

7. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

7.1 Single exposure

7.1.1 *Lethality*

In a study to determine the acute toxicity of four zinc compounds (acetate, nitrate, chloride and sulfate) administered by the oral or intraperitoneal route to male Swiss mice and male Sprague Dawley rats (Table 20), the majority of deaths occurred during the first 48 h. The clinical and physical signs of toxicity included miosis, conjunctivitis, decreased food and water consumption, haemorrhage and haematosis in the tail. These effects were reported to diminish with time, indicating rapid elimination of zinc from the body. The acute toxicity of zinc varied with the zinc salt used, and ranged from 237 to 623 mg/kg in rats and from 86 to 605 mg/kg in mice after oral administration; the acute toxicity following an intraperitoneal dose ranged from 28 to 73 mg/kg in rats and from 32 to 115 mg/kg in mice (Domingo et al., 1988). LC_{50} values following inhalation exposure to zinc chloride were 11 800 mg/min per m^3 in mice (Schenker et al., 1981) and 2000 mg/ m^3 in rats (Karlsson et al., 1991).

7.1.2 *Acute studies: summary of key findings*

Zinc chloride can produce significant lung damage in rats when instilled directly into the lung; zinc oxide, by contrast, does not produce lung damage even when administered at relatively high concentrations. This is possibly due to the respective solubilities of the two compounds: zinc chloride is readily soluble in water whereas zinc oxide is not. Zinc chloride induces intra-alveolar oedema which closely resembles the effects of inhaled zinc oxide/hexachloroethane smoke in experimental animals. (Zinc oxide/hexachloroethane when burned produces zinc chloride with a residue of zinc oxide.) The oedema correlates with increased levels of protein in the lavage fluid fraction, which represents a plasma exudate. Onset is very rapid, with the greatest effects generally being noted within 3 days when high doses are used. The condition was found to regress between

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Table 20. Acute lethal dose toxicity of various zinc compounds in rats and mice: LD₅₀ values (mg/kg)^a

Compound	Route of administration			
	Rats		Mice	
	Oral	Intraperitoneal	Oral	Intraperitoneal
Zinc acetate	794	162	287	108
as zinc:	237	48	86	32
Zinc nitrate	1330	133	926	110
as zinc:	293	39	204	32
Zinc chloride	1100	58	1260	91
as zinc:	528	28	605	44
Zinc sulfate	1710	200	926	316
as zinc:	623	73	337	115

^a Animals were observed for 14 days.
From: Domingo et al. (1988).

days 3 and 7. Key findings from these studies are summarized in Table 21.

7.2 Short-term exposure

7.2.1 Oral exposure

Reduced growth rates, reduced body weights and anaemia were observed in a number of rat studies and also in a mouse and a sheep study, following high oral or dietary intakes of zinc (Van Reen, 1953; Maita et al., 1981; Allen et al., 1983; Zaporowska & Wasilewski, 1992). Copper deficiency induced by high doses of zinc, was implicated in these effects, as copper supplementation reversed the zinc-induced anaemia (Smith & Larson 1946).

Exposure to high doses of zinc was associated with pancreatic atrophy and histological changes in kidneys, accompanied by changes in kidney function in rats, mice and sheep (Maita et al., 1981; Allen et al., 1983; Llobet et al., 1988). Changes in the liver, including decreased activities of cytochrome P450 and liver catalase,

Table 21. Key findings from acute studies in experimental animals

Species	Exposure	Compound	Effects	Reference
Rats (male)	inhalation, 1 h 0, 11, 580 mg/min/m ³	zinc oxide/hexachloro-ethane	11/40 deaths, pulmonary oedema	Brown et al. (1990)
Rats (male)	intratracheal 0, 2.5 mg/kg	zinc chloride	respiratory distress, alveolitis, parenchymal scarring	Brown et al. (1990)
Guinea-pigs and rats	inhalation (nose only), 3 h 2.5, 5 mg/m ³	zinc oxide (median diameter 0.06 µm)	inflammatory changes in the lung at both levels of exposure	Gordon et al. (1992)
Guinea-pigs (male, Hartley)	aerosol, 3 h 0, 7.8 mg/m ³	zinc oxide (projected area diameter 0.05 µm)	decrease in the lung volume capacity	Lam et al. (1982)
Rats (male, Porton Wistar)	intratracheal 0.3 mg/kg	zinc oxide, zinc chloride	elevated alveolar surface protein levels with zinc chloride exposure only	Richards et al. (1989)

Table 21 (contd.)

Species	Exposure	Compound	Effects	Reference
Rats (male, Porton Wistar)	intratracheal 0, 0.25, 0.5, 1, 2, 4, 5 mg/kg	zinc oxide, zinc chloride	intra-alveolar oedema at doses above 0.5 mg/kg	Richards et al. (1989)
Rats (male, Porton Wistar)	inhalation 2.5 mg/kg	zinc chloride	pulmonary oedema	Richards et al. (1989)
Sheep (weaner)	drench 3 g	zinc	14/100 deaths, oedema of abomasum and duodenum, fibrosing pancreatitis	Allen et al. (1986)
Rabbits (New Zealand)	inhalation 0, 0.6, 0.81 g/m ³	hexachloroethane/zinc	acute inflammation of lungs, pulmonary oedema at both doses	Marrs et al. (1983)

and decreased *de novo* synthesis of high-density lipoprotein, were seen in rats exposed to high levels of zinc (Van Reen, 1953; Woo, 1983; Cho et al., 1989). Minor degenerative changes in the brain, accompanied by elevated neurosecretion and increased activity in the neurohypophysis were seen in rats exposed intragastrically to zinc oxide for 10 days at 100 mg/day (Kozik, 1981).

Key findings from these studies are summarized in Table 22.

7.2.2 Inhalation exposure

Guinea-pigs (3 per group) were given 1, 2 or 3 consecutive, daily, 3-hour, nose-only exposures to freshly generated zinc oxide particles with a projected area diameter of 0.05 μm at concentrations of 0, 2.3, 5.9 or 12.1 mg/m^3 . Exposure to zinc oxide at 5.9 or 12.1 mg/m^3 was associated with increased protein and neutrophils and increased activities of β -glucuronidase, acid phosphatase, alkaline phosphatase, lactate dehydrogenase and angiotensin-converting enzyme in lavage fluid. These increases were concentration-dependent, were detected after the second exposure, and generally increased after the third exposure. Significant morphological changes observed at concentrations of 5.9 or 12.1 mg/m^3 consisted of inflammation and type 2 pneumocyte hyperplasia in the proximal alveolar ducts. No evidence of inflammation was present in animals exposed to zinc oxide at 2.3 mg/m^3 . It was concluded that exposure of guinea-pigs to ultrafine atmospheric zinc oxide at concentrations of 5.9 or 12.1 mg/m^3 causes significant pulmonary damage. Detection of injury was stated to correlate well with pulmonary lavage fluid changes (Conner et al., 1988).

Male Hartley guinea-pigs exposed to zinc oxide at a concentration of 7 mg/m^3 for 3 h/day for 5 consecutive days showed pulmonary impairment (as measured by lung oedema, lung volume carbon monoxide diffusing capacity and pulmonary mechanics). Exposures at 2.7 mg/m^3 using the same time frame did not cause pulmonary impairment (Lam et al., 1988). A single exposure at 8 mg/m^3 was also without effect (Lam et al., 1982). Guinea-pigs exposed to zinc oxide at a concentration of 5 mg/m^3 for 3 h/day for 6 consecutive days showed significant reductions in vital capacity, functional residual capacity, alveolar volume, and lung volume carbon monoxide diffusing capacity following the last exposure,

Table 22. Key findings from short-term exposure studies in experimental animals

Species	Exposure	Compound	Effects	Reference
Mice	0.6 g/kg of diet for 4 weeks	zinc sulfate	no adverse effects on immune responsiveness	Schiffer et al. (1991)
Mice, rats	0, 300, 3000, 30 000 µg/g diet for 13 weeks	zinc sulfate	NOEL for both species was set at 3000 µg/kg; retarded growth, anaemia and pancreatic atrophy at 3000 µg/kg level	Maita et al. (1981)
Rats	0, 0.5, 1% of diet for 15 days	zinc oxide	death at 1% level; reduced body weight, reduced fat content of the liver and impaired bone development at both doses	Van Reen (1953)
Rats	500–700 mg/100 g diet for 4–5 weeks	zinc in zinc carbonate	growth reduction, reduced levels of liver catalase and cytochrome oxidase activity, effects reversed by copper supplement	Van Reen (1953)
Rats	100 mg/day intra-gastrically for 10 days	zinc oxide	elevated neurosecretion in hypothalamus, increased release of antidiuretic hormone in neurohypophysis	Kozik (1981)

Table 22 (contd.)

Rats	100 mg/day intra-gastrically for 10 days	zinc oxide	morphological and histoenzymic changes in the brain	Kozik (1981)
Rats	0, 0.12 mg/ml drinking-water for 4 weeks	zinc as zinc chloride	decreased body weight, anaemia and increased lymphocyte count	Zaporowska & Wasilewski (1992)
Rats	0, 160, 320, 640 mg/kg body weight/day for 3 months	zinc acetate	no effect on weight gain or on red blood cells, histological changes in kidneys and increased concentrations of urea and creatinine in plasma at 640 mg/kg body weight per day	Llobet et al. (1988)
Rats	0, 0.7, 1% in diet for 4 weeks	zinc carbonate	subnormal growth, anaemia and reproductive failure at both dose levels, anaemia reversed by copper supplement, growth retardation reversed by liver extract supplement	Smith & Larson (1946)

which had not returned to normal values by 72 h, although increases to flow resistance and decreases in compliance and total lung capacity did return to normal (Lam et al., 1985)

Key findings from these studies are summarized in Table 23.

7.3 Long-term exposure

The long-term studies on the effects of zinc vary in quality and tend to be limited in their usefulness in determining chronic toxicity in animals, as the study design generally does not lend itself to elucidation of dose-related effects. The available studies do, however, provide some information on target organ toxicity resulting from zinc exposure. Key findings from these studies are summarized in Table 24.

7.3.1 Oral exposure

Osborne-Mendel rats (4 per sex per group) were fed diets containing zinc sulfate at 0, 100, 500 and 1000 µg/g for 21 months. While only minimal monitoring of toxic effects was carried out, it was reported that food intake, body weights, haemoglobin values, and erythrocyte, leukocyte and differential counts were unaffected by the treatment. Microcytosis coupled with polychromasia or hyperchromia appeared at 16 months in rats receiving the highest dose and at 17 months in the other zinc-treated groups. However, it was stated that the erythrocyte count returned to normal later in the study (time not specified). Counts of the bone marrow smears taken at autopsy revealed a dose-related decrease in the myeloid:erythrocyte ratio in all of the treated groups. The kidneys of male rats in the 500 and 1000 µg/g groups were enlarged and an increased incidence of nephritis was seen in male, but not female, rats (Hagan et al., 1953).

In a chronic study (Aughey et al., 1977), C3H mice were administered zinc sulfate in the drinking-water at a concentration of 0.5 g/litre for 14 months. Control and zinc-treated mice were removed from the colony in groups of five per sex, usually at monthly intervals, for estimation of plasma zinc, glucose and insulin, tissue zinc, and histological, histochemical and electron microscopy examinations. Plasma zinc increased to a plateau at levels about 1.5–2 times those in controls within the first 30 days. Levels of zinc

Table 23. Key findings from repeated dose inhalation studies in guinea-pigs

Species	Exposure	Compound	Effects	Reference
Guinea-pigs (male Hartley, 3 per group)	0, 2.3, 5.9 or 12.1 mg/m ³ , 3 h/day for 1, 2 or 3 days	zinc oxide (projected area diameter 0.05 µm)	inflammation and hyperplasia of the lung at 5.9 and 12.1 mg/m ³ after the second exposure; NOEL, 2.3 mg/m ³	Conner et al. (1988)
Guinea-pigs (male Hartley, 5–8 per group)	0, 2.7 or 7 mg/m ³ , 3 h/day for 5 days	zinc oxide (median diameter 0.05 µm)	oedema, decrease in total lung capacity and diffusing capacity for CO at 7 mg/m ³ , oedema; no effects observed at 2.7 mg/m ³	Lam et al. (1988)
Guinea-pigs (male Hartley, 18–38 per group)	0 or 5 mg/m ³ , 3 h/day for 6 days (nose only)	zinc oxide (projected area diameter 0.05 µm)	inflammation, decrease in vital capacity, functional residual capacity, total lung capacity and diffusing capacity for CO	Lam et al. (1985)

Table 24. Key findings from long-term exposure studies in experimental animals

Species	Exposure	Compound	Effects	Reference
Rats	0, 0.1, 0.5 or 1% in diet for up to 39 weeks	zinc carbonate	reduction of growth at 1% and indications of anaemia in the 0.5 and 1% groups	Sutton & Nelson (1937)
Rats	0, 100, 500 or 1000 µg/g in diet for 21 months	zinc sulfate	minimal monitoring; no effect on growth and no anaemia; dose-related decrease in myeloid/erythrocyte ratio in all treated groups; enlarged kidneys at 500 and 1000 µg/g in all male groups; NOEL, <100 µg/g	Hagan et al. (1953)
Mice	0.5 g/litre in drinking-water for 14 months	zinc sulfate	pancreatic hypertrophy, pituitary gland hypertrophy	Aughey et al. (1977)
Dogs	200 mg/kg body weight per day in diet reduced to 50 mg/kg body weight/day by week 35	zinc sulfate	emesis, 1/4 deaths, hypochromic anaemia, hyperplastic bone marrow	Hagan et al. (1953)
Rabbits	5 mg/g in diet for 22 weeks	zinc carbonate	no effects on growth, decrease in haemoglobin and serum copper concentrations	Bentley & Grubb (1991)

Table 24 (contd.)

Mink	0, 500, 1000 or 1500 mg/kg for 144 days	zinc sulfate	no effect on body weights, haematological parameters or survival; no histological lesions in liver, pancreas or kidney; NOEL, 1500 mg/kg	Aulerich et al. (1991)
Ferrets	0, 500, 1500 or 3000 mg/kg for up to 6 months	zinc oxide	body weight loss, reduced food intake and death at 3000 mg/kg on days 9–13 and at 1500 mg/kg on days 7–21; diffuse nephrosis and active haemopoiesis in bone marrow and spleen in the 3000 and 1500 mg/kg groups; pancreatitis in one animal in each group at 3000 and 1500 mg/kg; no toxicity observed at 500 mg/kg except some evidence of effect on red blood cells	Straube et al. (1980)
Mice, rats	0, 1.2, 12 or 120 mg/m ³ air for 1 h/day, 5 days per week for 20 weeks	zinc in smoke produced by ignition of zinc oxide/hexachloroethane	no effect on body weight; increase in mortality in mice at 120 mg/m ³ ; macrophage infiltration of the lung in rats and mice at the highest dose; significant increase in the frequency of alveologenic carcinoma in high dose mice Note: carbon tetrachloride may be present in smoke	Marrs et al. (1988)

in the liver, spleen and skin remained unchanged. The pancreatic islet cells in treated mice were hypertrophied and contained an increased number of secretory granules; however, plasma glucose and insulin levels remained comparable to those in control animals. Hypertrophy of the pituitary gland, suggestive of a pituitary feedback effect, was also observed.

Adult and juvenile mink (3 per sex per group) were fed diets supplemented with zinc as zinc sulfate at 0, 500, 1000 or 1500 mg/kg for 144 days. No adverse effects on food consumption, body weight gains, haematological parameters, fur quality or survival were observed. Zinc concentrations in the liver, kidneys and pancreas increased in direct proportion to the zinc content of the diet. No histological lesions indicative of zinc toxicity were detected in the liver, kidneys or pancreas (Aulerich et al., 1991).

Ferrets (3–5 per group) were fed diets containing zinc administered as zinc oxide at 0, 500, 1500 or 3000 mg/kg for up to 6 months. The three ferrets in the 3000 mg/kg group lost a significant amount of their body weights, had greatly reduced food intakes, and died or were killed *in extremis* between days 9 and 13 of the dosing period. The ferrets exposed to 1500 mg/kg zinc were killed at 7–21 days, by which time they presented with poor condition, weight loss and up to 80% reduction in food intake. Histological examination of the three animals from the 3000 mg/kg group and the four animals from the 1500 mg/kg group revealed diffuse nephrosis and active haematopoiesis in the bone marrow and the extramedullary area of the spleen. One animal from each dose group had acute pancreatitis. Haematograms indicated a severe but responding macrocytic hypochromic anaemia, with high reticulocyte counts in the two highest dose groups. In the liver and kidneys of treated animals, the zinc concentration was significantly increased and the copper concentration was lower than control values. These changes were associated with a high concentration of iron in the liver. Increased incidences of elevated serum urea and blood glucose concentrations and decreased serum ceruloplasmin oxidase activity were observed at the two highest doses, and protein, blood, glucose and bilirubin were present in the urine. None of the ferrets given zinc at 500 mg/kg in the diet developed clinical signs. These animals were killed on days 48, 138 and 191 respectively; they showed signs of extramedullary haematopoiesis in the spleen and slight increases in

kidney zinc concentration and decreases in liver copper concentration. Although the number of animals used was small, given the lack of dose–response studies, the threshold of zinc toxicity in ferrets was proposed to be between 500 and 1500 mg/kg, with the kidney identified as the target organ of toxicity in this species (Straube et al., 1980).

The consequences of copper deficiency may be relevant to some of the effects noted in studies using elevated zinc levels. The occurrence of anaemia in animals receiving high doses of zinc is generally attributed to induction of copper deficiency. Some otherwise unexplained effects of high doses of zinc may also be secondary to impaired copper utilization. Relevant studies are described in section 7.8.

7.4 Skin irritation

Zinc chloride, applied daily as a 1% aqueous solution in an open patch test for 5 days, was severely irritant in rabbits, guinea-pigs and mice, inducing epidermal hyperplasia and ulceration. Aqueous zinc acetate (20%) was slightly less irritant. Zinc oxide (20% suspension in dilute Tween 800), zinc sulfate (1% aqueous solution) and zinc pyrithione (20% suspension) were mildly irritant, and induced a marginal epidermal hyperplasia and increased hair growth. Zinc undecylenate (20% suspension) was not irritant. Epidermal irritancy was related to the interaction of zinc with epidermal keratin (Lansdown, 1991).

7.5 Reproductive toxicity, embryotoxicity and teratogenicity

The available studies are limited in their usefulness in determining the reproductive and developmental effects of zinc owing to poor study design and inadequate reporting, although they do provide an indication of the effects of zinc exposure. Key findings from these studies are summarized in Table 25.

In a study in mice (Mulhern et al., 1986), female weanling C57BL/6J mice were fed diets containing zinc as zinc carbonate at a concentration of 2000 mg/kg until they were mated at 6 weeks of

Table 25. Key findings from studies on reproduction, embryotoxicity and teratogenicity in experimental animals

Species	Exposure	Compound	Effects	Reference
Mice	2000 mg/kg until mating at 6 weeks of age, then in various combinations during gestation/lactation and after weaning; second generation killed at 8 weeks	zinc carbonate	in second generation mice exposed throughout gestation/lactation and after weaning, elevated levels of zinc in bones, decreased blood copper levels, signs of anaemia and reduced body weights; alopaecia at 5 weeks, reversed at 8 weeks	Mulhern et al. (1986)
Mice	0, 12.5, 20.5 or 25 mg/kg body weight i.p. on days 8, 9, 10 or 11 of gestation	zinc chloride	increases in skeletal defect incidence, usually ripple ribs; effects were dose-related and seen at all dose levels; no soft tissue anomalies attributed to zinc; greatest effect at 20.5 mg/kg on day 10 of gestation, causing 4/10 deaths	Chang et al. (1977)
Mouse embryos	100 µmol/litre <i>in vitro</i> for 24 h at the 1-, 2-, 4- and 8-cell stage	zinc	40% increase in cell death at 1-cell stage; embryo development affected more in early than late stages; relevance to fetal development uncertain	Vidal & Hidalgo (1993)

Table 25 (contd.)

Rats	0, 0.1%, 0.5% or 1% in diet for 39 weeks and during pregnancy	zinc carbonate	reproduction adversely affected in the 0.5% group: all second litters dead, no further pregnancies thereafter; no pregnancy achieved in the 1% group; anaemia in 0.5% and 1% groups; anaemia and sterility reversed in 0.5% group but sterility remained the 1% group when zinc removed from diet	Sutton & Nelson (1937)
Rats	0, 4 or 500 mg/kg in diet for 8 weeks to weanlings.	zinc chloride	testicular cell development examined only: excess zinc had no effect	Evenson et al. (1993)
Rats	0, 500, 1000 or 2000 µg/g diet during pregnancy	not given	significant decrease in total number of pups born and increased percentage of stillbirths at 2000 µg/g; no increase in the incidence of malformations	O'Dell (1968)
Rats	0 or 150 mg/kg in diet throughout gestation	zinc sulfate	effects on fetus assessed on day 18: incidence of resorption significantly increased in treated animals	Kumar (1976)

Table 25 (contd.)

Species	Exposure	Compound	Effects	Reference
Rats	0, 0.25 or 0.5% in diet during gestation and 14 days of lactation	zinc oxide	maternal body weight, gestation period and viable pups/litter were unattested at either dose level at birth or on day 14; no malformations observed in any pup; dose-related reduction in pup body weight; some changes in iron and copper distribution in newborn pups at both treatment levels	Ketcheson et al. (1969)
Rats	500 mg/kg in diet during gestation	zinc carbonate	no effect on maternal haematocrits; no effects on litter numbers, viability, implantation sites, fetal length and weight, placental weights or incidence of resorptions; no increase in the incidence of malformations or skeletal ossification	Uriu-Hare et al. (1989)
Rats	4000 mg/kg, 18 days post coitus	zinc sulfate	incidence of conception reduced; no increase in stillbirths or malformations in exposed groups	Pal & Pal (1987)

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age. The dams and offspring were distributed into 10 different dietary groups, exposing the second generation to various combinations during gestation, lactation and postweaning development. Second-generation mice were killed at 8 weeks of age. Second-generation mice exposed to high doses of zinc throughout the gestation, lactation and postweaning period had elevated levels of zinc in their bones, decreased blood copper levels, lowered haematocrit values and reduced body weights. Mice in this group began to lose fur at 2–4 weeks of age, with severe alopecia developing at 5 weeks of age, accompanied by thinner than normal skin. The fur grew back by 8 weeks of age, albeit lighter in colour.

The feeding of low (4 mg/kg of diet), normal (12 mg/kg) and high (500 mg/kg) levels of zinc as zinc chloride to weanling Sprague-Dawley rats (10 males per group) for 8 weeks indicated that a diet deficient in zinc is associated with a significant deviation in the ratio of testicular cell types present in the testes, including a reduction in the numbers of cells in S phase and total haploid cells. In rats fed zinc-deficient diets, about 50% of epididymal spermatozoa had a significant decrease in resistance to DNA denaturation *in situ*. Excess zinc in the diet had no effect on rat testicular cell development as defined by sperm resistance to DNA denaturation, distribution of testicular cell types and sperm chromatin structure integrity (Evenson et al., 1993).

A diet supplemented with zinc (source not identified) at 0, 500, 1000 and 2000 µg/g, and with adequate levels of copper (10 µg/g) was administered to pregnant rats (strain and number not given). There was a significant decrease in the total number of pups born and an increase in the percentage of stillbirths at the highest dose of zinc, but no effect on the survival of offspring allowed to nurse for 1 week. The data were reported to indicate that the incidence of hydrocephalus was increased in rat embryos of zinc-treated dams. However, there was no obvious correlation between dose and the incidence of hydrocephalic fetuses associated with the treatment: 0.1% in controls, 0.2% in the 500 µg/g group, 0.7% in the 1000 µg/g group, and 0.1% in the 2000 µg/g group (O'Dell, 1968).

Pregnant rats (10–12 per group, strain not identified) were fed a diet supplemented with zinc sulfate (150 µg/g) throughout the entire gestation period. On day 18 of pregnancy, the incidence of

resorptions in pregnant rats increased from 2/101 implantation sites in the 12 control rats (2 females affected) to 11/116 implantations in the supplemented rats (8 females affected) This difference was statistically significant, indicating that even moderately high levels of zinc in the diet of rats may be associated with harmful effects on pregnancy (Kumar, 1976).

Diets high in zinc (0.2 and 0.5%), added as zinc oxide, were fed to pregnant albino rats (10 per group) for the entire period of gestation and for the first 14 days of lactation. The zinc content of the basal diet was 9 mg/kg. Four pups from each litter were killed at birth and the remaining pups were killed and examined on day 14 of lactation. Maternal body weights, food intake, gestation period and the number of viable young per litter were unaffected by the increased zinc levels in the diet, either at birth or on day 14 of lactation. Two dams fed 0.5% zinc had stillborn litters containing oedematous pups. Four stillborn pups were born to dams fed 0.2% zinc (number of dams not given); these pups were not oedematous. Anatomical malformations were not observed in any pup. The body weights of the newborn and 14-day-old pups in the 0.5% group were significantly reduced whereas the size of newborn pups, but not the 14-day-old pups from the 0.2% group were significantly greater than pups from dams fed the basal diet. The dry liver weights of pups at birth were unaffected by the zinc treatment but were significantly reduced in day-14 pups in the 0.5% group. Total zinc in newborn pups and day-14 weanlings was elevated in a dose-related manner in pups from the dams exposed to 0.2% and 0.5% zinc. Bodies (viscera removed) of newborn and day-14 pups from mothers fed the zinc diets contained significantly lower total iron than those from mothers receiving the basal diet: the reduction was dose-dependent. In contrast, the livers of newborns from zinc-treated dams contained significantly elevated total iron than the basal diet pups. These changes in liver iron levels were not observed in day-14 pups. Total copper in the whole animal and body (viscera removed) of the newborn rats was not altered by the treatment. However, liver copper levels were significantly lower only in the newborns of mothers fed 0.5% zinc. After 14 days, total copper was significantly lower in the whole animal, liver and body (viscera removed) of pups from dams fed both zinc diets; this reduction was dose-dependent. Livers of dams fed excess zinc contained elevated zinc and reduced iron and copper levels (Ketcheson et al., 1969). Another study reported no

resorption in Sprague-Dawley rats receiving 0.5% zinc as zinc carbonate in the diet (Kinnamon, 1963).

