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1 HUMAN TOXICITY

Copper is an essential element for all organisms. In mammals it is a constituent of a number of enzymes involved in various oxidative reactions, and essential for the activity of other enzymes. Further it plays a role in the hematopoietic process, bone development, carbohydrate metabolism, pigment formation and possibly in preventing cardiovascular anomalies (Osterberg, 1980; JECFA, 1982).
With exception of specific conditions, for example in the work environment, the main route of exposure for humans to copper is via the intake of food and beverages, including drinking water. For this reason this part of the document is focused on **oral** exposure. Based on estimates on the daily intake in the Netherlands (see basis document, chapter 4) and data on human requirements, copper does not seem to be a problem to the general population; for this reason data on human toxicity and related data are not treated exhaustively.
Data on which there is consensus in the literature are derived mainly from the next reviews: "Drinking Water Criteria Document for Copper - Final Draft" (EPA, 1985a), "Copper", a monograph of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1982) and "A Conspectus of Research on Copper Metabolism and Requirements of Man" (Mason, 1979).
Toxicity data can be found mainly in the first two publications; the other publication is focused on the chemobiokinetics, metabolism and human requirements.
In another document ("Summary Review of the Health Effects Associated with copper") the emphasis is on health (and ecological) effects of copper in the atmosphere (EPA, 1987).
Unless stated otherwise all data in this chapter are expressed as the amount of copper(-ion).

1.1 Chemobiokinetics and metabolism

1.1.1 Animals

After oral intake or administration the absorption of copper has been found to depend on animal species, age, physiological status (e.g. copper body burden, pregnancy), chemical form of copper and a variety of dietary

compounds (Mason, 1979; JECFA, 1982; Hansard, 1983). Relative high absorption rates have been found in newborn, for example, as much as 100% of an intragastric dose was absorbed in 7-10 days old rat pups. At weanling and thereafter the rate of absorption is decreased to ≤ 10% in several animal species (JECFA, 1982; CCRX, 1986). In feed copper is present in the form of organic complexes, mainly with proteins and amino acids. In feeding experiments with rats Kirchgeβner and co-workers have shown that, within certain limits, the absorption of copper out of complexes with amino acids is more dependent on the specific amino acid than on the molecular size or the stability of the complex formed. The rate of absorption is also influenced by the molecular configuration, with a higher rate with L-amino acids compared with D-amino acids (Kirchgeβner, 1973). Some dietary factors which have been found to reduce the copper absorption are trace metals (especially molybdenum), sulfur, complexing agents like phytates (constituents of cereals) and calcium carbonate, and ascorbic acid. It is not possible to quantify the relative effect of all factors which are involved.

In most mammals the absorption primarily occurs from the upper gastrointestinal tract.

Inhalation of copper oxide aerosols by rats resulted in copper oxide particles in plasma and kidneys; quantitative data are not available (EPA, 1985a).

After dermal application of a saline solution of copper(II)bis(glycinate), about 3% of the applied copper had penetrated the skin of cats 24 hours after the treatment (EPA, 1985a).

Once absorbed, copper is bound predominantly to albumin and to a lesser extend to amino acids, in the portal blood; these copper complexes are called "labile" or "direct reacting" copper. In these forms copper is transported mainly to the liver - the key organ in the metabolism of this element - from where the largest fraction is excreted directly via the bile. The remaining part is bound initially to a metallothionein(-like) protein, followed by the formation of the cuproprotein ceruloplasmin and, to a lesser extend, other cuproproteins. The very stable copper-ceruloplasmin complex ("indirect reacting" copper; also known as the enzyme ferroxidase I) can enter the blood and transport copper to other tissues.

Most other cuproproteins only have an enzymatic function, for example cytochrome c oxidase and superoxide dismutase. The non-enzyme cuproproteins like metallothionein(s) probably contribute to the copper homeostatic mechanism.

More detailed information concerning the cuproproteins has been given by NRC (1977), Mason (1979) and Österberg (1980).
The highest copper concentrations are found in liver, brain, heart and kidneys.

The main excretion route for copper is via the bile to the feces; fecal copper also includes unabsorbed copper and copper resulting from salivary, gastric and duodenal secretion, and from replaced epithelial cells. Urinary excretion is very low, because only the amino acid fraction of the plasma is available for glomerular filtration and because of the efficient reabsorption of a part of this fraction (Hansard, 1983; Aaseth and Norseth, 1986).
Minor excretion routes are milk, hair, nails and, in monkeys, menses.
Mammalian homeostatic mechanisms are effective in maintaining proper copper balance despite a wide range of dietary intake.

1.1.2 Humans

Chemobiokinetics and metabolism in humans and other mammals are essentially the same. Especially data which differ from animal data will be mentioned here.

After oral administration copper is absorbted primarily in the stomach and duodenum. The amount of copper absorbed can notably vary between individuals. For example, a range of 15-97% was estimated in 7 persons who has been fasting and received an oral dose of copper acetate; the mean value being 60%. Other reported (mean) values range from 25% to 65%. (JECFA, 1982; EPA, 1985a).
The daily requirement of copper for humans is estimated to be 0.025-0.1 $mg.kg^{-1}$ bw, with the highest relative requirement in premature infants, and young and adolescent children.
Balance-studies (in which the daily intake and loss are measured during a certain period) indicate a daily requirement of about 1-5 mg (0.02-0.08 $mg.kg^{-1}$ bw) for adults. Based on the well comparable results of a number of balance studies conducted between 1934 and 1954, a daily amount of 2-3 mg (0.03-0.05 $mg.kg^{-1}$ bw) is widely accepted to be adequate, for example by the JECFA. More recent balance studies indicate that a daily intake below 2 mg or even not much in excess of 1 mg (0.02 $mg.kg^{-1}$ bw) is sufficient to equal the daily loss. Information provided by parenteral nutrition studies

also indicate a daily requirement of approximately 1 mg, assuming a 40% to 60% absorption of ingested copper (Mason, 1979).
Data on the inhalation of copper (compounds) - especially quantitative data - are scarce. Based on industrial data, copper fumes, dusts and mists can be absorbed, resulting in systemic effects (Stokinger, 1981; Davies and Bennett, 1985; EPA, 1985a). According to Davies and Bennett (1985) the retention of inhaled copper is 20%.
Copper can penetrate both burned and intact human skin, even from bracelets (EPA, 1985a).

Liver and brain together account for about one-third of the total copper body content. Relative low levels are found in muscle tissue and bones, but due to the great masses these tissues account for about 50% of total copper in adults. In blood plasma, most copper is found in ceruloplasmin, which represents approximately 93% of total plasma copper. Whole blood and plasma levels are fairly constant, due to homeostatic mechanisms.
Estimates of whole body contents in adults range between 50 and 150 mg. According to Davies and Bennett (1985) this amount is more likely ranging from 50 to 70 mg (no details or references are given by these authors to back up this statement).

Fecal excretion is by far the most important route to eliminate copper. Urinary excretion is only 1-3% of fecal excretion.
Sweat can contribute substantially to copper excretion under humid and hot conditions. For example, in a study in which 3 men were exposed to an environment of 38 ^{o}C and 50% humidity, about 45% of the dietary intake was lost in the sweat during a 10 day observation period (Mason, 1979, citation).

1.2 Animal toxicity

Data in this subchapter do not include those concerning livestock (ruminants, pigs, poultry). For data on these animals, see chapter 3.

1.2.1 Short-term

Most acute oral tests with inorganic copper compounds have been conducted with rats, resulting in a range of LD50-values of 66 to 416 mg Cu.kg^{-1} bw.

The lowest values (66 to 82 mg $Cu.kg^{-1}$ bw) were reported for cupric chloride, cupric sulfate pentahydrate and cupric carbonate which all are Cu(II)-salts, but the highest value of 416 mg $Cu.kg^{-1}$ bw has been reported both for Cu4(Cl)2H6O6 (basic cupric chloride) and Cu2O, a Cu(II)- and a Cu(I)-salt, respectively. Based on these and other values, for example another acute oral LD50 of 244 mg $Cu.kg^{-1}$ bw for hydrated cupric sulfate, the differences does not seem to correlate with speciation of inorganic compounds.
With cupric acetate LD50-values of 209 and 248 mg $Cu.kg^{-1}$ bw were found for rats.
For mice and Guinea pigs oral LD50-values of 90 and 15 mg $Cu.kg^{-1}$ bw, respectively, were found with cupric chloride as test substance. For rabbits and birds LD50-values of 91 and 465 mg $Cu.kg^{-1}$ bw, respectively, were found with basic copper carbonate - CuCO3Cu(OH)2 - as test substance (JECFA, 1982; Sax, 1984; RTECS, 1986).

In a study in which weanling rats were fed copper sulfate (530 and 1,600 mg $Cu.kg^{-1}$ feed) or copper gluconate (1,600 mg $Cu.kg^{-1}$ feed) for 9 months, the level of 1,600 $mg.kg^{-1}$ caused a variety of toxic effects on internal organs, growth retardation and mortality. Copper gluconate was more toxic than copper sulfate, consistent with a higher rate of accumulation. The feed level of 530 $mg.kg^{-1}$ (~26-53 mg $Cu.kg^{-1}$ $bw.day^{-1}$) resulted in varying degrees of testicular degeneration; other effects were not found (Harrison et al., 1954; see also long-term). In another feeding study in which male weanling rats were fed a diet containing 2,000 mg $Cu.kg^{-1}$ (as copper sulfate; ~100-200 mg $Cu.kg^{-1}$ $bw.day^{-1}$) for 15 weeks, both accumulation in liver and kidneys and gross and histological changes (e.g. necrosis) of these organs were at the maximum after 6 weeks, demonstrating the development of a tolerance at prolonged exposure (Haywood, 1980).
Male rats (90 days old) receiving 25 mg $Cu.kg^{-1}$ $bw.day^{-1}$ (as hydrated copper sulfate) by gavage for 3 weeks showed several effects, for example decreased body weight gain, necrosis of liver and kidneys, and changes in blood parameters.
In male Wistar rats (90-100 g) a dietary supplement of 50 mg $Cu.kg^{-1}$ feed (as copper sulfate, corresponding with ~2.8-5.5 mg $Cu.kg^{-1}$ $bw.day^{-1}$) during 5 weeks did not affect feed intake, weight gain, relative liver weight and activities of aspartate aminotransferase, alanine aminotransferase and alkaline fosfatase (Miranda et al., 1981).
In rabbits a daily oral dose of 0.25 mg copper acetate (~44 mg $Cu.kg^{-1}$ $bw.day^{-1}$) during 33 to 41 weeks resulted in hemochromatosis and liver

cirrhosis (Hall and Butt, 1928). In another feeding experiment with rabbits 2,200 mg $Cu.kg^{-1}$ as copper acetate in the diet (~66 mg $Cu.kg^{-1}$ $bw.day^{-1}$) for up to 15 weeks, caused mortality in 16 out of the 21 exposed animals. Almost all exposed rabbits showed one or a combination of the following effects on the liver: pigmentation (especially hemofuscin accumulation), cirrhosis and necrosis (Hall and Mackay, 1931).
In a one year study with male and female beagle dogs the highest dietary level of 2,400 mg copper gluconate per kg feed (equivalent to 60 mg copper $gluconate.kg^{-1}$ $bw.day^{-1}$, corresponding with about 8 mg $Cu.kg^{-1}$ $bw.day^{-1}$) caused accumulation of copper in liver, kidneys and spleen, but no compound-related deaths or gross or microscopic pathological lesions were found (Shanaman, 1972; cited in JECFA, 1982; report not available).

1.2.2 Long-term

With exception of a specific carcinogenicity study with mice (see further on) only one chronic toxicity study was available. In this study groups of 40 weanling Sprague-Dawley rats (males and females) were fed diets with 0.1%, 1% and 3% "potassium sodium copper chlorophyllin" (approximately 2.6-5.3, 26-53 and 80-160 mg $Cu.kg^{-1}$ $bw.day^{-1}$) for two years. No effects on mortality, growth, organ weights, and blood and urine parameters were found, as little as gross or microscopic pathology of internal organs (Harrison et al., 1954; see also short-term).
In rabbits, every second day orally dosed with a cupric sulfate solution (~12 mg $Cu.kg^{-1}$ bw) during 16 months, cirrhosis-like hepatic damage was found (Tachibana, 1952; cited in JECFA, 1982).

1.2.3 Mutagenicity

Both inorganic and organic copper compounds have been tested for genotoxic and mutagenic effects in a variety of short-term assays, with diverse results even with the same test substance (e.g. Hansen and Stern, 1984; EPA, 1985a).
For example, in reverse mutation assays a negative respons was found with copper sulfate using Salmonella typhimurium strains TA 98 and TA 100 (Moriya, 1983). Using Escherichia coli a positive respons was found, but only at highly toxic concentrations (Demeric, 1951). In other, both in vivo and in vitro tests, both positive and negative results with copper sulfate

have been found. In several reverse mutation tests with copper gluconate and copper 8-quinolinolate, only the latter gave a weak positive reaction in S. typhimurium strain TA 100 after metabolic activation (JECFA, 1982; Moriya, 1983).
Based on the screened results the mutagenic potency of copper is equivocal.

1.2.4 Carcinogenicity

The reviewed data only revealed one specific carcinogenicity study in which copper was given orally. Groups of 18 male and 18 female B6C3F1 and B6AKF1 mice (7 days old) were treated daily by gavage with 1,000 mg copper hydroxyquinoline per kg bw, (~181 mg $Cu.kg^{-1}$ $bw.day^{-1}$) during the first 3 weeks of the study, followed by a dietary dose level of 2,800 $mg.kg^{-1}$ feed, approximately 25-50 mg $Cu.kg^{-1}$ $bw.day^{-1}$. At termination after 1.5 years, no statistically significant increases in the incidence of tumors were found. After a single subcutaneous injection of 1,000 mg copper hydroxyquinoline per kg bw using groups of 18 male and 18 female mice (28-days old) of the same strains, a significantly ($p < 0.001$) increased incidence of reticulum cell sarcomas (6/17 and 8/141 in treated, and in vehicle and untreated animals, respectively) was found in male B6C3F1 mice after the same exposure time; in the other groups of mice no increased tumor incidences were found (Bionetics Research Labs; cited in EPA, 1985a).
Based on these results, those of the earlier mentioned feeding studies and those of other studies (cited in JECFA, 1982 and EPA, 1985a) in which copper compounds were administered orally or by injection - in some experiments together with known animal carcinogens - there is no reason to consider copper to be carcinogenic to animals.

1.2.5 Teratogenicity and reproductive toxicity

In mature C57BL and DBA female mice, doses of 500 to 2,000 mg copper sulfate (anhydrous ?) in the diet (~ 26 to 104 mg $Cu.kg^{-1}$ $bw.day^{-1}$) from 1 month prior to mating up to termination on day 19 of gestation did not result in adverse effects. Only the highest doses (3,000 and 4,000 $mg.kg^{-1}$ feed, ~155 and 207 $mgCu.kg^{-1}$ $bw.day^{-1}$) led to increased fetal mortality, decreased litter sizes and to skeletal malformations in 2-9% of the foetuses, while no malformations were found in the control and other treatment groups (Lecyk, 1980).

In embryotoxicity and teratogenicity ("segment II-type") studies with oral administration of copper gluconate up to 30 mg.kg^{-1} bw.day^{-1} (4 mg Cu.kg^{-1} bw.day^{-1}), neither effects on implantation data (corpora lutea, implantation sites, implantation loss) nor embryotoxic or teratogenic effects were found in mice and rats. In male rats oral administration of 3 mg (0.42 mg Cu.kg^{-1} bw.day^{-1}) during 60 days did not affect fertility (JECFA, 1982).

In a reproduction study groups of female rats were given copper sulfate in the drinking water at levels of 0.1, 1 and 10 mg Cu.l^{-1}, corresponding with 0.0012-0.0015, 0.012-0.015 and 0.12-0.15 mg Cu.kg^{-1} bw(.day^{-1} ?), respectively, from 5 months prior to mating up to termination at day 21 of gestation. All test concentrations resulted in an increased embryonic mortality, decreased weight and length of the fetuses and a delayed ossification. At the highest concentration hydrocephaly and enlargement of the brain ventricles was found in 2/39 (controls: 0/30) and 5/39 (controls: 1/30) of the exposed animals, respectively (Nadeenko et al., 1980; in Russian). The lowest effective dose in this study is very low compared with no-effect-levels found in the earlier mentioned oral reproduction/teratogenicity studies. The lowest total dose (~180 x 0.0015 = 0.27 mg Cu.kg^{-1} bw) is similar to the lowest dose that resulted in embryotoxic and teratogenic effects after a single i.v. injection in pregnant hamsters (see below, Ferm and Hanlon, 1974). For these reasons the study of Nadeenko et al. will be left out of consideration.

Copper has been found to be embryotoxic (reduced number of blastocysts) in rats recieving a single i.p. injection of 7.5 mg CuSO4.kg^{-1} bw (3 mg Cu.kg^{-1} bw) on day 3 of gestation (JECFA, 1982).

In studies with golden hamsters a single i.p. injection of 2.7 mg copper citrate per kg bw (1.1 mg Cu.kg^{-1} bw) on day 8 of gestation resulted in embryotoxic and teratogenic effects: increased embryonic death, edematous embryos, cardiovascular malformations, and tail and limb defects (DiCarlo Jr, 1979, 1980). Copper citrate was found to be more toxic to hamsters than copper sulfate after a single i.v. injection during day 8 of gestation, resulting in higher rates of resorption and higher percentages of embryos with malformations at similar copper doses. With copper citrate the lowest dose of 0.25-1.50 mg Cu.kg^{-1} bw already resulted in adverse effects. The relative high copper concentrations in placenta and embryos (compared with maternal blood and uterus) after injection of ^{64}Cu(II) citrate showed that the placenta is permeable to copper (Ferm and Hanlon, 1974).

In <u>in vitro</u> tests with preimplantation mouse embryos and developing chick embryos copper compounds also have been found to be embryotoxic and/or

teratogenic. Metallic copper liberated from fine pieces of wire was also found to be lethal to mouse preimplantation embryos.
(Metallic) copper from intrauterine devices (IUDs) has been found to prevent implantation and blastocyst development in rats, hamsters and rabbits (Tatum, 1973; Fern and Hanlon, 1974). When a copper ring-IUD was inserted in these animals after the implantation process has been completed and was left in utero throughout gestation and lactation, neither effects on reproductive function and reproductive tissues of treated females were found nor teratogenic and other effects in the F1 and F2 generation (Chang and Tatum, 1973).
In a variety of animals (sheep, goat, rat, guinea pig, dog, chicken) copper deficiency also has been found to result in a teratogenic respons (EPA, 1985a).

1.3 Human toxicity

Most information on the toxicity of copper is known from clinical cases from patients who ingested copper accidentally (relatively low doses) or intentionally (relatively high doses). Sources of accidental cases of poisoning are mainly contaminated drinking water and (other) beverages, especially acidic, carbonated drinks. In the cases of intentional poisoning copper sulfate has been ingested most frequently.
Occupational exposure (see furthur on) is giving additional information, mainly on effects after inhalation of copper and copper compounds.

1.3.1 Short-term

The first signs and symptoms of an acute poisoning after ingestion of copper (salts) are: a metallic taste, epigastric pain, headache, nausea, dizziness, vomiting and diarroea. These effects have been found already in women at ingested copper quantities of 5.3 to 32 mg copper, after drinking of different quantities of a whisky cocktail kept in a shaker for some time (Wyllie, 1957). In men the consumption of a total amount of $\geq$ 7 mg copper in tea caused symptoms of an acute poisoning (EPA, 1985a). So, the lowest amount of copper that may result in an acute poisoning is about 0.1 mg $Cu.kg^{-1}$ bw. In 1-4 year old children signs of an acute poisoning were found after consuming an orange-flavored drink which was kept overnight in a

brass pot. The drink contained 34 mg $Cu.l^{-1}$, so the total ingested dose of the youngest children was likely less than 8.5 mg (NRC, 1977).
Copper sulfate has been used historically in medical practice as an emitic in the treatment of intoxications, at a total oral dose of at least 25-75 mg Cu, but this use is now very restricted because of the risk on gastrointestinal and/or other (systemic) effects (EPA, 1985a; Aaseth and Norseth, 1986).
In severe cases of copper intoxications (after ingestion of up to about 100 g copper sulfate according to the patients) the following effects have been found: ulcerations and hemorrhage in the gastrointestinal tract, intravascular hemolysis, hypotension, and irreversible effects on liver and kidneys, resulting in collapse, coma and ultimately in death (e.g. Chuttani et al., 1965).
The fatal oral human dose of various inorganic copper salts has been estimated by the World Health Organization to be 200 $mg.kg^{-1}$ bw, with a considerable variability in individual sensitivity (JECFA, 1982). According to Gosselin (1976) the lethal dose of copper(II) salts is 50-500 $mg.kg^{-1}$ bw for adults. Data cited in EPA (1985a) are in the same range.
In one reported case, 2 oral doses of 10 cc of a 10%-solution of hydrated cupric sulfate (total dose 2 g, i.e. 40 mg cupric sulfate per kg bw) administered to an adult female to treat an alcohol-diazepam intoxication resulted in death (JECFA, 1982; EPA, 1985a).

Several cases of acute copper poisoning during or after recurrent hemodialysis have been reported, due to copper in parts of the equipment. The effects resemble those of "metal fume fever" (see "occupational exposure"). Hemolytic anemia has been reported at copper concentrations in the dialysate of 22 to 50 $\mu g\ Cu.l^{-1}$ (EPA, 1985a).
Treatment of burned skin with a copper sulfate solution (≥ 3%) or with copper sulfate crystals can result in hemolysis and may be fatal (NRC, 1977; EPA, 1985a).

