Copper : an environmental hazard ?

S. C. Monteiro (APC Ltd), M. Hale (APC Ltd), and S. Durosoy (Animine)

Introduction

The EU authorities have for decades had a policy of reducing copper levels in animal diets, and especially in pig feeds. Back in 1982, the Scientific Committee for Animal Nutrition (SCAN) concluded that maximal level in total dietary copper should not exceed 125 mg/kg in complete feeds for piglets and pigs. In another Opinion from 1983, SCAN already expressed the concern of higher selection of *E.coli* strains resistant to one antibiotic (chloramphenicol) with higher dietary copper in pig feeds. However, they acknowledged that specific measures could be authorized in some regions where environmental concerns are lower. Until the early 2000's, maximum authorized levels took into account animal production densities to assess the risk of copper load due to pig manure spraying. At that time, maximum Cu dietary concentration was 175 mg/kg for piglets up to 16 weeks of age. But regulation differed among European countries for pigs after the 17th week of age: in Member States where the mean density of the porcine population was equal to or higher than 175 pigs per 100 ha of utilizable agricultural land, maximum Cu level in the complete feed was 35 mg/kg instead of 100 mg/kg. The SCAN Opinion from 2003 proposed a compromise to reduce copper burden without affecting performance of farm animals, especially when its usage as a growth promotor is well documented. Thus, Regulation 1334/2003 of 25 July 2003 defined new maximum copper levels in pig feeds:

— piglets up to 12 weeks: 170 (total) mg/kg

— other pigs: 25 (total) mg/kg

The most recent (2016) EFSA report on copper in animal nutrition recommended to modify maximum content of copper in complete feeds for some animal species. The strongest consequence on animal performance would be induced by the reduction from 170 mg/kg to 25 mg/kg Cu in the post-weaning phase of piglets. This article summarizes current challenges and suggests scenarios for a more sustainable usage of copper in pig feeds.

Environmental risk assessment of feed additives

Following the administration of Cu as feed additive to livestock and their metabolism in the animal, the remaining Cu will be excreted in manure, slurry or litter. As a common practice, these are applied to soils as fertilisers due to their high nutrient content, hence Cu sequestered in manures will also be applied to soils. Cu residues in soil can potentially runoff to surface waters and/or leach to groundwater following rain events. Furthermore, in surface waters Cu will partition into sediment.

Copper is a transition metal and has more than one oxidation state, existing in cuprous (Cu (I), Cu^{+}) and cupric (Cu (II), Cu^{2+}) forms. When Cu (II) is introduced in the environment, it binds to inorganic and organic ligands contained within water, soil, and sediments (Monteiro *et al*., 2010).

According to the feed additive regulations (EC 1831/2003), an environmental risk assessment (ERA) is required for any substances that enter the environmental compartments. The ERA consists on estimating the exposure and hazard substances might pose in the environment. To assess the exposure, the concentrations of each substance in each environmental compartment are estimated. Therefore, predicted environmental concentrations (PECs) are calculated for soil, surface water, groundwater and sediment. The Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008) presents the requirements for calculating the amounts of feed additives applied in manures to land. The amount of feed additive being released into the environment is estimated based on feed intake and the nitrogen content of the manure, which are different for each animal. The amount of manure containing feed additives allowed to be spread on land depends on the nitrogen content of the manure and the nitrogen emission standard, which is currently 170

Kg nitrogen per hectare (EU Nitrate Directive 91/676/EEC). Initially a worst-case approach is used for exposure calculations, *e.g.* the calculations assume that the feed additive is continuously given to the animal and that the total intake of active substance is excreted in the manure. Potential toxicity is estimated using laboratory and/or field studies combined with relevant safety factors to determine the predicted no-effect concentrations (PNECs) that Cu poses to soil, aquatic and sediment dwelling organisms. The risk is then calculated by comparing the calculated exposure (PECs) to the hazard (PNECs) for each environmental compartment.

