ESTABLISHING A REFERENCE DOSE RESPONSE RELATIONSHIP FOR CARCINOGENICITY OF FIVE COBALTS SALTS

Background

At the 22nd meeting of the Committee for Risk Assessment (RAC) in September 2012, the ECHA Secretariat presented a proposal to set DNELs and dose response relationships for substances prior to receiving applications for authorisation (AfAs). This was approved by RAC as a trial exercise. However, in early 2015, ECHA agreed to continue supporting the practice for Annex XIV substances, recognizing its value to the Authorisation process and its efficiency[1].

The DNELs and dose response relationships so derived are intended as non-legally binding 'reference values'. They provide applicants with a clear signal as to how RAC is likely to evaluate these important elements of the risk assessment of AfA.

Reference values in the form of DNELs for threshold substances and/or dose response relationships for non-threshold substances (mainly carcinogens) are published in advance of applications, for authorisation, so providing greater consistency and better use of the legally defined periods of opinion-development in the Committee for Risk Assessment (RAC).

The five cobalt salts addressed in this report were prioritised for inclusion in Annex XIV by the ECHA recommendation of 20 December 2001. On 21 December 2012, the Commission requested ECHA to conduct an investigation on the uses of the five cobalt salts. This investigation was to assess the need to develop a restriction proposal for the substances to address risks which are not adequately controlled. In the context of this study and as a result of the uncertainty surrounding the mode of action of the cobalt salts (threshold/non-threshold), ECHA requested the contractor to analyse the existing evidence and determine the dose – response relationship for the carcinogenicity effect. The results of this study are presented in this note for RAC consideration and agreement. They will be taken forward in the context of chemical risk management procedures under REACH.

Annex 1: Reference dose response relationship for carcinogenicity of five cobalt salts

[1] At the Conference on "*Lessons learnt on Applications for Authorisation*" co-organised by ECHA and the European Commission that took place on 10-11 February 2015.

Annex 1 Reference dose response relationship for carcinogenicity of water soluble cobalt salts

Five cobalt salts have been identified as substances of very high concern (SVHCs) under REACH and have been placed on the candidate list for authorisation:

cobalt(II) sulphate (EC#:233-334-2),

cobalt dichloride (EC#: 231-589-4),

cobalt(II) dinitrate (EC#:233-402-1),

cobalt(II) carbonate (EC#:208-169-4),

cobalt(II) diacetate (EC#:200-755-8)

Relevance of endpoints

The objective of this document is to support the assessment of remaining cancer risks related to the industrial use of cobalt(II) sulphate (EC#:233-334-2), cobalt dichloride (EC#: 231-589-4), cobalt(II) dinitrate (EC#:233-402-1), cobalt(II) carbonate (EC#:208-169-4), and cobalt(II) diacetate (EC#:200-755-8) in the context of chemical risk management procedures under REACH.

All the above-mentioned cobalt salts are subject to identical harmonised classification for the following endpoints: Skin Sens. 1 (H317); Resp. Sens. 1 (H334); **Carc. 1B (H350i); Muta. 2 (H341)**; Repr. 1B (H360F). It should be noted that all the mentioned cobalt salts are subject to classification as Muta.2 and further that the classification as Carc. 1B is exposure route specific and only pertains to inhalation exposure.

Due to the water solubility profiles of the substances, they are all considered soluble substances in biological systems. Thus, the five cobalt salts are described and evaluated as a category, and the divalent cobalt cation (Co^{2+}) is considered the common critical entity of the salts in relation to the carcinogenic and mutagenic potential. Thus, the different counter ions of the cobalt salts (i.e. sulphate, nitrate, chloride, acetate, and carbonate) are not considered further with regard to these effects.

Carcinogenicity

Very limited human data are available on the cobalt salts with regard to carcinogenicity.