1.3.2 Long-term

Data on chronic toxicity in healthy humans due to ingestion of excess copper are very limited. However, "Wilson's disease", an inherited autosomal recessive disorder of copper metabolism/homeostasis is considered to represent a form of chronic copper toxicity (e.g. EPA, 1985a), although there are indications that copper alone is not responsible for all

histopathological changes seen in this disease (Uzman, 1957; Wolff, 1960). Wilson's disease, also known as "hepatolenticular degeneration", has a general prevalence of 1 in 160,000 - 200,000 and is characterized by a life-long low ceruloplasmin level, a decreased fecal excretion and increased copper levels in liver, kidneys, brain and cornea. Symptoms of the disease are liver damage (cirrhosis), a variety of renal and neurological disordes, the formation of copper containing "Kayser-Fleischer rings" around the cornea and occasionally cataracts as well as episodes of hemolysis. Severe cases of the disease may result in anemia, jaundice and death.

Two cases of poisoning in very young children (most probably) due to a prolonged exposure to copper have been reported, one non-fatal and one fatal case. In the first case, a 15-month old boy had to be treated after an exposure for 3 months to food and beverages prepared with drinking water containing copper up to 0.8 $mg.l^{-1}$, a level not normally assumed to cause adverse effects. This level might have been toxic in this case due to the young age of the child, resulting in a relative greater rate of uptake. The fatal case - a 14-month old boy - occurred after exposure <u>in utero</u> and post-partum due to copper levels up to 9.7 $mg.l^{-1}$ in the drinking water. Wilson's disease was indicated, but could not be established for certain.
Recurrent episodes of abdominal pain - almost daily in the 7-year old daughter - and emesis occured in three of four persons of a family exposed to copper levels above 2 $mg.l^{-1}$ up to 7.8 $mg.l^{-1}$ in the drinking water for about 1.5 years (Spitalny et al., 1984).
The American Centers for Disease Control have reported 112 cases of intoxications from drinking water with copper levels from 4 to 70 $mg.l^{-1}$ in the years 1977-1982; data on exposure times are not given (EPA, 1985a).
Fragments of metallic copper and copper alloys in the eye may result in visible copper accumulation in the cornea (Kayser-Fleischer rings), cataract and ultimately in loss of the eye (Rosen,1949; NRC, 1977).
Mallory (1925) has mentioned a prolonged inhalation and/or ingestion of copper as one of the possible causes of human hemochromatosis (pigment cirrhosis); this hypothesis is consistent with the results of animal experiments, for example those with rabbits (Hall and Butt, 1928; Hall and MacKay, 1931). In 10 of 16 earlier described cases of hemochromatosis there was also a history of contact with copper or brass for many years (Mills, 1924; cited in Hall and Butt, 1928).

1.3.3 Other data

The mutagenic potency of copper is equivocal (see 1.2.3). With exception of a cancer mortality correlation study in which a possitive correlation was found between the dietary intake of copper (and other trace elements) and mortality rates due to a number of common cancers (Schrauzer et al., 1977), and data on occupational exposure (see further on), no human data on the carcinogenic potency of copper after ingestion were found. Also no data on the teratogenic potency of ingested copper with regard to humans have been found. Based on animal data humans are not likely at risk with regard to carcinogenic and teratogenic effects due to the dietary intake of copper.

IUDs with a wrap of copper wire have a higher contraceptive efficiency than plastic ones, due to the release of copper (Barkoff, 1976; NRC, 1977). In women wearing a copper T-IUD for 6 to 10 months, the copper concentration in the secretory endometrium was 2-times higher than in women without IUD; this (together with changes in the concentration of other elements) probably initiates the prevention of the implantation of the fertilized ovum (Hernández, 1975). It is not yet fully known if the relative small amount of parenteral copper lost from a copper IUD (about 0.05-0.10 mg per day) can lead to (systemic) effects, but on the basis of animal experiments and human data there are no reasons to expect toxic effects in users and teratogenic effects in cases of pregnancy (Tatum, 1973; Guillebaud, 1976; NRC, 1977). Metallic copper has also been found to have spermicidal properties which may contribute to the contraceptive properties of copper IUDs (Tatum, 1973; Aaseth and Norseth, 1986).
Allergic reactions have been ascribed to copper, for example due to the use of a copper IUD, ingestion of or skin contact with copper salts, and the filling of a tooth with black copper cement. The frequency of copper allergy is very low (Barkow, 1976; Aaseth and Norseth, 1986).

1.3.4 Occupational exposure

After short-term exposure to copper fumes, mists or fine dusts, an influenza-like syndrome ("metal fume fever" - also called "copper fume fever" or "brass chill") can develop, but the number of reported cases is low. This syndrome can include the following symptoms: irritation of the upper respiratory tract and eyes, dryness of mouth and throat, chills, fever, headache, aching muscles, and digestive disorders, such as diarroea.

The symptoms disappear 24-48 hours after termination of the exposure. Copper dusts and aerosols have an objectionable taste, which mostly limits exposure. Metal fume fever has been reported in several occupational activities, for example copper welding and polishing, and in workers exposed to dusts or aerosols of one or more copper salts (Cohen, 1974; NRC, 1977; Stokinger, 1981; EPA, 1985a; Aaseth and Norseth, 1986). Gleason (1968) reported mild symptoms of metal fume fever in workers exposed to copper dust of "extreme fineness" due to the polishing of copper plates. These symptoms already came through at a copper concentration of 0.1 - 0.3 $mg.m^{-3}$, which is well below the occupational standard for copper dust in the Netherlands of 1 $mg.m^{-3}$ (see basis document, chapter 1). According to Stokinger (1981) no effects occur at copper fume concentrations up to 0.4 $mg.m^{-3}$.
Copper - metallic copper, copper salts - can cause (allergic) contact dermatitis, but the number of known cases is very limited (NRC, 1977; Stokinger, 1981).

In copper and brass (copper/zinc 2:1) workers a "slow copper poisoning" has been reported, resulting in laryngitis, bronchitis, intestinal colic with catarrh and diarroea, general emaciation and anemia (Chatterji and Ganguly, 1950; cited in Mason, 1979).
In 1969, Pimentel and Marques described for the first time an occupational disease - "vineyard sprayer's lung" - which they attributed to the use of "Bordeaux mixture", a 1-2.5% solution of basic copper sulfate neutralized by lime. This mixture is used to control mildew on grapes, for example in Portugal. Pimentel and Marques were able to induce similar lung lesions (the presence of desquamation, intra-alveolar macrophages and inter-alveolar septal histiocytic granulomas and the scars of these lesions) in Guinea pigs exposed to the mixture by inhalation. These lesions could also be reproduced in Guinea pigs exposed to copper oxychloride or organic fungicides like maneb and zineb. The disease may remain in a subclinical form for several decades or may reveal itself by symptoms as dyspnoea, mucoid sputum, weakness, loss of apetite and weight, coughing, fever, chills, and muscular and joint pains (Villar, 1974; Pimentel and Menezes, 1975).
The use of Bordeaux mixture is not allowed in the Netherlands, but that of copper oxychloride (and of maneb and zineb) is permitted to protect crops.
Roentgenograms of 15 patients - all with a history of exposure to Bordeaux mixture - in which the disease was diagnosed histologically and histochemically, showed lung condensations, varying from diffuse

reticulonodular shadows to massive, tumor-like opacities. In all 5 patients who died within the period of investigation, copper containing granulomas were found in the lungs, in one case together with a bronchogenic carcinoma. In all 15 patients copper containing hyaline scars were present in the lungs (Villar, 1974). In three fatal cases of vineyard sprayer's lung described by Pimentel and Menezes (1975) lung roentgenograms showed reticular and micronodular shadows; histologic examinations of the lungs showed histiocytic granulomatosis and fibrohyaline nodular scars, with abundant inclusions of copper. In all 3 cases these lesions were found together with a proliferation and swelling of Kupffer cells and with hepatic granulomatosis, with histiocytic, sarcoid and transitional types of granulomas. Effects on the liver were also found in 30 rural workers with (in most cases) a fatal form of vineyard sprayer's lung, ascribed to the use of Bordeaux mixture for 3 to 45 years. The morphological changes were: proliferation of Kupffer cells, with small granular copper inclusions (30 cases), the presence of histiocytic or sarcoid type copper containing granulomas (7 cases), fibrosis (8 cases), cirrhosis (3 cases), and the presence of an angiosarcoma in 1 case (Pimentel and Menezes, 1977).

With exception of vineyard sprayer's disease no significant chronic effects of copper have been reported due to occupational exposure. The increased incidences of lung cancer among workers in copper refineries and copper-ore mines have been ascribed to the co-exposure to arsenic compounds; no etiological role of copper itself has been suggested (NRC, 1977; EPA, 1985a; Aaseth and Norseth, 1986).
In summary, despite the widespread use of copper, industral-linked copper intoxications appear to be of little concern with regard to health hazards.

2 ECOTOXICITY

2.1 Aquatic organisms

From all heavy metals copper is probably the element which has been investigated most extensively to clarify the impact on aquatic life. The vast amount of data has resulted in many reviews of which the next ones have been used as basis for this paper.
Data on freshwater organisms have been reviewed by Alabaster and Lloyd (1980), Skidmore and Firth (1983) and Harrison (1986); data on both freshwater and marine organisms by Demayo et al. (1982), Mance et al. (1984), Moore and Ramamoorthy (1984) and EPA (1985b). An extensive review on the biological importance of copper in the marine environment has been written by Lewis and Cave (1982). Other sources have been screened for additional information, especially the annual literature-reviews of the (U.S.) Water Pollution Control Federation, from 1979 up to 1986.
At collecting primary literature a selection has been made with regard to short-term data; emphasis has been layed on data on long-term toxicity.
According to data on bivalve molluscs reviewed by Verburgh (1987), there is a small margin (about a factor of 10) between the water copper level which is essential to prevent deficiency and that which results in toxic effects.
All data on copper levels in water and sediment are expressed as the amount of copper(-ion).

2.1.1 Bioaccumulation / bioavailability

In a variety of organisms the bioaccumulation of copper has been studied, for example for monitoring purposes or to study factors influencing the bioavailability. The bioaccumulation can be influenced by a variety of both abiotic and biotic factors, which have been reviewed by Lewis and Cave (1982) and Luoma (1983). Up to now a basic understanding of the complex relationships between all factors which influence this process is lacking and data are inconsistent.
Many investigators have studied the effect of the chemical speciation (see basisdocument, chapter 3) on the uptake, by using copper-complexing agents like EDTA, NTA, humic and fulvic acid, and amino acids. From these studies (conducted with organisms from several trophic levels: algae, macrophytes, invertebrates, vertebrates) it appears that primarily "free" copper (-ion)

is accumulated from solution, independent of the organism studied (e.g. Zamuda and Sunda, 1982; Luoma, 1983; Nor, 1985; Zamuda et al., 1985; Blust et al., 1986). The uptake of copper from solution by the water hyacinth Eichornia crassipes could be prevented by relative high amounts of EDTA or humic acid but not of amino acids or fulvic acid (Nor and Cheng, 1986). In the presence of amino acids or fulvic acid the total amount of copper taken up by the plant was exceeding the amount of free copper-ion in solution. The difference between the stability constants of humic acid and fulvic acid appears to be too small to explain the difference in uptake of copper. The same relative amount of humic acid (25 times the amount of copper on a weight/weight basis) which was sufficient to prevent the accumulation of copper by the water hyacinth did not influence the accumulation of copper by the water flea Daphnia magna (Winner, 1984a). These data illustrate that also ligand-bound copper can be accumulated, possibly after dissociation of the complex before uptake, and that the degree of accumulation can differ with species.

According to data on both inorganic and organic copper complexes, reviewed by Verburgh (1987), the bioavailability of lipid-soluble complexes is higher than that of water-soluble complexes.

In bioaccumulation studies in the laboratory, positive correlations have been found between the total copper level of the exposure water and the copper level in organisms, for example in coelenterata, molluscs and arthropods (a crayfish and insect larvae) (Nehring, 1976; Stebbing and Pomroy, 1976; Evans, 1980; Zaroogian and Johnson, 1983). However, in the natural environment the situation is much more complicated as will be demonstrated in the next examples. In a study in a freshwater system in the Netherlands (rivers with lakes downstream), metals were analysed in the "soluble" (< 0.45 μm filter) and "particulate" (> 0.45 μm filter) fraction of the water, in the sediment and the enclosed porewater, and in organisms, both filter-feeders (zooplankton, mussels) and deposit-feeders (insect larvae, worm, mussel and fish). In both abiotic and biotic components the same gradient for copper and other metals was found, with decreasing levels downstream, but, in general, no significant correlations were found between copper levels in organisms and in the abiotic components collected from the same site. If there was any correlation, it was more frequently with the soluble fraction than with the particulate fraction of the water or with the sediment; a relationship with the porewater was found never (Hueck-van der Plas, 1984). In a study in the marine environment narrow size ranges of two molluscs (one grazing and one carnivorous species) were collected from a small environmental homogeneous site. In the first species the copper

concentration ranged from 0.5 to 5.5 $\mu M.g^{-1}$; in the second species from 2.5 to 7.5 $\mu M.g^{-1}$, illustrating the variability (Lobel, 1982).

Especially in more recent years much attention has been focused on the role of sediments within the aquatic environment, because it is the greatest potential source for metals like copper. Although copper is bound very tightly to sediments, a part may be or become available for aquatic organisms, in particular for organisms which are living and/or feeding at the sediment. From bioaccumulation studies with bottom organisms (worms, snails, mussels) in water-sediment systems it has become clear that at least the sediment-type (particle-size distribution) influences the uptake, but that there are great differences between species (e.g. De Kock and Marquenie, 1982; De Kock, 1985; Simmers, 1985). Bryan and Hummerstone (1971) found a tendency of increasing levels in the estuarine worm Nereis diversicolor with increasing sediment levels, but a comparison of animal and sediment data from specific sites did not show a straightforward relationship. In two correlation studies with the lugworm Arenicola marina no significant correlation could be established although there was also a trend of higher levels in the worm at higher sediment levels in one study (Packer et al., 1980; Everaarts, 1986). According to Everaarts the different results of both studies with A. marina may be due to different sediment levels and particle-size distributions.

Data on the bioavailability of copper and other metals from sediments have been reviewed by De Kock and Marquenie (1982). Their conclusion: "Considering the dynamics and variability of natural systems, it is doubtfull wether descriptive correlation studies based on field data of pollutant concentrations in sediments and in tissues of properly chosen organisms (deposit feeding invertebrates) will ever lead to meaningful results". This statement is supported by the variable results of additional data. Also in rooted aquatic and marsh plants the situation is inconsistent, with both positive and no correlations between copper levels in plants and sediments (e.g. Mudroch and Capobianco, 1979; Demayo et al., 1982; Baars et al., 1986). In some, but not all, cases better correlations have been found with a certain extractable fraction compared with total copper (or other metals) in both plants and animals, with a specific fraction of the sediment or when the major complexing components were taken into account, indicating that not all the metal is available for uptake (e.g. De Kock and Marquenie, 1982; Luoma, 1983; O'Donnel et al., 1985; Tessier et al., 1985).

In the common carp Cyprinus carpio exposed for 3 months to a lethal level, both accumulation and mortality decreased in the presence of the complexans EDTA, NTA, or DTPA (Muramoto, 1982). However, in one series of experiments with the waterflea D. magna humic acid had no effect on the accumulation, but decreased both acute and chronic toxicity (Winner, 1984). In another series of experiments with D. magna (Winner, 1985) most combinations of increased hardness and humic acid concentration tend to decrease the accumulation, so the meaning of the first results is not clear. Based on a study with the marine worm Cirriformia spirabrancha in which the total accumulated amount of copper did not increase further above a certain water concentration while the median survival time was decreasing with increasing water level, Milanovich et al. (1976) concluded that lethality is rather related to the rate of uptake than to the total accumulated amount. According to Harrison (1986) there is often no correlation between copper body burden and biological respons due to deposition in special depots; regulation mechanisms have been found at several trophic levels, for example in molluscs, crustaceans and fish. In the flowing aquatic weed Lemna paucicostata the absorption of copper was not influenced within a certain pH range, but growth was inhibited more strongly at higher pH (Nasu et al., 1983).
Based on the data presented here and on other screened data it must be concluded, that in many cases the copper level in organisms can not be simply predicted from that in abiotic components of the environment (and conversely) and, that the copper level in organisms can not be used to predict the impact on aquatic life.

2.1.2 Bioconcentration and biomagnification

An overview of bioconcentration factors (BCF = concentration in organism : concentration in the water) has been published by Demayo et al., 1982; Mance et al., 1984; EPA, 1985b and Harrison, 1986. These data together with data from primary sources have been summarized in Table 2.1.
The reviewed data are difficult to compare because in many cases important information about exposure time and exposure level ("background" or "elevated") is lacking and in some cases it is not clear whether the data are on a wet or a dry weight basis. In some cases the BCF-value has been calculated from the net gain ($C_{exposed} - C_{controls}$); in other cases no correction has been made for the level in controls. Because of the high variability in BCF-values (also within related groups of organisms) and the

lack of data on how most values have been established, only combined data are given in Table 2.1, based on both field and laboratory studies. The presented values are just indicative for the possible rate of accumulation. Although the bioconcentration factor is based on the water level, it is the result of all exposure routes, including food. The dietary intake may be an important factor, for example in filter-feeders like oysters and mussels.

Table 2.1. Bioconcentration factors (BCF)

Group	fresh water	seawater
Algae	400-21,000	75-27,000
Macrophytes	30-54,000	10,000-20,000
(Zoo)plankton	35,000	250-2,700
Annelids	23,000	200-2,550
Molluscs	1,700-23,000	10-28,000
Crustaceans	80- 6,000	7,000-10,000
Insects	200-14,000	-
Fish	1-450	150-700

BCF = *concentration in organism : concentration in water.*
\- no data

There are no consistent data on the influence of the environmental concentration on the bioconcentration factor. For example, in an accumulation study with the mussel Mytilus edulis, Calabrese et al. (1984) found an increase of the BCF with increasing seawater concentration (ranging from 3 to 13 $\mu g.l^{-1}$) after 12 and 21 months; however, this was not found after 18 months when the highest tissue levels were found. Young et al. (1979) found a decrease of the BCF with increasing water copper concentrations (0.5 to 10 $\mu g.l^{-1}$) in shrimp (Pandalus danae) larvae after an exposure of 7 weeks, but it is not clear wether this exposure time was long enough to reach equilibrium.

The highest bioconcentration factor (28,000) in invertebrates at elevated copper concentration in the water has been found in the eastern oyster Crassostrea virginica after a continuous exposure for 4.5 months to 50 $\mu g.l^{-1}$, resulting in a bluish-green color, but little mortality (EPA, 1985b). The same "green sick" has been found for other bivalve molluscs collected from the field (Lewis and Cave, 1982). These data illustrate the high accumulation potency of these invertebrates. In the earlier mentioned accumulation study with M. edulis exposed for 21 months in a continuous-flow system to 3 (controls), 4, 8, or 13 $\mu g.l^{-1}$, the mussels accumulated

more copper at the highest two concentrations. At the highest concentration the body level was 3 to 13 times higher than that in the controls; the highest accumulation was found after about 18 months. The BCF was depending both on the copper concentration and the time of exposure; both the highest and lowest value were found at the background concentration. When exposed to background copper levels together with an elevated silver concentration, copper accumulation significantly increased with increasing silver concentrations, resulting in BCF-values of up to 59,000 (Calabrese et al., 1984). In a 1-yr field study in which M. edulis and seawater were collected at monthly intervals at two locations no relationship could be established between tissue copper levels and dissolved or particulate copper concentrations in the water; however, there was a positive relation of tissue copper levels with lead and zinc concentrations in the water, indicating an interelemental effect (Popham and D'Auria, 1982).
For larval shrimp P. danae a bioconcentration factor of 80,000 and 7,000 was found after exposure to 0.5 and 10 $\mu g.l^{-1}$, respectively, with total tissue levels of 70 and 40 $mg.kg^{-1}$ for exposed and unexposed animals, respectively (Young et al., 1979). These and other data illustrate the fact that the bioconcentration factor can not be used to predicted the actual level in exposed organisms compared with unexposed ones, because concentration also happens at very low environmental concentrations.
Generally the lowest BCF-values have been reported in fish, both in fresh- and seawater.

When the copper levels in organisms are compared with sediment levels, much lower bioconcentration factors (BCF = concentration in the organism : concentration in the sediment) are found, with the majority of the values < 5. Both for benthos and other organisms the bioconcentration factor is often < 1, so in these cases copper is accumulated but not concentrated. An example of this was found for the worm N. diversicolor collected from estuarine sides with low to very high copper-sediment levels, with BCF-values ranging from 0.24 to 0.68 (Bryan and Hummerstone, 1971).

Little information is available on the possible biomagnification - the accumulation in foodchains, resulting in higher tissue copper levels at higher trophic levels - of copper. Schmidt (1978) reported high copper levels in digestive organs of several groups of organisms (echinoderms, molluscs, fish), indicating an important role of dietary intake in the accumulation proces. Field data on a short food chain (zooplankton, stickleback, trout) in freshwater showed a decreasing copper level in

organisms at higher trophic level (Roch et al., 1985). Other field data show that biomagnification may have occurred from moss to an isopod, but not from snail to trout (Dallinger and Krautzky, 1985). Also in an artificial food chain (algae -> daphnids -> fish) in the laboratory starting with algae exposed to a mixture of heavy metals, no biomagnification was found (Tarifeno-Silva, et al., 1982). This is in agreement with the low BCF-values reported in fish.
Data on food chains in the marine environment also show (in general) no biomagnification at higher trophic levels; the primary effect seems to be a change in species composition at low trophic levels, resulting in an alteration of food for higher levels (Lewis and Cave, 1982).

