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.

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	$CoCl_2 \cdot 6H_2O$	238.0	24.79

hexahydrate			
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 '	0	0	7 '	7 '
.... 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/m³	5 mg/m³	10 mg/m³	20 mg/m³	40 mg/m³
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/m³ males or 40 mg/m³ 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 ≥ 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/m³	1.25 mg/m³	2.5 mg/m³	5 mg/m³
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 ($P \leq 0.05$) 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 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/m³	5 mg/m³	10 mg/m³
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 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/m³	1.25 mg/m³	2.5 mg/m³	5 mg/m³	10 mg/m³
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

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/m³	2.5 mg/m³	5 mg/m³
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/m³	2.5 mg/m³	5 mg/m³
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/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	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/m^3		0.3 mg/m^3		1.0 mg/m^3		3.0 mg/m^3	
	♂	♀	♂	♀	♂	♀	♂	♀
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≤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/m³ 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

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.

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/m^3	1.0 mg/m^3	3.0 mg/m^3
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.

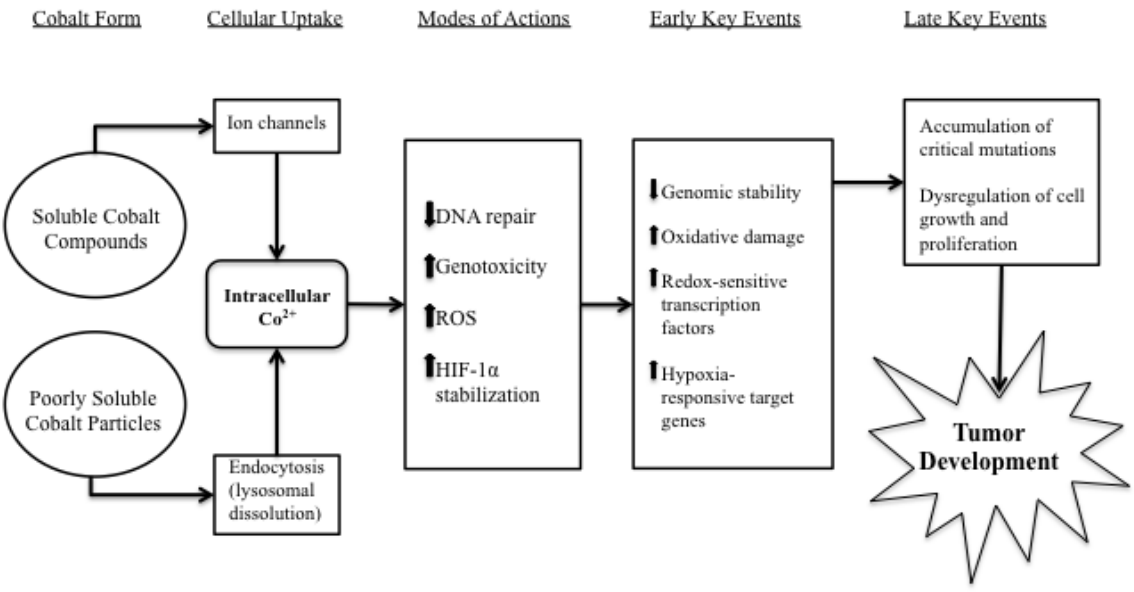

Figure 1: Proposed modes-of-action of cobalt carcinogenicity NTP (2015)

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/m^3)				
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/m^3)				

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.

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	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

(females)					
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 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	LOAEL: 40 mg/m^3: Decreased testis weight	[a]	NTP 2014

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 ≥ 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	≥ 2.5 mg/m^3: Reduced sperm motility ≥ 5 mg/m^3: Reduced sperm count, decreased testis weight 10 mg/m^3: Degeneration testes epithelium, exfoliated germ cells, hypospermia, vacuolization and atrophy epididymis	a, b, c	NTP 2014

		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			
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	5 mg/m^3: Testes infarct	[a, b]	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	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	50 mg/kg	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 $p \leq 0.05$
**: Significantly different from the controls at $p \leq 0.01$

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.

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