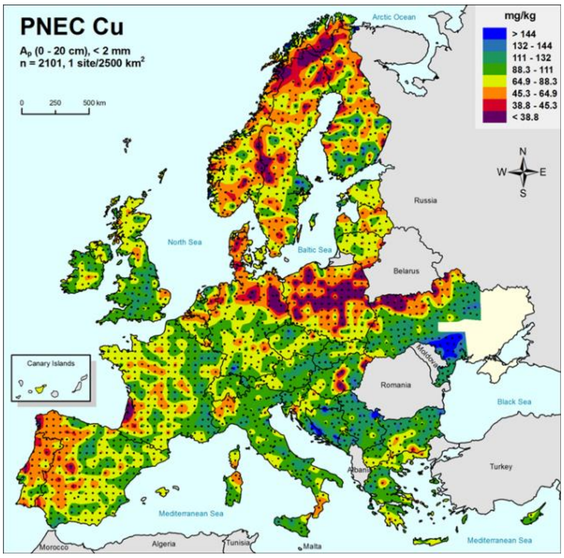
$$\text{Risk characterisation ratio (RCR)} = \frac{\text{exposure}}{\text{effects}} = \frac{\text{PEC}}{\text{PNEC}}$$

If the ratio of PEC/PNEC is above 1, the substance poses a risk to the environment and is considered unacceptable unless further refinements are proposed, or new studies are performed.

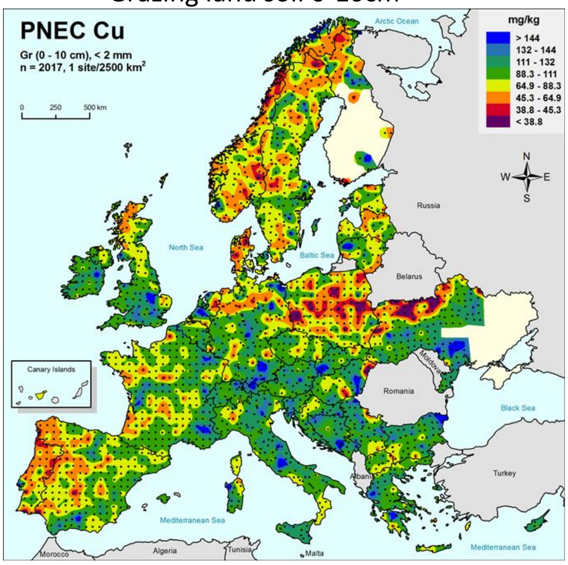
Copper in the environment: soils

The risks of potentially toxic metal additions to agricultural soil are not immediate but are associated with the slow accumulation of a pool of soil-bound metal, on a timescale of decades or longer, to a point where its concentration is sufficient to either affect soil organisms directly (Monteiro *et al.,* 2010). More specifically, for the use of Cu in feed additives for piglet rearing, a risk was identified due to accumulation in the long-term exposure simulations (50 years; Monteiro *et al*., 2010). However, four years later, the GEMAS project (Geochemical Mapping of Agricultural Soils)) performed a realistic risk assessment of trace elements in more than 4000 soil samples at the European scale (Reimann, 2014). For copper, only a few, isolated sites were predicted at risk: 1.6% and 1.3% of sites for agricultural and grazing land, respectively. Such exceptional situations were found in some Mediterranean areas with high natural background concentrations and applications of Cu fungicides in vineyards.