Pregnant Sprague Dawley rats (8 per group) were exposed to basal (24.4 mg/kg of diet) or high levels of zinc (500 mg/kg) in the diet, supplemented as zinc carbonate, for the duration of the gestation period. Ingestion of high zinc levels had no effect on maternal food intake or on body weight throughout the pregnancy. Maternal haematocrits on gestational day 20 were similar in the basal and high-zinc groups. High dietary zinc levels had no effect on litter numbers, litter viability, implantation sites, fetal lengths and weights, placental weights or number of resorptions. There was no significant increase in the incidence of malformations associated with high zinc exposure or in the ossification of the fetal skeleton. Zinc, copper and iron content of the maternal liver, and maternal kidney weights in the basal and high-zinc groups remained comparable. Plasma of dams exposed to the high-zinc diet contained significantly increased zinc levels and significantly decreased iron levels, whereas copper levels remained similar to those found in rats fed the basal diet. The absolute concentrations of zinc bound to albumin and α_2 -macroglobulin proteins were significantly increased in the high-zinc group as were maternal liver metallothionein concentrations (Uriu-Hare et al., 1989).

Exposure of Charles Foster rats (12 per group) to diets containing zinc as zinc sulfate at a concentration of 4000 mg/kg reduced the incidence of conception in females treated for 18 days post coitus, indicating that high zinc intake interferes with implantation of fertilized ova. However, exposure to this level of zinc 21–26 days before mating and throughout gestation for 18 days did not affect the incidence of conception. This apparently contradictory finding was interpreted to be due to an adaptation to zinc feeding, which is known to decrease the body burden of zinc. No stillborn or malformed fetuses were observed in zinc-treated animals in either study (Pal & Pal, 1987).

7.6 Mutagenicity and related end-points

Genotoxicity studies conducted in a variety of test systems have failed to provide evidence that zinc is mutagenic. However, there are indications of some weak clastogenic effects following zinc

exposure. The findings from genotoxicity studies are detailed below and are summarized in Table 26.

7.6.1 In vitro studies

Exposure to zinc does not increase mutation frequencies in the majority of bacterial or mammalian cell culture test systems (Nishioka, 1975; Amacher & Paillet, 1980; Kada et al., 1980; Gocke et al., 1981; Marzin & Vo, 1985; Rossman et al., 1987; Thompson et al., 1989; Karlsson et al., 1991). However, gene mutation effects following exposure to zinc were observed in the TK^{+/+} mouse lymphoma and Chinese hamster ovary cells *in vitro* cytogenetic assays (Thompson et al., 1989), and chromosomal effects were obtained in human lymphocyte cultures (Deknudt & Deminatti, 1978). Zinc chloride did not induce mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells (Amacher & Paillet, 1980) and did not induce mispairing between nucleic acid bases *in vitro* (Murray & Flessel, 1976).

Zinc sulfate inhibited the activity of DNA polymerase-1 activity *in vitro*, but had no effect on the fidelity of DNA synthesis in an assay measuring misincorporation of nucleotides into the new strand of DNA (Sirover & Loeb, 1976; Miyaki et al., 1977). Zinc chloride at concentrations of up to 20 µg/ml did not cause morphological transformation of Syrian hamster embryo cells *in vitro* (Di Paolo & Casto, 1979); however, zinc chloride and zinc sulfate gave equivocal results in an *in vitro* test for the capacity of these metal salts to enhance viral transformation of Syrian hamster embryo cells, producing enhancement in 3/6 and 3/7 trials respectively (Casto et al., 1979). Exposure to zinc had no effect on the induction of unscheduled DNA synthesis in primary cultures of rat hepatocytes (Thompson et al., 1989).

7.6.2 In vivo studies

The induction of chromosome aberrations has been studied in bone marrow cells harvested from animals exposed to elevated levels of zinc. Taken as a whole, studies of this end-point yield equivocal and sometimes contradictory results—a likely reflection of inter-study differences in routes, levels and duration of zinc exposure, the nature of lesions scored (gaps compared to more accepted structural

alterations) and great variability in the technical rigour of individual studies. Increased aberrations have been reported in rats after inhalation exposure (zinc oxide at 0.5–1.0 mg/m³ for 5 months; Voroshilin et al., 1978), in rats after oral exposure (zinc chloride in water at 249 mg/litre for 14 days; Kowalska-Wochna et al., 1988) and in mice after multiple intraperitoneal injections of zinc chloride (at 2–5 mg/kg body weight; Gupta et al., 1991). In contrast, other studies have produced negative findings, for example, after intraperitoneal injection of mice (zinc chloride at 15 mg/kg body weight; Vilkina et al., 1978), or have suggested that the induction of aberrations is contingent upon concomitant calcium deficiency. Those studies do not provide compelling evidence for significant clastogenic activity. Negative results have also been reported in the mouse micronucleus test (intraperitoneal injection of zinc sulfate; Gocke et al., 1981). Negative micronucleus test results are consistent with a lack of significant clastogenic activity.

There was no increase in the frequency of dominant lethal mutation in germ cells of mice injected by the intraperitoneal route with zinc chloride at 15 mg/kg body weight (Vilkina et al., 1978).

Zinc sulfate (5 mmol/litre), which is an almost-lethal dose) fed to adult *Drosophila melanogaster* did not increase the incidence of sex-linked recessive lethal mutations when tested in three successive broods (Gocke et al., 1981). In contrast, zinc chloride fed to adult *D. melanogaster* at 0.247 mg/ml significantly increased the incidence of dominant lethal mutations and sex-linked recessive lethal mutations in treated flies (Carpenter & Ray, 1969).

7.7 Carcinogenicity

No adequate experimental evidence has been found to indicate that zinc salts administered orally or parenterally are tumorigenic.

Deficiency and supplements of zinc can have an influence on carcinogenesis, possibly as a result of the influence of zinc on cell growth (Petering et al., 1967; Barr & Harris, 1973; Phillips & Sheridan, 1976; Rath et al., 1991), although zinc has also been reported to inhibit tumour induction (Kasprzak et al., 1988). Zinc has been demonstrated to inhibit the mutagenic action of some genotoxic carcinogens (Francis et al., 1988; Leonard & Gerber, 1989) but has

Table 26. Genotoxicity studies with zinc

Test	Zinc source doses	Concentrations of zinc	Results	Reference
Non-mammalian systems				
Prokaryotes				
Gene mutation				
<i>Salmonella typhimurium</i> (TA102)	zinc sulfate	10–1000 nmol/litre per plate	- (no S9)	Marzin & Vo (1985)
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	zinc acetate	50–7200 µg/plate	- (with and without S9)	Thompson et al. (1989)
<i>S. typhimurium</i> (TA98, TA1538)	zinc 2,4-pentanedione	400 µg/plate	+ (with and without S9)	Gocke et al. (1981)
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	zinc sulfate	up to 3600 µg/plate	- (with and without S9)	Karlsson et al. (1991)
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	zinc oxide/hexachloroethane smoke		- (with and without S9)	Karlsson et al. (1991)
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537)	zinc chloride	0.05 mol/litre	-	Nishioka (1975)
<i>Bacillus subtilis</i> H17, M45	zinc chloride	not given	-	Kada et al. (1980)
<i>Bacillus subtilis</i> H17, M45	zinc chloride	0.4 mmol/litre	-	Rossman et al. (1987)
<i>Escherichia</i> microscreen assay	zinc chloride	0.4 mmol/litre	-	Rossman et al. (1987)
λ prophage induction	zinc chloride	0.4 mmol/litre	-	Rossman et al. (1987)
Trp+ reversion				
comutagenesis				

Table 26 (contd.)

Fidelity of DNA synthesis	zinc sulfate	0.2 mmol/litre	-	Miyaki et al. (1977)
DNA polymerase	zinc chloride	0.4 mmol/litre	-	Sirover & Loeb (1976)
Plants				
Chromosomal aberrations				
Vicia faba	zinc sulfate	0.1% solution	+	Herich (1969)
Insects				
Sex-linked recessive lethal test	zinc sulfate	5 mmol/litre	-	Gocke et al. (1981)
Sex-linked recessive lethal test	zinc chloride	0.247 mg/ml of food	+	Carpenter & Ray
Dominant lethal test	zinc chloride	0.247 mg/ml of food	+	(1969)
Mammalian systems				
<i>In vitro</i> animal cells				
Gene mutation				
mouse lymphoma	zinc chloride	0.12–12.13 µg/ml	-	Amacher & Paillet (1980)
mouse lymphoma	zinc acetate	0–13 µg/ml and 4.2–42 µg/ml	+ (with and without S9)	Thompson et al. (1989)
Chromosomal aberration				
Chinese hamster ovary cells	zinc acetate	25–45 µg/ml and 45–80 µg/ml	+ (with and without S9)	Thompson et al. (1989)

Table 26 (contd.)

Test	Zinc source doses	Concentrations of zinc	Results	Reference
Cell transformation				
Syrian hamster embryo cells	zinc chloride	0–20 µg/ml	-	DiPaolo & Casto (1979)
enhancement of cell transformation	zinc sulfate	0.05–0.6 mmol/litre	+/-	Casto et al.
enhancement of cell transformation	zinc chloride	0.05–0.6 mmol/litre	+/-	(1979)
Unscheduled DNA synthesis				
rat hepatocytes	zinc acetate	10–1000 µg/ml	-	Thompson et al.
rat hepatocytes	zinc 2,4-pentanedione	10–1000 µg/ml	-	(1989)
<i>In vitro</i> human cells				
Chromosomal aberration				
human lymphocytes	zinc chloride	$3 \times 10^{-4} - 3 \times 10^{-5}$ mol/litre	+	Deknudt & Deminatti (1978)
<i>In vivo</i> animal				
Sister chromatid exchange				
sheep bone marrow cell	emission dust	32 g/day	+	Bires et al. (1995)
rat bone marrow	zinc chloride	240 mg/kg	+	Kowalska-Wochna et al. (1988)

Table 26 (contd.)

Micronucleus test					
mouse	zinc sulfate	0.1–0.3 mmol/litre per kg	-	Gocke et al. (1981)	
mouse	zinc oxide/hexachloroethane smoke	0.1 ml smoke condensate	-	Karlsson et al. (1991)	
Chromosomal aberration					
rat bone marrow	zinc oxide	0.5–1 mg/m ³	+	Voroshilin et al. (1978)	
mouse	zinc chloride	15 g/kg	-	Vilkina et al. (1978)	
mouse bone marrow	zinc chloride	0.5% zinc for 1 month	-	Deknudt & Gerber (1979)	
mouse bone marrow	zinc chloride	0–15 mg/kg	+	Gupta et al. (1991)	
rat bone marrow	zinc chloride	240 mg/kg for 14 days	+	Kowalska-Wochna et al. (1988)	
sheep bone marrow cell	emission dust	32 g/day	-	Bires et al. (1995)	
Dominant lethal mutation					
mouse	zinc chloride	15 mg/kg	-	Vilkina et al. (1978)	
<i>In vivo</i> human					
Chromosomal aberration	zinc smelter dust cadmium plant fumes/dust		+	Bauchinger et al. (1976)	
			-	Deknudt & Leonard (1975)	

also been shown to be co-carcinogenic in other studies (Wallenius et al., 1979).

7.8 Interactions with other metals

In general, zinc shows a low toxicity to animals, but at high exposure levels it can interact with other trace elements, especially copper, resulting in toxicity, which is usually due to depletion of these elements, and leading to nutritional deficiencies. It has been postulated (Hill & Matrone, 1970) that elements with similar properties will act antagonistically to each other biologically, as a result of their competition for binding sites on proteins that require metals as cofactors. The interaction of zinc with other metals, such as copper, iron and calcium, has been reviewed in some detail elsewhere (Walsh et al., 1994; Bremner & Beattie, 1995).

7.8.1 Zinc and copper

Copper deficiency induced by excess zinc intake in experimental animals is manifested by reduced copper concentrations in liver, serum and heart, and decreased activities of copper metalloenzymes (Duncan et al., 1953; Van Reen, 1953; Cox & Harris, 1960; L'Abbe & Fischer, 1984).

Excessive zinc intake has been shown to inhibit intestinal absorption, hepatic accumulation and placental transfer of copper, as well as to induce clinical and biochemical signs of copper deficiency (Campbell & Mills, 1974; Bremner et al., 1976; Hall et al., 1979; L'Abbe & Fischer, 1984). Results of an isotope experiment suggest that zinc interferes with copper metabolism by decreasing utilization and increasing excretion of copper in the rat, but has little effect on copper absorption (Magee & Matrone, 1960). High levels of zinc in the diet have been shown to induce *de novo* synthesis of metallothionein in a dose-related fashion. It has been suggested that the induced metallothionein sequesters copper, reducing its bioavailability (Hall et al., 1979). Animals deficient in copper are infertile (Mertz, 1987). Richmond (1992) decreased the mortality of pups delivered of copper-deficient dams by injection of oxytocin at term. Atrophy of the exocrine pancreas in copper deficiency (Alvarez et al., 1989) may be secondary to vascular changes (Weaver, 1989). Allen et al. (1982) found that copper-deficient rats responded poorly

to injection of thyrotropin-releasing hormone. Deficient, non-anaemic rats at 24 °C became hypothermic with, *inter alia*, decreased concentration of triiodothyronine in plasma. Mice deficient in copper (Lynch & Klevay, 1992) have a bleeding tendency characterized, *inter alia*, by increased activated partial thromboplastin time, prothrombin time.

Findings of infertility, thyroid abnormalities, pancreatic changes, coagulation defects and bone pathology in experiments using increasingly high doses of zinc may impair copper utilization. Characterization of the copper contents of diets and the copper levels in organs is important in understanding the relevance of these effects. If the effects of high doses of zinc are not accompanied by decreased copper in target organs, it seems likely that they are related to zinc intoxication.

In a study designed to measure the level of zinc at which copper metabolism begins to be affected, Wistar rats (10 per group) were fed diets containing zinc as zinc sulfate at 0, 15, 30, 60, 120 or 240 mg/kg. Ceruloplasmin activity is significantly reduced at doses of 30 mg/kg and greater, and the number of rats with extremely low ceruloplasmin activity increases with increased zinc levels in the diet. The level of zinc at which 50% of animals would have abnormally low ceruloplasmin activity was calculated to be 125–129 mg/kg. Liver superoxide dismutase and heart cytochrome *c* oxidase activities were significantly reduced at 120 and 240 mg/kg respectively, as compared to controls (L'Abbe & Fischer, 1984).

Mink (11 females and 3 males per group) were exposed to a basal diet or to a diet supplemented with zinc as zinc oxide at 1000 µg/g throughout the mating, gestation and lactation periods. The basal diet contained zinc at 20.2 µg/g and copper at 3.1 µg/g. Supplementation of the basal diet with zinc had no significant effect on the body, liver, spleen or kidney weights of the adult female mink. No significant differences from control females were seen in the haematological parameters measured. Clinical signs consistent with copper deficiency (alopecia, anaemia or achromotrichia) were not observed in the adult mink. All females on the basal diet whelped, but only 8 females on the zinc-supplemented diets produced offspring. The body weights of male kits born to dams consuming the zinc supplemented diet were significantly lower than those of

controls at 12 weeks of age. No significant differences were noted in erythrocyte or leukocyte count, haemoglobin concentration, mean corpuscular haemoglobin concentration, mean corpuscular volume or the leukocyte differential count between the zinc-treated and control kits bled at 8 weeks of age. There was a significant decrease in haematocrit value in the zinc-exposed kits. The T-cell mitogenic response was significantly reduced in the zinc-treated mink kits; however, the immunosuppression was reversible, as a normal response was seen approximately 14 weeks after the kits were weaned and placed on basal diets. In 3- to 4-week-old kits, whelped and nursed by females, that were fed a zinc-supplemented diet, greying of the fur developed around the eyes, ears, jaws and genitals, with a concomitant hair loss and dermatosis in these areas. The condition was stated to be consistent with copper deficiency; it spread over much of the body within a few weeks and persisted for several weeks after the kits were removed from the supplemented diets (Bleavins et al., 1983).

Pregnant sheep (5–12 per group) were fed diets containing zinc as zinc sulfate at 0, 30, 150 and 750 mg/kg for approximately 110 days. The diet contained copper at 2.5 mg/kg. Food consumption, weight gain and efficiency of food utilization were reduced in ewes in the 750-mg dose group. The reduction in feed intake began within 10 days of the beginning of the treatment. Some 20 days prior to parturition, copper status in the highest dose group was severely depressed, with reductions in plasma copper, ceruloplasmin and amine oxidase activity when compared to the group on the basal diet. The concentration of zinc in plasma was greatly increased in the 750 mg dose group only. Reproductive performance was severely impaired in the highest dose group, with increased incidence of non-viable lambs, defined as lambs which were aborted, stillborn or died within 7 days of birth. The cause of death in these lambs was not determined. Lambs born alive in the high dose group were weak, did not suckle, displayed ataxia and died following convulsions within 48 h of birth. Of 20 lambs conceived in the high-dose group, only one survived longer than 5 weeks. Two findings common to all non-viable lambs from the high-dose group were high tissue zinc concentrations and low tissue copper concentrations; radiographs of these lambs revealed arrested growth in the long bones. Addition of copper (10 mg/kg of diet) to another group of pregnant sheep fed diets containing zinc at 750 mg/kg, prevented the development of

copper deficiency, but failed to prevent the adverse effect of high zinc on weight gain, feed consumption, efficiency of feed utilization and lamb viability. The doses of zinc in pregnant ewes were calculated to be 20 mg/kg body weight per day at the start of the study, declining to 10 mg/kg per day with reduced food intake. It was postulated that the reduced viability of lambs may have been due to fetal malnutrition caused by the reduced maternal food intake and food utilization, or alternatively to direct toxicity of zinc to the fetus (Campbell & Mills, 1979).

The reverse interaction, namely the effect of copper on zinc status, is less clear. Excessive copper can affect zinc metabolism in some species, but zinc absorption does not appear to be seriously affected. The intestinal absorption of zinc in the rat was decreased by 20% when the dietary copper level was increased from 3 to 24 mg/kg, with no further decreases seen at copper levels of 300 mg/kg (Hall et al., 1979). However, there is some evidence for competition and/or inhibition of copper or zinc uptake into intestinal cells when the luminal concentration of the respective metal is very high (Oestreicher & Cousins, 1985).

7.8.2 Zinc and other metals

High levels of zinc (0.5–1%) fed to rats have been shown to interfere directly with iron metabolism (Magee & Matrone, 1960). The occurrence of hypochromic, macrocytic anaemia in rats following the ingestion of excessive zinc and the reversal of this anaemia by iron supplementation demonstrate the interaction between iron and zinc (Cox & Harris, 1960; Magee & Matrone, 1960). Zinc intoxication affects iron metabolism by increasing the iron turnover, decreasing the life span of erythrocytes and decreasing the hepatic accumulation of iron as ferritin (Settlemyre & Matrone, 1967a,b).

Zinc appears to be a less effective inhibitor of iron absorption than iron is of zinc absorption. In iron-deficient mice and rats, the oral absorption of zinc is greatly increased, which has been interpreted to indicate a shared transport pathway (Pollack et al., 1965; Forth & Rummel, 1973; Hamilton et al., 1978). Iron absorption and distribution is altered by zinc deficiency. A marked increase in iron and a decrease in zinc concentration in various

organs, such as the liver, bone, pancreas and testes, have been observed in zinc-deficient animals in comparison to pair-fed controls. These changes are reversed following zinc supplementation (Prasad et al., 1967; Prasad et al., 1969; Petering et al., 1971).

Excess dietary zinc administered to pregnant rats and also to weanling and adult rats lowers the tissue iron content of the treated animals (Duncan et al., 1953; Cox & Harris, 1960; Magee & Matrone, 1960; Cox et al., 1969). In another study, reduced tissue iron and copper levels in weanling rats and reductions in calcium and phosphorus deposition in bones of young rats were observed following feeding with excess zinc (Sadasivan, 1951). High levels of dietary zinc have been also been shown to interfere with the metabolism of calcium and to increase total calcium and concentrations of calcium in the liver, but to decrease these levels in the body of exposed fetuses (Cox et al., 1969). Elevated magnesium concentrations (mg/kg) but not total magnesium content were detected in the liver and body of fetuses from mothers fed excess zinc (Cox et al., 1969).

Interaction between zinc and cadmium in animals has been reviewed elsewhere (IPCS, 1993). Supplementation with zinc has been shown to prevent the teratogenic and carcinogenic effects of cadmium: the induction of severe facial abnormalities in hamster embryos induced by cadmium (2–4 mg/kg administered intravenously) was prevented by the simultaneous administration of zinc (as zinc sulfate at 992 mg/kg) (Ferm & Carpenter, 1968); and the induction of interstitial cell tumours in rats and mice was prevented by concurrent zinc supplementation (Gunn et al., 1963).

7.9 Zinc deficiency in animals

Zinc is essential for DNA replication, RNA polymerases, protein synthesis and many metabolic processes. All cell replication, protein synthesis and growth processes are partially dependent upon zinc. Systemic depletion of this element therefore inevitably leads to deleterious effects.

The essentiality of zinc for growth has been described elsewhere (Todd et al., 1934; Hove et al., 1937; Hove et al., 1938). In experimental animals, restriction of zinc in the diet leads to an

immediate decline in plasma zinc levels, followed by a loss of appetite and poor growth, which are evident within a few days of zinc depletion. Further symptoms can include dermatitis, alopecia and testicular atrophy (Macapinlac et al., 1966, 1968; Chesters & Quarterman, 1970; Wallwork et al., 1981). Zinc deficiency in experimental animals is characterized by rash, alopecia, hyperkeratosis, parakeratosis and hypopigmentation (O'Dell et al., 1959; Oberleas et al., 1962). In monkeys, as the deficiency progresses, animals stand in a hunched position, have an unsteady gait and unkempt fur, become emaciated and eventually die (Macapinlac et al., 1967; Sandstead et al., 1978; Swenerton & Hurley, 1980). It has been observed that the healing of wounds is retarded in zinc-deficient rats and that healing can be accelerated with zinc supplementation (Sandstead et al., 1970).

Zinc deficiency has an adverse effect on the pancreas of experimental animals. *In vitro* assays in pancreatic preparations from rats fed a zinc-deficient diet showed a marked impairment of the insulin response to glucose, which was directly proportional to the degree of zinc deficiency (Huber & Gershoff, 1973; Jhala & Baly, 1991). Plasma insulin levels in response to glucose injection were decreased in obese but not in lean rats fed a zinc-deficient diet over 8 weeks (Zwick et al., 1991). Additionally, a markedly zinc-deficient diet in rats produced a significant reduction in the total pancreatic content of zinc within 2 days and was associated with a more than 50% reduction in the activity of γ -glutamyl hydrolase (an indicator of pancreatic activity) in pancreatic tissue (Canton & Cremin, 1990). Rapid loss of pancreatic carboxypeptidase activity has been demonstrated under similar conditions (Mills et al., 1967).

Zinc deficiency has been shown to be correlated with a diminished activity of some enzymes. The level of a serum enzyme alkaline phosphatase decreased in zinc-deficient animals and increased with zinc replenishment (Sadasivan, 1952; Van Reen, 1953). It has been postulated that the promoter region of the gene for intestinal alkaline phosphatases contains a metal-responsive element, and that zinc deficiency leads to suboptimal transcription of this type of enzyme (Stuart et al., 1985; Millan, 1987). Zinc deficiency has also been reported to impair the activity of intracellular hepatic enzymes. The biotransformation of some pharmacological agents was reduced in zinc-deficient rats and was also associated with a

decrease in the cytochrome P450 content of microsomes (Becking & Morrison, 1970).

Serum lipid concentrations were shown to be lower than normal in zinc-deficient rats, and this was postulated to be caused by the impairment of intestinal absorption of lipids by zinc deficiency (Koo et al., 1987).

Adverse reproductive effects were seen in rats when their diets were low in zinc (Hurley & Swenerton, 1966; Hurley & Shrader, 1974). Spermatogenesis was shown to be arrested in weanling rats and the germinal epithelium of the testes was atrophic (Barney et al., 1968). The menstrual cycle of rats (Apgar, 1970) and monkeys (Swenerton & Hurley, 1980) was also reported to be impaired, and ovarian follicular development appeared to be retarded (see also Evenson et al., 1993 for another study in rats).

Zinc deficiency is lethal or injurious to the embryos and fetuses of experimental animals. Evidence indicates that adequate levels of zinc are essential for conception (Swenerton & Hurley, 1980), blastula development and implantation (Hurley & Shrader, 1974), organogenesis (Blamberg et al., 1960; Kienholz et al., 1961; Hurley & Swenerton, 1966), fetal growth (McKenzie et al., 1975; Fosmire et al., 1977), prenatal survival (Hurley & Swenerton, 1966) and parturition (Apgar, 1973). Severe zinc deficiency results in high fetal resorption, with malformation of the skeleton, nervous system and viscera found in most of the surviving fetuses (Hurley & Swenerton, 1966; Hurley et al., 1971; Hurley & Shrader, 1972). Impaired synthesis and/or metabolism of DNA is postulated to cause these abnormalities (Swenerton et al., 1969; Dreosti et al., 1972; Dreosti & Hurley, 1975).

