586	40.049 ± 0.671	39.753 ± 1.032	40.301 ± 0.698	38.159 ± 0.723	35.457 ± 0.668**
Lung						
Absolute	0.20 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.01*	0.27 ± 0.01**	0.30 ± 0.01**
Relative	5.416 ± 0.116	6.051 ± 0.235	5.737 ± 0.147	6.234 ± 0.088**	7.436 ± 0.262**	9.142 ± 0.177**
R. Testis						
Absolute	0.118 ± 0.002	0.119 ± 0.002	0.114 ± 0.002	0.114 ± 0.002	0.104 ± 0.003**	0.033 ± 0.001**
Relative	3.136 ± 0.058	3.131 ± 0.037	3.019 ± 0.078	3.073 ± 0.056	2.825 ± 0.082**	1.004 ± 0.025**
Female						
n	10	10	10	9	10	10
Necropsy body wt	30.9 ± 1.0	31.6 ± 1.1	31.4 ± 0.9	30.1 ± 0.7	29.0 ± 1.1	26.8 ± 1.0**
R. Kidney						
Absolute	0.21 ± 0.01	0.22 ± 0.00	0.21 ± 0.01	0.20 ± 0.00	0.17 ± 0.00**	0.16 ± 0.00**
Relative	6.887 ± 0.184	6.849 ± 0.155	6.661 ± 0.126	6.689 ± 0.254	6.031 ± 0.132**	6.142 ± 0.185**
Liver						
Absolute	1.46 ± 0.06	1.51 ± 0.07	1.46 ± 0.05	1.30 ± 0.03*	1.16 ± 0.04**	1.01 ± 0.03**
Relative	47.051 ± 0.808	47.552 ± 0.952	46.455 ± 1.046	43.092 ± 0.773**	39.831 ± 0.459**	38.045 ± 1.246**
Lung						
Absolute	0.21 ± 0.01	0.22 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.28 ± 0.01**	0.33 ± 0.01**
Relative	6.904 ± 0.227	6.884 ± 0.176	7.300 ± 0.274	7.555 ± 0.184	9.787 ± 0.241**	12.602 ± 0.487**

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test

** $P \leq 0.01$

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table 29: Summary of reproductive tissue evaluations for male mice in the 3 month inhalation study of cobalt metal

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.7 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
L. Cauda epididymis	0.0217 ± 0.0014	0.0210 ± 0.0008	0.0231 ± 0.0018	0.0168 ± 0.0006*
L. Epididymis	0.0603 ± 0.0022	0.0578 ± 0.0019	0.0614 ± 0.0035	0.0429 ± 0.0021**
L. Testis	0.1185 ± 0.0017	0.1132 ± 0.0023	0.1027 ± 0.0036**	0.0316 ± 0.0014**
Spermatid measurements				
Spermatid heads (10^6/testis)	22.34 ± 0.84	22.22 ± 0.65	18.90 ± 1.20*	0.53 ± 0.10**
Spermatid heads (10^6/g testis)	210.84 ± 6.85	227.74 ± 7.16	205.67 ± 7.43	24.27 ± 4.78**
Epididymal spermatozoal measurements				
Sperm motility (%)	86.0 ± 1.1	82.0 ± 0.8*	82.2 ± 1.1*	2.6 ± 1.2**
Sperm (10^6/cauda epididymis)	11.55 ± 0.39	10.53 ± 0.43	9.62 ± 0.49**	0.71 ± 0.06**
Sperm (10^6/g cauda epididymis)	551.1 ± 37.9	505.9 ± 23.3	439.9 ± 40.3*	43.4 ± 3.7**

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test (cauda epididymis weight) or Shirley's test (spermatid and epididymal spermatozoal measurements)
** Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test (body and tissue weights) or Shirley's test (spermatid and epididymal spermatozoal measurements)
[a] Data are presented as mean ± standard error.

Table 30: Incidences of selected nonneoplastic lesions of the respiratory system in mice in the 3 month inhalation study of cobalt metal

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Male						
Lung[a]	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte[b]	0	10** (1.0)[c]	10** (1.0)	10** (1.0)	10** (2.0)	10** (3.0)
Bronchiole, Epithelium, Hyperplasia	0	0	0	10** (1.0)	10** (1.9)	10** (3.0)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	10** (1.0)	10** (1.0)	10** (1.5)	10** (2.7)	10** (3.9)
Alveolus, Proteinosis	0	0	0	0	10** (1.0)[c]	10** (2.0)
Alveolar/bronchiolar, Epithelium, Karyomegaly	0	0	0	0	10** (1.0)	10** (3.0)
Hemorrhage	0	1 (1.0)	0	1 (1.0)	7** (1.1)	6** (1.0)
Nose	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	0	8** (1.4)	10** (2.5)
Olfactory Epithelium, Degeneration	0	2 (1.0)	10** (1.0)	10** (1.0)	10** (2.0)	10** (3.0)
Olfactory Epithelium, Hyperplasia	0	0	1 (1.0)	5* (1.0)	2 (1.0)	3 (1.3)
Respiratory Epithelium, Degeneration	0	0	6** (1.0)	9** (1.0)	10** (1.9)	10** (2.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	2 (1.0)	5* (1.0)	10** (1.3)	10** (1.9)
Turbinate, Atrophy	0	0	0	0	8** (2.1)	10** (3.0)
Larynx	10	10	10	10	10	10
Metaplasia, Squamous	0	10** (1.8)	10** (1.8)	10** (1.9)	10** (1.9)	10** (2.1)

	Chamber Control	0.625 mg/m³	1.25 mg/m³	2.5 mg/m³	5 mg/m³	10 mg/m³
Female						
Lung	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (2.1)	10** (3.0)
Bronchiole, Epithelium, Hyperplasia	0	0	0	10** (1.0)	10** (1.9)	10** (3.0)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	10** (1.0)	10** (1.0)	10** (1.1)	10** (2.6)	10** (3.9)
Alveolus, Proteinosis	0	0	0	0	10** (1.0)	10** (1.8)
Alveolar/bronchiolar, Epithelium, Karyomegaly	0	0	0	0	10** (1.7)	10** (3.0)
Hemorrhage	0	0	0	0	8** (1.0)	2 (1.0)
Nose	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	1 (1.0)	10** (2.5)	10** (2.4)
Olfactory Epithelium, Degeneration	0	1 (1.0)	7** (1.0)	9** (1.0)	10** (2.5)	10** (2.9)
Olfactory Epithelium, Hyperplasia	0	0	0	3 (1.0)	0	0
Respiratory Epithelium, Degeneration	0	0	1 (1.0)	8** (1.0)	10** (1.9)	10** (1.9)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	9** (1.0)	10** (2.0)	10** (2.0)
Turbinate, Atrophy	0	0	0	0	10** (2.2)	10** (2.9)
Larynx	10	10	10	10	10	10
Metaplasia, Squamous	0	10** (1.3)	10** (1.4)	10** (1.6)	10** (1.8)	10** (2.2)

* Significantly different (P≤0.05) from the chamber control group by the Fisher exact test
** P≤0.01
[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

Table 31: Incidences of selected nonneoplastic lesions of the genital system in male mice in the 3 month inhalation study of cobalt metal

	Chamber Control	0.625 mg/m³	1.25 mg/m³	2.5 mg/m³	5 mg/m³	10 mg/m³
Testes[a]	10	10	10	10	10	10
Germinal Epithelium, Degeneration[b]	2 (1.0)[c]	0	0	0	1 (1.0)	10** (4.0)
Epididymis	10	10	10	9	10	10
Exfoliated Germ Cell	0	0	0	0	0	10** (2.7)
Hypospermia	0	0	0	0	0	10** (2.9)
Vacuolization Cytoplasmic	0	0	0	0	0	9** (1.0)
Atrophy	0	0	0	0	0	10** (1.0)

** Significantly different (P≤0.01) from the chamber control group by the Fisher exact test
[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

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In a carcinogenicity study, F344/NTac rats (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for up to **105 weeks**. No haematology was performed in this study. Survival of female rats exposed to 2.5 mg/m^3 was significantly less than that of the chamber control group. Mean body weights of ≥ 2.5 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 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.

Table 32: Survival and body weight of rats in the 105 weeks inhalation study of cobalt metal

	0 mg/m^3		**1.25 mg/m^3**		**2.5 mg/m^3**		**5 mg/m^3**	
	♂	♀	♂	♀	♂	♀	♂	♀
No survivors w 105	17	35	20	26	16	24	16	24
Mean survival (days)	663	688	670	685	677	663	669	672
Mean body weight w105	467	349	459	338	414	292	333	244

The incidences of alveolar epithelium hyperplasia, alveolar proteinosis, chronic active inflammation, and bronchiole epithelium hyperplasia in all exposed groups were significantly greater than those in the chamber control groups (for details, see tables 33 and 34 below).
A spectrum of nonneoplastic lesions occurred in the nose of exposed male and female rats including chronic active and suppurative inflammation, respiratory metaplasia, atrophy, hyperplasia, basal cell hyperplasia, and necrosis of the olfactory epithelium; hyperplasia, squamous metaplasia, and necrosis of the respiratory epithelium; and atrophy of the turbinate.
Incidences of hyperplasia of the adrenal medulla were significantly increased in female rats exposed to 1.25 or 2.5 mg/m^3.
The incidence of infarct in the testes was significantly increased in male rats exposed to 5 mg/m^3.
Cobalt concentrations in the lung increased with increasing exposure concentration. The LOAEC of this study is 1.25 mg/m3 (lowest dose administered) (NTP 2014).

Table 33: Incidences of selected nonneoplastic lesions of the respiratory system in rats in the 105 week inhalation study of cobalt metal

	Chamber Control	**1.25 mg/m^3**	**2.5 mg/m^3**	**5 mg/m^3**
Male				
n	50	50	50	50
Alveolar Epithelium, Hyperplasia[a]	3 (1.0)[b]	47** (2.8)	49** (3.3)	49** (3.6)
Alveolus, Proteinosis	0	48** (2.6)	49** (2.9)	49** (3.1)
Inflammation, Chronic Active	22 (1.1)	50** (3.0)	50** (2.9)	50** (2.9)
Bronchiole, Epithelium, Hyperplasia	0	44** (1.5)	47** (2.7)	50** (3.7)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Female				
n	50	50	50	50
Alveolar Epithelium, Hyperplasia	9 (1.1)	49** (2.8)	50** (2.7)	49** (3.4)
Alveolus, Proteinosis	0	50** (2.7)	50** (2.7)	50** (2.9)
Inflammation, Chronic Active	20 (1.0)	50** (3.0)	50** (2.9)	50** (2.9)
Bronchiole, Epithelium, Hyperplasia	0	47** (1.5)	46** (2.1)	48** (3.8)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Number Examined Microscopically	48	47	45	50
Inflammation, Chronic Active[a]	28 (1.2)[b]	35* (1.3)	40** (1.7)	49** (2.6)
Inflammation, Suppurative	9 (1.0)	12 (1.7)	24** (2.2)	46** (2.6)
Olfactory Epithelium, Metaplasia, Respiratory	12 (1.1)	26** (1.7)	37** (1.5)	50** (2.2)
Olfactory Epithelium, Atrophy	2 (1.0)	21** (1.0)	34** (1.0)	29** (1.2)
Olfactory Epithelium, Hyperplasia	0	1 (1.0)	2 (1.5)	7** (1.1)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	1 (1.0)	0	13** (1.0)
Olfactory Epithelium, Necrosis	0	1 (1.0)	5* (1.6)	5* (1.8)
Respiratory Epithelium, Hyperplasia	20 (1.3)	35** (1.2)	45** (1.7)	50** (2.2)
Respiratory Epithelium, Metaplasia, Squamous	0	1 (1.0)	11** (1.2)	35** (1.3)
Respiratory Epithelium, Necrosis	1 (1.0)	4 (1.8)	5 (1.4)	13** (1.6)
Turbinate, Atrophy	1 (1.0)	35** (1.0)	35** (1.0)	41** (1.0)
Female				
Number Examined Microscopically	50	50	49	50
Inflammation, Chronic Active	22 (1.3)	42** (1.1)	39** (1.1)	50** (2.4)
Inflammation, Suppurative	6 (1.2)	4 (1.3)	4 (1.0)	42** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory	6 (1.0)	18** (1.3)	24** (1.2)	47** (2.1)
Olfactory Epithelium, Atrophy	0	22** (1.1)	35** (1.0)	35** (1.2)
Olfactory Epithelium, Hyperplasia	0	0	3 (1.0)	5* (1.0)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	1 (1.0)	19** (1.0)
Olfactory Epithelium, Necrosis	0	2 (1.5)	0	1 (3.0)
Respiratory Epithelium, Hyperplasia	15 (1.2)	43** (1.0)	48** (1.0)	49** (2.1)
Respiratory Epithelium, Metaplasia, Squamous	2 (1.0)	0	3 (1.0)	45** (2.0)
Respiratory Epithelium, Necrosis	1 (3.0)	1 (2.0)	1 (1.0)	15** (1.6)
Turbinate, Atrophy	1 (1.0)	38** (1.0)	27** (1.0)	45** (1.0)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table 34: Incidences of selected nonneoplastic lesions of the adrenal medulla in rats in the 105 week inhalation study of cobalt metal

	Chamber Control	1.25 mg/m³	2.5 mg/m³	5 mg/m³
Male				
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	19 (2.3)[b]	21 (2.5)	9* (3.0)	9** (2.4)
	Chamber Control	**1.25 mg/m³**	**2.5 mg/m³**	**5 mg/m³**
Female				
Number Examined Microscopically	50	50	50	50
Hyperplasia	12 (1.8)	27** (2.0)	27** (2.3)	10 (2.8)

In a carcinogenicity study, B6C3F1/N mice (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for up to **105 weeks**. No haematology was performed in this study. Survival of males exposed to 2.5 or 5 mg/m3 was significantly less than that of the chamber control group. Mean body weights of 5 mg/m^3 males and females 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.

Table 35: Survival and body weight of mice in the 105 week inhalation study of cobalt metal

	0 mg/m³		1.25 mg/m³		2.5 mg/m³		5 mg/m³	
	♂	♀	♂	♀	♂	♀	♂	♀
No survivors w 105	39	36	31	34	28	27	22	26
Mean survival (days)	715	686	695	695	672	680	668	668
Mean body weight w105	51.2	58.5	51.8	57.3	47.0	56.3	39.5	40.6

The incidences of alveolar/bronchiolar epithelium hyperplasia and cytoplasmic vacuolization, alveolar epithelium hyperplasia, proteinosis, and alveolus infiltration cellular histiocyte were significantly increased in all exposed groups of males and females (for details, see table 36 below). The incidences of bronchiole epithelium hyperplasia were significantly increased in males exposed to 5 mg/m^3 and females exposed to ≥ 2.5 mg/m^3. The incidence of bronchiole epithelium erosion was significantly increased in males exposed to 2.5 mg/m^3. The incidences of suppurative inflammation were significantly increased in males exposed to ≥ 2.5 mg/m^3 and females exposed to 5 mg/m^3. In the nose, the incidences of suppurative inflammation; olfactory epithelium atrophy, hyperplasia, and respiratory metaplasia; cytoplasmic vacuolization and squamous metaplasia of the respiratory epithelium; and atrophy of the turbinate were significantly increased in all exposed groups of males and females. The incidences of atypical respiratory metaplasia of the olfactory epithelium and hyaline droplet accumulation of the respiratory epithelium were significantly increased in 1.25 and 2.5 mg/m^3 males and females.
The incidences of respiratory epithelium squamous metaplasia and cytoplasmic vacuolization of the larynx in all exposed groups of males and females were significantly greater than those in the

chamber control groups. The incidences of squamous epithelium hyperplasia were significantly increased in all exposed groups of females and in males exposed to 5 mg/m^3. In the trachea, the incidences of epithelium cytoplasmic vacuolization were significantly increased in all exposed groups of males and females.
The incidence of germinal epithelium degeneration in the testes was significantly increased in male mice exposed to 5 mg/m^3.
Cobalt concentrations in the lung increased with increasing exposure concentration. The LOAEC of this study is 1.25 mg/m^3 (lowest dose administered) (NTP 2014).

Table 36: Incidences of selected nonneoplastic lesions of the respiratory system in mice in the 105 week inhalation study of cobalt metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Lung[a]	50	49	50	50
Alveolar/bronchiolar Epithelium, Hyperplasia[b]	0	46** (1.0)[c]	49** (1.6)	50** (2.3)
Alveolar/bronchiolar Epithelium, Vacuolization Cytoplasmic	0	49** (1.1)	47** (1.9)	48** (3.1)
Alveolar Epithelium, Hyperplasia	4 (2.3)	29** (1.7)	24** (1.8)	43** (2.0)
Bronchiole, Epithelium, Hyperplasia	4 (2.5)	7 (1.3)	9 (1.3)	11* (1.5)
Bronchiole, Epithelium, Erosion	0	4 (1.0)	10** (1.3)	2 (1.0)
Proteinosis	2 (1.0)	46** (1.7)	49** (3.1)	50** (3.9)
Alveolus, Infiltration Cellular, Histiocyte	10 (1.8)	49** (1.8)	48** (2.5)	48** (3.1)
Inflammation, Suppurative	1 (1.0)	2 (2.0)	6* (1.5)	16** (2.3)
	Chamber Control	**1.25 mg/m^3**	**2.5 mg/m^3**	**5 mg/m^3**
Female				
Lung	49	50	50	50
Alveolar/bronchiolar Epithelium, Hyperplasia	0	49** (1.1)	49** (1.9)	50** (2.7)
Alveolar/bronchiolar Epithelium, Vacuolization Cytoplasmic	0	48** (1.1)	49** (1.9)	48** (3.5)
Alveolar Epithelium, Hyperplasia	2 (2.5)	27** (1.6)	26** (1.4)	41** (1.4)
Bronchiole, Epithelium, Hyperplasia	0	3 (1.0)	12** (1.1)	26** (1.2)
Bronchiole, Epithelium, Erosion	0	0	4 (1.0)	3 (1.0)
Proteinosis	0	45** (1.4)	50** (2.6)	50** (3.9)
Alveolus, Infiltration Cellular, Histiocyte	10 (1.7)	49** (1.6)	50** (2.5)	49** (3.1)
Inflammation, Suppurative	0	3 (1.3)	2 (1.0)	15** (1.7)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Nose[a]	50	49	50	50
Inflammation, Suppurative[b]	16 (1.1)[c]	32** (1.9)	49** (2.7)	50** (3.1)
Olfactory Epithelium, Atrophy	3 (1.0)	46** (1.2)	42** (1.2)	31** (1.2)
Olfactory Epithelium, Hyperplasia	0	25** (1.2)	17** (1.0)	8** (1.1)
Olfactory Epithelium, Metaplasia, Respiratory	5 (1.4)	24** (1.3)	44** (2.3)	50** (3.1)
Olfactory Epithelium, Respiratory Metaplasia, Atypical	0	14** (2.0)	9** (1.1)	1 (1.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	13 (1.2)	29** (1.1)	29** (1.1)	7 (1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	41** (1.2)	39** (1.2)	37** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	3 (1.0)	45** (1.0)	35** (1.1)	33** (1.2)
Turbinate, Atrophy	3 (1.3)	25** (1.3)	49** (2.1)	50** (3.3)
Larynx	48	47	49	50
Respiratory Epithelium, Metaplasia, Squamous	7 (1.0)	47** (1.0)	49** (1.0)	49** (1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	20** (1.0)	24** (1.0)	32** (1.1)
Squamous Epithelium, Hyperplasia	2 (1.0)	5 (1.0)	5 (1.0)	8* (1.0)
Trachea	48	47	48	50
Epithelium, Vacuolization Cytoplasmic	0	14** (1.4)	31** (1.6)	37** (1.4)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Female				
Nose	50	50	50	50
Inflammation, Suppurative	3 (1.0)	47** (2.3)	50** (3.1)	50** (3.3)
Olfactory Epithelium, Atrophy	4 (1.0)	44** (1.2)	39** (1.2)	24** (1.2)
Olfactory Epithelium, Hyperplasia	1 (1.0)	22** (1.1)	16** (1.0)	8* (1.0)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	26** (1.8)	44** (2.7)	50** (3.3)
Olfactory Epithelium, Respiratory Metaplasia, Atypical	0	18** (1.6)	14** (1.5)	1 (1.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	12 (1.0)	38** (1.1)	40** (1.2)	10 (1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	40** (1.0)	47** (1.1)	47** (1.1)
Respiratory Epithelium, Metaplasia, Squamous	0	49** (1.2)	49** (1.4)	50** (1.5)
Turbinate, Atrophy	0	44** (2.2)	50** (2.9)	50** (3.4)
Larynx	47	50	50	47
Respiratory Epithelium, Metaplasia, Squamous	2 (1.0)	49** (1.0)	50** (1.0)	47** (1.1)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	24** (1.0)	31** (1.0)	34** (1.0)
Squamous Epithelium, Hyperplasia	2 (1.0)	13** (1.1)	21** (1.0)	21** (1.0)
Squamous Epithelium, Erosion	1 (1.0)	2 (1.0)	7* (1.0)	4 (1.0)
Trachea	48	50	48	49
Epithelium, Vacuolization Cytoplasmic	0	26** (1.4)	37** (1.6)	39** (1.8)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test
** $P \leq 0.01$
[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

Pigs were exposed to dose levels up to 1.0 mg cobalt/m^3 as cobalt metal for 3 months. Decreased lung compliance was found in pigs exposed to 0.1 mg cobalt/m^3. Electrocardiogram abnormalities that may reflect ventricular impairment were observed at 0.1 mg cobalt dust/m^3. No histological effects on the kidneys or liver were found (Kerfoot, 1975) (ATSDR, 2004).

Studies with soluble cobalt compounds

Cobalt sulphate (heptahydrate)

Fischer rats (5/sex/dose) were exposed to air containing cobalt sulphate heptahydrate at concentrations of 0 (chamber controls), 0.1, 0.5, 5, 50 or 200 mg/m³ (calculated on the basis of the anhydrous salt) 6 hours per day, for 12 exposures over **16 days** (whole body).
Exposure to 200 mg/m³ cobalt sulphate heptahydrate as an aerosol resulted in deaths of all rats within 5 days. Several male rats exposed to 50 mg/m³ also died somewhat later. Rats exposed to 50 mg/m³ lost weight.

Table 37: Survival and body weight of rats in the 2 week inhalation study of cobalt sulphate

	0 mg/m³		**0.1 mg/m³**		**0.5 mg/m³**		**5 mg/m³**		**50 mg/m³**		**200 mg/m³**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
No survivors d16	5	5	5	5	5	5	5	5	3	5	0	0
BW (g) d16 (% of control)	242 ± 3	155 ± 3	250 ±4 (103)	164 ± 5 (106)	252 ± 8 (104)	158 ± 2 (102)	234 ± 9 (97)	157 ± 3 (101)	128 ± 9 (53)	120 ± 11 (77)	-	-
Bw gain (g) (% of control)	52 ± 2	27 ±3	59 ± 5 (113)	33 ±3 (122)	62 ± 4 (119)	29 ±2 (107)	50 ±3 (96)	26 ±2 (96)	-61 ±6 (-117)	-10 ±9 (-37)	-	-

At the two highest exposure concentrations, inflammation and necrosis of the respiratory epithelium were seen in the larynx, trachea, bronchioles, and the respiratory turbinates of the nose. Degeneration of the olfactory epithelium was also present. In the 50 mg/m³ groups, hyperplasia and squamous metaplasia in the epithelium of the respiratory turbinates and hyperplasia (acanthosis) of the squamous epithelium of the larynx occurred in rats that survived at least 9 days or were killed at the end of the 16-day exposure period. Inflammation in the nose at 50 mg/m³ consisted of a serous exudate in the lumen of the nasal cavity. In the lungs, oedema and haemorrhage into alveolar spaces were seen at the 200 mg/m³ exposure concentration. At the 50 mg/m³exposure concentration, inflammation and histiocytic (macrophage) infiltration in the lungs were present. Fibrosis around bronchioles and mild-to-moderate ectasia (dilatation) of bronchioles were also present at this concentration.
Other lesions observed in exposed rats that died during the exposure period consisted of lymphoid necrosis in the thymus and congestion of vessels in the brain/meninges. At the highest concentration, centrilobular congestion and necrosis were present in the liver of both male and female rats. Atrophy of the testis, characterized by a decreased number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts, was observed in rats exposed to 50 mg/m³.
Cardiomyopathy of minimal severity, characterized by mononuclear inflammatory cell infiltrates, hyalinised myocardial fibres, and/or fibrosis in the myocardium, was observed primarily in animals that died but was also seen in 2/5 male controls and thus was not clearly compound related. The LOAEC of this study is 50 mg/m^3 (10.5 mg cobalt/m^3), the NOAEC 25 mg/m^3 (1.1 mg cobalt/m^3) (NTP, 1991).

B6C3F1 mice (5/sex/dose) were exposed to air containing cobalt sulphate heptahydrate at concentrations of 0 (chamber controls), 0.1, 0.5, 5, 50 or 200 mg/m³ (calculated on the basis of the anhydrous salt) 6 hours per day, for 12 exposures over **16 days** (whole body). All mice exposed to 200 mg/m^3 and 4/5 males and 1/5 females exposed to 50 mg/m^3 died before the end of the study. Mice exposed to 50 mg/m³ lost weight. Exposure to ≥ 50 mg/m^3 resulted in clinical signs, including hyperactivity, chromodacryorrhea, hypothermia, rapid & shallow breathing and reduced body tone.

Table 38: Survival and body weight of mice in the 2 week inhalation study of cobalt sulphate

	0 mg/m^3		**0.1 mg/m^3**		**0.5 mg/m^3**		**5 mg/m^3**		**50 mg/m^3**		**200 mg/m^3**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
No survivors d16	5	5	5	5	5	5	5	5	1	4	0	0
BW (g) d16 (% of control)	28.4 ± 0.8	24.2 ± 0.3	29.0 ± 1.0 (102)	24.3 ± 0.2 (100)	29.1 ± 0.7 (102)	24.9 ± 0.5 (103)	29.7 ± 0.7 (104)	24.0 ± 0.9 (99)	19.0 ± 0.0 (67)	19.4 ± 0.4 (80)	-	-
Bw gain (g) (% of control)	2.2 ± 0.5	3.0 ± 0.3	2.0 ± 0.8 (91)	2.7 ± 0.3 (90)	2.1 ± 0.4 (95)	2.8 ± 0.2 (93)	2.4 ± 0.6 (109)	2.2 ± 0.5 (73)	-7.4 ± 0.0 (-336)	-2.7 ± 0.3 (-90)	-	-

In mice exposed to ≥ 5 mg/m^3 gray discoloration of the lung was observed as well as fluid in the larynx and trachea. In addition, absolute and relative lung weight was increased in both sexes exposed to 50 mg/m^3, whereas absolute and relative thymus weight was decreased at this dose. Lesions attributed to cobalt sulphate exposure were seen at all levels of the respiratory tract in mice. At the three highest concentrations, inflammation and necrosis of the respiratory epithelium were seen in the larynx, trachea, bronchioles, and respiratory turbinates of the nose. Degeneration of the olfactory epithelium was also present. In the 50 mg/m³ group, mice that survived more than 1 week or were killed at the end of the 16-day exposure period had hyperplasia (acanthosis) of the squamous epithelium in the larynx and regeneration of the bronchiolar epithelium in the lung. Also at the 50 mg/m³ exposure concentration, an inflammatory response in the lung was characterized by fibrosis around bronchioles and infiltration of histiocytes into alveolar spaces.
Other lesions observed in exposed mice that died during the exposure period consisted of lymphoid depletion and necrosis in the thymus and congestion of vessels in the brain/meninges. In the liver, necrosis of hepatocytes was present in all mice that died during the exposure period; minimal necrosis was present in the liver of one male mouse (50 mg/m³) that was killed at the end of the study. The LOAEC of this study is 5 mg/m^3 (1.1 mg cobalt/m^3), the NOAEC 0.5 mg/m^3 (0.1 mg cobalt/m^3) (NTP, 1991).

Groups of F344 rats (10/sex/dose) were exposed to aerosols containing 0, 0.3, 1.0, 3.0, 10, or 30 mg/m³ cobalt sulphate heptahydrate 6 hours per day, 5 days per week, for **13 weeks**. Mortality was not observed. Final mean body weight of male rats exposed to 30 mg/m³ was 14% lower than that of controls. Relative kidney weight (males exposed to all doses) and absolute and relative lung weights (both sexes exposed to 1 mg/m^3 or more) were increased.

Table 39: Survival and body weight of rats in the 13 week inhalation study of cobalt sulphate

Organ	Control	0.3 mg/m³	1 mg/m³	3 mg/m³	10 mg/m³	30 mg/m³
MALE						
Body weight (grams)	331 ± 8.0	330 ± 8.9	325 ± 6.2	328 ± 10.4	327 ± 8.4	**282 ± 8.8
Kidney						
Absolute	1,093 ± 36	1,150 ± 37	1,140 ± 27	1,145 ± 35	1,145 ± 39	1,042 ± 35
Relative	3.3 ± 0.06	*3.5 ± 0.04	*3.5 ± 0.03	*3.5 ± 0.07	*3.5 ± 0.05	**3.7 ± 0.06
Lung						
Absolute	1,364 ± 45	1,451 ± 33	*1,506 ± 53	**1,690 ± 79	**1,951 ± 78	**2,008 ± 75
Relative	4.1 ± 0.11	*4.4 ± 0.11	**4.6 ± 0.11	**5.1 ± 0.12	**6.0 ± 0.11	**7.1 ± 0.11
FEMALE						
Body weight (grams)	188 ± 4.7	173 ± 4.8	181 ± 5.9	196 ± 4.8	191 ± 4.2	175 ± 2.8
Kidney						
Absolute	668 ± 25	617 ± 14	646 ± 19	691 ± 19	666 ± 22	673 ± 19
Relative	3.5 ± 0.09	3.6 ± 0.07	3.6 ± 0.07	3.5 ± 0.05	3.5 ± 0.06	3.8 ± 0.08
Lung						
Absolute	935 ± 28	904 ± 18	*1,035 ± 24	**1,282 ± 38	**1,344 ± 40	**1,573 ± 47
Relative	5.0 ± 0.10	5.2 ± 0.10	**5.7 ± 0.11	**6.6 ± 0.13	**7.0 ± 0.16	**9.0 ± 0.21

(a) Mean ± standard error in milligrams (absolute) or milligrams per gram (relative) for groups of 10 animals; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

Significant increases were observed in erythrocytes, in the mean hemoglobin concentration, and in the hematocrit value (≥10 mg/m^3 in females and in ≥3 mg/m^3 in males) and reticulocyte count (females at 30 mg/m^3). Significant decreases were observed in platelet count (≥10 mg/m^3), serum cholesterol (≥10 mg/m^3 in males and 30 mg/m^3 in females), T3 concentration (≥10 mg/m^3 in females) and TSH (30 mg/m^3 in males). Granular casts were observed in the urine from many exposed males (3-7 animals per dose group, but not in controls. A dose-related increase in epithelial cells in the urine was observed in males (≥3 mg/m^3).

No statistically significant effects were observed on sperm parameters. The estrous cycle was longer in females exposed to 30 mg/m^3, but not statistically significant.

The larynx appeared to be the most sensitive tissue, showing metaplastic and inflammatory lesions after exposure at concentrations as low as 0.3 mg/m³ cobalt sulphate heptahydrate (see table 40 below for details). Cardiomyopathy was observed in 3/10 male controls (minimal severity) and 3/10 males of the 30 mg/m^3 dose group (minimal-mild severity) and in 1/10 females of the 30 mg/m^3 dose group (minimal severity) (other doses not examined).

Based on the results of this study, a LOAEC of 0.3mg/m³ (0.06 mg cobalt/m^3)for local effects in the respiratory tract can be derived (lowest dose administered) (NTP, 1991).

Table 40: Incidences of selected nonneoplastic lesions of the respiratory system in rats in the 13 week inhalation study of cobalt sulphate

Site/Lesion	Control	0.3 mg/m³	1 mg/m³	3 mg/m³	10 mg/m³	30 mg/m³
MALE						
Nose						
Acute inflammation	0	0	0	0	0	3
Olfactory epithelium degeneration	0	0	0	0	**7	**10
Respiratory epithelium hyperplasia	0	0	1	1	2	*5
Respiratory epithelium squamous metaplasia	0	0	0	1	*5	**9
Larynx (step sections)						
Mineralization	0	(b) 0	0	2	**10	**10
Chronic inflammation	0	(b) 2	**8	**9	**9	**9
Suppurative inflammation	0	(b) 0	0	0	*4	2
Ulcer	0	(b) 0	0	0	**7	**7
Necrosis	0	(b) 1	0	0	**10	**10
Inflammatory polyp	0	(b) 0	0	2	**10	**8
Squamous metaplasia	0	**(b) 9	**10	**10	**10	**10
Lung						
Histiocytic infiltrates	1	0	*6	**10	**10	**10
Inflammation, subacute	0	0	1	*5	**10	**10
Fibrosis	0	0	0	0	1	**10
Bronchiolar epithelium regeneration	0	0	0	0	0	**7
Bronchiolar ectasia	0	0	0	0	**8	**10
Alveolar emphysema	0	0	0	0	1	2
Alveolar epithelium hyperplasia	0	0	0	3	**6	**6
FEMALE						
Nose						
Olfactory epithelium degeneration	0	0	0	0	**6	**10
Respiratory epithelial hyperplasia	0	0	0	3	**9	**9
Respiratory epithelial squamous metaplasia	0	0	0	1	3	**6
Larynx (step sections)						
Mineralization	0	(c) 0	0	1	**8	**10
Chronic inflammation	1	(c) 2	**7	**10	**10	**10
Ulcer	0	(c) 0	0	0	3	**6
Necrosis	0	(c) 0	0	2	**9	**10
Inflammatory polyp	0	(c) 0	0	1	**10	**9
Squamous metaplasia	1	**(c) 7	**10	**10	**10	**10
Lung						
Histiocytic infiltrates	0	3	**10	**10	**10	**10
Inflammation, subacute	0	0	2	**9	**10	**10
Fibrosis	0	0	0	1	*4	*5
Bronchiolar epithelium regeneration	0	0	0	0	0	*5
Bronchiolar ectasia	0	0	0	2	**8	**10
Alveolar emphysema	0	0	0	1	2	**7
Alveolar epithelium hyperplasia	0	0	0	3	1	1

(a) Ten rats were examined in each group unless otherwise specified.
(b) Nine rats were examined.
(c) Eight rats were examined.
*P<0.05 by Fisher exact test
**P<0.01 by Fisher exact test

Groups of B6C3F1 mice (10/sex/dose) were exposed to aerosols containing 0, 0.3, 1.0, or 3.0, 10, 30 mg/m³ cobalt sulphate heptahydrate 6 hours per day, 5 days per week, for **13 weeks**. Two males of the highest dose group died prematurely. Mean body weight of males exposed to 30 mg/m^3 and females exposed to ≥ 10 mg/m^3 were significantly lower than controls (14% for males, 22% for females at top dose). Absolute and relative lung weight were increased at ≥ 10 mg/m^3 and absolute and relative testes weight and absolute epididymal weight were decreased at 30 mg/m^3.

Table 41: Survival and body weight of mice in the 13 week inhalation study of cobalt sulphate

Organ	Control	0.3 mg/m³	1 mg/m³	3 mg/m³	10 mg/m³	30 mg/m³
MALE						
Body weight (grams)	37.5 ± 1.54	37.1 ± 1.28	39.9 ± 1.28	35.7 ± 0.88	35.8 ± 0.98	**(b) 32.5 ± 0.81
Lung						
Absolute	181 ± 4.3	179 ± 9.6	186 ± 6.5	187 ± 4.2	**213 ± 4.5	**(b) 321 ± 6.7
Relative	4.9 ± 0.13	4.8 ± 0.18	4.7 ± 0.08	5.2 ± 0.09	**6.0 ± 0.15	**(b) 9.9 ± 0.32
Testis						
Absolute	(c) 120 ± 1.9	125 ± 2.7	123 ± 2.3	120 ± 2.4	121 ± 2.1	**57 ± 6.8
Relative	(c) 3.3 ± 0.11	3.4 ± 0.07	3.1 ± 0.09	3.4 ± 0.10	3.4 ± 0.05	**1.7 ± 0.19
FEMALE						
Body weight (grams)	33.2 ± 1.31	33.8 ± 1.25	34.7 ± 1.33	33.3 ± 0.94	31.6 ± 0.74	**26.1 ± 0.59
Lung						
Absolute	194 ± 9.0	192 ± 4.2	187 ± 4.7	198 ± 4.7	**232 ± 7.3	**327 ± 5.8
Relative	5.9 ± 0.28	5.8 ± 0.26	5.4 ± 0.12	6.0 ± 0.22	**7.3 ± 0.11	**12.6 ± 0.40

(a) Mean ± standard error in milligrams (absolute) or milligrams per gram (relative) for groups of 10 animals unless otherwise specified; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Eight animals were weighed.
(c) Nine animals were weighed.
*P<0.05
**P<0.01

Microscopic lesions were generally limited to the respiratory tract. Lesions were concentration related and similar in incidence and severity in males and females (for details see table 42 below). Lymphoid hyperplasia was present in the mediastinal lymph nodes at 30 mg/m^3.

The number of abnormal sperm was increased at 30 mg/m^3 and sperm motility was decreased at ≥ 3 mg/m^3 (lower concentrations not analysed). At the highest dose, atrophy of the testis was observed, which consisted of a loss of germinal epithelium in the seminiferous tubuli; more severely affected testes also contained foci of mineralization. The estrous cycle was significantly longer in females exposed to 30 mg/m^3.

Based on the results of this study, a LOAEC of 0.3mg/m³ (0.06 mg cobalt/m^3) for local effects in the respiratory tract can be derived (lowest dose administered) (NTP, 1991).

