European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on safety and efficacy of coated granulated cobaltous carbonate monohydrate as feed additive for all species[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Cobalt(III) is a component of cobalamin. Its essentiality as trace element results from the capacity of certain animal species to synthesise cobalamin by the gastrointestinal microbiota. Feeding cobalt(II) carbonate hydroxide (2:3) monohydrate up to the maximum authorised total cobalt in feed is safe for the target animals. Cobalt is predominantly excreted via the faecal route. Absorbed cobalt follows aqueous excretion routes. About 43 % of body cobalt is stored in muscle; however, kidney and liver are the edible tissues containing the highest cobalt concentrations and are most susceptible to reflect dietary cobalt concentrations. In animals with the capacity to synthesise cobalamin, cobalt is also deposited in tissues as vitamin B_{12}. Cobalt(II) cations are genotoxic under *in vitro* and *in vivo* conditions. Cobalt(II) carbonate has carcinogen, mutagen and reproduction toxicant (CMR) properties. No data are available on the potential carcinogenicity of cobalt(II) following oral exposure. However, oral exposure may potentially entail adverse threshold-related effects in humans. The estimated population intake of cobalt most likely includes the contribution of foodstuffs from animals fed cobalt-supplemented feedingstuffs. An increase in cobalt exposure by the use of cobalt-containing feed additives is therefore not expected. Considering the population exposure to cobalt, about 4–10 times lower than the health-based guidance value, no safety concern for the consumer is expected for threshold effects of oral cobalt. Cobalt(II) carbonate is a skin and eye irritant, and a dermal and respiratory sensitiser. Its dust is a hazard to persons handling the substance. Exposure by inhalation must be avoided. The use of cobalt from any source at the authorised maximum content in feed does not provide a risk to the environment. The coated granulated cobalt(II) carbonate hydroxide (2:3) monohydrate is available for cobalamin synthesis in the rumen and therefore effective in ruminants; this conclusion is extrapolated to horses and rabbits.

KEY WORDS

Nutritional additive, compounds of trace elements, cobaltous carbonate monohydrate, cobalt(II) carbonate hydroxide (2:3) monohydrate, coated granulated preparation, safety, health-based guidance value, efficacy

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of coated granulated cobaltous carbonate monohydrate, as feed additive for all animal species.

The only physiological role of cobalt in target animals is that of a component of vitamin B_{12}. The significance of cobalt(II) as an essential trace element results from the capacity of certain animal species to synthesise sufficient quantities of vitamin B_{12} by the gastrointestinal microbiota.

Feeding supplemental cobalt from cobalt(II) carbonate hydroxide (2:3) monohydrate up to the maximum total cobalt content in feed currently set by European Union (EU) legislation is considered safe for the target animals. The margin of safety is around 10.

The apparent absorption of cobalt from cobalt chloride measured in laboratory animals was in the range of 13 to 34 %. Cobalt is predominantly excreted via the faecal route. Absorbed cobalt follows aqueous excretion routes, via kidney but also via milk. About 43 % of body cobalt is stored in muscle; however, kidney and liver are the edible tissues containing the highest cobalt concentrations and most susceptible to increase such concentrations as a response to increased cobalt concentrations in feed. In animals with the capacity to synthesise cobalamin, cobalt is also deposited in tissues in the form of vitamin B_{12}. Most cobalt in offal and muscle tissues of ruminants can be attributed to vitamin B_{12}. The fraction of vitamin B_{12}-bound cobalt is smaller in hindgut fermenters (horses and rabbits) and considerably smaller in poultry and pork meat, and particularly in milk and eggs indicating dietary supply of cobalt as such.

Correlations between cobalt/vitamin B_{12} intake at physiological feed concentrations and tissue deposition as cobalt(II)/cobalt(III) could not be established owing to lack of data. Any prediction of the cobalt content of food of animal origin from dietary cobalt is therefore not possible at present.

Cobalt(II) cations are considered genotoxic under *in vitro* and *in vivo* conditions. Cobalt(II) carbonate has CMR (carcinogen, mutagen and reproduction toxicant) properties. No data are available on the potential carcinogenicity of cobalt(II) following oral exposure either in humans or in experimental animals. However, oral exposure may potentially entail a number of adverse effects in humans (effects on heart, erythropoiesis and thyroid and prenatal development, as well as allergic dermatitis). For these threshold effects, the FEEDAP Panel developed a health-based guidance value of 0.0016 mg/kg body weight (bw) and day.

The estimated population average intake of cobalt was reported to be 0.005–0.04 mg Co/day in the USA, 0.011 mg Co/day in Canada, 0.012 mg/day in the UK, and 0.029 mg Co/day in France. These figures most likely include already the contribution of foodstuffs from animals fed feedingstuffs that are routinely supplemented with cobalt(II) compounds. An increase of cobalt exposure resulting from the use of cobalt-containing feed additives is therefore not to be expected.

Considering the population exposure to cobalt, which is about 4–10 times lower than the health-based guidance value, no safety concern for the consumer is expected for threshold effects of oral cobalt at the current intake level. However, considering the toxicological profile of cobalt(II) and its salts, and the uncertainties regarding the deposition and the speciation of cobalt (cobalt(II) or vitamin B_{12}) in foodstuffs of animal origin, the FEEDAP Panel confirms its previous position that it would be prudent to limit the cobalt (cobalt(II) cation) supplementation of feedingstuffs to a level lower than the current maximum authorised.

Cobalt(II) carbonate hydroxide (2:3) monohydrate is considered as a skin and eye irritant and as dermal and respiratory sensitiser. No information was available for the coated granulated additive. Considering the toxicological profile of cobalt(II) carbonate, its dust is a hazard to persons handling the substance. Exposure by inhalation must be avoided.

The use of cobalt from any source at the currently maximum authorised concentration in feed will not result in a substantial increase of the concentration in the environment and no further environmental risk assessment is deemed necessary.

The coated granulated cobalt(II) carbonate hydroxide (2:3) monohydrate is considered as a source of available cobalt for microbial synthesis of vitamin B_{12} in the gastrointestinal tract. The additive is therefore considered as an effective nutritional additive for ruminants. This conclusion can be extrapolated to horses and rabbits.

The FEEDAP Panel made some recommendations particularly for (i) the restriction of the use of cobalt(II)-containing additives to ruminants, horses and rabbits, (ii) the reduction of the maximum total cobalt content to 1 mg/kg complete feed and (iii) the limitation of handling the additive to premixture industry.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Doxal Italia S.p.A.[5] for re-evaluation of authorisation of coated granulated cobaltous carbonate monohydrate, when used as feed additive for all animal species (category: Nutritional additives; functional group: compounds of trace elements). However, it is noted that the application does not exactly correspond to the existing authorisation (basic cobaltous carbonate monohydrate).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the applications to the European Food Safety Authority (EFSA) as a grouped application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossiers in support of this application.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 16 November 2011.