In experimental animals, carcinogenicity data are only available in relation to the inhalational exposure route. Thus, two studies using inhalational exposure of rats and mice were available from NTP (1998). In these studies, rats and mice were exposed to 0, 0.3, 1.0 and 3.0 mg/m^3 of cobalt sulphate heptahydrate 6h/day, 5d/week during 105 weeks. The exposure resulted in increased incidences of bronchoalveolar neoplasms in both sexes of both species at all dose levels, including 0.3 mg/m^3 cobalt sulphate heptahydrate (equivalent to 0.067 mg Co/m^3). In male/female mice, the following incidences of bronchoalveolar adenomas or carcinomas were observed: 22 %/8 %, 28 %/14 %, 38 %/26 %, and 56 %/36 % at the dose levels of 0, 0.3, 1.0 and 3.0 mg/m^3 of cobalt sulphate heptahydrate, respectively. In rats, the incidences in males/females were 2 %/0%, 8 %/6 %, 8 %/32 %, and 14 %/32 %.

Thus, from these two studies there was clear evidence for a carcinogenic potential from inhalation exposure to water soluble cobalt salts in relation to induction of local tumours in the respiratory tract. Because of the local nature of the carcinogenic response, the carcinogenic potential of the cobalt salts may be specific for the inhalation route; however, due to lack of data in relation to other exposure routes this has not been confirmed. In the context of this document, dose response considerations can only be made for the inhalational exposure route.

For the background documentation for this document, a review was provided (Larsen et al. 2015) covering the registration data from the REACH registrations and the most recent (since 2004) national and international expert assessments regarding the toxicology of the cobalt substances. Furthermore, the background document took into account data received from the Cobalt Development Institute (CDI/CoRC 2015).

Table 1: Overview of expert group findings of the carcinogenic potential and mode of action of the cobalt salts (Larsen et al. 2015) below gives an overview of the outcome from these assessments regarding carcinogenicity, genotoxicity, mode of action, threshold/non-threshold considerations, critical studies, and low-dose extrapolation.

Table 1: Overview of expert group findings of the carcinogenic potential and mode of action of the cobalt salts (Larsen et al. 2015)

Expert evaluation	Carc.	Muta. *In vitro/ in vivo**	*Mode of action***	Carc. threshold / non-threshold	Cancer POD; Reference	Critical effect; POD; (Reference)
ATSDR (2004)	+ inhalation	+/+ oral + i.p.	*ROS*	No discussion	-	Reduced lung function, humans NOAEL: 0.0058 mg Co/m^3 Occupational exposure, metallic cobalt (Nemery et al. 1992)
Swedish Work and Health SWH (2005a+b)	+ inhalation	+/+ oral + i.p.	*ROS (DNA repair)*	No discussion	-	Respiratory tract irritation, humans LOAEL: 0.003 mg Co/m^3 Occupational exposure, hard metal (Alexanderson 1979)
IARC (2006)	+ inhalation; + i.p.	+/+ i.p.	*ROS* *DNA repair*	No discussion	-	Not assessed
WHO/CICAD (2006)	+ inhalation	+/+ oral + i.p.	*ROS* *DNA repair*	No discussion but attempt was made regarding	BMDL10 (male mice): 0.358 mg Co/m3 (NTP 1998)	Reduced lung function, humans NOAEL: 0.0058 mg Co/ m^3 Occupational exposure, metallic

Expert evaluation	Carc.	Muta. *In vitro/ in vivo**	*Mode of action***	Carc. threshold / non-threshold	Cancer POD; Reference	Critical effect; POD; (Reference)
				low-dose risk estimation		cobalt (Nemery et al. 1992)
MAK (2007) + MAK (2009)	+ inhalation, also relevant for dermal exposure route	+/+ oral + i.p.	*ROS (DNA repair)*	No threshold could be derived in relation to genotox and cancer	-	Various effects on the respiratory tract: various LOAELs presented No specific POD
EFSA (2009) + EFSA (2012)	+ inhalation	+/+ oral + i.p.	*ROS (DNA repair)*	No discussion	-	Polycythaemia LOAEL (oral): 1 mg Co/kg (ATSDR 2004)
Environment Canada, Health Canada (2011)	+ inhalation	+/+ oral + i.p.	*ROS (DNA repair)*	No direct interaction between Co(II) and genetic material. MoE approach to be used	-	Reduced lung function, humans NOAEL: 0.0058 mg Co/ m^3 Occupational exposure, metallic cobalt (Nemery et al. (1992)) Cardiomyopathy, humans, LOAEL (oral): 0.04 mg/kg-bw/day (ATSDR 2004); (WHO/CICAD 2006)
Danish EPA (2013)	+ inhalation, other exposure routes not excluded	+/+ oral + i.p.	*ROS; (DNA repair)*	No discussion	-	Polycythemia, humans LOAEL (oral): 1 mg/kg/d (Davis and Fields 1958)
NTP (2013)	+ inhalation (cobalt metal)	+/+ inhalation	*ROS (K-ras mutations)*	No discussion	-	-
NTP 2014	+ inhalation (cobalt sulphate)	+/not addressed	*ROS DNA repair*	No discussion	-	-