Table 42: Incidences of selected nonneoplastic lesions of the respiratory system in mice in the 13 week inhalation study of cobalt sulphate

Site/Lesion	Control	0.3 mg/m³	1 mg/m³	3 mg/m³	10 mg/m³	30 mg/m³
MALE						
Nose						
Acute inflammation	0	--	0	1	**10	**9
Olfactory epithelium degeneration	0	--	0	0	**9	**8
Respiratory epithelium squamous metaplasia	0	--	0	0	**8	**8
Larynx						
Inflammation	0	0	0	(b) 0	1	**9
Necrosis	0	0	0	(b) 0	0	3
Squamous metaplasia	0	**7	**10	*(b) 5	**9	**10
Trachea						
Squamous metaplasia	0	--	--	--	0	2
Lung						
Histiocytic infiltrates	0	**10	**9	**10	**10	**10
Chronic inflammation	0	0	0	0	1	**10
Bronchiolar epithelium regeneration	0	0	0	0	0	**10
Alveolar epithelium hyperplasia	0	0	0	0	3	**8
Mediastinal lymph nodes						
Hyperplasia	0	--	--	--	(c) 0	**(b) 6
Testis						
Atrophy	0	--	--	--	0	**9
Mineralization	0	--	--	--	0	*4
FEMALE						
Nose						
Acute inflammation	0	0	1	*4	**10	**10
Olfactory epithelium degeneration	0	0	0	1	**10	**10
Respiratory epithelium squamous metaplasia	0	0	0	1	**9	**9
Larynx						
Inflammation	0	0	0	(b) 0	**6	**(b) 8
Necrosis	0	0	0	(b) 0	0	**(b) 6
Squamous metaplasia	0	**8	**8	**(b) 8	**9	**(b) 9
Trachea						
Squamous metaplasia	0	--	--	--	0	3
Lung						
Histiocytic infiltrates	0	0	**9	**10	**10	**10
Chronic inflammation	0	0	0	0	*5	**10
Bronchiolar epithelium regeneration	0	0	0	0	0	**10
Alveolar epithelium hyperplasia	0	0	0	0	**10	**10
Mediastinal lymph nodes						
Hyperplasia	0	--	--	(d) 0	(e) 1	**7

(a) Ten mice were examined in each group unless otherwise specified; -- indicates tissue not examined.
(b) Nine mice were examined.
(c) Seven mice were examined.
(d) Five mice were examined.
(e) Six mice were examined.
*P<0.05 by Fisher exact test
**P<0.01 by Fisher exact test

In a carcinogenicity study, Fischer 344 rats (50/sex/dose) were exposed (whole body) to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m³ cobalt sulphate heptahydrate for 6 hours per day, 5 days per week, for **105 weeks**. No haematology was performed in this study. There was no effect on survival or body weight. Irregular breathing was observed more frequently in female rats exposed to 3.0 mg/m³.

Table 43: Survival and body weight of rats in the 105 week inhalation study of cobalt sulphate

	0 mg/m³		**0.3 mg/m³**		**1.0 mg/m³**		**3.0 mg/m³**	
	♂	♀	♂	♀	♂	♀	♂	♀
No survivors w 104	18	32	16	27	22	32	15	31
Body weight (g) w104 (% of control)	476	326	454 (95)	337 (103)	481 (101)	331 (101)	459 (96)	334 (102)

In all exposed groups, the incidences of proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation, and interstitial fibrosis in the lung were significantly increased. The incidence of squamous metaplasia in 1.0 mg/m³ females was significantly increased. The incidences of alveolar epithelial hyperplasia in all groups of exposed males and in females exposed to 3.0 mg/m³ and atypical alveolar epithelial hyperplasia in 3.0 mg/m³ females were significantly greater than in control groups.

Many of the lesions were highly cellular and morphologically similar to those observed spontaneously, but others were predominantly fibrotic, squamous, or mixtures of alveolar/bronchiolar epithelium and squamous or fibrous components. Hyperplasia generally represented an increase in numbers of epithelial cells along alveolar walls with maintenance of normal alveolar architecture. Multiple hyperplastic lesions were often observed in animals receiving higher concentrations of cobalt sulphate heptahydrate.

While squamous epithelium is not normally observed within the lung, squamous metaplasia of alveolar/ bronchiolar epithelium is a relatively common response to pulmonary injury and occurred in a number of rats in this study. In general, diagnoses of squamous lesions were made only when the lesion composition was almost entirely squamous epithelium. However, squamous metaplasia/ differentiation was a variable component of other alveolar/bronchiolar proliferative lesions, including the fibroproliferative lesions, and was clearly a part of the spectrum of lesions resulting from exposure to cobalt sulphate heptahydrate. LOAEC was 0.3 mg/m^3 (0.06 mg cobalt/m^3, lowest dose administered) (NTP 1998).

Table 44: Incidences of selected nonneoplastic lesions of the respiratory system in rats in the 105 week inhalation study of cobalt sulphate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Number Examined Microscopically	50	50	48	50
Alveolar Epithelium, Hyperplasia[a]	9 (1.8)[b]	20* (2.0)	20* (2.1)	23**(2.0)
Alveolar Epithelium, Hyperplasia, Atypical	0	1 (2.0)	2 (3.0)	2 (4.0)
Metaplasia, Squamous	0	1 (1.0)	4 (2.0)	2 (3.0)
Alveolar Epithelium, Metaplasia	0	50**(1.9)	48**(3.1)	49**(3.7)
Inflammation, Granulomatous	2 (1.0)	50**(1.9)	48**(3.1)	50**(3.7)
Interstitium, Fibrosis	1 (1.0)	50**(1.9)	48**(3.1)	49**(3.7)
Proteinosis	0	16**(1.4)	40**(2.3)	47**(3.4)
Cyst	0	0	0	1 (4.0)
Female				
Number Examined Microscopically	50	49	50	50
Alveolar Epithelium, Hyperplasia	15 (1.4)	7 (1.6)	20 (1.8)	33**(2.0)
Alveolar Epithelium, Hyperplasia, Atypical	0	0	3 (3.7)	5* (3.2)
Metaplasia, Squamous	0	1 (2.0)	8**(2.3)	3 (1.7)
Alveolar Epithelium, Metaplasia	2 (1.0)	47**(2.0)	50**(3.6)	49**(3.9)
Inflammation, Granulomatous	9 (1.0)	47**(2.0)	50**(3.6)	49**(3.9)
Interstitium, Fibrosis	7 (1.0)	47**(2.0)	50**(3.6)	49**(3.9)
Proteinosis	0	36**(1.2)	49**(2.8)	49**(3.9)
Cyst	0	0	1 (4.0)	0

* Significantly different ($P \leq 0.05$) from the chamber control by the logistic regression test
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Nose[a]	50	50	49	50
Lateral Wall, Hyperplasia[b]	2 (1.5)[c]	14**(1.4)	21**(1.5)	20**(1.6)
Lateral Wall, Metaplasia, Squamous	1 (1.0)	3 (1.3)	5 (1.4)	8* (2.0)
Olfactory Epithelium, Atrophy	8 (1.1)	24**(1.4)	42**(1.5)	48**(2.5)
Olfactory Epithelium, Metaplasia	5 (1.2)	1 (3.0)	5 (1.8)	30**(1.9)
Larynx	50	49	48	50
Epiglottis, Metaplasia, Squamous	0	10**(1.3)	37**(1.8)	50**(2.8)
Female				
Nose	50	49	50	50
Lateral Wall, Hyperplasia	1 (1.0)	8* (1.3)	26**(1.4)	38**(1.7)
Lateral Wall, Metaplasia, Squamous	1 (1.0)	1 (3.0)	4 (1.3)	10**(1.4)
Olfactory Epithelium, Atrophy	5 (1.4)	29**(1.2)	46**(1.6)	47**(2.9)
Olfactory Epithelium, Metaplasia	2 (2.0)	2 (1.5)	3 (1.7)	40**(2.3)
Larynx	50	49	50	50
Epiglottis, Metaplasia, Squamous	1 (1.0)	22**(1.1)	39**(1.4)	48**(2.6)

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
[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

Table 45: Incidences of selected nonneoplastic lesions of the adrenal medulla in rats in the 105 week inhalation study of cobalt sulphate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Number Examined Microscopically	50	50	49	50
Hyperplasia[a]	34 (2.0)[b]	23* (2.5)	29 (2.1)	30 (2.1)
Female				
Number Examined Microscopically	48	49	50	48
Hyperplasia	8 (1.6)	7 (2.3)	11 (2.1)	13 (2.0)

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
[a] Number of animals with lesion
[b]

In a second carcinogenicity study, B6C3F1 mice (50/sex/dose were exposed (whole body) to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m³ cobalt sulphate heptahydrate for 6 hours per day, 5 days per week, for **105 weeks**. No haematology was performed in this study. There was no effect on survival. Mean body weights of 3.0 mg/m³ male mice were less than those of controls from week 96 until the end of the study. The mean body weights of all exposed female mice were generally greater than those of controls from week 20 until the end of the study. Irregular breathing was observed more frequently in female rats exposed to 1.0 mg/m³.

Table 46: Survival and body weight of mice in the 105 week inhalation study of cobalt sulphate

	0 mg/m³		0.3 mg/m³		1.0 mg/m³		3.0 mg/m³	
	♂	♀	♂	♀	♂	♀	♂	♀
No survivors w 104	23	35	32	40	24	35	20	33
Body weight (g) w104	42.6	46.9	43.2	49.7	41.6	46.9	38.5	47.7

The incidences of atrophy of the olfactory epithelium in 1.0 and 3.0 mg/m³ males and females and hyperplasia of the olfactory epithelium in 3.0 mg/m³ males and females were significantly greater than in controls. The incidences of suppurative inflammation in 3.0 mg/m³ males and in 1.0 mg/m³ females were significantly greater than in controls.

The incidences of squamous metaplasia in the larynx were significantly increased in all exposed groups. Squamous metaplasia was limited to the base of the epiglottis and was not a severe lesion in exposed mice.

In all exposed groups, the incidences of cytoplasmic vacuolization of the bronchi were significantly greater than those in control groups. The incidences of diffuse histiocytic cell infiltration in 3.0 mg/m³ males and of focal histiocytic cell infiltration in 3.0 mg/m³ females were also significantly greater than in controls. The histiocyte infiltrate was very commonly seen in lungs with alveolar/bronchiolar neoplasms, and the increased incidences of infiltrate in the lungs of exposed animals were considered to reflect the higher incidences of lung neoplasms in these animals rather than a primary effect of cobalt sulphate heptahydrate exposure.

High incidences of chronic inflammation, karyomegaly, oval cell hyperplasia, and regeneration occurred in all groups of male mice and were usually observed together in the same liver. These changes were generally mild to moderate in severity and observed throughout the liver (usually not within proliferative lesions), but they appeared most pronounced in the portal regions. Similar lesions were observed in only a few females, and the severity was also much less than that observed in most males. This spectrum of lesions is consistent with those observed with Helicobacter hepaticus infection. Liver sections from four of five male mice with liver lesions were positive for bacterial organisms consistent with H. hepaticus when examined using Steiner's modification of the Warthin Starry silver stain. LOAEC was 0.3 mg/m^3 (0.06 mg cobalt/m^3, lowest dose administered) (NTP 1998).

Table 47: Incidences of selected nonneoplastic lesions of the respiratory system in mice in the 105 week inhalation study of cobalt sulphate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Number Examined Microscopically	50	50	50	50
Infiltration Cellular, Diffuse, Histiocyte[a]	1 (3.0)[b]	2 (3.0)	4 (2.3)	10**(1.5)
Infiltration Cellular, Focal, Histiocyte	10 (2.7)	5 (2.6)	8 (3.0)	17 (2.7)
Bronchus, Cytoplasmic Vacuolization	0	18**(1.0)	34**(1.0)	38**(1.0)
Alveolar Epithelium Hyperplasia	0	4 (2.3)	4 (1.8)	4 (2.3)
Female				
Number Examined Microscopically	50	50	50	50
Infiltration Cellular, Diffuse, Histiocyte	0	0	0	4 (3.3)
Infiltration Cellular, Focal, Histiocyte	2 (2.0)	5 (1.8)	7 (2.9)	10* (2.4)
Bronchus, Cytoplasmic Vacuolization	0	6* (1.0)	31**(1.0)	43**(1.0)
Alveolar Epithelium Hyperplasia	2 (1.5)	3 (1.3)	0	5 (2.0)

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Nose[a]	50	50	48	49
Olfactory Epithelium, Atrophy[b]	0	0	29**(1.2)[c]	48**(1.8)
Olfactory Epithelium, Hyperplasia	0	0	0	10**(1.0)
Inflammation, Suppurative	0	1 (3.0)	0	6* (2.2)
Larynx	48	49	48	49
Metaplasia, Squamous	0	37**(1.0)	48**(1.0)	44**(1.0)
Female				
Nose	50	50	49	48
Olfactory Epithelium, Atrophy	0	2 (1.5)	12**(1.0)	46**(1.5)
Olfactory Epithelium, Hyperplasia	0	0	0	30**(1.3)
Inflammation, Suppurative	0	1 (1.0)	5* (1.6)	4 (1.5)
Larynx	50	49	47	50
Metaplasia, Squamous	0	45**(1.0)	40**(1.0)	50**(1.1)

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
[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

Table 48: Incidences of selected nonneoplastic lesions of the liver in mice in the 105 week inhalation study of cobalt sulphate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Number Examined Microscopically	50	50	50	50
Inflammation, Chronic[a]	33 (1.3)[b]	36 (1.6)	40 (1.7)	39 (1.3)
Karyomegaly	39 (2.3)	35 (2.8)	39 (2.7)	43 (2.7)
Regeneration	32 (2.3)	30 (2.7)	35 (2.4)	38 (2.8)
Bile Duct, Hyperplasia	0	3 (1.3)	6* (1.7)	4 (2.5)
Oval Cell, Hyperplasia	38 (2.6)	36 (2.8)	40 (2.7)	44 (2.7)
Female				
Number Examined Microscopically	50	50	50	49
Inflammation, Chronic	6 (1.7)	1 (1.0)	1 (1.0)	2 (2.0)
Karyomegaly	4 (2.8)	2 (1.5)	0	1 (2.0)
Oval Cell, Hyperplasia	2 (2.0)	1 (2.0)	0	0

* Significantly different ($P \leq 0.05$) from the chamber control by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Studies with cobalt oxide

Prolonged exposure (3–4 months) of rats and rabbits to mixed cobalt oxides (0.4–9 mg cobalt/m^3) resulted in lesions in the alveolar region of the respiratory tract characterized histologically by nodular accumulation of Type II epithelial cells, accumulations of enlarged highly vacuolated macrophages, interstitial inflammation, and fibrosis (Johansson *et al.* 1984, 1987, 1991, 1992; Kyono *et al.* 1992; Palmes *et al.* 1959). In at least one instance, the lesions appeared to regress when exposure was terminated (Palmes *et al.* 1959 as summarised by ATSDR, 2004).

Lifetime exposure of hamsters to 7.9 mg cobalt/m^3 as cobalt oxide resulted in emphysema. No reduction in body weight was observed (Wehner *et al.* 1977 as summarised by ATSDR, 2004).

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

A cohort study was performed to determine which respiratory effects and symptoms are associated with long-term (at least 10 years) exposure in cobalt production. Among the exposed workers (mean cumulative exposure to cobalt 1000 µg-year), there was a significantly increased prevalence of suspected work-related asthma (15 subjects), phlegm, cough with wheezing, shortness of breath with wheezing and breathlessness on exertion than among controls. No chronic respiratory diseases, except asthma, were found among non-smoking cobalt production workers. FEV1 and the respiratory flow rates MEF25 and MEF50 were significantly lower among exposed smokers compared to smoking controls. One new case of occupational asthma (cobalt) with positive reaction in a provocation test and one case of allergic asthma were diagnosed. At concentrations lower than 100 µg Co/m^3 cobalt metal or cobalt sulphate exposure increased the risk of asthma by about five times in exposed workers. However, all cases of cobalt asthma diagnosed referred to workplace exposure conditions where additional irritant gases like sulphur dioxide, hydrogen sulphide or ammonia were present in the ambient air in addition to cobalt (Linna, A.; *et al.*2003).

A cross-sectional study on the effects of cobalt exposure in the Kokkola cobalt plant on the cardiovascular system of workers was conducted with 203 male workers with at least one year of exposure to cobalt. No clinically significant cardiac dysfunction due to cobalt exposure was found. Two echocardiography parameters (isovolumic relaxation time, deceleration time) which were considered to be the most important outcome variables because of their ability to reflect the earliest changes in the cardiac function were significantly changed due to cumulative exposure to cobalt. The clinical significance of these changes, however, remains to be evaluated (Linna, A.; *et al.*2004).

The following information is extracted from the ATSDR toxicological profile for cobalt (ATSDR, 2004)

The effects of chronic occupational exposure to cobalt and cobalt compounds on the respiratory system in humans are well-documented. These effects include respiratory irritation, diminished pulmonary function, wheezing, asthma, pneumonia, and fibrosis and occurred at exposure levels ranging from 0.007 to 0.893 mg cobalt/m^3 (exposure from 2 to 17 years). These effects have been observed in workers employed in cobalt refineries, as well as hard metal workers, diamond polishers, and ceramic dish painters (painting with cobalt blue dye).

Acute exposure of 15 healthy young men to atmospheres of hard metal dust containing 0.038 mg cobalt/m^3 for 6 hours resulted in reduced forced vital capacity (FVC), but no dose-response relation could be discerned. By contrast, 42 workers occupationally exposed to hard metal showed no decrease in ventilatory function at 0.085 mg cobalt/m^3, but significant changes in FEV1 (forced expiratory volume in 1 second) at 0.126 mg cobalt/m^3. Several other studies of hard metal workers have shown respiratory effects, including decreased ventilatory function, wheezing, asthma, and fibrosis, but have had less complete reports of exposure.

Swennen *et al.* (1993) performed a cross-sectional study on 82 workers in a cobalt refinery. Workers were examined for cobalt in blood and urine, a number of erythropoietic variables, thyroid metabolism, pulmonary function, skin lesions, and several serum enzymes. The concentrations of cobalt in blood and in urine after the shift were significantly correlated with those in air. Workers exposed to airborne cobalt metal, salts, or oxides (mean concentration 0.125 mg/m^3, range 0.001–7.7 mg/m^3) showed an increased ($p<0.05$) prevalence of dyspnea and wheezing and had significantly more skin lesions (eczema, erythema) than control workers. A dose-effect relation was found between the reduction of the FEV1 and the intensity of the current exposure to cobalt, as assessed by measurement of cobalt in blood, air, or urine.

Gennart and Lauwerys (1990) examined the ventilatory functions of 48 diamond polishing workers, relative to 23 control workers. Exposure occurred mainly in one of two rooms, with mean airborne concentrations of 0.0152 and 0.1355 mg cobalt/m3; control subjects worked in other areas of the facilities, where no exposure to cobalt occurred. Significant decreases in ventilatory function were found in the exposed workers relative to the control workers. Duration of exposure played a significant factor, with no significant differences in workers who had been exposed for ≤5 years; reported decreases in ventilatory function were noted in workers exposed for > 5 years. Inhalation exposure to cobalt salts (exposure levels not reported) among glass bangle workers resulted in decreases in decreased ventilatory function, generally restrictive in nature, relative to controls (Rastogi *et al.* 1991).

Nemery *et al.* (1992) conducted a cross-sectional study of cobalt exposure and respiratory effects in diamond polishers. Exposure occurred mainly from the generation of airborne cobalt resulting from the use of cobalt-containing polishing discs. The study groups were composed of 194 polishers working in 10 different workshops, and were divided into control, low-, and high-exposure groups. The low-exposure group (n=102) was exposed to an average of 0.0053 mg cobalt/m^3, based on personal sampling measurements, while the exposure level for the high dose group (n=92) was 0.0151 mg cobalt/m^3; there was considerable overlap in the total range of concentrations for the low- and high-exposure groups. Workers in the high-exposure group were more likely than those in the other groups to complain about respiratory symptoms; the prevalence of eye, nose, and throat irritation and cough, as well as the fraction of these symptoms related to work, were significantly increased in the high-exposure group. Workers in the high-exposure group also had significantly reduced lung function compared to controls and low-exposure group workers, as assessed by FVC, FEV1, MMEF (forced expiratory flow between 25 and 75% of the FVC) and mean PEF (peak expiratory flow rate). Results in the low-exposure group did not differ from controls.

Occupational exposure of humans to cobalt-containing dust, either as cobalt metal or as hard metal, has been shown to result in cardiomyopathy, characterized by functional effects on the ventricles (Horowitz *et al.* 1988) and/or enlargement of the heart (Barborik and Dusek 1972; Jarvis *et al.* 1992), but the exposure levels associated with cardiac effects of inhaled cobalt in humans have not been determined.

Beer-cobalt cardiomyopathy was observed in people who heavily consumed beer that contained cobalt sulphate as a foam stabilizer. The beer drinkers ingested an average of 0.04 mg Co/kg/day to

0.14 mg Co/kg/day for a period of years. The cardiomyopathy was characterized by sinus tachycardia, left ventricular failure, cardiogenic shock, diminished myocardial compliance, absence of a myocardial response to exercise or catecholamine, enlarged heart, pericardial effusion, and extensive intracellular changes (changes in the myofibers, mitochondria, glycogen, and lipids). The beer-cobalt cardiomyopathy appeared to be similar to alcoholic cardiomyopathy and beriberi, but the onset of beer-cobalt cardiomyopathy was very abrupt. It should be noted, however, that the cardiomyopathy may have also been due to the fact that the beer-drinkers had protein-poor diets and may have had prior cardiac damage from alcohol abuse. Studies in animals, and limited human data, have supported this possibility, as much greater oral exposure levels (on the order of 8-30 mg Co/kg-day) are necessary to induce cardiac effects.

Swennen *et al.* (1993) reported slightly, but statistically significantly, decreased levels of red cells and total hemoglobin (~4–5% decreases) in a group of 82 workers occupationally exposed to a mean concentration of 0.125 mg cobalt/m^3 as cobalt metal dust.

Exposure to cobalt and cobalt compounds has been demonstrated to increase levels of erythrocytes and hemoglobin in both humans and animals. Davis and Fields (1958) reported increased (~16–20%) erythrocyte levels in six of six healthy men exposed orally to cobalt chloride (~1 mg Co/kgday); erythrocyte counts returned to normal 9–15 days after cessation of cobalt administration. Increased levels of erythrocytes were also found following oral treatment of anephric patients (with resulting anemia) with cobalt chloride. The increase in hemoglobin resulted in a decreased need for blood transfusions. Treatment of pregnant women for 90 days with cobalt chloride, however, did not prevent the reduction in hematocrit and hemoglobin levels often found during pregnancy.

4.7.1.6 Other relevant information

HIF-1 is a heterodimer composed of HIF-1α and HIF-1β subunits and is the key mediator of hypoxia response (Davidson *et al.* 2015, Galanis *et al.* 2008, Salnikow *et al.* 2004). There is strong experimental support that HIF-1 activation is involved in cobalt-induced effects. Cobalt metal particles, cobalt chloride, and cobalt sulphate heptahydrate promote a hypoxia-like state *in vivo* and *in vitro*, even with normal molecular oxygen pressure, by stabilizing HIF-1α (Nyga *et al.* 2015, Galán-Cobo *et al.* 2013, Gao *et al.* 2013, Saini *et al.* 2010b, Saini *et al.* 2010a, Galanis *et al.* 2009, Qiao *et al.* 2009, Xia *et al* 2009, Beyersmann and Hartwig 2008, Maxwell and Salnikow 2004). This has been demonstrated in several human cell lines, including cancer cell lines (Fu *et al.* 2009, Ardyanto *et al.* 2008, Wang and Semenza 1995). Further, Wang and Semenza (1995) demonstrated that HIF-1 induction either from hypoxia or cobalt chloride treatment was indistinguishable with respect to DNA binding specificity and contacts with target DNA sequences.
Possible mechanisms by which cobalt ions activate HIF-1 include replacing iron in the regulatory oxygenases or depleting intracellular ascorbate (a cofactor for prolyl hydroxylase activity), thus, deactivating these enzymes (Davidson *et al.* 2015, Qiao *et al.* 2009, Maxwell and Salnikow 2004, Salnikow *et al.* 2004). Oxidative stress has also been investigated as a possible mechanism of cobalt-induced HIF activation; however, Salnikow *et al.* (2000) showed that activation of HIF-1-dependent genes was independent from ROS formation. Nyga *et al.* (2015) also reported evidence that HIF-1α stabilization in human macrophages treated with cobalt metal nanoparticles or cobalt ions occurred via an ROS-independent pathway (extracted from NTP 2016a).

The HIF-1 α RNA and/or protein was detected in 28 of 77 analyzed normal tissue cell types including lung, heart muscle, testis and adrenal gland although in variable degree (http://www.proteinatlas.org/ENSG00000100644-HIF1A/tissue).

4.7.1.7 Summary and discussion of repeated dose toxicity

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The observed effects in rats and mice after oral exposure to soluble cobalt compounds are characterised by an increase in Hb, RBC and/or Htc occurring after prolonged exposure to 2.5 mg Co/kg bw/day but not at 0.7 mg Co/kg bw/day. This effect was used in humans to treat patients with anaemia and most likely caused by stabilizing HIF-1α. In humans also effects on the heart were observed which are also observed in an animal study which focussed specifically on this effect. However, oral gavage exposure to cobalt powder seems to induce other effects which cannot be specified but may be caused by local gastro intestinal irritation. These other effects limit the possible external dose level. Seen the absence of the typical effects (increase of Hb) of Co^{2+} in the cobalt powder study on day 15, the bioavailability after oral gavage exposure also seems limited.

Inhalation exposure of rats and mice to cobalt powder and cobalt sulphate in multiple tests with different duration induces effects on the respiratory system (mainly lungs and larynx) and at somewhat higher concentrations also effects on the nose and an increase in Hb, RBC and/or Htc. Also effects on the testes were observed with both species and both substances.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

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).

4.9 Germ cell mutagenicity (Mutagenicity)

De table below includes only mutagenicity information on cobalt and soluble cobalt compounds as these are considered the most relevant for read-across.

Table 49: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Test substance	Lowest effective dose or highest ineffective dose	Results	Remarks	Reference
In vitro					
Bacteria					
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 471	NTP 2014
Ames test (TA 98)	Cobalt powder.	5000 µg/plate	-S9: negative +S9: negative	OECD 471 3 test labs	Kirkland 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) +S9: negative	No guideline	Wong, P.K. 1988
Ames test (TA 97)	Cobalt chloride	13 µg/mL	-S9:**positive**	No guideline, methodical and reporting deficiencies	Pagano, D.A.; Zeiger, E. 1992
Ames test (TA 98, TA 100, TA 1537, TA 2637)	cobalt(II)chloride	130000 µg/plate	-S9:negative	No guideline,	Ogawa, H.I. *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 471 3 test labs	Kirkland 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 471	Publication 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, E. *et al.* 1992
Ames test (TA 100)	Cobalt sulphate	5000 µg/plate	-S9: negative +S9: negative	OECD 471 3 test labs	Kirkland 2015
Mammalian cells					
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

Mammalian cell gene mutation test (hprt locus)	Cobalt metal powder	30 µg/mL	-S9: negative +S9: **positive**	OECD 476	Kirkland 2015
Mammalian cell gene mutation test (tk locus)	cobalt dichloride hexahydrate	57.11 µg/mL	negative	No guideline. Treatment was only 3 hours	Amacher, D.A.; Paillet, S.C. 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, A.; *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, M. *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 476	Kirkland 2015
Mammalian cell gene mutation test (hprt locus)	Cobalt oxide	120 µg/mL	-S9: negative +S9: negative	OECD 476	Kirkland 2015
Mammalian cell gene mutation test (hprt locus)	Cobalt sulfide	922 µg/mL	-S9: negative +S9: negative	OECD 476	Kirkland 2015
Mammalian cell gene mutation test (Gpt locus and transgenic G12, Gpt locus)	Cobalt sulfide (CoS_2 and CO_3S_4) particles	1 µg/mL 0.5 µg/mL	Negative (Gpt locus) **Positive** (transgenic G12)		Kitahara *et al.*, 1996
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
Comet assay (human peripheral blood leukocytes)	Cobalt chloride	100 µM	negative		Colognato *et al.*, 2008
Comet assay (human lung	Cobalt chloride	150µM	**positive**		Patel *et al.*,

epithelial cells)					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	5mM	**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, S.; *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, G.R.; Allison, A.C. 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, F.; *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, A.C.; *et al.* 2001
mammalian cell micronucleus test (human cells)	Cobalt metal	3 µg/mL	**positive**		De Boeck *et al.*, 2003b
mammalian cell micronucleus test (BALB/c bone marrow)	cobalt(II) dichloride hexahydrate	50 µg/mL	negative		Suzuki Y. *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, D.P.; *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, S.; *et al.* 1995
In vivo					
DNA damage in renal, hepatic and pulmonary chromatin in rat, ip	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 in testicular cells of hamster (n=10, 6 examined), ip	cobalt chloride	400 mg/kg bw (99 mg cobalt/kg bw)	**Positive** (bone marrow and testes)	No guideline, experimental and reporting deficiencies	Farah, S.B., 1983
chromosome aberration test in bone marrow of rat (5-15/sex/dose), oral	cobalt chloride hexahydrate	600 mg/kg bw (149 mg cobalt/kg bw)	Negative	OECD 475	Study report 1998
Spermatogonial chromosome aberration test in rat	cobalt chloride hexahydrate	30 mg/kg bw/day (7.4 mg cobalt/kg bw)	Negative		Kirkland 2015
chromosome aberration test in bone marrow of male mice (5/dose), oral	cobalt chloride hexahydrate	4.96 mg/kg (1.2 mg cobalt/kg bw)	**Positive**	No guideline	Palit, S.; *et al.* 1991
chromosome aberration test in bone marrow of rat (2/sex/dose), oral	Cobalt sulphate heptahydrate	320 mg/kg bw (67 mg cobalt/kg bw)	Negative	OECD 475, minor deviations	Study report 2009
chromosome aberration test in bone marrow of	Cobalt sulphate heptahydrate	1000 mg/kg bw (210 mg	Negative		Kirkland 2015

rat (5/sex/dose), oral		cobalt/kg bw)			
chromosome aberration test in rat (2/sex/dose), oral	Cobalt oxide	1000 mg/kg bw (786 mg cobalt/kg bw)	Negative	OECD 475, minor deviations	Study report 2009
chromosome aberration test in rat (5/sex/dose), oral	Cobalt oxide	2000 mg/kg bw (1573 mg cobalt/kg bw)	Negative		Kirkland 2015
micronucleus assay in peripheral blood of mice (10/sex/dose), inhalation	cobalt	10 mg/m^3	Negative	OECD 474	NTP 2014
micronucleus assay in bone marrow of male mice (5/dose), ip	cobalt(II)chloride hexahydrate	50 mg/kg bw (12.4 mg cobalt/kg bw)	**Positive**	No guideline	Suzuki 1993
micronucleus assay in bone marrow of rat (5-15/sex/dose), oral	cobalt chloride hexahydrate	600 mg/kg bw (149 mg cobalt/kg bw)	Negative	OECD 474	Study report 1998
micronucleus assay in bone marrow of male mice (5/dose), ip	Cobalt chloride hexahydrate	11.25 mg/kg bw (2.8 mg cobalt/kg bw)	**Positive**		Rasgele *et al.*, 2013
Dominant lethal assay in male mice (n=10)	Cobalt chloride hexahydrate	400 ppm (approximately 67 mg Co/kg bw)	**Positive**		Pedigo, N.G.; Vernon, M. W. 1993
Dominant lethal assay in male mice (10/dose)	Cobalt chloride hexahydrate	46.9 mg /kg bw (11.6 mg cobalt/kg bw/day)	**Positive**		Elbetieha, A. *et al.*, 2008

4.9.1 Non-human information

4.9.1.1 In vitro data

Studies with cobalt metal

Cobalt metal (100 to 5,000 µg/plate) gave an equivocal response in *Salmonella typhimurium* strain TA100 in the absence of S9 activation mix; with 10% rat liver S9, doses up to 7,500 µg/plate did not induce an increase in mutant colonies in TA100. In *S. typhimurium* strain TA98 without S9, cobalt metal (100 to 3,500 µg/plate) was mutagenic (NTP, 2014), although the responses observed were weak and not well correlated with dose level; with S9, no mutagenic activity was observed. This result was not confirmed in another Ames test in *Salmonella typhimurium* strain 98 as no mutagenic response was observed in three independent tests in different laboratories (Kirkland 2015). In *Escherichia coli* strain WP2 uvrA/pKM101, doses of cobalt metal up to 450 µg/plate were not associated with mutagenic activity, with or without S9 (NTP 2014).

In mammalian cells, DNA strand breaks were observed in an alkaline elution test in 3T3 cells (Anard, 1997). A HPRT mouse lymphoma assay was weakly positive in the presence of S9, but negative in the absence of S9. Also in human lymphocytes, leukocytes, PBMCs and HepG2 cells, all 6 available studies with cobalt metal report DNA damage as shown by positive comet assays. In addition, micronuclei were induced in all 3 studies using human cells.

Studies with cobalt salts

Varying results are reported in bacterial gene mutation tests with cobalt chloride (hexahydrate) and cobalt sulphate heptahydrate. All studies with S9 were negative. For studies without S9, positive results were observed in some studies with *S. typhimurium* strain TA 97, TA98 and *E. coli* SY1032/pKY241 for cobalt chloride (hexahydrate). Other studies with *S. typhimurium* strain TA 98 however were negative, as were studies with *S. typhimurium* strain TA 97a, TA 100, TA 1535, TA 1538, TA 2637 and *E. coli* WP2. For cobalt sulphate heptahydrate, mutagenic activity was observed in one study with *S. typhimurium* strain TA 100. However, this was not confirmed in four independent tests. In strain TA98 and TA 1535, no mutagenicity was observed.

Also cobalt salts induced DNA damage (strand breaks, mitochondrial DNA damage) in mammalian cells: 4 out of 5 studies were positive (the negative study used a different assay). In addition, gene mutations were induced in the *hprt* locus (cobalt (di)chloride, but not cobalt sulphate, oxide or sulphide) and transgenic G12 *gpt* locus (cobalt chloride and sulphide), but not in the *tk* (cobalt dichloride), *gpt* (cobalt chloride and sulphide) and v79-*8AG* locus (cobalt dichloride). DNA damage was also shown in comet assays in human lymphocytes, fibroblasts, hep G2 cells and epithelial cells (5 out of 7 assays positive). Two sister chromatid exchange assays are available, both with cobalt chloride. Both were positive. Chromosome aberration assays in human fibroblasts were found positive (3 with cobalt chloride, 1 with cobalt oxide), whereas no increase in chromosome aberrations was found in human lymphocytes (cobalt sulphate, nitrate, acetate and oxide). Two micronucleus assays in mouse bone marrow and 3T3 cells with cobalt chloride were negative, whereas one in human lymphocytes was positive, although with a high variability in response of donors. Also a micronucleus test with cobalt sulphate heptahydrate in Syrian hamster embryo cells was positive.

4.9.1.2 In vivo data

Studies with cobalt metal

In a micronucleus assay in male and female B6C3F1 mice, cobalt (0, 0.625, 1.25, 2.5, 5 or 10 mg/m^3 by inhalation for 13 weeks, 5d/week), cobalt did not induce micronuclei in peripheral blood of mice. However, also no significant alterations in the percentages of reticulocytes (polychromatic erythrocytes) were seen in male or female mice, suggesting that exposure to cobalt metal under these conditions did not cause bone marrow toxicity (NTP, 2014).

Studies with soluble cobalt salts

DNA base damage was studied in renal, hepatic, and pulmonary chromatin of male and female F344/NCr rats that had been given either 50 or 100 μmol of Co(II) acetate/kg body wt (~2.9 or 5.9 mg cobalt/kg bw) in a single ip dose and killed 2 or 10 days later. Control rats received 200 μmol of sodium acetate/kg body wt. The response was organ-specific. Eight of the DNA base products in renal chromatin of Co(II)-treated rats (mostly 5-OH-Cyt and other pyrimidine products), five in hepatic chromatin (mostly FapyGua and other purine products), and two in pulmonary chromatin (5-OHMe-Ura > FapyAde) were increased by 30% to more than 200% over control levels with increasing Co(II) dose. The renal and hepatic, but not pulmonary, DNA base damage tended to

increase with time. No significant differences in response were found between male and female rats. The bases determined were typical products of hydroxyl radical attack on DNA, suggesting a role for this radical in the mechanism(s) of DNA damage caused by Co(II) in vivo (Kasprzak *et al.* 1994).

In a mammalian germ cell cytogenetic assay in male Syrian hamsters (10, only 6 analysed), the effects of cobalt chloride for induction of CA in metaphase I and II meiotic testicular cells were studied. Animals were dosed intraperitoneally on 5 consecutive days with 400 mg/kg bw (not clear whether this is the daily or the total dose). At least 50 cells in metaphase I and 40 cells in methaphase II were analysed from testicular tissue per animal. An increase in cells with at least 23 bivalents (instead of the normal 22) was seen in metaphase I preparations from the treated group. This is an unconventional study design and the biological relevance of the results is therefore difficult to assess (Farah et al 1983 as summarised in the registration of cobalt dichloride).

A spermatogonial chromosomal aberration assay was performed in Sprague Dawley CD rats with 0, 3, 10 and 30 mg/kg bw/day (equivalent to 0, 0.7, 2.5 and 7.4 mg cobalt /kg bw by gavage, for 28 days). In the cobalt chloride groups all group mean structural CA frequencies fell within the historical control range (see table 50). Also, there were no polyploid cells found from 1000 metaphases scored in each of the groups (Kirkland 2015).

Table 50: Chromosomal aberration frequencies in spermatogonia of rats treated with cobalt dichloride

Dose (mg/kg/day)	MI	No. of cells examined (M+F)	Group mean % cells with CA (range)		% polyploid cells
			Including gaps	Excluding gaps[a]	
0	1.00	1000	2.8 (1.5–4.0)	1.1 (1.0–1.5)	0.0
3	1.48	1000	1.3 (0.5–3.0)	0.7 (0.5–1.5)	0.0
10	1.26	1000	2.2 (1.5–2.5)	0.7 (0.5–1.0)	0.0
30	1.11	1000	2.2 (1.5–2.5)	0.9 (0.5–1.5)	0.0

[a] Historical control range = 0.7–1.5% for group mean CA, excluding gaps.

In a combined chromosome aberration and micronucleus assay in male and female Sprague Dawley rats, cobalt chloride hexahydrate (0, 50, 200 or 600 mg/kg bw equivalent to 0, 12.4, 49.6 or 149 mg Co/kg bw by gavage) did not induce a significant increase in cells with structural and numerical chromosome aberrations or micronucleated polychromatic erythrocytes. PCE/NCE ratio was reduced, indicating that the substance did reach the bone marrow (Study report 1998).

