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Preface

Between 1993 and 1997, the World Health Organization (WHO) published the second edition of *Guidelines for drinking-water quality* in three volumes: Volume 1, *Recommendations,* in 1993, Volume 2, *Health criteria and other supporting information,* in 1996, and Volume 3, *Surveillance and control of community supplies,* in 1997. As with the first edition, the development of these guidelines was organized and carried out jointly by WHO headquarters and the WHO Regional Office for Europe.

At the Final Task Group Meeting (Geneva, Switzerland, 21 - 25 September 1992), when the second edition of the *Guidelines* was approved, it was agreed to establish a continuing process of updating of the guidelines, with a number of chemical substances and microbiological agents subject to periodic evaluation. Addenda containing these evaluations will be issued as necessary until the third edition of the *Guidelines* is published, approximately 10 years after the second edition.

In 1995, a Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* agreed on the framework for the updating process and established three working groups to support the development of addenda and monographs on chemical aspects, microbiological aspects, and protection and control of water quality.

The Committee selected the chemical substances to be evaluated in the first addendum, designated coordinators for each major group of chemicals, and identified lead institutions for the preparation of health criteria documents evaluating the risks for human health from exposure to the particular chemicals in drinking-water. Institutions from Canada, Finland, France, Germany, the Netherlands, Sweden, the United Kingdom, and the USA, as well as the ILO/UNEP/WHO International Programme on Chemical Safety (IPCS), prepared the requested health criteria documents.

Under the responsibility of the designated coordinators for each chemical group, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the 1997 Working Group Meeting on Chemical Substances in Drinking-Water. The Working Group reviewed the health risk assessments and, where appropriate, decided upon guideline values.

During the preparation of draft health criteria documents and at the 1997 Working Group Meeting, careful consideration was always given to previous risk assessments carried out by IPCS in its Environmental Health Criteria monographs, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues, and the Joint FAO/WHO Expert Committee on Food Additives, which evaluates contaminants such as nitrate and nitrite in addition to food additives.

Evaluations of chemical substances given in this Addendum supersede evaluations previously published in Volume 1 and Volume 2 of the *Guidelines.*

Acknowledgements

The work of the following coordinators was crucial in the development of this first addendum on chemical substances in drinking-water:

P. Chambon, Health Environment Hygiene Laboratory of Lyon, Lyon, France (inorganic constituents)

U. Lund, Water Quality Institute, Horsholm, Denmark (organic constituents)

H. Galal-Gorchev, Urban Environmental Health, World Health Organization, Geneva, Switzerland (pesticides)

E. Ohanian, Environmental Protection Agency, Washington, DC, USA (disinfectants and disinfectant by-products)

The coordinators for the overall administrative and technical aspects of this addendum were, respectively, J. Kenny and H. Galal-Gorchev, Urban Environmental Health, WHO, Geneva, Switzerland.

Ms Maria Sheffer of Ottawa, Canada, was responsible for the scientific editing of the addendum.

Special thanks are due to the authors and their institutions for the preparation of draft health criteria documents. The following institutions prepared such health criteria documents: Health Canada; Water Research Centre, England; National Public Health Institute, Finland; Health Environment Hygiene Laboratory of Lyon, France; Fraunhofer Institute for Toxicology and Aerosol Research, Germany; National Institute of Public Health and Environmental Protection, Netherlands; National Food Administration, Sweden; International Programme on Chemical Safety, Switzerland; and the Environmental Protection Agency, USA.

The preparation of this addendum was made possible by the financial support afforded to WHO by the following countries: Canada, Japan, and the USA.

A financial contribution for the convening of the Working Group Meeting on Chemical Substances in Drinking-Water and for printing the addendum was received from the European Commission and is gratefully acknowledged.

The preparation of the first addendum to the *Guidelines for drinking-water quality* involved the participation of numerous institutions and experts. The work of these institutions and scientists, whose names appear in Annex 1, was central to the completion of this addendum and is much appreciated.

Acronyms and abbreviations used in the text[1]

[1] These are the acronyms and abbreviations that are used without definition in the text.

ADI	acceptable daily intake
ATPase	adenosine triphosphatase
CAS	Chemical Abstracts Service
CI	confidence interval
DNA	deoxyribonucleic acid
EEC	European Economic Community
EPA	Environmental Protection Agency (USA)
FAO	Food and Agriculture Organization of the United Nations
IARC	International Agency for Research on Cancer
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMP	Joint Meeting on Pesticides
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC_{50}	lethal concentration, median
LD_{50}	lethal dose, median
LOAEL	lowest-observed-adverse-effect level
LOEC	lowest-observed-effect concentration
MTD	maximum tolerated dose
NCI	National Cancer Institute (USA)
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program (USA)
PMTDI	provisional maximum tolerable daily intake
RNA	ribonucleic acid
TDI	tolerable daily intake
UNEP	United Nations Environment Programme
USA	United States of America
USP	US Pharmacopoeia
WHO	World Health Organization
w/v	weight/volume

Introduction

Chemical substances evaluated in this addendum were selected by the 1995 Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* for one or more of the following reasons:

- adequate data were not available to allow a guideline value to be derived, or only a provisional guideline value could be derived, in the second edition of the *Guidelines;*

- the substance was recommended for evaluation by the Task Group convened to finalize the second edition of the *Guidelines;*

- new health risk assessments were available from IPCS through its Environmental Health Criteria monographs, JMPR, or JECFA;

- a new evaluation of the carcinogenic risk of the chemical was available from IARC;

- requests to evaluate the chemical were made to the WHO Secretariat.

Concepts of guideline value and provisional guideline value, assumptions made, and scientific principles for the assessment of risk to human health from exposure to chemicals in drinking-water used in this addendum are described in Volume 1, *Recommendations,* of the second edition of the *Guidelines.* Only a brief summary of the approaches used to derive the guideline values is given here.

In developing the guideline values for potentially hazardous chemicals, a daily consumption of 2 litres of drinking-water by a person weighing 60 kg was generally assumed. Where it was judged that infants and children were at a particularly high risk from exposure to certain chemicals, the guideline values were derived on the basis of a 5-kg infant consuming 0.75 litre per day or a 10-kg child consuming 1 litre per day.

For compounds showing a threshold for toxic effects, a tolerable daily intake (TDI) approach was used to derive the guideline value. A portion of the TDI was allocated to drinking-water, based on potential exposure from other sources, such as food and air. Where information on other sources of exposure was not available, an arbitrary (default) value of 10% of the TDI was allocated to drinking-water.

For compounds considered to be genotoxic carcinogens, guideline values were determined using a mathematical model. The guideline values presented in the addendum to Volume 1 are the concentrations in drinking-water associated with an estimated excess lifetime cancer risk of 10^{-5} (one additional cancer case per 100 000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years). In this addendum to Volume 2, concentrations associated with excess lifetime cancer risks of 10^{-4}, 10^{-5}, and 10^{-6} are presented to emphasize the fact that each country should select its own appropriate risk levels.

It is emphasized that the guideline values recommended are not mandatory limits. Such limits should be set by national or regional authorities, using a risk-benefit approach and taking into consideration local environmental, social, economic, and cultural conditions.

Inorganic constituents

Aluminium

First draft[1] prepared by H. Galal-Gorchev
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[1] This document is essentially a synopsis of the evaluation of aluminium contained in the IPCS Environmental Health Criteria monograph for aluminium (WHO, 1997).]

No health-based guideline value for aluminium was recommended in the second edition of the WHO *Guidelines for drinking-water quality*. It was concluded that although further studies were needed, the balance of epidemiological and physiological evidence did not support a causal role for aluminium in Alzheimer disease. An aluminium concentration of 0.2 mg/litre in drinking-water provided a compromise between the practical use of aluminium salts in water treatment and discoloration of distributed water.

The Coordinating Committee for the updating of the WHO *Guidelines* recommended that a health criteria document be prepared for aluminium, based on the IPCS Environmental Health Criteria monograph that was finalized in 1995.

1. GENERAL DESCRIPTION

1.1 Identity

Aluminium is the most abundant metallic element and constitutes about 8% of the Earth's crust. It occurs naturally in the environment as silicates, oxides, and hydroxides, combined with other elements, such as sodium and fluoride, and as complexes with organic matter.

Compound	*CAS no.*	*Molecular formula*
Aluminium	7429-90-5	Al
Aluminium chloride	7446-70-0	$AlCl_3$
Aluminium hydroxide	21645-51-2	$Al(OH)_3$
Aluminium oxide	1344-28-1	Al_2O_3
Aluminium sulfate	10043-01-3	$Al_2(SO_4)_3$

1.2 Physicochemical properties (Lide, 1993)

Property	*Al*	*$AlCl_3$*	*$Al(OH)_3$*	*Al_2O_3*	*$Al_2(SO_4)_3$*
Melting point (°C)	660	190	300	2072	770 (d)
Boiling point (°C)	2467	262 (d)	-	2980	-
Density at 20°C (g/cm^3)	2.70	2.44	2.42	3.97	2.71
Water solubility (g/litre)	(i)	69.9	(I)	(i)	31.3 at 0°C

d, decomposes; i, insoluble

1.3 Organoleptic properties

Use of aluminium salts as coagulants in water treatment may lead to increased concentrations of aluminium in finished water. Where residual concentrations are high, aluminium may be deposited in the distribution system. Disturbance of the deposits by change in flow rate may increase aluminium levels at the tap and lead to undesirable colour and turbidity (WHO, 1996). Concentrations of aluminium at which such problems may occur are highly dependent on a

number of water quality parameters and operational factors at the water treatment plant.

1.4 Major uses

Aluminium metal is used as a structural material in the construction, automotive, and aircraft industries, in the production of metal alloys, in the electric industry, in cooking utensils, and in food packaging. Aluminium compounds are used as antacids, antiperspirants, and food additives (ATSDR, 1992). Aluminium salts are also widely used in water treatment as coagulants to reduce organic matter, colour, turbidity, and microorganism levels. The process usually consists of addition of an aluminium salt (often sulfate) at optimum pH and dosage, followed by flocculation, sedimentation, and filtration (Health Canada, 1993).

1.5 Environmental fate

Aluminium is released to the environment mainly by natural processes. Several factors influence aluminium mobility and subsequent transport within the environment. These include chemical speciation, hydrological flow paths, soil-water interactions, and the composition of the underlying geological materials. Acid environments caused by acid mine drainage or acid rain can cause an increase in the dissolved aluminium content of the surrounding waters (ATSDR, 1992; WHO, 1997).

Aluminium can occur in a number of different forms in water. It can form monomeric and polymeric hydroxy species, colloidal polymeric solutions and gels, and precipitates, all based on aquated positive ions or hydroxylated aluminates. In addition, it can form complexes with various organic compounds (e.g. humic or fulvic acids) and inorganic ligands (e.g. fluoride, chloride, and sulfate), most but not all of which are soluble. The chemistry of aluminium in water is complex, and many chemical parameters, including pH, determine which aluminium species are present in aqueous solutions. In pure water, aluminium has a minimum solubility in the pH range 5.5-6.0; concentrations of total dissolved aluminium increase at higher and lower pH values (CCME, 1988; ISO, 1994).

2. ANALYTICAL METHODS

Aluminium is reacted with pyrocatechol violet followed by spectrometric measurement of the resulting coloured complex. The method is restricted to the determination of the aquated cations and other forms of aluminium readily converted to that cationic form by acidification. The limit of detection is 2 µg/litre (ISO, 1994). The limit of detection for the determination of aluminium by inductively coupled plasma atomic emission spectroscopy ranges from 40 to 100 µg/litre (ISO, 1996).

Flame and graphite furnace atomic absorption spectrometric (AAS) methods are applicable for the determination of aluminium in water at concentrations of 5-100 mg/litre and 0.01-0.1 mg/litre, respectively. The working range of the graphite furnace AAS method can be shifted to higher concentrations either by dilution of the sample or by using a smaller sample volume (ISO, 1997).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Aluminium enters the atmosphere as a major constituent of atmospheric particulates originating from natural soil erosion, mining or agricultural activities, volcanic eruptions, or coal combustion. Atmospheric aluminium concentrations show widespread temporal and spatial variations. Airborne aluminium levels range from 0.0005 µg/m^3 over Antarctica to more than 1 µg/m^3 in industrialized areas (WHO, 1997).

3.2 Water

The concentration of aluminium in natural waters can vary significantly depending on various physicochemical and mineralogical factors. Dissolved aluminium concentrations in waters with near-neutral pH values usually range from 0.001 to 0.05 mg/litre but rise to 0.5-1 mg/litre in more acidic waters or water rich in organic matter. At the extreme acidity of waters affected by acid mine drainage, dissolved aluminium concentrations of up to 90 mg/litre have been measured (WHO, 1997).

Aluminium levels in drinking-water vary according to the levels found in the source water and whether aluminium coagulants are used during water treatment. In Germany, levels of aluminium in public water supplies averaged 0.01 mg/litre in the western region, whereas levels in 2.7% of public supplies in the eastern region exceeded 0.2 mg/litre (Wilhelm & Idel, 1995). In a 1993-1994 survey of public water supplies in Ontario, Canada, 75% of all average levels were less than 0.1 mg/litre, with a range of 0.04-0.85 mg/litre (OMEE, 1995). In a large monitoring programme in 1991 in the United Kingdom, concentrations in 553 samples (0.7%) exceeded 0.2 mg/litre (MAFF, 1993). In a survey of 186 community water supplies in the USA, median aluminium concentrations for all finished drinking-water samples ranged from 0.03 to 0.1 mg/litre; for facilities using aluminium sulfate coagulation, the median level was 0.1 mg/litre, with a maximum of 2.7 mg/litre (Miller et al., 1984). In another US survey, the average aluminium concentration in treated water at facilities using aluminium sulfate coagulation ranged from 0.01 to 1.3 mg/litre, with an overall average of 0.16 mg/litre (Letterman & Driscoll, 1988; ATSDR, 1992).

3.3 Food

Aluminium is present in foods naturally or from the use of aluminium-containing food additives. The use of aluminium cookware, utensils, and wrappings can increase the amount of aluminium in food; however, the magnitude of this increase is generally not of practical importance. Foods naturally high in aluminium include potatoes, spinach, and tea. Processed dairy products, flour, and infant formula may be high in aluminium if they contain aluminium-based food additives (FAO/WHO, 1989; Pennington & Schoen, 1995; WHO, 1997).

Adult dietary intakes of aluminium (mg/day) have been reported in several countries: Australia (1.9-2.4), Finland (6.7), Germany (8-11), Japan (4.5), Netherlands (3.1), Sweden (13), Switzerland (4.4), United Kingdom (3.9), and USA (7.1-8.2). Intake of children 5-8 years old was 0.8 mg/day in Germany and 6.5 mg/day in the USA. Infant intakes of aluminium in Canada, the United Kingdom, and the USA ranged from 0.03 to 0.7 mg/day (WHO, 1997).

3.4 Estimated total exposure and relative contribution of drinking-water

Aluminium intake from foods, particularly those containing aluminium compounds used as food additives, represents the major route of aluminium exposure for the general public, excluding persons who regularly ingest aluminium-containing antacids and buffered analgesics, for whom intakes may be as high as 5 g/day (WHO, 1997).

At an average adult intake of aluminium from food of 5 mg/day and a drinking-water aluminium concentration of 0.1 mg/litre, the contribution of drinking-water to the total oral exposure to aluminium will be about 4%. The contribution of air to the total exposure is generally negligible.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

In experimental animals, absorption of aluminium via the gastrointestinal tract is usually less than 1%. The main factors influencing absorption are solubility, pH, and chemical species. Organic complexing compounds, notably citrate, increase absorption. Aluminium absorption may interact with calcium and iron transport systems. Aluminium, once absorbed, is distributed in most organs within the body, with accumulation occurring mainly in bone at high dose levels. To a limited but as yet undetermined extent, aluminium passes the blood-brain barrier and is also distributed to

the fetus. Aluminium is eliminated effectively in the urine (WHO, 1997).

In humans, aluminium and its compounds appear to be poorly absorbed, although the rate and extent of absorption have not been adequately studied. The mechanism of gastrointestinal absorption has not yet been fully elucidated. Variability results from the chemical properties of the element and the formation of various chemical species, which is dependent upon the pH, ionic strength, presence of competing elements (e.g. silicon), and presence of complexing agents within the gastrointestinal tract (e.g. citrate). The urine is the most important route of aluminium excretion (WHO, 1996, 1997).

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

The oral LD_{50} of aluminium nitrate, chloride, and sulfate in mice and rats ranges from 200 to 1000 mg of aluminium per kg of body weight (WHO, 1997).

5.2 Short-term exposure

Groups of 25 male Sprague-Dawley rats were fed diets containing basic sodium aluminium phosphate or aluminium hydroxide at 0, 5, 67, 141, or 288/302 mg of aluminium per kg of body weight per day for 28 days. No treatment-related effects on organ and body weights, haematology, clinical chemistry parameters, and histopathology were observed, and there was no evidence of deposition of aluminium in bones. The NOELs were 288 and 302 mg of aluminium per kg of body weight per day for sodium aluminium phosphate and aluminium hydroxide, respectively (Hicks et al., 1987).

In a study in which a wide range of end-points was examined, groups of 10 female Sprague-Dawley rats received drinking-water containing aluminium nitrate for 28 days at 0, 1, 26, 52, or 104 mg of aluminium per kg of body weight per day. The only effects noted were mild histopathological changes in the spleen and liver of the high-dose group. Although tissue aluminium concentrations were generally higher in treated animals, the increases were significant only for spleen, heart, and gastrointestinal tract of the high-dose group. The NOAEL was 52 mg of aluminium per kg of body weight per day (Gomez et al., 1986).

Groups of 10 female Sprague-Dawley rats received aluminium nitrate in their drinking-water at doses of 0, 26, 52, or 260 mg of aluminium per kg of body weight per day for 100 days. Organ and body weights, histopathology of the brain, heart, lungs, kidney, liver, and spleen, haematology, and plasma chemistry were examined. The only effect observed was a significant decrease in body-weight gain associated with a decrease in food consumption at 260 mg of aluminium per kg of body weight per day. Aluminium did not accumulate in a dose-dependent manner in the organs and tissues examined. The NOAEL in this study was 52 mg of aluminium per kg of body weight per day (Domingo et al., 1987a).

Sodium aluminium phosphate, a leavening acid, was administered to groups of six male and six female beagle dogs at dietary concentrations of 0, 0.3, 1.0, or 3.0% for 6 months. Statistically significant decreases in food consumption occurred sporadically in all treated groups of female dogs, but there was no associated decrease in body weight. No significant absolute or relative organ-weight differences were found between any of the treated groups and controls. Haematological, blood chemistry, and urinalysis data showed no toxicologically significant trend. The NOAEL was the highest dose tested, approximately 70 mg of aluminium per kg of body weight per day (Katz et al., 1984).

Beagle dogs (four per sex per dose) were fed diets containing basic sodium aluminium phosphate at 0, 10, 22-27, or 75-80 mg of aluminium per kg of body weight per day for 26 weeks. The only treatment-related effect was a sharp, transient decrease in food consumption and concomitant

decrease in body weight in high-dose males. The LOAEL was 75-80 mg/kg of body weight per day (Pettersen et al., 1990).

5.3 Long-term exposure

No adverse effects on body weight or longevity were observed in Charles River mice (54 males and 54 females per group) receiving 0 or 5 mg of aluminium (as potassium aluminium sulfate) per kg of diet during their lifetime (Schroeder & Mitchener, 1975a; FAO/WHO, 1989).

Two groups of Long-Evans rats (52 of each sex) received 0 or 5 mg of aluminium (as potassium aluminium sulfate) per litre of drinking-water during their lifetime. No effects were found on body weight; average heart weight; glucose, cholesterol, and uric acid levels in serum; and protein and glucose content and pH of urine. The life span was not affected (Schroeder & Mitchener, 1975b; FAO/WHO, 1989).

5.4 Reproductive and developmental toxicity

Aluminium nitrate was administered by gavage to groups of pregnant Sprague-Dawley rats on day 14 of gestation through day 21 of lactation at doses of 0, 13, 26, or 52 mg of aluminium per kg of body weight per day. These doses did not produce overt fetotoxicity, but growth of offspring was significantly delayed (body weight, body length, and tail length) from birth to weaning in aluminium-treated groups (Domingo et al., 1987b).

Aluminium nitrate was administered by intubation to male Sprague-Dawley rats at 0, 13, 26, or 52 mg of aluminium per kg of body weight per day for 60 days prior to mating and to virgin females for 14 days prior to mating, with treatment continuing throughout mating, gestation, parturition, and weaning of the litters. No reproductive effects on fertility (number of litters produced), litter size, or intrauterine or postnatal offspring mortality were reported. There was a decrease in the numbers of corpus lutea in the highest dose group. However, a dose-dependent delay in the growth of the pups was observed in all treatment groups; female offspring were affected at 13 mg of aluminium per kg of body weight per day and males at 26 and 52 mg of aluminium per kg of body weight per day. Because of the design of this study, it is not clear whether the postnatal growth effects in offspring represented general toxicity to male or female parents or specific effects on reproduction or development. However, the reported LOAEL in females in this study was 13 mg of aluminium per kg of body weight per day (Domingo et al., 1987c).

The developmental toxicity of aluminium by the oral route is highly dependent on the form of aluminium and the presence of organic chelators that influence bioavailability. Aluminium hydroxide did not produce either maternal or developmental toxicity when it was administered by gavage during embryogenesis to mice at doses up to 92 mg of aluminium per kg of body weight per day (Domingo et al., 1989) or to rats at doses up to 265 mg of aluminium per kg of body weight per day (Gomez et al., 1990). When aluminium hydroxide at a dose of 104 mg of aluminium per kg of body weight per day was administered with ascorbic acid to mice, no maternal or developmental toxicity was seen, in spite of elevated maternal placenta and kidney concentrations of aluminium (Colomina et al., 1994); on the other hand, aluminium hydroxide at a dose of 133 mg of aluminium per kg of body weight per day administered with citric acid produced maternal and fetal toxicity in rats (Gomez et al., 1991). Aluminium hydroxide (57 mg of aluminium per kg of body weight) given with lactic acid (570 mg/kg of body weight) to mice by gavage was not toxic, but aluminium lactate (57 mg of aluminium per kg of body weight) produced developmental toxicity, including poor ossification, skeletal variations, and cleft palate (Colomina et al., 1992).

5.5 Mutagenicity and related end-points

Aluminium can form complexes with DNA and cross-link chromosomal proteins and DNA, but it has not been shown to be mutagenic in bacteria or induce mutation or transformation in

mammalian cells *in vitro*. Chromosomal aberrations have been observed in bone marrow cells of exposed mice and rats (WHO, 1997).

5.6 Carcinogenicity

There is no indication that aluminium is carcinogenic. JECFA evaluated the limited studies of Schroeder and Mitchener (1975a, b; section 5.3) and concluded that there was no evidence of an increase in tumour incidence related to the administration of potassium aluminium sulfate in mice or rats (FAO/WHO, 1989).

5.7 Neurotoxicity

Behavioural impairment has been reported in laboratory animals exposed to soluble aluminium salts (e.g. lactate, chloride) in the diet or drinking-water in the absence of overt encephalopathy or neurohistopathology. Both rats (Commissaris et al., 1982; Thorne et al., 1987; Connor et al., 1988) and mice (Yen-Koo, 1992) have demonstrated such impairments at doses exceeding 200 mg of aluminium per kg of body weight per day. Although significant alterations in acquisition and retention of learned behaviour were documented, the possible role of organ damage (kidney, liver, immunological) due to aluminium was incompletely evaluated in these studies (WHO, 1997).

In studies on brain development in mice and rats, grip strength was impaired in offspring of dams fed 100 mg of aluminium (as aluminium lactate) per kg of body weight per day in the diet, in the absence of maternal toxicity (WHO, 1997).

6. EFFECTS ON HUMANS

There is little indication that aluminium is acutely toxic by oral exposure despite its widespread occurrence in foods, drinking-water, and many antacid preparations (WHO, 1997).

In 1988, a population of about 20 000 individuals in Camelford, England, was exposed for at least 5 days to unknown but increased levels of aluminium accidentally distributed to the population from a water supply facility using aluminium sulfate for treatment. Symptoms including nausea, vomiting, diarrhoea, mouth ulcers, skin ulcers, skin rashes, and arthritic pain were noted. It was concluded that the symptoms were mostly mild and short-lived. No lasting effects on health could be attributed to the known exposures from aluminium in the drinking-water (Clayton, 1989).

It has been hypothesized that aluminium exposure is a risk factor for the development or acceleration of onset of Alzheimer disease (AD) in humans. WHO (1997) has evaluated some 20 epidemiological studies that have been carried out to test the hypothesis that aluminium in drinking-water is a risk factor for AD. Study designs ranged from ecological to case-control. Six studies on populations in Norway (Flaten, 1990), Canada (Neri & Hewitt, 1991), France (Michel et al., 1991; Jacqmin et al., 1994), Switzerland (Wettstein et al., 1991), and England (Martyn et al., 1989) were considered of sufficiently high quality to meet the general criteria for exposure and outcome assessment and the adjustment for at least some confounding variables. Of the six studies that examined the relationship between aluminium in drinking-water and dementia or AD, three found a positive relationship, but three did not. However, each of the studies had some deficiencies in the study design (e.g. ecological exposure assessment; failure to consider aluminium exposure from all sources and to control for important confounders, such as education, socioeconomic status, and family history; the use of surrogate outcome measures for AD; and selection bias). In general, the relative risks determined were less than 2, with large confidence intervals, when the total aluminium concentration in drinking-water was 0.1 mg/litre or higher. Based on current knowledge of the pathogenesis of AD and the totality of evidence from these epidemiological studies, it was concluded that the present epidemiological evidence does not support a causal association between AD and aluminium in drinking-water (WHO, 1997).

In addition to the epidemiological studies that examined the relationship between AD and

aluminium in drinking-water, two studies examined cognitive dysfunction in elderly populations in relation to the levels of aluminium in drinking-water. The results were again conflicting. One study of 800 male octogenarians consuming drinking-water with aluminium concentrations up to 98 µg/litre found no relationship (Wettstein et al., 1991). The second study used "any evidence of mental impairment" as an outcome measure and found a relative risk of 1.72 at aluminium drinking-water concentrations above 85 µg/litre in 250 males (Forbes et al., 1994). Such data are insufficient to show that aluminium is a cause of cognitive impairment in the elderly.

