Opinion of the Scientific Committee for Animal Nutrition on the use of copper in feedingstuffs

(Adopted on 19 February 2003)

1. Background

Copper is authorised for all species under Directive 70/524/EEC concerning additives in feedingstuffs under the category "trace elements", as presented in annex I.

The Commission has the intention to review the maximum content of trace elements authorised in feedingstuffs in order to adapt these levels to the requirements and to minimise negative effects on the environment. In regard with these objectives, particular attention should be paid to the use of copper where negative effects on the environment have been described.

2. Terms of reference

2.1. For the various food producing animal species, what is the relation between the content in copper of the ration of the animal and the quantity of residual copper in tissues and organs? Do the residues resulting from the permitted conditions of use represent a risk for the consumer?

2.2. As regards pigs and calves, does the addition of compounds of copper to feedingstuffs involve significant effects on the growth rate of the animals or on the conversion rate of the ration? In this respect are the permitted maximum contents in copper justified?

2.3. In the case of pigs and bovines, what is the relation between the content in copper of the ration of the animal and the quantity excreted in the environment? Can the excreted quantities of copper, resulting from the permitted conditions of use, be prejudicial to the environment? In the affirmative, what is the nature of the risks?

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3. COPPER RECOMMENDATIONS FOR LIVESTOCK AND OTHER FOOD PRODUCING ANIMALS

Copper is an essential trace element that plays a vital role in the physiology of animals: for foetal growth and early post-natal development, for haemoglobin synthesis, connective tissue maturation especially in the cardiovascular system and in bones, for proper nerve function and bone development, and inflammatory processes.

It is involved in different biochemical processes of animal metabolism such as
- enzyme-coenzyme catalytic reactions. It is associated with the function of a number of enzymes such as oxygenases including cytochrome C oxidase, and copper-zinc superoxide dismutase
- ion transport, for instance with ceruloplasmin (ferroxidase I), a putative copper transport protein required for the incorporation of iron into transferrin for its transport in plasma

Copper deficiency leads to physiological disturbance. Symptoms include depression of growth, anaemia, bowing of the legs, spontaneous fractures, ataxia of newborns, cardiac and vascular disorders and depigmentation, decrease in some organs weight, depressed reproductive performance including egg production.

Despite of the broad knowledge on copper, some aspects of the metabolism of copper are still poorly understood. The present knowledge is summarised by Mac Dowell (1992) and Underwood and Suttle (1999).

- Copper is absorbed in the upper small intestine. Its absorption as that of all trace elements is difficult to measure and published results are consequently highly variable. Copper absorption is affected by the physiological stage of the animal, dietary level of copper (Jenkins and Hidiroglou, 1989) and interactions with phytate, ascorbic acid, fibre, tannin etc. which appear to complex with copper (Cousins, 1985) and other trace elements. Lönnerdal *et al.* (1985) report that copper absorption is higher in neonates than in adults. Thus, absorption rate hovered 60-70 % of dietary copper in calves compared to less than 5% in adult ruminants (ARC, 1980; Bremner and Dalgarno, 1973).

- Bile has been shown to be the major pathway for copper excretion in many animal species (Aoyagi *et al.*, 1995). Faeces are therefore the main route for excretion of copper, the urinary excretion representing only small losses through kidneys.

3.1. Requirements and recommendations

Requirements for trace elements are mainly identified by dose response curves. In most cases, there are not enough experimental data available for a factorial approach.

Studies on trace element requirements lead to variable results due to the numerous factors impacting on the outcome of experiments: criteria used to assess results, composition of the basal diet and animal breed.

For practical reasons and considering the above difficulties, various national scientific bodies express rather recommendations than trace element requirements. Recommendations include a safety margin to ensure an appropriate coverage of the animal requirements, even in the case of high performing animals.

Recommendations are expressed as mg per kg feed or as mg per kg dietary dry matter (DM). The expression as a concentration in the feed, as opposed to mg per animal and day is based on the assumption that feed values, feed intake and feed efficiency ratio data are averaged.

3.1.1. Pigs

Some requirements of and recommendations for pigs are listed in table 1. Highest values are generally established for piglets because of their high growth rate, and the concomitant low copper supply by sow's milk (Hill *et al*, 1983b). Values for pigs remain however within a rather small range of 4 to 10 mg copper per kg dietary dry matter.

Table 1: Copper requirements of and recommendations for pigs expressed by scientific bodies

Requirements expressed in mg/kg feed (90 % DM)				Recommendations expressed in mg/kg dietary DM							
NRC 1998			ARC 1981	INRA 1989 a)			AFRC 1990		GfE 1987		
Piglets up to 20 kg	Growing pigs	Breeding animals	Growing pigs up to 90 kg live weight	Piglets	Growing pigs	Sows	Growing boars	Adult boars	Piglets	Growing pigs	Breeding sows and boars
5-6[a)]	3-4[a)]	5	4	10	10	10	4	4	6	4-5[a)]	8-10

[a)] the higher values are valid for the animals with the lower live weight.

3.1.2. Calves, growing cattle and dairy cows

The intrauterine retention of copper during the last four weeks of pregnancy of cows is estimated at 2.1 mg/d (ARC 1980). Milk normally contains 0.10 (ARC 1980) to 0.15 mg copper per kg (Kirchgessner *et al*. 1978) but varies according to breed up to 0.30 mg/ kg fresh milk (Williams, 1959) and remains independent from the copper supplementation of the diet (Schwarz and Kirchgessner, 1978).

In milk replacers for calves the absorption rate is estimated at 70% (ARC, 1980). Therefore, for calves fed milk replacers, 2-4 mg copper/kg DM have been considered sufficient (ARC, 1980).

Copper absorption declines considerably when the function of the reticulorumen develops. The microbial metabolism of inorganic and organic sulphur compounds in the rumen results in the production of sulphide and, when additionally molybdenum is present, also in the production of thiomolybdates, which bind copper with a high affinity. As a result the absorbability of copper is markedly reduced (Gooneratne *et al*., 1989, Suttle, 1991). The recommendations for growing ruminants approximate therefore 10 mg/kg dry matter (Table

2). These requirements increase up to 16 mg copper/kg dry matter feed in late gestation and early lactation due to the reduction of dry matter intake (NRC, 2001).

Table 2: Copper requirements (NRC) of and recommendations (INRA, GfE) for growing cattle and dairy cows expressed by scientific bodies

NRC (2001)		INRA (1989 b)	GfE (2001)	
mg/kg feed (90 % DM)		mg/kg DM		
Growing Heifers	Dairy cows	Ruminants	Growing heifers	Dairy cows
9-10[a])	9-11[a])	10	10	10

a) Up to 16 mg copper/kg diet in late gestation and early lactation with reduced dry matter intake

3.1.3. *Sheep and goat*

For small ruminants, like for cattle, copper absorption varies with age and the development of the rumen and depends on the presence of molybdenum and sulphur. Recommendations (Table 3) take into account that absorption from feed is also highly variable from 1.5 % for very young grass to 7 to 9 % for hay and cereals (Underwood and Suttle, 1999).

Table 3: Some copper requirements (ARC, NRC) of and recommendations (INRA) for sheep and goat (mg/kg DM) expressed by scientific bodies

Authority	INRA (1989b)	ARC (1985)	NRC (1985)
Lambs and kids	7	5	7-11
Lactating ewes, goats	10	-	7-11

Recommended dietary concentration of copper for goats amounted 10-15 mg/kg dietary DM for goats (GfE, 2003b). In the specific case of sheep, there is only a narrow margin of safety between recommendations and toxic levels.

3.1.4. *Poultry*

Copper requirements of and recommendations for poultry are usually established for the Gallus species (Table 4).

Table 4: Copper requirements (NRC) of and recommendations (GfE) for poultry by scientific bodies

Animal category	NRC (1994)	GfE (1999)
	mg/kg diet	mg/kg DM
Growing chickens	4	7
Laying hens	2.5	7
Broilers	8	7

Copper for other avian species (ducks, guinea fowl) are generally extrapolated from these recommendations.

In the case of turkey, requirements and recommendations from NRC (1994) and GfE (2003a) are generally higher than those for the Gallus species (Table 5).

Table 5. Some copper requirements (NRC) of and recommendations(GfE) for turkeys (mg/ kg) by scientific bodies

Authority	NRC (1994) (90% DM)	GfE (2003a) (DM)
Turkey poults 0-4 weeks	8	15
5-8 weeks	8	6
9-24 weeks	6	6

3.1.5. *Fish*

Fish, unlike most terrestrial animals, can absorb minerals from their aquatic environment as well as from their diets. However this source only covers part of the requirement of fish.

Different fish species have been shown to have varying dietary copper requirements, as given in Table 6. Requirement also appears to be dependent on stage in the life cycle. Berntssen *et al.* (1999b) concluded that the growth suppression observed in Atlantic salmon fry fed a diet containing 7.2 mg Cu/kg for 12 weeks, indicated a dietary copper requirement in juveniles slightly higher than that previously suggested in the literature.

Table 6. Copper requirements (NRC, 1993) of some cultured fish species

Fish species	(mg Cu /kg diet)
Atlantic salmon (*Salmo salar*)	5
Channel catfish (*Ictalurus punctatus*)	5
Rainbow trout (*Oncorhynchus mykiss*)	3
Common carp (*Cyprinus carpio*)	3

Recommendations are in the range of 12 to 15 mg / kg (Lorentzen *et al.*, 1998).