A chromosome aberration assay in bone marrow of rats (Hsd:SD) was performed with cobalt sulphate (100, 300 and 1000 mg/kg bw/day equivalent to 21, 63 and 210 mg Co/kg bw/day), tricobalt tetraoxide (200, 600 and 2000 mg/kg bw/day equivalent with 47, 141 and 470 mg Co/kg bw/day) and cobalt oxide (200, 600 and 2000 mg/kg bw/day equivalent with 157, 472 and 1573 mg Co/kg bw/day) by gavage using 1% methyl cellulose in water as vehicle for 5 consecutive days. All samples were taken 16 hours after the last treatment. In addition, the presence of cells with nuclear analomies/abberations as an indicator for DNA damage and decreases in the mitotic index were determined in histological sections of several organs. General toxicity was observed after exposure to cobalt sulphate and cobalt oxide resulting in mortalities and a reduction in the number of exposed days of the remaining animals. No CA frequency could be determined for some groups. There was evidence of bone marrow toxicity with both cobalt sulphate and cobalt monoxide based on decreases in mitotic index. The mitotic index was increased after multiple exposure to tricobalt tetraoxide. Some increased CA frequencies were seen in the top dose groups treated with cobalt sulphate and cobalt monoxide (treatment only twice and some mortalities). Because CA frequencies in vehicle control animals were low (historical controle data in the range of 0-2%), the finding of 1.8% cells with CA in the high dose sulphate and monoxide groups of males and the presence of a dose-effect relation for cobalt oxide could be indicative of a clastogenic response (Table 51).

Animals treated with cobalt sulphate showed clear increases in nuclear anomalies (NA) in all regions of the intestine and, at the high dose only, a small increase in anomalies in the liver (Table 52). The mitotic index was decreased in most of these tissues. Slight increases in NA were also seen in lung and bladder. However, as these tissues show a very low and variable mitotic index, the apparent increases were not considered conclusive. A slight increase in NA in the testes of animals tested with high dose cobalt sulphate could be due to indirect toxic effects or normal variation, given the relatively low number of animals involved. Animals treated with cobalt monoxide showed clear increases in NA in both regions of the gastrointestinal tract examined and in the glandular stomach. Effects were seen to a lesser extent in the liver. No or only marginal effects were observed with tricobalt tetraoxide except for the gut at the highest dose (Kirkland 2015). Overall, the available data show mainly local effects after gavage exposure and some indication of more systemic effects.

Table 51: Bone marrow CA results for the multi dose phase

Treatment	Dose (mg/kg/day)	Relative MI	No. of cells examined (M+F)	% cells with CA (excluding gaps, polyploidy and endoreduplication)		
				M	F	M+F
Vehicle control	0	100	1000	0.2	0.2	0.2
Cobalt sulphate	100	97	900	0.0	0.3	0.1
	1000	**65**	900	***1.8***	0.8	**1.2**
Cobalt monoxide	600	**73**	1000	0.8	0.6	0.7
	2000	**39**	500	***1.8***	ND	**1.8**
Cobalt tetraoxide	200	**139**	1000	0.2	0.8	0.5
	600	**154**	1000	0.2	0.8	0.5
	2000	113	1000	0.6	0.0	0.3
CPA	10*	117	1000	***7.2***	***11.2***	**9.2**
DMH	10*	123	900	0.3	0.2	0.2

M = male.
F = female.
ND = no data due to mortalities.
* Single dose given on day prior to euthanasia.
Bold figures indicate statistical significance ($p < 0.05$).
Bold italics indicate values exceed laboratory historical control range.

Table 52: Results of analysis of NA and mitotic rate in various tissues during the multi dose phase of the *in vivo* study

Tissue	Cobalt sulphate		Cobalt monoxide		Tricobalt tetraoxide		CPA		DMH	
	Increased NA cells	Decreased mitotic rate	Increased NA cells	Decreased mitotic rate	Increased NA cells	Decreased mitotic rate	Increased NA cells	Decreased mitotic rate	Increased NA cells	Decreased mitotic rate
Non-glandular stomach	0	++	0	++	0	0	0	**	0	0
Glandular stomach	+++	++*	+++	++	+/−	0	0	**	0	0
Duodenum	+++	+	+++	+++	+/−	0	0	0	0	0
Ileum	+++	+++	ND	ND	ND	ND	0	0	0	+/−
Caecum	+++	+++	ND	ND	ND	ND	0	0	0	0
Colon	+++	++	+++	+++	+	0	+/−	0	0	0
Rectum	+++	+	ND	ND	ND	ND	+	0	++	0
Liver	+	+++	++	+++	0	+	0	**	+/−	+/−
Lungs	+	ND	ND	ND	ND	ND	0	ND	0	ND
Urinary bladder	+	ND	ND	ND	ND	ND	+/−	ND	+/−	ND
Testis	+/−	0	ND	ND	ND	ND	0	0	0	0

0 = no change.
+/− = possible increase in NA or decrease in mitotic rate.
+ = slight increase in NA or decrease in mitotic rate.
++ = substantial increase in NA or decrease in mitotic rate.
+++ = very substantial increase in NA or decrease in mitotic rate.
ND = no data.
* = possible stimulation of mitosis at low dose, but decrease at high dose.
** = possible stimulation of mitosis at only dose tested.

In a chromosome aberration assay in male Swiss mice, cobalt chloride (single dose of 0, 20, 40 or 80 mg/kg bw by gavage, equivalent to 4.96-19.8 mg cobalt/mg bw, post exposure periods of 6, 12, 18 and 24 hours) dose- and time-related increases in CA frequency were seen in all treated groups (Palit, S.; *et al.* 1991).

Table 53: Data on bone marrow Chromosome aberrations

Duration (h)	Concentration (mg/kg body wt)	Log dose	Total Chromosomal aberrations (CA) G′	G′′	B′	B′′	Polyploids and others	Percentage of CA[a] Mean ± SD Including gap	Without gap	Break/Cell
	Control	1	3	0	2	0	1	2.4 ± 1.67	1.2 ± 1.095	0.008
6	20	1.30	5	1	7	0	7	8 ± 2	5.6 ± 2.19	0.028
	40	1.60	8	0	10	0	10	11.2 ± 2.28	7.6 ± 3.28	0.04
	80	1.90	11	0	16	0	9	15.6 ± 6.22	10 ± 2.44	0.064
	Trend test *p* value[b]							***4.96	***4.08	***3.38
	Control	1	2	1	2	1	2	4.4 ± 2.60	2 ± 2	0.016
12	20	1.30	5	0	7	2	10	9.6 ± 1.67	7.6 ± 3.84	0.044
	40	1.60	9	1	11	1	8	12 ± 6.32	8 ± 2.44	0.052
	80	1.90	13	0	25	0	4	16.8 ± 3.033	11.6 ± 3.57	0.1
	Trend test *p* value[b]							***4.28	***3.29	***3.99
	Control	1	4	0	4	0	1	3.6 ± 2.60	2 ± 2.82	0.016
18	20	1.30	5	1	17	3	11	14.8 ± 10.6	12.4 ± 8.29	0.092
	40	1.60	3	1	19	0	25	19.2 ± 8.3	17.6 ± 7.40	0.076
	80	1.90	6	0	24	1	27	23.2 ± 9.54	20.8 ± 10.44	0.104
	Trend test *p* value[b]							***5.73	***5.57	***3.26
	Control	1	7	1	5	0	4	6.8 ± 1.78	3.6 ± 1.6	0.02
24	20	1.30	4	0	10	3	14	12.4 ± 2.6	10.8 ± 3.03	0.064
	40	1.60	11	1	14	2	21	19.6 ± 7.53	14.8 ± 6.41	0.072
	80	1.90	7	0	29	1	24	24.4 ± 10.03	21.6 ± 7.92	0.124
	Trend test *p* value[b]							***5.33	***9.04	***8.038

[a]Abbreviations: G′, G′′ = Chromatid and isochromatid gaps, respectively; B′, B′′ = Chromatid and isochromatid breaks respectively.
[a]Mean percent of CA ± SD of the mean among 5 animals per set.
[b]*p* value determined by a one-tailed trend test.
***Significantly different at $p \leq 0.001$.

In a chromosome aberration assay in male and female Sprague Dawley rats, cobalt sulphate heptahydrate (0, 80, 160 or 320 mg/kg bw, equivalent to 0, 17, 34 or 67 mg cobalt/kg bw, by gavage) had no mutagenic effects in the chromosome aberration tests. A multi-dose phase test conducted with three cobalt compounds (including cobalt sulphate heptahydrate) showed that under given experimental conditions cobalt ions reaches the bone marrow. (Study report, 2009).

Cobalt chloride (0, 25, 50 or 90 mg/kg bw, equivalent to 0, 6.2, 12 or 22 mg cobalt/kg bw) was administered once by intraperitoneal injection to male BALB/c AnNCrj mice (5/dose). After 30 hours, the bone marrow was processed for analysis. Treatment with the test item induced a dose-dependent increase in MPCE frequency. The P/N ratio was the lowest at 90 mg/kg bw. (Suzuki *et al.*, 1993).

Table 54: Results micronucleus test Suzuki et al 1993 (Results show mean ± SD)

Dose of CoCl2*6H2O [mg/kg bw]	MPCE frequency [%]	P/N ratio
90	0.75±0.43**	0.87±0.19**
50	0.46±0.09**	1.44±0.44
25	0.12±0.13	2.47±0.17
0	0.18±0.15	1.98±0.32

** P < 0.05 statistically different from the solvent control

In a micronucleus assay, cobalt chloride (11.25, 22.5 and 45 mg/kg bw, equivalent to 2.79, 5.6 or 11 mg cobalt/kg bw) was administered by intraperitoneal injection to male Swiss albino mice (5/dose). This induced a significant increase in frequency of micronucleated polychromatic erythrocytes (MNPCE) at 24 and 48 hours when compared with the control. No reduction of the PCE/NCE ratio was observed both 24 and 48 hours as compared to the negative control, indicating no cytotoxicity occurred at these doses (Rasgele 2013).

Cobalt toxicity was evaluated in a dominant lethal assay (DLA) to determine whether the detrimental effects of cobalt on spermatozoa would have an impact on offspring. Ten male B6C3F1 mice were treated with cobaltous chloride (400 ppm Co) (estimated as 67 mg Co/kg bw/day) in drinking water for 10 weeks and mated. Neither the stage nor rate of development in vitro of 2-cell embryos to blastocyst from cobalt-treated males was affected. Although all males were fertile, the number of pregnant females was decreased in the group mated with males treated with cobalt. 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 from litters at day 19 of gestation. 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 treatment), and recovered over the next 6 weeks. There was a decrease in testes weight. Sperm parameters at the end of DLA and the recovery period showed that cobalt decreased all parameters measured at 12 weeks, but these parameters, except concentration, recovered to control levels by 18 weeks. For further details on sperm parameters, see paragraph 4.11. 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 (Pedigo, N.G.; Vernon, M. W., 1993).

Table 55: Results dominant lethal assay in mice

	0 ppm	**400 ppm Co**
Number of pregnant females	29/32 (91%)	18/31 (58%)*
Number of fertile males (at 10 weeks)	10/10	10/10
Average total implantations per pregnant female	8.3 ± 0.4	6.5 ± 0.8*
Average dead implantations per pregnant female	0.4 ± 0.1	0.4 ± 0.1
Average preimplantation loss per pregnant female	0.43 ± 0.2	2.4 ± 0.7*

Sexually mature male mice were exposed to 200, 400 and 800 ppm cobalt chloride hexahydrate (25.7, 46.9 and 93.0 mg/kg bw/day) in their drinking water for 12 weeks. Males were than mated with untreated female mice. Average body weight gain was significantly reduced in all dose groups (final body weights were 95, 94 and 93% of the control group). Two animals out of 10 and one out of 10 died during the 10^{th} weeks of the exposure to 800 and 400 ppm cobalt chloride, respectively. There were no other signs of clinical toxicity observed in the survived animals.Testicular sperm count was decreased at ≥ 400 ppm. Epididymal sperm count was decreased at all doses. Testicular weight was reduced at ≥ 400 ppm. Epididymal weight was reduced at 800 ppm. Histological examination of the testes showed hypertrophy of the interstitial Leydig cells, congested blood vessels, degeneration of the spermatogonial cells and necrosis of both the seminiferous tubules and the interstitial tissue (doses unknown). For further details on sperm parameters, see paragraph 4.11. At ≥ 400 ppm number of pregnancies and number of implantation sites was significantly reduced. Resorptions were increased at all doses whereas the number of viable foetuses was decreased. The increase in resorptions at 200 ppm in the absence of a significant effect on pregnancy could be considered as a positive result. No information on positive control groups and the laboratory's historical negative control data is available (these authors have multiple publications on the effects of substances on fertility in male mice) and mating was only performed at week 12. (Elbetieha, A. *et al.* 2008).

Table 56: Results dominant lethal assay with mice exposed to cobalt chloride

Treatment (ppm)	Number of males	Number of mated females	Number (%) of pregnant females	Number of implantation sites[a]/female	Number of viable fetuses[a]	Total Number of resorptions/ Total No. of implantation sites	Number (%) of animals with resorptions
Control (Tap water)	10	20	19/20 (95.0)	7.89 ± 2.38	7.74 ± 2.40	3/150	3/19 (16)
Cobalt chloride (200)	10	20	15/20 (75.0)	5.67 ± 2.02^{++}	5.00 ± 2.14^{++}	9/81***	10/15** (67)
Cobalt chloride (400)	9	18	12/18 * (66.7)	5.42 ± 1.68^{++}	4.67 ± 1.83^{+++}	9/65***	10/16** (63)
Cobalt chloride (800)	8	16	7/16*** (43.8)	6.43 ± 2.23	5.83 ± 1.94^{+}	10/45****	5/7* (70)

[a] Results are expressed as mean ± S.D.

$^{+}$ $p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ (Student *t* test).

* $p<0.05$, **$p<0.005$, ***$p<0.001$, ****$p<0.0001$ (Fisher's exact test).

4.9.2 Human information

In a cross sectional study, 35 workers were exposed to cobalt dust from three refineries and 35 matched control subjects recruited from the respective plants. The study design integrated complementary methodologies to assess damage on lymphocytes and definitive chromosome breakage/loss (micronuclei in lymphocytes). 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 TWA exposure of 20 µg/m³ Co (De Boeck, M.; *et al.* 2000).

4.9.3 Other relevant information

In the NTP carcinogenicity study of cobalt sulphate heptahydrate in B6C3F1 mice (NTP, 1998) (see also paragraph 4.10) *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. This provides supportive evidence that cobalt sulphate heptahydrate may indirectly damage DNA by oxidative stress.

Filtered and unfiltered extracts of cobalt sulphate heptahydrate and cobalt di(2-ethylhexanoate) induced comparable ROS formation in A549 cells. However, ROS production by cobalt sulphate was not associated with cytotoxicity whereas ROS production by cobalt di(2-ethylhexanoate) was (Kirkland 2015). Cobalt di(2-ethylhexanoate) and cobalt sulphate induced an increase in Comet tail intensity which was further enhanced by pretreatment with hOGG1 to detect oxidative base lesions. No difference was observed between filtered and unfiltered fractions. However in both studies the level of the dissolved fraction was high. Therefore, these studies are not considered conclusive for the effect of undissolved cobalt compounds.

Five soluble cobalt compounds have a harmonised classification as Muta. 2. However, additional information has been provided after this advice by TC-C&L from May 2004.

Information on the possible mechanisms for the induction of genotoxicity and carcinogenicity is provided in chapter 4.10.3.

The studies with soluble cobalt (2+) salts are considered relevant for cobalt, as cobalt is transformed into Co^{2+} in biological systems as shown in the NTP studies with cobalt. Formation of Co^{2+} from Co after oral and inhalation exposure is considered likely as inhalation exposure to Co results in the same systemic effect (increase in RBC and hemoglobin) as after exposure to soluble Co^{2+} salts.
A review of the mutagenicity of cobalt and cobalt compounds by Kirkland *et al.* (2015) on request by the Cobalt Development Institute and the Cobalt Research Consortium was recently published. They concluded that there is no evidence of genetic toxicity with relevance for humans of cobalt substances and cobalt metal.

4.9.4 Summary and discussion of mutagenicity

Bacterial mutation assays were all negative (except 1) when S9 was added to the mixture. Without S9, results varied for cobalt, cobalt chloride and cobalt sulphate. Whereas overall the results were negative, positive effects were observed in some studies with *S. typhimurium* strain TA 97, TA98 and *E. coli SY1032/pKY241*.

Different types of cobalt compounds caused DNA damage (especially DNA strand breaks) after exposure *in vitro* in rodent as well as in human cells in elution assays and comet assays. Results of gene mutation assays showed contradicting results. Mutation studies in rodent cells are positive for some gene loci (i.e. hprt), but negative for others (i.e tk). However, recent gene mutation studies (hprt locus) performed according to OECD guidelines have not reproduced any positive effect. In cytogenetic assays, cobalt chloride induced sister chromatid exchange (SCE) in mouse macrophage-like cells as well as in human lymphocytes.

Most micronuclei studies in rodent cells were negative, whereas overall, in human cells positive effects were observed. Chromosomal aberrations were evaluated only in human cells, after exposure to various forms of cobalt. Mixed results were reported, possibly related to cell type or exposure level and not compound solubility. Nevertheless, it can be concluded that overall, *in vitro* results indicate that cobalt and cobalt compounds are genotoxic.

In vivo exposure to cobalt or cobalt compounds resulted in DNA damage, chromosome aberrations and micronuclei in all studies when administered intraperitoneally, showing that cobalt and soluble cobalt salts (chloride and sulphate) indeed have mutagenic potential. However, all oral and the single inhalation chromosome aberration and micronucleus studies were negative, even though in some of these studies a reduced PCE/NCE ratio indicated that the test compound did reach the bone marrow. There was one exception: dose-dependent increases in chromosomal breaks and aberrations were reported in Swiss mouse bone marrow after oral exposure to a single dose of cobalt chloride (≥4.96 mg/kg bw). It is not clear why a relatively low dose oral study shows chromosomal aberrations, whereas others do not. However, also the evaluation of lung tumours in the carcinogenicity study shows that cobalt (as cobalt sulphate) is capable of inducing mutations. The results of the available in vivo studies could also be split between rats and mice with all rat studies being negative and mice studies positive. No explanation can be given for this possible species difference. A possible explanation for the presence of an increase in CA in bone marrow in mice may be the increase in erythropoiesis in the bone marrow due to the pseudohypoxic effect of Co2+. However, it is unclear from the available data whether a short exposure period as applied in these types of test is sufficient to induce relevant erythropoiesis. The decrease in the P/N ratio as observed by Suzuki *et al.* (1993) and comparable results in the micronucleus tests with cobalt resinate in rats (data not shown) does not suggest such an effect.

Two dominant lethal assays with cobalt chloride, both in mice, were positive, resulting in reduced fertility and increased implantation loss. The increase in implantation loss may indicate an increase in mutations of the germ cells, although it is also possible that the implantation loss is secondary to the strong effects on sperm cells via a reduction in the selection of the best sperm cells. Most tested dose levels were above the MTD for a dominant lethal test defined (amongst others) as not affecting mating success (percentage of pregnancies were 58 vs 91% in the first and 67 and 44 vs 95% in the second study). However, in the study by Elbetieha (2008) a reduction in viable foetuses was also observed at a dose level (200 ppm) without a significant reduction in pregnant females (75 vs 95%).

4.9.5 Comparison with criteria

Overall, *in vitro* studies indicate a genotoxic potential for cobalt and cobalt compounds. However, whereas also *in vivo* studies with intraperitoneal administration clearly show genotoxicity, most results with oral and inhalation exposure are negative. Only 1 chromosomal aberration study (without guideline, but well conducted) with oral administration was positive. A reduction in viable foetuses was observed in a dominant lethal test at a dose level without a significant decrease in the percentage of pregnant females. In addition, in the carcinogenicity study with inhalation exposure in mice, specific gene mutations in the *K-ras* gene were reported in the lung tumours induced by cobalt sulphate.

There is no in vivo information on germ cells with cobalt. The available oral dominant lethal tests with soluble cobalt compounds show a reduction in implantations and an increase in resorption but mainly at dose levels with reduced pregnancy and sperm counts. However, in one study at one dose level (Elbetieha, 2008 at 200 ppm), a statistically significant increase in resorptions was observed without decrease in pregnancy. Seen the limitations of this study which was not set up as a dominant lethal test, the results a not considered clear evidence. Therefore, classification of soluble cobalt compounds in category 1B for mutagenicity is not proposed.

Since there is no information on humans, it is concluded that there is not enough evidence for classification in Muta. 1A.

According to the guidance, 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.
Note: 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.

It is clear that *in vitro* and *in vivo* (although after ip. injections), soluble cobalt (compounds) is able to induce genotoxicity in somatic cells. In addition, the increased implantation loss in 2 dominant lethal assays may point towards genotoxicity in germ cells. However, it is questioned whether the studies most relevant for classification, i.e. oral and inhalation *in vivo* studies, provide enough strength for classification. Nevertheless, considering the positive in vivo study by Palit *et al.*, the results of the oral dominant lethal test and the K-ras mutations observed by the NTP, it is considered likely that cobalt and cobalt compounds are able to induce genotoxicity after oral or inhalation exposure. The difference between IP and oral studies may be due to a difference in local dose between these two routes. As it is indicated that the mutagenicity of cobalt may be indirect, the local dose level must reach a certain level to induce such effects. This is possibly shown by the strong increase in nuclear anomalies in the gastro-entero tract as shown by Kirkland (2015).

Therefore, especially local mutagenicity at the port-of-entry cannot be excluded. In addition, it is noted that 5 cobalt salts (cobalt sulphate, cobalt nitrate, cobalt chloride, cobalt carbonate and cobalt acetate) are classified as Muta 2. Overall, classification of soluble cobalt compounds as Muta Cat 2 is warranted.

There are several positive mutagenicity *in vitro* studies with cobalt itself (DNA strand breaks, comet assay, Hprt mutation assay, micronucleus test) which can be used as supplemental information but no in vivo tests. Read-across from the soluble cobalt compounds to cobalt is considered scientifically correct because it is shown that after inhalation exposure to cobalt, Co^{2+} is systemically available in many organs including the testes. However, the note to the criteria for category 2 states that read-across can only be applied if there is a structural relationship to known germ cell mutagens which can be interpreted as Category 1A or 1B. Therefore, read-across from the other category 2 classified soluble cobalt compounds to cobalt seems to be not in line with the criteria.

Nevertheless, on scientific grounds, 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 are considered strong enough for read across for Cat 2 and therefore, it is concluded that also cobalt metal should be considered suspected of causing genetic defects.

4.9.6 Conclusions on classification and labelling

Cobalt metal should be classified as Muta. 2; H341: suspected of causing genetic defects.

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.

Additional key elements

OECD/CoCAM (2014) concluded on soluble cobalt salts, that they do not elicit any mutagenic activity either in bacterial or mammalian test systems. However, they induce some genotoxic effects *in vitro*, mainly manifest as DNA strand or chromosomal breaks, which are consistent with a reactive oxygen mechanism. A WoE approach was applied, considering positive as well as negative *in vivo* clastogenicity studies and the absence of such chromosome damage in humans that are occupationally exposed to inorganic cobalt substances. OECD/CoCAM (2014) concluded that effective protective processes exist *in vivo* to prevent genetic toxicity both in animal and in human. However, it cannot be excluded that the occupational exposure was too low to cause significant and dectable effects in humans.

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) +S9: negative	No guideline	Wong, 1988
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 (CoS_2 and CO_3S_4) 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
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	cobalt nitrate	0.15 µg/mL	negative	No guideline	Paton and Allison, 1972

mononuclear leukocytes)					
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
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/m^3	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	cobalt chloride	4.96 mg/kg (1.2 mg	**Positive**	No guideline	Palit *et al.,*

aberration mice, oral	hexahydrate	cobalt/kg bw)			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
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**.

4.10 Carcinogenicity

Table 57: Summary table of relevant carcinogenicity studies

Method	Test substance	Results	Remarks	Reference
combined repeated dose and carcinogenicity inhalation study in rats (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	Cobalt Purity >98% MMAD: 1.4-2.0 µm GSD: 1.6-1.9	≥1.25 mg/m^3: increased incidences of alveolar/bronchiolar neoplasms and cystic keratinising epitheliomas of the lung (m/f); mononuclear cell leukemia (f) ≥2.5 mg/m^3: increased incidences of benign/malignant pheochromocytomas of the adrenal medulla (m/f); neoplasms of the pancreatic isles (m)		NTP, 2014
combined repeated dose and carcinogenicity inhalation study in mice (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	Cobalt Purity >98% MMAD: 1.5-2.0 µm, GSD: 1.6-1.9	≥1.25 mg/m^3: increased incidences of alveolar/bronchiolar neoplasms of the lung (m/f)		NTP, 2014
combined repeated dose and carcinogenicity inhalation study in rats (50/sex/dose) 0, 0.3, 1.0, or 3.0 mg/m³ 6 hours per day, 5 days per week, for 105 weeks)	cobalt sulphate heptahydrate; purity 99% MMAD: 1.1-1.8 µm GSD: 1.9-2.6	≥1 mg/m^3(0.2 mg cobalt/m^3): increased incidences of alveolar/bronchiolar neoplasms of the lung (m/f); increased incidences of pheochromocytomas of the adrenal medulla (m) 3 mg/m^3 (0.7 mg cobalt/m^3): increased incidences of pheochromocytomas of the adrenal medulla (f)		NTP, 1998
combined repeated dose and carcinogenicity inhalation study in mice (50/sex/dose) 0, 0.3, 1.0, or 3.0 mg/m³ 6 hours per day, 5 days per week, for 105 weeks)	cobalt sulphate heptahydrate; purity 99% MMAD: 1.1-2.0 µm GSD: 2.1-3.0	≥1 mg/m^3 (0.2 mg cobalt/m^3): increased incidences of hemangiosarcoma in the liver (m); increased incidences of alveolar/bronchiolar neoplasms of the lung (f) 3 mg/m^3 (0.7 mg cobalt/m^3): increased incidences of alveolar/bronchiolar neoplasms of the lung (m);		NTP, 1998

carcinogenicity inhalation study in hamster (51/group) 0 or 10 g/L 7h/day, 5 days/week, for 17-21 months	Cobalt(II) oxide (particle size distribution unknown)	10 g/L (7864 g cobalt/m^3): No increase in lung tumors observed	This concentration in air is considered unrealistic and supposed to be 10 **m**g/L	Wehner, 1977
carcinogenicity study (intratracheal instillation)in rat (50/sex/dose) 0, 2, or 10 mg/kg bw/day 1 dose/2 wk × 18 doses, then 1 dose/4 weeks × 11 doses (up to 30th dose), then 1 dose/2 weeks × 9 doses; total 39 doses in 1,5 y	Cobalt(II) oxide	10 mg/kg bw (7.9 mg cobalt /kg bw): significant increase in incidence of bronchioalveolar adenomas/carcinomas combined in males		Steinhoff and Mohr, 1991

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

No oral carcinogenesis studies are available for cobalt.

4.10.1.2 Carcinogenicity: inhalation

In a carcinogenicity study, F344/NTac rats (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for up to 105 weeks. Survival of female rats exposed to 2.5 mg/m³ was significantly less than that of the chamber control group. Mean body weights of ≥ 2.5 mg/m³ males were at least 10% less than those of the chamber control group after weeks 99 and 12, respectively, and those of ≥ 2.5 mg/m³ 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.

Nonneoplastic effects are described under 4.7: repeated dose toxicity.

In the lung, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with positive trends in male and female rats and with the exception of the incidence of alveolar/bronchiolar adenoma in 1.25 mg/m³ females, the incidences were significantly greater than those in the chamber controls. The incidences of multiple alveolar/bronchiolar adenoma and carcinoma generally increased with increasing exposure concentration, and the incidences of multiple carcinoma were significantly increased in all exposed groups of males and in 5 mg/m³ females. The incidences of cystic keratinizing epithelioma were increased in exposed groups of female rats; cystic keratinizing epithelioma also occurred in two exposed males. One female rat exposed to 5 mg/m³ had a squamous cell carcinoma (table 58).

There was a higher frequency and different spectrum of point mutations within hot spot regions of Kras, Egfr, and Tp53 genes within alveolar/bronchiolar carcinomas from cobalt metal-exposed male

and female rats compared to spontaneous alveolar/bronchiolar carcinomas. Kras mutations and G→T transversions were most frequent in rats chronically exposed to cobalt metal.

In the adrenal medulla, incidences of benign pheochromocytoma, malignant pheochromocytoma, and benign or malignant pheochromocytoma (combined) occurred with positive trends in male and female rats, and with the exception of the incidence of malignant pheochromocytoma in 2.5 mg/m³ females, the incidences in rats exposed to 2.5 or 5 mg/m³ were significantly greater than those in the chamber controls. The incidences of bilateral benign and malignant pheochromocytoma were significantly increased in the 5 mg/m³ groups (table 59).

Pancreatic Islets: The incidences of carcinoma and adenoma or carcinoma (combined) occurred with positive trends in male rats and the incidences of adenoma, carcinoma, and adenoma or carcinoma (combined) generally exceeded the historical control ranges for all routes of administration (table 57). The incidences of adenoma in 2.5 mg/m^3 males and of adenoma or carcinoma (combined) in males exposed to 2.5 or 5 mg/m3 were significantly greater than those in the chamber controls. Incidences of adenoma, carcinoma, and adenoma or carcinoma (combined) in 5 mg/m^3 females were slightly increased; the increases were not statistically significant but did exceed the historical control ranges for all routes of administration (table 60).

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia were significantly increased in all exposed groups of female rats and exceeded the historical control range for all routes of administration (table 61)

Kidney: In the standard evaluation of the kidney, the incidences of renal tubule adenoma, carcinoma, and adenoma or carcinoma (combined) were slightly increased in male rats exposed to 5 mg/m^3 (table 62). Although not statistically significant, the incidences in this group exceeded the historical control ranges for all routes of administration (table 62). In the standard evaluation, a single section of each kidney is routinely examined microscopically. Because the incidences of renal tubule neoplasms in the standard evaluation suggested the possibility of a treatment-related carcinogenic effect, an extended evaluation of the kidney was performed in male rats to explore this possibility. For the extended evaluation, kidneys of male rats were step-sectioned at 1 mm intervals to obtain three to four additional sections from each kidney, and these sections were examined microscopically. In the extended evaluation, additional renal tubule adenomas and renal tubule hyperplasias were identified but no additional renal tubule carcinomas (table 62); a renal tubule oncocytoma was identified in one male exposed to 2.5 mg/m^3. In the combined standard and extended evaluation, the incidences of renal tubule hyperplasia in the exposed groups were similar to that in the chamber controls. The incidence of renal tubule adenoma in the 5 mg/m^3 group was greater than that in the chamber control group, but the increase was not statistically significant. The incidences of renal tubule carcinomas were unchanged (NTP, 2014).

Table 58: Incidences of neoplastic lesions of the lung in rats in the 2 year inhalation study of 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%

Historical control incidences from the NTP historical database (containing all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period) for F344/NTac rats for all routes and all vehicles are used, since the current study is the only inhalation study in F344/NTac rats in the historical control database.

Table 59: Incidences of neoplastic lesions of the adrenal medulla in rats in the 2 year inhalation study of cobalt metal.

	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%

Historical control incidences from the NTP historical database (containing all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period) for F344/NTac rats for all routes and all vehicles are used, since the current study is the only inhalation study in F344/NTac rats in the historical control database

Table 60: Incidences of neoplastic lesions of the pancreatic islets in rats in the 2 year inhalation study of 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%

Historical control incidences from the NTP historical database (containing all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period) for F344/NTac rats for all routes and all vehicles are used, since the current study is the only inhalation study in F344/NTac rats in the historical control database

Table 61: Incidences of mononuclear cell leukemia in female rats in the 2 year inhalation study of cobalt metal.

	Chamber Control	1.25 mg/m³	2.5 mg/m³	5 mg/m³
All Organs: Mononuclear Cell Leukemia[a]				
Overall rate[b]	16/50 (32%)	29/50 (58%)	28/50 (56%)	27/50 (54%)
Adjusted rate[c]	35.7%	62.4%	60.5%	58.9%
Terminal rate[d]	12/35 (34%)	15/26 (58%)	12/24 (50%)	13/25 (52%)
First incidence (days)	663	590	117	473
Poly-3 test[e]	P=0.118	P=0.007	P=0.013	P=0.019

[a] Historical control incidence for 2-year studies (all routes) (mean ± standard deviation): 35/100 (35.0% ± 4.2%), range 32%-38%
[b] Number of animals with mononuclear cell leukemia per number of animals necropsied
[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.

Table 62: Incidences of neoplastic lesions of the kidneys in male rats in the 2 year inhalation study of cobalt metal.

	Chamber Control	1.25 mg/m³	2.5 mg/m³	5 mg/m³
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.

In a carcinogenicity study, B6C3F1/N mice (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for up to 105 weeks. Survival of males exposed to 2.5 or 5 mg/m³ was significantly less than that of the chamber control group. Mean body weights of 5 mg/m³ males and females 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.

Nonneoplastic effects are described under 4.7: repeated dose toxicity.

In the lung, incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with positive trends in male and female mice, and the incidences were all significantly greater than those in the chamber controls. The incidences of alveolar/bronchiolar adenoma were significantly increased in 2.5 mg/m³ males and in 5 mg/m³ females. The incidences of multiple alveolar/bronchiolar carcinoma were significantly increased in all exposed groups of males and females (table 63).

There was a higher frequency and different spectrum of point mutations within hot spot regions of Kras, Egfr, and Tp53 genes within alveolar/bronchiolar carcinomas from cobalt metal-exposed male and female mice compared to spontaneous alveolar/bronchiolar carcinomas. Kras mutations and G→T transversions were most frequent in mice chronically exposed to cobalt metal (NTP, 2014).

Table 63: Incidences of neoplastic lesions of the lung in mice in the 2 year inhalation study of 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 a carcinogenicity study, Fischer 344 rats (50/sex/dose were exposed (whole body) to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m³ cobalt sulphate heptahydrate for 6 hours per day, 5 days per week, for 105 weeks. There was no effect on survival or body weight.

Nonneoplastic effects are described under 4.7: repeated dose toxicity (incidences can be found in the table below).

The combined incidence of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) was significantly increased in 3.0 mg/m³ males and exceeded the historical control range. Although the incidences of alveolar/bronchiolar adenoma in 3.0 mg/m³ males and alveolar/bronchiolar carcinoma in 1.0 mg/m³ males were not significantly increased, they exceeded the historical control ranges for inhalation studies. In females exposed to ≥ 1.0 mg/m³, the incidences of alveolar/bronchiolar adenomas, carcinomas and adenomas/carcinomas combined were significantly increased and exceeded the historical control ranges. Also the incidences of carcinomas and adenomas/carcinomas combined in the low dose group exceeded historical controls, although not being significantly increased compared to the control group (table 64).

In addition, the incidence of benign pheochromocytoma in 3.0 mg/m³ females was significantly increased and exceeded the historical range for inhalation studies (for incidences, see table below). The incidences of benign, complex, or malignant pheochromocytoma (combined) in 1.0 mg/m³ males and in 3.0 mg/m³ females were also significantly increased and exceeded the historical control ranges (table 65).
The incidence of hyperplasia was not significantly increased in exposed males or females. Benign pheochromocytomas were well-delineated masses often with altered architecture and variable compression of surrounding parenchyma. Neoplastic cells were arranged in variably sized aggregates, clusters, and/or variably thick trabecular cords. Larger neoplasms usually exhibited greater cellular pleomorphism and atypia than smaller neoplasms. Malignant pheochromocytomas were identified when there was invasion of or beyond the adrenal capsule or when distant metastases were observed. Although a very common spontaneous neoplasm in male F344/N rats, pheochromocytomas have a lower spontaneous occurrence in females. In this study, the incidence of pheochromocytoma in 3.0 mg/m³ females was considered related to the administration of cobalt sulphate heptahydrate. The marginally increased incidence of pheochromocytoma in males was considered an uncertain finding because it occurred only in the 1.0 mg/m³ group and was not supported by increased incidence or severity of hyperplasia (NTP, 1998).

