SCIENTIFIC OPINION

Scientific Opinion on the use of cobalt compounds as additives in animal nutrition[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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ABSTRACT

In the light of genotoxicity of cobalt(II) compounds and their presumed carcinogenicity after inhalatory exposure, the use of cobalt compounds as additives in animal nutrition has been assessed, considering (i) the necessity of its use for all animal species, (ii) its implications for consumer safety and (iii) on the safety of persons handling cobalt compounds. Since the only known role of cobalt in animals is that of the central atom of vitamin B_{12}, only animals with the capacity of synthesizing vitamin B_{12} in the intestinal tract like ruminants, horses and coprophagous rabbits can utilise cobalt. Consequently, there is no necessity for cobalt supplementation of feed for other animals. No data are available in the open literature on the potential carcinogenicity of cobalt following the exposure via the oral route either in humans or in experimental animals. The exposure of consumers to total dietary cobalt has been considered. There was no indication that the thresholds for non-carcinogenic events (i.e. cardio-myopathy, polycythaemia, goiter, developmental) would be exceeded at present. Users are exposed to cobalt compounds. In the re-evaluation of cobalt compounds their dusting potential deserves therefore particular attention. As a first step, the FEEDAP Panel recommends minimizing exposure of users. The FEEDAP Panel recommends modifying the authorisation of cobalt compounds in feedstuffs by (i) restricting the use of cobalt compounds as additives to feed for ruminants (except milk replacer), horses and rabbits, (ii) limiting cobalt supplementation in feed for ruminants (except milk replacer), horses and rabbits to a maximum of 0.3 mg Co/kg complete feed, and (iii) reducing the authorised maximum cobalt content from all sources from 2 to 1 mg/kg complete feed for all species except fish. Any negative consequences of these measures on animal health and the efficiency of animal production are not expected.

KEY WORDS

Nutritional additive, compound of trace elements, cobalt, cobalt compounds, vitamin B_{12}, safety

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SUMMARY

According to Regulation (EC) No 790/2009 amending Regulation (EC) No 1272/2008, cobalt dichloride and cobalt sulfate are of low acute toxicity (category 4), but are classified as respiratory and skin sensitizers (category 1), as acute and chronic toxicants to the aquatic environment (category 1) and as presumed human carcinogens by the inhalatory route (class 1B). Cobalt(II) cations are also considered genotoxic under *in vitro* and *in vivo* conditions.

In the light of the above properties of cobalt and since several cobalt compounds are authorised by EU legislation as feed additives, a risk assessment of the use of cobalt compounds in animal nutrition was undertaken considering (i) the necessity of cobalt supplementation for the target species including potential adverse effects of minimising/withdrawing cobalt supplementation on animal health, (ii) the safety for consumers of foods from animals treated with cobalt salts, and (iii) the safety of persons handling cobalt compounds as feed additives.

Monogastric animals (excluding horses and rabbits) do not require cobalt but they require vitamin B_{12}. Consequently, there is no need for any cobalt supplementation to their feed.

The ruminal microflora can synthesize vitamin B_{12}, provided dietary cobalt is available in sufficient quantities. Consequently, the vitamin B_{12} requirement of these animals can be covered by dietary cobalt. The host metabolism of ruminants also requires only vitamin B_{12}. But for a potential replacement of cobalt by vitamin B_{12}, there are not enough data to evaluate the consequences on health and performance for these species under field conditions. Such a replacement is also considered inefficient because of the high ruminal degradation rate of oral vitamin B_{12}. A withdrawal of cobalt (supplementation) from the diet would also affect ruminal microbiota, its composition and function. Some small beneficial effects observed in ruminants after cobalt supply are likely to be related to an unspecific cobalt effect on the microbiota rather than to vitamin B_{12}. An optimal micronutrient supply of ruminants would therefore include cobalt. A comparable conclusion may be drawn for horses and coprophagous rabbits (hindgut fermentation of vitamin B_{12}), although there is a lack of quantitative data.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the potential cobalt supplementation to diets for ruminants, horses and rabbits should be maintained. A cobalt supplementation of 0.3 mg/kg dry matter (DM) and, taking into account cobalt background concentrations of feed material not exceeding 0.5 mg/kg DM of complete feed, a maximum content of 1 mg Co/kg complete feed, is considered appropriate. For fish diets, the existing maximum content of 2 mg Co/kg complete feed should be maintained due to the higher cobalt background levels of fish meal, a major part of fish diets.

The tolerance of ruminants to cobalt is very high and greatly in excess of the requirements. Therefore, it is considered unlikely that cobalt toxicity in target animals could be a major problem in practice.

Among foodstuffs of animal origin, offal shows the highest cobalt content, liver with about 0.02-0.07 mg/kg fresh weight followed by kidney with about 0.001-0.01 mg/kg FW. Meat is in the range of 0.001-0.02 mg/kg fresh weight as are fillets of freshwater fish. Milk and eggs contain about 0.004-0.005 mg Co/kg; dairy products like cheese and butter are relatively rich in cobalt (0.02 mg/kg FW).

Virtually all cobalt in offal and beef meat can be attributed to vitamin B_{12}. The fraction of vitamin B_{12}-bound cobalt is considerably smaller in poultry and pork meat (about 20 to 40 %) indicating dietary supply of cobalt as such. Eggs and milk contain even higher amounts of vitamin B_{12} unrelated cobalt (about 70 and 95 %, respectively) indicating excretion of absorbed soluble cobalt. However, these are estimates with several uncertainties due to methodological reasons (e.g. poor data set, analytical methods).

No data are available in the open literature on the potential carcinogenicity of cobalt following the exposure via the oral route either in humans or in experimental animals. The exposure to cobalt via the oral route may potentially entail a number of adverse in effects in humans (cardiac effects, effects on erythropoiesis, effects on thyroid, developmental effects, and allergic dermatitis).

A daily oral intake of 600 μg Co (based on a LOAEL of 1 mg/kg for polycythaemia) appears a minimum risk level for humans that would protect from the known threshold-related adverse effects.

The estimated population average intake of cobalt was reported to be 0.012 mg/day in the UK, 0.005–0.04 mg Co/day in the US, 0.011 mg Co/day in Canada, and 0.029 mg Co/day in France. All these intake values are quite below the oral threshold of 600 μg per person.

The FEEDAP Panel, based on its own calculations, concluded that the potential cobalt intake of consumers from food of animal origin would not exceed 14 μg/day and is therefore not of safety concern.

Users are exposed to cobalt compounds (dichloride and sulfate are skin and respiratory sensitizers and carcinogenic by inhalatory route). For pulmonary effects, the Agency for Toxic Substances and Disease Registry (ATSDR) has developed a minimum risk level of 0.1 μg Co/m^3 air. At present, no data on dusting potential of the authorised cobalt compounds have been available to the FEEDAP Panel. During re-evaluation of cobalt compounds, the dusting potential of cobalt additives deserves particular attention.

Since the contact of persons handling the additive cannot be fully avoided, the FEEDAP Panel recommends, as a first step, minimizing exposure to cobalt compounds.

The FEEDAP Panel also recommends modifying the authorisation of cobalt compounds in feedstuffs by (i) restricting the use of cobalt compounds as additives to feed for ruminants (except milk replacer), horses and rabbits, (ii) limiting cobalt supplementation in feed for ruminants (except milk replacer), horses and rabbits to a maximum of 0.3 mg Co/kg complete feed, and (iii) reducing the authorised maximum cobalt content from all sources from 2 to 1 mg/kg complete feed for all species except fish.

No negative consequences of these measures on animal health and the efficiency of animal production are expected.

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BACKGROUND AS PROVIDED BY EFSA

Cobalt is a component of Vitamin B_{12}. Cobalt salts have been commonly used for many years in animal nutrition and six cobalt salts are currently authorised in the EU as feed additives (Co-carbonate; Co-acetate; Co-dichloride; Co-nitrate; Co-sulfate (mono- and hepta- hydrate)) at a maximum content of 2 mg Co/kg complete feedingstuff.[4]

The International Agency for Research on Cancer has classified cobalt, cobalt compounds[5], Co-sulfate and other soluble Co (II) salts[6] as possibly carcinogenic to humans (group 2B).

The European Commission has recently classified Co-sulfate and Co-dichloride in the Category 1B (presumed to have carcinogenic potential for humans, classification largely based on animal evidence).[7]

In 2009, the Panel on Food Additives and Nutrient Sources Added to Food (ANS) was requested by the European Commission to deliver an opinion on the safety of cobalt(II) chloride hexahydrate added for nutritional purposes as a source of cobalt in food supplements and the bioavailability of cobalt from this source. The ANS panel concluded that "Given the toxicological profile of cobalt(II) chloride hexahydrate, including genotoxicity and carcinogenicity, the proposed uses of cobalt(II) chloride hexahydrate added for nutritional purposes in food supplements as a source of cobalt are of safety concern".[8]

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) identified the need to perform an assessment on the safety of the use of cobalt compounds as additives in animal nutrition.

TERMS OF REFERENCE AS PROVIDED BY EFSA

Considering the given genotoxic and carcinogenic properties of cobalt compounds, the FEEDAP Panel is requested to deliver an opinion on safety for the consumer and the user related to cobalt compounds used as feed additives as well as on the potential impact on target animal health.

[4] OJ L 187, 26.7.2003, p. 11
[5] IARC Monograph 52; 1991
[6] IARC Monograph 86; 2006
[7] OJ L 353, 31.12.2008, p. 1
[8] The EFSA Journal (2009) 1066, 1-8

ASSESSMENT

1. Introduction

Cobalt (Co) is a metallic element occurring in the most common compounds in +2 or +3 oxidation states. Co(III) is the central atom of cobalamin (vitamin B_{12}), representing about 4.3 % of the molecular weight.

The toxicological profile of Co and its impact in the terrestrial food chain have been extensively reviewed (ATSDR, 2004; IARC, 2006; WHO, 2006; Lison, 2007; Gál et al., 2008). A comprehensive overview is given in Appendix A.

Concern has been raised by the potential genotoxic and carcinogenic activity of Co(II). The results of *in vitro*-assays performed with soluble Co(II) salts including acetate, chloride, and sulfate (IARC 2006) clearly demonstrate the full mutagenic potential of the several salts in mammalian cells, whereas most of the tests performed in bacteria were negative. Two major mechanisms seem to be involved, namely (i) the generation of reactive oxygen species through a Fenton-like mechanism and (ii) the inhibition of DNA repair mechanisms (Beyersmann and Hartwig, 2008). Several published *in vivo* studies indicate that Co salts (chloride or acetate) are capable of inducing a variety of genotoxic alterations (DNA damage, gene mutations, micronuclei formation, chromosomal aberration) in laboratory species exposed by oral or parenteral routes. Although experimental data indicate some evidence of a genotoxic potential for Co in human lymphocytes *in vitro* (De Boeck et al., 1998; Lison et al., 2001), inconclusive evidence of Co-mediated genotoxicity in humans has been provided by studies conducted in workers exposed to Co dusts (De Boeck et al., 2000) or in individuals bearing orthopaedic joint replacements made of Co-containing alloys (Keegan et al., 2008).

Several experiments performed in laboratory animals support the *in vivo* carcinogenicity of Co salts when administered by different routes, and namely local tumours (sarcomas) at injection sites and lung tumours after intratracheal instillation (Bucher et al., 1999). No published studies were found concerning oral route.

Lison et al. (2001) concluded that the genotoxic potential of Co(II) cations is demonstrated *in vitro* and there is substantial evidence that Co(II) cations exert genotoxic as well as carcinogenic effects in animals; moreover, it seems reasonable to consider that all soluble Co(II) salts (chloride, sulfate, acetate) share this carcinogenic potential.

The available data in humans rely almost exclusively on the follow up of workers employed in Co production plants. Several reports addressing cancer risks among workers in hard-metal production facilities provide evidence of an increased lung cancer risk related to exposure to hard-metal dust containing Co and tungsten carbide.