3.2. Interactions (see also SCAN opinion on zinc; SCAN 2003)

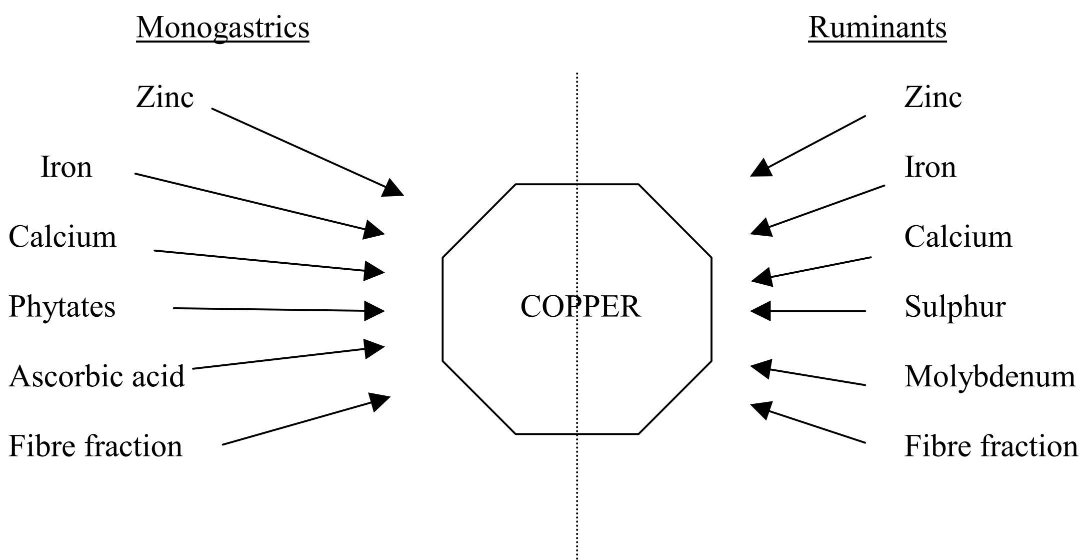
In monogastrics, there is an antagonistic interaction between copper and elements such as zinc and iron. High dietary zinc induces copper deficiency and *vice versa* as both elements compete for absorption. High calcium intake is reported to reduce zinc availability, whereby the possibility of copper toxicity in pigs fed high copper diets is enhanced (Davis and Mertz, 1987). In pigs, high levels of dietary copper decreased iron storage in the liver (Bradley *et al.*, 1983) and could lead to anaemia in the absence of adequate iron supplementation (Dove and Haydon, 1991). The addition of high levels of copper in pig diets (175 mg/kg) has been shown to significantly increase the retention of cadmium in kidneys, liver and muscle (Rothe *et al.*, 1994).

Copper naturally present in feedingstuffs is generally covalently bound to phytic acid (myoinositol 1,2,3,4,5,6-hexakis phosphate) forming stable complexes called phytates. Its availability for monogastric animals is therefore low. On the other hand, the use of phytase in animal nutrition, in order to improve phosphorus utilisation, can as a side effect enhance copper absorption (Pallauf *et al.*, 1992; Adeola, 1995 Adeola *et al.*, 1995; Pallauf and Rimbach, 1997 and Gebert *et al.*, 1999). In ruminants, phytates are degraded by the rumen microflora, and the copper / phytate interaction is therefore not relevant.

Molybdenum and sulphur are the two most important dietary factors that reduce copper absorption in ruminants (Simpson *et al.*, 1981; Suttle, 1991). Despite of sufficient dietary copper, deficiency may occur when the copper/ molybdenum ratio reaches 3/1 or when more than 2.5 g of sulphur /kg dry matter are present in the diet.

Diets containing more than 350 mg Fe/kg dry matter can also markedly inhibit copper utilisation by ruminants. Higher iron levels decreased significantly liver copper concentration (Grün *et al.*, 1978). Diets based on silage or winter herbage contaminated with soil can sometimes contain more than 350 mg Fe /kg. Copper deficiency can develop when the ratio of Fe/Cu is higher than 50 to 100/1.

Figure 1: Illustration of some interactions with copper, for monogastrics and for ruminants, respectively



3.3. Consequences of excess of copper

Deficit and excess of copper can occur because of copper content in the feed but also due to a number of other factors that impact on the bioavailability of copper, as mentioned in 3.2. This situation can have serious consequences for the health of animals.

Species sensitivity to copper varies. In particular, toxic levels can be reached rapidly in sheeps which are known to be sensitive animals, followed by non ruminant calves. Pigs and poultry are the most tolerant animals. In the case of fish, induction of apoptosis, cell proliferation and metallothionein was shown in the intestine of Atlantic salmon (*Salmo salar*) exposed to a relatively low dietary concentration of 34 mg/kg for 4 weeks (Berntssen *et al.*, 1999a), whereas other studies did not show any growth suppression at dietary copper concentrations up to 350-691 mg/kg on rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (Lanno *et al*., 1985; Julshamn *et al*., 1988; Miller *et al*., 1993; Mount *et al*., 1994; Berntssen *et al*., 1999a).

Copper is stored in some target organs such as liver and kidney. High amount of copper in liver (concentration above 1000 mg /kg DM liver) can lead to the sudden death of sensitive animals (Zervas *et al.*, 1990).

Excess of copper leads to the reduction in the number of erythrocytes and consequently to macrocytic anaemia, in particular in young animals naturally deprived of iron (Lillie *et al.*, 1977; Bremner, 1998;). Excess of dietary copper increases intracellular copper, leading to hepatic cell lysis and release of cell content in the liver, causing jaundice and ascites.

Finally, long term excess of dietary copper generally reduces performance of all animals. A reduction in feed intake results in a decrease in growth and in an increase in the mortality. An elevation of the level of plasma copper and iron has also been described. A decrease in the weight of testes and accessory sex organs, a reduction in the weight of lymphoid organs such as thymus have also been observed (Underwood and Suttle, 1999).

Increase of deposition of cadmium in the kidney and liver was reported in pigs fed up to a slaughter weight of 80 kg with 175 mg copper/kg feedingstuff (Rothe *et al.*, 1994).

The use of high copper in swine and poultry nutrition has been shown to cause accidental copper poisoning in more sensitive animals, sheep grazing on pasture fertilised with swine manure (Kerr and McGavin, 1991) or cattle fed litter of copper treated poultry (Tokarnia *et al*., 2000).

3.4. Sources of copper

3.4.1. Common levels of copper in some feed materials

An indication of the copper content of the most common feed materials, established by different institutes in some Member States, is summarised in the Table 7 below.

Table 7: Copper in some feed materials (mg/kg DM)

Feed materials	Range of copper content	Feed materials	Range of copper content
Alfalfa hay	7.3	Rapeseed, solv. Extr.	5-7
Alfalfa meal CP = 12 20 %	6-7	Rapeseed whole	7
Barley	3.5-7	Rye	4-6
Barley dist. grains	37	Skimmed milk	0.9-6.5
Brewer's grain	20-22	Sorghum	2.7-10
Brewer's yeast	25-57	Soybean meal 44	14-20
Citrus pulp dried	4.3-6	Soybean meal 50	15-18
Cotton seed	14	Soybean whole	12-15
Cotton, dehulled solvent.	3.2-18	Straw Barley	1.7-5
Fish meal	5.6-10	Straw Wheat	7
Grass hay	9.0	Sugar beet pulps	10
Horse beans	10.7-13	Sugar molasses	8.4-12
Linseed	12-16	Sunflower	25
Linseed solv.extr.	18	Sunflower Dehulled	28
Lupins	6	Torula yeast	15
Maize	1.9-3.3	Triticale	3.7-10
Maize dist. grains	45-75	Wheat bran	10-30
Maize dist. sol. dehyd.	17-72	Wheat hard, soft	4.2-8
Maize germ meal	7-12	Wheat middlings	5.8-9
Maize gluten feed, meal	6-31	Wheat middlings	12-13
Maize silage	7.6	Whole milk powder	1.3
Oats	2.8-5		

INRA= Institut National de la Recherche Agronomique (France),
DLG= Deutsche Landwirtschafts-Gesellschaft (Germany),
ACV= Afnemers Controle op Veevoeder (The Netherlands),
ADAS= Agricultural Development and Advisory Service (UK),
NRC = National Research Council (USA)

The availability of copper present in different feeds is not well known. The absorption is higher than 50 % for the milk replacer in young calves and less than 5 % in growing ruminants mainly fed with roughages (Pfeffer and Flachowsky, 2002).

3.4.2. *Copper supplementation, availability of different copper sources*

Because of the low copper content in some feedingstuffs compared with the requirements and recommendations (see Tables 1-6) and varying bioavailability, copper supplementation (see annex) is necessary for most species. In the light of the requirements identified above and considering the specific animal sensitivity, the current level of copper supplementation could/should be lowered to better reflect the real animal physiological needs and tolerance. However, adaptation should consider the natural level of copper present in feedingstuffs and all factors impacting on copper availability and an appropriate safety margin should therefore be included in the levels fixed for this trace element.

Numerous copper compounds have been experienced as copper supplements, particularly in the diet of pigs and piglets (Jondreville *et al.*, 2002).

The bioavailability of different sources of copper, mostly measured as liver storage was compared to that of sulphate in broilers, pig and ruminants (Table 8).

Table 8 Bioavailability relative to copper sulphate (%) of different sources of copper in broilers, pigs and ruminants, mostly measured as liver storage (Jongbloed *et al.*, 2002).

Species	Broiler	Pig	Ruminants
Sulphate	100 (5)	100 (11)	100 (11)
Carbonate	64 (3)	100 (3)	93 (2)
Oxide	24 (4)	74 (4)	76 (3)
Methionine	91(1)	100 (1)	-
Lysine	100 (4)	94 (3)	104 (6)

( ) Number of studies

4. INTEREST OF COPPER AS GROWTH PROMOTER IN PIGS AND CALVES

Several investigations have shown that the addition of copper to the diets of pigs increases their growth performance. This positive effect on growth seems to be dependent on a simultaneous increase in feed intake. The mechanisms involved remain not well understood (Zhou *et al.,* 1994a).

4.1. Effect on the gastro-intestinal flora and interactions with other feed additives

4.1.1. Effect on the gastro-intestinal flora

Several observations revealed that levels of copper sulphate incorporated in diet modified quantitatively some Gram positive bacterial populations of the gut as demonstrated for *Streptococcus* spp. These results concerning the reduction of the *Streptococcus* spp. populations in the gut are in agreement with the observations of Dunning and Marquis (1998) that streptococci are susceptible to copper under anaerobic conditions while most lactobacilli and *E. coli* are insensitive.

Evidence that copper produces a growth promoting effect through the microbial gut flora is supported by the results of Shurson *et al.* (1990), who observed a positive effect of high concentration (283 ppm) of copper in the diet on the daily growth rate and feed conversion rate in conventional pigs and a negative effect in germ-free pigs. On the other hand, a similar growth-promoting effect was also obtained when copper histidinate or histidine solution, simulating absorption rates in pigs fed 250 ppm dietary copper, were administered by intravenous injection and thus bypass the gastrointestinal tract (Zhou *et al.,* 1994b).

No effect on the digestibility of dietary nitrogen has been observed (Luo and Dove, 1996 ; Roof and Mahan, 1982).

4.1.2. *Bacterial resistance to copper and to antibiotics*

Until several years, copper resistant bacterial strains belonging to various bacterial species have been isolated from environmental sources. For toxic metal ions, including copper, the most resistant systems are based on the energy-dependent efflux of toxic ions (Silver, 1996).