Table 64: Incidences of neoplastic lesions of the lung in rats in the 2 year inhalation study of cobalt sulphate heptahydrate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Alveolar/bronchiolar Adenoma[c]				
Overall rate[d]	1/50 (2%)	4/50 (8%)	1/48 (2%)	6/50 (12%)
Adjusted rate[e]	2.3%	17.7%	2.4%	28.4%
Terminal rate[f]	0/17 (0%)	2/15 (13%)	0/21 (0%)	2/15 (13%)
First incidence (days)	568	589	611	638
Logistic regression test[g]	P=0.051	P=0.179	P=0.753	P=0.055
Alveolar/bronchiolar Carcinoma[h]				
Overall rate	0/50 (0%)	0/50 (0%)	3/48 (6%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	11.3%	6.7%
Terminal rate	0/17 (0%)	0/15 (0%)	1/21 (5%)	1/15 (7%)
First incidence (days)	—[i]	—	652	734 (T)
Logistic regression test	P=0.360	—	P=0.136	P=0.475
Alveolar/bronchiolar Adenoma or Carcinoma[j]				
Overall rate	1/50 (2%)	4/50 (8%)	4/48 (8%)	7/50 (14%)
Adjusted rate	2.3%	17.7%	13.4%	33.9%
Terminal rate	0/17 (0%)	2/15 (13%)	1/21 (5%)	3/15 (20%)
First incidence (days)	568	589	611	638
Logistic regression test	P=0.032	P=0.179	P=0.163	P=0.029
Female				

Alveolar/bronchiolar Adenoma[k]				
Overall rate	0/50 (0%)	1/49 (2%)	10/50 (20%)	9/50 (18%)
Adjusted rate	0.0%	3.4%	36.4%	30.0%
Terminal rate	0/28 (0%)	0/25 (0%)	9/26 (35%)	9/30 (30%)
First incidence (days)	—	714	692	735 (T)
Logistic regression test	P=0.001	P=0.480	P < 0.001	P=0.003
Alveolar/bronchiolar Carcinoma[l]				
Overall rate	0/50 (0%)	2/49 (4%)	6/50 (12%)	6/50 (12%)
Adjusted rate	0.0%	8.0%	20.2%	17.5%
Terminal rate	0/28 (0%)	2/25 (8%)	4/26 (15%)	4/30 (13%)
First incidence (days)	—	735 (T)	694	610
Logistic regression test	P=0.023	P=0.213	P=0.015	P=0.017
Alveolar/bronchiolar Adenoma or Carcinoma[m]				
Overall rate	0/50 (0%)	3/49 (6%)	15/50 (30%)	15/50 (30%)
Adjusted rate	0.0%	11.2%	50.6%	46.1%
Terminal rate	0/28 (0%)	2/25 (8%)	12/26 (46%)	13/30 (43%)
First incidence (days)	—	714	692	610
Logistic regression test	P < 0.001	P=0.096	P < 0.001	P < 0.001
Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/49 (0%)	1/50 (2%)	1/50 (2%)
Alveolar/bronchiolar Adenoma, Alveolar/bronchiolar Carcinoma, or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	3/49 (6%)	16/50 (32%)	16/50 (32%)
Adjusted rate	0.0%	11.2%	54.1%	49.2%
Terminal rate	0/28 (0%)	2/25 (8%)	13/26 (50%)	14/30 (47%)
First incidence (days)	—	714	692	610
Logistic regression test	P < 0.001	P=0.096	P < 0.001	P < 0.001

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[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 incidence for 2-year inhalation studies with chamber controls (mean ± standard deviation): 17/654 (2.6% ± 3.6%); range 0%-10%
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal.
[h] Historical incidence: 6/654 (0.9% ± 1.0%); range 0%-2%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 23/654 (3.5% ± 3.7%); range 0%-10%
[k] Historical incidence: 7/650 (1.1% ± 1.6%); range 0%-4%
[l] Historical incidence: 0/650
[m] Historical incidence: 7/650 (1.1% ± 1.6%); range 0%-4%

Neoplasm incidences from the NTP historical control database, which is updated yearly, are used as historical control

Table 65: Incidences of neoplastic lesions of the adrenal medulla in rats in the 2 year inhalation study of cobalt sulphate heptahydrate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Benign Bilateral Pheochromocytoma				
Overall rate	1/50 (2%)	4/50 (8%)	6/49 (12%)	5/50 (10%)
Benign Pheochromocytoma (includes benign bilateral pheochromocytoma)[c]				
Overall rate[d]	14/50 (28%)	19/50 (38%)	23/49 (47%)	20/50 (40%)
Adjusted rate[e]	51.0%	70.0%	71.9%	71.4%
Terminal rate[f]	6/17 (35%)	8/15 (53%)	13/21 (62%)	8/15 (53%)
First incidence (days)	534	541	526	526
Logistic regression test[g]	P=0.172	P=0.226	P=0.069	P=0.126
Benign, Complex, or Malignant Pheochromocytoma (includes benign bilateral pheochromocytoma)[h]				
Overall rate	15/50 (30%)	19/50 (38%)	25/49 (51%)	20/50 (40%)
Adjusted rate	52.1%	70.0%	74.1%	71.4%
Terminal rate	6/17 (35%)	8/15 (53%)	13/21 (62%)	8/15 (53%)
First incidence (days)	534	541	526	526
Logistic regression test	P=0.218	P=0.295	P=0.045	P=0.180
Female				
Benign Pheochromocytoma[i]				
Overall rate	2/48 (4%)	1/49 (2%)	3/50 (6%)	8/48 (17%)
Adjusted rate	5.1%	3.1%	9.3%	26.4%
Terminal rate	0/27 (0%)	0/25 (0%)	1/26 (4%)	7/29 (24%)
First incidence (days)	666	702	694	709
Logistic regression test	P=0.004	P=0.498N	P=0.512	P=0.043
Benign, Complex, or Malignant Pheochromocytoma[j]				
Overall rate	2/48 (4%)	1/49 (2%)	4/50 (8%)	10/48 (21%)
Adjusted rate	5.1%	3.1%	11.7%	31.5%
Terminal rate	0/27 (0%)	0/25 (0%)	1/26 (4%)	8/29 (28%)
First incidence (days)	666	702	685	663
Logistic regression test	P < 0.001	P=0.498N	P=0.323	P=0.014

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
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 incidence for 2-year inhalation studies with chamber controls (mean ± standard deviation): 163/623 (26.2% ± 13.2%); range 0%-50%
d Number of animals with neoplasm per number of animals with adrenal medulla examined microscopically
e Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
f Observed incidence in animals surviving until the end of the study
g In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by **N**.
h Historical incidence: 176/623 (28.3% ± 12.0%); range 8%-50%
i Historical incidence: 35/608 (5.8% ± 4.9%); range 0%-14%
j Historical incidence: 39/608 (6.4% ± 4.4%); range 2%-14%

Neoplasm incidences from the NTP historical control database, which is updated yearly, are used as historical control

In a second carcinogenicity study, B6C3F1 mice (50/sex/dose were exposed (whole body) to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m³ cobalt sulphate heptahydrate for 6 hours per day, 5 days per week, for 105 weeks. There was no effect on survival. Mean body weights of 3.0 mg/m³ male mice were less than those of controls from week 96 until the end of the study. The mean body weights of all exposed female mice were generally greater than those of controls from week 20 until the end of the study.

Nonneoplastic effects are described under 4.7: repeated dose toxicity (incidences can be found in the table below).

The incidences of alveolar/bronchiolar neoplasms (adenoma and/or carcinoma) in 3.0 mg/m³ males and females and the combined incidence of alveolar/ bronchiolar neoplasms in 1.0 mg/m³ females were significantly greater than those in the chamber control groups and generally exceeded the historical control ranges for inhalation studies (table 66). Although similar in appearance to "spontaneous" lung neoplasms in chamber controls, alveolar/ bronchiolar neoplasms in mice exposed to cobalt sulphate heptahydrate had different molecular lesions in the Kras gene. Of the K-ras mutations detected at the second base of codon 12, a higher frequency (5/9, 55%) of G to T transversions was detected compared to concurrent (0/1) and historical control lung neoplasms (1/24, 4%). K-ras codon 61 CTA or CGA mutations were not present in cobalt sulphate heptahydrate-induced lung neoplasms.

The incidences of hemangiosarcoma in all exposed groups of male mice and in 1.0 mg/m³ in female mice exceeded the range observed in historical controls for inhalation studies. In addition, the incidence of hemangiosarcoma in 1.0 mg/m³ males was significantly greater than in controls (table 67). Hemangiosarcomas were morphologically similar to those observed spontaneously and consisted of multiple variably sized blood-filled spaces that were separated by cords of hepatocytes and lined by plump endothelial cells (NTP, 1998).

Table 66: Incidences of neoplastic lesions of the lung in mice in the 2 year inhalation study of cobalt sulphate heptahydrate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Alveolar/bronchiolar Adenoma[c]				
Overall rate[d]	9/50 (18%)	12/50 (24%)	13/50 (26%)	18/50 (36%)
Adjusted rate[e]	30.4%	30.9%	41.1%	54.6%
Terminal rate[f]	4/22 (18%)	6/31 (19%)	7/24 (29%)	7/20 (35%)
First incidence (days)	600	460	548	524
Logistic regression test[g]	P=0.018	P=0.353	P=0.256	P=0.027
Alveolar/bronchiolar Carcinoma[h]				
Overall rate	4/50 (8%)	5/50 (10%)	7/50 (14%)	11/50 (22%)
Adjusted rate	13.2%	16.1%	25.3%	43.7%
Terminal rate	2/22 (9%)	5/31 (16%)	4/24 (17%)	7/20 (35%)
First incidence (days)	449	733 (T)	687	552
Logistic regression test	P=0.006	P=0.528	P=0.273	P=0.033
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	11/50 (22%)	14/50 (28%)	19/50 (38%)	28/50 (56%)
Adjusted rate	35.5%	36.5%	56.5%	78.8%
Terminal rate	5/22 (23%)	8/31 (26%)	10/24 (42%)	13/20 (65%)
First incidence (days)	449	460	548	524
Logistic regression test	P < 0.001	P=0.345	P=0.071	P < 0.001
Female				

Alveolar/bronchiolar Adenoma[j]				
Overall rate	3/50 (6%)	6/50 (12%)	9/50 (18%)	10/50 (20%)
Adjusted rate	8.8%	15.0%	25.2%	32.8%
Terminal rate	3/34 (9%)	4/37 (11%)	6/32 (19%)	8/28 (29%)
First incidence (days)	734 (T)	664	649	706
Logistic regression test	P=0.024	P=0.287	P=0.057	P=0.024
Alveolar/bronchiolar Carcinoma[k]				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	9/50 (18%)
Adjusted rate	2.9%	2.7%	9.2%	25.3%
Terminal rate	1/34 (3%)	1/37 (3%)	1/32 (3%)	4/28 (14%)
First incidence (days)	734 (T)	734 (T)	495	536
Logistic regression test	P<0.001	P=0.743N	P=0.201	P=0.009
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	4/50 (8%)	7/50 (14%)	13/50 (26%)	18/50 (36%)
Adjusted rate	11.8%	17.5%	32.6%	50.2%
Terminal rate	4/34 (12%)	5/37 (14%)	7/32 (22%)	11/28 (39%)
First incidence (days)	734 (T)	664	495	536
Logistic regression test	P<0.001	P=0.318	P=0.016	P<0.001

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[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 incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 141/947 (14.9% ± 7.0%); range 6%-36%
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by **N**.
[h] Historical incidence: 75/947 (7.9% ± 5.7%); range 0%-16%
[i] Historical incidence: 205/947 (21.7% ± 8.0%); range 10%-42%
[j] Historical incidence: 61/939 (6.5% ± 3.2%); range 0%-14%
[k] Historical incidence: 38/939 (4.1% ± 3.2%); range 0%-12%
[l] Historical incidence: 97/939 (10.3% ± 3.7%); range 0%-16%

Neoplasm incidences from the NTP historical control database, which is updated yearly, are used as historical control

Table 67: Incidences of neoplastic lesions of the liver in mice in the 2 year inhalation study of cobalt sulphate heptahydrate

	Chamber Control	0.3 mg/m³	1.0 mg/m³	3.0 mg/m³
Male				
Hemangiosarcoma[c]				
Overall rate[d]	2/50 (4%)	4/50 (8%)	8/50 (16%)	7/50 (14%)
Adjusted rate[e]	9.1%	11.5%	23.5%	25.0%
Terminal rate[f]	2/22 (9%)	2/31 (6%)	2/24 (8%)	3/20 (15%)
First incidence (days)	733 (T)	685	523	502
Logistic regression test[g]	P=0.078	P=0.441	P=0.050	P=0.069

Female				
Hemangiosarcoma[h]				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/49 (0%)
Adjusted rate	2.9%	0.0%	7.3%	0.0%
Terminal rate	1/34 (3%)	0/37 (0%)	1/32 (3%)	0/28 (0%)
First incidence (days)	734 (T)	—[i]	524	—
Logistic regression test	P=0.431N	P=0.483N	P=0.318	P=0.539N

* Significantly different (P≤0.05) from the chamber control by the logistic regression test
(T)Terminal sacrifice
[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 incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 12/947 (1.3% ± 1.7%); range 0%-6%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the chamber control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.
[h] Historical incidence: 5/937 (0.5% ± 1.0%); range 0%-3%
[i] Not applicable; no neoplasms in animal group

Neoplasm incidences from the NTP historical control database, which is updated yearly, are used as historical control

Cobalt(II) oxide

No carcinogenicity was observed after exposure of Syrian hamsters to CoO aerosol (10 g/L, 7 hrs/day, 5 days/week). This stated exposure level is considered unrealistically high and probably was 10 **m**g/L. Exposure did cause pneumoconiosis, which was evidenced by a variety of lesions including, e.g., interstitial pneumonitis, diffuse granulomatous pneumonia, fibrosis of alveolar septa, and bronchial and bronchiolar epithelial (basal cell) hyperplasia. Survival was poor, but not different between test and control group (Wehner *et al.* 1977).

Sprague Dawley rats (50/sex/dose) were intratracheally instilled with 0, 2 or 10 mg cobalt(II) oxide /kg bw (1 dose/2 wk × 18 doses, then 1 dose/4 weeks × 11 doses (up to 30th dose), then 1 dose/2 weeks × 9 doses; total 39 doses). Significant increases in lung neoplasms (alveolar/bronchiolar adenoma, benign squamous epithelial neoplasm, or alveolar/bronchiolar carcinoma combined) were observed in male rats. Non-significant increases in lung neoplasms (alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma) were seen in females. There were significant increases in alveolar/bronchiolar proliferation (types of lesions not described) in both sexes combined (table 68) (Steinhoff and Mohr 1991).

Table 68: Incidences of lung neoplasms in rats exposed to cobalt(II)oxide

	0 mg/kg bw	**2 mg/kg bw**	**10 mg/kg bw**
Male			
Bronchio/alveolar adenoma	0/50 (0%)	0/50 (0%)	2/50 (4%)
Bronchio/alveolar carcinoma	0/50 (0%)	0/50 (0%)	3/50 (6%)
Bronchioalveolar adenomas/carcinomas combined	0/50 (0%)	0/50 (0%)	5/50 (10%)*

Benign squamous epithelial tumor	0/50 (0%)	1/50 (2%)	0/50 (0%)
Female			
Bronchio/alveolar adenoma	0/50 (0%)	1/50 (2%)	0/50 (0%)
Bronchio/alveolar carcinoma	0/50 (0%)	0/50 (0%)	1/50 (2%)
Bronchioalveolar adenomas/carcinomas combined	0/50 (0%)	1/50 (2%)	1/50 (2%)

4.10.1.3 Carcinogenicity: dermal

4.10.2 Human information

The mortality between 1950 and 1980 of a cohort of 1143 workers in an electrochemical plant producing cobalt and sodium was investigated (Mur, J.M. *et al.*, 1987). The overall death rate was slightly but not significantly higher, than the national rate: SMR for cobalt production workers was 1.29. Mortality from malignant tumors was reported to be increased (SMR = 1.65), especially from lung cancer (SMR 4.66; $p < 0.05$; 4 cases). The relationship between cobalt production and lung cancer mortality was supported by a case-control study nested in the cohort study. Among cases (deaths from lung cancer) there were 44% of workers who had been ever employed at the cobalt production (all for more than 10 years), there were only 17% among the controls. However, the difference was not statistically significant. The authenticity of the occupational origin of this risk could not be established due to the low number of cases and because the role of smoking and of simultaneous exposure to arsenic and nickel could not be taken into account.

This investigation was followed by a follow-up study with the workers of the same electrochemical plant extending from 1981 - 1988 (Moulin, J.J. *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. With regard to 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. Any excess of mortality from diseases of the circulatory and of the respiratory systems did not appear among Cobalt production workers. Maintenance workers, however, exhibited an elevated SMR for lung cancer (1.80, 95% CI 0.78-3.55), reaching statistical significance for duration of exposure and time since first exposure ≥30 years (asbestos exposure may have been occurred).

Lasfargues *et al.* (1994) reported on the mortality of a cohort of 709 male workers in a French hard metal plant, using the national rates for French males for comparison. The overall mortality did not differ from expected, but there was a significant increase in mortality due to cancer of the trachea, bronchus, and lung (SMR=2.13, 95% CI=1.02–3.93). Smoking alone did not account for the lung cancer excesses, although the influence of smoking on the observed mortality could not be entirely ruled out (ATSDR 2004).

(Information below is copied from NTP 2016a)

Two publications reported on an overlapping population of hard-metal workers. The first was a historical mortality cohort and nested case-control study of lung cancer among 7,459 workers at 10 hard-metal producing factories in France (Moulin *et al.* 1998) where activities also included powder metallurgy processes. The second was a sub-study of lung cancer among 2,860 workers in the largest hard-metal producing factory in France (the factory was included in the Moulin *et al.* [1998] study, with an additional year of follow-up included) which also produced magnets and stainless steel with cobalt, and cobalt powders by calcination and reduction of cobalt hydroxide (Wild *et al.* 2000). In the internal nested case-control analysis (Moulin *et al.* 1998), based on 15 exposed cases, a borderline statistically significant increased risk of lung cancer was associated with exposure (levels 2 to 9) to "cobalt alone or simultaneously with agents other than tungsten carbide" compared with little or no exposure (levels 0 or 1) (OR = 2.21, 95% CI = 0.99 to 4.90). Regarding the presence of an exposure-response relationship, Moulin *et al.* reported two-fold elevated trend tests (although not reaching statistical significance) based on 15 cases across levels of exposure (OR = 2.05, 95% CI = 0.94 to 4.45), levels of duration (2.20, 95% CI = 0.99 to 4.87), cumulative weighted (1.83, 95% CI = 0.86 to 3.91), and cumulative un-weighted doses (2.03, 95% CI = 0.94 to 4.39). Numbers of cases and category-specific OR estimates for levels or categories of duration or cumulative dose were not provided. Wild *et al.* (2000) added years of follow-up to the cohort from the largest factory included in the multi-center study and found a statistically significant elevated SMR of lung cancer among those exposed to "cobalt except in hard metals" based on the JEM (SMR = 1.95, 95% CI = 1.09 to 3.22). Wild *et al.*, however, did not provide information on exposure-response relationships; and neither study provided an examination of latency.

In a historical cohort and nested case-control study of stainless and alloyed steel workers and lung cancer conducted in one factory in France (N = 4,897), no association between cobalt exposure and lung cancer was found in this study (Moulin *et al.* 2000a).

Two case-control studies (O'Rorke *et al.* 2012, Rogers *et al.* 1993) compared cobalt in toenails of cases of esophageal cancer and population-based controls. Rogers *et al.* (1993) reported elevated odds ratio for esophageal cancer for those with the highest levels (≥ 0.17 ppm) of cobalt concentration in toenails compared to those with the lowest level (< 0.05 ppm) of cobalt (OR = 9.0, 95% CI = 2.7 to 30.0). The OR was elevated but not significant for those with medium levels (0.05 to 0.17 ppm) of cobalt concentration compared to those with low levels (OR = 2.4, 95% CI = 0.8 to 7.2). The exposure-response test for trend was significant ($P < 0.001$).

O'Rorke *et al.* (2012) reported a non-significant elevated risk of esophageal adenocarcinoma among those with the highest cobalt levels (OR = 1.54, 95% CI = 0.84 to 2.85). In addition, they reported a significantly increased risk of Barrett's esophagus among participants with higher toenail concentrations of cobalt (≥ -4.4705, log transformed values equivalent to ≥ 0.011 μg/g) (OR = 1.97, 95% CI = 1.01 to 3.85), with a significant ($P = 0.05$) linear test for trend. Both of the estimates were adjusted for age, sex, smoking, location (Northern Ireland or Republic), energy intake, gastro-esophageal reflux, and *H. pylori* infection. O'Rorke *et al.* reported no information regarding the correlation of dietary intake of cobalt and nail concentration. In this study, a 2-fold risk of Barrett's esophagus was also associated with higher toenail concentrations of zinc.

4.10.3 Other relevant information

Several soluble cobalt compounds including cobalt chloride and cobalt sulphate have a harmonised classification as Carc. 1B including a specific concentration limit of 0.01%. This SCL was based on potency calculations performed by Norway which are not available to us. The method for deriving

SCL for carcinogenicity are described in EC: Guidelines for setting specific concentration limits for carcinogens in Annex I of directive 67/548/EEC. Inclusion of potency considerations. Commission working group on the classification and labelling of dangerous substances. Office for the Official Publications of the European Communities, Luxembourg, ISBN 92-828-7443-5, 1999.

From the inhalation studies with cobalt, a SCL can be derived according to the T25 method described in this guideline. The lowest dose with increased tumour incidence is 1.25 mg cobalt/m^3. The highest net tumour increase at this dose is 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%). Correction factors have to be applied for dosing at 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/day (=high potency). For high potency carcinogens classified in Carc 1B, an SCL of 0.01% should be applied.

NTP has recently published a monograph on the carcinogenesis of cobalt and cobalt compounds (NTP, 2016a). The listing recommendation was that "Cobalt and cobalt compounds that release cobalt ions *in vivo*" are reasonably anticipated to be human carcinogens based on sufficient evidence from studies in experimental animals and supporting mechanistic data. Mechanistic data indicate that the release of cobalt ions in vivo (whether from soluble or poorly water-soluble compounds and particles) is a key event for cobalt-induced carcinogenicity. Indeed, "Cobalt and cobalt compounds that release cobalt ions *in vivo*" have been included in the 14th Report of Carcinogens (RoC) of the NTP (NTP 2016b). In the NTP monograph, several possible mechanisms for the induction of tumours are described. Relevant mechanistic data have been summarised below.

Similar cytotoxic, genotoxic, and carcinogenic effects have been described for soluble and particulate forms of cobalt. Consistent with other metal compounds, three modes of action are proposed for the carcinogenic effects of cobalt: 1) genotoxicity and inhibition of DNA repair, 2) induction of reactive oxygen species (ROS) and oxidative stress, and 3) induction of hypoxia-like responses by activating hypoxia-inducible factor 1 (HIF-1). The three possibilities are discussed below.

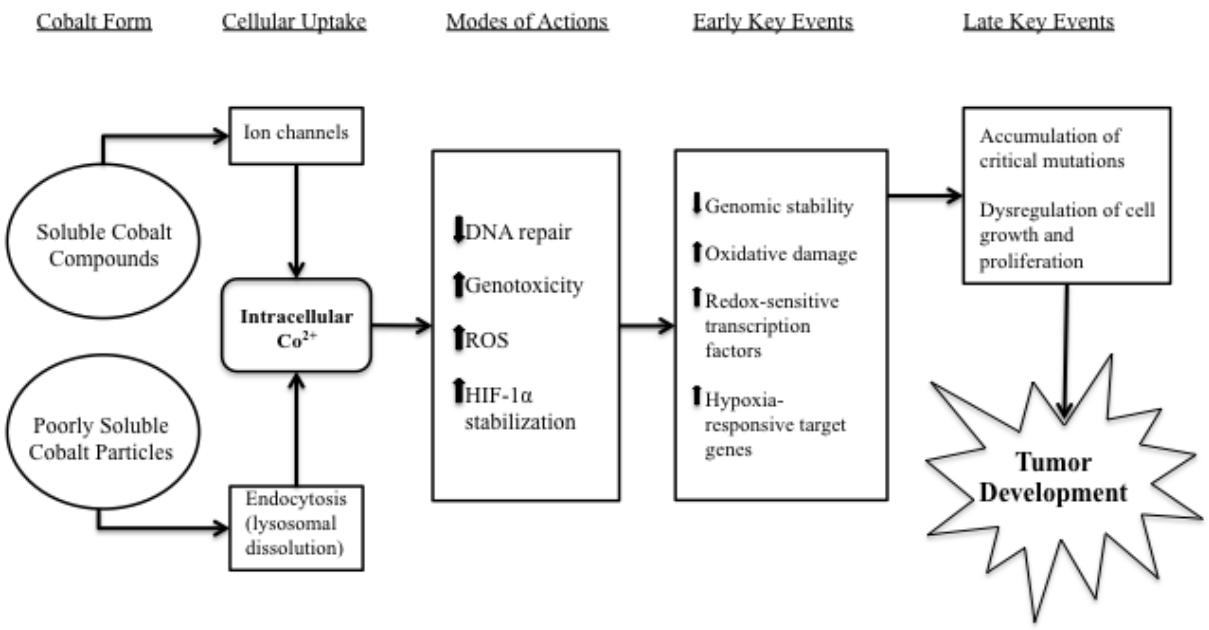

Figure 1: Proposed modes-of-action of cobalt carcinogenicity NTP (2015)

1) genotoxicity and inhibition of DNA repair

Genotoxicity assays with cobalt salts and cobalt metal demonstrate a mutagenic potential (*see 4.9 Germ cell mutagenicity*) and at least two molecular mechanisms seem to apply: (1) a direct effect of cobalt(II) ions to induce oxidative damage to DNA through a Fenton-like mechanism, and (2) an indirect effect of cobalt(II) ions through inhibition of repair of DNA damage caused by endogenous events or induced by other agents.

Possible mechanisms include substitution of cobalt ions for zinc ions resulting in proteins with modified catalytic activity (e.g., p53 tumor suppressor protein and zinc finger domains of DNA repair proteins) or substitution of cobalt for magnesium in DNA polymerases or topoisomerases (Beyersmann and Hartwig 2008, Witkiewicz-Kucharczyk and Bal 2006, Baldwin *et al.* 2004, Kopera *et al.* 2004, Asmuss *et al.* 2000, Hartwig 1998, Kasten *et al.* 1997, Hartwig *et al.* 1991). The DNA binding capacity of p53 protein can be modulated by cobalt(II) ions (Adámik *et al.* 2015, Lee *et al.* 2001, Méplan *et al.* 2000, Palecek *et al.* 1999). In addition to cell cycle arrest and apoptosis, p53 and its downstream genes also regulate DNA excision repair pathways, including repair of oxidative damage (Smith and Seo 2002). Kasten *et al.* (1997) reported that non-cytotoxic doses of cobalt enhanced DNA damage caused by ultraviolet radiation in human fibroblasts by inhibiting both the incision and polymerization steps of nucleotide excision repair. Kopera *et al.* (2004) and Asmuss *et al.* (2000) showed that cobalt reduced the DNA-binding ability of xeroderma pigmentosum group A (XPA) protein (a zinc finger protein involved in nucleotide excision repair). Further, poly(ADP-ribose)polymerase (PARP), a DNA strand break repair protein also was inhibited by cobalt (Hartwig *et al.* 2002). The co-mutagenic effects of cobalt observed in vitro are consistent with one study by Steinhoff and Mohr (1991) that reported co-carcinogenic effects of cobalt oxide and benzo[a]pyrene for squamous-cell carcinoma of the lung.

2) oxidative stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) induce oxidative and nitrative stress and are recognized as key contributors to carcinogenesis (Mates *et al.* 2010). In addition to generating DNA damage, ROS also activate redox-sensitive transcription factors (e.g., NF-κB, AP1, p53) (Beyersmann and Hartwig 2008, Valko *et al.* 2006, Valko *et al.* 2005). These transcription factors have been linked to carcinogenesis because of their role in regulating DNA repair, inflammation, cell proliferation, differentiation, angiogenesis, and apoptosis. Thus, depending on the dose and the extent and timing of interference, ROS may initiate tumor development by mutagenesis and/or promote tumor growth by dysregulation of cell growth and proliferation.

Both cobalt ions and cobalt metal can catalyze the formation of ROS in vivo and in vitro (Chattopadhyay *et al.* 2015, Annangi *et al.* 2014, Scharf *et al.* 2014, Alarifi *et al.* 2013, Patel *et al.* 2012, Papis *et al.* 2009, Qiao *et al.* 2009, Kotake-Nara and Saida 2007, Limbach *et al.* 2007, Peters *et al.* 2007, Dick *et al.* 2003, Pourahmad *et al.* 2003, Zou *et al.* 2001, Kawanishi *et al.* 1994, Hanna *et al.* 1992, Lewis *et al.* 1992, 1991, Kadiiska *et al.* 1989, Kawanishi *et al.* 1989, Moorhouse *et al.* 1985). Direct interactions between cobalt metal or ions and oxygen or lipids can generate ROS. High concentrations (10 mg/mL) of aqueous suspensions of Co(0) metal particles can react with dissolved oxygen to generate hydrogen peroxide and hydroxyl radicals in the presence of superoxide dismutase (SOD) (Lee *et al.* 2012, Jomova and Valko 2011, Leonard *et al.* 1998). The hydroxyl radical was not generated when catalase, a hydrogen peroxide scavenger, was added.

Cobalt(II) ions alone did not generate significant amounts of hydroxyl radicals from hydrogen peroxide except when bound to certain endogenous chelators such as glutathione and anserine (Leonard *et al.* 1998, Mao *et al.* 1996, Shi *et al.* 1993). Glutathione and anserine normally function as antioxidants; however, these data suggest that a cobalt(II)-mediated switch to pro-oxidants may occur and cause cellular damage (Valko *et al.* 2005). Cobalt(II) ions also are capable of reacting with lipid hydroperoxides to generate free radicals in the presence of proper chelating agents (Shi *et al.* 1993). Hydroxyl radicals and lipid hydroperoxide-derived free radicals are considered important intermediates in oxidative stress-induced genetic damage and as mediators of tumor initiation and promotion (Barrera 2012, Shi *et al.* 1993, Vaca *et al.* 1988). Thus, under certain conditions, both cobalt metal and cobalt ions are capable of generating ROS through Fenton-like reactions with the potential to increase oxidative stress and cellular injury through DNA damage, protein modification, induction of oncogene expression, and nuclear transcription factor activation.

3) induction of hypoxia-like responses

HIF-1 is a heterodimer composed of HIF-1α and HIF-1β subunits and is the key mediator of hypoxia response (Davidson *et al.* 2015, Galanis *et al.* 2008, Salnikow *et al.* 2004). There is strong experimental support that HIF-1 activation is involved in cobalt-induced carcinogenesis. Cobalt metal particles, cobalt chloride, and cobalt sulphate heptahydrate promote a hypoxia-like state *in vivo* and *in vitro*, even with normal molecular oxygen pressure, by stabilizing HIF-1α (Nyga *et al.* 2015, Galán-Cobo *et al.* 2013, Gao *et al.* 2013, Saini *et al.* 2010b, Saini *et al.* 2010a, Galanis *et al.* 2009, Qiao *et al.* 2009, Xia *et al* 2009, Beyersmann and Hartwig 2008, Maxwell and Salnikow 2004). This has been demonstrated in several human cell lines, including cancer cell lines (Fu *et al.* 2009, Ardyanto *et al.* 2008, Wang and Semenza 1995). Further, Wang and Semenza (1995) demonstrated that HIF-1 induction either from hypoxia or cobalt chloride treatment was indistinguishable with respect to DNA binding specificity and contacts with target DNA sequences. Possible mechanisms by which cobalt ions activate HIF-1 include replacing iron in the regulatory oxygenases or depleting intracellular ascorbate (a cofactor for prolyl hydroxylase activity), thus, deactivating these enzymes (Davidson *et al.* 2015, Qiao *et al.* 2009, Maxwell and Salnikow 2004, Salnikow *et al.* 2004). Oxidative stress has also been investigated as a possible mechanism of cobalt-induced HIF activation; however, Salnikow *et al.* (2000) showed that activation of HIF-1-dependent genes was independent from ROS formation. Nyga *et al.* (2015) also reported evidence that HIF-1α stabilization in human macrophages treated with cobalt metal nanoparticles or cobalt ions occurred via an ROS-independent pathway.
The evidence suggests that HIF-1α is a major regulator of the adaptation of cancer cells to hypoxia and may contribute to tumor 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 (Greim *et al.* 2009, Ardyanto *et al.* 2008, Hammond and Giaccia 2005, Maxwell and Salnikow 2004, Lee *et al.* 2001). HIF-1α overexpression, stabilization and transcriptional activation is found in more than 70% of human cancers (e.g., breast, ovarian, cervical, prostate, brain, lung, head and neck) and is associated with poor clinical outcomes (Cheng *et al.* 2013, Galanis *et al.* 2009, Galanis *et al.* 2008, Maxwell and Salnikow 2004, Paul *et al.* 2004). Greim *et al.* (2009) also identified hypoxia and HIF activation as a relevant mechanism for pheochromocytomas in rats. Further evidence for a role of HIF-1 in cancer is as follows: (1) enhanced glycolytic and angiogenic activities are hallmarks of many tumors and are consequences of HIF-1 activation, (2) immunolabelling for HIF-1α subunits confirms there is a common activation in solid tumors, (3) genetic studies comparing tumor growth with and without HIF-1 have generally shown that tumors without specific HIF subunits have decreased vascularization and growth, (4) a number of pathways implicated in cancer progression increase activation of the HIF-1 pathway in normoxia and hypoxia, and (5) the VHL tumor suppressor protein is required to regulate HIF-1 (Maxwell and Salnikow 2004). VHL loss of

function results in constitutive HIF activation and an increased risk of developing cancer (summarized from NTP 2016a).

4.10.4 Summary and discussion of carcinogenicity

Two carcinogenicity studies are available for cobalt, one in rat and one in mice. In addition, two carcinogenicity studies are available for cobalt sulphate heptahydrate (in rat and mice) and two for cobalt oxide (in rat and hamster). All studies are inhalation studies (except for the study with cobalt oxide in rats, in which cobalt is administered via intratracheal instillation).

Inhalation exposure of cobalt metal in rats and mice resulted in an increased incidence of alveolar/bronchiolar adenomas and carcinomas, in males as well as females. The incidence of carcinomas and adenomas/carcinomas combined was significantly increased in all dose groups in rats and mice (i.e. ≥1.25 mg/m^3). The incidence of adenomas was significantly increased at doses of ≥1.25, 2.5, 2.5 and 5 mg/m^3 in male and female rats and male and female mice, respectively (see table below). These incidences were dose related and exceeded the historical control ranges for these tumours in all cases. In both rats and mice, lesions were also observed in the alveolar, bronchiolar and nose epithelia after exposure to cobalt metal (in both subchronic and chronic studies). This included inflammation, alveolar epithelium hyperplasia, histiocytic cellular infiltration of the alveolus, cytoplasmic vacuolization of bronchiolar epithelium, necrosis of the bronchiolar epithelium, and interstitial fibrosis of the alveolar epithelium. Increased incidences of lesions of the nose occurred in exposed male and female rats and included olfactory epithelium necrosis, olfactory epithelium atrophy, respiratory epithelium necrosis, and respiratory epithelium squamous metaplasia. Depending on study duration and effect, effects were observed starting at doses as low as 0.625 mg/m^3.

In addition, several cystic keratinizing epitheliomas were observed in rats exposed to cobalt: 2 in males and 7 in females (not dose related). None were observed in controls, as could be expected for this rare (chemical induced) tumour type.

Table 69: Tumour incidence rates in rat and mouse bioassays after inhalation exposure to cobalt metal.

	Dose (mg/m³)				
RAT 2-year study	**0**	**1.25**	**2.5**	**5**	HC*
Lung					
♂ alveolar/bronchiolar					
adenoma	5.0%	**24.1%**	**23.3%**	**32.5%**	4-6%
carcinoma	0%	**38.2%**	**76.8%**	**80.6%**	0%
combined	5.0%	**58%**	**84.6%**	**93.6%**	4-6%
cystic keratinizing epithelioma	0	1	0	1	0%
♀ alveolar/bronchiolar					
adenoma	4.5%	16.2%	**22.1%**	**30.9%**	0-4%
carcinoma	0%	**21.3%**	**42.0%**	**69.2%**	0%
combined	4.5%	**34.7%**	**48.5%**	**86.2%**	0-4%
cystic keratinizing epithelioma	0	4	1	2	0%
Adrenal medulla					
♂ pheochromocytoma					
benign	35.8%	54.3%	**81.2%**	**76.4%**	20-30%
malignant	5.0%	5.0%	**21.4%**	**39.1%**	0-4%
combined	40.2%	54.3%	**82.7%**	**90.7%**	20-34%

♀ pheochromocytoma					
benign	13.6%	27.2%	**52.1%**	**80.6%**	2-12%
malignant	0%	4.7%	**7.5%**	**27.0%**	0-2%
combined	13.6%	29.4%	**54.5%**	**89.4%**	4-12%
Pancreatic islets					
♂ adenoma	0%	2.5%	**15.1%**	7.7%	0%
carcinoma	5.0%	2.5%	12.6%	15.1%	0-4%
combined	5.0%	4.9%	**24.7%**	**22.6%**	0-4%
♀ adenoma	0%	0%	0%	2.5%	0-2%
carcinoma	2.2%	0%	0%	7.2%	0-2%
combined	2.2%	0%	0%	7.2%	2%
Blood					
♀ mononuclear cell leukemia	35.7%	**62.4%**	**60.5%**	**58.9%**	32-38%
MOUSE 2 year study	**0**	**1.25**	**2.5**	**5**	HC*
Lung					
♂ alveolar/bronchiolar					
adenoma	14.7%	24.5%	**35.9%**	**7.3%**	2-26%
carcinoma	22.8%	**79.4%**	**87.6%**	**93.8%**	4-24%
combined	33.0%	**85.0%**	**89.7%**	**95.9%**	16-40%
♀ alveolar/bronchiolar					
adenoma	6.9%	19.9%	18.9%	**24.5%**	0-12%
carcinoma	11.3%	**53.8%**	**78.9%**	**87.7%**	0-14%
combined	18.0%	**63.7%**	**84.6%**	**91.6%**	2-22%

Values in **bold**: statistically significantly different from control
* Historical control values for 2-year studies (all routes)

In rats, tumours were also observed in the adrenal medulla. The incidence in benign, malignant and combined pheochromocytomas was significantly increased at doses ≥2.5 mg/m^3, in both sexes. It is noted that hyperplasia of the adrenal medulla was also observed in the carcinogenicity study, at ≥ 2.5 and ≥ 1.25 mg/m^3 in males and females, respectively. In males, the incidence in adenomas and adenomas/carcinomas combined of the pancreatic islets was significantly increased at 2.5 and ≥ 2.5 mg/m^3, respectively. In females, the incidence of mononuclear cell leukemia was significantly increased at al doses. These tumours were not observed in mice.

As observed for cobalt metal, inhalation exposure to cobalt sulphate heptahydrate resulted in increased incidences of alveolar/bronchiolar adenomas and carcinomas, in both sexes of rats and mice. A significant increase in tumours was observed at doses of ≥ 1 mg/m^3 in female rats and ≥ 3 mg/m^3 in mice and male rats. This is equivalent to 0.38 and 1.14 mg/m^3 when expressed as cobalt. The top dose is therefore comparable with the lowest dose used in the studies with cobalt metal. Also in (sub)chronic inhalation studies with cobalt sulphate, several non-neoplastic and pre-neoplastic lesions were observed, including inflammation, necrosis, fibrosis, degeneration, hyperplasia and squamous cell metaplasia of respiratory and olfactory epithelium.

In addition, the incidence in benign pheochromocytomas alone and the incidence in benign, complex or malignant pheochromocytomas combined was significantly increased at doses ≥3 mg/m^3, in females (equivalent to 1.14 mg/m^3 cobalt). This is comparable to the cobalt dose that induced pheochromocytomas after cobalt exposure to cobalt metal.

Table 70: Tumour incidence rates in rat and mouse bioassays after inhalation exposure to cobalt sulphate heptahydrate or cobalt oxide.