2.1.3 Other kinetic data

In both plants and animals copper is a component of many enzymes (Demayo et al., 1982; Lewis and Cave, 1982).
In molluscs and arthropods copper is also used for the synthesis of the respiratory blood pigment hemocyanin (e.g. Betzer and Yevich, 1975; Schmidt, 1978; Albrecht et al., 1981; Lewis and Cave, 1982).
Copper is bound to proteins in invertebrates and vertebrates and/or glycopeptides in algae and invertebrates (Coppellotti et al., 1985; Roch et al., 1985; Piccinni et al., 1985). An induction of copper-binding metallothioneins and/or other (hepato)proteins has been reported after exposure to copper, for example in mussels and fish (EPA, 1985b; Roch et al., 1985; Reish et al., 1986). In fish this results in concentrations in the liver which are more than 10-500 times the concentrations in muscle (Benoit, 1975; Dallinger and Kautzky 1985; Roch et al., 1985). Excess copper is stored in specific structures (e.g. lysosomes or granules) and may be excreted (in part), for example by fish (Lewis and Cave, 1982; Harrison, 1986).

2.1.4 Toxicity

Many studies (with or without organic or inorganic complexing agents) have been conducted to clarify the species of copper which can be related with toxicity. These studies used selective analysis-methods and/or computer models to specify the form(s) of copper in the water. In most studies cupric ion (Cu2+) and/or "labile" copper (free cupric ion(s) and easily

dissociable and exchangable organic and inorganic complexes) were found to be related with toxicity, in algae (e.g. Steemann and Wium-Andersen, 1970; Anderson and Morel, 1978; Jackson and Morgan, 1978; Sunda and Lewis, 1978; Peterson et al., 1984), invertebrates (Andrew et al., 1977; Young et al., 1979) and fish (e.g. Zitko et al., 1973; Pagenkopf et al., 1974; Shaw and Brown, 1974; Howard and Sprague, 1978; Waiwood and Beamish, 1978; Chakoumakos et al., 1979). O'Donnel et al. (1985) - who reviewed a number of studies (with bacteria, algae, crustaceans and fish) in which the concentration of the free copper ion has been related to the observed toxicity - reported the toxicity values expressed as free ion to differ maximally with a factor of 3. This is much lower than the range of toxicity values when expressed as total or dissolved copper (see further on).

Regarding the soluble inorganic copper species, a statistical analysis of a number of these data confirm the general picture that the hydroxides, especially the positively and neutrally charged species, are more toxic than carbonates (Cowan, 1986). Giesy et al. (1983) found the toxicity for the daphnid Simocephales serrulatus more related to total copper than to free copper, but based on the other data the toxicity is more related with soluble, in particular labile copper than with particulate or total copper.

In agreement with these data, both natural and artificial complexing agents decrease the toxicity of copper, for example amino acids, humic substances, sediment extracts, "yellow substance", EDTA , TRIS, NTA, and Fe(OH)3. This has been found for all kinds of organisms: bacteria (Milanovich et al., 1975; Nusch, 1977), algae (Steemann Nielsen and Wium-Andersen, 1970; Sunda and Lewis, 1978; Florence et al., 1983), molluscs (Stephenson and Taylor, 1975; Harrison, 1985), arthropods (Lewis et al., 1972, 1973; Biesinger et al., 1974), and fish (Sprague, 1968; Brown, 1974). The effect depends both on (the stability of) the formed complex and the exposed species; for example, for daphnids copper was much less toxic in the presence of TRIS compared with amino acids, and the protective effect of one specific amino acid was 4 times higher with guppies than with daphnids (Borgmann and Ralph, 1983). The toxicity of copper to early-life stages of the oyster Crassostrea gigas was found to decrease in the presence of the chelators oxalate, glycine, citrate and EDTA; the ability to reduce copper toxicity showed an increasing trend with increasing stability constant of the formed complex (Harrison, 1985).

Algae are capable of excreting complexing substances in response to copper stress; this also can counteract the toxic action of copper, especially in eutrophic waters (EPA, 1985b).

Although it is clear that many complexing agents from both biotic and abiotic origin have a protective effect, it is difficult to quantify this effect in the natural environment, because in general very high (e.g. equimolar or higher) quantities of the complexing agents are used in experimental studies.

Lipid-soluble ligands (test organisms: algae) and anionic detergents (test organisms: fish) were found to increase the toxicity of copper (Florence et al., 1983; Ahsanullah and Florence, 1984)).

Data on the joint action of copper with other heavy metals are inconsistent, sometimes even with the same combination of metals and the same organism. For example, Khangarot et al. (1984) found an additive effect of copper and zinc at low concentrations and a synergistic effect of these two metals at higher concentrations in common carp Cyprinus carpio. Based on the screened data a synergistic action of copper and other metals appears more common than a simple additive (= no interaction) and/or an antagonistic action. In chronic toxicity tests in reconstituted water with daphnids (D. pulex) the adverse effects of a copper concentration of 10 $\mu g.l^{-1}$ were abolished by the presence of 5 $\mu g.l^{-1}$ selenium, indicating that in this case the toxicity of copper could be ascribed to deficiency of another element (Winner, 1984b).

Most toxicity tests have been conducted with (hydrated) CuSO4 or Cu(Cl)2, which both are very soluble at the used concentrations. Although in some tests different (acute) toxicity values have been found between these two copper compounds and/or other anorganic copper compounds, these differences are to small to be of any biological significance. For this reason the different copper compounds will not be discussed separately. Organic copper complexes have been excluded, because of the possible reduction of toxicity due to strong complexation.

Unless stated otherwise, the following toxicity data in water are expressed as total (acid soluble) copper, in μg Cu(II).l^{-1}. However, some of the used test waters have been filtered, without specification of the used filter, so the total copper concentration may have been underestimated. Copper values in sediments (also "total") are expressed in mg Cu(II).kg^{-1}

Freshwater organisms - short-term

Skidmore and Firth (1983) reported a range of more than 20,000 for acute toxicity values for copper, based on 209 reviewed tests with all kinds of organisms. Even within one species there may be a high variability: for example, the 96-hr LC50-value for rainbow trout Salmo gairdneri was

reported to be 17-29 $\mu g.l^{-1}$ (alevins, smolts) and 890 $\mu g.l^{-1}$ (greater fish), respectively (Chapman, 1978; Calamari and Marchetti, 1973). Both were continuous-flow tests, with comparable test conditions. The difference can be (partly) explained by differences in developmental stage and hardness of the test water.

From all abiotic factors which have been found to influence the acute toxicity of copper, the **hardness** of the water appears to be the most consistent, although the ultimate effect is strongly species dependent. The acute toxicity of copper is inversely related with the hardness of the water; this statement is based especially on data on fish (e.g. Table 5.2, bold data), but the same relationship has been found for other organisms, for example plants, worms, crustaceans and insects (Brkovic'-Popovic' and Popovic', 1977; EPA, 1985b; Gauss et al., 1985). It is not possible to quantify the effect of hardness on toxicity because of the high variability between different organisms. In one series of tests with D. pulex the 72-hr LC50-value at a hardness of 230 $mg.l^{-1}$ (as CaCO3) was 2 times lower than that at a hardness of 58 and 115 $mg.l^{-1}$; in the presence of 0.75-1.5 mg humic acid per litre test water no difference was found (Winner, 1985).

A selection of relatively low acute toxicity data for species of some important groups of organisms is presented in Table 2.2, in soft ($\leq$ 100 mg $CaCO3.l^{-1}$) and hard (> 100 mg $CaCO3.l^{-1}$) water, respectively. All presented data are from tests which have been conducted in natural water without treatments as UV-sterilization. In soft waters the lowest acute toxicity values for most of the listed organisms do not or hardly differ. In hard waters the variability is more pronounced.

Some lower toxicity values have been reported from tests in treated or reconstituted water, both resulting in a relatively low organic matter content. For example, for D. magna a 48-hr EC50-value of 6.5 $\mu g.l^{-1}$ has been reported in hard (H = 250) reconstituted water (Dave, 1984).

Freshwater organisms - long-term

A selection of the results of long-term toxicity tests - conducted in waters of specified hardness - is listed in Table 2.3. With two exceptions only tests in which a No-Observed-Effect-Concentration (NOEC) was established, are listed in this table. For two organisms (C. carpio and Noemacheilus barbatulus) only a No-Observed-Lethal-Concentration (NOLC) was available and listed in the table. If more than one NOEC-value (resulting from different life-cycle or early-life-stage tests) for one species in either soft or hard water was available, usually only one has been listed,

but all available NOEC-values are used in one of the methods of extrapolation (Kooyman, 1985) which has been used to establish a "safe" copper level in water (see 4.4, risk evaluation).

From the results of long-term tests it can been seen that the influence of the hardness of the test water is much less consistent compared with acute tests. For young rainbow trout S. gairdneri EC25-values (25% reduction of growth rate) were 13 to 34 times higher in hard (H = 360) than in soft (H = 30) water, at pH 6 and 7.5 - 8.0, respectively (Waiwood & Beamish, 1978). In early-life-stage tests with three fish species and one life-cycle test with one fish species the NOEC-values in soft and hard water were equal or similar (within a factor of 2), see Table 2.3. The differences (if any) may be ascribed in part to differences in other factors like the concentration of organic matter which is lower in the softer waters due to a greater dilution factor. This is in agreement with the results of a life-cycle test conducted by Brungs et al (1976) who found a relatively high NOEC-value (compared with the values of 11 and 15 $\mu g.l^{-1}$ in soft and hard water, respectively - see Table 2.3) of 66 $\mu g.l^{-1}$ for the fathead minnow Pimephales promelas in test water with high total organic carbon and phosphate levels. In a 7-d life-cycle test with the daphnid Ceriodaphnia dubia in upstream river water with a background copper level of about 1 $\mu g.l^{-1}$, an actual copper concentration of 32 $\mu g.l^{-1}$ strongly reduced both survival and reproduction. When these daphnids were exposed to the same river water collected at sites below sewage treatment plants (resulting in higher copper, total organic carbon and suspended solids levels) actual levels of 67 and 97 $\mu g.l^{-1}$ were not or only slightly toxic, also in agreement with the data mentioned above (Carlson, et al., 1986).

Also for the daphnid D. pulex no different toxicity values were found in soft and hard water (Winner and Gauss, 1986). For D. magna the toxicity was somewhat (2-3 times) lower at a hardness of 100 compared with a hardness of both 50 and 200 (Chapman et al., cited in EPA, 1985b).

Most reported NOEC-values are in the range of 5 to 40 $\mu g.l^{-1}$, both in soft and hard water, with apparently no or little difference in sensitivity between specific groups of organisms. In life-cycle tests with four species of daphnids (D. magna, D. parvula, D. ambigua and D. pulex) in the same test water an equal NOEC-value of 40 $\mu g.l^{-1}$ was found for all species (Winner and Farrell, 1976).

In reconstituted, hard (H = 250) water a very low 21-days LC50-value of 1.4 $\mu g.l^{-1}$ was found for D. magna in a renewal test; the NOLC-value was about 0.4 $\mu g.l^{-1}$ (nominal concentrations). At 0.4 $\mu g.l^{-1}$ reproduction was strongly reduced, but both at some lower and higher copper concentrations

no adverse effect on this parameter was found. On the basis of both survival and reproduction the NOEC-value was 0.2 $\mu g.l^{-1}$ according to the author (Dave, 1984). The high toxicity of copper in this test may be explained by the absence of natural complexing materials in the test water. For this reason and because of the varying influence of increasing copper concentrations on the parameters tested (especially on reproduction), the significance of this test is considered to be doubtful and the result of this test is not used in the evalution.

In tests conducted in filtered (50 μm), UV-sterilized lake water, D. magna was found to be more sensitive to copper stress in population toxicity experiments under flow-through conditions (intermittent-flow system) than in semistatic life-table experiments. The difference may be the result of an additional stress because of food limitation in the population experiments or may be caused by changes in the speciation of copper due to the high population densities (Van Leeuwen et al., in press). However, the difference may also have been caused by the difference in test type (semistatic versus flow-through).

In flow-through bioassays in charcoal filtered, soft (H = 17) test water, which was from smelting snow, adult asiatic clams (Corbicula manilensis) showed high mortality within 10 days of exposure to 890 - 2,570 $\mu g.l^{-1}$, but at exposure to 6,000 - 12,000 $\mu g.l^{-1}$ no deaths occured until after 10 to 15 days; the clams responded to these very high exposure levels by closing the valves of their shells tightly. Under these test conditions the 75-d LC50 was below 11 $\mu g.l^{-1}$. In static tests, conducted in the same test water which was sterilized by UV light, with different larval stages trochophore larvae were most sensitive: all larvae died within 8 hours at exposure to 5 $\mu g.16^{1}$, while control mortality was below 25%. The apparent copper complexing capacity of the test water used in the experiments with adults was only 3 $\mu g.l^{-1}$, and the Chelex-100 labile copper fraction ranged from 57% to 89% of total copper; the Chelex-100 fraction includes DPASV-labile copper, moderately labile ligands and possibly slowly exchangable ligands bound in colloidal matter. In the larval assays the complexing capacity was 2 $\mu g.l^{-1}$ and the DPASV labile copper fraction (Cu-ion and very labile copper ligands) was 54% at 5 $\mu g.l^{-1}$ total copper (Harrison et al., 1984).

In waters of unspecified hardness exposure to 3 and 30 $\mu g.l^{-1}$ for 60 days resulted in toxic effects in snails (Biomphalaria glabrata) and eels (Anguilla anguilla), respectively (Rødsaether et al., 1977; Cardarelli, 1974, cited in Imlay and Winger, 1983).

In algae grow was delayed (Chlorella pyrenoidosa) or inhibited (Nitzschia palea) after addition of 1-5 $\mu g.l^{-1}$ in a medium without citric acid and

EDTA (Steemann Nielsen and Wium-Andersen, 1970). Much higher toxicity values (100 to 10,000 $\mu g.l^{-1}$) have been reported for other algae, but data on test water and exposure time are lacking (EPA, 1985b).
On macrophytes only two tests which have been conducted in natural water were available. In one test the growth of Elodea nuttallii was inhibited for more than 50% at a concentration of 635 $\mu g.l^{-1}$ after exposure for 7-10 days in water of unspecified hardness (van der Werff, 1984). In the other test a 7-d EC50-value of 600 $\mu g.l^{-1}$ was found for Lemna minor (Bishop and Perry, 1981). In artificial media the lowest toxicity values for macrophytes (10 - 100 $\mu g.l^{-1}$) were found for duckweed, L. minor valdiviana (Demayo et al., 1982; Hutchinson and Czyrska, 1975). The submerged, rooted Elodea canadensis was found to be less sensitive than the free-floating L. minor in unspecified water (EC50-values of 3,100 and 130 $\mu g.l^{-1}$, respectively). This difference may be in part due to the presence of a sediment layer in the case of E. canadensis (Brown and Rattigan, 1979). According to Nor and Cheng (1986) macrophytes are more tolerant to copper than many other aquatic populations, for example algae.

Freshwater-sediment systems

Sediments can accumulate great amounts of copper compared with the overlaying water. So, sediments are a potential source of copper, not only to organisms living in, and/or feeding at the sediment, but also to other aquatic organisms. Copper can be released from the sediment either as soluble or as sediment-bound (particulate) copper, for example by dredging or by (bio)turbation. An example of the last mentioned phenomenon was demonstrated by Malueg et al. (1983) in acute sediment toxicity tests in which daphnids in the water were exposed to polluted sediments: co-exposure with insect nymphs in the sediment could increase the mortality of daphnids compared to exposure of daphnids alone.
Data on short-term sediment toxicity tests are listed in Table 2.4. A comparison of the toxic copper levels in the water in the water-sediment systems with those of tests without sediment shows, that copper is less toxic in the presence of sediments. For example, Cairns et al. (1984) found 10-d LC50-values of 720 and 1,150 $\mu g.l^{-1}$ (total copper) in sediment toxicity tests with the crustaceans Hyalella azteca and Gammarus lacustris, respectively, while these values were 59 and 31 $\mu g.l^{-1}$ in comparable toxicity tests without sediment (Nebeker et al., unpublished; cited in Cairns et al., 1984). The last mentioned values are in good agreement with the 10-d LC50-values expressed as dissolved copper: 40 and 60 $\mu g.l^{-1}$,

respectively, indicating that mainly dissolved copper is responsible for the toxic action and that sediment-bound copper is not or hardly toxic.
From the data presented in Table 2.4 it is clear that the copper concentration in the sediment can not be used directly to predict the concentration in the overlaying water. It is also clear that sediment and water levels expressed as total copper are not very usefull to predict the toxicity. The found differences can be ascribed to sediment parameters (like organic carbon content, the presence of other metals or the particle-size distribution) and other factors, for example if the water-sediment system is already in equilibrium or not. Data for D. magna and H. limbata in the tests with polluted sediments containing 540 and 550 $mg.kg^{-1}$ indicate that a high organic carbon content has a protective effect (Malueg et al., 1984b). The available data are insufficient to establish or qualify a relation between organic carbon content of the sediment and the ultimate effect.
In these short-term sediment toxicity tests the lowest reported sediment and water copper levels: 200 $mg.kg^{-1}$ and 26-28 $\mu g.l^{-1}$, respectively, were not lethal to D. magna. The lowest lethal levels in these tests were 480 $mg.kg^{-1}$ (D. magna and (0 ->) 50 $\mu g.l^{-1}$ (D. magna and H. limbata).

In a long-term (7 months) laboratory test in which unpolluted sediments (32 $mg.kg^{-1}$ d.w., at start) with the native microcosm (periphyton, macroalgae, zooplankton, macroinvertebrates and emerged insects) received a continuous flow of copper enriched "outdoor" water (H = 200), an actual water level of 30 $\mu g.l^{-1}$ (corresponding with a sediment level of 57 $mg.kg^{-1}$ caused changes in the biological structure; the NOEC-value was 9 $\mu g.l^{-1}$, corresponding with a sediment level of 37 $mg.kg^{-1}$. At this level primary production of the whole system and growth of the macroalga Vaucheria terrestris were significantly reduced after 7 months; the NOEC-value for these parameters was 4 $\mu g.l^{-1}$, corresponding with a sediment level of 34 $mg.kg^{-1}$ (Hedtke, 1984).

Table 2.5 summarizes some effects of copper in the natural aquatic environment in which elevated copper levels in water and/or sediment were found or induced. At water levels of 10 and 65 $\mu g.l^{-1}$ a number of algal species was affected, but total biomass was not affected or only initially (McKnight, 1981; Leland and Carter, 1984). Other data indicate that water levels of about 20 $\mu g.l^{-1}$ and upwards are affecting several groups of organisms (algae, invertebrates, fish).

Correlation studies show that in general 1) the total number of organisms (abundance), 2) the number of taxa (diversity) and 3) the biomass (productivity) of benthic macro-invertebrates are inversely related to sediment copper levels. Increased copper levels also result in a shift to a number of tolerant species, for example chironomids (insects).

Marine organisms - short-term

A selection of relatively low acute toxicity values for marine organisms is presented in Table 2.6. The **bold** data are from tests which have been conducted in seawater without special treatment like sterilization or addition of complexing agents. In most cases it is not clear wether the test water is of natural origin or artificial.
In natural, but membrane-filtered (0.45 μm) seawater about 25% mortality was found in a static test with the prefeeding stages (eggs -> nauplius II) of the copepod Euchaeta japonica (crustaceans) after addition of 5.4 $\mu g.l^{-1}$, resulting in a total concentration of 6.0 $\mu g.l^{-1}$ (Lewis et al., 1972).
The available data on the influence of the salinity on the (acute) toxicity of copper indicate that for some organisms copper is somewhat less toxic at higher salinity, especially at low copper concentrations, but the differences are too small to be of any biological significance (McLeese, 1974; Jones et al., 1976; MacInnes and Calabrese, 1979; Mance et al., 1984).
The toxicity of copper to the softshell clam Mya arenaria was found to be strongly depending on temperature of the test water: the 7-d toxicity values (LC0, LC50 and LC100) were at least 30 and 60 times lower at 17 °C and 22 °C, respectively, compared with the values at 4 °C (Eisler, 1977).

Marine organisms - long-term

In Table 2.7 the available data on long-term tests with marine organisms, resulting in a NOEC-value, are listed. For two organisms (Nereis diversicolor and Busicon canaliculatum) only a NOLC-value was available. Data on tests which have been conducted in treated water (e.g. UV-sterilized, autoclaved, enriched with chelators) have been excluded because of the possible effects on the toxicity compared with untreated water. For example, UV-sterilization of natural water reduces the organic content and thus may increase the toxicity of copper; enrichment with chelators can have the opposite result.

Only one life-cycle test has been reported, with the crustacean Mysidopsis bahia, resulting in a NOEC-value of 38 $\mu g.l^{-1}$. A number of other organisms (invertebrates from several phyla) were found to be more sensitive, with NOEC-values of 2.5 - 5 $\mu g.l^{-1}$ in partial-life-cycle tests. No data were available on life-cycle tests or early-life-stage tests with fish. For 6 fish species L(E)C50-values in the range of 150-610 $\mu g.l^{-1}$ are reported after an exposure time of 2 to 4 weeks (Baker, 1969; Gardner and LaRoche, 1973; Engel et al., 1976, abstract). For embryos of the Atlantic cod (Gadus morhua) a 14-d LC50-value of 10 $\mu g.l^{-1}$ was reported (Swedmark and Granmo, 1981; cited in EPA, 1985b).