Zinc deficiency impairs development of the brain and has been shown to cause long-term behavioural consequences in rats. Evidence for the essentiality of zinc for the maturation of brain was provided by studies in rats (Hurley & Swenerton, 1966; Warkany & Petering, 1972), which demonstrated a variety of malformations in the brains of offspring that had been deprived of zinc early in gestation. Inhibition of DNA synthesis in neural crest cells is postulated to be one of the causes of such malformations (Swenerton et al., 1969). Zinc-deprived 10-day-old suckling rats showed

suppression of incorporation of thymidine into their DNA (Sandstead et al., 1972). The cerebellum of a 21-day-old rat showed histological evidence of retarded maturation (Buell et al., 1977), and impaired division and migration of external granular cell neurons (Dvergsten et al., 1983; Dvergsten et al., 1984). The long-term functional significance of zinc deficiency in the fetus and neonate was studied in rats deprived of zinc during late gestation and/or suckling. Severe maternal deprivation (zinc at < 1 mg/kg in the diet) on days 14–20 of gestation caused stunting and a decrease in brain cell number in fetuses (McKenzie et al., 1975). Nutritionally rehabilitated offspring subsequently showed active avoidance of shock and an increased aggressive response to shock (Halas et al., 1975, 1976, 1977). Severe maternal zinc deprivation throughout nursing impaired growth of suckling pups and subsequently increased errors by nutritionally rehabilitated offspring in maze tests (Lokken et al., 1973; Halas et al., 1983). Reference to or long-term memory of shock on days 18–21 of nursing was also impaired (Halas et al., 1979). In rats that were mildly zinc-deprived during gestation and lactation (zinc at 10 mg/kg) where there was only a minimum effect on the growth of pups, it was subsequently revealed that the zinc-rehabilitated offspring had deficits in working memory (Halas et al., 1986). Maternal zinc deprivation of rhesus monkeys throughout most of the third trimester (Sandstead et al., 1978) and throughout gestation and lactation (Golub et al., 1985) caused acrodermatitis in the dam and subsequent reduction of exploration and play in infants during weaning. Later study of these animals found impaired ability to solve complex learning sets at 300 and 700, but not at 1000 days (Strobel & Sandstead, 1984).

Immune function was shown to be adversely affected by zinc deficiency. Calves with an inborn error in zinc absorption display thymic hypoplasia, an increased susceptibility to infection, growth failure, diarrhoea, dermatitis and death. Treatment with zinc can prevent and cure the illness (Brummerstedt et al., 1977). However, it may be difficult to separate immune deficiency from malnutrition in this case. In rats and mice, zinc deficiency was reported to impair the growth of the thymus and to retard both cellular and humoral immunity (Fraker et al., 1978; Luecke et al., 1978; Fernandes et al., 1979; Pekarek et al., 1979; Lennard, 1980). Mice fed diets deficient in zinc for 30 days developed thymic atrophy, had markedly depleted numbers of lymphocytes and macrophages in the spleen, and showed

a markedly reduced ability to produce antibody-mediated responses to T-cell dependent and T-cell independent antigens. Delayed-type hypersensitivity responses, cell-mediated responses to tumour antigens and the function of natural killer cells were also significantly reduced (Fraker et al., 1978, 1986; Fernandes et al., 1979). However, in another study, it was shown that the ability of lymphocytes to proliferate and to produce interleukins and mitogenic-stimulated antibody responses was normal in zinc-deficient mice (Cook-Mills & Fraker, 1993). The reasons for this discrepancy are unclear, but mitogenic responses are a less reliable indicator of immune reactivity than antigen-specific responses. It has been established that, although the T-cell:B-cell ratio is unaffected, the total number of lymphocytes is significantly reduced in zinc-deficient mice (King & Fraker, 1991). The incidence of oesophageal tumours induced by methylbenzyl nitrosamine (MBN) was higher in rats fed diets low in zinc, at 3 mg/kg compared to rats fed diets containing 60 mg/kg (Fong et al., 1978). This effect may arise through the oesophageal epithelium being damaged by zinc deficiency, making it sensitive to MBN and/or its activated metabolite (Fong et al., 1984). The mechanism appears to be via the activation of a specific cytochrome P450 by zinc deficiency, with a resultant increase in MBN-induced formation of O⁶-methylguanine in oesophageal DNA (Barch & Fox, 1987). Studies investigating the effect of dietary zinc deficiency in oesophageal carcinogenesis are reported in section 8.3.7.

8. EFFECTS ON HUMANS

In the general population, essential elements have a range of acceptable exposures at which there are no untoward effects. Below this range there is the potential for effects associated with deficiency, and above it, effects associated with toxicity. The curve describing this concept of acceptable intake is shown in Chapter 10 (Fig. 1). As zinc is an essential component in a multiplicity of enzymatic reactions (see section 6.5.2), there is a need to define the range of acceptable intake to provide for biological requirements that balance the consequences of deficit and excess. In the position of balance, there is homeostasis, with optimum health. An additional factor is the consequence of interactions of zinc with other elements, which can introduce a toxicity mediated by zinc excess (Hill & Matrone, 1970). In this Chapter, the effects associated with zinc deficiency are described, along with the adverse effects associated with zinc excess, including those mediated by interaction with other elements.

8.1 Human dietary zinc requirements

8.1.1 *Estimation of zinc requirements*

There are inherent difficulties in estimating zinc requirements for humans, with a number of physiological, dietary and environmental factors affecting various populations. Estimates have been made using metabolic balance studies, in which zinc intake was compared with zinc excretion in the urine and faeces (Sandstead, 1984, 1985; Sandstead et al., 1990), and using additional factorial calculations that account for the zinc required for growth, losses (including zinc lost in sweat, shed hair and skin, semen and milk) and bioavailability (Sandstead, 1973; King & Turnlund, 1989). Growing infants, children, growing adolescents, and pregnant and lactating mothers require more zinc per kilogram of body weight than do mature adults (WHO, 1996b). The factorial estimates for zinc requirements are outlined in Tables 27 and 28.

A major factor affecting zinc requirements is the variation in the percentage absorption of zinc from differing dietary sources; this is discussed in section 6.1.2.

Table 27. Provisional dietary requirements for zinc in relation to estimates of retention, losses and availability^a

Age	Peak daily retention (mg)	Urinary excretion (mg)	Sweat excretion (mg)	Total required (mg)	Intake necessary (mg) in daily diet for available zinc content of		
					10%	20%	40%
Infants							
0–4 months	0.35	0.4	0.5	1.25	12.5	6.3	3.1
5–12 months	0.2	0.4	0.5	1.1	11.0	5.5	2.8
Males							
1–10 years	0.2	0.4	1.0	1.6	16.0	8.0	4.0
11–17 years	0.8	0.5	1.5	2.8	28.0	14.0	7.0
18+ years	0.2	0.5	1.5	2.2	22.0	11.0	5.5
Females							
1–9 years	0.15	0.4	1.0	1.55	15.5	7.8	3.9
10–13 years	0.65	0.5	1.5	2.65	26.5	13.3	6.6
14–16 years	0.2	0.5	1.5	2.2	22.0	11.0	5.5
17+ years	0.2	0.5	1.5	2.2	22.0	11.0	5.5

Table 27 (contd.)

Age	Peak daily retention (mg)	Urinary excretion (mg)	Sweat excretion (mg)	Total required (mg)	Intake necessary (mg) in daily diet for available zinc content of		
					10%	20%	40%
Pregnant women							
0–20 weeks	0.55	0.5	1.5	2.55	25.5	12.8	6.4
20–30 weeks	0.9	0.5	1.5	2.9	29.0	14.5	7.3
30–40 weeks	1.0	0.5	1.5	3.0	30.0	15.0	7.5
Lactating women	3.45	0.5	1.5	5.45	54.5	27.3	13.7

^a WHO, 1973. The above estimates were based on the assumption that the fat-free tissue concentration of zinc in humans is approximately 30 µg/g. This figure is equivalent to 2.0 g of zinc in the soft tissues of an adult male and 1.2 g in the soft tissues of an adult female, as determined from lean body mass. The zinc requirement at various ages was determined from the change in lean body mass with age. Bone zinc was not included in these calculations, because zinc in bone is relatively sequestered from the metabolically active pool of body zinc. The zinc content of sweat is based on an assumed zinc surface loss of 1 mg/litre. The estimated requirement for lactation is based on a zinc content in milk of 5 mg/litre and a daily milk secretion of 650 ml. The urinary excretion of zinc is based on reported levels.

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Table 28. Dietary reference values for zinc (mg/day)

Age	United Kingdom ^a			USA RDA ^b	WHO ^c	European DRI ^d
	LNRI	EAR	RNI			
Infants						
0–3 months	2.6	3.3	4.0	5.0		
4–6 months	2.6	3.3	4.0	5.0		
7–12 months	3.0	3.8	5.0	5.0	5.6	4.0
1–3 years	3.0	3.8	5.0	10.0	5.5	4.0
4–6 years	4.0	5.0	6.5	10.0	6.5	6.0
7–10 years	4.0	5.4	7.0	10.0	7.5	7.0
Males						
11–14 years	5.3	7.0	9.0	15.0	12.1	9.0
15–18 years	5.5	7.3	9.5	15.0	13.1	9.5
19–50+ years	5.5	7.3	9.5	15.0	9.4	9.5
Females						
11–14 years	5.3	7.0	9.0	12.0	10.3	9.0
15–18 years	4.0	5.5	7.0	12.0	10.2	7.0
19–50+ years	4.0	5.5	7.0	12.0	6.5	7.1
Pregnancy	^c	^c	^c	15.0	7.3–13.3	^b
Lactation						
0–4 months				19.0	12.7	+5.0
4+ months				16	11.7	+5.0

DRI = dietary reference intake; EAR = estimated average requirement; LNRI = lower reference nutrient intake; RDA = recommended daily allowance; RNI = recommended nutrient intake

^a UK (1991).

^b US National Academy of Sciences (1989).

^c WHO (1996b); normative requirement for diet of moderate zinc availability

^d EU (1993); no increment.

The effects of dietary supplementation on humans have recently been reviewed (Gibson, 1994). Tables 29, 30 and 31 provide a summary, taken from this review, of the effects of supplementation in infants, children and lactating women.

Table 29. Double-blind zinc supplementation studies in infants

Country	Subjects and treatment	Mean plasma zinc levels ($\mu\text{mol/litre}$)				Growth effects and other responses	Reference
		Zinc supplementation		Control			
		Start	End	Start	End		
USA	68 normal healthy full-term male infants at birth; studied for 6 months double-blind study; formula with zinc at 1.8 mg/litre or 5.8 mg/litre		119	110		improved weight and length in males only	Walravens & Hambidge (1976)
France	57 normal healthy infants at 5.4 months old, studied for 3 months double-blind study; zinc at 5 mg/day (25) or placebo (32)					improved weight gain; improved length in males only	Walravens et al. (1992)
USA	50 failure-to-thrive infants, 8–27 months old, studied for 6 months randomized double-blind trial, pair matched; zinc at 5.7 mg/day as syrup (25) or placebo (25)	10.7	9.8	10.7	10.4	improved weight especially in males; tendency to increased activity of serum alkaline phosphatase in zinc group	Walravens et al. (1989)

Table 29 (contd.)

Country	Subjects and treatment	Mean plasma zinc levels ($\mu\text{mol/litre}$)				Growth effects and other responses	Reference
		Zinc supplementation		Control			
		Start	End	Start	End		
Chile	32 marasmic infants, 7–8 months old, studied for 90 days randomized double-blind trial; zinc at 2 mg/kg daily in solution (16) or placebo (16)	14.7	15.6	16.1	15.6	weight-for-length effect; decrease in percentage of anergic infants; increase in serum IgA in zinc group	Castillo-Duran et al. (1987)
Chile	39 severely malnourished infants studied for 104 days double blind trial; zinc at 1.9 mg/kg (19) or 0.35 mg/kg in daily formula (20)	19.4	18.6	23.4	18.0	linear length effect; improved immune function	Schlesinger et al. (1993)

Table 29 (contd.)

Bangladesh	60 severely malnourished infants 5–60 months old studied for 3 weeks rice-based diet <i>ad lib</i> with vitamins and minerals; zinc at 10 mg/kg daily if < 6 kg or 50 mg/day if > 6 kg as zinc sulfate; non-supplemented group (30)	8.2	18.5	7.9	10.6	improved weight gain and weight for length	Khanum et al. (1988)
Bangladesh	65 children with AD and 152 with PD 3–24 months old supplemented for 2 weeks followed for 2 or 3 months in a double-blind randomized study after supplementation for 2 weeks with zinc at 15 mg /kg daily or placebo					improved length gain in AD group, and in PD with < 90% weight/age and 90% height/age; reduced no. of episodes of diarrhoea in AD and PD groups and attack rate of respiratory tract infections in AD group only	Roy et al. (1992)

AD = acute diarrhoea; PD = persistent diarrhoea; SGA = small for gestational age

Table 30. Double-blind zinc supplementation studies in children

Country, date	Subjects and treatment	Dietary zinc intake (mg)	Mean plasma zinc levels ($\mu\text{mol/litre}$)				Growth effects and other responses	Reference
			Zinc supplementation		Control			
			Start	End	Start	End		
Egypt 1965–1966	90 growth-retarded schoolboys, 11–18 years old studied for 5.5 months randomized trial; zinc at 14 mg (30) or placebo (30); capsules given at school	14	1–7	19–2	11–7	13–3	no weight or height effects; no difference in sexual maturation; no effect on serum alkaline phosphatase	Carter et al. (1969)
Iran 1967–1968	60 growth-retarded schoolboys, 12–18 years old, studied for 17 months (5 months trial, 7 months rest, 5 months trial); first 5 months, 28 mg of zinc (20), 67 mg of iron (20) or placebo (20); second 5 months, micro-nutrients (20), micronutrients + 40 mg of zinc (20) or placebo (20); capsules given at school	12	17–2	14–7	11–6	14–1	no weight or height effects; difference in sexual maturation	Ronaghy et al. (1968)

Table 30 (contd.)

Iran 1969–1971	50 growth-retarded schoolboys, 13 years old, studied for 17 months (5 months trial, 7 months rest, 5 months trial) non-randomized trial; micronutrients (20), micronutrients + 40 mg of zinc (20) or placebo (10); capsules given at school	12	8–2	10–2	10–5	10–7	weight and height effects; difference in bone age; tendency for faster sexual development; no effect on serum alkaline phosphatase	Ronaghy et al. (1974)
USA, Colorado	40 growth-retarded, low-zinc-status children, 2–6 years old, studied for 1 year randomized pair-matched trial; zinc at 10 mg/day (20) or placebo (20); syrup given by parents at home	4–6	10–7	10–8	11–3	11–3	height effect (especially in boys); increase in appetite	Walravens et al. (1983)
Canada 1986	60 growth-retarded boys, 5–7 years old, studied for 12 months randomized pair-matched trial; zinc at 10 mg/day (30) or with placebo (30); fruit juice drink given by parents at home	6–4	15–6	16–2	16–5	16–4	height effect only in subjects with low hair zinc (<1.68 $\mu\text{mol/g}$); increase in appetite perceived by parents	Gibson et al. (1989b)

Table 30 (contd.)

Country, date	Subjects and treatment	Dietary zinc intake (mg)	Mean plasma zinc levels ($\mu\text{mol/litre}$)				Growth effects and other responses	Reference
			Zinc supplementation		Control			
			Start	End	Start	End		
Thailand 1989–1990	133 children, 6–13 years old, with suboptimal zinc and vitamin A nutriture studied for 6 months randomized pair-matched trial; zinc at 25 mg/day (33), vitamin A (33), vitamin A + zinc (32) or with placebo (35); capsules taken on school days	4.3	13.2	19.0	13.2	14.3	no weight or height effects; increase in serum alkaline phosphatase activity; improved dark adaptation; improved conjunctival integrity	Udomkesmalee et al. (1992)
Gambia 1989–1990	109 apparently healthy children, 0.5–3 years old, studied for 15 months randomized group-matched trial; 70 mg of zinc (55) or placebo (54); drink given twice a week at clinic						no weight or height effects; increase in arm circumference; less malaria; improved intestinal permeability	Bates et al. (1993)

Table 30 (contd.)

Guatemala 1989	162 schoolchildren, 6–8 years old, studied for 25 weeks randomized pair-matched trial; micronutrients (82), micronutrients + zinc at 10 mg/day (80); chewable tablet given at school on weekdays	10	14.2	16.2	14.4	14.9	no weight or height effects; increase in triiceps skinfold; smaller decrease in mid-arm circumference; no increase in serum alkaline phosphatase	Cavan et al. (1993)
Chile 1991	46 short-stature schoolchildren, 6–12 years old, consuming diets providing 50–60% of normal daily zinc intake, studied for 12 months randomized study; zinc at 10 mg/day or placebo						no weight effect; height effect in males only; no difference in plasma zinc	Castillo-Duran et al. (1995)
Chile 1993	98 healthy pre-school children studied for 14 months zinc at 10 mg/day or placebo						height effect in males; trend towards improved immune function and reduced giardiasis	Ruz et al. (1997)

Table 31. Double-blind zinc supplementation studies in lactating women

Country	Subjects and treatment	Dietary zinc intake (mg)	Response	Reference
USA, Colorado	53 middle-income lactating women, studied for varying durations up to 9 months controlled trial; zinc at 15 mg/day (14), placebo (39) or control (8); tablets taken at home	12.2	decreased fall in milk zinc levels	Krebs et al. (1985)
USA, Indiana	49 middle-income mothers studied for first 6 months of lactation controlled trial; micronutrients (25) or micronutrients + zinc at 25 mg/day (24); different commercial supplements taken at home	11.2	higher milk zinc levels	Karra et al. (1986)
USA, Maryland	40 middle-income women studied for first 6 months of lactation randomized double-blind trial; micronutrients (20) or micronutrients + zinc at 25 mg/day (20); tablets taken at home	12	no effect on milk zinc levels	Moser-Veillon & Reynolds (1990)

Methods for the assessment of zinc status in humans are discussed in section 6.5.1.

8.2 Zinc deficiency

8.2.1 Clinical manifestations

Cases of severe zinc deficiency are now rare, but mild deficiency during periods of rapid growth, pregnancy, synthesis of new tissue, and in persons consuming plant-based diets, is not uncommon. Zinc deficiency also occurs in the presence of certain disease states such as malabsorption syndromes, renal and hepatic diseases, and in association with burns and alcoholism. Two genetic disorders, acrodermatitis enteropathica and sickle-cell disease, are associated with suboptimal zinc status.

The first cases of human zinc deficiency were reported in the Middle East among adolescent dwarves in the 1960s (section 8.2.4). Since those first reports, mild zinc deficiency has been reported in infants and younger children living both in developing and in industrialized countries.

The health effects associated with zinc deficiency in humans have been extensively reviewed (Prasad, 1988; Aggett, 1989; Clegg et al., 1989; Hambidge, 1989; Keen & Hurley, 1989; Walsh et al., 1994). Zinc deficiency has been classified into three syndromes (Henkin & Aamodt, 1983): acute, chronic and subacute zinc deficiency. The clinical symptoms range from neurosensory changes, oligospermia in males, decreased thymulin activity, decreased interleukin-2 production, hypogeusia and impaired neuropsychological functions (Prasad, 1988; Penland, 1991) in mild or marginal deficiency, through to growth retardation, male hypogonadism, and delayed wound healing with moderate deficiency, and alopecia, mental disturbances, cell-mediated immune disorders and pustular dermatitis in patients with severe zinc deficiency (Prasad, 1988). These conditions are generally reversible when the deficiency is corrected by zinc supplementation.

8.2.2 Brain function

In an experimental study (Henkin et al., 1975b) in which severe, acute zinc deficiency was induced in eight patients with scleroderma

by treatment with large doses of oral histidine, severe zincuria was produced, and plasma zinc levels decreased from 60–105 µg/dl to 40–60 µg/dl. Signs of zinc deficiency included anorexia, dysosmia, ataxia, tremor, loss of memory, impaired higher intellectual processes, paranoid ideation and receptive aphasia. Treatment with zinc by mouth improved signs within 24 h.

The effects of less severe zinc deficiency are less easily characterized and include reduced growth and impaired immune function (WHO, 1996b). In a study in which 14 men were fed diets providing zinc at a rate of 1, 2, 3, 4 or 10 mg/day for periods of 35 days in a 7-month study (Johnson et al., 1993), impaired neuromotor and cognitive function was observed (Penland, 1991), with significant decreases in sensory motor, attention, visual memory and spatial and perceptual tasks.

8.2.3 Immune function

In patients suffering from acrodermatitis enteropathica — a rare genetic defect affecting the assimilation of zinc — an increased incidence of secondary infections is seen, and T-cell numbers, thymic hormone levels and T-cell mediated cellular and humoral immunities are deficient (Aggett, 1989). Similar changes have been noted in other patients with zinc deficiency and with sickle-cell anaemia (Fraker et al., 1986; Endre et al., 1990), and patients with suboptimal zinc intakes have been reported to be at greater risk of infection and disease (Bogden et al., 1987). In an experimental study in which male volunteers with experimentally induced mild zinc deficiency had decreased interleukin-2 activity, a decreased T4+:T8+ ratio and increased T101 cells and serum immunoglobulin (Ig), these changes were corrected upon zinc repletion (Prasad et al., 1988). Immune function related to zinc deficiency has been reviewed by Keen & Gershwin (1990). It has been suggested that zinc may act as an antiviral agent. Possible mechanisms by which this could be achieved are inhibition of virus protein coat synthesis and prevention of virus entry into the cell (Korant & Butterworth, 1976; Prasad, 1996).

8.2.4 Growth

Growth retardation and hypogonadism were reported in adolescents in the Middle East, and these effects were believed to be

related to inadequate dietary zinc intake (Prasad et al., 1961, 1963b). The principal features of this syndrome were growth failure and delayed sexual maturation, giving 16- to 18-year-olds a physical appearance resembling that of prepubertal 9-year-olds, commonly associated with hepatosplenomegaly and iron deficiency. Zinc deficiency appears to be a major contributing factor in this syndrome. Administration of zinc, along with a balanced diet, produced accelerated growth, and enlargement of the penis and testes in males, and of breasts in females; a well-balanced diet alone was not followed by rapid improvement (Prasad et al., 1963a; Sandstead et al., 1967; Halsted et al., 1972). A subsequent series of zinc supplementation studies in Iran gave mixed results (Ronaghy et al., 1974; Mahloudji et al., 1975): there was a clear stimulation of growth after supplementation, but no significant stimulation of gonadal development (Ronaghy et al., 1974). Supplementation with zinc plus iron did not stimulate growth (Mahloudji et al., 1975).

Details of more recent double-blind zinc supplementation studies conducted on infants and children are reviewed in Gibson (1994).

8.2.5 Dermal effects

Severe zinc deficiency resulting from total parenteral nutrition without zinc (Arakawa et al., 1976; Kay et al., 1976), and in patients suffering from acrodermatitis enteropathica (Aggett, 1989) leads to dermatological effects, including erythematous scaling eruptions in the naso-labial and retro-auricular folds, with the dermatitis extending to the trunk and becoming exudative upon continued zinc deficiency (total parenteral nutrition), and bullous pustular dermatitis of the extremities and the oral, anal and genital areas, combined with paronychia and generalized alopecia (acrodermatitis enteropathica).

8.2.6 Reproduction

An association between low serum zinc levels and reproduction was made when one of 83 infants in a series of studies (Jameson, 1976) showed a congenital cardiac malformation, with a ventricular septum defect and coarctation of the aorta. The infant's mother had shown the lowest serum zinc level (12.2 $\mu\text{mol/litre}$) in the 13th week of gestation, but all other laboratory findings were normal. In women with complications such as abnormal labour or atonic bleeding,

serum zinc concentrations had been significantly reduced during early pregnancy. Additionally, of 316 pregnancies, a high proportion (60%) of the women who gave birth to infants with congenital defects had shown low serum zinc concentrations in the first trimester.

In a study in which 450 women were followed during and after pregnancy (Mukherjee et al., 1984), plasma zinc was reported to be an indicator of feto-maternal complications, including fetal distress and maternal infections, for those women in the lowest quartile for plasma zinc. In a study in low-income women, there was a significantly higher prevalence of low birth weight in the infants of mothers in the lowest quartile for plasma zinc at 16 weeks gestation than in those born to the other mothers (Neggers et al., 1990).

Studies to examine whether maternal zinc status is a useful predictor of pregnancy outcome have produced mixed results. Scholl et al. (1993), in a cohort study of pregnant girls and women of low socioeconomic status, reported that low dietary intakes of zinc (< 6.0 mg/day) were associated with increased risk of low-birth-weight infants, after controlling for energy intake and other variables known to influence outcome. Some studies (including Hunt et al., 1984; Cherry et al., 1989; Goldenberg et al., 1995), but not all double-blind supplementation trials have provided further support for this suggestion. In a study by Tamura & Goldenberg (1996) of 580 indigent African-American pregnant women, those randomly assigned to a zinc-supplemented group (25 mg of zinc daily as zinc sulfate) at 19.2 weeks of gestation had infants with a significantly higher birth weight (126 g; $P = 0.03$) and head circumference (0.4 cm; $P = 0.04$) than infants born to mothers in the placebo group. The results suggested that, by increasing the zinc intakes of pregnant women with suboptimal zinc nutriture, pregnancy outcomes could be improved. Recent reviews of this subject appear in Gibson (1994) and Tamura & Goldenberg (1996), and a summary of some of these findings is provided in Table 32.

8.2.7 Carcinogenicity

In a study in Chinese men aged between 45 and 75 years, the zinc levels in serum and hair were lower in those patients with oesophageal cancer (Lin et al., 1977). The results of these studies do

Table 32. Zinc supplementation studies in pregnant women

Country, date	Subjects	Treatment	Dietary zinc intake (mg)	Responses	References
United Kingdom 1985–1986	494 middle-class women studied for last 4 months of pregnancy	randomized double-blind trial; zinc at 20 mg/day (246) or placebo (248); capsules taken at home	9	no effect on birth weight; no differences in leukocyte zinc	Mahomed et al. (1989)
USA, New Orleans	556 low-income adolescent women studied for last 3 months of pregnancy	randomized double-blind trial; zinc at 30 mg/day (268) or placebo (288); tablets taken at home	30	no effect on birth weight; reduced rates of prematurity and neonatal morbidity	Cherry et al. (1989)
USA, Los Angeles 1981–1982	138 Hispanic teenagers studied for last 4 months of pregnancy	randomized double-blind trial; micronutrients (68) or micronutrients + zinc at 20 mg/day (70); capsules taken at home	9–8	no effect on birth weight	Hunt et al. (1995)

Table 32 (contd.)