Expert evaluation	Carc.	Muta. *In vitro/ in vivo**	*Mode of action***	Carc. threshold / non-threshold	Cancer POD; Reference	Critical effect; POD; (Reference)
OECD (2014a+b)	+ inhalation	+/- oral	*ROS*	Threshold approach as not genotoxic *in vivo*	BMDL10 (female rats): 0.414 mg/m^3 as cobalt sulfate heptahydrate (NTP 1998)	Cobalt asthma, humans NOAEC: 0.12 mg Co/m^3 (Sauni et al., 2010)
ANSES 2014	+ inhalation	Metallic cobalt concluded as a weak genotoxic substance	*ROS (DNA repair)*	Non-threshold	Uncertain	Cancer/ inflammation. *Pragmatic* 8-h occupational limit value of 2.5 µg Co/m3 based on a BMDL10 (inflammation, rats) of 0.07 mg Co/m3
REACH CSR (2014)	+; inhalation	+/- oral (inhalation metallic Co)	*ROS (Non-DNA damage*	Threshold approach as not genotoxic *in vivo*	BMDL10 (female rats): 0.414 mg/m^3 as cobalt sulphate heptahydrate (NTP 1998)	DNEL (workers, long-term): 0,105 mg/m^3 based on repeated dose toxicity DNEL (general population, long-term): 0.0166 mg/m^3 based on cancer - both as cobalt sulphate (DNEL values as reported in public version of REACH registration of cobalt sulphate)

**+/- indicates positive/negative conclusion regarding genotoxicity*
***Mode of action set in ( ) indicates that the mode of action was only briefly mentioned*

Genotoxicity

In vitro data

From the IARC (2006) evaluation on cobalt and cobalt substances, it can be generally seen that for the water soluble cobalt salts there was a lack of mutagenic activity in bacteria, although isolated positive findings occurred and a co-mutagenic potential was noted in connection with co-exposure to known mutagens, e.g. benzo(a) pyrene and napthtylamine.

In *saccharomyces cerevisiae,* gene conversion and petite *p*–mutation in mitochondrial DNA were seen, but no other types of mutation occurred.

IARC (2006) noted several positive results in mammalian cells cultured *in vitro* with respect to induction of DNA–protein cross-linkage, DNA strand breakage and sister chromatid exchange in most of the studies.

In cultured human cells *in vitro*, positive results were noted for inhibition of protein-DNA binding activities and inhibition of p53 binding to DNA, for induction of gene expression (in Cap43 in human lung cells), for induction of DNA strand breakage and sister chromatid exchange. In cultured human lymphocytes, induction of aneuploidy was noted (IARC 2006). When looking through the other different expert evaluations, there are no substantial differences in the interpretation of the *in vitro* mutagenicity data. Overall, it was acknowledged that cobalt metal particles and soluble cobalt (II) salts have the capacity to cause DNA damage and chromosomal damage in mammalian cells *in vitro*.

In vivo data

A number of published *in vivo* reports indicate that cobalt salts can induce a variety of genotoxic alterations (DNA damage, gene mutations and chromosomal aberrations), see Table 2: *In vivo* genotoxicity data on water soluble and sparingly soluble cobalt salts (Compiled from IARC 2006, MAK 2007, ECHC 2011, OECD 2014a, Kirkland et al. 2015).