Recently, a strain of *Enterococcus faecium* harbouring a conjugative plasmid conferring acquired copper resistance was isolated from a pig. The trcB resistance gene encodes a putative protein belonging to the ATPase family responsible of the efflux. Strains containing the trcB gene are able to grow in vitro in the presence of 28 mM of $CuSO_4$ versus 4 mM for the susceptible strains (Hasman and Aarestrup, 2002). Copper resistance was correlated to macrolide and glycopeptide resistance and the trcB gene was located on the same conjugative plasmid as the antibiotic resistant genes (Hasman and Aarestrup, 2002).

This copper resistance gene was also found in copper resistant strains of Enterococci isolated from pigs reared in Denmark, Spain and Sweden (Aarestrup *et al*. 2002).

The minimum concentration of copper necessary to select resistant bacterial strains is actually unknown.

4.1.3. *Interactions with other feed additives*

Interactions between copper and other feed additives can occur, as previously observed with carbadox, a growth promoter no longer authorised in the European Union. Carbadox administered alone in the feed of pigs reduced the percentages of intestinal *Escherichia coli* resistant to therapeutic antibiotics but the simultaneous administration of copper sulphate suppressed this effect and increased the percentages of *E. coli* resistant to antibiotics (Siebert, 1982).

Due to the antimicrobial activity of copper on some Gram positive bacterial species, strains of micro-organisms claiming authorisation for use as additives in animal feed must demonstrate compatibility with copper.

4.2. Effect on growth

With the exception of pigs and broilers (and probably laying hens), copper fed at high levels is not known to have any practical growth promoting effect in farm animals or in fish.

4.2.1. *Effect on calves*

High dietary levels of copper have never been used in calves for growth promotion purposes. The high ability of calves to absorb copper together with the high copper storage affinity of their liver results in reaching quickly toxic levels. Thus, copper poisoning is already described at 30 mg/kg of dry matter in milk replacer for this animal category (ARC, 1980; Jilg *et al*., 1997). This toxic level is below the current maximum permitted level in feed.

4.2.2. *Effect on pigs*

In pigs, copper added at substantially higher levels than the animal requirements has been shown to have growth promoting effects (Braude 1948; Barber *et al*., 1965). A considerable number of experiments has been performed on animals of different body weight, different ages and different levels with various results. In a review of the effect of an extra dietary supply of 250 mg/kg in feeds on an air dried basis from 25 kg up to slaughter, Braude 1967, recorded 83 such tests from all over the world involving a total of 1215 pigs in each treatment (0 versus 250 mg/kg supplement). Weighed for the number of growing-finishing pigs participating in each test, the improvement in daily body weight gain was on average +8.1 % (from -12 % to + 25 %). The efficiency of feed utilisation was in average 5.4 % (from -5.2 to + 12.6 %). In only three tests out of 83, there was no improvement in daily gain and in 5 tests, no improvement in the gain per feed ratio.

In the experiments, the most frequently used copper source was copper sulphate. The majority of the experiments has been conducted on only two diets, the control containing 15/30 mg/kg total copper and the test supplied with 250 mg/kg extra copper. In the absence of a sufficient number of studies done with the levels of copper inclusion in the feed currently allowed in the European Union, effects of copper on pigs growth used at 175 mg/kg feed will have to be extrapolated from regression equations.

The following approaches were used:

– Plotting graphically selected data according to the response of performance of animals to graded levels of supply and expressing the number of experiments demonstrating significant differences at least at $P < 0.05$;

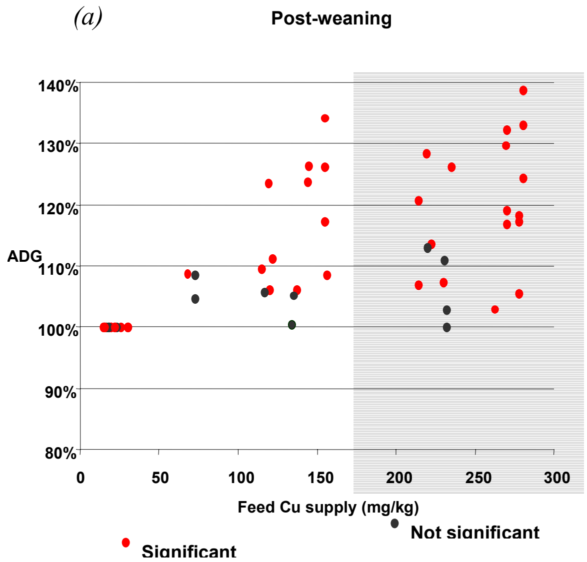
– Comparing in an analysis of variance the average performance (average daily body weight gain, ADG, and feed conversion, FC = feed intake/ADG) only in case of piglets from weaning to *ca* 25 kg body weight, splitting the experimental data into 6 different ranges of levels of supply 15-30mg/kg ; 68-73mg/kg ; 115-123mg/kg ; 140-162 mg/kg ; 214-235mg/kg ; 262-280mg/kg diet for which a sufficient number of data was available. The analysis compared the performances observed for the different levels to those of the control group, as well as the levels tested between them, on a step by step basis, using the method of contrasts.

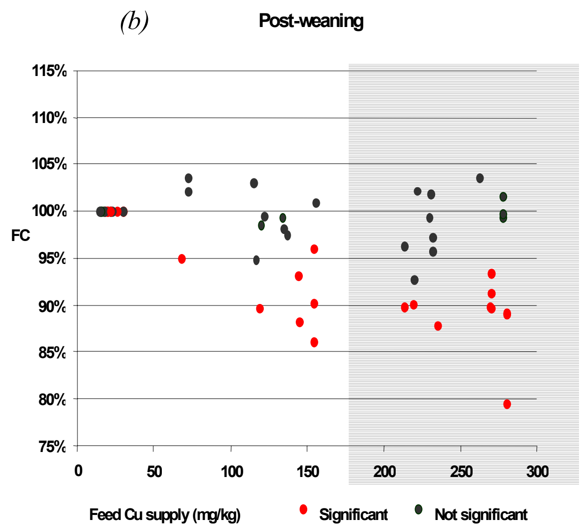
– Calculating the linear regression equation between the copper supplementation (x) and the percentage improvement of the average daily body weight gain (y) and feed conversion (y) respectively

4.2.2.1. Post weaning period

In the post weaning period up to 25 kg of body weight, results of 22 experiments have been considered: one experiment compared 4 levels of supply, 12 experiments 3 levels of supply and 9 experiments considered only two levels of supplementation (Original data are extracted from Armstrong *et al*., 2000; Stansbury *et al*., 1990; Cromwell *et al*., 1998; Cromwell *et al.,* 1989; Coffey *et al*., 1994; Roof and Mahan, 1982; Dove and Haydon, 1992; Bunch *et al* 1961 and 1965; Zhou *et al.,* 1994ab ; Apgar *et al*.,1995) . Piglets were weaned at a minimum of 12/14 day of age up to 35 days. The maximum range was 3.6 kg (initial weight) to 30 kg (final weight) of body weight for a period of treatment ranging from 14 to 63 days.

Figure 2 Effect of increasing the level of total copper in the weaning diet of piglets up to 25 kg body weight on average daily body weight gain (a) and on feed conversion (b).





The grey areas indicate the levels of copper inclusion higher than 175 mg/kg feed

Regression equations for the post weaning period (% means percentage units)

ADG (% of improvement):
y = 0. 08x (as mg/kg of supply) + 0.9934
(P<0.01) from 15 to 280 mg/kg;
y=0.12x + 0.9737
(P<0.01) from 15 to 162 mg/kg

FC (% of decrease)
y = - 0.03x (as mg/kg of supply) + 1.0047
(P< 0.05) from 15 to 280 mg/kg
y= -0.0367x + 1.01
(P<0.05) from 15 to 162 mg/kg.

Out of 22 experiments in which increasing amounts of copper ranging from ca 20 to ca 280 mg/kg total copper in the diet of piglets have been used, 15 demonstrated a significant ($P<0.05$) positive effect on growth rate (Figure 2a). Compared to that, only 8 showed a significant effect ($P<0.05$) for the improvement of the feed conversion.

Because of a large variation in experimental conditions, in particular in the initial body weight and experimental design throughout time, it was impossible to go further in the statistical analysis. In the absence of a significant curvilinear regression, a linear increase of the ADG has been observed with the increase in the level of dietary copper supply. The regression equation between the percentage units of improvement of ADG (y) and the level of total copper (x, mg/kg), calculated by including all average values from each of the 22 experiments, was highly significant ($P<0.01$). The regression between the percentage units of improvement of the FC and the level of supply was significant only with $P<0.05$ because of more variable responses of the animals to the level of supply between experiments (Figure 2b).

All the differences compared by an analysis of variance for the average daily body weight gain were statistically significant. The maximum stimulation of the growth of piglets was observed for the maximum level of supply, i.e. 262 to 280 mg/kg of total dietary copper ($P<0.001$). The addition of copper significantly improved the feed conversion up to 63 to73 mg total dietary copper /kg diet ($P<0.02$), no further improvement occurred at higher copper levels. These results are in full agreement with data issued from the regression equation.

It can be concluded for the post weaning period that the constant improvement (no breakpoint) amounted to an increase of 0.12 % in daily body weight gain for each 1 mg/kg of dietary copper supply between ca 10 to 35 mg/kg and 140 to 162 mg/kg diet. Similarly the decrease in the feed conversion amounted to 0.037 percentage unit for each increase of 1 mg/kg of dietary supply, within a restricted range of dose.

4.2.2.2. The growing pig (25 to *ca.* 60 kg body weight)

Results of 20 experiments published in the scientific literature have been analysed with the methods described in 4.2.2.

Five of the twenty studies demonstrated a significant overall improvement in the average daily body weight gain ($P<0.05$) consecutive to copper supplementation whereas two other studies, using levels of 150 and 250 mg/kg of total copper respectively, showed a significant detrimental effect (Figure 3a).

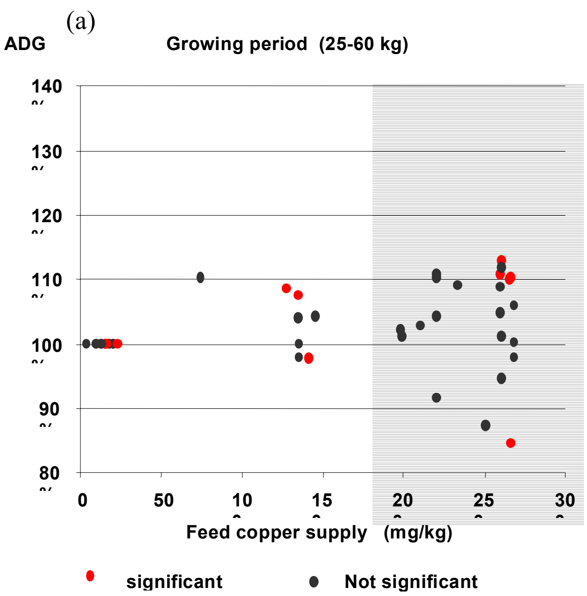
In addition, only 4 experiments showed a significant effect for the improvement of feed conversion, compared to the non-supplemented diets (Figure 3b).