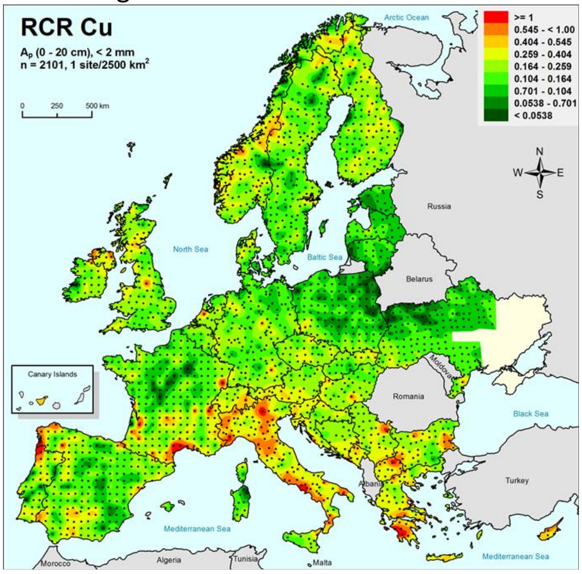
Agricultural soil 0-20cm

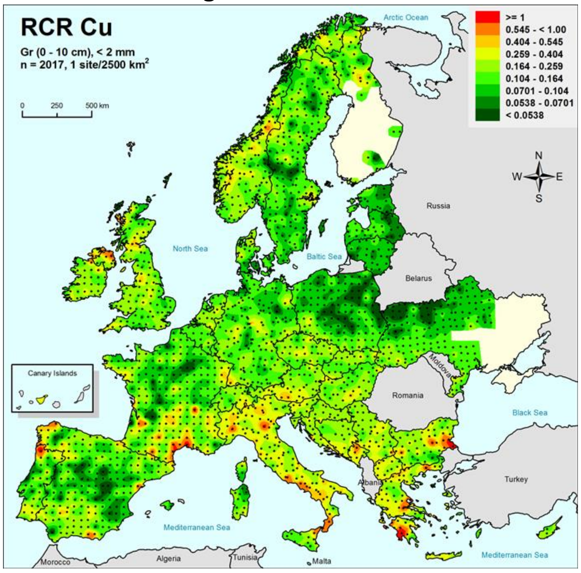


Grazing land soil 0-10cm



Agricultural soil 0-20cm | Grazing land soil 0-10cm





Copper in the environment: surface and ground water

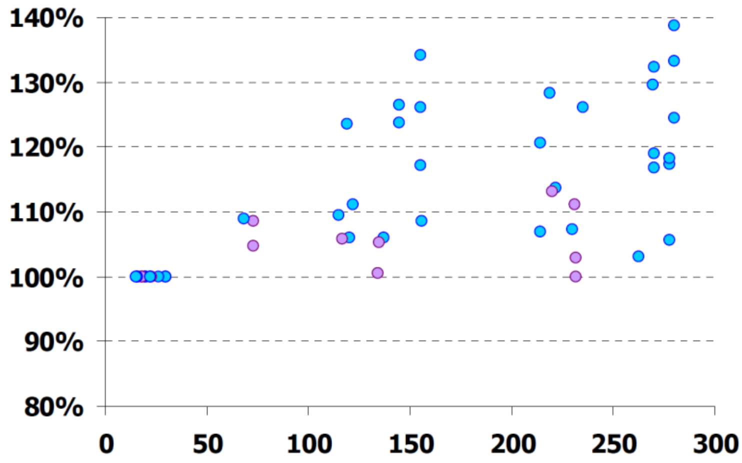
Cu residues in soil can potentially runoff to surface waters and/or leach to groundwater following rain events. Furthermore, in surface waters Cu will partition into sediment. Studies clearly demonstrated that a very rapid dissipation of Cu occurs in surface waters, where it rapidly binds to sediments or organic matter, or precipitate as insoluble inorganic salts (EFSA, 2018). In freshwater, the risk to aquatic organisms following the application of Cu in manures to soils has been identified as low. This is due to the strong retention of Cu by soils that result in small predicted increases in average surface water concentrations (Monteiro *et al*., 2010). Therefore, the risk to groundwater is also low since Cu binds to soils and therefore is unlikely to leach into groundwater.

Copper in the environment: microbial resistance

A growing number of scientific publications look at microbial resistance from pathogenic bacteria against heavy metals in the terrestrial environment. Antibacterial metals can also co-select for antibiotic resistance via the transfer of mobile gene elements, and metal resistance genes (MRGs) tend to occur together with antibiotic resistance genes (ARGs). Such potential risk is of both environmental and medical relevance (Pal *et al*., 2017). Copper specifically selected for resistance to vancomycin, the last-resort antibiotic for the treatment of resistant staphylococcal infections in humans. High Cu-exposure to the microbial community in pig manure selects for Cu resistance but also co-selects for antibiotic resistance. Some recent studies showed some significant positive correlations between some ARGs and copper (Zhu *et al*., 2013; Wang *et al*., 2017). However, most of these results were obtained in non-European animal manures, with elevated concentrations of heavy metals (Zn, Cu, As, Cd) and antibiotic residues. At the opposite, both resistance against copper and against antibiotics can be found in areas without a pollution history, such as Antarctic water. Consequently, it remains unclear whether heavy metals like copper are one of the drivers of developing antibiotic resistance : a sufficiently clear demonstration of this chain of events is currently not available (EFSA, 2016). As a metallic compound like copper is persistent in soil, preventive measures are necessary in a long term approach.