Table 2: *In vivo* genotoxicity data on water soluble and sparingly soluble cobalt salts (Compiled from IARC 2006, MAK 2007, ECHC 2011, OECD 2014a, Kirkland et al. 2015)

Substance and reference	**Assay**	**Exposure**	**Result**
Intraperitoneal exposure			
Cobalt(II) chloride Farah 1983	Aneuploidy Male hamsters – bone marrow – germ cells	400 mg/kg bw i.p. dosed over 9 days	 positive positive
Cobalt(II) chloride Suzuki et al. 1993	Micronuclei Mice – bone marrow	25-90 mg/kg bw i.p.	positive, dose related
Cobalt(II) chloride Rasgele et al. 2013	Micronuclei Mice – bone marrow	11.2, 22.5, 45 mg/kg bw i.p.	positive
Cobalt(II)acetate Kasprzak et al. 1994	Oxidative DNA base damage Rats – kidney, liver, lung	50 µM/kg bw i.p. (~2.9 mg Co/ kg bw)	positive

Substance and reference	Assay	Exposure	Result
Oral exposure			
Cobalt(II) chloride, Palit et al. 1991	Chrom. abb. Mice – bone marrow	0, 20, 40, 80 mg/kg bw oral	positive at all exposure levels and dose-related
Cobalt(II) chloride, Gudi 1998	Chrom. abb. Rats – bone marrow	50,200,600 mg/kg bw oral	negative
Cobalt(II)sulfate Legault 2009	Chrom abb. Rats – bone marrow	80, 160, 320 mg/kg/d single dose oral, and during 5 days oral	negative
Cobalt(II) chloride, Kirkland et al. (2015)	Chrom. abb. Rats - sperm cells	0, 3, 10, 30 mg/kg bw/d oral during 28 days	negative, no signs of toxicity noted apart from a small reduction in body weight
Inhalational exposure			
Cobalt (II)sulphate, heptahydrate NTP 1998	K-*ras* mutation Mice – from lung neoplasms	0, 0.3, 1, 3 mg/m^3 inhalation 2 years	positive

Overall, the data indicate that the water cobalt salts are genotoxic *in vivo* in connection with *i.p.* administration (Farah 1983, Suzuki et al. 1993, Rasgale et al. 2013, Kasprzak et al. 1994). Although these studies were acknowledged by the other expert assessments assessed, the relevance of the studies by Farah 1983, Suzuki et al. 1993, Rasgale et al. 2013 were questioned by OECD (2014) and Kirkland et al. (2015) as the exposure route was not considered relevant for human exposure. Furthermore, shortcomings of the studies were argued (i.e. poor reporting, too high dose level used) and the increase in micronuclei found by Rasgele et al. (2013) and Suzuki et al. (1993) was suggested to be a follow from increased erythropoiesis. Different interpretations of these studies have been made, but due to lack of a clear understanding of the mechanism involved, these consistently positive data cannot be dismissed or neglected as indications for a genotoxic potential of water soluble cobalt salts *in vivo*.

Also, i.p. micronucleus test data cannot be said to be irrelevant for the assessment of mutagenicity when it comes to a soluble *in vitro* genotoxic substance that is a potential lung

carcinogen. When assessing i.p. micronucleus test data, there are two different issues:

(i) testing for inherent potential to be mutagenic in whole animals, the hazards of concern being anything in any tissue that could be caused by chemically-induced mutagenic lesions in DNA; and

(ii) testing specifically for the ability of a chemical to produce heritable mutations in the germ cells.

The *in vivo* micronucleus test (or a comparable chromosome aberration test) has been the key study to investigate (i) for many years for substances that have been found to be genotoxic in *in vitro* systems. For all types of chemical, internationally, the i.p. route has been considered valid for this test since the early 1990s. Its use was routine in new substance dossiers, for example. The OECD test guideline No. 474, does not exclude the use of this exposure route, if justified, and such a justification could be that the target cells are to be regarded as a surrogate for any tissue in the body. In contrast, in tests such as COMET, UDS and transgenic mice gene mutations, the targets are in specific tissues and the i.p. route may not be justified.

For oral exposure, a positive dose-related finding regarding chromosome aberrations in bone marrow in mice was found (Pallit et al. 1991) with cobalt chloride, whereas more recent studies in rats gave negative results with exposure to either cobalt chloride or cobalt sulphate heptahydrate (Gudi 1998, Legault 2009). The reliability of the study by Palit et al. (1991) has, however, been questioned as it was considered most unusual for genotoxins to produce dose-related responses at all sampling times tested (OECD 2014a and Kirkland et al. 2015). Although the studies by Gudi (1998) and Legault (2009) were concluded negative based on the findings at the lowest dose levels in the studies, uncertainties apply to these studies due to mortality at the two highest dose levels.