The additive "Basic cobaltous carbonate monohydrate" had been authorised in the European Union (EU) under the element Cobalt-Co for all animal species "Without a time limit" (Commission Regulation (EC) No 1334/2003)[7] and amendments. Following the provisions of Article 10(1) of Regulation (EC) No 1831/2003 the compound was included in the EU Register of Feed Additives under the category "Nutritional additives" and the functional group "Compounds of trace elements".[8]

EFSA issued an opinion on the use of cobalt compounds as additives in animal nutrition (EFSA, 2009a). More recently the FEEDAP Panel has delivered an opinion on the safety and efficacy of cobalt carbonate 46 % as feed additive for ruminants, horses and rabbits (EFSA, 2012a).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animal(s), consumer, user and the environment and the efficacy of the coated granulated cobaltous carbonate monohydrate when used under the conditions described in Table 1.

[4] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[5] Doxal Italia S.p.A.Via Mascagni 6. 20050 Sulbiate (MI) – Italy.
[6] EFSA Dossier reference: FAD-2010-0371.
[7] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.
[8] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003, http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Film granulated preparation of basic cobaltous carbonate monohydrate (E3)
Registration number/EC No/No (if appropriate)	E8
Category(-ies) of additive	3. Nutritional additives
Functional group(s) of additive	b. Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Basic cobaltous carbonate monohydrate (E3)	**$2CoCO_3$. $3Co(OH)_2$. H_2O**	**Cobalt min. 46%**	**UV spectroscopy**

Trade name (if appropriate)	-
Name of the holder of authorisation (if appropriate)	-

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All animal species and categories	-	-	2 mg cobalt (total)/kg	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	- For use in animal nutrition only. - For use in premixtures and in feedingstuffs. - The additive shall contain cobalt in the form of basic cobaltous carbonate monohydrate prepared in order to limit dust emissions to a maximum of 2 mg (total dust) per filter (Stauber-Heubach test).
Specific conditions or restrictions for handling (if appropriate)	-
Post-market monitoring (if appropriate)	No specific requirements other than the traceability and complaint system implemented in compliance with the requirements of Regulation No 183/2005.
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

This opinion is based in part on data provided by a single company involved in the production/distribution of cobalt containing compounds. It should be recognised that this data covers only a fraction of the existing cobalt-containing additives. The Panel has sought to use the data provided together with data from other sources to deliver an opinion.

1. Introduction

Cobalt (Co) is a metallic element occurring in the most common compounds in the +2 or +3 oxidation states. Cobalt(III) is the central atom of cobalamin (vitamin B_{12}), representing about 4.3 % of the molecular weight. Cobalt salts have been commonly used for many years in animal nutrition. Six cobalt salts are currently authorised in the EU (Commission Regulation (EC) No 1334/2003)[9] as feed additives (cobalt acetate tetrahydrate, basic cobalt carbonate monohydrate, cobalt chloride hexahydrate, cobalt nitrate hexahydrate, cobalt sulphate monohydrate and cobalt sulphate heptahydrate) for all animal species with a total maximum content of 2 mg Co/kg complete feedingstuff.

The current application is for coated granulated cobaltous carbonate monohydrate. The cobalt compound which is the subject of the assessment has been submitted for re-evaluation.

EFSA commissioned a study by the University of Gent (Belgium) of selected trace and ultratrace elements who submitted a technical report to EFSA (Van Paemel et al., 2010); cobalt was included in this study. Information from this report has been used in this opinion.

A previous opinion of the FEEDAP Panel (EFSA, 2009a) on the risk assessment of the use of cobalt compounds in animal nutrition considered (i) the necessity of cobalt supplementation for the different 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. The current opinion summarises and updates the relevant sections, for details see the above-mentioned opinion.

In a previous opinion, the FEEDAP Panel (EFSA, 2009a) reviewed the toxicological data of cobalt available in the public literature, and adopted for threshold-related effects the health-based guidance value set by the US Agency for Toxic Substances and Disease Registry for intermediate oral exposure ($\geq$ 365 days) (ATSDR, 2010). In an opinion of the French Food Safety Agency (formerly AFSSA and now ANSES) regarding the migration of cobalt from porcelain oven dishes intended to come in contact with food (AFSSA, 2010), AFSSA considered also the FEEDAP's 2009 opinion. In the current opinion, the FEEDAP Panel considered this assessment together with others in the light of the recently adopted default values for uncertainty factors (UFs) of the EFSA's Scientific Committee (EFSA, 2012b). Since the proposal for a health-based guidance value is not in the focus of the terms of reference, this consideration is given in Appendix (Appendix B).

1.1. Other uses of cobalt

At present no cobalt sources/forms are authorised in the EU in the manufacture of food supplements. The EFSA's Scientific Panel on Additives and Nutrient Sources added to Food (ANS Panel) assessed the safety of cobalt(II) chloride hexahydrate for nutritional purposes (EFSA, 2009b). The Panel considered that the proposed uses of this compound in food supplements were of safety concern.

Cobalt is listed in the fertiliser Regulation as micro-nutrient essential for plant growth.[10]

In the USA, the following cobalt compounds are allowed in animal feeds: cobalt acetate, cobalt carbonate, cobalt chloride, cobalt choline citrate complex, cobalt glucoheptonate, cobalt gluconate,

[9] OJ L 187, 26.7.2003, p. 11.
[10] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers. OJ L 304, 21.11.2003, p. 1.

cobalt oxide, cobalt sulphate, cobalt amino acid complex, cobalt amino acid chelate and cobalt proteinate (AAFCO, 2010).

1.2. Biological role of cobalt

The FEEDAP Panel summarised in a previous opinion (EFSA, 2009a) the biological role of cobalt as follows:

"The only known essential role of cobalt in animals and humans is being a component of vitamin B_{12} as Co(III). Absorbed cobalt *per se*, Cobalt(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. The efficiency of incorporation of cobalt in vitamin B_{12} in ruminants is low and inversely related to cobalt intake. Incorporation rate may be characterized by a range of 3 to 15 %. Besides covering the requirements for vitamin B_{12} synthesis, cobalt may play a role in rumen fermentation by increasing fibre digestion from low quality forages.

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 of this vitamin by hindgut bacteria, which is why coprophagous animals may receive some supply of vitamin B_{12} from microbial fermentation."

2. Characterisation

For compounds of trace elements, the element itself is considered the active substance.

2.1. Characterisation and identity of the additive

The coated granulated preparation(s) of cobaltous carbonate monohydrate, based on basic cobalt carbonate monohydrate (chemical formula: $2CoCO_3 \times 3Co(OH)_2 \times H_2O$; molecular weight: 534.7 Da; CAS No 51839-24-8; min. 46 % Co), contains up to 5 % cobalt, products with 1, 2 and 5 % cobalt are exemplarily introduced in the dossier. The product consists further of coating agents and dispersants (poly-oxy-ethylene, sorbitan monolaurate, glycerol polyethyleneglycol ricinoleate, polyethyleneglycol 300, sorbitol, and maltodextrin) and a carrier granule (calcium magnesium carbonate, calcium carbonate, corn cobs). The coat-granulating agents comply with the EU requirements for food additives and/or the European Pharmacopeia and/or JECFA specifications (see Appendix C). The content of coat-granulating agents depends on the target concentration of cobalt in the additive (target concentrations of 1, 2 and 5 % cobalt may require the addition of 2.3, 2.4 and 3.0 % coating agents, respectively, to obtain the desired quality of the granulated preparation).

Batch to batch consistency was demonstrated for two production series of a 5 % cobalt-containing preparation.[11] Twelve and eight batches showed mean cobalt content of 5.22±0.14 % and 5.23±0.12 %, respectively.