7. CONCLUSIONS

The Environmental Health Criteria document for aluminium (WHO, 1997) concluded that:

> On the whole, the positive relationship between aluminium in drinking-water and AD, which was demonstrated in several epidemiological studies, cannot be totally dismissed. However, strong reservations about inferring a causal relationship are warranted in view of the failure of these studies to account for demonstrated confounding factors and for total aluminium intake from all sources.
>
> Taken together, the relative risks for AD from exposure to aluminium in drinking-water above 100 µg/litre, as determined in these studies, are low (less than 2.0). But, because the risk estimates are imprecise for a variety of methodological reasons, a population-attributable risk cannot be calculated with precision. Such imprecise predictions may, however, be useful in making decisions about the need to control exposures to aluminium in the general population.

The degree of aluminium absorption depends on a number of parameters, such as the aluminium salt administered, pH (for aluminium speciation and solubility), bioavailability, and dietary factors. These should be taken into consideration during tissue dosimetry and response assessment. Thus, the use of currently available animal studies to develop a guideline value is not appropriate because of these specific toxicokinetic/dynamic considerations.

Owing to the uncertainty surrounding the human data and the limitations of the animal data as a model for humans, a health-based guideline value for aluminium cannot be derived at this time.

The beneficial effects of the use of aluminium as a coagulant in water treatment are recognized. Taking this into account and considering the potential health concerns (i.e. neurotoxicity) of aluminium, a practicable level is derived based on optimization of the coagulation process in drinking-water plants using aluminium-based coagulants, to minimize aluminium levels in finished water.

A number of approaches are available for minimizing residual aluminium concentrations in treated water. These include use of optimum pH in the coagulation process, avoiding excessive aluminium dosage, good mixing at the point of application of the coagulant, optimum paddle speeds for flocculation, and efficient filtration of the aluminium floc (Letterman & Driscoll, 1988; WRc, 1997). Under good operating conditions, concentrations of aluminium of 0.1 mg/litre or less are achievable in large water treatment facilities. Small facilities (e.g. those serving fewer than 10 000 people) might experience some difficulties in attaining this level, because the small size of the plant provides little buffering for fluctuation in operation, and small facilities often have limited resources and access to expertise to solve specific operational problems. For these small facilities, 0.2 mg/litre or less is a practicable level for aluminium in finished water (WRc, 1997).

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Boron

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In the 1993 WHO *Guidelines for drinking-water quality*, a health-based guideline value for boron of 0.3 mg/litre was derived from a 2-year study in dogs published in 1972 (testicular atrophy was the critical end-point of toxicity). New scientific data have become available, including an IPCS Environmental Health Criteria monograph for boron which had been finalized by a Task Group in November 1996, and which was therefore available for the present evaluation.

1. GENERAL DESCRIPTION

1.1 Identity

Boron (CAS no. 7440-42-8) is never found in the elemental form in nature. It exists as a mixture of the ^{10}B (19.78%) and ^{11}B (80.22%) isotopes (Budavari et al., 1989). Boron's chemistry is complex and resembles that of silicon (Cotton & Wilkinson, 1988).

1.2 Physicochemical properties

Elemental boron exists as a solid at room temperature, either as black monoclinic crystals or as a yellow or brown amorphous powder when impure. The amorphous and crystalline forms of boron have specific gravities of 2.37 and 2.34, respectively. Boron is a relatively inert metalloid except when in contact with strong oxidizing agents.

Sodium perborates are persalts, which are hydrolytically unstable because they contain characteristic boron-oxygen-oxygen bonds that react with water to form hydrogen peroxide and

stable sodium metaborate ($NaBO_2 \cdot nH_2O$).

Boric acid is a very weak acid, with a pK_a of 9.15, and therefore boric acid and the sodium borates exist predominantly as undissociated boric acid [$B(OH)_3$] in dilute aqueous solution at pH <7; at pH >10, the metaborate anion $B(OH)_4^-$ becomes the main species in solution. Between these two pH values, from about 6 to 11, and at high concentration (>0.025 mol/litre), highly water soluble polyborate ions such as $B_3O_3(OH)_4^-$, $B_4O_5(OH)_4^-$, and $B_5O_6(OH)_4^-$ are formed.

The chemical and toxicological properties of borax pentahydrate $Na_2B_4O_7 \cdot 5H_2O$, borax $Na_2B_4O_7 \cdot 10H_2O$, boric acid, and other borates are expected to be similar on a molar boron equivalent basis when dissolved in water or biological fluids at the same pH and low concentration.

1.3 Major uses

Boric acid and borates are used in glass manufacture (fibreglass, borosilicate glass, enamel, frit, and glaze), soaps and detergents, flame retardants, and neutron absorbers for nuclear installations. Boric acid, borates, and perborates have been used in mild antiseptics, cosmetics, pharmaceuticals (as pH buffers), boron neutron capture therapy (for cancer treatment), pesticides, and agricultural fertilizers.

1.4 Environmental fate

Waterborne boron may be adsorbed by soils and sediments. Adsorption-desorption reactions are expected to be the only significant mechanism influencing the fate of boron in water (Rai et al., 1986). The extent of boron adsorption depends on the pH of the water and the concentration of boron in solution. The greatest adsorption is generally observed at pH 7.5-9.0 (Waggott, 1969; Keren & Mezuman, 1981; Keren et al., 1981).

In natural waters, boron exists primarily as undissociated boric acid with some borate ions. As a group, the boron-oxygen compounds are sufficiently soluble in water to achieve the levels that have been observed (Sprague, 1972). Mance et al. (1988) described boron as a significant constituent of seawater, with an average boron concentration of 4.5 mg/kg.

2. ANALYTICAL METHODS

A spectrometric method using azomethine-H is available for the determination of borate in water. The method is applicable to the determination of borate at concentrations between 0.01 and 1 mg/litre. The working range may be extended by dilution (ISO, 1990).

A widely used method for the analysis of boron in bone, plasma, and food is inductively coupled plasma atomic emission spectroscopy (Hunt, 1989). This method is also used for water (ISO, 1996) and wastewater (Huber, 1982). Detection limits in water range from 6 to 10 µg of boron per litre.

Inductively coupled plasma mass spectroscopy (ICP-MS) is a widely used non-spectrophotometric method for the analysis of boron, as it uses small volumes of sample, is fast, and applies to a wide range of materials (fresh and saline water, sewage, wastewater, soils, plant samples, and biological materials). ICP-MS can detect boron down to 0.15 µg/litre (WHO, in press). Using direct nebulization, ICP-MS can give a detection limit of 1 ng/g in human blood, human serum, orchard leaves, and total diet (Smith et al., 1991).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Boron is not present in the atmosphere at significant levels (Sprague, 1972). Because borates exhibit low volatility, boron would not be expected to be present to a significant degree as a vapour in the atmosphere. Atmospheric emissions of borates and boric acid in a particulate (<1-45 µm in size) or vapour form occur as a result of volatilization of boric acid from the sea, volcanic activity, mining operations, glass and ceramic manufacturing, the application of agricultural chemicals, and coal-fired power plants.

3.2 Water

The natural borate content of groundwater and surface water is usually small. The borate content of surface water can be significantly increased as a result of wastewater discharges, because borate compounds are ingredients of domestic washing agents (ISO, 1990).

Naturally occurring boron is present in groundwater primarily as a result of leaching from rocks and soils containing borates and borosilicates. Concentrations of boron in groundwater throughout the world range widely, from <0.3 to >100 mg/litre. In general, concentrations of boron in Europe were greatest in southern Europe (Italy, Spain) and least in northern Europe (Denmark, France, Germany, the Netherlands, and the United Kingdom). For Italy and Spain, mean boron concentrations ranged from 0.5 to 1.5 mg/litre. Values ranged up to approximately 0.6 mg/litre in the Netherlands and the United Kingdom, and approximately 90% of samples in Denmark, France, and Germany were found to contain boron at concentrations below 0.3, 0.3, and 0.1 mg/litre, respectively (WHO, in press). Monthly mean values of boron in the Ruhr River, Germany, ranged from 0.31 to 0.37 mg/litre in a survey conducted during 1992-1995 (Haberer, 1996).

The majority of the Earth's boron occurs in the oceans, with an average concentration of 4.5 mg/litre (Weast et al., 1985). The amount of boron in fresh water depends on such factors as the geochemical nature of the drainage area, proximity to marine coastal regions, and inputs from industrial and municipal effluents (Butterwick et al., 1989).

Boron concentrations in fresh surface water range from <0.001 to 2 mg/litre in Europe, with mean values typically below 0.6 mg/litre. Similar concentration ranges have been reported for water bodies within Pakistan, Russia, and Turkey, from 0.01 to 7 mg/litre, with most values below 0.5 mg/litre. Concentrations ranged up to 0.01 mg/litre in Japan and up to 0.3 mg/litre in South African surface waters. Samples taken in surface waters from two South American rivers (Rio Arenales, Argentina, and Loa River, Chile) contained boron at concentrations ranging between 4 and 26 mg/litre in areas rich in boron-containing soils. In other areas, the Rio Arenales contained less than 0.3 mg of boron per litre. Concentrations of boron in surface waters of North America (Canada, USA) ranged from 0.02 mg/litre to as much as 360 mg/litre, indicative of boron-rich deposits. However, typical boron concentrations were less than 0.1 mg/litre, with a 90th-percentile boron concentration of approximately 0.4 mg/litre.

Concentrations of boron found in drinking-water from Chile, Germany, the United Kingdom, and the USA ranged from 0.01 to 15.0 mg/litre, with most values clearly below 0.4 mg/litre. These values are consistent with ranges and means observed for groundwater and surface waters. This consistency is supported by two factors: (i) boron concentrations in water are largely dependent on the leaching of boron from the surrounding geology and wastewater discharges, and (ii) boron is not removed by conventional drinking-water treatment methods.

3.3 Food

The general population obtains the greatest amount of boron through food intake. Concentrations of boron reported in food after 1985 have more validity because of the use of more adequate

analytical methods.

The richest sources of boron are fruits, vegetables, pulses, legumes, and nuts. Dairy products, fish, meats, and most grains are poor sources of boron. Based on the recent analyses of foods and food products, estimations of daily intakes of various age/sex groups have been made (WHO, in press). The estimated median, mean, and 95th-percentile daily intakes of boron were 0.75, 0.93, and 2.19 mg/day, respectively, for all groups, and 0.79, 0.98 and 2.33 mg/day, respectively, for adults aged 17 and older. Using food included in US Food and Drug Administration Total Diet Studies, Iyengar et al. (1988) determined the mean adult male daily intake of boron to be 1.52 mg/day, whereas Anderson et al. (1994) determined the intake to be 1.21 mg/day. Based on the United Kingdom National Food Survey (MAFF, 1991), the dietary intake of boron in the United Kingdom ranges from 0.8 to 1.9 mg/day. It should be noted that increased consumption of specific foods with high boron content will increase boron intake significantly; for example, one serving of wine or avocado provides 0.42 and 1.11 mg, respectively (Anderson et al., 1994).

3.4 Estimated total exposure and relative contribution of drinking-water

The mean daily intake of boron in the diet is judged to be near 1.2 mg/day (Anderson et al., 1994). Concentrations of boron in drinking-water have wide ranges, depending on the source of the drinking-water, but for most of the world the range is judged to be between 0.1 and 0.3 mg/litre. Based on usage data, consumer products have been estimated to contribute a geometric mean of 0.1 mg/day to the estimate of total boron exposure (WHO, in press). The contribution of boron intake from air is negligible. The total daily intake can therefore be estimated from mean concentrations and concentration ranges to be between 1.5 and 2 mg.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Numerous studies have shown that boric acid and borax are absorbed from the gastrointestinal tract and from the respiratory tract, as indicated by increased levels of boron in the blood, tissues, or urine or by systemic toxic effects of exposed individuals or laboratory animals.

Clearance of boron compounds is similar in humans and animals. The ratio of mean clearance values as a function of dose in non-pregnant rats versus humans is approximately 3- to 4-fold - i.e. similar to the default value for the toxicokinetic component of the uncertainty factor for interspecies variation[1] (WHO, 1994). Elimination of borates from the blood is largely by excretion of >90% of the administered dose via the urine, regardless of the route of administration. Excretion is relatively rapid, occurring over a period of a few to several days, with a half-life of elimination of 24 hours or less. The kinetics of elimination of boron have been evaluated in human volunteers given boric acid via the intravenous and oral routes (Jansen et al., 1984; Schou et al., 1984). Absorption is poor through intact skin but is much greater through damaged skin.

[1] Report of informal discussion to develop recommendations for the WHO *Guidelines for drinking-water quality* - Boron. Cincinnati, OH, 28-29 September 1997. Report available from WHO, Division of Operational Support in Environmental Health, Geneva

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

The oral LD_{50} values for boric acid or borax in mice and rats are in the range of about 400-700 mg of boron per kg of body weight (Pfeiffer et al., 1945; Weir & Fisher, 1972). Oral LD_{50} values in the range of 250-350 mg of boron per kg of body weight for boric acid or borax exposure have been reported for guinea-pigs, dogs, rabbits, and cats (Pfeiffer et al., 1945; Verbitskaya, 1975). Signs of acute toxicity for both borax and boric acid in animals given single large doses orally include

depression, ataxia, convulsions, and death; kidney degeneration and testicular atrophy are also observed (Larsen, 1988).

5.2 Short-term exposure

In a 13-week study, mice (10 per sex per dose) were fed diets containing boric acid at approximately 0, 34, 70, 141, 281, or 563 mg of boron per kg of body weight per day. At the two highest doses, increased mortality was seen. Degeneration or atrophy of the seminiferous tubules was observed at 141 mg of boron per kg of body weight per day. In all dose groups, extramedullary haematopoiesis of the spleen of minimal to mild severity was seen (NTP, 1987).

In a study in which borax was given in the diet to male Sprague-Dawley rats (18 per dose) at concentrations of 0, 500, 1000, or 2000 mg of boron per kg of feed (approximately equal to 0, 30, 60, or 125 mg of boron per kg of body weight per day) for 30 or 60 days, body weights were not consistently affected by treatment. Organ weights were not affected by 500 mg of boron per kg of feed; at 1000 and 2000 mg of boron per kg of feed, absolute liver weights were significantly lower after 60 days, and epididymal weights were significantly lower (37.6% and 34.8%, respectively) after 60 days, but not after 30 days. Weights of prostate, spleen, kidney, heart, and lung were not changed at any dose (Lee et al., 1978).

In a 90-day study in rats (10 per sex per dose) receiving 0, 2.6, 8.8, 26, 88, or 260 mg of boron per kg of body weight per day in the diet as boric acid or borax, all animals at the highest dose died within 3-6 weeks (Weir & Fisher, 1972). In animals receiving 88 mg of boron per kg of body weight per day, body weights in males and females were reduced; absolute organ weights, including the liver, spleen, kidneys, brain, adrenals, and ovaries, were also significantly decreased in this group. Organ-to-body-weight ratios for the adrenals and kidneys were significantly increased, but relative weights of the liver and ovaries were decreased. A pronounced reduction in testicular weights in males in the 88 mg of boron per kg of body weight per day group was also observed.

Boric acid or borax was also fed to beagle dogs for 90 days or for 2 years. In the 90-day boric acid study (weight-normalized doses of 0, 0.44, 4.4, or 44 mg of boron per kg of body weight per day; five animals per sex per dose), testis weight was significantly lower than controls in the middle and upper dose groups (reduced by 25% and 40%, respectively). Although testicular microscopic structure was not detectably abnormal in the controls and middle dose group, four of five dogs in the high-dose group had complete atrophy, and the remaining high-dose dog had one-third of tubules showing some abnormality. In the borax study, testis weights in the low-, middle-, and high-dose groups were 80%, 85%, and 50% of controls, respectively; only the last was significantly different from controls. No mention was made of the testicular microscopic structure of the controls or low-dose animals; middle-dose animals were not detectably altered (aside from the considerable fixation-induced artifact in the outer third of the tissue), whereas four of five high-dose dogs had complete testicular atrophy, and the remaining high-dose dog had “partial” atrophy. No other clinical or microscopic signs of toxicity were reported in any animals (Weir & Fisher, 1972).

In the 2-year study, the dogs (four per sex per dose) received the boric acid or borax in the diet at weight-normalized doses of 0, 1.5, 2.9, or 8.8 mg of boron per kg of body weight per day. An additional group received 29 mg of boron per kg of body weight per day for 38 weeks. Testicular atrophy was observed in two test dogs receiving borax at 26 weeks and in the two and one dogs, respectively, killed after 26 or 38 weeks of boric acid consumption. The authors stated that boric acid caused testicular degeneration in dogs, including spermatogenic arrest and atrophy of the seminiferous epithelium. The study was terminated at 38 weeks. In these studies, the number of dogs was small and variable (one or two dogs at each of three time points) and inadequate to allow statistical analysis. All three treated dogs had widespread and marked atrophy in 25-40% of the seminiferous tubules. A common control group was used for both the borax and boric acid studies. Testicular lesions occurred in the controls (one of four controls had slight to severe

seminiferous tubular atrophy, another had moderate to severe atrophy, whereas a third had a detectable but insignificant reduction in spermatogenesis and 5% atrophic seminiferous tubules) (Weir & Fisher, 1972). These studies were conducted before the advent of Good Laboratory Practices (GLPs). Confidence in these studies is low, and they were considered not suitable for inclusion into the risk assessment because of 1) small and variable numbers of dogs, 2) variable background lesions in controls leading to uncertainty of the strength of the response to treatment, 3) lack of GLPs, and 4) other, more recent studies of greater scientific quality with findings at similar intake levels of boron (Ku et al., 1993; Price et al., 1996a).

5.3 Long-term exposure

A 2-year study in mice (50 per sex per dose) receiving approximately 0, 275, or 550 mg of boric acid per kg of body weight per day (0, 48, or 96 mg of boron per kg of body weight per day) in the diet (NTP, 1987; Dieter, 1994) demonstrated that body weights were 10-17% lower in high-dose males after 32 weeks and in high-dose females after 52 weeks. Increased mortality rates were statistically significant in males, with significant lesions in male mice appearing in the testes and no significant non-neoplastic lesions in female mice.

In a 2-year study, rats (35 per sex per dose) were administered weight-normalized boron doses of 0, 5.9, 18, or 59 mg/kg of body weight per day in the diet (Weir & Fisher, 1972). High-dose animals had coarse hair coats, scaly tails, hunched posture, swollen and desquamated pads of the paws, abnormally long toenails, shrunken scrotum, inflamed eyelids, and bloody eye discharge. The haematocrit and haemoglobin levels were significantly lower than controls, the absolute and relative weights of the testes were significantly lower, and relative weights of the brain and thyroid gland were higher than in controls. In animals in the mid- and low-dose groups, no significant effects on general appearance, behaviour, growth, food consumption, haematology, serum chemistry, or histopathology were observed.

5.4 Reproductive and developmental toxicity

Short- and long-term oral exposures to boric acid or borax in laboratory animals have demonstrated that the male reproductive tract is a consistent target of toxicity. Testicular lesions have been observed in rats, mice, and dogs administered boric acid or borax in food or drinking-water (Truhaut et al., 1964; Weir & Fisher, 1972; Green et al., 1973; Lee et al., 1978; NTP, 1987; Ku et al., 1993). The first clinical indication of testicular toxicity in dogs is shrunken scrota observed during treatment; significant decreases in absolute and relative testicular weight are also reported. After subchronic exposure, the histopathological effects range from inhibited spermiation (sperm release) to degeneration of the seminiferous tubules with variable loss of germ cells to complete absence of germ cells, resulting in atrophy and transient or irreversible loss of fertility, but not of mating behaviour.

In time-response and dose-response reproductive studies (Linder et al., 1990), adult male Sprague-Dawley rats were administered two doses in one day, with a total dose of 0 or 350 mg of boron per kg of body weight in the time-response experiment (animals were sacrificed at 2, 14, 28, or 57 days post-treatment) and a total dose of 0, 44, 87, 175, or 350 mg of boron per kg of body weight in the dose-response experiment (animals were sacrificed after 14 days). Adverse effects on spermiation, epididymal sperm morphology, and caput sperm reserves were observed during histopathological examinations of the testes and epididymis. The NOAEL for male reproductive effects in the dose-response study was 87 mg of boron per kg of body weight per day.

In a multi-generation study, doses of 0, 117, 350, or 1170 mg of boron per kg of feed (as borax or boric acid) were administered to male and female rats (Weir & Fisher, 1972). At the highest dose, rats were found to be sterile, males showed atrophied testes in which spermatozoa were absent, and females showed decreased ovulation. The NOAEL in this study was 350 mg of boron per kg of feed, equivalent to 17.5 mg of boron per kg of body weight per day.

To investigate the development of testicular lesions, boric acid was fed at 61 mg of boron per kg of body weight per day to male F344 rats; sacrifice of six treated and four control rats was conducted at intervals from 4 to 28 days. At 28 days, there was significant loss of spermatocytes and spermatids from all tubules in exposed rats, and basal serum testosterone levels were significantly decreased from 4 days on (Treinen & Chapin, 1991). In another study, the activities of enzymes found primarily in spermatogenic cells were decreased, and enzyme activities associated with premeiotic spermatogenic cells were significantly increased in rats exposed to 60 or 125 mg of boron per kg of body weight per day for 60 days (Lee et al., 1978). Mean plasma follicle-stimulating hormone levels were significantly elevated in a dose-dependent manner in all treatment groups (30, 60, or 125 mg of boron per kg of body weight per day) in this study after 60-day exposures.

Reversibility of testicular lesions was evaluated by Ku et al. (1993) in an experiment in which F344 rats were dosed at 0, 3000, 4500, 6000, or 9000 mg of boric acid per kg of feed (equivalent to 0, 26, 39, 52, or 78 mg of boron per kg of body weight per day) for 9 weeks and assessed for recovery up to 32 weeks post-treatment. Inhibited spermiation was exhibited at 3000 and 4500 mg of boric acid per kg of feed (5.6 µg of boron per mg of tissue), whereas inhibited spermiation progressed to atrophy at 6000 and 9000 mg of boric acid per kg of feed (11.9 µg of boron per mg of testes); there was no boron accumulation in the testes to levels greater than those found in the blood during the 9-week period. After treatment, serum and testis boron levels in all dose groups fell to background levels. Inhibited spermiation at 4500 mg of boric acid per kg of feed was reversed by 16 weeks post-treatment, but focal atrophy, which did not recover up to 32 weeks post-treatment, was detected.

Developmental toxicity has been demonstrated experimentally in rats, mice, and rabbits (NTP, 1990; Heindel et al., 1992; Price et al., 1996b). Rats were fed a diet containing 0, 14, 29, or 58 mg of boron per kg of body weight per day as boric acid on gestation days 0-20 (Heindel et al., 1992). An additional group of rats received boric acid at 94 mg of boron per kg of body weight per day on gestation days 6-15 only. Average fetal body weight per litter was significantly reduced in a dose-related manner in all treated groups compared with controls. The percentage of malformed fetuses per litter and the percentage of litters with at least one malformed fetus were significantly increased at $\geq$29 mg of boron per kg of body weight per day. Malformations consisted primarily of anomalies of the eyes, the central nervous system (CNS), the cardiovascular system, and the axial skeleton. The most common malformations were enlargement of lateral ventricles in the brain and agenesis or shortening of rib XIII. The LOAEL of 14 mg of boron per kg of body weight per day (the lowest dose tested) for rats occurred in the absence of maternal toxicity; a NOAEL was not found in this study.

Price et al. (1996a) did a follow-up to the Heindel et al. (1992) study in Sprague-Dawley (CD) rats to determine a NOAEL for fetal body-weight reduction and to determine whether the offspring would recover from prenatally reduced body weight during postnatal development. Boric acid was administered in the diet to CD rats on gestation days 0-20. Dams were terminated and uterine contents examined on gestation day 20. The intake of boric acid was 0, 3.3, 6.3, 9.6, 13, or 25 mg of boron per kg of body weight per day. Fetal body weights were 99, 98, 97, 94, and 88% of controls for the low- to high-dose groups, respectively. Incidences of short rib XIII (a malformation) or wavy rib (a variation) were increased in the 13 and 25 mg of boron per kg of body weight per day dose groups relative to control litters. There was a decreased incidence of rudimentary extra rib on lumbar 1 (a variation) in the high-dose group that was deemed biologically but not statistically significant. The NOAEL in this study was 9.6 mg of boron per kg of body weight per day, based on a decrease in fetal body weight at the next higher dose.

Developmental toxicity and teratogenicity of boric acid in mice at 0, 43, 79, or 175 mg of boron per kg of body weight per day in the diet were investigated (Heindel et al., 1992). There was a significant dose-related decrease in average fetal body weight per litter at 79 and 175 mg of boron per kg of body weight per day. In offspring of mice exposed to 79 or 175 mg of boron per

kg of body weight per day during gestation days 0-20, there was an increased incidence of skeletal (rib) malformations. These changes occurred at doses for which there were also signs of maternal toxicity (increased kidney weight and pathology); the LOAEL for developmental effects (decreased fetal body weight per litter) was 79 mg of boron per kg of body weight per day, and the NOAEL was 43 mg of boron per kg of body weight per day.

Developmental toxicity and teratogenicity of boric acid in rabbits were investigated by Price et al. (1996b) at doses of 0, 11, 22, or 44 mg of boron per kg of body weight per day, given by gavage. Frank developmental effects in rabbits exposed to 44 mg of boron per kg of body weight per day included a high rate of prenatal mortality, an increased number of pregnant females with no live fetuses, and fewer live fetuses per live litter on day 30. At the high dose, malformed live fetuses per litter increased significantly, primarily because of the incidence of fetuses with cardiovascular defects, the most prevalent of which was interventricular septal defect. Skeletal variations observed were extra rib on lumbar 1 and misaligned sternebra. The NOAEL for maternal and developmental effects was 22 mg of boron per kg of body weight per day.