In addition to this, Kirkland et al. (2015) reported data from a very recent study where chromosome aberrations in sperm cells were studied after 28 days of oral exposure of rats to 0, 3, 10 and 30 mg/kg/d of cobalt chloride. No signs of toxicity were noted in the study apart from a small reduction in body weight. At none of the dose levels, increased frequencies of chromosome aberrations or of polyploidy were observed. However, data on mitotic index did not indicate toxicity towards bone marrow cells in the animals.

In relation to inhalation exposure, NTP (1998) examined tissues from lung neoplasms in mice obtained from the 2-year inhalational carcinogenicity study for genetic alterations in the K-*ras* gene. A dose response relationship in the frequency of K-*ras* mutations was observed in cobalt sulphate heptahydrate-induced lung neoplasms: 14 %, 38 %, and 45 % at the dose levels of 0.3, 1.0, and 3.0 mg/m^3 doses, respectively. There were generally no differences in the mutation frequency or spectra between benign and malignant lung neoplasms. NTP (1998) noted that the higher number of k-*ras* mutations (G to T transversions at codon 12) is supportive evidence that cobalt sulphate heptahydrate may indirectly damage DNA by oxidative stress. According to NTP (1998), the observation of similar frequencies and spectra of mutations in cobalt sulphate heptahydrate-induced alveolar/bronchiolar adenomas and carcinomas is consistent with other studies showing that K-*ras* activation occurs as an early and initiating event. If mutations in the K-*ras* gene occurred later in the carcinogenic process, an increased frequency of K-*ras* mutations would have been expected in the carcinomas.

In conclusion:

- Several i.p. studies on water soluble cobalt salts have been positive for genotoxic effects after systemic uptake.
- Oral studies are non-conclusive i.e. no clear evidence on systemic genotoxicity after oral exposure.

- There may be local genotoxic effects, but these have not been really studied in appropriate studies (e.g. by in vivo comet assay in respiratory epithelial cells). NTP results on k-*ras* mutations in lung tumours suggest oxidative damage in lung tissue. In addition, i.p. data indicate oxidative damage on DNA.

Based on this evaluation it is concluded that genotoxicity as a mode of action behind lung tumours cannot to be ruled out. This is also supported by the in vitro studies discussed above.

Mode of action

Although the underlying mechanisms for the potential genotoxic and carcinogenic effects of the water-soluble cobalt salts have not been fully elucidated, the current evidence support the following primary modes of action as described by Beyersmann and Hartwig (2008):

Induction of ROS and oxidative stress:

The cobalt(II) ions are able to induce the formation of reactive oxygen species (ROS) both *in vitro* and *in vivo*, and further they catalyse the generation of hydroxyl radicals from hydrogen peroxide in a Fenton type reaction. The mechanism was supported by an i.p. study by Kasprzak et al. (1994) in which cobalt(II) resulted in the formation of oxidative DNA base damage in kidneys, liver and lungs. In addition, the analysis of mutations in tumour tissues in a carcinogenicity study with cobalt sulphate in mice (NTP 1998) revealed that five of nine mutations were G-T transversions in codon 12 of the K-*ras* oncogene, which might be due to oxidative DNA damage.

Inhibition of DNA repair:

Data have shown that the genotoxic effects of other mutagenic agents were enhanced by soluble cobalt salts as well as by cobalt metal dust. Further, cobalt(II) inhibited the nucleotide excision repair of DNA damage caused by UV-C radiation in human fibro-blasts. Both the incision and polymerisation steps were inhibited. In particular, cobalt inhibited the Xeroderma pigmentosum group A (XPA) protein, a zinc finger protein involved in nucleotide excision repair where cobalt(II) substituted the zinc ion.

These *in vitro* findings are coherent with the co-carcinogenic effect found *in vivo*, where cobalt(II) oxide enhanced the carcinogenicity of benzo[a]pyrene (Steinhoff and Mohr (1991) using intratracheally administration of the substances.