Three batches of the 5 % cobalt-containing additive were examined for heavy metals and arsenic, and one for dioxins.[12] The maximum levels for Cd (< 1 mg/kg), Pb (<10 mg/kg), and As (<10 mg/kg), for PCDD/F (0.32 ng WHO-TEQ/kg) and dioxin-like PCBs (0.021 ng WHO-TEQ/kg) appear to comply with EU legislation. But when considering that Directive 2003/32/EC refers to compounds of trace elements (As max 30, Pb max 100 and Cd max 10 mg/kg) and assuming that the basic cobalt carbonate is the only source of heavy metals and arsenic and dioxins, the upper bound values might be about 9 mg Cd, 91 mg Pb and 91 mg As/kg basic cobalt carbonate, the later value being above the threshold of the Directive. A comparable excess could be calculated for dioxins (2.9 ng/kg basic cobalt carbonate *vs*. 0.5 ng/kg of the Directive). A protocol for routine screening of heavy metals and arsenic,

[11] Technical Dossier/Section II/Annex 2.1.3.a.
[12] Technical Dossier/Section II/Annex 2.1.4.f.

as well as a proposal for specification of the coated granulated additive (< 5 mg Pb/kg, < 5 mg Cd/kg and < 15 mg As/kg) is attached to the dossier.

Mercury (4–8 mg/kg additive corresponding to 36–73 mg/kg basic cobalt carbonate) appears high (compared to other mercury thresholds, i.e. 0.1 mg/kg feed materials, 0.2 mg/kg mineral feed, 0.3 mg/kg $CaCO_3$), is, however, not regulated for compounds of trace elements by Directive 2003/32/EC. Upon request of EFSA, the applicant provided the internal specifications for the cobalt raw materials (<1 mg Co/kg) and for the carrier calcium magnesium carbonate (<0.3 mg/kg). Provided that internal control measures are in place, the values measured for mercury could hardly be explained. A second analysis of the same three batches in another certified laboratory resulted in <0.005 mg Hg/kg of the coated granulated preparation (5 % cobalt).[13]

Bulk density is 1290 g/L.[14] The median particle size determined by laser diffraction of three batches was 750 µm, no particles with a diameter less than 100 µm were found, whereas in the basic cobalt carbonate used for the preparation of the coated granulated material all particles were below 50 µm.[15] Dusting potential (Stauber-Heubach method; mg/50 g sample) was examined on the basis of the same 20 batches as used for batch to batch consistency;[16] the dust emission was 0.3±0.1 for the first 12 batches, and 0.2±0.1 for other eight batches (corresponding to about 0.015 g/m³) for the two production series. Mechanical stability of the granules under compression (5 t/cm^2) was principally demonstrated, however, dusting potential increased from 15 to about 45 mg/m³.

The feed additive grade basic cobaltous carbonate monohydrate is prepared by binding the cobalt-compound onto the surface of the carrier granule coated forming agents and dispersants applied as liquids in a low shear granulation process. The coating agents throw a "net" across the granule surfaces and the quantities used can vary from batch to batch. MSDS for the process ingredients were submitted.

2.2. Stability and homogeneity

Stability studies are not required for inorganic compounds of trace elements.

The capability of the coated granulated formulation of basic cobalt carbonate to form homogeneous mixtures was assessed in a complete feed for pigs for fattening (three batches, target concentration 2 mg Co/kg).[17] The additive was incorporated via premixtures containing vitamins and trace elements. Twenty samples were analysed. The CVs for two sets of 10 samples each were 7.8 %.

2.3. Physico-chemical incompatibilities in feed

According to the current knowledge, no incompatibilities resulting from the use of cobalt(II) carbonate, hydroxide (2:3) monohydrate in animal nutrition are likely to be expected.

2.4. Conditions of use

The cobalt(II) carbonate, hydroxide (2:3) monohydrate is intended to be used as feed additive for all animal species at a maximum (total) concentration in complete feed of 2 mg Co/kg.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of cobalt (three compounds, including basic cobaltous carbonate monohydrate) in animal feed. The Executive Summary of the EURL report is in Appendix A.

[13] Supplementary information/Annex 2-8.
[14] Technical Dossier/Section II/Annex 2.1.5.b.
[15] Technical Dossier/Section II/Annex 2.1.5.a.
[16] Technical Dossier/Section II/Annex 2.1.5.a.
[17] Technical Dossier/Section II/Annex I 20.2.4.2.a.

3. Safety

The paragraphs below on the safety of cobalt for target animals and consumers have been taken and summarised from a previous FEEDAP opinion (EFSA, 2009a). Additional information, which updates the mentioned opinion, has been considered and referenced in the current opinion.

3.1. Safety for the target species

3.1.1. Dietary requirement

A specific requirement for cobalt *per se* is not established. The dietary requirement/allowances of ruminants and horses for cobalt intended to optimise the vitamin B_{12} supply is in the range of 0.1–0.25 mg/kg complete feedingstuffs.

Considering the low cobalt average background levels in feedingstuffs (< 0.1–0.5 mg/kg dry matter (DM), plant feed materials showing lowest values), feed supplementation is generally needed to cover the requirement. In general, a cobalt supplementation of 0.3 mg/kg DM is considered sufficient.

Other animal species than ruminants, rabbits and horses do not need dietary cobalt, but do need vitamin B_{12}.

3.1.2. Tolerance

The tolerance of ruminants to cobalt is very high and greatly in excess of the requirements. The US National Research Council (NRC) set 25 mg/kg feed as maximum tolerable level of cobalt for cattle, poultry, sheep and horses and 100 mg/kg feed for swine (NRC, 2005). According to current EU legislation, feed for all animal species is not allowed to exceed 2 mg total Co/kg complete feed.

Typical signs of chronic cobalt toxicity in most species are reduced feed intake and body weight, emaciation, anaemia, hyperchromaemia, debility and increased liver cobalt (NRC, 1980). Cobalt toxicity in cattle is characterised by mild polycythaemia, excessive urination, defecation and salivation, shortness of breath and increased haemoglobin (Hb), red cell count and packed cell volume (NRC, 1996). Sheep seem to be more resistant to cobalt overdoses than cattle, and young ruminants without rumen function are more sensitive to cobalt overdoses than adults.

Therefore, it is considered unlikely that cobalt toxicity in target animals could be a major problem in practice. However, Murray (2010) reported some field cases of cobalt toxicosis in calves and concluded that supplementation of animals with minerals should be based on evidence of deficiency supported by laboratory confirmation. Signs of chronic cobalt toxicity are similar in many respects to deficiency. Administration of additional or higher doses in order to compensate the apparent deficiency would further deteriorate the clinical symptoms. The author concluded that supplementing diets of non-deficient animals, and failure to adhere to recommended dosages, is at best wasteful but is also potentially fatal.

Corrier et al. (1986) administered different cobalt doses from cobalt chloride hexahydrate (equivalent to 0, 120 and 180 mg/kg feed from days 1 to 70; and 0, 400 and 600 mg supplemental Co/kg diet from days 71 to 108) to adult sheep. The authors observed a numerical decrease in weight gain at both cobalt doses, but no differences in haematological or biochemical determinations. Neither gross nor microscopic lesions were found in the testes or other tissues. Cobalt concentration in liver and kidney increased from 0.09 (both tissues) to about 8.6 and 2.0 mg Co/kg wet tissue, respectively, without differences between the two cobalt doses administered. However, the results differ from other previous studies (Becker and Smith, 1951), which reported that daily doses of between 4 and 11 mg Co/kg bw caused anaemia, severe anorexia, weight loss, acute toxicosis and mortality.

