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Norwegian Scientific Committee for Food Safety

Zinc and copper in pig and poultry production – fate and effects in the food chain and the environment

Opinion of the Panel on Animal Feed of the Norwegian Scientific Committee for Food Safety

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Aksel Bernhoft (Chair)

Carl Einar Amundsen

Torsten Källqvist

Knut Rudi

Trine Sogn

Siamak Yazdankhah

Robin Ørnsrud

Contributors

Persons working for VKM, either as appointed members of the Committee or as project group, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The members of the project group are:

VKM members

Aksel Bernhoft (Chair), Panel on Animal Feed
Torsten Källqvist, Panel on Plant Protection Products
Siamak Yazdankhah, Panel on Biological Hazards
Robin Ørnsrud, Panel on Animal Feed

External experts

Carl Einar Amundsen, Norwegian Defence Estates Agency (NDEA)
Knut Rudi, Norwegian University of Life Sciences (NMBU)
Trine Sogn, Norwegian University of Life Sciences (NMBU)

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Evaluated and approved by

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Panel on Animal Feed:

Aksel Bernhoft (Chair), Marit Aursand, Live L. Nesse, Einar Ringø, Birger Svihus, Bente Torstensen, Robin Ørnsrud

Scientific coordinators from the secretariat: Tron Øystein Gifstad

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Summary

Zinc (Zn) and copper (Cu) are essential trace elements in animal and human nutrition and also necessary for normal growth and development of plants and other environmental organisms. However, farmed animals as pig and poultry receive additional Zn and Cu in their diets due to supplement of the elements in compound feed as well as Zn medical remedies. This usage results in considerably content of Zn and Cu in the animal manure.

On request from the Norwegian Food Safety Authority, the Norwegian Scientific Committee for Food Safety, Panel for Animal Feed has assessed the fate of Zn and Cu from feed and other sources, via pig and poultry intake to manure, and further to soil, and the long term effects in the food chain and environment. The assessment starts with a description of the requirement of Zn and Cu in pigs and poultry. The animals´ requirement is compared with their total exposure including the bioavailability of the compounds and the resulting concentrations in the manure.

In a model based on two selected levels of application of manure to the soil (20 or 70 kg phosphorous (P)/ha per year), the Zn and Cu concentrations in soil up to 100 years are estimated. Three selected areas in Norway, representing typical agricultural fields, are included in the model. The concentrations in plants from these fields are estimated. The estimated increment of Zn and Cu in plants together with knowledge on animal and human diets and intake levels are the basis for estimation of the exposure, which is used in the characterisation of health risk of Zn and Cu in animals and humans after up to 100 years of application of the manure. Furthermore are characterised the risks for effects of Zn and Cu for organisms in soil, water and water sediments. An assessment of the possible risk for development of bacterial resistance against Zn and Cu and antibiotics is also undertaken.

The requirement of Zn in pigs may vary within 25 – 80 mg/kg diet dependent on the type of production and the feed composition. The requirement of Zn in poultry may correspondingly vary within 30 – 60 mg/kg diet. Lower levels are sufficient in feed without whole-grain products and plant proteins containing phytic acid, a storage form of phosphorus which reduces the bioavailability of Zn. The requirement of Cu in pigs is 3.5 – 5 mg/kg and in poultry 4 – 8 mg Cu/kg diet, mainly dependent on type of production.

The data on Zn and Cu in complete compound feed in Norway indicate exposure of Zn and Cu to pigs and poultry at least two times higher than the requirement but most often several times above the required level. In addition, piglets are commonly fed complementary feed with Zn and Cu, as well as Zn in medical remedies. The estimated extra exposure via complementary feed is rather marginal but the medical use of Zn against diarrhoea and oedema disease in weaned piglets is considerable. The use of Zn as medical remedy is at a level of approximately 15-20 times higher than the exposure via complete feed and 30-60 times above the normally required level. The diseases Zn is meant to cure are associated with the management, housing and husbandry and may possibly be controlled otherwise.

The Zn and Cu in drinking water are normally at low concentrations and not considered to constitute a significant additional exposure. Zn-coated steel installations as mainly used in pig barns may release Zn at a considerable amount into the manure but will probably not contribute significantly to the total Zn exposure of the pigs.

The bioavailabilities of Zn and Cu and other trace elements interfere due to shared carriers for absorption from the gut, particularly at high exposure levels. Therefore, the feeding of animals with Zn and Cu in amounts greatly exceeding the required levels may reduce the bioavailability of Zn and Cu as well as of other essential trace elements. A reduction of Zn

and Cu exposure through feed and feed supplements will correspondingly reduce the concentrations in manure. This effect will be highest for Zn since this element is poorly retained in animals, particularly at high exposure levels, whereby high amounts end in the manure. Proper use, avoiding surplus dosing is therefore important in order to avoid possible environmental effects.

Several reports indicate that Zn and Cu from organic complexes have almost equal bioavailabilities as inorganic Zn and Cu, e.g. Zn-sulphate and Cu-sulphate. The bioavailabilities of Zn and Cu from organic complexes may, however, vary with i.e. pH. Experimental studies on the pharmacological effects of Zn or Cu to reduce post-weaning scouring and improve body weight gain have shown that formulations of Zn or Cu in organic form or lipid-encapsulated Zn may be effective at relatively low concentrations - achieving comparable effect as far higher concentrations of inorganic Zn or Cu. This indicates that the bioavailability and retention of Zn and Cu may be increased, and the concentrations in manure reduced by use of suitable formulations.

Zn and Cu adsorb strongly to soil, and repeated application of manure with a high content of these metals will cause accumulation in the soil. The amounts of accumulated metals have been calculated based on various levels of Zn and Cu in the manure and different application doses of manure applied annually for 100 years. Application of high doses,70 kg P/ha, of pig manure containing the present high concentrations of Zn may result in toxic effects to soil living organisms (invertebrates, plants and microorganisms) after long-term use (100 years). The risk of toxic effects on soil living organisms is somewhat less using poultry manure. The present level of Cu in pig and poultry manure will not result in toxic effects on soil living organisms even at high application rates of manure.

Leaching of Zn from fields that have been fertilised for 100 years with pig or poultry manure with a high content of Zn may pose a risk to aquatic organisms in local water bodies such as small streams or ponds where the dilution level is less than a factor 50. Also sediment dwelling organisms may be adversely affected by Zn under worst case conditions. Cu is not expected to reach concentrations that are harmful to aquatic or sedimental biota.

The predicted increase of Zn in plants grown in selected Norwegian areas intensively fertilised with manure from pigs for 100 years, varied from 2.4 to 5.8 times the background levels. The corresponding predicted increase of Cu in plants varied from 1.8 to 3 times the background levels. The increases of Zn and Cu by use of poultry manure were somewhat lower.

By intensive use of pig (or poultry) manure for 100 years the Cu concentration in feed plants may increase and give livestock a total exposure level of approximately 10 mg Cu/kg diet. Above this level an elevated risk for adverse accumulation and toxicity of Cu in sheep would be expected. Such Cu exposure is not of health concern for other livestock species. The elevation of Zn exposure from plant feed is not of toxicological concern in livestock.

A higher exposure of livestock with Zn and Cu due to intensive use of manure from pigs or poultry on fields is not considered to give significant increment in the concentrations of Zn or Cu in animal products such as meat, milk or eggs.

The consumption of vegetable food produced in areas where manure from pigs or poultry has been used over time leads to an increased human intake of Zn and Cu via particularly cereals and vegetables. By intensive use of pig manure for 100 years the predicted Zn intake of mean consumers will exceed the Upper Intake Limit (UL) and the Zn intake of high consumers will exceed NOAEL (No Observed Adverse Effect Level). Such exposure of Zn may be of concern for human health. For Cu the predicted intake of mean consumers is below UL, whereas high consumers exceed UL. Such exposure of Cu may be of low concern for human health.

Soil bacteria and enteral bacteria in farmed animals are shown to develop resistance to trace elements such as Zn and Cu. Resistance to Zn is often linked with resistance to methicillin in staphylococci and Zn supplementation to animal feed may increase the proportion of multi-resistant *E. coli* in gut microbiota. Resistance to Cu in bacteria, in particular enterococci, is often associated with resistance to antimicrobial drugs, like macrolide antibiotics and vancomycin. There is lack of data which could demonstrate whether Zn/Cu-resistant bacteria may acquire antibiotic resistance genes / become antibiotics-resistant, or if antibiotics-resistant bacteria are more capable to become Zn/Cu-resistant than antibiotics-susceptible bacteria. Data on dose-response relation for Zn/Cu exposure and resistance is also lacking. However, it seems more likely that a resistance driven effect occurs at high trace element exposure than at more basal exposure levels.

In conclusion, Zn and Cu are essential trace elements in plant, animal and human nutrition, and animal manure is a source of great value for fertilisation of agricultural soil. The results of the present suggested overload of Zn and Cu in feed for pigs and poultry and reduced bioavailability of Zn and Cu as well as of other essential trace elements in the animals are high levels of these elements in the manure. Zn and Cu exposure of bacteria in livestock and the environment may develop resistance to Zn and Cu and there are links to antibiotic resistance. In the long run, elevated concentrations of Zn and Cu in manure may adversely influence organisms in the environment and the food chain and be of health concern for human consumers. Reduction in animal intakes of Zn and Cu via feed, additives and medical remedies is possible to carry out respecting animal health and seems necessary respecting adverse effects in the environment and food chain.

Norsk sammendrag

Sink og kobber i produksjonen av gris og fjørfe – stoffenes skjebne og effekter i næringskjede og miljø

Sink (Zn) og kobber (Cu) er essensielle sporelementer i fôret til dyr og i menneskers mat. De er også nødvendige for normal vekst og utvikling av planter og andre organismer. Husdyr som griser og fjørfe får imidlertid ekstra tilførsel av Zn og Cu i fôret. Zn gis også som medisinsk tilskudd. Denne bruken resulterer i betydelige konsentrasjoner av Zn og Cu i dyras gjødsel.

På oppdrag fra Mattilsynet har Vitenskapskomiteen for mattrygghet, Faggruppen for fôr vurdert hva som skjer med Zn og Cu når gris og fjørfe får stoffene i fôret eller fra andre kilder, så via gjødsel fra disse dyra til jorda og deretter mulige effekter i næringskjeden og miljøet etter lang tids bruk av slik gjødsel.

Vurderingen starter med beskrivelse av behov for Zn og Cu hos gris og fjørfe, deretter dyras totale eksponering, inkludert hvor stor andel av stoffene som er biotilgjengelig for dyra og hvor mye som havner i gjødslen. I en modell basert på to gjødslingsnivåer (20 og 70 kg fosfor (P)/hektar pr år), er konsentrasjonene av Zn og Cu i jorda beregnet i opp til 100 år. Tre utvalgte områder i Norge, som representerer typiske jordbruksområder, er inkludert i modellen.

Konsentrasjonene av Zn og Cu i plantene som dyrkes på disse områdene er beregnet. Basert på disse resultatene, kjente data om diettens sammensetning, størrelse på husdyr og mennesker, er eksponeringen og helserisikoen vedrørende Zn og Cu for husdyr og mennesker vurdert ved bruk av slik gjødsel i opp til 100 år. Videre er risikoen for effekter av Zn og Cu for organismer i jorda og i vann og i sedimenter i nedslagsfeltet vurdert. Sist men ikke minst er det en vurdering av den mulige risikoen for utvikling av bakteriell resistens mot Zn og Cu, samt mot antibiotika.

Totalbehovet for Zn hos griser varierer mellom 25 – 80 mg/kg fôr avhengig av produksjonsform og fôrets sammensetning. Totalbehovet for Zn hos fjørfe varierer tilsvarende mellom 30 – 60 mg/kg fôr. Lavere konsentrasjoner vil være tilstrekkelig i fôr som ikke inneholder produkter av hele korn eller andre planteprodukter som inneholder fytinsyre. Dette er en lagringsform for fosfor i planter, somreduserer utnyttelsen av Zn. Totalbehovet for Cu hos griser er 3,5 – 5 mg/kg og hos fjørfe 4 – 8 mg/kg fôr, hovedsakelig avhengig av produksjonsform.

Opplysningene om Zn og Cu i fullfôr til gris og fjørfe indikerer at inntaket av Zn og Cu er minst to ganger høyere enn behovet, men som oftest betydelig høyere. I tillegg får smågriser ofte tilskudd av Zn og Cu, samt Zn som tilskuddsfôr. Den ekstra eksponeringen via legemidler er beregnet å være relativt liten. Den medisinske bruken av Zn mot diare og ødemsjuke hos avvente smågris innebærer imidlertid en betydelig ekstra eksponering – ca. 15-20 ganger høyere enn via fullfôret og 30-60 ganger over det som karakteriseres som normalt behov. Disse smågrissjukdommene er knyttet til driftsforhold som dyretetthet, renhold og stell og kan muligens kontrolleres på alternativ måte.

Konsentrasjonene av Zn og Cu i drikkevann er vanligvis lave og vurderes til ikke å være en betydelig tilleggseksponering for gris og fjørfe. Derimot kan innredninger av galvanisert stål i grisehus frigjøre Zn i betydelige mengder til gjødselen, men Zn fra denne kilden vil antakelig ikke gi noe betydelig bidrag til den totale Zn-eksponeringen hos gris.

Biotilgjengeligheten (utnyttelsen) av Zn og Cu og andre sporelementer er knyttet til samme mekanisme for opptak fra tarmen, som særlig interferer ved høye eksponeringsnivåer. Fôring av dyra med Zn og Cu i nivåer som langt overstiger behovet, kan derfor redusere opptak og biotilgjengelighet av Zn of Cu og andre essensielle sporelementer. En reduksjon av Zn- og Cu-eksponering via fôr og tilskudd vil derfor tilsvarende redusere nivået i gjødselen. Denne effekten vil være høyest for Zn, da stoffet har lav retensjon (tilbakeholdelse) i dyra, og ekstra lav retensjon ved høy eksponering. Detbetyr ekstra store mengder i gjødselen. Å bruke Zn og Cu i tilpassede r mengder i fôret og å unngå unødvendig høye doseringer vil derfor være viktig for å hindre mulige effekter i miljøet.

Flere rapporter viser at Zn og Cu fra organiske forbindelser ser ut til å ha omtrent tilsvarende biotilgjengelighet som uorganisk Zn og Cu, som for eksempel Zn-sulfat og Cu-sulfat. Biotilgjengeligheten til Zn og Cu i organiske komplekser kan imidlertid variere med bl.a. pH. Eksperimentelle studier av farmakologiske effekter av Zn og Cu for å redusere avvenningsdiare og øke tilveksten, har vist at moderate konsentrasjoner av Zn eller Cu i organisk form, eller som Zn innkapslet i lipider, kan være like effektive som langt høyere konsentrasjoner av Zn eller Cu i uorganisk form. Dette indikerer at biotilgjengeligheten og retensjonen av Zn og Cu kan økes med tilpassede formuleringer, som da vil innebære lavere total tilførsel til dyra og mindre mengder i gjødselen.

Zn og Cu bindes sterkt til jord og gjentatt tilførsel av gjødsel med høyt innhold av disse stoffene medfører at de akkumulerer i jorden. Mengden av Zn og Cu akkumulert i jorda er beregnet på bakgrunn av ulike nivåer av Zn og Cu i gjødselen og ulike tilførselsnivåer av gjødselen i opp til 100 år. Kraftig gjødsling, 70 kg P/ha i form av grisegjødsel med dagens innhold av Zn, kan resultere i toksiske effekter av Zn hos jordlevende organismer (planter, virvelløse dyr og mikroorganismer). Effektrisikoen er noe lavere ved bruk av gjødsel fra fjørfe. Dagens nivå av Cu i gjødsel fra gris og fjørfe vil ikke resultere i toksiske effekter hos jordlevende organismer, selv ved kraftig gjødsling.

Utvasking av Zn fra jord som har blitt tilført største mengde gjødsel fra gris eller fjørfe i 100 år med et høyt innhold av Zn, kan innebære risiko for vannlevende organismer i lokale vannområder som mindre bekker og tjern hvor fortynningsfaktoren er mindre enn 50. Beregningene fra største mengde gjødsel indikerer også risiko for effekter på organismer i sedimentene. For Cu er det ikke forventet at en vil nå konsentrasjoner som er skadelige for vann- eller sedimentlevende organismer.

Den beregnede økningen av Zn i planter fra de tre utvalgte geografiske områdene etter kraftig gjødsling med grisegjødsel i 100 år er 2,4 – 5,8 ganger dagens bakgrunnsnivå. Den tilsvarende beregnede økning av Cu i plantene i disse områdene er 1,8 – 3,0 ganger bakgrunnsnivået. Økningen av Zn og Cu ved å bruke tilsvarende gjødsling med fjørfegjødsel er noe mindre.

Økningen av Cu i plantene ved kraftig gjødsling med gjødsel fra gris (eller fjørfe) i 100 år innebærer at husdyr som beiter eller blir fôret kun på vekster fra disse arealene blir eksponert for en konsentrasjon av Cu på ca. 10 mg/kg fôr. Cu over 10 mg/kg fôr vil kunne innebære en økt risiko for skadelig Cu-akkumulering i leveren og Cu-forgiftning hos sau. Tilsvarende Cu-eksponering er ikke av helsemessig betydning for andre husdyrarter. Økning av Zn-innholdet i plantene vil ikke være av toksikologisk betydning for husdyra.

Den høyere Zn- og Cu-eksponeringen for husdyr som beiter eller blir fôret på vekster fra jord etter kraftig gjødsling med grise- eller fjørfegjødsel antas ikke å gi noen signifikant økning i konsentrasjonene av Zn eller Cu i animalske matprodukter som kjøtt, melk eller egg.

Humant konsum av vegetabilske matvarer fra råvarer dyrket i jord gjødslet over tid med grise- eller fjørfegjødsel, vil føre til økt inntak av Zn og Cu spesielt via korn, men også via grønnsaker. Beregningene viser at kraftig gjødsling med grisegjødsel i 100 år innebærer at inntaket av Zn for gjennomsnittskonsumenten vil overstige øvre inntaksgrense (UL) og inntaket av Zn for høykonsumenten vil overstige ikke-observert-skadelig-effekt-nivå (NOAEL). Slik eksponering vil være av helsemessig betydning for mennesker. Når det gjelder inntaket av Cu, vil det beregnede inntaket for gjennomsnittskonsumenten være under UL, mens høykonsumenten vil overstige UL. Slik eksponering vil være av liten helsemessig betydning.

Bakterier i jord og husdyr er vist å kunne utvikle resistens mot sporelementer som Zn og Cu. Resistens mot Cu i bakterier, spesielt enterokokker, har ofte blitt knyttet til resistens mot antimikrobielle midler som makrolid-antibiotika og vancomycin. Resistens mot Zn er ofte knyttet til resistens mot meticillin i stafylokokker, og Zn-tilskudd i husdyrfôret ser ut til å kunne ha sammenheng med økt andel av multi-resistente *E. coli* i pattedyrtarmen. Det mangler data som kunne vise om Zn/Cu-resistente bakterier erverver antibiotika-resistensgener og blir antibiotikaresistente, eller om antibiotika-resistente bakterier er mer utsatt for å bli Zn/Cu-resistente enn bakterier som er følsomme for antibiotika. Det mangler også data for dose-respons-sammenheng mellom Zn/Cu eksponering og resistens, men det kan vurderes som mer sannsynlig at en høy eksponering vil være mer resistensdrivende enn en moderat eksponering.

Samlet sett er Zn og Cu essensielle sporelementer for planter, dyr og mennesker, og husdyrgjødsel er en viktig kilde for gjødsling av landbruksarealer. Resultatene av dagens tilsynelatende overforbruk av Zn og Cu i fôret til griser og fjørfe er redusert biotilgjengelighet av Zn og Cu og av andre essensielle sporelementer i dyra. Det resulterer i høye nivåer av Zn og Cu gjødselen. Bakterier i husdyra og miljøet som eksponeres for Zn og Cu, kan utvikle resistens mot Zn og Cu, og det er sammenhenger med antibiotikaresistens. Lang tids bruk av gjødsel med høye konsentrasjoner av Zn og Cu kan skade organismer i miljøet og i næringskjeden og også bli av betydning for menneskers helse. En reduksjon av inntaket av Zn og Cu via fôr, fôrtilskudd og medisiner er forsvarlig når det gjelder dyrehelsen og synes nødvendig med tanke på skadelige effekter i miljø og næringskjede.

Literature search

The present report is an assessment of Zn and Cu in pig and poultry production and the fate and effects in the environment and food chain from this use of Zn and Cu. The assessment of Zn and Cu in pig and poultry production includes the requirement of Zn and Cu of these animals and their present exposure in Norwegian farming.

The main literature base on requirement including bioavailability, retention, excretion, deficiency and toxicity includes updated books and reports written by international experts and expert groups available from the library at Norwegian University of Life Sciences (NMBU) School of Veterinary Science, and publications from European Food Safety Authority (EFSA). In addition, literature searches made in Web of Science in January 2014 were necessary. [Zinc retention pigs] gave 87 hits [copper retention pigs poultry] gave 4 hits. Background information for assessment of Zn and Cu exposure in pigs and poultry was mainly collected from the feed industry and other agricultural industry, as well as the Norwegian Food Safety Authority (NFSA).

The assessment of the fate and effects in the environment and the food chain of Zn and Cu are mainly estimated by use of modelling where scientific recognised variables were used in the models. In addition, local reported variables were used by modelling the fate of Zn and Cu in the three selected agricultural regions.

Environmental effects of Zn and Cu have been exhaustively revised in connection with recent risk assessments under the European community programs for regulation of chemicals (EC1498/94 and EC 1907/2006), and the results are compiled in risk assessment reports (RAR), (ECI 2008, EU 2010). The sections on toxicity to freshwater and soil organisms are mainly based on information from these reports and the toxicity databases compiled for the RARs have been used as a basis for the current environmental risk assessment of Zn and Cu. Additionally a literature search on Zn and Cu application to soils, high soil concentrations and plant uptake was performed. The topic is well investigated connected to e.g. use of sewage sludge and pig manure in agricultural plant production.

For risk characterisation of Zn and Cu in animals internationally recognised maximum tolerable levels were used (NRC, 2005). For risk characterisation of Zn and Cu in humans updated upper intake levels (ULs) by international scientific bodies were used (references in text).

A literature search using relevant terms such as; Zn AND animal feed, bacteria, (antibiotic resistance OR antimicrobial resistance), manure, requirement, bioavailability using the Advanced Search Builder provided by PubMed (www.ncbi.nlm.nih.gov/pubmed) or Web of Science was performed. A similar search using the same terms, but Cu instead of Zn was also performed. The reference lists in the selected citation were scrutinized to identify additional articles or reports, overlooked by the searches. Titles and abstracts of all identified citations were screened and were excluded if they did not relate to the terms of reference. The titles of all hits were scanned, and for those that were of potential relevance, the abstracts were also scanned. Of these, for those of potential relevance, the full text was obtained and assessed whether it was of relevance to this Opinion. Original and review articles, and textbook content were included in this assessment.

A list of the articles on metal driven co-selection of antibiotic resistance, which fulfilled the inclusion criteria with summary of the findings and main conclusion, is presented in tables in **Appendix I**. We have not identified studies, which demonstrate a correlation between the concentration of Zn/Cu and development of Zn/Cu resistance in bacteria (*in vivo*). We have not identified investigations from Norway, which studied the effect of Zn and or Cu on microbiota in animal / human or on microorganisms in environment.

Abbreviations and definitions

AF	Assessment Factor
ARG:	Antimicrobial Resistant Gene
AVS	Acid-volatile sulphides
BCF	Bioconcentration Factor
BLM	Biotic Ligand Model
Ca	Calcium
CEC	Cation Exchange Capacity
Cu	Copper
$CuSO_4$	Copper (II) Sulfate
czrC:	One of the genes encoding resistance to Zn in bacteria
DM	Dry matter
DMT	Divalent Metal Transporter
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
dwt	Dry weight
EC	European Community
ECI	European Copper Institute
EEA	European Economic Area
EFSA	European Food Safety Authority
Eq	Equation
EQS	Environmental Quality Standard
EU	European Union
ermB:	Gene encoding resistance to erythromycin in bacteria
Fe	Iron
FIRFA	Federal Insecticide, Fungicide and Rodenticide Act
ha	Hectare
HC5	Hazardous Concentration (5-percentile of a SSD)
mefA:	One of the genes encoding resistance to erythromycin in bacteria
Mg	Magnesium
MIC:	Minimum Inhibitory Concentration
MLST:	Multilocus sequencing typing
Mn	Manganese
MRE	Metal Response Elements

mRNA	messenger Ribonucleic Acid
MRSA:	Methicillin resistant *S. aureus*
MSSA:	Methicillin susceptible *S. aureus*
MTL	Maximum Tolerable Levels
NFSA	Norwegian Food Safety Authority (Mattilsynet)
NMBU	Norges miljø og biovitenskapelige universitet (Norwegian University of Life Sciences)
NNR	Nordic Nutrition Recommendations
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOT	Norsk overflateteknologi
NRC	National Research Council of the National Academies
OC	Organic Carbon
P	Phosphorous
PCR:	Polymerase Chain Reaction
PEC	Predicted Environmental Concentration
PFGE:	Pulsed field Gel Electrophoresis
PNEC	Predicted No Effect Concentration
PRZM	Pesticide Root Zone Model
RAR	Risk Assessment Report
RCR	Risk Characterisation Ratio (PEC/PNEC)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIP	Riverine input program
ROS	Reactive oxygen species
SCF	Scientific Committee on Food
SOD	Superoxide dismutase
Spa-type:	The *spa* typing technique uses the sequence of a polymorphic VNTR in the 3' coding region of the *S. aureus*-specific staphylococcal protein A (*spa*).
SSCmec type:	A mobile genetic element that carries the central determinant for broad-spectrum beta-lactam resistance encoded by the mecA gene.
SSD	Species Sensitivity Distribution
sulA and *sulIII*:	Two of the genes encoding resistance to sulphonamid in bacteria
tcrB:	Gene encoding resistance to Cu in bacteria
tetB(P):	One of the genes encoding resistance to tetracycline in bacteria

tetM:	One of the genes encoding resistance to tetracycline in bacteria
TGD	Technical Guidance Document
TOC	Total Organic Carbon
UL	Upper Intake Limit
UK	United Kingdom
US	United States
USA	United States of America
VKM	Vitenskapskomiteen for mattrygghet (The Norwegian Scientific Committee for Food Safety)
VRE:	Vancomycin Resistant enterococci
WHO	World Health Organization
Zn	Zinc
ZnO	Zinc oxide

Background

The content of zinc (Zn) and copper (Cu) in manure has been shown to be especially high in farmed animals receiving a high portion of their diet from compound feed. This is typical for pig and poultry farming. A recent research project from Bioforsk (2012) has looked at the heavy metal contents in manure from farmed animals. The results show some variation between farms, but that pig and poultry manure often has high Zn and Cu contents. Bioforsk also pointed out that there might be other sources than feed to the Zn and Cu content in manure, like drinking water pipes and other building materials.

EU has established upper limits for Zn and Cu in feed. An adaption text allows Norway to further limit the amount of Cu in compound feed for piglets. Most of the basis feed materials in compound feed contain rather low concentrations of Zn and Cu. In practice, the amounts added constitute the dominant part of the Zn ad Cu in complete compound feed. In addition to what is added in feed, complementary feeds with Zn and Cu are available, and veterinarians can prescribe Zn supplements to pigs. Zn and Cu are essential trace elements and too low dietary contents for farmed animals may cause animal health and welfare problems. The labelling of feed declares the amount of Zn and Cu added, and not the total amount.

In areas with surplus manure from pigs or poultry, such manure may be added to the same fields every year. Zn and Cu may be accumulated in soil after the spreading of manure (EFSA, 2010). The long-term effects of such repeated application are sparsely known, and there are no studies from Norwegian agriculture. There are several exposure routes of Zn and Cu relevant for the environment and human and animal health when such manure is added to soil.

Recent research indicates that Zn and Cu in feed may play a role in the development of antibiotic resistance. This is both relevant for microorganisms living in the soil and disease-causing microorganisms in farmed animals.

The EU is currently preparing two new regulations which may be relevant for manure and the Zn and Cu content that is allowed in a fertiliser in the future: a new waste regulation for organic waste called «end of waste for compost» and a new fertiliser regulation. The limit values of Zn and Cu provided for in the feed additive regulation are also likely to be re-evaluated in 2014.

Terms of reference

The Norwegian Food Safety Authority would like to request a risk assessment on zinc and copper from feed to soil and food. Zinc and copper are added to feed as essential nutrients. Samples from manure have shown that there is a high content of copper and zinc in manure, and therefore it is a need to know the long-term effects of repeated addition of manure to agricultural land. At the same time there is a need to know the risk for animal health and welfare if the amount of copper and zinc in feed is reduced.

The Norwegian Food Safety Authority would like VKM to give their opinion on:

1. **Reduced exposure of zinc and copper to pigs and poultry to reduce the concentrations in manure**

1.1 The requirement of zinc and copper in the complete feed for pigs and poultry.

1.2 Are other sources (drinking water, barn installation) than compound feed of importance for the zinc and copper content in manure?

1.3 Is it possible to reduce zinc and copper in feed or veterinary prescriptions without adverse effects on growth, health and welfare of pigs and poultry? And what is the possible corresponding gain of reduced concentrations in manure?

1.4 Is it possible to use more biologically efficient chemical compounds of copper or zinc to increase the animal uptake and reduce the excretion of these elements in urine and excrements?

2. **Application of manure to soil**

2.1 Define a moderate and high level of yearly zinc and copper application from manure to soil under different crop rotations, and calculate the soil concentrations in 10, 50 and 100 years perspective. Addition of zinc and copper from other sources should also be taken into account.

2.2 Evaluation of the following exposure routes for the scenarios described in 2.1

Target organism	Description of scenario
Plants	
Plants	Plants growing on soil where pig or poultry manure has been used
Animals	
Soil organisms	Soil organisms living in soil where pig or poultry manure has been used
Aquatic organisms	Aquatic organisms living in a body of surface water influenced by soil where pig or poultry manure has been used
Grazing animals	Animals eating grass and/or soil and soil organism from fields where pig or poultry manure has been used
Animal eating feed	Animals eating feed grown in fields where pig or poultry manure has been used

The need for an assessment of human exposure depends on the results of the assessment of plant and animals and may be excluded.

Humans	
Humans eating plants	Humans eating plant products that have been grown on fields where pig or poultry manure has been used
Humans eating animal products	Humans eating products from grazing animals and/or products from animals eating feed grown in fields where pig or poultry manure has been used.
Humans drinking water	Humans drinking surface- and/or groundwater influenced by soil where pig or poultry manure has been used

3. Resistance to antibiotics

3.1. Can zinc and copper in feed play a role in the development of resistance to antimicrobial agents?

Terms of reference in Norwegian:

1. Reduksjon i tilførselen av sink og kobbertil fjørfe og svin for å redusere konsentrasjonen i gjødsel.

1.1. Behovet for kobber og sink i kraftfôr til gris og fjørfe.

1.2. Er det andre kilder (drikkevann, fjøsinnredning) enn kraftfôr som er av betydning for innholdet av sink og kobberi gjødsel?

1.3. Er det mulig å redusere innholdet av sink og kobberi fôret eller ved veterinær behandling uten negative effekter på vekst, helse og velferd hos gris og fjørfe? Hva vil den eventuelle respektive gevinsten i form av redusert innhold i gjødsel være?

1.4. Er det mulig å bruke kobber- og sinkforbindelser som har en høyere biologisk effektivitet for å øke opptaket i dyrene og for å redusere utskillelsen i avføring og urin?

2. Tilførsel av husdyrgjødsel til jord

2.1. Definer en moderat og en høy årlig tilførsel av sink og kobberfra husdyrgjødsel ved ulike vekstskifter, og beregn innholdet i jord i 10, 50 og 100 års perspektiv. Tilførsel av sink og kobberfra andre kilder skal også tas hensyn til.

2.2. Evaluering av de følgende eksponeringsveiene for scenarioene beskrevet i punkt 2.1.

Målorganismer	Beskrivelse av scenario
Planter	
Planter	Planter som vokser på jord der husdyrgjødsel fra fjørfe og svin har blitt benyttet.
Dyr	
Jordlevende organismer	Jordlevende organismer som lever der det er gjødslet med husdyrgjødsel fra fjørfe og svin.
Vannlevende organismer	Vannlevende organismer som lever i en vannforekomst som er påvirket av jord gjødslet med husdyrgjødsel fra fjørfe og svin.
Beitedyr	Dyr som spiser gress og/eller jord og jordorganismer fra beitemark gjødslet med husdyrgjødsel fra fjørfe og svin.
Dyr som spiser fôr	Dyr som spiser fôr høstet fra jorder gjødslet med husdyrgjødsel fra fjørfe og svin.

En vurdering av human eksponering er avhengig av resultatet av vurderingen for planter og dyr, og kan på bakgrunn av den muligens utelates.

Mennesker	
Mennesker som spiser planter	Mennesker som spiser planter som er dyrket på jord gjødslet med husdyrgjødsel fra fjørfe og svin.
Mennesker som spiser produkter av animalsk opphav	Mennesker som spiser produkter fra dyr som har beitet eller spist fôr som er dyrket på jord gjødslet med husdyrgjødsel fra fjørfe og svin.
Menneseker som drikker vann	Mennesker som drikker overflatevann- og/eller grunnvann som er påvirket av jord gjødslet med husdyrgjødsel fra fjørfe og svin.

3. Resistens mot antibiotika

3.1 Kan sink og kobber i fôr spille en rolle i utviklingen av antimikrobiell resistens?

Assessment

1. Introduction on Zn and Cu

Zinc (Zn) and copper (Cu) are essential trace elements for all forms of life and perform many biological functions. They are transition metals, Zn with atomic number 30 and Cu atomic number 29. Both Zn and Cu have a ubiquitous cellular distribution and are important structural component or regulatory co-factors of a wide range of different enzymes in many important biochemical pathways in both plants and animals.

Organisms have developed a homeostatic capacity that allows them to regulate the internal concentration of essential elements to a certain extent and to maintain it at optimal levels under varying external essential element availability. However, the capacity of this regulation is limited and when the external concentration becomes too high or too low, toxicity or deficiency can occur (MERAG 2007).

1.1 Zn and Cu in soil and soil organisms (microbiota and invertebrates)

1.1.1 Zn and Cu in soil

The natural source of Zn in soil is minerals, mainly the sulphides, and the total concentration is usually in the range from 10 to 300 mg/kg soil DM. In soil, Zn occurs as Zn^{2+}, adsorbed to soil cation exchange sites or complexed by organic compounds (Havlin et al. 2014). The bioavailability of Zn in soil may vary considerably, mainly due to changes in soil pH. At pH >7, Zn deficiency in plants often occurs even in soils with good Zn status. An eventually high bioavailability of Zn in soil at low pH can then simply be reduced by addition of lime. Increased content of organic matter has a positive effect on Zn bioavailability as chelated Zn (organic complex with Zn) is important to the transport of Zn to root surfaces and plant uptake (Havlin et al. 2014).

The natural source of Cu in soils is minerals, mainly the sulphides, and the total concentration is usually in the range from10 to 80 mg/kg soil DM. In soil solution, Cu might be available as both Cu^{+} and Cu^{2+} , but Cu^{2+} is the most common form. In contrast to Zn the mobility of Cu in soil is low, and thus the soil concentration of Cu^{2+} is low. Cu is strongly complexed to soil organic matter. Although plants might utilize some chelated Cu, high content of soil organic matter usually initiates Cu deficiency by plants (Havlin et al. 2014). The bioavailability of Cu in soil is usually optimal at pH 5-6 and higher pH´s reduce the bioavailability.

1.1.2 Zn and Cu toxicity in soil organisms including plants

The soil and environmental microbiota are tremendously complex, and it has been suggested that one gram of soil can contain from 10^4 to 10^7 bacterial species (Torsvik et al. 2002). A recent study has confirmed this high diversity estimate, by estimating 20 000 unique bacterial genomes in a model soil environment (Frisli et al. 2012). The majority of the diversity is still unknown, but there are now ongoing efforts in characterizing the soil and environmental diversity (Vogel et al. 2009).

Data on toxic effects of Zn on microbial processes, plants and invertebrates obtained from tests in natural and artificial soils with characteristics (pH, clay content, organic matter content and background concentration of Zn) within the boundaries of the ranges for these parameters in soils in EU were reviewed during the recent EU risk assessment of zinc and zinc compounds (EU 2010).

The selected data included 97 NOECs for effects on microbial processes (C-mineralisation, N-mineralisation and enzyme activity). The NOECs ranged from 17 to 1000 mg Zn/kg dw. For invertebrates 27 NOECs representing three species were selected with a range from 32 to 1000 mg Zn/kg dw. For plants 29 NOECs representing 16 species were included with a range of 32 to 400 mg Zn/kg dw. The large variation in toxicity of zinc is partly due to variation in the bioavailability of Zn in soil is caused by abiotic factors such as cation exchange capacity and pH in the soil. Furthermore, ageing processes reduce the available fraction of Zn in the soil over time.

Toxicity data for Cu in organisms associated to soil includes effects on microbial processes and chronic effects on various species of higher plants and invertebrates. The database compiled for the EU risk assessment by ECI (2008) contains 252 NOEC or EC10-values representing 19 species and microbial processes. Among plants NOEC values range from 18 mg Cu/kg for *Hordeum vulgare* to 698 mg Cu/kg for *Lycopersicon esculentum*. The range for invertebrates is from 8.4 mg Cu/kg (cocoon production of the earthworm *Eisenia andrei*) to 1460 mg Cu/kg (reproduction of the arthropod *Folsomia candida*). Tests with microbial multispecies communities showed NOECs for effects on respiration from 30 to 2402 mg/kg.

Much of the large observed variation in toxicity among, as well as between, species can be attributed to differences in bioavailability due to soil properties (pH, organic matter content and clay content) and to differences in ageing and application mode.