5.5 Mutagenicity and related end-points

The mutagenic activity of boric acid was examined in the *Salmonella typhimurium* and mouse lymphoma assays, with negative results. No induction of sister chromatid exchange or chromosomal aberrations was observed in Chinese hamster ovary cells (NTP, 1987). Sodium borate did not cause gene mutations in the *S. typhimurium* preincubation assay (Benson et al., 1984). Borax was not mutagenic in cell transformation assays with Chinese hamster cells, mouse embryo cells, and human fibroblasts (Landolph, 1985).

5.6 Carcinogenicity

Tumour incidence was not enhanced in studies in which $B6C3F_1$ mice received 0, 2500, or 5000 mg of boric acid per kg of feed for 103 weeks (NTP, 1987) and Sprague-Dawley rats received diets containing 0, 117, 350, or 1170 mg of boron per kg of feed (as borax or boric acid) for 2 years (Weir & Fisher, 1972).

6. EFFECTS ON HUMANS

Available human data on boron compounds for routes other than inhalation focus on boric acid and borax. According to Stokinger (1981), the lowest reported lethal doses of boric acid are 640 mg/kg of body weight (oral), 8600 mg/kg of body weight (dermal), and 29 mg/kg of body weight (intravenous injection). Stokinger (1981) stated that death has occurred at total doses of between 5 and 20 g of boric acid for adults and <5 g for infants. Litovitz et al. (1988) stated that potential lethal doses are usually cited as 3-6 g total for infants and 15-20 g total for adults. A case-series report of seven infants (aged 6-16 weeks) who used pacifiers coated with a borax and honey mixture for 4-10 weeks concluded that exposures ranged from 12 to 90 g, with a very crudely estimated average daily ingestion of 18-56 mg of boron per kg of body weight (O'Sullivan & Taylor, 1983).[2] Toxicity was manifested by generalized or alternating focal seizure disorders, irritability, and gastrointestinal disturbances. Although infants appear to be more sensitive than adults to boron compounds, lethal doses are not well documented in the literature.

[2] Estimates given here are corrected values, as intakes reported in this publication were underestimated by a factor of 3 (M. Taylor, personal communication to M. Dourson, in a letter dated 28 August 1997).

Goldbloom & Goldbloom (1953) reported four cases of boric acid poisoning and reviewed an additional 109 cases in the literature. The four cases were infants exposed to boric acid by repeated topical applications of baby powder. Toxicity was manifested by cutaneous lesions (erythema over the entire body, excoriation of the buttocks, and desquamation), gastrointestinal disturbances, and seizures. Approximately 35% of the 109 other case reports of boric acid

poisoning involved children <1 year of age. The mortality rate was 70.2% for children, compared with 55.0% for all cases combined. Death occurred in 53% of patients exposed by ingestion, 75% of patients subjected to gastric lavage with boric acid, 68% of patients exposed by dermal application for treating burns, wounds, and skin eruptions, and 54% of patients exposed by other routes. Information on signs and symptoms for 80 patients showed that gastrointestinal disturbances were prevalent (73%), followed by CNS effects (67%). Cutaneous lesions were prevalent in 76% of the cases and in 88% of cases involving children <2 years of age. Gross and microscopic findings were reported for 45% of fatal cases. In general, boric acid caused chemical irritation primarily at sites of application and excretion and in organs with maximum boron concentrations. The most common CNS findings were oedema and congestion of the brain and meninges. Other common findings included liver enlargement, vascular congestion, fatty changes, swelling, and granular degeneration.

In addition to case reports, poison centres have published case-series reports. Unlike the case reports reviewed by Goldbloom & Goldbloom (1953), more recent reports suggest that the oral toxicity of boron in humans is milder than previously thought. Litovitz et al. (1988) conducted a retrospective review of 784 cases of boric acid ingestion reported to the National Capital Poison Center in Washington, DC, USA, during 1981-1985 and the Maryland Poison Center in Baltimore, MD, USA, during 1984-1985; approximately 88.3% of the cases were asymptomatic. All but two of the cases had acute (single) ingestion, and 80.2% involved children <6 years of age. No severe toxicity or life-threatening effects were noted, although boric acid levels in blood serum ranged from 0 to 340 µg/ml. The most frequently occurring symptoms, which involved the gastrointestinal tract, included vomiting, abdominal pain, diarrhoea, and nausea. Other symptoms (primarily CNS and cutaneous) occurred in fewer cases: lethargy, rash, headache, light-headedness, fever, irritability, and muscle cramps. The average dose ingested was estimated at 1.4 g. According to Litovitz et al. (1988), 21 of the children <6 years of age, 15 of whom were <2 years of age, ingested the reported potential lethal dose of 3 g; eight adults ingested the reported potential lethal dose of 15 g without clinical evidence of lethal effects.

Linden et al. (1986) published a retrospective review of 364 cases of boric acid exposure reported to the Rocky Mountain Poison and Drug Center in Denver, CO, USA, between 1983 and 1984. Vomiting, diarrhoea, and abdominal pain were the most common symptoms given by the 276 cases exposed in 1983. Of the 72 cases reported in 1984 for whom medical records were complete, 79% were asymptomatic, whereas 20% noted mild gastrointestinal symptoms. One 2-year-old child died, presumably from repeated ingestion of an insecticide containing 99% boric acid.

Overall, owing to the wide variability of data collected from poisoning centres, the average dose of boric acid to produce clinical symptoms is still unclear, presumably in the range of 100 mg to 55.5 g, reported by Litovitz et al. (1988).

Findings from human experiments show that boron is a dynamic trace element that can affect the metabolism or utilization of numerous substances involved in life processes, including calcium, copper, magnesium, nitrogen, glucose, triglycerides, reactive oxygen, and estrogen. Although the first findings involving boron deprivation of humans appeared in 1987 (Nielsen et al., 1987), the most convincing findings have come mainly from two studies in which men over the age of 45, postmenopausal women, and postmenopausal women on estrogen therapy were fed a low-boron diet (0.25 mg/2000 kcal) for 63 days and then fed the same diet supplemented with 3 mg of boron per day for 49 days (Nielsen, 1989, 1994; Nielsen et al., 1990, 1991, 1992; Penland, 1994). These dietary intakes were near the low and high values in the range of usual dietary boron intakes. The major differences between the two studies were the intakes of copper and magnesium: in one experiment, they were marginal or inadequate; in the other, they were adequate. The marginal or inadequate copper and magnesium intakes caused apparent detrimental changes that were more marked during boron deprivation than during boron repletion. Although the function of boron remains undefined, boron is becoming recognized as an element of potential nutritional importance because of the findings from human and animal studies.

7. PROVISIONAL GUIDELINE VALUE

The TDI of boron is derived by dividing the NOAEL (9.6 mg/kg of body weight per day) for the critical effect, which is developmental toxicity (decreased fetal body weight in rats), by an appropriate uncertainty factor, which is judged to be 60. The value of 10 for interspecies variation (animals to humans) was adopted because of lack of toxicokinetic and toxicodynamic data to allow deviation from this default value. Available toxicokinetic data do support, however, reduction of the default uncertainty factor for intraspecies variation from 10 to 6 (WHO, 1994).

Interspecies (toxicokinetic) variations for boron relate primarily to clearance. The ratio of mean clearance values in non-pregnant rats versus non-pregnant humans for boron (based on all of the data considered suitable for inclusion) is 3-4. In view of the lack of adequate kinetic studies in rats and hence less than optimum confidence in much of the data that serve as the basis for the ratio, replacement of the default for the toxicokinetic component of the interspecies factor is considered premature at this time. The total uncertainty factor for interspecies variation is 10.

Intraspecies variation (toxicokinetics) for boron relates also primarily to variations in clearance. As the critical effect that serves as the basis for the TDI is developmental, pregnant women are the subgroup of interest in this regard. Based on pooled individual data from available studies, the mean glomerular filtration rate (GFR) in 36 healthy women was 145 ± 23 ml/minute in early pregnancy and 144 ± 32 ml/minute in late pregnancy. The standard deviation represented 22% of the mean value in late pregnancy. Based on division of the mean GFR (144 ml/minute) by the GFR at two standard deviations below the mean (80 ml/minute) to address variability for approximately 95% of the population, the ratio for the toxicokinetic component of interspecies variation is 1.8 (compared with the default value for this component of 3.2). As there are no data to serve as a basis for replacement of the default value for the toxicodynamic component of the uncertainty factor for intraspecies variation, the total uncertainty factor for intraspecies variation is $1.8 \times 3.2 = 5.7$ (rounded to 6)[3].

[3] Report of informal discussions to develop recommendations for the WHO *Guidelines for drinking-water quality* - Boron. Cincinnati, OH, 28-29 September 1997. Report available from WHO, Division of Operational Support in Environmental Health, Geneva

Using an uncertainty factor of 60, the TDI is therefore 0.16 mg/kg of body weight. With an allocation of 10% of the TDI to drinking-water and assuming a 60-kg adult consuming 2 litres of drinking-water per day, the guideline value is 0.5 mg/litre (rounded figure).

Conventional water treatment (coagulation, sedimentation, filtration) does not significantly remove boron, and special methods would have to be installed in order to remove boron from waters with high boron concentrations. Ion exchange and reverse osmosis processes may enable substantial reduction but are likely to be prohibitively expensive. Blending with low-boron supplies might be the only economical method to reduce boron concentrations in waters where these concentrations are high (WRc, 1997).

The guideline value of 0.5 mg/litre is designated as provisional, because it will be difficult to achieve in areas with high natural boron levels with the treatment technology available.

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Copper

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In view of uncertainties regarding copper toxicity in humans, a provisional guideline value for copper of 2 mg/litre was established in the 1993 WHO *Guidelines for drinking-water quality*, based on a 10% allocation of the 1982 JECFA PMTDI to drinking-water. The PMTDI was established as 10 times the normal copper intake of 0.05 mg/kg of body weight per day, which was considered to be without adverse effect.[1] Copper was selected for re-evaluation by the Coordinating Committee for the updating of the *Guidelines* because of the provisional guideline value and because the development of an IPCS Environmental Health Criteria monograph for copper was under way.

[1] It was erroneously assumed in the 1993 *Guidelines* that the PMTDI established by JECFA was based on a study in dogs.

1. GENERAL DESCRIPTION

1.1 Identity

Copper (CAS no. 7440-50-8) is a transition metal that is stable in its metallic state and forms monovalent (cuprous) and divalent (cupric) cations. Common copper compounds include the following:

Compound	*CAS no.*
Copper(II) acetate monohydrate [$Cu(C_2H_3O_2)_2 \cdot H_2O$]	6046-93-1
Copper(II) chloride [$CuCl_2$]	7447-39-4
Copper(II) nitrate trihydrate [$Cu(NO_3)_2 \cdot 3H_2O$]	10031-43-3
Copper(II) oxide [CuO]	1317-38-0
Copper(II) sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$]	7758-99-8

1.2 Physicochemical properties (Weast, 1983; ATSDR, 1990; Lewis, 1993)

Compound	*Density (g/cm^3)*	*Water solubility (g/litre)*
Copper(II) acetate monohydrate	1.88	72
Copper(II) chloride	3.39	706
Copper(II) nitrate trihydrate	2.32	1378
Copper(II) oxide	6.32	Insoluble
Copper(II) sulfate pentahydrate	2.28	316

1.3 Organoleptic properties

Dissolved copper imparts a light blue or blue-green colour and an unpleasant, metallic, bitter taste to drinking-water. The taste threshold is between 1 and 5 mg/litre and is influenced by the presence of other solutes (ATSDR, 1990; Olivares & Uauy, 1996a). Blue to green staining of porcelain sinks and plumbing fixtures occurs from copper dissolved in tap-water.

1.4 Major uses

Metallic copper is malleable, ductile, and a good thermal and electrical conductor. It has many commercial uses because of its versatility. Copper is used to make electrical wiring, pipes, valves, fittings, coins, cooking utensils, and building materials. It is present in munitions, alloys, and coatings. Copper compounds are used as or in fungicides, algicides, insecticides, wood preservatives, electroplating, azo dye manufacture, engraving, lithography, petroleum refining, and pyrotechnics. Fertilizers, animal feeds, and pharmaceuticals can contain copper compounds (ATSDR, 1990; Lewis, 1993). Copper compounds are also used as food additives (e.g. nutrient and/or colouring agent) (FAO/IPCS, 1994). Copper sulfate pentahydrate is sometimes added to surface water for the control of algae (NSF, 1996). Copper sulfate was once prescribed as an emetic, but this use has been discontinued owing to adverse health effects (Ellenhorn & Barceloux, 1988).

1.5 Environmental fate

The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen, and the presence of oxidizing agents and complexing compounds or ions (US EPA, 1995). Surface oxidation of copper produces copper(I) oxide or hydroxide. In most instances, copper(I) ion is subsequently oxidized to copper(II) ion. However, copper(I) ammonium and copper(I) chloride complexes, when they form, are stable in aqueous solution.

In pure water, the copper(II) ion is the more common oxidation state (US EPA, 1995). Copper(II) ions will form complexes with hydroxide and carbonate ions. The formation of insoluble malachite [$Cu_2(OH)_2CO_3$] is a major factor in controlling the level of free copper(II) ion in aqueous solutions. Copper(II) ion is the major species in water at pHs up to 6; at pH 6-9.3, aqueous $CuCO_3$ is prevalent; and at pH 9.3-10.7, the aqueous $[Cu(CO_3)_2]^{2-}$ ion predominates (Stumm & Morgan, 1981).

In one copper-polluted, organic-depleted lake, soluble copper speciated in the order $CuOH^+$ > Cu^{2+} > $CuCO_3$ based on equilibrium calculations (Lopez & Lee, 1977). Dissolved copper ions are removed from solution by sorption to clays, minerals, and organic solids or by precipitation (Callahan et al., 1979). Copper has been reported to strongly adsorb to clay materials in a pH-dependent fashion; such adsorption is increased by the presence of particulate organic materials (Payne & Pickering, 1975; Huang et al., 1977; Brown et al., 1983). Copper discharged to wastewater is concentrated in sludge during treatment. Sorption can be reversed in a reducing or acidic environment; thus, sediments and sludge can act as a reservoir for copper ions (Lopez & Lee, 1977). Copper ions form chelates with humic acids and polyvalent organic anions (Cotton & Wilkinson, 1980). The presence of chelating agents increases the solubility of copper in an aqueous medium.

2. ANALYTICAL METHODS

The most important analytical methods for the detection of copper in water are atomic absorption spectrometry (AAS) with flame detection, graphite furnace atomic absorption spectroscopy, inductively coupled plasma atomic emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and stabilized temperature platform graphite furnace atomic absorption (ISO, 1986, 1996; ASTM, 1992, 1994; US EPA, 1994). The ICP-MS technique has the lowest detection limit (0.02 µg/litre) and the AAS technique the highest (10 µg/litre). Detection limits for the other three techniques range from 0.7 to 3 µg/litre. Measurement of dissolved copper requires sample filtration; results from unfiltered samples include dissolved and particulate copper.

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Copper is present in the atmosphere from wind dispersion of particulate geological materials and particulate matter from smokestack emissions. In a nationwide study by the US EPA for the years 1977-1983, the range of copper concentrations in 23 814 air samples was 0.003-7.32 μg/m^3 (US EPA, 1987).[2] Median values for different cities and years ranged from 0.004 to 1.79 μg/m^3, and mean values ranged from 0.0043 to 1.96 μg/m^3. Concentrations of copper determined in over 3800 samples of ambient air at 29 sites in Canada over the period 1984-1993 averaged 0.014 μg/m^3. The maximum value was 0.418 μg/m^3 (WHO, in press). Atmospheric copper is removed by gravitational settling, dry disposition, rain, and snow.

[2] Additional source: Computer printout of frequency distribution listing of copper in air, 1977-1983. Research Triangle Park, NC, US Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

3.2 Water

Because copper is a naturally occurring element, it is found ubiquitously in surface water, groundwater, seawater, and drinking-water (ATSDR, 1990; US EPA, 1991). In a 1969 survey of 678 groundwater supplies in the USA, the maximum reported copper concentration was 0.47 mg/litre, whereas the mean concentration in samples exceeding the detection limit (0.010 mg/litre) was 0.075 mg/litre (US EPA, 1991). Comparative values for 109 surface water supplies were 0.304 and 0.066 mg/litre, respectively. Copper levels in surface water samples were reported to range from 0.0005 to 1 mg/litre, with a median of 0.01 mg/litre (ATSDR, 1990). In the United Kingdom, the mean copper concentration in the River Stour was 0.006 mg/litre (range 0.003-0.019 mg/litre). Background levels were 0.001 mg/litre, derived from an upper catchment control site. Fourfold increases in copper concentrations were apparent downstream of a sewage treatment plant. In an unpolluted zone of the River Periyar in India, copper concentrations ranged from 0.0008 to 0.010 mg/litre (WHO, in press).

Copper concentrations in drinking-water vary widely as a result of variations in pH, hardness, and copper availability in the distribution system. A number of studies indicate that copper levels in drinking-water samples can range from $\leq$0.005 to 18 mg/litre, with the primary source most often being the corrosion of interior copper plumbing (ATSDR, 1990; US EPA, 1991). Levels of copper in running or fully flushed water tend to be low, whereas those of standing or partially flushed water samples are more variable and can be substantially higher (frequently $\geq$1 mg/litre) (ATSDR, 1990). In the Netherlands, copper concentrations between 0.2 and 3.8 mg/litre were reported in water standing for 16 hours. Average copper levels in water from municipalities were between 0.04 and 0.69 mg/litre. In two cities in Sweden, the mean standing water copper level was 0.7 mg/litre, with a 90th percentile of 2.1 mg/litre; in water for consumption, the mean copper concentration was 0.6 mg/litre, with a 90th percentile of 1.6 mg/litre. In distributed water from 70 municipalities across Canada, mean concentrations of copper ranged from 0.02 to 0.075 mg/litre; maximum values ranged up to 0.56 mg/litre. In about 20% of the distributed water supplies, the level of copper was significantly higher than in the corresponding treated water samples. Furthermore, the increase was higher in those areas where the water was soft and corrosive (WHO, in press). In another survey in Canada, the median copper concentration in distributed water was 0.27 mg/litre (range >0.01-0.9 mg/litre) for 27 supplies with acid or neutral pH (Health Canada, 1992). Some of the copper in drinking-water may derive from treatment of surface water sources with copper sulfate algicides (US EPA, 1991).

3.3 Food

Food is a principal source of copper exposure for humans. Liver and other organ meats, seafood, nuts, and seeds are good sources of dietary copper (NAS, 1989). Vitamin/mineral preparations

for children and adults generally contain 2 mg of copper as copper oxide. Infant formula contains 0.6-2 µg of copper per kcal (Olivares & Uauy, 1996b).

Based on data collected during the US Food and Drug Administration's Total Diet Study (1982-1986), the average dietary intake of copper for adult males was 1.2 mg/day, whereas that for adult females was 0.9 mg/day. The average intake for infants (6 months to 1 year) was 0.45 mg/day, and that for 2-year-olds was 0.57 mg/day (Pennington et al., 1989).

In Scandinavian countries, intakes are in the range of 1.0-2.0 mg/day for adults, 2 mg/day for lactovegetarians, and 3.5 mg/day for vegans (Pettersson & Sandstrum, 1995; WHO, in press). The United Kingdom reported intakes of 1.2 and 1.6 mg/day for adult females and males, respectively, and 0.5 mg/day for children 1½ to 4½ years old. Australia reported intakes of 2.2 and 1.9 mg/day for adult females and males, respectively, and 0.8 mg/day for the 2-year-old. In Germany, the dietary intake of adults was 0.95 mg/day (WHO, in press).

Copper is an essential nutrient. In the USA, the estimated safe and adequate dietary intake for copper is 1.5-3 mg/day for adults, 0.4-0.6 mg/day for infants, and 0.7-2 mg/day for children; although average intakes for adults appear to be suboptimum, there is little evidence of copper deficiency in the population (NAS, 1989). Estimates of average copper requirements are 12.5 µg/kg of body weight per day for adults and about 50 µg/kg of body weight per day for infants (WHO, 1996).

3.4 Estimated total exposure and relative contribution of drinking-water

Food and water are the primary sources of copper exposure in developed countries. In general, dietary copper intakes for adults range from 1 to 2 mg/day (WHO, in press); use of a vitamin/mineral supplement will increase exposure by 2 mg/day. Drinking-water contributes 0.1-1 mg/day in most situations. Thus, daily copper intakes for adults usually range from 1 to 3 mg/day. Consumption of standing or partially flushed water from a system that contains copper pipes or fittings can considerably increase total daily copper exposure, especially for infants fed formula reconstituted with tap-water.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Absorption of orally administered copper in mammals occurs in the upper gastrointestinal tract and is controlled by a complex homeostatic process involving active and passive transport, plus binding sites on metallothionein and other proteins in the mucosal cells (Linder & Hazegh-Azam, 1996). Mucosal and serosal transport mechanisms differ. Movement of copper across the mucosa occurs by diffusion or facilitated transport. The presence of competing metals, dietary proteins, anions, fructose, and ascorbic acid influences copper uptake from the gastrointestinal tract (Lonnerdal, 1996). Using an *in vivo* intestinal perfusion system in rats, it was demonstrated that excess concentrations of cations having an electronic configuration similar to that of copper [i.e. iron(II), zinc, tin, and cobalt] in the presence of a high-affinity ligand such as *L*-histidine can significantly inhibit the intestinal absorption and/or retention of copper (Wapnir et al., 1993).

Within the mucosal cells, 80% of the copper is found in the cytosol bound to proteins, especially metallothionein. Transport from the mucosal cells is mediated by a *p*-type ATPase active transport system. Serosal transport of copper is inhibited in Menke's syndrome, one of several genetic diseases related to copper. Copper in the portal blood is bound to albumin or transcuprin; a small amount may be chelated by peptides and amino acids (Linder & Hazegh-Azam, 1996).

Copper uptake from the blood by the liver and distribution within the liver are not well understood. They are presumed to involve a transport process that differs from that in the intestines (Linder & Hazegh-Azam, 1996). Within the liver, copper becomes incorporated in ceruloplasmin, as well as the enzymes superoxide dismutase and cytochrome oxidase. Excess copper is bound to hepatic metallothionein. Ceruloplasmin is the primary copper transport protein in systemic circulation and

contains about 75% of the plasma copper (Luza & Speisky, 1996). Ceruloplasmin carries copper to the cells for uptake. It also has enzymatic activity as a ferrooxidase and functions in the synthesis of haemoglobin.

In humans, the highest concentrations of copper are found in the liver, brain, heart, kidney, and adrenal gland; moderate levels are found in the intestine, lung, and spleen; and low concentrations occur in endocrine glands, bone, muscle, larynx, trachea, aorta, and testes (Schroeder et al., 1966; Evans, 1973). Approximately 50% of the body's copper is found in muscle and bone tissue, whereas about 10% is stored in the liver (Evans, 1973; Luza & Speisky, 1996). The liver of newborn infants contains about 10 times the copper of the adult liver and accounts for 50-60% of the total body copper (Luza & Speisky, 1996).

Copper is an essential nutrient and is required for the proper functioning of many important enzyme systems. Copper-containing enzymes include ceruloplasmin, superoxide dismutase, cytochrome oxidase, tyrosinase, monoamine oxidase, lysyl oxidase, and phenylalanine hydroxylase (Linder & Hazegh-Azam, 1996). The activity of the enzyme superoxide dismutase can be used in the assessment of copper status (Olivares & Uauy, 1996b). Ceruloplasmin and serum copper concentrations are also employed as indicators of copper status.

Copper is excreted from the body in bile, faeces, sweat, hair, menses, and urine (Gollan & Deller, 1973; Luza & Speisky, 1996). In humans, the major excretory pathway for absorbed copper is bile, where copper is bound to both low-molecular-weight and macromolecular species. Biliary copper travels to the intestine; after minimal reabsorption, it is eliminated in the faeces. In normal humans, <3% of the daily copper intake is excreted in the urine (Luza & Speisky, 1996). Urinary copper may originate from amino acid-bound metal or from dissociated copper-albumin complexes; erythrocyte or ceruloplasmin-bound copper generally will not permeate the glomerulus, thus preventing its excretion (Evans, 1973).

There are several genetic disorders that affect copper utilization. The genetic abnormalities associated with Menke syndrome, Wilson's disease, and aceruloplasminaemia are fairly well understood. There is some evidence to suggest a genetic basis for Indian childhood cirrhosis and idiopathic copper toxicosis (Muller et al., 1996; Olivares & Uauy, 1996a). In the case of Menke syndrome and Wilson's disease, the affected genes have been identified. Data suggest that the predisposing genetic component for idiopathic copper toxicosis may be an autosomal recessive gene (Muller et al., 1996). Individuals with a glucose-6-phosphate dehydrogenase deficiency disorder have an increased susceptibility to copper-induced oxidation reactions and methaemoglobin formation (Moore & Calabrese, 1980), but there is no direct effect of the disorder on copper uptake, distribution, or metabolism.

In Menke syndrome, there is minimal copper absorption from the intestines, leading to death during early childhood (Harris & Gitlin, 1996; Olivares & Uauy, 1996a). Children suffering from Menke syndrome (an X chromosome-linked disorder) exhibit mental deterioration, failure to thrive, hypothermia, and connective tissue abnormalities (Harris & Gitlin, 1996). The defective protein is a *p*-type ATPase responsible for intestinal transport of copper; a child with Menke syndrome suffers from a profound copper deficiency, despite adequate dietary copper. There is no effective treatment for Menke syndrome, although administration of copper as the dihistidine complex delays the development of symptoms (Linder & Hazegh-Azam, 1996).

