the Cobalt Development Institute

Threshold Mechanism for Cobalt Compounds

Provided to ECHA through Public Consultation (15th June to 14th September 2011) Regarding Prioritisation of Cobalt Salts

NOTE: this document is a working draft and should therefore be treated accordingly

Overview: Weight of Evidence for Threshold versus non-Threshold Effects Related to the Health Endpoints of Genotoxicity and Carcinogenicity of Cobalt Substances

Introduction

A number of cobalt compounds have been classified *inter alia* as carcinogen category 1 B (305i by inhalation) and mutagen category 2 in the EU (EC regulation 790/2009). The Cobalt Development Institute (CDI) and the Cobalt REACH Consortium (CoRC) have performed a number of additional studies that were not available at the time when the decision on the classification was made. As a consequence there is an increasing weight of evidence from high quality guideline genotoxicity studies pointing to a likely threshold mode of action for the observed inhalation carcinogenicity of cobalt sulphate that was the basis for the classification for carcinogenicity. A number of study results have only recently become available and some studies are still being finalized. An update of the REACH dossiers for the registered cobalt substances with the new data is planned for June 2011. As considerations of a possible threshold mode of action are also of importance in the prioritization process for substances of very high concern, we are summarizing the new results below.

Genotoxicity of Cobalt Substances:

***In Vitro* Cytogenicity**: The effect of cobalt substances on cellular chromosomes and genes has been investigated by *in vitro* and *in vivo* studies. *In vitro* cytogenetic assays evaluate physical damage to chromosomes such as changes in number, aberrations, and clastogenicity (chromosome breakage), or micronucleus formation and indirect damage to DNA. Toxic responses or changes at the chromosome level are believed to be *threshold-related* effects while mutagenic effects or direct effects on DNA are considered to be *non-threshold- related* (for review see Bolt 2008).

Several *in vitro* assays, with various cobalt substances (for example: inorganic cobalt salts such as cobalt dichloride, cobalt carboxylates such as cobalt resinate, and cobalt metal powder) yielded positive results. Thus the evidence that cobalt substances are associated with effects at the chromosome level *in vitro* is reasonably well documented (CoRC Study EFF4, 2009 HPV studies, 2009, Haddouk, 2007,Sire 2007, Byersman *et al* 1992).

***In Vivo* Cytogenicity:** There are few *in vivo* cytogenetic studies reported in the published literature and none are considered to be guideline compliant. A number of newly performed guideline conform studies on chromosomal aberrations *in vivo*, did all reveal negative results, showing no *in vivo* clastogenicity of the tested compounds.

1) The positive results reported by Palit and co-workers (1992) as chromosome aberrations in a non-guideline compliant mouse bone marrow cell study cells were not confirmed by the results of a GLP cytogenetics study with CoCl2 **(**Guddi et al, 1998). In the subject study the oral administration of CoCl2 to rats was not associated with bone marrow chromosome aberrations or micronucleus formation.

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2) In two GLP studies, neither the highly (water) soluble cobalt acetylacetonate or the less water-soluble cobalt resinate (a cobalt carboxylic acid salt) were found to induce micronucleus formation in rat bone marrow cells (Sire, 2007, 2009).

3) A guideline compliant bone marrow micronucleus assay evaluating the oral exposure of mice to cobalt 2-ehylhexanoate did not find increased numbers of micronucleated cells in bone marrow (Richold et al 1981).

4) The US NTP reported no increases in micronucleus formation in circulating lymphocytes of rats after 90d inhalation of cobalt metal powder (NTP 2006).

Taken together , these studies indicate that a wide range of cobalt substances do not appear have *in vivo* cytogenetic toxicity in GLP-conducted studies, even though some were (+) in *in vitro* cytogenetic toxicity studies.

***In Vitro* Mutagenicity:** Mutation assays evaluate the direct alterations or damage to genes and/or DNA. Mutagenicity is considered to be a non-threshold effect (Bolt 2008).