According to Regulation (EC) No 1272/2008,[9] on classification, labelling and packaging of substances and mixtures, Co-dichloride and Co-sulfate are of low acute toxicity (category 4), but are classified as respiratory and skin sensitizers (category 1) as acute and chronic toxicants to the aquatic environment (category 1)[10] and as presumed human carcinogens (class 1B) by the inhalatory route.

In the light of the above properties of Co and since several Co compounds are authorised by EU legislation as feed additives (Co-carbonate, Co-acetate, Co-dichloride, Co-nitrate and Co-sulfate mono- and heptahydrate; maximum content for all species: 2 mg total Co/kg complete feed), a risk assessment of the further use of Co compounds in animal nutrition should consider:

- the necessity of Co supplementation for the different target species including potential adverse effects of minimising/withdrawing Co supplementation on animal health

[9] OJ L 353, 31.12.2008, p. 1, last amended by Regulation (EC) No 790/2009 (OJ L 235, 5.9.2009, p. 1)
[10] H400 and H410 (Very toxic to aquatic life with long lasting effects)

- the safety for consumers of foods from animals treated with Co salts
- the safety of persons handling Co compounds as feed additives.

Due to the direct relationship between Co and vitamin B_{12}, vitamin B_{12} is also considered in this opinion.

2. Cobalt and vitamin B_{12} in target species

The discovery that increased dietary Co can prevent the disease called unthriftiness in sheep and cattle was made in the thirties of the former century. The Co incorporation into the structural centre of vitamin B_{12} and therapeutic efficiency of vitamin B_{12} injection to ruminants with Co deficiency was demonstrated some 20 years later. A more detailed overview on the biological role of Co and vitamin B_{12} in animal nutrition can be found in Appendix B.

2.1. The biological role of cobalt

The only known essential role of Co in animals and humans is being a component of vitamin B_{12} as Co(III). Absorbed Co per se, Co(II), is not known to have any biological function.

Cobalt is an essential trace element for ruminants and horses, which can synthesise vitamin B_{12} in the digestive tract by microbial action (NRC, 1980 and 1989). Non-ruminants require the intake of vitamin B_{12} because they lack the ability to synthesise the vitamin in significant amounts by digestive tract microbiota. However, pigs and poultry are known to synthesise small amounts by hindgut bacteria, coprophagous animals may receive some supply of vitamin B_{12} from microbial fermentation.

The efficiency of incorporation of Co in vitamin B_{12} in ruminants is low and inversely related to Co intake. Incorporation rate may be characterized by a range of 3 to 15 % and an average range of 10 to 15 %. (Smith and Marston, 1970; Stemme et al, 2008; Girard et al, 2009).

Besides covering the requirements for vitamin B_{12} synthesis, Co may play a role in rumen fermentation by increasing fiber digestion from low quality forages (Lopez-Guisa and Satter, 1992; Zelenak et al., 1992).

Water soluble forms of Co are better absorbed than water insoluble forms. Cobalt is predominantly excreted via the faecal route, characterising mainly the unabsorbed fraction. Absorbed Co follows aqueous excretion routes, via kidney but also via the milk. Urinary Co is considered a good indicator of exposure to soluble Co, but not to insoluble Co compounds (Cornelis et al., 1995).

2.2. The biological role of vitamin B_{12} (cobalamin)

Cobalamin belongs to the structural class of corrinoids, which includes besides the vitamin active forms hydroxycobalamin (OH-cbl), adenosylcobalamin (ado-cbl) and methylcobalamin (me-cbl) several rather inactive analogues as cobamide and cobinamide.

While a number of vitamin B_{12}-dependent metabolic functions have been identified in micro-organisms, only two vitamin B_{12}-dependent enzymes have been described in animals (Kennedy et al., 1991). Methylmalonyl Coenzyme A mutase is involved in the conversion of methylmalonyl-CoA into succinyl-CoA, finally releasing energy from proteins and fatty acids. The 5-methyltetrahydrofolate-homocysteine methyltransferase, also known as methionine synthase, catalyses the conversion of homocysteine to methionine. Finally, a third coenzyme function (Leucine mutase) has been proposed (Underwood and Suttle, 1999; McDowell, 2000).

Vitamin B_{12} might also be essential for a proper function of ruminal microbiota. While some ruminal bacteria produce vitamin B_{12} from Co, others require vitamin B_{12} in ruminal fluid for their normal metabolic function. Namely, production of propionate, a key metabolite for energy metabolism of ruminants, at the expense of succinate has been shown *in vitro* (Chen and Wolin, 1981; Strobel, 1992) and confirmed by *in vivo* studies (Kennedy et al., 1991 and 1996).

Only about 40 % of the apparent ruminally synthesised cobalamin reaches the lower intestine. The degradation products without vitamin B_{12} activity reaching the intestine are poorly absorbed. The intestinal disappearance (difference between duodenal and ileal supply) indicated an apparent absorption of cobalamin of about 45 %. Orally supplied cobalamin is utilised at lower levels (higher degradation rate in the rumen, lower intestinal disappearance) (Girard et al, 2009).

2.3. Deficiency signs

In general, Co-vitamin B_{12} deficiency in animals results in loss of appetite, reduced growth rate and even loss of body weight in several cases, and anaemia (Underwood and Suttle, 1999)

Ruminants fed forages with Co concentrations <0.08 mg/kg develop signs of Co/vitamin B_{12} deficiency (McDowell, 1997) showing normocytic and normochromic anaemia, disturbances of lipid metabolism, reduced folate level, accumulation of iron and nickel in liver, compromised neutrophil function and reduced resistance to parasitic infections. Necropsy of severely affected animals shows emaciation, often with total absence of body fat, liver fatty changes and occasional spleen haemosiderosis (Paterson and McPherson, 1990; Kennedy et al., 1994; Stangl et al. 1989 and 2000). Sensitivity to Co deficiency is highest in cattle, followed by sheep and goats (McDowell, 2003).

Ruminants appear to be more sensitive to vitamin B_{12} deficiency than non-ruminants (NRC, 2001). In pigs vitamin B_{12} deficiency affects reproduction, litter size and survival of the progeny. Similarly, in poultry, the lack of vitamin B_{12} impairs hatchability (Squires and Naberl, 1992; McDowell, 2003).

2.4. Occurrence of cobalt in feed materials

A study in Sweden from 1983 to 1990 evaluated Co levels in various feedstuffs (Jorhem & Sundström, 1993). Cobalt levels were highest in seeds (alfalfa 0.86 mg/kg FW; linseed, 0.56 mg/kg FW). More recent data (CVB, 2009) confirm these findings principally, although at higher levels with 1.99 mg Co/kg linseed and 1.73 mg Co/kg alfalfa meal. Comparably high values were found for rice with hulls (2.03 mg/kg), corn gluten meal (2.02 mg/kg) and fish meal (1.88 mg/kg). The CVB data are listed in Appendix C.

2.5. Requirement

2.5.1. Cobalt

The dietary requirement of Co was estimated tobe for dairy cattle 0.11 (NRC, 2001), for cattle for fattening 0.1 (NRC, 2000), for sheep 0.11 and for goats 0.1-0.2 (NRC, 2006), and for horses 0.1 mg Co/kg DM (NRC, 1989). More recent allowances for maximum growth and maximum feed intake of cattle for fattening were estimated to be 0.12 and 0.16-0.18 mg/kg DM (Schwarz et al., 2000), for biochemical end points (e.g. liver vitamin B_{12} and plasma homocysteine) 0.15 to 0.20 mg and for maximum vitamin B_{12} synthesis 0.25 mg/kg DM (Stangl et al., 2000).

Considering low Co average background levels in feedingstuffs (<0.1 to 0.5 mg/kg DM, plant feed materials showing lowest values), feed supplementation is generally needed to cover the requirement.

It should be noted that all the above requirement data are intended to optimise the vitamin B_{12} supply to some animal species. A specific requirement for Co per se is not established.

2.5.2. Vitamin B_{12}

In general, requirements of vitamin B_{12} for various species are relatively low and depend also on dietary levels of other nutrients like choline, methionine, folacin and ascorbic acid. With an abundance of methyl groups, the vitamin B_{12} (and folacin) requirements are reduced (McDowell, 2000).

The vitamin B_{12} requirement for pigs, poultry and fish is in the range of 3-20 µg/kg complete feed (NRC 1993, 1994, 1998), for dogs and cats 20 and 26 µg/complete feed (NRC, 2006), respectively. Only milk replacer fed calves show a higher requirement (70 µg/kg milk replacer, NRC 1998).

In ruminants, the requirements of vitamin B_{12} for dairy cows have been estimated between 0.34 and 0.68 µg/kg body weight (NRC, 1989a), corresponding to approximately 10-20 µg/kg DM.

2.6. Fate of cyanocobalamin supplemented to feed for ruminants

The utilisation of vitamin B_{12} supplemented to feed for ruminants has been shown to be rather ineffective. A study on dairy cows fed diet supplemented with an extremely high dose of vitamin B_{12} (500 mg/day) showed an extensive ruminal destruction of added vitamin; only 20 % of supplemented cyano-cobalamin reached the duodenum (Girard et al., 2009). Due to further apparent degradation of ingested cyanocobalamin along the small intestine before reaching the ileal level (the site of vitamin B_{12} absorption), only 0.27 % were absorbed within 24 hours; total absorption over a longer period could result in a higher amount (Girard et al., 2001).

2.7. Conclusions on the necessity of cobalt for target animals

According to the knowledge of the FEEDAP Panel, compound feedingstuffs in the EU are currently supplemented with Co compounds irrespective of the intended target species. The supplementation rate may range from 0.1 to 0.6 mg/kg complete feed.

Monogastric animals (including poultry and fish) do not require Co but vitamin B_{12}. Consequently, there is no need for any Co supplementation to the feed for these animals.

The ruminal microflora can synthesize vitamin B_{12}, provided dietary Co is available in sufficient quantities. Consequently, the vitamin B_{12} requirement of these animals can be covered by dietary Co. The host metabolism of ruminants requires also only vitamin B_{12}. However, there are not enough data to evaluate the consequences on health and performance for these species under field conditions for a complete replacement of Co by vitamin B_{12}. Such a replacement is also considered inefficient because of the high ruminal degradation rate of oral vitamin B_{12}. A withdrawal of Co (supplementation) from the diet would also affect ruminal microbiota, its composition and function. An optimal micronutrient supply of ruminants would therefore include Co. A comparable conclusion may be drawn for horses and coprophagous rabbits (hindgut fermentation of vitamin B_{12}), although there is a lack of quantitative data.

The FEEDAP Panel concludes that the potential Co supplementation to diets for ruminants, horses and rabbits should be maintained. A Co supplementation of 0.3 mg/kg DM and, taking into account Co background concentrations of feed material not exceeding 0.5 mg/kg DM of complete feed, a maximum content of 1 mg Co/kg complete feed is considered appropriate. For fish diets, the existing maximum content of 2 mg Co/kg complete feed should be maintained due to the higher background levels of fish meal being a major part of fish diets.

3. Safety

3.1. Safety for the target animals

The tolerance of ruminants to Co is very high and greatly in excess of the requirements. Therefore, it is considered unlikely that Co toxicity in target animals could be a major problem in practice. Sheep apparently tolerate higher doses than cattle.

The NRC (2005) set 25 mg/kg feed as maximum tolerable levels of Co for cattle, poultry, sheep and horses and 100 mg/kg feed for swine. According to EU legislation, feed for all animal species is not allowed to exceed 2 mg total Co/kg complete feed.

3.2. Safety for the consumer

3.2.1. Occurrence of cobalt and vitamin B_{12} in foodstuffs

3.2.1.1. Total Co concentrations in foodstuffs

In a report from WHO (2006), the largest potential source of Co exposure for the general population is food. They concluded that most of the ingested Co is inorganic; vitamin B_{12} contains Co but occurs in foods of animal origin and represents only a small fraction of total Co intake. Green vegetables and fresh cereals have been reported to be the richest sources of Co (0.2–0.6 mg/kg DM), whereas dairy products, refined cereals, and sugar were found to contain the least Co (0.01–0.03 mg/kg DM) (IARC, 1991; Cobalt Development Institute, 2003).