Wilson's disease is an autosomal recessive disorder that affects the hepatic intracellular transport of copper and its subsequent inclusion into ceruloplasmin and bile. A *p*-type ATPase is again affected, but the enzyme is different from that affected in Menke syndrome. Because copper is not incorporated into ceruloplasmin, its normal systemic distribution is impaired, and copper accumulates in the liver, brain, and eyes (Pennington et al., 1989; Harris & Gitlin, 1996). Wilson disease generally appears in late childhood and is accompanied by hepatic cirrhosis, neurologic degeneration, and copper deposits in the cornea of the eye (Kayser-Fleischer rings). Patients with Wilson disease are treated with chelating agents, such as penicillamine, to promote copper

excretion (Yarze et al., 1992). Patients that follow their therapeutic regime can expect to live a normal life (Scheinberg & Sternleib, 1996). Restriction of dietary copper alone cannot influence the progression of the disease.

Aceruloplasminaemia is an autosomal recessive disorder caused by changes in the ceruloplasmin gene that affect the ability of ceruloplasmin to bind copper (Harris & Gitlin, 1996). The symptoms of aceruloplasminaemia do not become apparent until adulthood. They include dementia, diabetes, retinal degeneration, and increased tissue iron stores.

The etiologies of Indian childhood cirrhosis and idiopathic copper toxicosis are complex and may involve a combination of genetic, developmental, and environmental factors (Muller et al., 1996; Pandit & Bhave, 1996). Both disorders are characterized by liver enlargement, elevated copper deposits in liver cells, pericellular fibrosis, and necrosis. Both disorders are generally fatal. Exposure to elevated levels of copper in milk or infant formula prepared in copper-containing vessels or with water containing elevated copper concentrations is believed to contribute to the hepatic copper overload. Poor biliary excretion of copper may also play a role in the etiology of the disease.

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Acute responses to copper vary with species and copper compound. Sheep and dogs are more sensitive to copper than rodents, pigs, and poultry (Linder & Hazegh-Azam, 1996). Soluble copper salts are more toxic than insoluble compounds.

In a classic study, Wang & Borison (1951) evaluated the acute emetic response of 107 mongrel dogs to a single dose of copper sulfate pentahydrate in aqueous solution. In this group, 20 (19%) responded to 20 mg of copper sulfate [5 mg of copper(II)] with a mean response latency of 19 ± 11 minutes. Ninety-one dogs (85%) responded to 40 mg of copper sulfate [10 mg of copper(II)] with a response latency of 16 ± 9 minutes, and all animals responded to an 80-mg dose of copper sulfate [20 mg of copper(II)] (latency 19 ± 7 minutes). Some of the animals were then subjected to a vagotomy, sympathectomy, or vagotomy and sympathectomy. After severing of the neural pathways, the dogs were again exposed to copper sulfate. The acute dose required to elicit the emetic response increased in the vagotomized and in the vagotomized/sympathectomized dogs, as did the response latency time. The greatest effects on response threshold and latency time were seen in the vagotomized/sympathectomized dogs. The authors postulated that the emetic action of copper sulfate in dogs is biphasic. The initial rapid response event is caused by the action of copper sulfate on the peripheral nervous system and is the most sensitive response. The secondary response is elicited by the central nervous system and is responsive to absorbed copper. The requirement of the secondary response for absorption increases its latency time. More recent studies in dogs and ferrets (Bhandari & Andrews, 1991; Makale & King, 1992; Fukui et al., 1994) confirm the importance of gastrointestinal neural pathways and receptors in copper sulfate-induced emesis.

In one study, copper sulfate solution was infused into the stomach and duodenum of groups of four or five ferrets with ligated pyloric sphincters (Makale & King, 1992). In one group (four ferrets), the stomach infusion preceded the duodenal infusion; in the other group (five ferrets), the duodenal infusion preceded the stomach infusion. Infusion to the stomach resulted in vomiting in seven of nine ferrets with a mean latency of 4.4 minutes. Infusion to the duodenum resulted in vomiting in one of nine animals. The authors concluded that the primary site of the emetic response to copper sulfate is in the stomach of the ferret.

5.2 Short-term exposure

Several short-term studies of copper toxicity have been conducted in rats and mice. Effects were

largely the same in both species, but rats were slightly more sensitive than mice (Hébert et al., 1993).

Groups of five male and five female F 344/N rats were administered copper sulfate in their drinking-water for 2 weeks at estimated doses up to 97 mg of copper per kg of body weight per day (Hébert et al., 1993). A LOAEL of 10 mg of copper per kg of body weight per day observed in male rats was based on an increase in the size and number of protein droplets in the epithelial cells of the proximal convoluted tubules of the males. No renal effects were seen in the females receiving the same dose. There was no NOAEL for males in this study; the NOAEL for females was 26 mg of copper per kg of body weight per day.

When copper (as copper sulfate) was administered by gavage to 10 male albino rats at a dose of 25 mg/kg of body weight for 20 days, the kidneys displayed necrosis and tubular engorgement. Centrilobular necrosis, perilobular sclerosis, and periportal copper disposition were seen in the liver; haemoglobin and haematocrit values were decreased (Rana & Kumar, 1980).

Copper was less toxic to rats when administered in the diet than when administered in drinking-water or by gavage. This was true for 2-week and 13-week exposures. A dietary concentration of 1000 mg/kg of feed (estimated doses of 23 mg/kg of body weight per day for 2 weeks and 16 mg/kg of body weight per day for 13 weeks) had no adverse effects in male or female F 344/N rats (Hébert et al., 1993). Dietary concentrations of 2000 mg/kg of feed (44 mg/kg of body weight per day for 2 weeks and 33 mg/kg of body weight per day for 13 weeks) were associated with hyperplasia and hyperkeratosis of the squamous epithelium of the limiting ridge of the rat forestomach.

With the 13-week exposure and the 2000 mg/kg of feed (33 mg/kg of body weight per day) dietary concentration, protein droplets were present in the kidneys, and liver inflammation was noted (Hébert et al., 1993). Changes in liver and kidney histopathology were dose-related; males were affected more than females. Staining of the kidney cells for $\alpha_{2\mu}$ globulin was negative. Dose-related decreases in haematological parameters at 4000 mg/kg of feed (66 mg/kg of body weight per day) and 8000 mg/kg of feed (134 mg/kg of body weight per day) were indicative of a microcytic anaemia, whereas increases in serum enzymes were indicative of liver damage. There was some evidence of significant biochemical effects with the 2000 mg/kg of feed (33 mg/kg of body weight per day) exposure concentration and definitive effects at the 4000 mg/kg of feed (66 mg/kg of body weight per day) and 8000 mg/kg of feed (134 mg/kg of body weight per day) concentrations. Iron stores in the spleen were depleted, especially for the highest exposure concentration.

5.3 Long-term exposure

Male weanling Wistar rats (four per group) were given either a normal diet containing 10-20 mg of copper per kg of feed (controls) or diets supplemented with 3000, 4000, or 5000 mg of copper per kg of feed for 15 weeks (Haywood & Loughran, 1985). The animals receiving 3000 mg of copper per kg of feed were then allowed to continue the experimental regime for the remainder of the year. Assuming that rats consume 5% of their body weight per day in food, these dietary copper concentrations would correspond to approximate doses of 0.5-1.0, 150, 200, and 250 mg of copper per kg of body weight per day. All copper-supplemented groups exhibited reductions in body-weight gains relative to the control group that persisted until the end of the 15-week exposure period. For the 3000, 4000, and 5000 mg/kg of feed groups, copper concentrations in the liver peaked at 3-4 weeks, declined significantly by 6 weeks, but were still elevated at 15 weeks. Although the timing and duration varied somewhat, all supplemented groups evidenced hepatocellular necrosis during weeks 1-6, followed by a regeneration process that began after 3-5 weeks. The adaptation process noted during the latter part of the first 15 weeks of exposure continued during the 3000 mg/kg of feed group extension period. The average body weight recovered to 80% of that of the control group, and the copper concentration in the liver dropped from 1303 µg/g at 15 weeks to 440 µg/g at 52 weeks. However, even at 52 weeks, hepatic copper

was greater in the exposed animals than in the controls (23 µg/g).

5.4 Reproductive and developmental toxicity

Sperm morphology and motility analyses, testis and epididymis weight determination, and estrous cycle characterization were performed in rats and mice as part of a subchronic dietary study (Hébert et al., 1993). No significant differences from control values were found for any of the following reproductive parameters: testis, epididymis, and cauda epididymis weights, spermatid count, spermatid number per testis or per gram testis, spermatozoal motility and concentration, estrous cycle length, or relative length of time spent in the various estrous stages. A reproductive NOAEL of 4000 mg of copper sulfate pentahydrate per kg of diet (66 and 68 mg of copper per kg of body weight per day for male and female rats, respectively) was established for these parameters in this study.

There is some evidence that copper is a developmental toxicant. Embryotoxicity and teratogenicity were reported in hamsters when dams were injected intraperitoneally with copper citrate (DiCarlo, 1980) or intravenously with copper sulfate or copper citrate (Ferm & Hanlon, 1974). Such effects were noted at single injection doses as low as 2.13 mg of copper per kg of body weight for copper sulfate and 0.25-1.5 mg of copper per kg of body weight for copper citrate. When mice (7-22 females per group) were fed diets supplemented with 0, 500, 1000, 1500, 2000, 3000, or 4000 mg of copper sulfate per kg of diet for 1 month, fetal mortality and decreased litter size were observed in the 2000-4000 mg/kg of diet groups. Various skeletal and soft-tissue malformations were seen in 2-8% of the surviving fetuses from the two highest dose groups (Lecyk, 1980). The low concentrations of supplemental copper (500 and 1000 mg/kg of diet) had a beneficial effect on development.

5.5 Mutagenicity and related end-points

Copper sulfate has been reported to induce reverse mutations in *Escherichia coli* (Demerec et al., 1951) but not in TA98, TA100, TA1535, or TA1537 strains of *Salmonella typhimurium* in the presence or absence of microsomal activation (Moriya et al., 1983; Wong, 1988). Negative microbial mutation results have been reported for copper sulfate and copper chloride in *Saccharomyces cerevisiae* (Singh, 1983) and *Bacillus subtilis* (Nishioka, 1975; Kanematsu et al., 1980; Matsui, 1980).

Copper chloride or copper acetate concentrations of 20-150 mmol/litre induced errors in viral DNA synthesis from poly(c) templates (Sirover & Loeb, 1976), and copper sulfate concentrations of 1 mmol/litre, but not 0.03-0.3 mmol/litre, caused DNA strand breakage in rat hepatocytes (Sina et al., 1983). Results from *in vitro* tests are not transferable to the *in vivo* situation, where copper ions are generally bound to protein or amino acid ligands.

Male Wistar rats were implanted with subcutaneous osmotic pumps that continuously administered saline, copper(II) chloride, the copper chelate cupric nitrilotriacetate (Cu-NTA), or NTA for 3 or 5 days. Copper was delivered at a rate of 4 mg/kg of body weight per day, which maintained serum copper levels 30-70% higher than in the untreated controls for copper(II) chloride or 100-120% higher than in controls for Cu-NTA. Hepatic and renal DNA levels of 8-hydroxy guanosine, a deoxyguanosine oxidation product associated with mutagenesis and carcinogenesis, were significantly elevated in the copper-exposed animals (Toyokuni & Sagripanti, 1994). Chromosomal aberrations and micronuclei were observed in the bone marrow of inbred Swiss mice exposed to copper (from copper sulfate) concentrations of 0, 1.3, 2.6, or 5.1 mg/kg of body weight by intraperitoneal or subcutaneous injection (Bhunya & Pati, 1987).

5.6 Carcinogenicity

Copper and its salts do not appear to be animal carcinogens based on limited long-term exposure data. In one study, two strains of mice (18 per sex per group per strain) were exposed to copper

hydroxyquinoline (181 mg of copper per kg of body weight per day) by gelatin capsule until they were 28 days old, whereupon the compound was administered for an additional 50 weeks in the feed at 2800 mg/kg of feed (506 mg of copper per kg of body weight per day). No significant increases in tumour incidence were observed in either sex or either strain (BRL, 1968).

6. EFFECTS ON HUMANS

6.1 Acute exposure

The acute lethal dose for adults lies between 4 and 400 mg of copper(II) ion per kg of body weight, based on data from accidental ingestion and suicide cases (Chuttani et al., 1965; Jantsch et al., 1984; Agarwal et al., 1993). Individuals ingesting large doses of copper present with gastrointestinal bleeding, haematuria, intravascular haemolysis, methaemoglobinaemia, hepatocellular toxicity, acute renal failure, and oliguria (Agarwal et al., 1993).

At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting, diarrhoea). Records of published studies of gastrointestinal illness induced by copper from contaminated beverages plus public health department reports for 68 incidents indicate an acute onset of symptoms (Low et al., 1996). Symptoms generally appear after 15-60 minutes of exposure; nausea and vomiting are more common than diarrhoea. Copper concentrations were not available for all incidents. Among 24 outbreaks with quantitative data, the lowest copper concentrations were 3.5 and 3.8 mg/litre; background information on the samples analysed and analytical methods was limited. In 6 of the 24 cases (25%), the copper concentration was less than 10 mg/litre (Low et al., 1996). Data on the amount of beverage consumed were not available for any of these incidents.

An analysis of data from the US Centers for Disease Control regarding 155 reported cases of copper intoxication from drinking-water sources during the years 1977-1982 and 1991-1994 indicated that reported levels of copper in drinking-water were in the range of 4.0-156 mg/litre (US EPA, 1987; CDC, 1993, 1996).

6.2 Short-term exposure

Recurrent morning episodes of nausea, vomiting, and abdominal pain were reported by three of four family members exposed for over a year to drinking-water containing elevated levels of copper. Symptoms were associated with early-morning consumption of water, juice, or coffee. Copper concentrations measured on three occasions during the period covered by the complaint ranged from 2.8 to 7.8 mg/litre; only the 7.8 mg/litre sample was collected in the early morning (Spitalny et al., 1984). All samples were collected before the complaint was registered with the Department of Health. The median copper concentration for a series of samples collected after the complaint was 3.1 mg/litre, compared with a median value of 1.6 mg/litre for a series of samples collected from another house on the same service line but located closer to the beginning of the line.

Recurrent episodes of vomiting, diarrhoea, and abdominal cramps were reported among individuals consuming water from homes with water that exceeded the US EPA maximum contaminant level goal of 1.3 mg/litre in four case-studies (Knobeloch et al., 1994). Data on water use practices and symptoms were collected using a questionnaire. The incidence of symptoms was positively correlated with the copper concentration in the water samples, ingestion of first-draw water, and water intake. There was a negative correlation with consumer age and use of bottled water. The presence of various confounding factors suggests discretion when drawing conclusions based on the data presented.

A 26-year-old male presented with symptoms of cirrhosis, liver failure, and Wilson disease (Kayser-Fleischer rings) after more than 2 years of self-prescribed use of copper supplements (O'Donohue et al., 1993). The patient ingested 30 mg of supplemental copper per day for 2 years

and 60 mg/day for a poorly defined period of up to a year. Liver damage was extensive, and a transplant was required. The diseased liver had an average copper concentration of 3230 µg/g dry weight (normal 20-50 µg/g); tissue histopathology was similar to that seen in Indian childhood cirrhosis and Wilson disease. The patient's family medical history and evaluation of his parents and sisters for copper excretion suggested that he did not carry the Wilson disease gene. Liver damage apparently resulted from the prolonged daily exposure to over 10 times the US estimated safe and adequate daily intake for copper.

6.3 Long-term exposure

Long-term intake of copper in the diet in the range of 1.5-3 mg/day has no apparent adverse effects. Daily intake of copper below this range can lead to anaemia, neutropenia, and bone demineralization in malnourished children (NAS, 1989). Adults are more resistant than children to the symptoms of a copper deficiency. No studies of adverse effects of long-term exposure of humans to copper at concentrations greater than those that occur in the diet were identified.

7. PROVISIONAL GUIDELINE VALUE

The IPCS Environmental Health Criteria monograph for copper (WHO) concluded that:

> The upper limit of the AROI [acceptable range of oral intake] in adults is uncertain but it is most likely in the range of several but not many mg per day in adults (several meaning more than 2 or 3 mg/day). This evaluation is based solely on studies of gastrointestinal effects of copper-contaminated drinking-water. A more specific value for the upper AROI could not be confirmed for any segment of the general population....The available data on toxicity in animals were considered unhelpful in establishing the upper limit of the AROI, due to uncertainty about an appropriate model for humans.

A copper level of 2 mg/litre in drinking-water will be protective of adverse effects of copper and provides an adequate margin of safety. Owing to limitations of the epidemiological and clinical studies conducted to date, it is not possible to establish a clear effect level with any precision. Thus, it is recommended that this guideline value for copper of 2 mg/litre remain provisional as a result of uncertainties in the dose-response relationship between copper in drinking-water and acute gastrointestinal effects in humans. It is also noteworthy that copper is an essential element.

It is stressed that the outcome of ongoing epidemiological studies in Chile, Sweden, and the USA may, upon publication, shed some light on more accurate quantification of effect levels for copper-induced toxicity in humans, including sensitive subpopulations.

Staining of laundry and sanitary ware occurs at copper concentrations above 1 mg/litre. At levels above 5 mg/litre, copper also imparts a colour and an undesirable bitter taste to water.

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Nickel

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In the 1993 WHO *Guidelines for drinking-water quality*, a health-based guideline value for nickel of 0.02 mg/litre was derived from a 2-year study in rats published in 1976 (NOAEL of 5 mg/kg of body weight per day; depressed body-weight gain and altered organ-to-body-weight ratios were

the critical end-points of toxicity). The Coordinating Committee for the updating of the *Guidelines* recommended that the guideline value for nickel be re-evaluated in light of more recent experimental data.

1. GENERAL DESCRIPTION

1.1 Identity

Nickel is a lustrous white, hard, ferromagnetic metal. It occurs naturally in five isotopic forms: 58 (67.8%), 60 (26.2%), 61 (1.2%), 62 (3.7%), and 64 (1.2%).

1.2 Physicochemical properties

Nickel usually has two valence electrons, but oxidation states of +1, +3, or +4 may also exist. Nickel is not affected by water but is slowly attacked by dilute hydrochloric or sulfuric acid and is readily attacked by nitric acid. Fused alkali hydroxides do not attack nickel. Several nickel salts, such as the acetate, chloride, nitrate, and sulfate, are soluble in water, whereas hydroxides, oxides, carbonates, sulfides, disulfides, and subsulfides are practically insoluble in water. Alloys of nickel containing more than 13% chromium are to a high degree protected from corrosion in many media by the presence of a surface film consisting mainly of chromium oxide (Morgan & Flint, 1989; Haudrechy et al., 1994).

Property	*Value*
Specific density	8.90 g/cm^3 at 25°C
Melting point	1555°C
Boiling point	2837°C

1.3 Major uses

Nickel is used principally in its metallic form combined with other metals and non-metals as alloys. Nickel alloys are characterized by their hardness, strength, and resistance to corrosion and heat.

Nickel is used mainly in the production of stainless steels, non-ferrous alloys, and super alloys. Other uses of nickel and nickel salts are in electroplating, as catalysts, in nickel-cadmium batteries, in coins, in welding products, and in certain pigments and electronic products (IARC, 1990). It is estimated that 8% of nickel is used for household appliances (IPCS, 1991).

1.4 Environmental fate

Nickel occurs predominantly as the ion $Ni(H_2O)_6^{2+}$ in natural waters at pH 5-9 (IPCS, 1991). Complexes with ligands, such as OH^-, SO_4^{2-}, HCO_3^-, Cl^-, or NH_3, are formed to a minor degree in this pH range.

2. ANALYTICAL METHODS

The two most commonly used analytical methods for nickel in water are atomic absorption spectrometry and voltammetry. The reported detection limits are 10 ng/litre and 1 ng/litre, respectively (IPCS, 1991; ATSDR, 1996). Flame atomic absorption spectrometry is suitable in the range of 0.5-100 µg/litre (ISO, 1986), whereas inductively coupled plasma atomic emission spectroscopy can be used for the determination of nickel with a limit of detection of about 10 µg/litre (ISO, 1996).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Nickel concentrations in remote areas are in the range of 1-3 ng/m^3, whereas concentrations in rural and urban air range from 5 to 35 ng/m^3. It has been estimated that non-occupational exposure via inhalation is 0.2-1.0 µg/day in urban areas and 0.1-0.4 µg/day in rural areas (Bennett, 1984). The mainstream smoke of one cigarette contains about 0.04-0.58 µg of nickel (IARC, 1990).

3.2 Water

Nickel concentrations in groundwater depend on the soil use, pH, and depth of sampling. The average concentration in groundwater in the Netherlands ranges from 7.9 µg/litre (urban areas) to 16.6 µg/litre (rural areas). Acid rain increases the mobility of nickel in the soil and thus might increase nickel concentrations in groundwater (IPCS, 1991). In groundwater with a pH <6.2, nickel concentrations up to 980 µg/litre have been measured (RIVM, 1994).

In Canada, the median nickel level in drinking-water supplies was below the detection limit of 2 µg/litre; the maximum level observed was 69 µg/litre (Méranger et al., 1981). In US drinking-water, 90% of all samples (*n* = 2503) contained ≤10 µg/litre, and 97% had nickel concentrations of ≤20 µg/litre (ATSDR, 1996).

In Europe, reported nickel concentrations in drinking-water were generally below 10 µg/litre (IPCS, 1991). Nickel levels below 1 µg/litre have been reported from Denmark and Finland (Punsar et al., 1975; Gammelgaard & Andersen, 1985). Average dissolved nickel concentrations in surface water in the rivers Rhine and Meuse are below 7 µg/litre (RIWA, 1994).

Increased nickel concentrations in groundwater and municipal tap-water (100-2500 µg/litre) in polluted areas have been reported (McNeely et al., 1971; Hopfer et al., 1989). Water left standing overnight in nickel-containing plumbing fittings contained a nickel concentration of 490 µg/litre (Andersen et al., 1983).

Certain stainless steel well materials were identified as the source of increased nickel concentrations in groundwater wells in Arizona, USA. Mean nickel levels were 8-395 µg/litre; in some cases, nickel levels were in the range 1-5 mg/litre (Oakley & Korte, 1996).

Leaching of nickel from chromium-nickel stainless steel pipework into drinking-water diminished after a few weeks; as chromium was rarely found at any time in the water, this indicates that the leakage of nickel is not of corrosive origin, but rather attributable to passive leaching of nickel ions from the surface of the pipes (Schwenk, 1992).

Concentrations of nickel in water boiled in electric kettles may, depending on the material of the heating element, be markedly increased, especially in the case of new or newly decalcified kettles. Nickel concentrations in the range 100-400 µg/litre, with extreme values close to 1000 µg/litre, have been reported (Rasmussén, 1983; Pedersén & Petersén, 1995).

Levels of nickel in bottled mineral water were below the detection limit of 25 µg/litre (Allen et al., 1989).

3.3 Food

Nickel levels in food are generally in the range 0.01-0.1 mg/kg, but there are large variations (Booth, 1990; Jorhem & Sundström, 1993; Dabeka & McKenzie, 1995; Levnedsmiddelstyrelsen, 1995). Higher median levels of nickel (0.1-0.4 mg/kg) were found in wholemeal products (Smart & Sherlock, 1987; Levnedsmiddelstyrelsen, 1995), whereas markedly higher levels (1-6 mg/kg)

were found in beans, seeds, nuts, and wheat bran (Smart & Sherlock, 1987; Jorhem & Sundström, 1993). Even higher nickel levels (8-12 mg/kg) were found in cacao (Smart & Sherlock, 1987).

Stainless steel cooking utensils (e.g. oven pans, roasting pans) contributed markedly to the levels of nickel in cooked food, sometimes exceeding 1 mg/kg in meat (Dabeka & McKenzie, 1995). In contrast, Flint & Packirisamy (1995) found only minor increases in nickel concentrations in acid foodstuffs when new stainless steel pans were used.

Daily dietary intakes of nickel were 140-150 µg in the United Kingdom in 1981-1984 (Smart & Sherlock, 1987), 82 µg in Sweden in 1987 (Becker & Kumpulainen, 1991), 150 µg in Denmark (Flyvholm et al., 1984), and 160 µg in the USA (Myron et al., 1978). The dietary intake of nickel in a well controlled Canadian study ranged from 190 µg/day for 1- to 4-year-old children to 406 µg/day for 20- to 39-year-old males. The nickel intake for 20- to 39-year-old women was on average 275 µg/day (Dabeka & McKenzie, 1995). Dietary nickel intake by 0- to 12-month-old infants was on average 0.005 mg/kg of body weight per day (equal to 0.038 mg/day). Infants fed evaporated milk were exposed to 0.004 mg/kg of body weight per day, whereas infants fed soy-based formula were exposed to 0.010 mg/kg of body weight per day (Dabeka, 1989).

As nuts and beans are important sources of protein for vegetarians, this population group can be expected to have a markedly higher intake of nickel than reported in the studies cited above. The nickel intake of eight volunteers when they ingested normal diets averaged 0.13 mg/day (range 0.06-0.26 mg/day), compared with 0.07 mg/day when diets containing low nickel levels were consumed (range 0.02-0.14 mg/day). When food rich in nickel was ingested, the daily intake was 0.25 mg/day (range 0.07-0.48 mg/day) (Veien & Andersen, 1986).

3.4 Estimated total exposure and relative contribution of drinking-water

Food is the dominant source of nickel exposure in the non-smoking, non-occupationally exposed population. According to the 1981 United Kingdom Total Diet Study, the contribution from food is 0.22-0.23 mg/day per person. Water generally contributes 0.005-0.025 mg daily (i.e. 2-11% of the total daily oral intake of nickel) (MAFF, 1985).

The value of information on dietary intake of nickel based on market basket studies or on raw foodstuffs is limited because of the high impact that the cooking procedure may have on the nickel content in food. In studies including analyses of prepared food, water contributed, on average, only a minor part (<10%) of the daily dietary intake of nickel (Dabeka & McKenzie, 1995). However, in cases of heavy pollution, use of certain types of kettles, use of non-resistant material in wells, or use of water standing for an extended time in water pipes, the nickel contribution from water may be significant.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

4.1 Laboratory animals

Nickel is poorly absorbed from diets and is eliminated mainly in the faeces. Absorbed nickel is rapidly cleared from serum and excreted in urine (IPCS, 1991).