3.1.3. Interactions in vivo

There appears to be a considerable variation in cobalt tolerance in animals. Several dietary factors (e.g. protein, sulphydryl-compounds, iron, selenium and vitamin E) and age may affect the level of cobalt necessary to cause overt toxicosis (NRC, 2005).

3.1.4. Conclusions on the safety for species

Feeding supplemental cobalt from cobalt(II) carbonate hydroxide (2:3) monohydrate up to the maximum total cobalt content in feed set by current EU legislation is considered safe for the target animals. The margin of safety is around 10.

3.2. Safety for the consumer

3.2.1. Metabolic fate

The metabolic fate of cobalt and vitamin B_{12} (cobalamin) was already described in a previous opinion of the FEEDAP Panel (EFSA, 2009a), and it is summarised below.

In laboratory animals such as rats and mice, the reported apparent absorption of cobalt from cobalt chloride was in a range of 13–34 %. Cobalt is predominantly excreted via the faecal route, and includes mainly the unabsorbed fraction. Absorbed cobalt follows aqueous excretion routes, via kidney but also via milk. Urinary cobalt is considered a good indicator of exposure to soluble cobalt, but not to insoluble cobalt compounds.

The microflora of the rumen (of cattle, sheep and goat) and of the large hindgut fermenters (horses and rabbits) synthesize vitamin B_{12} if sufficient quantities of available cobalt were orally provided. 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 %.

In animals with the capacity to synthesise cobalamin, cobalt from orally administered cobalt(II) is also deposited in tissues in the form of vitamin B_{12}.

3.2.2. Tissue deposition

About 43 % of body cobalt is stored in muscles. However, kidneys and liver are the edible tissues containing the highest cobalt concentrations; these organs also reflect the cobalt intake.

Among foodstuffs of animal origin, offal shows the highest cobalt content: liver with about 0.02–0.07 mg/kg fresh weight (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). Most cobalt in offal and muscle tissue of ruminants can be attributed to vitamin B_{12}. This fraction of vitamin B_{12}-bound cobalt is probably already smaller in hindgut fermenters (such as horses and rabbits, since only cobalt not absorbed in the small intestine is available for vitamin B_{12} synthesis in the hindgut), and 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). Correlations between cobalt/vitamin B_{12} intake at physiological feed concentrations and tissue deposition could not be established by the FEEDAP Panel owing to lack of data. Any prediction of the cobalt content of food of animal origin from dietary cobalt is therefore not possible at present.

3.2.2.1. Quantitative relations

Male rats were given a protein restricted diets for eight weeks (Clyne et al., 1988), either non-supplemented or supplemented with 20 mg $CoSO_4x7H_2O$/kg body weight (~ 4 mg Co/kg). Cobalt supplementation resulted in a significant decrease in weight gain, and an increase of cobalt in the myocardium (1.615 vs. 0.051 mg/kg), muscle tissue (0.483 vs. 0.019 mg/kg) and serum (0.124 vs. 0.001 mg/kg).

Henry et al. (1997) studied on a total of 27 mature wethers (59.8 kg bw) the effect of increasing dietary cobalt concentrations (0, 20, and 40 mg supplemental Co/kg feed from $CoSO_4xH_2O$, the basal diet contained 0.17 mg Co/kg) on tissue deposition for 60 days. The addition of cobalt had no effect on feed intake. The cobalt concentration in liver (control 0.20 mg/kg DM) responded to increasing dietary cobalt (3.74 and 7.33 mg Co/kg DM with 20 and 40 mg supplemental dietary cobalt, respectively) as did kidney (0.77 *vs.* 3.27 and 4.82 mg/kg DM), muscle (0.13 *vs.* 0.59 and 1.26 mg/kg DM) and heart.

3.2.2.2. A survey of foodstuffs of animal origin in the USA

Coleman et al. (1992) published data on the cobalt concentration of muscle, liver and kidney specimens in the USA, collected at slaughter from 2314 animals of 17 animal species/categories for one year (1985-1986). They analysed muscle, liver and kidneys of about 328 calves, 286 heifers/steers, 95 bulls/cows, 162 lambs, 34 sheep, 318 pigs, 280 boars/sows, 311 young chickens, 306 mature chickens, 61 turkeys and 11 ducks. The limit of quantification (LOQ) was 0.15 mg Co/kg tissue FW. They found quantifiable cobalt levels in about 1.9 % of muscle, 24 % of liver, and 15 % of kidney specimens.[18] Mean values were between 0.20 and 0.23 mg Co/kg muscle (exception 1.92 mg/kg for four specimens from heifer/steer), 0.22 and 0.27 mg Co/kg liver (exception 1.15 mg/kg for 54 specimens from bull/cow) and between 0.22 and 0.28 mg Co/kg kidney without essential differences between animal species/categories. When calculating the specimens without quantifiable cobalt concentrations, considering the LOQ (worst case assumption), mean cobalt concentrations in muscle, liver, and kidney of 0.15, 0.17, and 0.16 mg Co/kg, respectively, could be suggested.

3.2.3. Toxicological studies

The FEEDAP Panel previously reviewed the available toxicity data (EFSA, 2009a). The Panel summarised:

"According to Regulation (EC) No 790/2009 amending Regulation (EC) No 1272/2008, cobalt dichloride and cobalt sulphate 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." 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 EFSA´s ANS Panel concluded in 2009 that "Given the toxicological profile of cobalt(II) chloride hexahydrate, including genotoxicity and carcinogenicity, the Panel concludes that the proposed uses of cobalt(II) chloride hexahydrate added for nutritional purposes in food supplements as a source of cobalt are of safety concern" (EFSA, 2009b).

The most recent assessment has been published by the European Chemicals Agency (ECHA) in 2010 as support documents for the identification of cobalt(II) carbonate, cobalt(II) diacetate, cobalt(II)

[18] Exact figures as follows:
- Muscle specimens (quantifiable/total): calf (5/327), heifer/steer (4/287), bull/cow (2/95), lamb (1/165), sheep (2/34), pig (10/324), boar/sow (2/280), young chicken (9/311), mature chicken (3/308), turkey (2/61), duck (4/111).
- Liver specimens (quantifiable/total): calf (107/327), heifer/steer (91/287), bull/cow (54/95), lamb (40/164), sheep (12/34), pig (58/324), boar/sow (82/281), young chicken (29/309), mature chicken (51/309), turkey (16/60), duck (17/111).
- Kidney specimens (quantifiable/total): calf (70/328), heifer/steer (31/286), bull/cow (9/95), lamb (25/162), sheep (7/34), pig (34/318), boar/sow (15/280), young chicken (47/311), mature chicken (43/306), turkey (5/61), duck (66/111).

dinitrate, cobalt(II) sulphate and cobalt dichloride as substances of very high concern (SVHC) because of their carcinogen, mutagen and reproduction toxicant (CMR) properties (ECHA 2008; ECHA 2010a–d). The support documents cover also the hydrated forms of the cobalt salts.