In a field experiment with M. edulis on a mussel bed the 6-w NOLC was 21 (range 8-32) $\mu g.l^{-1}$, but about 6 months after termination of the experiment all exposed animals had died (Table 2.8; De Wolf et al., 1972).
In a "controlled experimental ecosystem" (1,700 m^3 water) with 5-m old salmonids (Oncorhynchus keta) no observable effect on growth and mortality was found after 6 weeks at 2.5 $\mu g.l^{-1}$ seawater, a level 10 times above background; no other concentrations were used (Koeller and Parsons, 1977).

Seawater-sediment systems

As in freshwater the toxicity of copper is less in the presence of sediment (Neanthes arenaceodentata; Pesch and Morgan, 1978) or a sediment-extract (Euchaeta japonica, Lewis et al., 1973) in the test water. In continuous-flow tests with the worm N. arenaceodentata the LT50 (median survival time) increased at least a factor of 4.5 in the presence of sediments, while only up to 5% of the total amount of copper that entered the system was adsorbed by the sediments (Pesch, 1979). So, not all the copper is bound to sediment, but a part of the copper in the water phase is not or less toxic due to a protective effect of substances released from the sediment.
In Table 2.8 some data on the toxicity of copper in seawater-sediment systems are summarized. In laboratory experiments the burrowing activity of the mollusc Protothaca staminea was reduced in an enriched sediment containing 23 $mg.kg^{-1}$ after enrichment, but no reduction was found in the presence of a polluted sediment with the same amount of copper (Phelps et al., 1983). In another test with this species there was no delay in burrowing time on aged enriched sediment. In a long-term test 25% of the exposed animals died on sediments with a copper level of 38 $mg.kg^{-1}$ after enrichment ; the NOLC-value was 19 $mg.kg^{-1}$ (Phelps et al., 1985).

Table 2.2. A selection of (low) acute toxicity values for freshwater organisms in natural, untreated water

Organism	Hardness	Criterion	Result $\mu g\ Cu.l^{-1}$	Reference
Soft water				
Algae:				
Phytoplankton	10	$EC50^{20}$	13	Hongve et al., 1980
Molluscs:				
Corbicula fluminea	51-76	$LC50^{96}$	40	Rodgers et al., 1980
Physa integra	"soft"	$EC50^{96}$	39	Arthur and Leonard, 1970
Crustaceans:				
Ceriodaphnia reticulata	45	$LC50^{48}$	17	Mount and Norberg, 1984
Daphnia hyalina	66	$LC50^{48}$	5	Baudouin and Scoppa, 1974
Daphnia magna	44-53	$EC50^{48}$	10	Biesinger and Christensen, 1972
Gammarus pseudolimnaeus	"soft"	$EC50^{96}$	20	Arthur and Leonard, 1970
Simocephalus serrulatus	"soft"	$LC50^{24}$	7-24	Giesy et al., 1983
Insects:				
Chironomus tentans-larvae	71	$LC50^{96}$	298	Nebeker et al., 1984a
Fish:				
Ptychocheilus oregonensis	20-56	$LC50^{96}$	18-23	Andros and Garton, 1980
Lepomis macrochirus	**20[1]**	**$LC50^{96}$**	**66**	**Pickering and**
Pimephales promelas	**20[1]**	**$LC50^{96}$**	**22-25**	**Henderson, 1966**
Salmo clarki	**15-34[1]**	**$LC50^{96}$**	**16-74[2]**	**Chakoumakos et**
	66-96[1]	**$LC50^{96}$**	**44-186[2]**	**al., 1979**
Salmo gairdneri	**30[1]**	**$LC50^{96}$**	**20-30[2]**	**Howarth and**
	100[1]	**$LC50^{96}$**	**31-86[2]**	**Sprague, 1978**
Hard water				
Molluscs:				
Potamopyrgus jenkinsi	250-380	$LC50^{96}$	54-79	Watton and Hawkes, 1984
Viviparus bengalensis	160-210	$LC50^{96}$	88	Gupta et al., 1981
Crustaceans:				
Daphnia magna	130-160	$LC50^{72}$	86	Winner and Farrell,
Daphnia pulex	130-160	$LC50^{72}$	54-86	1976
Fish:				
Lepomis macrochirus	**360**	**$LC50^{96}$**	**10200**	**Pickering and**
Pimephales promelas	**360**	**$LC50^{96}$**	**1140-1760**	**Henderson, 1966**
Salmo clarki	**165-224[1]**	**$LC50^{96}$**	**232-376[2]**	**Chakoumakos et al., 1979**
Salmo gairdneri	**365**	**$LC50^{96}$**	**70-516[2]**	**Howarth and Sprague, 1978**

[1]) Test water partly deionized or mixed with deionized water to reduce hardness

[2]) Dissolved (0.45 μm filter) copper

Hardness: $mg.l^{-1}$, as CaCO3

Table 2.3. Long-term toxicity values for freshwater organisms based on partial-life-cycle (plc), life-cycle (lc) and early-life-stage (els) tests

Organism	Cu-conc. analized	Test type	Test water, Hardness $mg.l^{-1}$ CaCO3	Exp. time	Reference Criterion	Result $\mu g\ Cu.l^{-1}$
SOFT WATER						
Algae:					Blaylock et al., 1985	
Chlorella vulgaris	A	S	well, H = 85	4-d	NOEC	± 80
Selenastrum capricornutum	A	S	well, H = 85	4-d	NOEC	± 100
Molluscs:						
Campeloma decisum					Arthur & Leonard, 1970	
shell length 11-27 mm	A	CF	-, H = 35-55	6-w	NOEC	8 α
Physa integra					Arthur & Leonard, 1970	
shell length 4-7 mm	A	CF	-, H = 35-55	6-w	NOEC	8 α
Crustaceans:						
Ceriodaphnia dubia					Carlson et al., 1986	
< 4 hours, P -> F	A	R	river, H = 20-44	1-w lc	NOEC	12 α
Daphnia magna			-		Blaylock et al., 1985	
≤ 24 hours, P -> F (brood 6)	A	R	well, H = 85	2-w lc	NOEC	10
Daphnia pulex					Winner, 1985	
24 hours	A	R	art., H = 58	7-w lc	NOEC	6
Gammarus pseudolimnaeus					Arthur & Leonard, 1970	
P adults -> F1 adults	A	CF	-, H = 35-55	15-w lc	NOEC	5 α
Insects:						
Chironomus tentans					Nebeker et al., 1984a	
4th instar larvae -> adults	A	CF	well, H = 36	3-w	NOEC	34 α
Clistoronia magnifica					Nebeker et al., 1984b	
5th instar larvae P -> F2	A	CF	well, H = 26	8-m lc	NOEC	8
Paratanytarsus parthenogeneticus					Hatakeyama & Yasuno, 1981	
2nd instar larvae, P -> F1	A	S	art., H = 25	3-w lc	NOEC	40 α
Fish:						
Catostomus commersoni					McKim et al., 1978	
eyed eggs -> larv.-juv.	A	CF	lake, H = 44-50	6-w els	NOEC	13 α
Coregonus artedi					Mckim et al., 1978	
eyed eggs -> larv-juv.	A	CF	lake, H = 44-50	9-w els	NOEC	43 α
Cyprinus carpio					Muramoto, 1982	
7-9 cm; 10-12 g	-	R	tap, H = 11	3-m	NOLC	50
Esox lucius					McKim et al., 1978	
green eggs -> larv.-juv.	A	CF	lake, H = 44-50	6-w els	NOEC	35 α
Ictalurus punctatus					Sauter et al., 1976	
eggs -> fry	A	CF	well, H = 36	9-w els	NOEC	12 α
Lepomis macrochirus					Benoit, 1975	
2 years, P -> F1	A	CF	[1]lake, H = 44-50	22-m lc	NOEC	77 α
Lepomis macrochirus					Benoit, 1975	
newly hatched larvae	A	CF	[1]lake, H = 44-50	3-m els	NOEC	21 α
Micropterus dolomieui					McKim et al., 1978	
eyed eggs -> larv.-juv.	A	CF	lake, H = 44-50	5-w els	NOEC	37 α
Oncorhyngus tschawytscha					Hazel & Meith, 1970	
eyed eggs -> fry	A	CF	river, H = 44	1-m els	NOEC	21
Pimephales promelas					Mount & Stephan, 1969	
fry, 10-20-mm, P -> F1	A	CF	[2]pond, H = 31	11-m lc	NOEC	11
Pimephales promelas					Norberg and Mount, 1985	
newly hatched larvae,	A	R	lake, H = 48	1-w	NOEC	10 α
Salmo gairdneri					McKim et al., 1978	
eyed eggs -> larv.-juv.	A	CF	lake, H = 44-50	7-w els	NOEC	11 α
Salmo trutta					McKim et al., 1978	
green eggs -> larv.-juv.	A	CF	lake, H = 44-50	18-w els	NOEC	22 α

Salvelinus fontinalis						McKim & Benoit, 1971		
yearlings, P -> F1	A	CF	lake, H =	40-48	8-m lc	NOEC	17	
Salvelinus fontinalis						McKim et al., 1978		
eyed eggs -> larv.-juv.	A	CF	lake, H =	44-50	11-w els	NOEC	22	α
Salvelinus fontinalis						Sauter et al., 1976		
eggs -> fry	A	CF	well, H =	38	9-w els	NOEC	3	α
Salvelinus namaycush						McKim et al., 1978		
eyed eggs -> larv.-juv.	A	CF	lake, H =	44-50	13-w els	NOEC	22	α
Stizostedion vitreum						Sauter et al., 1976		
eggs -> fry	A	CF	well, H =	35	4-w els	NOEC	13	

HARD WATER

Algae:								
Phytoplankton, natural population						Horne & Goldman, 1974		
Anabaena, Aphanizomenon	-	S	lake, H =	115	10-d	NOEC	5	
Crustaceans:								
Daphnia magna						Winner & Farrell, 1976; Winner,'81		
< 24 hours, P -> F	-	R	pond, H =	130-160	>4-m lc	NOEC	40	
Daphnia magna						Van Leeuwen et al., in press		
P -> F	A	R	lake, H =	225	3-w lc	NOEC	13	α
P -> F	A	CF	lake, H =	225	2-w lc	NOEC	5	α
Daphnia pulex						Ingersoll & Winner, 1982		
≤ 24 hours, P -> F	A	R	art., H =	106	10-w lc	NOEC	5	
Orconectes rusticus						Hubschmann, 1967		
newly hatched	-	CF	tap, H =	100-125	4-w	NOEC	15	
Fish:								
Ictalurus nebulosis						Christensen et al., 1972		
2 years	A	CF	-, H =	190-215	1-m	NOEC	27	
Ictalurus punctatus						Sauter et al., 1976		
eggs -> fry	A	CF	well, H =	186	9-w els	NOEC	13	
Noemacheilus barbatulus			bore-			Solbe' and Cooper, 1976		
9-12 cm	A	CF	hole, H =	232-266	2-m	NOLC	120	α
Pimephales promelas						Mount, 1968		
fry, 7 weeks P -> F1	A	CF	[2]pond, H =	182-216	11-m lc	NOEC	15	α
Salmo gairdneri						Seim et al., 1984		
embryos, 6-d post-fert.	A	CF	well, H =	120	11-w els	NOEC	16	α
Salvelinus fontinalis						Sauter et al., 1976		
eggs -> fry	A	CF	well, H =	187	9-w els	NOEC	5	

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[1]) UV-sterilized

[2]) mixed with deionized water or tap water to reduce hardness

α) actual concentration

lc) life-cycle test with 2 generations (P -> F)

els) early-life-cycle test (embryo-larval test)

NOEC) no-observed-effect-concentration

NOLC) no-observed-lethal-concentration

-) no data

Table 2.4. Short-term sediment toxicity tests with freshwater organisms - laboratory experiments

Organism / Dosing type	Exp. time	Criterion	Sediment <--Result--> Water mg Cu.kg^{-1}	μg Cu.l^{-1}
Crustaceans:				
Daphnia magna <24-hr old				Malueg et al., 1984a
Polluted sediments, covered with recirculating well water (H = 21-31)				
Co-exposure with Hexagenia limbata nymphs in the sediment				
3.1% org. C	2-dβ	LC(40)	480 (dry)	0->130 α
2.3% org. C	2-dβ	NOLC	930 (dry)	0->350 α
3.1% org. C	2-dψ	LC(97)	480 (dry)	230->190 α
3.7% org. C **(control sed.)**	2-dψ	NOLC	200 (dry)	26-> 28 α
1.4% org. C	2-dψ	NOLC	140 (dry)	260->330 α
		water concentrations: at t = 0 -> 2 d		
Daphnia magna <24-hr old				Malueg et al., 1984b
Polluted sediments, covered with recirculating well water (H = 21)				
Co-exposure with Hexagenia limbata nymphs in the sediment				
1.4% org. C	2-dψ	LC(100)	550 (dry)	0-> 50 α
14.2% org. C	2-dψ	NOLC	540 (dry)	0->100 α
Daphnia magna 4-7 day old				Cairns et al., 1984
Enriched sediments, equilibrated with overlaying well water (H = 34-65)				S
1.8% org. C; 11.8% clay	2-d	LC50	937 (dry)	~100 α
3.0% org. C; 55.9% clay	2-d	LC50	681 (dry)	~300 α
Hyalella azteca - juveniles and adults				Cairns et al., 1984
Enriched sediment equilibrated with overlaying well water (H = 34-65)				S
3.0% org. C; 55.9% clay	10-d	LC50	1,078 (dry)	720 α
Gammarus lacustris - juveniles and adults				Cairns et al., 1984
Enriched sediment equilibrated with overlaying well water (H = 34-65)				S
3.0% org. C; 55.9% clay	10-d	LC50	964 (dry)	1,150 α
Insects:				
Hexagenia limbata-nymphs				Malueg et al., 1984a
Polluted sediments, covered with recirculating well water (H = 21-31)				
2.3% org. C	10-d	NOLC	930 (dry)	0->350 α
1.8% org. C	10-d	NOLC	650 (dry)	0->500 α
Hexagenia limbata-nymphs				Malueg et al., 1984b
Polluted sediments, covered with recirculating well water (H = 21)				
14.2% org. C	10-d	NOLC	540 (dry)	0->100 α
1.4% org. C	10-d	LC(100)	550 (dry)	0-> 50 α
2.2% org. C	10-d	LC(40)	1,800 (dry)	0->1,200 α
Chironomus tentans-2nd and 3th instar larvae				Cairns et al., 1984
Enriched sediments equilibrated with overlaying well water (H = 34-65)				S
1.8% org. C; 11.8% clay	10-d	LC50	2,296 (dry)	250 α
3.0% org. C; 55.9% clay	10-d	LC50	857 (dry)	430 α

Polluted sediments: contain copper (and other metals) due to long-term pollution in the field ("native copper")
Enriched sediments: contain copper due to addition in the laboratory
α: actual concentration

β: start of test directly after preparing the test system
ψ: start of test 6 days after preparing the test system
R: renewal test
S: static test

Table 2.5. Toxicity values in freshwater-sediment systems, field data

"Ecosystem"	Exp. time	Criterion	Sediment <--Result--> Water mg Cu.kg^{-1}	 μg Cu.l^{-1}
Plankton and plants				
Algae-natural population of benthic, epiphytic algae Experiment in creek with **enriched water** (H = 57). Total biomass	12-m	**NOEC**		Leland & Carter, '84 **≥10** β,α
Reduced population density of 1, 4, and 16 taxa of the 22 most abundant taxa at a level of 3, 5, and 10 μg.l^{-1} (β), respectively.				
Algae-natural population **Enriched water** Application of copper sulfate in a lake (background copper concentration 9 μg.l^{-1}), resulting in a copper concentration of ..... 24-28 The application prevented a water bloom, but the development of green algae was more or less normal.				Steemann Nielsen & Wium-Andersen, '70ψ
Plankton-natural populations **Enriched water** One application of copper sulfate in a lake (alkalinity 5x10^{-4}, background copper concentration 6 μg.l^{-1}), resulting in an average total copper concentration (from surface to 4 m depth) of ..... 65 α **Phytoplankton:** Initially inhibition of primary production and biomass; no inhibition after 10 days; shift from dominant species Ceratium hirundinella to green algae. **Zooplankton:** strongly reduced and some species no longer observed; after 1 month the strongly reduced rotifer Keratella sp. had increased to 3 times pretreatment levels; Daphnia sp. not observed for half a year.				McKnight, 1981
Plants-natural populations **Enriched water** One application of copper sulfate in ponds (H = 110-170), at nominal copper concentrations of 1,000 and 3,000 μg.l^{-1}.				McIntosh, 1974
Algae:	3-m	EC		1,000
Macrophytes:	3-m	EC		3,000
	3-m	**NOEC**		**1,000**
Recovery target species after 14 days to 3 months.				
Invertebrate-populations				
Invertebrates-natural population **Enriched stream** (H = 185-328), copper concentration 9 μg.l^{-1} upstream and 120 μg.l^{-1} (treatment site) -> 23 μg.l^{-1} (recovery side).				Winner et al., 1975, 1980
	2.5-yr	EC		23 α
Negative correlation between both number of species and number of individuals, and the copper concentration; reduction of insect densities at all sites compared with the control site (-> **NOEC < 23 μg.l^{-1}**); reduction to disappearance of other invertebrates at concentrations of 37-120 μg.l^{-1}.				
Macro-invertebrates-natural populations **Polluted sediments**, mean level 589, range 243-1,229 (---)			--- α	Kraft and Sypniewski 1981
On the average, reduced number of taxa and organisms compared with "control" sediments with an average level of 33 (range 6 to 54) mg.kg^{-1}.				

Molluscs, mayflies and crustaceans were abundant in the control sediments but were rare or absent in the polluted sediments, in which chironomids (insects) were predominant.

Macro-invertebrates-natural populations Malueg et al., 1984a
Polluted sediments range 140-930 (dry) --- α
Reduced number of taxa, number of organisms and biomass of benthic invertebrates compared with "control" sediments with sediment levels of 17-37 mg.CU2+.kg^{-1}; different dominant species.

Macro-invertebrates-natural populations Malueg et al., 1984b
Polluted sediments, range 16-2,700 (dry) --- α
In general, number of organisms, biomass and species diversity of benthic invertebrates inverse related with sediment level; shift to other dominant species, but data are not consistent.

Macro-invertebrates-natural populations Brown, 1977
Polluted sediments range 1,000-5,000 (---) 200-800 α
At higher concentrations: insect larvae dominant, with low diversity. Both diversity and numbers of other benthic invertebrates strongly reduced at the most polluted sites.

Macro-invertebrates-natural population LaPoint et al., 1984
Polluted streams 10-100 α
Both number of taxa and number of individuals of benthic invertebrates negatively correlated with copper (and other metals) water levels.

Ecosystems

Lake-ecosystem Hanson & Stefan, '84
Enriched lake **water** (H = 200-300) treated for 58 years with copper sulfate, 3-5 times every year, to prevent algal bloom.
range 120-5,600 (dry) 20-530 α
Short-term effects: mortality algae, O2-depletion, fish mortality.
Long-term effects: increasing tolerance some algal species resulting in a shift from green to blue-green algae, shift from gamefish (e.g. walleye and bass) to rough fish (e.g. carp, bullhead, sheepshead), strong reduction of benthic macro-invertebrate fauna and of macrophytes.

River-ecosystem Carlson et al., '86
Polluted river **water** (H = 20-101) recieving sewage treatment plant water.
1-140 α
Diverse and healthy fish and periphyton populations at up to about 20 $\mu g.l^{-1}$; stressed community at > 40 $\mu g.l^{-1}$.