1.2 Zn and Cu in aquatic organisms

Aquatic organisms are exposed to the metals mainly as ions (Cu^{2+} and Zn^{2+}) dissolved in the surrounding water phase. Interactions from various abiotic factors in the water affect the chemical speciation of the metals in water by forming complexes that are less available for uptake in the organisms. The most important factors in this respect are dissolved organic carbon, water hardness (mainly calcium content) and pH value. Furthermore, the uptake of the metal ions in the organism is affected by the concentration of other cations that competes in the binding to the receptor on the uptake site (e.g. gills in fish). Due to these effects of abiotic factors, the toxicity of metals to aquatic organisms differs between waters with different chemical composition.

Based on laboratory studies of toxicity of metals on aquatic organisms exposed in waters of different chemical composition, models have been developed to quantitatively describe the effect of abiotic factors on the toxic response. Such models are known as Biotic Ligand Models and are available for both Zn (De Schamphelaere et al. 2005) and Cu (De Schamphelaere and Janssen 2004).

Organisms living in the bottom sediments are exposed to metals mainly through the pore water but uptake may also take place through direct transfere from the solid phase, in particular in organisms that ingest sediment. Also in the sediment environment, the biological availability of metals is affected by abiotic factors, mainly the content of organic carbon and sulphide.

1.2.1 Zn toxicity to freshwater organisms

Environmental effects of Zn have been exhaustively revised in connection with recent risk assessments under the European community programs for regulation of chemicals

(EC1498/94 and EC 1907/2006), and the results are compiled in a risk assessment report (RAR), (EU 2010). The following paragraphs on environmental effects are mainly based on this report. References to other sources of information are included where appropriate.

In the risk assessment of Zn, the toxicity data was selected to represent the major physico-chemical characteristics (in particular pH and hardness) that are encountered in natural European freshwaters using the criteria below:

- pH range: 6-9
- Hardness range: 24 – 250 mg $CaCO_3$/L
- Zn (background concentration): >1 µg/L

The lower limit for background concentration of Zn in the test water was included to exclude tests where Zn deficiency may have occurred.

The background concentration of Zn was subtracted from the reported total exposure concentrations in order to obtain NOECs as added concentrations.

The selected data included chronic NOEC values for 18 species representing algae, invertebrates and fish. Algae appears to be the most sensitive group with the lowest NOEC (geometric mean value) for the green alga *Pseudokirchneriella subcapitata* of 17 µg/L. Among the invertebrates the NOEC range from 37 µg/L for the crustacean *Ceriodaphnia dubia* to 400 µg/L (zebra mussel, *Dreissena polymorpha*). The fish NOECs range from 44 µg/L *(Jordanella floridae*) to 660 µg/L (*Brachydanio rerio*).

1.2.2 Zn toxicity to sediment dwelling organisms

The database on chronic toxicity of Zn to sediment dwelling organisms, which was compiled for the RAR contains only four useful chronic NOEC values representing three species. The lowest NOEC is for the crustacean *Hyalella azteca* (488 mg/kg dw) and the highest for the oligochaete *Tubifex tubifex* (1101/kg dw). These NOEC-values are expressed as added-Zn concentrations.

1.2.3 Cu toxicity to freshwater organisms

In the risk assessment of Cu (ECI 2008), the selection criteria for toxicity data included information on abiotic factors in the test medium. The background concentration of Cu in the test media was not used as a selection criterion.

The selected data included 139 chronic NOEC values for 27 species representing algae, macrophytes, invertebrates and fish. Among algae, the green alga *Pseudokirchneriella subcapitata* showed the lowest species NOEC (geometric mean value 54 µg/L). NOECs for invertebrates range from 6 µg/L (snail, *Juga plicifera*) to 54.3 µg/L amphipod *Hyalella azteca*, geometric mean value). Among fish, the lowest NOEC was found for rainbow trout (*Onchorynchus mykiss*, 11.6 µg/L, geometric mean value) and the highest for *Noemacheilus barbatulus* (120 µg/L).

1.2.4 Cu toxicity to sediment dwelling organisms

The database on toxicity to freshwater sediment dwelling organisms compiled for risk assessment of Cu contains 109 NOEC values representing 6 species. The intra-species variation of NOECs is large and mainly caused by differences in the content of organic carbon and acid volatile sulphide (AVS) in the sediments. After rejection of the tests with

AVS >0.77 mmol/kg the database contained 62 NOECs representing 6 species. The geometric mean values of the organic carbon (OC)-normalised NOECs for the most sensitive endpoint were calculated for each species. The species $NOEC_{OC,\ normalised}$ ranged from 1.970 mg Cu/g OC for The amphipod *Gammarus pulex* to 6.228 mg Cu/g OC for a mayfly (*Hexagenia* sp).

1.3 Zn and Cu in plants

During the late 19th century many investigations revealed the regular presence of Zn in plants as well as in animals in concentrations often comparable with those of iron and usually much higher than those of most other trace elements. The essentiality of Zn for higher plants was established in the 1920s (Hambidge et al. 1986).

Zn is taken up in plants as Zn^{2+} via facilitated diffusion or by specific Zn transporters. The Zn uptake is strongly inhibited by high concentrations of Cu, Iron (Fe), manganese (Mn), magnesium (Mg), calcium (Ca), as well as high concentrations of phosphate. Similarly to Mg and Ca, Zn is present in the Xylem sap (plant sap where nutrients are translocated from root to leaves) as free 2-charges positive cation. Redistribution of Zn from the vegetative to the generative plant parts has been found to be relatively high (Mengel and Kirkby 2001). At high concentrations, Zn has been found to deposit in older leaves, and thus the Zn concentrations in grains were hardly influenced by a high Zn uptake. At high Zn load major amounts of Zn have also been found to accumulate in plant roots (Aasen 1997). For most plant species, Zn concentrations in the range of 20 to 100 µg Zn/g DM are sufficient for plant growth and development.

Zn is an essential component of several enzymes, mainly Zn-metalloenzymes. Among them is e.g. Cu-Zn superoxide dismutase (Cu-Zn SOD), which is required for the detoxification of the superoxide radical (Bowler et al. 1992). Zn-containing enzymes are also involved in the carbohydrate metabolism and N metabolism of plants. In Zn deficient plants, protein synthesis and the protein levels are markedly reduced, and amino acids and amides are accumulated.

Cu is taken up by plants in very small quantities and the Cu concentrations in most plant species are low, i.e. in the range 5-50 µg Cu/g DM. The plant Cu uptake mechanism is still not understood. As already mentioned, Cu strongly inhibits the uptake of Zn and *vica versa.* However, the plant Cu uptake is largely independent of competitive ions and relates mostly to the level of available Cu in the rhizosphere. In roots, Cu is known to effectively replace other ions from root exchange sites and is strongly bound in the root apoplast. Roots are thus frequently higher in Cu concentrations than other plant tissues. Cu is not readily mobile in plants. However, when the plant concentration of Cu is high translocation of Cu within the plant takes place. Cu has a strong affinity for nitrogen (N) in amino groups and the soluble N component of the amino groups acts as Cu carriers in both Xylem and Phloem (transport tissues in plants).

The biochemical role of Cu in plants is prominently connected to it’s valency change abilities. Enzymatically bound Cu participates in redox reactions. It’s role in Cu-Zn SOD is already mentioned above. Furthermore, Cu influences both the carbohydrate and the N metabolism.

1.4 Zn and Cu in animals and humans

1.4.1 Zn in animals and humans

The biochemical role of Zn is as an essential part of more than 300 enzymes involved in synthesis, metabolism and turnover of proteins, carbohydrates, lipids, nucleic acids and some of the vitamins. Well known Zn-containing enzymes include superoxide dismutase (SOD), alkaline phosphatase and alcohol dehydrogenase. Zn is essential for several body functions and has three general physiological roles: catalytic, structural and regulatory. Zn-dependent enzymes can be found in all known classes of enzymes (McCall et al. 2000) and Zn appears to be part of more enzyme systems than all of the other trace minerals combined. Zinc plays an important role in the structure of proteins and cell membranes. A structure resembling a finger, referred to as a zinc finger domain, stabilizes the structure of a number of proteins. Proteins with Zn-binding domains are estimated to represent approximately 10 % of the human proteome, and the same would be expected to be true in production animals (Andreini et al. 2006). Cell membrane integrity is also affected by Zn. Loss of Zn from biological membranes increases their susceptibility to oxidative damage and impairs their function (O'Dell 2000). Zn plays a major role in regulating gene transcription, as Zn finger-containing transcription factors bind to DNA and affect the transcription of genes. Furthermore, Zn finger-containing transcription factors bind to regulatory DNA sequences called metal response elements (MRE) in the promotor region of genes and enhances or repress transcription.

Due to the many biochemical functions of Zn, symptoms of deficiency are varied and occur in several organ systems. A main symptom in most species is retarded growth, but species-specific symptoms are also found. For poultry, frayed feathers, shortened leg and wing bones, enlarged hock joint and reduced egg production and hatchability have been reported. In 1955, Tucker and Salmon presented the discovery that Zn cures and prevents parakeratosis in pigs. During the next decade the importance of Zn for growth and development in various species including humans applied to prenatal as well as postnatal development was demonstrated (Hambidge et al. 1986). In all species, Zn deprivation is characterized by loss of appetence, retardation or cessation of growth and lesions of the integument and its outgrowths – hair, hoof, horn, wool or feathers (Suttle 2010). However, well-defined cases of absolute clinical Zn deficiency are not common in animals and humans in European countries and North America (Hambidge et al. 1986; NNR 2012). In animals the disease is mostly known from experimental conditions or diets containing f.i. high calcium or phytate, which reduce the Zn absorption (Suttle 2010). In general, both the Zn absorption and excretion are under homeostatic control (Pond et al. 2005). In humans Zn deficiency is most often related to incomplete parenteral nutrition, cases of malabsorption and use of drugs (NNR 2012).

A wide margin of safety exists between the required Zn intake and the amount that will produce toxic effects. Pigs and poultry are more tolerant to excess of Zn than f.ex. ruminants, where large amounts may change rumen metabolism due to a toxic effect of Zn on rumen microbiota (Pond et al. 2005).

1.4.2 Cu in animals and humans

Cu was first shown to be essential for growth and haemoglobin formation in laboratory rats by Hart and coworkers in 1928 as they recognized that rats on a milk diet required Cu in addition to iron to cure the anemia, which developed. Subsequently, it became evident that Cu is as a critical element in a range of metabolic pathways of great importance in practical husbandry and is affected be diets with deficient or toxic concentrations of Cu. In addition to Cu deficiency anemia, which may occur in all species of animals and humans, Cu deficiency may also produce bone, nerve, skin/hair, reproductive as well as cardiovascular disorders in several species (Davis and Mertz 1987).

Its main role is as a co-factor in Cu-containing enzymes, e.g. amine oxidases, ferroxidases and superoxide dismutases (Turnlund 2006). Thus, many of the physiologic functions of Cu can be predicted based on the biochemical role of the Cu containing enzymes (Prohaska 1997). In connective tissue formation, the Cu-containing enzyme lysyl oxidase is essential for the cross-linking of collagen and elastin, which is necessary for the proper maturation of connective tissue. Thus, Cu plays a role in bone formation, skeletal mineralization and integrity of the connective tissue in the heart and vascular system (Werman et al. 1996). Cu is involved in iron metabolism through ceruloplasmin and ferroxidase II proteins and is possibly also involved in the formation of bone marrow cells, and hence the production of red blood cells. Cu plays multiple roles in the central nervous system; it is required for myelin production through cytochrome c oxidase activity in phospholipid synthesis, and is involved in normal neurotransmission through Cu-proenzymes such as monoamine oxidase in the catecholamine metabolism. Furthermore, evidence also suggests a role in immune functions. Cellular and humoral factors of the immune system are suppressed by Cu deficiency including changes in T-lymphocytes, T-helper cells, B-cells, monocytes and interleukin-2 (Failla et al. 1998).

In humans, it has been demonstrated that suboptimal Cu intake compared to recommended intake might be associated with increased risk of colorectal cancer (NNR 2012).

Recent studies have advanced the understanding of the complex ways in which Cu is absorbed, transported and incorporated into functional enzymes and proteins involved in energy metabolism, formation of connective tissue and defense against free radicals (Suttle 2010; NNR 2012). The absorption of Cu is basically regulated by the amount of Cu in the diet. However, it is shown that the Cu absorption is inhibited at high dietary levels of Zn, as Zn induces the production of metallothionein, which chelates Cu (NNR 2012). Furthermore, the Cu absorption interacts with the uptake of molybdenum (Mo) and sulfur (S) due to shared carriers. This connection has been observed in most outbreaks of Cu deficiency as well as poisoning disorders in grazing livestock, also in Norway. Clinical disorders of deficiency as well as Cu poisoning may frequently occur in grazing ruminants. Sheep are particularly sensitive to Cu resulting in various deficiency symptoms in lambs as well as poisoning due to Cu accumulation in the liver and release resulting in haemolysis. Cu responsive disorder rarely occurs naturally in non-ruminants as pigs and poultry (Suttle 2010). Chronic Cu poisoning may occur in pigs and poultry given Cu supplements as growth stimulants in proportions that are not suitably balanced with other minerals. Furthermore, the Cu tolerance is also influenced by the composition of diet.

Cu deficiency in humans is rare, but has been found in a number of circumstances, first of all in premature infants fed milk formula and in patients with prolonged parenteral nutrition without Cu supplementation. Intake of high doses of Cu leads to acute toxicity, with symptoms of gastric pain, nausea, vomiting and diarrhea (NNR 2012).

1.5 Zn and Cu in animal and human microbiota

1.5.1 The human, porcine and chicken microbiota

The microbiota associated with humans and animals represent a complex assemblage of microorganisms covering all three domains of life (Bacteria, Achaea and Eukarya) (Ley et al. 2008). All body sites are colonized, with the lower gastrointestinal tract being the most densely populated. In number of cells the microbiota generally outnumbers the host by a factor of 10, while with respect to number of genes the microbiota contains 100 times more genes than the host. Therefore, the gut microbiota can be considered an organ in itself (O'Hara et al. 2006).

The function of the microbiota is to protect the host from pathogen invasion, to train the immune system, to extract energy from low accessible nutrients, and in addition to produce essential vitamins and metabolites needed by the host (Nicholson et al. 2012).

Generally, gut microbes are strictly anaerobic due to the anaerobic conditions in the gut. The anaerobic conditions are mainly created by microbial respiration (Backhed et al. 2005). The human and porcine gut microbiota have similar composition with a dominance of the phyla *Firmicutes* and *Bacteriodetes* (Castillo et al. 2007, Louzupone et al. 2012), while the chicken microbiota are lower in *Bacteroidetes*, and higher in *Lactobacillus* and *Proteobacteria* (Sekelja et al. 2012). It has been proposed that diet is the main driver for the composition and functioning (nutrient breakdown and metabolite production) of the gut microbiota since microorganisms in the gut can utilize nutrient compounds the host cannot break down (Muegge et al. 2011). For humans, however, it has been shown that the gut microbiota is highly individual – that is the microbiota varies in different individuals. The humans are separated into three main clusters based on individual differences in the gut microbiota composition. These clusters have been denoted enterotypes, suggesting that they represent the gut microbiota counterpart of blood types (Arumugam et al. 2011).

Mainly due to the complexity we have very limited knowledge of what shapes the host associated microbiota composition (Louzupone et al. 2012). The main unresolved questions are if the host-associated microbiota is shaped by bacterial-bacterial competition, or if the host can shape the composition (Ley et al. 2006).

1.5.2 Effect of Zn and Cu on pathogenic microorganisms in pigs and poultry

In microbial ecosystems there is a delicate balance between trace metals such as Zn and Cu as limiting factors, and the toxic effect of these (Gielda et al. 2012). These trace elements are common cofactors in enzymes, while the toxic effects are more diverse, ranging from the replacement of other trace elements, binding to enzymes, and oxidation.

It has been hypothesized that the antimicrobial effect of Zn and Cu leads to growth promotion in a similar manner as for the effect of antibiotic-based growth promoters (Jensen et al. 2006). For, Cu, although it has been reported that there is a shift in the microbiota associated with exposure (Dunning et al. 1998), it could be that the growth promoting effect is mainly through increased tolerance for bacterial antigens (Namkung et al. 2006).

A range of pathogens can exploit host responses through inflammation induction (Lupp et al. 2007, Stecher et al. 2007). For instance, upon inflammation the host will produce chelating agents such as calprotectin that will limit the availability of the trace elements and thereby bacterial growth (Corbin et al. 2008). For Zn, however, recent evidence suggests that pathogens can have a competitive advantage over the commensal microbiota under limiting conditions, thereby being promoted under an inflamed state (Gielda et al. 2012, Liu et al. 2012). Since diarrhea in itself can lead to Zn depletion this could also promote the pathogen survival. Therefore, for Zn a potential growth promoting effect can be to limit the pathogen competitive advantage through Zn depletion, enabling the commensal microbiota to be competitive.

1.5.3 Mechanisms of antibacterial activity

The potential mechanisms of growth promotional effects of trace elements are attributed to their antimicrobial activities, similar to that of antibiotics, in that gut microbiota are altered to reduce fermentation loss of nutrients and to suppress gut pathogens (Højberg et al. 2005). The data from that study illustrate that elevated doses of dietary ZnO and $CuSO_4$ reduced the sizes of major groups of bacteria among the porcine gastrointestinal commensals, namely, the lactobacilli and streptococci. The reduced level of these commensals in the proximal part of the gastrointestinal tract may benefit the host animal by allocating more feed components for its growth performance. Furthermore, feeding the animals high dietary ZnO doses resulted in an altered pattern of organic acid accumulation, with lower levels of lactate and succinate in the stomach and small intestine and an accumulation of these compounds in the cecum and colon. How this influences the physiology of the animals needs further elucidation in detail, since lactic acid produced in the stomach is normally considered a part of the natural defense mechanism of the host, whereas lactate accumulation in the large intestine has mainly been observed in connection with various disorders. $CuSO_4$ reduced the number of coliforms in the large intestine, which may be a part of other mechanisms, such as the suppression of specific pathogens and induced resistance of the animal to pathogen adhesion and invasion as well as pathogen-produced toxins (Carlson et al. 2004).

Trace elements like Zn and Cu may be toxic to bacteria and the microbial toxicity of trace elements is due to their chemical affinity to the thiol groups of macrobiomolecules but also depends on the solubility of the metal compounds under physiological conditions.

2. Requirement and exposure of Zn and Cu in pigs and poultry

2.1 Requirement of Zn

The requirement of micronutrients depends to a large extent on the bioavailability of the nutrient in question (see below). Whole-grain products and plant proteins contain phytic acid, a storage form of phosphorus. Phytic acids may form complexes with divalent cations such as Cu, Zn and Fe and is poorly hydrolyzed by pigs and poultry (Woyengo and Nyachoti 2013). These complexes are insoluble and may lead to decreased absorption of the minerals. This has a profound effect on the dietary levels needed to support growth. Furthermore, phytic acid may also increase the endogenous nutrient losses in pigs and poultry increasing the requirement due to the lost nutrients (Woyengo and Nyachoti 2013). Thus, dietary divalent cation availability could be greatly improved by reducing the phytic acid content, e.g. by adding phytase as an animal feed supplement (Kornegay et al. 1997; Revy et al. 2006).

2.1.1 Pigs

The Zn requirement is influenced by several diet-related factors, including phytic acid or plant phytates, Ca, Cu and others, as well as protein level and source (reviewed by National Research Council, NRC 2012). In young pigs fed a conventional weanling diet, which would contain phytate, 80 mg Zn/kg diet was determined to be adequate (van Heugten et al. 2003, referred by NRC 2012). However, in young pigs fed a casein-glucose diet considerably lower Zn concentration is required (15 mg/kg diet) because this diet does not contain factors as phytate that reduce Zn availability (reviewed by NRC 2012). For growing pigs fed diets containing isolated soybean protein or corn-soybean meal (both diets contain significant amounts of phytate) that contain the recommended level of Ca, the Zn requirement is about 50 mg/kg diet. The Zn requirement of breeding animals is not well established, but may be somewhat higher than for growing pigs due to fetal growth, milk production, tissue repair during uterine involution, and sperm production in boars (reviewed by NRC 2012). According to the comprehensive book Mineral Nutrition of Livestock by Suttle (2010) from UK, the Zn requirement for growth in pigs is placed from around 46 mg/kg DM in weanling piglets to about 27 mg/kg DM at finishing stage (40-24 mg/kg diet). These figures are based on experimental data as well as results of a hypothetical factorial model.

In conclusion, the US estimated data on requirement of Zn in pigs is somewhat higher than those from Europe. NRC (2012) indicates a requirement of 50-80 mg/kg diet and Suttle (2010) indicates 24-49 mg/kg diet. More specifically, piglet may need 40-80 mg/kg of Zn in feed with phytate and without phytase (15 mg/kg in feed without phytate), growing pigs 25-50 mg/kg feed, and breeding pigs somewhat higher that in feed for growing pigs (probably up to piglet feed levels?). The estimated requirements are also summarized in Table 3.

Interest in **pharmacological use** of Zn to reduce post-weaning scouring and improve body weight started about 25 years ago (reviewed by NRC 2012). Several studies have shown such effects by using Zn levels at 2000-6000 mg/kg diet for some weeks. However, there are also some studies, which have not observed beneficial effect of such high Zn levels. Studies of pharmacological levels of Zn (3000 mg/kg as Zn oxide) and Cu (250 mg/kg as Cu sulphate) have shown that both were efficacious individually as weight promoters, but not additive in combination. From other studies additive effects of combinations of pharmacological levels of

Zn and Cu from available sources are reported. Hill et al. (2001) reported that improved performance with high Zn levels could be additive with antibiotics. Inhibited expression of stem cell factor in the small intestine, leading to reductions in the number of mast cells and histamine release is a proposed effect mechanism (Ou et al. 2007).

Experiments have been conducted to determine whether feeding lower concentrations of Zn in organic form to pigs would maintain growth performance and decrease Zn excretion in manure compared with feeding several thousand mg/kg of inorganic Zn. A study on comparison of Zn oxide and Zn methionine indicated that supplementing starter diets with 250 mg Zn/kg from methionine gave equal performance improvements as 2000 mg Zn/kg from the oxide salt (Ward et al 1996). The growth performance of weanling pigs fed diets containing Zn at 300 or 450 mg/kg as Zn-polysaccharide did not differ from that of pigs fed 2000 mg/kg as Zn oxide, but feeding 300 mg/kg as the polysaccharide decreased Zn excretion by 76 % compared with feeding 2000 mg/kg as Zn oxide (Buff et al. 2005).

Recently, experiments by using lipid-encapsulated Zn at lower doses to prevent diarrhoea in weaned piglets have been conducted. Lipid encapsulated Zn oxide at 100 mg Zn/kg diet was shown to be as effective as supplementation of non-encapsulated Zn oxide at 3000 mg Zn/kg diet for suppression of post-weaning diarrhoea in pigs in an Australian study (Kim et al. 2010 unpublished report). The idea with the lipid encapsulated product is to deliver Zn^{2+} ions to the intestine where the lipid coating is broken down by lipase enzymes. The plasma Zn concentration of pigs fed with encapsulated Zn was not increased from the control (normal feed, approximately 200 mg Zn/kg) level, whereas the plasma concentrations in pigs given the high Zn feed concentration at 3000 mg/kg was above 3 fold higher. The faecal Zn concentrations reflected the dietary concentrations.

In a Norwegian study of piglet health, lipid-encapsulated, low concentration Zn oxide in peat dust (375 mg/L) was compared with non-encapsulated Zn oxide (3750 mg/L), basic peat dust without Zn oxide supplement or no peat dust (Oropeza-Moe et al. 2013 unpublished report). The estimated intake of Zn in lipid-encapsulated and non-encapsulated peat was approximately 4.2 and 42 %, respectively, of the Zn intake when used the recommended Zn concentration in feed against weaning diarrhoea at 2400 mg/kg. Statistical significant effects were not shown in this study but the results indicated poorest faeces score and performance in control groups with no peat dust at all, some improvement with basic peat dust and further and similar improvement with lipid-encapsulated, low concentration Zn oxide and non-encapsulated Zn oxide.

In conclusion, most studies on pharmacological use of Zn to reduce post-weaning scouring and improve body weight show improved performance at concentrations of inorganic Zn above 2000 mg/kg diet. In some experimental studies using considerably lower concentrations of Zn in organic (Zn methionine) or lipid-encapsulated forms, similar pharmacological effects have been shown as well as considerably lower faecal Zn concentrations.

2.1.2 Poultry

According to Suttle (2010), the Zn requirement decreases with age, primarily due to a gradually improved phytate metabolism, from 60-65 mg/kg DM (53-57 mg/kg diet) the first week of broiler growth to 45-50 mg/kg DM (40-44 mg/kg diet) the next 3 weeks, and then 30-35 mg/kg DM (26-31 mg/kg diet).

For turkey poults the requirement of Zn is regarded somewhat higher (about 20 % higher) (Suttle 2010).

These figures are based on a corn/soy bean diet, and may be up to 20 % lower for wheat-based or phytase-supplemented diets (reviewed by Suttle 2010).

For laying hens the Zn requirement is relatively high (probably 50-60 mg/kg DM corresponding to 44-53 mg/kg diet) due to low Zn availability on diets high in calcium (reviewed by Suttle 2010).

The most recent NRC Nutrient Requirements of Poultry (1994) gives some lower requirement figures. However, the requirement has probably increased over the years (Suttle 2010), and the recent report contains more updated data. The estimated requirements are summarized in Table 3.

2.2 Requirement of Cu

2.2.1 Pigs

NRC (2012) describes that a level of 5-6 mg Cu/kg diet is adequate for the neonatal pig, and that the requirement of Cu is probably not higher for pig in later growth stages. NRC (2012) further maintains that definitive information on requirements during gestation and lactation are scarce. Suttle (2010) describes that suckling baby pigs meet their need at 6 mg Cu/kg DM (5 mg/kg diet), that 4 mg/kg DM (3.5 mg/kg diet) is considered sufficient for growing pigs, and also that 4 mg/kg DM cover the need for sows and their offspring.

In conclusion, there is general agreement between US and European experts that approximately 5 mg Cu/kg diet is adequate for piglets, and 3.5 mg/kg diet seems to be sufficient for growing pigs and sows. The estimated requirements are also summarized in Table 3.

Pharmacological treatment: Growth stimulation in pigs when fed at 100-250 mg Cu/kg diet (as Cu sulphate) is well documented through more than 50 years (reviewed by NRC 2012). Furthermore, it has been shown in several reports that the growth response to Cu in young pigs is independent of the growth response to other antibacterial agents. With similarly high dietary Cu concentrations to sows during gestation and lactation are also reported beneficial effects on offspring (litter size, birth weight and weight gain). However, other studies have shown no such response (reviewed by NRC 2012). The mechanisms for beneficial effects from higher than normal supplementation levels of Cu are mainly unknown (reviewed by NRC 2012). Growth-stimulating action of dietary Cu due to its antimicrobial actions may be a factor. However, various experimental studies in weanling pigs have shown that systemic acting mechanisms are also reasonable. These include: stimulated growth and serum mitogenic activity after intravenous exposure of Cu (Zhou et al. 1994), stimulated lipase and phospholipase A activities with improvement of dietary fat digestibility after dietary exposure (Luo and Dove, 1996) as well as affected mRNA expression of appetite-regulating genes in the hypothalamus after dietary exposure (Zhu et al. 2011). Increased growth performance in pigs fed pharmacological Cu levels from a Cu lysine complex compared with pigs fed Cu sulphate is shown (Coffey et al. 1994; Zhou et al. 1994b).

2.2.2 Poultry

Definitive updated data on the minimum Cu requirement of chicken for growth and of hens for egg production have not been reported (Suttle 2010). He refers to NRC (1994), which gives the following Cu requirements: Starting Leghorn type chicks 5 mg/kg and growing chicken 4 mg/kg, broilers and starting turkey poults 8 mg/kg, growing and breeding turkeys 6 mg/kg diet. For laying hens there is an older but not questioned recommendation at 3-4 mg/kg diet (Reviewed in Suttle 2010). The estimated requirements are summarized in Table 3.

2.3 Bioavailability, retention and excretion of Zn and Cu in pigs and poultry

Bioavailability is defined here as the proportion of an ingested nutrient that is absorbed in a form that can be utilized in the metabolism by a normal animal (Ammerman et at 1998). Most studies determine the relative bioavailability of different Zn forms, for Zn mainly with Zn sulphate bioavailability as the reference value. The absolute apparent bioavailability in pigs has been established to be about 23 % for Zn sulphate, 22 % for Zn oxide and 19 % for Zn acetate (EC 2003).

The influence of the chemical composition on Zn bioavailability has been intensively investigated. Jongbloed et al. (2002) selected relative bioavailability studies based on appropriateness of the used bioavailability response criterions. The relative bioavailability values for several inorganic and organic Zn compounds in different animal species are summarized in Table 1.

Table 1. Relative bioavailability assessment of Zn compounds compared with Zn sulphate (from Jongbloed et al. 2002).

Zn compound	Pigs	Poultry	Ruminants
Zn sulphate	100	100	100
Zn carbonate	98	93	58
Zn chloride		107	42
Zn oxide	92	67	95
Zn amino acid chelate	102	131	102

In growing pigs fed a diet supplied with Zn oxide at 0, 30, 60 120 and 200 mg/kg DM in addition to the basic Zn concentration at 42 mg/kg DM, about 20 % of the Zn amount in the feed were retained by the animals. However, Zn retention decreased with an additional increase of exposure (Poulsen and Larsen 1995). Reduced percentage retention with increased feed concentrations of inorganic Zn as well as of Cu was also shown in other studies on pigs, and of Zn in broiler chicks (Veum et al. 2009; Linares et al. 2007).

The absolute apparent bioavailability of Cu is variable. Monogastric species absorb Cu more efficiently than ruminants and young more efficient than mature animals. The degree of absorption in humans exceeds 30 %, but is somewhat lesser in other species (Pond et al. 2005). Data on the influence of the Cu form on Cu availability for different animal species have been reviewed by Jongbloed et al. (2002) (Table 2).

Table 2. Relative bioavailability assessment of Cu compounds compared with Cu sulphate (from Jongbloed et al. 2002).

Cu compound	Pigs	Poultry	Ruminants
Cu sulphate	100	100	100
Cu carbonate	100	64	93
Cu oxide	74	24	76
Cu methionine	100	91	95
Cu lysine	94	100	104

Bioavailabilities of Zn from Zn^{+} salts in the diet are influenced by the dietary ingredients (from NRC 2012). Historically, Zn and Cu have been added to animal diets using inorganic salts such as oxides and sulphates. However, trace mineral salts tend to dissociate after uptake in the low pH environment of the anterior gastrointestinal tract, allowing interaction with other nutrient and ingredient potentially leading to impaired absorption, and thus decreased bioavailability (Suttle 2010). Chelation of trace minerals has the advantage that the binding of the organic ligand to the mineral can provide stability to the complex in the anterior gastrointestinal system, thus minimizing mineral losses and allowing the complex to be delivered to the absorptive epithelium in the small intestine (Richards et al. 2010). However, several studies and reports show that Zn and Cu from organic complexes seem to have approximately equal bioavailability's as the references Zn sulphate and Cu sulphate (NRC 2012; Schlegel et al. 2013).

The bioavailability of Zn and Cu seems, however, to be highly different in various organic complexes. This may be due to different stabilities at low pH, and unstable complexes can therefore not considerably increase bioavailability (Richards et al. 2010).

In a study on broilers by Manangi et al. (2012), a supplement of Zn, Cu and Mn as methionine hydroxyl chelates at 32, 8 and 32 mg/kg diet, respectively, were compared with supplements of Zn, Cu and Mn as sulphates at 100, 125 and 90 mg/kg, respectively. The birds fed the low mineral levels as chelates showed improved footpad health as well as reduced trace mineral concentrations in the litter. Richards et al. (2010) showed lower lipid hydroperoxide concentrations in plasma, indicating lower oxidative stress, in broilers fed diets supplied with Zn, Cu and Mn as methionine hydroxyl chelates or glycine chelated at 30, 5 and 20 mg/kg, respectively, compared with broilers fed with the same mineral concentrations as inorganic salts, as amino acid complexes or no supplements. No significant difference was found between the groups supplied with inorganic forms, amino acid complexes or no supplements. Improved immune development and response to vaccination was shown in broilers fed Zn methionine in place of inorganic Zn sources (Moghaddam and Jahanian 2009). Wedekind et al. (1992) compared bioavailability of Zn methionine, Zn sulphate and Zn oxide in chicks and indicated that Zn methionine provided more bioavailable Zn than Zn sulphate and Zn oxide, regardless of the diet employed.

In pigs, Nitrayova et al. (2012) showed increased retention of Zn methionine and Zn-yeast compared with Zn oxide, Zn proteinate and Zn glycine. Weanling pigs fed an equal mixture of methionine hydroxyl chelated and inorganic Zn, Cu and Mn showed a faster immune response to vaccination compared to pigs fed the same mineral concentrations in inorganic form only (Richards et al. 2010). Studies on pharmacological exposure levels of Zn and Cu are primarily discussed in Chapters 2.1 and 2.2.

Mineral availability may be affected by several other dietary constituents. Antagonisms occur between minerals, e.g. high levels of Zn reduce the availability of Cu and vice versa. Zn stimulates the synthesis of metallothionein, which has a higher affinity for Cu than for Zn (Waalkes et al. 1984). Induction of intestinal metallothionein concentrations may lead to increased trapping of dietary Cu in the enterocyte with a subsequent lowering of the Cu passage into plasma. O'Hara et al. (1960) showed that feeding high dietary levels of Cu (250 mg Cu/kg diet) led to parakeratosis, a condition linked to Zn deficiency. Furthermore, Zn counteracted the effects of Cu toxicosis in pigs (Suttle et al. 1966). On the other hand, feeding pregnant sows with high dietary Zn resulted in Cu deficient piglets, a condition that was reversible by Cu supplementation to the diet (Hill et al. 1983).

Zn can be absorbed through a variety of calcium (Ca) channels (Bouron et al. 2013). High dietary Ca intake may reduce Zn absorption and prevent adverse effects of excessive Zn intakes in pigs (Hsu et al. 1975). Vitamin D, a well-known regulator of Ca homeostasis, increases Zn uptake in chicken (Roberson et al. 1994) exemplifying the integration of Ca and Zn uptake mechanisms. Furthermore, excessive Cu and Zn intake may influence iron (Fe) absorption. Several minerals, among them Cu and Zn, compete with Fe uptake through the apical divalent metal transporter 1 (DMT1) at the enterocyte membrane, the main route of uptake for non-heme Fe (Gunshin et al. 1997). Fe can compete with Zn for uptake through Zn channels (SLC39) (Jeong et al. 2013), high dietary Zn can thus compete with Fe and vice versa. Thus, the availability of one metal is mutually dependant on the intake of other metals.

Zn from grains and plant proteins has a low bioavailability, which is enhanced by microbial phytase addition to the diet. Phytic acid, present in small grains, corn and soy, is able to form complexes with trace minerals that are very stable and highly insoluble, rendering the minerals unavailable for absorption. Phytate strongly reduces Zn sulphate availability in pigs (Oberleas et al. 1962) and broilers (O'Dell & Savage 1960). The formation of phytic acid-mediated complexes is amplified in the presence of Ca (Richards et al. 2010). However, Schlegel et al. (2013) performed a meta-analysis to investigate the bioavailability of Zn from native ingredients and supplements in dependence to dietary phytates and suggested that phytates mainly affect the availability of native Zn present in plant feedstuffs and do not interact with supplemented Zn. Fiber is another factor present in vegetable feed ingredients that may affect mineral bioavailability. Insoluble chelates may form with fiber and thus lead to decreased absorption, particularly in chicks. Mohanna et al. (1999) showed that increased intestinal viscosity due to water-soluble non-starch polysaccharides reduced Zn availability in chicks. Raw materials containing viscous fibers such as triticale, rye or barley at high levels in chick diets may contribute to decreased absorption and increased excretion of Zn, and the authors suggested that mineral availability could be increased in diets for chicks by adding enzymes to degrade non-starch polysaccharides. For pigs, there does not seem to be a significant effect of fibers on mineral availability, possibly because the viscosity of digest is higher in poultry than in pigs (Bedford and Schulze, 1998).

Zn is absorbed principally in the duodenum by an active saturable process at normal dietary concentrations (Suttle, 2010). In mono-gastric animals as pig and poultry, dietary antagonists may set a ceiling on maximum absorption, where phytate is of major potency in forming unabsorbable complexes with Zn. At concentrations above physiological requirements, the relative absorption declines.

Most of the total body Zn is in muscles and bones. Excess intake of Zn leads to an increased deposition in the bone, liver, pancreas and kidney. Concentrations in muscle, milk and egg are more stable (NRC 2005). Excretion of Zn occurs predominantly via pancreatic secretions and the faeces, with little Zn voides in the urine (Suttle 2010).

The liver contains the highest Cu concentration. Liver and kidney Cu concentrations are related to the dietary intake whereas muscle, milk and egg concentrations are more stable and seem to be little influenced by differences of exposure (Van Paemel et al. 2010). Adjustment to fluctuations in Cu supply is achieved by control of absorption, hepatic storage and biliary Cu secretion. These processes vary in their significance between animal species (Suttle 2010).

2.4 Deficiency and toxicity of Zn and Cu

Zn plays essential role in a wide array of processes including cell proliferation and animal growth, immune development and response, reproduction, gene regulation, and defence against oxidative stress and damage (Richards et al. 2010). The role of Zn in gene regulation is based on its incorporation into the structure of various transcription factors and hormone receptor proteins. Reflecting its role in gene regulation, Zn is required for the synthesis of a variety of enzymes and other proteins. The key structural proteins, collagen and keratin, both require Zn for their synthesis (Suttle 2010). Collagen is the major structural protein of the extracellular matrix and connective tissues in internal tissue, including cartilage and bone. Keratin is the major structural protein of the hoof and claw horn, skin, feathers, hair and beaks. Decreases in collagen and keratin synthesis and turnover rates in Zn-deficiency can lead to a variety of defects including bone abnormalities, decreased tissue strength and dermatite, and poor feathering (Suttle 2010).