For cobalt(II) carbonate (EC No: 208-169-4, CAS No: 513-79-1), cobalt(II) diacetate (EC No: 200-755-8, CAS No: 71-48-7) and cobalt(II) sulphate (EC No: 233-334-2, CAS No: 10124-43-3), the following classification according to Regulation (EC) No 1272/2008[19] was made:

May cause cancer by inhalation (carcinogen 1B), suspected of causing genetic defects (mutagen 2), may damage fertility (reproduction toxicant 1B). May cause allergy or asthma symptoms or breathing difficulties if inhaled, may cause an allergic skin reaction. Very toxic to aquatic life, very toxic to aquatic life with long lasting effects.

On 20th December 2011, the ECHA published a recommendation[20] for the inclusions of substances in Annex XIV ("List of substances subject to authorisation") of Regulation (EC) No 1907/2006,[21] prioritising thirteen substances from the Candidate List of SVHC. Among them is cobalt(II) carbonate, cobalt(II) sulphate, cobalt(II) diacetate, cobalt(II) dinitrate and cobalt dichloride.

3.2.4. Assessment of consumer safety

The FEEDAP Panel summarised in 2009 (EFSA, 2009a):

"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. 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."

The estimated population intakes most likely include already the contribution of foodstuffs from animals fed feedingstuffs routinely supplemented with cobalt(II) compounds. An increase of cobalt exposure by the use of cobalt containing feed additives is therefore not to be expected.

Considering that dietary exposure to cobalt is about 4–10 times lower than the health-based guidance value (see Appendix B), no safety concern for the consumer is expected for threshold effects of oral cobalt at the current intake level.

However, considering (i) the toxicological profile of cobalt(II) and its salts, (ii) the scarcity of deposition data, particularly for milk and eggs, and (iii) the uncertainties regarding the speciation of cobalt (cobalt(II) or vitamin B_{12}) in foodstuffs of animal origin, the FEEDAP Panel confirms its previous position that it would be prudent to limit the cobalt (cobalt(II) cation) supplementation of feedingstuffs to a level lower than the current maximum authorised.

3.3. Safety for the users/workers

Any exposure of users to the cobalt(II) salt under application may be of concern, considering the toxicological profile of cobalt(II) carbonate and, particularly, its CMR properties. It should be considered as a skin and eye irritant as well as a potential sensitiser to the skin and the respiratory tract. No information was available for the coated granulated additive.

[19] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1.

[20] http://echa.europa.eu/documents/10162/17232/backgroundoc_cobalt_carbonate_en.pdf

[21] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1.

Particle size (inhalable and respirable fraction) and dusting potential of the cobalt(II) salt under application are of particular relevance.

3.3.1. Hazard by inhalation

Laser diffraction analysis did not identify in three batches on the coated granulated basic cobalt carbonate particles of inhalable size (diameter of ≤ 100 µm). The dusting potential is consequently very low (highest value of 20 batches 0.5 mg/50 g, corresponding to 0.025 g/m³ air).

For pulmonary effects, the ATSDR has developed a minimum risk level of 0.1 µg Co/m^3 air (ATSDR, 2010). The data submitted indicate a dusting potential of the coated granulated basic cobalt carbonate (20 batches) of about 1 250 µg Co/m³ air.

3.3.2. Conclusions

The coated granulated basic cobalt(II) carbonate is considered as a skin and eye irritant and as dermal and inhalatory sensitiser.

The compound has a low dusting potential. However, it exceeds the thresholds set by the ATSDR. Due to the CMR properties of the additive, its dust is a hazard to persons handling the substance. Exposure by inhalation must be avoided.

3.4. Safety for the environment

Based on the calculation method provided in the relevant EFSA technical guidance (EFSA, 2008), the highest increase of cobalt in soil is around 40 µg/kg after a one year application of manure assuming that 100 % of a dose (2 mg Co from any source/kg complete feed) will be excreted. According to the database of the Forum of European Geological Surveys,[22] the median total cobalt content in Europe is 8.97 mg/kg in subsoil and 7.78 mg/kg in topsoil, with a range varying from < 3 to 170 mg/kg in subsoils and up to 249 mg/kg in topsoils.

The FEEDAP Panel therefore concludes that the use of cobalt from any source at the currently maximum authorised dose will not result in a substantial increase of the concentration in the environment and no further environmental risk assessment is deemed necessary.

4. Efficacy

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

The FEEDAP Panel previously concluded that monogastric animals (including poultry and fish) do not require Co but vitamin B_{12} (EFSA, 2009). Consequently, there is no need for any cobalt supplementation to the feed for these animals.

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 Co. The host metabolism of ruminants requires also only vitamin B_{12}. 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.

4.1. Bioavailability of cobalt compounds

Kawashima et al. (1997) studied on the basis of a previous experiment (Henry et al., 1997) in three trials the relative bioavailability of seven cobalt sources in ruminants (sheep) compared to reagent grade $CoSO_4x6/7H_2O$ (23.1 % cobalt). The basal diet provided adequate cobalt to the animals. Cobalt deposition in liver and kidney was taken as the parameter. The cobalt sources were cobalt carbonate

[22] http://weppi.gtk.fi/publ/foregsatlas/article.php?id=15

feed grade (FG, 45.2 % cobalt), cobalt carbonate reagent grade (RG, 48.4 % cobalt), cobalt heptonate (4.1 % cobalt), cobalt oxide (FG, 70 % cobalt), cobalt oxide (RG, 72.5 % cobalt) and a cobalt oxide by-product (BP) (25.6 % cobalt, > 21.7 % manganese, 10 % zinc and 9 % calcium).

Both carbonates were more available than the oxides. The ranges of the relative figures for bioavailability (cobalt sulphate= 100) were for carbonate FG 87–141 %, carbonate RG 68–133 %, for the oxide BP 37-58 %, for oxide FG 7–24 %, for oxide RG 0–7 %, and for the heptonate 80–92 %.

4.2. Efficacy studies/trials

Efficacy trials are not required for compounds of trace elements already authorised as feed additives (Annex III, 3.4. Regulation (EC) No 429/2008).[23]

To demonstrate the availability of cobalt from the coated granulated additive, the applicant performed in vitro studies comparing the dissolution of cobalt(II) from the coated granulated form with the original basic cobalt carbonate after 1, 2,5 and 10 minutes in buffer solutions at a pH values of 3.0, 6.0 and 7.4. No differences between the two test substances were observed. It can therefore be concluded that the coated granulated additive releases the cobalt(II) salt under the pH conditions which can be found in the gastrointestinum.[24]

4.3. Conclusions on efficacy

Taking into account in vitro data provided by the applicant and the respective in vivo literature, the coated granulated cobalt(II) carbonate hydroxide (2:3) monohydrate is considered as an efficacious source of cobalt which is turn used for microbial fermentation of vitamin B_{12}. Evidence of the efficacy of the compound under assessment is therefore given for ruminants and can be extrapolated to horses and rabbits.

The FEEDAP Panel reiterates its previous conclusion (EFSA, 2009a) that the potential cobalt supplementation to diets for ruminants, horses and rabbits of 0.3 mg/kg DM and a maximum content of 1 mg Co/kg complete feed is considered appropriate, taking into account that the cobalt background concentrations in feed materials do not exceed 0.5 mg/kg DM of complete feed.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[25] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

The additive under application is a cobalt(II) carbonate hydroxide (2:3) monohydrate, placed in the market as a coated granulated preparation.