Country, date	Subjects	Treatment	Dietary zinc intake (mg)	Responses	References
USA, Los Angeles	213 Hispanic low-income women enrolled at gestation age < 27 weeks	randomized double-blind trial; micronutrients (106) or micronutrients + zinc at 20 mg/day (107)	9–3	no effect on birth weight; reduced incidence of pregnancy-induced hypertension	Hunt et al. (1995)
United Kingdom	56 pregnant women at risk of small-for-gestational age infants studied for last 15–25 weeks of pregnancy	randomized double-blind trial; zinc at 22.5 mg/day (30) or placebo (26)	22.5	lower incidence of Intra-uterine growth retardation; reduction in induced labours and Caesarean sections	Simmer et al. (1991)
USA	46 pregnant middle-income women studied for 7–9 months	double-blind study; zinc at 15 mg/day (10) or placebo (36); tablet taken 2 h after dinner	11	no effect on birth weight; no other effects observed	Hambidge et al. (1983)

not provide evidence for any causal relationship between low plasma/serum zinc levels and an increased incidence of cancer in humans. Similarly, in another study by Lipman et al. (1987), mean plasma zinc and mean plasma vitamin A in the 21 oesophageal cancer patients were significantly lower than in the 17 patients with oesophagitis, or the 12 normal controls. However, there were no differences in oesophageal zinc content between the cancerous tissue and adjacent normal tissue, the oesophagitis tissue and adjacent normal tissue, and normal oesophageal tissue.

8.3 Zinc toxicity: general population

8.3.1 Poisoning incidents

A number of reports outline the effect of acute exposure to zinc in humans. However, these reports are generally old and poorly documented, with inadequate characterization of the actual exposure levels, although some estimates of exposure have been made. For example, high concentrations of zinc in drinks (up to 2500 mg/litre) have been linked with effects such as severe abdominal cramping, diarrhoea, tenesmus, bloody stools, nausea, and vomiting in 300 people, and symptoms of dryness of the mouth, nausea, vomiting and diarrhoea in more than 40 people (Brown et al., 1964). The amount of zinc ingested was estimated to be approximately 325–650 mg. Lethargy, along with drowsiness, unsteady gait, and increased serum lipase and amylase levels, was seen in an individual who had ingested 12 g of elemental zinc, equivalent to 150 mg/kg body weight, resulting in increases in blood zinc concentrations (Murphy, 1970). No gastrointestinal distress was reported and chelation therapy was effective in achieving clinical improvement and reducing blood zinc levels. Severe local burns, metabolic acidosis, hepatic damage, hyperamylasaemia, lethargy and hypertension resulting from the ingestion of zinc chloride/ammonium chloride soldering flux were reported in a 16-month-old boy who developed pancreatic exocrine insufficiency 5 months later (Knapp et al., 1994).

Excess hepatic copper and zinc levels in a small number of Cree and Ojibwa-Cree children were associated with severe chronic cholestatic liver disease progressing to end-stage biliary cirrhosis in these children (Phillips et al., 1996). It was postulated that the effects might have been due to an inborn error of metal metabolism,

secondary dietary or environmental factors, or genetic factors. Zinc and copper also appeared to be accumulated in transplanted livers, but these findings were not quantitative and there were no detectable histological effects following transplantation. There were no data to indicate that any exposure to excess zinc had occurred in these children.

8.3.2 Dermal effects

Contact dermatitis has been reported following use of shampoos containing zinc pyrithione (Nigam et al., 1988). The specific etiological role for zinc was not clear, and the dermal application of zinc as zinc oxide has not been associated with any adverse dermal effects in humans.

8.3.3 Immune function

An adverse lymphocytic response was reported in 11 healthy adult men who ingested 150 mg of elemental zinc twice a day for 6 weeks; the subjects also showed a reduction in the lymphocytic stimulation response to phytohaemagglutinin (up to 70% reduction at 6 weeks), chemotaxis (50% reduction) and phagocytosis of bacteria by polymorphonuclear leukocytes (50% reduction). There were no control groups in this study and the copper status of the subjects was not measured. The absolute number of lymphocytes and the proportions of T- and B-cells were not altered. However, the measurement of immune status conducted *in vitro* may not be a true reflection of the immune responses in the subjects, in whom a two-fold elevation in serum zinc was measured (Chandra, 1984).

Conversely, when 103 apparently healthy elderly subjects were randomly assigned to one of three treatments and given supplementary daily doses of placebo, or 15 or 100 mg of zinc for 3 months, none of the treatments significantly altered delayed dermal hypersensitivity to a panel of seven recall antigens or *in vitro* lymphocyte proliferative responses to mitogens and antigens. A modest increase in plasma zinc was not accompanied by a decrease in plasma copper levels (Bogden et al., 1988). Subjects also received a daily supplement of 2 mg of copper above dietary intake.

Phagocytic fungicidal capacity was evaluated in a double-blind study in which marasmic infants received formula fortified with zinc and iron at 15 mg/litre for up to 105 days, with a mean daily zinc intake of 1.9 mg/kg (Schlesinger et al., 1993). A decrease in the number of infants whose monocyte phagocytic activity was above the median was observed after 60 days of zinc supplementation (63% upon admission compared to 32% after 60 days; $P < 0.05$). There was also a decrease in the number of infants whose monocyte fungicidal activity was above the median after 105 days of zinc supplementation (61% upon admission compared to 39% after 105 days; $P < 0.04$). The number and duration of impetigo episodes were greater in the group of infants fed the zinc-supplemented formula (1.31 ± 1.1 infectious episodes/infant compared to 0.55 ± 0.8 in controls).

However, in another study in marasmic infants (Castillo-Duran et al., 1987) in which 16 subjects received a daily elemental zinc supplement as zinc acetate of 2 mg/kg and 16 subjects received a placebo for 90 days, the incidence of infection, especially pyoderma, was significantly decreased in the zinc-supplemented group (3/16 in the supplemented group compared to 10/16 in controls; $P < 0.025$). The proportion of anergic infants decreased (from 50% to 25% between days 0 and 90) and serum IgA increased significantly (from 81 ± 32 to 111 ± 26 mg/100 ml) only in the zinc-supplemented group.

8.3.4 Reproduction

Dietary supplementation with zinc at a rate of 20 mg/day did not result in adverse effects on pregnancy progress or outcomes in healthy pregnant women in a number of large, controlled trials (Hunt et al., 1984; Kynast & Saling, 1986; Mahomed et al., 1989). In a double-blind trial in low-income pregnant adolescents thought to be at risk for poor zinc nutriture, supplementation with zinc at 30 mg/day did not result in adverse pregnancy outcomes (Cherry et al., 1989). Of the women, one-third received the zinc for the first trimester and the remainder from the second trimester. Similarly, dietary supplementation for the last 15–25 weeks of pregnancy with 22.5 mg/day to women at risk of delivering a small-for-gestational-age baby did not result in adverse reproductive effects (Simmer et al., 1991).

When seven pregnant women with low serum zinc levels ($< 11.5 \mu\text{mol/litre}$) were given a zinc supplement of 90 mg/day for the last 13–25 weeks of pregnancy, no adverse effects were associated with the supplementation (Jameson, 1976). In a follow-up study (Jameson, 1982) in which 133 women with low serum zinc levels ($< 10 \mu\text{mol/litre}$) were randomly assigned zinc supplementation at 45 mg/day or no supplementation, no adverse effects were associated with zinc supplementation, and serum copper levels were unaffected.

8.3.5 Zinc-induced copper deficiency

Elevated intakes of zinc have been shown to induce copper deficiency in humans (Prasad et al., 1978a; Fischer et al., 1984; Hoffman et al., 1988). The level of intestinal metallothionein may be important in the development of this zinc-induced hypocupraemia. As metallothionein has a greater affinity for copper than zinc, and zinc induces high levels of metallothionein in the intestinal mucosa (Richards & Cousins, 1975), the proposed mechanism for this copper deficiency is a reduction in copper absorption followed by sequestration of copper in a stable copper-metallothionein complex, which is returned to the intestinal lumen by the desquamation of the intestinal mucosal cells (Richards & Cousins, 1976a; Fischer et al., 1983). Balance studies indicate that, as the amount of zinc in the diet increases, so does the amount of dietary copper required, so that persons on a diet high in zinc may have an increased risk of copper deficiency (Sandstead, 1982b). The ingestion of zinc at levels near the recommended daily allowance of 15 mg (see section 5.2.2) may result in increased copper requirements, increased copper excretion, impaired copper status, or reduced copper retention (Greger et al., 1978a,b,c; Burke et al., 1981; Festa et al., 1985). The effect of dietary zinc on copper utilization depends markedly on the amount of dietary copper and the copper status of the individual (see section 8.3.5.1).

8.3.5.1 Controlled human studies

In a study in which adolescent females received dietary copper at a rate of 1.2 mg/day, faecal copper excretion was increased by approximately 14% (0.9 compared to 0.79 mg/day) in subjects receiving zinc at 14.7 mg/day compared with those fed 11.5 mg/day

during 10-day periods (Greger et al., 1978c), with all subjects in positive copper balance. The standard error in the estimate for zinc was 2.18 mg/day. In another study in adolescent females, no increased copper excretion was reported in subjects given dietary zinc at 7.4 or 13.4 mg/day and copper at 2.8 mg/day for 18 days, again with all subjects in positive copper balance (Greger et al., 1978b). Other studies are cited in Table 33.

A group of 18 female volunteers participated in a 10-week, single-blind dietary supplementation study designed to investigate the effect of zinc supplementation on iron, copper and zinc status. When subjects were given zinc at 50 mg/day (administered as two gelatin tablets daily, each containing 25 mg of zinc as zinc gluconate), there was a significant reduction ($P < 0.05$) compared with pretreatment levels in serum ferritin (23%), haematocrit (4%) and erythrocyte copper,zinc-superoxide dismutase (ESOD; 47%). Serum zinc was increased by approximately 25% ($P < 0.01$), but there were no changes in serum ceruloplasmin or haemoglobin. When subjects received iron at 50 mg/day in addition to the zinc, similar reductions in ESOD were observed (47%), while there were increases in serum ferritin (25%) and serum zinc (21%); there were no changes in haemoglobin, haematocrit or ceruloplasmin (Yadrick et al., 1989). No indication of dietary intake of zinc or copper was noted in this study.

The effects of zinc supplementation on the copper status of two groups of healthy adult men were investigated for 6 weeks. A test group containing half of the subjects received 25 mg of elemental zinc as zinc gluconate twice daily in gelatin capsules (50 mg/day), while the control group received placebo capsules (Fischer et al., 1984). No significant differences in plasma copper levels or ferroxidase activities were observed between the groups. Increases in plasma zinc (approximately 20%) and decreases in ESOD (approximately 20%) were observed in the zinc-supplemented group, the differences becoming statistically significantly ($P < 0.05$) in week 6 of the study. No indication of dietary intake of zinc or copper was noted in this study.

In a 12-week, double-blind cross-over study (Samman & Roberts, 1988), 47 healthy adult volunteers received 50 mg of elemental zinc (as 220 mg of zinc sulfate) or placebo, in a capsule,

Table 33. Summary of effects of zinc on copper homeostasis in humans^a

Copper intake (mg/day)	Subjects, duration	Zinc dose (mg/day)	Effects	Described health effects	Reference
1.2	adolescents 10 days	14.7	increase in faecal copper excretion; positive copper balance		Greger et al. (1987c)
2.8	adolescents (females) 18 days	7.4 or 13.4	no effect on copper excretion; positive copper balance; copper intake 2.8 mg/day		Greger et al. (1978b)
2.0	healthy adult females 12 days	9.5 or 19.9	no effect on faecal copper excretion; positive copper balance; copper intake 2 mg/day		Colin et al. (1983)
2.0	adult females 12 days	8 or 24	no effect on copper excretion; negative copper balance; copper intake 2 mg/day		Taper et al. (1980)
2.6	adult males 0.5 weeks	1.8–18.5	no effect on serum copper concentration; sudden increase in zinc intake from 4–8 mg/day to 18.5 mg/day resulted in a temporary increase in faecal copper excretion	none	Festa et al. (1985)
2.33	elderly adults (5 male, 6 female) 30 days	7.8 or 23.26	reduced copper retention and increased faecal copper excretion at higher zinc dose compared with lower dose; most subjects in positive copper balance	none	Burke et al. (1981)

^a The interpretation of studies is difficult because in many supplemental studies the total intake of diet and supplement of zinc is not given.

three times a day for 6 weeks. The zinc supplementation resulted in reductions in the ferroxidase activity of serum ceruloplasmin (from 13.0 to 11.3 U/ml), and ESOD activity (from 2184 to 1672 U/g of haemoglobin), but only in females. The change in plasma zinc levels was greater in females ($8.4 \pm 1.5 \mu\text{mol/litre}$) than males ($5.5 \pm 1.1 \mu\text{mol/litre}$), and no changes were reported in plasma copper or haematocrit. No indication of dietary intake of zinc or copper was noted in this study.

A study of five men and six women aged 56–83 years showed that zinc intakes of about 23 mg daily (about 6 mg from the diet and 17 mg from zinc sulfate given in a beverage consumed at breakfast) significantly lowered retention of copper (about 1 mg from the diet and 1.3 mg from copper sulfate given in the breakfast beverage) compared to copper retention when the diet plus zinc sulfate in the beverage provided 7.8 mg of zinc daily (Burke et al., 1981). When the amount of copper lost in sweat (≥ 0.3 mg daily) is considered in the interpretation of the data (Jacob et al., 1979; Milne et al., 1983), it is evident that intakes of 23 mg of zinc daily placed the subjects at risk of negative copper retention. A zinc:copper ratio of about 10 had an adverse effect on copper retention that was not evident with a ratio of about 3.5. Of note in this study is that the increased intake of zinc was from a zinc sulfate supplement.

8.3.5.2 Case reports

In case studies, effects associated with long-term, excessive zinc intakes (ranging from 150 mg/day to 1–2 g/day) have included sideroblastic anaemia, hypochromic microcytic anaemia, leukopenia, lymphadenopathy, neutropenia, hypocupraemia and hypoferraemia. Patients recovered to normal blood patterns after cessation of zinc intake with or without copper supplementation (Porter et al., 1977; Prasad et al., 1978b; Hoffman et al., 1988; Simon et al., 1988; Broun et al., 1990; Forman et al., 1990; Gyorffy & Chan, 1992; Ramadurai et al., 1993).

8.3.6 Serum lipids and cardiovascular disorders

Following the induction of hypercholesterolaemia in rats by administration of a high ratio of ingested zinc to copper (Klevay, 1973) and the identification of an association between the mortality

rate for coronary heart disease and the zinc:copper ratio in cows' milk in 47 cities in the USA (Klevay, 1975), it was hypothesized that the zinc:copper ratio has important influences on processes related to coronary heart disease (Klevay, 1975, 1980, 1983).

As a partial investigation of these concepts, a number of studies have been conducted to examine the effects of zinc intake on blood lipid levels. The lowest dose of zinc that affects lipid metabolism is ill-defined, but it was approximately twice the US recommended daily allowance. Doses of zinc of 50–300 mg in excess of dietary amounts generally have potentially harmful effects on lipid metabolism.

Effects resulting from zinc-induced copper deficiency are discussed in section 8.3.5.

In a 12-week, double-blind study in adult males, subjects received daily a placebo tablet ($n = 9$), or tablets containing 50 ($n = 13$) or 75 ($n = 9$) mg of elemental zinc, as zinc gluconate. Dietary analysis revealed that subjects in the 75 mg group consumed significantly less total fat, saturated fatty acids and protein than those in the other groups. Serum total cholesterol, low-density lipoprotein (LDL) cholesterol, very-low-density lipoprotein (VLDL) cholesterol and triglycerides were not affected by zinc supplementation. Serum high-density lipoprotein (HDL) cholesterol was significantly decreased ($P \leq 0.05$) at zinc doses of 75 mg/day (reductions of 11% and 15% at weeks 6 and 12, respectively) and 50 mg/day (15% at week 12) compared with placebo, and was also lower than baseline values ($P \leq 0.05$) at weeks 6, 8 and 12 at 75 mg/day (reductions of 13%, 15% and 13%, respectively) and week 12 at 50 mg/day (11%). Serum copper levels did not change with zinc supplementation (Black et al., 1988). The dietary intake of nutrients including copper and zinc were monitored throughout the study.

A study was conducted to investigate the relationship between level of exercise, zinc supplementation, and serum HDL cholesterol in men and women over the age of 60 years (Goodwin et al., 1985). There was a significant positive correlation between levels of exercise and serum HDL cholesterol in the 180 subjects not supplemented with zinc ($r = 0.26$; $P = 0.005$), but not for those subjects taking supplemental zinc. Following discontinuation of zinc

supplementation (24 mg/day; median 17–52 mg/day), there was a significant increase in HDL cholesterol levels (2.0 mg/100 ml; approximately 4%; $P = 0.04$) after 8 weeks in 22 subjects. This change was positively correlated with the level of exercise of the subjects. The authors noted that in young runners, HDL is unchanged by zinc administered at 50 mg/day for 8 weeks. They suggested that age and sex differences may be important in the relationship between zinc and lipid metabolism in humans, but no data were provided to support this hypothesis.

Reiser et al. (1985) described a diet mainly of conventional food but low in copper, and containing an amount of fructose similar to that consumed by many Americans. The effects of this diet on more than 20 male subjects have been described in a number of papers (Reiser et al., 1985, 1987; Bhathena et al., 1986; Holbrook et al., 1989). Prominent among these effects were decreased plasma encephalins and dyslipidaemia characterized by decreased HDL cholesterol and increased LDL cholesterol. The experiment was interrupted because of fear of adverse cardiac effects. Evidence of copper deficiency assessed by traditional means was minimal, but included decreased activity of ESOD.

In a study in which 12 healthy male subjects received 440 mg of zinc sulfate (160 mg of elemental zinc) daily for 5 weeks, HDL cholesterol levels decreased to 25% below baseline values (30.1 compared with 40.5 mg/100 ml; $P = 0.0001$), while total cholesterol, triglyceride and LDL cholesterol levels remained unchanged (Hooper et al., 1980). No indication of dietary intake of zinc or copper was noted in this study.

When 11 healthy male subjects ingested 150 mg of elemental zinc twice a day, serum HDL concentrations decreased significantly compared with baseline values after 4 weeks (20% reduction; $P < 0.01$) and 6 weeks (30% reduction; $P < 0.001$), while LDL levels increased slightly (by 10–15% at 4–6 weeks; $P < 0.05$); however, this study lacked a placebo control (Chandra, 1984). Dietary zinc estimates were made using 24-h dietary recall interviews.

Not all studies show that zinc supplementation affects serum HDL levels. In a double-blind, cross-over trial involving 26 women and 21 men, the diets of healthy volunteers were supplemented with

zinc at a rate of 150 mg/day for 6 weeks. Plasma total cholesterol and HDL levels remained unchanged in both sexes, while in women only, the LDL level decreased by 9%. There was also a trend for HDL to be redistributed in women, with slight increases in HDL₂ and slight decreases in HDL₃ (Samman & Roberts, 1988). When groups of eight women were given dietary supplementation of zinc at doses of 0, 15, 50 or 100 mg/day for 2 months, a transient 8% decrease in HDL cholesterol was seen at 4 weeks at the highest zinc level, but no uniform or sustained response of plasma cholesterol or HDL cholesterol was observed (Freeland-Graves et al., 1980). Records of the dietary nutrients, including zinc and copper, were obtained from the 3-day dietary records kept throughout this study.

8.4 Occupational exposure

8.4.1 Acute toxicity

Inhalation exposure to zinc chloride following the military use of “smoke bombs” has been reported to result in various effects, including interstitial oedema, interstitial fibrosis, pneumonitis, bronchial mucosal oedema and ulceration, and changes in the mucous membrane of the larynx and trachea (Pare & Sandler, 1954; Johnson & Stonehill, 1961; Milliken et al., 1963; Schenker et al., 1981; Matarese & Matthews, 1986). Acute injury has been associated with mortality under extreme exposure conditions, sometimes attributed to the effects upon the respiratory tract mucosa due to the hygroscopic and astringent nature of the zinc chloride particles released by such devices (Evans, 1945; Milliken et al., 1963; Hjortso et al., 1988; Homma et al., 1992).

8.4.2 Short-term exposure

The term “metal-fume fever” describes an acute industrial illness characterized by a variety of symptoms, including fever, chills, dyspnoea, muscle soreness, nausea and fatigue, which occur in workers following the inhalation of finely dispersed particulate matter formed when certain metals are volatilized. The oxides of a number of metals, including zinc, can cause this acute, reversible syndrome (Drinker et al., 1927a–d; Rohrs, 1957; Doig & Challen, 1964; Gordon et al., 1992). The description of the effects has been cited extensively, and the condition has variously been called

brassfounder's ague, zinc chills, zinc fever, Spelter's shakes and metal shakes (Batchelor et al., 1926; McCord & Friedlander, 1926; Mueller & Seger, 1985; Blanc et al., 1991).

Metal-fume fever is common in welders who work on various types of non-ferrous metals or ferrous metals alloyed with or coated with other metals. Zinc fume from galvanized coatings is a common cause. While the disease is generally short, transient and severe, serious complications are not common and individuals tend to develop a tolerance (Drinker et al., 1927a-d). Symptoms might occasionally be followed by pulmonary oedema or pneumonia (Doig & Challen, 1964). The size of the ultrafine zinc oxide particles appears to be critical in the development of the syndrome, with the particles needing to be small enough to reach the alveoli when inhaled (Brown, 1988). Recent studies in humans following occupational exposure to zinc oxide fumes have demonstrated some changes in pulmonary function and/or radiological abnormalities, which are reversible following cessation of exposure.

A cross-sectional analysis, conducted on spirometric lung-function parameters in zinc welders, non-welders with exposure to welding fumes and control subjects (Marquart et al., 1989), revealed no differences in lung function between groups, and changes in lung function over five consecutive work shifts were not related to the exposure level. The highest measured concentrations of welding fumes were 5.1 and 8.0 mg/m³ for an 8-h time-weighted average.

In a study designed to examine the pathogenesis of metal-fume fever in humans (Blanc et al., 1991), 14 subjects welded galvanized mild steel over a period of 15–30 min in special environmental exposure chambers, with controlled ventilation, humidity and temperature, designed to produce an exposure level in excess of 10 mg/m³ over 15 min. The mean cumulative exposure to zinc oxide for the 14 participants was reported to be 2.3 ± 1.7 g/min per m³ (range 0.6–5.1 g/min per m³), resulting in a range of mean exposure levels of 77–153 mg/m³, and a minimum exposure of 20–40 mg/m³, depending upon whether duration was 15 or 30 min. Pulmonary function and airway responsiveness were measured after 1 h ($n = 14$), 6 h ($n = 5$) and 20 h ($n = 9$), while bronchoalveolar lavage was conducted 8 h or 22 h after welding. A marked, dose-dependent inflammatory response was observed in the lungs, with a positive

correlation between cumulative zinc exposure and polymorphonuclear leukocyte count in bronchoalveolar lavage fluid at early ($r = 0.93$; $P < 0.05$) and late ($r = 0.87$; $P < 0.01$) follow-up. The proportion of polymorphonuclear leukocytes in the late follow-up sample, 37% (range 19–63%), was increased compared with the early follow-up figure of 9% (2–21%). There was only a minimal effect on pulmonary function, and no statistically significant correlation was observed between cumulative zinc exposure and pulmonary function. In the late follow-up group, the four participants with the highest cumulative exposures (> 3.5 g/min per m^3) all had myalgia. Two of the participants (with exposure of > 5 g/min per m^3 , i.e., approximately 150 mg/ m^3) also had fever (38 °C).

In a subsequent paper (Blanc et al., 1993), further information from the same subjects was reported together with additional data from a total of 23 volunteers adding a 3-h post-exposure time-point for bronchoalveolar lavage fluid (zinc exposure 1.8 ± 0.2 mg/ m^3). Increased concentration of tumour necrosis factor (TNF) in bronchoalveolar lavage fluid was prominent at 3 h, and less marked at 8 h or 22 h after exposure, exhibiting a statistically significant exposure–response relationship to airborne zinc at each time-point ($P < 0.05$). There were also significant changes in the concentrations of interleukin-6 and interleukin-8, but not of interleukin-1. The findings are consistent with a role of these cytokines in the pathogenesis of the inflammatory changes in metal-fume fever. Although these short-term exposures (15–30 min) were to zinc concentrations well above 10 mg/ m^3 , it should be noted that they do not exceed an 8-h time-weighted average of 5 mg/ m^3 if recalculated to an 8-h time interval; however, it is unlikely that an acute reaction of the type observed would occur if the same cumulative exposures were given over 8 h.

A number of case reports have demonstrated the acute effects of zinc fume inhalation in occupational settings. Reversible clinical signs and radiological effects, including aches and pains, dyspnoea, dry cough, lethargy, neutrophil leukocytosis, pyrexia, and widespread abnormality of both lung fields, with multiple nodules measuring 3–4 mm and becoming confluent and ill-defined in some areas, were seen when an individual was exposed to zinc fumes in a shipyard over a 3-week period (Brown, 1988). A systemic reaction and a self-limiting response in the periphery of the lung were

reported when a patient with a clinical history of recurring zinc fume fever underwent experimental welding exposures of 1 h using zinc-coated tubing (Vogelmeier et al., 1987). An acute lung reaction was also seen in an individual working with heated zinc who experienced chills, muscle ache and dyspnoea; radiographic examination revealed diffuse nodular infiltrates, which cleared after 10 days away from the job (Malo et al., 1990).

8.4.3 Long-term exposure

The complex environment encountered by workers in galvanizing and metal plating plants results in exposure to a variety of compounds, including zinc and zinc compounds.

A causal association between the exposure to zinc and any occupational asthma is difficult to establish. Occasional cases of occupational asthma have been reported among workers using soft solder fluxes containing ammonium chloride and zinc chloride. A causative relationship with zinc could not be concluded. The most suggestive case was a subject who developed asthma symptoms 2.5 years after being employed at a plant where metals were galvanized in heated zinc (Malo et al., 1993). Positive immediate skin tests to zinc sulfate at concentrations of 1 and 10 mg/ml were obtained, although no specific IgE antibodies to zinc were observed. An immediate asthmatic reaction was elicited after the subject inhaled nebulized zinc sulfate at a concentration of 10 mg/ml for 6 min.