Similarly as Zn, Cu is essential for a wide variety of health and performance-related functions in all animal species. Functions were Zn is involved, are often enhanced by Cu-dependent enzymes (Richards et al. 2010). For example, Cu promotes skin, bone, tendon and intestinal strength via the Cu-dependent enzyme lysyl oxidase which crosslinks collagen subunits into mature protein forms to increase their strength. This enzyme also crosslinks the structural protein elastin, which is present in connective tissue primarily in the cardiovascular system, intestine and other tissues that need to change size due to fill. Also keratin crosslinking is Cu-dependent.

Zn and Cu also play a key role in managing oxidative stress. Zn and Cu together are essential elements of the cytoplasmatic superoxide dismutase (SOD) which forms a first-line defence that converts reactive oxygen species (ROS) to the less toxic hydrogen peroxide, which are further inactivated by the Se-dependent glutathione peroxidase (Suttle 2010). Decreases in SOD activity, as in a mineral deficiency, can lead to increased damage of lipids, proteins and nucleic acids, which can induce cellular death via apoptosis. Zn may manage oxidative stress in other ways as well, including through induction of metallothionein, and as a cofactor in the p53 transcription factor, which mediates repair of DNA damaged f.e x. by oxidative stress (Richards et al. 2010). Poor oxidative stress management in production animals can result in decreased performance compromised immune function, increased morbidity and poor meat quality (Richards et al 2010; Suttle 2010).

In livestock, reduced feed intake and growth retardation or cessation are the first effects of Zn deprivation. Appetite for solid foods is sensitive to Zn, and may reflect the pivotal role of Zn in cell replication. Many of the adverse effects of severe Zn deficiency are secondary to loss of appetite. Deficiency is characterized by lesions of the integument and its outgrowths: hair, wool and feathers. More explicitly it leads to scaled skin in pigs (parakeratosis) and poor feathering in chicks. Bone growth is also affected, and impaired immunity is a generally observed characteristic (Van Paemel et al. 2010).

High dietary Zn intake may have a preventive effect on diarrhoea in piglets. It has been observed that these high supplementation levels support a large diversity of coliforms in weaned piglets and that they may reduce the pigs´ susceptibility to *E. coli* infections. These observations may contribute to the growth promoting effect of high dietary Zn in weaned piglets (Poulsen and Carlson, 2008).

Initial signs of Zn toxicosis in animals usually consist of reduced feed intake, growth rate, and other measures of performance or signs of secondary deficiencies of other minerals, such as Cu (NRC 2005).

Cu deficiency results in a wide range of signs in different animal species. Common signs in various animals include anemia, growth depression, bone disorders, depigmentation of hair, wool and feathers, demyelination of the spinal cord, fibrosis of the myocardioum, and diarrhoea. Usually depigmentation of the coat is the earliest clinical sign, whereas anemia usually develops only when deficiency is severe or prolonged (Van Paemel et al 2010).

Cu sulphate has been observed to quantitatively reduce some Gram positive bacterial populations in the gut e. g. *Streptococcus* spp. Investigations into the contribution of this antimicrobial property to the growth promoting effect has produced inconsistent results (Van Paemel et al. 2010).

Liver and kidney are target organs for Cu toxicosis in all species. Particularly in young animals, excess of Cu leads to reduced number of erythrocytes and anemia (NRC 2005). Sheep are particularly susceptible for copper toxicosis as Cu easily accumulates in the liver. At exceeded capacity of Cu storage in liver, liver necrosis occurs with release of Cu and Fe to blood characterized by hemolysis, hemoglobinemia and hemoglobinuria.

2.5 Exposure of Zn and Cu to pigs and poultry

Commercial compound feed is the main feed for pigs and poultry in Norway. The Norwegian industry, which produces compound feed for terrestrial animals including pigs and poultry, consists of companies grouped in three organisations, Felleskjøpet, Norgesfôr and Fiskå mølle. Their feed products are fortified with Zn and Cu. In Norway compound feed with standardized norms for nutrients including supplements of vitamins and minerals has been produced for pigs and poultry from 1957 and ca 1962, respectively (Flatlandsmo, 2012). The main Norwegian producer of complementary feed, feed premixes and medical remedies containing Zn and Cu is Normin AS. Felleskjøpet also produces some complementary feed for pigs based on premixes from Normin and others. It is known that treatment of pigs with Zn oxide made for technical use has occurred to a certain but unknown extent. Without control of product purity, possible contaminants or dosage given, such use is unacceptable and forbidden. To avoid uncontrolled use of nonregistered Zn oxide, a medical remedy of pure Zn oxide (VetZin) is registered in Norway but not yet marketed. The animal exposure of Zn and Cu via feed, medical remedies as well as drinking water and barn installations is discussed below.

2.5.1 Zn and Cu in complete compound feed

The approximate total amount of complete compound feed produced for pigs in Norway in 2012 based on the reported production from the three main industry organisations is 469,000 tons. The corresponding total amount of compound feed for poultry in 2012 is 428,000 tons.

The concentrations of Zn and Cu in the complete compound feed reported by the different producers are fairly similar. The mean concentrations of added and total concentrations of Zn and Cu reported by the feed producers are given in Table 3.

Table 3. Reported mean concentrations (mg/kg) of Zn and Cu added and total in complete compound feed for pigs and poultry produced by the main Norwegian feed companies, and estimated requirements (based on NRC 1994; NRC 2012; Suttle 2010).

Feed category	Zn added in feed	Zn total in feed	Zn total in feed DM	Zn requirement in feed	Cu added in feed	Cu total in feed	Cu total in DM	Cu requirement in feed
Piglet feed	120-130	150-155	170-176	40-80 (15 without phytate)	15-30	18-35	20-40	5
Growing pig feed	105-113	136-145	155-165	24-50	15-21	18-25	20-28	3.5
Pregnant sow feed	80-105	124-146	141-166		15-18	18-23	20-26	3.5
Lactating sow feed	91-105	129-139	147-158	40-80	15-18	19-23	22-26	3.5
Broiler chicken feed	83-100	113-134	128-152	30-55 (24-44 without phytate)	15-16	20-21	23-24	8
Laying hen feed	65-70	90-100	102-114	44-53	5-12	8-15	9-17	3-4
Broiler parent feed	105-109	135-140	153-159		11-15	16-20	18-23	
Turkey feed	83-100	122-135	139-153	35-65	15	20-21	23-24	6-8

In complete compound feed for pigs in Norway 2012, the estimated total amounts of Zn and Cu are 66,733 and 10,886 kg, respectively.

In complete compound feed for poultry in Norway 2012, the corresponding total amounts of Zn and Cu are 48,369 and 7,980 kg, respectively.

These figures are based on turnover of the various categories of compound feed and their concentrations of Zn and Cu reported from the industry organisations.

In general, the feed for piglets contains higher concentrations of Zn than any other feed, with 150-155 mg Zn/kg as mean concentrations reported from the producers. The feed for piglets may also contain more Cu than other feed. The amount of piglet feed turnover in 2012 was approximately 40,756 tons, which constitutes 8.7 % of total amount pig feed. The estimated amount of Zn in the piglet feed was 6,158 kg, which is 9.2 % of the total amount of Zn pig feed.

The estimated amount of Cu in the piglet feed was 1,209 kg, which is 11.1 % of the total amount of Cu in pig feed.

The Norwegian Food Safety Authority (NFSA) organises annual surveillance programs where the concentrations of several nutrients and undesirable components in animal feed are reported. The sampling frequency was one sample per 2000 tonnes of feed for the years between 2005-2007, but this has decreased in the subsequent years. The results comprise analyses from complete feed to production animals, feed materials, premixes, protein concentrates, additives and medical remedies (NFSA 2006, 2007, 2008, 2009, 2010, 2011). As a member state of the The European Economic Area (EEA), Norway enacts parts of the legislation passed in the European Union. The EU legislation concerning feed additives is fully adopted by Norway. The maximum permitted content of Zn and Cu is stated in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 and in “Forskrift om fôrvarer” (FOR-2002-11-07-1290). The current maximum permitted content is currently 25 mg Cu/kg (35 mg Cu/kg for piglets) and 150 mg Zn/kg of complete feeding stuff for pigs and poultry. The results for content of Zn and Cu in complete compound feed for pigs and poultry in the years 2005-2010 from the NFSA’s annual surveillance program are summarised in Figure 1 and Figure 2.

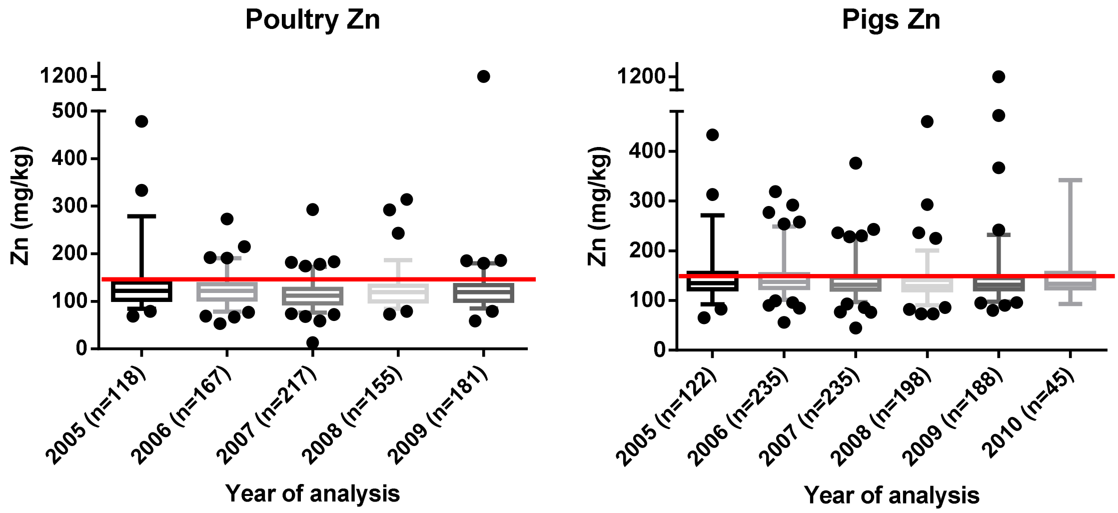


Figure 1. Concentrations (mg/kg) of Zn in complete compound feed to poultry (2005-2009) and pigs (2005-2010). Boxes show median (line), 25 and 75 percentile, whiskers show 2.5 and 97.5 percentile, “•” shows single values beyond 2.5 and 97.5 percentile. Red line shows maximum permitted content of Zn in complete feed for pigs and poultry.

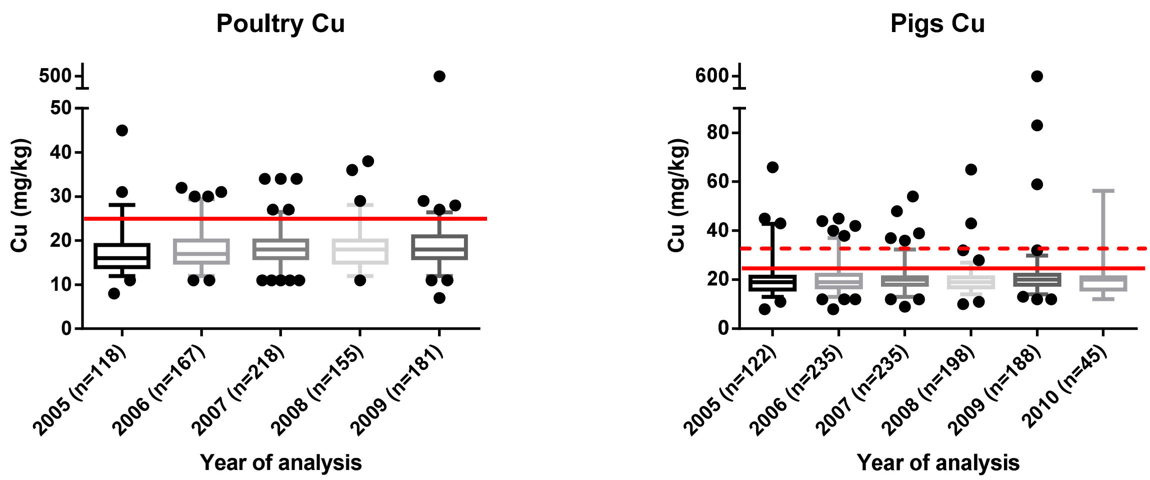


Figure 2. Concentrations (mg/kg) of Cu in complete compound feed to poultry (2005-2009) and pigs (2005-2010). Boxes show median (line), 25 and 75 percentile, whiskers show 2.5 and 97.5 percentile, “•” shows single values beyond 2.5 and 97.5 percentile. Red line shows maximum permitted content of Cu in complete feed for pigs and poultry. Red dotted line shows maximum permitted content of Cu in complete feed for piglets up to 12 weeks of age.

The results show median Zn and Cu concentrations for both pig and poultry feed below the EU upper limits. Several samples showed concentrations of Zn or Cu above the limits, particularly for pig feed. Considerable number of samples of feed for pigs exceeding the limits of Zn and is also reported from Denmark (Fødevarestyrelsen, 2012). In the years 2005-2009 and 2005-2010 the yearly production of poultry and pig feed ranged between 446 – 486 000 tons and 293 – 384 000 tons, respectively (www.slf.dep.no).

2.5.2 Zn and Cu in complementary feed

There are several feed additive products on the Norwegian marked containing Zn and Cu. Most products are used to piglets. The products we have obtained at the Norwegian market contain Zn oxide and Cu disulfate, and are as follows: VitaMineral Svin Mehus (4000 mg Zn and 260 mg Cu/kg), Provital ABDE (5000 mg Zn and 300 mg Cu/kg), VitaMineral Svin (2500 mg Zn and 275 mg Cu/kg), Ferro-Pel (1300 mg Zn and 550 mg Cu/kg), Pluss Multitilskudd Svin Felleskjøpet (4000 mg Zn and 260 mg Cu/kg), Pluss Jerntilskudd Felleskjøpet (936 mg Zn and 585 mg Cu/kg), Normin Ferro-Torv (150 mg Zn and 50 mg Cu/L), Normin A-V Torv (375 mg Zn/L) and Normin Ferro-Pasta (530 mg Zn and 8000 mg Cu/L).

Based on sales turnover of these products in 2012 and their concentrations of Zn and Cu, the estimated total amounts of Zn and Cu in these complementary feed are 433 kg Zn and 51 kg Cu. The amounts of Zn and Cu in complementary feed for pigs constitute low amounts (in addition 7 % and 4 %, respectively) compared with the amounts of these trace elements in complete feed.

No feed additives are used as complementary feed for poultry in Norway.

Furthermore, there is the special category of complementary feed to be used for mixing into the feed at farms which produce their own feed, the farm premixes. These are primarily used in farms which make wet-feed for pigs. In 2012 Normin AS produced farm premix with Zn and Cu for 39,000 tons wet-feed for pigs, corresponding to 10,500 tons compound feed. The normal concentrations of added Zn and Cu in the complete wet-feed are 118 mg Zn/kg DM and 15 mg Cu/kg DM. The approximate total amounts of Zn and Cu used in these farm premixes are 1,070 kg Zn and 133 kg Cu.

2.5.3 Use of Zn in medical remedies

The main medical remedy based on Zn is Normin Sink, which consists of 8 % Zn (10 % Zn oxide). VetZin, which is pure Zn oxide, is a registered remedy for import from Denmark but not yet marketed in Norway. The extent of use in pig herds of pure Zn oxide meant for technical use is not known. The indication for use of these Zn based remedies is to prevent diarrhoea and oedema disease in weaned piglets. The remedy is blended into the peat dust or the feed. In Norway, the recommended treatment of Zn against weaning diarrhoea is 2,400 mg/kg feed for 2 weeks, and against oedema disease 2,800 mg/kg feed for 2 weeks and 1 week with gradual reduction (Baustad et al. 2002; Lium and Baustad, 2002).

The estimated amount of Zn from the medical remedies is 4,130 kg in 2012 (data for Normin Sink, only). This amount of Zn represents an addition of 67 % to the Zn in complete feed for piglets.

No medical remedies based on Cu are registered in Norway.

2.5.4 The high Zn exposure to piglets via feed and medical remedies

The estimated total amount of Zn in complete feed for piglets was 6,158 kg in Norway in 2012. In comparison with the estimated total amount of Zn in complementary feed at 433 kg, where most complementary feed is used for piglets, show that the Zn exposure via complementary feed constitutes a supplement at a rather low total amount. On the other hand, the estimated use of Zn in medical remedies is much higher. The available figure for Zn in medical remedies at 4,130 kg indicates that the total Zn exposure to piglets via medical remedies is almost at a similar level as the total Zn exposure via complete compound feed. The use of Zn as medical remedy is used at narrower period of time (about 2 weeks) compared with use of piglet feed, commonly used for about 4 weeks from weaning.

2.5.5 Zn and Cu in drinking water

In very general terms pigs consume 2-2.5 L water per kg dry feed at moderate temperatures. Corresponding figures for poultry are 2-3 L water (Pond et al. 2005). The Zn concentration in Norwegian drinking-water is not regulated by rules. In natural surface waters, the concentration of Zn is usually below 10 µg/L, and in groundwaters 10-40 µg/L. In tap water, the Zn concentration can be much higher (about 1 mg/L) as a result of the leaching of Zn from piping and fittings (WHO, 2003). In comparison with the Zn exposure via the feed, the exposure via drinking water will usually represent a negligible amount.

The concentration of Cu is regulated in Norway in "Drikkevannsforskriften- for water for human consumption" with a limit 0.1 mg/L. Cu is found in surface water, groundwater, seawater and drinking-water, but it is primarily present in complexes or as particulate matter. Cu concentrations in surface water ranged from 0.0005 to 1 mg/L, with median value 0.01 mg/L in several studies from the USA (referred by WHO, 2004). Cu concentrations in drinking-water vary widely as a result of water characteristics, such as pH, hardness and availability in the distribution system. Results from a number of studies from Europe, Canada and USA indicate that Cu levels in drinking-water can range from ≤0.005 to >30 mg/L, with the primary source most often being the corrosion of interior Cu plumbing (referred by WHO, 2004). Levels of Cu in running or flushed water tend to be low, whereas those of standing or partially flushed water samples are more variable and can be substantially higher. As a conclusion, Cu exposure from tap water for Norwegian pigs and poultry is assumed normally not to contribute significantly to the total Cu exposure. However, in certain situations the Cu content in drinking-water may probably represent a certain contribution.

2.5.6 Zn in barn installations

Zn is used as coating of the steel in barn installations. In Norway this galvanizing is used by Norsk overflateteknologi (NOT). The thickness of the Zn coat is usually 80-100 µm. In addition to Norwegian barn equipment, such products are also imported and may have been galvanized in Poland, Denmark, France, Baltic countries and others. The process may differ and also the possible inclusion of other metals. In Norway, warm galvanizing with high grade Zn content (>99.9 % Zn) is used. Some of the products are treated with a sealer outside the Zn coating. The recent development in barn installations is use of metal as a frame for plastic components (or glass fibre or hard wood). Furthermore, concrete and stainless steel are used in barn installations to a considerable extent. This implies less amounts of Zn in newer products compared with older barn installations.

The general fair wear and tear of the Zn coat is 1 µm per year (personal communication with Geir Ove Salte, NOT). Use of detergents with low pH implies faster corrosion of the Zn coat.

In barns for pigs the duration of the Zn coat is about 15-20 years (personal communication with Odd Jan Dybing, Reime Agri As, Norway), which means a yearly wear at about 5 μm per year. The estimated surface of metal barn around an adult sow is about 0.5 m^2 (in new barns) to about 1 m^2 (in older barns).

The estimated worst case yearly release of Zn per sow pen may be as follows: 5 μm x 1 m^2 = 5 cm^3. With a density of Zn at 7.133 g/cm^3 this corresponds to 36 g/year, which gives a daily release of 100 mg. Some of this Zn release may be ingested by the sow due to licking and biting on the barn but the ratio of the released Zn available for exposure is probably low. Compared to the Zn exposure via diet (a feed intake of a non-lactating sow at 2 kg/day with an average Zn content at 135 mg/kg) the oral exposure of Zn via barn installation, is probably not significant. The sow is here used as an example, and a similarly low Zn exposure via barn installations compared with Zn exposure via the diet is also expected for other pigs as well as for poultry. However, most of the Zn release from the barns, ingested or not, ends in the manure. In the example of sows, Zn release from the barn may constitute a considerable part (approximately 25%).

2.5.7 Comparison of exposure and requirement of Zn and Cu for pig and poultry

The exposure of Zn and Cu to pig and poultry via complete compound feed in Norway and the corresponding knowledge of requirement are shown in Table 3. The data indicate exposure of Zn and Cu to pigs and poultry at least 2 times higher than the requirement but most often several times above required level. The Zn exposure via complete compound feed may be up to 6 times higher that required to growing pigs and up to 4 times higher to broiler chicks and turkeys. The exposure of Cu via complete feed exposure may be up to 6-7 times higher than required to pigs, and up to 5 times higher than required to laying hens.

In piglets, the Zn and Cu exposure via complete compound feed is 2-3 times higher than the Zn requirement and 3-7 timed higher than the Cu requirement. Piglets are commonly also exposed to complementary feed with Zn and Cu, as well as Zn in medical remedies. The estimated extra exposure via complementary feed is rather marginal, in average 7 % and 4 % more than exposure via complete feed for Zn and Cu, respectively. However, the medical used of Zn is considerable. The use of Zn as medical remedy against diarrhoea and oedema disease in weaned piglets is at a level of approximately 15-20 times higher than the exposure via complete feed, which is 30-60 times higher than the reported requirement.

The Zn and Cu in drinking water are normally at low concentrations which are considered to constitute an insignificant exposure. In pig barns with Zn-coated installations may Zn from this source constitute a significant contribution to Zn in manure but probably not influence significantly on the Zn exposure to the individual pig.

In sum, as the bioavailability of trace elements as Zn and Cu influence each other, particularly at high exposure levels, a “security” of feeding the animals with exposure levels far above required levels may not be the optimal strategy. The results of such assumed overload are reduced bioavailability of Zn, Cu and other essential trace elements and elevated levels in the manure. Furthermore, as shown in the following chapters, in the long run elevated concentrations in manure may influence on organisms in the food chain, ranged from the soil microbiota to humans.

3. Zn and Cu in manure

3.1 Estimated concentrations in manure

The concentration of Zn and Cu in manure is determined by three main factors:

1. Concentration of Zn and Cu in feed
   From Table 3, the mean concentrations of Zn and Cu in complete compound feed for piglets are approximately 170 mg Zn and 31 mg Cu/kg DM (Table 4). For other pigs the mean figures are 155 mg Zn and 25 mg Cu/kg DM, and for poultry 142 mg Zn and 20 mg Cu/kg DM. Piglets primarily, may also receive some complementary feed with Zn and Cu as well as medical remedies with Zn. The total amounts of Zn and Cu via complementary feed represent approximately 7 and 4 % in addition to the total amounts via complete feed for piglets, whereas the total amount of Zn in medical remedies is estimated to represent an addition of approximately 67 % to the Zn in complete feed for piglets (see details in Chapter 2). Also drinking water contains Zn and Cu but the concentrations are usually very low and negligible compared with the feed.

2. Retention of Zn and Cu in the body
   As mentioned above (chapter 2.3), the retention factors for Zn and Cu in the animal are approximately 20 % and 30 %. These percentages are used both for pigs and poultry in the calculations. Zn shows lower retention at high feed concentrations far above the requirement as when used complementary feed and medical remedies. For such high Zn exposure via complementary feed and medical remedies 10 % retention is used in the calculations.

3. The retention of feed in the body
   It is assumed that 70% of the feed is utilized in the animal (i.e. 1000 grams of feed becomes 300 grams of manure). This factor is used both for pigs and poultry.

Also a fourth factor may be of concern; the Zn in barn installations. In some cases the release of Zn from the barn may constitute a considerable part (approximately 25%; see Chapter 2.9).

The calculated concentrations of Zn and Cu in manure using the mean concentrations in complete feed, are shown in Table 4. The calculated supplement of Zn and Cu in piglet manure via use of complementary feed to piglets is 36 mg Zn/kg and 3 mg Cu/kg, i.e. 8 and 4 % increments. The calculated supplement of Zn in piglet manure via use of medical remedies to piglets is 342 mg/kg, i.e. 75 % increase from the calculated concentration in manure from complete feed for piglets. The calculations indicate that the concentrations of Zn and Cu in manure may vary considerably, depending on the amount of complementary and medicinal feed being used at the farm.

Table 4. Average concentrations of Zn and Cu in complete compound feed used for pigs and poultry and correspondingly calculated concentrations in manure.

	Complete feed mg/kg DM		In manure from complete feed mg/kg DM	
	Zn	Cu	Zn	Cu
Piglets	170	31	453	72
Other pigs	155	25	413	58
Poultry	142	20	379	47

3.2 Measured concentrations in manure

The most recent investigation on the content of Zn and Cu in pig and poultry-manure are from analyses of manure collected in 7 counties in 2010 and 2011 (Daugstad et al. 2012).

Table 5. Concentrations of Zn and Cu (mg/kg DM) in pig and poultry manure from 2010 and 2011 (Daugstad et al. 2012). The concentrations for poultry are mean concentrations for hen, chicken and turkey.

Manure	Zinc			Copper			No of samples analysed
	10 %	Mean	90 %	10 %	Mean	90 %	
Pig	263	636	1078	54	95	148	14
Poultry	201	343	561	41	62	115	14

According to Daugstad et al. (2012), the results are somewhat higher than earlier Norwegian data for Zn and Cu in pig manure, and at the same level as Zn and Cu in Swedish poultry manure.

3.3 Comparison of calculated and measured concentrations

The calculated mean manure concentrations using complete feed (Table 4) are within the range of measured concentrations of Zn and Cu in manure (Table 5). With the supplement of Zn from medical remedies and Zn and Cu from complementary feed for piglets, as well as Zn in barn installations the calculated concentration are quite similar the measured concentrations.

In conclusion, the calculated and measured concentrations of Zn and Cu pig and poultry manure are in the same concentration range. In the assessment of Zn and Cu in the environment and the food chain the measured concentrations in manure are used (mean and 90th percentile) (Table 5).

4. Selected agricultural regions used for the risk assessment

The risk posed by Zn and Cu in manure to plants, soil fauna and aquatic organisms is influenced by regional differences in agricultural practice, , climate and characteristics of soil and surface water. In order to account for these regional differences, the risk assessment has been performed for three major agricultural regions where pig and poultry manure is applied; 1) Region Jæren in southwestern Norway, 2) Region Solør in Southeastern Norway and 3) Sør-Trøndelag in central Norway.These regions are represented by the municipalities Klepp, Melhus and Åsnes (see figure 3).

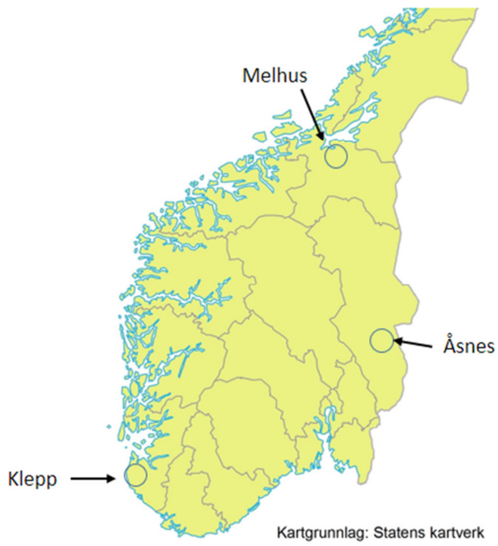


Figure 3. Map of southern Norway showing the location of the scenarios used in risk assessment.

4.1 Region Jæren (Klepp)

Klepp municipality is located in the coastal lowland area of Rogaland in soutwestern Norway. Agricultural land covers 72% of the municipality.

The usual crop rotations in the region are 4 years with meadow. After the 4th year the meadow is ploughed. In the coming spring new meadow is sown mixed with grain (1-2 kg barley/daa) or legumes (13-14 kg legumes/daa). This crop is harvested and utilized as animal feed. A second harvest with meadow may often be cut later the same year. After this alteration the practise of 1.-2.-3.-4. years with meadow is repeated. Manure is used on grain, green feed and meadow, rare on potato and vegetables. The manure is applied on meadow in the spring and also after the first harvest in June. Additionally, cultivated pasture is used as spreading area of manure in the early spring, i.e. the end of March (2 tonns/daa). The soil type in the community Klepp is mainly sandy loam. Further characteristics of the soil type are presented in Table 8.

River Figgjo drains an area of 228 km^2 south of Stavanger. The lower reach of the river runs through the Klepp municipality. River Figgjo is included in the Norwegian riverine input program (RIP) to the North Sea, and is sampled on a monthly basis for a number of water quality parameters (Høgåsen et al. 2011). The data from the period 2004-2009 has been used to calculate mean and percentile values for pH and concentrations of TOC, Zn and Cu. Calcium is not included in RIP but one observation (at Bore Bridge 2012) from the database “Vannmiljø” (vannmiljø.miljødirektoratet.no) has been used as calcium concentration for River Figgjo.Water quality characteristics relevant for the risk assessment are shown in Table 6.

4.2 Region Solør (Åsnes)

The Solør region is located in the valley of River Glomma. Much of the valley floor in Åsnes municipality is cultivated.

The usual crop rotations are either 1. -2.grain and -3.potato or 1.-2.-grain and 3. -4. -5. meadow. For those farms where manure is available the manure is spread in spring before sowing of grain. Manure is also spread before new establishment of meadow, but rare on established meadow. Manure is not used on potato. The soil type is silt. Åsnes municipality has been selected as representative for the Solør region. The dominating soil type in the municipality is silt. Further characteristics of the soil type are presented in Table 8.

River Hasla has been selected to represent surface waters in the agricultural areas of Åsnes. River Hasla drains an area of 200 km^2 west of River Glomma. Agriculture covers approximately 17% of the area, mainly in the lower range in the Åsnes municipality near the outlet into River Glomma. Physico-chemical data are available from one sampling only. The sample was taken near the outlet in August 2012 (vannmiljø.miljødirektoratet.no). Water quality characteristics relevant for the risk assessment are shown in Table 6.

4.3 Region Sør-Trøndelag (Melhus)

The Melhus municipality in Gauldalen area in Sør-Trønderlag has a broad variation in agronomic practise, but usual crop rotations are also there either 1. -2.grain and -3.potato or 1.-2.-grain and 3. -4. -5. meadow. Manure is applied in addition to mineral fertilizer at grain and meadow, rare at established meadow and not on potato and vegetables. The soil type is silty clay loam. Melhus municipality has been selected to represent the region. Melhus is located south of the Trondheim Fjord. The arable areas in Melhus are mainly on the valley floor of River Gaula. The soil type is silty clay loam. Further characteristics of the soil type are presented in Table 8.

River Gaula has a drainage area of 3659 km^2 extending from the mountain areas at the Swedish boarder in the west to the mouth in the Trondheim fjord. Agricultural areas are mainly located in Melhus community in the lower part of the drainage area. Due to the high discharge, influence of agriculture on the water quality is limited. However, no complete data sets have been found for smaller local streams in the region and therefore River Gaula will be used as a basis for aquatic risk assessment for the Melhus region. River Gaula is included in the Norwegian riverine input program (RIP) to the North Sea, and is sampled four times per year (Høgåsen et al. 2011). Data from the period 2004-2009 have been used to calculate mean and percentile values for flow and pH and concentrations of TOC, Zn and Cu. The calcium concentration has been derived from the “Elveserien” program (Saksgård and Schartau 2011), (mean value for the period 2000-2010). Water quality characteristics relevant for the risk assessment are shown in Table 6.

Table 6. Water quality parameters for rivers in the Klepp, Melhus and Åsnes municipalities used for the aquatic risk assessment (annual 50-percentiles)

Scenario	River	pH	SPM	TOC	Ca	Cu	Zn
			mg/L	mg/L	mg/L	µg/L	µg/L
Klepp	Figgjo	7.1	2.8	2.9	6.39[a]	1.0	4.9
Åsnes	Hasla	6.75[a]		16.4[a]	5.26[a]	0.11[a]	4.17[a]
Melhus	Gaula	7.3	8.2	3.7	7.40[b]	1.7	3.8

a) single value

b) annual mean value

5. Zn and Cu in soil

5.1 Background

The aim of this chapter is to estimate the soil concentrations of Zn and Cu in 10, 50 and 100 years perspective after annual application of manure from pigs and poultry. The material flows affecting the amount of Zn and Cu in soil, the soil accumulation, include input from manure, surface runoff, leaching, plant uptake and harvest. The soil factors which mainly influence the bioavailability and mobility of Zn and Cu are pH, soil organic matter and clay content.

The soil concentrations of Zn and Cu are estimated for each year in a 100 year timeframe. This timeframe should be sufficient to uncover undesirable effects of soil accumulation after use of manure.

The model calculations of future soil concentrations involve three main sections:

1. Input of Zn and Cu to soils from pig and poultry manure and atmospheric deposition
2. Soil processes and constants that influence the fate of Zn and Cu in soils
3. Removal processes of Zn and Cu from soils (leaching and plant uptake)

5.2 Input of Zn and Cu to soils

5.2.1 Manure application

Manure has been applied to agricultural soils during centuries, but it is only during the last 50-60 years Zn and Cu have been added to feeds resulting in elevated concentrations of these metals in manure.

To calculate the amount of Zn and Cu applied to soil using manure, two application rates were selected: 20 kg phosphorous (P) and 70 kg P/ha year. 20 kg P/ha is the “normal” (or average) amount of P added annually to cover the plant requirement. 70 kg P/ha is a high dose, but in some areas with high density of pig- and poultry farms, this amount of P could be applied.

Pig and poultry manure contains about 17 gram P/kg DM (Daugstad et al. 2012). To add a dose of 20 kg P/ha, 22 tons of pig manure or 2.3 tons of poultry manure (having dry matters of 5% and 53%, respectively) have to be applied. Correspondingly, a dose of 70 kg P/ha means application of 77 tons of pig manure or 8.1 tons of poultry manure.

Table 7. Annual application rates of Zn and Cu to soils when applying 20 and 70 kg P/ha as pig or poultry manure. 10 %, mean and 90 %: 10th percentile, mean and 90th percentile of Zn and Cu concentrations in manure (Table 5).

	Application rate: 20 kg P/ha						Application rate: 70 kg P/ha					
	Zn (g/ha/year)			Cu (g/ha/year)			Zn (g/ha/year)			Cu (g/ha/year)		
	10 %	Mean	90 %	10 %	Mean	90 %	10 %	Mean	90 %	10 %	Mean	90 %
Pig	308	744	1260	63	111	172	1077	2603	4411	219	390	603
Poultry	265	451	739	54	81	151	927	1580	2587	189	285	530

5.2.2. Atmospheric deposition and other sources

Atmospheric deposition is considered a significant source of Zn and Cu to soils i.e. more than 1% of what is applied in areas fertilised with pig and poultry manure.

Mineral fertilisers and liming products are not considered to be significant sources of Zn and Cu in areas fertilised with pig and poultry manure.

Of Zn or Cu containing pesticides on the Norwegian market are a preparation based on Cu oxide and mankozeb with Zn. Cu oxide is used in the production of fruits, berries and Christmas trees at a maximal dose of 3,050 g Cu/ha year. Mankozeb is used on potatoes (approximately 300 g Zn/ha per year) and vegetables (approximately 40 g Zn/ha per year). The Norwegian sales of the pesticide preparations correspond to approximately 2,750 kg Cu and 24,000 kg Zn/year (Norwegian Food Safety Authority, personal communication). The pesticide use does not necessary cover the same areas as fertilised with pig or poultry manure, and is not calculated in the models of the present assessment. However for comparison, the pesticidal use of Cu oxide constitutes a highly significant contribution of Cu to soil and corresponds to approximately 8 times higher yearly dose than that from a high dose application of pig manure. The total amount of the pesticide Cu oxide covers however only about 900 ha of Norwegian agricultural soil. The contribution of Zn from mankozeb on potatoes and vegetables constitutes about 11.5 % and 1.5 % of the mean Zn from high dose application of pig manure.

Concerning the calculations of the contribution of atmospheric deposition of future soil concentrations of Zn and Cu following long term manure application, an annual atmospheric input of 40 grams of Zn and 11 grams of Cu per ha is used for all three regions. These amounts are average values based upon monitoring data from the rural air- and precipitation chemistry monitoring network in Norway during the last years (2006-2012; eg. Aas et al. 2012).

The input from atmospheric deposition is calculated according to equation 1:

$$Catm_{soil1} = \frac{Atmospheric\ deposition_{Flux}}{DEPTHsoil \cdot RHOsoil} \qquad \textbf{Eq. 1}$$

Where

$Catm_{soil1}$	= concentration in soil after first year of atmospheric deposition [mg kg^{-1}]
Atmospheric $deposition_{Flux}$	= annual average atmospheric deposition flux [mg·m^{-2}]
$DEPTH_{Soil}$	= mixing depth of soil [0,2m]
RHO_{Soil}	= bulk density of soil [kg m^{-3}] (Table 8)

5.3 Soil processes and constants that influence the fate of Zn and Cu in soils

5.3.1 Soil depth

A soil depth of 20 cm is used when calculating soil concentrations after manure application. The change in soil density and pH due to manure application is not accounted for.

5.3.2 Soil background concentration

There exist no historic Norwegian data indicating the premanure soil concentrations. In this assessment we therefore consider the past application of manure as part of the soil background concentration. Zn and Cu soil background (Table 8) is the present concentration of these metals in the local soils and are considered a result of all former applications of Zn and Cu to these soils.

The soil background concentrations used are from a survey performed in 1993 including agricultural soil samples from southeastern (Hedmark), southwestern (Agder/Rogaland) and central parts (Nord- and Sør-Trøndelag) of Norway (Esser 1996).

5.3.3 Organic matter, pH, density and texture

The soil content of organic matter, soil pH and soil density varies much less than soil texture between the sites. Fine textured soils dominate at Melhus, and sandy/silty soils dominate at Klepp and Åsnes (Table 8). The soil data are taken from "Jorddatabanken" (Bioforsk 2013), a large agricultural soil database including several 100 000 samples. The soil types are the most frequent of the soils in each region (Table 8), but there are considerable variations in soil properties within the municipalities (especially texture).