The mechanism for intestinal absorption of nickel is not clear. Iron deficiency increased intestinal nickel absorption *in vitro* and *in vivo*, indicating that nickel is partially absorbed by the active transfer system for iron absorption in the intestinal mucosal cells (Tallkvist et al., 1994). In perfused rat jejunum, saturation of nickel uptake was observed at high concentrations of nickel chloride (Foulkes & McMullen, 1986). Iron concentrations in rat tissues were increased by dietary nickel exposure (Whanger, 1973). Nickel is bound to a histidine complex, albumin, and alpha-2-macroglobulin in serum (Sarkar, 1984).

Absorption of soluble nickel compounds from drinking-water is higher than that from food. After 24 hours, 10-34% of a single oral dose of water-soluble nickel compounds (i.e. $NiSO_4$, $NiCl_2$, $Ni(NO_3)_2$) was absorbed, whereas less than 2% of a single oral dose of insoluble or scarcely soluble nickel compounds (i.e. NiO, Ni, Ni_3S_2, NiS) was absorbed. It is not known if the animals were fasted before treatment. The highest nickel concentrations were found in the kidneys and lungs, whereas nickel concentrations in the liver were low (Ishimatsu et al., 1995).

Whole-body retention in mice after oral exposure to Ni^{2+} was less than 1% of the administered dose 5 days after exposure (Nielsen et al., 1993). Severa et al. (1995) observed an accumulation of nickel in organs in rats orally exposed to nickel in drinking-water at concentrations of 100 mg/litre for 6 months. The nickel concentration in liver was 10 times higher in exposed rats than in unexposed rats; in the kidney, the nickel level was only twice as high in exposed rats as in unexposed rats. Nickel levels in the kidney and blood were similar. There was no increase in nickel levels in organs between 3 and 6 months of exposure. Biliary excretion of nickel subcutaneously administered to rats as nickel chloride was less than 0.5% of the given dose (Marzouk & Sunderman, 1985).

Several reports indicate that transplacental transfer of nickel occurs in animals (IPCS, 1991). Elevated concentrations of nickel were detected in fetuses after intramuscular administration of nickel chloride to rats. The fetal organ with the highest nickel concentration was the urinary bladder (Sunderman et al., 1978).

A dose-dependent increase in nickel concentrations in rat milk was observed after a single subcutaneous injection of nickel chloride. The milk/plasma ratio was 0.02 (Dostal et al., 1989).

4.2 Humans

Following a 12-hour fast, a volunteer ingested 20 µg of ^{61}Ni-enriched nickel per kg of body weight as $Ni(NO_3)_2$ in 1 litre of water. The serum nickel concentration peaked at 2 hours at 34 µg/litre. By 96 hours, 27% of the ingested dose was excreted in the urine (Templeton et al., 1994a). These findings are consistent with the observations made by Sunderman and co-workers, who reported an absorption of 27 ± 17% of the given nickel dose (as nickel sulfate) added to drinking-water in 10 volunteers after a 12-hour fast. Intestinal absorption was only 1% of the given dose when nickel as sulfate salt was added to scrambled eggs. The half-time for absorbed nickel averaged 28 ± 9 hours (Sunderman et al., 1989). Plasma levels in fasting human subjects did not increase above fasting levels when 5 mg of nickel were added to an American breakfast or a Guatemalan meal rich in phytic acids (Solomons et al., 1982). The same amount of nickel added to water elevated the plasma nickel levels 4- to 7-fold. The absorption of nickel added to milk, tea, coffee, or orange juice was significantly less than the absorption of nickel from water.

A fatal case of nickel intoxication indicates that biliary excretion of nickel is of minor importance in humans (Grandjean et al., 1989).

According to the above studies, the daily amount of absorbed nickel in humans will be, on average, about 5 µg each from water and food.

Nickel has been detected in fetal tissues at levels similar to the levels found in adults (McNeely et al., 1972; Casey & Robinson, 1978).

Serum levels in the range 1.5-19 µg/litre were found in patients undergoing regular haemodialysis (Hopfer et al., 1989; Nixon et al., 1989). Significantly higher serum nickel levels were observed in non-occupationally exposed subjects from a heavily nickel-polluted area compared with levels in subjects living in a control area (nickel concentrations in tap-water 109 ± 46 vs 0.6 ± 0.2 µg/litre; serum nickel levels 0.6 ± 0.3 vs 0.2 ± 0.2 µg/litre) (Hopfer et al., 1989). Tentative reference values for nickel in serum and urine have been proposed: 0.2 µg/litre or lower in serum, and 1-3 µg/litre in urine of healthy adults (Templeton et al., 1994b).

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO SYSTEMS

5.1 Acute exposure

Effects on kidney function, including tubular and glomerular lesions, have been reported by several authors after parenteral administration of high nickel doses of between 1 and 6 mg/kg of body weight intraperitoneally in rabbits and rats (IPCS, 1991). Intramuscular injection of the insoluble compound nickel subsulfide caused acute kidney damage in mice (Rodriguez et al., 1996). Increased hepatic lipid peroxidation and increased serum levels of aspartate and alanine aminotransferase activity were observed when rats were parenterally exposed to nickel chloride at nickel doses of 7-44 mg/kg of body weight (Donskoy et al., 1986). Levels of hepatic monooxygenases decreased when mice were administered 10 mg of nickel per kg of body weight subcutaneously (Iscan et al., 1995).

5.2 Short-term exposure

Body-weight gain, haemoglobin, and plasma alkaline phosphatase were significantly reduced in weanling rats exposed to nickel (as nickel acetate) at concentrations of 500 or 1000 mg/kg in the diet (equivalent to 25 or 50 mg/kg of body weight per day) for 6 weeks compared with controls (Whanger, 1973). No effects were observed in rats exposed to 100 mg/kg in the diet (equivalent to 5 mg/kg of body weight per day).

5.3 Long-term exposure

Rats (25 per sex per dose) were exposed to nickel (as nickel sulfate) in the diet at doses of 0, 100, 1000, or 2500 mg/kg (equivalent to 0, 5, 50, or 125 mg/kg of body weight per day) for 2 years (Ambrose et al., 1976). Growth was depressed in rats at 1000 and 2500 mg/kg of diet, but there were indications that decreased food consumption might explain the decreased body-weight gains, particularly at 2500 mg/kg of diet. However, no statistical analysis seems to have been performed. Survival was overall very poor, especially in the control groups and the 2500 mg/kg of diet groups. In females at 1000 and 2500 mg/kg of diet, the mean relative liver weights were decreased by about 20%, and the mean relative heart weights were increased by about 30% compared with the control group. No histological or gross pathological findings related to nickel exposure were observed. The highest nickel concentrations were found in the kidneys. The NOAEL in this study was 5 mg/kg of body weight per day. However, the study does not meet current standards for long-term studies, mainly because of the low survival rate. The observed changes in organ weights in female rats might in part be due to changes in food and water consumption. Also, both gross and histopathological examinations of the animals were negative, although there were 20-30% changes in relative organ weights. It can thus not be excluded that the observed changes in relative organ weights were related to changes in food and/or water consumption rather than to a toxic effect of nickel.

Increased relative kidney weight was observed in rats exposed to nickel (as nickel sulfate) in drinking-water at daily doses of about 7 mg/kg of body weight for up to 6 months (Vyskocil et al., 1994). There was an increased excretion of albumin in urine in females, but there were no changes in total protein, beta-2-microglobulin, *N*-acetyl-beta-*D*-glucosaminidase, or lactate dehydrogenase in urine due to nickel exposure.

In a 2-year study, dogs (three per sex per dose) were exposed to 0, 100, 1000, or 2500 mg of nickel per kg of diet (equivalent to 0, 2.5, 25, and 62.5 mg/kg of body weight per day). In the 2500 mg/kg of diet group, decreased weight gain and food consumption, higher kidney- and liver-to-body-weight ratios, and histological changes in the lung were observed. The NOAEL in this study was 25 mg/kg of body weight per day (Ambrose et al., 1976).

5.4 Reproductive and developmental toxicity

Intraperitoneal administration of nickel nitrate (12 mg of nickel per kg of body weight) to male mice resulted in reduced fertilizing capacity of spermatozoa; no effects were seen at 8 mg of nickel per kg of body weight (Jacquet & Mayence, 1982).

Reduced number of live pups and reduced body weight of fetuses were observed in rats exposed to single doses of nickel chloride (16 mg of nickel per kg of body weight) or nickel subsulfide (80 mg of nickel per kg of body weight) administered intramuscularly on day 8 and day 6, respectively (Sunderman et al., 1978). No congenital anomalies were found in the fetuses.

In a three-generation study in rats at dietary levels of 250, 500, or 1000 mg of nickel (administered as nickel sulfate) per kg of diet (equivalent to 12.5, 25, or 50 mg/kg of body weight per day), a higher incidence of stillborn in the first generation was observed compared with the control group (Ambrose et al., 1976). Body weights were decreased in weanlings at 1000 mg/kg of diet in all generations. The number of pups born alive per litter and the number of pups weaned per litter were progressively fewer with increasing nickel dose, but no statistical analysis of the results is presented. Decreased weanling body weight is a clear-cut effect in the 1000 mg/kg of diet dose group. No teratogenic effects were observed in any generation at any dose level. No histological lesions were observed in the third generation at weaning.

Decreased litter sizes were observed in a small-scale three-generation study in rats administered nickel in drinking-water at 5 mg/litre, corresponding to 0.2 mg/kg of body weight per day (Schroeder & Mitchener, 1971).

Velazquez & Poirer (1994) and ATSDR (1996) described a two-generation study in rats. Nickel chloride was administered in drinking-water at concentrations of 0, 50, 250, or 500 mg/litre (equal to 0, 7, 31, and 52 mg of nickel per kg of body weight per day) from 90 days before breeding. Along with changes in maternal body weight and liver weight at the 500 mg/litre dose level in the P_0 generation, there were also a dose-related decrease in live litter size and pup weight and increased neonatal mortality. In the F_1 generation, there was dose-related mortality between 3 and 7 weeks of age at the 250 and 500 mg/litre dose levels. For the F_1 matings, there were also dose-related decreases in live litter size and increased mortality per litter, but this was significant only in the high-dose group. Decreased food intake and water intake were observed in the exposed animals. Also, the room temperature was up to 6°C higher than normal at certain times during gestation and the early postnatal days. Lower than normal levels of humidity were also recorded. Thus, the NOAEL in this study is considered to be 7 mg of nickel per kg of body weight per day; however, because of the problems referred to, it is difficult to make a direct association between the effects reported in this study and nickel exposure.

Female Long-Evans rats were exposed for 11 weeks prior to mating and then during two successive gestation periods (G1 and G2) and lactation periods (L1 and L2) to 0, 10, 50, or 250 ppm nickel as nickel chloride (equal to 0, 1.3, 6.8, and 31.6 mg of nickel per kg of body weight per day) in drinking-water (Smith et al., 1993). Dams drinking water containing nickel at 31.6 mg/kg of body weight per day consumed less liquid and more food per kg of body weight than did controls. Maternal weight gain was reduced during G1 in the mid- and high-dose groups. There were no effects on pup birth weight, and weight gain was reduced only in male pups from dams in the mid-dose group. The proportion of dead pups per litter was significantly elevated at the high dose in L1 and at the low and high dose in L2 (the increase at the middle dose in L2 approached statistical significance), with a dose-related response in both experimental segments. The number of dead pups per litter was significantly increased at each dose in L2. It was noted that the number of litters with dead pups and the total number of dead pups per litter in the control group were less in L2 than in L1. Plasma prolactin levels were reduced in dams at the highest dose level 1 week after weaning of the second litter. The authors concluded that 1.3 mg/kg of body weight per day represented the LOAEL in this study, although this is considered to be conservative, owing to variations in response between the successive litters.

Alterations in milk composition were observed in lactating rats exposed to four daily subcutaneous injections of nickel at doses of 3-6 mg/kg of body weight (Dostal et al., 1989). Liver weights were decreased in pups whose dams received 6 mg of nickel per kg of body weight. These findings may explain the effects seen on litter size and body weights of the pups in studies described above.

5.5 Mutagenicity and related end-points

Nickel compounds are generally inactive in bacterial mutation assays but active in mammalian cell systems (IPCS, 1991). It was concluded that there was cell toxicity in all gene mutation studies indicating nickel-dependent lesions using mammalian cells.

Chromosomal gaps, deletions and rearrangements, DNA-protein cross-links, and sister chromatid exchanges are reported in mammalian systems, including human cell systems. Chromosomal aberrations occur in all chromosomes but with preference to the heterochromatic centromeric regions (IPCS, 1991; Rossman, 1994).

In several experimental systems, nickel ions have been shown to potentiate the effects of other mutagenic agents, which may be explained by the capacity of nickel to inhibit DNA repair (Lynn et al., 1994; Rossman, 1994).

5.6 Carcinogenicity

A number of studies on the carcinogenicity of nickel compounds in experimental animals are available (IARC, 1990; Aitio, 1995). Generally, tumours are induced at the site of administration of the nickel compound. For instance, several nickel compounds induce injection-site sarcomas (Sunderman, 1984). Recently, a marked variation in the incidence of injection-site sarcomas between different strains of mice was reported (Rodriguez et al., 1996).

There are only a limited number of studies on carcinogenic effects after oral exposure to nickel compounds. The incidence of tumours was not higher in rats exposed to drinking-water containing nickel at 5 mg/litre during their lifetime compared with control rats (Schroeder et al., 1974). As well, no difference in tumour incidence was observed in a lifetime study in rats exposed to 5, 50, or 125 mg of nickel per kg of body weight per day in the feed compared with controls (Ambrose et al., 1976). Owing to the high death rate and lack of information on cause of death, this study is of minor value in evaluating carcinogenicity after oral exposure to nickel.

5.7 Other effects

Nickel salts affect the T-cell system and suppress the activity of natural killer cells in rats and mice (IPCS, 1991). Mitogen-dependent lymphocyte stimulation was inhibited in human lymphocytes (Sikora & Zeromski, 1995) and in spleens of mice exposed to nickel (IPCS, 1991). Dose-related decreased spleen proliferative response to lipopolysaccharide was observed in mice exposed to nickel sulfate in drinking-water for 180 days. At the lowest dose (44 mg of nickel per kg of body weight per day), decreased thymus weight was observed, but there was no nickel-induced immunosuppression NK cell activity or response to T-cell mitogens.

Parenteral administration of nickel to rabbits, chickens, and rats and oral administration of nickel to rabbits induce hyperglycaemia and reduce the levels of prolactin releasing factor in rats (IPCS, 1991).

The myeloid system was affected (i.e. decrease in bone marrow cellularity and dose-related reductions in the bone marrow proliferative response) when mice were exposed to nickel sulfate in drinking-water at doses of 0, 44, 108, or 150 mg of nickel per kg of body weight per day for 180 days (Dieter et al., 1988). The LOAEL in this study was 44 mg of nickel per kg of body weight per

day.

6. EFFECTS ON HUMANS

6.1 Acute exposure

A 2½-year-old girl died after ingesting about 15 g of nickel sulfate crystals. Cardiac arrest occurred after 4 hours; the autopsy revealed acute haemorrhagic gastritis (Daldrup et al., 1983).

Thirty-two industrial workers accidentally drank water contaminated with nickel sulfate and nickel chloride (1.63 g of nickel per litre). The nickel doses in persons who developed symptoms were estimated to range from 7 to 35 mg/kg of body weight. Twenty workers developed symptoms, including nausea, vomiting, diarrhoea, giddiness, lassitude, headache, and shortness of breath. In most cases, these symptoms lasted for a few hours, but they persisted for 1-2 days in seven cases. Transiently elevated levels of urine albumin suggesting mild transient nephrotoxicity were found in two workers 2-5 days after exposure. Mild hyperbilirubinaemia developed on day 3 after exposure in two subjects, and elevated levels of blood reticulocytes were observed in seven workers on day 8 post-exposure. It is known from animal studies that nickel after intrarenal injection enhances the renal production of erythropoietin, which may explain the reticulo-cytosis, and that nickel induces microsomal haem oxygenase activity in liver and kidney, leading to a secondary hyperbilirubinaemia. Serum nickel concentrations ranged between 13 and 1340 µg/litre in persons with symptoms (Sunderman et al., 1988).

Seven hours after ingesting nickel sulfate in drinking-water (50 µg of nickel per kg of body weight), a 55-year-old man developed left homonymous haemianopsia, which lasted 2 hours (Sunderman et al., 1989).

Nickel intoxication in 23 patients receiving haemodialysis was reported (Webster et al., 1980). The dialysate was contaminated by leachate from a nickel-plated stainless steel water heater tank. Symptoms such as nausea, vomiting, headache, and weakness occurred rapidly after exposure at plasma nickel concentrations of about 3 mg/litre and persisted for 3-13 hours after dialysis.

6.2 Skin irritation and hypersensitivity

Allergic contact dermatitis is the most prevalent effect of nickel in the general population. A recent epidemiological investigation showed that 20% of young (15-34 years) Danish women and 10% of older (35-69 years) women were nickel-sensitized, compared with only 2-4% of Danish men (15-69 years) (Nielsen & Menné, 1992). The prevalence of nickel allergy was found to be 7-10% in previously published reports (Menné et al., 1989). Edetic acid (EDTA) reduced the number and severity of patch test reactions to nickel sulfate in nickel-sensitive subjects (Allenby & Goodwin, 1983).

Systemically induced flares of dermatitis are reported after oral challenge of nickel-sensitive women with 0.5-5.6 mg of nickel as nickel sulfate administered in a lactose capsule (Veien, 1989). At the highest nickel dose (5.6 mg), there was a positive reaction in a majority of the subjects; at 0.5 mg, only a few persons responded with flares. Responses to oral doses of 0.4 or 2.5 mg of nickel did not exceed responses in subjects given placebos in double-blind studies (Jordan & King, 1979; Gawkrodger et al., 1986).

After an oral dose of 1 mg of nickel, significantly higher levels of nickel were found in the urine of atopic patients (i.e. persons with a history of flexural dermatitis) compared with controls, indicating a higher gastrointestinal absorption of nickel in atopic persons (Hindsén et al., 1994). No such difference was found between nickel-allergic patients and controls. The small number of patients may explain these unexpected findings.

There are several reports on the effects of diets low or high in nickel, but it is still a matter of discussion whether naturally occurring nickel in food may worsen or maintain the hand eczema of nickel-sensitive patients, mainly because results from dietary depletion studies have been inconclusive (Veien & Menné, 1990). In a single-blind study, 12 nickel-sensitive women were challenged with a supplementary high-nickel diet (Nielsen et al., 1990). The authors concluded that hand eczema was aggravated during the period (i.e. days 0-11) and that the symptoms thus were nickel-induced. However, it should be noted that in some subjects the severity of the eczema (i.e. the number of vesicles in the palm of the hand) varied markedly between day 14 or 21 before the challenge period and the start of the challenge period.

Oral hyposensitization to nickel was reported after six weekly doses of 5 mg of nickel in a capsule (Sjöwall et al., 1978) and 0.1 ng of nickel sulfate daily for 3 years (Panzani et al., 1995). Cutaneous lesions were improved in eight patients with contact allergy to nickel after oral exposure to 5 mg of nickel weekly for 8 weeks (Bagot et al., 1995). Nickel in water (as nickel sulfate) was given to 25 nickel-sensitive women in daily doses of 0.01-0.04 mg/kg of body weight per day for 3 months after they had been challenged once with 2.24 mg of nickel (Santucci et al., 1988). In 18 women, flares occurred after the challenge dose, whereas only 3 out of 17 subjects had symptoms during the prolonged exposure period. Later, Santucci and co-workers (1994) gave increasing oral doses of nickel in water (0.01-0.03 mg of nickel per kg of body weight per day) to eight nickel-sensitive women for up to 178 days. A significant improvement in hand eczema was observed in all subjects after 1 month.

6.3 Carcinogenicity

The identification of nickel species hazardous to humans was investigated by the International Committee on Nickel Carcinogenesis in Man by analysing 10 previously studied cohorts of men occupationally exposed to nickel (ICNCM, 1990). It was concluded that occupational exposure to sulfidic and oxidic nickel at high concentrations causes lung and nasal cancers. There was no correlation between metallic nickel exposure and cancer in lung or nose. Soluble nickel exposure increased the cancer risk and may also enhance the risk associated with exposure to less-soluble nickel compounds. The Committee also concluded that there was no substantial evidence that nickel compounds may produce cancers other than in the lung or nose in occupationally exposed persons.

Inhalation is an important route of exposure to nickel and its salts in relation to health risks. IARC (1990) concluded that nickel compounds are carcinogenic to humans (Group 1), whereas metallic nickel is possibly carcinogenic to humans (Group 2B). However, there is a lack of evidence of a carcinogenic risk from oral exposure to nickel.

7. PROVISIONAL GUIDELINE VALUE

In several limited studies, NOAELs were approximately 5 mg of nickel per kg of body weight per day. In a well conducted recent two-generation study on rats, dose-related increases in perinatal mortality were observed, giving a LOAEL of 1.3 mg of nickel per kg of body weight per day in the second litter, whereas the lowest-observed-effect level in the first litter was 31.6 mg of nickel per kg of body weight per day. These variations in response between successive litters make it difficult to draw firm conclusions. Also, a NOAEL of 7 mg of nickel per kg of body weight per day was derived from a more limited two-generation study in rats.

The guideline value for nickel of 0.02 mg/litre is maintained because, on the basis of the available data, it is considered to be protective of public health. Owing to uncertainties about the effect level for perinatal mortality, the value is considered to be provisional.

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Nitrate and nitrite

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A health-based guideline value for nitrate of 50 mg/litre (expressed as nitrate ion) was recommended in the second edition of the WHO *Guidelines for drinking-water quality* to prevent methaemoglobinaemia, a condition that occurs when nitrate is reduced to nitrite, in infants. Most clinical cases of methaemoglobinaemia occur at nitrate levels of 50 mg/litre and above, and almost exclusively in infants under 3 months of age. Accepting a relative potency for nitrite and nitrate with respect to methaemoglobin (metHb) formation of 10:1 (on a molar basis), a provisional guideline value for nitrite of 3 mg/litre (expressed as nitrite ion) was proposed. Because of the possibility of simultaneous occurrence of nitrite and nitrate in drinking-water, the sum of the ratios of the concentrations of each to its guideline value was not to exceed one.

In 1995, JECFA established ADIs for nitrate and nitrite and the US National Research Council published an evaluation of nitrate and nitrite in drinking-water. In light of this new information, the Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* determined that there was a need to reassess the existing guideline values for nitrate and nitrite.

1. GENERAL DESCRIPTION

1.1 Identity

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion (NO_3^-) is the stable form of combined nitrogen for oxygenated systems. Although chemically unreactive, it can be reduced by microbial action. The nitrite ion (NO_2^-) contains nitrogen in a relatively unstable oxidation state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (ICAIR Life Systems, Inc., 1987).

1.2 Physicochemical properties (ICAIR Life Systems, Inc., 1987)[1]

[1] Conversion to nitrogen: 1 mg NO_3^-/litre = 0.226 mg NO_3^--N/litre; 1 mg NO_2^-/litre = 0.304 mg NO_2^--N/litre

Property	*Nitrate*	*Nitrite*
Acid	Conjugate base of strong acid HNO_3; pK_a = -1.3	Conjugate base of weak acid HNO_2; pK_a = 3.4
Salts	Very soluble in water	Very soluble in water
Reactivity	Unreactive	Reactive; oxidizes antioxidants, Fe^{2+} of haemoglobin (Hb) to Fe^{3+}, and primary amines; nitrosates several amines and amides

1.3 Major uses

Nitrate is used mainly in inorganic fertilizers. It is also used as an oxidizing agent and in the production of explosives, and purified potassium nitrate is used for glass making. Sodium nitrite is used as a food preservative, especially in cured meats. Nitrate is sometimes also added to food to serve as a reservoir for nitrite.

1.4 Environmental fate

In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are first decomposed to give ammonia, which is then oxidized to nitrite and nitrate. The nitrate is taken up

by plants during their growth and used in the synthesis of organic nitrogenous compounds. Surplus nitrate readily moves with the groundwater (US EPA, 1987; van Duijvenboden & Matthijsen, 1989).

Under aerobic conditions, nitrate percolates in large quantities into the aquifer because of the small extent to which degradation or denitrification occurs. Under anaerobic conditions, nitrate may be denitrified or degraded almost completely to nitrogen. The presence of high or low water tables, the amount of rainwater, the presence of other organic material, and other physicochemical properties are also important in determining the fate of nitrate in soil (van Duijvenboden & Loch, 1983). In surface water, nitrification and denitrification may also occur, depending on the temperature and pH. The uptake of nitrate by plants, however, is responsible for most of the nitrate reduction in surface water.

Nitrogen compounds are formed in the air by lightning or discharged into it from industrial processes, motor vehicles, and intensive agriculture. Nitrate is present in air primarily as nitric acid and inorganic aerosols, as well as nitrate radicals and organic gases or aerosols. These are removed by wet and dry deposition.

2. ANALYTICAL METHODS

Spectrometric techniques are used for the determination of nitrate in water. Detection limits range from 0.01 to 1 mg/litre (ISO, 1986, 1988). A molecular absorption spectrometric method is available for the determination of nitrite in potable water, raw water, and wastewater. The limit of detection lies within the range of 0.005-0.01 mg/litre (ISO, 1984). A continuous-flow spectrometric method for the determination of nitrite, nitrate, or the sum of both in various types of water is suitable at concentrations ranging from 0.05 to 5 mg/litre for nitrite and from 1 to 100 mg/litre for nitrite/nitrate, both in the undiluted sample (ISO, 1996).

Nitrate and nitrite can also be determined in water by liquid chromatography, down to a level of 0.1 mg/litre for nitrate and 0.05 mg/litre for nitrite (ISO, 1992).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Atmospheric nitrate concentrations ranging from 0.1 to 0.4 $\mu g/m^3$ have been reported, the lowest concentrations being found in the South Pacific (Prospero & Savoie, 1989). Higher concentrations ranging from 1 to 40 $\mu g/m^3$ have also been reported, with annual means of 1-8 $\mu g/m^3$. Mean monthly nitrate concentrations in air in the Netherlands range from 1 to 14 $\mu g/m^3$ (Janssen et al., 1989). Indoor nitrate aerosol concentrations of 1.1-5.6 $\mu g/m^3$ were found to be related to outdoor concentrations (Yocom, 1982).