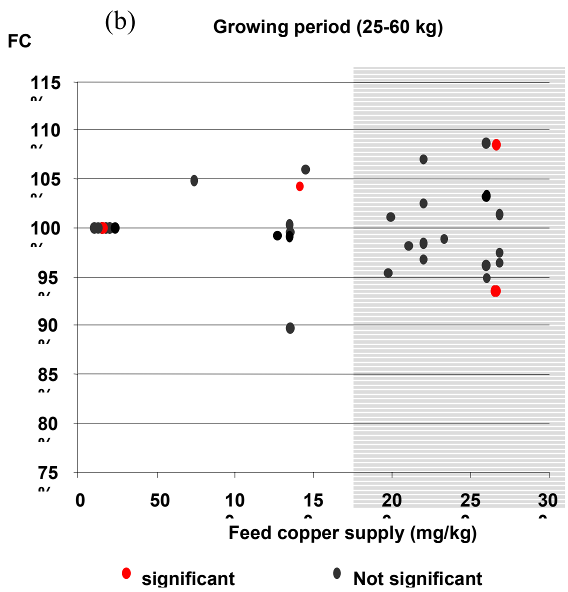
No significant correlation (P>0.05) could be found between the level of copper supply and the improvement in average daily body weight gain or the feed conversion.

As a consequence, it is not possible to conclude on the growth promoting effect of copper when fed to growing pigs at any levels above those covering the animal requirements.

Figure 3 Effect of increasing levels of copper in the diet of growing pigs (25 to *ca* 60kg of body weight) on average daily body weight gain (3a) and feed conversion (3b).

The grey areas indicate the levels of copper inclusion higher than 175 mg/kg feed





4.2.2.3. The growing finishing pig

Results of 17 experiments have been analysed in the same way for animals supplied the same level of dietary copper during the whole period of growing finishing *ca.* 25 and 100kg body weight.

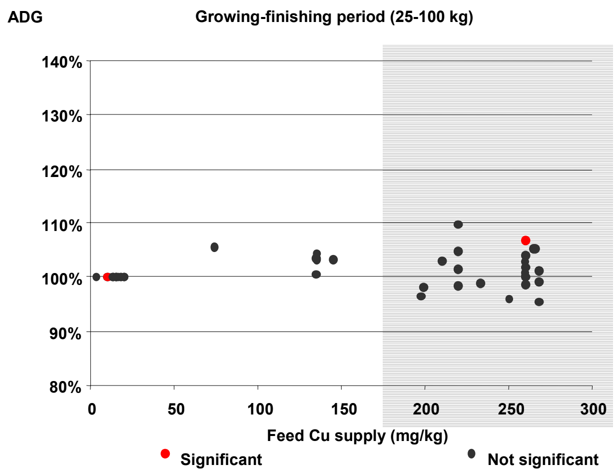
Out of these 17 experiments, none of them demonstrated a significant effect of the extra copper supply in the average daily body weight gain of animals. (Figure 4a)

Two of them only demonstrated a significant improvement of the feed conversion (Figure 4b).

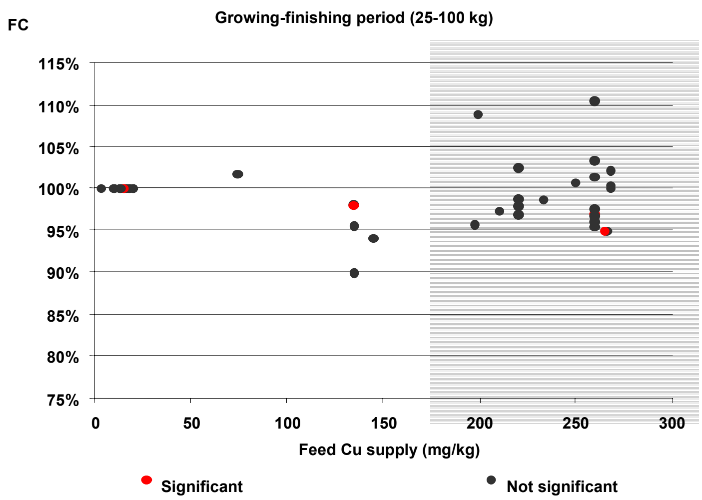
Figure 4 Effect of increasing levels of copper in the diet of growing finishing pigs (25 to *ca.* 100 kg body weight) on average daily body weight gain (4a) and feed conversion (4b).

The grey areas indicate the levels of copper inclusion higher than 175 mg/kg feed

(a)



(b)



As for the growing period, the dose-response effect of copper supplementation of finishing pig diet is not significant (P>0.05), either for the average daily body weight gain, or for the feed conversion.

4.2.2.4. Conclusion

When used at levels higher than those covering animal requirements and up to 175 mg and further to 250 mg/kg feed, copper impacts on the growth and feed conversion of young pigs, from weaning to a body weight of approximately 25 kg (8 to 10 weeks of age).

No significant effect is demonstrated in growing and finishing pigs.

4.2.3. Effect on poultry

Copper between 125 and 250 mg/kg feedingstuff is also effective in promoting growth of broiler chicken (Pesti and Bakalli, 1996; Ewing *et al.*, 1998; Skrivan *et al.*, 2000). In a study by Pesti and Bakalli (1996), copper (125-250mg/kg feedingstuff) has been shown to enhance the laying performance of hens and reduce egg cholesterol. There is obviously no comparative effect of copper on turkeys.

5. IMPACT OF THE USE OF COPPER IN ANIMAL DIETS ON CONSUMER SAFETY

5.1. Copper levels in animal tissues

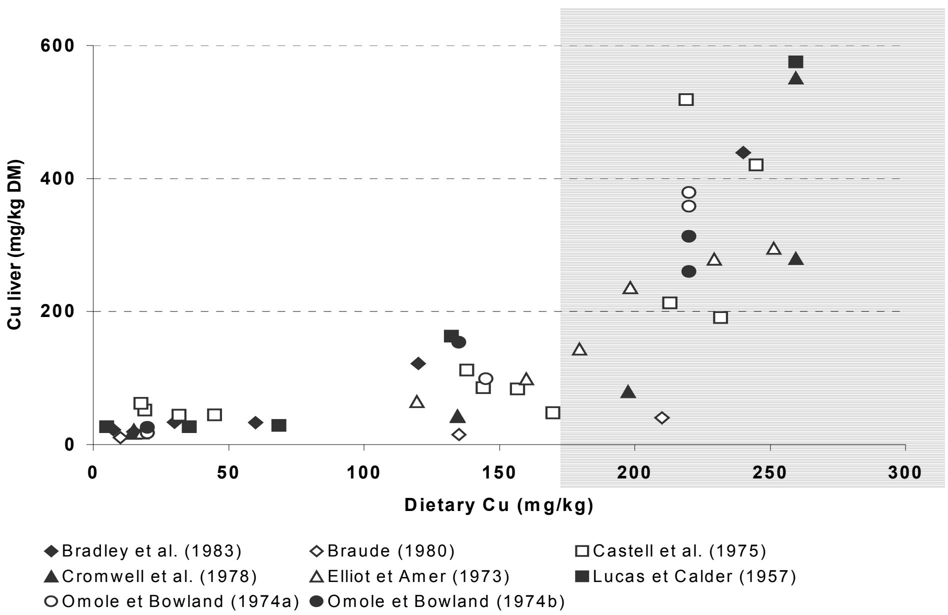
In the absence of appropriate dose dependent studies, estimations of copper levels in animal edible tissues were derived from experiments which were not conducted with this aim. The level of other trace elements (zinc and iron) interacting with copper is also highly variable. Because of the number and the consistency of the data issued from the scientific literature, the statistical analysis has been focused on results observed in experiments conducted with copper sulphate.

The distribution of total copper in the body varies with species, age and copper status of the animal. In general, levels in new-born animals are maintained throughout the suckling period, followed by a steady fall during growth to the time when adult values are reached (Davis and Mertz, 1987). The main target organ for copper is liver. Other edible tissues containing high concentrations of copper are heart, brain and kidney. Lower levels are found in muscle. Liver and kidney copper concentrations are related to the dietary intake whereas muscles are less affected. Copper level values are in general expressed on a dry matter basis in organs and tissues. Corresponding values for fresh weight could represent about one third of the values observed in dry matter (25% for muscle and kidney and 30% for liver).

5.1.1. Pigs

Data from different studies published on the copper storage in the liver of pigs for fattening at slaughter are summarised in Figure 5.

Figure 5. Effect of the level of dietary copper (x) (sulphate) supply to growing and fattening pigs on the liver copper content (y) in the pig at slaughter. ($y = 0.00005\ x^3 - 0.0105\ x^2 + 0.944\ x + 14.1$; $P < 0.001$)



There is no significant increase in the copper storage in liver up to 75 mg/kg of total copper in the diet supplied until slaughter**.** Liver copper starts to increase dramatically when dietary copper exceeds 120 mg/ kg feed.

Similar high values for copper concentrations in the liver of pigs fed diets supplemented with high levels of inorganic (sulphate) or organic copper have been observed in more recent experiments (Coffey *et al.* 1994; Apgar *et al.*, 1996; Cromwell *et al.*, 1989 and 1998).

The copper content in kidney reflects the dietary copper supply to a minor extent than liver. For copper supply between 200 and 250 mg/kg feed, kidney copper concentrations were between 40 and 100 mg/kg kidney dry matter (Omole and Bowland, 1974 a and b; Cromwell *et al.*, 1978, Bradley *et al.*, 1983,)

Copper content in muscle ranged between 2 and 5 mg/kg /DM for dietary supplies ranging from 180 to 240 mg/kg in the diet (Castell and Bowland, 1968; Meyer *et al.*, 1977; Madsen and Mortensen, 1982; Bradley *et al.*, 1983).

These levels were observed with administration of copper to the animals until slaughter. If copper is withdrawn, levels are affected as shown in Table 9.