5.3.4 Distribution coefficient for Zn and Cu in soil

The distribution of an element between the solid phase and water phase (Kd,soil) in soil is a constant that describes the soil binding strength. The higher the Kd-value, the stronger is the binding to soil solid phase. The binding or sorption to the solid phase is highly dependent on the properties of both the compound itself and the soil (pH, organic matter and clay content).

In this risk assessment of Zn and Cu in soil, Kd-values have been calculated using algorithms suggested by Degryse et al. (2009).

$$\log K_d(Zn) = 0.66 \cdot pH + 0.79 \cdot \log(\%Org.C) - 1.77 \qquad \text{Eq. 2}$$

$$\log K_d(Cu) = 0.34 \cdot pH + 0.65 \cdot \log(\%Org.C) + 0.45 \qquad \text{Eq. 3}$$

The values for pH and % org C (loss on ignition) used in the calculations are given in Table 8.

5.3.5 Rainfall and leaching

There are large variations in annual precipitation across Norway. Due to humid and cold climate in Norway, the precipitation exceeds the evaporation in all major agricultural areas.

In the southeastern parts of Norway, the precipitation varies from 300-800 mm. At Åsnes the annual mean precipitation the last 10 years (2003-2012) was 672mm (Table 8). This region of Norway is characterised by relatively high mean temperature, little wind and low air humidity. It is assumed that 40% of the precipitation infiltrates the soil on an annual basis (rate of infiltration 0,4).

Most of the agricultural areas in the western part of Norway have a precipitation in the range of 1200-1500 mm annually. In general, this region is the windiest in Norway and has the highest relative air humidity. An annual precipitation of 1464mm has been used for Klepp and 1236 for Melhus municipalities (mean precipitation during the last 10 years) and an infiltration rate of 0,7 has been at both sites (Table 8).

Table 8. Input data for the calculations of soil concentrations in Klepp, Melhus and Åsnes municipalities. See text for explanations.

	Municipality	Klepp	Melhus	Åsnes
	County	Rogaland	Sør-Trøndelag	Hedmark
Soil properties	Cu soil background	9	19	21
	Zn soil background	25	50	77
	LOI (%)	3.4	7.1	3.7
	OC (%)	2.0	4.2	2.2
	pH ($CaCl_2$)	5.9	6.2	5.9
	Soil dry density (kg/m3)	1100	1100	1200
	Soil type	Sandy loam (clay 6%)	Silty clay loam (clay 32%)	Silt (clay 6%)
	K_{dSoil} Cu (L/kg)	448	915	474
	K_{dSoil} Zn (L/kg)	230	649	246
Precipitation and infiltration	mm/year	1464	1236	672
	Rate of infiltration (F)	0.7	0.7	0.4
	Infiltration (mm/year)	1025	865	269
Crop rotation*		Gras-gras-gras-gras-barley	Barley-barley-barley-gras-gras-gras	Barley-barley-barley-gras-gras-gras
			Barley-barley-potatoes	Barley-barley-potatoes

*For Melhus and Åsnes the plant removal of Zn and Cu are calculated using the mean of the two crop rotations.

5.4 Removal of Zn and Cu from the soil

5.4.1 Leaching of Zn and Cu from soil

The leaching rate from the soil is calculated according to equation 4:

$$k_{leach} = \frac{F\inf_{soil} \cdot RAINrate}{K_{soil-water} \cdot DEPTH_{soil}} \qquad \textbf{Eq. 4}$$

Where

$Finf_{soil}$	= fraction of rain-water that infiltrates into soil (Table 8)
RAINrate	= rate of wet precipitation (mm/year) [m day^{-1}] (Table 8)
$K_{soil-water}$	= soil-water partitioning coefficient [$m^3 \cdot m^{-3}$]
$DEPTH_{soil}$	= mixing depth of soil (0.2m)
k_{leach}	= first order rate constant for leaching from soil layer [day^{-1}]

The soil-water partitioning coefficient, $K_{soil\text{-}water}$, can be calculated using equation 5:

$$K_{soil-water} = Fair_{soil} \cdot K_{air-water} + Fwater_{soil} + Fsolid_{soil} \cdot \frac{Kd_{soil}}{1000} \cdot RHOsoil \qquad \textbf{Eq. 5}$$

Where

$Fwater_{soil}$	= volume fraction of water in soil compartment [$m^3 \cdot m^{-3}$] (0.2, EC 2003)
$Fsolid_{soil}$	= volume fraction of solid in soil compartment [$m^3 \cdot m^{-3}$] (0.6, EC 2003)
$Fair_{soil}$	= volume fraction of air in soil compartment [$m^3 \cdot m^{-3}$] (0.2, EC 2003)
RHOsolid	= density of the solid phase [kg m^{-3}] (2500)
Kd_{soil}	= solids-water partition coefficient in soil [l kg^{-1}]
$K_{air\text{-}water}$	= air-water partitioning coefficient [$m^3 \cdot m^{-3}$]
$K_{soil\text{-}water}$	= soil-water partitioning coefficient [$m^3 \cdot m^{-3}$]

5.4.2 Uptake and elimination processes in plants

In the calculation of plant removal of Zn and Cu from soils, the concentration in the crop and harvested yield has been taken into account. Three scenarios for crop rotations have been assessed:

1. Gras-gras-gras-gras-barley (Klepp)
2. Barley-barley-barley-gras-gras-gras (Melhus and Åsnes)
3. Barley-barley-potatoes (Melhus and Åsnes)

The plant removal rate, k_p (day^{-1}), was calculated according to equation 6:

$$k_{plant} = \left(\frac{CP \cdot C_{crop}}{DEPTH_{soil} \cdot RHO_{soil} \cdot C_{Manure-soil} \cdot 0{,}001} \right) \cdot \frac{1}{365} \qquad \textbf{Eq. 6}$$

Where

k_{plant}	= plant removal rate, k_{plant} [day^{-1}]
CP	= crop production [kg DW $\cdot m^{-2} \cdot$ $year^{-1}$]
C_{plant}	= concentration in plant [$g \cdot kg^{-1}$ DW]
$DEPTH_{Soil}$	= mixing depth of soil [0.2m]
RHO_{Soil}	= bulk density of soil [kg m^{-3}] (Table 8)
$C\text{-}_{Manure\text{-}soil}$	= concentration in soil due to manure application t=5, 25 and 50 years [mg kg^{-1}]+background

The concentrations of Zn and Cu in agricultural crops are calculated using bioconcentration factors (BCFs) (Table 9), an approach assuming that plant concentrations (stem, leaf, grain etc.) are proportional to soil concentrations. In the calculations of future soil concentrations at 10, 50 and 100 years, the annual removal rate through crops was calculated using the soil concentration after respectively 5, 25 and 50 years. This is assumed to be the best estimate of average removal rate in the periods (i.e. the periods of 10, 50 and 100 years).

The BCFs are based upon empirical data where different plant species are grown on soil with varying properties. BCFs may differ not only between soils and plant varieties, but also between plants within the same plant variety (i.e. between geno types within the same variety).

In this risk assessment BCFs for potatoes, cereals and gras have been used. The BCFs are similar to the BCFs used in the risk assessment of contaminants in sewage sludge (VKM 2009).

$$Cplant_{potatoe,cereal,gras} = Csoil \cdot BCF_{potatoe,cereal,gras} \quad \textbf{Eq. 7}$$

Where

$Cplant_{potatoe,\ cereal,\ gras}$ = concentration in potatoe, cereal, gras [mg kg^{-1} dw]

Csoil = total concentration in soil [mg kg^{-1} dw]

$BCF_{potatoe,\ cereal,\ gras}$ = bioconcentration factor for the actual crop type [dw plant/dw soil]

Table 9. Bioconcentration factors (BCF) for Zn and Cu in potatoes, cereals and grass (VKM 2009).

BCF:	Zn	Cu
Potato	0.12	0.09
Cereals	0.17	0.26
Grass	0.28	0.21

The removal processes of contaminants included in the calculations of soil concentrations for Zn and Cu are leaching and plant uptake (assuming that the metal is translocated to plant parts that are removed from soil). The overall first order removal constant (k) is given by equation 8:

$$k = k_{Leach} + k_{Plant} \quad \textbf{Eq. 8}$$

Where:

k = first order rate constant for removal from top soil [day^{-1}]

k_{Leach} = first order rate constant for leaching from top soil [day^{-1}]

k_{Plant} = first-order rate constant for plant uptake from soil [day^{-1}]

The higher the individual rate constant the more important is the process for the removal of the contaminant. The relative importance of the different processes may be compared by ranking their rate constants.

Long term application of manure with high Zn and Cu concentration will increase the soil concentration of these metals (Table 11). Gräber et al. (2005) studied accumulation of Zn and Cu in Danish agricultural soils in intensive pig production areas. The application of manure was found to cause an accumulation of Zn and Cu in soil and the accumulation rates corresponded to the Zn and Cu concentrations in the manure. The accumulation was strongest in the soil top layer (0-20 cm) but was also found down to 50 cm. However, increased soil concentrations do not necessarily lead to high plant uptake. High soil pH (pH>6.5) usually restrict plant Zn uptake. Application of manure may lead to decreased soil pH which might increase plant Zn uptake. Zn forms chelates with soil organic matter. These chelates are mobile and may increase the transport to and uptake of Zn in plants. As long term manure application usually increases the content of organic matter in soil, increased concentrations of mobile Zn-chelates may occur. Cu is strongly bound to soil organic matter. As long term application of manure increases the soil organic matter, applied Cu most probably will be bound to soil organic matter forming stable complexes not available for plants. Concentrations of Zn and Cu in grain and fodder rapes were investigated in Swedish long-term field experiments with sewage sludge application (Börjesson 2014). While elevated soil concentrations of both Zn and Cu were found, only the plant Zn concentrations increased. However, both for Zn and Cu the plant concentrations: 80-83 mg Zn/kg DM and 20-25 mg Cu/kg DM were within the range of recommended plant concentrations. Also Lipoth and Schoenau (2007) reported elevated Zn and Cu concentrations in barley plants after 5 to 7 years application of pig manure with high Zn and Cu load in Canada. For Cu the increase was only found in straw, not in grain, while for Zn an increase was found both in grain and straw. However, both for Zn and Cu the measured concentrations were well below levels considered to be a risk for plants and primary consumers of the crop. However, also in this study the importance of soil pH and organic matter content on plant availability of Zn and Cu were stressed. A pH >7 renders the metals little plant available. Lower pHs and for Zn, also addition of fresh organic matter, might increase availability in plants.

5.4.3 Summary of removal processes of contaminants in soil

In general, the removal constants are small, clearly indicating that only a minor fraction of added metals through manure amendments are removed from the soil (i.e. high accumulation rate). Leaching is about 10 times more important than plant uptake for the removal of both Zn and Cu from soil at all sites (Table 10). The removal constants are highest at Klepp and smallest at Solør.

Table 10. Removal constants for Zn and Cu used in the future soil concentrations at Klepp, Melhus and Åsnes.

	Klepp. Jæren		Melhus. Trøndelag		Åsnes. Solør	
Removal process	**Zn**	**Cu**	**Zn**	**Cu**	**Zn**	**Cu**
kleach	3.4E-05	1.7E-05	1.2E-05	8.5E-06	1.0E-05	5.2E-06
kpluptake	2.28E-06	1.79E-06	1.25E-06	1.22E-06	1.14E-06	1.12E-06
SUM k	3.6E-05	1.9E-05	1.3E-05	9.8E-06	1.1E-05	6.3E-06

The leaching rate calculated by equation 4 is used only for the calculation of removal from soil and not for calculation of surface and groundwater concentrations.

5.5 Model calculation of future soil concentrations

To calculate the soil concentration after a certain number of applications of manure and annual atmospheric deposition (i.e. after 1-100 years), equation 9 is used (EC 2003):

$$CSoil_{soil\,X}(0) = \left(Catm_{soil1}(0) + Cmanure_{soil1}(0)\right) \cdot \left[1 + \sum_{n=1}^{X-1} Facc^{n}\right] \qquad \textbf{Eq. 9}$$

Where

$CSoil_{soil\,X}$ = concentration in soil after x-years applications of manure and atm. deposition [mg kg^{-1}]

$Cmanure_{soil\,1}$ = concentration in soil after first application of manure at t=0 [mg kg^{-1}]

$Catm_{soil1}$ = concentration in soil after first year of atmospheric deposition [mg kg^{-1}]

Facc = fraction of a substance that remains in the top soil at the end of a year (Eq. 10)

The fraction of the substance that remains in the upper 20 cm of soil at the end of a year is given by equation 10:

$$Facc = e^{-365\,k} \qquad \textbf{Eq. 10}$$

Where:

Facc = fraction accumulation in one year

K = first order rate constant for removal from top soil [day^{-1}] (Eq. 8)

The calculated future soil concentrations are presented in chapter 5.6.

5.6 Estimated concentrations in soil

Based upon the constants, processes and equations presented in chapter 5.1-5.4, the soil concentrations of Zn and Cu at Klepp, Melhus and Åsnes municipalities using pig or poultry manure have been calculated (Table 11). The excess (input-removal) of Zn and Cu are added to the soil background concentrations, resulting in higher future concentrations at Åsnes than at the other sites.

Since the concentrations of Zn and Cu in pig manure are higher than in manure from poultry, the future concentration will be higher in soils receiving pig manure (Table 11). With intensive use of pig manure (70 kg P) for 100 years, the estimated average increases of Zn concentrations in soil are 2.4, 3.4 and 5.8 times, respectively, at Åsnes, Melhus and Klepp. The corresponding increases of Cu concentrations are 1.8, 2.1 and 3.0 times.

In the risk assessment for the soil compartment the PNEC-values are compared to the calculated values given in Table 11. Risk assessment for water bodies and sediments is made by using the highest calculated soil concentrations application of pig manure (rate 70kg P/ha) at Solør for 100 years (Table 11). Maximum leaching has been estimated for the aquatic exposure routes, while maximum uptake in plants based on BCF has been used in other exposure routes. This approach implies an inconsistent mass balance for Zn and Cu, but makes sure that possible undesirable effects of these metals in feed and food are not over looked.

Table 11. Estimated soil concentrations (mg/kg DM) of Zn and Cu at Klepp, Melhus and Åsnes based on the use of pig or poultry manure (concentrations in manure: 10th perc, mean and 90th perc, Table 5) for 10, 50 and 100 years. Annual application rate of manure: 20 and 70 kg P/ha.

Amount P	Time		Klepp, Jæren				Melhus, Trøndelag				Åsnes, Solør			
			Zinc		Copper		Zinc		Copper		Zinc		Copper	
			Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry
		Soil back-ground	**25**	**25**	**9**	**9**	**50**	**50**	**18**	**18**	**77**	**77**	**21**	**21**
20 kg P	10y	10th perc	27	27	9	9	52	52	19	19	78	78	22	22
		Mean	29	28	9	9	54	53	19	19	80	79	22	22
		90 perc	32	29	9	9	57	54	19	19	83	80	22	22
	50y	10th perc	33	32	10	10	58	57	20	20	84	83	23	23
		Mean	43	37	11	11	68	61	21	21	93	87	24	23
		90 perc	55	43	13	12	80	68	23	22	104	93	25	25
	100y	10th perc	41	39	12	12	66	64	22	21	91	90	24	24
		Mean	61	48	14	13	86	73	24	23	110	97	26	25
		90 perc	84	61	17	16	110	86	27	26	131	109	29	28
70kg P	10y	10th perc	31	30	10	10	56	55	20	19	82	81	22	22
		Mean	38	33	11	10	63	58	20	20	89	84	23	23
		90 perc	47	38	12	11	72	63	22	21	97	89	24	24
	50y	10th perc	51	47	14	13	76	72	24	23	100	97	26	26
		Mean	86	62	18	15	111	88	28	25	133	111	30	28
		90 perc	127	86	23	21	153	111	33	31	171	132	34	33
	100y	10th perc	76	69	19	18	101	94	29	28	124	117	31	30
		Mean	145	99	27	22	171	124	37	32	188	145	38	34
		90 perc	227	145	37	33	254	170	47	43	263	187	47	44

5.7 Predicted no effect concentrations (PNECs) and risk characterization for soil organisms

Metal toxicity depends on the total metal dose (PEC), the physic-chemical properties of the soil, as well as the time since application of metal to the soil. For some elements models have been developed for calculation of site specific PNEC for soil organisms. Arche (www.arche-consulting.be) has developed a model for calculating site-specific soil PNEC-values for Zn and Cu.

The calculations are based on data and methods selected for the EU REACH dossiers (Registration, Evaluation, Authorisation and Restriction of Chemicals (Regulation EC No 1907/2006) for Cu (ECI 2008) and Zn (2010). The PNEC values are based on reliable chronic toxicity data for terrestrial organisms (plants, invertebrates and microbial processes). Bioavailability correction of the toxicity data is performed using:

i) a leaching-ageing factor (an empirically derived factor used to account for the reduced toxicity of metals observed in the field compared to the same samt total concentration of metal in laboratory toxicity tests with soluble metal salts) and

ii) normalisation of toxicity thresholds (NOECs) towards soil properties of the target soil based on regression of toxicity data with these soil properties.

The soil properties required for calculation of site specific PNECs for Cu are cation exchange capacity (CEC), % organic carbon, % clay and pH. For Zn, CEC, pH and background concentration of Zn are required. The leaching-ageing factors are 3 and 2 for Zn and Cu respectively.

The normalised NOEC-values are subjected to an analysis of the Species Sensitivity Distribution analysis (SSD). SSDs are constructed using a cumulative plot of logarithmically transformed NOECs against rank as-signed percentiles for each value to which a statistical distribution is fitted. A log-normal model is used to obtain the best fit. From the selected model the concentration that is hazardous to 5% of the species (HC5) is extrapolated. For Cu and Zn the calculated HC5 is used as PNEC.

The site-specific PNEC-values for Zn and Cu in soil from Melhus are higher than at the other sites due to higher pH, total organic carbon (TOC) and clay content (Table 12).

The result of the risk characterisation for soil organisms is shown in Table 12. For Zn the PEC/PNEC-ratios are higher than 1 at Solør and Klepp, while the ratios are below or equal to 1 (1.07 when using the high (90^{th} percentile) concentration in pig manure) at Melhus.

For Cu all the PEC/PNEC-ratios are below 1 which shows that the content of Cu in pig- and poultry manure will not pose any threat to soil living organisms even when applying high amounts of manure (equal to 70 kg P/ha year) for 100 years.

Table 12. Input parameters used in the PNEC-calculations, calculated site-specific PNECs and risk characterisation ratios (PEC/PNEC) for the three regions.

Region		Soil properties			Metal background		PEC total*		PNEC		PEC/PNEC	
		pH	TOC	Clay	mg/kg DM		mg/kg DM		mg/kg DM		Total approach	
			%	%	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu
Åsnes	Mean	5.9	2.2	7	77	21	188	38	117	64	**1.61**	0.59
	90% perc	5.9	2.2	7	77	21	263	47	117	64	**2.25**	0.73
Klepp	Mean	5.9	2	6	25	9	145	27	95	58	**1.53**	0.47
	90% perc	5.9	2	6	25	9	227	37	95	58	**2.39**	0.64
Melhus	Mean	6.2	4.2	32	50	18	171	37	237	150	0.72	0.25
	90% perc	6.2	4.2	32	50	18	254	47	237	150	**1.07**	0.31

*PEC total: the modelled soil concentration at the three sites after having applied pig manure (mean and 90 % concentrations, Table 5) corresponding to 70 kg P per ha, for 100 years i.e. the highest possible concentrations of Zn and Cu in soils after manure application.

Comparing the calculated soil concentrations following manure application (Table 11) with the site-specific PNEC-values for the three localities (Table 12), show that the PNEC values are exceeded after 50 years of manure application at Klepp and Åsnes, and after 100 years of 20 kg P application at Åsnes at the highest (90^{th} percentile) concentration in manure.

Application of 20 kg P/ha year as pig manure with a mean zinc concentration of 636 mg/kg (Table 5) will not result in soil accumulation exceeding the PNEC for the soils in any region in a long term perspective (100 years).

6. Zn and Cu in surface water

6.1 Predicted concentrations in runoff water

To address the potential risk to the surface water environment, the concentration of metals in a body of surface water that will result from leakage of metals from soil after application of manure for a 100 year period has been estimated. Separate assessments have been performed for the three scenarios. Two models were initially used to calculate the leakage of Zn and Cu from the soil to surface water. Leakage to drainage water and groundwater was predicted using the model MACRO_GV. MACRO is a one-dimensional, mechanistic model, which describes transport of water and chemicals as well as temperature distribution in a vertical soil profile (Larsbo & Jarvis 2003). The model accounts for the presence of macropores, which allows fast transport of water and chemicals in the soil. However, the MACRO model did not predict significant leakage of metals for any of the scenarios and therefore only the runoff model PRZM was used.

PRZM (Pesticide Root Zone Model) is a one-dimensional non-deterministic compartment model for prediction of transport of chemicals by chromatographic washout in un-saturated soil. (http://focus.jrc.ec.europa.eu/gw/models/PRZM/index.html). The PRZM3 version is a standard model for use in environmental exposure and risk assessment by the Environmental Protection Agency in USA and is in Federal Insecticide, Fungicide, and Rodenticide Act, FIRFA's list of recommended regulatory models for registry of pesticides in USA. The model is also used in the surface water scenarios for risk assessment of pesticides in the EU. PRZM simulates chemical movement in soil within and immediately below the plant root zone. It accounts for uptake in plants, binding to soil, degradation, volatilisation, erosion and surface runoff (See Figure 4). Input parameters are soil properties, crop rotation, physical/chemical properties of the chemical, applied amount of chemical and daily climatological data for the simulation period.

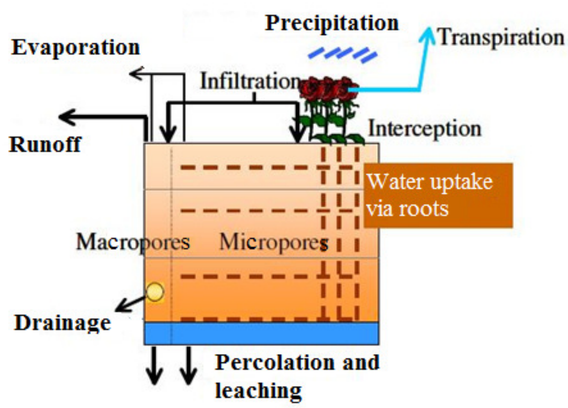


Figure 4. Concept of the model MACRO_GV (Source: Stenemo et al. 2005).

6.1.1 Soil profiles

In order to perform model calculations a complete soil profile is required for description of the water balance. The profiles are selected to fit to the available data on soil properties for the three scenarios as shown in section 5.3. The profiles are shown in Table 13. – Table 15.

For the profile used for the Klepp scenario has a slightly higher content of organic material than shown in Table 8

Table 13. Description of the soil profile used in the simulations for the Klepp scenario.

Layer	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Org. C%)	Density (kg/dm^3)
Ap	0-35	63	32	5	5.1	1.01
Bw	35-50	70	28	2	1.6	1.59
Bg	50-65	65	33	2	1.1	1.59
BCg	65+	67	32	1	0.3	1.50

Table 14. Description of the soil profile used in the simulations for the Melhus scenario.

Layer	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Org. C (%)	Density (kg/dm^3)
Ap	0-20	1.2	74.9	24	2.94	1.57
Bw	20-48	1.2	75.6	23.3	0.75	1.51
E	48-70	1	71.5	27.6	0.24	1.51
Btg	70-94	0.7	62.8	36.6	0.16	1.51
BCg	94-120	0.8	59.8	39.4	0.17	1.51
Cg	120+	0.8	61.9	37.3	0.19	1.51

For the Åsnes scenario a soil profile representing the Solør region with river deposits and a high content of silt was selected.

Table 15. Description of the soil profile used in the simulations for the Åsnes scenario.

Layer	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Org. C (%)	Density (kg/dm^3)
Ap	0-30	46.8	49.0	4.3	0.9 / 0.67	1.48
Bw	30-56	12.6	82.0	5.2	0.5 / 0.30	1.37
Bw/Cg	56-69	6.4	88.9	4.6	0.5 / 0.36	1.29
Apb/C	69-84	22.6	74.6	3.3	0.5 / 0.55	1.27
C1	84-92/110	36.2	60.9	2.9	0.1 / 0.10	1.33
Apb2	92/110-100/118	5.6	87.0	7.4	0.9 / 0.69	1.20
C	120+	0.3	93.0	6.7	0.9 / 0.49	1.25

The type of crop affects the results of the modelling, particularly by influence on the erosion rate. Loss of topsoil by erosion is higher in corn cultivation than in grass. In the modelling only corn production has been applied in Klepp and Åsnes, while a crop rotation with two years of grass followed by one year with corn was applied in Melhus.

Climate files with daily values of precipitation, evaporation, temperature, wind speed and radiation for the period 1991 to 2011 have been compiled based on observations from climatological stations in each of the three regions. For the model calculations it was assumed that the Zn and Cu were homogenously distributed in the upper 20 cm of the soil profile in May 1991. The model calculated the flux of water and the distribution of metals in the soil profile as well as the loss to runoff on a daily basis for 20 years (1991-2011). The application rate was adjusted to obtain the calculated concentrations after 100 years application of pig manure (corresponding to 70 kg P/ha) with a high content of metals (90 perc.) as shown in Table 5 and 7.

6.1.2 Result on runoff calculations

The Klepp scenario had the highest precipitation and as a consequence the highest runoff volume and loss of soil in runoff. The proportion of precipitation that occurred as runoff was also higher at Klepp (32%), than at Åsnes (23%) and Melhus (9%). The average concentration of soil in runoff water over the total simulation period was lowest at Melhus (233 mg/L) and highest at Åsnes (336 mg/L).

The vertical distribution of Cu and Zn that was added and mixed into the upper 20 cm of the soil profiles in May 1991 showed that the mobility of both metals was low in all scenarios. The profiles from Klepp are shown in Figure 5. The figures show that almost no Zn has reached below 50 cm after 20 years. For Cu, almost no copper is found below 40 cm. The concentrations near the surface decline with time as a result of loss with runoff and downward leaching. The profiles from Åsnes and Melhus show as similar pattern, although the changes in the vertical distributions over time are less pronounced at Melhus, where the higher clay content causes a stronger adsorption of the metals to the soil. Furthermore, the crop rotation at Melhus which included grass production reduced the loss by erosion.

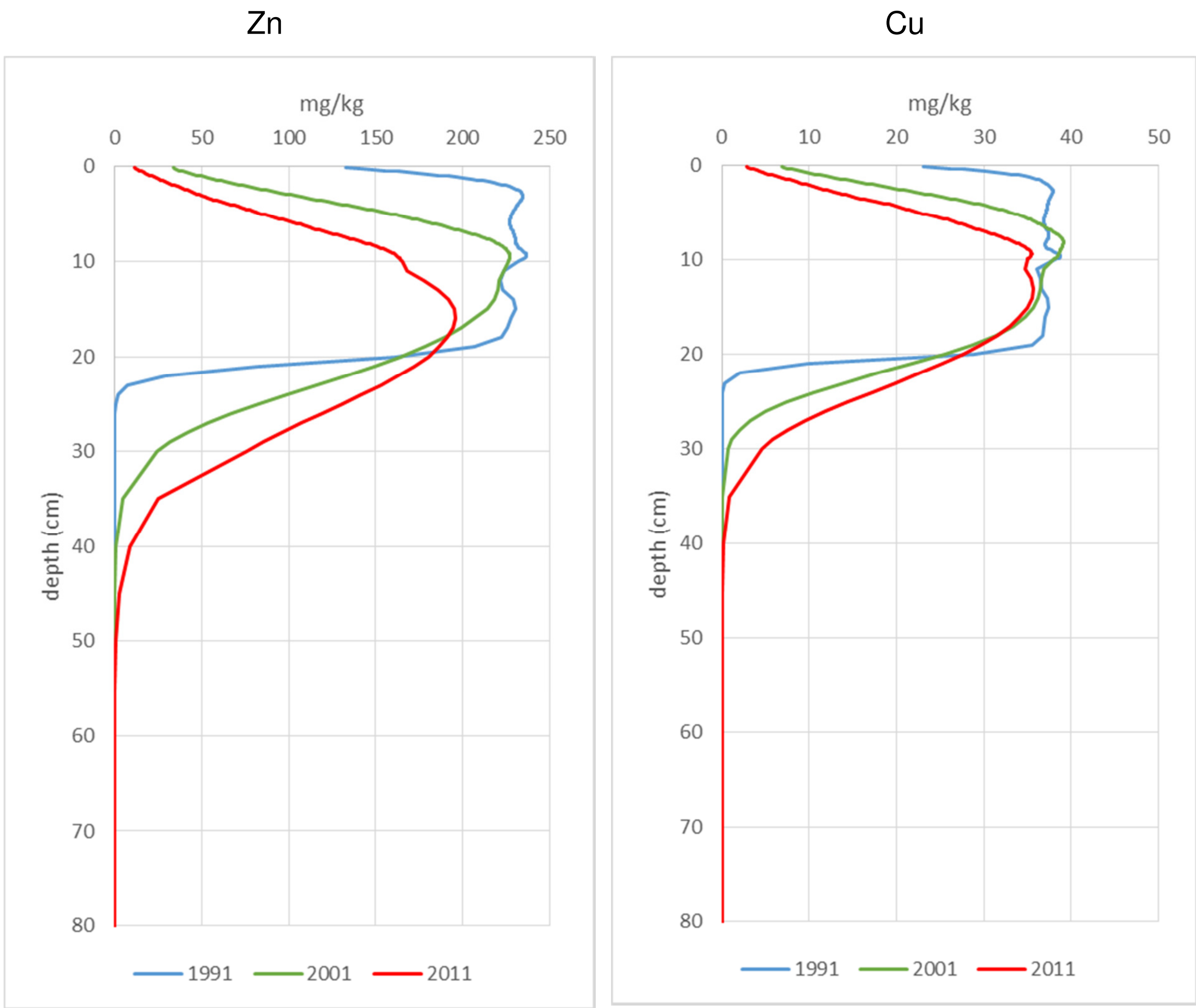


Figure 5. Profiles showing the distribution of added Zn and Cu at 7 months (1991), 10 years (2001) and 20 years (2011) after application of Zn to the upper 20 cm soil.

Due to the low vertical mobility of the metals, the model did not indicate any leakage to ground water and drainage water and the only significant loss from the soil profiles was by surface runoff. Generally, the loss was highest at Klepp and lowest at Melhus, and in two of the scenarios, (Åsnes and Melhus), the loss percentage was higher for Zn than for Cu. (See Table 16).

Table 16. Amount of applied metal and loss of metal after 20 years of simulation.

Scenario	Zn applied kg/da	Zn loss kg/da	Zn loss %	Cu applied kg/da	Cu loss kg/da	Cu loss %
Klepp	45.4	2.5	5.5	7.4	0.44	5.9
Åsnes	63.1	2.5	4.0	11.2	0.4	3.6
Melhus	79.7	1.0	1.3	14.7	0.1	0.7

The concentration of dissolved metals in the runoff water declined gradually over the 20 years simulation period as shown for copper at Klepp in Figure 6. This is a result of the depletion of metals in the top layer. For the risk assessment of surface waters it is therefore considered most relevant to focus on the initial period after application of metals. The amount of dissolved and suspended Cu in runoff water during the first full year after application of metals (1992) at Klepp, is shown together with precipitation and runoff volume in Figure 7. The corresponding amounts of dissolved and suspended Zn are shown in Figure 8.

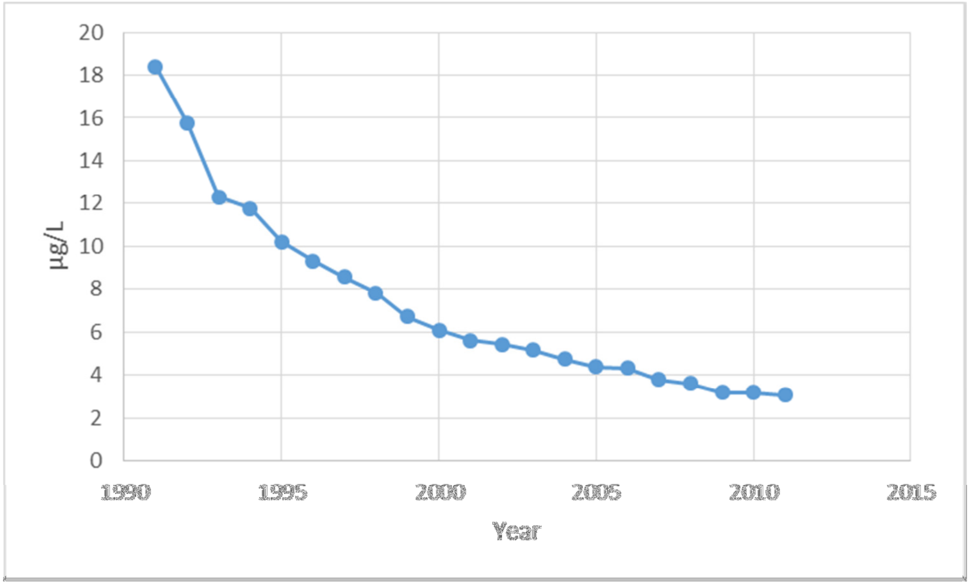


Figure 6. Maximum concentration of dissolved Cu (4-days average) in runoff water at Klepp.

The figures show, as expected, that runoff and metal loads in runoff water is correlated with episodes of heavy precipitation. While the load of metal is strongly fluctuating, the concentration of metals in the runoff water during episodes of runoff is more stable as shown in Figure 9. The figure shows that the concentrations of dissolved metals are slowly declining during the year as a result of depletion of metals from the soil surface layer. The concentrations of metals adsorbed to soil particles are much more variable due to variations in the amount of soil in the runoff water.

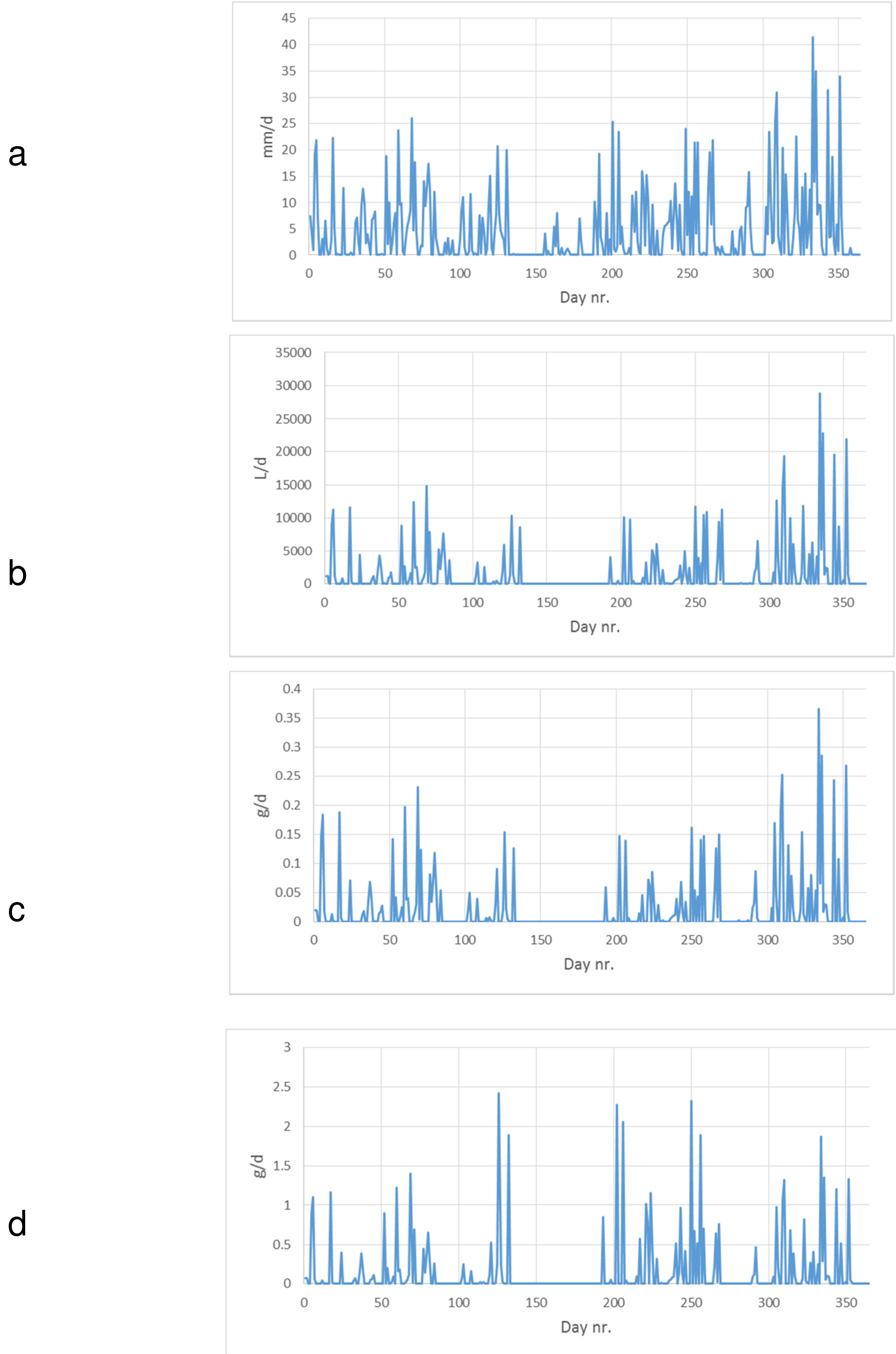


Figure 7. Precipitation (a), runoff volume (b), and dissolved (c) and sorbed (d) Cu in runoff water at Klepp 1992.