α: actual concentration
β: dissolved (0.2 μm filter) copper
ψ: secundary literature source
Polluted: contains copper (and other metals) due to long-term pollution in the field ("native copper")
Enriched: contains copper due to addition of copper to the water

Table 2.6. A selection of (low) acute toxicity values for marine organisms

Organism	Criterion	Result $\mu g\ Cu.l^{-1}$	Reference
Algae:			
Oscillatoria theibautii	$EC50^{3-5}$	**20**	Rueter et al., 1979
Scrippsiella faeroense	$EC50^{103}$	5	Mance et al., 1984[1]
Thalassiosira pseudonana	$EC50^{72}$	5	Mance et al., 1984[1]
Annelids:			
Neanthes arenaceodentata	$LC50^{96}$	**77**	Pesch and Morgan, 1978
Nereis diversicolor	$LC50^{96}$	**≥ 200**	Jones et al., 1976
Nereis diversicolor	$LC50^{96}$	100	Mance et al., 1984[1]
Phyllodoce maculata	$LC50^{96}$	120	Mance et al., 1984[1]
Molluscs:			
Crassostrea gigas-eggs->larv.	$EC50^{48}$	12	Knezovich et al., 1981[2]
Crassostrea gigas-embryos	$EC50^{48}$	5	Martin et al.,1981[2]
Crassostrea virginica-embryos	$LC50^{48}$	**103**	Calabrese et al., 1973
Haliotus sp.-adults	$LC50^{96}$	30	Harrison, 1985[1]
Mytilus edulis-embryos	$EC50^{48}$	6	Martin et al., 1981[2]
Mya arenaria	$LC50^{96}$	**39**	Eisler 1977
Crustaceans:			
Acartia tonsa-adults	$LC50^{72}$	**9-78**	Sosnowski et al., 1979
Acartia tonsa	$LC50^{96}$	**17-55**	Sosnowski and Gentile, 1978
Homarus americanus-450 g	$LC50^{96}$	**11-45**	McLeese, 1974
Fish:			
Clupea harengus pallasi-embr.	$LC50^{96}$	**236**	Rice & Harrison, 1978
	$LC50^{145}$	**38**	
Menedia menedia-larvae	$LC50^{96}$	136	Mance et al., 1984[1]
Paralichtus dentatus-embr.	$L(E)C50^{-}$	12-16	EPA, 1985b[1]

bold data: untreated seawater (no sterilization or addition of complexing agents)
[1]: review; no data water characteristics
[2]: UV-sterilized sea water
$^{-}$: no time specified, but "acute"

Table 2.7. Long-term toxicity values for marine organisms

Organism	Cu-conc. analized	Test type	TW	Exp. time	Criterion	Result $\mu g\ Cu.l^{-1}$	Reference
Coelenterata:							
Campanularia flexuosa	-	R	as	2-w	NOEC	10 δ	Stebbing, 1976
Hydra littoralis	-	S	as	2-w	NOEC	2.5	Stebbing & Pomroy, 1978
Eirene viridula	-	R	-	3-m	NOEC	10	Karbe, 1972
Annelida							
Nereis diversicolor	-	R	s	6-w	NOLC	100	Bryan & Hummerstone, 1971
Molluscs:							
Argopecten irradians-juveniles	A	CF	s	6-w	NOEC	5 α	Pesch et al., 1979
Busicon canaliculatum	-	R	ns	8-w	NOLC	100	Betzer & Yevich,1975
Crassostrea virginica-larvae	-	R	s	2-w	NOEC	10	Calabrese et al., 1977
Mercenaria mercenaria-larvae	-	R	s	1-w	NOEC	5	Calabrese et al., 1977
Mytilus edulis	A	-	s	21-m	NOEC	3 α	Calabrese et al., 1984
Crustaceans:							
Artemia salina-larvae	-	R	s	2-w	NOEC	25	Saliba & Ahsanullah,1973
Mysidopsis bahia P -> F	A	CF	-	lc	NOEC	38	EPA, 1985b~
Pandalus danae-larvae	A	CF	s	6-w	NOEC	10	Young et al., 1979

α) actual concentration
δ) dissolved (0.45 μm filter) copper
~) secundary literature source
lc) life-cycle test; no exposure time specified
TW) test water: s = seawater, origin unknown; as = artificial seawater; ns = natural seawater
NOEC) no-observed-effect-concentration
NOLC) no-observed-lethal-concentration
-) no data

Table 2.8. Seawater-sediment systems, field and laboratory data

"Ecosystem"	Exp. time	Criterion	**Sediment** <--Result--> **Water** mg Cu.kg^{-1}		μg Cu.l^{-1}
Laboratory data					
Molluscs:					
Protothaca staminea-length 15-35 mm				Phelps et al., 1983	
Enriched sediment (rinsed twice) with a flow of clean natural seawater 0.1% org. C; no clay and silt (coarse sand/gravel).					CF
Red. burrowing activity	1.5-hr	EC	23 (dry)	---	α
Red. reburrowing activity	2-d	EC	18 (dry)	---	α
No difference in burrowing time on the unenriched sediment (12 mg.kg^{-1}) compared with another natural sediment with a background con. of 23 mg.kg^{-1}					
Protothaca staminea-length 16-25 mm				Phelps et al., 1985	
Enriched sediment (rinsed twice) submerged in flowing seawater 0.1% org. C; no clay and silt (coarse sand/gravel).					CF
Red. (re)burrowing act.	2-d	EC	15 (dry)	---	α
	2-d	**NOEC**	**10 (dry)**	---	α
No delay of burrowing time on aged (> 1 day) enriched sediments.					
	7-w	LC(25)	38 (dry)	---	α
	7-w	NOLC	19 (dry)	---	α
Field data:					
Annelids:					
Capitella capitata				Oyenekan, 1983	
Higher numbers and annual production at an estuarien site with actual sediment copper levels ranging from 150 to 850 mg.kg^{-1} compared with a site with levels ranging from 135 to 400 mg.kg^{-1}, during a 20-month sampling period. Its abundance was related to silt and hydrocarbon contents of the sediments. Both sites were situated nearby refinery outfalls.					
Nereis diversicolor				Bryan & Hummerstone, 1971	
These worms were found to be present in **(polluted)** estuarine **sediments** with copper levels in the range of .....41-3,020 (dry)				---	α
Molluscs:					
Mytilus edulis-natural population				De Wolf et al., 1972	
Field experiment in open basins (flooded by the tides) built around parts of a mussel bed. The seawater was enriched with copper during high tide. Background concentration seawater: range 1-10 μg.l^{-1}; average 5 μg.l^{-1}.					
	6-w	NOLC	--	21	α
The seawater concentration of 21 (range 8-32) μg.l^{-1} resulted in delayed mortality.					
Invertebrates:					
Macro-invertebrates-natural populations				Rygg, 1985	
Polluted sediments		range	20-500 (dry)	---	α
Fauna diversity and sediment copper concentration strongly negative correlated; a 50% reduction in diversity at 200 mg.kg^{-1}. Concentrations of 100-150 mg.kg^{-1} may already have an effect compared with background levels of 20-30 mg.kg^{-1}. Carnivores (annelids) relatively more tolerant compared with deposit feeders.					
Benthic macro-fauna-natural populations				Oyenekan, 1983	
Polluted sediments		range	135-800	---	α

Significantly negative correlation between copper levels and species diversity during the 20-month period of investigation nearby refinery outfalls. Since copper levels correlated significantly with hydrocarbon levels, the species diversity is probably also related to hydrocarbon.

α: actual concentration
CF: continuous flow test
Polluted sediments: contain copper (and other metals) due to long-term pollution in the field ("native copper")
Enriched sediments: contain copper due to addition in the laboratory

2.2 Terrestrial organisms

Most data on the impact of copper on terrestrial organisms and ecosystems are available in combination with data on other heavy metals, which hinders the retrieval of appropiate data. No reviews - which are focussed entirely on this subject - on copper alone have been found. The less specific reviews on copper published by NRC (1977) and Demayo et al., (1982) only contain some general, limited information on plants and invertebrates which are living in or on the soil. Some useful reviews on more specific subjects (e.g. soil micro-organisms, soil processes, earthworms) will be mentioned later on in this chapter.
All data on copper levels in the soil are expressed as the amount of copper (-ion).

2.2.1 Bioaccumulation, bioconcentration and biomagnification

Most bioaccumulation studies with animals have been conducted with lumbricid earthworms (especially Lumbricus sp. and Allolobophora sp.). In woodland and grassland these worms can form up to 80% of the total biomass of the soil fauna, are important for the cycling of nutrients and the improvement of the soil structure, and are an important food source for wildlife, especially birds and small mammals (Ma, 1983b). In the Netherlands Ma and co-workers of the Research Institute for Nature Management have made an extensive study of the bioaccumulation in, and effects of copper on earthworms; most of their data have been published by Ma (1983a), together with a survey of other published data on earthworms.
In all studies by Ma and co-workers the earthworms were analysed with empty gut to prevent bias by ingested soil.
In a field study in which A. caliginosa was collected from 6 different agricultural soils containing heavy metals due to treatment with municipal waste compost (3 different treatments per soil -> totally 18 plots), the copper level in worms was positively correlated with the soil concentration; the regression slope was significantly smaller than unity, which means a lower concentration factor (CF = concentration in worms : concentration in soil) at higher soil concentration. This implicates that CF-values from different sources cannot be simply compared. The concentration in the worms was negatively correlated with the cation-exchange-capacity (CEC, ranging from 5 to 29 meq/100 g) and, to a lesser

extend, positively correlated with the soil copper concentration. An extension of the multiple regression analysis with the parameters organic matter content (% OM, range 3-14%) and pH (range 4.7-7.1) did not improve this regression model, but a linear regression analysis showed a significant correlation between the CF and both CEC and % OM (Ma, 1982; Ma, 1983a). A multiple regression analysis of copper content of adult L. rubellus collected at different distances from a zinc-smelter as function of soil parameters only showed a significant influence of soil copper content; pH (3.5-6.1) and % OM (2.2-8.6%) did not influence the worm content. In both earthworms species the copper level of adults and subadults collected from the same spot were similar (Ma, 1983a; Ma et al., 1983).

In both studies and in a field experiment in which a sandy soil (5% OM) was treated with different amounts of sewage sludge and adult L. rubellus were analysed (Ma, 1983a) always the same bioaccumulation pattern was found: at background soil copper concentrations the CF was mostly greater than unity (up to about 15 at very low concentrations) and at increased soil concentrations, the CF was smaller than unity. This means that copper is accumulated, but not concentrated (enriched) in the worms when exposed to increased soil copper levels.

In laboratory experiments with adult L. rubellus in a sandy loam soil and a sandy soil, both treated with copper chloride, also the same accumulation pattern was found: in both soils the CF was about unity in untreated soils and decreased with increasing soil levels. An extension of the experiments from 12 to 28 weeks did not further increase the copper level in the worms. Accumulation was up to 3 times higher in the sandy soil compared with the sandy loam soil; this can be explained by the differences in soil parameters and possibly by the higher activity of the worms in the sandy soil (Ma, 1982; Ma, 1983a).

These data on the accumulation pattern of (lumbricid) earthworms are confirmed by other, both field and laboratory, studies, in which also no concentration of copper was found at elevated soil levels (e.g. Van Rhee, 1977; Hartenstein et al.,1980; Eijsackers, 1981; Carter, 1983; Vossen, 1983).

Earthworms (L. rubellus, A. caliginosa) collected from unpolluted soils contain background copper levels of about 15-40 $mg.kg^{-1}$ b.w. (dry weights). In accumulation studies with lethal copper levels (≥200 $mg.kg^{-1}$ soil) the level in L.rubellus was never exceeding 100 $mg.kg^{-1}$ b.w. Based on the results of these studies Ma predicted a maximum level of 70 $mg.kg^{-1}$ b.w. in earthworms in polluted sandy soils and lower levels in other soils (Ma,

1983a). This is in agreement with field data, for example with data of Van Rhee (1977) who reported a level in earthworms (mostly L.rubellus and A. caliginosa) of 63 $mg.kg^{-1}$ b.w. in soil containing a copper level of 110 $mg.kg^{-1}$.
In a 28-d bioaccumulation study lumbricid worms collected from unpolluted sites were kept in 8 different soils with at least one heavy metal at an elevated level. The copper concentration in the worms was positively correlated with both total and DTPA-extractable copper in two species, only related with total copper in one species, and only with DTPA-extractable copper in two species (Stafford, 1986). Ash and Lee (1980) analysed 2 native species in 5 different soils; in 3 soils copper levels were highest in L. terrestris and in 2 soils in A. chlorotica. These data illustrate the complex relationship between the concentrations in earthworms and soil.

Data on the bioaccumulation of copper in other invertebrates at elevated environmental concentrations are limited and much less detailed. A lot of work on this subject has been done with isopods (crustaceans), mainly woodlice, which are feeding on litter. In a field study in which woodlice (Porcellio scaber), soil and litter from 89 sites, both polluted and unpolluted with heavy metals, were analysed, a significant positive correlation between whole body contents and both soil and litter levels has been found. However, at individual sites no prediction of the body content could be made on the basis of the soil or litter content, due to the high variability of the concentration factor. For example, woodlice collected from 2 different spots contained the same copper level (1,000 $mg.kg^{-1}$) while soil levels were 257 and 92 $mg.kg^{-1}$, and litter levels were 168 and 20 $mg.kg^{-1}$, respectively (Hopkin, 1986). In a field study in which another woodlouse species (Oniscus asellus) was kept on litter from their collection site the CF was 6-16 when kept on unpolluted litter (6 sites) and 2.6-3.3 when kept on polluted litter (2 sites) (Hopkin and Martin, 1982). In a field study with other isopods (mainly Tracheoniscus rathke) collected from 7 sites the concentration factor based on litter copper levels was similar in all cases: 6-9, despite differences in litter levels with a factor of 11. The CF based on soil levels ranged from 0.5 to 14, while soil levels maximally differed with a factor of 7. Both based on litter and soil content the highest CF was found at the lowest substrate content and vice versa (Wieser et al., 1976). From these studies it is clear that isopods concentrate copper from soil and/or litter, with in general a lower concentration factor at higher copper levels, but also that there is a high variability due to other factors. The high coppper level

often found in isopods (and other arthropods) is most probably related with the presence of the respiratory pigment haemocyanin and the presence of cuprosomes in the hepatopancreas; this organ contains about 85% of the total body burden (Hughes et al., 1980; Carter, 1983).

Apart from the work of Wieser and co-workers with isopods there is little information on food chain transport of copper in terrestrial ecosystems.
In an experiment in which centipedes (Lithobius variegatus) were fed hepatopancreas of woodlice collected from both unpolluted and polluted sites, the centipedes relatively assimilated less copper from the feed in the latter case; centipedes from the polluted site assimilated more copper compared with those from unpolluted sites, especially when fed polluted hepatopancreas, and were also less sensitive to copper poisoning (Hopkin and Martin, 1984). In another feeding experiment in which spiders (Dysdera crocata) were starved or fed on woodlice no significant differences in body level were found, and no biomagnification occurred (Hopkin and Martin, 1985).
Analyses of animals from different trophic levels - collected in a metal-polluted grassland - showed diet based concentration factors for copper of 0.1-2.9 for invertebrates (highest, intermediate and lowest values in herbivores, carnivores and detritivores, respectively) and of 0.06-0.43 for mice with different feeding habits, indicating a low food chain transfer potential (Hunter and Johnson, 1982).
The CF for plants collected from metal-polluted grassland was 0.06-0.11 for fine-leaved grasses and 0.15-0.20 for the composite ground vegetation (Hunter and Johnson, 1982). According to data reviewed by Hughes et al., (1980) and by Hague and Subramanian (1982) there is a high variability in levels of heavy metals in plants; the availability depends both on soil factors and on water fluxes within soil/plant systems. In some studies no relation has been found between copper levels in plants and soil, but in most studies plant levels increase with increasing soil levels. Many investigators have noted a marked accumulation of metals in the roots of plants, but transport to other parts may occur as has been noted for copper, for example in Acer sp. and agricultural crops like spinach and radish.
More extensive information on accumulation, distribution, speciation (forms and behaviour) and physiological functions of copper in plants can be found in the Proceedings of the Golden Jubilee International Symposium on "Copper in Soils and Plants" (Loneragan et al., 1981).
For information on agricultural crops: see chapter 3.

2.2.2 Toxicity

Microbe-mediated soil processes

Extensive literature reviews on the effects of copper and other metals on the microbial numbers and species diversity and, especially, microbe-mediated processes have been published by Doelman and Haanstra (1983) and Babich and Stotzky (1985). The first review also contains the results of experiments conducted at the Research Institute for Nature Management in the Netherlands.
The effects of copper and other metals have been investigated most extensively in short-term tests (duration some days to about 2 months) with experimentally polluted soils. Data on long-term effects are mostly derived from field data, for example on the basis of correlations between microbial activity and distance from a source of pollution (e.g. Freedman and Hutchinson, 1980).
The reviewed data and additional data from primary sources show a very high variability, resulting from differences in soil parameters, copper speciation and/or experimental design, for example the use of fresh or dried soil. This will be illustrated with a few examples.

Premi and Cornfield (1969) found no effect at 1,000 and a decreased net N-mineralization at 10,000 mg $Cu.kg^{-1}$ d.w.(as CuSO4) after aerobic incubation of a sandy loam soil for 3 weeks, while incubation of the same soil with CuCO3 resulted in an increased N-mineralization at both 1,000 and 10,000 mg $Cu.kg^{-1}$. The difference at the highest concentrations may have been the result of the difference in soil pH. In another sandy loam soil with practically the same characteristics and test conditions, 100-10,000 mg $Cu.kg^{-1}$ (as CuO or CuHPO4) resulted in an increased net N-mineralization (nitrification); at 1,000 $mg.kg^{-1}$ the stimulation of nitrification was at the maximum. No difference was found between the effect of both test substances, while CuHPO4 is more soluble, resulting in higher (2-3 times) EDTA-extractable copper levels (Quraishi and Cornfield, 1971). In a silt loam soil amended with sludge, alfalfa and (NH4)2SO4 an aerobic incubation of 12 weeks resulted in an EC50-value (reduced nitrification) between 100 and 200 mg $Cu.kg^{-1}$ d.w. with copper sulfate as test substance. Less than 1% and about 50% of these amounts of copper could be extracted from the soil after 2 weeks of incubation with 1 M KNO3 and 1 M HNO3, respectively, illustrating the binding capacity of the soil. The

solubility in KNO3 (and in DTPA) further decreased with time (Chang and Broadbent, 1982).

Doelman and Haanstra (1983) studied the effect of up to 8,000 mg Cu.kg^{-1} d.w. (as copper chloride) on soil respiration, enzyme activities and composition of microbial populations in 5 different representative soils in the Netherlands. The effects were measured for 8 weeks, starting only a few days and about 1-1.5 year after mixing of the soil with copper, respectively, to measure both short- and long-term effects. In all soils copper inhibited soil respiration after short-term exposure. After long-term exposure the results were more variable: Levels of 150-400 mg.kg^{-1} were inhibitory in two sandy soils, with more inhibition at higher levels. In a sandy peat soil inhibition occured at ≥ 1,000 mg.kg^{-1}. In a sandy loam soil and a clay soil there was no inhibition, or even a slight stimulation. Of the abiotic parameters of the soils, the clay-content (19% and 60% in the clay soils; 2-9% in the other 3 soils) and/or the Fe-, Mg- and Mn-content (much higher in the two clay soils compared with the other soils) appeared to have the greatest influence on the effect of copper. The organic matter content (13% in the sandy peat soil; 2-6% in the other soils) seemed to have less influence; the same can be concluded for other parameters (pH, % CaCO3, sand and silt content), which differed little or seemed to be less correlated with the copper effect. The CEC was lowest in the sandy soils, but highest in the sandy peat soil, so this parameter was also not useful in this study to predict the effect of copper in all soils. Copper inhibited enzyme activities at different levels in different soils. The extra-cellular enzymes fosfatase, urease and arylsulfatase were more sensitive than the intra-cellular enzymes β-glucosidase and protease. In the sandy soils the most sensitive enzyme was inhibited at a level of 150-550 mg.kg^{-1}. In the clay soils the lowest effective level was 730-1080 mg.kg^{-1}, and in the sandy peat soil 2310 mg.kg^{-1}. In contrast with soil respiration the effect of copper on enzyme activities appeared to be most correlated with the CEC.

Copper levels of 1,000 mg.kg^{-1} showed no effects on the total number and diversity of micro-organisms in the 5 soils, but lower levels decreased the numbers of specific (groups of) micro-organisms able to perform a specific proces, for example the nitrification of NH4+.

On the basis of all data which were available it must be concluded that elevated copper levels mostly have an inhibitory effect on microbe-mediated soil processes (respiration, mineralization, ammonification, nitrification, enzyme activities, litter decomposition). The data show a

high variability which often cannot be explained on the basis of the most common descriptors of the soils. However, in most cases the lowest concentrations of metals causing measurable effects on soil processes were found in sandy soils and the highest in clay soils and in organic soils (Doelman, 1985).

There is also an influence of elevated levels of copper and/or other metals on number and diversity of soil micro-organisms (bacteria, including actinomycetes, and fungi), but data are scarce compared with those on soil-processes. Within the group of bacteria Gram-negatives are more resistent than Gram-positives; the ecological consequences of the shift towards Gram-negatives resulting from elevated metal levels are not known. Fungi are more resistant than bacteria. In general the total number of micro-organisms decreases at higher metal levels and there will be a shift from prokaryotes (bacteria) to eukaryotes (fungi). This shift may lead to both a qualitative and quantitative change in metabolic activity, since the metabolic activities of eukaryotes are less diverse and their growth and reproduction rates are lower. Ultimately this may result in a visible accumulation of organic matter (Doelman, 1985).

The lowest added copper concentration (as copper sulfate) which has been found to have an effect on soil micro-organisms and soil processes (reduced bacterial growth and soil ATP content, shift in fungi genera;) is 11 mg $Cu.kg^{-1}$, after an exposure of 10 weeks (Zibilske and Wagner, 1982). In some studies reviewed by Doelman (1985) inhibition of soil respiration, nitrogen mineralization and nitrification did not occur below a copper level of 100 $mg.kg^{-1}$; between 100 and 1,000 $mg.kg^{-1}$ the results were inconsistent and above this level inhibition always occurred.

Invertebrates

Most toxicity studies have been conducted with earthworms. The available results of laboratory tests are summarised in Table 2.9. If more sublethal effects (cocoon production, growth, litter breakdown) have been studied in one test, only the toxicity value of the most sensitive effect has been listed.

Ma extensively studied the toxicity of copper to L. rubellus (Ma, 1982, 1983a, 1984).