Table 9 Effects of withdrawal of copper supplementation on liver and tissue storage in the growing pig

	Total dietary supply mg/kg		Body weight in kg at		Copper mg/kg DM in			
Ref.	Growing	Finishing	Withdrawal	Slaughter	Liver	Kidney	Muscle	Fat
(1)	22	22	-	91	22	-	6	-
	250	22	57	91	51	-	9	-
	250	250	-	91	278	-	7	-
(2)	12	12	-	92	19	-	-	-
	250	250	-	92	452	-	-	-
	250	18	46	92	48	-	-	-
	250	18	69	92	281	-	-	-
(3)	35	35	-	90	42	-	2.8	-
	150	150	-	90	67	-	2.8	-
	150	35	68	90	45	-	2.7	-
	255	255	-	90	238	-	2.8	-
	255	35	68	90	80	-	4.5	-
	255	35	79	90	79	-	3.0	-
(4)	14.4	10.2	-	100	56	34	3.9	0.7
	175	182	-	100	256	56	3.5	0.8
	175	17	80	100	117	-	-	-
	15	17	-	100	91	27	4.3	1.7
	198	216	-	100	577	35	5.4	1.8

(1) Bunch et al. (1963)
(2) Elliot and Amer (1973)
(3) Castell *et al*. (1975)
(4) Meyer *et al*. (1977)

High levels of copper in the liver of the pig at slaughter are prevented by a withdrawal of the extra supply of the trace element used in the feed during an appropriate period prior to slaughter (Lillie *et al.* 1977). Thus, if a continuous supply of 250 mg/kg until slaughter generates high levels of storage in selected organs, the withdrawal of the supply since 50 to 60 kg of body weight leads to similar levels as those observed when animals are fed only 20 mg/kg all along their life (Table 9). A progressive clearance from the organ and a dilution effect due to the development in size and weight of the liver explain these results.

It should be underlined that piglets fed high levels of copper (240 to 283 mg/kg feed) from weaning to 7 to 9 weeks of age store copper up to 505 to 537 mg/kg dry matter in their liver and up to 130 mg/kg dry matter in their kidneys (Gipp *et al.*, 1973b; Hedges *et al.*, 1973; Shurson *et al.*, 1990).

5.1.2. Cattle

The copper retention in growing cattle is estimated to 0.5 to 1.3 copper / kg body weight gain (ARC, 1980; CSIRO, 1990; Kirchgessner *et al*., 1994; Underwood and Suttle, 1999). As for pigs, dietary copper supplementation markedly enhance copper retention in the liver and to a lesser extent in the other edible tissues (Simpson *et al*. 1981). Twenty eight to 48 mg copper /kg diet dry matter increased the copper liver content from 63 (8 mg/kg feed) to 290 to 380 mg/kg dry matter (Engle *et al.*, 2000). Muscle content remained independent from copper supply and ranged between 3 and 4.2 mg/kg dry matter. These findings were in agreement with a previous experiment done by Bohman *et al.* (1984).

5.1.3. Calves

Values for the level of copper in the liver of calves are divergent between authors. High values of 910 mg/kg dry matter have been found when milk substitute was supplied with 15 mg copper/kg dry matter (Jilg *et al.*, 1997; Jenkins and Hidiroglou, 1989,). The level in muscles remained low, 5 to 6 mg/kg dry matter.

5.1.4. Small ruminants

The concentration of copper in the liver of the sheep is strongly correlated to copper intake because of the high affinity of sheep liver to copper, which is greater than in other ruminant species (Table 10). The level of copper storage in the kidney and even in the muscle is increased with the level in the diet. The goat species is proportionally less efficient in body storage of copper. In particular, the level of copper in muscle remained low whatever the level in the diet.

Table 10: Effect of high doses of dietary copper on tissue storage in sheep and goats

	Sheep	Goats		
Dietary copper mg/kg DM	7	8	38	12
Liver copper mg/kg DM	300	100	350	315
Kidney "	15	8	20	-
Muscle "	5	4	8	1.8
Author	1	1	1	2

1 Zervas *et al*., 1990*,* 2.Solaiman *et al*., 2001

5.1.5. Poultry

Chickens are characterised by a high level of copper clearance (Aoyagi *et al*., 1993). This explains why the level of copper in the liver and kidney of birds, ranging from 17 to 23 mg/kg dry matter and from 16 to 24 mg/kg dry matter respectively, is only marginally affected by the level of dietary supply. Copper levels in muscle remain very low (0.2 to 1.6 mg/kg) (Jackson 1977; Stevenson and Jackson, 1981; Ledoux *et al*., 1991).

The copper content in eggs is low ranging on average from 0.66 mg / kg for a dry matter content of 25.6 % (Kirkpatrick and Coffin, 1975) to 1.1 mg/kg (Naber, 1979) and remains relatively constant over a wide range of dietary copper (Naber, 1979).

5.1.6. Fish

In fish, copper is primarily stored in the liver and levels are not dependent on the dietary levels within the range allowed in fish diet (Table 11). At feed copper concentrations below 109 mg/kg diet (Lorentzen et al., 1998; Berntssen *et al*., 1999a), the resulting content in muscle, liver and whole body suggests that copper homeostasis is maintained.

Table 11. Effect of graded levels of dietary copper in the diet of Atlantic salmon on tissue copper storage (mg/kg dry matter).

Tissue	Cu supply (mg/kg diet)		Exposure (weeks)	Reference
	3.5 to 7	34 to 37		
Kidney	3.5	4	4	(2)
Muscle	4.4		12	(3)
Liver	100	105	4	(2)
	109		12	(3)
Whole body	2	2	12	(1)
	2.2		12	(3)

Adapted from (1) Berntssen *et al*. (1999b), (2) Berntssen *et al*. (2000) and (3) Lorentzen *et al*. (1998).

Copper concentrations in the liver of lean fish, such as cod (*Gadus morhua*) and saith (*Gadus virens*), are more than one order of magnitude lower than in salmonids. Cod fed a commercial diet were found to have a liver copper concentration of 5.3 mg/kg dry matter (Lie *et al.*, 1989).

Increasing dietary copper concentration from 7 to 37 mg/kg dry matter leads to a significant reduction of copper retention in salmon, decreasing from 25% to 6% (Berntssen *et al*., 1999b).

5.2. Contribution to human exposure

Many western human diets are low in copper. They commonly provide 1.0 – 2.5 mg copper per day. An EU population reference intake of 1.1 mg/day was established in 1992 (SCF, 1993). New guidelines for recommended intakes have been recently published in the USA that recommend adult males and females should consume 0.9 mg copper/day (Institute of Medicine, Food and Nutrition Board, 2001, personnal communication). As many minerals, copper is also a toxic element and the EU Scientific Committee for Food has recommended a maximum level of 10-35 mg/person/day (SCF, 1993). A provisional maximum tolerable daily intake (PMTDI) of 0.05-0.5 mg/kg bw (3 to 30 mg per day for a 60 kg bw individual) has been proposed (WHO, 1982) and is still valid.

Food is the major source of copper intake. Plants are contaminated through aerial deposition (copper from mines, smelters and foundries, but also from burning of coal or incinerating municipal waste) but this contribution is negligible compared to copper absorbed from the soil. Considering data from the UK (MAFF, 1997), but also a compilation of data from the USA, Australia and The Netherlands (IPCS, 1998), copper contents of food commodities of plant origin (cereals, sugar and preserves, potatoes, vegetables and fruits) and animal origin (meat, fish, eggs and milk) are in the range of 0.1-2.4 mg/kg wet weight. Lower levels are encountered in oils/fats (0.05 mg/kg). The highest concentrations are found in increasing order in nuts, offal and shellfish (10 to 200 mg/kg). It has been found that copper intake in adults varies depending on the type of diet, ranging from 1-1.5 mg/day for omnivore diets to 2.1-3.9 mg/kg for vegetarian diets which indicates the prevalence of vegetables as copper contributors to the human exposure, at least for the Canadian consumer (Gibson, 1994). An evaluation of copper intake in several EU countries compiled from recent publications indicates a mean intake ranging from 1.0 to 2.0 mg/day with a highest 97.5 percentile of 4.2 mg/day (SCF, unpublished data), the intake of children being about half that of adults (IPCS, 1998). These values include copper amounts ingested from drinking water, usual values ranging from 0.05 to 0.2 mg/L for running water but which may reach 4 mg/L in standing water.

Considering copper contents in tissues of farm animals fed diets covering only the animal requirements (10 to 22 mg/kg feed) until slaughter at commercial body weight (Chapter 5.1), the following considerations can be drawn. For poultry, pig, cattle (steers) and fish, copper concentrations expressed as mg/kg dry matter ranged from 5 to about 100 in liver, 3.5 to 40 in kidney, 0.2 to 5.0 in muscle and 0.7 to 1.7 in fat. Higher values were found in sheep and calf liver (about 300 and 400 mg/kg, respectively, see Figure 5 and Tables 9 to 11).

The very limited data available concerning tissue contents in pigs that received until slaughter (100 kg bw) a copper diet containing 175 mg/kg feed (Table 9), indicate that liver contained 256 mg/kg dry matter, but that kidney and muscle concentrations were in the same range as those found in pigs fed copper non-supplemented feed. When considering the highest and most prolonged copper dosage allowed as feed additive in pig feed, i.e. 175 mg/kg until 4 months (about 60 kg bw), no data on tissue content are available. However, referring to the regression equation relating to dietary levels of copper and copper concentration in liver (Figure 5), it can be calculated that a 175 mg/kg feed supplementation until slaughter at 100 kg bw instead of 60 kg bw would lead to a residual value of about 120 mg/kg dry matter liver. Considering the incidence of withdrawal of supplementary copper on the decrease of liver storage (Table 9), it is clear that the dosage and conditions set for the use of copper as feed additive for pigs would lead to copper concentrations significantly lower in the liver, that would not exceed the range of values already identified in the case of pigs fed diets covering only the animal requirements, *i.e.* 40 to 100 mg/kg dry matter. It can be concluded that the copper status in pig tissues is not significantly modified by copper supplementation of feeds at levels in accordance with the current regulation on feed additives. On the basis of the consumption figures retained by the SCAN to evaluate the consumer exposure[1] which represent a worst case scenario, and considering copper concentration in tissues expressed as mg/kg fresh weight, the maximum copper load resulting from the consumption of pig would be 3.7 mg/person/day which represent about 12% of the PMTDI.

Data from surveys performed in Germany (standard food composition table) indicate a range of copper concentrations of 9 to 84, 1.7 to 7.9 and 0.4 to 0.9 mg/kg fresh weight for pig liver, kidney and muscle respectively (Souci *et al.*, 2000). That would lead, considering the highest values, to a consumer exposure of about 9 mg/person/day.