Cobalt sulphate heptahydrate	Dose (mg/m³)				

RAT 2-year study	**0**	0.3	1	**3**	HC*
Lung					
♂ alveolar/bronchiolar					
adenoma	2.3%	17.7%	2.4%	28.4%	0-10%
carcinoma	0%	0%	11.3%	6.7%	0-2%
combined	2.3%%	17.7%	13.4%	**33.9%**	0-10%
♀ alveolar/bronchiolar					
adenoma	0%	3.4%	**36.4%**	**30.0%**	0-4%
carcinoma	0%	8.0%	**20.2%**	**17.5%**	0%
combined	0%	11.2%	**50.6%**	**46.1%**	0-4%
Adrenal medulla					
♂ pheochromocytoma					
Benign	51.0%	70.0%	71.9%	71.4%	0-50%
combined	52.1%	70.0%	74.1%	71.4%	8-50%
♀ pheochromocytoma					
Benign	5.1%	3.1%	9.3%	**26.4%**	0-14%
combined	5.1%	3.1%	11.7%	**31.5%**	2-14%
MOUSE 2 year study	**0**	0.3	1	**3**	HC*
Lung					
♂ alveolar/bronchiolar					
adenoma	30.4%	30.9%	41.1%	**54.6%**	6-36%
carcinoma	13.2%	16.1%	25.3%	**43.7%**	0-16%
combined	35.5%	36.5%	56.5%	**78.8%**	10-42%
♀ alveolar/bronchiolar					
adenoma	8.8%	15.0%	25.2%	**32.8%**	0-14%
carcinoma	2.9%	2.7%	9.2%	**25.3%**	0-12%
combined	11.8%	17.5%	32.6%	**50.2%**	0-16%
Liver					
♂ hemangiosarcoma	9.1%	11.5%	23.5%	25%	0-6%
♀hemangiosarcoma	2.9%	0%	7.3%	0%	0-3%
Cobalt oxide		Dose (mg/kg bw)			
Rat study	0	2	10		
Lung					
♂ alveolar/bronchiolar					
adenoma	0%	0%	4%		
carcinoma	0%	0%	6%		
combined	0%	0%	**10%**		
benign squamous epithelial	0%	2%	0%		
♀ alveolar/bronchiolar					
adenoma	0%	2%	0%		
carcinoma	0%	0%	2%		
combined	0%	2%	2%		

Values in **bold**: statistically significantly different from control
* Historical control values for 2-year studies (all routes)

Cobalt oxide also induced alveolar/bronchiolar adenomas and carcinomas in rats, although this was only significant when combined in males after intratracheal instillation of 10 mg/kg bw. In hamsters, no evidence of carcinogenesis was observed after inhalation of 10 g/L cobalt oxide. This dose level is considered unrealistic.

Several epidemiological studies suggest a correlation between cobalt exposure and lung cancer. However, in all these studies there is co-exposure to other carcinogens, limiting the usability of these studies for classification purposes.

Inhalation exposure to soluble (cobalt sulphate heptahydrate) and insoluble (cobalt metal and cobalt oxide) cobalt compounds result in similar toxic effects in the respiratory tract, and in carcinogenicity in lung and adrenal medulla (rats only).

The mechanisms of cobalt-induced neoplasms are not completely understood but the available data provide strong support that intracellular cobalt ions are the principle toxic entity. Cobalt ions are actively transported inside the cell via metal ion transport systems while cobalt particles with low solubility are readily taken up by cells via endocytosis. Once inside the cell, cobalt particles are partially solubilized at the low pH within lysosomes and release cobalt ions that can react with DNA, proteins, and lipids. Three possible modes of action (all relevant for humans) are proposed for the carcinogenic effects of cobalt: 1) genotoxicity and inhibition of DNA repair, 2) induction of reactive oxygen species (ROS) and oxidative stress, and 3) induction of hypoxia-like responses by activating hypoxia-inducible factor 1 (HIF-1) (NTP 2016a).

Cobalt and several cobalt compounds have been shown to induce genotoxicity in rodent and human cells in vitro. In vivo, such effects are also observed, although mostly after intraperitoneal administration.

Both cobalt ions and cobalt metal have been shown to catalyze the formation of ROS in vivo and in vitro through Fenton-like reactions. This can result in an increase in oxidative stress and tumor development by mutagenesis and/or promote tumor growth by dysregulation of cell growth and proliferation. Evaluation of the lung tumours in the carcinogenicity studies with cobalt metal and cobalt sulphate heptahydrate revealed a distinct pattern of G→T transversion of *K-ras* mutations in tumors from exposed animals, whereas such mutations were not noted in tumors of control animals. G→T transversions are associated with reactive oxygen species during oxidative damage to DNA (Behl *et al.*, 2015).

Several experimental studies indicate that cobalt activates HIF-1, a key mediator of hypoxia response. This may contribute to tumor development and progression via several pathways.

4.10.5 Comparison with criteria

Due to co-exposure to other carcinogens, epidemiological studies are not useful to conclude whether cobalt is carcinogenic in humans. Therefore, cobalt metal should not be classified as Carc. 1A.

According to CLP a substance should be classified in Category 1B if a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of a combination of benign and malignant neoplasms in at least two species or in two independent studies in one species. Substances may also be classified in Category 1B according to CLP if they produce an increased incidence of tumours in both sexes of a single species in a well-conducted study or if the substance leads to an unusual degree of malignant of neoplasms in one species and sex. Cobalt metal (as well as cobalt sulphate heptahydrate) induces benign and malignant lung (in rats and mice) and adrenal tumors (in rats) after inhalation exposure. Cobalt metal also induces adenomas/carcinomas in pancreatic islets of male rats. Three possible modes of action are proposed, all of which are relevant for human. Classification as Carc 1B is therefore required.

The criteria in table 3.6.3 of CLP state that the route of exposure should be stated if it is conclusively proven that no other routes of exposure cause the hazard. It is recognized that the cobalt compounds that currently have a harmonised classification for carcinogenicity are limited to the inhalation route (H350i). However, there are no carcinogenicity studies using other routes of exposure. However, the adrenal tumors, together with toxicity to internal organs as the testes and the results of tissue burden studies indicate that cobalt is distributed through the body after inhalation exposure. In our opinion, this means that it cannot be excluded that exposure via for example the oral route, may result in carcinogenesis, provided that the internal concentration of cobalt is high enough. Therefore, inclusion of a specific exposure route (i.e. inhalation) is not advised.

The proposed classification is mainly based on inhalation studies using cobalt with an MMAD of approximately 2 μm with a GSD of 2 μm. This particle size may not be representative for the particle size of cobalt as put on the market. Larger particles may not be inhalable or may deposit in the higher parts of the lung limiting the exposure of the alveoli. However, limiting the classification for carcinogenicity to a certain particle size is considered incorrect because it has not been shown that other exposure routes cannot result in carcinogenesis and because particle size may change during use of the substance like grinding, drilling and sanding. According to article 9.5, classification should be based on the form as put on the market and in which it can reasonably expected to be used. Therefore, no size depended classification is proposed.

Since cobalt is a carcinogen with high potency (T25<1 mg/kg bw/day) and is proposed to be classified in Carc 1B, an SCL of 0.01% should be applied.

4.10.6 Conclusions on classification and labelling

Cobalt metal induces lung and adrenal tumors after inhalation exposure in both sexes of rats and mice. Since it cannot be excluded that oral exposure may result in carcinogenesis, cobalt should be classified as Carc 1B; H350, without specification for an exposure route. In addition, an SCL of 0.01% should be applied.

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 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)

[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.

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/m^3	2.5 mg/m^3	5 mg/m^3
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 ($P \leq 0.05$) from the chamber control group by the Poly-3 test
** $P \leq 0.01$
(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/m³	2.5 mg/m³	5 mg/m³
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.**

4.11 Toxicity for reproduction

Table 71: Summary table of relevant repeated dose and reproductive toxicity studies

Method	Test substance	General toxicity	Reproductive effects	Remarks	Reference
fertility					
Combined repeated dose toxicity and reproduction screening study in rats (10/sex/dose) 0, 30, 100, 300 or 1000 mg/kg bw 2 weeks before mating – 2 weeks after mating (males) or ppd 3 (females)	Cobalt powder	≥ 100 mg/kg bw: Mortality, clinical effects, macroscopic intestinal changes	≥ 300 mg/kg bw: Decreased implantation sites and life birth index	a,b, c	CDI/CORC 2015
16 days inhalation in rats (5/sex/dose) 0, 2.5, 5, 10, 20 or 40 mg/m3	Cobalt Purity >98% MMAD: 1.8-1.9 µm GSD: 1.7-1.8	≥ 2.5 mg/m^3: decreased liver weight, atrophy and necrosis olfactory epithelium, cytoplasmic vacuolization bronchioli ≥ 5 mg/m^3: pale lungs, lung infiltration ≥ 10 mg/m^3:decreased body weight, decreased kidney and thymus weight, increased lung weight, fibrosis and necrosis in the lung ≥ 20 mg/m^3: mortality	≥ 10 mg/m^3: Decreased testis weight m^3	a	NTP 2014
17 days inhalation in mice (5/sex/dose) 0, 2.5, 5, 10, 20 or 40 mg/m3	Cobalt Purity >98% MMAD: 1.8-1.9 µm	≥ 2.5 mg/m^3: decreased liver weight, vacuolization lung and resp	LOAEL: 40 mg/m^3: Decreased testis weight	a	NTP 2014

	GSD: 1.7-1.8	epithelium, atrophy olfactory epithelium ≥ 5 mg/m^3: increased lung weight, infiltration and karyomegaly in the lung, inflammation resp epithelium, necrosis olfactory epithelium ≥ 10 mg/m^3: squamous metaplasia resp. epithelium ≥ 20 mg/m^3: decreased body weight 40 mg/m^3: mortality			
14 weeks inhalation in rat (10/sex/dose) 0, 0.625, 1.25, 2.5 or 5 mg/m3	Cobalt; Purity >98% MMAD: 1.6-2.0 µm GSD: 1.7-2.0	≥ 0.625 mg/m^3: increased lung weight, decreased sperm motility, inflammation lung, proteinosis alveoli ≥ 1.25 mg/m^3: hyperplasia bronchioli, degeneration olfactory epithelium ≥ 2.5 mg/m^3: hyperplasia olfactory and resp. epithelium, turbinate atrophy ≥ 5 mg/m^3: decreased body weight	≤ 0.625 mg/m^3: Decreased sperm motility	a, b, c	NTP 2014
14 weeks inhalation in mice (10/sex/dose) 0, 0.625, 1.25, 2.5, 5 or 10 mg/m3	Cobalt; Purity >98% MMAD: 1.6-2.0 µm GSD: 1.7-2.0	≥ 0.625 mg/m^3: infiltration lung, vacuolization bronchiole, squamous metaplasia larynx	≥ 2.5 mg/m^3: Reduced sperm motility ≥ 5 mg/m^3: Reduced sperm count,	a, b, c	NTP 2014

		≥ 1.25 mg/m^3: degeneration olfactory and resp. epithelium ≥ 2.5 mg/m^3: decreased liver weight, increased lung weight, decreased sperm motility, hyperplasia bronchiole and resp. epithelium, squamous metaplasia resp. epithelium ≥ 5 mg/m^3: tan lungs, decreased kidney and testis weight, decreased sperm activity, proteinosi and karyomegaly alveoli, tubinate atrophy, lung hemorrhage, inflammation lung and nose ≥ 10 mg/m^3: decreased body weight, degeneration testes, atrophy and cytopl. vacuolization epididymis, hypospermia, exfoliated germ cells	decreased testis weight 10 mg/m^3: Degeneration testes epithelium, exfoliated germ cells, hypospermia, vacuolization and atrophy epididymis		
combined repeated dose and carcinogenicity inhalation study in rats (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	Cobalt; Purity >98% MMAD: 1.4-2.0 µm GSD: 1.6-1.9	≥ 2.5 mg/m^3: decreased survival, decreased body weight, necrosis olfactory epithelium ≥ 1.25 mg/m^3: hyperplasia, proteinosis, inflammation, atrophy, squamous	5 mg/m^3: Testes infarct	[a, b]	NTP 2014

		metaplasia in nose and lung			
combined repeated dose and carcinogenicity inhalation study in mice (50/sex/dose) 0, 1.25, 2.5, or 5 mg/m³, 6 hours plus T90 (12 minutes) per day, 5 days per week for 105 weeks	Cobalt; Purity >98% MMAD: 1.5-2.1 µm GSD: 1.6-1.9	5 mg/m^3: decreased body weight ≥ 2.5 mg/m^3: decreased survival, inflammation and erosion lung ≥ 1.25 mg/m^3: hyperplasia, cytoplasmic vacuolization, proteinosis, infiltration, atrophy, metaplasia in lung, nose, larynx and trachea	LOAEL: 5 mg/m^3: Degeneration germinal epithelium testes	[a, b]	NTP 2014

16 days inhalation in rats (5/sex/dose) 0, 0.1, 0.5, 5, 50 or 200 mg cobalt sulphate heptahydrate/m3, 6h/day, 12 exposures over 16 days	Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm	≥ 50 mg/m^3 mortality, decreased body weight, inflammation and necrosis of respiratory epithelium, necrosis of thymus, testis atrophy. 200 mg/m^3: necrosis in liver	≥ 50 mg/m^3 (10.5 mg cobalt/m^3): Testes atrophy	a, b	NTP, 1991
16 days inhalation in mice (5/sex/dose) 0, 0.1, 0.5, 5, 50 or 200 mg cobalt sulphate heptahydrate/m3, 6h/day, 12 exposures over 16 days	Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm	≥ 5 mg/m^3 , inflammation and necrosis of the respiratory epithelium. ≥ 50 mg/m^3 mortality	NOAEL: ≥ 50 mg/m^3 (10.5 mg cobalt/m^3)	a, b	NTP, 1991
13 weeks inhalation in rats (10/sex/dose) 0, 0.3, 1, 3, 10 or 30 mg cobalt sulphate heptahydrate/m3, 6h/day, 5 days/week	Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm	≥ 0.3 mg/m^3: respiratory metaplasia. At higher doses, also inflammation, hyperplasia, necrosis and fibrosis are observed	NOAEL: ≥ 30 mg/m^3 (6.3 mg cobalt/m^3)	a, b, c	NTP, 1991
13 weeks inhalation in mice (10/sex/dose) 0, 0.3, 1, 3, 10 or 30 mg cobalt sulphate heptahydrate/m3, 6h/day, 5 days/week	Cobalt sulphate heptahydrate purity 99%; MMAD: 0.8-1.1 µm	≥ 0.3 mg/m^3: respiratory respiratory metaplasia. At higher doses, also inflammation, hyperplasia, necrosis and fibrosis are observed	≥ 3 mg/m^3 (0.6 mg cobalt/m^3): Decreased sperm motility 30 mg/m^3 (6.3mg cobalt/m^3): Decreased testes and epididymal weight, increased abnormal sperm count, testes atrophy	a, b, c	NTP, 1991
combined repeated dose and carcinogenicity inhalation study in rats and mice (50/sex/dose) 0, 0.3, 1.0, or 3.0 mg	Cobalt sulphate heptahydrate purity 99%; MMAD: 1.1-2.0 GSD: 1.9-3.0	Rats: ≥ 0.3 mg/m^3 respiratory hyperplasia, inflammation, metaplasia and	NOAEL: ≥ 3 mg/m^3 (0.6 mg cobalt/m^3)	a, b	NTP 1998

cobalt sulphate heptahydrate /m³ 6 hours per day, 5 days per week, for 105 weeks)		fibrosis Mice: ≥ 0.3 mg/m^3 respiratory hyperplasia, inflammation, metaplasia and fibrosis Liver inflammation, karyomegaly, oval cell hyperplasia, and regeneration			
Dominant lethal assay in male mice (10/dose), oral administration in drinking water for 84 days 0 or 67 mg cobalt/kg bw/day	Cobalt chloride hexahydrate	General toxicity or other effects were not determined in this study	400 ppm (approximately 67 mg Co/kg bw): Reduced fertility, increased preimplantation loss, reduced sperm parameters.	a, c	Pedigo, N.G.; Vernon, M. W. 1993
Dominant lethal assay in male mice (10/dose), 84 days oral administration in drinking water 0, 25.7, 46.9 or 93 mg cobalt chloride hexahydrate/kg bw/day	Cobalt chloride hexahydrate	≥ 200 ppm or 25.7 mg /kg bw (6.4 mg cobalt/kg bw/day): decreased body weight ≥ 400 ppm or 46.9 mg /kg bw (11.6 mg cobalt/kg bw/day): mortality	≥ 200 ppm or 25.7 mg /kg bw (6.4 mg cobalt/kg bw/day): Decreased sperm count ≥ 400 ppm or 46.9 mg /kg bw (11.6 mg cobalt/kg bw/day): Reduced testes weight, reduced pregnancies, reduced implantation sites 800 ppm or 93.0 mg /kg bw (23.1mg cobalt/kg bw/day): Reduced epididymal weight	a,b,c	Elbetieha, A. *et al.*, 2008
69 days study in male rats, oral diet administration 0, 5 or 20 mg cobalt/kg bw/day	Cobalt chloride	no information on general toxicity provided	20 mg cobalt/kg bw: decreased testis weight, testicular atrophy	a, b	Nation, J.R.; *et al.* 1983
3 months oral in male	Cobalt chloride		≥ 23 mg/kg bw/day	a, c	Pedigo, N.G.

mice (5/dose) 0, 23, 42 or 72.1 mg cobalt chloride hexahydrate/kg bw/day (drinking water)	hexahydrate	72 mg/kg bw (18 mg Co/kg bw/day): reduced body weight, reduced fluid intake	(6 mg Co/kg bw/day): Reduced testicular weight, reduced sperm concentration 72 mg/kg bw (18 mg Co/kg bw/day): Reduced fertility		*et al.*, 1988
13 weeks study in male mice (10/dose), oral administration 0 or 400 ppm cobalt chloride/day (drinking water)	Cobalt chloride	no information on general toxicity provided	400 ppm 92 mg/kg bw/day, 24 mg Co/kg bw/day): Reduced testicular weight, degeneration seminiferous tubuli, altered testicular vessel epithelium	a, b	Anderson, M.B., 1992
98 day oral administration in rats 0 or 265 ppm diet cobalt chloride/day	Cobalt chloride	no information on general toxicity provided	265 ppm (20 mg cobalt/kg bw/day): Degenerative changes testes	b	Mollenhauer, H.H *et al.*, 1985
3 months oral toxicity study in male rats 0 or 20 mg cobalt/kg bw/day(diet)	Cobalt chloride hexahydrate	≥ 265 ppm 20 mg Co/kg bw/day:Increased erythrocyte count, packed cell volume, and haemoglobin concentration	265 ppm (20 mg cobalt/kg bw/day): Degenerative, non-necrotic and necrotic lesions were present in the seminiferous tubules	b, c	Corrier, D.E.; *et al.*, 1985
3 months oral (gavage) toxicity study in rats (10/sex/dose)	Cobalt chloride hexahydrate	≥ 10 mg/kg bw: decreased body weight gain, changed hematological parameters 30 mg/kg bw: erythroid hyperplasia of the femur	NOAEL: 30 mg/kg bw/day 7.4 mg Co/kg bw/day	a, b	CDI/CORC 2015
development					

Combined repeated dose toxicity and reproduction screening study in rats (10/sex/dose) 0, 30, 100, 300 or 1000 mg/kg bw (gavage: undissolved) 2 weeks before mating – 2 weeks after mating (males) or ppd 3 (females)	Cobalt powder	≥ 100 mg/kg bw: Mortality, clinical effects, macroscopic intestinal changes NOAEL: 30 mg/kg bw/day	≥ 100 mg/kg bw: decreased viability index 300 mg/kg bw: increased pre- and post implantation loss, decreased live birth index, decreased pup weight		*CoRC/CDI* , 2015
Oral developmental study in female rats (15/dose) 0, 12, 24 or 48 mg cobalt chloride/kg bw/day (GD14-PND21)	Cobalt chloride	no general toxicity reported	≥ 12 mg/kg bw (3 mg cobalt/kg bw/day): Decreased number of litters, increased dead pups/litter, decreased fetal weight		Domingo, J.L.; *et al.* 1985
Oral developmental study in female rats (20/dose) (gavage) 0, 25, 50 or 100 mg cobalt dichloride/kg bw/day (GD6-15)	Cobalt chloride hexahydrate	≥ 25 mg/kg bw: decreased body weight gain ≥ 50 mg/kg bw: decreased GOT and creatinine ≥ 100 mg/kg bw: increased Hb, Ht, MCV, MCH and reticulocytes; increased cholesterol	NOAEL: ≥ 100 mg/kg bw/day (24.79 mg cobalt/kg bw/day)		Paternain, J.L. *et al.* 1988
Dominant lethal assay in male mice (10/dose), 84 days oral administration in drinking water 0, 25.7, 46.9 or 93 mg cobalt chloride hexahydrate/kg bw/day	Cobalt chloride hexahydrate	≥ 200 ppm or 25.7 mg /kg bw (6.4 mg cobalt/kg bw/day): decreased body weight ≥ 400 ppm or 46.9 mg /kg bw (11.6 mg cobalt/kg bw/day): mortality	≥ 200 ppm or 25.7 mg /kg bw (6.4 mg cobalt/kg bw/day): increased resorptions, decreased number of viable pups		Elbetieha, A. *et al.*, 2008
Oral developmental study in female rats (25/dose) (gavage) 0, 25, 50 or 100 mg cobalt dichloride hexahydrate/kg bw/day (GD6-19)	Cobalt dichloride hexahydrate	≥ 100 mg/kg bw/day: Reduced bw gain ≥ 50 mg/kg bw/day: Gastro-intestinal lesions	NOAEL: ≥ 100 mg/kg bw/day (24.8 mg cobalt/kg bw)		*CoRC/CDI* , 2015
Oral developmental study in female mice	cobalt sulphate heptahydrate	no relevant maternal	50 mg/kg bw (10.5 mg cobalt/kg bw):		Szakmáry, E. *et al.* 2001

(20 or 25/dose) (gavage) 0 or 50 mg cobalt sulphate /kg bw/day (GD6-15)		toxicity	Retarded body weight gain, increased skeletal retardation, increased malformations		
Oral developmental study in female rats (3-18/dose) 0, 25, 50 or 100 mg cobalt sulphate /kg bw/day (GD1-20/21) (gavage)	cobalt sulphate heptahydrate	no relevant maternal toxicity	≥ 25 mg/kg bw (5.2 mg cobalt/kg bw): Skeletal retardation ≥ 50 mg /kg bw (10.5 mg cobalt/kg bw): Retarded bw gain, visceral retardation, increased malformations		Szakmáry, E. *et al.* 2001
Oral developmental study in female rabbits (8-25/dose) 0, 20, 100 or 200 mg cobalt sulphate/kg bw (GD6-20) (gavage)	cobalt sulphate heptahydrate	≥ 20 mg/kg bw: mortality, circulatory failure, reduced bw gain	≥ 20 mg/kg bw (4.2 mg cobalt/kg bw): Increased resorptions, skeletal retardation		Szakmáry, E. *et al.* 2001

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

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Studies with cobalt metal

Oral studies

An oral screening study (OECD 422) is available with cobalt powder. No other fertility studies performed according to OECD guidelines are available for soluble cobalt compounds. However, there are several peer reviewed publications that show effects of cobalt compounds on fertility. In addition in several repeated dose studies, effects were found on the male reproductive system, although, in other repeated dose studies, such effects were not observed (see table above). Parts of the studies relevant for reproductive toxicology are described below, for more details, see 4.7 repeated dose toxicity.

In a study conform OECD TG 422, rats (SD) (n=10 / dose / sex) were treated by gavage with powdered cobalt (0, 30, 100, 300 or 1000 mg/kg bw/day, purity >99.8%) (vehicle 0.5% hydroxypropyl methylcellulose gel) (particle size : D50=12.8 µm) from 2 weeks before mating until approximately 2 weeks after mating (males) or 3 days post-partum (females). All females and 9 out of 10 males died at 1000 mg/kg bw/day. No mortality occurred in males at lower dose levels. Eight out of 10 females treated with 300 mg/kg bw/day and five out of 10 females treated with 100 mg/kg bw/day died during the mating, gestation or lactation period. No mortality was observed in females treated with 30 mg/kg bw/day. In the gestation period, the body weight of the female rats treated with 300 mg Cobalt Powder/kg b.w./day was marginally below the control (by 6%) on gestation day 14 and more distinctly below the control (by 11%) on gestation day 20. The body weight in rats treated with 1000 mg Cobalt Powder/kg b.w./day was slightly or distinctly below the control (by 8% or 17%, no statistical comparison) in the two females surviving gestation days 7 and 14. The body weight at autopsy was within the range of the control for the ten females at 30 mg Cobalt Powder/kg b.w./day and the five surviving females at 100 mg Cobalt Powder/kg b.w./day. The body weight of the female rats treated with 300 mg Cobalt Powder/kg b.w./day was reduced during the lactation period, being minus 21% below the control for the six survivors on lactation day 1 (statistically significant at $p \leq 0.01$). The body weight of the two surviving females was still reduced on lactation day 4 and at autopsy (20% or 19% below the control value).

The fertility of the female rats was not influenced (see table 72). No effects were noted on the sperm number, viability and morphology at any of the tested dose levels (1000 mg/kg bw group not examined). There were no test item-related differences in the number of corpora lutea between the control group and the treated animals. Pre- and postimplantation loss and live birth index was only altered at 300 mg/kg bw, a dose at which most animals died. The viability index of the offspring of the 5 remaining dams at 100 mg/kg bw was significantly reduced but within the range of the historical controls. Mean pups weight was dose relatedly decreased at day 0 and day 4 but this change was only significant at 300 mg/kg bw ($p \leq 0.01$) but within the historical control range. (CoRC/CDI , 2015).

Table 72 Reproductive toxicity parameters

parameter	0 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d	300 mg/kg bw/d
males				
Absolute weight epididymides (left and right)	0.705±0.118 0.690±0.081	0.554±0.048** 0.556±0.060**	0.674±0.089 0.676±0.089	0.624±0.044 0.672±0.088
Number of ultrasound-resistant spermatids per g testicular tissue x 10 6	83.35 ± 11.70	97.85 ± 15.64	101.09 ± 17.98	103.60 ±15.74
Motile spermatozoa in the epididymal cauda (%)	71.83 ± 7.74	71.38 ±8.57	64.67 ±14.30	74.32 ±3.88
Morphologically normal spermatids in the cauda epididymis (%)	99.95	99.40	99.83	99.95
females				

Pre-coital time (days)	4.0 ±4.8	2.8 ±1.5	6.8 ±7.4	3.5 ±4.0
Number of pregnant females	10/10	9/10	8/9	6/6
Fertility index	100	90	89	100
Gestation length	21.5 ±0.5	21.0 ±0	21.0 ±0	21.0 ±0
Number of dams with live pups	10/10	9/9	8/8	6/6
Number of dams with stillborn pups	0	0	1	3
Number of stillbirths	0	0	2	15
Number of live born pups (mean)	12.1±4.2	14.2 ±2.0	11.8 ±2.1	8.2 ±3.3
LIVE BIRTH INDEX	100	100	98.2	75.9**
Pre-implantation loss	16.3 ±16.3	15.4 ±11.4	17.2 ±18.6	24.1 ±22.6*
Post-implantation loss	12.6 ±18.2	6.7 ±8.1	9.9 ±7.7	30.9 ±24.9**
Number of runts	1	1	0	1
Number of malformed pups	0	0	0	0

* P≤0.05, ** P≤0.01, not all parameters tested at P≤0.05.

Inhalation studies

F344/N rats (5/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **16 days**. All rats exposed to 40 mg/m^3 and all male and three female rats exposed to 20 mg/m^3 died before the end of the study; the majority of deaths occurred by study day 7. Absolute testis weights were significantly decreased in the group exposed to 10 mg/m^3 (0.59 g vs 0.886 g in controls). Relative testis weight was also reduced but not significantly (NTP 2014).

B6C3F1/N mice (5/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **17 days**. Three male and three female mice exposed to 40 mg/m^3 died before the end of the study. Absolute testis weights were significantly decreased in the group exposed to 40 mg/m^3 (0.070 g vs 0.098 g in controls) (NTP, 2014).

Groups of F344/N rats (10/sex/dose) were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for **14 weeks**. All male and female rats survived to the end of the study. Sperm motility was significantly decreased in all males exposed to cobalt (2.8-7.9% lower than control) with a clear dose effect relation. No effects on testis and epididymis weight, spermatid and sperm counts and testis histopathology were observed (NTP, 2014).

Groups of 10 male and 10 female B6C3F1/N mice were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3, 6 hours plus T90 (12

minutes) per day, 5 days per week for **14 weeks.** Testes weights (absolute and relative) of males exposed to ≥ 5 mg/m^3 were significantly decreased. In addition, exposure concentration-related decreases in spermatid and epididymal spermatozoa counts, and sperm motility in combination with histopathologic findings in both the testis and epididymis were observed (see table 73 and 74) (NTP, 2014).

Table 73: Summary of reproductive tissue evaluation for male mice in the 3 month study of cobalt metal.

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.7 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
L. Cauda epididymis	0.0217 ± 0.0014	0.0210 ± 0.0008	0.0231 ± 0.0018	0.0168 ± 0.0006*
L. Epididymis	0.0603 ± 0.0022	0.0578 ± 0.0019	0.0614 ± 0.0035	0.0429 ± 0.0021**
L. Testis	0.1185 ± 0.0017	0.1132 ± 0.0023	0.1027 ± 0.0036**	0.0316 ± 0.0014**
Spermatid measurements				
Spermatid heads (10^6/testis)	22.34 ± 0.84	22.22 ± 0.65	18.90 ± 1.20*	0.53 ± 0.10**
Spermatid heads (10^6/g testis)	210.84 ± 6.85	227.74 ± 7.16	205.67 ± 7.43	24.27 ± 4.78**
Epididymal spermatozoal measurements				
Sperm motility (%)	86.0 ± 1.1	82.0 ± 0.8*	82.2 ± 1.1*	2.6 ± 1.2**
Sperm (10^6/cauda epididymis)	11.55 ± 0.39	10.53 ± 0.43	9.62 ± 0.49**	0.71 ± 0.06**
Sperm (10^6/g cauda epididymis)	551.1 ± 37.9	505.9 ± 23.3	439.9 ± 40.3*	43.4 ± 3.7**

* Significantly different (P≤0.05) from the chamber control group by Dunnett's test (cauda epididymis weight) or Shirley's test (spermatid and epididymal spermatozoal measurements)
** Significantly different (P≤0.01) from the chamber control group by Williams' test (body and tissue weights) or Shirley's test (spermatid and epididymal spermatozoal measurements)
[a] Data are presented as mean ± standard error.

Table 74: Incidences of selected nonneoplastic lesions of the genital system in male mice in the 3 month inhalation study of cobalt metal.

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Testes[a]	10	10	10	10	10	10
Germinal Epithelium, Degeneration[b]	2 (1.0)[c]	0	0	0	1 (1.0)	10** (4.0)
Epididymis	10	10	10	9	10	10
Exfoliated Germ Cell	0	0	0	0	0	10** (2.7)
Hypospermia	0	0	0	0	0	10** (2.9)
Vacuolization Cytoplasmic	0	0	0	0	0	9** (1.0)
Atrophy	0	0	0	0	0	10** (1.0)

** Significantly different (P≤0.01) from the chamber control group by the Fisher exact test
[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

In a carcinogenicity study, F344/NTac rats (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for up to **105 weeks**. Survival of female rats exposed to 2.5 mg/m^3 was significantly less than that of the chamber control group. The incidence of infarct in the testes was

significantly increased in male rats exposed to 5 mg/m^3. In affected testes, there was complete effacement of the parenchyma due to necrosis with loss of differential staining and cellular detail (NTP, 2014).

In a carcinogenicity study, B6C3F1/N mice (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T90 (12 minutes) per day, 5 days per week for up to 105 weeks. Survival of males exposed to 2.5 or 5 mg/m^3 was significantly less than that of the chamber control group. The incidence of germinal epithelium degeneration in the testes was significantly increased in male mice exposed to 5 mg/m^3 (chamber control, 9/50; 1.25 mg/m^3, 14/49; 2.5 mg/m^3, 8/50; 5 mg/m^3, 21/50) (NTP, 2014).

Studies with cobalt compounds

Oral studies

Cobalt toxicity was evaluated in a dominant lethal assay (DLA) to determine whether the detrimental effects of cobalt on spermatozoa would have an impact on offspring. Ten male B6C3F1 mice were treated with cobaltous chloride (400 ppm Co) (estimated as 67 mg Co/kg bw/day) in drinking water for 10 weeks and mated. Neither the stage nor rate of development in vitro of 2-cell embryos to blastocyst from cobalt-treated males was affected. Although all males were fertile, the number of pregnant females was decreased in the group mated with males treated with cobalt. 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 from litters at day 19 of gestation. 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 treatment), and recovered over the next 6 weeks (table 75). There was a decrease in testes weight. Sperm parameters at the end of DLA and the recovery period showed that cobalt decreased all parameters measured at 12 weeks, but these parameters, except concentration, recovered to control levels by 18 weeks (table 76). 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 (Pedigo, N.G.; Vernon, M. W., 1993).

Table 75: Results dominant lethal assay in mice

	0 ppm	**400 ppm Co**
Number of pregnant females	29/32 (91%)	18/31 (58%)*
Number of fertile males (at 10 weeks)	10/10	10/10
Average total implantations per pregnant female	8.3 ± 0.4	6.5 ± 0.8*
Average dead implantations per pregnant female	0.4 ± 0.1	0.4 ± 0.1
Average preimplantation loss per pregnant female	0.43 ± 0.2	2.4 ± 0.7*

Table 76: Testicular function in mice (% of control)

	12 weeks	**18 weeks (6 weeks recovery)**
Sperm concentration	15.3	63.8

Sperm motility	18.3	Control level
Path velocity	30.8	Control level
Progressive velocity	22.2	Control level
Linear index	75.7	Control level
Progressive motility	17.2	Control level
Track speed	43.7	Control level
Testicular weight	41	60

Sexually mature male mice were exposed to 200, 400 and 800 ppm cobalt chloride hexahydrate (25.7, 46.9 and 93.0 mg/kg bw/day) in their drinking water for 12 weeks. Males were than mated with untreated female mice. Average body weight gain was significantly reduced in all dose groups (final body weights were 95, 94 and 93% of the control group). Two animals out of 10 and one out of 10 died during the 10th weeks of the exposure to 800 and 400 ppm cobalt chloride, respectively. There were no other signs of clinical toxicity observed in the survived animals. Testicular sperm count was decreased at ≥ 400 ppm. Epididymal sperm count was decreased at all doses (table 77). Testicular weight was reduced at ≥ 400 ppm. Epididymal weight was reduced at 800 ppm. Histological examination of the testes showed hypertrophy of the interstitial Leydig cells, congested blood vessels, degeneration of the spermatogonial cells and necrosis of both the seminiferous tubules and the interstitial tissue (doses unknown). At ≥ 400 ppm number of pregnancies and number of implantation sites was significantly reduced. (Elbetieha, A. *et al.* 2008).

Table 77: Effects on sperm count in mice after 12 weeks exposure to cobalt chloride

Treatment (ppm)	Epididymal Sperm Count/mg Epididymis[a] ($X 10^3$)	Testicular Sperm Count/g testis[a] ($X 10^3$)	Daily Sperm Production/Testis[a] ($X 10^5$)
Control (Tap water)	192.79 ± 15.68	44.94 ± 2.72	10.12 ± 0.74
cobalt chloride (200)	167.23 ± 23.10*	45.36 ± 3.11	9.77 ± 1.20
cobalt chloride (400)	166.23 ± 25.41*	34.78 ± 2.31**	6.50 ± 0.39 ***
cobalt chloride (800)	150.62 ± 12.40***	33.31 ± 2.23 ***	5.81 ± 0.42 ****

[a] Results are expressed as mean ± S.D.

* p<0.05, **p<0.01, ***p<0.005, ****p<0.0001 (Student *t* test).

Table 78: effects on genital organ weight in mice after 12 weeks exposure to cobalt chloride

Numbers in brackets represent relative organ weights.

Treatment (ppm)	Number of Males	Epididymis weight (mg)	Testes weight (g)	Seminal vesicles weight (g)	Preputial gland weight (g)
Control (Tap water)	10	32.31± 1.66	0.21 ± 0.01 (57.75 ± 3.89)	0.13 ± 0.02 (36.36 ± 6.82)	0.096 ± 0.01 (26.07 ± 2.51)
Cobalt chloride (200)	10	31.88 ± 1.46	0.19 ± 0.01** (55.45 ± 3.18)	0.13 ± 0.03 (35.57 ± 7.46)	0.107 ± 0.015 (30.44 ± 4.86)*
Cobalt chloride (400)	9	31.75 ± 1.45	0.18 ± 0.01*** (53.08 ± 3.33)*	0.20 ± 0.02** (61.91 ± 8.24)***	0.096 ± 0.016 (29.32 ± 5.90)
Cobalt chloride (800)	8	29.80 ± 0.93***	0.15 ± 0.02 **** (46.28 ± 7.31)***	0.23 ± 0.07 *** (68.32 ± 21.94)***	0.094 ± 0.023 (28.81 ± 7.13)

[a] Results are expressed as mean ± S.D.
Relative organ weights, expressed as mg/10g body weight.

* $p<0.05$, **$p<0.01$, ***$p<0.005$, ****$p<0.0001$ (Student *t* test).

Table 79: Results dominant lethal assay in male mice exposed to cobalt chloride

Treatment (ppm)	Number of males	Number of mated females	Number (%) of pregnant females	Number of implantation sites[a]/female	Number of viable fetuses[a]	Total Number of resorptions/ Total No. of implantation sites	Number (%) of animals with resorptions
Control (Tap water)	10	20	19/20 (95.0)	7.89 ± 2.38	7.74 ± 2.40	3/150	3/19 (16)
Cobalt chloride (200)	10	20	15/20 (75.0)	5.67 ± 2.02^{++}	5.00 ± 2.14^{++}	9/81***	10/15** (67)
Cobalt chloride (400)	9	18	12/18 * (66.7)	5.42 ± 1.68^{++}	4.67 ± 1.83^{+++}	9/65***	10/16** (63)
Cobalt chloride (800)	8	16	7/16*** (43.8)	6.43 ± 2.23	5.83 ± 1.94^{+}	10/45****	5/7* (70)

[a] Results are expressed as mean ± S.D.

$^{+}$ $p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ (Student *t* test).

* $p<0.05$, **$p<0.005$, ***$p<0.001$, ****$p<0.0001$ (Fisher's exact test).

Male Sprague Dawley rats (6/dose) received cobalt chloride in chow (0, 5 or 20 mg cobalt/kg bw) for 69 days. No information on general toxicity was provided. Significant decreased testis weight and testicular atrophy at 20 mg/kg bw, but not at 5 mg/kg bw. Testicular weights were only 42% of control testis weights (Nation, J.R.; *et al.* 1983).