The only physiological role of cobalt(II) is being a component of vitamin B_{12}. The significance of cobalt(II) as essential trace element results from the capacity of certain animal species to synthesise sufficient quantities of vitamin B_{12} by gastrointestinal microbes.

CONCLUSIONS

Feeding supplemental cobalt from cobalt(II) carbonate hydroxide (2:3) monohydrate up to the maximum total cobalt content in feed currently set by EU legislation is considered safe for the target animals. The margin of safety is around 10.

[23] OJ L 333, 22.05.2008. p.1.
[24] Technical Dossier/Section IV/Annex 4.1.2.a.
[25] OJ L 35, 8.2.2005, p. 1.

The apparent absorption of cobalt from cobalt chloride measured in laboratory animals was in a range of 13 to 34 %. Cobalt is predominantly excreted via the faecal route, and includes mainly the unabsorbed fraction. Absorbed cobalt follows aqueous excretion routes, via kidney but also via milk. About 43 % of body cobalt is stored in muscle; however, kidney and liver are the edible tissues containing the highest cobalt concentrations and most susceptible to increase such concentrations as a response to increased cobalt concentrations in feed. In animals with the capacity to synthesise cobalamin, cobalt from orally administered cobalt(II) is also deposited in tissues in the form of vitamin B_{12}.

Most cobalt in offal and muscle tissues of ruminants can be attributed to vitamin B_{12}. The fraction of vitamin B_{12}-bound cobalt is smaller in hindgut fermenters (horses and rabbits) and considerably smaller in poultry and pork meat, and particularly in milk and eggs indicating dietary supply of cobalt as such. Correlations between cobalt/vitamin B_{12} intake at physiological feed concentrations and tissue deposition as Co(II)/Co(III) could not be established due to lack of data. Any prediction of the cobalt content of food of animal origin from dietary cobalt is therefore not possible at present.

Cobalt(II) cations are considered genotoxic under *in vitro* and *in vivo* conditions. Cobalt(II) carbonate has CMR (carcinogen, mutagen and reproduction toxicant) properties. No data are available on the potential carcinogenicity of cobalt(II) following oral exposure either in humans or in experimental animals. However, oral exposure may potentially entail a number of adverse effects in humans (cardiac effects, effects on erythropoiesis, effects on thyroid, developmental effects and allergic dermatitis). For these threshold effects, the FEEDAP Panel developed a health-based guidance value of 0.0016 mg/kg bw and day (see Appendix B).

The estimated population average intake of cobalt was reported to be 0.005–0.04 mg Co/day in the USA, 0.011 mg Co/day in Canada, 0.012 mg/day in the UK, and 0.029 mg Co/day in France. These figures most likely include already the contribution of foodstuffs from animals fed feedingstuffs routinely supplemented with cobalt(II) compounds. An increase of cobalt exposure by the use of cobalt containing feed additives is therefore not to be expected.

Considering the population exposure to oral cobalt which is about 4–10 times lower than the health-based guidance value, no safety concern for the consumer is expected for threshold effects of oral cobalt.

Cobalt(II) carbonate hydroxide (2:3) monohydrate is considered as a skin and eye irritant and as dermal and respiratory sensitiser. No information was available for the coated granulated additive. Considering the toxicological profile of cobalt(II) carbonate, its dust is a hazard to persons handling the substance. Exposure by inhalation must be avoided.

The FEEDAP Panel concludes that the use of cobalt from any source at the currently maximum authorised concentration in feed will not result in a substantial increase of the concentration in the environment and no further environmental risk assessment is deemed necessary.

The coated granulated cobalt(II) carbonate hydroxide (2:3) monohydrate, is considered as a source of available cobalt for microbial synthesis of vitamin B_{12} in the gastrointestinal tract. The additive is therefore considered as an effective nutritional additive for ruminants. This conclusion can be extrapolated to horses and rabbits.

RECOMMENDATIONS

The FEEDAP Panel recommends the following corrections and improvements in the description of the additive under application:

- The denomination of the additive should be changed to coated granulated cobalt(II) carbonate hydroxide (2:3) monohydrate, to describe a distinctive identity.

- The authorisation should reflect the real cobalt content of the additive (1, 2 or 5 %) instead of the value proposed by the applicant (46 %).

Particular specifications for heavy metals and arsenic in the coated granulated additive are not considered appropriate, since the amount of cobalt compound in the additive may vary and the cobalt(II) carbonate, hydroxide (2:3) monohydrate must comply with the thresholds set by Directive 2002/32. Moreover, it is recommended to introduce a maximum threshold for mercury (0.3 mg Hg/kg cobalt compound).

The FEEDAP Panel reiterates its recommendations from the previous opinion. Considering the toxicological profile of cobalt(II) containing compounds, the FEEDAP Panel recommends to minimize the exposure of users to cobalt(II) compounds at several levels of feed formulation and animal nutrition.

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;
- reducing the authorised total maximum cobalt content from 2 to 1 mg/kg complete feed for all species except fish;
- limiting cobalt supplementation in feed for ruminants (except milk replacer), horses and rabbits to a maximum of 0.3 mg Co/kg complete feed.

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

The FEEDAP Panel recommends further a modification of the conditions of use of cobalt(II) containing compounds:

- The incorporation into feed should be only allowed via premixtures considering (i) the low incorporation rate into feed and (ii) the better equipment of premixture manufacturers for handling hazardous substances.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Cobaltous carbonate, monohydrate (film granulated preparations) for all animal species. October 2010. Submitted by Doxal Italia S.p.A.
2. Dossier Cobaltous carbonate, monohydrate (film granulated preparations) for all animal species. Supplementary information. April 2012. Submitted by Doxal Italia S.p.A.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Cobalt (E3).
4. Comments from Member States received through the ScienceNet.

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APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Cobalt (E3)[26]

In the current application authorisation is sought under articles 4(1) and 10(2) for *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate; Cobaltous sulphate, heptahydrate* under the category of "nutritional additives" functional group 3b (compounds of trace elements), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all categories and species.

The Applicants stated minimum *total cobalt* contents of 23% in *Cobaltous acetate, tetrahydrate*; 45% in *Cobaltous carbonate* and/or *basic Cobaltous carbonate, monohydrate;* and 20% in *Cobaltous sulphate, heptahydrate.*

The *feed additives* are intended to be incorporated into *premixtures*, *feedingstuffs* and *water*. Applicants (FAD-2010-0227 and FAD-2010-0371) suggested maximum levels of total cobalt of 2 mg/kg *feedingstuffs* and 1 mg/L water to comply with the limits set in Regulations (EC) No 1334/2003. Applicant (FAD-2010-0402) took into consideration the EFSA Scientific opinion recommending to reduce the maximum level of Cobalt to 1 mg/kg *feedingstuffs*.