Upregulation of hypoxia-inducible factor HIF-1α:

Data have shown that cobalt(II) ions induce upregulation of hypoxia-inducible factor HIF-1α. Such upregulation is known to induce hypoxia and promote tumour growth.

As indicated in Table 1, there is *a general consensus* among the expert group assessments that especially ROS generation and impaired DNA repair are relevant modes of action for the genotoxic effects of the Co(II)-ion.

However, to which extent the available knowledge suffice to conclude on a threshold or non-threshold mechanism in a REACH context is less clear.

Overall, sufficient documentation has not been presented to make firm conclusions to whether the cobalt salts can be considered threshold or non-threshold carcinogens. This is reflected in the assessments by the various expert groups (Table 1). Most of the assessments do not discuss or conclude whether the carcinogenic mode of action has a threshold or not. MAK (2007) and ANSES (2014) indicate a non-threshold mode of action, however, giving very little,

if any discussion on this.

Environment Canada, Health Canada (2011), OECD (20145a+b), the REACH CSR´s (2014) and the recent review by Kirkland et al. (2015) did not consider the cobalt salts to be genotoxic *in vivo*. In general, they concluded on a threshold mode-of-action as they considered ROS generation and impaired DNA repair as mechanisms with a threshold (however, without giving further specific data/documentation for this assumption).

In the information further provided by CDI/CoRC (2015), it was acknowledged that specific data demonstrating a threshold for carcinogenic effects were lacking. However, it was argued (based on general assumptions) that the ROS initiating process in relation to DNA damage should be considered a threshold mode of action. For DNA-repair impairment, specific data on cobalt salts were forwarded indicating a threshold mechanism. Also, they found that the histopathological findings in the NTP (1998) studies could be explained by a cascade of effects, all of which could be considered as events with a threshold. Thus, alveolar proteinosis, chronic inflammation, hyperplasia of the alveolar epithelium, and hyperplasia of the bronchiolar epithelium could be interpreted to represent site-specific, steps in the formation of tumours. The sequential occurrence of these key events was also considered to be in accordance with the assumed MoA regarding ROS generation and oxidative DNA damage. Nevertheless, it was stated that uncertainties remain to the exact mechanisms of the alterations in the alveolar and bronchiolar epithelia and to the disturbances of the control of regenerating cell proliferation leading to carcinogenesis.

Overall, it has to be noted that specific thresholds remain to be identified for the Co(II)-ion with respect to tumour formation. Mechanistically, uncertainties pertain to whether the initial event of a catalytic effect of the cobalt(II) ions leading to oxidative DNA damages through a Fenton-like mechanism can be considered a threshold or a non-threshold effect. Further it is not clear whether the induction of alveolar proteinosis, chronic inflammation, hyperplasia (all of which may be considered as thresholded events) are prerequisite for the development of a carcinogenic response of Co(II).

When considering the REACH Guidance R.7a[2], it is stated that impairment of DNA repair *may* lead to genotoxicity via a non-linear or threshold dose response. In addition, it is stated that thresholds *may* be present for certain carcinogens that cause genetic alterations via indirect effects on DNA as a result of interaction with other cellular processes, e.g. cellular processes where the compensatory capacity or physiological or homeostatic control are exceeded. Also, it is recognised that for certain genotoxic carcinogens causing genetic alterations, a practical threshold *may* exist for the underlying genotoxic effect. For example, this has been shown to be the case for aneugens (agents that induce aneuploidy – the gain or loss of entire chromosomes to result in changes in chromosome number), or for chemicals that cause indirect effects on DNA that are secondary to another effect (e.g. through oxidative stress that overwhelms natural antioxidant defence mechanisms). The word "may" in the wording of the sentences indicates that a threshold cannot be concluded *per se,* but that such a conclusion has to be supported by data in a specific case.