The exposure of groups of volunteers to a polydisperse aerosol of zinc ammonium sulfate in an environmental control chamber at a nominal concentration of 20 µg/m³ produced minimal or no short-term respiratory effects, even in subjects diagnosed as asthmatics prior to the study (Linn et al., 1981).

8.4.4 Epidemiological studies

In general, well-conducted epidemiological studies in the workplace with adequate characterization of zinc exposure values are lacking, and there are inadequate data available to make an association between occupational exposure to zinc and disease states.

8.5 Subpopulations at special risk

8.5.1 Dialysis patients

Acute zinc toxicity has been reported in patients following kidney dialysis (Gallery et al., 1972; Petrie & Row, 1977). A patient who, for home dialysis, used rainwater draining from a painted galvanized iron roof, which had been stored in a galvanized iron tank, developed severe nausea and vomiting within 2 h of starting the procedure, with similar symptoms at subsequent dialyses. The tank water contained zinc at a concentration of 625 µg/100 ml. The patient's plasma and red cell zinc concentrations were 700 and 3500 µg/100 ml, respectively, haemoglobin was 3.5 g/100 ml, and a blood film showed moderate polychromatophilia; 6 weeks after rehospitalization, plasma zinc was still moderately raised. Intercurrent hospital dialyses were uneventful, and subsequent deionization of the patient's home water supply resulted in asymptomatic dialyses (Gallery et al., 1972). The use of water drawn through galvanized iron piping resulted in a fall in the haemoglobin levels of two home dialysis patients; these effects were eliminated after the installation of carbon filtration of the dialysis water (Petrie & Row, 1977). Severe anaemia was also seen in 9/10 patients dialysed in a hospital dialysis unit, following the installation of a new galvanized iron water softener in the dialysate water supply system. The dialysate contained zinc at a concentration of 4.89 µmol/litre (32 µg/100 ml). The installation of an activated carbon filter in the system reduced the zinc concentration to < 0.15 µmol/litre (< 1 µg/100 ml), resulting in a rise in haemoglobin levels in the patients towards previous values (Petrie & Row, 1977).

8.5.2 People with diabetes

Non-infective furunculoid skin lesions were reported in an insulin-dependent diabetic subject, apparently induced by the zinc acetate component of an intermediate-acting insulin preparation. This rare complication of insulin therapy was attributed to a reparative granulomatous phase arising from tissue damage caused by the zinc in the preparation (Jordaan & Sandler, 1989; Sandler & Jordaan, 1989). In two patients using insulin preparations containing zinc, pruritic, erythematous, papular lesions were observed at the injection site. Intradermal skin tests for zinc were positive in both

patients. Zinc-free insulin did not produce any allergic reactions in the patients (Feinglos & Jegasothy, 1979).

8.5.3 Hospital patients

An elderly woman died after she received 46 mmol (7.4 g) of zinc sulfate intravenously over 60 h, owing to a prescribing error; her serum zinc concentration was 640 $\mu\text{mol/litre}$ (4184 $\mu\text{g}/100\text{ ml}$). Zinc intoxication was characterized by hypotension, pulmonary oedema, diarrhoea, vomiting, jaundice and oliguria (Brocks et al., 1977). In another incident, seven hospital patients undergoing intravenous feeding with fluid containing elemental zinc at a concentration of 227 $\mu\text{g}/100\text{ ml}$ were inadvertently given fluid containing 10 times that amount (2270 $\mu\text{g}/100\text{ ml}$) for 26–60 days. Mortality was high (5/7). While the clinical manifestation of the zinc overdose was hyperamylasaemia (unaccompanied by clinical signs of pancreatitis), the authors concluded that all deaths had resulted from septic complications already present before the appearance of this symptom (Faintuch et al., 1978).

To investigate the effects of zinc administration on the healing of chronic leg ulcers, a double-blind trial was conducted in 27 subjects; 13 patients received 200 mg of zinc sulfate three times a day (approximately 135 mg of elemental zinc daily) for 18 weeks, while 14 patients received placebo. No signs of toxicity associated with the zinc treatment were reported in the study (Hallbook & Lanner, 1972). Similarly, in another study investigating the effect of oral zinc treatment on leg ulcers, no clinical signs of toxicity were reported in 18 patients administered 220 mg of zinc sulfate three times daily (approximately 150 mg of elemental zinc daily) for 16–26 weeks (Greaves & Skillen, 1970). Mild diarrhoea was reported in 3/52 patients receiving three daily doses of 220 mg of zinc sulfate for up to 71 days (Husain, 1969), while diarrhoea was reported in 6/16 geriatric patients receiving a similar zinc dose for 24 weeks (Czerwinski et al., 1974).

8.5.4 Other populations

No adverse effects were observed as a result of ingestion of 300–1200 mg of zinc sulfate heptahydrate daily for 3 years or 150 mg of zinc as zinc acetate daily for several weeks to 2 years by

Wilson disease patients (Hoogenraad et al., 1979, 1983, 1984; Brewer et al., 1983, Hill et al., 1987), doses of zinc of 1–2 mg/kg daily by infants and children with acrodermatitis enteropathica (Hambidge & Walravens, 1982), and 68–102 mg of zinc daily during pregnancy by a woman with acrodermatitis enteropathica (Jones & Peters, 1981). Few long-term studies of the effects of high oral zinc in healthy adults have been reported. In 11 female and 13 male patients with Wilson disease, the administration of 50 mg of elemental zinc as zinc acetate three times a day for about 2 years resulted in a decrease in total cholesterol of about 10% in both sexes and a reduction of HDL cholesterol of about 20% in male patients. The authors concluded that the coronary heart disease risk factor was not changed significantly in either sex (Brewer et al., 1991).

Recently, a controlled, randomized double-blind study showed that oral zinc therapy, 100 mg of zinc sulfate twice daily taken with food, significantly reduced visual loss in individuals with macular degeneration (Newsome et al., 1988).

8.6 Interactions

8.6.1 Copper

Impaired copper nutriture in humans has been noted following chronic elevated intake of zinc; these effects are reported in section 8.3.5.

8.6.2 Iron

The effect of inorganic zinc on the absorption of inorganic iron from a solution was investigated in two studies in healthy male volunteers (Crofton et al., 1989). Simultaneous administration of 344 μmol of zinc had no effect on the absorption of 842 μmol of radiolabelled iron (^{59}Fe) in the first study, based upon the area under the plasma iron concentration–time curve at 3 h and 6 h, the whole body retention of ^{59}Fe , and plasma content of ^{59}Fe . However, the authors noted a reduction in 4/9 subjects of the areas under the curve at 3 h and 6 h for iron, and suggested that there was a trend (not statistically significant) for zinc to inhibit the intestinal absorption of iron. The second study was conducted without a radiolabel, and the results indicated that the simultaneous administration of iron with

zinc at molar ratios of 1:1 (421 μmol) and 2.5:1 (1048 μmol) significantly reduced increments in the concentrations of iron in the plasma.

In a study described in section 8.3.5.1, in which women were supplemented with zinc at 50 mg/day for 10 weeks, competitive interactions between iron and zinc were suggested by the authors (Yadrick et al., 1989). Serum ferritin, the level of which is proportional to tissue iron stores, was reduced following zinc supplementation alone, but when iron at 50 mg/day was administered together with the zinc, serum zinc and serum ferritin increased.

In human subjects, the presence of inorganic iron in solution with ionic zinc at molar ratios of between 2:1 and 3:1 resulted in significant inhibition of zinc absorption (Solomons & Jacob, 1981; Solomons et al., 1983; Valberg et al., 1984; Sandstroem et al., 1985), while the presence of haem iron in the same molar excess did not inhibit the absorption of zinc (Solomons & Jacob, 1981). In healthy, non-pregnant woman, a progressive decrease in plasma zinc was seen as the ratio of iron to zinc was increased from 0.1 to 3.1, while the intake of zinc remained constant at 25 mg (Solomons & Jacob, 1981).

In studies in which iron was given with food, no inhibitory effect on zinc uptake was observed when the iron intake was not unusually high. The consumption of 54 mg of "organic" zinc in oysters with 100 mg of ferrous iron did not alter plasma uptake of zinc (Solomons & Jacob, 1981). Neither the addition of ferrous iron (at an iron:zinc ratio of 25) to a composite meal containing 2.6 mg of zinc (Sandstroem et al., 1985), nor the consumption of turkey meat containing 4 mg of zinc with ferric iron (17 or 34 mg) (Valberg et al., 1984) significantly changed the absorption of zinc. No effects of iron-fortified infant foods on zinc absorption of zinc from natural sources were demonstrated in adults or children (Fairweather-Tait, 1995) or in healthy infants given an iron supplement (30 mg of iron as ferrous sulfate) before a meal. However, dietary supplementation with large amounts of iron may impair zinc absorption, and this was observed in four human volunteers fed a zinc-deficient (zinc at 3.5 mg/day), protein-based, semisynthetic soy diet for 4 months (Prasad et al., 1978a); the two subjects receiving 130 mg of iron daily displayed a more rapid reduction in plasma zinc than did the two volunteers fed 20.3 mg of iron daily.

The effect of iron on zinc absorption may depend upon zinc status. For example, serum copper or zinc levels were not affected in healthy infants who were fed a zinc- sufficient diet supplemented with 30 mg of iron as ferrous fumarate daily given 30 min before a meal (Yip et al., 1985).

Three pregnant women, whose daily diets were supplemented with iron at rates of 100 mg/day or more, had lower plasma zinc than other pregnant women whose iron supplementation was less than 100 mg/day (Campbell-Brown et al., 1985), and daily multivitamin supplements containing 60–65 mg of iron inhibited zinc absorption in first-trimester pregnant women, compared with pregnant women receiving no iron supplementation, or with iron supplementation of less than 30 mg/day (Breskin et al., 1983). It is not known whether the iron supplements in these studies were taken in the presence or absence of food.

8.6.3 Calcium

Human subjects with a constant zinc intake of 14.5 mg/day and calcium intakes of 200–2000 mg/day showed no changes in zinc absorption (Spencer et al., 1983). Conversely, the intake of high zinc levels (140 mg/day) reduced calcium absorption in men with low calcium intakes (200 mg/day) but calcium absorption was not affected when calcium intake was 800 mg/day (Spencer et al., 1987).

9. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

Zinc is an essential micronutrient in all biota owing to its involvement in many physiological processes. It is essential in the maintenance of plasma membrane stability (Bettger & O'Dell, 1981; Cakmak & Marschner, 1988), in the activation of more than 300 enzymes, in transcription factors and in hormone receptors (see section 6.5.2).

Generally, organisms growing in natural terrestrial environments do not show symptoms of zinc deficiency. However, species introduced by humans into the environment may show these deficiencies. Zinc toxicity is observed in organisms exposed to anthropogenic zinc enrichment (Ernst, 1972) and in crops grown in naturally enriched environments (Chaney, 1993). More often than toxicity, zinc deficiency is reported from environments where humans have grown plants that are not adapted and/or have not been properly selected, ranging from crops and pastures in Australia (Donald & Prescott, 1975), Africa (Cottenie et al., 1981), Asia (Katyal & Ponnampereuma, 1974) and North America (Lingle & Holmberg, 1957), to fruit trees (SSSA, 1990) and forest trees. Application of various types of zinc fertilizers to soil or onto leaves can help to overcome these problems (Takkar & Walker, 1993). Another approach is to increase the zinc efficiency of cultivated plant species (El Bassam et al., 1990). Animals fed or feeding on zinc-deficient plants will also show symptoms of zinc deficiency (Blamberg et al., 1960, Elinder & Piscator, 1979).

Nutritional zinc deficiency is relatively rare for aquatic organisms. A possible exception may be the low zinc environments that characterize open oceans. Extremely low concentrations of zinc, iron and copper have been observed in open oceans and it has been suggested that these are rate limiting for phytoplankton growth (Anderson et al., 1978; Reuter & Morel, 1981; Bruland, 1993). In most other circumstances, organisms appear to have developed appropriate physiological mechanisms to ensure adequate uptake of zinc from the concentrations present in their native environment. Organisms not capable of doing this would of course have

disappeared from a particular ecosystem. Information concerning zinc deficiency in aquatic organisms must thus be obtained primarily from laboratory experiments. There are several reports of zinc deficiency under experimental conditions in protozoa (Falchuk, 1988), algae (Vymazal, 1986), daphnids (Keating & Caffrey, 1989), fish (Spry et al., 1988) and amphibians (Herkovits et al., 1989). White & Rainbow (1985) calculated theoretical estimates for the minimum metabolic requirements of zinc in molluscs and crustaceans. Enzymatic requirements for zinc in both groups were estimated to be 34.5 mg/kg dw. The possession of haemocyanin as a respiratory pigment adds a further non-enzymatic metabolic requirement of 58.3 mg/kg for certain gastropod molluscs and 36.3 mg/kg for some crustaceans such as decapods. However, Depledge (1989) recalculated the amount of zinc required by decapod crustaceans to be 67.9 mg/kg dw.

9.1 Laboratory experiments

Many experiments performed in laboratories give insufficient information on the speciation of zinc, especially when zinc is added to a medium rich in complexing agents such as sewage sludge and agar (for example, Codina et al., 1993). In the case of soils, there is a lack of information on the time period between the zinc application and the start of the experiments, i.e., the time necessary for an equilibrium to be reached between the metal application and the soil solution (Spurgeon & Hopkin, 1996). The lack of this information adversely affects the reliability and utility of toxicity determinations. A similarly inadequate procedure is followed in many experiments with animals in which zinc added to the feed is only adsorbed, whereas in the natural situation it is processed by the organism and incorporated into organic compounds. The difference between adsorbed and metabolically processed zinc has clearly been shown in experiments with Japanese quail fed spinach and lettuce (McKenna et al., 1992).

To be useful, toxicity testing requires, at a minimum, the following information: actual exposure concentrations (nominal concentrations are unacceptable); acceptable control results (i.e., an acceptably low level of mortalities and/or effects); physicochemical conditions (at a minimum, temperature, pH, dissolved oxygen and hardness); and a concentration–response relationship. Studies that

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met these criteria are so indicated where appropriate in the text and tables that follow.

9.1.1 Microorganisms

9.1.1.1 Water

Studies on the effect of zinc on microorganisms in the aquatic environment generally measure either growth or survival. However, the zinc concentrations added in these tests are often too high to be of environmental relevance (Codina et al., 1993, 50–432 mg/litre; Tijero et al., 1991, 200–600 mg/litre). Values for the EC₅₀ (the concentration producing effects in 50% of the tested organisms) and LC₅₀ (the concentration killing 50% of the tested organisms) in other experiments varied in a species-specific manner (Table 34).

Table 34. Zinc toxicity (LC₅₀ or EC₅₀ values in mg/litre) for microorganisms in the aquatic environment

Species	Duration of exposure (h)	LC ₅₀	Reference
<i>Drepanomonas revoluta</i>	24	0.25	Madoni et al. (1994)
<i>Spirostomum teres</i>	24	0.67	Madoni et al. (1994)
<i>Blepharisma americanum</i>	24	1.05	Madoni et al. (1994)
<i>Tetrahymena pyriformis</i>	56	5.77	Carter & Cameron (1973)
<i>Tetrahymena pyriformis</i>	8	< 1.00	Chapman & Dunlop (1981)
<i>Zoogloea ramigera</i>	24	approximately 3.0 ^a	Norberg & Molin (1983)
<i>Euplotes patella</i>	24	50.0	Madoni et al. (1992)

^a EC₅₀

9.1.1.2 Soil

Laboratory experiments are often carried out without equilibrium between the added zinc and the soil, which is a critical drawback in short-term experiments (< 3 weeks). Three parameters of microbial activities in soil have been studied: mineralization of

macronutrients (N, S); soil respiration as a parameter of the mineralization of organic compounds; and general soil activity (dehydrogenase). Microbial activity is less affected by zinc in soils rich in organic materials than in sandy and loamy soils (this situation was found for N-mineralization (Doelman & Haanstra, 1984), soil respiration (Frostegård et al., 1993) and dehydrogenase activity (Rogers & Li, 1985). These results can be explained by differences in zinc speciation.

More recent literature confirms the importance of organic matter in reducing the effects of zinc in microbial processes, such as the breakdown of glutamic acid, and phosphatase activity. Increasing exposure time lowers the EC₅₀ value (Table 35).

9.1.2 Aquatic organisms

9.1.2.1 Plants

Acute toxicity of zinc is often determined in short-term experiments of 24–96 h (Table 36). In the case of unicellular algae, these experiments cover 1–4 cell-division cycles. EC₅₀ values range from 0.058 to 10 mg/litre (nominal concentration) in a species-specific manner. The toxicity of zinc depends on the external concentration, the zinc speciation, and the pH and hardness of the water (Starodub et al., 1987, Stauber & Florence, 1989). Aquatic macrophytes are generally insensitive to zinc.

Most of the data for unicellular algae were obtained using culture media as the assay solutions. These results should be used with caution, since complexing agents, e.g., EDTA in the culture media, may reduce zinc bioavailability and lower its toxicity (Stauber, 1995). Crucial information with respect to physicochemical parameters (e.g., water hardness, dissolved organic carbon, dissolved oxygen) is not generally provided in most of the cited references.

Acute toxicity values tend to be lower for marine unicellular algae than for freshwater species. Only one set of experiments satisfies the ideal criteria as previously specified. In these tests, no-observed-effect concentrations, obtained under standardized test conditions (OECD 201 algae growth-inhibition test) for *Selenastrum capricornutum*, ranged between 30 µg/litre and 50 µg/litre (measured as dissolved concentration; hardness 16 mg/litre, CaCO₃).

Table 35. Impact of zinc (mg/kg) on nitrogen mineralization in relation to soil types

Process	Duration	EC ₅₀				Reference
		Sand	Sandy loam	Silty loam	Clay	
Urease	6 weeks	420	480	1030	1780	Doelman & Haanstra (1986)
	18 months	230	110	–	90	
Nitrification	–	–	100 ^a 1000	–	ca. 80	Wilson (1977)

^a No-observed-effect concentration.

Table 36. Toxicity of zinc to algae and aquatic plants in static conditions^a

Organism	Temp (°C)	Zinc compound tested	Hardness (CaCO ₃ mg/litre)	Parameter	End-point	EC ₅₀ (mg/litre)	Reference
Unicellular algae: freshwater							
Green algae							
<i>Chlorella vulgaris</i>	15.5	sulfate	n.g.	96-h EC ₅₀	culture growth	2.4 (n)	Rachlin & Farran (1974)
<i>Scenedesmus quadricauda</i>	20	sulfate	n.g.	24-h EC ₅₀	photosynthesis	> 0.225 (n)	Starodub et al. (1987)
<i>Selenastrum capricornutum</i>	25	zinc powder	16	72-h EC ₅₀	culture growth	0.15 (m,d)	van Woensel (1994)
				NOEC	culture growth	0.05 (m,d)	van Woensel (1994)
<i>Selenastrum capricornutum</i>	25	oxide	16	72-h EC ₅₀	culture growth	0.17 (m,d)	van Ginneken (1994)
				NOEC	culture growth	0.03 (m,d)	van Ginneken (1994)
Diatoms							
<i>Navicula incerta</i>	19	chloride	n.g.	9-h EC ₅₀	culture growth	10.0 (n)	Rachlin et al. (1983)
Unicellular algae: marine							
Marine diatoms							
<i>Asterionella japonica</i>	23	sulfate	n.g.	72-h EC ₅₀	culture growth	0.058 (n)	Fisher & Jones (1981)
<i>Nitzschia closterium</i>	15.5	sulfate	n.g.	96-h EC ₅₀	culture growth	0.271 (n)	Rosko & Rachlin (1975)
<i>Nitzschia closterium</i>	21	chloride	n.g.	96-h EC ₅₀	culture growth	0.065 (n)	Stauber & Florence (1990)

Table 36 (contd.)

Macrophytes: freshwater

Acute toxicity							
<i>Elodea canadensis</i> (segments)	24	sulfate	10	24-h EC ₅₀	photosynthetic O ₂	8.1 (n)	Brown & Rattigan (1979)
Prolonged tests							
<i>Elodea canadensis</i>	n.g.	sulfate	n.g.	28-d EC ₅₀	plant damage	22.5 (n)	Brown & Rattigan (1979)
<i>Lemna minor</i>	n.g.	sulfate	n.g.	28-d EC ₅₀	plant damage	67.7 (n)	Brown & Rattigan (1979)
<i>Lemna minor</i>	25–28	chloride	n.g.	7-d EC ₅₀	frond growth inhibition	10 (n)	Dirilgen & Inel (1994)
<i>Elodea nuttallii</i>	21	sulfate	n.g.	14 d	no toxic symptoms	32.7 (n)	Van der Werff & Pruyt
<i>Callitriche platycarpa</i>	21	sulfate	n.g.	28 d	no toxic symptoms	32.7 (n)	(1982)
<i>Callitriche platycarpa</i>	21	sulfate	n.g.	73 d	no toxic symptoms	0.654 (n)	Van der Werff & Pruyt
<i>Spirodela polyrhiza</i>	21	sulfate	n.g.	73 d	no effects observed	0.654 (n)	(1982)
<i>Lemna gibba</i>	21	sulfate	n.g.	73 d	no effects observed	0.654 (n)	Van der Werff & Pruyt (1982)

d = measurements expressed as dissolved zinc; m = measured concentrations; n = nominal concentrations; n.g. = not given

^a Many of the older test results should be regarded with caution because the assays were carried out in culture media containing complexing agents like EDTA, which could affect the bioavailability of zinc. Crucial information concerning physicochemical factors such as hardness, DOC and DO is lacking in most of the papers.

Floating aquatic plants can take up zinc by the roots and shoots (the lower surface with water contact). Zinc uptake is governed not only by the zinc concentration in the water but also by evapotranspiration, which is not taken into account in most experiments with duckweed (*Lemna minor*) (Hutchinson & Czyska, 1975; Brown & Rattigan, 1979; Dirilgen & Inel, 1994). EC₅₀ values vary from 10 to 67.7 mg/litre depending on the test period and conditions. Submerged aquatic plants, e.g., pondweeds (*Elodea* sp.), are more sensitive than floating aquatic plants (Brown & Rattigan, 1979).

Permanent high exposure to zinc gives rise to the selection of zinc-tolerant genotypes, e.g., *Lemna minor* (Van Steveninck et al., 1990) which detoxifies zinc as zinc phytate in vacuoles, and in several algal species (Say et al., 1977; Harding & Whitton, 1981). Zinc tolerance in plants and other organisms is discussed further in section 9.2.

9.1.2.2 *Invertebrates and vertebrates*

Information on the acute toxicity of zinc to freshwater and marine invertebrates is summarized in Tables 37 and 38, respectively, and to freshwater and marine fish is summarized in Tables 39 and 40, respectively. Studies that meet the criteria specified above so indicated in these tables.

The toxicity of zinc can be influenced both by intrinsic and by extrinsic factors. Numerous studies with aquatic animals have demonstrated that zinc toxicity decreases with increasing water hardness (Sinley et al., 1974; Bradley & Sprague, 1985; Winner & Gauss, 1986; Paulauskis & Winner, 1988; Everall et al., 1989) and decreasing temperature (McLusky & Hagerman, 1987; Hilmy et al., 1987; Zou & Bu, 1994). However, Berglind & Dave (1984) reported that, hardness over the range 50–300 mg/litre, CaCO₃, had no significant effect on the toxicity of zinc to daphnids. Similarly, Rehwoldt et al. (1972) found no effect of temperature (15–28 °C) on the toxicity of zinc to freshwater fish. Smith & Heath (1979) reported that the effect of temperature on zinc toxicity was species specific. While increased temperature resulted in an increase in toxicity of zinc to goldfish (*Carassius auratus*) and bluegill (*Lepomis macrochirus*), it had no effect on the toxicity of zinc to golden shiners (*Notemigonus crysoleucas*) or rainbow trout (*Oncorhynchus mykiss*).

Table 37. Toxicity of zinc to freshwater invertebrates^a

Organism	Size/age	Stat/flow	Temp (°C)	Hardness (mg/litre)	pH	Zinc compound tested	Parameter	Concentration (mg/litre)	Reference
Snail	eggs	stat	17	50	7.6		24-h LC ₅₀	28.1 (m)	Rehwoldt et al. (1973)
<i>Amnicola</i> sp.	eggs	stat	17	50	7.6		96-h LC ₅₀	20.2 (m)	(1973)
	adult	stat	17	50	7.6		24-h LC ₅₀	16.8 (m)	Rehwoldt et al. (1973)
	adult	stat	17	50	7.6		96-h LC ₅₀	14 (m)	(1973)
Mollusc	<2 mm	stat	10			sulfate	96-h LC ₅₀	3.2 (n)	Willis (1988)
<i>Ancylus fluviatilis</i>	>3 mm	stat	10			sulfate	96-h LC ₅₀	4.5 (n)	Willis (1988)
Annelid	<4 mg	stat	10			sulfate	96-h LC ₅₀	2.05 (n)	Willis (1989)
<i>Erpobdella oculata</i>	>15 mg	stat	10			sulfate	96-h LC ₅₀	8.8 (n)	Willis (1989)
Bristle worm		stat	17	50	7.6		24-h LC ₅₀	21.2 (m)	Rehwoldt et al. (1973)
<i>Nais</i> sp.		stat	17	50	7.6		96-h LC ₅₀	18.4 (m)	(1973)
Water flea		stat	17–19	44–53	7.4–8.2	chloride	48-h EC ₅₀	0.1 (n)	Biesinger & Christensen (1972)
<i>Daphnia magna</i>		stat	17–19	44–53	7.4–8.2	chloride	48-h EC ₅₀	0.28 (n)	(1972)
	<48 h	stat		175	6.0	sulfate	48-h LC ₅₀	0.24 (n)	LeBlanc (1982)
	<24 h	stat	20		6.5	sulfate	48-h LC ₅₀	0.151 (n)	Oikari et al. (1992)
	<24 h	stat	20		6.5	sulfate	48-h LC ₅₀	0.244 (n, hw)	Oikari et al. (1992)
	<24 h	stat		45	7.2–7.4		48-h LC ₅₀	0.068 (n)	Mount & Norberg (1984)

Table 37 (contd.)