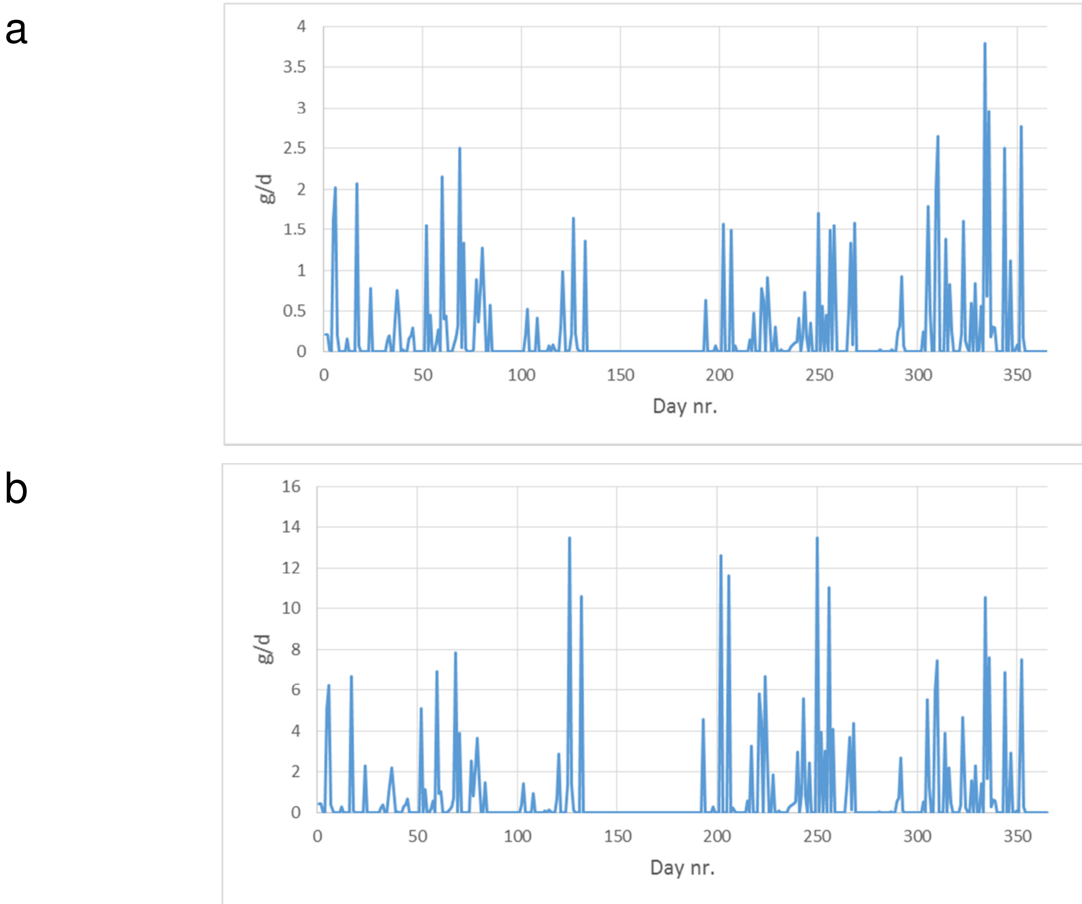
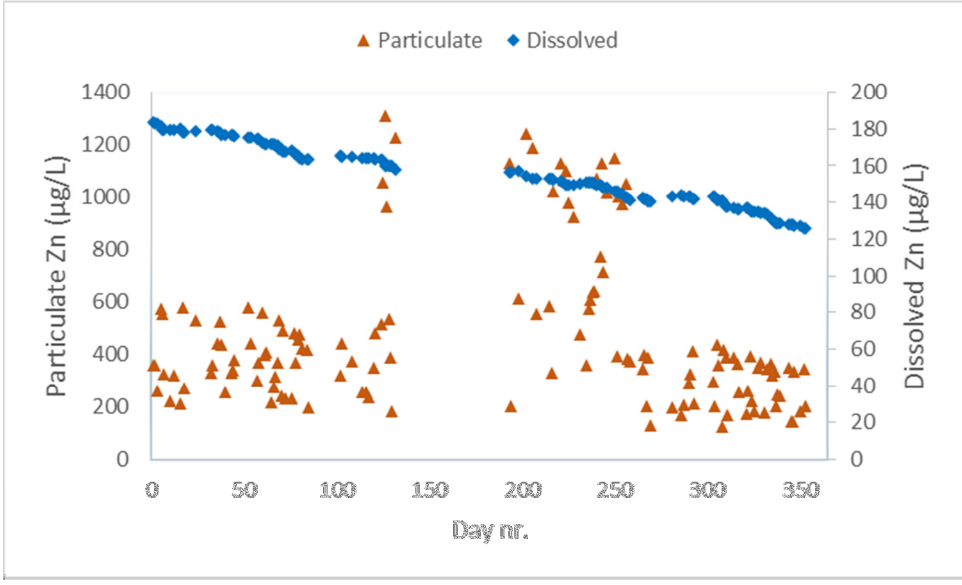


Figure 8. Dissolved (a) and sorbed (b) Zn in runoff water at Klepp 1992.

a



b

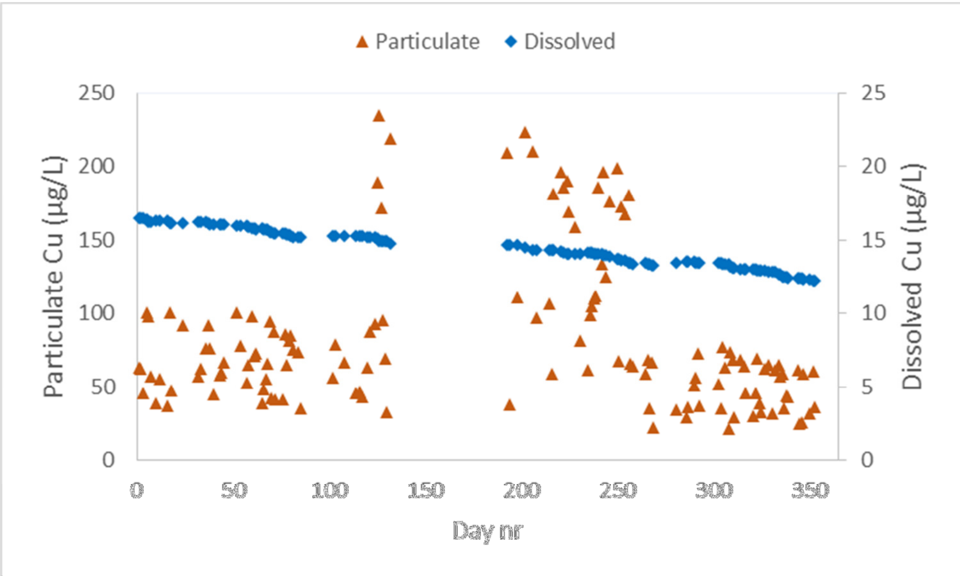


Figure 9. Concentrations of particulate and dissolved Zn (a) and Cu (b) in runoff water at Klepp 1992.

6.1.3 Predicted concentrations of Zn and Cu in surface water

The purpose of using specific streams as a basis for the risk assessment is to account for the effects of abiotic factors in the water on the bioavailability of metals. The selected streams are assumed to be representative also for smaller local streams in the area in terms of abiotic factors. In such, smaller streams the dilution of runoff water from fertilised soil will be much lower than in the selected reference streams. The predicted concentrations (PEC) of dissolved Zn and Cu in the surface water has been calculated for each of the scenarios, assuming that the runoff water from the soil is diluted a factor 10 or 50 in a surface water body with a water quality corresponding to the selected regional reference stream (See section 4).

In the risk characterisation, the PEC is compared to the predicted no effect concentration (PNEC) for Zn and Cu. PNEC is the concentration below which long term exposure to a substance is not expected to cause adverse effects to species in the environment. This implies that sporadic exposure to higher (but not acute toxic) concentrations can be tolerated for short periods. The exposure situation in surface water bodies receiving metal contaminated runoff water is extremely complex, with concentration peaks of varying duration and frequency. In order to account for these fluctuations, but still ensure a conservative estimate of the exposure concentrations, four-days moving average of the daily load of metals in runoff have been calculated, and the highest 4-d average value has been used as basis for calculation of PEC in the receiving water.

Predicted concentrations (PEC) of Zn and Cu in the local aquatic scenarios are calculated according to the Technical Guidance Document (EC 2003).

The local concentration in surface water is calculated as:

$$PEClocal_{SW} = Cregional + \left(\frac{C_{leakage}}{(1 + Kp_{susp} \cdot SUSP_{water} \cdot 10^{-6}) * DILUTION} \right)$$

Where:

Cregional	ambient background concentration in surface water	$mg \cdot L^{-1}$
$PEClocal_{sw}$	local concentration (dissolved) in surface water	$mg \cdot L^{-1}$
$C_{leakage}$	concentration (dissolved) in runoff water	$mg \cdot L^{-1}$
Kp_{susp}	solids-water partitioning coefficient for suspended matter	$L \cdot kg^{-1}$
DILUTION	dilution factor	

Kp_{susp} for the suspended matter in the receiving water are adopted from the risk assessment reports on Zn and Cu:

Metal	Kp_{susp}	Ref.
Cu	30 246	ECI 2008
Zn	110 000	EU 2010

Kp_{susp} for the suspended soil in runoff water is assumed to be equal to the Kd in soil used for calculation of dissolved and adsorbed metal in the PRZM model:

Metal	Klepp	Åsnes	Melhus
Cu	448	474	915
Zn	230	246	649

The KP_{susp} for the mixture of runoff soil and suspended matter in the receiving water is calculated from the proportion of mixing.

The local concentrations in sediment are calculated as:

$$PEClocal_{sed} = \frac{K_{susp-water}}{RHO_{susp}} * PEClocal_{sw} * 1000$$

Where:

$PEClocal_{sed}$	predicted environmental concentration in sediment	mg·kg
$K_{susp-water}$	suspended matter-water partitioning coefficient	$m^3 \cdot m^{-3}$
RHO_{susp}	bulk density of suspended matter	$kg \cdot m^{-3}$
$PEClocal_{sw}$	concentration in surface water	$mg \cdot L^{-1}$

$K_{susp-water}$ is calculated as:

$$K_{susp-water} = 0.9 + 0.1 * \frac{Kp_{susp}}{1000} * RHO_{solid}$$

Where:

RHO_{solid}	density of solid phase	2.5 $kg \cdot m^{-3}$	(TGD, EC 2003)

The concentrations of dissolved metals in surface waters ($PEClocal_{sw}$) and of total metals in sediments ($PEClocal_{sed}$) are calculated using two levels (10x and 50x) of dilution of runoff water in receiving water. The periods of highest 4d-average of total load of metals were used for calculation of the concentration of metals in the runoff water. These periods are shown in Table 17.

Table 17. The highest loads of metals (4 d. average) and concentrations in runoff (4 d. average) in runoff from a 1 da area, derived from PRZM model calculations for the three scenarios.

Scenario	4d period	Zn (g/d)	Zn (mg/L)	Cu (g/d)	Cu (mg/L)
Klepp	13.-16.09.1991	11.5	0.741	1.87	0.118
Åsnes	19-22.06.1991	17.7	1.717	3.07	0.294
Melhus	29.08 – 01.09.1993	8.97	0.705	1.71	0.133

The results of PEC-calculations using a 10x dilution factor are shown in Table 18.

Table 18. Total concentrations of metals and suspended solids (SPM) in runoff, and the calculated PECs at 10x dilution level in the receiving waters.

Scenario	Runoff			Zn		Cu	
	Tot. Zn mg/L	Tot. Cu mg/L	SPM mg/L	PECsw µg/L	PECsed[a] mg/kg	PECsw µg/L	PECsed[a] mg/kg
Klepp	0.741	0.118	2210	73.4	63.2	11	10.5
Åsnes	1.717	0.294	3670	137	131	18.2	20.7
Melhus	0.705	0.133	2220	60.9	61.1	10.9	13.1

[a] PECsed are expressed as added concentrations

The results of PEC-calculations using a 50x dilution factor are shown in Table 19.

Table 19. Total concentrations of metals and suspended solids (SPM) in runoff, and the calculated PECs at 50x dilution level in the receiving waters.

Scenario	Runoff			Zn		Cu	
	Total Zn mg/L	**Total Cu** mg/L	**SPM** mg/L	**PECsw** µg/L	**PECsed**[a] mg/kg	**PECsw** µg/L	**PECsed**[a] mg/kg
Klepp	0.741	0.118	2210	19.2	18.6	3.1	4.0
Åsnes	1.717	0.294	3680	33.2	40.9	4.1	8.1
Melhus	0.705	0.133	2220	16.3	20.8	3.8	7.4

[a] PEC_{sed} are expressed as added concentrations

The PECs for both metals were higher for Åsnes than at the two other locations. This is mainly due to the higher concentrations in the runoff water, and reflects a higher leaching potential in the silty soil at Åsnes.

6.2 Predicted no effect concentrations (PNECs) in aquatic organisms

For characterisation of the risk posed by the predicted exposure concentrations of Zn and Cu in the surface water and sediment, the Predicted No Effect Concentration (PNEC) for the metals in the two compartments has to be derived. The PNECs were derived as outlined by the Technical Guidance Document, TGD (EC 2003). After compilation of the No Observed Effect Concentrations (NOEC) in chronic toxicity tests on various organisms, the TGD describes various procedures for deriving PNEC, depending on the number of species and taxonomic groups that are available in the database. For the metals Zn and Cu, the databases are extensive enough to allow deriving the PNEC from the Species Sensitivity Distribution (SSD) for the surface water environment.

SSDs are constructed using a cumulative plot of logarithmically transformed NOECs against rank as-signed percentiles for each value to which a statistical distribution is fitted. Usually a log-normal model is used, but also other models may be used to obtain the best fit. From the selected model the concentration that is hazardous to 5% of the species (HC5[1]) is extrapolated. An additional assessment factor (AF) in the range 1-10 is used to calculate the PNEC from the HC5 as PNEC = HC5/AF. Criteria for selecting the appropriate AF are included in the TGD.

Since the toxicity of metals to aquatic organisms is influenced by abiotic factors as described in Chapter 1.2, PNECs should preferably be derived for the local or regional scenario. This can be done by applying BLM models to normalise the available NOECs in the toxicity database to the abiotic conditions in the scenario and perform a SSD analysis of the normalised NOECs. A software tool to perform these calculations has been developed by ARCHE and WCA under the name Biomet (http://www.arche-consulting.be/en/home/). Biomet contains the toxicity databases for Zn and Cu compiled during the EU risk assessments of these metals (EU 2010, ECI 2008). Some new data has been added using the same selection criteria as for the original data bases. BLM models and the algorithm for SSD

[1] HC5-50 may be used to denote the 5 percentile with 50% confidence

analysis are integrated in the Biomet tool. The necessary input parameters to run the models are pH, dissolved organic carbon (DOC) and Ca. The output of the software tool is site specific Environmental Quality Standards (EQS) for Zn and Cu as defined in the Water Framework Directive (2000/60/EC). The procedure for derivation of the EQS is the same as for PNEC according to the TGD, and therefore the site specific EQS can be used as a site specific PNEC in risk assessment.

Biomet uses an assessment factor, AF=1, for calculation of PNEC from the HC5 obtained from the SSD-analysis. This means that PNEC is identical to the HC5. It should be noted that the EU risk assessment report for Zn (EU 2010), proposed an AF=2. This has, however been questioned in a recent work by Van Sprang et al. (2009). Furthermore, more toxicity data has been included in the database used in Biomet for calculation of PNEC, which justifies the use of AF=1. Consequently, the UK has adopted an AF=1 in the proposal of regional Environmental Quality Standards for Zn. (WFD-UKTAG 2010).

The required input values for the different scenarios and the resulting PNECs are shown in Table 20. The PNEC for Zn calculated by Biomet refers to the added concentration only. To derive PNEC for the total concentration, the background concentrations of Zn have been added to the $PNEC_{add}$.

Table 20. Input values used for derivation of surface water PNEC with the Biomet software and PNECs for dissolved Zn and Cu in receiving water in the different scenarios.

Scenario	pH	SPM	TOC	Ca	Cu	Zn	$PNEC_{sw}$ Cu	$PNEC_{sw}$ Zn
		mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L
Klepp	7.1	2.8	2.9	6.39	1.0	4.9	14.57	24.23[a]
Åsnes	6.75	15	16.4	5.26	0.11	4.17	47.96	86.79[a]
Melhus	7.3	8.2	3.7	7.40	1.7	3.8	17.08	20.43[a]

[a] The background concentration has been added to the $PNEC_{add}$ for Zn

6.2.1 PNEC for sediment dwelling organisms

The database on chronic toxicity of Zn to sediment dwelling organisms, which was compiled for the RAR contains only four useful chronic NOEC values representing three species. The lowest NOEC is for the crustacean *Hyalella azteca* (488 mg/kg dw) and the highest for the oligochaete *Tubifex tubifex* (1101/kg dw). These NOEC-values are expressed as added Zn concentrations. The number of species is too limited to apply a SSD analysis and therefore the $PNEC_{add, sediment}$ was derived from the lowest NOEC applying an AF=10. Hence, the **$PNEC_{add, sediment}$ of 49 mg/kg** was proposed.

The bioavailability of Zn in sediments is influenced by abiotic factors and particularly the content of acid volatile sulphide (AVS). In the RAR no bioavailability correction is made on the PNEC, but bioavailability is addressed at the risk characterisation step by making corrections on the PEC in those cases when the initial PEC/PNEC ratios are >1. The bioavailability factor is calculated from the excess AVS concentration in the sediment. In case information on AVS and other abiotic parameters are not available, a generic bioavailability factor of 0.5 is applied on the PEC_{add}. (EU 2010).

In the Cu RAR, 62 chronic toxicity NOECs, representing 6 species were normalised for organic carbon before analysis of species sensitivity distribution (SSD). The HC5-50 derived from the SSD-analysis (log-normal model) was 1.741 mg Cu/g OC. It was concluded that an

AF = 1 is adequate for derivation of $PNEC_{sediment}$ from the HC5-50. Hence the $PNEC_{sediment, OC normalised}$ = 1.741 mg Cu/g OC. This corresponds to a **$PNEC_{sediment}$ of 87 mg Cu/kg** dry weight for a sediment containing 5 % organic carbon. (ECI 2008).

6.3 Risk characterization of aquatic organisms

The risk characterization ratios (RCR=PEC/PNEC) have been calculated for surface water and sediment for the three local scenarios and with two dilution levels of the runoff water.

For the surface water scenarios with the lowest dilution level (10x), a risk for effect of Zn is indicated by RCR>1 at all three scenarios, while no risk is indicated for Cu. (See Table 21).

At the higher dilution level (50x) all RCRs are <1, which indicates no risk in any of the scenarios. (See Table 22).

Table 21. Risk characterisation ratios for surface water scenarios with 10x dilution of runoff water. All concentrations are expressed as µg/L.

Scenario	Zn PEC	Zn $PNEC_{sw}$	Cu PEC	CU $PNEC_{sw}$	RCR Zn	RCR Cu
Klepp	73.4	24.2	11.0	14.6	**3.0**	0.75
Åsnes	137	91	18.2	48	**1.5**	0.38
Melhus	60.9	24.2	10.9	17.1	**2.5**	0.64

Table 22. Risk characterisation ratios for surface water scenarios with 50x dilution of runoff water. All concentrations are expressed as µg/L.

Scenario	Zn PEC	Zn $PNEC_{sw}$	Cu PEC	CU $PNEC_{sw}$	RCR Zn	RCR Cu
Klepp	19.2	24.2	3.1	14.6	0.79	0.21
Åsnes	33.2	91	4.1	48	0.36	0.09
Melhus	16.3	24.2	3.8	17.1	0.67	0.22

The calculated PECs for sediments are expressed as added concentrations of Zn and Cu. For the sediment assessments, the calculated PEC_{add} for Zn (See Table 19), have been corrected for bioavailability by a factor 0.5 as recommended in the RAR (EU 2010). The $PNEC_{sediment}$ for Zn is also expressed as added concentration and have been used directly in the RCR calculations. For Cu, the $PNEC_{sediment}$ is expressed as total concentration. Therefore the sediment background concentration must be subtracted from to the PNEC. However, no information is available on the metal background concentrations in sediments for the three scenarios. As an alternative, data from a national survey of metal concentration in lake sediments have been used to derive a general background concentration (Rognerud et al. 1999). The median concentration of Cu in the upper sediment layer in 235 lakes was 41.7 mg/kg and has been used as sediment background concentration in all scenarios. The result of the risk characterisation for sediment organisms is shown in Table 23. (dilution 10x) and Table 24. (dilution 50x).

Table 23. Risk characterisation ratios for sediment scenarios with 10x dilution of runoff water. All PECs and PNECs are added concentrations (mg/kg).

Scenario	Zn PEC	Zn $PNEC_{sed}$	Cu PEC	Cu $PNEC_{sed}$	RCR Zn	RCR Cu
Klepp	31.6	48	10.5	45	0.66	0.23
Åsnes	65.5	48	20.7	45	**1.36**	0.46
Melhus	30.6	48	13.1	45	0.64	0.29

Table 24. Risk characterisation ratios for sediment scenarios with 50x dilution of runoff water. All PECs and PNECs are added concentrations (mg/kg).

Scenario	Zn PEC	Zn $PNEC_{sed}$	Cu PEC	CU $PNEC_{sed}$	RCR Zn	RCR Cu
Klepp	9.3	48	4	45	0.19	0.09
Åsnes	20.5	48	8.1	45	0.43	0.18
Melhus	10.4	48	7.4	45	0.22	0.16

The sediment PNEC for Zn is only exceeded at the Åsnes scenario with a factor 10 dilution. No risk is indicated for Cu.

The predictions indicate that leakage of Zn from soil having received the highest load of Zn (manure with the highest 90th percentile concentration and application rate corresponding to 70 kg P/ha) may pose a risk to aquatic organisms in surface water and sediment in water bodies where the dilution rate of the runoff water from the fertilized area is less than a factor 50. The maximum daily runoff volumes during periods with the highest leakage of metals were between 18 and 20 m^3/da, day. This means that the volume of dilution water in the water body receiving leakage from a 1da area must be 1000 m^3/day or 11.6 L/s.

Another way to visualize the dilution requirement is to consider the drainage area of the receiving water, upstream of the area where manure is applied. In the Klepp scenario, which showed the highest RCR, the average annual volume of runoff water was 450 m^3/da, which means that the required annual volume of dilution water to achieve 50x dilution is 22 500 m^3. The average annual precipitation in the area was 1480 mm. Normally, the proportion of surface water drainage in this area of Norway is approximately 70% of the precipitation and, consequently, the upstream drainage area will produce approximately 1030 m^3/da of dilution water. Thus, an upstream drainage area of 22 500/1030=22 da, where manure is not applied, would be required to provide sufficient dilution water for the runoff water from a 1 da fertilised field.

Assessment of leakage of Zn and Cu from soil to surface water and effects of the metals on surface water biota has only been based on calculated levels of metals after 100 years of maximum (70 kg P/ha) application of pig manure containing the 90th percentile concentrations of metals. It is reasonable to assume that the resulting exposure concentration in surface water is approximately proportional to the accumulated concentration of metals in the soil. The effect of a shorter period of manure application or lower concentrations of metals in the manure may therefore be indicated by the soil concentrations as shown in

Table 11. At Klepp, where the assessment was based on an initial concentration of 227 mg Zn/kg in the soil, the RCR for effects in surface water was 3.0. This indicates that the soil concentration must be less than approximately 75 mg Zn/kg to avoid a risk to aquatic organisms. Even after 50 years application of pig manure at a high (70 kg P/ha) application rate and with a high concentration of Zn, the soil concentration is expected to reach above 75 mg/kg. The same is true for use of high Zn manure but at a lower application rate (20 kg P/ha) for 100 years. After 50 years application at this lower rate, however, the calculated soil concentration was 55 mg Zn/kg.

Similarly, comparing the concentrations of Zn in soil after 100 years application of manure (high rate, high concentration) shows that poultry manure gives Zn concentrations that are 60-70% of those from pig manure. Assuming again that the resulting concentrations in surface water is roughly proportional to the concentrations in soil, also use of poultry manure will produce concentrations of Zn above the PNEC in all three scenarios at the 10x dilution level.

The calculated maximum Zn concentrations in soil after 100 years application of pig manure with mean (50^{th} percentile) content of Zn are only a factor 1.4-1.6 lower than for the 90^{th} percentile Zn content. (See Table 11). This difference in soil Zn concentration indicates that a risk for adverse effects on aquatic organisms will also apply to use of manure with 50^{th} percentile content of Zn at the 10x dilution level in surface water.

7. Zn and Cu in grazing and fed animals

7.1 Exposure

7.1.1 Estimated concentration in plants

Table 25 shows the background concentrations and the predicted concentrations of Zn and Cu in feed and pasture plants fertilised with pig or poultry manure for 100 years. The mean concentrations of Zn and Cu in manure are used in the calculations (Table 5, not the 90th percentile). The highest Zn concentrations are shown in grass, and the highest Cu concentrations in cereals, whereas potato contains the lowest concentrations of both elements.

With intensive use of pig manure (70 kg P) for 100 years, the Zn concentration in plants is estimated to increase 2.4 to 5.8 times the background levels. The corresponding increases of Cu concentrations are 1.8 to 3.0 times. With use of poultry manure somewhat lower increases are expected. Due to various soil factors (see Chapter 5), Klepp area may show the highest increase of Zn and Cu, and Åsnes area may show the lowest increase. However, Åsnes has the highest background concentrations of Zn and Cu in the soil, and at a 100-year perspective Åsnes area is estimated to end up with the highest soil Zn and Cu concentrations. Thus, soil, feed and pasture plants from Åsnes is used as a worst case scenario for estimation of risk to animal health.

Table 25. Estimated concentrations in mg/kg DM (Eq. 7) in potato, cereals and grass.

Plant Concentration	Food item	Klepp, Jæren				Melhus, South Trøndelag				Åsnes, Solør			
		Zn		Cu		Zn		Cu		Zn		Cu	
		Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry
Plant concentration Background	Potato	3.0	3.0	0.8	0.8	6.0	6.0	1.7	1.7	9.2	9.2	1.9	1.9
	Cereals	4.3	4.3	2.2	2.2	8.5	8.5	4.7	4.7	13.0	13.0	5.4	5.4
	Grass	7.1	7.1	1.8	1.8	14.0	14.0	3.9	3.9	21.5	21.5	4.5	4.5
Plant concentration 20 kg P. 100 years	Potato	7.3	5.7	1.3	1.1	10.3	8.7	2.2	2.0	13.2	11.7	2.4	2.3
	Cereals	10.4	8.1	3.6	3.3	14.6	12.3	6.1	5.8	18.6	16.5	6.8	6.4
	Grass	17.1	13.3	3.0	2.7	24.1	20.3	5.1	4.8	30.7	27.3	5.6	5.3
Plant concentration 70 kg P. 100 years	Potato	17.4	11.9	2.4	2.0	20.5	14.9	3.3	2.9	22.5	17.4	3.4	3.0
	Cereals	24.7	16.8	6.8	5.6	29.1	21.1	9.4	8.2	31.9	24.6	9.7	8.6
	Grass	40.7	27.7	5.6	4.6	47.9	34.8	7.7	6.7	52.5	40.5	8.0	7.1

7.1.2 Animals at pasture and animals receiving feed

For herbivore domestic animals as cattle, sheep, goats and horses at pasture, their whole ration may be the pasture plants. However, dairy cows and goats usually also receive grain-based feed (compound feed) when at pasture ranging from 0 to about 1/3 of total dry matter intake. As manure is mixed into the soil it is not used in rough grazing areas. Cultivated species of pasture plants (grass species) grow in soil where manure may have been used. According to Norwegian legislation, cows have to be kept outdoors at least 8 weeks per year, and sheep and goats at least 16 weeks per year. Sheep, goats and horses are usually grazing rough grass but cattle may often graze on manure treated areas.

Animals at pasture usually also ingest some soil. The soil intake may depend on the pasture quality and the mineral need of the animals. The intake of soil is supposed to constitute up to some percentages (5%) of the dry matter ration.

Omnivore animals such as poultry and pigs moving outside may ingest grass and other vegetables. They may also ingest considerable amounts of soil and soil organisms as earthworms.

For herbivore domestic animals like cattle, sheep, goats and horses receiving feedstuff, the roughage use to constitute a main part of the ration. In addition, compound feed (grain based feed) or in some cases, potatoes etc. are given at a certain ratio (up to about 50 % of the dry matter ration to these species).

Small grains, oil seeds, array of seed legumes and some maize are commonly main feed ingredients in pig feed and may be grown on manure fertilized soil. The main feed ingredients in poultry feeds are maize and small grains.

Table 26 shows the normal amounts of dietary intake relative to the body weight of different livestock species at some physiological stages. The table also shows common composition of the diets for animals at pasture and when fed at home.

Table 26. Dry matter (DM) intake of farm animals related to body weight and their relative intake of compound feed, roughage and soil.

Animal species	Percent DM intake related to body weight	At pasture Ratio of total intake (DM)			Fed animals Ratio of total intake (DM)		
		Compound feed	Roughage	Soil	Compound feed	Roughage	Soil
Cattle							
Calves	3	0.1	0.85	0.05	0.25	0.75	0
Young heifers	2.7	0.1	0.85	0.05	0.25	0.75	0
Dry cows	1.7	0.1	0.85	0.05	0.15	0.85	0
High lactation cows	4	0.5	0.45	0.05	0.5	0.5	0
Sheep							
Early weaned lambs	5	0	0.95	0.05	0.25	0.75	0
Finishing lambs	4	0	0.95	0.05	0.25	0.75	0
Adult sheep maintenance	2	0	0.95	0.05	0.25	0.75	0
Adult sheep with twins	4	0	0.95	0.05	0.25	0.75	0
Goats							
Kids	3.5	0.1	0.85	0.05	0.25	0.75	0
Adult goats maintenance	2	0.1	0.85	0.05	0.25	0.75	0
Adult lactating goats	6	0.5	0.45	0.05	0.5	0.5	0
Horses							
Adult maintenance	1.5	0.25	0.7	0.05	0.3	0.7	0
Mares in lactation	3	0.25	0.7	0.05	0.3	0.7	0
Pigs							
Piglet	10				1	0	0
Growing pig	4				1	0	0
Adult pigs maintenance	1.2				1	0	0
Lactating sow	3.2				1	0	0
Poultry							
Growing chickens	10				1	0	0
Laying hens	6				1	0	0
Broiler parent	6				1	0	0
Turkey	6				1	0	0

7.2 Hazard and risk characterization in animals

The livestock exhibit considerable tolerance to high intakes of Zn. The extent of tolerance depends particularly on the species but mainly on the nature of the diet as discussed in Chapter 2. Maximum tolerable levels (MTL) for Zn given by NRC (2005) are for pigs 1000 mg/kg DM, for poultry, cattle and horses 500 mg/kg DM, and for sheep 300 mg/kg DM.

Maximum tolerable levels (MTL) for Cu given by NRC (2005) are for pigs, poultry and horses 250 mg/kg DM, for cattle 40 mg/kg DM, and for sheep 15 mg/kg DM.

The animal risk characterisation concerning Zn and Cu comprises evaluation of health risk for pasturing animals and animals receiving feed grown in soil where manure from pigs or poultry has been used for up to 100 years. Pig manure contained the highest levels of Zn and Cu, and out of the three selected scenario districts, the soil in Åsnes area showed the highest concentrations of Zn and Cu. The worst case scenario is thus evaluation of animal health after intensive use of pig manure for 100 years at the level of 70 kg P/ha in Åsnes. The estimated Zn exposure via pasture/roughage and cereals grown at these areas is 2.4 times higher after 100 years. The estimated Cu exposure via pasture/roughage and cereals from these areas is 1.8 times higher after 100 years. The estimated figures for increment of exposure via feed plants correspondingly grown in the other areas are higher than in Åsnes. For Melhus the increments are 3.4 times higher Zn and 2.0 times higher Cu, and for Klepp 5.8 times higher Zn and 3.0 times higher Cu. However, the background concentrations were considerably lower than in Åsnes.

The higher Zn and Cu concentrations in soil than in plants after 100 years implies that ingestion of some soil may easily contribute considerably to the total exposure. Usually pasturing includes ingestion of some soil, thus grazing may increase the total exposure more than if the animals were fed with plants grown in the same area.

The increase of Zn and Cu exposure will certainly be different (lower) if some of the animal ration is grown at other areas. Use of commercial compound feed will also influence the exposure. Table 27 shows the background intake of Zn and Cu for ruminants and horses at pasture and when fed, and the estimated intake of pasture/feed after 100 years of intensive use of pig manure. In this table commercial compound feed with Zn and Cu at the present 2014 levels as background and the same levels after 100 years were included. For animals at pasture with no compound feed in the ration (sheep) the Zn and Cu exposure reflects the concentrations in the grass plus some contribution from the soil. The estimated increments of total Zn and Cu exposure are about 3 and 2 times, respectively. For animals with low levels of compound feed supplied with Zn and Cu (calves, young heifers, dry cows, kids and maintenance goats) the increments are somewhat less. Only marginal increase of Zn and Cu (1.2-1.3 x) is shown for high lactation cows and goats at high amounts of compound feed in the situation with maintained present high concentrations of Zn and Cu in the compound feed.

If the Zn and Cu concentrations in feed plants increase by the use of fertilisation, supply of Zn and Cu by other means will likely be adjusted downwards. Thus, an adequate total exposure for animals fed on plants with elevated levels of Zn and Cu would be maintained. The situation where all feed ingredients are produced at the same soil with the present background concentrations of Zn and Cu, and after 100 years of intensive use of pig manure is shown in Table 28. For grazing animals the Zn exposure are estimated to increase up to 3 times and the Cu exposure about 2 times, and some lower increments at inside feeding.

The Tables 27 and 28 show the exposure for cattle, sheep, goats and horses. If pigs and poultry are given a diet correspondingly grown at a pig manure fertilised soil they will be similarly exposed to Zn and Cu. However, most pigs and poultry are fed commercial compound feed supplied with Zn and Cu. Therefore, these species are not shown in the tables for exposure scenarios.

Either we base the calculations on all ingredients of the animal ration grown at a soil fertilised with high amounts of pig manure, or on some parts of the ration obtained from outside as commercial compound feed supplied with maximum limits of Zn and Cu from 2014, the estimated exposure will be below the MTL for all species. For Zn exposure in livestock a 3 times higher exposure in 100 years is still far below MTL, and the health risk for livestock is negligible. The most critical combination of compound and species is Cu in sheep. The estimated worst case exposure of Cu in sheep is about 10 mg/kg complete ration. Such an exposure is still regarded to imply a low health risk. However, an increase of Cu exposure above 10 mg/kg diet increases the risk for Cu toxicity (Humphreys 1988), particularly with the Norwegian low soil molybdenum levels. It is worth to mention that Cu poisoning is described in sheep grazing pastures fertilised with pig manure (Kerr and McGavin, 1991). For Cu exposure in other species the risk for health effects is negligible.

In conclusion, by intensive use of pig (or poultry) manure for 100 years the Cu concentration in the feed plants may increase and give livestock a total exposure level at approximately 10 mg Cu/kg complete ration, which implies a low health risk for sheep and negligible for other livestock animals. Above this level a considerable risk for Cu toxicity in sheep would be expected.

Table 27. Estimated intake of Zn and Cu in ruminants and horses at pasture or when fed roughage (grass, see Table 25.) grown before and after intensive fertilizing of the soil with pig manure (application rate: 70 kg P/ha) for 100 years in Åsnes area, as well as compound feed with present maximum concentrations of Zn and Cu.

Animal species	Zinc								Copper							
	At pasture				Fed animals				At pasture				Fed animals			
	Background		Pig manure 100 years		Background		Pig manure 100 years		Background		Pig manure 100 years		Background		Pig manure 100 years	
	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet
Cattle																
Calves	1.04	35	2.07	69	1.61	54	2.31	77	0.23	7.6	0.37	12.2	0.36	12.1	0.44	14.8
Young heifers	0.93	35	1.86	69	1.45	54	2.08	77	0.21	7.6	0.33	12.2	0.33	12.1	0.40	14.8
Dry cows	0.59	35	1.17	69	0.69	41	1.14	67	0.13	7.6	0.21	12.2	0.15	9.1	0.21	12.1
High lactation cows	3.44	86	4.32	108	3.43	86	4.05	101	0.79	19.8	0.92	23.0	0.79	19.8	0.86	21.5
Sheep																
Early weaned lambs	1.08	22	2.96	59	2.68	54	3.85	77	0.23	4.5	0.48	9.5	0.36	7.1	0.49	9.8
Finishing lambs	0.87	22	2.37	59	2.15	54	3.08	77	0.18	4.6	0.38	9.5	0.29	7.1	0.39	9.8
Adult sheep maint.	0.43	22	1.19	59	1.07	54	1.54	77	0.09	4.6	0.19	9.5	0.14	7.1	0.20	9.8
Adult sheep w. twins	0.87	22	2.37	59	2.15	54	3.08	77	0.18	4.6	0.38	9.5	0.29	7.1	0.39	9.8
Goats																
Kids	1.21	35	2.42	69	1.88	54	2.69	77	0.23	6.6	0.39	11.2	0.34	9.6	0.43	12.3
Adult goats maint.	0.69	35	1.38	69	1.07	54	1.54	77	0.13	6.6	0.22	11.2	0.19	9.6	0.25	12.3
Adult lactating goats	5.16	86	6.48	108	5.15	86	6.08	101	0.89	14.8	1.08	18.0	0.89	14.8	0.99	16.5
Horses																
Adult maintenance	0.81	54	1.26	84	0.90	60	1.23	82	0.15	9.7	0.21	13.8	0.16	10.7	0.20	13.1
Mares in lactation	1.61	54	2.51	84	1.80	60	2.45	82	0.29	9.7	0.41	13.8	0.32	10.7	0.39	13.1

Table 28.Estimated intake of Zn and Cu in ruminants and horses at pasture or when fed inside roughage and cereals (see Table 25.) grown before and after intensive fertilising of the soil with pig manure (application rate: 70 kg P/ha) for 100 years in Åsnes area. In this table both roughage and cereals are from the same background and 100-year fertilised soil.