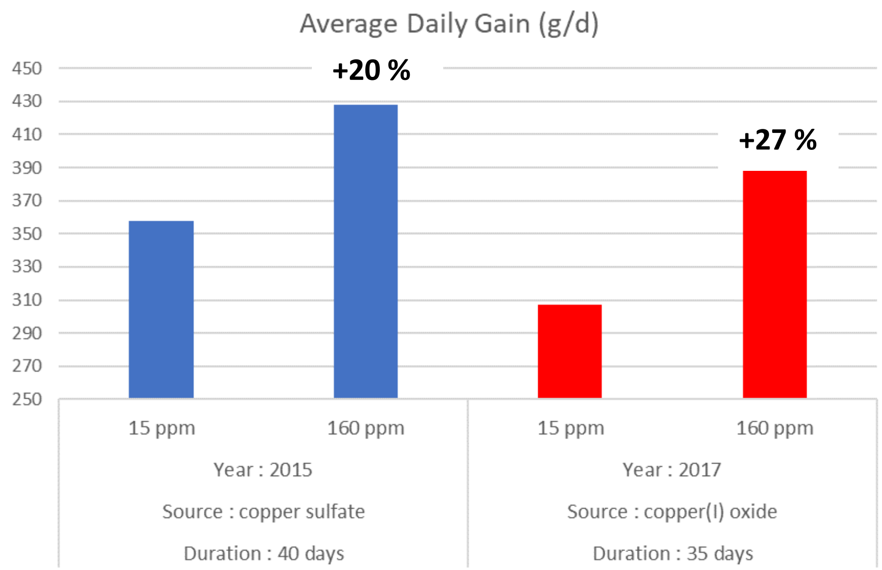
Growth promoting effect of copper on piglets

Literature review performed by SCAN in 2003 had clearly demonstrated the significant growth promoting effect of copper on young pigs (graph 1).



Graph 1 Effect of dietary Cu (mg/kg) on weight gain improvement of weaned piglets (rose : non significant; blue : significant)

More than ten years later, it is questionable if such improvement is maintained in modern conditions. Most recent studies (Bikker *et al.*, 2015; Bikker, 2017) have been performed in Wageningen University and are summarised in graph 2. They involved a high number of animals, 800 piglets per experiment.



Graph 2 : Effect of supplemented copper on body weight gain of weaned piglets ($p<0.05$)

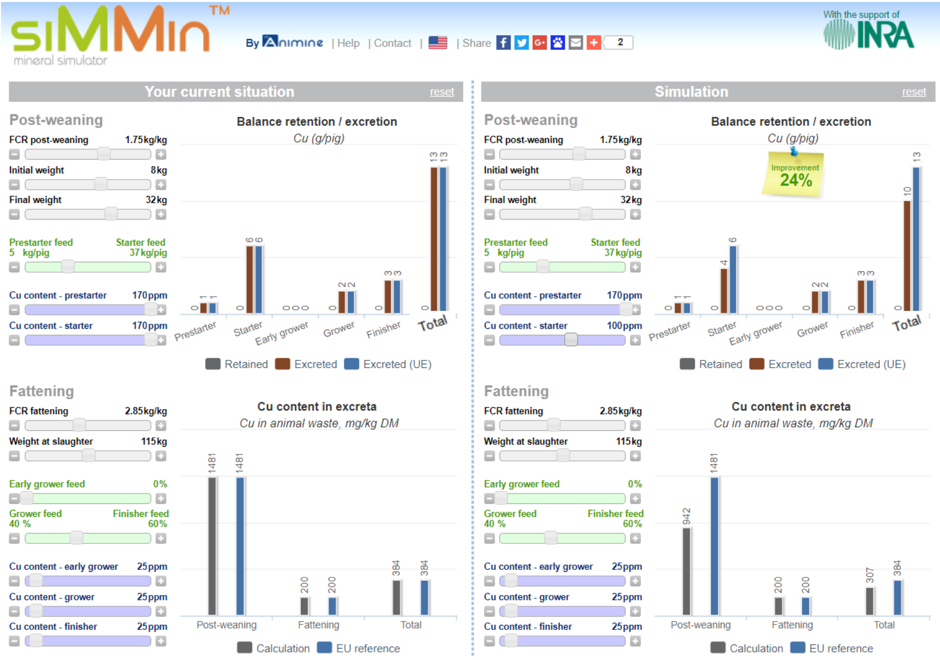
Gaining almost 3 kg body weight in the post-weaning phase due to one single feed additive is exceptional. Reducing copper concentrations from 170 to 25 mg/kg in feeds for weaned piglets would impair technical and economical performance of pig farms.