Organism	Size/age	Stat/flow	Temp (°C)	Hardness (mg/litre)	pH	Zinc compound tested	Parameter	Concentration (mg/litre)	Reference	
<i>D. magna</i> (contd.)	<24 h	stat*	20				sulfate	48-h LC ₅₀	0.75 (n)	Arambasic et al. (1995)
		stat					bromide	48-h LC ₅₀	1.22 (m)	Magliette et al. (1995)
<i>D. pulex</i>	<24 h	stat		45	7.2–7.4		48-h LC ₅₀	0.107 (n)	Mount & Norberg (1984)	
<i>Ceriodaphnia dubia</i>		stat	25				bromide	48-h LC ₅₀	0.50 (m)	Magliette et al. (1995)
<i>C. reticulata</i>	<24 h	stat		45	7.2–7.4			48-h LC ₅₀	0.076 (n)	Mount & Norberg (1984)
<i>D. hyalina</i>	1.27 mm	stat	10		7.2	sulfate	48-h LC ₅₀	0.04 (n)	Baudouin & Scoppa (1974)	
<i>D. lumholtzi</i>		stat*	28.5	200	7.9			48-h LC ₅₀	2.29 (n)	Vardia et al. (1988)
		stat*	28.5	200	7.9			96-h LC ₅₀	0.44 (n)	

Table 37 (contd.)

<i>Moina irrasa</i>	<24 h	stat	20	<5	8.0	chloride	48-h LC ₅₀	0.059 (n)	Zou & Bu (1994)
<i>M. macrocopa</i>		stat*	24–27		6.5	sulfate	48-h LC ₅₀	1.17 (n)	Wong (1992)
Copepod	1.27 mm	stat	10		7.2	sulfate	48-h LC ₅₀	5.5 (n)	Baudouin & Scoppa (1974)
<i>Cyclops abyssorum</i>									
<i>Eudiaptomus padanus</i>	1.27 mm	stat	10		7.2	sulfate	48-h-LC ₅₀	0.50 (n)	Baudouin & Scoppa (1974)
<i>Parastenocaris germanica</i>	adult	stat	10.5	10*	6.8	sulfate	48-h LC ₅₀	4.5 (m)	Notenboom et al. (1992)
	adult	stat	10.5		6.8	sulfate	96-h LC ₅₀	1.7 (m)	
Amphipod		stat	17	50	7.6		24-h LC ₅₀	10.2 (m)	Rehboldt et al. (1973)
<i>Gammarus</i> sp.		stat	17	50	7.6		96-h LC ₅₀	8.1 (m)	
<i>Crangonyx pseudogracilis</i>	4 mm	stat	13	50	6.75	sulfate	48-h LC ₅₀	121 (n)	Martin & Holdich (1986)
Isopod	4 mm	stat	13	50	6.75	sulfate	96-h LC ₅₀	19.8 (n)	
<i>Asellus aquaticus</i>	7 mm	stat	13	50	6.75	sulfate	96-h LC ₅₀	18.2 (n)	
Ostracod		stat*	28.5	200	7.9		48-h LC ₅₀	34.99 (n)	Vardia et al. (1988)
<i>Cypris subglobosa</i>		stat*	28.5	200	7.9		96-h LC ₅₀	8.35 (n)	Vardia et al. (1988)

Table 37 (contd.)

Organism	Size/age	Stat/flow	Temp (°C)	Hardness (mg/litre)	pH	Zinc compound tested	Parameter	Concentration (mg/litre)	Reference
Harpacticoid <i>Nitocra spinipes</i>	adult	stat	21	7	7.8	sulfate	96-h LC ₅₀	4.3 (n)	Lindén et al. (1979)
Rotifer <i>Brachionus calyciflorus</i>	juvenile	stat	20	36.2	7.3	chloride	24-h LC ₅₀	1.32 (n)	Couillard et al. (1989)
		stat	25				24-h LC ₅₀	1.3 (n)	Snell et al. (1991)
Midge <i>Chironomus</i> sp.		stat	17	50	7.6		24-h LC ₅₀	21.5 (m)	Rehwoldt et al. (1973)
<i>C. tentans</i>	3rd instar	stat	17	50	7.6		96-h LC ₅₀	18.2 (m)	Rehwoldt et al. (1973)
		stat	13	25	6.3	sulfate	48-h EC ₅₀	8.2 (n)	Khengarot & Ray (1989)
Caddis fly Unidentified		stat	17	50	7.6		24-h LC ₅₀	62.6 (m)	Rehwoldt et al. (1973)
		stat	17	50	7.6		96-h LC ₅₀	58.1 (m)	Rehwoldt et al. (1973)
Damsel fly Unidentified		stat	17	50	7.6		24-h LC ₅₀	32 (m)	Rehwoldt et al. (1973)
		stat	17	50	7.6		96-h LC ₅₀	26.2 (m)	Rehwoldt et al. (1973)

hw = humic water; m = measured concentrations; n = nominal concentrations; stat = static conditions (water unchanged for duration of test); stat* = static renewal conditions (water changed at regular intervals)

^a EC₅₀ values based on immobilization; hardness expressed as mg/litre O₃.

Table 38. Toxicity of zinc to marine invertebrates^a

Organism	Size/age	Stat/flow	Temp (°C)	Salinity (‰)	pH	Zinc salt	Parameter	Concentration (mg/litre)	Reference
Starfish <i>Asterias forbesi</i>	11.2 g	stat	20	20	7.8	chloride	96-h LC ₅₀	39 (n)	Eisler & Hennekey (1977)
American oyster <i>Crassostrea virginica</i>	embryo	stat	26	25		chloride	48-h LC ₅₀	0.31 (n)	Calabrese et al. (1973)
Mussel <i>Mytilus edulis</i>		stat	12	7		chloride	24-h LC ₅₀	20.8 (n)	Hietanen et al. (1988)
<i>M. edulis planulatus</i>		stat flow	20.6 17.6	34	8.0 7.8	chloride chloride	96-h LC ₅₀ 96-h LC ₅₀	2.5 (m) 3.6 (m)	Ahsanullah (1976) Ahsanullah (1976)
Bay scallop <i>Argopecten irradians</i>	juvenile	stat*	20	25		chloride	96-h LC ₅₀	2.25 (n)	Nelson et al. (1988)
Surf clam <i>Spisula solidissima</i>	juvenile	stat*	20	25		chloride	96-h LC ₅₀	2.95 (n)	Nelson et al. (1988)
Soft-shell clam <i>Mya arenaria</i>	4.6 g	stat	20	20	7.8	chloride	96-h LC ₅₀	7.7 (n)	Eisler & Hennekey (1977)

Table 38 (contd.)

Organism	Size/age	Stat/flow	Temp (°C)	Salinity (•)	pH	Zinc salt	Parameter	Concentration (mg/litre)	Reference
Squid <i>Loligo opalescens</i>	larvae	stat	8.6	30	8.1	chloride	96-h LC ₅₀	>1.92 (m)	Dinnel et al. (1989)
Cabezon <i>Scorpaenichthys marmoratus</i>	larvae	stat	8.3	27	7.9	chloride	96-h LC ₅₀	0.191 (m)	Dinnel et al. (1989)
Eastern mud snail <i>Nassarius obsoletus</i>	0.4 g	stat	20	20	7.8	chloride	96-h LC ₅₀	50 (n)	Eisler & Hennekey (1977)
Amphipod <i>Allorchestes compressa</i>	0.06 g	stat	20.5	34.5	7.9	chloride	96-h LC ₅₀	0.58 (m)	Ahsanullah (1976)
Harpacticoid copepod <i>Nitocra spinipes</i>						chloride	96-h LC ₅₀	0.85 (n)	Bengtsson & Bergström (1987)
						chloride	96-h LC ₅₀	1.3 (n)	Bengtsson & Bergström (1987)
						sulfate	96-h LC ₅₀	2.4 (n)	Bengtsson & Bergström (1987)
						sulfate	96-h LC ₅₀	2.8 (n)	Bergström (1987)
Ragworm <i>Nereis virens</i>	7.6 g	stat	20	20	7.8	chloride	96-h LC ₅₀	8.1 (n)	Eisler & Hennekey (1977)

Table 38 (contd.)

Sandworm <i>Neanthes vaalii</i>	0.33 g	stat	18.7	34.2	7.9	chloride	96-h LC ₅₀	5.5 (m)	Ahsanullah (1976)
Dungeness crab <i>Cancer magister</i>	larvae	stat	8.5	30	8.1	chloride	96-h LC ₅₀	0.586 (m)	Dinnel et al. (1989)
Fiddler crab <i>Uca annulipes</i>	24–29 mm	stat	29	25		sulfate	96-h LC ₅₀	31.9 (n)	Devi (1987)
	24–29 mm	stat	29	25		sulfate	96-h LC ₅₀	77 (n) (polluted)	Devi (1987)
<i>U. triangularis</i>	24–29 mm	stat	29	25		sulfate	96-h LC ₅₀	39.1 (n)	Devi (1987)
	24–29 mm	stat	29	25		sulfate	96-h-LC ₅₀	66.4 (n) (polluted)	Devi (1987)
Hermit crab <i>Pagurus longicarpus</i>	0.5 g	stat	20	20	7.8	chloride	96h LC ₅₀	0.4 (n)	Eisler & Hennekey (1977)
Grapsid crab <i>Paragrapsus quadridentatus</i>	1.44 g	stat	19.6	34.2	8.1	chloride	96-h LC ₅₀	11 (m)	Ahsanullah (1976)
Crab <i>Portunus pelagicus</i>	zoeae	stat*	25–27	35		chloride	48-h LC ₅₀	0.56–0.77 (n)	Greenwood & Fielder (1983)
<i>P. sanguinolentus</i>	zoeae	stat*	25–27	35		chloride	48-h LC ₅₀	0.62 (n)	Greenwood & Fielder (1983)
<i>Charybdis feriatus</i>	zoeae	stat*	25–27	35		chloride	48-h LC ₅₀	0.96 (n)	Greenwood & Fielder (1983)

Table 38 (contd.)

Organism	Size/age	Stat/flow	Temp (°C)	Salinity (•)	pH	Zinc salt	Parameter	Concentration (mg/litre)	Reference
Copepod <i>Tisbe holothuriae</i>		stat	22	38		sulfate	48-h LC ₅₀	0.62 (n)	Verriopoulos & Dimas (1988)
Grass shrimp <i>Palaemonetes pugio</i>	juvenile	stat*	20	10		chloride	48-h LC ₅₀	11.3 (m)	Burton & Fisher (1990)
Shrimp <i>Palaemon</i> sp.	0.28 g	stat	19.5	35.5	7.8	chloride	96-h LC ₅₀	9.5 (m)	Ahsanullah (1976)
Mysid <i>Holmesimysis costata</i>	juvenile	stat	13–15.5	34–36		sulfate	48-h LC ₅₀	0.458 (m)	Martin et al. (1989)
	juvenile	stat*	13–16	34–40		sulfate	96-h LC ₅₀	0.097 (m)	Martin et al. (1989)
Prawn <i>Metapenaeus dobsoni</i>	30–50 mm	stat*	27.5		7.5	sulfate	48-h LC ₅₀	3 (n)	Sivadasan et al. (1986)
	30–50 mm	stat*	27.5		7.5	sulfate	96-h LC ₅₀	0.84 (n)	(1986)

flow = flow-through conditions (zinc concentration in water continuously maintained); m = measured concentrations; n = nominal concentrations; stat = static conditions (water unchanged for duration of test); stat* = static renewal conditions (water changed at regular intervals)

^a EC₅₀ values based on immobilization; hardness expressed as mg/litre O₃.

Table 39. Toxicity (96-h LC₅₀) of zinc to freshwater fish^a

Organism	Size/age	Stat/flow	Temp (°C)	Hardness (mg/litre)	pH	Zinc salt	Concentration (mg/litre)	Reference
Chinook salmon <i>Oncorhynchus tshawytscha</i>	1.03 g	stat	12	211	7.4–8.3	chloride (47.3%)	1.27 (n)	Hamilton & Buhl (1990)
	juvenile	flow	11–13	20–21	7.1–7.2	sulfate	0.084 (m)	Finlayson & Verrue (1982)
	alevin	flow	12	23	7.1		>0.66 (n)	Chapman (1978b)
	swim-up	flow	12	23	7.1		0.097 (n)	Chapman (1978b)
	parr	flow	12	23	7.1		0.46 (n)	Chapman (1978b)
	smolt	flow	12	23	7.1		0.7 (n)	Chapman (1978b)
Coho salmon <i>O. kisutch</i>	alevin	stat	12	41	7.1–8.0	chloride	0.73 (n)	Buhl & Hamilton (1990)
	0.47 g	stat	12	41	7.1–8.0	chloride	0.82 (n)	Buhl & Hamilton (1990)
	0.63 g	stat	12	41	7.1–8.0	chloride	1.81 (n)	Buhl & Hamilton (1990)
	2.7 kg	flow	14	25	7.4	chloride	0.91 (n)	Chapman & Stevens (1978)
Rainbow trout <i>O. mykiss</i>	alevin	stat	12	41	7.1–8.0	chloride	2.17 (n)	Buhl & Hamilton (1990)
	0.60 g	stat	12	41	7.1–8.0	chloride	0.17 (n)	Buhl & Hamilton (1990)
	juvenile	flow			6.4–8.3	acetate	0.550 (m)	Hale (1977)
	alevin	flow	12	23	7.1		0.815 (n)	Chapman (1978b)
	swim-up	flow	12	23	7.1		0.093 (n)	Chapman (1978b)

Table 39 (contd.)

Organism	Size/age	Stat/flow	Temp (°C)	Hardness (mg/litre)	pH	Zinc salt	Concentration (mg/litre)	Reference
<i>Rainbow trout</i> (contd.)	parr	flow	12	23	7.1		0.136 (n)	Chapman (1978b)
	smolt	flow	12	23	7.1		>0.651 (n)	Chapman (1978b)
	2.7 kg	flow	10	83	7.45	chloride	1.76 (n)	Chapman & Stevens (1978)
	juvenile	flow	15	26	6.8	sulfate	0.43 (n)	Sinley et al. (1974)
	juvenile	flow	15	333	7.8	sulfate	7.21 (n)	Sinley et al. (1974)
	25–70 g	flow	12.7	137	7.3	sulfate	2.6 (m)	Meisner & Quan Hum (1987)
	160–290 g	flow	12.9	143	7.1	sulfate	2.4 (m)	(1987)
Cutthroat trout	0.6 g	stat	10	38	7.5	sulfate	0.152	Mayer & Ellersieck (1986)
<i>Salmo clarki</i>	0.9 g	stat	15	43	7.5	sulfate	0.600	Mayer & Ellersieck (1986)
	0.9 g	stat	10	40	7.8	sulfate	0.130	Mayer & Ellersieck (1986)
	1.0 g	stat	10	40	8.5	sulfate	0.061	Mayer & Ellersieck (1986)
	1.0 g	stat	10	38	6.5	sulfate	0.100	Mayer & Ellersieck (1986)
	1.0 g	stat	5	38	7.5	sulfate	0.074	Mayer & Ellersieck (1986)
Fathead minnow	79 mg	flow	25	220	7.8	sulfate	2.61 (n)	Broderius & Smith (1979)
<i>Pimephales promelas</i>	1–2 g	stat	25	20	7.5	sulfate	0.77–0.96 (n)	Pickering & Henderson (1966)
	1–2 g	stat	25	360	8.2	sulfate	33.4 (n)	

Table 39 (contd.)

	1-2 g	stat	25	20	7.5	acetate	0.88 (n)	Pickering & Henderson (1966)
	1-2 g	stat	15	20	7.5		2.33 and 2.55 (n)	
Arctic grayling <i>Thymallus arcticus</i>	fry	stat	12	41	7.1-8.0	chloride	0.32 (n)	Buhl & Hamilton (1990)
	alevin	stat	12	41	7.1-8.0	chloride	2.92 (n)	Buhl & Hamilton (1990)
	0.20 g	stat	12	41	7.1-8.0	chloride	0.14 (n)	Buhl & Hamilton (1990)
	0.85 g	stat	12	41	7.1-8.0	chloride	0.17 (n)	Buhl & Hamilton (1990)
Bluegill <i>Lepomis macrochirus</i>	1-2 g	stat	25	20	7.5	sulfate	4.85-5.82 (n)	Pickering & Henderson (1966)
	1-2 g	stat	25	360	8.2	sulfate	40.9 (n)	
	1-2 g	stat	15	20	7.5		6.44 (n)	
Pumpkinseed <i>Lepomis gibbosus</i>		stat	28	55	8.0		20.1 (m)	Rehwoldt et al. (1972)
Banded killifish <i>Fundulus diaphanus</i>		stat	28	55	8.0		19.2 (m)	Rehwoldt et al. (1972)
Striped bass <i>Morone saxatilis</i>		stat	28	55	8.0		6.8 (m)	Rehwoldt et al. (1972)

Table 39 (contd.)

Organism	Size/age	Stat/flow	Temp (°C)	Hardness (mg/litre)	pH	Zinc salt	Concentration (mg/litre)	Reference
White perch <i>Roccus americanus</i>		stat	28	55	8.0		14.4 (m)	Rehwoldt et al. (1972)
American eel <i>Anguilla rostrata</i>		stat	28	55	8.0		14.5 (m)	Rehwoldt et al. (1972)
Carp <i>Cyprinus carpio</i>		stat	28	55	8.0		7.8 (m)	Rehwoldt et al. (1972)
	3.2 cm	stat*	15		7.1	sulfate	0.45–1.34 (n)	Alam & Maughan (1992)
	6.0 cm	stat*	15		7.1	sulfate	1.64–2.25 (n)	Alam & Maughan (1992)
	47–62 mm	stat*	15	19	6.3	sulfate	3.12 (n)	Khargarot et al. (1983)
Goldfish <i>Carassius auratus</i>	1–2 g	stat	25	20	7.5	sulfate	6.44 (n)	Pickering & Henderson (1966)
Guppy <i>Poecilia reticulata</i>	0.1–0.2 g	stat	25	20	7.5	sulfate	1.27 (n)	Pickering & Henderson (1966)

Table 39 (contd.)

Flagfish <i>Jordanella floridae</i>	juvenile	flow	25	44	7.1–7.8	sulfate	1.5 (n)	Spehar (1976)
Channelfish <i>Nuria denricus</i>	500 mg	stat		4	6.1		6.06 (n)	Abbasi & Soni (1986)
Tilapia <i>Tilapia zilli</i>	subadult	stat	9.3	20–22	6.7	sulfate	33 (n)	Hilmy et al. (1987)
	subadult	stat	25	20–22	6.7	sulfate	13 (n)	Hilmy et al. (1987)
Catfish <i>Clarius lazera</i>	subadult	stat	9.3	20–22	6.7	sulfate	52 (n)	Hilmy et al. (1987)
	subadult	stat	25	20–22	6.7	sulfate	26 (n)	Hilmy et al. (1987)

flow = flow-through conditions (zinc concentration in water continuously maintained); m = measured concentrations; n = nominal concentrations; stat = static conditions (water unchanged for duration of test); stat* = static renewal conditions (water changed at regular intervals)

^a Hardness expressed as CaCO₃ in mg/litre.

Table 40. Toxicity of zinc to marine fish

Organism	Size/age	Stat/flow	Temp (°C)	Salinity (•)	pH	Zinc salt	Parameter	Concentration (mg/litre)	Reference
Chinook salmon <i>Oncorhynchus tshawytscha</i>	2.6 g	stat	11–13	brackish	7.6–8.1	chloride (47.3%)	96-h LC ₅₀	2.88 (n)	Hamilton & Buhl (1990)
Atheriniform fish <i>Rivulus marmoratus</i>	0.03–0.1 g	flow	26–27	14			96-h LC ₅₀	119.3–176.6	Lin & Dunson (1993)
Mummichog <i>Fundulus heteroclitus</i>	0.02–0.1 g juvenile	flow stat*	26–27 20	14 10		chloride	96-h LC ₅₀ 48-h LC ₅₀	129.5 (n) 96.5 (m)	Lin & Dunson (1993) Burton & Fisher (1990)
	1.3 g	stat	20	20	7.8	chloride	96-h LC ₅₀	60 (n)	Eisler & Hennekey (1977)
Grey mullet <i>Chelon labrosus</i>	0.87 g	flow	12	34.6	7.7	nitrate	96-h LC ₅₀	21.5 (m)	Taylor et al. (1985)

Table 40 (contd.)

English sole <i>Parophrys vetulus</i>	larvae	stat	12			sulfate	96-h LC ₅₀	14.5 (n)	Shenker & Cherr (1990)
Bleak <i>Alburnus alburnus</i>	8 cm	stat	10	7	7.8	chloride	96-h LC ₅₀	32 (n)	Lindén et al. (1979)
	8 cm	stat	10	7	7.8	sulfate	96-h LC ₅₀	41.9 (n)	Lindén et al. (1979)
Tidewater silverside <i>Menidia peninsulæ</i>	larvae	stat	25	20		sulfate	9-6h LC ₅₀	5.6 (n)	Mayer (1987)
Spot <i>Leiostomus xanthurus</i>	adult	stat	26	25		sulfate	96-h LC ₅₀	38 (n)	Mayer (1987)

flow = flow-through conditions (zinc concentration in water continuously maintained); m = measured concentrations; n = nominal concentrations; stat = static conditions (water unchanged for duration of test); stat* = static renewal conditions (water changed at regular intervals)

Zinc toxicity is also influenced by water pH and salinity, although the dose–response relationship is not necessarily monotonic (McLusky & Hagerman, 1987; Meinel & Krause, 1988; Reader et al., 1989). Notenboom et al. (1992) found no effect of reducing dissolved oxygen concentration (5.4 mg/litre to 0.1 mg/litre) on the toxicity of zinc to the copepod, *Parastenocanis germanica*. Paulauskis & Winner (1988) reported that the toxicity of zinc to *Daphnia magna* decreased with increasing concentrations of humic acids.

Bengsston (1974a) reported that yearling minnow (*Phoxinus phoxinus*) were more sensitive to zinc than adults, and Naylor et al. (1990) reported that juvenile *Gammarus pulex* and *Asellus aquaticus* were more sensitive than large adults. However, other studies have found little effect of organism age on zinc toxicity (Martin et al., 1989; Collyard et al., 1994).

Acute and short-term toxicity

Of the studies reported in Table 37, the results from five freshwater crustaceans meet the minimal data requirements. For four species the LC₅₀ values for zinc at 48–96 h range from 0.5 to 10 mg/litre; *Asellus aquaticus* was less sensitive to zinc (194–575 mg/litre). Other acute toxicity test results reported range from 0.04 to 2.29 mg/litre zinc for daphnids (*Daphnia*, *Ceriodaphnia* and *Moina*) to 28.1 and 62.6 mg/litre for a snail species and a caddisfly, respectively.

Acute toxicity results for eight marine invertebrate species were acceptable, in accordance with the minimal data requirements (Table 38). The 96-h LC₅₀ values for four species (including cabezon, amphipod, crab and mysid species) ranged from 0.191 to 0.586 mg/litre; those for the remaining species ranged from 2.5 to 11.3 mg/litre. Other results ranged from 0.31 (American oyster) to 77 mg/litre (fiddler crab).

Dinnel et al. (1989) reported on short-term zinc toxicity tests with the early life stages of echinoderms. Threshold values (EC₅₀) for the purple sea urchin (*Strongylocentrotus purpuratus*) were 23 and 262 µg/litre for embryo development (120 h) and gamete fertilization (80 min), respectively. Using the latter end-point, these

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authors also report EC₅₀ values of 383 and 28 µg/litre for the green sea urchin (*S. droebachiensis*) and the sand dollar (*Dendraster excentricus*).

Baird et al. (1991) found that the 48-h EC₅₀ for zinc for different clones of *Daphnia magna* ranged from 0.76 to 1.83 mg/litre. Hietanen et al. (1988) exposed the common mussel *Mytilus edulis* to increased zinc concentrations in brackish water (salinity 7‰) at a temperature of 12 °C. The 24-h EC₅₀ values, based on an increased opening response and on byssal attachment, were found to be 1.35 and 0.64 mg/litre respectively. Kraak et al. (1994a) calculated the 48-h EC₅₀, based on filtration rate, to be 1.35 mg/litre for the zebra mussel (*Dreissena polymorpha*). The no-observed-effect concentration (NOEC) for the same parameter was 0.19 mg/litre.

Acute zinc toxicity data for two species of freshwater fish met the minimal requirements (Table 39). The 96-h LC₅₀ values for *Oncorhynchus tshawytscha* and *O. mykiss* were 1.27 and 2.6 mg/litre, respectively. Other results for freshwater fish ranged from 0.061 to 52 mg/litre.

Data on three marine fish species were acceptable (Table 40). The 96-h LC₅₀ values ranged from 21.5 mg/litre for grey mullet (*Chelon labrosus*) to 176.6 mg/litre for *Rivulus marmoratus*. A 96-h LC₅₀ range of 2.88 to 129.5 mg/litre was found in the other reported data.

Norberg & Mount (1985) calculated the 7-day LC₅₀ for the fathead minnow (*Pimephales promelas*) to be 0.238 mg/litre in Lake Superior water (hardness 48 mg/litre, CaCO₃). No zinc-induced growth inhibition was observed at 0.18 mg/litre but survival was significantly lower at that concentration. The maximum acceptable toxicant concentration was estimated to be 0.125 mg/litre. Magliette et al. (1995) exposed larval fathead minnow (*Pimephales promelas*) to zinc bromide in 7-day static renewal tests. The 7-day LC₅₀ and EC₅₀ (growth), based on measured concentrations, were 0.78 and 0.76 mg/litre respectively. The lowest-observed-effect concentration (LOEC) for growth was 0.63 mg/litre.

Reader et al. (1989) found that mortality in brown trout (*Salmo trutta*) exposed to zinc at a concentration of 281 µg/litre at pH 6.5 in

soft water (calcium 22 µmol/litre) remained low during 30-day exposures, while in fish exposed to 0.316 mg/litre at pH 4.5, mortality was greater than 80%.