3.2 Water

Concentrations of nitrate in rainwater of up to 5 mg/litre have been observed in industrial areas (van Duijvenboden & Matthijsen, 1989). In rural areas, concentrations are somewhat lower.

The nitrate concentration in surface water is normally low (0-18 mg/litre) but can reach high levels as a result of agricultural runoff, refuse dump runoff, or contamination with human or animal wastes. The concentration often fluctuates with the season and may increase when the river is fed by nitrate-rich aquifers. Nitrate concentrations have gradually increased in many European countries in the last few decades and have sometimes doubled over the past 20 years. In the United Kingdom, for example, an average annual increase of 0.7 mg/litre has been observed in some rivers (Young & Morgan-Jones, 1980).

The natural nitrate concentration in groundwater under aerobic conditions is a few milligrams per

litre and depends strongly on soil type and on the geological situation. In the USA, naturally occurring levels do not exceed 4-9 mg/litre for nitrate and 0.3 mg/litre for nitrite (US EPA, 1987). As a result of agricultural activities, the nitrate concentration can easily reach several hundred milligrams per litre (WHO, 1985b). For example, concentrations of up to 1500 mg/litre were found in groundwater in an agricultural area of India (Jacks & Sharma, 1983).

In the USA, nitrates are present in most surface water and groundwater supplies at levels below 4 mg/litre, with levels exceeding 20 mg/litre in about 3% of surface waters and 6% of groundwaters. In 1986, a nitrate concentration of 44 mg/litre (10 mg of nitrate-nitrogen per litre) was exceeded in 40 surface water and 568 groundwater supplies. Nitrite levels were not surveyed but are expected to be much lower than 3.3 mg/litre (US EPA, 1987).

The increasing use of artificial fertilizers, the disposal of wastes (particularly from animal farming), and changes in land use are the main factors responsible for the progressive increase in nitrate levels in groundwater supplies over the last 20 years. In Denmark and the Netherlands, for example, nitrate concentrations are increasing by 0.2-1.3 mg/litre per year in some areas (WHO, 1985b). Because of the delay in the response of groundwater to changes in soil, some endangered aquifers have not yet shown the increase expected from the increased use of nitrogen fertilizer or manure. Once the nitrate reaches these aquifers, the aquifers will remain contaminated for decades, even if there is a substantial reduction in the nitrate loading of the surface.

In most countries, nitrate levels in drinking-water derived from surface water do not exceed 10 mg/litre. In some areas, however, concentrations are higher as a result of runoff and the discharge of sewage effluent and certain industrial wastes. In 15 European countries, the percentage of the population exposed to nitrate levels in drinking-water above 50 mg/litre ranges from 0.5 to 10% (WHO, 1985b; ECETOC, 1988); this corresponds to nearly 10 million people. Individual wells in agricultural areas throughout the world especially contribute to nitrate-related toxicity problems, and nitrate levels in the well-water often exceed 50 mg/litre.

Nitrite levels in drinking-water in the Netherlands are usually below 0.1 mg/litre. In 1993, a maximum value of 0.21 mg/litre was detected (RIVM, 1993).

Chloramination may give rise to the formation of nitrite within the distribution system, and the concentration of nitrite may increase as the water moves towards the extremities of the system. Nitrification in distribution systems can increase nitrite levels, usually by 0.2-1.5 mg of nitrite per litre, but potentially by more than 3 mg of nitrite per litre (AWWARF, 1995).

3.3 Food

Vegetables and cured meat are in general the main source of nitrate and nitrite in the diet, but small amounts may be present in fish and dairy products. Meat products may contain <2.7-945 mg of nitrate per kg and <0.2-6.4 mg of nitrite per kg; dairy products may contain <3-27 mg of nitrate per kg and <0.2-1.7 mg of nitrite per kg (ECETOC, 1988). Several vegetables and fruits contain 200-2500 mg of nitrate per kg (van Duijvenboden & Matthijsen, 1989). The nitrate content of vegetables can be affected by processing of the food, the use of fertilizers, and growing conditions, especially the soil temperature and (day)light intensity (Gangolli et al., 1994; WHO, 1995). Vegetables such as beetroot, lettuce, radish, and spinach often contain nitrate concentrations above 2500 mg/kg, especially when they are cultivated in greenhouses. Nitrite levels in food are very low (generally well below 10 mg/kg) and rarely exceed 100 mg/kg. Exceptions to this are vegetables that have been damaged, poorly stored, or stored for extended periods as well as pickled or fermented vegetables. In such circumstances, nitrite levels of up to 400 mg/kg have been found (WHO, 1995).

3.4 Estimated total exposure and relative contribution of drinking-water

Air pollution appears to be a minor source of nitrate exposure. In general, vegetables will be the main source of nitrate intake when nitrate levels in drinking-water are below 10 mg/litre (Chilvers et al., 1984; US EPA, 1987; ECETOC, 1988).

When nitrate levels in drinking-water exceed 50 mg/litre, drinking-water will be the major source of total nitrate intake, especially for bottle-fed infants. In the Netherlands, the average population exposure is approximately 140 mg of nitrate per day (including the nitrate in drinking-water). The contribution of drinking-water to nitrate intake is usually less than 14%. For bottle-fed infants, daily intake from formula made with water containing 50 mg of nitrate per litre would average about 8.3-8.5 mg of nitrate per kg of body weight per day.

The mean dietary intakes determined by the duplicate portion technique (WHO, 1985a) range from 43 to 131 mg of nitrate per day and from 1.2 to 3 mg of nitrite per day. Estimates of the total nitrate intake based on the proportion of nitrate excreted in the urine (Bartholomew et al., 1979) range from 39 to 268 mg/day, the higher values applying to vegetarian and nitrate-rich diets (ECETOC, 1988). The estimated total daily intake of nitrate ranged in the United Kingdom from 50 to 81 mg per person (Bonnell, 1995; Schuddeboom, 1995), in Denmark from 70 to 172 mg per person (Bonnell, 1995), and in Germany from 70 to 110 mg per person (Bonnell, 1995). According to the US EPA, the average nitrate intake from food is approximately 40-100 mg/day for males. The daily nitrite intake ranges from 0.3 to 2.6 mg/day, primarily from cured meat (NAS, 1981). Nitrite present in cured meat has been reported to account for up to 70% of total dietary intake of this substance, depending on the intake of such meat and the origin and type of cured meat consumed. Mean dietary nitrite intake from all food sources has been reported to range from <0.1 to 8.7 mg of nitrite per person per day for European diets (WHO, 1995).

4. KINETICS AND METABOLISMS IN LABORATORY ANIMALS AND HUMANS

4.1 Absorption, distribution, and elimination

Ingested nitrate is readily and completely absorbed from the upper small intestine. Nitrite may be absorbed directly from both the stomach and the upper small intestine. Part of the ingested nitrite reacts with gastric contents prior to absorption.

Nitrate is rapidly distributed throughout the tissues. Approximately 25% of ingested nitrate is actively secreted into saliva, where it is partly (20%) reduced to nitrite by the oral microflora; nitrate and nitrite are then swallowed and re-enter the stomach. Bacterial reduction of nitrate may also take place in other parts of the human gastrointestinal tract, but not normally in the stomach; exceptions are reported in humans with low gastric acidity, such as artificially fed infants, certain patients in whom hydrochloric acid secretion is slower than normal, or patients using antacids (Colbers et al., 1995). In rats, active secretion and reduction of nitrate in saliva are virtually absent (Walker, 1995). Total nitrate reduction in rats is probably less than in humans.

Absorbed nitrite is rapidly oxidized to nitrate in the blood. Nitrite in the bloodstream is involved in the oxidation of Hb to metHb: the Fe^{2+} present in the haem group is oxidized to its Fe^{3+} form, and the remaining nitrite binds firmly to this oxidized haem. The Fe^{3+} form does not allow oxygen transport, owing to the strong binding of oxygen (Jaffé, 1981; US National Research Council, 1995). Therefore, methaemoglobinaemia can lead to cyanosis.

Nitrite has been shown to cross the placenta and cause the formation of fetal methaemoglobinaemia in rats. It may react in the stomach with nitrosatable compounds (e.g. secondary and tertiary amines or amides in food) to form *N*-nitroso compounds. Such endogenous nitrosation has been shown to occur in human as well as animal gastric juice both *in vivo* and *in vitro*, mostly at higher pH values, when both nitrite and nitrosatable compounds were present simultaneously (Shephard, 1995; WHO, 1996).

The major part of the ingested nitrate is eventually excreted in urine as nitrate, ammonia, or urea, faecal excretion being negligible. Little nitrite is excreted (WHO, 1985b; ICAIR Life Systems, Inc., 1987; Speijers et al., 1989).

4.2 Endogenous synthesis of nitrate and nitrite

The excess nitrate excretion that has often been observed after low nitrate and nitrite intake originates from endogenous synthesis, which amounts, in normal healthy humans, to 1 mmol/day on average, corresponding to 62 mg of nitrate per day or 14 mg of nitrate-nitrogen per day. Gastrointestinal infections greatly increase nitrate excretion, as a result, at least in part, of increased endogenous (non-bacterial) nitrate synthesis, probably induced by activation of the mammalian reticuloendothelial system (WHO, 1985b, 1996; Speijers et al., 1989; Wishnok et al., 1995). This endogenous synthesis of nitrate complicates the risk assessment of nitrate.

Increased endogenous synthesis of nitrate, as reported in animals with induced infections and inflammatory reactions, was also observed in humans. Infections and non-specific diarrhoea played a role in the increased endogenous synthesis of nitrate (Tannenbaum et al., 1978; Green et al., 1981; Hegesh & Shiloah, 1982; Bartholomew & Hill, 1984; Lee et al., 1986; Gangolli et al., 1994). These observations are all consistent with the induction of one or more nitric oxide synthases by inflammatory agents, analogous to the experiments described in animals and macrophages. This induction in humans has been difficult to demonstrate directly, but administration of [^{15}N]arginine to two volunteers resulted in the incorporation of ^{15}N into urinary nitrate in both individuals, confirming the arginine-nitric oxide pathway in humans (Leaf et al., 1989).

Nitrate excretion in excess of nitrate intake by humans was reported in 1916, but this result remained obscure until the end of the 1970s, when it was re-examined because of the potential involvement of nitrate in endogenous nitrosation. A relatively constant daily production of about 1 mmol of nitrate was confirmed. A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by oxidation of the nitric oxide to nitrous anhydride and then reaction of nitrous anhydride with water to yield nitrite. Nitrite is rapidly oxidized to nitrate through reaction with Hb. In addition to macrophages, many cell types can form nitric oxide, generally from arginine. Under some conditions, bacteria can form nitric oxide by reduction of nitrite. These processes can lead to nitrosation of amines at neutral pH, presumably by reaction with nitrous anhydride. The question of whether the arginine-nitrate pathway can be associated with increased cancer risk via exposure to *N*-nitroso compounds remains open. Nitric oxide is mutagenic towards bacteria and human cells in culture; it causes DNA strand breaks, deamination (probably via nitrous anhydride), and oxidative damage; and it can activate cellular defence mechanisms. In virtually all of these cases, the biological response is paralleled by the final nitrate levels. Thus, while endogenously formed nitrate may itself be of relatively minor toxicological significance, the levels of this substance may potentially serve as indicators for those potentially important nitric oxide-related processes that gave rise to it (Wishnok et al., 1995).

As mentioned above, both *in vitro* and *in vivo* studies showed that nitrate can be reduced to nitrite by bacterial and mammalian metabolic pathways, via the widespread nitrate reductase (Gangolli et al., 1994). In humans, saliva is the major site for the formation of nitrite. About 5% of dietary nitrate is converted to nitrite (Spiegelhalder et al., 1976; Eisenbrand et al., 1980; Walters & Smith, 1981; Gangolli et al., 1994). A direct correlation between gastric pH, bacterial colonization, and gastric nitrite concentration has been observed in healthy people with a range of pH values from 1 to 7 (Mueller et al., 1983, 1986). In individuals with gastrointestinal disorders and achlorhydria, high levels of nitrite can be reached (6 mg/litre) (Rudell et al., 1976, 1978; Dolby et al., 1984). The situation in neonates is not clear. It is commonly accepted that infants younger than 3 months may be highly susceptible to gastric bacterial nitrate reduction, as the pH is generally higher than in adults (Speijers et al., 1989). However, the presence of acid-producing lactobacilli in the

stomach may be important, as these organisms do not reduce nitrate and may maintain a pH low enough to inhibit colonization by nitrate-reducing bacteria (Bartholomew et al., 1980). As mentioned above, nitrite may also be produced via the arginine-nitric oxide pathway but would be undetectable because of the rapid oxidation to nitrate. One possible example of nitrite production by this route, however, is the methaemoglobinaemia observed in infants suffering from diarrhoea (Gangolli et al., 1994).

5. EFFECTS ON LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

The acute oral toxicity of nitrate to laboratory animals is low to moderate. LD_{50} values of 1600-9000 mg of sodium nitrate per kg of body weight have been reported in mice, rats, and rabbits. Ruminants are more sensitive to the effects of nitrate as a result of high nitrate reduction in the rumen; the LD_{50} for cows was 450 mg of sodium nitrate per kg of body weight. Nitrite is more toxic than nitrate: LD_{50} values of 85-220 mg of sodium nitrite per kg of body weight have been reported for mice and rats (Speijers et al., 1989; WHO, 1996).

5.2 Short-term exposure

In a 13-week study in which nitrite was given to rats in drinking-water, a dose-related hypertrophy of the adrenal zona glomerulosa was observed at all dose levels (100, 300, 1000, or 3000 mg of potassium nitrite per litre). Increased metHb levels were seen only in the highest dose group (Til et al., 1988). WHO (1995) concluded that the NOEL in this study was 100 mg of potassium nitrite per litre (equivalent to 5.4 mg/kg of body weight per day expressed as nitrite ion), because the hypertrophy seen at this dose was not significantly different from the controls.

An additional 13-week study in which nitrite was also given in drinking-water, including lower doses of potassium nitrite and two doses of sodium nitrite (equimolar to the low and high doses of potassium nitrite), confirmed the finding of the adrenal hypertrophy of the zona glomerulosa for potassium nitrite and also revealed hypertrophy in the animals given sodium nitrite. The NOEL for the adrenal hypertrophy of the zona glomerulosa was 50 mg of potassium nitrite per litre (equivalent to 5 mg of potassium nitrite per kg of body weight per day) (Kuper & Til, 1995). Since then, studies designed to clarify the etiology of this hypertrophy and to establish its significance for human health have been partly performed and are currently in progress. The studies already performed confirmed the adrenal hypertrophy in another rat strain. However, the effects were seen only at higher dose levels. It was also seen that the hypertrophy was still present after a 30-day recovery period but had disappeared after a 60-day recovery period. At present, the mechanism of hypertrophy induced by nitrite is not clear (Boink et al., 1995).

A variety of experimental and field studies in different mammals identified inorganic nitrate as a goitrogenic agent. It could be shown in rats by oral and parenteral application of potassium nitrate (Wyngaarden et al., 1953; Bloomfield et al., 1961; Alexander & Wolff, 1966; Wolff, 1994), of nitrate in hay (Lee et al., 1970), and of sodium nitrate (Höring et al., 1985; Seffner & Höring, 1987a, b). Antithyroid effects of nitrate were also found in sheep (Bloomfield et al., 1961) and in pigs by application of potassium nitrate (Jahreis et al., 1986, 1987). Furthermore, nitrate was goitrogenic to livestock: pigs (Körber et al., 1983), cattle (Körber et al., 1983, 1985), sheep (Körber et al., 1983), and goats (Prassad, 1983).

5.3 Long-term exposure

The only observed effect of nitrate in rats after 2 years of oral administration was growth inhibition; this was seen at dietary concentrations of 5% sodium nitrate and higher. The NOEL in this study was 1%, which corresponds to 370 mg of nitrate per kg of body weight per day (Speijers et al., 1989; WHO, 1996). A more recent long-term study was solely a carcinogenicity study, in which the highest dose levels of 1820 mg of nitrate per kg of body weight per day did not

show carcinogenic effects. However, this level could not be considered as a NOEL, because complete histopathological examinations were not performed (WHO, 1996).

One of the long-term effects of nitrite reported in a variety of animal species is vitamin A deficiency; this is probably caused by the direct reaction of nitrite with the vitamin. The most important effects reported in long-term animal studies were an increase in metHb level and histopathological changes in the lungs and heart in rats receiving nitrite in drinking-water for 2 years. The LOAEL, which gave a metHb level of 5%, was 1000 mg of sodium nitrite per litre; the NOEL was 100 mg of sodium nitrite per litre, equivalent to 10 mg of sodium nitrite per kg of body weight per day (or 6.7 mg/kg of body weight per day expressed as nitrite ion) (Speijers et al., 1989).

5.4 Reproductive and developmental toxicity

The reproductive behaviour of guinea-pigs was impaired only at very high nitrate concentrations (30 000 mg of potassium nitrate per litre); the NOEL was 10 000 mg/litre (Speijers et al., 1989; WHO, 1996). In rabbits, dose levels of 250 or 500 mg of nitrate per litre administered during 22 weeks revealed no detrimental effects on reproductive performance after successive gestations. In sheep and cattle, no abortions were observed at dose levels causing severe methaemoglobinaemia (Speijers et al., 1989; WHO, 1996).

Nitrite appeared to cause fetotoxicity in rats at drinking-water concentrations equivalent to 200 and 300 mg of sodium nitrite per kg of body weight per day, causing increased maternal metHb levels. However, after similar doses in feed in other studies, no embryotoxic effects were observed in rats. In a reproductive toxicity study in guinea-pigs at dose levels of 0, 50, or 60 mg of sodium nitrite per kg of body weight per day given by subcutaneous injection, fetal death followed by abortion occurred at the highest dose level. Teratogenic effects were not observed in reported studies in mice and rats (Speijers et al., 1989; WHO, 1996).

5.5 Mutagenicity and related end-points

Nitrate is not mutagenic in bacteria and mammalian cells *in vitro*. Chromosomal aberrations were observed in the bone marrow of rats after oral nitrite uptake, but this could have been due to exogenous *N*-nitroso compound formation. Nitrite is mutagenic. It causes morphological transformations in *in vitro* systems; mutagenic activity was also found in a combined *in vivo-in vitro* experiment with Syrian hamsters. The results of *in vivo* experiments were controversial (Speijers et al., 1989; WHO, 1996).

5.6 Carcinogenicity

Nitrate is not carcinogenic in laboratory animals. Some studies in which nitrite was given to mice or rats in the diet showed slightly increased tumour incidence; however, the possibility of exogenous *N*-nitroso compound formation in these studies could not be excluded. In studies in which high levels of nitrite and simultaneously high levels of nitrosatable precursors were administered, increased tumour incidence was seen (Speijers et al., 1989; WHO, 1996). These types of tumours could be characteristic of the presumed corresponding *N*-nitroso compound endogenously formed. However, this increase in tumour incidence was seen only at extremely high nitrite levels, in the order of 1000 mg/litre of drinking-water. At lower nitrite levels, tumour incidence resembled those of control groups treated with the nitrosatable compound only. On the basis of adequately performed and reported studies, it may be concluded that nitrite itself is not carcinogenic to animals (Speijers et al., 1989; WHO, 1996).

6. EFFECTS ON HUMANS

6.1 Methaemoglobinaemia

The toxicity of nitrate to humans is mainly attributable to its reduction to nitrite. The major biological effect of nitrite in humans is its involvement in the oxidation of normal Hb to metHb, which is unable to transport oxygen to the tissues. The reduced oxygen transport becomes clinically manifest when metHb concentrations reach 10% of normal Hb concentrations and above; the condition, called methaemoglobinaemia, causes cyanosis and, at higher concentrations, asphyxia. The normal metHb level in humans is less than 2%; in infants under 3 months of age, it is less than 3%.

The Hb of young infants is more susceptible to metHb formation than that of older children and adults. This higher susceptibility is believed to be the result of the large proportion of fetal Hb still present in the blood of these infants. This fetal Hb is more easily oxidized to metHb. In addition, there is a deficiency in the metHb reductase responsible for the reduction of metHb back to Hb. The net result is that a dose of nitrite causes a higher metHb formation in these infants than in adults. With respect to exposure to nitrate, these young infants are also more at risk because of a relatively high intake of nitrate and, under certain conditions, a higher reduction of nitrate to nitrite by gastric bacteria due to the low production of gastric acid (Speijers et al., 1989; WHO, 1996). The higher reduction of nitrate to nitrite in the young infants is not quantified very well, and it appears that gastrointestinal infections increase the risk of higher yield of nitrite and thus a higher metHb formation (ECETOC, 1988; Speijers et al., 1989; Möller, 1995; Schuddeboom, 1995; WHO, 1996).

Other groups especially susceptible to metHb formation include pregnant women and people deficient in glucose-6-phosphate dehydrogenase or metHb reductase (Speijers et al., 1989).

6.1.1 Adults and children above the age of 3 months

Cases of methaemoglobinaemia have been reported in adults consuming high doses of nitrate by accident or as a medical treatment. Fatalities were reported after single intakes of 4-50 g of nitrate (equivalent to 67-833 mg of nitrate per kg of body weight) (Speijers et al., 1989; WHO, 1996), many of which occurred among special risk groups in whose members gastric acidity was reduced. Toxic doses - with metHb formation as a criterion for toxicity - ranged from 2 to 9 g (equivalent to 33-150 mg of nitrate per kg of body weight) (WHO, 1996). In a controlled study, an oral dose of 7-10.5 g of ammonium nitrate and an intravenous dose of 9.5 g of sodium nitrate did not cause increased metHb levels in adults, although vomiting and diarrhoea occurred (Speijers et al., 1989; WHO, 1996).

Accidental human intoxications have been reported as a result of the presence of nitrite in food. The oral lethal dose for humans was estimated to range from 33 to 250 mg of nitrite per kg of body weight, the lower doses applying to children and elderly people. Toxic doses giving rise to methaemoglobinaemia ranged from 0.4 to 200 mg/kg of body weight (WHO, 1996).

Another source of information with respect to nitrite toxicity in humans is the use of sodium nitrite as medication for vasodilation or as an antidote in cyanide poisoning. Doses of 30-300 mg per person (equivalent to 0.5-5 mg/kg of body weight) were reported not to cause toxic effects (WHO, 1996).

Few cases of methaemoglobinaemia have been reported in older children. A correlation study among children aged 1-8 years in the USA showed that there was no difference in metHb levels between 64 children consuming high-nitrate well-water (22-111 mg of nitrate-nitrogen per litre) and 38 children consuming low-nitrate water (<10 mg of nitrate-nitrogen per litre). These concentrations correspond to 100-500 and <44 mg of nitrate per litre, respectively. All the metHb levels were within the normal range, suggesting that older children are relatively insensitive to the

effects of nitrate (Craun et al., 1981).

6.1.2 Infants under 3 months of age

Cases of methaemoglobinaemia related to low nitrate appear to be restricted to infants. In infants under the age of 3 months, the conversion of nitrate to nitrite and metHb formation are high, as discussed above. Gastrointestinal disturbances play a crucial role, the reduction of nitrate to nitrite in the stomach being enhanced by bacterial growth at the high pH in the stomach of these infants. Toxic effects can therefore be induced at a much lower dose of nitrate than in adults. According to Corré & Breimer (1979), assuming an 80% reduction of nitrate to nitrite in these young infants, the toxic dose ranged from 1.5 to 2.7 mg of nitrate per kg of body weight, using 10% formation of metHb as a toxicity criterion. However, in reported cases of methaemoglobinaemia, the amounts of nitrate ingested were higher: 37.1-108.6 mg/kg of body weight, with an average of 56.7 mg of nitrate per kg of body weight (WHO, 1996). In studies in which a possible association between clinical cases of infantile methaemoglobinaemia or subclinically increased metHb levels and nitrate concentrations in drinking-water was investigated, a significant relationship was usually found, most clinical cases (97.7%) occurring at nitrate levels of 44.3-88.6 mg/litre or higher (Walton, 1951; WHO, 1996), and almost exclusively in infants under 3 months of age (Walton, 1951). Some cases of infant methaemoglobinaemia have indeed been described in which increased endogenous nitrate (nitrite) synthesis as a result of gastrointestinal infection appeared to be the only causative factor (WHO, 1996). As most cases of infantile methaemoglobinaemia reported in the literature have been associated with the consumption of private and often bacterially contaminated well-water, the involvement of infections is highly probable. Most of these studies may be therefore less suitable from the point of view of the quantitative assessment of the risk of nitrate intake for healthy infants. On the other hand, bottle-fed infants under 3 months of age have a high probability of developing gastrointestinal infections because of their low gastric acidity, which is another important reason to consider these infants as a risk group.

6.2 Carcinogenicity

Nitrite was shown to react with nitrosatable compounds in the human stomach to form *N*-nitroso compounds. Many of these *N*-nitroso compounds have been found to be carcinogenic in all the animal species tested, although some of the most readily formed compounds, such as *N*-nitrosoproline, are not carcinogenic in humans. The *N*-nitroso compounds carcinogenic in animal species are probably also carcinogenic in humans. However, the data from a number of epidemiological studies are at most only suggestive. The endogenous formation of *N*-nitroso compounds is also observed in several animal species, if relatively high doses of both nitrite and nitrosatable compounds are administered simultaneously. Thus, a link between cancer risk and endogenous nitrosation as a result of high intake of nitrate and/or nitrite and nitrosatable compounds is possible (Speijers et al., 1989; WHO, 1996).