For the identification of the various cobalt compounds in the *feed additives* the EURL recommends the general identification tests of acetates, carbonates and sulphates, described in the European Pharmacopoeia (Monograph 01/2008:20301). Additionally the EURL recommends crystallographic techniques, such as X-Ray diffraction, for the characterisation of crystalline structure of *Cobaltous acetate, tetrahydrate; Cobaltous carbonate, basic Cobaltous carbonate, monohydrate* and *Cobaltous sulphate, heptahydrate.*

For the *determination* of total cobalt in the *feed additive*, *premixtures* and *feedingstuffs* the Applicants submitted internationally recognised ring trial validated methods EN 15510 and CEN/TS 15621. Both methods are based on inductively coupled plasma atomic emission spectroscopy (ICP-AES), with or without pressure digestion. Similar performance characteristics were reported for the two methods mentioned above: – a relative standard deviation for *repeatability* (RSD_r) ranging from 2.5 to 12%; - a relative standard deviation for *reproducibility* (RSD_R) ranging from 11 to 26 %; and – a limit of quantification (LOQ) around 0.7 mg/kg *feedingstuffs*. Based on these performance characteristics the EURL recommends for official control the two CEN methods (EN 15510 and CEN/TS 15621), based on ICP-AES, to determine total cobalt content in the *feed additive*, *premixtures* and *feedingstuffs*. Furthermore, the EURL recommends the ring-trial validated CEN method EN ISO 11885, based on inductively coupled plasma optical (atomic) emission spectroscopy (ICP-AES) for the quantification of total cobalt in *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[26] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-CobaltGroup.pdf

APPENDIX B

The FEEDAP Panel of EFSA adopted in 2009 a scientific opinion on the use of cobalt compounds as additives in animal nutrition (EFSA, 2009). In this opinion, the Panel also reviewed the literature on threshold related adverse effects of oral cobalt intake. The Panel noted that the exposure to cobalt via the oral route may potentially entail a number of adverse in effects in humans (effects on heart, erythropoiesis, thyroid and prenatal development as well as allergic dermatitis). An increase in the erythrocyte number in humans, obtained by orally administering cobalt (as cobalt chloride) at an average daily rate of about 1 mg/kg bw to volunteers for 22 days (Davis and Fields, 1958), was considered the most sensitive biological effect linked to repeated oral exposure to the metal. The FEEDAP Panel adopted the conclusion of the ATSDR that the lowest observed effect level (LOAEL) obtained from this study could be taken to derive a minimum risk level of 0.01 mg Co/kg bw and day for intermediate duration (≤ 365 days) of cobalt exposure, applying a UF of 100 (10 for intra-human variability and 10 for the use of a LOAEL) (ATSDR, 2004). The LOAEL of 1 mg Co/kg bw and day was considered as confirmed by an 8-week study in rats administered cobalt chloride which established a no observed adverse effect level (NOAEL) of 0.6 mg Co/kg bw and day (Stanley et al., 1947).

In an opinion of the French Food Safety Agency (formerly AFSSA and now ANSES) considering the migration of cobalt from porcelain oven dishes intended to come in contact with food, it was concluded: *With respect to the health-based guidance values applicable to cobalt and in light of the few available studies on oral exposure, the Tolerable Daily Intake could be between 0.0016 and 0.008 mg/kg bw/day, for threshold toxic effects* (AFSSA, 2010).

In its opinion, AFSSA considered also the FEEDAP opinion (EFSA, 2009) and mentioned that *an uncertainty factor could however have been applied to the ATSDR Minimum Risk Level value to take into account subacute to chronic exposure extrapolation*. AFSSA continued indicating that *the value used in the REACH guidance documents for this extrapolation is 6, which brings the* (tolerable) *daily oral intake* (from 0.01 mg/kg bw down) *to 0.0016 mg/kg bw*.

The AFSSA noted further that *the use of the rat study by Stanley et al. (1947) with an inter-species factor of 4 (rats to human) and an intra-species factor of 10 leads to a final value of 0.016 mg/kg bw/day. An additional factor of at least 2 should however be applied to take into account the extrapolation from subchronic exposure (90 days) to chronic exposure (lifetime). This leads to a final value smaller than 0.008 mg Co/kg bw/day (the rat study lasted 56 days)*.

The AFFSA added: *However the genotoxicity data do not allow the possibility of non-threshold toxic effects to be ruled out. Lacking oral carcinogenesis studies, the Threshold of Toxicological Concern could be applied for this type of effect.*

Other health-based guidance values

The Dutch National Institute of Public Health and the Environment (RIVM) derived a Tolerable Daily Intake of 0.0014 mg Co/kg bw and day from a LOAEL of 0.04 mg Co/kg bw and day for cardiomyopathy after intermediate exposure in beer drinkers applying a safety factor of 30 (3 for intra-human variability and 10 for extrapolation to a NOAEL) (RIVM, 2001).

The Expert Group on Vitamins and Minerals (EVM) based its guidance level of 0.023 mg/kg bw and day - which would not be expected to result in any adverse effects - on a LOAEL of 23 mg/kg bw and day obtained in a 13-week study in mice using spermatogenesis as endpoint (EVM, 2003). An UF of 1000 was applied (10 for inter-species variation, 10 for inter-individual variation and 10 for LOAEL to NOAEL extrapolation). However, AFSSA considered that *according the REACH technical documents, the LOAEL-to-NOAEL extrapolation factor should be 3 (or 10), the inter-species factor 7 (for mice), the subchronic-to-chronic exposure extrapolation factor 2 and the intra-species factor 10, which leads to a total factor 1400 to 420. The guideline value would then be between 0.0164 and 0.0548 mg Co/kg bw/day.*

Cobalt health-based guidance values and population exposure to cobalt

Table B1 shows the different health-based guidance values adopted by various scientific bodies and calculated for a 70-kg adult person (EFSA, 2012).

Table B1. Cobalt health-based guidance values for a 70-kg person

Scientific body	mg Co/day
ATSDR/FEEDAP	0.700
AFSSA lower value	0.112
AFSSA higher value	0.560
RIVM	0.098
EVM	1.610
AFSSA corrected EVM, low value	1.148
AFSSA corrected EVM, high value	3.836

Some available estimates of the population intake for cobalt are 0.012 mg Co/day and person from a Total Diet Study in the UK (MAFF, 1997), and about 0.011 mg Co/day and person for the US population (ATSDR, 2004). All exposure data are considerably below the health-based guidance values (Table B1).

Assessment

It should be noted that the minimum risk level derived by ATSDR/FEEDAP was not foreseen for lifetime exposure. Its use was expressively limited to an intermediate duration (≤ 365 days) of exposure. The AFSSA calculation intends to extrapolate for lifetime exposure, which is different to the above and not comparable. However, the FEEDAP Panel acknowledges the need to derive a harmonised guidance value for chronic exposure to cobalt.

The FEEDAP Panel wishes to highlight that any extrapolation from a subacute study to a health-based guidance value for lifetime exposure is difficult and increases the margin of uncertainty. A recent guidance for this extrapolation from the EFSA's Scientific Committee is now available (EFSA, 2012). AFSSA, ATSDR and the FEEDAP Panel agree that polycythaemia is the most sensitive endpoint for threshold cobalt effects.

For the extrapolation from subchronic to chronic study duration in rodents, the EFSA's Scientific Committee recommends the use of an UF of 2, considering the extent of investigations usually performed in 90-day studies. The EFSA's Scientific Committee indicated in its opinion that it was not in a position to propose default values for the extrapolation from subacute to chronic study duration in rodents, due to differences in the respective study designs.