For piglets fed diets supplemented with the highest level of copper allowed (175 mg/kg) and slaughtered at 25 kg body weight without withdrawal period, no data on copper levels in tissues are available. A worst case scenario has been considered that refers to the tissue concentrations measured in animals supplemented the same dosage but until 100 kg body weight, *i.e.* 256, 56, 3.5 and 0.8 mg/kg dry matter for liver, kidney, muscle and fat, respectively (see Table 9). On the basis of the SCAN consumption figures, the copper load would reach 8.2 mg/person/day that represents 28% of the PMTDI.

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[1] Muscle: 300g, liver: 100g, kidney: 50g , fat: 50g in accordance with Council Directive 2001/79/EC.

For calf, the high contents of copper measured in the liver in experimental conditions (400 and 900 mg/kg dry matter, see *5.1.3*) would lead to a consumer exposure of 12 and 26 mg/person/day, representing 40% and 87% of the PMTDI, respectively. Data from surveys performed in Germany (standard food composition table) indicate a range of copper concentrations of 35 to 79 mg/kg fresh weight liver that would lead, considering the highest value, to a consumption load of 7.9 mg/person/day only (Souci et al., 2000).

Whole-body metabolism of copper in the human has shown that a 10-fold increase in dietary copper (in the range 0.8-8 mg/day) results in only twice as much copper absorbed (Turnlund, 1991). Moreover, biliary excretion represents the major and highly effective elimination pathway of endogeneous copper. Copper turnover is slow when dietary intake is low, and quick when intake is high. It appears that the regulatory mechanisms, especially of copper excretion, are very efficient, and that copper status indexes are resistant to change except under extreme dietary conditions (Turnlund, 1998). Consequently, chronic copper poisoning is very rare. Only disorders in homoeostatic mechanisms such as the hereditary copper metabolic disorders of Menkes disease and Wilson disease may result in deficiency or toxicity from exposure to copper at levels which are tolerated by the general population.

As a conclusion, copper supplementation of pig diet performed in accordance with the current legislation does not increase copper levels in tissues significantly when compared to the usual concentrations found in tissues from animals fed diets covering only the animal requirements. It does not therefore contribute to increase the exposure of the human consumer.
In the case of piglet (highly supplemented) and calf (having a high liver affinity for copper), the exposure of human consumers is increased, although remaining within the PMTDI.

6. EFFECT ON THE ENVIRONMENT

The use of copper in animal nutrition as dietary supplement contributes to the overall environment exposure to that trace element. Farm animal copper intake is almost entirely excreted and slurry is spread onto arable and grass lands, as common agricultural practice. In the case of aquaculture, copper comes from fish excreta but also from the supplementation itself as the feed is in direct contact with the aquatic environment.

The terms of reference limit the assessment to pigs and bovines. The Committee considered that these should be widened to include the impact of the use of copper in the different farm animal species as well as in fish.

Adequate models should be used to establish this impact.

In the particular case of fish, well-designed and suitable models are necessary to reflect the European production systems. One ton fish consumes circa 10 kg feed per day. Based on the current legislation, and considering that all copper consumed is released, 350 mg copper are released in the environment per day. This should be considered with different aquaculture production scenarios in Europe: pond, raceways, sea-cages, etc. and will not be further addressed in the present document.

For farm animals, the model is based on the calculation of the net quantities of copper finally added to the environment and implies therefore consideration of
- the copper quantity excreted by animals, based on their respective intake,
- the quantity spread on lands
- the disappearance due to crop uptake or transport within soil

Data on copper release from soil by crops or mobilisation are scarce. The available data would not allow consideration of crop sequence, influence of harvest and of climate as well as the degree of erosion. Therefore the worst case scenario was chosen by the Committee. All data for the calculation of element emissions from terrestrial animals are reported in annex.

6.1. Methodology

For the calculation, the values of copper concentration in feed authorised under Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs[2] were used i.e. 35 mg Cu/kg feed, for all animals, with the exception of the following animal categories or species:
- pigs up to 4 months of age: 175 mg Cu/kg feed
- ovines: 15 mg Cu/kg feed
- calves: 50 mg Cu/kg feed

These values do not consider the dry matter content of feedingstuffs. Considering a dry matter content of 88% of the feedingstuffs, the following corrected values have been used for the calculations:

- for all species, 39.7 mg/kg dry matter, except for
- pigs up to 4 months of age: 198.9 mg Cu/kg dry matter feed
- ovines: 17.0 mg Cu/kg dry matter feed
- calves: 56.8 mg Cu/kg dry matter feed

As copper is almost entirely excreted by the animals, although variations can occur and although excretion varies over time, all copper ingested by animals has been considered excreted and found in manure.

For soils, the assessment of impact of copper application has been calculated on the basis of copper/nitrogen ratio in manure and is based on the Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources ("European nitrate directive")[3] which establishes the nitrogen amount

[2] E.C.O.J. n° L 270 of 14/12/1970, page 1

[3] E.C.O.J. n° L 375 of 31/12/1991 p.1

applicable on sensitive agricultural soil (170 kg N/ha y). The copper/nitrogen ratio in manure has been calculated as the ratio between the total copper and the total nitrogen excreted in the life cycle period taken into account for each animal and assuming a nitrogen loss by evaporation from the manure of 25%, mainly due to ammonia volatilisation. For non sensitive soils, the maximum nitrogen amount allowed in European Member States has been used (France: 350 kg N/ha y) (Spaepen *et al.*, 1997)[4]. The soil concentration is calculated after one-year application, for the top soil layer (5 cm thick), assuming a default soil density of 1.5 g/cm³. For long term application of manure (20 years), maximum accumulation of copper in soil are referred to a depth of 20 cm of soil (the minimum layer involved in tillage) assumed as a more realistic scenario.

Calculated values for annual application on soil and resulting soil concentrations were compared with limit values for copper fixed in Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture[5].

6.2. Results

On the basis of the diets established by SCAN (see table under chapter 10 hereafter), the annual load of copper spread onto soil could be calculated.

Calculations are presented for different animal categories in chapter 11 at the end of the document).

The table 12 hereafter summarises the results for copper annual load and calculates the resulting increase in copper concentration of soils on the basis of the amount of nitrogen excreted by the animals, considering the levels of nitrogen allowed per hectare respectively on vulnerable and non vulnerable areas.

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4 In the Spaepen et al. paper a value of 600 kg N/ha is reported for Italy. This value, quoted as “Personal communication”, is not reliable. A maximum value of 350 kg N/ha is recommended for the Po valley by the Po Basin National Authority and lower value are recommended for other Italian agricultural areas.

5 E.C.O.J. n° L 181 of 04/07/1986 p.6

Table 12. Values of copper annual load and resulting metal concentrations in soil after one year (short term) and 20 years (long term) application of animal manure for different animals.

Calculations are performed for two levels of nitrogen application on soil: 170 and 350 kg/ha/y, respectively for vulnerable and not vulnerable areas.

Animals	Calculation based on application on soil of two levels of nitrogen : 170 and 350 kg/ha/y, respectively for vulnerable and non vulnerable areas					
	170	350	170	350	170	350
	Copper annual load on soil in g/ha/y		Increase of copper concentration in soil, in mg/kg,			
			Over one year		Over 20 years	
Veal calves	736	1516	0.98	2.02	4.91	10.11
Replacement calves	573	1179	0.76	1.57	3.82	7.86
Fattening steers	535	1101	0.71	1.47	3.56	7.34
Replacement heifers	532	1095	0.71	1.46	3.55	7.30
Dairy cow	524	1079	0.70	1.44	3.49	7.19
Piglets	2434	5011	3.25	6.68	16.23	33.41
Fattening pigs	481	990	0.64	1.32	3.21	6.60
Sows	305	628	0.41	0.84	2.03	4.19
Sheep-goats	222	458	0.30	0.61	1.48	3.05
Fattening lambs[1]	310	638	0.41	0.85	2.07	4.25
Broilers 5 weeks	641	1319	0.85	1.76	4.27	8.79
Broilers 8 weeks	579	1192	0.77	1.59	3.86	7.94
Replacement pullets	490	1009	0.65	1.34	3.27	6.72
Layers	524	1079	0.70	1.44	3.49	7.19
Turkey poults[2]	425	875	0.57	1.17	2.83	5.83
Turkey female[3]	462	951	0.62	1.27	3.08	6.34
Turkey male[4]	512	1054	0.68	1.41	3.41	7.03

[1] 2 runs/year;
[2] average of both sexes, middle and heavy breeds, age at slaughter: 11 weeks;
[3] females, age at slaughter: 16 weeks;
[4] males, age at slaughter: 22 weeks

The Directive 86/278/EEC fixed the following limit values for copper:

- amount which may be added annually to agricultural land, based on a 10 year average: 12 kg/ha/y
- concentration in soil: 50-140 mg/kg DM.

The Table 13 hereafter shows how much of the total load of copper allowed is consumed for the different animal species and categories, respectively for vulnerable areas and non sensitive soils. Results are expressed in percentage of the maximum amount that may be added annually to agricultural land, in compliance with the current legislation. In addition, two simulations are proposed to anticipate possible reduction of the maximum load of copper on soil. Two figures have been arbitrarily chosen: a fifth and a tenth of the current limit *i.e.* respectively 2.4 kg and 1.2 kg per hectare and per year.