Several reviews of epidemiological studies have been published; most of these studies are geographical correlation studies relating estimated nitrate intake to gastric cancer risk. The US National Research Council found some suggestion of an association between high nitrate intake and gastric and/or oesophageal cancer (NAS, 1981). However, individual exposure data were lacking, and several other plausible causes of gastric cancer were present. In a later WHO review (WHO, 1985b), some of the earlier associations appeared to be weakened following the introduction of individual exposure data or after adjustment for socioeconomic factors. No convincing evidence was found of an association between gastric cancer and the consumption of drinking-water in which nitrate concentrations of up to 45 mg/litre were present. No firm evidence was found at higher levels either, but an association could not be excluded because of the inadequacy of the data available. More recent geographical correlation and occupational exposure studies also failed to demonstrate a clear relationship between nitrate intake and gastric cancer risk, although these studies were well designed. A case-control study in Canada, in which dietary exposure to nitrate and nitrite was estimated in detail, showed that exogenous nitrite

intake, largely from preserved meat, was significantly associated with the risk of developing gastric cancer (ECETOC, 1988). On the other hand, case-control studies based on food frequency questionnaires tend to show a protective effect of the estimated nitrate intake on gastric cancer risk. Most likely this is due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer (Möller, 1995; WHO, 1996). Studies that have assessed the effect of nitrate from sources other than vegetables, such as the concentration in drinking-water or occupational exposure to nitrate dusts, have not shown a protective effect against gastric cancer risk. For other types of cancer, there are no adequate data with which to establish any association with nitrite or nitrate intake (Gangolli et al., 1994; Möller, 1995; WHO, 1996).

It has been established that the intake of certain dietary components present in vegetables, such as vitamins C and E, decreases the risk of gastric cancer. This is generally assumed to be at least partly due to the resulting decrease in the conversion of nitrate to nitrite and in the formation of *N*-nitroso compounds. It is possible that any effect of a high nitrate intake *per se* is masked in correlation studies by the antagonizing effects of simultaneously consumed dietary protective components. However, the absence of any link with cancer in occupational exposure studies is not in agreement with this theory.

The known increased risk of gastric cancer under conditions of low gastric acidity could be associated with the endogenous formation of *N*-nitroso compounds. High mean levels of *N*-nitroso compounds, as well as high nitrate levels, were found in the gastric juice of achlorhydric patients, who must therefore be considered as a special risk group for gastric cancer from the point of view of nitrate and nitrite (NAS, 1981; WHO, 1985b, 1996; ECETOC, 1988; Speijers et al., 1989).

6.3 Other effects

Congenital malformations have been related to high nitrate levels in drinking-water in Australia; however, these observations were not confirmed. Other studies also failed to demonstrate a relationship between congenital malformations and nitrate intake (WHO, 1985b; ECETOC, 1988).

Studies relating cardiovascular effects to nitrate levels in drinking-water gave inconsistent results (WHO, 1985b).

Possible relationships between nitrate intake and effects on the thyroid have also been studied, as it is known that nitrate competitively inhibits iodine uptake. In addition to effects of nitrate on the thyroid observed in animal studies and in livestock, epidemiological studies revealed indications for an antithyroid effect of nitrate in humans. If dietary iodine is available at an adequate range (corresponding to a daily iodine excretion of 150-300 µg/day), the effect of nitrate is weak, with a tendency to zero. The nitrate effect on thyroid function is strong if a nutritional iodine deficiency exists simultaneously (Höring et al., 1991; Höring, 1992).

Hettche (1956a,b) described an association between high nitrate concentrations in drinking-water and goitre incidence in 1955. As well, Höring & Schiller (1987), Sauerbrey & Andree (1988), Höring et al. (1991), Höring (1992), and van Maanen et al. (1994) found that inorganic nitrate in drinking-water is a manifested factor of endemic goitre. A dose-response relationship could be demonstrated by Höring et al. (1991) (nitrate in drinking-water vs incidence of goitre) as well as by van Maanen et al. (1994) (nitrate in drinking-water vs thyroid volume). Both the experimental and epidemiological studies give the impression that nitrate in drinking-water has a stronger effect on thyroid function than nitrate in food. The differences in nitrate kinetics after ingestion through drinking-water and through food could be the cause of the difference in thyroid effects. However, no adequate studies regarding this question exist at present. Furthermore, some of the above-mentioned studies demonstrate that dietary iodine deficiency is much more effective than nitrate exposure in causing goitre.

In addition to the effect of nitrite on the adrenal zona glomerulosa in rats, a study in humans

indicated that sodium nitrite (0.5 mg of sodium nitrite per kg of body weight per day, during 9 days) caused a decreased production of adrenal steroids, as reflected by the decreased concentration of 17-hydroxysteroid and 17-ketosteroids in urine (Til et al., 1988; Kuper & Til, 1995). Similar results were also found in rabbits (Violante et al., 1973). Although the mechanism is not clear, the effects of nitrite seen in rats seem relevant for the hazard assessment for humans, unless mechanistic studies prove otherwise.

7. GUIDELINE VALUES

With respect to chronic effects, JECFA recently re-evaluated the health effects of nitrate/nitrite, confirming the previous ADI of 0-3.7 mg/kg of body weight per day for nitrate ion and establishing an ADI of 0-0.06 mg/kg of body weight per day for nitrite ion (WHO, 1995). However, it was noted that these ADIs do not apply to infants below the age of 3 months. Bottle-fed infants below 3 months of age are most susceptible to methaemoglobinaemia following exposure to nitrate/nitrite in drinking-water.

For methaemoglobinaemia in infants (an acute effect), it was confirmed that the existing guideline value for nitrate ion of 50 mg/litre is protective. For nitrite, human data reviewed by JECFA support the current provisional guideline value of 3 mg/litre, based on induction of methaemoglobinaemia in infants. Toxic doses of nitrite responsible for methaemoglobinaemia range from 0.4 to more than 200 mg/kg of body weight. Following a conservative approach by applying the lowest level of the range (0.4 mg/kg of body weight), a body weight of 5 kg for an infant, and a drinking-water consumption of 0.75 litre, a guideline value for nitrite ion of 3 mg/litre (rounded figure) can be derived. The guideline value is no longer provisional.

Because of the possibility of the simultaneous occurrence of nitrite and nitrate in drinking-water, the sum of the ratios of the concentrations (C) of each to its guideline value (GV) should not exceed one, i.e.:

$$\frac{C_{nitrite}}{GV_{nitrite}} + \frac{C_{nitrate}}{GV_{nitrate}} \leq 1$$

It seems prudent to propose a guideline value for nitrite associated with chronic exposure based on JECFA's analysis of animal data showing nitrite-induced morphological changes in the adrenals, heart, and lungs. Using JECFA's ADI of 0.06 mg/kg of body weight per day, assuming a 60-kg adult ingesting 2 litres of drinking-water per day, and allocating 10% of the ADI to drinking-water, a guideline value of 0.2 mg of nitrite ion per litre (rounded figure) can be calculated. However, owing to the uncertainty surrounding the relevance of the observed adverse health effects for humans and the susceptibility of humans compared with animals, this guideline value should be considered provisional.

Because of known interspecies variation in the conversion of nitrate to nitrite, the animal model was not considered appropriate for use in human risk assessment for nitrate.

Chloramination may give rise to the formation of nitrite within the distribution system, and the concentration of nitrite may increase as the water moves towards the extremities of the system. All water systems that practise chloramination should closely and regularly monitor their systems to verify disinfectant levels, microbiological quality, and nitrite levels. If nitrification is detected (e.g. reduced disinfectant residuals and increased nitrite levels), steps should be taken to modify the treatment train or water chemistry in order to maintain a safe water quality. Efficient disinfection must never be compromised.

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Uranium[1]

[1] This review addresses only the chemical aspects of uranium toxicity. Information pertinent to the derivation of a guideline based on radiological effects is presented in the second edition of the Guidelines for drinking-water quality.

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At the time of publication of the 1993 *Guidelines for drinking-water quality*, adequate short- and long-term studies on the chemical toxicity of uranium were unavailable, and therefore a guideline value for uranium was not derived. Instead, it was recommended that the limits for radiological characteristics of uranium be adopted. The equivalent for natural uranium, based on these limits, is approximately 140 µg/litre.

As new data on the chemical toxicity of uranium are now available for use in the derivation of a guideline value, the 1995 Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* recommended that uranium be included in the 1998 Addendum.

1. GENERAL DESCRIPTION

1.1 Identity

Uranium occurs naturally in the +2, +3, +4, +5, and +6 valence states, but it is most commonly found in the hexavalent form. In nature, hexavalent uranium is commonly associated with oxygen as the uranyl ion, UO_2^{2+}. Naturally occurring uranium (^{nat}U) is a mixture of three radionuclides (^{234}U, ^{235}U, and ^{238}U), all of which decay by both alpha and gamma emissions (Cothern & Lappenbusch, 1983; Lide, 1992-93). Natural uranium consists almost entirely of the ^{238}U isotope, with the ^{235}U and ^{234}U isotopes respectively comprising about 0.72% and 0.0054% of natural uranium (Greenwood & Earnshaw, 1984). Uranium is widespread in nature, occurring in granites and various other mineral deposits (Roessler et al., 1979; Lide, 1992-93).

Compound	*CAS no.*	*Molecular formula*
Uranium	7440-61-1	U
Uranyl ethanoate	541-09-3	$C_4H_6O_6U$
Uranyl chloride	7791-26-6	Cl_2O_2U
Uranyl nitrate	36478-76-9	N_2O_8U
Uranium dioxide	1344-57-6	UO_2

1.2 Physicochemical properties (Lide, 1992-93)

Compound	*Melting point (°C)*	*Boiling point (°C)*	*Density at 20°C (g/cm^3)*	*Water solubility (g/litre)*
U	1132	3818	19.0	insoluble
$C_4H_6O_6U$	110	275 (decomposes)	2.9	76.94
Cl_2O_2U	578	(decomposes)	-	3200
N_2O_8U	60.2	118	2.8	soluble
UO_2	2878	-	10.96	insoluble

1.3 Major uses

Uranium is used mainly as fuel in nuclear power stations, although some uranium compounds are also used as catalysts and staining pigments (Berlin & Rudell, 1986).

1.4 Environmental fate

Uranium is present in the environment as a result of leaching from natural deposits, release in mill tailings, emissions from the nuclear industry, the combustion of coal and other fuels, and the use of phosphate fertilizers that contain uranium.

2. ANALYTICAL METHODS

Uranium in water is most commonly measured by solid fluorimetry with either laser excitation or ultraviolet light following fusion of the sample with a pellet of carbonate and sodium fluoride (detection limit 0.1 µg/litre) (Kreiger & Whittaker, 1980). Sample preparation for this method is tedious, however, and there is interference from other metals. Uranium can also be determined by inductively coupled plasma mass spectrometry, which has the same detection limit (0.1 µg/litre) and a between-run precision of less than 6% (Boomer & Powell, 1987). Alpha-spectrometry has been used for the determination of uranium in bottled waters (Gans, 1985) and environmental media (Singh & Wrenn, 1988), although the recovery is often highly variable owing to the low specific activity of natural uranium (Singh & Wrenn, 1988).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Mean levels of uranium in ambient air have been reported to be 0.02 ng/m^3 in Tokyo (based on a 1979-1981 survey) (Hirose & Sugimura, 1981) and 0.076 ng/m^3 in New York (based on two samples, each a composite of two weekly air filter collections, from 1985 and 1986) (Fisenne et al., 1987). On the assumption of a daily respiratory volume of 20 m^3 and a mean urban airborne concentration of 0.05 ng/m^3, the daily intake of uranium from air would be about 1 ng. Tobacco smoke (from two packages of cigarettes per day) contributes less than 50 ng of inhaled uranium per day (Lucas & Markun, 1970).

3.2 Water

In a survey of 130 sites (approximately 3700 samples) in Ontario, Canada, conducted between 1990 and 1995, the mean of the average uranium concentrations (range 0.05-4.21 µg/litre; detection limit 0.05 µg/litre) in treated drinking-water was 0.40 µg/litre (OMEE, 1996). Uranium concentrations of up to 700 µg/litre have been found in private supplies in Canada (Moss et al., 1983; Moss, 1985). The mean concentration of uranium in drinking-water in New York City, USA, ranged from 0.03 to 0.08 µg/litre (Fisenne & Welford, 1986). A mean uranium concentration of 2.55 µg/litre was reported in drinking-water from 978 sites in the USA in the 1980s (US EPA, 1990, 1991). In five Japanese cities, the mean level in potable water supplies was 0.9 ng/litre (Nozaki et al., 1970).

The daily uranium intake from water in Finland has been estimated to be 2.1 µg (Kahlos & Asikainen, 1980). The daily intake from drinking-water in Salt Lake City, USA, is estimated to be 1.5 µg (Singh et al., 1990). On the basis of the results of the survey from Ontario (OMEE, 1996), the daily intake of uranium from drinking-water in Canada is estimated to be 0.8 µg.

3.3 Food

Uranium has been detected in a variety of foodstuffs. The highest concentrations are found in shellfish, and lower levels have been measured in fresh vegetables, cereals, and fish. The average per capita intake of uranium in food has been reported to be 1.3 µg/day (Fisenne et al., 1987) and 2-3 µg/day (Singh et al., 1990) in the USA and 1.5 µg/day in Japan (Nozaki et al., 1970).

In a review of naturally occurring sources of radioactive contamination in food, dietary intakes of ^{238}U were found to range from 12 to 45 mBq/day in several European countries, from 11 to 60 mBq/day in Japan (the higher values were found in uranium mining areas), and from 15 to 17 mBq/day in the USA. The average daily dietary intake was in the order of 20 mBq, or about 4 µg. It was often difficult to determine whether these dietary intakes included intake from drinking-water, and it was emphasized that intake from drinking-water has sometimes been found to be equal to intake from the diet (Harley, 1988).

In a study by Cheng et al. (1993), the mean uranium concentration in nine different beverages was 0.98 µg/litre (range 0.26-1.65 µg/litre), and the mean concentration of uranium in mineral water was 9.20 µg/litre.

Landa & Councell (1992) performed leaching studies to determine the quantity of uranium leaching from 33 glass items and two ceramic items in which uranium was used as a colouring agent. Uranium-bearing glasses leached a maximum of 30 µg of uranium per litre, whereas the ceramic-glazed items released approximately 300 000 µg of uranium per litre.

3.4 Estimated total exposure and relative contribution of drinking-water

The daily intake of uranium from each source for adults is estimated to be: air, 0.001 µg; drinking-water, 0.8 µg; food, 1.4 µg. Thus, the total daily intake is approximately 2.2 µg, or 0.037 µg/kg of body weight for a 60-kg adult, the majority of which originates from food.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Although ubiquitous in the environment, uranium has no known metabolic function in animals and is currently regarded as non-essential (Berlin & Rudell, 1986). Absorption of uranium from the gastrointestinal tract depends upon the solubility of the uranium compound (Berlin & Rudell, 1986), previous food consumption (Sullivan et al., 1986; La Touche et al., 1987), and the concomitant administration of oxidizing agents, such as the iron(III) ion and quinhydrone (Sullivan et al., 1986). The average human gastrointestinal absorption of uranium is 1-2% (Wrenn et al., 1985).

The absorption of a uranium dose of approximately 800 mg/kg of body weight in starved female Sprague-Dawley rats increased from 0.17 to 3.3% when iron(III) (190 mg/kg of body weight) was administered simultaneously (Sullivan et al., 1986). Absorption of uranium in starved rats administered doses of uranium by gavage was reported to increase with dose; the degree of absorption ranged from 0.06 to 2.8% for doses between 0.03 and 45 mg of uranium per kg of body weight (La Touche et al., 1987). Only 0.06% of ingested uranium was absorbed in Sprague-Dawley rats and New Zealand white rabbits fed *ad libitum* and having free access to drinking-water containing up to 600 mg of uranyl nitrate hexahydrate per litre for up to 91 days (Tracy et al., 1992).

Following ingestion, uranium rapidly appears in the bloodstream (La Touche et al., 1987), where it is associated primarily with the red cells (Fisenne & Perry, 1985); a non-diffusible uranyl-albumin complex also forms in equilibrium with a diffusible ionic uranyl hydrogen carbonate complex (UO_2HCO^{3+}) in the plasma (Moss, 1985). Because of their high affinity for phosphate, carboxyl, and hydroxyl groups, uranyl compounds readily combine with proteins and nucleotides to form stable complexes (Moss, 1985). Clearance from the bloodstream is also rapid, and the uranium subsequently accumulates in the kidneys and the skeleton, whereas little is found in the liver (La Touche et al., 1987). The skeleton is the major site of uranium accumulation (Wrenn et al., 1985); the uranyl ion replaces calcium in the hydroxyapatite complex of bone crystals (Moss, 1985).

Based on the results of studies in experimental animals, it appears that the amount of soluble uranium accumulated internally is proportional to the intake from ingestion or inhalation. It has

been estimated that the total body burden of uranium in humans is 40 µg, with approximately 40% of this being present in the muscles, 20% in the skeleton, and 10%, 4%, 1%, and 0.3% in the blood, lungs, liver, and kidneys, respectively (Igarashi et al., 1987).

Once equilibrium is attained in the skeleton, uranium is excreted in the urine and faeces. Urinary excretion in humans has been found to account for approximately 1% of total excretion, averaging 4.4 µg/day (Singh et al., 1990), the rate depending in part on the pH of tubular urine (Berlin & Rudell, 1986). Under alkaline conditions, most of the uranyl hydrogen carbonate complex is stable and is excreted in the urine. If the pH is low, the complex dissociates to a variable degree, and the uranyl ion may then bind to cellular proteins in the tubular wall, which may then impair tubular function.

The half-life of uranium in the rat kidney has been estimated to be approximately 15 days. Clearance from the skeleton is considerably slower; half-lives of 300 and 5000 days have been estimated, based on a two-compartment model (Wrenn et al., 1985). In another study using a 10-compartment model, overall half-lives for the clearance of uranium from the rat kidney and skeleton were determined to be 5-11 and 93-165 days, respectively (Sontag, 1986). The overall elimination half-life of uranium under conditions of normal daily intake has been estimated to be between 180 and 360 days (Berlin & Rudell, 1986).

5. EFFECTS ON EXPERIMENTAL ANIMAL AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Reported oral LD_{50}s of uranyl ethanoate dihydrate for rats and mice are 204 and 242 mg/kg of body weight, respectively (Domingo et al., 1987). Among the most common signs of acute toxicity are piloerection, significant weight loss, and haemorrhages in the eyes, legs, and nose.

The most common renal injury caused by uranium in experimental animals is damage to the proximal convoluted tubules, predominantly in the distal two-thirds (Berlin & Rudell, 1986; Anthony et al., 1994; Domingo, 1995); the rate of effects varies with dosage level (Leggett, 1989). It has recently been shown that uranyl inhibits both Na^+ transport-dependent and Na^+ transport-independent ATP utilization as well as mitochondrial oxidative phosphorylation in the renal proximal tubule (Leggett, 1989; Domingo, 1995). At doses not high enough to destroy a critical mass of kidney cells, the effect appears to be reversible, as some of the cells are replaced; however, the new epithelial lining differs morphologically, and possibly functionally, from normal epithelium (Wrenn et al., 1985; Berlin & Rudell, 1986). Histopathologically, the regenerated cells are simple flattened cells with no microvilli on luminal surfaces and with reduced numbers of mitochondria (Leggett, 1989).

There is some evidence that tolerance may develop following repeated exposure to uranium (Yuile, 1973; Durbin & Wrenn, 1976; Campbell, 1985). This tolerance does not, however, prevent chronic damage to the kidney, as the regenerated cells are quite different; although histopathologically it may appear that the repair process is well advanced, the urinary biochemical changes return to normal only slowly (Leggett, 1989). Alterations causing thickening of the glomerular basement membrane of the kidney, which results from the storage of uranium in the kidney, can be prolonged and severe enough to cause permanent damage (McDonald-Taylor et al., 1992). Persistent ultrastructural changes in the proximal tubules of rabbits have also been reported to be associated with the kidney's ability to store uranium (McDonald-Taylor et al., 1997). Cell damage in the proximal tubules was significantly more severe in animals allowed up to a 91-day recovery period than in animals in the no-recovery group.

5.2 Short-term exposure

Forty male Sprague-Dawley rats given 0, 2, 4, 8, or 16 mg of uranyl ethanoate dihydrate per kg of body weight per day (equivalent to doses of 0, 1.1, 2.2, 4.5, or 9.0 mg of uranium per kg of body

weight per day) in drinking-water for 2 weeks exhibited a variety of biochemical effects, including increases in blood glucose levels at ≥4 mg of uranyl ethanoate dihydrate per kg of body weight per day, decreases in aspartate aminotransferase and alanine aminotransferase values at ≥8 mg of uranyl ethanoate dihydrate per kg of body weight per day, increases in several other haematological parameters at 16 mg of uranyl ethanoate dihydrate per kg of body weight per day, and increases in total protein levels in all treated groups (Ortega et al., 1989). The authors considered the NOAEL to be 2 mg of uranyl ethanoate dihydrate per kg of body weight per day (1.1 mg of uranium per kg of body weight per day).

Groups of 15 male and 15 female weanling Sprague-Dawley rats consumed water containing <0.001 (control), 0.96, 4.8, 24, 120, or 600 mg of uranyl nitrate hexahydrate per litre (equivalent to doses of <0.0001, 0.06, 0.31, 1.52, 7.54, and 36.73 mg of uranium per kg of body weight per day in males and <0.0001, 0.09, 0.42, 2.01, 9.98, and 53.56 mg of uranium per kg of body weight per day in females) for 91 days (Gilman et al., 1997a). Histopathological changes were observed mainly in the liver, thyroid, and kidney. In the liver, treatment-related lesions were seen in both sexes at all doses and were generally non-specific nuclear and cytoplasmic changes. The thyroid lesions were not considered specific to the uranium treatment. The kidney was the most affected tissue. In males, statistically significant treatment-related kidney lesions (reported at all doses) included nuclear vesiculation, cytoplasmic vacuolation, and tubular dilation. Other statistically significant lesions in males (≥4.8 mg of uranyl nitrate hexahydrate per litre) included glomerular adhesions, apical displacement of the proximal tubular epithelial nuclei, and cytoplasmic degranulation. In females, statistically significant changes in the kidney included nuclear vesiculation of the tubular epithelial nuclei (all doses) and anisokaryosis (all doses except 4.8 mg of uranyl nitrate hexahydrate per litre). However, the most important changes in the female were the capsular sclerosis of glomeruli and reticulin sclerosis of the interstitial membranes; these changes occurred in all dose groups and are considered to be “nonreparable lesions.” Significant treatment-related liver changes were also reported in hepatic nuclei and cytoplasm in both sexes at the lowest exposure level. The LOAEL for adverse effects on the kidney and liver of male and female rats, based on the frequency of degree of degenerative lesions in the renal proximal convoluted tubule, was considered to be 0.96 mg of uranyl nitrate hexahydrate per litre (equivalent to 0.09 mg of uranium per kg of body weight per day in females and 0.06 mg of uranium per kg of body weight per day in males). The reason for the difference in sensitivity between males and females is not clear, but it did not appear to be due to differences in pharmacokinetics, as accumulation of uranium in renal tissue did not differ significantly between the two sexes at all doses.

In a similar study, groups of 10 male New Zealand white rabbits were given uranyl nitrate hexahydrate in drinking-water at concentrations of <0.001 (controls), 0.96, 4.8, 24, 120, or 600 mg/litre (determined to be equivalent to doses of 0, 0.05, 0.2, 0.88, 4.82, and 28.7 mg of uranium per kg of body weight per day) for 91 days (Gilman et al., 1997b). Histopathological changes were observed in the kidney tubule, liver, thyroid, and aorta. Histopathological findings were observed in the kidney tubules at doses above 0.96 mg of uranyl nitrate hexahydrate per litre. When compared with controls, significant treatment-related changes included cytoplasmic vacuolation, anisokaryosis, nuclear pyknosis, and nuclear vesiculation; the incidence of nuclear vesiculation and anisokaryosis appeared to be dose-related, with nuclear vesiculation having the higher frequency and severity. Other treatment-related changes included tubular dilation, hyperchromicity, tubular atrophy, changes in the interstitium collagen, and reticulin sclerosis. In total, 11 different morphological indicators of tubular injury were observed in the highest exposure group. The LOAEL, based on the nuclear changes in the kidney, was considered to be 0.96 mg of uranyl nitrate hexahydrate per litre (equivalent to 0.05 mg of uranium per kg of body weight per day). It should be noted, however, that these rabbits were not *Pasteurella*-free, and four of them contracted a *Pasteurella* infection during the course of the study. In the same study, 10 *Pasteurella*-free female rabbits were exposed to drinking-water containing <0.001 (controls), 4.8, 24, or 600 mg of uranyl nitrate hexahydrate per litre (equivalent to doses of 0, 0.49, 1.32, and 43.02 mg of uranium per kg of body weight per day) for 91 days. Dose-related and treatment-related nuclear changes in the kidney tubule included anisokaryosis and vesiculation, which were

significantly different from effects observed in controls at all doses. Other treatment-related changes in the kidney included cytoplasmic vacuolation, tubular atrophy, and nuclear pyknosis. In general, histopathological changes in the kidney in females were generally less marked than in males. The LOAEL was considered to be 4.8 mg of uranyl nitrate hexahydrate per litre (equivalent to 0.49 mg of uranium per kg of body weight per day). In both sexes, histopathological changes in the liver, thyroid, and aorta were similar. In the liver, changes may have been treatment-related, although very mildly affected animals were seen in all groups, and changes in the thyroid were mild. Changes in the aorta were not dose-dependent. It should be noted that no similar aortic changes were observed in the 91-day uranyl nitrate hexahydrate studies in rats (Gilman et al., 1997a). It is interesting to note, however, that even though the female rabbits consumed on average 65% more water than the males and their average uranium intake was approximately 50% greater on a mg/kg of body weight per day basis, their average tissue levels were not similarly raised. The differences between the males and females, both qualitative and quantitative, suggest pharmacokinetic parameter differences, which contrasts with the findings in the rat study by the same authors (Gilman et al., 1997a).