Since Co is only regarded an essential element as a component of vitamin B_{12} and has not been considered a priority food contaminant, the literature on Co contents in European foods is relatively scarce. Between 1983 and 1990 Co levels of various foodstuffs in Sweden were evaluated by Jorhem and Sundström (1993). Cobalt levels were highest in beef liver (0.043 mg/kg), and milk chocolate (0.34 mg/kg), whereas fish, fruit, and leafy vegetables contained <0.01 mg/kg fresh weight.

Two comprehensive studies on the topic have been published focussing on total Co concentrations in foodstuffs from the Swedish and French markets (Jorhem and Sundström, 1998; LeBlanc, 2005). Some mushrooms appear to contain remarkably high concentrations of Co with levels in different species reported to average between 0.04 and 3 mg/kg FW (Jorhem and Sundström, 1998; Ouzouni et al., 2007). Other products of plant origin with particularly high Co contents include seeds (0.15 – 0.86 mg/kg FW), chocolate (0.05 – 0.34 mg/kg FW), chick peas (0.11 mg/kg FW), and beans (0.034 – 0.084 mg/kg FW) (Jorhem and Sundström, 1998; LeBlanc, 2005). In Spain, Co concentrations in 20 brands of beer ranged from 0.16 to 0.56 µg/L, with a median concentration of 0.39 µg/L (Cameán et al., 1998).

Among foodstuffs of animal origin, high levels of Co have been found in "shellfish" and offal products. Three samples of "shellfish" (crustaceans and molluscs) from regional markets in France showed an average Co concentration of 0.046 mg/kg FW (no measure of variability provided; LeBlanc et al., 2005). Jorhem and Sundström (1998) reported levels of beef and pork liver from Sweden to average 0.043 mg/kg FW (range: 0.019 – 0.074, n=3) and 0.030 mg/kg FW (range: 0.002 – 0.023), respectively. The content of Co in kidney was much lower and was determined to 0.008 mg/kg FW (range: 0.003 – 0.01) for cattle and 0.004 mg/kg FW (range: 0.001 – 0.011) for pig (Jorhem and Sundström, 1998). Meats from beef, pig and poultry are similarly low in Co with concentrations of 0.001 – 0.018 mg/kg FW. Cheeses, butter, margarine and oils from the French market showed uniform Co concentrations 0.017 – 0.018 mg/kg FW (LeBlanc et al., 2005). Fillets of some freshwater fish (Arctic char, whitefish and trout) from Sweden contained relatively high levels of Co (overall range: 0.001 – 0.02 mg/kg FW) (Jorhem and Sundström, 1998). Fillets from species such as pike, perch and walleye were within this range, showing maximum Co concentrations below 0.004 mg/kg FW, which was similar to the level found in most of the several seawater fish sampled. As fish were sampled from different areas (Jorhem and Sundström, 1998), some of the variation in Co levels may reflect local environmental concentrations rather than species-specific differences.

A tabulated summary is given in Appendix D.

3.2.1.2. Vitamin B_{12} concentrations in foodstuffs

Whilst there is little published information on total Co concentrations in European foodstuffs, there is plenty of data on their cobalamin contents and this information is listed in several national food composition tables (Appendix E). Vitamin B_{12} is primarily found in food of animal origin and the concentrations in plant-derived foods are very low. Only the former is considered here. Offal products generally have high levels of vitamin B_{12} with the highest concentrations present in liver from ruminants (range: 0.39 – 2 mg/kg). Vitamin B_{12} concentrations in livers from other food animals, such

as pork, cod and duck, are somewhat lower (range: 0.1 – 0.54 mg/kg). Kidney and heart have also relatively high, but more uniform vitamin B_{12} levels among species (range: 0.03 – 0.4 mg/kg). Average vitamin B_{12} concentrations in meat are also similar between species, ranging from averages of 0.008 mg/kg in pork meat to 0.02 mg/kg in meat from beef and lamb. Eggs and dairy products have likewise modest vitamin B_{12} contents with average levels varying one order of magnitude from 0.003 µg/100g in cream to 0.03 mg/kg in eggs. Molluscs (mussels, clams, snails, octopus, etc) contain high vitamin B_{12} levels and concentrations of vitamin B_{12} in some bivalves (e.g. clams) are similar to those in liver (up to 1.53 mg/kg). There is substantial variation in vitamin B_{12} levels of different fish species with some of the highest average levels found in fillet of pike (0.13 mg/kg), herring (0.12 mg/kg) and sardine (0.11 mg/kg). However, most species have considerably lower vitamin B_{12} levels in muscle with a typical range of 0.01 – 0.07 mg/kg tissue. Fish roe is also a source of vitamin B_{12} with concentrations ranging from 0.04 to 0.2 mg/kg.

3.2.1.3. Contribution of Co from cobalamin to total Co in food

Vitamin B_{12} contains about 4.3 % Co by weight. Calculating the Co content that can be attributed to vitamin B_{12} and subtracting it from total Co would reveal the amount of Co that is not incorporated in vitamin B_{12}. The results are shown in Table 1. The data in this table originate from different sources for the same foodstuff and are based on different sample size (see Appendixes D and E) and unknown differences in the analytical approach. The ratio is therefore an approximation but indicates potential differences in the content of non-vitamin B_{12}-related Co.

Ratios of about one (a range of 0.5 to 2, taking into account the above-mentioned uncertainties) would suggest that all Co comes from vitamin B_{12}. Vitamin B_{12} related Co shows in most foods of animal origin, including liver, kidney, meat of beef and fish, a good agreement with total Co concentrations. Deviations from this pattern in pork and poultry meat are considered as indicators that Co is fed as trace element and deposited in the meat as such. Milk, including dairy products, and to a lesser extent eggs, including egg products, considerably exceed the ratio of about one, indicating a significant excretion route for the absorbed dietary Co.

Table 1: Comparison of measured Co concentrations in different foods of animal origin compared with calculated Co contents associated with vitamin B_{12} in the same products

	Cobalt (µg/kg)		Ratio
FOOD CATEGORY	**Total**	**Vitamin B_{12}**	**Total Co/Co from vitamin B12**
OFFAL			
Beef liver	43	51	0.8
Pig liver	10	14	0.7
Beef kidney	8	10	0.8
Pig kidney	4	10	0.4
MEAT			
Beef	1	0.8	1.3
Pork	1	0.4	2.5
Poultry and game	2	0.4	5.0
EGGS AND EGG PRODUCTS	5	1.4	3.6
FISH			
Artic char	8	4	2.0
Baltic herring	5	5	1.0
Trout	4	3	1.3
Cod	2	0.4	5.0
Perch	2	2	1.0
Mackerel Atlantic	1	4	0.3
Pike	1	6	0.2
SHELLFISH	3	12	0.3
DAIRY PRODUCTS			
Milk	4	0.2	20
Cheese	18	0.6	30

3.2.1.4. Correlation between intake and deposition of Co

Literature data on the effects of Co supplementation to feed on Co tissue deposition in animals is very scarce. Henry at al. (1997) showed on sheep fed diets supplemented with high Co levels (0, 20 and 40 mg Co/kg as sulfate for 60 days), highly significant correlation between Co in the diet and in animal tissues. Highest accumulation was found in liver, followed by kidney, heart, spleen and muscle. Response to increasing oral Co of pigs and chickens showed the highest accumulation of the element in kidney followed by liver (Huck and Clawson, 1976; Blalock, 1985). In rats, the absorbed Co is primarily retained in liver (Ayala-Fierro et al., 1999).

Regarding tissue Co speciation, published data are even scarcer. Recent analysis of corrinoids in ovine tissues (Kelly et al, 2006) showed that in liver ado-cbl dominated followed by OH-cbl, me-cbl and cobalamin analogues while in blood OH-cbl predominated followed by ado-cbl, analogues and me-cbl. Dietary Co supplementation of ruminating sheep led to an increase in liver ado-cbl and analogues. Contrary to humans, in sheep the amount of ado-cbl was consistently higher in all tissues than methylcobalamin.

3.2.1.5. Conclusions

Among foodstuffs of animal origin, offal shows the highest Co content, liver with about 0.02-0.07 mg/kg FW, followed by kidney with about 0.001-0.01 mg/kg FW. Meat is in the range of 0.001-0.02 mg/kg FW as are fillets of freshwater fish. Milk and eggs contain about 0.004-0.005 mg Co/kg; dairy products like cheese and butter are relatively rich in Co (0.02 mg/kg FW).

Virtually all Co in offal and beef meat can be attributed to vitamin B_{12}. The fraction of vitamin B_{12}-bound Co is considerably smaller in poultry and pork meat (about 20 to 40 %) indicating dietary

supply of Co as such. Eggs and milk contain even higher amounts of vitamin B_{12}-unrelated Co (about 70 and 95 %, respectively) indicating excretion of absorbed soluble Co. However, these are estimates with several uncertainties due to methodological reasons (e.g. poor data set, analytical methods).

Correlations between Co/vitamin B_{12} intake at physiological feed concentrations and tissue deposition could not be established by the FEEDAP Panel due to lack of data. Any prediction of the Co content of food of animal origin from dietary Co/vitamin B_{12} is therefore not possible at present.

3.2.2. Guidance values for cobalt intake

There is some evidence that the occupational exposure to Co compounds via the inhalatory route could result in an increased lung cancer risk. However, no data are available in the open literature on the potential carcinogenicity of Co following the exposure via the oral route either in humans or in experimental animals (IARC, 2006; WHO, 2006).

As outlined in detail in the Appendix A the exposure to Co via the oral route may potentially entail a number of adverse in effects in humans, the most important of which are a) cardiac effects, b) effects on erythropoiesis, c) effects on thyroid, d) developmental effects and e) effects on the immune system (allergic dermatitis). A Minimal Risk Level of 0.01 mg Co/kg body weight/day has been derived for intermediate duration (≤ 365 days) of Co exposure based on a LOAEL of 1 mg/kg for polycythaemia (ATSDR, 2004).

A Co-related cardiomyopathy has been reported in the heavy drinkers consuming large quantities of beer in which Co-sulfate or chloride have been added as foam stabilizer at 1-2 mg/kg resulting in an average intake of 0.04 to 0.14 mg Co/kg/day for years, although other factors (poor protein diet, heart damage from alcohol abuse) may have acted as confounders (WHO, 2006).

An increase in the erythrocyte number in anaemic patients may be obtained by administering Co at an average daily rate of about 1 mg/kg bw by the oral route, the effective doses ranging from 0.7 to 2.0 mg/kg bw; this is considered the most sensitive biological effect linked to repeated oral exposure to the metal (ATSDR, 2004). Higher doses have been associated to thyroid effects in addition to the desired polycythaemia.

Goiter is a well known side effect of Co therapy in the medical treatment of certain anemias. A LOAEL of 0.54 mg/kg/day (WHO, 2006) has been derived for the effects on thyroid, consisting in a decrease of iodine uptake; this was based on a study in which Co-chloride (37.5 mg per day) was administered once a day for up to 25 days (Paley et al., 1958).

Developmental toxicity was seen in animal studies. A NOAEL was derived from a study in which no effects were observed in the children of 78 women given orally Co-chloride up to 0.6 mg Co/kg/day for 90 days during pregnancy for treatment of anaemia. However, only a limited examination of offspring was reported, and details of examined end points were not described (WHO, 2006).

A Co related systemic but reversible allergy referred to as dyshidrotic eczema has been reported in patients usually sensitive also to dietary nickel. Although this kind of pathology is clearly a non-threshold effect, most of the Co sensitive patients are reported to have an oral challenge of the metal far in excess of levels seen in a normal diet (assuming an average exposure of 12 μg Co per day). However it may be estimated that 1 % of these patients will develop flare with Co exposure equal to the average daily intake (Stuckert and Nedorost, 2008).

3.2.2.1. Conclusions on the safety of dietary cobalt for consumer

A daily oral intake of 600 μg Co (based on a LOAEL of 1 mg/kg for polycythaemia) appears an acceptable safe amount for humans that would protect from the known threshold-related adverse effects.