Table 13: Contribution in percentage of the maximum copper load allowed in soil to the contamination of lands, for vulnerable and non vulnerable areas, with copper. The contribution is evaluated against the current legal limit and two alternative reduced limits (20% and 10% of the existing limit)

			Legal situation		Alternative scenario with lower limit			
	Vulnerable areas	Non vulnerable areas	Based on current maximum copper soil concentration		Based on a fifth of the current maximum copper soil concentration		Based on a tenth of the current maximum copper soil concentration	
	Copper applied (g/ha and year)		Percentage of copper allowed per hectare					
			Max. 12 kg		Max. 2.4 kg		Max. 1.2 kg	
Animals	170 kg N/ha	350 kg N/ha	170 kg N/ha	350 kg N/ha	170 kg N/ha	350 kg N/ha	170 kg N/ha	350 kg N/ha
Veal calves	736	1516	6 %	13 %	31 %	63 %	61 %	**126 %**
Replacement calves	573	1179	5 %	10 %	24 %	49 %	48 %	98 %
Fattening steers	535	1101	4 %	9 %	22 %	46 %	45 %	92 %
Replacement heifers	532	1095	4 %	9 %	22 %	46 %	44 %	91 %
Dairy cow	524	1079	4 %	9 %	22 %	45 %	44 %	90 %
Piglets	2434	5011	20 %	42 %	**101 %**	**209 %**	**203 %**	**418 %**
Fattening pigs	481	990	4 %	8 %	20 %	41 %	40 %	82 %
Sows	305	628	3 %	5 %	13 %	26 %	25 %	52 %
Sheep-goats	222	458	2 %	4 %	9 %	19 %	19 %	38 %
Fattening lambs[1]	310	638	3 %	5 %	13 %	27 %	26 %	53 %
Broilers 5 weeks	641	1319	5 %	11 %	27 %	55 %	53 %	**110 %**
Broilers 8 weeks	579	1192	5 %	10 %	24 %	50 %	48 %	99 %
Replacement pullets	490	1009	4 %	8 %	20 %	42 %	41 %	84 %
Layers	524	1079	4 %	9 %	22 %	45 %	44 %	90 %
Turkey poults[2]	425	875	4 %	7 %	18 %	36 %	35 %	73 %
Turkey female[3]	462	951	4 %	8 %	19 %	40 %	39 %	79 %
Turkey male[4]	512	1054	4 %	9 %	21 %	44 %	43 %	88 %

[1] 2 runs/year;
[2] average of both sexes, middle and heavy breeds, age at slaughter: 11 weeks;
[3] females, age at slaughter: 16 weeks;
[4] males, age at slaughter: 22 weeks

The copper load appears to be within the limit of 12 kg/ ha and year, and averages at approximately 10% of the total amount allowed across animal species and categories, except in the case of piglets where the use of high amounts of copper in the feed leads to a higher exposure of environment, almost half of the maximum amount authorised. In the case of pig slurry, attention should be paid to avoid spreading on pastures accessed by sheep as this species is extremely sensitive and as exposure to copper can be fatal.

It can be concluded that on the basis of the present limits, the impact of copper use on environment is not of concern.

Although the current legal limit is complied with, in the case where this limit would be reviewed and reduced by risk managers, then the current levels of copper load could exceed maximum copper concentration of soil. Reduction of the limits for soil concentration would then probably mean revision of the amounts allowed in animals or appropriate controls of the amount of copper present in the slurry before spreading onto a land.

7. CONCLUSION

Copper is an essential trace element necessary to all animals. It has to be provided to animals to cover their requirements.

Currently, copper is authorised for all species including fish. Levels allowed in the diet of animals vary (see Council Directive 70/524/EEC). The current levels cover largely the above mentioned animal requirements. In the case of calves the levels authorised are even close to toxicity levels.

In terms of growth promotion and improvement of animal performances, copper is not known to have any practical effect in fish or farm animals, with the exception of pigs up to 8 to 10 weeks of age. High levels of copper (175 mg/kg) as authorised for the piglets are efficient in promoting growth, but efficacy for growing finishing pigs could not be demonstrated. Some studies have also shown some effects in poultry.

Copper is preferably stored in the liver. The other storage organ is kidney but to a lesser extent. Lower levels are observed in muscle and fat whatever the level used in the feed.

Use of copper at the current levels authorised in feed does not increase significantly the exposure of human consumers to copper. In the case of piglet (highly supplemented) and calf (having a high liver affinity for copper), the exposure of human consumers is increased, although remaining within the PMTDI.

No particular risk for the environment has been identified consecutive to the use of copper in pig and ruminant diets at the current allowed levels.

8. RECOMMENDATIONS

(1) Current copper levels allowed in diets should be reviewed to better reflect animal requirements.

(2) If reviewed, the current levels of copper in animal feed should consider the natural level of copper present in feedingstuffs and all factors impacting on copper availability. An appropriate safety margin should therefore be included in the levels fixed for this trace element. A total copper of 25 mg/kg complete feedingstuff would appear appropriate for all animals considered except calf fed milk replacer and sheep.

(3) The present level allowed for sheep (15 mg/kg) should be retained.

(4) The present level allowed for pre-ruminant calves could jeopardise their health due to the high accumulation of copper in their liver. Consequently, copper level authorised in feed should ideally be reduced to 5 mg/kg in milk replacer to protect calves' health. This would in addition allow reduction of the human consumer exposure.

(5) Because the growth promoting effect of 175 mg copper /kg diet could only be demonstrated for the piglets, SCAN would recommend to reduce the period of authorisation of a level of 175 mg/kg to the first 10 weeks of life instead of til 4 months of age.

(6) As long as 175 mg/kg feed remains authorised, comparably high levels of zinc and iron should be kept to prevent adverse effects of copper.

(7) The impact on aquatic environment of the use of copper in fish feed should be considered and assessed using adequate models for different fish production systems in Europe.

(8) Levels of copper presently allowed in feed of farm animals should be kept under scrutiny in the light of the possible evolution of the authorised load of copper on soil. For some animal categories, copper may indeed replace nitrogen as limiting factor for spreading of manure onto lands, if the allowed load of copper on soil was to be reduced.

(9) Attention should be paid to avoid spreading of pig slurry on pastures accessed by sheep as this species is extremely sensitive and as exposure to copper can be fatal.

(10) A gene encoding resistance to copper has been located on a plasmid derived from a gut bacterium which also carries a number of antibiotic resistance genes. Further work is needed to establish whether the use of copper, particularly at the highest permitted level in feed, can inadvertently co-select for antibiotic resistance.

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10. FARM ANIMAL DIETS USED BY SCAN AS A BASIS FOR ITS ASSESSMENT CALCULATIONS

* average of both sexes, middle and heavy breeds
** two runs/year

Animal category	Body weight				Production period (days)	Feed intake			Feed conversion (feed/gain)	Protein g / kg feed	Protein intake			CP/product	
	Range (kg)	Gain (kg)	ADG (g)	kg FCM /year		kg DM / day	kg DM / animal	kg / year			kg / day	kg / animal	kg / year	g/kg	g/kg gain or
Veal calves	45 - 200	155	1100		141	1,9	263,5	682	1,7	220	0,411	57,97	150		187,5
Replacement calves	45 - 125	80	702		114	1,83	209	669		202	0,370	42,22	135	160	
Fattening steers	125 - 600	475	1100		432	7	3023	2555		130	0,910	392,95	332	160	
Replacem. heifers	125 - 500	375	543		690	6,5	4485	2373		120	0,780	538,20	285	160	
Dairy cows	600		*Milk*	*5000*	365	14	5110	5110		140	1,960	715,40	715		34
	and calf	45												180	
			Total												
Dairy cows	650		*Milk*	*8000*	365	18	6570	6570		150	2,700	985,50	986		34
	and calf	45												180	
			Total												
Suckler cows	not calculated, because of no extra input on pasture or arable land except 20 kg mineral feed/year														
Piglets	8 - 25	17	378		45	0,600	27	219	1,6	227	0,136	6,13	50	170	170
Fattening pig	25 - 110	85	700		121,4	1,85	224	675	2,6	182	0,337	40,77	123	170	170
Sows, ear. wean.		25			49	4,400	215,6			204,5	0,900	44,10		170	170
		160			316	2,381	752,4	968		136	0,323	102,10	146,2	170	170
Sows, conv. wean.		25			80	4,400	352			204,5	0,900	72,00		170	170
		200			285	2,47	704	1056		136	0,336	95,76	167,76	170	170
Sheep - Goats	70	30	1,5 lambs		365	1,78	650	650		120	0,213		78		180
		4	wool												444
Fattening lambs	20 - 40	20	357		56	1,23	68,88			125	0,154	8,62			170
Broiler 5 weeks	40 - 1750	1,71	49		35	0,078	2,736	29	1,60	220	0,017	0,602	6,3	214	
Broiler 8 weeks	40 - 2650	2,61	47		56	0,093	5,2	34	1,99	205	0,019	1,066	6,9	214	
Replac. Pullets	38 - 1600	1,562	11		140	0,054	7,5	20	4,80	160	0,009	1,200	3,1	218	
Layers	2000		*Eggs*	*308*	365	0,118	43,0	43		160	0,019	6,880	6,9		121
Turkey 11 weeks*	50 - 5300	5,25	68		77	0,129	9,9	47	1,89	250	0,032	2,475	11,7	218	
Turkey fem. 16 w	50 - 9900	9,85	88		112	0,220	24,6	80	2,50	210	0,046	5,166	16,8	218	
Turkey male 22 w	50 - 20700	20,65	134		154	0,369	56,8	135	2,75	190	0,070	10,792	25,6	218	

Animal category	Product g N/kg	N-intake			N-retained		N in product		N-excreted		P	P	P-intake	P-excretion	
		kg / day	kg / animal	kg / year	kg / animal	kg / year	kg / animal	kg / year	kg / animal	kg / year	g/kg feed	g/kg gain g/kg	kg / animal	kg / animal	kg / year
Veal calves	30	0,066	9,275	24	4,65	12			4,625	**12**	6	7	1,581	0,496	**1,28**
Replacement calves	25,6	0,059	6,755	22	2,048	7			4,707	**15**	4,8	7	1,003	0,443	**1,42**
Fattening steers	25,6	0,146	62,873	53	12,16	10			50,713	**43**	3,5	7	10,580	7,255	**6,13**
Replacem. heifers	25,6	0,125	86,112	46	9,6	5			76,512	**40**	3,5	7,5	15,698	12,885	**6,82**
Dairy cows	5,44	0,314	114,464	114							3,5	1	17,885		
	28,8				1,296	1,296						7			
					1,296	1,296			113,168	**113**				12,57	**13**
Dairy cows	5,44	0,432	157,680	158			43,52	43,52			3,5	1	22,995		
	28,8				1,296	1,296						7			
					44,816	44,816			112,864	**113**				14,68	**15**
Suckler cows	not calculated, because of no extra input on pasture or arable land except 20 kg mineral feed/year														
Piglets	27,2	0,022	0,981	8	0,4624	3,751	0,462	3,751	0,518	**4**	7	4,9	0,21	0,127	**1,03**
Fattening pig	27,2	0,054	6,523	20	2,312	7			4,211	**13**	6,5	6	1,658	1,148	**3,45**
Sows, ear. wean.	27,2	0,144	7,056		0,68					**0**	6	6			
	27,2	0,052	16,331	23,4	4,352	5,032		5,032	18,4	**18,4**	6	4,9	6,6		**5,67**
Sows, conv. wean.	27,2	0,144	11,520		0,68						6	6			
	27,2	0,054	15,390	26,91	5,44	6,12		6,12	20,79	**20,79**	6	4,9	7,2		**6,07**
Sheep - Goats	28,8	0,034		12,44	1,148		0,864				3	5,2			
	160						0,284	1,148	11,29	**11,29**		0,3	1.915	1,76	**1,76**
Fattening lambs	27,2	0,025	1,400		0,544				0,856	**1,7****	3,5	5,2	0,241	0,137	**0,27****
Broiler 5 weeks	34,24	0,003	0,096	1,00	0,059	0,61			0,038	**0,394**	6,50	5,5	0,018	0,008	**0,087**
Broiler 8 weeks	34,24	0,003	0,171	1,11	0,089	0,58			0,081	**0,529**	6,25	5,5	0,033	0,018	**0,118**
Replac. Pullets	34,88	0,001	0,192	0,50	0,054	0,14			0,138	**0,359**	5,50	5,5	0,041	0,033	**0,085**
Layers	19,37	0,003	1,101	1,10			0,358	0,358	0,743	**0,743**	6,00	6,9	0,258	0,130	**0,130**
Turkey 11 weeks*	34,88	0,005	0,396	1,88	0,183	0,87			0,213	**1,009**	6,50	5,5	0,064	0,035	**0,168**
Turkey fem. 16 w	34,88	0,007	0,827	2,69	0,344	1,12			0,483	**1,574**	6,50	5,5	0,160	0,106	**0,345**
Turkey male 22 w	34,88	0,011	1,727	4,09	0,720	1,71			1,006	**2,385**	6,50	5,5	0,369	0,256	**0,606**