Animal species	Zinc								Copper							
	At pasture				Fed animals				At pasture				Fed animals			
	Background		Pig manure 100 years		Background		Pig manure 100 years		Background		Pig manure 100 years		Background		Pig manure 100 years	
	mg/kg bw per day	mg/kg diet	mg/kg bw perday	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet	mg/kg bw per day	mg/kg diet
Cattle																
Calves	0.63	21	1.72	57	0.58	19	1.42	47	0.14	4.6	0.29	9.7	0.14	4.7	0.25	8.4
Young heifers	0.56	21	1.55	57	0.52	19	1.28	47	0.13	4.6	0.26	9.7	0.13	4.7	0.23	8.4
Dry cows	0.35	21	0.97	57	0.34	20	0.84	49	0.08	4.6	0.17	9.7	0.08	4.7	0.14	8.4
High lactation cows	0.70	17	1.96	49	0.69	17	1.69	42	0.20	5.0	0.42	10.4	0.20	5.0	0.36	8.9
Sheep																
Early weaned lambs	1.08	22	2.96	59	0.97	19	2.37	47	0.23	4.5	0.48	9.5	0.24	4.7	0.42	8.4
Finishing lambs	0.87	22	2.37	59	0.78	19	1.90	47	0.18	4.6	0.38	9.5	0.19	4.7	0.34	8.4
Adult sheep maint.	0.43	22	1.19	59	0.39	19	0.95	47	0.09	4.6	0.19	9.6	0.10	4.7	0.17	8.4
Adult sheep w. twins	0.87	22	2.37	59	0.78	19	1.90	47	0.18	4.6	0.38	9.5	0.19	4.7	0.34	8.4
Goats																
Kids	0.73	21	2.00	57	0.68	19	1.66	47	0.16	4.7	0.34	9.7	0.17	4.7	0.30	8.5
Adult goats maint	0.42	21	1.14	57	0.39	19	0.95	47	0.09	4.7	0.19	9.7	0.10	4.7	0.17	8.5
Adult lactating goats	1.05	17	2.94	49	1.04	17	2.53	42	0.30	5.0	0.62	10.4	0.30	5.0	0.53	8.9
Horses																
Adult maintenance	0.29	20	0.81	54	0.28	19	0.70	46	0.07	4.8	0.15	9.9	0.07	4.8	0.13	8.5
Mares in lactation	0.59	20	1.62	54	0.57	19	1.39	46	0.14	4.8	0.30	9.9	0.14	4.8	0.26	8.5

8. Zn and Cu in humans

8.1 Exposure via animal products

Most of the total body Zn is in muscles and bones (approximately 85 %). Excess intake of Zn leads to an increased deposition in the bone, liver, pancreas and kidney. Concentrations in muscle, milk and egg are more stable (NRC, 2005). A higher exposure of Zn to livestock on pasture or receiving feed from soil highly fertilised with pig manure for 100 years is not considered to significantly increase the Zn concentrations in animal products as meat, milk or eggs.

The liver contains the highest Cu concentrations. Liver and kidney Cu concentrations are related to the dietary intake whereas muscle, milk and egg concentrations are more stable and seem to be little influenced by differences of exposure (Van Paemel et al. 2010). Normal liver Cu concentrations are higher in ruminants than in pigs and poultry, and relatively small amounts of dietary Cu may greatly increase liver Cu concentrations in ruminants, particularly in sheep. A higher exposure of Cu to livestock on pasture or receiving feed from soil highly fertilized with pig manure for 100 years is not considered to significantly increase the Cu concentrations in animal products as meat, milk or eggs. A possible increase in liver is not considered to significantly influence the total human intake of Cu.

In conclusion, absorbed Zn is mostly deposited in bone, liver, pancreas and kidney, and Cu in liver and kidney. Significantly increased Zn and Cu in animal products as meat, milk and eggs are not expected.

8.2 Exposure via plant food

The background concentrations of Zn and Cu in Zn and Cu containing foods from The Norwegian Food Composition Table (Matvaretabellen 2014) are used in this assessment. Together with the human intake (mean and high consumers) of these foods from Norkost 3 (Totland et al. 2012), the background intakes of Zn and Cu for mean and high consumers (95 percentile) are estimated (Table 29). The background exposure of Zn for mean consumers is 13.1 mg/day and the 26.7 mg/day for high consumers. The background exposure of Cu for mean consumers is 1.4 mg/day and 3.0 mg/day for high consumers. Cereals are the primary sources of Zn and Cu.

Norkost 3 is based on two 24-hour recalls by telephone at least one month apart. Food amounts were presented in household measures or estimated from photographs (Totland et al. 2012). The study was conducted in 2010/2011, and 1787 adults (925 women and 862 men) aged 18-70 participated.

For estimation of the human intakes of Zn and Cu after 100 years with pig and poultry manure the predicted increments of Zn and Cu in plants in the selected agricultural regions were used. More specifically, mean concentrations of Zn and Cu in pig manure (as pig manure averagely contains some higher Zn and Cu concentrations than poultry manure) at a rate of application of 70 kg P/ha was included. The estimations are based on intake of all plant food from such fertilised area. As the manure is spread locally, repeated spreading at the same areas year by year (100 years) is a realistic scenario.

It is assumed that the concentrations of Zn and Cu in meat, milk, cheese, butter and eggs are not influenced by manure application to soils. The intake from these foods is therefore considered constant during the 100 year period (see 8.1).

After 100 years the predicted range of increment (in Åsnes (lowest increase) and Klepp (highest increase)) in plants of Zn was 2.4 – 5.8 and of Cu 1.8 – 3.0. The estimated intake after 100 years for Zn was 24.9-52.5 mg/day and for Cu 2.4-3.9 mg/day. For the high consumers the figures are approximately doubled. As intake of Zn and Cu via animal products is supposed to be constant and cereals show relatively high bioconcentation factors (BCF) for Zn and Cu, cereals are estimated to become even more dominant sources of Zn and Cu after 100 years of pig/poultry manure.

Table 29. Mean and high human intake of zinc (Zn) and copper (Cu) from plants grown on soils where pig manure (application rate 70 kgP/ha) have been used for 100 years. Background intakes are based on Zn and Cu concentrations in The Norwegian Food Composition Table, and food intake from Norkost 3 conducted among adults. Intakes after 100 years are based on estimated ranges of increments for Zn and Cu by use of pig manure in Åsnes (lowest increase) and Klepp (highest increase) areas.

Food item	Food intake based on Norkost 3		Food item content of Zn and Cu		Zinc (Zn)				Copper (Cu)			
					Intake mean consumer		Intake high consumer		Intake mean consumer		Intake high consumer	
	Mean food consumption	High food consumption (95%-tile)	From 'The Norwegian food composition table'		Background	Pig manure 100 years	Background	Pig manure 100 years	Background	Pig manure 100 years	Background	Pig manure 100 years
	g/day	g/day	Zn mg/kg ww	Cu mg/kg ww	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day
Cereals	150	308	52.4	7.5	7.86	19.18 – 45.59	16.14	39.38 – 93.61	1.13	2.01 – 3.38	2.31	4.13 – 6.93
Potato	66	199	3	1	0.20	0.48 – 1.15	0.60	1.46 – 3.46	0.07	0.12 – 0.20	0.20	0.36 – 0.60
Carrot	23	98	3	0.5	0.07	0.17 – 0.40	0.29	0.72 – 1.71	0.01	0.02 – 0.03	0.05	0.09 – 0.15
Swede	4	30	1	0.2	0.00	0.01 – 0.02	0.03	0.07 – 0.17	0.00	0.00 – 0.00	0.01	0.01 – 0.02
Cabbage	4	27	1	0.2	0.00	0.01 – 0.02	0.03	0.07 – 0.16	0.00	0.00 – 0.00	0.01	0.01 – 0.02
Cauliflower	4	25	3	0.4	0.01	0.03 – 0.07	0.08	0.18 – 0.44	0.00	0.00 – 0.00	0.01	0.02 – 0.03
Chinese cabbage	12	51	2	0.3	0.02	0.06 – 0.14	0.10	0.25 – 0.59	0.00	0.01 – 0.01	0.02	0.03 – 0.05
Cucumber	12	50	1	0.2	0.01	0.03 – 0.07	0.05	0.12 – 0.29	0.00	0.00 – 0.01	0.01	0.02 – 0.03
Tomato	16	61	1	0.3	0.02	0.04 – 0.09	0.06	0.15 – 0.35	0.00	0.01 – 0.01	0.02	0.03 – 0.05
Peppers	7	34	1	0.1	0.01	0.02 – 0.04	0.03	0.08 – 0.20	0.00	0.00 – 0.00	0.00	0.01 – 0.01
Meat	87	253	24	0.4	3.10	3.10	5.89	5.89	0.14	0.14	0.27	0.27
Milk	312	895	4	0.1	0.7	0.7	1.3	1.3	0.04	0.04	0.08	0.08
Cheese	39	104	46	0.4	1.1	1.1	2.1	2.1	0.02	0.02	0.04	0.04
Butter	6	27	0	0								
Total intake of Zn and Cu through the food items					**13.1**	**24.9 – 52.5**	**26.7**	**51.8 – 110.3**	**1.4**	**2.4 – 3.9**	**3.0**	**5.1 – 8.3**

8.3 Exposure via drinking water

The Zn concentration in Norwegian drinking water is not regulated. In natural surface waters, the concentration of Zn is usually below 10 µg/L, and in groundwaters 10-40 µg/L. In tap water, the Zn concentration can be much higher (about 1 mg/L) as a result of leaching from piping and fittings (WHO, 2003).

The concentration of Cu is regulated in Norway in "Drikkevannsforskriften- for water for human consumption" with a limit 0.1 mg/L. Copper is found in surface water, groundwater, seawater and drinking water, but it is primarily present in complexes or as particulate matter. Cu concentrations in surface water ranged from 0.0005 to 1 mg/L, with a median value of 0.01 mg/L in several studies from the USA (referred by WHO, 2004). A survey of 985 Norwegian lakes in 1995 showed a median concentration of Cu at 0.33 µg/L and 90-percentile concentration 0.90 µg/L (Skjelkvåle et al. 1999). Cu concentrations in drinking water vary widely as a result of water characteristics, such as pH, hardness and availability in the distribution system. Results from a number of studies from Europe, Canada and USA indicate that Cu levels in drinking water can range from ≤0.005 to >30 mg/L, with the primary source most often being corrosion of the interior Cu plumbing (referred by WHO, 2004). Levels of Cu in running or flushed water tend to be low, whereas those of standing or partially flushed water samples are more variable and can be substantially higher.

In Norway, only approximately 15 % of the drinking water is abstracted from ground water, while surface water is the dominating source. The modelling of the fate of Zn and Cu from manure in soil that was performed to predict leakage to water showed that leakage to groundwater is insignificant, while leakage to surface water takes place, mainly with surface runoff from fertilized fields (See 6.1).

8.4 Hazard and risk characterization in humans

SCF (2003) has presented an Upper Intake Level (UL) for Zn for adults at 25 mg/day. The critical endpoint is the impact on the Cu status, with a NOAEL in humans at 50 mg/day. Lower ULs are given for children, with 7 mg/day for 1-3 years children.

The estimated intake of Zn by consumers of food produced in areas intensively fertilised with pig manure for 100 years is 24.9-52.5 mg/day for mean consumers and 51.8-110.3 mg/day for high consumers. The increase results from cereals and other plant food. The concentrations in animal products are not assumed to increase significantly. The results indicate that mean consumers would be exposed to Zn in food between UL and NOAEL, and high consumers would normally be exposed to Zn above NOAEL. The elevated exposure of Zn may be of concern for human health.

SCF (2003) has presented an UL for CU for adults at 5 mg/day. The critical endpoint is liver damage with a NOAEL in humans at 10 mg/day (SCF, 2003). Lower ULs are given for children, with 1 mg/day for 1-3 years children. The estimated intake of Cu by consumers of food produced in areas intensively fertilised with pig manure for 100 years is 2.4-3.9 mg/day for mean consumers and 5.1-8.3 mg/day for high consumers. The increase results from cereals and other plant food. The concentrations in animal products are not assumed to increase significantly. For mean consumers of food from these areas the Cu intake may lie below the UL. The estimated Cu intake for high consumers is above UL but below NOAEL. The elevated exposure of Cu may be of low concern for human health.

The maximum concentrations calculated for local surface water bodies receiving runoff water from fertilized soils were 137 µg Zn/L and 18 µg Cu/L (see Table 18). These concentrations are below the levels that may cause practical inconveniencies and human health effects in drinking water. Furthermore, surface water for domestic water supply is only extracted from larger water bodies where the influence of runoff water from fertilized agricultural fields is much lower. Thus, leakage of Cu and Zn from soil fertilized with manure to drinking water is not considered a risk to human health.

In conclusion, food produced in areas where manure from pigs or poultry has been used over time can lead to increased human intake of Zn and Cu via particularly cereals and vegetables. By intensive use of pig manure (highest content of Zn and Cu) for 100 years the Zn intake by mean consumers will exceed UL, whereas the Zn intake of high consumers will exceed NOAEL. The elevated exposure of Zn may be of concern for human health.

For Cu the corresponding intake of mean consumers will be below UL, whereas high consumers will exceed UL. The elevated exposure of Cu may be of low concern for human health.

9. Microbial resistance

9.1 Resistance mechanisms

Although overuse of antibiotics in agriculture and medicine is partially responsible for increased level of antibiotic resistance in bacteria, environmental exposure to trace metals may also contribute to antibiotic resistance, even in the absence of antibiotics themselves.

To avoid cellular damage caused by trace elements, bacteria have evolved mechanisms of metal tolerance. Both the mechanisms of toxicity and tolerance to trace elements in bacteria are discussed extensively in the review article of Seiler and Berendock (2012). It has been proved that antimicrobial agents other than antibiotics have the ability to promote a co-selection process, indirectly selecting for antibiotic resistance (Baker-Austin et al. 2006). The trace elements like Zn and Cu seems to have potential to act as a selective pressure that forces the proliferation and evolution of Zn/Cu and antibiotic resistance not only at the farm level, but also in the environment.

Zn and Cu are used in animal feed in concentrations in excess of the nutritional requirements of the animals and for prevention of diarrheal disease, and also as an alternative to in-feed antibiotics for growth promotion (Amachawadi et al. 2011; Cavaco et al. 2011). The total amounts and concentrations used in feed might be differing among countries, due to restrictions imposed by national legislation. As a consequence different selective pressure might be exerted within different countries.

Since data from Norway, regarding resistance development to Zn and Cu, is lacking, our assessment has been based on the published studies from other countries. Conditions from other countries are not necessarily relevant for Norway, however, the concentration of Zn and Cu in animal feed in EU countries is similar in Norway. Data regarding development of resistance against Zn and Cu in bacteria of human origin is deficient. The average exposure of Zn and Cu in humans, from food, are probably lower than the exposure of the supplement of these trace elements in animal feed.

9.2 Resistance in animal microbiota

In bacterial isolates found in **animals (appendix I)**, elevated Minimum Inhibitory Concentration (MIC)-values to Zn and Cu was detected in several opportunistic bacterial species compared to background isolates. The exposure of bacterial isolates to these trace metals may be the reason for the elevated MIC-values in these bacterial isolates. The data presented in these studies indicate that such elevation in (MIC)-values may be due to use of these trace elements in animal feed. MIC is defined as the lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions. Testing for susceptibility against Zn/Cu in various bacterial species was performed using either a micro-dilution technique or an agar-dilution technique and under different methodological conditions (studies listed in Appendix I). There is currently no standardized and approved method to determine the MIC values for Zn/Cu.

Among the examined bacterial species, the development of resistance to Cu in enterococci is associated with presence of resistance gene (*tcrB*), which is often located on a plasmid (Amachawandi et al. 2001, Freitas et al. 2011; Jacob et al. 2010; Kim et al. 2012). In enterococci, Cu resistance gene (*tcrB)* was shown to be associated with resistance to erythromycin (*ermB*). A conjugation study demonstrated co-transfer of *tcrB* and *ermB* genes between *E. faecium* and *E. faecalis* (Amachawadi et al. 2011). Transferable Cu resistance (*tcrB*) has been reported in *E. faecium* and *E. faecalis* isolated from piglets, calves, poultry, and human in Denmark (Aarestrup et al. 2002). Several studies performed in Denmark show a link between resistance to Cu and resistance to macrolides and glycopepetides (vancomycin) in enterococcal isolates of pig origin. The authors concluded that there is frequent occurrence of Cu resistance gene in these isolates, where Cu sulphate is being used in large amounts as feed additive. This may have contributed to co-selection resistance against macrolides and glycopepetides (Hasman and Aarestrup 2002; Hasman et al. 2005; Hasman and Aarestrup 2002; Hasman et al. 2006). Macrolides like erythromycin are commonly used in veterinary and human medicine and glycopeptide antibiotics (avoparcin) have been used as growth promoters for animal production by adding to feed, in many European countries, including in Norway, in the past (from mid-1970s). However, it is prohibited sine 1990s because of development of vancomycin resistance in bacteria, in particular in enterococci. The discontinued use of avoparcin in animal feed has resulted in a reduction in the number of vancomycin-resistant organisms isolated from animals (Aarestrup et al. 2001, Bonten et al. 2001). Because avoparcin and vancomycin are similar in structure, bacteria resistant to avoparcin are resistant to vancomycin as well.

The possibility of transfer of vancomycin resistance genes from enterococci to other Gram-positive bacteria, like staphylococci, raises significant concerns about the emergence of vancomycin-resistant *Staphylococcus aureus*. Nowadays, vancomycin constitutes one of the last resort antibiotics for treatment of MRSA infection in human.

Several studies in Appendix I have demonstrated an association between resistance to Zn and resistance to methicillin in staphylococci (Aarestrup et al. 2010; Cavaco et al. 2011; Cavaco et al. 2010). The study performed by Cavaco et al. (2011) found that MRSA strains from pigs from European countries, Canada, and China had a high prevalence of Zn resistance (mainly associated with *czrC* gene), whereas the corresponding MRSA were susceptible. Similar association between resistance to Zn and resistance to methicillin was also observed in samples from veal farms from the Netherlands. Methicillin is not the drug of choice for treatment of infection in veterinary medicine, neither in Norway nor in any countries within EU. There is lack of knowledge regarding the source of methicillin-Zn resistant staphylococci in animals. It is not clear whether the methicillin resistant staphylococci in animals are of human origin and have been resistant to Zn after exposure to feed or the Zn-resistant staphylococci have been resistant to methicillin, due to exposure to antibiotic(s).

A recent publication from Germany (Bednoz et al. 2013) showed an higher diversity of *E. coli* clones in animals supplemented with zinc compared to the background control group. The proportion of multi-resistant *E. coli* was significantly increased in the zinc group compared to control group. The authors suggested two possible mechanisms for their results; a) co-selection via Zn resistance as some of the isolates were both Zn and antimicrobial resistant, b) enhanced plasmid uptake under the influence of Zn, as the authors detected several resistance plasmids in isolates of the Zn feeding group. Identical clones were not present in in the control group.

There is lack of data which can demonstrate whether Cu/Zn-resistant bacteria may acquire antibiotic resistance genes / be antibiotic resistant or antibiotic resistant bacteria are more capable to be Cu/Zn-resistant than antibiotic susceptible bacteria.

9.3 Resistance in environmental microbiota

In bacterial isolates found in **environment (Appendix I**), elevated tolerance (higher MIC)-values to Zn and Cu was detected in several bacterial species like *Pseudomonas*, *E. coli*, enterococci, and Gram-negative bacteria of soil origins compared to bacteria of animal origin. The examined bacteria in all eight studies presented shows higher tolerance to either Zn or Cu or both. A link between resistance to Zn/Cu in bacteria found in environment and resistance to the examined antibiotics were observed in the all studies included in appendix I. This may indicate a co-selection of resistance to antibiotics in Zn/Cu resistant bacteria found in the environment.

The combined expression of antibiotic and metal resistance in bacteria isolated from environment may be caused by selection resulting from metals present in environments rich in Zn and Cu (de Vincent et al. 1990). In the environmental studies, the source of Zn/Cu is not always identified, but Zn/Cu is currently accumulating in many soils as a result of current agricultural practices, where these trace elements are often present in animal manure and sewage sludge that are spread on agricultural soils. While, antibiotics present in animal manure and sewage sludge may be degraded in short time, the metals are persistent and may accumulate in soil). Generally, bacterial isolates of environmental origin, with resistance to Zn or Cu, may be frequently resistant to more antibiotics than the bacterial isolates of animal origin (See appendix 1).

The data presented in in appendix I show that resistance to Zn/Cu is frequently encoded by genes located on plasmids and transposons and is often transferable between bacterial species. Metal resistance is often associated with single or multiple antibiotic resistance. Such resistance genes may be transferred to soil bacteria via manure containing Zn/Cu-resistant bacteria. The trace element driven co-selection of antibiotic resistance is of great importance in water and soil environments and seems to be influenced by agriculture and aquaculture (Seiler and Berendonk 2012). Co-selection mechanisms include co-resistance (different resistance determinants present on the same genetic element) and cross-resistance (the same genetic determinant responsible for resistance to antibiotics and metals) (Baker-Austin et al. 2006). However, the origins of trace elements from other sources than manure are not identified.

A significant positive correlation was found between some ARGs and typical trace elements in the some studies. In those studies molecular methods like polymerase chain reaction (PCR) was used to identify these genes (i. e. in manure) rather than to culture and isolate bacteria (Ji et al., 2012; Zhu et al., 2013).

According to Seiler and Berendonk (2012), the knowledge about the natural background of antibiotic resistance gene abundance (resistome) in the different environments is also limited. Therefore, it is difficult to distinguish between the natural resistome and an elevated abundance of resistance genes in different environmental samples. Hence, it is difficult to detect an increase of antibiotic resistance genes in field studies.

To conclude on bacterial resistance, bacteria in animals and environment may develop resistance to trace elements as Zn and Cu. The degree of exposure of Zn or Cu may be of importance but there is lack of data on dose-response relations. However, it seems more likely that a resistance driven effect occurs at high trace element exposure than at more basal exposure levels. Resistance to Zn is often linked with resistance to methicillin in staphylococci. Zn supplementation to animal feed may increase the proportion of multi-resistant *E. coli* in gut microbiota compared with control group. Resistance to Cu in bacteria, in particular enterococci, is often associated with resistance to antimicrobial agents, like macrolide and or vancomycin. There is lack of data which can demonstrate whether Zn/Cu-resistant bacteria may acquire antibiotic resistance genes / be antibiotic resistant or the other way around that antibiotic resistant bacteria are more capable to be Zn/Cu-resistant than antibiotic susceptible bacteria.

10. Uncertainty and sensitivity analysis

10.1 Accumulation and effects of Zn and Cu in soil

Sensitivity analysis involves calculations on how various constants and algorithms influence the results of the risk assessment. In every step of the model calculations, several values may be used (i.e. the concentration in manure, partitioning coefficients, etc).

10.1.1 Changes in soil properties with time

Total organic matter

An increase in TOC is expected after 100 years of manure application to an agricultural soil. Increased TOC content will increase the sorption of Zn and Cu in soils, reducing leaching and increase the fraction of contaminants accumulating in soils.

Increasing total organic carbon over time results in increased distribution coefficients (Kd) for Zn and Cu (Table 8). The Kd-value is of major importance for the leaching process and thereby the potential leaching to drainage and groundwater. Since the leaching rate (kleaching) is very small, the Kd-values have no effect on the total concentration in soil over time (100 years).

The significance of an increase in soil organic matter over time for the calculated soil PNEC-values is however significant/quite large (Table 12). Increasing TOC by 50% and 100% (from present level) results in a 40-50% increase in calculated PNEC-values. This has no significance for Cu since the PEC/PNEC-ratio (RCR) for all scenarios are <1. For Zn the increase in Kd-values is more important for the risk characterisation, especially for soils having a low clay content (Åsnes and Klepp). At these sites the RCR decrease from 1.6 to 1.1 and 1.5 to 1.2 at Åsnes and Klepp, respectively, when increasing the soil TOC by 100%.

Table 30. Calculated PNEC-values for soils at Åsnes, Klepp and Melhus using different values of TOC (present – I, 50% increase – II and 100% increase –III).

			Åsnes		Klepp		Melhus	
			Mean	90% perc	Mean	90% perc	Mean	90% perc
Zn	Metal background	mg/kg dwt	77	77	25	25	50	50
Zn	PEC total	mg/kg dwt	188	263	145	227	171	254
Cu	Metal background	mg/kg dwt	21	21	9	9	18	18
Cu	PEC total	mg/kg dwt	38	47	27	37	37	47
Soil properties	pH		5.9	5.9	5.9	5.9	6.2	6.2
	Clay	%	7	7	6	6	32	32
	TOC – I	%	2.2	2.2	2	2	4.2	4.2
	TOC – II	%	3.3	3.3	3	3	6.3	6.3
	TOC - III	%	4.4	4.4	4	4	8.4	8.4
PNEC	mg/kg dwt	Zn – I	117	117	95	95	237	237
	mg/kg dwt	Zn – II	146	146	111	111	251	251
	mg/kg dwt	Zn – III	171	171	123	123	261	261
	mg/kg dwt	Cu – I	64	64	58	58	150	150
	mg/kg dwt	Cu – II	78	78	71	71	170	170
	mg/kg dwt	Cu – III	90	90	82	82	188	188
RCR	Total approach	Zn – I	1.6	2.3	1.5	2.4	0.7	1.1
		Zn – II	1.3	1.8	1.3	2.1	0.7	1.0
		Zn – III	1.1	1.5	1.2	1.9	0.7	1.0
		Cu – I	0.6	0.7	0.5	0.6	0.2	0.3
		Cu – II	0.5	0.6	0.4	0.5	0.2	0.3
		Cu – III	0.4	0.5	0.3	0.4	0.2	0.2

In the calculation of soil concentrations (exposure concentrations), three steps are crucial for the final result:

1. Setting of overall parameters (precipitation excess (soil infiltration), soil depth, soil density)
2. Input of Zn and Cu to soils (content of in pig and poultry manure)
3. Removal of contaminants from the soil after manure application (leaching, plant uptake)

The significance of various parameters for the final result of the risk assessment should be evaluated. In the sensitivity analysis below the significance of the following are discussed:

1. Soil density
2. Precipitation excess (soil infiltration)
3. Soil distribution coefficient (K_d/K_{oc}-values)
4. Removal processes (k)

Examples of how these parameters influence soil concentrations and plant concentrations and thereby human exposure are shown below:

1. Soil density

Table 31. Calculated soil concentrations (mg/kg) after 100 years of pig manure application for using different soil densities.

Solør	**Zn**		**Cu**	
Pig manure	**Soil density**		**Soil density**	
	1200	1500	1200	1500
Soil conc, 20 kg P, 10 years	80	80	22	22
Soil conc, 20 kg P, 50 years	93	90	24	23
Soil conc, 20 kg P, 100 years	110	103	26	25
Soil conc, 70 kg P, 10 years	89	86	23	23
Soil conc, 70 kg P, 50 years	133	122	30	28
Soil conc, 70 kg P, 100 years	188	165	38	35

Soil density effects for calculated concentrations of Zn and Cu in plants are shown in Table 32.

Table 32. Calculated concentrations of Zn and Cu in plants (background-present level) and after 100 years of pig manure application (70 kg P, 100y). Soil densities of 1200 and 1500 kg/m^3 have been used in the calculations.

Solør	**Zinc**			**Copper**		
Pig manure	**Background**	**1200 kg/m^3**	**1500 kg/m^3**	**Background**	**1200 kg/m^3**	**1500 kg/m^3**
		70kg P, 100 y	70kg P, 100 y		70kg P, 100 y	70kg P, 100 y
Leafy vegetables	23.0	56.3	49.6	1.9	3.4	3.1
Carrot	9.2	22.5	19.8	1.9	3.4	3.1
Potato	9.2	22.5	19.8	1.9	3.4	3.1
Cereals	13.0	31.9	28.1	5.4	9.7	8.9
Gras	21.5	52.5	46.3	4.5	8.0	7.3

Since the exposure for the various endpoints is proportional to soil concentration, the human dietary exposure to Zn and Cu after 100 years of manure application also decreases (Table 33.). The decrease for Zn and Cu is 11% and 4%, respectively, and do not influence the main conclusions.

Table 33. Human mean dietary intake (mg/day) of Zn and Cu using soil density 1200 (used in the model) and 1500 kg/m^3 for Åsnes area (calculation explanations are given in Chapter 8.2)

Soil density	**Zinc**				**Copper**			
	Average consumer		**High consumer**		**Average consumer**		**High consumer**	
	Background	**Pig manure 100 years**	**Background**	**Pig manure 100 years**	**Background**	**Pig manure 100 years**	**Background**	**Pig manure 100 years**
	mg/dag	mg/dag	mg/dag	mg/dag	mg/dag	mg/dag	mg/dag	mg/dag
1200	13.1	24.9	26.7	51.8	1.4	2.4	3.0	5.1
1500	13.1	22.1	26.7	45.8	1.4	2.3	3.0	4.9

2. Precipitation excess

Precipitation excess (i.e. the amount of precipitation that infiltrates the soil) of 0,4 (Åsnes) and 0,7 (Klepp and Melhus) has been used in the soil, plant, human and animal calculations. Due to high sorption of Zn and Cu to soils, the removal rate due to leaching are small (Table 10) and the precipitation excess has nearly no effect on soil concentrations in a 100 years time scale. As can be seen, the infiltration rate has almost no effect on soil heavy metal concentrations.

3. Soil distribution coefficient

The distribution coefficient is important for leaching of contaminants from the soil, but as for the precipitation excess, this parameter has relatively little effect on future soil concentrations compared to variations in soil density.

The model calculations of future soil concentrations following manure application is to a limited extent influenced by input parameters soil density, precipitation excess and distribution coefficients. The input levels of Zn and Cu to soils are therefore the main uncertainty when it comes to conclusions, especially since only 14 samples of pig and poultry manure have been used as input. Mean and 90th percentile have however been used as inputs in the calculations, and should give representative outputs when it comes to evaluations of risk. The 14 samples have more or less the same level of Zn and Cu as former investigations of manure from Norway and Denmark.

4. Removal processes (k)

Calculations of PEC_{water} (chapt.6) showed that surface runoff is more important than drainage as a removal process for Zn and Cu in soils. In the calculation of future soil concentrations following manure application, runoff is not included as one of the removal processes which leads to a slight overestimation of future soil concentrations.

Calculations using MACRO GV showed that the runoff of Zn and Cu at the three sites varied from 1-6% of added amount during a 20 year period after application. Since the manure is mixed into the upper 20cm of soil, runoff of Zn and Cu is limited, and the annual soil loss do not influence the calculated soil concentrations (10, 50 and 100 years and manure application) to a significant extent. Calculations indicate that including runoff will have reduced future soil concentrations by about 3%, which is well within the uncertainties in the calculations.

10.2 Surface water assessment

In the surface water assessment, the effect of Zn and Cu on aquatic organisms in a water body receiving runoff and drainage from fields to which manure has been added for 100 years has been predicted. Several factors and constants involved in the models and calculations affect the outcome of the predictions. In general, a realistic worst case concept has been used in selection of values of input parameters. The effects of varying some of the critical factors are shown below.

1. Selection of crops in PRZM modelling.

Corn cultivation implies that the soil surface is less protected against soil erosion than permanent grass cultivation. In all the modelling scenarios, corn has been included as the only crop (Klepp and Åsnes) or in rotation with grass (Melhus). The soil concentration in the runoff water was high in scenarios with corn. A separate modelling with only grass showed that the annual soil loss was reduced with approximately 90 %. Thus, since most of the metals in the runoff water are adsorbed to soil particles, the amount of metals that reach the receiving water body is much lower from a grassland fields than from a corn field. In most areas, however, grassland cultivation is not permanent and rotation with corn occurs sporadically. Therefore the worst case scenario of corn cultivation has been included for all locations and the PECs used in risk characterization apply to episodes of high runoff from corn fields which are likely to occur in a 20 years perspective.

2. Abiotic factors in receiving waters

The PNECsw, which is the highest concentration that is assumed to have no harmful effects of surface water organisms, is affected by several abiotic factors as discussed in chapter 1.2. In the risk assessment, reference streams/rivers have been selected to represent the abiotic conditions in local water bodies which may be exposed to runoff and drainage from cultivated fields, fertilized with manure. No alteration of these factors by runoff from the fields has been assumed to occur. However, at the lowest dilution rats (10x) that has been used for calculation of PEC it is likely that also the pH value or the concentrations of Ca and DOC in the receiving water will be significantly altered. The pH-values are most likely to become reduced by addition of runoff water since the pH-values in the soils (See Table 8) is lower than in the receiving waters (Table 6). For Ca, and DOC, runoff water will likely cause increased concentrations. The effect of variations in these abiotic factors on the PNEC in the three receiving water is demonstrated in Table 34. (Zn) and Table 35. (Cu).

The lowering of the pH-value with 0.5 units has no significant effect on the PNECs for Zn, while the PNECs for Cu is increased in Klepp and Melhus and decreased in Åsnes. Increasing DOC concentration results in higher PNECs for both Zn and Cu in all receiving waters. Increased Ca concentrations have no effect on PNECs for Cu, and variable but rather small effects on Zn PNECs. Finally, the combined effect of decreased pH (0.5 units), and increased DOC (5mg/L) and Ca (2 mg/L) is shown in the last column of the tables. This combination gives higher PNEC values than the "original" PNECs in the receiving water ("reference") except for Cu at Åsnes. In the tables all PNECs that are below the calculated PECs at 10x dilution of the runoff water (See Table 21) are indicated in bold. The variations in abiotic factors does not influence the outcome of the risk assessments in most of the scenarios. For Zn all PNECs are below the PECs (i.e. an indication of risk) except for at Melhus when DOC is increased with 10 mg/L. For Cu, no risk is indicated.

Table 34. Effect on PNECs for Zn of variation in abiotic factors pH (units), DOC and Ca (mg/L). The reference PNEC refers to the receiving water conditions (See Table 12) and "Combination" shows the PNECs at pH-0.5, DOC+5 and Ca+2.

Scenario	Reference	pH -0.5	DOC+5	DOC+10	Ca+2	Ca+5	Combination
Klepp	19.3	19.3	47.7	67.4	20.4	14.6	20.6
Åsnes	87	87	96.5	125.6	73.8	36.8	106
Melhus	20.4	20.4	47.7	73.8	21.5	16.2	23.6

Table 35. Effect on PNECs for Cu of variation in abiotic factors pH (units), DOC and Ca (mg/L). The reference PNEC refers to the receiving water conditions (See Table 12) and "Combination" shows the PNECs at pH-0.5, DOC+5 and Ca+2.

Scenario	Reference	pH -0.5	DOC+5	DOC+10	Ca+2	Ca+5	Combination
Klepp	14.6	33	32.9	62.2	14.6	14.6	19.6
Åsnes	48	6.7	69.6	102	48	48	32.8
Melhus	17.1	43	50.7	73.3	17.1	17.1	33.1

At the higher dilution level (50x) of runoff water, significant changes of the abiotic factors in the receiving water are less likely and changes in the outcome of the risk assessment are not expected.

10.3 Uncertainty of plant concentrations

The background and future concentrations of Zn and Cu in agricultural crops are calculated using bioconcentration factors (BCFs) (Table 9), an approach assuming that plant concentrations (stem, leaf, grain etc.) are proportional to soil concentrations. In the calculations of future soil concentrations at 10, 50 and 100 years, the annual removal rate through crops was calculated using the soil concentration after respectively 5, 25 and 50 years. This is assumed to be the best estimate of average removal rate in the periods (i.e. the periods of 10, 50 and 100 years).

The BCFs are based upon empirical data where different plant species are grown on soil with varying properties. BCFs may differ not only between soils and plant varieties, but also between plants within the same plant variety (i.e. between geno types within the same variety).

In this risk assessment BCFs for potatoes, cereals and gras have been used. The BCFs are similar to the BCFs used in the risk assessment of contaminants in sewage sludge (VKM 2009).

$$Cplant_{potatoe,cereal,gras} = Csoil \cdot BCF_{potatoe,cereal,gras} \qquad \textbf{Eq. 7}$$

Where

$Cplant_{potatoe,\ cereal,\ gras}$ = concentration in potatoe, cereal, gras [mg kg^{-1} dw]

Csoil = total concentration in soil [mg kg^{-1} dw]

$BCF_{potatoe,\ cereal,\ gras}$ = bioconcentration factor for the actual crop type [dw plant/dw soil]

However, BCF may represent a maximal uptake in plants in particular for Cu as low plant uptake of Cu is reported in several studies. The uptakes of Zn and Cu are highly dependent of soil pH and organic matter content (see chapter 5.4.2).

10.4 Uncertainty in animal risk characterisation

The animal risk characterisation is primarily based on estimated background concentrations and predicted concentrations of Zn and Cu in feed and pasture plants fertilised with pig or poultry manure for 100 years. The *mean* concentrations of Zn and Cu in manure and soil are used in the calculations for animal exposure. Mean yearly concentrations are assumed to represent the realistic average total levels in the situation of repeated application for 100 year, as the long term effects are the relevant effect scenario for farmed animals. For soil and water organisms where more acute effects are the realistic scenarios 90 percentiles are included.

The background Zn and Cu concentrations are estimated on the basis of published bioconcentration factors (BCFs) for grass, potatoes and cereals.

As pig manure shows some higher Zn and Cu concentrations than poultry manure, *pig manure* was selected as the manure for in depth animal risk characterisation.

Intensive use of manure (70 kg P/ha) for 100 years was included in the characterisation. A moderate use of manure (20 kg P/ha) would have reduced the risk significantly. The estimated average increments of Zn concentration in plants were 2.4 to 5.8 times the background levels based on data from the three selected areas covering main agricultural areas of Norway. The corresponding increases of Cu concentrations were 1.8 to 3.0 times. The modelling showed the highest increase at Klepp area and the lowest increase at Åsnes area. However, due to higher background concentrations Åsnes showed to highest concentrations in plants after 100 years, and this area was used for risk characterisation of animal health.

The total intake of compound feed and roughage for various animal species were based on figures in Pond et al. (2005), and relevant reports and presented in Table 26. As soil contains relatively high concentrations of Zn and Cu the animal intake of soil will easily influence on the results.

The hazard characterisation for animal health was based on Maximum tolerable levels (MTL) for Zn and Cu given by NRC (2005).

The risk characterisation of animal health contains uncertainty in concentrations of Zn and Cu in manure, in the level of manure application, in Zn and Cu in soil and in plants, as well as of intake of various feedstuffs including ingestion of soil. The effects of the various uncertainties may certainly influence on the figures presented but the main conclusion of the animal characterisation should give a realistic impression of the health risk.