3.2.3. Consumer exposure

Plant products have been estimated to contribute up to 88 % of the total Co in the Japanese diet (Yamagata et al., 1963; IARC, 1991). The Total Diet Study in the United Kingdom in 1994 estimated the population average intake of Co to be 0.012 mg/day (MAFF, 1997; EVM, 2002). Cobalt intake in the United States has been estimated to be 0.005–0.04 mg/day (Jenkins, 1980), with relatively high concentrations of Co occurring in fish and vegetables (Barceloux, 1999). More recent data (ATSDR, 2004) give the average dietary consumption in the USA with about 0.011 mg Co per person and day.

In Canada, the estimated average daily intake is 0.011 mg/day (Dabeka, McKenzie, 1995). Bakery goods/cereals and vegetables contributed most to this daily intake, at 29.8 % and 21.9 %, respectively. The Co intake of Canadian children (age 1–19 years) has been estimated to range from 0.007 to 0.014 mg/day (Dabeka & McKenzie, 1995). In France, the estimated average daily intake is 0.029 mg/day (Biego et al., 1998). Foodstuffs that contributed most to this intake were milk and dairy products (32 %), fish/crustaceans (20 %), and condiments/sugar/oil (16 %). All these intake values are well below the oral threshold of 600 µg per person.

A worst case calculation, taking 300 g muscle, 100 g liver and 50 g kidney, 1.5 L milk and 100 g eggs (Regulation (EC) No 429/2008) and applying proximate mean Co concentrations[11] for muscle, liver and kidney of 0.01, 0.045 and 0.006 mg Co/kg tissue and 0.004 mg/L milk and 0.005 mg Co/kg eggs (see section 3.2.1.5) results in a Co intake of 14 µg/day.

3.3. Safety for the user

Occupational exposure to Co occurs in several industries, including hard metal manufacturing, welding and grinding (WHO, 2006). Air concentrations of Co in occupational settings generally range from 0.01 to 1.7 mg/m^3 (IARC, 1991; Barceloux, 1999), compared to normal atmospheric levels of 0.4–2.0 ng/m^3 (ATSDR, 2004). No data specific to the feed industry is available.

The respiratory tree is the critical target of the exposure via inhalation route. Aside from a potential increase in lung cancer risk (Co-dichloride and sulfate are presumed carcinogens by inhalatory route, Regulation (EC) No 790/2009), workers chronically exposed to Co-containing dusts are reported to develop a wide array of adverse effects including respiratory irritation, diminished pulmonary function, wheezing, asthma, pneumonia and fibrosis (ATSDR, 2004). A LOAEC of 0.038 mg hard metal/m^3 has been calculated for respiratory distress upon acute exposure (6 h) (Kusaka et al., 1986). As regards the occupational exposure, a NOAEC of 0.0053 mg/m^3 could be derived for pulmonary effects (decreased forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC), increased cough and upper airway irritation) from a study performed in diamond polishers (Nemery et al., 1992), while a LOAEC of 0.007 mg hard metal/m^3 has been calculated for asthma (Shirakawa et al., 1988). The ATSDR (2004) derived 0.1 µg Co/m^3 air as Minimum Risk Level for chronic inhalatory exposure to Co based on pulmonary effects.

Cobalt sulfate and dichloride are classified in Regulation (EC) No 790/2009 as skin and respiratory sensitizers, Co oxide as skin sensitizer only.

The dermal exposure to Co results in an allergic dermatitis in a variable percentage of population, occurring with all forms of the metal (i.e. metal particles and salts) (IARC, 2006). Exposure levels associated with the development of dermatitis have not been identified (ATSDR, 2004).

3.3.1. Conclusions

Cobalt compounds are classified as skin and respiratory sensitizers (Co-sulfate and dichloride, oxide only skin sensitizer) and presumed carcinogens by inhalatory route (Co sulfate and dichloride).

[11] Arithmetic mean of ranges, 0.02-0.07 mg/kg FW liver, 0.001-0.01 mg/kg FW kidney, 0.001-0.02 mg/kg FW muscle. Milk and eggs contain about 0.004 and 0.005 mg Co/kg, respectively.

For pulmonary effects, the ATSDR has developed a Minimum Risk Level of 0.1 µg Co/m^3 air. At present, no data on dusting potential of the authorized Co compounds have been available to the FEEDAP Panel. During re-evaluation of Co compounds, the dusting potential of Co additives deserves particular attention.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Monogastric animals (excluding horses and rabbits) do not require Cobalt but they require vitamin B_{12}. Consequently, there is no need for any cobalt supplementation to their feed.

The ruminal microflora can synthesize vitamin B_{12}, provided dietary cobalt is available in sufficient quantities. Consequently, the vitamin B_{12} requirement of these animals can be covered by dietary cobalt. The host metabolism of ruminants also requires only vitamin B_{12}. But for a potential replacement of cobalt by vitamin B_{12}, there are not enough data to evaluate the consequences on health and performance for these species under field conditions. An optimal micronutrient supply of ruminants would therefore include cobalt. A comparable conclusion may be drawn for horses and coprophagous rabbits (hindgut fermentation of vitamin B_{12}), although there is a lack of quantitative data.

The FEEDAP Panel concludes that the potential cobalt supplementation to diets for ruminants, horses and rabbits should be maintained. A cobalt supplementation of 0.3 mg/kg DM and, taking into account cobalt background concentrations of feed material not exceeding 0.5 mg/kg DM of complete feed, a maximum content of 1 mg Co/kg complete feed is considered appropriate. For fish diets, the existing maximum content of 2 mg Co/kg complete feed should be maintained due to the higher cobalt background levels of fish meal, a major part of fish diets.

Among foodstuffs of animal origin, offal shows the highest cobalt content, liver with about 0.02-0.07 mg/kg FW, followed by kidney with about 0.001-0.01 mg/kg FW. Meat is in the range of 0.001-0.02 mg/kg FW as are fillets of freshwater fish. Milk and eggs contain about 0.004-0.005 mg Co/kg; dairy products like cheese and butter are relatively rich in cobalt (0.02 mg/kg FW).

Virtually all cobalt in offal and beef meat can be attributed to vitamin B_{12}. The fraction of vitamin B_{12}-bound Co is considerably smaller in poultry and pork meat (about 20 to 40 %) indicating dietary supply of Co as such. Eggs and milk contain even higher amounts of vitamin B_{12} unrelated cobalt (about 70 and 95 %, respectively) indicating excretion of absorbed soluble cobalt. However, these are estimates with several uncertainties due to methodological reasons (e.g. poor data set, analytical methods).

No data are available in the open literature on the potential carcinogenicity of cobalt following the exposure via the oral route either in humans or in experimental animals. Exposure by this route may potentially entail a number of adverse in effects in humans (cardiac effects, effects on erythropoiesis, effects on thyroid, developmental effects, and allergic dermatitis). A daily oral intake of 600 µg cobalt (based on a LOAEL of 1 mg/kg for polycythaemia) appears an minimum risk level for humans that would protect from the known threshold-related adverse effects.

The estimated population average intake of cobalt was reported to be 0.012 mg/day in the UK, 0.005–0.04 mg/day in the US, 0.011 mg Co/day in Canada, and 0.029 mg/day in France. All these intake values are well below the oral threshold of 600 µg per person.

The FEEDAP Panel, based on its own calculations, concludes that the potential cobalt intake of consumers from food of animal origin would not exceed 14 µg/day and is therefore not of safety concern.

Users are exposed to cobalt compounds (dichloride and sulfate are skin and respiratory sensitizers and carcinogenic by inhalatory route). For pulmonary effects, the ATSDR has developed a minimum risk level of 0.1 µg Co/m^3 air. At present, no data on dusting potential of the authorised cobalt compounds have been available to the FEEDAP Panel. During re-evaluation of cobalt compounds, the dusting potential of cobalt additives deserves particular attention.

RECOMMENDATIONS

Since the contact of persons handling the additive cannot be fully avoided, the FEEDAP Panel recommends, as a first step, minimizing exposure to cobalt compounds.

The FEEDAP Panel recommends modifying the authorisation of cobalt compounds in feedstuffs by:

- Restricting the use of cobalt compounds as additives to feed for ruminants (except milk replacer), horses and rabbits.
- Limiting cobalt supplementation in feed for ruminants (except milk replacer), horses and rabbits to a maximum of 0.3 mg Co/kg complete feed.
- Reducing the authorised maximum cobalt content from all sources from 2 to 1 mg/kg complete feed for all species except fish.

No negative consequences of these measures on animal health and the efficiency of animal production are expected.

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Appendix A

Cobalt: Toxic mechanisms and effects

The toxicological profile of Co and Co compounds and its impact in the terrestrial food chain have been reviewed (ATSDR, 2004; WHO, 2006; Gál et al., 2008).

Aside from few cases of intolerance to cyanocobalamin leading to urticaria (James and Warin, 1971), no reports of adverse effects resulting from vitamin B_{12} overdosage are available in the open literature (Lane and Rojas-Fernandes, 2002)

Toxic mechanisms

Although a number of Co-mediated toxicity endpoints are well recognised, the mechanisms by which Co exerts its toxic effects are not fully understood. The attenuation or the reversal of several Co-mediated toxic manifestations provided by sulfhydryl (-SH)-containing compounds (Huck and Clawson, 1976; Kumar et al., 1990) point to the ability of the metal to form complexes with critical–SH groups (including lipoic acid), which results in the inhibition of key processes such as oxidative phosphorylation (Webb, 1964) and hence cell energy production. Another generally accepted Co-mediated mechanism of toxicity is the induction of oxidative stress *in vitro* and *in vivo*. Cobalt(II) catalyses the generation of hydroxyl radicals ($OH^{\bullet}$) from hydrogen peroxide in a Fenton type reaction (Lloyd et al., 1997) causing a fall in reduced glutathione (GSH) levels and an increase in lipid peroxidation products such as malondialdehyde and other thiobarbituric reacting substances (Gonzales et al., 2005).

The alteration of calcium homeostasis achieved by blocking Ca^{++} channels is another well recognised action of soluble Co compounds that has been related to impairment of both the steroidogenesis and the neuromuscular transmission (WHO, 2006).

Toxic effects

Heme synthesis and polycythemia

There is an apparently contrasting effect on heme synthesis. On the one hand, Co stimulates heme oxidation in many organs by inducing heme-oxygenase and causing a reduction in the levels of hemoglobin and other hemoproteins like cytochrome P450 (Elbirt and Bonkosky, 1999). On the other hand, it is reported to enhance erythropoietin synthesis through a complex mechanism and hence cause polycythaemia, which is considered one of the toxic endpoints of Co. Cobalt (II) is known to evoke a hypoxia-like state *in vivo* and *in vitro* even in the presence of normal molecular oxygen pressure. The underlying mechanism involves the stabilization of hypoxia-inducible factor HIF-1α, which normally is degraded when sufficient oxygen is present: in the hypoxic state, HIF-1α acts as a subunit of a transcription factor inducing the expression of genes controlling erythropoietin synthesis (Maxwell and Salnikow, 2004). Due to the property of inducing a number of hypoxia-like responses including erythropoiesis, it is not excluded that Co supplementation (presently not banned and therefore not included in the current anti-doping tests) could be used an illegal and unfair way of enhancing athletic performance (Lippi et al., 2005).

Cardiomyopathy

Cardiomyopathy has been observed in heavy drinkers consuming large quantities of beer in which Co-sulfate was used as foam stabilizer; it has been calculated that those individuals ingested 0.04 to 0.14 mg Co/kg/day for years (ATSDR, 2004). On autopsy, enlarged heart, pericardial effusion and severe myocardial degeneration were consistently recorded (Barceloux, 1999). It has been suggested that other factors, such as poor nutritional status and excessive alcohol consumption, likely contributed to the development of cardiomyopathy in the affected beer drinkers (Lison, 2007). Similar heart lesions have been observed in several laboratory species exposed to Co, but in the dog substantially higher Co doses were required than those in the human cases of beer drinkers, to elicit cardiomyopathy (Lison, 2007). The mechanisms underlying this toxic effect are presently not fully understood. A decrease in

cell energy production possibly related to the irreversible chelation of lipoic acid, an increase in lipid peroxidation products and inhibition of thyroid hormone synthesis may be involved in the pathogenesis of the cardiac lesions (Roy et al., 1968; Diaz et al, 1994; ATSDR, 2004).