In laboratory tests with exposure times of ≥ 6 weeks all NOLC-values for L.rubellus were similar, with lowest values of 131-165 $mg.kg^{-1}$ soil d.w.; regarding this value, no difference was found between juveniles and adults or between exposure times of 6 weeks and 7 months. Long-term sublethal

parameters (cocoon production, growth) are affected at lower levels (35-60 $mg.kg^{-1}$ d.w.). Regarding the most sensitive sublethal parameters in comparative tests in a loamy sand and a sandy loam, copper was more toxic in the latter, despite a higher clay content; on the basis of accumulation studies mentioned earlier the opposite was expected. This discrepancy may be explained by the higher (1.7 times) organic matter content in the loamy sand. Although the pH did not seem to influence the accumulation of copper, adjustment of this parameter from 5 to 7 in the loamy sand eliminated the adverse effect (Ma, 1984). A comparable result was found for Dendrobaena rubida (Gunnarsson and Rundgren, 1986).

In experiments with Octolasium cyaneum in different soils the 4-d LC50-value showed the highest correlation with the carbon content and a lower correlation with both nitrogen and calcium levels; there was no correlation with pH or C/N-ratio (Jäggy and Streit, 1982).

In solutions of copper chloride the 4-d LC50-value for juvenile L. rubellus was 0.3 mg $Cu.l^{-1}$; this value was about threshold.

From the data by Ma (1983a, 1984) copper chloride seems somewhat more toxic than copper sulfate. However, in laboratory experiments in which the effect of different copper salts (nitrate, chloride, sulfate, acetate, carbonate and oxide) on Eisenia foetida was tested in two soils covered with either enriched manure or enriched activated sludge for 8 weeks, the relative toxicity of the different salts was not consistent. Only the insoluble copper oxide was much less toxic than the other, all well soluble copper salts (Hartenstein et al, 1981; Malecki et al., 1982).

The few laboratory tests with other earthworm species than L.rubellus do not indicate a higher sensitivity to copper for these species.

In field experiments by Ma (1983a) unspecified sandy soils were treated with CuS, CuSO4 or sewage sludge for 2 or 4 years, and worms were collected 3 or 4 years after the last treatment. All treatments resulted in a reduction of the number of collected worms (mainly L. rubellus in the copper treated soil and both L. rubellus and A. caliginosa in the sewage sludge treated soil). The numbers of L. rubellus decreased with 45-65% at soil copper levels of 135-154 $mg.kg^{-1}$ d.w. and with 35% at 69 $mg.kg^{-1}$ d.w. In the sewage sludge treated soil A. caliginosa was slightly more reduced than L. rubellus at elevated copper levels (69-265 $mg.kg^{-1}$ d.w.). The much more soluble CuSO4 seemed to have a greater effect than CuS, but the number of observations was too small for statistics (Ma, 1983a).

Other field data show that soil treatment with copper-fungicides or animal slurry can lead to a lower density of earthworms (resulting in a lower rate

of litter breakdown) and/or changes in the composition of earthworm populations, due to differences in tolerance; these effects have been found at soil copper levels in the range of about 50 - 150 $mg.kg^{-1}$ (Nielsen, 1951; Van Rhee, 1977; Niklas and Kennel, 1978; Eijsackers, 1981). Vossen (1983) found no effect on the total number of adult A. caliginosa in plots (sandy loam, 17% clay, 8% OM, pH 8) treated with copper chloride after an exposure of 3 months to nominal levels of 55 and 150 $mg.kg^{-1}$ d.w., but a significant part of these adults had lost the ability to reproduce. Due to other factors (e.g. the presence of other metals, species-specific sensibility or a specific biotope it is not always possible to establish a relationship between the copper level in the soil and the number of worms in the field (Van Rhee, 1977; Marinissen, 1982; Bengtsson et al., 1983).
The data resulting from laboratory tests are in good agreement with those from the field. The lowest long-term toxic levels are in the range from 35 to 50 $mg.kg^{-1}$ d.w.

Data on soil copper levels which are detrimental to other invertebrates are lacking.
Freedman and Hutchinson (1980) found a trend of decreased numbers of Collembola (springtails) and Acarina (mites) nearest to a nickel-copper smelter but the data were not consistent. Insects appeared not to be affected. Bengtsson et al. (1985) reported lower population densities of Collembola species near a brass mill, at a soil copper level of 400 $mg.kg^{-1}$. In a study with several groups of invertebrates kept on conifer litter enriched with copper, springtails and mites were less sensitive than earthworms (Heungens, 1970).

Other organisms

Data on copper toxicity to other animals living in the terrestrial environment are lacking.
Most data on plants deal with agricultural crops (see chapter 3). Soil copper levels of about 6-30 $mg.kg^{-1}$ wet weight are essential for "normal" plant growth and development. Toxic levels range from 25 to 50 $mg.kg^{-1}$ for the most sensitive plants, and from 150 to 400 $mg.kg^{-1}$ for most others (NRC, 1977; Demayo et al., 1982). Some plants tolerate much higher copper levels (Hughes et al., 1980). According to these autors dramatic alterations in plant productivity and species diversity of natural populations exposed to metals are rare.

According to Lexmond et al. (1982) the toxicity of copper to plants depends primarily on the copper-ion activity, which is reduced at higher pH and higher organic matter content. Clay content and CEC are less important in determining the copper toxicity.

Table 2.9. Toxicity values for earthworms (laboratory tests)

Organism	Test subst. / Exp. time	Soil / Criterion	Result (mg Cu.kg^{-1} soil d.w.)	Ref.
Short-term (up to 2 weeks)				
Eisenia foetida	Cu-sulfate < 1-w	LC100 (in sludge)	≤2,500 (d.w.?)	1
Eisenia foetida	Cu-oxychloride 2-w	LC50 (in soil)	536-637	2
Eisenia foetida	Cu-nitrate 2-w	LC50 (in soil)	217	3
Lumbricus terrestris	Cu-oxychloride 2-w	LC50 (in soil)	58-69	4
Octolasium cyaneum	Cu-sulfate 4-d	LC50 (in different soils)	180-2,600	5
Long-term				
Dendrobaena rubida adults	Cu-nitrate 4-m **pH 5.5 or 6.5:** **pH 4.5:**	Sandy soil, mixed with cow dung; 8-12% OM, pH = 4.5, 5.5 or 6.5 EC(100) (no cocoon production) NO(A)EC β **EC(90)** (red. cocoon production)	 500 100 **100**	7
Eisenia foetida	Cu-sulfate 4-m	Activated sludge **NOEC** (E not specified)	 **1,500**	1
Lumbricus rubellus adults	Cu-chloride 6-w	Sandy loam-17% clay, 3.4% OM, pH 7 NOLC **NO(A)EC** β (cocoon prod.) Loamy sand-2% clay, 5.7% OM, pH 5 NOLC **NOEC** (cocoon production)	 136 α **30** α 131 α **54** α	8,9
Lumbricus rubellus adults	Cu-sulfate 6-w **All pH values:** **pH 6 or 7:** **pH 5:**	Loamy sand-2% clay, 5.7% OM, pH 5 (native), 6 or 7 (adjusted) NOLC NOEC (coc. prod., growth) **NOEC** (growth)	 278 α 278 α **148** α	9
Lumbricus rubellus adults	Cu-chloride 6-w 3-m	Sandy loam-17% clay, 8% OM, pH 7 LC(50), **NOLC** 1,000, LC(50), **NOLC** < 1,000,	 **150** α **150** α	10
Lumbricus rubellus juveniles	Cu-chloride 3-m	Sandy loam-17% clay, 8% OM, pH 8 NOLC **EC** (sexual devel. & growth inh.)	 60 α **60** α	6
Lumbricus rubellus adults	Cu-chloride ? 3-m	Sandy loam-no data soil parameters NOLC **EC(70)** (red. cocoon production)	 165 α **35** α	8

Lumbricus rubellus juveniles	Cu-chloride 7-m	Sandy loam-no data soil parameters		8
		NOLC	145 α	
		EC(50)	145 α	
		(growth inhibition)		
Octolasium cyaneum	Cu-sulfate 1-m	"Brown", 5.4% OM, pH 4.2-4.8		5
		NOLC	100 α	
		Peat, 72% OM, pH 4.5		
		NOLC	1,200 α	

α) Actual concentration
β) Higher cocoon production compared with unenriched (without copper added) soil
EC) Effective-concentration
NOEC) No-observed-effect0concentration
NOLC) No-observed-lethal-concentration

References:

1) Hartenstein et al., 1980
2) Haque and Ebing, 1983
3) Heimbach, 1985
4) Neuhauser, 1985
5) Jäggy and Streit, 1982
6) Vossen, 1983
7) Gunnarsson and Rundgren, 1986
8) Ma, 1983a
9) Ma, 1984
10) Ma, 1982

3 AGRICULTURAL CROPS AND LIVESTOCK

3.1 Agricultural crops

3.1.1 Requirements

In the Netherlands a minimum soil copper level of 4-5 mg HNO3-Cu.kg^{-1} (d.w.) has been recommended for arable soils, to prevent adverse effects on "normal" growth and development of crops due to deficiency. For grassland this level is 5 mg.kg^{-1} d.w., primarily based on the copper requirement of livestock (Henkens, 1975; Van Luit, 1975; CCRX, 1986). HNO3-Cu is the amount of copper extractable with 0.43 M HNO3, expressed as mg Cu.kg^{-1} soil, on a dry weight basis. Lexmond et al. (1982) found HNO3-Cu to represent in general 60-80% of total copper levels in soils (HNO3-Cu = 0,6-0,8 x total copper); in some soils lower values (33-55%) were found. Most soils which were analysed, were sandy soils. After addition of soluble copper salts 90-100% of total copper is extractable in this way. Copper not extractable with diluted nitric acid is considered to be not directly available for plants.

3.1.2 Accumulation

On arable land most crops remove up to about 80 g Cu.ha^{-1}.yr^{-1} from the soil; 50 g Cu.ha^{-1}.yr^{-1} is considered as the average. On grassland the net amount of copper removed is only about 15 g.ha^{-1}.yr^{-1} because most of the copper taken up will return to the soil via the manure (Henkens, 1975; Lexmond et al., 1982; SCAN, 1984). No data on soil copper levels are given, but these data are probably valid for unpolluted soils.
In pot experiments with food crops grown on four harbour sediments with total copper levels of 103-233 mg.kg^{-1} d.w., the following copper levels (in mg.kg^{-1} fresh weight) were found in edible parts of mature crops: potato 2.2-4.9, carrot 1.0-1.1; radish 0.4-0.5 and lettuce 0.8-1.0; there was no apparent relationship with the substrate copper level. In one sediment the level in potatoes was exceeding the current residu limit of 3 mg.kg^{-1} fresh weight, valid in law in the Netherlands; that in potatoes grown on the other sediments was below this limit. The level in the other vegetables was well below the residu limit of 20 mg.kg^{-1} fresh weight for

these crops (see also chapter 1 and 5, basis document). In the edible parts of the crops grown on these polluted sediments 1.6-2.4 times more copper was accumulated compared with the same crops grown on reference soils containing 12-14 mg $Cu.kg^{-1}$. When the same crops were grown on a clay soil (31 mg $Cu.kg^{-1}$ d.w.) or on different mixtures of this soil with up to 30% dredged sediment, resulting in total copper levels of 45-74 $mg.kg^{-1}$ d.w., the crops accumulated more copper at higher substrate concentrations, but the increase in the copper level was ≤ 30%. In both pot experiments the highest copper levels in food crops were found in leafy vegetables (Smilde et al., 1982). In another pot experiment the copper level of lettuce, radish and spring wheat grown on other contaminated sediments (112-269 $mg.kg^{-1}$ d.w., total copper) also stayed well below the current standard of 20 $mg.kg^{-1}$ (Van Driel et al., 1985).

Potatoes grown on 3 sandy soils and 3 clay soils (average background levels 22 and 32 mg $Cu.kg^{-1}$ d.w., respectively) which were treated for many years with municipal waste compost (resulting in average levels of about 70 and 100 mg $Cu.kg^{-1}$ d.w. in sandy soils and clay soils, respectively), accumulated significantly more copper than potatoes grown at background levels, but the increase did not exceed 30%. Potatoes grown on all enriched soils contained an average level of 1.3 mg $Cu.kg^{-1}$ fresh weight; potatoes grown at background levels contained 1.0 to 1.1 mg $Cu.kg^{-1}$ fresh weight. Potatoes grown on dewatered sewage sludges with 197 to 3,422 mg $Cu.kg^{-1}$ d.w. contained 1.4 to 2.2 mg $Cu.kg^{-1}$ d.w.; there was no relationship between sludge and potato levels. In these and other experiments using soils mixed with liquid or dewatered sewage sludge, or contaminated fluvial sediments containing up to 206 mg $Cu.kg^{-1}$ d.w., the level in potatoes grown in 140 litre vessels always stayed well below the current standard of 3 mg $Cu.kg^{-1}$ fresh weight. Based on the results of the experiments mentioned before and data from the literature reviewed by De Haan and Lubbers potato (tubers) copper levels of 1.3 and 2.5 mg $Cu.kg^{-1}$ fresh weight are considered to be "normal" and "(too) high", respectively, by these authors. The leaves of potatoes grown on the contaminated sediments contained about 20 mg $Cu.kg^{-1}$ d.w., a level 2 to 3 times above background (De Haan and Lubbers, 1983).

In pot experiments grass grown on the harbour sediments mentioned before contained 18-23 mg $Cu.kg^{-1}$ d.w., which is somewhat higher than the current maximum level (15 $mg.kg^{-1}$, on a 12% moisture basis) in complete feed for sheep (see chapter 1, basis document). In other pot experiments the level in grass grown on the mixtures of the clay soil and the dredged sediment (see above) remained in the first growing season below 15 $mg.kg^{-1}$ d.w. when

the substrate level was not exceeding 50 mg.kg^{-1} d.w. In the second growing season the grass from the first cut grown on the soil contained 15 mg Cu.kg^{-1} d.w. and that grown on the mixtures contained up to 23 mg Cu.kg^{-1} d.w.; the level in grass from the second cut was always below 15 mg Cu.kg^{-1} d.w. (Smilde et al., 1982). In grass grown on other sediments (pot experiment) contaminated with heavy metals (112-269 mg.kg^{-1} d.w. total copper) copper levels were ranging from 11-20 mg.kg^{-1} d.w.; the highest level was always found in grass from the first cut. These copper levels were hardly increased (10%-23%) compared to those in grass grown on sediment with a copper level of 26 mg.kg^{-1} in which levels ranged from 11 mg Cu.kg^{-1} d.w. (grass from the 4th cut) to 17 mg Cu.kg^{-1} (grass from the 1st cut (Van Driel et al., 1985). In a field experiment the level in grass, grown on a sandy soil with top layer copper levels of 60-208 mg.kg^{-1} d.w. due to a 5-year treatment with sewage sludge, increased about 2 times (from 7 to 15 mg Cu.kg^{-1} d.w.) compared with that in grass grown on the same soil without treatment (soil copper level 5 mg.kg^{-1} d.w.). Generally the grass level was somewhat increasing with increasing N-gift, at fixed soil copper levels. The level decreased with increasing age (Hemkes and Kemp, 1983). In grass grown under high-tension cables of copper (soil HNO3-Cu levels 7 to 59 mg.kg^{-1} d.w.) at 11 locations, copper levels did not exceed 15 mg.kg^{-1} d.w. in most cases, neither in may nor in september. Only at one location (soil HNO3-Cu level 28 mg.kg^{-1} d.w.) the grass level was exceeding 20 mg.kg^{-1} d.w. According to the authors there is no relationship between grass and soil copper levels when soil HNO3-Cu exceeds 5 mg.kg^{-1}. In this case the grass copper level will probably be the result of both soil copper accumulated by the grass and the deposition on the grass of airborne copper (Hemkes and Hartmans, 1973).

Based on the results of these and other experiments (see CCRX, 1986) it must be concluded that most crops accumulate more copper when grown on substrates with elevated copper levels, but the results strongly depend on plant species and substrate parameters. In many crops the maximum increase is about two-fold.

Most copper is accumulated in the roots of crops (Smilde, 1976; Graham, 1981).

In a pot experiment using 3 clay soils (clay content 12%, 40% and 58%, respectively; % OM 1.6-3.2) and 3 sandy soils (OM content 3.4%, 6.8% and 19.4%, respectively; % clay 4-5) oat was grown at supplemented copper levels of 25 to 400 mg Cu.kg^{-1} d.w., added as cupric acetate. For the green crop a significantly ($p < 0.05$) negative relationship was found between the transfer coefficients (c_t = ratio increase in concentration in crop /

increase in concentration in soil) and soil CEC. In the sandy soils the accumulation was increasing with lower OM content; In the clay soils the highest accumulation was found in the soil with the highest clay content (possibly due to the high background level of 58 mg Cu.kg^{-1} d.w.), but the differences between the 3 soils were small (De Haan et al., 1985).

3.1.3 Toxicity

In a pot experiment a yield reduction of at least 25% was found in agricultural crops (maize, sugar beet, spinach, common bean) grown on a sandy soil (OM 2.5%; pH-KCl 4.8) enriched with copper, resulting in a soil HNO3-Cu level of 82 mg.kg^{-1}. At a level of 67 mg.kg^{-1} only spinach productivity was clearly reduced. It is not reported in which form copper was added (Henkens, 1975). A number of data on agricultural crops including grass reviewed by Henkens (1975) and Smilde (1976) indicate toxic effects at (HNO3-)Cu levels of about ≥ 80 mg.kg^{-1} for most crops. According to Henkens (1975) Leguminosae are adversely affected at HNO3-Cu levels of 20-40 mg.kg^{-1}. In a 2-year field experiment with snapbeans (Phaseolus vulgaris) grown on a loamy sand (0.7% OM; pH 6.7) treated with cupric sulfate or cupric chloride in the first year before planting, a significant yield reduction at the 10% level of probability was found (based on the regression equation between yield and extractable soil copper) when soil EDTA-Cu was about 20 mg.kg^{-1} d.w. In the second year the yield reduction was not lower compared with the first year, so the toxicity of copper was not reduced over this period of time (Walsh et al., 1972). Analyses of soils in the Netherlands resulted in the equation EDTA-Cu = 0.8 x HNO3-Cu (Henkens, 1975). Based on this equation the aforementioned threshold level of 20 mg EDTA-Cu (from Walsh et al., 1972) is corresponding with a HNO3-Cu level of about 25 mg.kg^{-1} d.w. According to the relation between HNO3-Cu and total copper found in sandy soils in the Netherlands by Lexmond et al. (1982), this HNO3-Cu level of 25 mg.kg^{-1} corresponds with a total copper level of 30-40 mg.kg^{-1}.

In acidic sandy soils (pH 4-5) a 1 N ammoniumacetate-extractable Cu level of 25 to 50 mg.kg^{-1} may cause damage to the most sensitive crops. In clay soils sensitive crops are affected at about 100 mg Cu.kg^{-1} and upwards (Smilde, 1976; citations).

In a pot experiment with food crops (potato, carrot, radish, lettuce, spring weat) and grass grown on reference soils (one loam and one clay soil) and harbour sediments containing total copper levels of 12-14 and

103-233 mg.kg^{-1} d.w., respectively, no yield reduction was found at higher copper levels. In another pot experiment in a clay soil (31 mg.kg^{-1}) and mixtures of this soil with dredged sediment (resulting in total copper levels of 45-74 mg.kg^{-1}) no adverse effect on yield of potato, carrot, lettuce, spring barley and grass was found; only the yield of radish and endive was somewhat (13% and 17%, respectively) reduced at the highest copper level (Smilde et al., 1982). In a series of pot experiments in fluvial clay soils (reclaimed or dredged sediments) with comparable soil texture (5-10% OM; 27-31% clay) the yield of food crops (lettuce, spring wheat) and grass was not related to sediment total copper levels which were 26 and 112-269 mg.kg^{-1} d.w. in the control sediment and the contaminated sediments, respectively. The yield of radish was reduced in the four contaminated sediments, but this may (in part) be the result of the presence of elevated levels of other metals in these sediments, for example cadmium which was present in concentrations of 0.6 and 5-41 mg.kg^{-1} in control and contaminated sediments, respectively (Van Driel et al., 1985).
In a pot experiment using 3 clay soils (1.6%-2.4%-3.2% OM, 12%-40%-58% clay, CEC 15-21-33 meq.100 g^{-1} d.w., respectively) and 3 sandy soils (3.4%-6.8%-19.4% OM, 4%-5%-4% clay, CEC 9-19-47, respectively) there was no yield reduction of oat with a copper supplement (as copper acetate) of 200 mg Cu.kg^{-1} d.w.; at the highest supplement of 400 mg Cu.kg^{-1} d.w. grain and straw yields were reduced except in the sandy soil with the highest OM content. A significantly ($p < 0.05$) negative relationship was found between maximum copper application rates without yield reduction and soil CEC. In sandy soils the yield reduction at the highest copper concentration was decreasing with increasing OM content, consistent with the accumulation data. In the clay soil yield and accumulation were little dependent on clay content (De Haan et al., 1985).
Several authors consider plant tissue levels between 14 and 25 mg.kg^{-1} d.w. to be the threshold concentration in agricultural crops; above this threshold level crop yield will be reduced (Demayo et al., 1982).
Based on own research and some literature data Lexmond et al. (1982) consider the HNO3-Cu/C-ratio, in dependance of the pH, a suitable parameter to establish toxic levels in soils. A HNO3-Cu/C-ratio of 2-3 is considered to be safe for all crops. In fluvial clay soils and sandy soils this ratio corresponds with copper levels of 40-60 and 50-70 mg.kg^{-1}, respectively (CCRX, 1986). For more details, see basis document, chapter 3.