10.5 Uncertainty in human risk characterisation

The background concentrations of Zn and Cu in Zn and Cu containing foods from The Norwegian Food Composition Table (Matvaretabellen 2014) are used together with the human intake (mean and high consumers) of these foods from Norkost 3 (Totland et al. 2012) for estimation of the background intakes of Zn and Cu for mean and high consumers (Table 29). The Norwegian Food Composition Table and Norkost 3 are based on amounts of data and low degree of uncertainty is suggested. However, the presentation of our data is a rather rough estimate based on the average Norwegian, not age or gender specified.

For estimation of the human intakes of Zn and Cu after 100 years with pig and poultry manure the range of estimated increments of Zn and Cu in plants in the selected agricultural regions were used. As for animal characterisation, these figures were based on *mean* concentrations of Zn and Cu in *pig manure* (as pig manure averagely contains somewhat higher Zn and Cu concentrations than poultry manure) at a *high rate of manure application* of 70 kg P/ha.

The estimations are based on a 100 % intake of the plant food from the fertilised area. As the manure is spread locally, repeated spreading at the same areas year by year (100 years) and that is a realistic scenario. However a 100 % intake of plant food from such fertilized area is may not be the situation for an average consumer.

It is assumed that the concentrations of Zn and Cu in meat, milk, cheese, butter and eggs are not influenced by manure application to soils. The intake from these foods is therefore considered constant during the 100 year period.

The risk characterisation of human health contains uncertainty in concentrations of Zn and Cu in manure, in the level of manure application, in Zn and Cu in soil and in plants, in drinking water, in animal products, and in the level of intake of food from the fertilized areas. The effects of the various uncertainties may certainly influence on the figures presented but the main conclusion of the human characterisation is suggested to give a realistic impression of the health risk.

11. Answer to the terms of reference

1. **Reduced exposure of zinc and copper to pigs and poultry to reduce the concentrations in manure**

1.1 The requirement of zinc and copper in the complete feed for pigs and poultry.

The requirement of Zn and Cu in pigs may vary within 25 – 80 mg Zn/kg and a 3.5-5 mg Cu/kg total diet.

The requirement of Zn and Cu in poultry may vary within 30 – 60 mg Zn/kg and 4 – 8 mg Cu/kg total diet.

1.2 Are other sources (drinking water, barn installation) than compound feed of importance for the zinc and copper content in manure?

Zn exposure via drinking water will usually represent a negligible amount of the total Zn exposure for pigs and poultry.

Cu exposure from drinking water for pigs and poultry in Norway is considered in general not to contribute significantly to the total Cu exposure.

Most of the Zn release from barn installations made of Zn coated steel, ingested or not, ends in the manure. The roughly estimated Zn release from the barn installations may constitute up to approximately 25 % of the total manure concentration.

1.3 Is it possible to reduce zinc and copper in feed or veterinary prescriptions without adverse effects on growth, health and welfare of pigs and poultry? And what is the possible respective gain of reduced concentrations in manure?

In pigs and poultry, the present maximum levels of Zn and Cu compound feed are 150 and 25 mg/kg respectively, except 35 mg Cu/kg in feed for piglets. The data from the industry and results from the Norwegian Food Safety Authority show that the feed commonly contains Zn and Cu up to the limits. The data indicate exposure of Zn and Cu to pigs and poultry at least 2 times higher than the requirement and most often several times above the required level. The relatively large margins between the levels in compound feed and the animals' requirement indicate that reduction of the present level should be possible.

Furthermore, piglets are commonly also fed complementary feed with Zn and Cu, and Zn in medical remedies. The normally preventive use of Zn as medical remedy against diarrhoea and oedema disease in weaned piglets is approximately 15-20 times higher than the exposure via complete feed, and 30-60 times above the normally required level. These diseases are associated with the management, housing and husbandry and may possibly be controlled otherwise.

The bioavailability of Zn and Cu and other trace elements influences each other, particularly at high exposure levels. Therefore the way of feeding animals with Zn and Cu far above required levels may reduce their bioavailability.

A reduction of exposure of Zn and Cu in feed and supplements would correspondingly reduce their concentrations in manure. In fact, for Zn the effect on reduction in manure will be further enhanced as high exposure show particularly low animal retention. Proper use, avoiding surplus dosing is also important in order to avoid possible effects in the food chain and environment.

1.4 Is it possible to use more biologically efficient chemical compounds of copper or zinc to increase the animal uptake and reduce the excretion of these elements in urine and excrements?

Several reports tell that Zn and Cu from organic complexes seem to have approximately equal bioavailability to Zn in Zn sulphate and Cu in Cu sulphate. The bioavailability of Zn and Cu seems however to be different for various organic complexes, due to f.ex. different pH stability among various organic trace minerals. From experimental studies of pharmacological effects of Zn or Cu to reduce post-weaning scouring and improve body weight gain, rather low concentrations of Zn or Cu in organic form or Zn in lipid-encapsulated form were shown similarly effective as far higher concentrations of inorganic Zn or Cu. As the bioavailability and retention of Zn and Cu in the body is normally rather low and even lower at high exposure, biologically more efficient chemical forms of Zn and Cu will reduce the excretion and manure concentrations correspondingly efficient.

2. Application of manure to soil

2.1 Define a moderate and high level of yearly zinc and copper application from manure to soil under different crop rotations, and calculate the soil concentrations in 10, 50 and 100 years perspective. Addition of zinc and copper from other sources should also be taken into account.

To calculate the amount of Zn and Cu applied to soil using manure, two application rates were selected: 20 kg phosphorous (P) and 70 kg P/ha year. 20 kg P/ha is an average amount of P added annually to cover the plant requirement. 70 kg P/ha is a high dose, but in some areas with high density of pig and poultry farms, this amount of P could be applied.

Pig and poultry manure contains about 17 gram P/kg dry manure. To add a dose of 20 kg P/ha, 22 tons of pig manure or 2.3 tons of poultry manure (having dry matters of 5 % and 53 %, respectively) have to be applied. Correspondingly, a dose of 70 kg P/ha means application of 77 tons of pig manure or 8.1 tons of poultry manure.

The assessment is based on Zn and Cu concentrations in manure from pigs and poultry in a Norwegian report (Daugstad et al, 2012). The reported levels were in accordance with estimated levels based on sources (complete feed, complementary feed, medical remedies, drinking water, and barn installations).

Realistic soil depth, coefficients for Zn and Cu distribution in soil, rainfall and leaching as well as uptake in plants via crop rotations at three selected regions in Norway, Klepp (Jæren), Åsnes (Solør) and Melhus (Southern Trøndelag) were used in the assessment.

Mean and 10 and 90 percentiles of Zn and Cu from use of pig or poultry manure at high or moderate levels for 10, 50 and 100 years were estimated.

2.2 Evaluation of the following exposure routes for the scenarios described in 2.1

Plants and soil organisms in soil where pig or poultry manure has been used:

Application of high doses (70 kg P/ha) of pig manure may result in toxic effects to soil living organisms (invertebrates, plants and microorganisms) after long term use (100 years). The risk of negative effects on soil living organisms is somewhat less using poultry manure.

The present level of Cu in pig and poultry manure will not result in negative effects on soil living organisms even at high application rates of manure.

Aquatic organisms living in a body of surface water influenced by soil where pig or poultry manure has been used:

Leaching of Zn from fields that have been intensively fertilised for 100 years with pig or poultry manure with a high content of Zn may pose a risk to aquatic organisms in local water bodies such as small streams or ponds where the dilution level is less than a factor 50. Also sediment dwelling organisms may be adversely affected by Zn under worst case conditions. Cu is not expected to reach concentrations that are harmful to aquatic biota.

Animals eating grass and/or soil and soil organism and animals eating feed from fields where pig or poultry manure has been used:

By intensive use of pig (or poultry) manure for 100 years, the Cu concentration in the feed plants may increase and give livestock a total exposure level at approximately 10 mg Cu/kg complete ration. Above this level elevated risk for Cu, adverse accumulation and toxicity in sheep would be expected. Such exposure level is not of health concern for other livestock species. The elevation of Zn exposure is not of toxicological concern for livestock.

Humans eating plant products that have been grown on fields where pig or poultry manure has been used:

The consumption of food produced in areas where manure from pigs or poultry has been used over time leads to an increased human intake of Zn and Cu via particularly cereals and vegetables. Intensive use of pig manure (70 kg P/ha) for 100 years means that the Zn intake of mean consumers will exceed the Upper Intake Limit (UL) and the Zn intake of high consumers will exceed NOAEL. Such exposure of Zn may be of concern for human health. For Cu the estimated intake of mean consumers is below UL, whereas high consumers exceed UL. Such exposure of Cu may be of low concern for human health.

Humans eating products from grazing animals and/or products from animals eating feed grown in fields where pig or poultry manure has been used:

Zn and Cu are rather poorly retained in the animal body. Absorbed Zn is mostly deposited in bone, liver, pancreas and kidney, and Cu in liver and kidney. Increased Zn and Cu in animal products as meat, milk and eggs are not expected.

Humans drinking surface- and/or groundwater influenced by soil where pig or poultry manure has been used:

Leaching of Zn and Cu from soil where pig or poultry manure has been used may cause increased concentrations of these metals in local bodies of surface waters. The expected concentrations in surface waters used as drinking water are, however, below the levels that may cause practical inconveniencies and human health effects. Leakage of Zn and Cu to groundwater is considered to be insignificant.

3. Resistance to antibiotics

3.1 Can zinc and copper in feed play a role in the development of resistance to antimicrobial agents?

Bacteria in animals and the environment may develop resistance to trace elements as Zn and Cu. Resistance to Cu in bacteria, in particular enterococci, is often associated with resistance to antimicrobial agents, like macrolide and vancomycin. Resistance to Zn is often linked with resistance to methicillin in staphylococci, and Zn supplementation to animal feed may increase the proportion of multi-resistant *E. coli* in gut microbiota. However, there is lack of data which can demonstrate whether Zn/Cu-resistant bacteria may acquire antibiotic resistance genes / be antibiotic resistant or the other way around, antibiotic resistant bacteria are more capable to be Zn/Cu-resistant than antibiotic susceptible bacteria. The degree of exposure of Zn or Cu may be of importance but there is also lack of data on dose-response relations.

12. Conclusions

12.1 Requirement for pig and poultry

The requirement of Zn and Cu in pigs may vary within 25 – 80 mg Zn/kg and 3.5 – 5 mg Cu/kg total diet.

The requirement of Zn and Cu in poultry may vary within 30 – 60 mg Zn/kg and 4 – 8 mg Cu/kg total diet.

12.2 Exposure for pig and poultry

The data on Zn and Cu in complete compound feed in Norway indicate exposure of Zn and Cu to pigs and poultry at least 2 times higher than the requirement but most often several times above required level.

Piglets are commonly also exposed to complementary feed with Zn and Cu, as well as Zn in medical remedies. The estimated extra exposure via complementary feed is rather marginal but the medical use of Zn against diarrhoea and oedema disease in weaned piglets is considerable and at a level of approximately 15-20 times higher than the exposure via complete feed, and 30-60 times above what is normally required.

The Zn and Cu in drinking water are normally at low concentrations considered to constitute an insignificant exposure. In pig barns with Zn-coated installations, such Zn may constitute a considerable contribution to Zn in manure but probably not significantly influencing the Zn exposure of the average pig.

12.3 Effects on soil organisms

Application of high doses (70 kg P/ha) of pig manure may result in toxic effects to soil living organisms (invertebrates, plants and microorganisms) after long term use (100 years). The risk of negative effects on soil living organisms is somewhat less using poultry manure.

The present level of Cu in pig and poultry manure will not result in negative effects on soil living organisms even at high application rates of manure.

12.4 Effects on aquatic organisms

Intensive fertilisation with pig manure containing a high level of Zn may pose a risk of toxic effects on aquatic and sediment dwelling organisms in small streams and ditches which receive runoff water from fields where manure has been applied for 100 years. In larger steams, rivers and lakes, where the runoff water dilution exceeds a factor 50, no risk is foreseen.

Cu is not expected to reach levels that are harmful to aquatic organisms in surface water.

12.5 Increase of Zn and Cu in plants

The estimated increase of Zn in plants from three selected areas intensively fertilized with manure from pigs varied from 2.4 to 5.8 times the background levels after 100 years. The corresponding estimated increase of Cu in plants varied from 1.8 to 3 times the background levels. The increases of Zn and Cu by use of poultry manure would be somewhat lower.

12.6 Animal health risk

The increased level of Zn in soil from intensive use of manure from pigs or poultry implies a negligible health risk for livestock grazing or receiving feed from these areas.

The critical endpoint for animal health effects from the increased level of Cu in soil via intensive use of manure from pigs or poultry is Cu poisoning in sheep. However, the risk for sheep health from intensive use of such manure up to 100 years is considered to be low, and for other species negligible.

12.7 Human health risk

A higher exposure of livestock with of Zn and Cu due to intensive use of manure from pigs or poultry on fields is not considered to increase the concentrations of Zn or Cu in animal products such as meat, milk or eggs.

The consumption of food produced in areas where manure from pigs or poultry has been used over time leads to an increased human intake of Zn and Cu via particularly cereals, and vegetables. Intensive use of pig manure (70 kg P/ha) for 100 years means that the Zn intake of mean consumers will exceed the Upper Intake Limit (UL) and the Zn intake of high consumers will exceed NOAEL. Such exposure of Zn may be of concern for human health. For Cu the estimated intake of mean consumers is below UL, whereas high consumers exceed UL. Such exposure of Cu may be of low concern for human health.

12.8 Bacterial resistance

Bacteria in animals and environment may develop resistance to Zn/Cu. The resistance genes to these trace elements are identified in some bacterial species from animals and environment.

Resistance to Zn/Cu and its link to antibiotics resistance in bacterial species originated from animal and environment are identified. Resistance genes to Zn/Cu are often located on plasmids, which may be transferable to other bacteria, intra and inter-species.

A resistance link between Zn and methicillin is identified in staphylococci of animal origin.

Resistance to Cu is often linked to resistance to macrolides (e.g. erythromycin) or glycopeptides (e. g. vancomycin) in enterococci.

Zn supplementation to animal feed may increase the proportion of multi-resistant *E. coli* in gut microbiota.

The transmission of Zn/Cu resistant bacteria with resistance to antimicrobial agents to human microbiota cannot be discounted. Our knowledge regarding mechanisms of induction of Zn/Cu resistance in bacteria is limited and knowledge on dose-response relations are lacking. However, it seems more likely that a resistance driven effect occurs at high trace element exposure than at more basal exposure levels.

12.9 Overall conclusion

Zn and Cu are essential trace elements in plant, animal and human nutrition, and animal manure is a source of great value for fertilisation of agricultural soil. The results of the present suggested overload of Zn and Cu in feed for pigs and poultry and reduced bioavailability of Zn and Cu as well as of other essential trace elements in the animals are high levels of these elements in the manure. Zn and Cu exposure of bacteria in livestock and the environment may develop resistance to Zn and Cu and there are links to antibiotic resistance. In the long run, elevated concentrations of Zn and Cu in manure may adversely influence organisms in the environment and the food chain and be of health concern for human consumers. Reduction in animal intakes of Zn and Cu via feed, additives and medical remedies is possible to carry out respecting animal health and seems necessary respecting adverse effects in the environment and food chain.

13. Data gaps and recommendations

- High levels of Zn in manure seems to be the main problem when it comes to long term effects in soil and aquatic organisms and humans, whereas high Cu may be a future problem for sheep. There are, however, little data on Zn and Cu in manure from pig, poultry and other livestock animals. More efforts should be made to monitor the actual level of Zn and Cu in manure at a regular basis or, more specifically, the Zn and Cu flow at farm level – feed-manure-soil-plants-water.

- There are some results from livestock studies indicating that low concentrations of Zn or Cu in organic forms or Zn in lipid-encapsulated form give similar animal efficiency as far higher concentrations of the commonly used inorganic Zn or Cu. More studies on the possibilities to use biologically more efficient chemical forms of Zn and Cu should be conducted.

- Knowledge on the development, mechanisms and dose-response-relations of the microbiological resistance against Zn and Cu, and its link to antibiotic resistance is needed.

- Methods for determination of MIC-values against Zn/Cu in bacteria should be standardised.

- There is lack of knowledge on the occurrence of the microbiological resistance against Zn and Cu in livestock, environment and humans. Surveys should be conducted.

- Reduction of intakes of Zn and Cu to pigs and poultry via feed, additives and medical remedies is possible to carry out respecting animal health. Comparison of requirement and exposure of Zn and Cu should similarly be conducted for other livestock, pet and farmed fish species. Reduction of Zn and Cu seems necessary respecting adverse effects in the environment and food chain.

14. References

Aarestrup FM, Cavaco L, Hasman H. 2010. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. Vet Microbiol. 142(3-4):455-7.

Aarestrup FM, Hasman H, Jensen LB, Moreno M, Herrero IA, Domínguez L, Finn M, Franklin A. 2002. Antimicrobial resistance among enterococci from pigs in three European countries. Appl Environ Microbiol. 68(8):4127-9.

Aarestrup FM, Hasman H. 2004. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Vet Microbiol. 100(1-2):83-9.

Aarestrup FM, Seyfarth AM, Emborg H-D, Pedersen K, Hendriksen RS, Bager F. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother. 45:2054–9.

Aasen, I. 1997. Mangelsjukdomar og andre ernæringsforstyringar hos kulturplanter. Landbruksforlaget. 95 p.

Aktan Y, Tan S, Icgen B. 2013. Characterization of lead-resistant river isolate *Enterococcus faecalis* and assessment of its multiple metal and antibiotic resistance. Environ Monit Assess. 185(6):5285-93.

Amachawadi, R.G., Shelton, N. W., Shi, X., Vinasco, J., Dritz, S. S., Tokach, M. D., Nelssen, J. L., Scott, H. M., Nagaraja, T. G. 2011. Selection of Fecal Enterococci Exhibiting *tcrB*-Mediated Copper Resistance in Pigs Fed Diets Supplemented with Copper Appl Environ Microbiol. 77(16): 5597–5603.

Ammerman C, Baker D, Lewis A. 1995. Bioavailability of nutreients for animals. Academic Press Inc. San Diego.

Andreini C, Banci L, Bertini I, Rosato A. 2006. Counting the zinc-proteins encoded in the human genome. J Proteome Res. 5:196-201.

Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, et al. 2011. Enterotypes of the human gut microbiome. Nature advance online publication.

Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-Bacterial Mutualism in the Human Intestine. Science 307:1915-1920.

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. 2006. Co-selection of antibiotic and metal resistance. Trends Microbiol. 14(4):176-82.

Baustad B, Djønne B, Haukvik IA. 2002. Tarmsjukdommer hos spedgris og smågris. Nor Vet Tidsskr 114:172-177.

Bedford MR & Schulze H. 1998. Exogenous enzymes for pigs and poultry. Nutr Res Rev*.*, 11**,** 91-114.

Bednorz C, Oelgeschläger K, Kinnemann B, Hartmann S, Neumann K, Pieper R, Bethe A, Semmler T, Tedin K, Schierack P, Wieler LH, Guenther S. 2013. The broader context of antibiotic resistance: zinc feed supplementation of piglets increases the proportion of multi-resistant Escherichia coli in vivo. Int J Med Microbiol. 303(6-7):396-403.

Berg J, Thorsen MK, Holm PE, Jensen J, Nybroe O, Brandt KK. 2010. Cu exposure under field conditions coselects for antibiotic resistance as determined by a novel cultivation-independent bacterial community tolerance assay. Environ Sci Technol. 44(22):8724-8.

Berg J, Tom-Petersen A, Nybroe O. 2005. Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. The objective of this study was to determine whether Cu-amendment of field plots affects the frequency of Cu resistance, and antibiotic resistance patterns in indigenous soil bacteria. Lett Appl Microbiol. 40(2):146-51.

Bioforsk 2012. Næringsinnhald i husdyrgjødsel – Analyser av husdyrgjødsel frå storfe, sau, svin og fjørfe 2006-2011. Bioforsk Report Vol.7, nr.24, 2012 - http://www.bioforsk.no/ikbViewer/Content/97342/Naeringsinnhald%20i%20husdyrgjodsel%20rapport%20til%20SLF%202822012.pdf

Bioforsk 2013. Norwegian Institute for Agricultural and Environmental Research, Fr. A. Dahlsvei 20, NO-1430 Ås (ref arne.gronlund@bioforsk.no).

Bonten MJM, Willems R, Weinstein RA. 2001. Vancomycin-resistant enterococci: why are they here, and where do they come from? Lancet Infect Dis. 1:314–25.

Bouron, A. & Oberwinkler, J. 2013. Contribution of calcium-conducting channels to the transport of zinc ions. Pflugers Arch - Eur J Physiol**,** 1-7

Bowler C, Montague M van and Inze D. 1992. Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physol. Plant Mol. Biol. 43: 83-116.

Börjesson G, Kirchmann H and Kätterer, T. 2014. Four Swedish long-term field experiments with sewage sludge reveal a limited effect on soil microbes and on metal uptake by crops. J Soils Sediments 14: 164-177.

Buff CE, Bollinger DW, Ellersieck MR, Brommelsiek WA, Veum TL. 2005. Comparison of growth performance and zinc absorption, retention, and excretion in weanling pigs fed diets supplemented with zinc-plysaccharide or zinc oxide. J Anim Sci 83:2380-2386.

Cardonha AM, Vieira RH, Peirano G, Rodrigues Ddos P. 2005. Resistance to antibiotics and heavy metals from Escherichia coli isolated from sea water and pluvial galeries. Acta Cir Bras.20 Suppl 1:253-6.

Carlson D, Poulsen HD and Sehested J. 2004. Influence of weaning and effect of post weaning dietary zinc and copper on electrophysiological response to glucose, theophylline and 5-HT in piglet small intestinal mucosa. Comp. Biochem. Physiol. A 137:757-765.

Castillo M, Skene G, Roca M, Anguita M, Badiola I, et al. 2007. Application of 16S rRNA gene-targetted fluorescence in situ hybridization and restriction fragment length polymorphism to study porcine microbiota along the gastrointestinal tract in response to different sources of dietary fibre. FEMS Microbiol Ecol 59: 138-146.

Cavaco LM, Hasman H, Aarestrup FM. 2011. Zinc resistance of Staphylococcus aureus of animal origin is strongly associated with methicillin resistance. Vet Microbiol. 150(3-4):344-8.

Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, Ito T, Aarestrup FM. 2010. Cloning and occurrence of czrC, a gene conferring cadmium and zinc resistance in methicillin-resistant Staphylococcus aureus CC398 isolates. Antimicrob Agents Chemother. 54(9):3605-8.

Coffey RD, Cromwell GL, Monegue HJ. 1994. Efficacy of a copper-lysine complex as a growth promotant for weanling pigs. *J Anim Sci* 72:2880-2886.

Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, et al. 2008 Metal chelation and inhibition of bacterial growth in tissue abscesses. Science 319: 962-965.

Daugstad K, Kristoffersen AØ. and Nesheim L. 2012. Næringsinnhald i husdyrgjødsel – Analyser av husdyrgjødsel frå storfe, sau, svin og fjørfe 2006-2011. Bioforsk-report 7/2012, ISBN.no 978-82-17-00897-2. Bioforsk, Fredrik A Dahlsvei 20, N-1432 Ås.

Davis GK, Mertz W. 1987. Copper. In Mertz W (ed): Trace Elements in Human and Animal Nutrition 5th ed, Vol 1, Academic Press Inc. Orlando, pp 301-364.

Degryse F, Smolders E & Parker DR 2009. Partitioning of metals (Cd, Co, Cu, Ni, Pb,Zn) in soils: concepts, methodologies, prediction and applications – a review. *EuropeanJournal of Soil Science* 60: 590-612.

De Schamphelaere KAC and Janssen CR 2004. Effects of dissolved organic carbon concentration and source, pH and water hardness on the chronic toxicity of copper to daphnia magna. Environmental Toxicity and Chemistry. 23(5): 1115-1122.

De Schamphelaere KAC, Lofts S and Janssen CR 2005: Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids and fish in natural surface waters. Environmental Toxicity and Chemistry 24:1190-1197.

De Schamphelaere KAC, Heijerick DG and Janssen CR. 2003. Development and Validation of Biotic Ligand Models for Predicting Chronic Zinc Toxicity to Fish, Daphnids and Algae (Final report of ILZRO project ZEH-WA-1). Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium. (Sponsor: International Lead and Zinc Research Organization, ILZRO, United States).

de Vicente A, Avilés M, Codina JC, Borrego JJ, Romero P. 1990. Resistance to antibiotics and heavy metals of Pseudomonas aeruginosa isolated from natural waters. J Appl Bacteriol. 68(6):625-32

Dunning JC, Ma Y, Marquis RE 1998 Anaerobic killing of oral streptococci by reduced, transition metal cations. Appl Environ Microbiol 64: 27-33.

EC 2003. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances; Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances; Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Available at http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/doc/tgd

EC 2003. Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs. European Commission. Available at http://esis.jrc.ec.europa.eu/

ECI (European Copper Institute). 2008. Voluntary Risk Assessment on Copper. Available online at http://www.eurocopper.org/copper/copper-ra.html

EFSA, 2010 Pre-assessment of environmental impact of zinc and copper used in animal nutrition. Prepared by Monteiro SC, Lofts S, Boxall AB. 138 pp.

Esser, K. 1996. Reference concentrations for heavy metals in mineral soils, oat, and orchard grass (*Dáctylis glomerata*) from three agricultural regions in Norway, Water, Air, and Soil Pollution, 89: 375-397.

EU 2010 European Union Risk Assessment Report - zinc metal EUR 24587. Available at http://esis.jrc.ec.europa.eu/.

Failla ML & Hopkins RG. 1998. Is Low Copper Status Immunosuppressive? Nutr Rev. 56: S59-S64.

Flatlandsmo K. 2012. Giftnemnda og beslektede organer under Landbruksdepartementet. In: Frøslie A (ed), Norsk Veterinærhistorisk Selskap Årbok, pp 55-71.

Fôranalyser (2005-2011) Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). Mattilsynet, seksjon Landdyr og dyrehelsepersonell, Ås

Freitas AR, Coque TM, Novais C, Hammerum AM, Lester CH, Zervos MJ, Donabedian S, Jensen LB, Francia MV, Baquero F, Peixe L. 2011.Human and swine hosts share vancomycin-resistant Enterococcus faecium CC17 and CC5 and Enterococcus faecalis CC2 clonal clusters harboring Tn1546 on indistinguishable plasmids. J Clin Microbiol. 49(3):925-31.

Frisli T, Haverkamp TH, Jakobsen KS, Stenseth NC, Rudi K. 2012. Estimation of metagenome size and structure in an experimental soil microbiota from low coverage next generation sequence data. J Appl Microbiol.

Fødevarestyrelsen 2012. Kobber og zink i dansk svinefoder. Ministeriet for Fødevarer, Landbruk og Fiskeri. No. 2012107, 15 pp.

Gielda LM, DiRita VJ.2012. Zinc competition among the intestinal microbiota. MBio 3: e00171-00112.

Gräber I, Hansen JF, Olesen SE., Petersen J, Østergaard HS and Krogh L. 2005. Accumulation of Copper and Zinc in danish Agricultural Soils in Intensive Pig production Areas. Danish J Geogr. 105: 15- 22.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF., Boron WF., Nussberger S, Gollan JL. & Hediger MA. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature, 388**,** 482-488

Hambidge KM, Casey CE, Krebs NF. 1986. Zinc. In Mertz W (ed): Trace Elements in Human and Animal Nutrition 5th ed, Vol 2, Academic Press Inc. Orlando, pp 1-138.

Hart EB, Steenbock H, Waddell J, Elvehjem CA. 1928. J Biol Chem 77:797.

Hasman H, Aarestrup FM. 2005. Relationship between copper, glycopeptide, and macrolide resistance among Enterococcus faecium strains isolated from pigs in Denmark between 1997 and 2003. Antimicrob Agents Chemother. 49(1):454-6.

Hasman H, Aarestrup FM. 2002. tcrB, a gene conferring transferable copper resistance in Enterococcus faecium: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. Antimicrob Agents Chemother. 46(5):1410-6.

Hasman H, Kempf I, Chidaine B, Cariolet R, Ersbøll AK, Houe H, Bruun Hansen HC, Aarestrup FM. 2006. Copper resistance in Enterococcus faecium, mediated by the tcrB gene, is selected by supplementation of pig feed with copper sulfate. Appl Environ Microbiol. 72(9):5784-9.

Havlin, JL, Tisdale S L, Nelson WL and Beaton JD0. 2014. Soil fertility and fertilizers. 8th ed. Pearson. Pp. 516.

Hill GM, Mahan DC, Carter SD, Cromwell GL, Ewan RC, Harrold RL, Lewis AJ, Miller PS, Shurson GC, Veum TL. 2001. Effect of pharmacological concentrations of zinc oxide with and without the inclusion of an antibacterial agent on nursery pig performance. J Anim Sci 79:934-941.

Hill, GM., Ku PK, Miller ER, Ullrey DE., Losty TA. & Odell BL. 1983. A copper deficiency in neonatal pigs induced by a high zinc maternal diet. J Nutr, 113**,** 867-872

Hsu FS., Krook L, Pond WG. & Duncan JR. 1975. Interactions of dietary calcium with toxic levels of lead and zinc in pigs. J Nutr, 105, 112-118

Humphreys DJ. 1988. Veterinary Toxicology. 3rd ed. Bailliere Tindall. London.

Høgåsen T, Skarbøvik E, Stålnacke P, Selvik JR, Aakerøy PA and Karle Ø. 2011. Elvetilførselprogrammet (RID) – 20 års overvåking av tilførsler til norske kystområder (1999-2009). Datarapport 1 – vannkjemi i RIP-elver. Miljødirektoratet, Rapport TA-2868/2011.

Højberg O, Canibe N, Poulsen HD, Hedemann MS, Jensen BB. 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Appl Environ Microbiol. 2005 May;71(5):2267-77.

Hölzel CS, Müller C, Harms KS, Mikolajewski S, Schäfer S, Schwaiger K, Bauer J. 2012. Heavy metals in liquid pig manure in light of bacterial antimicrobial resistance. Environ Res. 2012 Feb;113:21-7.

Jacob ME, Fox JT, Nagaraja TG, Drouillard JS, Amachawadi RG, Narayanan SK. 2010. Effects of feeding elevated concentrations of copper and zinc on the antimicrobial susceptibilities of fecal bacteria in feedlot cattle. Foodborne Pathog Dis. 7(6):643-8.

Jensen HM. 2006. Health management with reduced antibiotic use - Experiences of a Danish pig vet. Animal Biotechnology 17: 189-194.

Jeong J & Eide DJ. 2013. The SLC39 family of zinc transporters. Mol Asp Med, 34, 612-619

Ji X, Shen Q, Liu F, Ma J, Xu G, Wang Y, Wu M. 2012. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. J Hazard Mater. 15;235-236:178-85.

Kerr LA, Mcgavin HD. 1991. Cronic copper poisoning in sheep grazing pastures fertilized with swine manure. JAVMA 198(1):99-101.

Kim JC, Hansen CF, Pluske JR, Mullan BP. 2010. Evaluating the replacement if zinc oxide with an encapsulated zinc oxide product as a means of controlling post-weaning diarrhoea in piglets. Report prepared for the Co-operative Research Centre for an Internationally Competetive Pork Industry. Government of Western Australia – Murdoch University, 13 pp.

Jongbloed AW, Kemme PA, De Groote G, Lippens M, Meschy F. 2002. Bioavailability of major and trace minerals. EMFEMA International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials), Brussels.

Kim J, Lee S, Choi S. 2012. Copper resistance and its relationship to erythromycin resistance in Enterococcus isolates from bovine milk samples in Korea. J Microbiol. 50(3):540-3.

Kornegay ET. & Harper AF. 1997. Environmental Nutrition: Nutrient Management Strategies to Reduce Nutrient Excretion of Swine. Prof Anim Sci., 13, 99-111.

Larsbo M & Jarvis NJ. 2003. MACRO 5.0. A model of water flow and solute transport in macroporous soil. Technical description. Emergo 2003:6, Department of Soil Sciences, Division of Environmental Physics, SLU.

Lazăr V, Cernat R, Balotescu C, Cotar A, Coipan E, Cojocaru C. 2002. Correlation between multiple antibiotic resistance and heavy-metal tolerance among some E.coli strains isolated from polluted waters. Bacteriol Virusol Parazitol Epidemiol. 47(3-4):155-60.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, et al. 2008 Evolution of mammals and their gut microbes. Science 320: 1647-1651.

Ley RE, Peterson DA, Gordon JI 2006 Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124: 837-848.

Linares LB, Broomhead JN, Guaiume EA, Ledoux DR, Veum TL, Raboy V. 2007. Effects of low phytate barley (*Hordeum vulgare* L.) on zinc utilization in young broiler chicks. Poult Sci 86:299-308.

Lipoth SL. and Schoenau JJ. 2007. Copper, zinc, and cadmium accumulation in two prairie soils and crops as influenced by repeated application of manures. J. Plant Nutr. Soil Sci. 170: 378-386.

Liu Janet Z, Jellbauer S, Poe AJ, Ton V, Pesciaroli M, 2012. Zinc Sequestration by the Neutrophil Protein Calprotectin Enhances Salmonella Growth in the Inflamed Gut. Cell Host Microbe 11: 227-239.

Lium B, Baustad B. 2002. Noen spesifikke infeksjoner hos gris. Nor Vet Tidsskr 114:218-225.

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489: 220-230.

Luo XG, Dove CR. 1996. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. J Anim Sci 74:1888-1896.

Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, et al. 2007. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2: 119-129.

Malik A, Aleem A. 2011. Incidence of metal and antibiotic resistance in Pseudomonas spp. from the river water, agricultural soil irrigated with wastewater and groundwater. Environ Monit Assess. 178(1-4):293-308.

Manangi MK, Vazquez-Anon M, Richards JD, Carter S, Buresh RE, Christensen KD. 2012. Impact of feeding lower levels of chelated trace minerals versus industry levels of inorganic trace menerals on broiler performance, yield, foodpad health, and litter mineral concentration. J Appl Poult Res. 21(4):881-890.¨

Matvaretabellen 2014. The Norwegian Food Composition Table. http://www.matvaretabellen.no/.

McCall KA., Huang C & Fierke CA. 2000. Function and mechanism of zinc metalloenzymes. J Nutr, 130, 1437-1446

Mengel, K. and Kirkby, E.A. 1992. Principles of plant nutrition. 5th ed. Kluwer academic press. 849p.

MERAG 2007. Metals Environmental Risk Assessment Guidance. EuroMetaux (Brussels) Available at http://www.icmm.com/page/1185/publications/documents/metals-environmental-risk-assessment-guidance-merag

Moghaddam HN, Jahanian R. 2009. Immunological responses of broiler chicks can be modulated by dietary supplementation of zinc-methionine in place of inorganic zinc sources. Asian-Aust J Anim Sci. 22:396-403.

Minesteriet for fødevarer, Landbrug og Fiskeri 2012. Kobber og sink i dansk svinefoder, Rapport om niveauet af kobber og zink i dansk svinefoder, produceret hos landbrug og foderstofvirksomheder ved offentlig kontrol i 2007-2011.

Mohanna, C., Carré, B. & Nys, Y. 1999. Incidence of dietary viscosity on growth performance and zinc and manganese bioavailability in broilers. Anim Feed Sci Technol., 77, 255-266.

Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, et al. 2011. Diet Drives Convergence in Gut Microbiome Functions Across Mammalian Phylogeny and Within Humans. Science 332: 970-974.

Namkung H, Gong J, Yu H, de Lange CFM. 2006. Effect of pharmacological intakes of zinc and copper on growth performance, circulating cytokines and gut microbiota of newly weaned piglets challenged with coliform lipopolysaccharides. Canadian Journal of Animal Science 86: 511-522.

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, et al. 2012 Host-gut microbiota metabolic interactions. Science 336: 1262-1267.

Nitrayova S, Windisch W, von Heimendahl E, Müller A, Bartelt J. 2012. Bioavailablity of zinc from different sources in pigs. *J Anim Sci* 90:185-187.

NNR. 2012. Nordic Nutrition Recommendations. (PDF available online at: http://dx.doi.org/10.6027/Nord2014-002).

Norwegian Food Safety Authority. 2006. Fôranalyser 2005 Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). In: Norwegian Food Safety Authority, S. L. O. D. (ed.). Ås.

Norwegian Food Safety Authority. 2007. Fôranalyser 2006 Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). In: Norwegian Food Safety Authority, S. L. O. D. (ed.). Ås.

Norwegian Food Safety Authority. 2008. Fôranalyser 2007 Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). In: Norwegian Food Safety Authority, S. L. O. D. (ed.). Ås.

Norwegian Food Safety Authority. 2009. Fôranalyser 2008 Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). In: Norwegian Food Safety Authority, S. L. O. D. (ed.). Ås.

Norwegian Food Safety Authority. 2010. Fôranalyser 2009 Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). In: Norwegian Food Safety Authority, S. L. O. D. (ed.). Ås.

Norwegian Food Safety Authority. 2011. Fôranalyser 20010 Kjemiske og mikrobiologiske analyser. Mikroskopiundersøkelser. (In Norwegian). *In:* Norwegian Food Safety Authority, S. L. O. D. (ed.). Ås

NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. National Research Council of the National Academies. The National Academies Press, Washington DC

NRC. 2005. Mineral tolerance of animals. 2nd rev. ed. National Research Council of the National Academies. The National Academies Press, Washington DC.

NRC. 2012. Nutrient requirements of swine. National Research Council of the National Academies. The National Academies Press, Washington DC.

Oberleas D, Muhrer ME & O'dell BL. 1962. Effects of Phytic Acid on Zinc Availability and Parakeratosis in Swine. J. Anim. Sci., 21, 57-61.

O'Dell BL. 2000. Role of zinc in plasma membrane function. J Nutr, 130, 1432-1436.

O'Dell BL, Savage JE. 1957. Poult Sci 36:489

O'Dell BL. & Savage JE. 1960. Effect of phytic acid on zinc availability. Exp Biol Med., 103, 304-306.