Male CD1-mice (5/dose) were dosed with 100, 200 and 400 ppm of cobalt chloride hexahydrate in drinking water (23.0, 42.0 and 72.1 mg/kg bw) for 12 weeks in a dose response study or with 400 ppm for 13 weeks, followed by 20 weeks of recovery in a time course study. Fluid intake for the 400 ppm groups decreased to 77% of control in the time course study and to 81% of control in the dose response study. Body weight of the high dose group was slightly but significantly decreased in most weeks of the study. No further information on general toxicity was provided. Cobalt demonstrated a time and dose-dependent decrease in testicular weight and epididymal sperm concentration. Relative testicular weight was decreased to 71%, 52%, and 30% of control value. Epididymal sperm concentration was decreased to 66, 29 and 8% of control values. At 400 ppm,

this resulted in decreased fertility (7.8% vs 82.3% in controls). Serum testosterone concentrations in all cobalt groups were significantly ($p < 0.05$) elevated five- to seven-fold above control serum concentrations. FSH and LH serum levels were normal. In a time schedule study with 400 ppm (13 weeks treatment followed by 20 weeks recovery), sperm concentration declined from 81% at 9 weeks to 15% at 11 and 13 weeks. Fertility was decreased to 22% of control values in the dose response study at 400 ppm (no significant difference at ≤ 200 ppm) and in the time course study at 13 weeks (not at 7, 9 and 11 weeks). The fertility of cobalt treated mice, remained statistically depressed below control values throughout the recovery period. Other measured parameters substantiated this finding. Testicular weight also remained significantly depressed (Pedigo, 1988).

Table 80: Effects on testicular weight and sperm concentration in CD 1 mice after 13 weeks oral administration of cobalt chloride

	0 ppm	**100 ppm**	**200 ppm**	**400 ppm**
Testicular weight (% control value)	100	71	52	30
epididymal sperm concentration (% control value)	100	66	29	8
fertility	82.3			7.8

Male CD-1 mice (10/dose) were exposed to 0 or 400 ppm cobalt chloride via drinking water for 13 weeks. Evaluations were performed after 7, 9, 11 and 13 weeks and after 20 weeks of recovery. No information on general toxicity was provided. Reduction of testicular size, vascular congestion of various degrees and progressive degeneration of seminiferous tubules was observed from week 9 onwards. Changes of vessel epithelium in the testes were observed at all time points. No recovery of testicular weight was observed (Anderson, M.B. 1992).

Adult male Sprague-Dawley rats were maintained on a diet containing 0 or 265 ppm cobalt (as cobalt chloride) (20 mg Co/kg bw/day) for up to 98 days. Three rats of each dose group were sacrificed weekly and assayed for testicular damage by light and electron microscopy. Testicular congestion became apparent after 35 days of treatment. Degenerative changes became first apparent after 70 days of treatment, followed by a progressive deterioration of cell architecture and decrease in testicular volume. The degenerative changes were of a very general nature; e.g., thickening of basal lamina and basement membranes, increased packing of red blood cells in veins and arteries, formation of "giant" cells, loss of sperm tail filaments, and degeneration of sperm mitochondria. No cobalt residues could be detected by energy dispersive x-ray microanalysis. These data indicate that testicular degeneration was not a primary response to cobalt and suggest that the testes become hypoxic due both to blockage of veins and arteries by red blood cells and to changes in permeability caused by thickening of basal lamina and basement membranes. No information on general toxicity was provided in these studie (Mollenhauer, H.H *et al.*, 1985).

Male Sprague Dawley rats were given a daily diet containing 0 or 265 ppm Co as cobalt chloride hexahydrate (20 mg cobalt/kg bw/day) during a 98 day study period. Rats were sacrificed on day 1, 2, 4, 7, 14, 21, 28, 35, 42, 56, 63, 70, 84 and 98 (3 rats/dose/time point). No effects on body weight were observed. At 265 ppm, an increased erythrocyte count (141% of control), packed cell volume (156% of control), and haemoglobin concentration (128% of control) were observed. No lesions were found in control animals or in test groups killed on day 1-28. In rats killed on day 35 and thereafter, the testes were moderately to markedly congested. On day 70, lesions were present in the

seminiferous tubules (percentage damaged tubuli in the rats killed at day 70 was 20, 24 and 70%). Degenerative and non-necrotic lesions were present in the seminiferous epithelium of the less markedly affected tubules. Degenerative changes were present in Sertoli cells, spermatogonia, primary spermatocytes, and round spermatids. In severely damaged tubules, the changes were characterized by advanced degeneration and necrosis. The testes of the rats killed on day 98 were dark, congested, and reduced in size. Degeneration and necrosis of the germinal epithelium was present in 27%, 40%, and 90% of the seminiferous tubules of the three rats, respectively. An increased number of tubules were collapsed and devoid of germinal cells except for occasional spermatogonia and Sertoli cells along the basement membrane. Lesions were not observed in Leydig cells, epididymis nor in seminal vesicles of the cobalt-fed rats at any time during the study. Based on an increase in RBC a mechanism is suggested consisting of hypoxia caused by reduced blood flow(Corrier, D.E.; *et al.* 1985).

Inhalation studies

Fischer rats (5/sex/dose) were exposed to air containing cobalt sulphate heptahydrate at concentrations of 0 (chamber controls), 0.1, 0.5, 5, 50 or 200 mg/m³ (calculated on the basis of the anhydrous salt) 6 hours per day, for 12 exposures over **16 days** (whole body). Exposure to 200 mg/m³ cobalt sulphate heptahydrate as an aerosol resulted in deaths of all rats within 5 days. Several male rats exposed to 50 mg/m³ also died somewhat later. Atrophy of the testis, characterized by a decreased number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts, was observed in rats exposed to 50 mg/m³ (NTP, 1991).

B6C3F1 mice (5/sex/dose) were exposed to air containing cobalt sulphate heptahydrate at concentrations of 0 (chamber controls), 0.1, 0.5, 5, 50 or 200 mg/m³ (calculated on the basis of the anhydrous salt) 6 hours per day, for 12 exposures over **16 days** (whole body). All mice exposed to 200 mg/m^3 and 4/5 males and 1/5 females exposed to 50 mg/m^3 died before the end of the study. No effects on the testes were reported (NTP, 1991).

Groups of F344 rats (10/sex/dose) were exposed to aerosols containing 0, 0.3, 1.0, 3.0, 10, or 30 mg/m³ cobalt sulphate heptahydrate 6 hours per day, 5 days per week, for **13 weeks**. Mortality was not observed. No statistically significant effects were observed on sperm motility, counts or incidence of abnormal sperm and testis and epididymis histopathology (NTP, 1991).

Groups of B6C3F1 mice (10/sex/dose) were exposed to aerosols containing 0, 0.3, 1.0, or 3.0, 10, 30 mg/m³ cobalt sulphate heptahydrate 6 hours per day, 5 days per week, for **13 weeks**. Two males of the highest dose group died prematurely. Absolute and relative testes weight and absolute epididymal weight were decreased at 30 mg/m^3. The number of abnormal sperm was increased at 30 mg/m^3 and sperm motility was decreased at ≥ 3 mg/m^3 (lower concentrations not analysed). At the highest dose, atrophy of the testis was observed (n=9, vs 0 in controls), which consisted of a loss of germinal epithelium in the seminiferous tubuli; more severely affected testes also contained foci of mineralization (n=4 vs 0 in controls). The estrous cycle was significantly longer in females exposed to 30 mg/m^3(NTP, 1991).

In a carcinogenicity study, Fischer 344 rats (50/sex/dose) were exposed (whole body) to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m³ cobalt sulphate heptahydrate for 6 hours per day, 5 days per week, for **105 weeks**. There was no effect on survival or body weight. In a second carcinogenicity study, B6C3F1 mice (50/sex/dose were exposed (whole body) to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m³ cobalt sulphate heptahydrate for 6 hours per day, 5 days per week, for **105 weeks**. There

was no effect on survival. In both studies, no histopathological effects on reproductive organs were reported (NTP 1998).

4.11.1.2 Human information

4.11.2 Developmental toxicity

Cobalt metal

Rats (SD) (n=10 / dose / sex) were treated by gavage with powdered cobalt (0, 30, 100, 300 or 1000 mg/kg bw/day) (vehicle 0.5% hydroxypropyl methylcellulose gel) (no information on particle size available) from 2 weeks before mating until approximately 2 weeks after mating (males) or 3 days post-partum (females). All females and 9 out of 10 males died at 1000 mg/kg bw/day. No mortality occurred in males at lower dose levels. Eight out of 10 females treated with 300 mg/kg bw/day and five out of 10 females treated with 100 mg/kg bw/day died during the mating, gestation or lactation period. No mortality was observed in females treated with 30 mg/kg bw/day. There were no test item-related differences in the number and sex of pups, runts or malformed pups. No test item-related influence was noted in the values calculated for the gestation length, the birth index and the live birth index between the control group and the animals treated with 30 or 100 mg Cobalt Powder/kg b.w./day. Treatment with 300 mg Cobalt Powder/kg b.w./day resulted in a statistically significant (at $p \leq 0.01$) increase of the post-implantation loss (30.9%, control 12.6%) and significant decrease (at $p \leq 0.01$) in the live birth index (75.9%, control: 100 %). From 100 mg Cobalt Powder/kg b.w./day onwards, an increased F1-offspring mortality rate (stillbirths, prematurely deceased and cannibalised pups) was noted due to complete loss of litters of prematurely deceased dams. The mean viability index (group 3: 95.4%, group 4: 95.5%) was slightly decreased (control: 100). The mean litter weight of pups was slightly below the control weights on lactation day 0/1 (by up to 11%) and on lactation day 4 (by up to 18%) in groups 5 and 2 (30 or 100 mg Cobalt Powder/kg b.w./day). Distinct reductions were noted for the mean litter weight of pups in group 3 (300 mg Cobalt Powder/kg b.w./day (statistically significant at $p \leq 0.01$ in male and total pups) on lactation day 0/1 (up to 20% below the control) and on lactation day 4 (up to 27% below the control). The total litter weight of pups was below that of the control in group 2 (100 mg Cobalt Powder/kg b.w./day; female animals and total pups) and in group 3 (300 mg Cobalt Powder/kg b.w./day) due to the lower number of pups. No external abnormalities were observed in any of the pups examined (CoRC/CDI , 2015).

Cobalt sulphate (heptahydrate)

Szakmary *et. al* (2001) administered cobalt sulphate to pregnant CD1 mice, Wistar rats and New Zealand White rabbits.

Pregnant female C57BL mice (25 or 20/dose) were given 0 or 50 mg/kg bw of cobalt sulphate heptahydrate by gavage daily during GD6-15. Maternal weight gain was nonsignificantly decreased by the administered doses of cobalt. An increased frequency of foetuses with retarded body weight gain and skeletal retardation was observed. In addition, cobalt increased certain malformations (major anomalies of eyelids, kidneys, cranium and spine) although the increase was not statistically significant (table 81).

Table 81: Effects of cobalt on development of mice

Parameters	Control	Cobalt sulfate 50 mg/kg
Number of litters studied	25	19
Number of live fetuses	164	134
External malformations (major anomalies)		
Exencephalia	—	—
Ablepharia	—	1
Number of fetuses dissected	75	64
Visceral retardation	2	2
Visceral anomalies (major anomalies)		
Ectopia testis	—	—
Ectopia ovaries	—	—
Dilated pelvis renalis	—	1
Dilated ureter	—	—
Duplication of kidney	—	1
Alizarin-stained fetuses	89	70
Skeletal retardation[a]	27	58[b]
Skeletal anomalies (minor)		
Supernumerary ribs	—	—
Skeletal malformation (major anomalies)		
Cranium	3	15
Sternum	—	—
Ribs	1	—
Vertebra	7	12
Total number of fetuses	164	134
Number (%) of malformed fetuses	9 (10.1)	19 (27.1)[b]

Note. Doses are given as mg/kg bw.
[a] sternum hypoplasia, double vertebral ossification centers, shortened rib 13, dilated cranial sutures (rabbit).
[b] significant at $p<.05$ (Kruskal-Wallis test).

Pregnant female Sprague Dawley rats (3-18/dose) were given 0, 25, 50 or 100 mg/kg bw of cobalt sulphate heptahydrate by gavage daily during GD1-20 and were sacrificed on GD 21. In a second study, rats were treated until GD 21 (only 0 and 25 mg/kg bw/day) and were allowed to give birth. In this study, the development of the pups was followed up until pnd 21. Cobalt concentration in maternal blood, fetal blood and amniotic fluid (24 hours after the last exposure on day 20) increased in a dose dependent matter. The cobalt concentration in fetal blood was higher than in maternal blood showing placental transfer. Maternal body weight gain was not significantly affected. The relative liver, adrenal and spleen weight were increased at the highest dose level. Several clinical chemical parameters were changed statistically significant compared to the controls at the highest dose. RBC and Hb were increased at the highest dose but not statistically significant (n= 5 or 6). There were no effects on litter size, resorptions or post-implantation loss. The frequency of foetuses with retarded body weight and skeletal and visceral retardation significantly increased with the dose of cobalt sulphate. The two higher doses increased the frequency of malformations of the skeleton and the urogenital system (dilated ureter) (table 82). No statistically significant increase in a particular type of malformation was observed. The number of dams that died during delivery increased dose-dependently (0, 1, 5, 12 in the 0, 25, 50 and 100 mg/kg bw group, respectively). However, it is unclear how these dams can die during delivery as the protocol state that these dams were processed (meaning opening of the uterus) on day 21 of gestation. The perinatal index decreased from 92 ± 7 in the control group to 73 ± 9 in the treated group (25 mg/kg bw/day). The presence of post-natal maternal toxicity is not stated. Survival index was not affected. Fetal bw was significantly reduced on pnd 1 and 7, but not on pnd 14 and 21 (table 83). Some effects on the

maturation of the nervous system in the pups was observed at 25 mg/kg bw/day but this may be related to the lower body weights.

Table 82: Effects of cobalt on development of rats

Parameters	Control	Cobalt sulfate 25 mg/kg	Cobalt sulfate 50 mg/kg	Cobalt sulfate 100 mg/kg
Number of litters studied	15	18	17	14
Number of live fetuses	168	241	235	190
External malformations (major anomalies)				
Exencephalia	—	—	—	—
Ablepharia	—	—	—	—
Number of fetuses dissected	81	116	113	91
Visceral retardation	5	8	13[b]	18[b]
Visceral anomalies (major anomalies)				
Ectopia testis	—	—	1	—
Ectopia ovaries	—	—	1	—
Dilated pelvis renalis	—	—	1	—
Dilated ureter	—	1	1	4
Duplication of kidney	—	—	—	—
Alizarin-stained fetuses	87	125	122	99
Skeletal retardation[a]	18	35[b]	62[b]	66[b]
Skeletal anomalies (minor)				
Supernumerary ribs	—	—	4	2
Skeletal malformation (major anomalies)				
Cranium	—	—	1	—
Sternum	—	—	—	—
Ribs	—	—	—	—
Vertebra	1	2	3	3
Total number of fetuses	168	241	235	190
Number (%) of malformed fetuses	1 (0.6)	3 (1.2)	7 (2.9)[b]	7 (3.7)[b]

Note. Doses are given as mg/kg bw.
[a] sternum hypoplasia, double vertebral ossification centers, shortened rib 13, dilated cranial sutures (rabbit).
[b] significant at p<.05 (Kruskal-Wallis test).

Table 83: Effects on postnatal development of offspring in rats

	Control	25 mg/kg $CoSO_4$
Treated mothers (number)	15	15
Postnatal tested litters (number)	11	13
Live offspring (number)	104	103
Perinatal index (%)	92.0 ± 7.0	73.3[a] ± 6.6
Survival index (%)	85.1 ± 8.5	87.5 ± 4.2
Body weights of offspring		
Postnatal d 1	6.6 ± 0.09	5.7[a] ± 0.09
Postnatal d 7	14.5 ± 0.34	12.5[a] ± 0.47
Postnatal d 14	29.2 ± 0.95	28.2 ± 1.13
Postnatal d 21	51.7 ± 1.33	48.2 ± 1.87

[a]Significant at $p < .05$.

Perinatal index: 100*(number of live pups on d 5)/(number of live newborns)
Survival index: 100*(number of live pups on d 21)/(number of live pups on d5)

Pregnant New Zealand White rabbits (8-25/dose) were treated daily with cobalt sulphate (0, 20, 100, or 200 mg/kg bw) by gavage during GD6-20. All doses resulted in mortality (5/25, 4/13 and 7/8 dams died), due to circulatory failure. Total resorption was found in the only surviving dam of the group treated with a dose of 200 mg/kg, in all the 9 survivors out of 13 dams treated with a dose of 100 mg/kg, and in 6 of the 20 surviving dams treated with a dose of 20 mg/kg cobalt sulphate. It is noted that according to table 84, 20 litters were studied for effects on development. However, due to the death of 5, and total resorption in 6 animals, this should probably be 14 litters. Cobalt sulphate at 20 mg/kg proved to be embryotoxic for the surviving foetuses with inhibition of skeletal development Cobalt sulphate did not induce malformations in rabbits (table 80) (Szakmáry, E. *et al.* 2001).

Table 84: Effects of cobalt on development of rabbits

Parameters	Control	Cobalt sulfate 20 mg/kg
Number of litters studied	20	20
Number of live fetuses	165	102
External malformations (major anomalies)		
Exencephalia	—	1
Ablepharia	—	—
Number of fetuses dissected	165	102
Visceral retardation	—	1
Visceral anomalies (major anomalies)		
Ectopia testis	—	—
Ectopia ovaries	—	—
Dilated pelvis renalis	—	1
Dilated ureter	—	—
Duplication of kidney	—	—
Alizarin-stained fetuses	87	55
Skeletal retardation[a]	14	22[b]
Skeletal anomalies (minor)		
Supernumerary ribs	50	46
Skeletal malformation (major anomalies)		
Cranium	—	—
Sternum	1	—
Ribs	—	—
Vertebra	—	—
Total number of fetuses	165	102
Number (%) of malformed fetuses	1 (0.6)	2 (1.9)

Note. Doses are given as mg/kg bw.
[a] sternum hypoplasia, double vertebral ossification centers, shortened rib 13, dilated cranial sutures (rabbit).
[b] significant at $p<.05$ (Kruskal-Wallis test).

Cobalt chloride (hexahydrate)

Pregnant Wistar rats (15/group) were administered 0, 12, 24 or 48 mg cobalt chloride/kg bw/day on GD 14 to PND 21. Toxic effects in the dams are not described, although it is noted that toxic effects were observed in previous studies at doses of ≥ 24 mg/kg bw. The number of litters was reduced at all doses (see table below) although it is unclear whether these dams died, were not pregnant, did not give birth or gave birth to dead pups only. The ratio of living young/litter was statistical significant decreased and the ratio of dead young/litter increased at 48 mg/kg. A statistical

significant decrease of body weight, body length and tail length was observed at all dose levels (dose-dependent). No effects were observed on liver and renal function. No external malformations were observed (Domingo, 1985).

Table 85: summary of data from rat pups nursed by cobalt-treated mothers during a period of 21 days.

Day	Dose levels (mg/kg/day)	N.° of litters	N.° of living young	N.° of dead young	Dead / Living ratio (× 100)	Male / female ratio	Living young / litter	Dead young / litter	Average body weight / litter
1	0	12	120	4	3.33	0.93	10.0 ± 3.4	0.3 ± 0.9	74.8 ± 19.2
	12	5	64	3	4.68	1.06	12.8 ± 1.1	0.5 ± 0.9	80.9 ± 14.4
	24	6	56	7	12.50	0.93	9.3 ± 4.4	1.2 ± 1.5	54.8 ± 27.7
	48	7	60	15	25.00	1.00	8.6 ± 4.2	2.1 ± 1.7*	57.4 ± 17.0
4	0	12	114	6	5.26	1.00	10.7 ± 2.2	0.5 ± 0.4	110.8 ± 21.2
	12	5	61	3	4.92	1.10	12.1 ± 1.1	0.6 ± 0.4	111.5 ± 12.2
	24	6	51	5	9.80	0.88	8.5 ± 3.9	0.6 ± 0.3	80.8 ± 38.5*
	48	7	30	30	100.00	1.30	4.3 ± 5.4**	4.3 ± 3.7**	86.1 ± 14.2
21	0	12	106	8	7.55	1.02	8.8 ± 3.6	0.3 ± 0.2	392.2 ± 102.5
	12	5	57	4	7.02	1.10	11.8 ± 2.4	0.8 ± 0.6	357.1 ± 62.6
	24	6	44	7	15.91	0.90	8.3 ± 4.3	1.2 ± 2.4	279.0 ± 52.4**
	48	7	29	1	3.45	1.23	4.1 ± 2.0**	0.1 ± 0.0**	245.7 ± 16.8**

Table 86: Average body weight, body length and tail length of rat pups nursed by cobalt-treated mothers

Day	Dose levels (mg/kg/day)	Body weight (g) Males	Body weight (g) Females	Body length (mm) Males	Body length (mm) Females	Tail length (mm) Males	Tail length (mm) Females
1	0	7.18 ± 1.24 (41)	6.61 ± 1.16 (43)	53.8 ± 0.5	50.9 ± 0.5	18.2 ± 0.3	17.4 ± 0.3
	12	5.68 ± 0.69 (33)***	5.69 ± 1.02 (31)***	49.1 ± 0.3**	49.5 ± 0.3	15.7 ± 0.1***	15.8 ± 0.2**
	24	5.61 ± 1.13 (27)***	6.04 ± 0.82 (29)**	50.1 ± 0.4*	48.3 ± 0.3**	16.6 ± 0.2***	16.9 ± 0.2
	48	5.34 ± 1.17 (26)***	5.56 ± 1.08 (28)***	47.2 ± 0.4**	47.5 ± 0.3***	15.3 ± 0.2***	15.1 ± 0.1***
4	0	10.82 ± 2.14 (38)	10.00 ± 2.09 (39)	64.1 ± 0.5	61.3 ± 0.5	25.4 ± 0.4	25.3 ± 0.4
	12	9.03 ± 0.87 (30)***	8.78 ± 1.03 (29)**	62.1 ± 0.3*	61.3 ± 0.3	24.2 ± 0.2	24.1 ± 0.3
	24	8.64 ± 1.19 (27)***	8.75 ± 0.94 (29)**	62.0 ± 0.3*	60.9 ± 0.3	24.3 ± 0.2	24.5 ± 0.2
	48	8.65 ± 0.43 (18)***	8.83 ± 0.77 (18)**	59.8 ± 0.3***	58.5 ± 0.2*	22.7 ± 0.3***	23.0 ± 0.2*
21	0	43.06 ± 8.07 (35)	41.24 ± 8.59 (35)	109.6 ± 0.9	106.8 ± 0.8	75.8 ± 1.3	73.3 ± 1.2
	12	30.75 ± 7.97 (30)***	29.82 ± 7.34 (28)***	101.4 ± 0.7***	100.1 ± 0.7***	70.0 ± 0.6*	70.8 ± 0.6
	24	26.69 ± 5.12 (23)***	27.33 ± 4.28 (28)***	94.7 ± 0.9***	95.8 ± 0.9***	67.9 ± 1.2***	65.2 ± 1.1**
	48	25.70 ± 3.22 (14)***	28.73 ± 6.65 (13)***	91.6 ± 0.8***	88.3 ± 0.7***	59.1 ± 0.9***	56.6 ± 0.8***

Pregnant Sprague-Dawley rats were given by gavage a daily dose of 0, 25, 50, and 100 mg/kg cobalt(ll) chloride hexahydrate on gd 6–15. Females were sacrificed on d 20. Maternal body weight gain was significantly reduced, particularly at 100 mg/kg bw. In addition, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and reticulocytes were increased significantly in the 100-mg/kg bw group. No treatment-related changes were recorded in the number of corpora lutea, total implants, resorptions, the number of live and dead fetuses, fetal size parameters, or fetal sex distribution data. An increased (not statistically significant) incidence of stunted fetuses per litter was observed at 50 and 100 mg/kg·d group. No gross external abnormalities, skeletal malformations, or ossification variations were observed (Paternain, 1988).

Sexually mature male mice were exposed to 200, 400 and 800 ppm cobalt chloride hexahydrate (25.7, 46.9 and 93.0 mg/kg bw/day) in their drinking water for 12 weeks. Males were than mated with untreated female mice. Average body weight gain was significantly reduced in all dose groups (final body weights were 95, 94 and 93% of the control group). Two animals out of 10 and one out of 10 died during the 10th weeks of the exposure to 800 and 400 ppm cobalt chloride, respectively.

There were no other signs of clinical toxicity observed in the survived animals. Resorptions were increased at all doses whereas the number of viable foetuses was decreased (table 79). It is noted that the number of pregnant females at 400 ppm is lower than the number of animals with resorptions according to table 79. This is not explained (Elbetieha, A. *et al.* 2008).

Pregnant Crl:CD(SD) rats (25/group) were given by gavage a daily dose of 0, 25, 50, and 100 mg/kg Cobalt dichloride hexahydrate (in tap water) on gd 6–19. The study was performed according to OECD GL 414. 20 litters per dose group were analysed. No mortality was observed in the dams. At doses ≥ 50 mg/kg bw, piloerection was observed, as well as reduced motility and salivation. At 100 mg Cobalt dichloride hexahydrate/kg b.w./day a haemorrhagic nose/snout was additionally noted for 3 of 20 dams on gestation days 19 or 20. Net body weight change was significantly reduced in all dose groups (52.8, 127.7 and 136.8%, respectively). No effects were observed on gravid uterus weight (see table 87). Food consumption was decreased at 50 and 100 mg/kg bw. A significant reduction in food consumption was also observed for the low dose group at day 19 to 20. Gastro-intestinal lesions in form of haemorrhagic foci in the stomach and intestines were noted in a dose related way for the dams dosed with 50 or 100 mg/kg bw/day. Effects on hematological parameters (HGB, RBC, Reti, PLT, HCT, MCHC, abs Lym, Mono, Eos and Baso) were also observed in these dose groups. No test item-related changes were noted for number of resorptions and post-implantation loss (see table 88). There was a slight but statistically significant reduction on mean fetal weights by 8% in the mid and high dose groups (although within LPT background levels, see table 89)). No effects were observed on number of dead fetuses, and number of malformations, retardations or variations (CDI/CORC 2015).

Table 87 Effects on body weight

	control	25 mg/kg bw	50 mg/kg bw	100 mg/kg bw
Body weight gain in gram gestation day 0 to 20 (% of controls)	153.1	138.9 (-9.3%)	102.7** (-32.9%)	98.3** (-35.8%)
BW at GD20 (% of controls)	374.68	354.97 (-5.3%)	321.61** (-14.2%)	318.05** (-15.1%)
Net bw change GD6-20 (% of controls)	38.4	18.1* (-52.8%)	-10.6* (-127.7%)	-14.1* (-136.8%)
Gravid uterus weight	75.55	81.78	76.91	73.11

Table 88 Effects on reproductive parameters.

		TEST GROUP 1 Control	TEST GROUP 2 25 mg/kg	TEST GROUP 3 50 mg/kg	TEST GROUP 4 100 mg/kg
Females Pregnant	N	20	20	20	20
Aborted	N	0	0	0	0
Premature Birth	N	0	0	0	0
Dams with Viable Fetuses	N	20	20	20	20
Dams with all Resorptions	N	0	0	0	0
Female Mortality	N	0	0	0	0
	%	0	0	0	0
Pregnant at C-section	N	20	20	20	20
	%	100	100	100	100
Corpora Lutea	MEAN	14.6	15.2	15.0	14.8
	S.D.	2.6	2.4	1.8	1.9
	TOTAL	292	304	299	295
Implantation Sites	MEAN	13.5	14.0	14.7	13.9
	S.D.	1.5	2.4	1.6	2.4
	TOTAL	270	280	293**	277 ##
Pre-implantation Loss	MEAN%	6.2	7.9	1.8	6.1
	S.D.	10.1	10.3	3.5	10.5
Post-implantation Loss	MEAN%	3.9	1.8	2.0	2.7
	S.D.	5.8	4.0	3.2	4.2

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P≤0.05 ** = P≤0.01 (Fisher or Chi-square test)
INCLUDING ONE SET OF TWINS (ONE MALE FETUS / ONE LATE RESORPTION)

		TEST GROUP 1 Control	TEST GROUP 2 25 mg/kg	TEST GROUP 3 50 mg/kg	TEST GROUP 4 100 mg/kg
Pregnant at C-section	N	20	20	20	20
Resorptions: Total	MEAN	0.6	0.2	0.3	0.4
	S.D.	0.8	0.5	0.5	0.6
	TOTAL	11	4 *	6	8 ##
	MEAN%	3.9	1.5	2.0	2.7
	S.D.	5.8	3.8	3.2	4.2
Early	MEAN	0.6	0.2	0.2	0.4
	S.D.	0.8	0.5	0.4	0.6
	TOTAL	11	4 *	3 *	7
	MEAN%	3.9	1.5	1.0	2.4
	S.D.	5.8	3.8	2.5	4.1
Late	MEAN	0.0	0.0	0.2	0.1
	S.D.	0.0	0.0	0.4	0.2
	TOTAL	0	0	3	1 ##
	MEAN%	0.0	0.0	1.0	0.3
	S.D.	0.0	0.0	2.5	1.4
Dead fetuses	N	0	1	0	0

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P≤0.05 ** = P≤0.01 (Fisher or Chi-square test)
##: ONE LATE RESORPTION RESULTING FROM ONE SET OF TWINS (DAM NO. 95).

		TEST GROUP 1 Control	TEST GROUP 2 25 mg/kg	TEST GROUP 3 50 mg/kg	TEST GROUP 4 100 mg/kg
Dams with Viable Fetuses	N	20	20	20	20
Live fetuses	MEAN	13.0	13.8	14.4	13.5
	S.D.	1.5	2.5	1.6	2.3
	TOTAL	259	275	287	270
	MEAN%	96.1	98.2	98.0	97.3
	S.D.	5.8	4.0	3.2	4.2
Females	MEAN	6.4	7.0	7.5	6.0
	S.D.	2.0	2.4	2.0	2.7
	TOTAL	128	139	150	119
	MEAN%	47.5	50.9	51.3	42.3
	S.D.	14.3	17.7	13.3	17.2
Males	MEAN	6.6	6.8	6.9	7.6
	S.D.	1.8	2.4	2.1	2.4
	TOTAL	131	136	137	151
	MEAN%	48.6	47.7	46.6	54.9
	S.D.	12.9	17.0	12.9	15.8
PER CENT LIVE FEMALES		49	51	52	44
PER CENT LIVE MALES		51	49	48	56

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P≤0.05 ** = P≤0.01 (Fisher or Chi-square test)

Table 89 Effects on foetal body weight

Parameter	Mean value observed in this study [mean weight (g) per dam]	LPT background data range of individual values [fetal incidence in mean %] (n = 56 control or n = 143 test item groups data taken from 2000 - 2014)#[1-2]
All viable fetuses (g)	Control: 3.6 Group 2: 3.7 Group 3: 3.3 * Group 4: 3.3 *	3.2 - 4.0 (control) 3.1 - 4.0 (test item groups)
Male fetuses (g)	Control: 3.7 Group 2: 3.8 Group 3: 3.4** Group 4: 3.4 *	3.2 - 4.1 (control) 3.2 - 4.2 (test item groups)
Female fetuses (g)	Control: 3.5 Group 2: 3.6 Group 3: 3.2** Group 4: 3.2 *	3.1 - 3.8 (control) 3.0 - 3.9 (test item groups)

#[1]: data not audited by QAU
#[2]: the dosing duration of the historical data sets was similar to the scheme in the present study up to gestation day 19
*: Significantly different from the controls at p ≤ 0.05
**: Significantly different from the controls at p ≤ 0.01

4.11.2.1 Non-human information

4.11.2.2 Human information

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Fertility

Inhalation exposure of rats and mice to cobalt resulted in a decrease in sperm motility at 0.625 mg Co/m^3 in rats and at least 2.5 mg Co/m^3 in mice in the 14-week studies. This parameter was not determined in the 17-day and the 2-year study. More severe effects were observed in the chronic study in rats at 5 mg Co/m^3 (testes infarction) and in the mice at 5 mg/m^3 in the 14-week and chronic study. These effects in mice became very severe only at 10 mg Co/m^3 (dose not tested in rats).

Inhalation exposure of rats and mice to cobalt sulphate resulted in effects on the testes in rats only at 10.5 mg/m3. In mice, effects were already observed at 0.63 Co mg/m^3 (sperm motility) but became severe at 6.3 mg Co/m^3.

These inhalation studies show a consistent effect between cobalt and cobalt sulphate in mice with a reduction in sperm motility observed at 0.6 mg Co/m^3 and more severe effects at 6-10 mg Co/m^3. In rats exposed to cobalt this decrease in sperm motility was also observed but not with cobalt sulphate. Also no severe effects were observed at higher dose levels but this may be caused by the somewhat lower dose levels tested.

In an oral combined toxicology and reproductive screening study in rats with cobalt powder, no effects on male reproductive organs and fertility were observed up to the highest tested dose level of 1000 mg/kg bw/day (gavage). The absence of or presence of very limited haematological effects

(typical for systemic Co^{2+}) may indicate limited bioavailability of Co^{2+} via the oral route with gavage exposure. This low bioavailability is in contrast with the results of the bioelution study in artificial gastric fluid of 61% which indicated a good bioavailability. Due to the short duration of this study, observation of structural and functional effects on male fertility is unlikely as these effects are normally observed only after a longer exposure period.

In oral (diet and drinking water) studies designed to study the effect of cobalt on the testis in rats and mice, the effects were observed with a significant delay of 35 days before structural effects on the testes were observed in rats. In mice structural effects were observed after 56 days but were not studied earlier. Reduced fertility did not appear until week 11 in mice (Pedigo 1993, Elbetieha, 2004 and Pedigo 1988) (no fertility data available for rats). All effects were observed at around 10 to 20 mg Co/kg bw/day. A 90-day study with cobalt chloride did not induce any effect on the testes. However, the applied dose levels (30 mg/kg bw/day = 7.5 mg Co/kg bw/day) were below the dose level of approximately 10-20 mg Co/kg bw/day that induces structural and functional effects on male fertility in rats.

Structural and functional effects on male fertility were mainly observed in the presence of clear effects on the lung and an increase in RBC/Hb in the inhalation studies and an increase in RBC/Hb in the oral studies. As comparable effects were seen via both routes it is unlikely that the fertility effects are secondary to the lung effects. It is 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. The increase in RBC is most likely caused by the effect of Co^{2+} on the hypoxia inducing factors. However, this mechanism would also be applicable to all other tissues which are supplied with oxygen using the same blood. Also, the slower blood flow contains more Hb and therefore more oxygen which could compensate the lower blood flow. A direct effect of Co^{2+} on the hypoxia inducing factors in the testes can also be considered. Overall, no mechanism has been identified. Also, reduced sperm mobility was already observed in the 14-week inhalation study in mice at a dose level of 2.5 and 5 mg/m^3 at which no statistical increase in RBC was observed and at 10 mg/m^3 there were severe effects (including a strong reduction in sperm counts and motility) with only a very limited (4%) but statistically significant increase in RBC concentration. Therefore, it is unlikely that the structural effects on the male reproductive organs and sperm parameters are secondary to the increase in RBC. As a result, the effect on male fertility is not considered secondary to general toxic effects.

It is noted that rats and mice have a sperm reserve that is much larger than in humans. In humans, adverse effects on sperm caused by cobalt chloride, but also by cobalt metal or other cobalt compounds, may result in decreased fertility sooner than in laboratory animals. Actual studies on fertility are only performed with cobalt and cobalt chloride and show contradicting results. However, the effects on sperm parameters observed with cobalt metal, cobalt chloride and cobalt sulphate are very similar and observed in two species and via two exposure routes. The severity of these effects in the 14-week inhalation study with cobalt powder in mice (NTP, 2014) with a reduction of sperm counts by 92-94% with a sperm motility of the remaining sperms of 2.6% is comparable to the structural effects in the study by Pedigo and Vernon (1993) which resulted in a clear decrease in fertility in mice It can therefore be expected that the effects on the male reproductive system as observed with cobalt metal and cobalt sulphate, may also reduce fertility.

Development

The combined repeated dose toxicity and reproductive screening study with cobalt powder (oral exposure) shows no developmental effects at dose levels without maternal mortality (CDI 2015). However, oral developmental studies with cobalt sulphate in rats and mice result in a reduced foetal body weight and skeletal retardation at dose levels that did not significantly affect maternal body

weight. In addition, malformations of eyelids, kidneys, cranium and spine (mice) and vertebra and urogenital system (rats) are reported but without statistical significance (Szakmary 2001). In rats treated until day 21 of gestation, the number of litters and perinatal index were adversely affected. Although there was a slight reduction in maternal body weight, this was not statistically significant. Treatment until day 21 of gestation resulted in a clear increase in dams died during delivery (Szakmary 2001). Also a developmental study with cobalt chloride in rats resulted in decreased foetal weight, as well as in reduced length, already visible at doses that do not induce maternal toxicity (Domingo *et al.*, 1985). In rats as well as mice, the effects on viability are only observed in the early postnatal period. In another developmental study with cobalt chloride (according to OECD TG) however, no effects on pre-natal viability was observed, although maternal toxicity (gastrointestinal lesions and severely reduced body weight gain) was observed (Paternain *et al.*, 1988). No developmental effects except for reduced foetal body weight in the presence of reduced maternal body weight were observed in the OECD TG 414 and GLP compliant study with cobalt dichloride hexahydrate with dosing up to 100 mg/kg bw/day (24.8 mg Co/kg bw/day) (CDI/CORC, 2015).

4.11.5 Comparison with criteria

No useful human information on the effects of cobalt on fertility or development is available. Classification as Rep 1A is therefore not possible.

Substances should be classified as Rep 1B based on clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Substances should be classified as Rep 2 when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Although actual effects on fertility are only tested and shown in 1 species (mice), the preceding effects on sperm parameters are consistently observed in mice as well as in rats, and with several cobalt compounds (including cobalt metal). The absence of such effects in the two recent regulatory studies can be explained by the low dose level in the oral 90-day study with cobalt chloride and by the short exposure duration and the possibly low bioavailability of Cobalt after gavage exposure. Since adverse effects on the reproductive system that may also be relevant for humans are observed in multiple studies and in two species, it is concluded that there is clear evidence for an effect on (male) fertility and cobalt metal should be classified in Repr. 1B.