3.2. Livestock

3.2.1 Requirements and current maximum allowable feed copper levels

Mammals (cattle, sheep, pigs) require approximately 4 to 10 mg Cu.kg^{-1} feed on a dry weight basis for a "normal" health. The precise quantity is depending both on animal and diet factors of which trace metal content and type and quantity of proteins strongly can influence the copper status (Hill, 1977b; Hansard, 1983; SCAN, 1984; Vreman, 1986).
In the Netherlands and other countries of the European Communities the maximum allowable total copper concentration in complete feed for these animals is ranging from 15 mg.kg^{-1} for sheep to 50 mg.kg^{-1} for calves, with exception of feed for piglets which may contain up to 175 mg.kg^{-1} (Schumer, 1986; see also basis document, chapter 1).
For poultry a requirement of 4 mg Cu.kg^{-1} feed has been estimated by the National Research Council of America (Jenkins et al., 1970). The maximum allowable concentration for these animals is 35 mg.kg^{-1} feed (Schumer, 1986; basis document, chapter 1).

3.2.2 Accumulation

In ruminants especially molybdenum and sulfur/sulfate reduce the accumulation of copper; in pigs high molybdenum and sulfate levels don't or hardly influence the copper status when fed a diet containing up to 500 mg Cu.kg^{-1} feed (Kline et al., 1971; Hill, 1977a). In pigs zinc and iron reduce the retention of supplementary copper in the liver (Suttle and Mills, 1966).
With the exception of suckling animals which can absorb 50% or more of the copper intake when fed an (artificial) milk diet, the absorption is generally below 10% (JECFA, 1982; CCRX, 1986). Cows held on river forelands with soil levels ranging from 30 to 170 mg.kg^{-1} did not show different meat levels, but average grass levels only ranged from 13 to 18 mg.kg^{-1}. In all animals most copper (up to 90%) is accumulated in the liver (CCRX, 1986).
In growing pigs a dietary copper level of 250 or 500 mg Cu.kg^{-1} feed during 8 weeks did not result in a significantly higher meat (loin) copper level; liver copper levels increased about 4-fold and 100-fold, respectively (Kline et al., 1971).

3.2.3 Toxicity

Toxicity data on livestock have been reviewed in several publications, for example in Hill (1977a,b), JECFA (1982), SCAN (1984) and EPA (1985a). Most data concern sheep and pigs.

Sheep are the most sensitive animals, at least on the basis of feed content; copper levels as low as 20-27 mg $Cu.kg^{-1}$ feed (~0.8-1.1 mg $Cu.kg^{-1}$ $bw.day^{-1}$) may already lead to toxic effects, including mortality. In a study in which lambs (127 days old) were fed diets with 0 or 15 mg added $Cu.kg^{-1}$ feed (actual concentrations 14-16 and 29-33 mg $Cu.kg^{-1}$, respectively) 2 of 4 exposed animals died when no addition of sulfate and molybdenum was given; with addition of these elements there was no mortality after an exposure time of 88 days (Kline et al., 1971). Based on data of "Animal Health Services" in the Netherlands a copper level in concentrates of 15 $mg.kg^{-1}$ d.w. is considered to be safe for sheep; a concentrate level of $\geq$ 20 $mg.kg^{-1}$ d.w. may be hazardous to these animals (Van Ulsen, 1972; Hartmans, 1975). According to Hill (1977b) maximum levels of 8 to 20 $mg.kg^{-1}$ have been recommended in concentrate diets for growing sheep. Also grass and soil levels of 15 $mg.kg^{-1}$ d.w. are considered to be safe for sheep (Hemkes and Hartmans, 1973). According to data reviewed by McDowell (1985) grazing sheep ingest approximately up to 200 g soil per day, corresponding with 14% of the daily dry matter intake or 2% of the daily intake on a fresh weight basis, in periods of scarcity of herbage. So, if a feed level of 15 $mg.kg^{-1}$ is considered to be safe, a soil level which is somewhat higher is not expected to cause adverse effects via the intake of this soil. Sheep of the Texel breed are especially sensitive, consistent with more accumulation of copper in these animals compared with other breeds (Hill, 1977b). Non-acute copper poisoning in sheep occurs in two phases: at first a pre-hemolytic phase characterized by the accumulation of copper in liver and other organs, but no significant clinical signs, followed by a hemolytic crisis after about four or more weeks. The hemolytic crisis is often fatal; if not the proces will be repeated at continued exposure (JECFA, 1982).

According to an estimation of Hansard (1983) cattle can tolerate a feed copper level of 70-100 $mg.kg^{-1}$ (~1.1-1.5 and ~2.1-3.0 mg $Cu.kg^{-1}$ $bw.day^{-1}$ in maintenance and fattening cattle, respectively) over an extended period. According to Rosenberger (1970; cited in Smilde, 1976) chronic intoxications may be expected at a feed copper level of 50-80 $mg.kg^{-1}$ (~0.8-1.2 and 1.5-2.4 mg $Cu.kg^{-1}$ $bw.day^{-1}$. This statement is based on a daily feed intake of 15 kg d.w. per animal. Calves can tolerate several

hundred mg copper per kg diet, but when given milk substitutes 50 mg $Cu.l^{-1}$ is already toxic, (Hill, 1977b).

For pigs feed levels of up to 270 mg $Cu.kg^{-1}$ (~11-13.5 mg $Cu.kg^{-1}$ $bw.day^{-1}$) have been reported to increase weight gain and to improve feed conversion. A statistical analysis of data in 129 publications indicate an optimum level of 224 mg $Cu.kg^{-1}$ feed (~9-11 mg $Cu.kg^{-1}$ $bw.day^{-1}$) (SCAN, 1984). The effect of copper is strongly influenced by the diet. For example, a copper supplement of 600 $mg.kg^{-1}$ feed (as basic cupric carbonate, ~24-30 mg $Cu.kg^{-1}$ $bw.day^{-1}$) caused severe toxic effects in 4 of 6 weanling female pigs fed a maize-fish meal-diet, but was hardly toxic in a maize-soya bean-diet or a maize-dried skim milk-diet, in a 7-w study. In the maize-fish meal-diet a copper (as cupric sulfate) supplement of 425 $mg.kg^{-1}$ feed (~17-21 mg $Cu.kg^{-1}$ $bw.day^{-1}$) for 11 weeks resulted in a significantly higher growth rate, food consumption and food conversion efficiency and no signs of toxicity when also a supplement of zinc and iron was given; without additional zinc and iron a dietary copper (as cupric sulfate) level of 250 $mg.kg^{-1}$ (~10-12.5 mg $Cu.kg^{-1}$ $bw.day^{-1}$) resulted in jaundice in 3 of 6 exposed pigs, and to increased aspartate transaminase and copper levels in serum; In the latter case no stimulation of growth compared with unexposed pigs was found at termination (Suttle and Mills, 1966). A level of 250 mg $Cu.kg^{-1}$ feed even has been reported to cause severe anemia in pigs, when the animals were fed a semi-purified diet (Hill, 1977b). In feeding studies with growing pigs (exposure time 54-88 days) neither an adverse effect on growth nor effects on hemoglobin and hematocrit levels or on plasma and loin copper levels were found at copper (as cupric sulfate) levels up to 250 $mg.kg^{-1}$ feed (~10-12.5 mg $Cu.kg^{-1}$ $bw.day^{-1}$); a feed copper level of 500 $mg.kg^{-1}$ reduced weight gain, also when supplementary molybdenum (up to 200 $mg.kg^{-1}$ feed) together with sulfate (1,000 $mg.kg^{-1}$ feed) was given (Kline et al., 1971). In general weight gain may be reduced but no adverse effects are observed in pigs fed conventional diets containing up to 500 mg $Cu.kg^{-1}$ (Kline et al., 1971; Hill, 1977a,b).

In chickens copper supplements of up to about 200 $mg.kg^{-1}$ feed usually produce no adverse effects; the optimum level for growth promotion and feed conversion is about 150 mg $Cu.kg^{-1}$. Supplements of 250 mg $Cu.kg^{-1}$ can promote or reduce weight gain, dependent on the diet used. Feed copper levels above 200-250 $mg.kg^{-1}$ mostly reduce feed intake and weight gain, and can also lead to other adverse effects, for example to lesions of gizzard lining (Jenkins et al., 1970; Fisher et al., 1973; Hill, 1977b; Jackson et al., 1979). As in pigs copper is more toxic in a semi-purified diet (depression of weight gain at addition of only 80 mg $Cu.kg^{-1}$ feed) compared

with a conventional diet, probably due to differences in complexing agents content.

Based on the administration of single doses of either one crystal of copper sulfate or copper carbonate powder, pigeons seem somewhat less and ducks somewhat more sensitive compared with chickens, but the differences which have been found, are small (Pullar, 1940).

4 SUMMARY AND CONCLUSIONS

4.1 Human toxicity

Copper is an essential element for all organisms. In mammals it plays a role in a number of physiological processes, for example oxidative reactions, the hematopoietic process, bone development and carbohydrate metabolism. The chapter on "human toxicity" is focussed on oral exposure, because this is the main route of exposure for the general population.

4.1.1 Animals

Chemobiokinetics and metabolism

After oral intake the absorption of copper is depending on a variety of both animal and dietary factors. In newborns the highest rate of absorption (up to 100%) has been found. Once absorbed copper is predominantly bound to albumin and transported mainly to the liver, the key organ in copper metabolism, from where the largest fraction is excreted directly via the bile into the feces. The remaining part is bound initially to a metallothionein(-like) protein, followed by the formation of ceruloplasmin (ferroxidase I) and, to a lesser extent, of other cuproproteins. The very stable ceruloplasmin can enter the blood and transport copper to other tissues.

Toxicity

The lowest reported acute oral LD50-values are 15, 66-82, and 90 mg $Cu.kg^{-1}$ bw for Guinea pigs, rats, and mice and rabbits, respectively. These values are all from tests with inorganic Cu(II)-salts.
In rats a level of (about) 25 mg $Cu.kg^{-1}$ bw resulted in toxic effects after subacute or subchronic oral exposure to copper gluconate or copper sulfate. In a feeding study (33-41 weeks) with rabbits an oral dose of about 44 mg $Cu.kg^{-1}$ $bw.day^{-1}$ (as copper acetate) resulted in hemochromatosis and liver cirrhosis. No subchronic oral studies with lower doses were available with regard to these animals.

In a one year study with beagle dogs the highest dietary level of copper gluconate (corresponding with about 8 mg $Cu.kg^{-1}$ $bw.day^{-1}$) did not cause compound related deaths or pathological lesions. In rabbits, orally dosed every second day with a cupric sulfate solution (~12 mg $Cu.kg^{-1}$ bw) during 16 months, hepatic damage (cirrhosis-like) was found.
In the only available chronic toxicity study (Sprague-Dawley rats, exposure time 2 years) the highest dietary level of "potassium sodium copper chlorophyllin" (corresponding with 80-160 mg $Cu.kg^{-1}$ $bw.day^{-1}$) was without effect, so this copper compound was less toxic than compounds tested in short-term studies.
Copper compounds have been tested for genotoxic and mutagenic effects in a variety of tests. Because of the diverse results - even with the same copper compound - the mutagenic potency of copper is equivocal.
Only one specific oral carcinogenicity study was available. In this study with male and female mice copper hydroxyquinoline did not result in carcinogenic effects. Based on this and other (less specific) studies, in which copper was administered orally or by injection, copper is not considered to be carcinogenic to animals.
In oral embryotoxicity/teratogenicity studies with mice and rats the highest dose of 4 mg $Cu.kg^{-1}$ $bw.day^{-1}$ (as copper gluconate) did not affect implantation data and did not result in embryotoxic or teratogenic effects. In a study in which mature female mice received copper sulfate in the diet from 1 month prior to mating up to termination on day 19 of gestation, skeletal malformations were only found at the two highest doses (about 155 and 207 mg $Cu.kg^{-1}$ $bw.day^{-1}$), which also led to increased fetal mortality and decreased litter sizes. In *in vivo* tests with single i.p. or i.v. injections during gestation, copper compounds have been found to be embryotoxic to rats and to be teratogenic (resulting in skeletal and cardiovascular malformations) to hamsters at embryotoxic concentrations.
Also in *in vitro* tests with preimplantation mouse embryos and developing chick embryos copper compounds were found to be embryotoxic and/or teratogenic. Copper from IUDs is able to prevent implantation and blastocyst development in experimental mammals, but insertion of a copper-IUD after the implantation process has no effects.

4.1.2 Humans

Chemobiokinetics and metabolism

Chemobiokinetics and metabolism in humans are essentially the same as in other mammals. Mean values of gastrointestinal absorption of copper are reported in the range from 25% to 65%, but individual values show a greater variability. Newborns and infants have a relative high rate of absorption. Absorption can also occur after exposure to airborn copper or after skin contact with metallic copper or copper compounds. Also in humans fecal excretion is usually by far the most important route to 'eliminate copper, but in a hot and humid environment sweat can contribute substantially to copper excretion.
The daily requirement of copper for humans is estimated to be 0.025-0.1 $mg.kg^{-1}$ bw, dependent on age. For adults a daily amount of 2 to 3 mg (0.03-0.05 $mg.kg^{-1}$ $bw.day^{-1}$) is widely accepted to be adequate, for example by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1982). There are also data which indicate that approximately 1 mg (0.02 $mg.kg^{-1}$ $bw.day^{-1}$) is sufficient to meet the daily requirement for adults.
Estimates of whole body copper contents in adults range between 50 and 150 mg.

Toxicity

Cases of acute copper poisoning have already been reported at doses of about 0.1 mg $Cu.kg^{-1}$ bw, after ingestion of copper containing drinks (a cocktail, tea). The lethal oral dose for adults is 50-500 mg copper(II)salt per kg bw. Data on effects of ingested copper in healthy persons after long-term exposure are hardly available.
Human data on the carcinogenic or teratogenic potency of copper after ingestion were not available but based on animal data humans are not likely at risk with regard to these effects.
Allergic reactions have been ascribed to copper, for example due to the use of a copper IUD and ingestion of, or skin contact with copper salts, but the number of reported cases is very low.

In the work environment short-term exposure to copper fumes, mists, or dusts occasionally results in the occurrence of an influenza-like syndrome called "metal fume fever" or more specific "copper fume fever" or "brass chill". Mild symptoms of this syndrome have already been reported at a

copper dust concentration of 0.1 to 0.3 $mg.m^{-3}$, well below the occupational standard for copper dust (1 $mg.m^{-3}$) in the Netherlands. Despite its widespread use in industry, copper intoxications appear to be of little concern with regard to health hazards, even at long-term exposure.
An occupational disease called "vineyard sprayers lung" has been attributed to the inhalation of "Bordeaux mixture", a solution of basic copper sulfate neutralized by lime. In patients with this disease a variety of lung and liver lesions has been found which may be lethal. The use of Bordeaux mixture is not allowed in the Netherlands. However, similar lesions as found in patients exposed to Bordeaux mixture have also been reproduced in Guinea pigs exposed to copper oxychloride, the use of which is allowed in the Netherlands.

4.2 Ecotoxicity

4.2.1 Aquatic organisms

Accumulation

Copper is highly accumulated by a number of organisms of different trophic levels: algae, macrophytes, annelids, molluscs, crustaceans and insects, with bioconcentration factors (BCF = concentration in the organism : concentration in the water) in excess of 1,000. Based on BCF-values and the persistence of copper, a high degree of accumulation is expected to occur in the aquatic environment, at least in invertebrates. In fish the bioaccumulation of copper is much lower.
Data on the accumulation of copper in foodchains are limited, but biomagnification only appears to be of any significance at lower trophic levels; in the case of fish no biomagnification has been reported.
Much effort has been made to correlate copper levels in organisms with environmental water and/or sediment levels. In many cases no correlation could be established, so the copper level in organisms cannot be simply predicted from copper levels in abiotic components of the environment, and conversely. In general, the level in organisms appears to be more related with the "soluble" (< 0.45 μm filter) copper fraction of the water than with the "particulate" (> 0.45 μm filter) copper fraction of the water or with the total copper level of water and sediment. Based on a number of studies "free" copper(-ion) primarily appears to be accumulated,

independent on the organism. Up to now a basic understanding of the complex relationships between all factors - both biotic and abiotic - which influence the accumulation of copper is lacking. The total accumulated amount of copper (body burden) is often not useful to predict the biological respons.

Toxicity to freshwater organisms

For different kinds of organisms the toxicity of copper was found to be related with "labile" copper (free cupric ion(s) and easily dissociable and exchangeable copper complexes). The toxicity of copper is reduced in the presence of a variety of both natural and artificial complexing agents. The acute toxicity of copper is inversely related with the hardness. The degree of the influence of the hardness is not similar for different organisms, so no general quantification of the effect of this paramater can be given.

For relatively sensitive organisms the acute L(E)C50-values in soft water (hardness $\leq$ 100 $mg.l^{-1}$, as CaCO3) are in the range from 5 to 50 μg $Cu.l^{-1}$. In hard water (hardness > 100 $mg.l^{-1}$, as CaCO3) these values exceed 50 μg $Cu.l^{-1}$.

NOEC-values from long-term tests are in the range from 10 to 100 μg $Cu.l^{-1}$ for most freshwater organisms tested; tests with some freshwater organisms resulted in NOEC-values below 10 μg $Cu.l^{-1}$. These data are for both soft and hard water. The most sensitive organisms are from different taxonomic groups (algae, molluscs, crustaceans, fish). The influence of the hardness of the water on long-term toxicity is less consistent compared with that on acute toxicity; the differences, if any, are not considered to be of biological significance.

Based on the results of toxicity tests and the persistence of this element, copper should be regarded as a "black-list substance" according to a proposal in the Netherlands (BCW, 1985).

In acute sediment toxicity tests copper has been found to be less toxic to aquatic organisms compared with tests without sediment. The results of these tests indicate that mainly dissolved copper is responsible for the toxic action and that sediment-bound copper is much less toxic.

On the basis of the results of field studies with water-sediment systems a concentration of about 10 to 20 μg $Cu.l^{-1}$ is not expected to cause serious damage to aquatic ecosystems at long-term exposure, although this level will be too high for the most sensitive organisms, for example some algal species.

The available data on sediment toxicity are limited. Correlation studies show that abundance, diversity and biomass of benthic macro-invertebrates are inversely related to sediment copper levels, and that other species are predominant at elevated copper levels compared with background levels.

Toxicity to marine organisms

For relatively sensitive marine organisms the acute L(E)C50-values are in the range from 5 to 100 $\mu g\ Cu.l^{-1}$. Most available long-term tests have resulted in NOEC-values between 2.5 and 50 $\mu g\ Cu.l^{-1}$. The most sensitive organisms are from different taxonomic groups (coelenterata, molluscs, fish).
As in freshwater the toxicity of copper is reduced in the presence of sediment or a sediment-extract. Data on sediment toxicity were hardly available. In laboratory tests freshly enriched sediments containing 15 to 38 mg $Cu.kg^{-1}$ led to toxic effects.
From a field study it appears that sediment levels of 100 to 150 mg $Cu.kg^{-1}$ d.w. may reduce fauna diversity.

4.2.2 Terrestrial organisms

Accumulation

Most data on copper accumulation (and toxicity) concern earthworms. At elevated copper levels these invertebrates have been found to accumulate but not to concentrate copper, with concentration factors (CF = concentration in worms : concentration in soil) below unity. Isopods (crustaceans) like woodlice have been found to concentrate copper from soil and/or litter at elevated copper concentrations (CF-values up to 15 and 50 on the basis of soil and litter, respectively). In general the CF-value decreases with increasing soil (and litter) copper levels.
Although usually higher tissue levels are found at higher soil concentrations in both animals (invertebrates) and plants the relationships are complex. The influence of other factors like soil parameters is poorly understood.
The few available data indicate that copper has a low potential for biomagnification in terrestrial ecosystems.

Toxicity

High copper levels mostly have an inhibitory effect on microbe-mediated soil processes (respiration, mineralization, ammonification, nitrification, enzyme activities, litter decomposition) and affect both microbial numbers and species diversity, but the results are highly variable and effective concentrations are often very high compared with background levels. At soil levels up to 100 mg $Cu.kg^{-1}$ no adverse effects are expected.
Most data on toxicity to earthworms concern Lumbricus rubellus. In laboratory experiments with exposure times of at least 6 weeks NOLC-values ranged from 130 to 165 mg $Cu.kg^{-1}$ soil (dry weight). Sublethal effects (reduced cocoonproduction, reduced growth) were found at soil levels of 35 to 60 mg $Cu.kg^{-1}$ d.w.; the lowest reported soil level without adverse effects on this earthworm species was 30 mg $Cu.kg^{-1}$ d.w. The few available data on other earthworm species do not indicate a significantly higher sensitivity to copper compared with L. rubellus. The data from laboratory studies are in good agreement with those from field studies in which adverse effects on earthworms were reported at soil levels of 50 to 70 mg $Cu.\ kg^{-1}$.
Data on soil copper levels which are detrimental to other invertebrates and to vertebrates living in the terrestrial environment are lacking.
For plants toxic soil levels range from 25 to 50 mg $Cu.kg^{-1}$ for the most sensitive species and from 150-400 mg $Cu.kg^{-1}$ for most other species.