O'hara PJ., Newman AP. & Jackson E. 1960. Parakeratosis and copper poisoning in pigs fed a copper supplement. Austral Vet J., 36, 225-229

O'Hara AM, Shanahan F. 2006. The gut flora as a forgotten organ. EMBO Rep 7: 688-693.

Oropeza-Moe M, Grøntvedt CA, Framstad T. 2013. Evaluering av sinkkilder som tilsetningsstoff i avveningstorv til gris. Forsøksrapport skrevet til SINTEF, Fossli & Co AS. Norges veterinærhøgskole. 18 s.

Ou D, Li D, Cao Y, Li X, Yin J, Qiao S, Wu G. 2007. Detary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J Nutrit Biochem. 18:820-826.

Peltier E, Vincent J, Finn C, Graham DW. 2010. Zinc-induced antibiotic resistance in activated sludge bioreactors. Water Res. 44(13):3829-36.

Pond WG, Church DC, Pond KR, Schoknecht PA. 2005. Basic animal nutrition and feeding. 5th ed. John Wiley & Sons, New York.

Poulsen HD, Larsen T. 1995. Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. *Livestock Prod Sci* 43:235-242.

Poulsen HD, Carlson D. 2008. Zinc and copper for piglets – how do high dietary levels of these minerals function. In: Schlegel et al. eds. Trace elements in animal production systems. Wageningen Academic Publishers, Wageningen, pp. 151-160.

Prohaska JR. 1997. Responses of rat cuproenzymes to variable dietary copper. J Nutr Biochem 8: 316-321.

Rajkumar M, Freitas H. 2008. Influence of metal resistant-plant growth-promoting bacteria on the growth of Ricinus communis in soil contaminated with heavy metals. Chemosphere. 71(5):834-42.

Revy PS., Jondreville C, Dourmad JY. & Nys Y. 2006. Assessment of dietary zinc requirement of weaned piglets fed diets with or without microbial phytase. J Anim Physiol Anim Nutr., 90, 50-59.

Roberson KD. & Edwards HM. 1994. Effects of 1,25-dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. Poult Sci., 73, 1312-1326.

Richards JD, Zhao J, Harrell RJ, Atwell CA, Dibner JJ. 2010. Trace mineral nutrition in poultry and swine. *Asian-Aust J Anim Sci*. 23(11):1527-1534.

Saksgård R and Schartau AK. 2011. Kjemisk overvåking av norske vassdrag – Elveserien 2010. Norsk institutt for naturforskning, Rapport 748.

SCHER. 2007. Scientific opinion on the risk assessment report on zinc, environmental part. Scientific Committee on Health and Environmental Risks. Brussels, Belgium: European Commission.

Schlegel P, Sauvant D & Jondreville C. 2013. Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. Animal, 7, 47-59

SCF. 2003. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Zinc (expressed on 5 March 2003). SCF/CS/NUT/UPPLEV/62 Final.

Seiler C, Berendonk TU. 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol. 3:399.

Sekelja M, Rud I, Knutsen SH, Denstadli V, Westereng B, et al. 2012. Abrupt temporal fluctuations in the chicken fecal microbiota are explained by its gastrointestinal origin. Appl Environ Microbiol 78: 2941-2948.

Skjelkvåle BL, Henriksen A, Steinn Jónsson G, Mannio J, Wilander A, Jensen JP, Fjeld E and Lien L. 2001. Chemistry of lakes in the Nordic region – Denmark, Finland with Åland, Iceland, Norway with Svalbard and Bear Island, and Sweden. Norwegian Institute for Water Research Report SNO 4391-2001.

Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, et al. 2007. Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biol 5: 2177-2189.

Stenemo, F., Jarvis, N., Jonsson. 2005. MACRO_GV – ett simuleringsverktyg för platsspecifika bedömningar av bekämpningsmedelsläckage till grundvatten. Emergo 2005:3. Department of Soil Sciences, Division of Environmental Physics, SLU.

Suttle NF. 2010. Mineral nutrition of livestock. 4th ed. CABI, Oxfordshire.

Suttle NF. & Mills CF. 1966. Studies of toxicity of copper to pigs .I. Effects of oral supplements of zinc and iron salts on development of copper toxicosis. Brit J Nutr. 20, 135-&

Torsvik V, Ovreas L, Thingstad TF 2002. Prokaryotic diversity--magnitude, dynamics, and controlling factors. Science 296: 1064-1066.

Totland TH, Melnæs BK, Lundberg-Hallèn N, Helland-Kigen KM, Lund-Blix NA, Myhre JB, Johansen AMW, Løken EB & Andersen LF. 2012. En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11. Helsedirektoratet. Oslo.

Tucker HF, Salmon WD. 1955. Proc Soc Exp Biol Med 88:613.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, et al. 2009. A core gut microbiome in obese and lean twins. Nature 457: 480-484.

Turnlund JR. 2006. Copper in *Modern nutrition in health and disease,*(Shils ME, Shike M, Ross AC, Caballero B & Cousins R, eds.), pp. 1951. Williams & Wilkins, Baltimore

Van Heugten E, Spears JW, Kegley EB, Ward JD, Qureshi MA. 2003. Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. J Anim Sci 81:2063-2071.

Van Paemel M, Dierick N, Janssens G, Fievez V, De Smet S. 2010. Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment. Technical Repprt submitted to EFSA. Copper 71 pp. Zinc 71 pp.

Van Sprang PA, Verdonck FAM, Van Assche F, Regoli L, De Schamphelaere KAC. 2009. Environmental risk assessment of zinc in European freshwaters: A critical appraisal. Sci Tot Env 407: 5373-5391.

Veum TL, Ledoux DR, Shannon MC, Raboy V. 2009. Effect of graded levels of iron, zinc, and copper supplementation in diets with low-phytate or normal barley on growth performance, bone characteristics, hematocrit volume, and zinc and copper balance of young swine. J Anim Sci 87:2625-2634.

VKM. 2009. Eriksen GS, Amundsen CE., Bernhoft A, Eggen T, Grave K, Halling-Sørensen B, Källqvist T, Sogn T and Sverdrup L. Risk assessment of contaminants in sewage sludge applied on Norwegian soils. Opinion of the Panel on Contaminants in the Norwegian Scientific Committee for

Foof Safety. the Norwegian Scientific Committee for Foof Safety, P.O.Box 4404 Nydalen, N-0403 Oslo.

Vogel TM, Simonet P, Jansson JK, Hirsch PR, Tiedje JM, et al. 2009. TerraGenome: a consortium for the sequencing of a soil metagenome. Nat Rev Micro 7: 252-252.

Ward TL, Asche GL, Louis GF, Pollmann DS. 1996. Zinc-methionine improves growth performance of starter pigs. J Anim Sci 74 (Suppl 1):182 (Abstr.)

Waalkes MP, Harvey MJ & Klaassen CD. 1984. Relative in vitro affinity of hepatic metallothionein for metals. Toxicol. Lett., 20, 33-39.

Wedekind KJ, Hortin AE, Baker DH. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J Anim Sci. 70:178-187.

Werman MJ & David R. 1996. Lysyl oxidase activity, collagen cross-links and connective tissue ultrastructure in the heart of copper-deficient male rats. J Nutr Biochem 7: 437-444

WFD-UKTAG. 2010. Proposed EQS for Water Framework Directive Annex VIII substances: zinc. Water Framework Directive - United Kingdom Technical Advisory Group. Report available at www.wfduk.org

WHO. 2003. Zinc in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. World Health Organization, Geneva, 5 pp.

WHO. 2004. Copper in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. World Health Organization, Geneva, 23 pp.

Woyengo TA. & Nyachoti CM. 2013. Review: Anti-nutritional effects of phytic acid in diets for pigs and poultry - current knowledge and directions for future research. Can. J. Anim. Sci., 93, 9-21.

www.slf.dep.no/no/produksjon-og-marked/korn-og-kraftfor/marked-og-pris/statistikk?index=40&metaKey=11220 accessed April 2014

Zhou W, Kornegay ET, Lindemann MD, Swinkels JWGM, Welten MK, Wong EA. 1994. Stimulation and growth by intravenous injection of copper in weanling pigs. J Anim Sci 72:2395-2403.

Zhou W, Kornegay ET, Lindemann MD. 1994b. The role of feed intake and copper source on copper-stimulated growth in weanling pigs. J Anim Sci 72:2385-2394.

Zhu D, Yu B, Ju C, Mei S, Chen D. 2011. Effects of high copper on the expression of hypothalamic appetite regulators in weanling pigs. J Anim Feed Sci 20:60-70.

Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashsham SA, Tiedje JM. 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc Natl Acad Sci USA. 110(9):3435-40.

APPENDIX I

Zn/Cu and possible antimicrobial resistance in bacterial isolates of animal origin.

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
Aarestrup *et al*., 2010	Denmark	n= 91 bacterial isolates isolated from fecal samples swines	MRSA (n=31), MSSA (n=60)	Zn	Methicillin, erthyromycin, penicillin, tetracyclin	This study provides biological evidence to suggest that the use of Zn compounds may be partly implicated in the emergence of some MRSA clones among swine in Denmark.
Aarestrup and Hasman, 2004	Denmark	n=569 bacterial isolates tested against Cu n= 177 bacterial isolates tested against Zn	*Salmonella* (n=156), *S. aureus* (n=43), *S. hyicus* (n=38), *E. faecalis* (n=52), *S. faecium* (n=78)	Heavy metals including Zn, Cu	Benzalkonium chloride, hydrogen peroxide, chlorhexidine	This study showed that Danish bacterial isolates from livestock so far have not or have only to a limited degree developed resistance to antimicrobial compounds commonly used for disinfection. Acquired Cu resistance was only found in enterococci. There were large differences in the intrinsic susceptibility of the different bacterial species to these compounds, and Salmonella especially seems intrinsically less susceptible than the other bacterial species, which might have human health implications.
Amachawadi et al., 2011	USA	The study was done with weaned piglets, housed in 10 pens with 6 piglets per pen. n= 180 Enterococci were tested,	*Enterococcus* sp.	Cu	Erythromycin	The higher prevalence of *tcrB*-positive enterococci in piglets fed elevated Cu compared to that in piglets fed normal Cu suggests that supplementation of Cu in swine diets selected for resistance. A conjugation assay demonstrated cotransfer of *tcrB* and erythromycin *erm*(B) genes between *E. faecium* and *E. faecalis* strains.
Bendorz et al., 2013	Germany	A total of 36 piglets (n=6 per proup and each time point) were euthanized on 32±2, 32±2, 53±2 of age, treatment groups were balanced for litter and gender. N=1481 bacterial isolates (181 clones)	*E. coli*	Zn	Ampicillin, streptomycin, chlroramphenicol, gentamicin, tetracycline, enrofloxacin, cetotaxime	The proportion of multi-resstant *E. coli* was significantly ncreased in the zinc group compared to the control group (18.6% vs. 0%).
Cavaco et al., 2010	Denmark	Bacterial isolates from pigs and human	*S. aureus*	Zn	Methicillin	Seventy-four percent (n = 23) of the animal isolates and 48% (n = 24) of the human MRSA isolates of CC398 were resistant to Zn chloride and positive for *czrC*. All 48 MSSA strains from both human and pig origins were found to be susceptible to Zn chloride and negative for czrC. Our findings showed that *czrC* is encoding Zn and cadmium resistance in CC398 MRSA isolates, and that it is widespread both in humans and animals. Thus, resistance to heavy metals such as Zn and cadmium may play a role in the co-selection of methicillin resistance in *S. aureus*.

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
Cavaco et al., 2011	Denmark	476 porcine MRSA isolates from ten European countries, 18 porcine MRSA isolates from Canada and seven MRSA from China, 92 MRSA and 60 methicillin-susceptible S. aureus (MSSA) isolates from veal calves in the Netherlands and 88 porcine MSSA isolates from four European countries.	*S. aureus*	Zn, Cu, *czrC* gene encoding Zn resistance	Methicillin	Almost all (99%) Zn-resistant MRSA carried *czrC*. Of the 37 European non-CC398 MRSA, 62% were resistant to Zn, but only 46% of them carried *czrC*. The MICs of the MSSA isolates to Zn chloride ranged from 1 to 4mM and none carried *czrC*. The MICs of Cu sulphate were associated neither with methicillin resistance nor with the detection of *czrC*. This study showed that Zn resistance and the *czrC* gene are widespread among CC398 MRSA isolates. This suggests that the use of Zn in feed might have contributed to the emergence of MRSA.
Freitas et al., **2011**	Portugal	Vancomycin resistant enterococci isolates from pigs (n = 29) and healthy persons (n = 12) recovered during wide surveillance studies performed in Portugal, Denmark, Spain, Switzerland, and the United States (1995 to 2008) were compared with outbreak /prevalent VRE clinical strains (n = 190; 23 countries; 1986 to 2009)	Enterococci	Cu	Vancomycin	*E. faecium* CC17 (ST132) isolates from pig manure and two clinical samples showed identical PFGE profiles and contained a 60-kb mosaic plasmid carrying diverse Tn1546-IS1216 variants. The only *Enterococcus faecalis* isolate obtained from pigs (CC2-ST6) corresponded to a multidrug-resistant clone widely disseminated in hospitals in Italy, Portugal, and Spain, and both animal and human isolates harbored an indistinguishable 100-kb mosaic plasmid (containing the whole Tn1546 backbone. The results indicate a current intra- and international spread of *E. faecium* and *E. faecalis* clones and their plasmids among swine and humans.
Hasman and Aarestrup 2002	Denmark	Bacterial isolates from pigs, broilers, calves, sheeps, and humans	*E. faecium*	Cu	Glycopeptides, macrolides	The *tcrB* gene was found in *E. faecium* isolated from pigs (75%), broilers (34%), calves (16%), and humans (10%) but not in isolates from sheep. Cu resistance, and therefore the presence of the *tcrB* gene, was strongly correlated to macrolide and glycopeptide resistance in isolates from pigs, and the *tcrB* gene was shown to be located on the same conjugative plasmid as the genes responsible for resistance to these two antimicrobial agents. The frequent occurrence of this new Cu resistance gene in isolates from pigs, where Cu sulfate is being used in large amounts as feed additive, suggests that the use of Cu has selected for resistance
Hasman et al., 2006	Denmark	Bacterial isolates	Enterococcal species; *E. faecium,* *E. mundtii,* *E. casseliflavus,* *E. gallinarum*	Cu	Glycopeptides, macrolides	A significant relationship between Cu resistance (*tcrB*), glycopeptide resistance (Tn1546), and macrolide resistance [*erm(B)*] in *Enterococcus faecium* isolated from pigs was found. The *tcrB* gene was located closely upstream of the Tn1546 element.

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
Hasman et al., 2005	Denmark		Enterococci	Cu	Glycopeptides	The *tcr* gene cluster mediates in vitro Cu resistance in *Enterococcus faecium*. Here we describe the selection of *tcr*-mediated Cu resistance in E. faecium in an animal feeding experiment with young pigs fed 175 mg Cu/kg feed (ppm), which is the concentration commonly used for piglets in European pig production. tcr-mediated Cu resistance was not selected for in a control group fed low levels of Cu (6 ppm). We also show coselection of macrolide- and glycopeptide-resistant *E. faecium* in the animal group fed the high level of Cu. Finally, we identify the tcr genes in the enterococcal species *E. mundtii, E. casseliflavus, and E. gallinarum* for the first time.
Hölzel et al., 2012	Germany	n=305 pig manure samples	*E. coli* (n=613)	Zn, Cu, Mercury	Beta-lactams, aminoglycosides (total 29 differen antimicrobial agents)	Concentrations of heavy metals were determined by atomic spectroscopic methods in 305 pig manure samples and were connected to the phenotypic resistance of *Escherichia coli* (n=613) against 29 antimicrobial drugs. Antimicrobial resistance in the porcine microflora might be increased by Zn and Cu. By contrast, the occurrence of mercury in the environment might, due to co-toxicity, act counter-selective against antimicrobial resistant strains.
Jacob *et al.*, 2010	USA	n=20 fecal samples from heifer	*E. coli*, *Enterococcus* spp.	Zn, Cu	*E. coli*: Clindamycin, Erythromycin, penicillin, tiamulin, tylosin *Enterococcus*: Chloramphenicol, ciprofloxacin, gentamcin, linezolid, penicillin, streptomycin, vancoomycin	The *tcrB* gene was not detected in feces or in enterococcal isolates. Proportions of *erm(B)* and *tet(M)* were unaffected by Cu or Zn supplementation. However, the proportion of *tet(M)* increased ($p < 0.05$) between days 0 and 14. Feeding elevated Cu and/or Zn to feedlot cattle had marginal effects on antimicrobial susceptibilities of fecal *E. coli* and enterococci.
Ji et al., 2012	China	n= Samples from manures, and soils collected from multiple feedlots		Zn, Cu	Chloramphenicol, tetracyclines, sulfonamide	The results revealed the presence of chloramphenicol, sulfonamides and tetracyclines. Typical heavy metals, such as Cu, Zn, and As, were detected. All ARGs tested were detected in the collected samples except *tetB(P)*, which was absent in animal manures. Overall, sulfonamide ARGs were more abundant than tetracycline ARGs. Except for *sulII*, only a weak positive correlation was found between ARGs and their corresponding antibiotics. On the contrary, significant positive correlations ($p<0.05$) were found between some ARGs and typical heavy metals. For example, *sulA* and *sulIII* were strongly correlated with levels of Cu, Zn and Hg. The data demonstrated that the

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
						presence of ARGs was relatively independent of their respective antibiotic inducer. In addition to antibiotics, toxic heavy metals, such as Hg, Cu, and Zn, exerted a strong selection pressure and acted as complementary factors for ARG abundance.
Kim *et al*., 2012	Republic of Korea	n=245 Milk samples from bovine	*Enterococcus* spp.	Cu	Erythromcin	Of the 245 enterococcal isolates, 79.2% (n=194) displayed erythromycin resistance (≥8 µg/ml). Of the erythromycin-resistant isolates, 97.4% (n=189) possessed *erm(B)*, 73.7% (n=143) possessed mef(A), and 71.6% (n=139) possessed both genes. Of the 245 enterococcal isolates, only 4.5% (n=11) displayed Cu resistance (≥28 mM) and the Cu resistance gene*, tcr(B),* was detected in seven isolates that all possessed *erm(B).*
Zhu et al., 2013	China	Manure and compost samples from swine farms (n= 36) Soil samples	Anitibiotic resistance genes detected by PCR	Heavy metals (Zn, Cu)	149 antibiotica resistance genes,	Quantitative PCR arrays detected 149 unique resistance genes among all of the farm samples, the top 63 ARGs being enriched 192-fold (median) up to 28,000-fold (maximum) compared with their respective antibiotic-free manure or soil controls. Antibiotics and heavy metals used as feed supplements were elevated in the manures, suggesting the potential for coselection of resistance traits. The potential for horizontal transfer of ARGs because of transposon-specific ARGs is implicated by the enrichment of transposases--the top six alleles being enriched 189-fold (median) up to 90,000-fold in manure--as well as the high correlation (r(2) = 0.96) between ARG and transposase abundance. In addition, abundance of ARGs correlated directly with antibiotic and metal concentrations, indicating their importance in selection of resistance genes. Diverse, abundant, and potentially mobile ARGs in farm samples suggest that unmonitored use of antibiotics and metals is causing the emergence and release of ARGs to the environment.

Zn/Cu and possible antimicrobial resistance in bacterial isolates from environment.

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
Aktan et al., 2013	Turkey	Water samples from river n= no information	*Enterococcus faecalis*	Heavy metals including Zn	Amikacin, azteronam, gentamicin	Of the 33 lead-resistant isolates, one isolate with a minimal inhibitory concentration of 1,200 mg L(-1) was isolated and identified as *Enterococcus faecalis* by using biochemical tests and 16S rRNA sequencing. Lead-resistant *E. faecalis* isolate was found out to be resistant to other heavy metals like aluminum, lithium, barium, chromium, iron, silver, tin, nickel, Zn, and strontium and to drugs like amikacin, aztreonam, and gentamicin. *E. faecalis* harbored four plasmids with the molecular sizes of 1.58, 3.06, 22.76, and 28.95 kb.
Berg et al., 2010	Denmark	Soil samples n= no information	Soil bacterial resistome	Cu	Tetracycline, vancomycin, olaquindox, nalidixic acid, chloramphenicol, ampicillin	High Cu exposure selected for Cu-tolerant bacterial communities but also coselected for increased community-level tolerance to tetracycline and vancomycin. Cu-resistant isolates showed significantly higher incidence of resistance to five out of seven tested antibiotics (tetracycline, olaquindox, nalidixic acid, chloramphenicol, and ampicillin) than Cu-sensitive isolates. Our BrdU-PICT data demonstrate for the first time that soil Cu exposure co-selects for resistance to clinically important antibiotics (e.g., vancomycin) at the bacterial community-level.
Berg et al., 2005	Denmark	Soil samples n= no information	Gram-negative bacteria	Cu	Different antibiotics	More than 95% of the Cu-resistant isolates were Gram-negative. Cu-resistant Gram-negative isolates had significantly higher incidence of resistance to ampicillin, sulphanilamide and multiple (> or =3) antibiotics than Cu-sensitive Gram-negative isolates. Furthermore, Cu-resistant Gram-negative isolates from Cu-contaminated plots had significantly higher incidence of resistance to chloramphenicol and multiple (> or =2) antibiotics than corresponding isolates from control plots. The results of this field experiment show that introduction of Cu to agricultural soil selects for Cu resistance, but

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
						also indirectly selects for antibiotic resistance in the Cu-resistant bacteria.
Cardonha et al., 2005	Brazil	Storm sewers and the seawater n= no information	98 strains of *E. coli* 50 from storm sewers and 48 from the seawater	Cu	nitrophurantoine, nalidixic acid, sulfatomexazol-trimethoprin and chloramphenicol	A total of 98 strains of *E. coli*, 50 from storm sewers and 48 from the seawater were analyzed resistance to several antimicrobials and to heavy metals. Among the twelve antimicrobials tested, 28 (28.5%) of *E. coli* strains showed resistance to different antimicrobials drugs to seven. The greatest resistance rate was to tetracycline (46.4%), ampicillin (39.3%) and cephalothin (32.1%), with the remainder (nitrophurantoine, nalidixic acid, sulfatomexazol-trimethoprin and chloramphenicol) at lower percentages. Among the heavy metals, all the strains (100%) were resistant to Zn and to Cu in the largest concentration (250 ig/mL), and 18.4% were resistant to HgCl the 50 ig/mL.
Lazăr et al., 2002	Romania	Polluted waters n= no information	*12 E. coli* strains isolated from chronically	Cu, Zn	ampicillin, tetracycline, gentamycin, kanamycin, chloramphenicol, ceftazidime and cefotaxime	In soil and water, multiple antibiotic resistances is clearly associated with resistance/tolerance to heavy-metals (Hg2+, Cu2+, Pb2+, Zn2+, Ca2+). Possible correlation between resistance against heavy-metal s and antibiotics of 12 *E. coli* strains isolated from polluted waters was investigated. All strains are multiple antibiotic resistant, 16% of them being resistant to 3, 4 and 6 antibiotics, 32% to 5 and 8% to all 7 antibiotics, respectively. Multiple tolerances to high levels of $Cd^{2+,}$ Cu^{2+}, Cr^{3+} and Ni^{2+} was common among multiple antibiotic resistant strains. The phenotypic data shows the direct association between multiple antibiotic and heavy-metal resistance for *E. coli* strains in polluted water.
Malik *et al*., 2011	India	n=48 river water samples, 144 bacterial isolates	*Pseudomonas* spp.	Zin, Cu	Tetracycline, sulphadiazine, polymixin B, ampicillin, erythromycin	A high level of resistance against tetracycline and polymyxin B (81.2%) was observed in river water isolates. However, 87.5% of *Pseudomonas* isolates from soil irrigated with wastewater showed resistance to sulphadiazine, whereas 79.1% were resistant to both ampicillin and

Reference	Country	Sample	Bacterial species	Susceptibility to Zn / Cu	Susceptibility to antimicrobial agents	Conclusion
						erythromycin. Isolates from soil irrigated with groundwater exhibited less resistance towards heavy metals (Cu, Zn) and antibiotics as compared to those of river water and wastewater irrigated soil. Majority of the *Pseudomonas* isolates from water and soil exhibited resistance to multiple metals and antibiotics.
Peltier et al., 2010	USA	Samples from lab-scale activated sludge reactors Experimental study	Culturable bacteria	Zn	Oxytetracycline, ciprofloxacin, tylosin	The study show that sub-toxic levels of Zn can cause increased antibiotic resistance in waste treatment microbial communities at comparatively low antibiotic levels, probably due to developed cross-resistance resulting from pre-exposure to Zn.
Rajkumar and Freitas, 2008	Portugal	Soil samples n= no information	*Pseudomonas* spp.	Heavy metals including Zn	N.d.	The metal resistant- plant growth-promoting bacterial (PGPB) strains *Pseudomonas* sp. (PsM6) and *Pseudomonas jessenii* (PjM15) could serve as an effective metal sequestering and growth-promoting bioinoculant for plants in metal-stressed soil. The study has provided a new insight into the phytoremediation of metal-contaminated soil.

APPENDIX II

Soil properties in Åsnes, Klepp and Melhus municipalities

Data from "Jorddatabanken" show that the variations in soil properties within the municipalities are large. At all three sites there are a substantial fraction of the soils were soil organisms are more susceptible (i.e. lower pH, organic matter and lower silt and clay content) to Zn and Cu accumulation than the soils selected as input in the risk assessment. This is especially the case at Melhus, where about 70% of the soils have a more course texture than silty clay loam (most frequent soil texture). This mean that when considering the effects Zn and Cu accumulation have on soil living organisms, an extra margin of safety (i.e. PEC/PNEC ratio) should be included.

Location	Soil texture	Number of samples	Mean loss on ignition (%)	Mean soil density (kg/l)	Mean pH
Åsnes	Sand	28	4.4	1.2	5.6
	Loamy sand	41	3.3	1.4	5.8
	Fine sand	3	1.9	1.4	6.0
	Fine sandy loam	426	4.9	1.2	5.8
	Sandy loam	862	5.4	1.2	5.9
	Sandy loam	16	4.0	1.1	6.0
	Silt loam	1487	3.1	1.3	5.9
	Silt	2622	4.2	1.2	5.9
	Sandy clay loam	9	4.6	1.3	5.9
	Silt loam	151	6.8	1.2	6.0
Klepp	Sand	14	1.0	No data	5.9
	Fine sandy loam	4062	3.4	No data	5.9
	Silt	641	3.5	No data	5.9
	Silty clay loam	36	2.9	No data	6.1
	Loamy sand	8	8.0	1.1	6.5
	Fine sandy loam	16	8.8	1.1	5.6
	Silt loam	1	3.7	1.4	5.9
Melhus	Sand	3	0.6	1.2	5.8
	Sand	108	8.8	1.1	6.0
	Loamy sand	3	3.8	1.3	6.1
	Fine sand	6	2.3	1.0	6.5
	Sandy loam	11	12.4	1.3	6.0
	Fine sandy loam	58	9.3	1.1	6.0
	Sandy loam	123	7.4	1.1	6.0
	Sandy loam	9	8.9	1.1	6.0
	Silt loam	25	3.6	1.2	5.9
	Silt	5	5.4	1.1	6.4
	Silt	181	11.2	1.2	6.5
	Sandy clay loam	12	5.9	1.3	6.1
	Silty clay loam	62	6.2	1.2	6.0
	Silty clay loam	213	7.8	1.1	6.2
	Clay loam	2	5.3	1.1	7.9
	Clay	13	0.0	0.7	5.5
	Clay	13	5.1	0.9	5.7

APPENDIX IIIa

Predicted intake of Zn and Cu in ruminants and horses only via the diet portions of gras at pasture or roughage inside grown before and after intensive fertilising of the soil with pig manure (application rate: 70 kg P/ha) for 100 years in Åsnes area.

Zinc	Pasture						Inside					
µg/kg bw per day	Background			Pig manure 100 years			Background			Pig manure 100 years		
	Roughage	Soil	Sum	Roughage	Soil	Sum	Roughage	Soil	Sum	Roughage	Soil	Sum
Cattle												
Calves	548	38	585	1339	281	1621	483	0	483	1182	0	1182
Young heifers	493	34	527	1205	253	1459	435	0	435	1064	0	1064
Dry cows	310	21	332	759	159	918	310	0	310	759	0	759
High lactation cows	387	50	437	945	375	1321	430	0	430	1050	0	1050
Sheep												
Early weaned lambs	1020	63	1083	2495	469	2964	806	0	806	1970	0	1970
Finishing lambs	816	50	867	1996	375	2371	645	0	645	1576	0	1576
Adult sheep maint.	408	25	433	998	188	1186	322	0	322	788	0	788
Adult sheep with twins	816	50	867	1996	375	2371	645	0	645	1576	0	1576
Goats												
Kids	639	44	683	1563	328	1891	564	0	564	1379	0	1379
Adult goats maint.	365	25	390	893	188	1080	322	0	322	788	0	788
Adult lactating goats	580	75	655	1418	563	1981	645	0	645	1576	0	1576
Horses												
Adult maintenance	226	19	244	551	141	692	226	0	226	551	0	551
Mares in lactation	451	38	489	1103	281	1384	451	0	451	1103	0	1103
Copper	Pasture						Inside					
µg/kg bw per day	Background			Pig manure 100 years			Background			Pig manure 100 years		
	Roughage	Soil	Sum	Roughage	Soil	Sum	Roughage	Soil	Sum	Roughage	Soil	Sum
Cattle												
Calves	114	9	123	204	57	262	101	0	101	180	0	180
Young heifers	103	8	111	184	52	236	91	0	91	162	0	162
Dry cows	65	5	70	116	32	148	65	0	65	116	0	116
High lactation cows	81	11	92	144	76	221	90	0	90	160	0	160
Sheep												
Early weaned lambs	213	14	227	381	95	476	168	0	168	301	0	301
Finishing lambs	170	11	182	305	76	381	135	0	135	241	0	241
Adult sheep maint.	85	6	91	152	38	191	67	0	67	120	0	120
Adult sheep with twins	170	11	182	305	76	381	135	0	135	241	0	241
Goats												
Kids	133	10	143	239	67	305	118	0	118	210	0	210
Adult goats maint.	76	6	82	136	38	174	67	0	67	120	0	120
Adult lactating goats	121	17	138	216	115	331	135	0	135	241	0	241
Horses												
Adult maintenance	47	4	51	84	29	113	47	0	47	84	0	84
Mares in lactation	94	9	103	168	57	226	94	0	94	168	0	168

APPENDIX IIIb

Predicted intake of Zinc (Zn) and copper (Cu) in ruminants and horses at pasture or when fed inside roughage grown before and after intensive fertilizing of the soil with pig manure (application rate: 70 kg P/ha) for 100 years in Åsnes area, as well as compound feed with present maximum concentrations of Zn and Cu.

Zinc µg/kg bw per day	**Pasture**								**Inside**							
	Background				**Pig manure 100 years**				**Background**				**Pig manure 100 years**			
	Concentrates	**Roughage**	**Soil**	**Sum**	**Concentrates**	**Roughage**	**Soil**	**Sum**	**Concentrates**	**Roughage**	**Soil**	**Sum**	**Concentrates**	**Roughage**	**Soil**	**Sum**
Cattle																
Calves	450	548	38	1035	450	1339	281	2071	1125	483	0	1608	1125	1182	0	2307
Young heifers	405	493	34	932	405	1205	253	1864	1013	435	0	1448	1013	1064	0	2076
Dry cows	255	310	21	587	255	759	159	1173	383	310	0	693	383	759	0	1141
High lactation cows	3000	387	50	3437	3000	945	375	4321	3000	430	0	3430	3000	1050	0	4050
Sheep																
Early weaned lambs	0	1020	63	1083	0	2495	469	2964	1875	806	0	2681	1875	1970	0	3845
Finishing lambs	0	816	50	867	0	1996	375	2371	1500	645	0	2145	1500	1576	0	3076
Adult sheep maintenance	0	408	25	433	0	998	188	1186	750	322	0	1072	750	788	0	1538
Adult sheep with twins	0	816	50	867	0	1996	375	2371	1500	645	0	2145	1500	1576	0	3076
Goats																
Kids	525	639	44	1208	525	1563	328	2416	1313	564	0	1876	1313	1379	0	2691
Adult goats maintenance	300	365	25	690	300	893	188	1380	750	322	0	1072	750	788	0	1538
Adult lactating goats	4500	580	75	5155	4500	1418	563	6481	4500	645	0	5145	4500	1576	0	6076
Horses																
Adult maintenance	563	226	19	807	563	551	141	1255	675	226	0	901	675	551	0	1226
Mares in lactation	1125	451	38	1614	1125	1103	281	2509	1350	451	0	1801	1350	1103	0	2453

Copper µg/kg bw per day	**Pasture**								**Inside**							
	Background				**Pig manure 100 years**				**Background**				**Pig manure 100 years**			
	Concentrates	**Roughage**	**Soil**	**Sum**	**Concentrates**	**Roughage**	**Soil**	**Sum**	**Concentrates**	**Roughage**	**Soil**	**Sum**	**Concentrates**	**Roughage**	**Soil**	**Sum**
Cattle																
Calves	105	114	9	228	105	204	57	367	263	101	0	363	263	180	0	443
Young heifers	95	103	8	205	95	184	52	330	236	91	0	327	236	162	0	399
Dry cows	60	65	5	129	60	116	32	208	89	65	0	154	89	116	0	205
High lactation cows	700	81	11	792	700	144	76	921	700	90	0	790	700	160	0	860
Sheep																
Early weaned lambs	0	213	14	227	0	381	95	476	188	168	0	356	188	301	0	488
Finishing lambs	0	170	11	182	0	305	76	381	150	135	0	285	150	241	0	391
Adult sheep maintenance	0	85	6	91	0	152	38	191	75	67	0	142	75	120	0	195
Adult sheep with twins	0	170	11	182	0	305	76	381	150	135	0	285	150	241	0	391
Goats																
Kids	88	133	10	231	88	239	67	393	219	118	0	337	219	210	0	429
Adult goats maintenance	50	76	6	132	50	136	38	224	125	67	0	192	125	120	0	245
Adult lactating goats	750	121	17	888	750	216	115	1081	750	135	0	885	750	241	0	991
Horses																
Adult maintenance	94	47	4	145	94	84	29	207	113	47	0	160	113	84	0	197
Mares in lactation	188	94	9	290	188	168	57	413	225	94	0	319	225	168	0	393

APPENDIX IIIc

Predicted intake of Zn and Cu in ruminants and horses at pasture or when fed inside roughage and cereals grown before and after intensive fertilising of the soil with pig manure (application rate: 70 kg P/ha) for 100 years in Åsnes area. In this table both roughage and cereals are from the same background and 100-year fertilised soil.

Zinc	Pasture								Inside							
µg/kg bw per day	Background				Pig manure 100 years				Background				Pig manure 100 years			
	Natural conc.	Roughage	Soil	Sum	Natural conc.	Roughage	Soil	Sum	Natural conc.	Roughage	Soil	Sum	Natural conc.	Roughage	Soil	Sum
Cattle																
Calves	39	548	38	625	96	1339	281	1716	98	483	0	581	239	1182	0	1421
Young heifers	35	493	34	562	86	1205	253	1545	88	435	0	523	215	1064	0	1279
Dry cows	22	310	21	354	54	759	159	973	33	310	0	344	81	759	0	840
High lactation cows	261	387	50	698	638	945	375	1958	261	430	0	691	638	1050	0	1688
Sheep																
Early weaned lambs	0	1020	63	1083	0	2495	469	2964	163	806	0	969	399	1970	0	2368
Finishing lambs	0	816	50	867	0	1996	375	2371	130	645	0	775	319	1576	0	1895
Adult sheep maintenance	0	408	25	433	0	998	188	1186	65	322	0	387	159	788	0	947
Adult sheep with twins	0	816	50	867	0	1996	375	2371	130	645	0	775	319	1576	0	1895
Goats																
Kids	46	639	44	729	112	1563	328	2002	114	564	0	678	279	1379	0	1658
Adult goats maintenance	26	365	25	416	64	893	188	1144	65	322	0	387	159	788	0	947
Adult lactating goats	391	580	75	1047	957	1418	563	2938	391	645	0	1036	957	1576	0	2532
Horses																
Adult maintenance	49	226	19	293	120	551	141	812	59	226	0	284	143	551	0	695
Mares in lactation	98	451	38	587	239	1103	281	1624	117	451	0	569	287	1103	0	1390

Copper	Pasture								Inside							
µg/kg bw per day	Background				Pig manure 100 years				Background				Pig manure 100 years			
	Natural conc.	Roughage	Soil	Sum	Natural conc.	Roughage	Soil	Sum	Natural conc.	Roughage	Soil	Sum	Natural conc.	Roughage	Soil	Sum
Cattle																
Calves	16	114	9	139	29	204	57	291	41	101	0	142	73	180	0	253
Young heifers	15	103	8	125	26	184	52	262	37	91	0	128	66	162	0	228
Dry cows	9	65	5	79	17	116	32	165	14	65	0	79	25	116	0	141
High lactation cows	109	81	11	201	195	144	76	415	109	90	0	199	195	160	0	355
Sheep																
Early weaned lambs	0	213	14	227	0	381	95	476	68	168	0	236	122	301	0	422
Finishing lambs	0	170	11	182	0	305	76	381	54	135	0	189	97	241	0	338
Adult sheep maintenance	0	85	6	91	0	152	38	191	27	67	0	95	49	120	0	169
Adult sheep with twins	0	170	11	182	0	305	76	381	54	135	0	189	97	241	0	338
Goats																
Kids	19	133	10	163	34	239	67	339	48	118	0	165	85	210	0	296
Adult goats maintenance	11	76	6	93	19	136	38	194	27	67	0	95	49	120	0	169
Adult lactating goats	163	121	17	302	292	216	115	623	163	135	0	298	292	241	0	533
Horses																
Adult maintenance	20	47	4	72	37	84	29	149	25	47	0	72	44	84	0	128
Mares in lactation	41	94	9	144	73	168	57	299	49	94	0	143	88	168	0	256