Effects on the thyroid

Cobalt prevents thyroxine iodination by the inhibition of tyrosine iodinase, resulting in a drop in circulating thyroxine levels which may lead to clinical hypothyroidism (ATSDR, 2004). Because of the low thyroxine levels, the excretion of thyroid stimulating hormone is increased with resultant hyperplasia (goiter). Depression of thyroid function associated with cardiomyopathy occurred in humans consuming large quantities of beer containing Co as anti foaming agent (Roy et al., 1968). Clinical treatments for sickle cell anaemia in children utilizing 12 mg Co/day for 90 days were associated with the impairment of thyroid function (thyroid enlargement, decreased iodine uptake) (Washburn and Kaplan, 1964). A reduced ^{131}I concentration capacity has been observed in patients given 20-30 mg Co as antianemic (Schirrmacher, 1967), but smaller Co intake (5–10 mg per day) has been also associated to thyroid pathology in heavy beer drinkers (Roy et al., 1968). As compared to unexposed reference controls, female platers exposed to semi-soluble Co dyes (Co-zinc silicate) in Danish porcelain factories showed an increase in both urinary Co content and serum thyroxine as well as free thyroxine (Prescott et al., 1992).

Effects on lungs

Aside from occupational asthma, the inhalation of a large array of Co compounds (oxides, salts or the so called "hard metal dust" where Co is associated with tungsten carbide) by Co-exposed workers results in a pathological process of the pulmonary parenchyma ranging from an intense alveolitis to end-stage pulmonary fibrosis, although it is questionable whether Co alone could be responsible for the severe lung damage (Barceloux, 1991).

Effects on the immune system

Like nickel, Co is believed to function as a hapten, resulting in the generation of antibodies against Co-protein complexes (Thierse et al., 2005), and multiple reports have recognised an immunological correlation with specific immunoglobulin IgE and IgG antibodies to a complex of Co and albumin (Shirakawa et al., 1988; Shirakawa and Morimoto, 1997). The most common hypersensitivity reaction to Co is allergic contact dermatitis, a type IV reaction; many patients are also allergic to nickel (Garner, 2004) arising from Co exposure whether by inhalation, orally, or topically. The ingestion of foods rich in Co, especially Brazil nuts and cow liver, but also lamb liver, has been implied in the genesis of a particular form of systemic allergy called dyshidrotic eczema, a type of chronic intermittent dermatitis affecting hands and feet; a small percentage of patients, however, were reported to develop a Co-related flare with "normal" dietary intake of the metal (Stuckert and Nedorost, 2008). Occupational Co-related asthma may occur in hard metal workers displaying cough, wheezing and dyspnoea that often improves during weekends and holidays (Cirla, 1994).

Genotoxicity

The genotoxicity of Co was recently reviewed (De Boeck et al., 2003; IARC, 2006; WHO, 2006).

In vitro studies -The results of assays performed with soluble Co(II) salts including acetate, chloride, and sulfate (IARC, 2006) clearly demonstrate the full mutagenic potential of the several salts in mammalian cells, although most of the tests performed in bacteria were negative. Two major mechanisms seem to be involved, namely i) the generation of reactive oxygen species through a Fenton-like mechanism and ii) the inhibition of DNA repair mechanisms (Beyersmann and Hartwig, 2008). The latter has been related to the competition with Mg(II) ions and with Zn for binding to zinc finger domains in repair proteins. It has also been reported that the DNA binding capacity of the p53 protein, which is a Zn-dependent mechanism, can be modulated by Co(II) ions (Méplan et al., 2000). In this respect, it is worth noting that Co-oxide enhanced the carcinogenicity of benzo[a]pyrene in a rat study (Steinhoff and Mohr, 1991). More recently, Li et al. (2009) reported that the exposure of human

lung carcinoma A549 cells to Co concentrations ≥ 200 μM (as $CoCl_2$) for 24 h altered the expression of hundreds of genes involved in different cellular functions, including tumorigenesis, and this finding was related to several observed histone modifications brought about by the metal.

In vivo studies - Several published reports indicate that Co salts (chloride or acetate) are capable of inducing a variety of genotoxic alterations (DNA damage, gene mutations, micronuclei formation, chromosomal aberration) in laboratory species exposed by oral or parenteral routes. Male Swiss mice administered a single oral dose of Co (as Co-chloride) at 0, 4.96, 9.92 or 19.8 mg/kg bw exhibited a dose–response increase in percentages of chromosomal breaks and chromosomal aberrations in bone marrow cells (Palit et al., 1991a, 1991b, 1991c, 1991d). Typical oxidative, free radical-mediated DNA damage was observed in lung, liver and kidney at 2 and 10 days after a single i.p. dosing with 50 or 100 μmol Co as Co-acetate (corresponding to 5.89 or 2.95 mg Co) per kg bw to F344 rats of either sex (Kasprzak et al., 1994). A single i.p. injection of Co-chloride at 12.4 or 22.3 but not 6.19 mg/kg body weight in BALB/c mice caused an increase in micronucleus formation after 30 h (Suzuki et al., 1993).

Although experimental data indicate some evidence of a genotoxic potential for Co in human lymphocytes *in vitro* (De Boeck et al., 1998; Lison et al., 2001), inconclusive evidence of Co-mediated genotoxicity in humans has been provided by studies conducted in workers exposed to Co dusts (De Boeck et al., 2000) or in individuals bearing orthopaedic joint replacements made of Co containing alloys (Keegan et al., 2008).

Carcinogenicity

Animal carcinogenicity - The carcinogenic potential of Co and its compounds was evaluated by IARC (2006) and reviewed by WHO (2006). Several experiments performed in laboratory animals support the *in vivo* carcinogenicity of Co compounds when administered by different routes, namely local tumours (sarcomas) at injection sites and lung tumours after intratracheal instillation. For example, a concentration-related increase in alveolar and bronchiolar carcinomas and other neoplastic (i.e. adrenal tumours in female rats) and non-neoplastic lesions (pulmonary edema, acute pneumonia) were reported in F344/N rats and B6C3F1 rats exposed to 0.3, 1.0 or 3 mg/m^3 Co-sulfate for three years (Bucher et al., 1999). The evaluation of the carcinogenic potential of Co in laboratory species suffers from some limitations, mainly linked to the rather unrealistic administration routes which are of doubtful relevance for assessing the risk of cancer in humans. Oral data on the carcinogenic effects of Co and Co compounds are not available (ATSDR, 2004). Nonetheless, based on the results of the available studies, IARC (2006) has concluded that there is sufficient evidence for the carcinogenicity of Co-sulfate in experimental animals .

According to Lison et al. (2001), it can be overall concluded that the genotoxic potential of Co(II) cations is demonstrated *in vitro* and there is substantial evidence that Co(II) cations exert genotoxic as well as carcinogenic effects in animals; moreover, it seems reasonable to consider that all soluble Co(II) salts (chloride, sulfate, acetate) share this carcinogenic potential.

Human carcinogenicity - The available data rely almost exclusively on the follow up of workers employed in Co production plants. Several reports addressing cancer risks among workers in hard-metal production facilities provide evidence of an increased lung cancer risk related to exposure to hard-metal dust containing Co and tungsten carbide. The study of workers in hard-metal factories in France also allowed estimation of lung cancer risk in relation to exposures to Co in the absence of tungsten carbide, although the presence of cofounding factors could not be excluded (Lison, 2007). The overall evaluation performed by IARC (2006) was the following: Co metal with tungsten carbide is probably carcinogenic to humans (Group 2A), whereas Co metal without tungsten carbide as well as Co-sulfate and other soluble Co(II) salts are possibly carcinogenic to humans (Group 2B).

Such conclusions are in line with the specialised experts of the European Union who have classified Co-chloride and sulfate as C2 human carcinogens by the inhalatory route (class 1B) (Regulation (EC) No 1272/2008).[12]

Effects on reproduction

Exposure to Co has resulted in effects on reproductive endpoints in experimental animals, although not all the performed experiments gave unequivocal results (WHO, 2006). Rats exposed to dietary Co (268 mg Co/kg feed as Co-chloride) up to 98 days showed polycythaemia and consistent degenerative and necrotic lesions in the seminiferous tubules (Corrier et al., 1985). Testicular atrophy was reported in rats, but not in mice, exposed to 19 mg Co/m^3 as Co-sulfate over 16 days (Bucher et al., 1990). Cobalt produced dose-dependent maternal toxicity and was found to be embryotoxic in female rats, mice and rabbits orally administered Co-sulfate doses ranging from 20 to 300 mg/kg bw by gavage (single or multiple doses), as evidenced by elevated frequency of fetuses with body weight or skeletal retardation and embryolethality. There was also an increase in the frequency of major anomalies in mice and rats, with anomalies of the eyes, kidneys, skull, spine and sternum in mice, and anomalies of the urogenital system in rats, while no teratogenic effects were observed in rabbits (Szakmáry et al., 2001). A NOAEL was derived from a study in which no developmental effects were observed in the children of 78 women given Co-chloride up to 0.6 mg Co/kg/day for 90 days orally during pregnancy for treatment of anemia; however, only a limited examination of offspring was reported, and details of examined end points were not described (WHO, 2006). Fetotoxicity[13] was noted in mice and rats following the administration of 50 mg Co/kg bw as Co-sulfate (lowest effective dose) to dams on days 6 to 15 and 1 to 21, respectively. Under the same experimental conditions, the lowest dose of Co required to produce statistically significant teratogenic effects[14] was 25 mg/kg bw in the rat. As regards humans, there is no information about adverse effects on reproduction related to professional exposure, including the hard metal industry (Keegan et al., 2008).

Toxicity in target species

Excessive Co intake may cause adverse effects in target species. However, there is a limited database and most of the available information is mostly derived from experimental studies. The best-characterised toxic responses are believed to be polycythaemia and cardiomyopathy (Gál et al., 2008), although these manifestations are not always encountered in all affected species.

The toxic level has proven to be very high in ruminants and greatly in excess of the requirements, so that, under field conditions, it is considered unlikely that Co toxicity could represent a major problem. Ely et al. (1948) reported that the daily exposure of dairy calves to 0.9 mg/kg bw by the oral route resulted in a toxic syndrome characterised by lacrimation, salivation, dyspnea, incoordination, and excessive defecation and urination. The injection of methionine prior to Co administration markedly reduced the severity of the reaction. In another trial performed in dairy calves (Keener et al., 1949), the untoward symptoms appeared in animals daily exposed to Co doses greater than 1 mg/kg bw by the oral route and consisted in loss of appetite, decreased water consumption, lack of muscular coordination and some increase in erythrocyte concentration, i.e., a mild polycythaemia. In a case report (McLaren et al., 1964), adult cattle deaths were recorded over the week following the administration of about 30 g of Co-sulfate in solution, with liver Co content ranging from <5 to 288 ppm (dry weight). According to Becker and Smith (1951), sheep appear even less susceptible than cattle, being able to tolerate a daily dose of 160 mg/kg bw of Co-chloride for a period of eight weeks; upon the increasing of the dosage, anorexia and weight loss were the predominant symptoms, with no evidence of polycythaemia.