Copper balance in the pig farm : siMMin

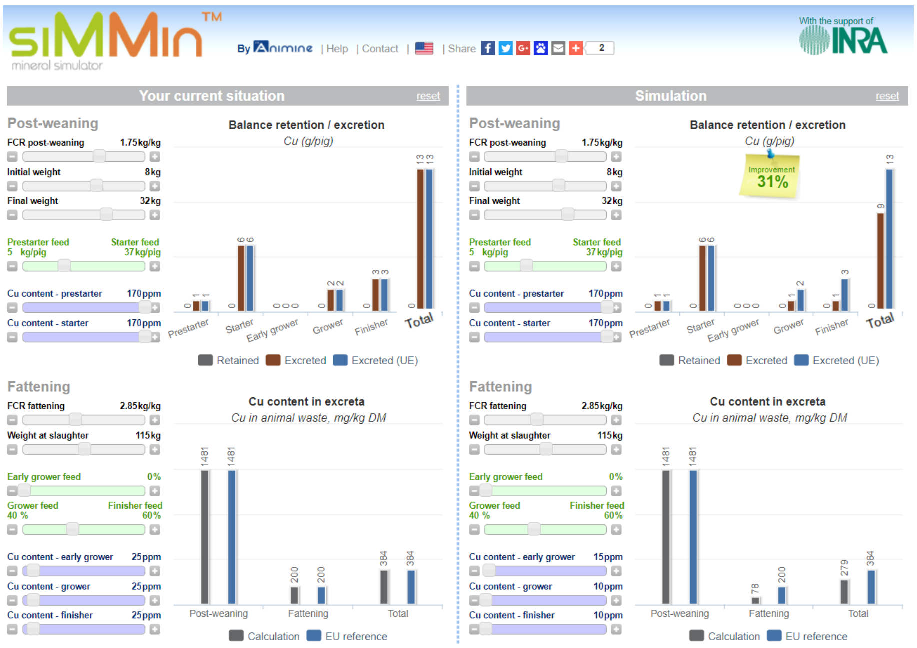
Calculating copper balance for growing pigs is possible with the mass balance method. There is a linear relationship between dietary Cu content and concentration in the manure due to homeostatic regulation. As copper retention can be calculated based on the difference in mineral body content between the beginning and the end of a defined period, mineral excretion can be deduced by the difference between Cu intake and Cu retention. INRA (French National Institute for Agricultural

Research) has defined mathematical models for critical nutrients. With siMMin™, an online software available at https://animine.eu/mineral-simulator-software/ it is easy to model the copper balance related to feeding program.

Following EFSA recommendations, a compromise has been suggested to reduce total Cu content, in the second feed after weaning, from 170 to 100 mg/kg. It would reduce Cu load in the environment by approximately 25%, but is likely to have negative effects on piglet growth performance.



siMMin™ enables to simulate other scenarios. For example, by keeping existing Cu levels in the post-weaning phase and reducing maximal Cu concentration of fattening diets at 10 ppm, the reduction of excreted Cu quantities would be even higher. Withdrawing Cu supplementation in the fattening phase would have no impact on pig performance and welfare, as copper nutritional requirements are not exceeding 7 mg/kg. Such regulatory change would preserve farm productivity and improve sustainability of pig production systems.



Conclusion

A balance needs to be reached on the levels of Cu added as feed additives, especially in the post-weaning phase of piglets where the proposed reduction is significant, to ensure that the growth promoting effect is still maintained without triggering an environmental risk. Because the mode of action of dietary copper on pigs is not fully elucidated, more research is needed on the effect of copper doses and sources at the intestinal and systemic levels. Metals do not degrade in the environment, but they can be transformed by environmental processes to either increase or decrease the availability of toxic species (Monteiro *et al.*, 2010). Ecotoxicity is not only related to total copper concentrations but also to metal speciation. For Cu^{2+} this is clearly demonstrated in studies showing a very rapid dissipation in surface waters, rapidly binding to sediments or organic matter, or precipitation as insoluble inorganic salts (EFSA, 2018). Therefore, a large majority of the Cu^{2+} present in the environment will not be in its free ionic form and will not be bioavailable to organisms. Nevertheless, in the more recent risk assessment of copper compounds to be used as plant protection products (EFSA, 2018), a worst-case approach has been used that considered that all Cu in the environment is equally bioavailable, regardless of its speciation. This is expected to significantly overestimate the risk of Cu compounds in the environment. Therefore, even if some progress has been made in last years, research is warranted on metal speciation in animal wastes, so that regulators have a quantitative evaluation not only of total copper but also of copper species. Eurostars funded program SUMINAPP will investigate the effect of feed grade copper sources on copper chemical forms present in fresh manure and after anaerobic digestion.

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