Mount et al. (1994) fed rainbow trout (*Oncorhynchus mykiss*) on a brine shrimp (*Artemia* sp.) diet containing zinc at concentrations of 920, 930 or 1900 mg/kg dw for up to 60 days. No significant mortality or effect of zinc on growth was observed during the experiment. Spry et al. (1988) fed rainbow trout (*O. mykiss*) on a purified diet containing zinc concentrations of 1, 90 and 590 mg/kg, which ranged from deficient to excessive. Fish were simultaneously exposed to zinc concentrations in water of up to 0.5 mg/litre for 16 weeks. There was no significant difference in the physical condition of fish in any treatment compared with controls.

Chronic and long-term toxicity

Data that meet the selection criteria are presented in Table 41 for freshwater invertebrates and in Table 42 for freshwater fish. No data are presented for marine and estuarine species. Table 41 contains data for four invertebrate species, two crustaceans, an insect and a snail, tested under a variety of experimental conditions and in waters of different pH (6.9–8.39), hardness (15–197 mg/litre, CaCO₃) and humic acid concentration. The threshold zinc concentrations range from 25 to 225 µg/litre (both values for *D. magna*) and clearly illustrate the influence of water hardness and humic acid concentration on zinc toxicity.

With respect to freshwater fish (Table 42), there are primary chronic toxicity data for six species covering water hardness ranging from 35 to 374 mg/litre, CaCO₃. For water hardness of ≥ 100 mg/litre, CaCO₃, all NOECs are ≥ 500 mg/litre, except in one behavioural study (Korver & Sprague, 1989), which reported a NOEC of 60 mg/litre. For studies in which water hardness was ≤ 100 mg/litre, CaCO₃, all NOECs were ≤ 50 mg/litre.

Freshwater studies

Farris et al. (1989) studied growth and cellulase activity in the Asiatic clam (*Corbicula* sp.) during a 30-day exposure to zinc sulfate concentrations ranging from 0.034 to 1.1 mg/litre. The cellulase

Table 41. Long-term and chronic toxicity to freshwater invertebrates^a

Species	Life stage/age	End-point	pH	Hardness (mg/litre)	Humic acid (mg/litre)	Temp (°C)	Duration (days)	Threshold (zinc in µg/litre)	Reference
Water flea <i>Daphnia magna</i>	< 24 h	survival (LC ₅₀)	7.74	45.3	–	18	21	158	Biesinger & Christensen (1972)
		production of young (EC ₅₀)	7.74	45.3	–	18	21	102	
		production of young (MATC)	8.39	51.9	–	20	50	25	Paulauskis & Winner (1988)
			8.32	101.8	–	20	50	87.5	
			8.29	197	–	20	50	175	Paulauskis & Winner (1988)
			8.29	197	1.5	20	50	225	
8.39	51.9	1.5	20	50	100				
<i>Ceriodaphnia dubia</i>	< 24 h	production of young (MATC)	8	97.6	–	25	7	22	Belanger & Cherry (1990a)
				113.6				71	
				182				71	
Midge <i>Tanytarsus dissimilis</i>	eggs	survival (LC ₅₀)	7.5	46.8	–	22	10	36.8	Anderson et al. (1980)
Snail <i>Ancylus fluviatilis</i>	adults	eggs per capsule (NOEC–LOEC)	6.9	15–15.3	–	–	31	105–187	Willis (1988)

LOEC = lowest-observed-effect concentration; MATC = maximum acceptable toxicant contamination; NOEC = no-observed-effect concentration

^a Measured zinc concentrations were ± 15% at nominal concentrations.

Table 42. Long-term and chronic toxicity to freshwater fish

Species	Life stage/ age	End-point	pH	Hardness (mg/litre)	Humic acid (mg/litre)	Temp (°C)	Duration (days)	Threshold (µg zinc/litre)	Reference
<i>Brachydanio rerio</i>	embryo- larval	hatchability (NOEC)	7.5	100	–	25	16	500	Dave et al. (1987)
<i>Phoxinus phoxinus</i>	yearling	growth (MATC)	7.5	(3.9 dH; alkalinity; 64 mg/litre)	–	12	150	80.6	Bengtsson (1974a)
<i>Oncorhynchus mykiss</i>	yearling (45 g)	growth and hypo- glycaemia (MATC)	7.3	374	–	10	100	763	Watson & McKeown (1976)
<i>Pimephales promelas</i>	males (4.6 g)	avoidance (MATC)	8.1	318	–	20	7.5	130.5	Korver & Sprague (1989)

Table 42 (contd.)

<i>P. promelas</i>	full life cycle	critical end-point (MATC)	7-8	46	-	25	154	106	Benoit & Holcombe (1978)
<i>O. nerka</i>	adult embryo-juvenile	survival, fertility, fecundity, growth, osmoregulation, acclimation (MATC)	7.2	35	-	9-14	21 months	164.6	Chapman (1978a)
<i>Salvelinus fontinalis</i>	3-generation life-cycle	all life-cycle parameters (egg fragility was critical end-point) (MATC)	7.0-7.7	45	-	9	3 generations	852	Holcombe et al. (1979)

MATC = maximum acceptable toxicant contamination ; NOEC = no-observed-effect concentration

index declined following weight and shell loss between days 20 and 30 at the lower dose and by day 30 the growth rate was only 50% of controls. At the higher dose, animals did not grow after 5 days and had a rapidly declining cellulase index; 50% of the clams at this exposure concentration died.

Münzinger & Guarducci (1988) exposed the freshwater snail *Biomphalaria glabrata* to increased zinc concentrations (0.5 to > 5.0 mg/litre) for 33 days. At a zinc concentration of 1.5 mg/litre, 60% of young snails and 20% of adults died; at concentrations of ≥ 3.0 mg/litre no snails survived. Egg capsules were produced at zinc concentrations of up to 1.5 mg/litre. The number of eggs per capsule and the fecundity of the molluscs were significantly reduced by zinc exposure.

Mirenda (1986) calculated the 2-week LC₅₀ for zinc for the crayfish (*Orconectes virilis*) to be 84 mg/litre in soft water (26 mg/litre, CaCO₃). Bodar et al. (1989) exposed parthenogenetic eggs of *Daphnia magna* to zinc concentrations of 10, 50 and 100 mg/litre. Exposure at 10 and 50 mg/litre had no significant effect on death rates in the six early life stages. There was no effect of zinc on survival, even at the highest exposure concentration, during developmental stages 1 and 2 (these stages take about half of the development time from egg to juvenile). The toxicity of zinc at 100 mg/litre was exerted during stages 3–6. Winner (1981) studied the toxicity of zinc to *Daphnia magna* in lifetime exposure tests. Zinc caused a significant reduction in body length of primiparous animals and longevity at concentrations of ≥ 0.1 mg/litre; however, mean brood sizes of animals reared at 0.2 mg/litre were not significantly different from those of control animals. Winner & Gauss (1986) found that an increase in water hardness from 52 to 102 mg/litre (CaCO₃) resulted in a significant reduction in zinc toxicity as estimated from survival curves over a 50-day exposure to zinc at 0.125 mg/litre. The addition of humic acid (1.5 mg/litre) to soft water (52 mg/litre, CaCO₃) significantly increased survival.

Paulauskis & Winner (1988) studied the effect of zinc on the brood size of *Daphnia magna* in chronic (50-day) toxicity tests. An increase in water hardness from 50 to 200 mg/litre (CaCO₃), and the addition of humic acid (1.5 mg/litre) significantly reduced the toxic effect of zinc on brood size. NOEC values were 0.1 and

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0.025 mg/litre in soft water with and without humic acid and 0.225 and 0.175 mg/litre in hard water with and without humic acid, respectively.

Belanger & Cherry (1990) exposed *Ceriodaphnia dubia* to zinc in reproductive toxicity tests at three pH levels (6, 8 and 9) in three different surface waters from Virginia and Louisiana, USA. In New River water (hardness, 97.6 mg/litre, CaCO₃) significant reproductive impairment, as measured by the number of young per female, was found at a zinc concentration of 0.025 mg/litre at pH 6 and 8, while in Amy Bayou water (hardness, 113.6 mg/litre, CaCO₃) significant reproductive impairment was noted at 0.1 mg/litre. Reproductive impairment was found at 0.05 mg/litre in Clinch River water at pH levels of 6 and 9 but not 8.

Biesinger & Christensen (1972) exposed *Daphnia magna* to zinc chloride for 3 weeks. The 3-week LC₅₀ was 0.16 mg/litre; the 3-week EC₅₀, based on reproductive impairment, was 0.10 mg/litre. Enserink et al. (1991) calculated the 21-day LC₅₀ for *Daphnia magna* to be 0.84 mg/litre in Lake Ijssel water (background zinc concentration < 0.01 mg/litre; hardness, 225 mg/litre, CaCO₃). An EC₅₀ based on population growth was 0.57 mg/litre. Münzinger & Monicelli (1991) carried out 21-day tests on *Daphnia magna* at added zinc concentrations of 0.05, 0.10 or 0.15 mg/litre in lake water (total zinc < 6 µg/litre). No significant effects on survival or reproduction were reported at the two lower concentrations. At 0.15 mg/litre, mortality was 80%, the number of progeny was reduced by more than 50%, and primiparous individuals were significantly smaller and produced significantly fewer eggs.

Wong (1993) studied the effect of zinc on the longevity and reproduction of the cladoceran *Moina macrocopa* reared in aquarium water with a zinc content of less than 1 µg/litre. A significant reduction in survival was observed at < 0.5 mg/litre within 1 day. The LT₅₀ (time taken for 50% of animals to die) was reduced by more than 2 days at a zinc concentration of > 0.45 mg/litre and the average life span was reduced by more than 50% at > 0.70 mg/litre compared to controls. The net reproductive rate decreased abruptly at 0.7 mg/litre.

Maltby & Naylor (1990) exposed *Gammarus pulex* to zinc concentrations of 0.1, 0.3 or 0.5 mg/litre in 7-day tests. Zinc had no significant effect on either the number or size of offspring released from the current brood or on the number of offspring released from the subsequent brood, incubated under non-stressed conditions. The metal did cause a significant reduction in the size of offspring released from the subsequent brood and a positive correlation between zinc concentration and the number of broods aborted.

Anderson et al. (1980) calculated a 10-day LC₅₀ for zinc for the midge *Tanytarsus dissimilis* reared in unfiltered Lake Superior water (background zinc concentration 5.1 µg/litre) to be 0.037 mg/litre. The midges were exposed to zinc during embryogenesis, hatching and larval development to the 2nd or 3rd instar. In flow-through life-cycle tests with caddisfly (*Clistoronia magnifica*), the highest zinc concentration tested, 5.2 mg/litre, had no significant effect on any life stage (Nebeker et al., 1984).

Dave et al. (1987) reported the results of a ring test of the 16-day embryo-larval toxicity test on zebrafish (*Brachydanio rerio*) using zinc sulfate as the toxicant. Hatching time delay was found to be the most sensitive parameter, with an NOEC of 0.5 mg/litre. Dawson et al. (1988) studied the effect of zinc on fathead minnow (*Pimephales promelas*) and South African clawed toad (*Xenopus laevis*) in embryo-larval assays. Static renewal tests were conducted for 6 days with minnow embryos to allow for hatching to take place and for 4 days with toad embryos. LC₅₀ values were found to be 3.6 mg/litre for fathead minnows and 34.5 mg/litre for toad embryos. EC₅₀ values, based on malformation, were 0.8 and 3.6 mg/litre for the two species, respectively; the minimum concentrations that significantly inhibited growth were 0.6 and 4.2 mg/litre, respectively.

Sayer et al. (1989) exposed yolk-sac fry of brown trout (*Salmo trutta*) to zinc concentrations of 4.9, 9.8 and 19.5 µg/litre (75, 150 and 300 nmol/litre) at pH 4.5 and calcium concentrations of 20 or 200 µmol for 30 days. Mortalities were high (70–100%) at the lower calcium concentration for all three zinc concentrations. No deaths or significant effects on mineral uptake were observed for zinc at the higher calcium exposure.

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Bengtsson (1974a) exposed both yearling and adult minnows (*Phoxinus phoxinus*) to zinc as zinc nitrate in freshwater (< 0.02 mg/litre) over a 150-day period. Yearlings were the most sensitive, with growth significantly reduced at 0.13 mg/litre. Suppressed growth was associated with reduced feeding activity.

Chapman (1978a) studied the chronic toxicity of zinc to sockeye salmon (*Oncorhynchus nerka*) in a 22-month adult-to-smolt toxicity test. Fish were exposed to zinc concentrations ranging from 30 to 242 µg/litre in well-water (background zinc concentration 2 µg/litre; hardness, 35 mg/litre, CaCO₃). No adverse effects on survival, fertility, fecundity, growth or the subsequent survival of smolts transferred to seawater were observed.

Spehar (1976) exposed flagfish (*Jordanella floridae*) to zinc concentrations ranging from 28 to 267 µg/litre during a complete life-cycle test in untreated Lake Superior water (background zinc concentration 10 µg/litre; hardness, 44 mg/litre, CaCO₃). The 30-day survival of larvae previously exposed to zinc as embryos was significantly reduced at 267 µg/litre, while growth (100 days) was significantly reduced at 139 µg/litre. Reproduction was unaffected at zinc concentrations of up to 139 µg/litre. In a second experiment, fish were not exposed as embryos and significant reductions were observed in survival at 85 µg/litre after 30 days. The growth of female fish (100 days) was significantly reduced at 51 µg/litre. It should be noted that background zinc concentrations were less than 1 µg/litre in the second experiment. Spehar et al. (1978) found that cadmium (4.3–8.5 µg/litre) did not influence the mode of action of zinc under the same experimental conditions. The joint action of the toxicants on survival was little different from the toxicity of zinc alone.

Benoit & Holcombe (1978) carried out fathead minnow (*Pimephales promelas*) life-cycle tests in Lake Superior water (mean total zinc 2 µg/litre) at total zinc concentrations ranging from 44 to 577 µg/litre. The most sensitive parameters were egg adhesiveness and fragility, which were significantly affected at 145 µg/litre but not at 78 µg/litre. Hatchability and survival of larvae were significantly reduced, and deformities at hatching significantly increased at ≥ 295 µg/litre.

Holcombe et al. (1979) found no significant harmful effects on brook trout (*Salvelinus fontinalis*) exposed to zinc concentrations ranging from 2.6 (control) to 534 µg/litre for three generations in Lake Superior water. In a second experiment, a zinc concentration of 1368 µg/litre significantly reduced both the survival of embryos and 12-week-old larvae.

Kumar & Pant (1984) studied the toxic effects of zinc on the gonads of the fish *Puntius conchoni* exposed to one-third of the 96-h LC₅₀ for zinc (which is 33.26 mg/litre) for up to 4 months. Male fish showed dilation in the testicular blood capillaries with necrosis and disintegration of the seminiferous tubules. Significant atresia in the ovary and damage to younger oocytes was found in female fish.

Watson & McKeown (1976) exposed yearling rainbow trout (*Oncorhynchus mykiss*) to zinc concentrations ranging from < 0.1 (control) to 1.12 mg/litre for up to 63 days. Growth was significantly inhibited at 1.12 mg/litre. Significant hyperglycaemia was found at all zinc exposure concentrations after 7 days but the condition remained significant by the end of the experiment only at 1.12 mg/litre.

Nemcsók et al. (1984) found that zinc chloride (1, 10 or 50 mg/litre) did not decrease acetylcholinesterase activity in serum, brain, heart or muscle of common carp (*Cyprinus carpio*) exposed for 2 h.

Korver & Sprague (1989) analysed the ability of male fathead minnows (*Pimephales promelas*) to avoid zinc concentrations ranging from 0.02 (control) to 13.5 mg/litre for up to 180 min. The LOEC was found to be 0.284 mg/litre; however, when fish were exposed in the presence of a shelter, the LOEC was 1.83 mg/litre.

Bengtsson (1974b) studied the effect of zinc on the ability of minnows (*Phoxinus phoxinus*) to compensate for a rotating water mass. Fish were exposed for approximately 100 days and significant adverse effects were found at 0.06 mg/litre for under yearlings, 0.16 mg/litre for yearlings and 0.2 mg/litre for adults.

Seawater studies

Calabrese et al. (1973) exposed eggs of American oyster (*Crassostrea virginica*) from within one hour of fertilization for 42–48 h to zinc chloride under static conditions (26 °C; salinity 25‰). An EC₅₀ based on embryonic development was calculated to be 0.31 mg/litre. In similar tests, Calabrese & Nelson (1974) found the EC₅₀ for the hard clam (*Mercenaria mercenaria*) to be 0.166 mg/litre. Calabrese et al. (1977) found the 8–10 day LC₅₀ and an EC₅₀, based on growth, for hard clam larvae (*Mercenaria mercenaria*) to be 195.4 and 61.6 µg/litre, respectively, when tested in natural seawater. The values do not include the background zinc concentration of 17.7 µg/litre. Strömberg (1982) studied the effects of zinc on growth of the common mussel *Mytilus edulis* in tests of 10–22 days. Zinc concentrations ranging from 0.01 to 0.20 mg/litre were added to local seawater (background zinc concentration 5 µg/litre). Significant reductions in growth were observed at 0.01 mg/litre; an EC₅₀ of 0.06 mg/litre was calculated for days 2–6.

Hunt & Anderson (1989) exposed the red abalone *Haliotis rufescens* to increased zinc concentrations in natural seawater. A 48-h EC₅₀, based on larval development, and a 9-day EC₅₀, based on metamorphosis, were found to be 0.068 and 0.050 mg/litre, respectively; NOEC values for the two parameters were 0.037 and 0.019 mg/litre, respectively.

Dinnel et al. (1989) exposed the purple sea urchin (*Strongylocentrotus purpuratus*), green sea urchin (*Strongylocentrotus droebachiensis*) and red sea urchin (*Strongylocentrotus franciscanus*) to zinc in 120-h sperm/fertilization tests. The sand dollar (*Dendraster excentricus*) was exposed to zinc in a 72-h test. EC₅₀ values were found to be 0.26, 0.38, 0.31 and 0.028 mg/litre in the sperm test for the four species, respectively.

Reish & Carr (1978) found that the reproduction of the polychaetous annelids *Ctenodrillus serratus* and *Ophryotrocha diadema* was significantly inhibited at zinc concentrations of ≥ 0.5 mg/litre in 21-day tests.

Bengtsson & Bergström (1987) found that the 13-day EC₅₀, based on fecundity, for the harpacticoid copepod *Nitocra spinipes*

ranged from 0.17 to 0.43 mg/litre. There was no significant effect of salinity, which ranged from 7• to 25•.

Price & Uglow (1979) found the LT_{50} for the marine shrimp *Crangon crangon* to be 130 h at a zinc concentration of 14.4 mg/litre. When the test was carried out on various moult stages, LT_{50} values were 64, 140 and 152 h for the post-moult, inter-moult and pre-moult stages respectively.

Macdonald et al. (1988) exposed embryos of yellow crab (*Cancer anthonyi*) to zinc in 7-day tests. Zinc concentrations of ≥ 0.1 mg/litre significantly reduced survival. Hatching of embryos and larval survival were significantly reduced at 0.01 mg/litre; no embryos hatched at zinc concentrations of ≥ 1.0 mg/litre.

Redpath & Davenport (1988) reported that the pumping rate in the common mussel (*Mytilus edulis*) decreased with increasing zinc concentration and stopped completely at zinc concentrations in the range 0.47–0.86 mg/litre.

Weeks (1993) found a significant reduction in the feeding rate of the talitrid amphipod *Orchestia gammarellus* at dietary zinc concentrations ranging from 63 to 458 mg/kg during 48-h tests. However, no significant effect was found in 20-day exposures.

Somasundaram et al. (1984a) exposed the Atlantic herring (*Clupea harengus*) to zinc concentrations ranging from 0.1 to 6 mg/litre for up to 408 h. Zinc concentrations of ≥ 2 mg/litre significantly decreased total egg and yolk volumes throughout the study. At zinc concentrations of 0.1, 0.5 and 2.0 mg/litre, the development rate of eggs was faster than controls but at 6 mg/litre the rate was slower.

Somasundaram et al. (1984b) incubated eggs of Atlantic herring (*Clupea harengus*) at four zinc concentrations (0.5, 2.0, 6.0 and 12.0 mg/litre). The ultrastructural changes in the trunk muscle tissue of larvae hatched from the eggs were examined by morphometric analysis. The mean relative volumes of mitochondria, sarcoplasmic reticulum and muscle fibre were significantly increased and the surface:volume ratio of the mitochondrial cristae was significantly reduced. The ultrastructural changes in brain cells of larvae were also

examined (Somasundaram et al., 1984c). All zinc exposures caused significant swelling of the nuclear membranes and rough endoplasmic reticulum, an increase in intracellular spaces and a decrease in the relative volumes of mitochondria. Somasundaram et al. (1985) studied the ultrastructural changes in the posterior gut and pronephric ducts of the herring larvae. Significant changes were observed only at zinc concentrations of 6.0 and 12.0 mg/litre; the endoplasmic reticulum, perinuclear space and mitochondria were swollen and there was a reduction in the surface:volume ratio of the mitochondrial cristae. At the highest zinc concentration, the posterior gut cells showed signs of necrosis. Examination of the epidermal structure revealed more vesicles and intracellular spaces in the epidermal cells, swollen mitochondria and signs of necrosis at zinc concentrations of 6.0 or 12.0 mg/litre (Somasundaram, 1985).

9.1.2.3 *Effects on communities*

Mesocosms

Belanger et al. (1986) exposed the Asiatic clam (*Corbicula* sp.) to zinc concentrations ranging from 0.025 to 1 mg/litre for 30 days in outdoor artificial stream systems. Background total zinc concentrations ranged from 0.02 to 0.094 mg/litre. Zinc concentrations of ≥ 0.05 mg/litre significantly reduced weight gain between days 20 and 30. Exposures to zinc at 1 mg/litre resulted in mortality of 10–50% by day 30.

Genter et al. (1988) added zinc at a concentration of 0.5 mg/litre to a flow-through stream mesocosm and studied the effects on an established periphyton community for 30 days. Seven diatoms and a coccoid green alga were significantly inhibited by zinc exposure. The algal total biovolume-density was reduced to < 5% of control levels by zinc from days 5–30. Zinc addition reduced protozoan numbers by more than 50%.

Marshall et al. (1983) conducted *in situ* experiments in Lake Michigan (background zinc concentration ~ 1 $\mu\text{g/litre}$) to determine the responses of the plankton community to added zinc for 2 weeks. Total zinc concentrations of 17.1 $\mu\text{g/litre}$ significantly reduced chlorophyll *a*, primary productivity, dissolved oxygen, specific zooplankton populations and zooplankton species diversity.

Niederlehner & Cairns (1993) studied the effect of zinc on a naturally-derived periphyton community collected from a 195-ha lake (pH 7.1; hardness, 12.6 mg/litre; background zinc concentration 13.3 µg/litre). Toxicity tests with added zinc at concentrations of 73 and 172 µg/litre were carried out in dechlorinated tap water (pH 7.78; hardness, 73.8 mg/litre; zinc 1.3 µg/litre) for 21 days. Species richness was significantly impaired at the higher zinc exposure; primary production and community respiration were impaired at both zinc concentrations. The community was then exposed to pH levels ranging from 3 to 4.5 for 48 h. The pH stress significantly reduced species richness from the initial levels in controls and at both zinc concentrations. No significant differences between zinc treatments were observed at pH < 4.0.

Colwell et al. (1989) studied the effect of zinc on epilithic communities in artificial streams. Zinc concentrations of 0.05 and 1 mg/litre were added to the streams (background zinc concentration 0.02 mg/litre). After 30 days, greater biomass and lower protein:carbohydrate ratios were evident in epilithon exposed to the highest zinc concentration compared to the controls. Metal-tolerant populations had replaced metal-sensitive organisms by the end of the experiment at the higher zinc exposure.

Kiffney & Clements (1994) exposed benthic macroinvertebrate communities from two different sites to zinc (130 µg/litre) for 7 days. The background zinc concentrations at the two collection sites and in the artificial stream were below detection limits. Significant effects were observed at the community and population level following the addition of zinc. Specifically, mayflies from both sites were sensitive to zinc, but the magnitude of the response varied between sites. The results indicated that benthic macroinvertebrate communities from different stream orders may vary in sensitivity to zinc.

Field observations

Etxeberria et al. (1994) reported that increasing environmental levels of bioavailable zinc are associated with enlarged digestive lysosomes in mussels.

Solbé (1977) found that macroinvertebrates and fish were adversely affected by effluent from a steel works entering a hard

water stream that had its source in the neighbouring limestone hills. The observed concentration of dissolved zinc in the river was 25 mg/litre; ammonia was the only other contaminant found at concentrations toxic to aquatic life, and it was quickly oxidized.

Graham et al. (1986) found increased mortality of rainbow trout (*Oncorhynchus mykiss*) at zinc-contaminated sites on the Molonglo River, New South Wales, Australia when compared with non-contaminated sites on the same river. Zinc concentrations of up to 2.32 mg/litre in the water and 1016 mg/kg dw in gill tissue were reported. The authors concluded that the concentrations of copper measured in the water were not sufficiently high to be lethal to the fish, although copper could have acted synergistically with zinc.

Hogstrand et al. (1989) reported that hepatic levels and metallothionein in perch (*Perca fluviatilis*) caught downstream from a brass works in Sweden reflected the water concentration of zinc (0.56–59 µg/litre). A significant correlation was found between hepatic zinc and metallothionein levels.

Clements & Kiffney (1995) examined benthic macroinvertebrate community responses to heavy metals at 33 sites in six Colorado streams (USA) in which zinc concentrations ranged from 2 to 691 µg/litre. The number of taxa and species richness of mayflies (Ephemeroptera), and the abundance of most mayfly and stonefly taxa were significantly reduced at sites where the zinc concentration exceeded the hardness-based criterion .

Van Tilborg (1996) reported on a freshwater stream in Belgium (the Kleine Nete) which contained total zinc at an average of 60 µg/litre (range <20–140 µg/litre). According to the Belgian Biotic Index, this stream has a high quality ecosystem.