4.3 Agricultural crops and livestock

4.3.1 Agricultural crops

Requirements and accumulation

In the Netherlands a minimum soil level of 4-5 mg $HNO3\text{-}Cu.kg^{-1}$ (d.w.) is considered to be sufficient for "normal" growth and development of crops.
On the average, an amount of 50 g copper per hectare is removed from the soil every year via the accumulation in crops; an amount of 80 g per hectare per year is considered as maximum (in nonpolluted soils).
In general crops grown on soils with elevated copper levels accumulate more copper than crops on reference soils, but the level in the plants is

dependent on species and on soil paramaters. Often no clear relationship between the level in plants and soil is found.
In food crops grown on harbour dredge spoils or on a clay soil mixed with dredge spoils (pot experiments) at total copper levels up to 270 $mg.kg^{-1}$ d.w., the level in edible parts of food crops was ≤ 2.4 times the level in crops grown on reference soils with copper levels of about 15 $mg.kg^{-1}$. With one exception (potatoes grown on one of the harbour dredge spoils) the levels did not exceed the current residu limits (3 $mg.kg^{-1}$ for potatoes and 20 $mg.kg^{-1}$ for other vegetables, fresh weight) in the Netherlands.
In pot experiments the copper level in grass grown on sediments containing 100 to 270 mg $Cu.kg^{-1}$ d.w. was (in a number of cases) exceeding the current Dutch maximum level (15 mg $Cu.kg^{-1}$, on a 12% moisture basis) in complete feed for sheep, the mammals which are most sensitive for copper, at least on the basis of feed copper level. On a soil mixed with dredge spoils resulting in substrate copper levels of 45 to 74 $mg.kg^{-1}$ d.w. the grass level was also exceeding this 15 $mg.kg^{-1}$ standard in a number of cases (pot experiment). In a field experiment the level in grass, grown on a sandy soil with copper levels of 60 to 208 $mg.kg^{-1}$ d.w. due to treatment with sewage sludge, increased about two-fold (from 7 to 15 mg $Cu.kg^{-1}$ d.w.) compared with the reference soil containing 5 $mg.kg^{-1}$. At locations below high-tension cables of copper (soil HNO_3-Cu levels 7 to 59 $mg.kg^{-1}$ d.w.) the level in grass was generally below 15 $mg.kg^{-1}$ d.w. In all these studies the maximum level found in grass was about 25 mg $Cu.kg^{-1}$ d.w., well below the current Dutch standards of ≥ 35 $mg.kg^{-1}$ (on a 12% moisture basis) for complete feed for livestock other than sheep (see basis document, chapter 1).

Toxicity

In a pot experiment the yield of agricultural crops (maize, butter-bean, spinach, sugar beet) was reduced at a soil HNO_3-Cu level of 82 $mg.kg^{-1}$ in a sandy soil with 2.5% organic matter (OM); at a level of 67 $mg.kg^{-1}$ only the yield of spinach was clearly reduced. Leguminosae are more sensitive, with toxic soil HNO_3-Cu levels of 25-40 $mg.kg^{-1}$. The lowest toxic level of approximately 20 mg EDTA-$Cu.kg^{-1}$ d.w. (corresponding with 25 mg HNO_3-$Cu.kg^{-1}$ or 30-40 $mg.kg^{-1}$ total copper) has been reported for snapbeans grown on a loamy sand with 0.7% OM (field experiment).
Food crops and grass grown on harbour dredge spoils, fluvial clay soils or a clay soil mixed with dredge spoil were not adversely affected at total substrate copper levels up to 270 $mg.kg^{-1}$ d.w. (pot experiments). The yield

of radish was reduced in four contaminated sediments containing ≥ 112 $mg.kg^{-1}$ total copper, but it is questionable if copper was the causative factor.

4.3.2 Livestock

Requirements and accumulation

Mammals (cattle, sheep, pigs) and poultry require a minimum copper level of 4 to 10 mg $Cu.kg^{-1}$ in feed on a dry weight basis, to prevent deficiency. In mammals the absorption of copper is generally below 10% of the intake; suckling animals absorb more copper.
The absorption is depending on dietary factors of which especially molybdenum and sulfate are reducing the accumulation in ruminants. In pigs the accumulation is more dependent on dietary zinc and iron.
Most accumulated copper is stored in the liver. Within certain limits the copper level in plasma and meat is not increasing with increasing levels in the diet, but that in liver increases to a very high degree. For example, in growing pigs fed a diet containing 500 mg $Cu.kg^{-1}$ for 10 weeks the level in meat hardly increased, while the copper level in the livers increased about 100-fold.

Toxicity

Of mammals, ruminants are more sensitive to copper than nonruminants (pigs). Sheep are most sensitive, at least on the basis of the copper level in feed. In sheep a dietary level of about 20 mg $Cu.kg^{-1}$ (corresponding with ~0.8 mg $Cu.kg^{-1}$ $bw.day^{-1}$) and upwards may already lead to toxic effects, including mortality due to a hemolytic crisis. The toxicity of copper can be counteracted by high dietary molybdenum and sulfate levels, consistent with a reduced accumulation of copper. A feed level of 15 mg $Cu.kg^{-1}$ is considered as safe for this animal species.
Cattle can tolerate a feed level of 70 to 100 mg $Cu.kg^{-1}$, corresponding with ~1.1-1.5 and ~2.1-3.0 mg $Cu.kg^{-1}$ $bw.day^{-1}$ in fattening and maintenance cattle, respectively, over an extended period.
Based on a large number of studies, for growing pigs an optimum (with regard to feed conversion and growth rate) copper level in the feed of 225 $mg.kg^{-1}$ (~9 mg $Cu.kg^{-1}$ $bw.day^{-1}$) has been established. The effect of copper

is strongly dependent on the composition of the diet. A feed level of 250 mg $Cu.kg^{-1}$ may lead to severe anemia when given in one diet, while a feed level of 500 to 600 mg $Cu.kg^{-1}$ only causes a slightly reduced weight gain but no obvious toxic effects in other diets. Signs and symptoms of copper toxicity are reduced or eliminated at high dietary zinc and iron levels. In general weight gain may be reduced but no adverse effects are observed in pigs fed conventional diets enriched with up to 500 mg $Cu.kg^{-1}$.
For chickens the optimum copper level in the feed is about 150 mg $Cu.kg^{-1}$. Levels above 200 to 250 mg $Cu.kg^{-1}$ mostly reduce feed intake and weight gain, and can also lead to other adverse effects. As in pigs, copper is more toxic in a semi-purified diet (suppression of weight gain at addition of only 80 mg $Cu.kg^{-1}$ feed) than in conventional diets.

4.4 Risk evaluation

4.4.1 Humans

Based on copper levels in food, drinks (including drinking water) and environmental air, the contribution of the inhalation pathway to the body content is neglectible, even when all copper inhaled will be absorbed. For this reason only the ingestion pathway will be used in the risk evaluation for the general population.
There are insufficient human data on copper toxicity to establish an maximum acceptable daily intake, as little as a suitable chronic animal toxicity study resulting in a dose-without-effect (DWE). The only available DWE: 8 mg $Cu.kg^{-1}$ $bw.day^{-1}$ is from a subchronic dietary study, a 1-year study with beagle dogs. Extrapolation of this value to an acceptable daily intake for humans for a life-time exposure using a margin of safety of 100 (Gezondheidsraad, 1985) results in a value of 0.08 mg $Cu.kg^{-1}$ $bw.day^{-1}$; this values is only slightly higher than the - on the basis of balance-studies - estimated minimum requirement of 0.02-0.05 mg $Cu.kg^{-1}$ $bw.day^{-1}$ for adults.
Based on data on copper levels in food and drinking water (see basis document, chapter 4) it is concluded that the total dietary daily intake of copper for adults will not easily exceed 10 mg, corresponding with 0.17 mg $Cu.kg^{-1}$ $bw.day^{-1}$. This "maximum" daily intake is about 50 times lower than the DWE derived from the dietary study with beagle dogs. Taking into account the life-time exposure to varying background levels of copper

without known adverse effects, this margin of safety is considered sufficient, so 0.17 mg $Cu.kg^{-1}$ $bw.day^{-1}$ is regarded as a safe level for a life-time exposure.

A considerable part of the general population will have an average daily intake below 0.17 mg $Cu.kg^{-1}$ $bw.day^{-1}$. The daily intake of these persons is similar to the widely accepted requirement of 0.03 to 0.05 mg $Cu.kg^{-1}$ $bw.day^{-1}$.

Exceeding the amount of 0.17 mg $Cu.kg^{-1}$ $bw.day^{-1}$ occasionally, will not lead to irreversible adverse effects due to the homeostatic mechanism. The intake of a relatively high amount of copper due to a high copper level in food and (especially) drinks may lead to acute gastrointestinal disturbances when the intake takes place within a short time. However, in most cases the intake of a large amount of copper will be prevented by the adverse effect on palatability.

For comparison the point of view of the Joint FAO/WHO Expert Committee on Food Additives follows below. In 1967 this committee has tentatively proposed a maximum acceptable copper load of 0.5 $mg.kg^{-1}$ $bw.day^{-1}$, provided that the dietary levels of those substituents such as molybdenum and zinc, which are known to affect copper metabolism, are within normal levels (JECFA, 1967). This value is about 10 times higher than the estimated daily intake, but no further arguments for the choice of this value were given. Based on human epidemiological and nutritional data (no further details given) which indicate that a daily copper intake of 2 to 3 mg (0.03-0.05 $mg.kg^{-1}$ $bw.day^{-1}$) is likely to be exceeded by significant sections of the population with no apparent deleterious effects, the previous tentative value of 0.5 $mg.kg^{-1}$ $bw.day^{-1}$ was reaffirmed as a provisional value for a maximum tolerable intake from all sources (JECFA, 1974, 1982).

Because it is not clear on which data this value (0.5 $mg.kg^{-1}$ $bw.day^{-1}$) has been based, the value of <u>0.17 mg $Cu.kg^{-1}$ $bw.day^{-1}$</u> (see above) is recommended as a value for a maximum daily intake at a life-time exposure.

4.4.2 Aquatic organisms

At present there are no generally accepted methods for extrapolation of the results of laboratory single-species toxicity studies to natural ecosystems. Provisionally two different theoretical procedures will be used to calculate "safe levels" for the aquatic environment (Kooyman, 1985; Slooff et al., 1986). In this way a lot of information can be gathered on

the possible usefulness of these extrapolation methods, but the results of these practices should only be considered to be indicative.
Both methods can use either acute toxicity values (LC50-values) and chronic no-observed-effect-concentrations (NOEC-values). Because sufficient long-term NOEC-values are available, only these were used to calculate "safe levels" for freshwater organisms (both in "soft" and "hard" water: hardness ≤ 100 and > 100 mg.l^{-1}, as CaCO3, respectively) and for marine organisms.
In figure 4.1 all available NOEC-values are shown. Table 4.1 summarizes the results of both methods of extrapolation.

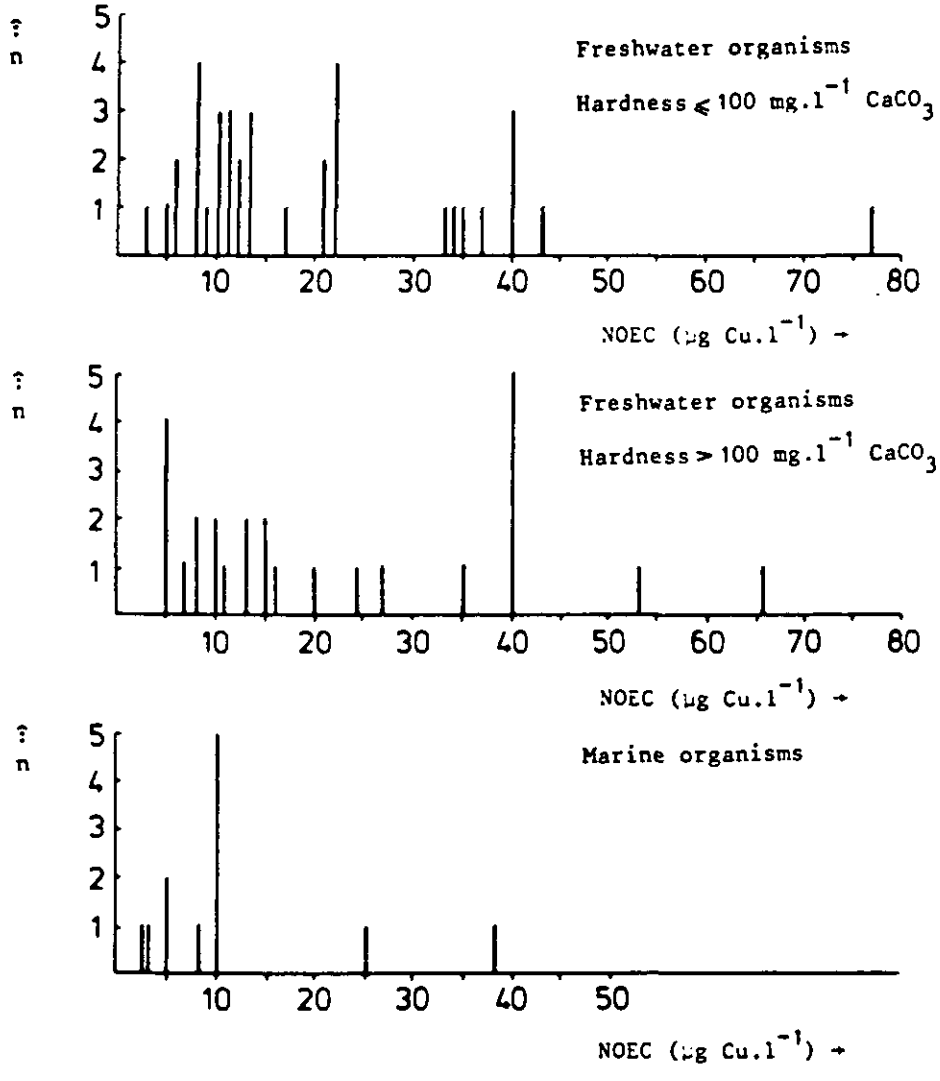

Figure 4.1 Distribution of NOEC-values for freshwater organisms (in soft and hard water, respectively) and for marine organisms

Table 4.1 "Safe" copper concentrations in the aquatic environment according to the methods of extrapolation proposed by Kooyman (1985) and Slooff et al. (1986)

	freshwater H ≤ 100	freshwater H > 100	seawater	
Slooff:				
Lowest $NOEC_{ss}$	3	5	2.5	$\mu g\ Cu.l^{-1}$
---> $NOEC_{eco}$	10.8	16.7	9.3	$\mu g\ Cu.l^{-1}$
UF = 33.5 --->	**0.3**	**0.5**	**0.3**	$\mu g\ Cu.l^{-1}$
Kooyman:				
--->	**0.05-0.1**[α]	**0.007**	**0.001**	$\mu g\ Cu.l^{-1}$

ss: "single species"; eco: "ecosystem"; UF: "uncertainty factor"
α: As "safe" chronic value 0.05 $\mu g.l^{-1}$ has been calculated based on all available NOEC-values, and 0.1 $\mu g.l^{-1}$ based on all NOEC-values minus those on 2 species of algae which were much less sensitive compared with the other organisms tested.

Freshwater

Data from "field" toxicity studies in the aquatic environment (both in soft and hard waters) indicate that the $NOEC_{eco}$-values as calculated by the method proposed by Slooff et al. (about 10 to 15 $\mu g.l^{-1}$) will have only marginal effects on the freshwater ecosystem. Some sensitive species, for example a number of algae species, will be adversely affected or even completely disappear at these concentrations, but due to a shift to less sensitive species the ecosystem as a whole appears not to be threatened seriously.
So, the calculated $NOEC_{eco}$-values are in good agreement with data from field studies.
The application of the uncertainty factor of 33.5 (which is common for this method of extrapolation) on the calculated $NOEC_{eco}$-values results in calculated "safe levels" of 0.3 and 0.5 $\mu g.l^{-1}$ for soft and hard water, respectively. These levels are similar to the lowest reported background levels and lower than effective concentrations derived both from field and laboratory toxicity studies. It is concluded that the application of the uncertainty factor is not reasonable in the case of copper. The use of the method of Kooyman results in even lower levels: 0.01 to 0.1 $\mu g.l^{-1}$ and is therefore not suitable to establish a realistic freshwater criterion for copper.
The lowest $NOEC_{ss}$-value: 3 $\mu g.l^{-1}$, is considered to be safe, not only to freshwater ecosystems as a whole, but also to sensitive single species.

This value ("total copper") is based on a flow-through test in soft water (H = 38). For this reason it is likely that most copper was present in solution, so the value of 3 $\mu g.l^{-1}$ represents "soluble" copper. Many of the other long-term test were also flow-through tests, with a continuous flow of freshly prepared copper enriched water; in a number of these tests the used water was reconstituted, or the used natural water was filtered and/or mixed with tap or deionized water. It is assumed that also in these cases most copper was present as soluble copper. According to data on surface water in the Netherlands the soluble fraction of the copper is about one-third of the total amount.
Based on these data and because of the fact that especially the soluble copper fraction of the water appears to be related with toxicity, a maximum value of 3 $\mu g.l^{-1}$ "soluble" copper - corresponding with about 10 $\mu g.l^{-1}$ "total" copper is provisionally recommended for freshwater. This value is in agreement with data from field studies.
Because of the similarity between the $NOEC_{ss}$-values in soft and hard waters, no different levels on the basis of hardness are recommended.

Seawater

Analogous to the reasoning used to establish a safe level for freshwater and because of the similarity between the $NOEC_{ss}$-values in seawater and freshwater, also for seawater a maximum level of 3 $\mu g.l^{-1}$ "soluble" copper (10 $\mu g.l^{-1}$ "total" copper) is provisionally recommended. This level cannot be related to data from toxicity studies in the field because suitable data are lacking.

Additional remarks fresh- and seawater

The interaction of copper with other heavy metals or other substances has been left out of account because of the variable results in tests which studied this interaction. Also, a possible adaptation of aquatic organisms to elevated copper levels has been left out of account because suitable data were not available.
On the basis of the screened data it is not possible to establish a minimum water copper concentration which is sufficient to meet the requirements of aquatic organisms. For bivalves a small margin (about a factor of 10) between the level of copper which is essential and that which is toxic has been reported. If this margin is similar for other aquatic organisms, than

the lowest reported background copper concentrations may be to low for some of these organisms.
The available data on sediment toxicity are limited. Most data are on correlation studies which are not suitable to establish an acceptable sediment copper level. The situation is also complicated by elevated levels of other metals in polluted sediments in the "natural" environment.

4.4.3 Terrestrial organisms

Agricultural crops require a minimum soil HNO3-Cu level of approximately 5 $mg.kg^{-1}$ for normal growth and development, corresponding with a total copper level of 6-10 $mg.kg^{-1}$. This copper level is probably also sufficient to prevent copper deficiency in livestock.
No suitable methods of extrapolation, which can be used to establish a maximum acceptable soil copper level, are available. The method proposed by Kooyman (see 4.4.2, risk evaluation aquatic organisms) was considered to be not suitable because of the highly variable results of the toxicity studies.
For earthworms the lowest reported toxic soil level is 35 mg $Cu.kg^{-1}$ (total copper) after 6 weeks exposure, in a sandy loam soil (no data soil parameters). The lowest NOEC-value is 30 mg $Cu.kg^{-1}$, in a sandy loam soil with 17% clay and 3.4% organic matter.
The most sensitive plants (see 3.1, agricultural crops) are adversely affected at soil HNO3-Cu levels of 25-40 $mg.kg^{-1}$. For example, the yield of snapbeans was reduced at a soil HNO3-Cu concentration of approximately 25 $mg.kg^{-1}$ - corresponding with a total copper level of 30-40 $mg.kg^{-1}$ - in a loamy sand with 0.7% organic matter.
Adverse effects on microbe-mediated soil processes are not expected at soil copper levels below 100 $mg.kg^{-1}$.
Data on other soil organisms are lacking.

Recently some proposals have been made in the Netherlands to establish criteria for environmental pollutants in soils in dependence of the soil parameters "organic matter" and "clay" (see also basis document, 5.4.3) . For copper this has tentatively resulted in the next equation which can be used to establish an acceptable copper level ("reference-value", indicative for a "good" soil quality) in different soils (VROM, 1987):

$$[Cu] = (15 + 0.6 \times (\% \text{ organic matter} + \% \text{ clay}))$$

Using this equation the tentative reference-value for a hypothetical "standard-soil" (organic matter content 10%; clay content 24%) is 35 mg $Cu.kg^{-1}$.
Unfortunately the available data are much to limited to check the usefulness of this proposal. Up to now the effects of different parameters, which are commonly used to characterize soils (organic matter content, clay content, cation-exchange-capacity, pH), on copper accumulation in and toxicity to terrestrial organisms are poorly understood. There are no consistent data which are valid for all organisms studied. However, the toxicity of copper appears to be more dependent on the organic matter content than on the clay content of the soil, which is not expressed in the most recent proposal.

Based on all available data a minimum level of 5 mg $HNO3\text{-}Cu.kg^{-1}$ soil is recommended, in view of the requirements of organisms. For most soils this level corresponds with about 10 mg $total\text{-}Cu.kg^{-1}$ (dry weight).
In view of the toxicity data a maximum level of 30 mg $total\text{-}Cu.kg^{-1}$ (dry weight) is recommended for soils with an organic matter content below 5%, as well as for soils which are used as grassland for sheep and agricultural soils which are used to grow grass or fodder plants for sheep.
At this copper level the accumulation of copper in crops will not involve a risk to livestock (including sheep) and humans. With regard to the ingestion of soil by grazing animals this copper level is also safe.
On the basis of data on experimental animals including livestock, there will also be no risk to wildlife (mammals, birds) at this soil copper level.
For other soils, with an organic matter content above 5% and/or a relatively high pH, a higher level is acceptable (about 50 mg $total\text{-}Cu.kg^{-1}$ and upwards), but on the basis of the available data it is not possible to establish precise copper levels in dependence of these parameters.

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