Growing-finishing pigs were offered a corn-soy diet containing Co (as Co-chloride • $6H_20$) at a level of 0, 25, 50, 100, 200, 400 or 600 mg/kg. The pigs tolerated up to 200 mg Co/kg diet in the presence of adequate level of iron and toxic effects consisting in anorexia, growth depression, stiff-leggedness,

[12] OJ L 353, 31.12.2008, p. 1, last amended by Regulation (EC) No 790/2009 (OJ L 235, 5.9.2009, p. 1)
[13] Body weight retardation
[14] Sternum hypoplasia, double vertebral ossification centers, shortened rib 13

humped back and muscular tremors and incoordination were recorded upon the addition of 400 or 600 mg/kg diet, the severity of the symptoms being consistently alleviated by methionine addition (0.5 to 1 %); not only polycythaemia was never observed, but even the opposite effect occurred, i.e. lowered hemoglobin and hematocrit values (Huck and Clawson, 1976). In another study, pigs daily dosed with Co-sulfate (100 mg/kg bw) for three consecutive days developed cardiomyopathy with moderate mortality; gross examination revealed hydropericardium and mottled or pale appearance of the myocardium, with prominent involvement of the atria (Van Vleet et al., 1977)

Cobalt toxicity in chickens is well documented. Depressed growth rate has been reported as the most relevant sign of excessive Co intake in chickens. The lack of adequate iron supply in the diet is considered a predisposing factor, while methionine or cysteine are reported to alleviate Co toxicity (Southern and Baker, 1981). More recently, Diaz et al. (1994) reported a dose-related polycythaemia in one-day-old meat-type chickens fed 0, 10, 100 or 500 mg/kg of added cobaltous chloride for 42 days, with increase in both erythrocyte count and haemoglobin levels reaching the statistical significance mostly in the group administered the highest level of the trace element; birds from such group also showed a significant higher incidence of right ventricular hypertrophy and ascites.

As detailed in Marr et al (1998), LC_{10} and LC_{50} values of 120 and 490 mg/L, respectively, were reported for rainbow trouts (4 days post hatch) exposed to Co for 28 days, and comparable values were found with different water quality conditions.

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APPENDIX B

The biological role of cobalt and vitamin B_{12} in target animals

Cobalt is a metallic element occurring in the most common compounds in +2 or +3 oxidation states. Cobalt can be found in copper and nickel minerals and in combination with sulfur and arsenic in ores. Co(II) is major form in salts and is stable in aqueous solution. Co(III), the form of the element bound in structural centre of vitamin B_{12}, has strong oxidising properties, is unstable in aqueous solution and is involved in coordination complexes with nitrogen-donor ligands.

Kinetics of cobalt

Absorption

Data on Co absorption rates from digestive tracts of farm animals are rather scarce. In ruminants, the apparent absorption rate was estimated to be very low, being in range 1 to 2 % (Looney et al., 1976; Van Bruwaene et al., 1984). Oral or intraruminal administration of labeled Co to sheep or cattle resulted in 84 to 98 % faecal recovery of metal within 5 to 14 days (Smith, 1987). Such a low Co absorption in ruminants may relate to binding of Co by ruminal microorganisms (NRC, 2005). Part of Co ingested by ruminant is used by ruminal bacteria for a synthesis of vitamin B_{12}.

In laboratory animals such as rats and mice, the reported apparent absorption of Co from Co-chloride was in a range 13 to 34 % whereas Co from insoluble Co oxide showed 1 to 3 % absorption rate only (Kirchgessner et al., 1994; Ayala-Fierro et al., 1999). In rats and guinea pigs it has been suggested that Co absorption may decline with age (Naylor and Harrison, 1995). In healthy human volunteers given Co doses between <1 and 1.2 mg metal, the gastrointestinal absorption was found to vary from 5 to >20 % (Smith et al., 1972).

In monogastric mammalians Co and iron appear to share a common intestinal transport system and Co absorption is greatly increased in case of iron deficiency (Thomson et al., 1971). This may be due to the competition for the divalent metal transporter, DMT1 (Kwong and Niyogi, 2009). It has been shown that high supplementation doses of Co or iron are able to reciprocally reduce tissue deposition and plasma levels of these elements in pigs and chickens (Huck and Clawson, 1976; Blalock, 1985). High doses of the dietary sulfur-containing amino acids cysteine and methionine were also shown to reduce Co tissue deposition in chickens (Southern and Baker, 1981). This may be due to the formation of stable complexes of sulphur amino acids with Co (Baker and Czarnecki-Maulden, 1987).

Tissue distribution of cobalt

Since Co is a structural component of vitamin B_{12}, the metal can be found in all animal tissues. The Co concentrations in tissues are generally low being less than 1 mg per kg tissue DM. Liver and kidney followed by heart show the highest Co contents. Liver is considered a major store tissue of Co in a form of vitamin B_{12} in adult animals.

Literature data on Co dietary supplementation effects on Co tissue deposition in animals are very scarce. Henry et al. (1997) showed on sheep fed for 60 days diet supplemented with Co levels up to 40 mg Co/kg feed in a form of $CoSO_4$, that liver and kidney Co contents increased proportionally with dose reaching respective values 7.3 and 4.8 mg/kg DM but muscle Co content increased from 0.1 only to 0.3 mg/kg DM. Response to increasing oral Co of pigs and chickens showed the highest accumulation of element in kidney followed by liver (Huck and Clawson, 1976; Blalock, 1985). In rats the absorbed Co is primarily retained in liver (Ayala-Fierro et al., 1999).

Regarding tissue Co speciation, published data are even scarcer. Recent analysis of corrinoids in ovine tissues (Kelly et al., 2006) showed that in liver 5`-deoxyadenosylcobalamine (ado-cbl) predominated followed by hydroxycobalamin (OH-cbl), methylcobalamin (me-cbl) and cobalamin analogues while in blood OH-cbl predominated followed by ado-cbl, analogues and me-cbl. Dietary Co

supplementation of ruminating sheep led to increase in liver ado-cbl and analogues. Contrary to humans, in sheep the amount of ado-cbl was consistently higher in all tissues than me-cbl.

Excretion of cobalt

Faecal elimination is the major route of Co excretion following oral intake. Absorbed Co is primarily excreted by urine but small amounts appear to be excreted by endogenous Co recycling into digestive tract (Kirchgessner et al., 1994). Within 70 days after oral administration of ^{60}Co in a form of Co-chloride to lactating dairy cows, the excretion of labeled metal by faeces, urine and milk was 97, 0.26 and 0.01 %, respectively (Van Bruwaene et al., 1984). In rats Co from dietary supplementation with Co(II) chloride was excreted primarily by faeces (70-to 83 % of administered dose) with urinary excretion accounting for the remaining (Barnaby et al., 1968; Hollins and McCullough, 1971; Ayala-Fierro et al., 1999).

Biological function of cobalt

Essentiality

The discovery that increased dietary Co can prevent the disease called unthriftiness in sheep and cattle was made in the thirties of the former century. The Co incorporation into the structural centre of vitamin B_{12} and therapeutic efficiency of vitamin B_{12} injection to ruminants with Co deficiency was demonstrated some 20 years later.

The only known essential role of Co in animals and humans is being a component of vitamin B_{12}. Absorbed Co *per se* does not have any known biological function.

Cobalt is a dietary essential trace element for ruminants and horses, which can synthesize vitamin B_{12} in the digestive tract by microbial action (NRC, 2000, 2001, 2007a, 2007b). The efficiency of incorporation of Co in vitamin B_{12} in ruminants is low and inversely related to Co intake. Incorporation rate may be characterized by a range of 3 to 15 % and an average range of 10 to 15 %. (Smith and Marston, 1970; Stemme et al., 2008; Girard et al., 2009).

Non-ruminants require intake of vitamin B_{12} because they lack the ability to synthesize it in significant amounts through digestive tract microbiota. Coprophagous animals and poultry on deep litter receive some supplies of vitamin B_{12} from microbial fermentation. However, the amounts of vitamin B_{12} delivered from this source are not reliable in poultry (McDowell, 2003).

Role of vitamin B_{12}

Chemically, vitamin B_{12} belongs to a the structural class of corrinoids. They are compounds containing four reduced pyrrole rings joined into a macrocyclic ring by links between their α-positions; three of these links are formed by one-carbon units (methylidyne radicals) and the other by a direct Ca-Ca bond. The formula is based upon the skeleton of corrin, $C_{19}H_{22}N_4$. Besides the vitamin B_{12} active forms OH-cobalamin, adenosyl cobalamin and methyl cobalamin, corrinoids include also several rather inactive analogues (factors and derivates) as cobamic acid, its hexaamid cobamide, cobinamide, cobyrinic acis, cobyric acid and others (CBN, 1976).

While a number of vitamin B_{12}-dependent metabolic functions have been identified in microorganisms, only two vitamin B_{12}-dependent enzymes have been discovered in animals: methylmalonyl Coenzyme A mutase (EC 5.4.99.2) which requires adenosylcobalamin, 5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13), also known as methionine synthase, which requires methylcobalamin (Kennedy et al., 1991). Methylmalonyl CoA mutase is involved in reaction converting methylmalonyl-CoA into succinyl-CoA which serves to release energy from proteins and fatty acids. Methionine synthase is a methyl transfer enzyme, which can catalyse the conversion of homocysteine into methionine. Leucine mutase requiring adenosylcobalamin involved in lesions of central nervous system during vitamin B_{12} deficiency was described too (Underwood and Suttle, 1999; McDowell, 2000).

Specific potential benefits of supplementary Co in ruminants

Specifically in ruminants, it seems that vitamin B_{12} might also be essential for a proper function of rumen microflora. It is well established that some rumen bacteria produce vitamin B_{12} from Co while the others require delivery of vitamin B_{12} for their normal metabolic function, namely for propionate production which is a key metabolite for energy metabolism of ruminants. For example, it has been shown that *Prevotella ruminicola* and some *Bacteroides* species form propionate at the expense of succinate, and this result is dependent on vitamin B_{12} supplementation (Chen and Wolin, 1981; Strobel, 1992). It should be stressed that more than 95 % of ruminal vitamin B_{12} was found to be associated with the bacterial fraction and only less than 4 % (2.3 µg/L) was recovered in the remaining part of rumen fluid after centrifugation (Smith and Marston, 1970). This free vitamin B_{12} presumably would be utilised by vitamin B_{12} dependent rumen bacteria. Relevant consequences of low rumen vitamin B_{12} production on rumen metabolism were clearly demonstrated in experiments with sheep fed Co deficient diet: due to low vitamin B_{12} ruminal production the mean rumen succinate concentration increased more than 100-fold with a concomitant equimolar decrease in rumen propionate concentrations within 2 days. The similar response to a low dietary Co was found also in lambs (Kennedy et al., 1991 and 1996).

It has been suggested that s with Co content higher than ruminant requirements (0.11 mg/kg) may have some beneficial effects in terms of enhanced ruminal digestion of fibre from lower quality forages, increased total number of anaerobic bacteria in rumen and increased production of lactic acid in rumen (Lopez-Guisa and Satter, 1992; Zeleňák et al., 1992; Paragon, 1993). These findings could not be replicated by Hussein et al. (1994). More recently, positive effects of supplementary Co were reported based on finding that minimum dietary Co required to maximise feed intake and growth performance of growing cattle finished on a corn silage-based diet was in a range between 0.16 and 0.18 mg Co/kg DM (Schwarz et al., 2000). Similarly in dairy cows, increasing the dietary supply of Co from 0.19 to 0.93 mg/kg DM had no effects on plasma concentration of vitamin B_{12}, milk production and milk components yield (Kincaid and Socha, 2007). Although recent experiment with supplementation of 0.3 mg Co/kg DM to basal diet for lactating cows did not result in any change of ruminal pH, ammonia level, molar proportion of short chain fatty acids or microbial protein flow, the Co content of basal diet used (0.17 mg Co/kg DM) was already well above currently set requirements (Stemme et al., 2008).

The fate of vitamin B_{12} supplemented to feed for dairy cows

Besides passive diffusion appearing at high vitamin doses, an active saturable process with intrinsic factor involved in absorption of vitamin B_{12} was detected also in cows (Schneider and Stroiński, 1987). Consequently, intestinal absorption is likely to be similar to that in humans, the binding of the complex cobalamin-intrinsic factor with specific receptors at the ileal level being an essential step for the absorption of the vitamin. This process is rather slow.