9.1.3 Terrestrial organisms

9.1.3.1 Plants

Geochemical differences in zinc concentrations in soils and autonomic selection processes during the evolution of plants result in a great variation in zinc demand and zinc content between plant species and between plant genotypes of the same species. As a

general rule, plants from environments poor in zinc are characterized by low zinc concentrations, those from zinc-enriched environments by high concentrations (Ernst, 1996). Within each ecosystem, biodiversity can only be maintained if species differ in their various ecological niches; zinc-demand is one variable. There is no convincing explanation as to why certain plant species have a higher uptake rate and accumulation pattern of zinc than others, although one possible reason may be to develop a defence against herbivores by accumulating high metal levels (Ernst et al., 1990). A great variation of zinc content is well known in forest ecosystems growing on soils with a normal zinc content. This variation is due to a number of factors including changes in the degree of infestation with endomycorrhizal fungi during a growing season and changes in ectomycorrhizal partners during the life history of the plant. When comparing zinc-sensitive and zinc-tolerant genotypes, it was found that, in zinc-tolerant genotypes only, the rapid compartmentation of zinc in the vacuole is one reason for an increased demand for zinc (Mathys, 1977). Zinc-activated enzymes, such as carbonic anhydrase, therefore reach the same activity in tolerant plants at higher external zinc concentrations than in zinc-sensitive plants. This response pattern has to be interpreted as a decrease in zinc efficiency in zinc-tolerant plants given the same level of zinc uptake by both genotypes (Harmens et al., 1993a). Zinc tolerance in plants is coded by only two major genes (Schat et al., 1996). Whether these genes are related to the zinc-efficiency genes reported from soybean varieties (Hartwig et al., 1991) remains to be investigated.

Therefore critical zinc levels cannot be established by analysing only the zinc content of leaves or other plant tissues; it is necessary to test the zinc demand of the plant genotype, its potential for physiological flexibility (allocation and retranslocation) (Ernst, 1995), siderophore exudation (Von Wirén et al., 1996), and cellular zinc compartmentation. The aim should be to establish the range of effects on the physiological processes under consideration from no effect up to 100% effect.

Toxicity to plants grown hydroponically and in soil

All such experiments involve acute toxicity, defined as toxicity over less than one life cycle in duration (seed to seed: cereals, rape, trees) or a harvest cycle (spinach, lettuce, cabbage). Zinc toxicity is

first expressed in reduced root growth, a parameter that is used routinely in testing zinc-resistance in plants (Antonovics et al., 1971; Wainwright & Woolhouse, 1977; Schat et al., 1996). In higher plants the toxicity of zinc increases with exposure time, and therefore increasing zinc concentration in the plant and translocation from root to shoot (Mitchell & Fretz, 1977; Rauser, 1978; Dijkshoorn et al., 1979; Davies, 1993; Sheppard et al., 1993).

Zinc toxicity affects general physiological processes, e.g., transpiration, respiration and photosynthesis, and plant development in general can be visibly inhibited. Stunted growth, leaf epinasty and chlorosis of the younger leaves are striking symptoms of strong zinc toxicity. However, at lower degrees of zinc toxicity, these visible symptoms are less pronounced or can even be absent, whereas at the cellular level several processes are affected, owing to increases in local metal concentrations. Several mechanisms of metal action at the physiological and biochemical level have been described (for a review see Chaney, 1993; Vangronsveld & Clijsters, 1994), ranging from disturbance of cell division (Powell et al., 1986a,b; Davies et al., 1991) and ion balance (Ernst, 1996) to inhibition of photosynthesis (Van Assche & Clijsters, 1986). In *Phaseolus vulgaris*, growth inhibition and stress enzyme induction were both observed to occur when exactly the same internal zinc concentration was exceeded (Van Assche et al., 1988).

The critical leaf tissue concentration of zinc at which growth is affected was found for many plant species to be between 200 and 300 mg/kg dry matter (Davis & Beckett, 1978; Van Assche et al., 1988; Balsberg Pålsson, 1989; Vangronsveld & Clijsters, 1992; Mench et al., 1994; Marschner, 1995). However, zinc phytotoxicity in leaves can depend to a large extent on the plant species, the age of the leaf and other factors, such as exposure period and exposure concentration.

Evaluations of phytotoxicity of zinc-polluted substrata are generally made by chemical analysis of the substratum itself. These results give rise to misinterpretations since availability of zinc to plants in and consequently metal uptake from the substratum are functions of the chemical form of the element in the soil, several soil parameters (e.g., pH, organic matter content, soil type) and plant species. Moreover, soils are frequently contaminated by a mixture of

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metals. Each of these materials separately can be phytotoxic, or they can interact in a synergistic, antagonistic or cumulative way (Beckett & Davis, 1978) (see section 9.3). The physiological and metabolic responses of test plants can be considered as a biological criterion for the total phytotoxic effect, since they are the result of the interactions of the metals present in the soil with other soil factors (biotic or abiotic) and with the plant. Phytotoxicity responses of test plants grown under controlled environmental conditions only reflect further the interference with metabolic processes of metals assimilated through the roots. Morphological responses (e.g., root growth, stem elongation, leaf expansion, biomass) and physiological and biochemical parameters (respiration, photosynthesis, capacities of enzymes and isozyme patterns, but not phytochelatin levels) can be used for the evaluation of phytotoxicity (Van Assche & Clijsters, 1990; Vangronsveld & Clijsters, 1992; Harmens et al., 1993b) (Tables 43–44).

Table 43. Impact of zinc-enriched sewage sludge added to non-dried sassafras sandy loam on crop plants (after Chaney, 1993)

Crop species	Yield as % of control	Geometric mean of zinc shoot (mg/kg dry weight)	Chlorosis ^a
Red fescue	17.2	965	1.8
Tall fescue	69.8	1060	1.7
Canadian blue grass	16.4	898	2.3
Cyperus	33.6	580	3.1
Barley	57.6	1060	1.4
Soybean	11.4	1140	4.2
Lettuce	1.2	3620	4.6

^a Range of 1–5 with 5 being severe chlorosis.

Concentrations of zinc that are subtoxic or non-toxic to plants may have metabolic effects higher up the food chain. The disappearance of herbivorous insects on zinc-tolerant plants is one example of differences in species-specific tolerances (Ernst et al., 1990). Similarly, the zinc-content of zinc-efficient plants may be

Table 44. Acute toxicity of zinc to plants grown in hydroponic culture or soil

Plant species	Exposure		End-point	Toxicity data	Parameter	Reference
	Concentration (mg/litre)	Time (days)				
Hydroponic culture <i>Hordeum vulgare</i>	0–450	16	biomass, concentration	NOEC LD ₁₀₀	8.3–27.2 mg/litre 168–460 mg/kg dw 150–800 mg/litre 1000–8000 mg/kg dw	Davis & Beckett (1978)
<i>Phaseolus vulgaris</i>	0.975–26	4	biomass, starch content	NOEC EC ₅₀	0.975 mg/litre 26 mg/litre	Rausser (1978)
<i>Phaseolus vulgaris</i>	0–1990	16	primary leaf physiology and zinc concentration	NOEC EC ₅₀	189–266 mg/kg dw 500 mg/kg dw (for shoot length)	Van Assche et al. (1988)
<i>Allium cepa</i>	6.5–65	2	root length	NOEC EC ₅₀	6.5 mg/litre 25.9 mg/litre	Arambasic et al. (1995)
<i>Lepidium sativum</i>	65–164	2	root length	NOEC EC ₅₀	65 mg/litre 547 mg/litre	Arambasic et al. (1995)

Table 44 (contd.)

Plant species	Exposure		End-point	Toxicity data	Parameter	Reference
	Concentration (mg/litre)	Time (days)				
<i>Lolium perenne</i>	0–30	14	root length	NOEC EC ₅₀	< 0.1 mg/litre 1.6 mg/litre	Wong & Bradshaw (1982)
<i>Festuca rubra</i> zinc-sensitive zinc-tolerant	0–0.2	4	mitosis	EC ₁₇ +NOEC	0.2 mg/litre 0.2mg/litre	Powell et al. (1986a)
<i>Acer rubrum</i>	0–0.4	78	root growth, zinc content	EC ₅₀ zinc root zinc leaf	0.05 mg/litre 2190 mg/kg dw 381 mg/kg dw	Mitchell & Fretz (1977)
<i>Picea abies</i>	0–0.4	66	root growth, zinc content	EC ₅₀ zinc root zinc needle	0.2 mg/litre 4125 mg/kg dw 1440 mg/kg dw	Mitchell & Fretz (1977)
<i>Pinus strobus</i>	0–0.4	78	root growth, zinc content	EC ₅₀ zinc root zinc needle	0.2 mg/litre 9375 mg/kg 1005 mg/kg	Mitchell & Fretz (1977)

Table 44 (contd.)

Soil						
<i>Acer rubrum</i>	0–165 mg/kg pH 6.7		root growth, zinc content	NOEC zinc root zinc leaf	165 mg/kg in soil 618 mg/kg dw 209 mg/kg dw	Mitchell & Fretz (1977)
<i>Picea abies</i>	0–165 mg/kg pH 6.7		root growth, zinc content	NOEC zinc root zinc needle	165 mg/kg in soil 615 mg/kg dw 127 mg/kg dw	Mitchell & Fretz (1977)
<i>Pinus strobus</i>	0–165 mg/kg pH 6.7		root growth, zinc content	+NOEC zinc root zinc needle	165 mg/kg in soil 1430 mg/kg 314 mg/kg	Mitchell & Fretz (1977)
<i>Plantago lanceolata</i>	9.5–614 mg/kg pH 4.4, 4% organic matter	6 weeks	zinc content	EC ₅₀	>1010 mg/kg in leaf	Dijkshoorn et al. (1979)
<i>Trifolium repens</i>	9.5–614 mg/kg pH 4.4, 4% organic matter	6 weeks	zinc content	EC ₅₀	800 mg/kg leaf	Dijkshoorn et al. (1979)
<i>Lolium perenne</i>	9.5–614 mg/kg pH 4.4, 4% organic matter	6 weeks	zinc content	EC ₅₀	500–600 mg/kg leaf	Dijkshoorn et al. (1979)

NOEC = no-observed-effect concentration; dw = dry weight

insufficient for optimum performance of herbivorous animals and humans, especially if all the cellular zinc is present in a form which is not readily bioavailable.

9.1.3.2 Invertebrates

Haight et al. (1982) calculated 24-h, 48-h and 72-h LC₅₀ values for zinc of 82, 39.4 and 20 mg/litre (added as zinc sulfate to the growth medium), respectively, for juvenile free-living nematodes (*Panagrellus silusiae*) and 255, 95.1 and 47.5 mg/litre for adults.

Neuhauser et al. (1985) exposed earthworms (*Eisenia fetida*) to zinc in contact and artificial soil toxicity tests. In 48-h contact tests LC₅₀ values were 13 µg/cm² for zinc acetate, 12 µg/cm² for zinc chloride, 10 µg/cm² for zinc nitrate and 13 µg/cm² for zinc sulfate. There were no significant differences between the toxicities of the different zinc salts. In an artificial soil test, the 2-week LC₅₀ was found to be 662 mg/kg. Spurgeon et al. (1994) reported the 14-day LC₅₀ for *E. fetida* to be 1010 mg/kg. The 56-day LC₅₀ and NOEC were 745 and 289 mg/kg respectively; the EC₅₀ and NOEC based on cocoon production were 276 and 199 mg/kg respectively.

Neuhauser et al. (1984) exposed earthworms (*E. fetida*) to zinc concentrations of 1000, 2500, 5000 and 10 000 mg/kg of manure (dry weight) for 6 weeks. Zinc at ≥ 5000 mg/kg significantly reduced growth and cocoon production. Similar results were obtained with four different zinc salts (acetate, chloride, nitrate and sulfate). The growth rate and reproduction had returned to normal after a subsequent 6-week period without zinc. Malecki et al. (1982) exposed earthworms (*E. fetida*) to six different zinc salts for 8 weeks. Significant reductions in cocoon production were observed at zinc carbonate and sulfate concentrations of 500 mg/kg dw. A zinc concentration of 2000 mg/kg adversely affected reproduction (acetate, chloride and nitrate) and growth (chloride, nitrate and sulfate). Zinc oxide significantly affected both growth and reproduction at 4000 mg/kg. Zinc carbonate did not adversely affect growth at the highest exposure (40 000 mg/kg). Long-term studies (20 weeks) with zinc acetate revealed significant reductions in cocoon production at 5000 mg/kg. Van Gestel et al. (1993) exposed earthworms (*E. andrei*) to zinc as zinc chloride at concentrations in dry artificial soil of 100–1000 mg/kg. Zinc significantly reduced

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reproduction at soil concentrations of 560 and 1000 mg/kg and induced the production of malformed cocoons. EC₅₀ values for the effect of zinc on cocoon production and the number of juveniles per worm per week were 659 and 512 mg/kg dry soil, respectively. At the end of a 3-week recovery period, reproduction had returned to normal.

Marigomez et al. (1986) fed terrestrial slugs (*Arion ater*) for 27 days on a diet containing zinc concentrations ranging from 10 to 1000 mg/kg. No treatment-related effect on mortality was observed. Zinc concentrations of 1000 mg/kg significantly reduced feeding activity and growth.

Beyer & Anderson (1985) fed woodlice (*Porcellio scaber*) for 64 weeks on soil litter containing zinc at concentrations of up to 12 800 mg/kg. Soil litter containing ≥ 1600 mg/kg had adverse effects on reproduction; adult survival was reduced at ≥ 6400 mg/kg. Woodlice fed diets containing up to 20 000 mg/kg for 8 weeks showed decreased survival at concentrations of ≥ 5000 mg/kg (Beyer et al., 1984).

9.1.3.3 Vertebrates

High dietary levels of zinc are frequently fed to poultry to force moulting and reduce egg deposition (Hussein et al., 1988). Stahl et al. (1990) fed hens on a diet containing zinc at concentrations of 48, 228 or 2028 mg/kg for 12 or 44 weeks. Zinc treatments had no effect on overall egg production, feed conversion, feed consumption, hatchability, or progeny growth to the age of 3 weeks. Zinc levels were elevated in eggs from hens fed the diet containing 2028 mg/kg, but chick performance and tissue zinc content were unaffected by maternal zinc nutritional status. Stahl et al. (1989) fed chicks on a diet containing zinc at 37 (control), 100 or 2000 mg/kg for 21 days. There were no zinc-related deaths; at the highest exposure growth rate was decreased, anaemia was evident, tissue copper and iron decreased and tissue zinc increased.

Japanese quail (*Coturnix coturnix japonica*) fed a diet containing zinc (as zinc oxide) at 15 000 mg/kg for 7 days showed significant reductions in body weight. Egg production approached zero on day 3, eggshell breaking strength was reduced and moulting was induced (Hussein et al., 1988).

Dewar et al. (1983) fed 2-week-old chicks on a diet containing zinc at 74 (controls), 2000, 4000 or 6000 mg/kg for 4 weeks. High mortality was noted at the highest dose; all groups receiving zinc-supplemented food showed an increased incidence of gizzard and pancreatic lesions. Similar results were found when 1-day-old chicks were fed a diet containing zinc at 1000, 2000 or 4000 mg/kg for 4 weeks and when hens were fed diets containing 10 000 or 20 000 mg/kg for only 4 days. No lesions were found in hens exposed for 4 days to 10 000 mg/kg followed by 28 days on a control diet. Dean et al. (1991) fed day-old male chicks a diet containing zinc at 73 (controls) or 5280 mg/kg for 4 weeks. The zinc-supplemented feed significantly decreased body weight but did not affect food consumption compared with controls. Serum cholesterol, thyroxine and triiodothyronine levels were significantly reduced; serum growth hormone was significantly reduced but had recovered by the end of the experiment.

Gasaway & Buss (1972) maintained young mallard duck (*Anas platyrhynchos*) on a diet containing zinc concentrations ranging from 3000 to 12 000 mg/kg for 60 days. Food intake and body weight showed decreases as the level of zinc in the diet increased. Zinc caused reductions in pancreas and gonad weight in relation to body weight. The ratio of adrenals and kidney to body weight increased significantly. No significant changes in the liver:body weight ratios were observed. In ducks exposed to zinc there was partial paralysis of the legs, diarrhoea and weight loss within 10 days; severe paralysis was noted in some ducks within 20 days. Slight anaemia was found after 30 days but by day 45 extreme anaemia was observed in most of the exposed birds. High mortality was noted in all groups during the 60-day experiment with only 2 of the 45 exposed ducks surviving the whole time period. Zinc toxicosis consisted of paralysis of the legs, high concentrations of zinc in pancreas and kidney, and yellowish-red kidneys.

Zinc poisoning of birds has been reported as a result of the ingestion of zinc, for instance, from wire mesh cages (Van der Zee et al., 1985; Reece et al., 1986). Grandy et al. (1968) dosed mallard ducks (*A. platyrhynchos*) orally with eight No. 6 zinc shot and observed them for 30 days: three of the 15 birds died within the observation period. The average weight loss among surviving birds was 22%, significantly more than in control birds. Only three of the

mallards dosed retained the zinc shot until the end of the study. Signs of intoxication in order of increasing severity were stumbling, an inability to run, complete loss of muscular control of the legs, loss of swimming ability and spasmodic wing movements. However, it should be noted that the zinc pellets were found not to be pure zinc but contained 92% zinc, 0.16% lead, a trace of iron and 7% not determined. French et al. (1987) dosed mallard ducks with five or ten No. 6 zinc shot (99.9% purity) and observed the birds for 28 days. Observation during the experimental period, post-mortem examination and histopathological examination revealed no effects of zinc on the dosed birds.

Mammals can also die from ingestion of zinc. Straube & Walden (1981) reported that 20 of 25 ferrets (*Mustela putorius furo*) being used in an experiment died of renal failure after eating raw meat that was accidentally contaminated with zinc from the wire cages. Zinc poisoning was diagnosed after autopsy and laboratory investigation.

Straube et al. (1980) fed ferrets (*M. putorius furo*) on a basal diet (zinc content 27 mg/kg) with zinc supplements of 500, 1500 or 3000 mg/kg for up to 6 months. The ferrets fed the two highest concentrations showed severe signs of toxicity between weeks 1 and 2, with the animals at the highest exposure dying within 2 weeks. Lesions included diffuse nephrosis, haemorrhages in the intestine and severe macrocytic hypochromic anaemia.

Bleavins et al. (1983) fed mink (*M. vison*) on a diet supplemented with zinc at 500 mg/kg for 2.5 months. No clinical signs of zinc toxicity were observed and the zinc supplement was increased to 1000 mg/kg. Again, no signs of zinc toxicity were observed; however, the offspring of zinc-treated females showed achromotrichia, alopecia, lymphopenia and a reduced rate of growth suggesting copper deficiency, although other signs of this latter condition (anaemia and neurotopenia) were not observed. Aulerich et al. (1991) fed adult and kit male and female mink on a basal diet (zinc content 40 mg/kg) supplemented with zinc at 500, 1000 or 1500 mg/kg for 144 days. No marked adverse effects on food consumption, body weight gains, haematological parameters, fur quality or survival were observed. Histopathological examination of the liver, kidneys and pancreas did not reveal any lesions indicative of zinc toxicosis.

Racey & Swift (1986) housed pipistrelle bats (*Pipistrellus pipistrellus*) in roosting cages treated with zinc octoate. The pregnant female bats (three groups of 10), collected from nursery roosts, were trained to feed on mealworms before transfer to the experimental cages. The cages were metal, lined with plywood and painted with zinc octoate as a solution in white spirit at 0.5 litre/m² and containing 8% zinc as metal (as recommended by the manufacturers), or with white spirit. Treatment of the wood was conducted 2 months before introduction of the bats into the cages. During the course of the 142-day experiment, there were 2 deaths in the zinc octoate group, 2 in the white spirit control group and 3 in the untreated control group.

9.2 Tolerance to zinc

Tolerance to zinc (and other metals) has been documented in a wide variety of plants and animals. Tolerance may occur in two ways (Miller & Hendricks, 1996): through acclimatization at some early stage in the life cycle, or through natural selection. The latter process is heritable, the former is not. However, as noted by Bervoets et al. (1996), few studies have discriminated even the effects of acclimatization on metal uptake or toxicity. Some key studies that have done so are detailed below.

Free-living fungi, as well as those associated in mycorrhiza with higher plants (vesicular-arbuscular mycorrhiza, ectomycorrhiza), have enhanced zinc resistance in zinc-enriched soils (Colpaert & Van Assche, 1992; Gadd, 1993). All tested actinomycetes and non-spore forming bacteria isolated from a site contaminated with metals were zinc tolerant, growing normally in media containing zinc concentrations of 39–130 mg/litre (600–2000 µmol/litre) (Jordan & Lechevalier, 1975). Shehata & Whitton (1981) reported that blue-green algae are often frequent in waters with very high levels of zinc, and laboratory assays have shown that these algae are much more resistant to zinc than most isolates from sites lacking zinc enrichment. Hornor & Hilt (1985) determined the distribution of zinc-tolerant bacteria from three stream sites containing high (3125 µg/litre), medium (291 µg/litre) and low (109 µg/litre) concentrations of zinc. Zinc tolerance was estimated by the ability of bacteria to grow on media amended with zinc (4–512 mg/litre). The presence of zinc-tolerant bacteria was correlated with the degree of heavy metal contamination. Zinc concentrations ranging from 4 to

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16 mg/litre were stimulatory to growth of bacteria from contaminated sites while concentrations of 4 mg/litre were inhibitory to bacteria from the control site.

Antonovics et al. (1971) reviewed metal tolerance in plants and found several examples of plant communities that show high tolerance to zinc. Most of the studies that relate to zinc tolerance are associated with soils enriched with zinc either naturally or by metal mining activity. All plants growing for a long time on zinc-enriched soils have evolved a zinc resistance regardless of whether they are fungi, mosses, ferns or angiosperms. Shaw (1990) noted that the development of metal tolerance in plants is among the best observed examples of evolution related to natural and anthropogenic stress.

Acclimatization and adaptation to zinc have been demonstrated by Miller & Hendricks (1996) for *Chironomus riparius*. Other examples of zinc resistance in aquatic organisms are provided by Klerks & Weis (1987), Klerks & Levinton (1989) and Klerks (1990).

Pre-exposure of rainbow trout to zinc at a concentration of 2 mg/litre for more than 5 days significantly decreased the acute toxicity of the metal (Bradley & Sprague, 1985). Anadu et al. (1989) found that acclimatization to a zinc concentration of 50 µg/litre increased the tolerance of juvenile rainbow trout by a factor of 3–5. Maximum tolerance was achieved within 7 days with no further change noted after 2–3 weeks. Further studies revealed that there was no increase in tolerance after acclimatization periods of less than 3 days. Tolerance to zinc was rapidly lost following return to control water, with almost complete reversion to control tolerance after only 7 days. Hobson & Birge (1989) found that tolerance to zinc in 96-h acute toxicity tests increased significantly after 14 days of exposure to zinc at 0.6 mg/litre but decreased significantly following exposure to 1.8 mg/litre for 7 and 14 days. After 21 days of pre-exposure to 0.6 or 1.8 mg/litre there was no significant effect on acute toxicity compared with controls. The authors found no correlation between changes in tolerance and observed changes in metallothionein-like proteins. Hogstrand & Wood (1995) reported that acclimatization to zinc in rainbow trout adapted to fresh water can develop without any detectable increase in zinc accumulation in the gills or liver. Acclimatization to zinc does not necessarily involve induction of metallothionein. The inhibition of the calcium influx by zinc is

mainly competitive in its nature, and persists during chronic exposure, indicating that zinc and calcium compete for the same uptake sites. Zinc-adapted fish have a decreased rate of zinc influx compared to controls. The authors therefore speculated that fish are able to regulate the uptake of zinc separately from calcium so that, in zinc-adapted fish, zinc influx can be markedly reduced without altering the influx of calcium.

Joosse et al. (1984) found that a tolerant population of the terrestrial woodlouse (*Porcellio scaber*) from a contaminated site regulated its body content of zinc at a higher level than a control population. Another population of *P. scaber* collected from a zinc-contaminated area was found to be adapted to high zinc and cadmium concentrations (Van Capelleveen, 1987). However, individuals from the contaminated site produced larger quantities of metalloproteins and showed lower growth efficiencies and drought resistance than individuals from a control site.

Differences in assimilation rates for zinc in two populations of centipede (*Lithobius variegatus*) were found to be related to the degree of contamination of the site from which the population was collected (Hopkin & Martin, 1984). Centipedes from a contaminated site survived longer than those from an uncontaminated site when both populations were fed on woodlice hepatopancreas with high concentrations of metals. A review of resistance in terrestrial invertebrates is provided by Posthuma & Van Straalen (1993).

9.3 Interactions with other metals

Zinc can behave antagonistically in combination with copper (Ahsanullah et al., 1988; Vranken et al., 1988; Kraak et al., 1994b) and synergistically with lead or iron alone or in combination (Ahsanullah et al., 1988; Konar & Mullick, 1993), and with mercury or nickel (Vranken et al., 1988). Zinc and cadmium can show additive toxicity (Negilski et al., 1981; Kraak et al., 1994b). Metal interactions can vary, however, depending on physicochemical conditions. For instance, Tomasik et al. (1995) found that zinc toxicity in soft water (50 mg/litre, CaCO₃) was lowered by magnesium and molybdenum and increased by cobalt or selenium. However, in hard water (100 mg/litre, CaCO₃) zinc was either inhibited by all metals (at a concentration of 1 mg/litre) or there was

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a weak synergism (at 0.5 mg/litre). Similarly, Biesinger et al. (1986) found that different zinc concentrations resulted in different interactions (with cadmium and mercury).

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Abbreviations

1. Summary and conclusions
2. Identity, physical and chemical properties, and analytical methods
3. Sources of human and environmental exposure
4. Environmental transport, distribution and transformation
5. Environmental levels and human exposure
6. Kinetics and metabolism in mammals

10. Evaluation of human health risks and effects on the environment
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References

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