In accounting for the absence of a NOAEL, the Scientific Committee recommends to use the benchmark procedure. If the benchmark procedure cannot be applied to the critical toxicological study (as is the case, individual data of the publication of Davis and Fields (1958) are not accessible), the LOAEL approach might be used and an additional UF will be needed, the size of which should be determined on a case-by-case basis and justified. Several organisations recommend in their guidelines, e.g. Guidelines for drinking water quality (WHO, 2011), the application of an additional UF of up to 10 to the LOAEL to derive a health-based guidance value.

Considering the study on human volunteers (Davis and Fields, 1958) with a LOAEL of 1 mg Co/kg bw and day and applying the recommendations and data given in the Scientific Committee guidance document, the following UFs can be applied: 10 for inter human variability, 10 for extrapolation from subacute to chronic and 6 for extrapolation from LOAEL to NOAEL. The total UF is then 600. A health-based guidance for chronic exposure for threshold related toxic effects would be 0.0016 mg Co/kg bw and day (equivalent to 0.12 mg Co/day for an adult 70-kg person, figure rounded following the recommendations of the Scientific Committee).

Considering the rat study (Stanley et al., 1947) with a NOAEL of 0.6 mg Co/kg bw and day and applying the Scientific Committee recommendations, the following UFs were applied: 10 for inter-species variability, 10 for intra-species variability and 4 for extrapolation from subchronic (8-week study) to chronic. The total UF is then 400. A health-based guidance for chronic exposure for threshold related toxic effects would be 0.0015 mg Co/kg bw and day (equivalent to 0.1 mg Co/day for an adult 70-kg person, figure rounded following the recommendations of the Scientific Committee).

The values for a health-based guidance level for chronic exposure to oral cobalt for threshold related toxic effects derived from the two relevant studies considered in the previous FEEDAP opinion (EFSA, 2009) are similar (0.0016 (derived from the human study, Davis and Fields, 1958) and 0.0015 mg/kg bw and day (derived from the rat study, Stanley et al., 1947)). The value derived from the human study is equal to the AFSSA proposal.

The FEEDAP Panel adopts 0.0016 mg Co/kg bw and day (corresponding to a daily intake of 0.12 mg for a 70-kg adult person) as a health-based guidance value. Considering the population exposure to oral cobalt which is about 4 to 10 times lower than the health-based guidance value, no safety concern for the consumer is expected for threshold effects of cobalt.

The FEEDAP Panel principally agrees with AFSSA when noting that the genotoxicity data do not allow to rule out the possibility of non-threshold toxic effects for cobalt(II). However, applying the threshold of toxicological concern (TTC) concept ($TTC_{genotoxic}$ 0.15 µg/person and day) would lead to 0.0025 µg Co/kg bw and day (about thousand folds lower than the proposed health-based guidance value); for several reasons this seems inappropriate. Cobalt occurs naturally in food of plant and animal origin. The estimated population average intake of cobalt is reported to be in the range of 5–40 µg/person and day. Even a complete withdrawal of cobalt from animal nutrition would not reduce the population exposure to the magnitude required for the $TTC_{genotoxic}$ to be met.

For some animal species (ruminants and, probably, horses and rabbits) cobalt is an essential trace element for vitamin B_{12} synthesis. A (not fully quantifiable) part of cobalt in animal tissues and products is cobalt(III) in cobalamin and, as such, is indispensable to human nutrition. Nevertheless, any reasonable measure to reduce cobalt(II) content in foodstuffs of animal origin should be taken. Considering (i) the toxicological profile of cobalt(II) and its salts, (ii) the scarcity of deposition data, particularly for milk and eggs, and (iii) the uncertainties regarding the speciation of cobalt (cobalt(II) or vitamin B_{12}) in foodstuffs of animal origin, it would be prudent to limit the cobalt (cobalt(II) cation) supplementation of feedingstuffs generally at a lower level and to animals which convert cobalt(II) to cobalamin. Therefore the FEEDAP Panel recommends to the European Commission to modify 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) reducing the authorised total maximum cobalt content from 2 to 1 mg/kg complete feed for all species except fish, and (iii) limiting cobalt supplementation in feed for ruminants (except milk replacer), horses and rabbits to a maximum of 0.3 mg Co/kg complete feed.

Conclusions

The FEEDAP Panel, following the recommendations of the Scientific Committee, applies to the LOAEL (based on polycythaemia) of about 1 mg/kg bw observed in a 22-day study on volunteers an UF of 600 (10 for inter human variability, 10 for extrapolation from subacute to chronic and 6 for extrapolation from LOAEL to NOAEL). This results in a health-based guidance value of 0.0016 mg/kg bw and day for threshold effects of oral cobalt, corresponding to a daily oral intake of 0.12 mg Co for a 70 kg adult person.

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APPENDIX C

Specific purity criteria for the coat-granulating agents

Polyoxolethylene (20) sorbitan monolaureate (E432) meets the purity criteria defined in Commission directive 98/86/EC of 11 November 1998 amending Commission Directive 96/77/EC laying down specific purity criteria on food additives other than colours and sweeteners.

Glycerol polyethyleneglycol ricinoleate (E484) meets the purity criteria specified in the United States Pharmacopoeia entry for Polyoxyl 35 castor oil and the European Pharmacopoeia entry for macrogolglyceryl ricinoleate (monograph 01/2005:1082).

Polyethylene glycol 300 comprises 100 % polyethylene glycol in accordance with Ph Eur monograph 1444 and JECFA Monograph 316.

Sorbitol meets the purity criteria specified for sorbitol liquid in Commission Directive 95/31/EC of 5 July 1995 laying down specific criteria of purity concerning sweeteners for use in foodstuffs.

Maltodextrin (food grade) has the specific purity criteria SO_2 max 10 mg/kg and heavy metals max 0.5 mg/kg.

ABBREVIATIONS

AAFCO	Association of American Feed Control Officials
AFSSA	Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency)
ALT	alanine transaminase
ANS	EFSA's Scientific Panel on Additives and Nutrient Sources added to Food
ANSES	Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail
As	arsenic
AST	aspartate transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
Ca	calcium
CAS	Chemical Abstracts Service
Cd	cadmium
CMR	carcinogen, mutagen and reproduction toxicant
Co	cobalt
Cr	chromium
DM	dry matter
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
EVM	Expert Group on Vitamins and Minerals
FEEDAP	EFSA's Scientific Panel on Additives and Products or Substances used in Animal Feed
FG	feed grade
FW	fresh weight
Hb	haemoglobin
HDL	high-density lipoprotein
Hg	mercury
Ht	haematocrit
ICP-AES	inductively coupled plasma atomic emission spectroscopy
LOAEL	lowest observed adverse effect level
LOQ	limit of quantification
MAFF	Ministry of Agriculture Food and Fisheries
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MRL	maximum residue limit
NRC	National Research Council
NOAEL	no observed adverse effect level
Pb	lead
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*para*-dioxin
PCDF	polychlorinated dibenzofuran
RBC	red blood cell
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	Dutch National Institute of Public Health and the Environment
RG	reagent grade
SVHC	substances of very high concern
TTC	threshold of toxicological concern
TEQ	toxic equivalent factor
UF	uncertainty factor
WBC	white blood cell
WHO	World Health Organization