A specific concentration limit for Repr. 1B H360F is not warranted as severe effects on male reproductive organs and on fertility are only seen at dose levels above 4 mg/kg bw/day.

Only few studies are available for development. The screening study with cobalt metal does not show developmental effects at the lowest dose level which induced limited maternal mortality. Although some maternal toxicity was observed in some of these studies, effects on development (including reduced body weight and body length, reduced viability and malformations of skeleton and urogenital system) are also observed in rats and mice at doses that are not toxic to the dams. However, no specific type of malformation was statistically significant and the effects were not observed in the OECD TG 414 study with cobalt chloride with comparable cobalt exposures. Therefore, these effects do not warrant classification. For the increase in dead dams during delivery

it is unclear whether this effect is a reproductive effect or maternal toxicity. This effect was also observed in the screening study with cobalt metal (mortality around day 20/21) but these dose levels also induced mortality at other time points. The increase in postnatal mortality in rats treated with soluble cobalt compounds were partly seen at dose levels with unknown maternal toxicity (Domingo, 1985) but also at dose levels without maternal toxicity but with some limitation (Szakmary, 2001). Classification could be considered However, such effects were not observed with cobalt powder (screening study). Therefore, these effects also do not warrant classification.

4.11.6 Conclusions on classification and labelling

Cobalt and soluble cobalt compounds induce adverse effects on the male reproductive system, resulting in decreased fertility. No clear teratogenic effects were observed in the available developmental studies with cobalt and cobalt compounds. Some studies with prolonged soluble cobalt exposure show death during delivery (dams) and postnatal mortality (foetuses). These tests have some limitations in reporting but classification could be considered. However, these effects were not observed in the screening study with cobalt powder. Therefore, these effects do not warrant classification for cobalt. Therefore, cobalt should be classified as Repr 1B; H360F.

No data are available to determine if effects through lactation occur. Therefore, no labelling is proposed for lactation, due to lack of data.

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. Studies with cobalt metal

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".

Additional key elements

A prospective, well-presented study on sperm parameters in male patients with hip arthroplasty has been recently published (Chen *et al.*, 2016). Comparing preoperation and postoperation values in patients with metal-on-metal articulations in total hip arthroplasty, the authors showed an increase in concentration of cobalt in blood and semen, and 24% reduction in percentage of morphologically normal sperm.

Nevertheless, several limitations are pointed out by the study authors. As a pilot study, only 50 patients (25 with metal-on-metal endoprothesis) were enrolled. The study period was only 1 year after operation (the running-in period for endoprothesis), so the long-term effects on seminal parameters could not be evaluated. Also, control subjects were men with metal-on-polyethylene (MoP) hip arthroplasty, and not healthy subjects.
Therefore, in the opinion of RAC, this study can only be considered as a supportive evidence for cobalt effects on male fertility.

"Abstract (Chen et al., 2016)
Purpose: The widespread usage of metal-on-metal (MoM) articulations in total hip arthroplasty (THA) has been tempered by concerns of increased metal ion production. The purpose of the study is to evaluate the influence of metal ion exposure on semen quality in young male patients undergoing THA. Methods: Male patients who were scheduled for unilateral THA and aged between 20 and 45 years were prospectively enrolled. Patients were sorted into MoM and metal-on-polyethylene (MoP) groups with equal case number. Semen and blood metal ion

levels were measured and sperm analysis was performed before, 6 months after, and 1 year after surgery. Results: Compared to preoperative baseline, patients (n=50) in both groups had increased cobalt (Co) and chromium (Cr) concentrations in blood and seminal fluid after surgery. Between-group comparisons at 6 months and 1 year after surgery showed that patients in the MoM group both had a greater Co concentration in blood and semen and a greater Cr concentration in blood and semen. Patients receiving MoM prosthesis had a reduced percentage of morphologically normal sperm, and decreases from the preoperative level (44.7%) were significant at 6 months (36.8%, p=0.03) and 1 year (33.8%, p=0.004). Conclusions: Our data shows a significantly greater concentration of metal ion in blood and semen in patients with MoM prosthesis with a reduced percentage of morphologically normal sperm. Despite small effects on sperm quality, some concerns remain. Further studies are necessary to determine sources of metal ion and to investigate effects on male fertility."

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	$CoSO_4 \times 7H_20$	Co metal	$CoSO_4 \times 7H_20$ **	Co metal	$CoSO_4 \times 7H_20$
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/m³)	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 11^{th} 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.

Summaries of the studies on reproductive toxicities presented in the CLH report

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.

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

1. STUDIES WITH COBALT METAL

1.1. Oral exposure

CDI/CORC 2015a - Combined repeated dose toxicity and reproduction screening study in rats with cobalt metal

The study was performed in line with OECD TG 422. SD rats (10 animals/dose/sex) were dosed by gavage with 0, 30, 100, 300 or 1000 mg/kg bw/d of **powdered cobalt**, (purity >99.8%; vehicle 0.5% hydroxypropyl methylcellulose gel; particle size: D50=12.8 µm) from 2 weeks before mating until approximately 2 weeks after mating (males) or 3 days post-partum (females).

General toxicity, neurotoxicity and reproductive toxicity were evaluated in the study (Table below). Detailed histopathologic examination was performed on one testicle and one epididymis, including evaluation of qualitative stages of spermatogenesis and histopathology of interstitial structure, of all adult males dosed at 0, 30, 100 and 300 mg/kg bw/d.

General toxicity

Mortality and other signs of general toxicity (decreased body weight and relative food intake, clinical signs, pathological organ changes) were more pronounced in females, starting at lower doses (≥ 100 mg/kg bw/d) compared to males (reduced body weight at ≥ 300 mg/kg bw/d, mortality, clinical signs and pathological organ changes at 1000 mg/kg bw/d). Mortality in females was observed during mating, gestation and lactation.

No exposure-related changes in haematological or biochemical parameters were observed (on day 15), although small increases in haemoglobin values were observed in males (4-8%; dose groups not stated).

Fertility
In males, no treatment related effects on fertility were observed (Table 72 in the CLH report, additional document "Repr. appendix"). There was a decrease in absolute weight of epididymides, but without any dose-effect relationship (the effect was observed only at the lowest dose, 30 mg/kg bw/d). There was no effect on sperm number, viability and morphology at any of the tested dose levels. However, the absence of (or presence of very limited) haematological effects typical for systemic cobalt toxicity may indicate limited bioavailability of cobalt given by gavage. In addition, it was pointed out by the DS that the effects on male fertility are expected after an exposure period longer that the one applied in this study (e.g. Pedigo *et al.*, 1988).
In females, fertility indices (including pre-coital time, number of pregnant females, fertility index, number of corpora lutea) were not affected.

Development
Number of dams with stillborn pups, number of stillbirths, and pre- and post-implantation losses were increased, and mean number of live born pups and live birth index decreased at 300 mg/kg bw/d, a dose at which significant general maternal toxicity (including mortality) occurred. At this dose an increase in placenta with mild or moderate congestion was also noted.
In exposed groups, mean pup weights were dose relatedly decreased at day 0 and day 4, but the decrease was statistically significant only at 300 mg/kg bw/da, and it was within the historical control range. Viability index was significantly reduced at 100 mg/kg bw/d but within the historical control range (data for viability index and historical control data not presented). No external abnormalities were observed at any dose level.

Table: CDI/CORC 2015a, Combined repeated dose toxicity and reproduction screening study in rats

	powdered cobalt (mg/kg bw/d)				
Males	**0**	**30**	**100**	**300**	**1000**
N	10	10	10	10	10
Mortality	0/10	0/10	0/10	0/10	9/10
Body weight (% of control)	-			87 (week 3)	84 (week 3)
Relative food intake (% of control)		no effect	no effect	no effect	no effect
Piloerection			+	+	+
Pre-lethal symptoms[a]					+
Reduced forelimb grip strength			+	+	+
Spleen relative weight		↑	↑	↑	↑
Pathological changes of organs[b]		NM			+

Fertility indices:					
Absolute weight	-	77*	94	87	NM
epididymides (left, right; % of control)		81*	99	97	
	powdered cobalt (mg /kg bw/d)				
Females	**0**	**30**	**100**	**300**	**1000**
N	10	10	10	10	10
Mortality	0/10	0/10	5/10	8/10	10/10
Body weight (% of controls)	-			89 (G20) 79 (L1) 80 (autopsy)	NA
Relative food intake (% of controls)				63 (L1)	32 (L1)
Piloerection			+	+	+
Pre-lethal symptoms[a]			+	+	+
Reduced forelimb grip strength				+	+
Spleen relative weight		↑	↑	↑	NA
Pathological changes of organs			+[c,d,f]	+[c,f,g]	+[c,e]
Fertility/development indices:					
N of pregnant females	10/10	9/10	8/9	6/6	NA
N of dams with stillborn pups	0	0	1	3	NA
N of stillbirths	0	0	2	15	NA
Mean N of live born pups (% of control)	-	117	98	68*	NA
Live birth index	100	100	98	76*	NA
Pre-implantation loss (% of control)	-	94	106	148*	NA
Post-implantation loss (% of control)	-	53	79	245*	NA
Viability index			↓*		NA

*Statistically significant difference compared to controls (P<0.05; Student *t*-test, Dunnett's test, Chi-square test); [a]Reduced motility, soft faeces, hunched posture, increased or decreased respiratory rate, reduced body temperature (animal cold at touch), dyspnoea, tremor, miosis, ptosis, a haemorrhagic nose, haemorrhagic urine, pale skin; [b]Reddened stomach, intestines, caecum and thymus, enlarged and/or reddened adrenals, oedematous lungs; [c]Reddened, haemorrhagic foci, filled with fluid in the gastro-intestinal tract, dose related changes; [d]Reddened thymus; [e]Enlarged and/or reddened adrenals, oedematous lungs; [g]Adrenal congestion, increased placentas with mild or moderate congestion; N – number; week – test week; G20 – gestation day 20; L1 – lactation day 1; NA – not applicable (100% mortality); NM – not measured (analysed).

Organs in females dosed at 30 mg/kg bw/d were not examined histopathologically. Food intake was not changed in males. No test item-related changes in haematological or biochemical parameters were observed on day 15, although

some small increases were observed in haemoglobin in males (4-8%). No biologically relevant effects on organ weights were reported with the exception of effects on the spleen. In females the relative weight increased in a dose related manner up to 165%, and in males up to 134%.

1.2. Inhalation exposure

Six NTP inhalation studies with cobalt metal are presented in the CLH report. Male and female F344/N or F344/NTac rats and B6C3F1/N mice were exposed to cobalt metal by inhalation (6 hours per day, 5 days per week) for approximately 2 weeks, 3 months, or 2 years. In all experiments animals were exposed for 6 hours plus T_{90} (12 minutes) per day (T_{90} = the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation), 5 days per week. The inhalation route was chosen by NTP because this is the most common route of exposure to cobalt metal dust in occupational settings in humans.

NTP 2014a: 16-day inhalation study in rats with cobalt metal

F344/N rats (5/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, for 16 days. No haematology was performed in this study.

General toxicity

Mortality occurred at ≥ 20 mg/m^3, with the majority of deaths occurring by day 7. Mean body weight and body weight gain were significantly decreased in males exposed to ≥ 10 mg/m^3, and in females exposed to ≥ 20 mg/m^3 (Table 14 and Table 19 in the CLH report). Clinical signs of toxicity (abnormal breathing, lethargy, and thinness) were present in males exposed to ≥ 20 mg/m^3 and in females exposed to 40 mg/m^3.

Absolute and relative lung weights were increased in males and females dosed at ≥ 10 mg/m^3, and histopathological changes in lungs and nose were present from the lowest dose tested (2.5 mg/m^3) (Table 14 and Table 20 in the CLH report), although the average severity of the lesions was minimal to mild up to the dose of 10 mg/m^3 (and minimal to moderate above 10 mg/m^3).

Fertility

Absolute testis weight was significantly decreased in the group exposed to 10 mg/m^3 (0.590 g *vs.* 0.886 g in controls). Relative testis weight was also reduced (5.05 g *vs.* 6.17 g in controls) but not significantly. Histopathology of testes was not performed at this dose level.

Table: NTP 2014a: 16-day inhalation study in rats

	Cobalt metal (mg/m^3)					
Males	**0**	**2.5**	**5**	**10**	**20**	**40**
Survival	5/5	5/5	5/5	5/5	0/5[a]	0/5[b]
Final weight (% of control)	-	100	97	80	NA	NA
Clinical signs[c]					+	+
Organ wt (% of controls)						
Kidney – relative wt	-	100	97	108*	NA	NA
– absolute wt		100	95	85*		
Liver – relative wt	-	87*	90*	92*	NA	NA
– absolute wt		87	87*	73*		
Lung – relative wt	-	102	107	141*	NA	NA
– absolute wt		102	104	112		
Left testis – relative wt	-	105	99	82	NA	NA

– absolute wt	-	105	96	67*	NA	NA
Thymus – relative wt	-	95	96	96	NA	NA
– absolute wt	-	96	96	76*	NA	NA
Histopathological changes of organs[d]						
Lungs						
Haemorrhage	0	0	0	1	5	5
Acute inflammation	0	0	0	0	4	5
Alveolar epithelial hyperplasia	0	0	0	0	3	5
Alveolar cellular infiltration, histiocytes	0	0	4	3	5	5
Bronchiolar epithelium, cytoplasmic vacuolisation	0	5	5	5	1	0
Bronchiolar epithelium, necrosis	0	0	0	0	2	3
Interstitial fibrosis	0	0	0	5	2	0
Nose						
Olfactory epithelium, necrosis	0	3	4	4	4	5
Olfactory epithelium, atrophy	0	5	5	5	3	3
Respiratory epithelium, necrosis	0	0	0	1	3	5
Respiratory epithelium, squamous metaplasia	0	0	0	1	2	1
		Cobalt metal (mg Co/m^3)				
Females	**0**	**2.5**	**5**	**10**	**20**	**40**
Survival	5/5	5/5	5/5	5/5	2/5[e]	0/5[f]
Final weight (% of control)	-	100	96	88	55	NA
Clinical signs[c]						+
Organ wt (% of controls)						
Kidney – relative wt	-	96	99	102	123*	NA
– absolute wt						
Liver – relative wt	-	92	93	97	116*	NA
– absolute wt						
Lung – relative wt	-	96	110	137*	215*	NA
– absolute wt						
Thymus – relative wt	-	102	116	104	36*	NA
– absolute wt						
Histopathological changes of organs[d]						
Lungs						
Haemorrhage	0	0	0	0	3	5
Acute inflammation	0	0	0	0	2	5
Alveolar epithelial hyperplasia	0	0	0	0	2	2
Alveolar cellular infiltration, histiocytes	0	0	0	0	5	5
Bronchiolar epithelium, cytoplasmic vacuolisation	0	4	5	5	3	0

Bronchiolar epithelium, necrosis	0	1	1	4	3	3
Interstitial fibrosis	0	0	0	4	3	0
Nose						
Olfactory epithelium, necrosis	0	5	3	5	5	5
Olfactory epithelium, atrophy	0	5	5	5	4	1
Respiratory epithelium, necrosis	0	0	0	0	5	5
Respiratory epithelium, squamous metaplasia	0	0	0	0	1	0

*Statistically significant difference compared to controls ($p \leq 0.05$, Williams' or Dunnett's test); [a]Days of deaths: 5, 5, 5, 9, 13; [b]Days of deaths: 5, 6, 6, 7, 7; [c]Abnormal breathing, lethargy, and thinness; [d]Average severity grade of lesions observed was minimal to mild at ≤ 10 mg/m^3, and minimal to moderate >10 mg/m^3; [e]Days of deaths: 5, 7, 13; [f]Days of deaths: 5, 6, 6, 6, 7; NA – not applicable (100% mortality); wt – weight; Relative organ weights were calculated in relation to body weight.

NTP 2014b: 14-week inhalation study in rats with cobalt metal

F344/N rats (10/sex/dose) were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, or 5 mg/m^3, for 14 weeks.

General toxicity

All animals survived to the end of the study. Body weight was lower in males and females exposed to 5 mg/m^3 (93% of controls). There were no clinical signs related to cobalt exposure.

In male rats, dose-related increases in the haemoglobin concentration, erythrocyte count, haematocrit value, and packed cell volume occurred in all exposed groups by week 14 (up to 27-30% increase in the top dose group compared to controls). At week 14, female rats also had an increase in these parameters (up to 22-23% increase in the top dose group compared to controls), although at the lowest dose only haemoglobin was increased. Certain biochemical parameters (decrease in cholesterol in both sexes, decrease in blood glucose in males) were also affected in a dose-related manner during the study.

The increased lung weight, which was related to lung histopathologic changes, was observed in all exposed groups of males and females.

Fertility

Sperm motility was slightly but statistically significantly decreased in all males exposed to cobalt (3-8% lower than control) with a dose-effect relationship. No effects on testis and epididymis weight, spermatid and sperm counts or testis histopathology were observed.

In top dose females (5 mg/m^3), a higher probability of extended dioestrous compared to controls was indicated. However, as pointed out in the NTP 2014 report, the toxicological significance of this subtle alteration in the oestrous cycle is not clear. Oestrous cycle length was not affected, and cobalt-related histopathologic findings were not observed in the female reproductive organs.

Table: NTP 2014b: 14-week inhalation study in rats

	Cobalt metal (mg/m^3)				
Males	**0**	**0.625**	**1.25**	**2.5**	**5**
Survival	10/10	10/10	10/10	10/10	10/10
Final weight (% of control)	-	105	102	102	93
Organ wt (% of controls)					
Lung – absolute wt	-	122	131*	125*	126*

– relative wt		116	128*	122*	135*
Testis – absolute wt	-	103	102	102	102
– relative wt	-	98	100	100	110*
Haematology (week 14; % of control)					
Haemoglobin	-	104*	120*	126*	130*
Red blood cells	-	105*	122*	128*	129*
Haematocrit	-	104*	119*	125*	129*
Platelets	-	95	90*	80*	84*
Histopathological changes of respiratory system[a]					
Lungs					
Chronic active inflammation	0	10	10	10	10
Alveolar proteinosis	0	10	10	10	10
Alveolar epithelial hyperplasia	0	0	0	0	2
Bronchiolar epithelium, hyperplasia	0	0	10	10	10
Nose					
Olfactory epithelium, degenerations	0	0	2	9	10
Olfactory epithelium, hyperplasia	0	0	2	6	10
Respiratory epithelium, hyperplasia	0	0	3	9	10
Turbinate, atrophy	0	0	0	3	9
Epididymal spermatozoal measurements (% of control)					
Sperm motility (%)	-	NM	97*	94*	92*
Sperm (10^6/cauda epididymis)	-	NM	94	98	90
Sperm (10^6/g cauda epididymis)	-	NM	92	94	93
		Cobalt metal (mg/m^3)			
Females	**0**	**0.625**	**1.25**	**2.5**	**5**
Survival	10/10	10/10	10/10	10/10	10/10
Final weight (% of control)	-	102	98	99	93
Organ wt (% of controls)					
Lung – absolute wt	-	125	127*	126*	130*
– relative wt	-	122	128*	127*	139*
Haematology (week 14; % of control)					
Haemoglobin	-	102*	109*	118*	123*
Red blood cells	-	102	109*	118*	122*
Haematocrit	-	101	109*	119*	123*
Platelets	-	94*	92*	82*	87*
Histopathological changes of respiratory system[a]					
Lungs					
Chronic active inflammation	2	10	10	10	10
Alveolar proteinosis	0	10	10	10	10
Alveolar epithelial hyperplasia	0	0	0	0	1

Bronchiolar epithelium, hyperplasia	0	0	10	10	10
Nose					
Olfactory epithelium, degenerations	0	0	5	10	10
Olfactory epithelium, hyperplasia	0	0	0	3	10
Respiratory epithelium, hyperplasia	0	1	0	9	10
Turbinate, atrophy	0	0	0	4	6

*Statistically significant difference compared to controls ($p \leq 0.05$, Williams', Dunnett's or Shirley's test); [a]Average severity grade of lesions observed was minimal to mild, except for mild to moderate alveolar proteinosis in males and degeneration of olfactory epithelium in both sexes at 5 mg/m^3; wt – weight; NM – not measured; Relative organ weights were calculated in relation to body weight.

NTP 2014c: combined repeated dose and carcinogenicity study in rats with cobalt metal

In a carcinogenicity study, F344/NTac rats (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, for up to 105 weeks. No haematology was performed in this study. Neoplastic changes are described in the section "RAC evaluation of carcinogenicity".

General toxicity

Survival in exposed male rats was comparable to controls, while in exposed female rats it was lower than in controls (24%-26% *vs.* 35% in controls). Decreased body weight compared to control values was observed in both sexes dosed at 2.5 mg/m^3 (11% in males and 16% in females) and 5 mg/m^3 (29% in males and 30% in females) at the end of the study (week 105).

Exposure-related clinical findings were observed in exposed males and females (group prevalence not reported), and included abnormal breathing and thinness.

An increase in non-neoplastic histopathological changes was noted in the lungs and nose of males and females in all exposed groups, as well as neoplastic changes in the lungs and other organs.

Fertility

The incidence of infarct in the testes was significantly increased in male rats exposed to 5 mg/m^3 (12 affected animals *vs.* 1 in controls). Infarcts were mostly unilateral, with complete effacement of the parenchyma due to necrosis with loss of differential staining and cellular detail. Multifocal intratubular mineralisation was described in a few of the affected testes.

Table: NTP 2014c: combined repeated dose and carcinogenicity study in rats

	Cobalt metal (mg/m^3)			
Males	**0**	**1.25**	**2.5**	**5**
Survival	17/50	20/50	16/50	16/50
Mean survival (days, % of control)	-	101	102	101
Final weight (% of control)	-	98	89	71
Non-neoplastic histopathological changes of respiratory system and testes				
Lungs[a]				
Chronic active inflammation	22	50	50	50

Alveolar proteinosis	0	48	49	49
Alveolar epithelial hyperplasia	3	47	49	49
Bronchiolar epithelium, hyperplasia	0	44	47	50
Nose[b]				
Chronic active inflammation	28	35	40	49
Suppurative inflammation	9	12	24	46
Olfactory epithelium, metaplasia	12	26	37	50
Olfactory epithelium, atrophy	2	21	34	29
Olfactory epithelium, hyperplasia	0	1	2	7
Olfactory epithelium, basal cell hyperplasia	0	1	0	13
Olfactory epithelium, necrosis	0	1	5	5
Respiratory epithelium, hyperplasia	20	35	45	50
Respiratory epithelium, squamous metaplasia	0	1	11	35
Respiratory epithelium, necrosis	1	4	5	13
Turbinate, atrophy	1	35	35	41
Testes				
Infarct	1	0	2	12
	Cobalt metal (mg/m^3)			
Females	**0**	**1.25**	**2.5**	**5**
Survival	35/50	26/50	24/50	24/50
Mean survival (days, % of control)	-	100	96	98
Final weight (% of control)	-	97	84	70
Non-neoplastic histopathological changes of respiratory system				
Lungs[a]				
Chronic active inflammation	20	50	50	50
Alveolar proteinosis	0	50	50	50
Alveolar epithelial hyperplasia	9	49	50	49
Bronchiolar epithelium, hyperplasia	0	47	46	48
Nose[b]				
Chronic active inflammation	22	42	39	50
Suppurative inflammation	6	4	4	42
Olfactory epithelium, metaplasia	6	18	24	47
Olfactory epithelium, atrophy	0	22	35	35
Olfactory epithelium, hyperplasia	0	0	3	5
Olfactory epithelium, basal cell hyperplasia	0	0	1	19
Olfactory epithelium, necrosis	0	2	0	1
Respiratory epithelium, hyperplasia	15	43	48	49
Respiratory epithelium, squamous metaplasia	2	0	3	45
Respiratory epithelium, necrosis	1	1	1	15
Turbinate, atrophy	1	38	27	45

[a]Average severity grade of lesions observed was mild to moderate at 1.25 and 2.5 mg/m^3, and moderate to marked at 5 mg/m^3; [b]Average severity grade of lesions observed was minimal to mild at 1.25 and 2.5 mg/m^3, and minimal to moderate at 5 mg/m^3; wt – weight; Relative organ weights were calculated in relation to body weight.

NTP 2014d: 17-day inhalation study in mice with cobalt metal

B6C3F1/N mice (5/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, for 17 days. No haematology was performed in this study.

General toxicity

Three male and three female mice exposed to 40 mg/m^3 died before the end of the study. Mean body weight and body weight gain were significantly decreased in male and female mice dosed at ≥ 20 mg/m^3. Body weight at the end of the study was reduced by 27% in males administered 40 mg/m^3. Exposure-related clinical findings (abnormal breathing, lethargy, and thinness) were noted in male rats exposed to ≥ 20 mg/m^3 and females exposed to ≥ 10 mg/m^3.

Lung weights of both sexes exposed to ≥ 5 mg/m^3 were significantly and dose-dependently increased. Compared to controls, higher incidences of non-neoplastic lesions of the lungs and nose occurred in male and female mice in all exposed groups, with average severity grade of lesions from minimal to moderate. Liver weights of exposed male and female mice were significantly decreased at all doses.

Fertility

Absolute testis weight of the 40 mg/m^3 group was significantly lower (by 29%) compared to controls.

Table: NTP 2014: 17-day inhalation study in mice

	Cobalt metal (mg/m^3)					
Males	**0**	**2.5**	**5**	**10**	**20**	**40**
Survival	5/5	5/5	5/5	5/5	5/5	2/5[a]
Final weight (% of control)	-	97	101	98	91	73
Clinical signs[b]					+	+
Organ wt (% of controls)						
Liver – absolute wt	-	87*	87*	88*	79*	73*
– relative wt	-	89*	86*	90*	86*	101
Lung – absolute wt	-	117	128*	133*	161*	200*
– relative wt	-	118	123	136*	178*	273*
Left testis – absolute wt	-	106	101	86	91	71*
– relative wt	-	109	99	87	99	97
Histopathological changes of organs						
Lungs[c]						
Alveolar cellular infiltration, histiocyte	0	2	5	5	5	5
Bronchiolar epithelium, cytoplasmic vacuolisation	0	4	3	5	3	3
Alveolar/bronchiolar epithelium karyomegaly	0	0	4	5	5	4
Interstitial fibrosis	0	0	0	3	5	3
Acute inflammation	0	0	0	0	0	3
Nose[d]						
Acute inflammation	0	0	1	5	5	5

Olfactory epithelium, atrophy	0	5	5	5	5	4
Olfactory epithelium, necrosis	0	2	3	0	5	5
Respiratory epithelium, cytoplasmic vacuolisation	0	4	5	4	5	5
Respiratory epithelium, squamous metaplasia	0	0	0	4	4	2
	Cobalt metal (mg Co/m^3)					
Females	**0**	**2.5**	**5**	**10**	**20**	**40**
Survival	5/5	5/5	5/5	5/5	5/5	2/5[e]
Final weight (% of control)	-	98	97	96	84	62
Clinical signs[c]				+	+	+
Organ wt (% of controls)						
Liver – relative wt	-	87*	86*	81*	74*	66*
– absolute wt	-	90*	89*	84*	89*	105
Lung – relative wt	-	100	116*	121*	153*	174*
– absolute wt	-	102	119*	126*	180*	275*
Histopathological changes of organs						
Lungs[f]						
Alveolar cellular infiltration, histiocyte	0	2	5	5	5	5
Bronchiolar epithelium, cytoplasmic vacuolisation	0	2	4	3	2	1
Alveolar/bronchiolar epithelium karyomegaly	0	3	4	5	4	4
Interstitial fibrosis	0	0	0	2	5	2
Acute inflammation	0	0	2	1	3	2
Nose[g]						
Acute inflammation	0	0	5	5	5	5
Olfactory epithelium, atrophy	0	5	5	5	5	3
Olfactory epithelium, necrosis	0	3	5	2	4	3
Respiratory epithelium, cytoplasmic vacuolisation	0	5	5	5	4	4
Respiratory epithelium, squamous metaplasia	0	0	0	1	3	1

*Statistically significant difference compared to controls ($p \leq 0.05$, Williams' or Dunnett's test); [a]Days of deaths: 5, 5, 8; [b]Abnormal breathing, lethargy, and thinness; [c]Average severity grade of lesions observed was minimal to mild at ≤ 20 mg/m^3, and minimal to moderate at 40 mg/m^3; [d]Average severity grade of lesions observed was minimal to mild; [e]Days of deaths: 6, 7, 9; [f]Average severity grade of lesions observed was minimal to mild at ≤ 10 mg/m^3, and minimal to moderate at 20 and 40 mg/m^3; [g]Average severity grade of lesions observed was minimal to mild; wt – weight; Relative organ weights were calculated in relation to body weight.

NTP 2014e: 14-week inhalation study in mice with cobalt metal

B6C3F1/N mice (10/sex/dose) were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3, for 14 weeks.

General toxicity

There was no substance-related mortality (one female dosed at 2.5 mg/m^3 was accidentally

killed during the first week of the study). Mean body weight of males and females exposed to 10 mg/m^3 was significantly lower (by 14% in males and 13% in females) than in control animals. At the same dose level, abnormal breathing was noted in approximately 50% of males and females. Statistically significant, but minimal (< 5%) increases were observed in haemoglobin concentration and erythrocyte count of 10 mg/m^3 males and in the erythrocyte count of 10 mg/m^3 females at 14 weeks.
Liver weight was decreased at 10 mg/m^3 in males and at ≥ 2.5 mg/m^3 in females, and kidney weight was decreased at ≥ 5 mg/m^3 in both sexes. Lung weight was increased in males exposed to ≥ 2.5 mg/m^3 and in females exposed to ≥ 5 mg/m^3. Histopathological changes in the respiratory system were present from the lowest dose tested (0.625 mg/m^3), with average severity increasing in a dose-dependent manner.

Fertility
Dose-related decreases in reproductive tissue weights, spermatid and epididymal spermatozoa counts and sperm motility, together with histopathologic findings in the testis and epididymis, were observed.
In females, the oestrous cycle was significantly (20%) longer in the 10 mg/m^3 group, compared to controls. However, the Markov transition matrix analyses (for each dose group, a transition probability matrix was estimated for transitions among the proestrus, oestrus, metoestrus, and dioestrus stages, and analysed using chi-square statistics) indicated no significant differences in oestrous cyclicity between exposed and control females.

Table: NTP 2014e: 14-week inhalation study in mice

	Cobalt metal (mg/m^3)					
Males	**0**	**0.625**	**1.25**	**2.5**	**5**	**10**
Survival	10/10	10/10	10/10	10/10	10/10	10/10
Final weight (% of control)	-	101	101	98	98	86
Clinical signs[a]						+
Organ wt (% of controls)						
Kidney – absolute wt	-	103	103	103	94*	74*
– relative wt	-	101	100	102	92*	86*
Liver – absolute wt	-	103	102	101	96	78*
– relative wt	-	102	101	103	97	90*
Lung – absolute wt	-	115	110	115*	135*	150*
– relative wt	-	112	106	115*	137*	169*
Right testis – absolute wt	-	101	97	97	88*	28*
– relative wt	-	100	96	98	90*	32*
Left testis – absolute wt	-	NM	NM	96	87*	27*
Left cauda epididymis – absolute wt	-	NM	NM	97	106	77*
Left epididymis – abs. wt	-	NM	NM	96	102	71*
Spermatid measurements (% of controls)						
Spermatid heads (10^6/testis)	-	NM	NM	99	85*	2*
Spermatid heads (10^6/g testis)	-	NM	NM	108	98	12*
Epididymal spermatozoal measurements (% of controls)						
Sperm motility (%)	-	NM	NM	95*	96*	3*
Sperm (10^6/cauda	-	NM	NM	91	83*	6*

epididymis)						
Sperm (10^6/g cauda epididymis)	-	NM	NM	92	80*	8*
Histopathological changes of organs						
Lungs[b]						
Alveolar cellular infiltration, histiocyte	0	10	10	10	10	10
Bronchiolar epithelium, hyperplasia	0	0	0	10	10	10
Bronchiolar epithelium, cytoplasmic vacuolisation	0	10	10	10	10	10
Alveolar proteinosis	0	0	0	0	10	10
Alveolar/bronchiolar epithelium karyomegaly	0	0	0	0	10	10
Haemorrhage	0	1	0	1	7	6
Nose[c]						
Chronic active inflammation	0	0	0	0	8	10
Olfactory epithelium, degeneration	0	2	10	10	10	10
Olfactory epithelium, hyperplasia	0	0	1	5	2	3
Respiratory epithelium, degeneration	0	0	6	9	10	10
Respiratory epithelium, squamous metaplasia	0	0	2	5	10	10
Turbinate, atrophy	0	0	0	0	8	10
Larynx[d]						
Squamous metaplasia	0	10	10	10	10	10
Testes[e]						
Germinal epithelium, degeneration	2	0	0	0	1	10
Epididymis[f]						
Exfoliated germ cell	0	0	0	0	0	10
Hypospermia	0	0	0	0	0	10
Cytoplasmic vacuolisation	0	0	0	0	0	9
Atrophy	0	0	0	0	0	10
		Cobalt metal (mg Co/m^3)				
Females	**0**	**0.625**	**1.25**	**2.5**	**5**	**10**
Survival	10/10	10/10	10/10	9/10[f]	10/10	10/10
Final weight (% of control)	-	102	102	97	94	87
Clinical signs[c]						+
Organ wt (% of controls)						
Kidney – absolute wt	-	105	100	95	81*	76*
– relative wt	-	99	97	97	88*	89*
Liver – relative wt	-	103	100	89*	79*	69*
– absolute wt	-	101	99	92*	85*	81*
Lung – relative wt	-	105	110	110	133*	157*
– absolute wt	-	100	106	109	142*	183*
Histopathological changes of organs						
Lungs[b]						

Alveolar cellular infiltration, histiocyte	0	10	10	10	10	10
Bronchiolar epithelium, hyperplasia	0	0	0	10	10	10
Bronchiolar epithelium, cytoplasmic vacuolisation	0	10	10	10	10	10
Alveolar proteinosis	0	0	0	0	10	10
Alveolar/bronchiolar epithelium karyomegaly	0	0	0	0	10	10
Haemorrhage	0	0	0	0	8	2
Nose[c]						
Chronic active inflammation	0	0	0	1	10	10
Olfactory epithelium, degeneration	0	1	7	9	10	10
Olfactory epithelium, hyperplasia	0	0	0	3	0	0
Respiratory epithelium, degeneration	0	0	1	8	10	10
Respiratory epithelium, squamous metaplasia	0	0	0	9	10	10
Turbinate, atrophy	0	0	0	0	10	10
Larynx[d]						
Squamous metaplasia	0	10	10	10	10	10

*Statistically significant difference compared to controls ($p \leq 0.05$, Williams', Dunnett's or Shirley's test); [a]Abnormal breathing; [b]Average severity grade of lesions observed was minimal to mild at ≤5 mg/m^3 (except for cytoplasmic vacuolisation of bronchiolar epithelium which was mild to moderate at 5 mg/m^3), and minimal to marked at 10 mg/m^3; [c]Average severity grade of lesions observed was minimal to mild at ≤5 mg/m^3, and minimal to moderate at 10 mg/m^3; [d]Average severity grade of lesions observed was minimal to mild; [e]Average severity grade of lesions observed was minimal at ≤5 mg/m^3 and marked at 10 mg/m^3; [f]Average severity grade of lesions observed was minimal to moderate; [f]One animal accidentally killed during the 1st study week; wt – weight; NM – not measured; Relative organ weights were calculated in relation to body weight.

NTP 2014f: combined repeated dose and carcinogenicity study in mice with cobalt metal

B6C3F1/N mice (50/sex/dose) were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, for up to 105 weeks. No haematology was performed in the study. Neoplastic changes are described in the section "RAC evaluation of carcinogenicity".

General toxicity

Survival of animals exposed to 2.5 or 5 mg/m^3 was significantly lower compared to controls. Final body weight at 5 mg/m^3 was lower in males (by 23%) and females (by 31%) compared to controls. Abnormal breathing and thinness were noted in exposed male and female mice (group prevalence not reported), as well as increased incidence of histopathological non-neoplastic and neoplastic changes, changes in respiratory system of both sexes, present already at the lowest dose tested (1.25 mg/m^3).

Fertility

In the testes of mice exposed to 5 mg/m^3 the incidence of minimal to mild germinal epithelium degeneration was significantly higher compared to controls. It was generally a minimal to mild lesion, usually affecting one to a few scattered seminiferous tubules. Affected tubules were characterised by partial to complete absence of spermatogenic cells, often with concurrent

swelling of the Sertoli cells with resultant hypocellularity and decreased height of the germinal epithelium. The lumens were generally empty but sometimes contained few spermatozoa, sloughed germinal epithelial cells, or cellular debris.