It has been shown that intramuscular injections of cyanocobalamin (10 mg/cow/week) to dairy cows with dietary supplementation with Co (0.66 mg/kg DM) and folate (4 mg/kg bw) resulted in an improved performance of dairy cows in term of energy-corrected milk yields, milk solids, fat and lactose as well as reduced serum methylmalonic acid compared with cows given only supplementary folate (Girard and Matte, 2005). When supplemented to feed, folate and cyanocobalamine significantly increased milk production and milk crude protein while cyanocobalamine supplemented alone had no effects in dairy cows (Graulet et al., 2007).

Explanation for such an ineffective feed vitamin B_{12} supplemention for dairy cows is that supplemented cyanocobalamine undergoes huge degradation in ruminant forestomachs. It was estimated (Smith and Marston, 1970) that in sheep fed 0.5 mg cyanocobalamin during 4 months, only 1-3 % of dose was absorbed. A study on dairy cows fed diet supplemented with vitamin B_{12} at dose 500 mg/day showed an extensive ruminal destruction of added vitamin; a disappearance of 63 % of added cyanocobalamin before the duodenal cannula was noted (Santschi et al., 2005). Recently the same Laboratory refined the method for vitamin B_{12} analysis in duodenal digesta and found in an

experiment with similar protocol on dairy cows that only 20 % of supplemented cyanocobalamin reached the duodenal level. Cobinamide appears to be a major product of supplementary cyanocobalamin degradation in rumen from several corrinoids observed. Consequently, these authors concluded that the use of dietary supplement of cyanocobalamine is not an efficient means to increase vitamin B_{12} supply to cows (Girard et al., 2009).

It appears that microbial degradation of supplemental cyanocobalamin continues also within its further passage along with digesta via duodenum and jejunum to ileum (the site of vitamin B_{12} absorption). Direct measurements of vitamin B_{12} in portal blood of dairy cows showed that only 0.27 % (1.3 mg) vitamin B_{12} from an ingested cyanocobalamin dose of 500 mg/day was absorbed within 24 hours (Girard et al., 2001). Since absorption of cobalamin is rather slow, lasting longer than only 24 hours, the sampling period in the experiment of Girard et al. (2001), the figure 0.27 % for absorbed vitamin B_{12} from a single *per os* dose to cows might be an underestimation. Nevertheless, the recent research clearly confirms that utilisation of supplemental vitamin B12 in ruminants is rather ineffective.

Deficiency

In general, the common signs of Co-vitamin B_{12} deficiency in animals are lack of appetite and consequently a reduction in body weight gain, feed intake and feed conversion, lack of thrift, severe emaciation, weakness, monoblastic anaemia, decreased fertility, and decreased milk and wool production (McDowell, 2003).

In ruminants fed forages with Co concentrations <0.08 mg/kg, the Co-vitamin B_{12} deficiency can be predicted with confidence (McDowell, 1997). Ruminants respond to deficient dietary Co by lack of apetite, signs of normocytic and normochromic anemia, disturbances of lipid metabolism, reduced folate level, accumulation of iron and nickel in liver, compromised neutrophiles function and reduced resistance to parasitic infections. Necropsy of severely affected animals show emaciation, often with total absence of body fat, white liver due to fatty degeneration and spleen is occasionally haemosiderised (Paterson and McPherson, 1990; Kennedy et al., 1994; Stangl et al., 1998, 1999, 2000a and 2000c; McDowell, 2003). Cattle are more sensitive to Co deficient intake than sheep whereas goats seem to be more resistant to insufficient Co intake than sheep (McDowell, 2003). Lambs were reported to be most sensitive to deficient Co under grazing conditions followed by mature sheep, calves and mature cattle (Andrews, 1956).

Ruminants appear to be more sensitive to vitamin B_{12} deficiency than non-ruminants since they are dependent on gluconeogenesis to meet their tissue requirements for glucose (NRC, 2001). While liver deposits of vitamin B_{12} in adult ruminants facing low Co intake are usually sufficient to last several months the young animals are very sensitive to Co deficiency due to low body stores of vitamin B_{12} (Underwood, 1981). Intake of diets deficient in Co leads to a decline of vitamin B_{12} synthesis in rumen (Underwood and Suttle, 1999). Without vitamin B_{12}, the hepatic conversion of propionate to succinate is disturbed leading to a rise of methylmalonic acid urinary excretion (Kennedy et al., 1991; Underwood and Suttle, 1999). The level of metabolic available folate in liver is also altered during vitamin B_{12} deficiency since this vitamin is a component of the enzyme converting methylfolate to the metabolically active form tetrahydrofolate (Stangl et al., 2000b). Both folate in the form of 5-methyl-tetrahydrofolate and vitamin B_{12} are involved in the conversion of homocysteine to methionine in the biosynthesis of methionine (McDowell, 2000).

In monogastric animals, such as pigs, which require intake of vitamin B_{12}, its deficiency affected reproduction, litter size and survival. Similarly, in poultry, the lack of vitamin B_{12} impaired hatchability with embryo dying at day 17 of incubation (McDowell, 2003).

Indicators of Co/vitamin B_{12} status

Generally, the urinary concentration of Co is considered a reliable indicator of Co supply.

Ruminant liver Co concentrations in a range 0.05 to 0.07 mg/kg DM are critical levels indicating deficiency, with levels from 0.05 to 0.12 mg/kg showing on marginal deficiency, and >0.12 considered as adequate (McDowell, 1997; Tokarnia and Döbereiner, 1978 – cited from McDowell, 2003).

For ruminants, low levels of Co and vitamin B_{12} in tissues and blood, increased serum pyruvate and methylmalonic acid (MMA) levels are considered the most appropriate indicators of Co deficiency (McDowell, 2003). However, MMA does not seem to be a good indicator of vitamin B_{12} status in lactating ruminants and their offsprings (Underwood and Suttle, 1999). Deficiency of vitamin B_{12} also impairs the conversion of formiminoglutamic acid to glutamic acid; therefore increased urinary excretion of formiminoglutamic acid may be a good indicator for calves (Quirk and Norton, 1988). Other indicators of vitamin B_{12} status such as plasma homocysteine, liver methionine synthase activity and liver folate have been also suggested (Kennedy et al., 1992; Kennedy et al., 1995; Schwarz et al., 2000; Stangl et al., 2000b). Plasma levels of folate are not indicative of a deficient or adequate supply of Co and vitamin B_{12}, respectively (Stangl et al., 2000b).

Liver vitamin B_{12} <0.1µg/g is considered indicative of Co deficiency (Underwood, 1977; Smith, 1987). Serum vitamin B_{12} level from 380 to 760 pmol/L was suggested for ruminants as indicator of marginal deficiency and levels below 380 pmol/L were considered to be deficient (Graham, 1991).

Regarding serum MMA as indicator of Co status in cattle, levels below 2 µmol/L were tentatively suggested as being normal, levels from 2 to 4 µmol/L as subclinically Co-deficient and levels >4 µmol/L indicating Co deficiency (Paterson and MacPherson, 1990).

Requirements

Cobalt

Plant and animal derived feedstuffs generally contains 0.1 to 0.5 mg Co/kg DM. Due to local occurrence of soils deficient in Co the forages may not meet the animal requirements for Co (Ammerman, 1970). Alkaline soils or liming can prevent adequate uptake of Co by plants (Mills, 1981). It has been suggested that 20 µg Co/L ruminal fluid is a critical Co level to provide adequate vitamin B_{12} synthesis. Ruminal fluid contains normally about 40 µg Co/L (Miller et al., 1988).

The efficiency of cobalamine synthesis in ruminants is low and inversely related to Co intake: in sheep cobalamine production falls from 15 % at Co-deficient diet to 3 % with a high Co supplementation (Smith and Marston, 1970). In dairy cows, the efficiency of Co utilization for cobalamine synthesis was recently estimated between 7.1 and 9.5 % in cows fed diet containing 0.17 and 0.29 mg Co/kg DM, respectively (Stemme et al., 2008).

Prolonged dietary supplementation of lactating cows (0.15-0.93 mg Co/kg DM) resulted in an increase of vitamin B_{12} in colostrum and milk and of Co in milk (Kincaid and Socha, 2007). In non lactating cows (Kincaid and Socha, 2007) doses up to 1.71 mg Co/kg DM did not cause any increase of Co in serum and liver.

The relative synthesis of cobalamine and its analogues with no B_{12} activity is affected by dietary composition. It has been suggested that diets largely consisting of roughages promote greater production of cobalamine while larger dietary inclusion of concentrates reduces cobalamine production and thus lowers the ratio of cobalamine to the various analogues of vitamin B_{12} (Sutton and Elliot, 1972). Increasing dietary Co leads to increased production of these analogs in rumen at the expense of the biologically active form of vitamin B_{12} (Kawashima et al., 1997). Based on experiments with rumen simulation technique (continuous-flow fermentors), it has been suggested that a total (background plus supplemental) Co concentration of 0.10 to 0.15 mg/kg DM is adequate for vitamin B_{12} production to meet the requirements of ruminal microbiota fed a high-concentrate diet (Tiffany et al., 2006).

Steers supplemented with Co (0, 0.05 and 0.15 mg Co/kg DM) showed increasing DM intake (8.53, 9.31 and 9.48 kg) and average daily gain (1.42, 1.57 and 1.61 kg) during the experimental period of

160 days. Supplemental Co increased vitamin B_{12} in plasma and liver and plasma glucose concentration and this increase was higher in steers fed a corn-based diet comparing to barley based diet (Tiffany and Spears, 2005).

The dietary requirement of dairy cattle for Co was estimated to 0.11 mg/kg DM (Ammerman, 1970; NRC, 2001). The Co requirements for cattle for fattening proposed by NRC (2000) is 0.1 mg/kg DM, based on value proposed by Smith (1987).

Levels of dietary Co from 0.3 to 0.5 mg/kg DM were also suggested for optimal ruminal fermentation and vitamin B_{12} synthesis (Paragon, 1993; Singh and Chhabra, 1995). In an experiment with Co supplemented cattle for fattening, Stangl et al. (2000b) assessed the Co status as a response of plasma and liver vitamin B_{12}, liver folacin, plasma homocysteine and MMA, haemoglogin and haematocrit to various Co doses. Based on plasma vitamin B_{12} as response criterion, the authors suggested the dietary Co requirement of growing cattle to be 0.25 mg/kg DM while dietary Co levels needed to maximise the liver vitamin B_{12} and liver folate were found to be 0.24 and 0.19 mg/kg DM, respectively. Co dietary concentrations required to minimise plasma concentrations of homocysteine and MMA were estimated to 0.16 and 0.12 mg/kg DM, respectively while haemoglobin and haematocrit were only decreased in cattle on diets containing less than 0.1 mg Co/kg DM. In their conclusions, Stangl et al. (2000b) recommended for cattle for fattening Co dietary supply of 0.15 to 0.20 mg/kg DM; the desired Co content for a maximum vitamin B_{12} synthesis proposed by these authors would be 0.25 mg/kg DM.

In a controlled field experiment, performed over three subsequent years, using Texel twin lambs it was shown that the Co supplemented group of lambs had higher vitamin B_{12} serum concentration, body weight gain and better survival rate (Vellema et al., 1997). Similar effect was observed in newly weaned Omani goats fed a diet covering minimum daily requirement of Co for sheep (c.a. 0.1 mg Co/kg DM) and then treated twice monthly with subcutaneous injection of 2 mg hydroxycobalamin. Control animals exhibit lower body gain, and lower mean haemoglobin concentration. Over 60 % of control animals developed hepatic lipidosis compared with 5.3 % among supplemented animals (Johnson et al., 2004). NRC (2007b) set Co requirements for sheep and goats 0.11 and 0.1 to 0.2 mg/kg DM, respectively.

Vitamin B_{12}

In general, the requirements of vitamin B_{12} for various species are relatively very low and depend also on dietary levels of other nutrients like choline, methionine, folacin and ascorbic acid. With abundance of methyl groups the vitamin B_{12} and folacin requirements are reduced (McDowell, 2000).

In ruminants, the requirements of dairy cows for vitamin B_{12} have been estimated between 0.34 and 0.68 µg/kg body weight (NRC, 1989). The higher requirements of vitamin B_{12} for ruminants than for non-ruminants are presumably associated with a function of vitamin B_{12} in the metabolism of propionic acid and its role in the overall energy metabolism in ruminants. Adult ruminants can fully cover their vitamin B_{12} requirements by rumen microbial synthesis; in young animals the rumen microbiota appears to be functional for a synthesis of all B vitamins at animal age of 6 to 8 weeks (McDowell, 2000).