In the context of a risk management decision under REACH, the scientific weight of evidence has to be weighed against the remaining uncertainties. The REACH Guidance R.8[3] emphasises that "*the decision on a threshold and a non-threshold mode of action may not always be easy to make, especially when, although a biological threshold may be postulated, the data do not allow identification of it. If not clear, the assumption of a non-threshold mode of action would be the prudent choice*". Thus, lack of sufficient documentation and existence of remaining

2 Guidance on information requirements and chemical safety assessment, Chapter R.7a: Endpoint specific guidance (version 3.0)

3 Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response for human health (version 2.1)

uncertainties would lead to the use of the most cautious approach for assessing genotoxic carcinogens, i.e. the non-threshold approach.

So, although the suggested mechanisms may have thresholds, the current data does not allow identification of this. Overall, it can be concluded:

- carcinogenicity data are only available for local tumours in the respiratory tract in relation to inhalation exposure, thus dose response estimations can only be made for inhalation exposure.

- the current scientific findings and mode of action considerations support the notion that water soluble cobalt substances may be threshold carcinogens although there are some uncertainties related to initiation by catalytic ROS generation and direct oxidative DNA damage. In addition, the genotoxicity data may indicate a non-threshold mechanism.

- thresholds have not been identified for the cobalt salts in relation to the carcinogenicity and genotoxicity in the respiratory tract.

Therefore at present, due to lack of identified thresholds and due to remaining uncertainties regarding the mechanisms involved, the water soluble cobalt salts are considered as genotoxic carcinogens and are to be assessed using a non-threshold approach.

Bioavailability

Further discussion regarding bioavailability aspects in relation to cancer risk is not considered necessary, as the carcinogenic response of the cobalt salts is not in relation to development of tumours after systemic uptake but pertain to local tumours in connection with direct exposure of the lung tissue.

Carcinogenicity risk assessment

It is concluded that cancer risk estimates can only be made in relation to the inhalational exposure route, as carcinogenicity data only pertain to inhalation exposure and local tumours of the respiratory tract. Also, it is concluded that the cobalt salts may be considered genotoxic carcinogens using a non-threshold approach for risk assessment.

The point of departure (POD) for the dose response assessment is based on the findings from the NTP (1998) inhalation studies in which mice and rats were exposed to cobalt sulphate heptahydrate by inhalation. From these data, OECD (2014a) calculated benchmark doses (BMD) using the US EPA BMD software (Version 2.0) with the Gamma Model (Version 2.13). The numbers of alveolar/bronchiolar adenoma or carcinoma in the lungs of rats and mice were selected as benchmark response. The 95 % lower confidence limit of the BMD for a treatment-related increase in response of 10 % was calculated (BMDL10). The lowest BMDL10 value of 0.414 mg/m³ was found for female rat tumours.

When converting this dose level to cobalt(II)-levels, it further has to be taken into account that chemical analysis showed that exposure in fact was to cobalt sulphate hexahydrate and *not* the heptahydrate (NTP 1998). Thus, using the molecular weights of cobalt sulphate hexahydrate (263.10 g/mol) and cobalt (58.83 g/mol) a BMDL10 of 0.093 mg Co/m^3 was derived by OECD (2014a).

As the animals in the NTP (1998) were exposed to cobalt sulphate particle with a MMAD (Mass Median Aerodynamic Diameter) in the range of 1 µm – 3 µm, and as the lung tumours from which the BMDL10 level were derived were located in the deeper part of the lung, the dose-response relationships below are related to the *respirable fraction* of the particles.

Inhalable particles would - for the particle fraction above the size of the respirable range – to a great extent be deposited in the upper part of the respiratory tract. Data from the NTP (1998) indicate that both rats and mice develop hyperplasia, metaplasia and atrophy in epithelial cells of the nose, and metaplasia of the squamous epithelium of the larynx. Although inhalable particles should also be considered as carcinogenic the dose-response related to this metric is far more uncertain as this will very much depend of the content of respirable particles. Thus, the most valid dose-response relationship for carcinogenicity is to be based on an exposure metric for respirable particles.

Dose response relationships were derived by linear extrapolation, which is to be considered as a very conservative approach, especially at very low exposure levels. It is acknowledged therefore that excess risks in the lower exposure range might be overestimated following this approach.

Inhalation exposure

Worker exposure, conversion of dose metric

The BMDL10 value of 0.093 mg Co/m^3 was calculated in association to lifetime exposure of female rats (6h/d, 5d/week, for 105 weeks).