11. Calculation of the amount of copper spread onto lands

Animals	Time	Weight range	Feed intake		Copper in feed		Total copper excretion		Nitrogen excreted			Copper / Nitrogen ratio per animal	Copper applied, in g/ha and year, on	
	days	kg	kg dm per animal	kg dm per year	mg / kg	mg / kg dm [(1)]	g / animal	g / year	kg / animal	kg / year	kg / year [(2)]	g / kg [(3)]	**vulnerable areas [(4)]**	**non sensitive soils [(5)]**
Veal calves	141	45-200	263	682	50,0	56,8	14,943	38,750	4,6	12,000	9,000	4,331	**736**	**1516**
Replacement calves	114	45-125	209	669	50,0	56,8	11,875	38,011	4,7	15,000	11,250	3,369	**573**	**1179**
Fattening steers	432	125-600	3025	2555	35,0	39,8	120,313	101,619	51	43,000	32,250	3,145	**535**	**1101**
Replacement heifers	690	125-500	4485	2373	35,0	39,8	178,381	94,381	76	40,000	30,000	3,129	**532**	**1095**
Dairy cow	365	650	6570	6570	35,0	39,8	261,307	261,307	113	113,000	84,750	3,083	**524**	**1079**
Piglets	45	8-25	27	219	175,0	198,9	5,369	43,551	0,5	4,000	3,000	14,318	**2434**	**5011**
Fattening pigs	121,4	25-110	224	675	35,0	39,8	8,909	26,847	4,2	13,000	9,750	2,828	**481**	**990**
Sows	80+285		704	1056	35,0	39,8	28,000	42,000	20,8	20,8	15,600	1,795	**305**	**628**
Sheep-goats	365	70	650	650	15,0	17,0	11,080	11,080	11,3	11,300	8,475	1,307	**222**	**458**
Fattening lambs	56	20-40	69	70	15,0	17,0	1,176	1,193	0,86	1,700	1,275	1,823	**310**	**638**
Broilers	35	0.04 - 1.75	2,7	29	35,0	39,8	0,107	1,153	0,038	0,390	0,293	3,768	**641**	**1319**
Broilers	56	0.04 - 2.65	5,2	34	35,0	39,8	0,207	1,352	0,081	0,530	0,398	3,404	**579**	**1192**
Replacement pullets	140	0.04 - 1.6	7,5	20	35,0	39,8	0,298	0,795	0,138	0,350	0,263	2,882	**490**	**1009**
Layers	365	2	43	43	35,0	39,8	1,710	1,710	0,74	0,740	0,555	3,081	**524**	**1079**
Turkey poults	77	0.05 - 5.3	9,9	47	35,0	39,8	0,394	1,869	0,21	1,000	0,750	2,500	**425**	**875**
Turkey female	112	0.05 - 9.9	24,6	80	35,0	39,8	0,978	3,182	0,48	1,570	1,178	2,718	**462**	**951**
Turkey male	154	0.05 - 20.7	56,8	135	35,0	39,8	2,259	5,369	1	2,390	1,793	3,012	**512**	**1054**

(1) 88% dry matter

(2) considering an average 25% loss

(3) the calculation includes the 25% nitrogen losses in accordance with [2]

(4) calculation based on the limit of 170 kg N / ha and year fixed in Council Directive 91/676/EEC

(5) calculation based on the limit of 350 kg N / ha and year extracted from the publication of Spaepen *et al.*

(6) It is assumed that the amount excreted corresponds to 100% of the copper ingested

12. CURRENT COPPER AUTHORISATIONS (CD 70/524/EEC)

Trace elements						
No. (or EC No.)	Element	Additive	Chemical formula	Maximum content of the element in mg/kg of complete feedingstuff	Other provisions	Period of authorisation
E4	Copper-Cu	Copper-lysine sulphate	$Cu(C_6H_{13}N_2O_2)_2.SO_4$	Pigs for fattening: - in Member States where the mean density of the porcine population is equal to or higher than 175 pigs per 100 ha of utilizable agricultural land: - up to 16 weeks: 175 (total) - in Member States where the mean density of the porcine population is lower than 175 pigs per 100 ha of utilizable agricultural land: - up to 16 weeks: 175 (total)	Not more than 50 mg/kg of copper in the complete feedingstuff may come from copper-lysine sulphate.	30.09.2001[e]
				Pigs for fattening : - in Member States where the mean density of the porcine population is equal to or higher than 175 pigs per 100 ha of utilizable agricultural land : - from 17th week up to slaughter: 35 (total) - in Member States where the mean density of the porcine population is lower than 175 pigs per 100 ha of utilizable agricultural land : - from 17th week up to six months: 100 (total) - over six months up to slaughter: 35 (total) Breeding pigs: 35 (total) Other species or categories of animals, with the exception of calves prior to the start of rumination and sheep: 35 (total)	Not more than 25 mg/kg of copper in the complete feedingstuffs may come from copper-lysine sulphate.	30.09.2001[e]

[e] First authorisation Commission Regulation(EC) N°639/1999 (OJ L 82, 26.3.1999, p. 6)
[e] First authorisation Commission Regulation(EC) N°639/1999 (OJ L 82, 26.3.1999, p. 6)

No. (or EC No.)	Element	Additive	Chemical formula	Maximum content of the element in mg/kg of complete feedingstuff	Other provisions	Period of authorisation
E 4	Copper-Cu	Cupric acetate, monohydrate	$Cu(CH_3COO)_2 . H_2O$	Pigs for fattening: -in Member States where the mean density of the porcine population is equal to or higher than 175 pigs per 100 ha of utilizable agricultural land: - up to 16 weeks: 175 (total) - from 17th week up to slaughter: 35(total) -in Member States where the mean density of the porcine population is lower than 175 pigs per 100 ha of utilizable agricultural land: - up to 16 weeks: 175 (total) - from 17th week up to 6 months: 100 (total) - over 6 months up to slaughter: 35 (total) Breeding pigs: 35 (total) Calves: - milk replacers 30 (total) - other complete feedingstuffs: 50 (total) Ovines: 15 (total) Other species or categories of animals: 35 (total)	-	Without a time limit
		Basic cupric carbonate, monohydrate	$CuCO_3 \cdot Cu(OH)_2 . H_2O$		-	Without a time limit
		Cupric chloride, dihydrate	$CuCl_2 . 2H_2O$		-	Without a time limit
		Cupric methionate	$Cu(C_5H_{10}NO_2S)_2$		-	Without a time limit
		Cupric oxide	CuO		-	Without a time limit
		Cupric sulphate, pentahydrate	$CuSO_4 . 5H_2O$		-	Without a time limit

<table>
<tr><th>No. (or EC No.)</th><th>Element</th><th>Additive</th><th>Chemical formula</th><th>Maximum content of the element in mg/kg of complete feedingstuff</th><th>Other provisions</th><th>Period of authorisation</th></tr>
<tr><td rowspan="3"></td><td rowspan="3"></td><td>Cupric sulphate, monohydrate</td><td>$CuSO_4 . H_2O$</td><td rowspan="2">Pigs for fattening:
-in Member States where the mean density of the porcine population is equal to or higher than 175 pigs per 100 ha of utilizable agricultural land:
- up to 16 weeks: 175 (total)
- from 17th week up to slaughter: 35(total)
-in Member States where the mean density of the porcine population is lower than 175 pigs per 100 ha of utilizable agricultural land:
- up to 16 weeks: 175 (total)
- from 17th week up to 6 months: 100 (total)
- over 6 months up to slaughter: 35 (total)
Breeding pigs: 35 (total)
Ovines: 15 (total)
Other species or categories of animals with the exception of calves: 35 (total)</td><td rowspan="2">Denatured skimmed milk powder and compound feedingstuffs manufactured from denatured skimmed milk powder
- subject to the relevant provisions of Commission Regulations (EEC) No 368/77 and (EEC) No. 443/77
- Declaration of the amount of copper added, expressed as the element, on the label or package or container of denatured skimmed milk powder</td><td rowspan="2">Without a time limit</td></tr>
<tr><td>Cupric sulphate, pentahydrate</td><td>$CuSO_4 . 5H_2O$</td></tr>
<tr><td>Cupric chelate of amino acids hydrate</td><td>$Cu (x)_{1-3} . nH_2O$
(x=anion of any amino acid derived from hydrolysed soya protein)
Molecular weight not exceeding 1500.</td><td>Pigs for fattening:
- in Member States where the mean density of the porcine population is equal to or higher than 175 pigs per 100 ha of utilizable agricultural land:
- up to 16 weeks: 175 (total)
- from 17th week up to slaughter: 35 (total)
- in Member States where the mean density of the porcine population is lower than 175 pigs per 100 ha of utilizable agricultural land:
- up to 16 weeks: 175 (total)
- from 17th week up to six months: 100 (total)
- over six months up to slaughter: 35 (total)
Breeding pigs: 35 (total)
Other species or categories of animals, with the exception of calves prior to the start of rumination and sheep: 35 (total)</td><td>Not more than 20 mg/kg of copper in the complete feedingstuff may come from cupric chelate of amino acids hydrate.</td><td>Without a time limit</td></tr>
</table>