For cobalt substances, the published scientific literature provides conflicting information with few guideline-compliant *in vitro* mammalian cell mutation assays reported. The CoRC following guideline-compliant studies on the mutagenicity of cobalt compounds in mammalian cell systems (Beyersmann *et al* 1992).

1) Mouse HPRT locus, (Lloyd 2010, Stone 2010,2011) conducted on 11 cobalt substances (inorganic or cobalt carboxylates). These substances were chosen based on low, moderate, and high levels of solubility in water, artificial tissue fluids, and culture medium. Several were also found to be (+) in *in vitro* cytogenetic assays. All of the forward mutation assay results were negative, with the possible exception of cobalt metal powder. The test sample was (+) at the highest two concentrations tested in 1 experiment, in the presence of liver enzyme fraction only (In the most conservative assumption this result is considered weak evidence). Substances tested to date are *CoSO4-7H20, CoS, CoOxalate, Cobalt oxide hydroxide, Cobalt 2-ethylhexanoate, CoLiO2, CoB Neodecanoate, CoO, Co Powder, Co3O4, Co diHydroxide*

2) Mouse TK/lymphoma test: In a guideline-compliant assay, cobalt acetylacetonate (a cobalt dione) was found to induce mutation at this locus however cobalt resinate (a carboxylate salt) did not induce mutations (Sarlang, 2010,2011)

Overall, these *in vitro* guideline compliant study data provide a weight of evidence to support to the position that cobalt substances possess only weak if any, mutagenic capacity in mammalian cells *in vitro*.

Experimental Carcinogenicity of Cobalt Sulfate by Inhalation:

The classification of 5 cobalt salts as inhalation carcinogens is based on a single lifetime rodent inhalation study of cobalt sulfate-heptahydrate (US NTP 1998). A lifetime rodent inhalation study of a cobalt powder is completed (US NTP 2010), however the results are not yet available.

Substances that are not considered to cause tumors by direct genotoxicity may be evaluated for risk by threshold calculation (See Bolt, 2008). The evidence from guideline- compliant *in vitro* and *in*

vivo genotoxicity studies indicates that cobalt substances may not be directly genotoxic. relationship to carcinogenicity, genotoxicity if it occurs may be indirect, from the condition of sustained local issue damage and associated increased cell proliferation. A scientifically plausible explanation for the lung carcinogenicity of cobalt sulfate can be based on the threshold-related mechanisms of the inflammatory response. The hallmark of the effects associated with cobalt in the few inhalation studies reported is the presence of overt tissue inflammation in the entire respiratory system (US NTP 1990, 1998, 2006). The effects of: a). inflammation, b) tissue hypoxia, and c) generation of activated oxygen species or peroxides can activate a cascade of mechanisms that may range in effects from decreased lung function, tissue fibrosis, and lung cancer (Cassarett and Doull 2001). For setting workplace exposures to cobalt, if the mechanism for carcinogenicity is based on secondary mechanisms such as inflammation, then human health-based exposure limits that consider a threshold for inflammation can be used.

Conclusion

The weight of evidence from guideline-conformed chromosomal aberration and mutagenicity studies with cobalt compounds available to date indicates that cobalt does not cause gene mutations in mammalian cells *in vitro.* Although cobalt compounds induced chromosomal aberrations in in vitro studies in mammalian cells, the positive *in vitro* results were not confirmed *in vivo.* It can therefore be concluded that cobalt substances are unlikely to be genotoxic or clastogenic *in vivo* and a threshold mode of action for carcinogenicity can reasonably be assumed.

Other Considerations

We believe it is also important to consider that cobalt is an element constantly present in the bodies of humans and animals either in the form of vitamin B12 or ionically bound to carrier proteins in blood, simply from dietary intake. Although the body burden of cobalt only amounts to a few micrograms, it's ubiquitous and constant presence in body tissues is indicative of the fact that low dietary levels of cobalt have no health impacts. Carcinogenicity and genotoxicity associated with cobalt ingestion have not been reported in humans or animals.

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