Table: NTP 2014f: combined repeated dose and carcinogenicity study in mice

	Cobalt metal (mg/m³)			
Males	**0**	**1.25**	**2.5**	**5**
Survival	39/50	31/50	28/50	22/50
Mean survival (days, % of control)	-	97	94	93
Final weight (% of control)	-	101	92	77
Non-neoplastic histopathological changes of respiratory system and testes	(50)	(49)	(50)	(50)
Lungs[a]				
Alveolar/bronchiolar epithelium, hyperplasia	0	46	49	50
Alveolar/bronchiolar epithelium, cytoplasmic vacuolisation	0	49	47	48
Alveolar epithelial hyperplasia	4	29	24	43
Bronchiolar epithelium, hyperplasia	4	7	9	11
Bronchiolar epithelium, erosion	0	4	10	2
Proteinosis	2	46	49	50
Alveolar cellular infiltration, histiocyte	10	49	48	48
Suppurative inflammation	1	2	6	16
Nose[b]				
Suppurative inflammation	16	32	49	50
Olfactory epithelium, atrophy	3	46	42	31
Olfactory epithelium, hyperplasia	0	25	17	8
Olfactory epithelium, respiratory metaplasia	5	24	44	50
Olfactory epithelium, atypical respiratory metaplasia	0	14	9	1
Respiratory epithelium, hyaline droplet accumulation	13	29	29	7
Respiratory epithelium, cytoplasmic vacuolisation	0	41	39	37
Respiratory epithelium, squamous metaplasia	3	45	35	33
Turbinate, atrophy	3	25	49	50
Larynx[c]				
Respiratory epithelium, squamous metaplasia	7	47	49	49
Respiratory epithelium, cytoplasmic vacuolisation	0	20	24	32
Squamous epithelium, hyperplasia	2	5	5	8
Trachea[d]				
Epithelium, cytoplasmic vacuolisation	0	14	31	37
Testes				
Germinal epithelium, degeneration	9	14	8	21
	Cobalt metal (mg/m³)			
Females	**0**	**1.25**	**2.5**	**5**
Survival	36/50	34/50	27/50	26/50
Mean survival (days, % of control)	-	101	99	97
Final weight (% of control)	-	98	96	69

Non-neoplastic histopathological changes of respiratory system (incidence)				
Lungs[a]	(49)	(50)	(50)	(50)
Alveolar/bronchiolar epithelium, hyperplasia	0	49	49	50
Alveolar/bronchiolar epithelium, cytoplasmic vacuolisation	0	48	49	48
Alveolar epithelial hyperplasia	2	27	26	41
Bronchiolar epithelium, hyperplasia	0	3	12	26
Bronchiolar epithelium, erosion	0	0	4	3
Proteinosis	0	45	50	50
Alveolar cellular infiltration, histiocyte	10	49	50	49
Suppurative inflammation	0	3	2	15
Nose[b]	(50)	(50)	(50)	(50)
Suppurative inflammation	3	47	50	50
Olfactory epithelium, atrophy	4	44	39	24
Olfactory epithelium, hyperplasia	1	22	16	8
Olfactory epithelium, respiratory metaplasia	1	26	44	50
Olfactory epithelium, atypical respiratory metaplasia	0	18	14	1
Respiratory epithelium, hyaline droplet accumulation	12	38	40	10
Respiratory epithelium, cytoplasmic vacuolisation	0	40	47	47
Respiratory epithelium, squamous metaplasia	0	49	49	50
Turbinate, atrophy	0	44	50	50
Larynx[c]	(47)	(50)	(50)	(47)
Respiratory epithelium, squamous metaplasia	2	49	50	47
Respiratory epithelium, cytoplasmic vacuolisation	0	24	31	34
Squamous epithelium, hyperplasia	2	13	21	21
Squamous epithelium, erosion	1	2	7	4
Trachea[d]	(48)	(50)	(48)	(49)
Epithelium, cytoplasmic vacuolisation	0	26	37	39

[a]Average severity grade of lesions observed was minimal to mild at 0 and 1.25 mg/m^3, minimal to moderate at 2.5 mg/m^3, and minimal to marked at 5 mg/m^3; [b]Average severity grade of lesions observed was minimal to mild at 0 and 1.25 mg/m^3, and minimal to moderate at 2.5 mg/m^3 and 5 mg/m^3; [c]Average severity grade of lesions observed was minimal; [d]Average severity grade of lesions observed was minimal to mild.

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:

1. Pedigo and Vernon, 1993: Dominant lethal assay in male mice
2. Elbetieha *et al.*, 2008: Dominant lethal assay in male mice
3. Nation *et al.*, 1983: 69-day diet study in male rats
4. Pedigo *et al.*, 1988: 3-month oral (drinking water) study in male mice
5. Anderson *et al.*, 1992: 13-week oral (drinking water) study in male mice

6. Mollenhauer *et al.*, 1985: 98-day diet study in rats
7. Corrier *et al.*, 1985: 3-month diet study in male rats
8. CDI/CORC 2015b: 3-month oral (gavage) study in rats.

Pedigo and Vernon, 1993: Dominant lethal assay in male mice

Ten male B6C3F1 mice were treated with one dose of 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. At the end of the dominant lethal assay (DLA), half of the males were maintained for 6 weeks for evaluation of recovery of fertility and sperm parameters. The growth and development of pre-implantation embryos from cobalt-treated males and untreated females was evaluated *in vitro* every other week during cobalt exposure. Two-cell embryos were cultured to the blastocyst stage of development to measure the rate of development and extent of growth.

No information on general toxicity, positive control groups or the laboratory's historical negative controls is available. Tissue concentrations of cobalt measured by atomic absorption analysis were increased in liver, kidney, testis, and epididymis after 12 weeks of cobalt treatment. Kidney and testis cobalt concentration decreased after the recovery period.

Fertility

Male fertilisation rate (percentage of 2-day old embryos that were 2-cell or greater after mating with superstimulated, cobalt-untreated females) was evaluated during the period of cobalt treatment (after 7 and 10 weeks of treatment), after the DLA (week 12), and during recovery period (weeks 16 and 18).

All males were fertile, but in the group of females mated with cobalt-treated males there was a decrease in the number of pregnancies, total and live implantations, and an increase in average pre-implantation losses.

Male fertilisation rate decreased after 12 weeks treatment (during the DLA period; 1.8% *vs.* 82.4% in controls), and, following the cessation of exposure, recovered over the next 6 weeks. There was a decrease in testes weight (59% compared to control). All analysed sperm parameters were decreased in cobalt-treated males after 12 weeks of exposure (Table 76 in the CLH report), and, except for sperm concentration, recovered to control levels by 18 weeks (during 6 weeks without exposure).

Development

In the group of females mated with cobalt-treated males there was a decrease in the number of live implantations and an increase in average pre-implantation losses (Table 18 and Table 75 in the CLH report). No change in post-implantation losses was observed (at gestation day 19) and *in vitro* development of 2-cell embryos to blastocyst from cobalt-treated males was not affected.

Table: Pedigo and Vernon, 1993 dominant lethal assay in mice

ppm Co (as **cobalt chloride hexahydrate**)

	0	**400**
Number of pregnant females	29/32 (91%)	18/31 (58%)*
Number of fertile males (at 10 weeks)	10/10	10/10
Average total implantations per pregnant	8.3 ± 0.4	6.5 ± 0.8*

female		
Average dead implantations per pregnant female	0.4 ± 0.1	0.4 ± 0.1
Average pre-implantation loss per pregnant female	0.43 ± 0.2	2.4 ± 0.7*

* Statistically significant difference compared to controls ($p<0.05$, Student *t*-test)

Elbetieha et al., 2008: Dominant lethal assay in male mice

In this non-guideline study, sexually mature male Swiss mice (10/dose) were exposed to 200, 400 and 800 ppm cobalt chloride hexahydrate (25.7, 46.9 and 93.0 mg cobalt chloride hexahydrate/kg bw/d; 6.4, 11.6 and 23.1 mg cobalt/kg bw/d) in their drinking water for 12 weeks (84 days). Males were then mated with untreated females (one male with two virgin untreated females, for 10 days to cover two oestrus cycles).

No information on positive control groups and the laboratory's historical negative control data is available. Haematologic parameters were not analysed.

General toxicity

Average body weight gain was reduced in all dose groups (up to 7%), and mortality occurred at the middle and high dose groups during the 10th week of the exposure. There were no other clinical signs of toxicity observed in surviving animals.

Fertility

Cobalt chloride hexahydrate treatment negatively affected male fertility: testicular sperm count was decreased at ≥ 11.6 mg Co/kg bw/d, epididymal sperm count was decreased at all doses, testicular weight was reduced at ≥ 11.6 mg Co/kg bw/d and epididymal weight at 23.1 mg Co/kg bw/d. There was an increase in the weight of the seminal vesicles in the middle and high dose groups, which the study authors hypothesised to be the consequence of interstitial Leydig-cell hypertrophy. In males dosed at the middle and high doses, hypertrophy of the interstitial Leydig cells, congested blood vessels, degeneration of the spermatogonial cells and necrosis of both the seminiferous tubules and the interstitial tissue were observed.

Increased numbers of resorptions were observed at ≥ 6.4 mg Co/kg bw/d, and a significant decrease in the number of pregnancies was observed at ≥ 11.6 mg Co/kg bw/d. The number of implantation sites was also decreased (at 6.4 and 11.6 mg/kg bw/d), but without any clear dose response relationship.

Development

Increased number of resorptions and decreased number of viable foetuses was observed at all tested doses.

Table: Elbetieha *et al.*, 2008: Dominant lethal assay in male mice

	Co (mg/kg bw/d as **cobalt chloride hexahydrate**)			
Males	**0**	**6.4**	**11.6**	**23.1**
N	10	10	10	10
Mortality	0	0	1/10	2/10
Body weight (end of experiment), % of control	-	95%*	94%*	93%*
Reproductive organ weights, absolute (g)				

Testis	-	90*	86*	71*
Epididymis	-	99	98	92*
Seminal vesicles	-	100	154*	177*
Sperm counts ($x10^3$)				
Epididymal (per mg tissue)	-	87*	86*	78*
Testicular (per g tissue)	-	101	78*	74*
Daily sperm production/testis	-	97	64*	57*
Fertility/developmental indices				
Pregnant/mated females (%)	19/20 (95)	15/20 (75)	12/18 (67)*	7/16 (44)*
N of implantation sites/female	-	72*	68*	81
N of viable foetuses	-	65*	61*	75*
N resorptions/N implantations	3/150	9/81*	9/65*	10/45*
N of females with resorptions (%)	3/19 (16)	10/15 (67)*	error?	5/7 (70)*

*Statistically significant difference compared to controls (P<0.05, Student *t*-test or Fisher's exact test); N – number, error? – 16 animals stated as pregnant, instead of 12

Nation et al., 1983: 69-day diet study in male rats

Male Sprague Dawley rats (6/dose) received cobalt chloride in chow (0, 5 or 20 mg cobalt/kg bw/d) for 69 days. No information on general toxicity was provided.

Fertility

Significantly decreased testis weight (42% of controls) and testicular atrophy were observed at 20 mg/kg bw/d, but not at 5 mg/kg bw/d.

Pedigo et al., 1988: 3-month oral (drinking water) study in male mice

Acute and chronic studies were performed in CD1-mice, but only chronic studies were presented in the CLH report (in acute studies cobalt chloride was administered intraperitoneally).

Male mice (5/dose) were dosed with 100, 200 and 400 ppm of cobalt chloride hexahydrate in drinking water (23.0, 42.0 and 72.1 mg Co/kg bw/d) for 12 weeks in a dose response study or with 400 ppm (58.9 mg Co/kg bw/d - fluid intake at 400 ppm group was lower than in 400 ppm group in the dose response study) for 13 weeks, followed by 20 weeks of recovery in a time course study.

General toxicity

Body weight of the high dose group (72.1 mg Co/kg bw/d) was slightly but significantly decreased during most weeks of the study (approximately 90% of controls at week 12). There was no information on clinical signs of cobalt toxicity. Cobalt treatment did not induce a statistically significant effect on haematocrit (numerical data not reported).

Fertility

Testicular, prostatic and seminal vesicle weight, epididymal sperm concentration, sperm motility, fertility (measured as % of ova fertilised *in vivo* when males were mated with super

ovulated females), and serum levels of testosterone, FSH and LH, were analysed in the studies.
Time and dose-dependent decrease in testicular weight and epididymal sperm concentration was observed.
In a dose response study, testicular relative weights were decreased to 71%, 52%, and 30% of control value in males dosed at 23, 42 and 72.1 mg Co/kg bw/d, respectively. In a time course study in males dosed at 58.9 mg Co/kg bw/d, testis weight started to be significantly decreased at week 9, and after 13 weeks of exposure it was 31% of control values. Weights of the prostate glands and seminal vesicles were not affected (data not reported).
Epididymal sperm concentration was decreased to 66, 29 and 8% of control values in a dose response study at 23, 42 and 72.1 mg Co/kg bw/d, respectively. In a time course study, a significant decrease in sperm concentration was observed at the 11th and 13th weeks of exposure (15% of controls). Sperm motility was also decreased in both studies.
Fertility was decreased at 400 ppm (10% and 22% of controls in the dose response study and time course study, respectively), and the time course study showed that the onset of this decrease was after the 11th exposure week.
Serum testosterone concentrations in all cobalt-treated groups were significantly elevated five- to seven-fold above control serum concentrations. FSH and LH serum levels did not significantly differ from control values, although a non-significant, increasing dose response trend was observed for LH.
Fertility indices that were affected during cobalt treatment did not show full recovery during 20 weeks of exposure cessation, and remained significantly lower than control values.

Table: Pedigo *et al.*,1988: 3-months oral (drinking water) study in male mice
a) Dose response study

	Cobalt chloride hexahydrate (mg Co/kg bw/d)		
Males	**23**	**42**	**72.1**
Final body weight (% of control)	96	95	90*
Fertility indices (% of control)			
Testis weight (relative to body wt)	71*	52*	30*
Epididymal sperm concentration	66*	29*	8*
Sperm motility (%)	104	63	42*
Fertility[a]	97	86	10*
Testosterone	594*	694*	511*
LH	100	122	151

*Statistically significant difference compared to controls ($p<0.05$, Student *t*-test, fertility: analysis of variance with *post hoc* test); [a]Percent ova fertilised; wt - weight.

b) Time course study

	Cobalt chloride hexahydrate 58.9 mg Co/kg bw/d				**Recovery**
Males	week 7	week 9	week 11	week 13	week 33
Fertility indices (% of control)					
Testis weight (relative to body wt)	85	75*	42*	31*	42*
Epididymal sperm concentration	74	81	15*	15*	21
Sperm motility (%)	98	90	17*	29*	47
Fertility[a]	88	100	100	22*	49*

*Statistically significant difference compared to controls ($p<0.05$, Student *t*-test); [a]Percent ova fertilised; wt - weight.

Anderson et al. 1992: 13-week oral (drinking water) study in male mice
Male CD-1 mice (10/dose) were exposed to 0 or 400 ppm cobalt chloride hexahydrate via drinking water for 13 weeks. Evaluations were performed after 7, 9, 11 and 13 weeks of exposure and after a 20-week recovery period. No information on general toxicity was provided, but histologic evaluation did not reveal any abnormalities in the morphology of kidneys and liver.

Fertility
Reduction of testicular size, vascular congestion of various degrees and progressive degeneration of seminiferous tubules was observed from week 9 onwards. Germinal epithelium and Sertoli cells of the seminiferous tubules were damaged, while interstitial Leydig cells were not. Changes in vessel epithelium in the testes were observed at all time points.
No recovery of testicular weight was observed. Histologic evaluation showed repopulation of tubules in a few animals, while in others little or no recovery was observed.

Mollenhauer et al. 1985: 98-day diet study in rats (cobalt chloride)
Adult male Sprague-Dawley rats were maintained on a diet containing 0 or 265 ppm cobalt (as cobalt chloride) (20 mg Co/kg bw/d) for up to 98 days. Three rats of each dose group were sacrificed weekly and assayed for testicular damage by light and electron microscopy. No information was provided about general toxicity.

Fertility
Testicular congestion became apparent after 35 days of treatment, and degenerative changes after 70 days of treatment, followed by a progressive deterioration of cell architecture and testicular volume. The degenerative changes were of a very general nature (e.g., thickening of the basal lamina and basement membranes, increased packing of red blood cells in veins and arteries, formation of "giant" cells, loss of sperm tail filaments, degeneration of sperm mitochondria), and no cobalt residues could be detected by energy dispersive x-ray microanalysis. It was concluded that these data indicate that testicular degeneration was not a primary response to cobalt and suggest that the testes become hypoxic due both to blockage of veins and arteries by red blood cells and to changes in permeability caused by thickening of basal lamina and basement membranes.

Corrier et al., 1985: 3-month diet study in male rats
Male Sprague Dawley rats were given a daily diet containing 0 or 265 ppm Co as cobalt chloride hexahydrate (20 mg cobalt/kg bw/d) during a 98-day study period. Rats were sacrificed on day 1, 2, 4, 7, 14, 21, 28, 35, 42, 56, 63, 70, 84 and 98 (3 rats/dose/time point), and tissue specimens from the testes, cauda epididymis, and seminal vesicles were examined histologically.

General toxicity
No effects on body weight were observed. At 265 ppm, an increased erythrocyte count (141% of control), packed cell volume (156% of control), and haemoglobin concentration (128% of control) were observed.

Fertility
No lesions were found in control animals or in test groups killed on day 1-28. In rats killed on day 35 and thereafter, the abdominal viscera, blood and testes of the exposed rats were dark-

red and cyanotic, with testes moderately to markedly congested. On day 70, degenerative and necrotic changes in the germinal epithelium and Sertoli cells were found, with spermatogonia, primary spermatocytes and round spermatids markedly affected, while elongated spermatids, spermatozoa and Sertoli cells were more resistant. Lesions were not observed in the Leydig cells, cauda epididymis or seminal vesicles. The authors hypothesised that described changes were due to cobalt-induced polycythemia that may have produced a prolonged state of tissue hypoxia.

CDI/CORC 2015b: 3-month oral (gavage) study in rats with cobalt chloride hexahydrate

In a 90-day repeated dose toxicity study according to OECD TG 408, Crl:CD(SD) rats (10/sex/dose) were given cobalt chloride hexahydrate at doses of 0, 3, 10 or 30 mg/kg bw/d by gavage (0.7, 2.5 or 7.4 mg Co/kg bw/d). Recovery (4-week period) was evaluated in control and high dose group in additional 5 animals per sex per dose. No justification for the test dose levels was provided. Additional sampling for cobalt determination was performed but these results were not yet available.

General toxicity

No substance-related mortality was observed. No relevant neurological changes were noted. Body weight was reduced at the end of the study (6% and 11% for males in the 10 and 30 mg/kg bw/d group and 9% for females in the 30 mg/kg bw/d group; statistically significant only in high dose males).

Haemoglobin, red blood cells and haematocrit were increased in the mid and high dose groups in males (by 10-11% and 20-26%, respectively, compared to controls), and in the high dose in females (by 11-14% compared to controls). Platelet count was decreased in the high dose group in males (by 31%). Plasma levels of bilirubin were increased by 14% for the male animals treated with 10 mg/kg bw/d of cobalt dichloride hexahydrate and by 29% to 34% for the male and 16% for the female animals treated with 30 mg/kg bw/d. No treatment related effects were observed on urinalysis, organ weights (except for a small increase in spleen relative weights) and macroscopic *post mortem* findings.

Microscopic evaluation showed an increase of erythroid hyperplasia (of marginal to slight severity) in the bone marrow of males and females in the mid and high dose groups.

Recovery: On test day 118, body weight of the male and female animals previously treated with 30 mg/kg bw/d of cobalt dichloride hexahydrate was still statistically significantly reduced compared to the control group. All changes previously observed in haematological and biochemical parameters and at histological examination had subsided.

Fertility

In males, histopathological examination of testes and epididymides did not show test-item related effects. In females no effects were observed on the oestrous cycle.

Some alterations were observed in the hormone serum levels of testosterone in both sexes and on serum 17β-estradiol levels in females (measured at 0, 6 and 13 weeks) (Table below). However, they were not considered treatment related and the control values varied over time, making assessment difficult.

Table: CDI/CORC 2015b: 3-month oral (gavage) study in rats

	Cobalt chloride hexahydrate (mg Co/kg bw/day)				**Recovery** in high dose
Males	**0**	**0.7**	**2.5**	**7.4**	group (7.4)
N	10	10	10	10	5

Body weight (90 days; % of control)	-	NS	94	89*	83*
Haematology (90 days; % of control)					
Haemoglobin	-	101	111*	126*	NS
Red blood cells	-	100	110*	120*	NS
Haematocrit	-	101	110*	124*	NS
Platelets	-	87	86	69*	NS
Plasma bilirubin	-	NS	114	129-134	NS
Bone marrow erythroid hyperplasia (incidence)	0	0	4	7	NS
	Cobalt chloride hexahydrate (mg Co/kg bw/day)				**Recovery** in high dose group (7.4)
Females	**0**	**0.7**	**2.5**	**7.4**	
N	10	10	10	10	5
Body weight (90 days; % of control)	-	NS	NS	91	87*
Haematology (90 days; % of control)					
Haemoglobin	-	101	100	114*	NS
Red blood cells	-	100	97	111*	NS
Haematocrit	-	101	100	114*	NS
Platelets	-	93	91	88	NS
Plasma bilirubin (% of control)	-	NS	NS	116	NS
Bone marrow erythroid hyperplasia (incidence)	0	0	7	7	NS

*Statistically significant difference compared to controls ($p<0.05$, Student *t*-test); NS – not significantly different from control

Table: Hormone levels in rats after 13 weeks exposure to cobalt chloride

	Cobalt chloride hexahydrate (mg Co/kg bw/d)		**Statistical significance**
Males	**0**	**30**	
Testosterone (ng/Ll serum)			
Predose	10.109 ± 4.410	6.307 ± 3.110	$p \leq 0.05$
42 day	6.304 ± 3.024	3.940 ± 2.104	$p \leq 0.05$
91/92 day	3.459 ± 1.541	1.477 ± 0.730	$p \leq 0.01$
119 day	2.587 ± 0.734	1.119 ± 0.394	$p \leq 0.01$
	Cobalt chloride hexahydrate (mg Co/kg bw/d)		**Statistical significance**
Females	**0**	**30**	
Testosterone (ng/mL serum)			
Predose	0.483 ± 0.041	0.652 ± 0.095	$p \leq 0.01$
42 day	0.603 ± 0.117	0.751 ± 0.244	$p \leq 0.05$
91/92 day	1.167 ± 0.333	0.713 ± 0.125	$p \leq 0.01$
119 day	1.338 ± 0.268	0.728 ± 0.216	$p \leq 0.01$
17β-Estradiol (pg/mL serum)			
Predose	6.68 ± 5.60	14.06 ± 9.80	$p \leq 0.05$
91/92 day	7.30 ± 6.94	29.53 ± 18.17	$p \leq 0.01$

2.2. INHALATION STUDIES WITH COBALT COMPOUNDS

Six NTP studies are presented in the CLH Report, in which F344/N rats and B6C3F1 mice were exposed to **cobalt sulphate heptahydrate** (99% pure) aerosol 6 hours per day (plus T_{90}), 5 days per week, for 16 days, 13 weeks or 105 weeks (NTP, 1991a, b, 1998).

NTP 1991a: 16-day inhalation studies with cobalt sulphate heptahydrate in rats and mice

Rats and mice (5/sex/group) were exposed to 0, 0.1, 0.5, 5, 50 or 200 mg/m³ of cobalt sulphate (calculated on the basis of the anhydrous salt: "Throughout this Report, atmospheric concentrations are expressed in milligrams of cobalt sulphate per cubic meter of air rather than in milligrams of the heptahydrate", NTP, 1991), i.e. to 0, 0.04, 0.2, 1.9, 19 or 76 mg Co/m^3. Histologic examinations were performed on controls and animals exposed to 50 or 200 mg cobalt sulphate/m^3 and on male mice exposed to 5 mg cobalt sulphate/m^3.

General toxicity

All rats and mice exposed at the top concentration (76 mg Co/m^3) died, while partial survival was seen at 19 mg Co/m^3. Both rats and mice exposed to 19 mg Co/m^3 lost weight, and clinical signs (hypoactivity, chromodacryorrhea, hypothermia, rapid and shallow breathing, and reduced body tone) were observed at 19 or 76 mg Co/m^3.

In animals of both species and sexes dosed at 19 mg Co/m^3 lung absolute and relative weights were significantly increased, and thymus absolute and relative weights were markedly decreased, compared to controls. Pathological changes in the respiratory organs were observed in rats at 19 or 76 mg Co/m^3 (lower doses not examined histologically), and in mice already at 1.9 mg Co/m^3 (lower doses not examined histologically).

Lesions in other organs were also observed (lymphoid necrosis in the thymus and congestion of vessels in the brain/meninges observed in exposed rats and mice that died during the study; centrilobular congestion and necrosis in the liver of male and female rats dosed at 76 mg Co/m^3, and necrosis of hepatocytes in all mice that died during the exposure period).

Fertility

Atrophy of the testis, characterised by a decreased number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts, was observed in rats exposed to 19 mg Co/m^3.

No effects on the testes were reported in mice.

NTP 1991b: 13-week inhalation studies with cobalt sulphate heptahydrate in rats and mice

Rats and mice (10/sex/group) were exposed to 0, 0.3, 1.0, 3.0, 10 or 30 mg cobalt sulphate/m³, i.e. to 0, 0.11, 0.4, 1.1, 3.8 or 11.4 mg Co/m^3. Sperm morphology and vaginal cytology were performed for rats and mice exposed to 0, 3, 10 or 30 mg/m^3 og cobalt sulphate.

General toxicity

All animals survived, except for 2 male mice exposed to the top concentration (11.4 mg Co/m^3).

Rats and mice exposed to 11.4 mg Co/m^3 had lower body weight compared to controls. Lung weights were increased (compared to controls) in rats exposed to ≥ 0.11 mg Co/m^3 and in mice exposed to ≥ 3.8 mg Co/m^3. At the top dose, clinical signs were observed in rats (ruffled fur, hunched posture), but not in mice.

Significantly increased values of haematocrit and red blood cell concentration were observed in exposed rats (but not in mice), at ≥ 1.1 mg Co/m^3 in males (up to 29-32% at the top dose, compared to controls) and ≥ 3.8 mg Co/m^3 in females (up to 26-27% at the top dose, compared to controls).
Lesions in the respiratory tract in exposed rats and mice were observed already at the lowest dose applied, i.e. 0.11 mg Co/m^3 (squamous metaplasia in the larynx).

Fertility
In mice, sperm motility was decreased in animals dosed at ≥ 1.1 mg Co/m^3 (lower concentrations were not evaluated), and increased numbers of abnormal sperm were found at the top dose. At the top dose also decreased testis and epididymal weights were observed (e.g. 52% decrease in absolute testis weight, compared to controls), with atrophy of the testis (loss of germinal epithelium in the seminiferous tubules). Oestrous cycle was significantly longer (by 19%) in female mice at the top dose.
In rats, no statistically significant effects were observed on sperm parameters. Oestrous cycle length was increasing with increasing doses, but the differences were not statistically significant.

NTP 1998: Toxicology and carcinogenesis inhalation studies of cobalt sulphate heptahydrate in rats and mice
Fischer 344 rats and B6C3F1 mice (50/sex/dose) were exposed (whole body) to aerosols containing 0, 0.3, 1.0 or 3.0 mg/m³ cobalt sulphate heptahydrate, i.e. to 0, 0.1, 0.3, 1 mg Co/m^3 (measured aerosol stoichiometry showed that "aerosol delivered to the exposure chambers is primarily cobalt sulphate hexahydrate"; NTP, 1998) for 6 hours per day, 5 days per week, for 105 weeks. No haematology and sperm analysis were performed in this study. Neoplastic changes are described in the section "RAC evaluation of carcinogenicity".

General toxicity
There was no effect on survival. Body weights of exposed male and female rats were similar to controls throughout the study. Body weights of the top dose male mice were lower than in control animals (from week 96 until the end of the study).
Exposure to cobalt sulphate heptahydrate caused a spectrum of inflammatory, fibrotic and proliferative lesions in the respiratory tract of male and female rats and mice, with increased incidence of non-neoplastic and neoplastic changes in the respiratory system already at the lowest dose tested.

Fertility
No histopathological effects on reproductive organs were reported.

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:

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

CDI/CORC, 2015a: Combined repeated dose toxicity and reproduction screening study in rats with cobalt metal was already described in the Fertility section (Table above). Developmental effects (increased number of dams with stillborn pups, number of stillbirths, and pre- and post-implantation losses; decreased mean number of live born pups and live birth index, and significant decrease in mean pup weight) were observed at 300 mg/kg bw/d, a dose at which significant general maternal toxicity (including mortality) occurred. The viability index was significantly reduced at 100 mg/kg bw/d, but it was within the historical control range (data for viability index and historical control data not presented). No external abnormalities were observed at any dose level.

Szakmáry et al., 2001: study with cobalt sulphate heptahydrate in pregnant mice, rats and rabbits

In a study in mice (pregnant female C57BL mice, 20 exposed and 25 control animals, exposed to 50 mg cobalt sulphate heptahydrate/kg bw/d during GD 6-15), an increased frequency of foetuses with retarded body weight was found (25% *vs.* 5%), although average foetal weight was similar in exposed and control group (1.02 g *vs.* 1.11 g). An increased incidence of skeletal retardation (sternum hypoplasia, double vertebral ossification centres, shortened rib 13) and certain malformations (skeletal malformation of cranium, and vertebra) was observed. Study authors stated that the incidence of other malformations (eyelids and kidneys) was also increased, but the incidence was one malformation type in 134 foetuses (*vs.* 0 in controls).
Maternal weight gain was 19% lower compared to controls (non-significant difference). No histopathological changes were found in exposed females.

Table: Effects of cobalt on development of mice

	cobalt sulphate heptahydrate (mg Co/kg bw/d)	
Parameters	**0**	**30**
Number of litter studied	25	19
Number of live foetuses	164	134
External malformations (major anomalies)		
Exencephalia	–	–
Ablepharia	–	1

Number of foetuses dissected	75	64
Visceral retardation	2	2
Visceral anomalies (major anomalies)		
Ectopia testis	–	–
Ectopia ovaries	–	–
Dilated pelvis renalis	–	1
Dilated ureter	–	–
Duplication of kidneys	–	1
Alizarin-stained foetuses	89	70
Skeletal retardation[a]	27	58[b]
Skeletal anomalies (minor)		
Supernumerary ribs	–	–
Skeletal malformations (major anomalies)		
Cranium	3	15
Sternum	–	–
Ribs	1	–
Vertebra	7	12
Total number of foetuses	164	134
Number (%) of malformed foetuses	9 (10.1)	19 (27.1)[b]

[a]Sternum hypoplasia, double vertebral ossification centres, shortened rib 13; [b]Significant at $p<0.5$ (Kruskal-Wallis test)

In the experiments in **rats** (pregnant female Sprague Dawley rats, 3-18/dose, exposed to 0, 25, 50 or 100 mg cobalt sulphate heptahydrate/kg bw/d during GD 1-20, or exposed to 0 or 25 mg cobalt sulphate heptahydrate/kg bw/d during GD 1-21), there were no effects on litter size, resorptions or post-implantation loss, but a dose-related increase in the frequency of foetuses with retarded body weight and skeletal and visceral retardation (type not stated) was observed. At the two higher doses there was an increase in the frequency of skeletal (vertebra) and urogenital (dilated ureter) malformations.

The perinatal index decreased by 25% in the treated group (25 mg/kg bw/d) and pup body weight was decreased by 16% during the first postnatal week, recovering to control values thereafter. Survival index was not affected. Some effects on the maturation of the nervous system in exposed pups was observed (e.g. 2-day delay in swimming ability, 1-day delay in auditory reflex), which, according to the DS could be related to lower body weight gain during the first postnatal week.

Maternal body weight gain was not significantly affected. The relative liver, adrenal and spleen weights were increased at the highest dose level, but the actual data were not given. Several clinical chemical parameters were changed at the highest dose (38% increased urea nitrogen, 31% increased creatinine, 18% decreased albumin, 28% decreased blood glucose, compared to the controls). The presence of post-natal maternal toxicity is not stated.

Cobalt concentration in maternal blood, foetal blood and amniotic fluid (24 hours after the last exposure on day 20) increased in a dose-dependent matter. The cobalt concentration in foetal blood was higher than in maternal blood showing placental transfer.

Table: Effects of cobalt on development of rats

	cobalt sulphate heptahydrate (mg Co/kg bw/day)			
Parameters	**0**	**25**	**50**	**100**
Number of litter studied	15	18	17	14
Number of live foetuses	168	241	235	190

External malformations (major anomalies)				
Exencephalia	–	–	–	–
Ablepharia	–	–	–	–
Number of foetuses dissected	81	116	113	91
Visceral retardation	5	8	13[b]	18[b]
Visceral anomalies (major anomalies)				
Ectopia testis	–	–	1	–
Ectopia ovaries	–	–	1	–
Dilated pelvis renalis	–	–	1	–
Dilated ureter	–	1	1	4
Duplication of kidneys	–	–	–	–
Alizarin-stained foetuses	87	125	122	99
Skeletal retardation[a]	18	35[b]	62[b]	66[b]
Skeletal anomalies (minor)				
Supernumerary ribs	–	–	4	2
Skeletal malformations (major anomalies)				
Cranium	–	–	1	–
Sternum	–	–	–	–
Ribs	–	–	–	–
Vertebra	1	2	3	3
Total number of foetuses	168	241	235	190
Number (%) of malformed foetuses	1 (0.6)	3 (1.2)	7 (2.9)[b]	7 (3.7)[b]

[a]Sternum hypoplasia, double vertebral ossification centres, shortened rib 13; [b]Significant at $p<0.5$ (Kruskal-Wallis test)

In pregnant New Zealand White **rabbits** (8-25/dose), all applied doses of cobalt sulphate (0, 20, 100 or 200 mg/kg bw/dy, during GD 6-20) resulted in mortality (5/25, 4/13 and 7/8), due to circulatory failure. Total resorptions were found in all surviving animals at 100 and 200 mg/kg bw/d, and in 30% of surviving dams at the lowest dose. In the surviving pups at the lowest dose skeletal retardation was observed, but malformations were not found.

Domingo et al., 1985: study with cobalt chloride in pregnant rats

Pregnant Wistar rats (15/group) were administered 0, 12, 24 or 48 mg cobalt chloride/kg bw/d on GD 14-21. The number of litters was reduced at all doses (by 42-58%), without a dose-related pattern. However, it is unclear whether the dams died, were not pregnant, did not give birth or gave birth to dead pups only. Pup survival was reduced during the postnatal exposure period, as was body weight and length, at all dose levels, in a dose-dependent manner. No effects were observed on liver and renal function, and no external malformations were noted.
Toxic effects in the dams are not described, although it is noted that toxic effects were observed in previous studies at doses of ≥ 24 mg/kg bw/d.

Table: Average body weight, body length and tail length of rat pups nursed by cobalt treated mothers

Day **Males**	**Dose levels** (mg/kg bw/d)	**Body weight** (g)	**Body length** (mm)	**Tail length** (mm)
1	0	7.18 ± 1.24 (41)	53.8 ± 0.5	18.2 ± 0.3
	12	5.68 ± 0.69 (33)***	49.1 ± 0.3**	15.7 ± 0.1***
	24	5.61 ± 1.13 (27)***	50.1 ± 0.4*	16.6 ± 0.2***

	48	5.34 ± 1.17 (26)***	47.2 ± 0.4**	15.3 ± 0.2***
4	0	10.82 ± 2.14 (38)	64.1 ± 0.5	25.4 ± 0.4
	12	9.03 ± 0.87 (30)***	62.1 ± 0.3*	24.2 ± 0.2
	24	8.64 ± 1.19 (27)***	62.0 ± 0.3*	24.3 ± 0.2
	48	8.65 ± 0.43 (18)***	59.8 ± 0.3***	22.7 ± 0.3***
21	0	43.06 ± 8.07 (35)	109.6 ± 0.9	75.8 ± 1.3
	12	30.75 ± 7.97 (30)***	101.4 ± 0.7***	70.0 ± 0.6*
	24	26.69 ± 5.12 (23)***	94.7 ± 0.9***	67.9 ± 1.2***
	48	25.70 ± 3.22 (14)***	91.6 ± 0.8***	59.1 ± 0.9***
Females				
1	0	6.61 ± 1.16 (43)	50.9 ± 0.5	17.4 ± 0.3
	12	5.69 ± 1.02 (31)***	49.5 ± 0.3	15.8 ± 0.2**
	24	6.04 ± 0.82 (29)**	48.3 ± 0.3**	16.9 ± 0.0.2
	48	5.56 ± 1.08 (28)***	47.5 ± 0.3***	15.1 ± 0.1***
4	0	10.00 ± 2.09 (39)	61.3 ± 0.5	25.3 ± 0.4
	12	8.78 ± 1.03 (29)**	61.3 ± 0.3	24.1 ± 0.3
	24	8.75 ± 0.94 (29)**	60.9 ± 0.3	24.5 ± 0.2
	48	8.83 ± 0.77 (18)**	58.5 ± 0.2*	23.0 ± 0.2*
21	0	41.24 ± 8.59 (35)	106.8 ± 0.8	73.3 ± 1.2
	12	29.82 ± 7.34 (28)***	100.1 ± 0.7***	70.8 ± 0.6
	24	27.33 ± 4.28 (28)***	95.8 ± 0.9***	65.2 ± 1.1**
	48	28.73 ± 6.65 (13)***	88.3 ± 0.7***	56.6 ± 0.8***

Paternain, 1988: Study in pregnant rats

Exposure to cobalt chloride (hexahydrate) in pregnant Sprague Dawley rats (by gavage, doses of 0, 25, 50 and 100 mg cobalt chloride hexahydrate/kg bw/d) on GD 6–15, resulted in a significant reduction in body weight gain, particularly at the dose of 100 mg/kg bw/d, at which haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, and reticulocytes were increased significantly. No treatment related effect was observed on corpora lutea number and implantation, foetal survival, size or sex distribution. A non-significant increase in the incidence of stunted foetuses per litter was observed at 50 and 100 mg/kg bw/d, and there were no gross external abnormalities, skeletal malformations or variations related to cobalt exposure.

Elbetieha et al., 2008: Dominant lethal assay in male mice

The study is already described in the Fertility section. Briefly, increased number of resorptions and decreased number of viable foetuses was observed in mice dams mated with males treated with cobalt chloride hexahydrate (the effects were noticed at all tested doses).

Pedigo and Vernon, 1993: Dominant lethal assay in male mice

The study is already described in the Fertility section. In females mated with cobalt-treated males there was a decrease in the number of live implantations and an increase in average pre-implantation losses. No change in post-implantation losses was observed (at gestation day 19) and *in vitro* development of 2-cell embryos to blastocyst from cobalt-treated males was not affected.

CDI/CORC, 2015c: PNDT study with cobalt chloride hexahydrate in pregnant rats
In this guideline study (according to OECD TG 414), pregnant Crl:CD(SD) rats (25/group) were given by gavage 0, 25, 50, and 100 mg cobalt chloride hexahydrate/kg bw/dy, on GD 6–19.
No mortality was observed in the dams, but at doses ≥ 50 mg/kg bw/d clinical signs were observed (piloerection, reduced motility, salivation), and at 100 mg/kg bw/d a haemorrhagic nose/snout (in 3/20 dams on GD 19 or 20). Net body weight gain was significantly reduced in all dose groups (53%, 128% and 137%, respectively), with decreased food consumption. At 50 and 100 mg/kg bw/d gastro-intestinal lesions (haemorrhagic foci in the stomach and intestines) and changes in haematological parameters were found. No effects were observed on gravid uterus weight.
No treatment related changes were observed for post-implantation loss, number of resorptions, dead foetuses, malformations, retardations or variations. The only effect was a slight (but statistically significant) reduction of mean foetal weights (by 8%) in 50 and 100 mg/kg bw/d dose groups. However, the values were still within historical control values.

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.

4.12 Other effects

Out of scope of this proposal

5 ENVIRONMENTAL HAZARD ASSESSMENT

Out of scope of this proposal

6 OTHER INFORMATION

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8 ANNEXES