For conversion of the daily exposure concentration, the converted BMDL10 value can be calculated according to REACH Guidance R.8 by use of the following factor:

BMDL10 conv (daily exposure) = BMDL10 (conc.) x (6h/d / 8h/d) x (6.7 m^3 * / 10m^3 **)

*average inhalation volume of humans during 8h (comparable to situation of the experimental animals)

**inhalation volume of worker during 8h light activity

BMDL10 conv (daily exposure) = 0.093 mg Co/m^3 x (6h/d / 8h/d) x (6.7 m^3/10m^3)

BMDL10 conv (daily exposure) = 0.047 mg Co/m^3

General population exposure, conversion of dose metric

The BMDL10 value of 0.093 mg Co/m^3 was calculated in association to exposure of female rats 6h/d, 5d/week, for 105 weeks (lifetime).

Thus, this dose metric has to be converted to daily lifetime exposure for the general population, i.e. the conversion shall consider population exposure 24h/d, 7d/week during lifetime.

For conversion of the daily exposure concentration, the converted BMDL10 value can be calculated according to REACH guidance R8 by use of the following factors:

BMDL10 conv (daily exposure) = BMDL10 (conc.) x (6h / 24h) x (5d / 7d)

BMDL10 conv (daily exposure) = 0.093 mg Co/m^3 x (6h / 24h) x (5d / 7d) = 0.017 Co/m^3

Non-threshold approach, dose-response

Non-threshold approach, Dose response, Workers

The linearized approach described by the REACH Guidance R.8 will be used for the non-threshold approach. When making risk calculations for occupational exposure levels, a correction has to be done to account for the fact that workers are only exposed during a fraction of their life (48 weeks per year during 40 years of work life) compared to the experimental animals that were exposed throughout their lifetime).

BMDL10 conv (occup exp) = BMDL10 (daily exp) x (52w / 48w) x (75y / 40y)

BMDL10 conv (occup exp) = 0.047 mg Co/m^3 x (52w / 48w) x (75y / 40y) = 0.095 mg Co/m^3

This BMDL10 conv (occup exp) should not be subject to the use of further assessment factors before scaling down to low level exposure, as an allometric assessment factor is only used for dose metrics expressed in mg/kg/d and not inhalational dose metrics expressed in mg/m^3.

Thus, from a risk level of 0.1 at a dose of 0.095 mg Co/m^3, a linear extrapolation for the dose response relationship for excess cancer risk can be made down to zero risk and zero exposure.

The risk can be calculated by the slope of the curve = 0.1 / 0.095 mg Co/m^3 = 1.05 (mg Co/m^3)$^{-1}$, thus

Excess risk = dose level x 1.05 (mg Co/m^3)$^{-1}$

Using this relationship, the following levels of excess risk can be calculated in relation to 8h average worker exposure:

8-h TWA cobalt concentration (mg/m^3) as respirable particles	**Excess lung tumour risk in workers (x10^{-4})**
0.1	1 050
0.095	1 000
0.01	105
0.005	53
0.001	10.5
0.0001	1.1

Non-threshold approach, Dose response, General population

The linearized approach described by the REACH Guidance R.8 will be used for the non-threshold approach. According to this method the BMDL10 (daily exposure) value calculated above should not be subjected to the use of further assessment factors before scaling down to low level exposure, as an allometric assessment factor is only used for dose metrics expressed in mg/kg/d and not inhalational dose metrics expressed in mg/m^3.

Thus, from a risk level of 0.1 at a dose of 0.017 mg Co/m^3, a linear extrapolation for the dose response relationship for excess cancer risk can be made down to zero risk and zero exposure.

The risk can be calculated by the slope of the curve = 0.1 / 0.017 mg Co/m^3 = 5.88 (mg Co/m^3)$^{-1}$, thus

Excess risk = dose level x 5.88 (mg Co/m^3)$^{-1}$

Using this relationship, the following levels of excess risk can be calculated in relation to 24h average population exposure:

24-h TWA cobalt concentration (mg/m^3) as respirable particles	**Excess lung tumour risk in the general population (x10^{-4})**
0.02	1176
0.017	1 000
0.01	588
0.001	59
0.0001	5.9
0.00001	0.6
0.000001	0.06

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