Table B1: Requirements of vitamin B12 (µg/kg feed) for various animal categories**.**

Animal category	Requirements	Reference
Calves (milk replacer)	70	NRC (2001)
Pigs for growing and finishing	5 – 20	NRC (1998)
Pigs for breeding and in lactation	15	NRC (1998)
Chickens for fattening	10	NRC (1994)
Laying hens (with feed intake 100 g)	4	NRC (1994)
Turkeys	3	NRC (1994)
Pacific Salmon	15 - 20	NRC (1993)
Dogs	26	NRC (2006)
Cats	20	NRC (2006)

Using breeding hens fed vitamin B_{12}-deficient diet (half requirement of 4 µg/kg diet) for 27-30 weeks and then fed recovery diet it was shown that maximum egg production, hen weight, egg weight, and hachability were observed in hens fed a diet containing 8 µg vitamin B_{12}/kg diet. The concentration in the egg yolk responded rapidly to dietary changes of this vitamin (Squires and Naber, 1992). In sows dietary treated with vitamin B_{12} up to 400 µg, a lowering effect on plasma homocysteine was observed (Simard et al., 2007).

In humans, the average dietary requirement for vitamin B_{12}, as established by the Scientific Committee for Food (SCF, 1993) was set to 1.0 µg/day, with a population reference intake for adults of 1.4 µg/day. More recently, the Report of a joint FAO/WHO expert consultation (FAO/WHO, 2002) set the estimated average requirements of vitamin B_{12} for adult humans to 2.0 µg/day and the recommended nutrient intake to 2.4 µg/day.

Maximum tolerable levels of cobalt for animals in feed

The NRC (2005) set 25 mg/kg feed as maximum tolerable levels of Co to cattle, poultry, sheep and horses and 100 mg/kg feed for swine. According to EU legislation, feed for all animal species is not allowed to exceed 2 mg total Co/kg complete feed.

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APPENDIX C

Table C1: Cobalt concentrations in feed materials (CVB, 2009)[15]

Feed materials	Dry Matter g/kg	Cobalt mg /kg DM
Dry feed materials		
Alf meal CP<140	917	2.38
Alf meal CP>180	904	2.38
Alf meal CP140-160	911	1.42
Alf meal CP160-180	910	1.42
Barley	869	0.29
Blood meal spray dr	937	0.11
Brewers' grains dr	903	0.11
Brewers' yeast dried	936	0.22
Buckwheat	865	0.07
Chicory pulp dried	897	0.22
Coconut exp CFAT<100	909	0.22
Coconut extr	898	0.22
Cottons extr with h	945	0.22
Fish meal CP<580	927	2.03
Fish meal CP>680	922	2.04
Fish meal CP580-630	913	2.05
Fish meal CP630-680	913	2.05
Lentils	874	0.18
Linseed	913	2.18
Linseed exp	901	0.34
Linseed extr	870	0.22
Maize	872	0.13
Maize chem-h treated	879	0.13
Maize gluten meal	901	2.24
Malt culms CP<200	920	0.11
Malt culms CP>200	913	0.11
Oats grain	889	0.26
Oats grain peeled	884	0.01
Palm kern exp CF<180	961	0.10
Palm kern exp CF>180	912	0.10
Peanut exp wtht sh	914	0.33
Peanut extr wtht sh	913	0.22
Peas	867	0.12
Rapes meal Mervobest	871	0.11
Rapeseed exp	894	0.22
Rapeseed extr CP<380	873	0.01
Rice wtht hulls	872	2.33
Semameseed meal extr	929	0.91
Soyb meal CF>70	874	0.30
Soyb meal Mervobest	872	0.10
Soyb meal Rumi S	872	1.12
Soybm CF<45 CP<480	873	0.30
Soybm CF<45 CP>480	874	0.30
Soybm CF45-70 CP<450	876	0.30

[15] Centraal Veevoeder Bureau, Den Haag, the Netherlands

Soybm CF45-70 CP>450	875	0.30
Sugarb p SUG100-150	903	0.21
Sugarb pulp SUG<100	898	0.33
Sugarbeet molasses	723	0.82
Sunfls exp w hulls	913	0.11
Wheat bran	883	0.11
Wheat feedfl CF<35	865	0.12
Wheat feedfl CF35-55	869	0.12
Wheat germ	877	0.11
Wheat middlings	865	0.13
Moisture reach feed materials		
Beetp pressed f+sil	218	0.21
Chicory pulp f+sil	232	0.26
Maize glutenf f+sil	418	0.23
Roughage and comparable feed materials		
Beet leaves sil	175	0.33
Clover red silage	378	0.15
Grass average	163	0.1
Grass bales ad	918	0.1
Grass fr April h y.	172	0.1
Grass fr April l y.	172	0.1
Grass fr April n y.	172	0.1
Grass fr Aug h y.	150	0.1
Grass fr Aug l y.	150	0.1
Grass fr Aug n y.	150	0.1
Grass fr July h y.	159	0.1
Grass fr July l y.	159	0.1
Grass fr July n y.	159	0.1
Grass fr June h y.	169	0.1
Grass fr June l y.	169	0.1
Grass fr June n y.	169	0.1
Grass fr May h y.	164	0.1
Grass fr May l y.	164	0.1
Grass fr May n y.	164	0.1
Grass fr Oct h y.	163	0.1
Grass fr Oct l y.	163	0.1
Grass fr Oct n y.	163	0.1
Grass fr Sept h y.	149	0.1
Grass fr Sept l y.	149	0.1
Grass fr Sept n y.	149	0.1
Grass hay av qual	845	0.16
Grass hay good qual	845	0.16
Grass hay horse crs	839	0.16
Grass hay horse fine	843	0.16
Grass hay horse midd	835	0.16
Grass hay poor qual	845	0.16
Grass horse gr past	161	0.1
Grass horse same fld	177	0.1
Grass sil average	474	0.16
Grass sil horse crs	645	0.16
Grass sil horse fine	568	0.16
Grass sil horse midd	605	0.16
Grass sil Ju-Au 2000	505	0.16

Grass sil Ju-Au 3000	505	0.16
Grass sil Ju-Au 4000	505	0.16
Grass sil June 2000	486	0.16
Grass sil June 3000	486	0.16
Grass sil June 4000	486	0.16
Grass sil May 2000	453	0.16
Grass sil May 3500	453	0.16
Grass sil May 5000	453	0.16
Grass sil Se-Oc 2000	426	0.16
Grass sil Se-Oc 3000	426	0.16
Green cereals silage	250	0.43
Lucerne (alfalfa) ad	910	0.1
Maize (Fodder) ad	909	0.18
Maize f fr DM240-280	263	0.18
Maize f fr DM280-320	300	0.18
Maize fod fr DM 320	336	0.18
Maize fod fr DM<240	225	0.18
Maize sil DM < 240	225	0.18
Maize sil DM 320	337	0.18
Maize sil DM240-280	268	0.18
Maize sil DM280-320	301	0.18
Whole crop sil(Cer)	373	0.07

APPENDIX D

Table D1: Cobalt concentrations in products of animal origin

Food Category	Cobalt (µg/kg) Average	Minimum	Maximum	N	Countries
OFFAL					
Beef liver	43	19	74	3	SWE[16]
Pig liver	10	2	23	36	SWE
Beef kidney	8	3	10	3	SWE
Pig kidney	4	1	11	36	SWE
Offals	33			3	FR[17]
MEAT					
Beef	1	1	1	3	SWE
Pork	1	1	12	36	SWE
Meat	8			8	FR
Poultry and game	2			4	FR
EGGS AND EGG PRODUCTS	5			5	FR
FISH					
Artic char	8	4	12	4	SWE
Whitefish	6	1	20	6	SWE
Baltic herring	5	2	12	6	SWE
Trout	4	1	10	3	SWE
Cod	2	1	4	5	SWE
Perch	2	1	4	8	SWE
Hake Chilean	1			1	SWE
Mackerel Atlantic	1			1	SWE
Pike	1	1	2	2	SWE
Pike-perch	1	1	1	3	SWE
Atlantic salmon		<1	10	56	NOR[18]
Fish	7			17	FR
SHELLFISH	3			46	FR
DAIRY PRODUCTS					
Milk	4	1	8	32	FR,POL[19]
Cheese	18			8	FR
Butter	18			1	FR

[16] Jorhem L., Sundström B. 1998. Journal of Food Composition and Analysis, 6, 223-248

[17] Leblanc J.C., Guérin T., Noël L., Calamassi-Tran G., Volatier J.L. , Verger P. 2005 Dietary exposure estimates of 18 elements from the 1st French Total Diet Study,Food Additives & Contaminants: Part A, 22,624- 641.

[18] Anne-Katrine Lundenbye Haldorsen, personal communication

[19] Dobrzañski Z., Koacz R., Górecka H., Chojnacka K., Bartkowiak A. The Content of Microelements and Trace Elements in Raw Milk from Cows in the Silesian Region. Polish Journal of Environmental Studies 14, 685-689.

APPENDIX E

Table E1: Vitamin B12 concentration in products of animal origin (FAO)[20]

	Vitamin B_{12} (µg/100g)				
Food Category	**Average**	**Minimum**	**Maximum**	**N**	**Countries**
LIVER					
Beef	116	65	200	7	DK, FIN, GER, ITA, NOR, UK
Horse	110	110	110	1	ITA
Rain deer	106	84	128	2	FIN, NOR
Lamb	72	48	114	3	NOR, ITA, UK
Calf	56	39	68	4	DK, GER, UK
Duck	33	21	54	8	DK, FIN, ITA, NOR, UK
Pork	31	23	40	5	DK, FIN, ITA, NOR, UK
Cod	13	10	22	5	DK, NOR
Paté and sausage	11	3	36	16	DK, FIN, GER, ITA, NOR, UK
KIDNEY	23	15	40	10	DK, GER, ITA, UK
HEART	10	3	13	15	DK, FIN, GER, ITA, NOR, UK
BEEF MEAT	2	1	5	119	DK, FIN, GER, ITA, NOR, UK
PORK MEAT	0.8	0.2	2	160	DK, FIN, GER, ITA, NOR, UK
LAMB MEAT	2	1	3	48	DK, FIN, GER, ITA, NOR, UK
POULTRY MEAT	1	0.0	4	67	DK, FIN, ITA, NOR, UK
EGGS	3	2	5	16	DK, FIN, ITA, NOR, UK
FISH					
Pike	13	2	24	4	DK, FIN, ITA, NOR
Herring	12	9	16	10	DK, FIN, GER, ITA, NOR, UK
Roe	12	4	20	16	DK, FIN, ITA, NOR, UK
Sardine	11	9	15	5	DK, FIN, ITA, UK
Arctic char	10	10	10	1	NOR
Mackerel	9	5	12	7	DK, GER, ITA, NOR, UK
Anchovies	5	1	11	8	DK, FIN, ITA, NOR, UK
Plaice	3	1	10	4	DK, NOR, UK
Atlantic salmon	5	1	8	47	NOR[21]
Other species	3	1	7	94	DK, FIN, GER, ITA, NOR, UK
MOLLUSCS					
Bivalves	29	2	153	21	FIN, GER, ITA, NOR, UK
Gastropods	16	2	36	12	FIN, ITA, NOR, UK
Cephalopods	11	2	20	5	DK, ITA
MILK	1	0.1	4	61	DK, FIN, GER, IRL, ITA, NOR, UK
CHEESE	1	0.2	4	256	DK, FIN, GER, IRL, ITA, NOR, UK
CREAM	0.3	0.1	1	34	DK, FIN, ITA, NOR, UK

[20] FAO. The International Network of Food Data System. Food Composition Tables – Europe (http://www.fao.org/infoods/tables_europe_en.stm)
[21] National Institute of Nutrition and Seafood research. www.nifes.no