In an additional study to observe the reversibility of renal injury in *Pasteurella*-free male New Zealand white rabbits, groups of 5-8 animals were given <0.001 (control), 24, or 600 mg of uranyl nitrate hexahydrate per litre (equivalent to 0, 1.36, and 40.98 mg of uranium per kg of body weight per day) in drinking-water for 91 days, with a recovery period of up to 91 days (Gilman et al., 1997c). Minor histopathological lesions were seen in the liver, thyroid, and aorta. In the kidney, tubular injury with degenerative nuclear changes, cytoplasmic vacuolation, and tubular dilation was observed in the high-dose group, which did not exhibit consistent resolution even after a 91-day recovery period. In general, the male rabbits did not respond as dramatically as those in the earlier study (Gilman et al., 1997b), although the histopathological changes observed in this study were similar to those noted in the female rabbits of the previous study. Animals in this study consumed approximately 33% more uranium per day than the males in the previous study (Gilman et al., 1997b), yet uranium residues in kidney tissue were 30% less, which would appear to indicate that *Pasteurella*-free rabbits are less sensitive than the non-*Pasteurella*-free strain to the effects of the uranyl ion in drinking-water. Based on the histopathological data in the kidney, a LOAEL for the male New Zealand rabbits in this study is estimated to lie between 24 and 600 mg of uranyl nitrate hexahydrate per litre.

5.3 Long-term exposure

In an early series of experiments, very high doses (up to 20% in the diet) of a variety of uranium compounds were fed to rats, dogs, and rabbits for periods ranging from 30 days to 2 years (Maynard and Hodge, 1949). On the basis of very limited histopathological investigations, renal damage was reported in each species.

5.4 Reproductive and developmental toxicity

Adverse reproductive effects, in terms of total number of litters and average number of young per litter, were reported in rats given 2% uranyl nitrate hexahydrate for 7 months (Maynard & Hodge, 1949). More recent studies have examined the teratogenic/embryotoxic effects and reproductive outcomes of uranyl acetate dihydrate in Swiss albino mice. Domingo et al. (1989a) evaluated the developmental toxicity of uranium by treating groups of 20 pregnant Swiss mice by gavage to doses of 0, 5, 10, 25, or 50 mg of uranyl acetate dihydrate per kg of body weight per day (equivalent to 0, 2.8, 5.6, 14, and 28 mg of uranium per kg of body weight per day) on days 6-15 of gestation; the animals were sacrificed on day 18. Although all dams survived, there was a dose-related reduction in maternal weight gain, a significant decrease in daily feed intake, and a significant increase in liver weights. Exposure-related fetotoxicity, including reduced fetal body weights and length, increased incidence of stunted fetuses per litter, increased incidence of both external and internal malformations, and increased incidence of developmental variations, was observed in the fetuses of mice at ≥2.8 mg of uranium per kg of body weight per day. At doses ≥14 mg of uranium per kg of body weight per day, specific malformations included cleft palate and

bipartite sternebrae, and developmental variations included reduced ossification and unossified skeletal variations. There was no evidence of embryolethality at any dose. Based on both the maternal and fetotoxic effects, a LOAEL of 2.8 mg of uranium per kg of body weight per day could be considered.

A second study by Domingo et al. (1989b) evaluated the effect of uranium on late fetal development, parturition, lactation, and postnatal viability. Groups of 20 female mice were treated by gavage from day 13 of pregnancy until day 21 of lactation to doses of 0, 0.05, 0.5, 5, or 50 mg of uranyl acetate dihydrate per kg of body weight per day (equivalent to 0, 0.028, 0.28, 2.8, and 28 mg of uranium per kg of body weight per day). Maternal deaths (2/20 at 2.8 mg of uranium per kg of body weight per day, and 3/20 at 28 mg of uranium per kg of body weight per day) were attributed to the treatment; however, maternal toxicity was not evident from changes in body weight or food consumption, although relative liver weight was significantly reduced in all treatment groups. Decreases in pup viability, as indicated by significant decreases in litter size on day 21 of lactation, and significant decreases in the viability and lactation indexes were observed in the high-dose group. Based on developmental effects in pups, a NOEL of 2.8 mg of uranium per kg of body weight per day was established.

Paternain et al. (1989) studied the effects of uranium on reproduction, gestation, and postnatal survival in mice. Groups of 25 mature male Swiss mice were exposed to oral doses of 0, 5, 10, or 25 mg of uranyl acetate dihydrate per kg of body weight per day (equivalent to 0, 2.8, 5.6, and 14 mg of uranium per kg of body weight per day) for 60 days prior to mating with mature females (25 per group). Females were exposed for 14 days prior to mating, and exposure continued through mating, gestation, parturition, and nursing of litters; half the treated dams were sacrificed on day 13 of gestation. No treatment-related effects on mating or fertility were observed. Embryolethality (number of late resorptions and dead fetuses) was significantly increased and the number of live fetuses was decreased in the high-dose group. Lethality in pups (at birth and at day 4 of lactation) was significantly increased at $\geq$5.6 mg of uranium per kg of body weight per day, and pup growth (decreases in weight and length) and development of offspring, from birth and during the entire lactation period, were significantly affected in the high-dose group.

Unspecified degenerative changes in the testes of rats have also been reported following chronic administration of uranyl nitrate hexahydrate and uranyl fluoride in the diet (Maynard and Hodge, 1949; Maynard et al., 1953; Malenchenko et al., 1978). In a more recent study, male Swiss mice were exposed for 64 days to uranyl acetate dihydrate in drinking-water at doses of 0, 10, 20, 40, or 80 mg/kg of body weight per day (equivalent to 0, 5.6, 11.2, 22.4, and 44.8 mg of uranium per kg of body weight per day) prior to mating with untreated females for 4 days (Llobet et al., 1991). With the exception of interstitial alterations and vacuolization of Leydig cells at the highest dose, no effects were observed in testicular function/spermatogenesis. There was, however, a significant, non-dose-related decrease in the pregnancy rate of these animals.

5.5 Mutagenicity and related end-points

Uranyl nitrate was cytotoxic and genotoxic in Chinese hamster ovary cells at concentrations ranging from 0.01 to 0.3 mmol/litre. There was a dose-related decrease in the viability of the cells, a decrease in cell cycle kinetics, and increased frequencies of micronuclei, sister chromatid exchanges, and chromosomal aberrations (Lin et al., 1993). The authors suggest that the data provide a possible mechanism for the teratogenic effects observed in the studies by Domingo et al. (1989a). The genotoxic effects in this study were thought to occur through the binding of the uranyl nitrate to the phosphate groups of DNA. Chromosomal aberrations have also been induced in male mouse germ cells exposed to enriched uranyl fluoride; however, these aberrations may have been produced by the radioactivity of the test compound (Hu & Zhu, 1990).

5.6 Carcinogenicity

Although bone cancer has been induced in experimental animals by injection or inhalation of

soluble compounds of high-specific-activity uranium isotopes or mixtures of uranium isotopes, no carcinogenic effects have been reported in animals ingesting soluble or insoluble uranium compounds (Wrenn et al., 1985).

6. EFFECTS ON HUMANS

Nephritis is the primary chemically induced effect of uranium in humans (Hursh & Spoor, 1973).

Little information is available on the chronic health effects of exposure to environmental uranium in humans. In Nova Scotia, Canada, clinical studies were performed on 324 persons exposed to variable amounts of naturally occurring uranium in drinking-water (up to 0.7 mg/litre) supplied from private wells. No relationship was found between overt renal disease or any other symptomatic complaint and exposure to uranium. However, a trend towards increasing excretion of urinary $ß_2$-microglobulin and increasing concentration of uranium in well-water was observed; this raises the possibility that an early tubular defect was present and suggests that this parameter might be useful as an index of subclinical toxicity. The group with the highest uranium concentrations in well-water failed to follow this trend, but this was attributed to the fact that most of the individuals in this group had significantly reduced their consumption of well-water by the time the measurements were made, leading to the conclusion that the suspected tubular defect might well be rapidly reversible (Moss et al., 1983; Moss, 1985).

In a pilot study conducted in 1993 in three communities in Saskatchewan, Canada, there was a statistically significant association (p = 0.03) between increasing but normal levels of urine albumin (measured as mg/mmol creatinine) and the uranium cumulative index. The cumulative index was calculated for each study participant as the product of the uranium concentration in drinking-water, the number of cups of water consumed per day, and the number of years lived at the current residence (Mao et al., 1995). The study was conducted with 100 participants in three different areas with mean uranium levels ranging from 0.71 (control) to 19.6 µg/litre. Urine albumin levels ranged from 0.165 to 16.1 mg/mmol creatinine, with eight participants having "elevated" urine albumin concentrations (>3.0 mg/mmol creatinine). Three participants had serum creatinine concentrations of >120 µmol/litre (range 50-170 µmol/litre), which is reportedly indicative of prevalent renal damage. It should be noted, however, that diabetics were not excluded from the study, although diabetic status and age, known risk factors for renal dysfunction, were factored into the statistical analysis of the results. According to the authors, microalbuminuria has been shown to be a sensitive indicator of early renal disease.

7. PROVISIONAL GUIDELINE VALUE

There are insufficient data regarding the carcinogenicity of uranium in humans and experimental animals. The guideline value for the chemical toxicity of uranium was therefore derived using a TDI approach. As no adequate chronic study was identified, the TDI was derived using the results of the most extensive subchronic study conducted to date in which uranium was administered in drinking-water to the most sensitive sex and species (Gilman et al., 1997a). In the 91-day study in rats, the LOAEL for degenerative lesions in the proximal convoluted tubule of the kidney in males was considered to be 0.96 mg of uranyl nitrate hexahydrate per litre, which is equivalent to 0.06 mg of uranium per kg of body weight per day.

A TDI of 0.6 µg/kg of body weight per day was derived using the LOAEL of 60 µg/kg of body weight per day and an uncertainty factor of 100 (for intra- and interspecies variation). There is no need to apply an additional uncertainty factor to account for the use of a LOAEL instead of a NOAEL because of the minimal degree of severity of the lesions being reported. Also, an additional uncertainty factor for the length of the study (91-day) is not required because the estimated half-life of uranium in the kidney is 15 days, and there is no indication that the severity of the renal lesions will be exacerbated following continued exposure.

This TDI yields a guideline value of 2 µg/litre (rounded figure), assuming a 60-kg adult consuming

2 litres of drinking-water per day and a 10% allocation of the TDI to drinking-water. This value would be protective, based on associations for subclinical renal effects reported in preliminary epidemiological studies.

Several methods are available for the removal of uranium from drinking-water, although some of these methods have been tested at laboratory or pilot scale only. Coagulation using ferric sulfate or aluminium sulfate at optimal pH and coagulant dosages can achieve 80-95% removal of uranium, whereas at least 99% removal can be achieved using lime softening, anion exchange resin, or reverse osmosis processes. In areas with high natural uranium levels, a value of 2 µg/litre may be difficult to achieve with the treatment technology available (WRc, 1997).

The guideline value for uranium is provisional because it may be difficult to achieve with the treatment technology available, because of limitations in the key study, namely the lack of a dose-response relationship (no NOEL) despite the wide range of administered doses, and because of insufficient information on the degree and severity of the pathological examinations. It should be noted that there are several human studies under way that may provide helpful additional data.

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Organic constituents

Cyanobacterial toxins: Microcystin-LR

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No guideline values were proposed for cyanobacterial toxins in the second edition of the WHO *Guidelines for drinking-water quality*. As microcystins (produced by cyanobacteria, or blue-green algae) are extremely toxic and are often associated with poisonings in humans and animals, the Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* decided that guideline values for cyanobacterial toxins were needed.

1. GENERAL DESCRIPTION

1.1 Identity

The cyanobacteria, also known as blue-green algae, owe their name to the presence of photosynthetic pigments. Cyanobacteria are a major group of bacteria that occur throughout the world. Freshwater cyanobacteria may accumulate in surface water supplies as “blooms” and may

concentrate on the surface as blue-green “scums.”

Some species of cyanobacteria produce toxins, which are classified according to their mode of action into hepatotoxins (e.g. microcystins), neurotoxins (e.g. anatoxins), skin irritants, and other toxins. Both hepatotoxins and neurotoxins are produced by cyanobacteria commonly found in surface water and therefore are of relevance to water supplies (Carmichael, 1992; Fawell et al., 1993).

The hepatotoxins are produced by various species within the genera *Microcystis*, *Anabaena*, *Oscillatoria*, *Nodularia*, *Nostoc*, *Cylindrospermopsis*, and *Umezakia*, although not all strains do so (Fawell et al., 1993; AWWA, 1995). Most hepatotoxins (all cyclic heptapeptides) are microcystins. At least 50 congeners of microcystins are known (Carmichael, 1994), and several of these may be produced during a bloom. The chemical structure of microcystins includes two variable amino acids and an unusual aromatic amino acid, ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), containing a substituted phenyldecadienoic acid (Botes et al., 1985). Different microcystins have different lipophilicities and polarities, which could affect their toxicity. Microcystin-LR was the first microcystin chemically identified; to date, most work has been conducted using this microcystin. It has been associated with most of the incidents of toxicity involving microcystins in most countries (Fawell et al., 1993). Microcystin-LR is a cyclic heptapeptide with a molecular weight of about 1000 daltons.

Neurotoxins are not considered as widespread in water supplies, and they do not appear to pose the same degree of risk from chronic exposure as microcystins (Fawell et al., 1993; AWWA, 1995). The neurotoxins, such as anatoxin-a and -a(s), are highly toxic nerve poisons but have short biological half-lives. On acute exposure, the neurotoxins cause death within minutes to a few hours, depending on the species, the amount of toxin ingested, and the amount of food in the stomach (Carmichael, 1992). Dog poisonings in Scotland were reported to be due to the consumption of *Oscillatoria* containing anatoxin-a (Codd, 1992).

Toxic cyanobacteria also produce cytotoxic alkaloids, the most recently described being from the species *Cylindrospermopsis raciborskii*, which occurs in freshwater lakes, rivers, and drinking-water storage reservoirs in tropical areas. The molecular structure is a tricyclic guanidine linked to a hydroxymethyl uracil with a molecular weight of 415 daltons (Ohtani et al., 1992). These alkaloids have been implicated in a variety of health effects, ranging from gastroenteritis to kidney disease (Falconer, 1994).

1.2 Occurrence and growth of cyanobacteria

The occurrence of a particular genus and species of cyanobacteria around the world is apparently influenced by regional differences in water chemistry and climatic conditions. For example, *Cylindrospermopsis* is produced in tropical waters but has not been found in temperate climates. Similarly, *Microcystis* and *Anabaena* blooms occur widely in the temperate regions of the world (AWWA, 1995). In general, 50-75% of bloom isolates can produce toxins, often with more than one toxin being present. Toxic and non-toxic blooms of the same species can be found together (Skulberg et al., 1993; AWWA, 1995; Codd & Bell, 1996). The overall toxicity of a bloom can be uncertain, because variations can occur in toxin concentration over a short time and spatially within a water body experiencing a bloom (Hrudey et al., 1994). There is no simple method to distinguish the toxic from the non-toxic forms. The unpredictability of toxin production within any given bloom “renders them potentially dangerous and suspect at all times” (Ressom et al., 1994), and prevention of cyanobacterial blooms is therefore the key to the control of toxic blooms.

The growth of cyanobacteria and the formation of blooms are influenced by physical, chemical, and biological factors, which were recently reviewed by Pearson et al. (1990), Ressom et al. (1994), and AWWA (1995) and are discussed below. As a result of the interplay of these factors, there may be large yearly fluctuations in the levels of cyanobacteria and their toxins. There is also a seasonal variation in predominating species.

Cyanobacterial blooms persist in water supplies that contain adequate levels of essential inorganic nutrients such as nitrogen and phosphorus, water temperatures generally between 15 and 30°C, and pH levels between 6 and 9. Blooms usually occur in late summer or early fall and are most common in eutrophic or hypereutrophic bodies of water.

The amount of daylight needed to optimize growth depends on the species. In addition, some cyanobacteria, such as *Microcystis aeruginosa*, can regulate their buoyancy in response to available light. This characteristic allows cyanobacteria to migrate through thermal gradients and use nutrients confined to cooler deeper water below. Buoyancy is controlled mainly through the production of carbohydrates from photosynthesis. This control mechanism breaks down if there is too little carbon dioxide available. Although buoyancy cannot be adjusted during the night, the organisms will float to the surface because of their reduced carbohydrate content as a result of respiration at night.

Turbulence and high water flows are unfavourable to the growth of cyanobacteria, as they interfere with the organisms' ability to maintain a position in the water column. Heavy rain storms can increase runoff and nutrient levels in the water, which encourages the formation of blooms.

The formation of surface scum is enhanced by calm weather conditions. Initially, there may be high barometric pressure and light to moderate winds, accompanied by constant circulation in a water body in which large numbers of cyanobacteria are maintaining their position in the water column to take advantage of those conditions. If the wind stops and circulation also stops, the cyanobacteria may suddenly become "overbuoyant." If they cannot adjust their buoyancy fast enough or at all (at night), then the blooms will float to the surface and form surface scum. Thus, scums are often formed overnight. The scum may drift downwind and may settle at lee shores and quiet bays, where the cyanobacteria may release their toxins and eventually die (Ressom et al., 1994).

1.3 Toxin production and persistence

The two main factors that have been shown to affect toxin production are light and temperature. The optimum temperature for toxin production in cyanobacteria is between 20 and 25°C (Gorham, 1964; van der Westhuizen & Eloff, 1985; Watanabe & Oishi, 1985), which suggests that cyanobacteria are most toxic during periods with warm weather and in areas with warm climates. However, the optimum temperature may change from country to country. Light intensity more than light quality is an important factor in toxin production in *M. aeruginosa*. Both toxicity and the ratio of the toxin to protein production are enhanced by both red and green light compared with white light (Utkilen & Gjølme, 1992). The toxicity of cyanobacteria increases with an increase in light intensity below 40 microeinsteins/m^2 per second (van der Westhuizen & Eloff, 1985; Watanabe & Oishi, 1985; Utkilen & Gjølme, 1992) and therefore decreases with water depth (Utkilen & Gjølme, 1992). However, when mixing of water from different depths occurs, especially during periods of high winds, this may not be true.

Some laboratory studies have shown that pH, nitrogen, phosphorus, and carbon dioxide could also influence the growth of microcystins. The presence of six different microcystins in floating scums of *M. aeruginosa* in Hartbeespoort Dam, South Africa, was monitored for 2.5 years. The toxins were either not detectable or in low concentrations during the winter and reached maximum concentrations during the summer. The total concentrations of four of the toxins (5-415 µg/g dry scum) were directly correlated to solar radiation, surface water temperature, pH, and per cent oxygen saturation. No significant correlations were found between total toxin concentrations in scum samples and organic and inorganic nutrient concentrations in surface water (Wicks & Theil, 1990).

Kotak et al. (1995) studied the patterns of occurrence of microcystin-LR (measured as µg/g biomass of *M. aeruginosa* by high-performance liquid chromatography, or HPLC) in three

hypereutrophic hard-water lakes in central Alberta, Canada, over three seasons. *Microcystis aeruginosa* was highly variable temporally (differences up to 3 orders of magnitude) within each lake over 1 year, between years in an individual lake, and between lakes in a year. Seasonal changes in microcystin-LR concentration were positively correlated to the abundance and biomass of the *M. aeruginosa,* total and total dissolved phosphorus concentration, pH, and chlorophyll. Surprisingly, there was a negative correlation between microcystin-LR concentration and nitrate concentration and no correlation with water temperature. Over a 24-hour period, the concentration of microcystin-LR in *M. aeruginosa* decreased more than 6-fold at night compared with concentrations during the day. Codd & Bell (1996) determined the effect of temperature and nutrient supply on microcystin levels in cultures of *M. aeruginosa* in water bodies in the United Kingdom. The amount of microcystin per unit cyanobacterial dry weight was higher when nitrate levels were in excess and highest between 20 and 25°C, but was reduced above 25°C and below 20°C.

As toxin production varies greatly among different strains of the same species, genetic differences and metabolic processes may also be important in the production of these toxins. Studies have shown that the ability to produce toxins can vary temporally and spatially at a particular site or within the bloom itself (Hrudey et al., 1994; Ressom et al., 1994).

Cyanobacterial toxins either are membrane-bound or occur free within the cells. In laboratory studies, most of the toxin release occurs as cells age and die and passively leak their cellular contents, although active release of toxins can also occur from young growing cells (Pearson et al., 1990). Watanabe & Oishi (1983) investigated the toxicity of a cultured strain of *M. aeruginosa* through lag, exponential, and stationary growth phases. Maximum toxicity was observed between the exponential and stationary growth phases. The maximum cellular content of the toxin in two cultured *Microcystis* strains occurred at the late stage of exponential growth (Watanabe et al., 1989).

Microcystins and alkaloid toxins are degraded in natural waters, but there may be a lag phase before significant degradation takes place. Studies conducted using microcystin-LR at 10 µg/litre in reservoir water in the United Kingdom suggest a half-life of less than a week (Cousins et al., 1996). Codd & Bell (1996) also found that microcystin was readily biodegraded in ambient waters, with a half-life of about 1 week. Generally, if a lag phase exists, it is about 9 or 10 days long. In one study, microcystin was present up to 21 days following treatment of a bloom with an algicide (Jones & Orr, 1994). This could have been due to the shock dose with copper sulfate. Microcystin-LR is very stable in water and resistant to pH extremes and temperatures up to 300°C (Wannemacher, 1989). Biodegradation and photolysis are means by which released microcystin-LR can naturally degrade in water (Kenefick et al., 1993; Tsuji et al., 1994).

2. ANALYTICAL METHODS

Ressom et al. (1994), Lambert et al. (1994b), and AWWA (1995) reviewed the methods available for the analysis of microcystins in drinking-water. In comparing the various analytical methods being used for microcystin-LR and other microcystins, including their detection limits, it may be useful to distinguish screening methods, such as the mouse bioassay, enzyme-linked immunosorbent assay (ELISA), and phosphatase bioassay, which are conducted before clean-up and indicate the presence of toxins in samples, from methods that are conducted for the identification and quantification of the various individual microcystins (Harada, 1994). Often, more than one toxin may be present in a sample. The consensus among those using analytical methods is that a single method will not suffice for the identification and accurate quantification of many microcystins. The best approach for monitoring is to use a combination of screening and more sophisticated quantification methods.

It is important to measure *total* microcystins, which includes microcystins occurring free in water and microcystins bound to or inside cyanobacterial cells and which includes all microcystins, not just microcystin-LR. Thus, sample preparation may need to include sonification (to break up cells)

and a variety of extraction procedures in order to isolate the different (i.e. more lipophilic or polar) microcystins. Most of the existing studies on the levels of microcystins in water supplies have not clearly indicated whether total or free microcystins were measured.

The mouse bioassay plays an important role as a screening tool, as it gives the total toxic potential of the sample within a few hours and it can distinguish hepatotoxins from neurotoxins. The assay determines the minimum amount of toxin required to kill a mouse and compares this value with lethal doses of a known amount of toxin. The disadvantage is that it does not detect toxins at low levels, especially in finished drinking-water, and it does not identify the specific toxic agent (Lambert et al., 1994a). As some cyanobacteria can produce both microcystin and anatoxin, the presence of microcystin can be overlooked with the mouse bioassay. The problem arises because anatoxin can kill the mouse within minutes, whereas microcystin can kill the animal within an hour. Bhattacharya and colleagues (1996) used a modified mouse liver slice culture technique for rapidly screening large numbers of cultures and bloom samples of cyanobacterial species for cytotoxicity and hepatotoxicity. Following microcystin-LR treatment, the hepatocytes were swollen with granulated cytoplasm. Congestion and haemorrhage were also evidenced by eosinophilic debris. The method is sensitive enough to detect toxins at the microgram level (Bhattacharya et al., 1996).

The protein phosphatase bioassay is another screening method for the quantification of microcystin-LR in water samples (Lambert et al., 1994a). This method is sensitive to subnanogram levels of microcystins in finished and raw water samples. This is a quick method, and many samples can be quantified in a few hours. However, it is not specific to microcystins and will indicate the presence of other substances inhibiting protein phosphatases. This should not be a problem when monitoring a particular area where the potentially occurring species and their possible toxins are known.

An ELISA using polyclonal antibodies and with a detection limit of 0.2 ng/ml has been published (Chu et al., 1990). It is likely that methods using monoclonal or polyclonal antibodies raised against a single toxin (e.g. microcystin-LR) will have problems of cross-reactivity with other microcystins.

There are several HPLC methods for the identification and quantification of microcystins and other cyanobacterial toxins. Many HPLC methods are variations on methods developed by Siegelman et al. (1984) and Harada et al. (1988). HPLC can distinguish between microcystin analogues, provided standards are available for reference (Lambert et al., 1994b). The HPLC-ultraviolet (UV) detection method is more sensitive than the mouse bioassay, but it does not detect microcystin at levels lower than 1 µg/litre in waters containing high levels of natural organic matter (Lambert et al., 1994b). In the United Kingdom, an official HPLC-UV method with a detection limit of 0.5 µg/litre has been developed by the Water Research Centre. This method is designed to measure only free microcystin-LR; it does not measure “total” microcystin (free plus cell-bound) or microcystins other than microcystin-LR. It could be modified, however, to measure total microcystins (Fawell et al., 1993).

Lawton et al. (1994) developed a reverse-phase HPLC method to determine numerous variants of microcystin and nodularin (a hepatotoxin) in both raw and treated water by a single procedure within 24 hours. This method involves filtration to separate cyanobacterial cells from water, allowing intracellular and extracellular toxin levels to be assessed. The filtered water is subjected to trace enrichment using a ^{18}C solid-phase extraction cartridge, followed by identification and determination by photodiode array HPLC. Recoveries of microcystin-LR, -RR, -LY, -LF, and nodularin were good when raw and treated water samples were spiked with a mixture of microcystins and nodularin at concentrations as low as 0.25 µg/litre.

Liquid chromatography-mass spectrometry can analyse microcystin-LR, -YR, and -RR separately at 37, 42, and 23 ng/litre, respectively. It is a simple method that does not require a clean-up procedure because of its high selectivity (Tomoyasu & Keiji, 1996).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

The major route of human exposure to cyanobacterial toxins is the consumption of drinking-water. A minor exposure route is the recreational use of lakes and rivers; for microcystin-LR, however, absorption through skin contact is unlikely, as the toxin does not readily cross cell membranes (Eriksson et al., 1990). Some people are also exposed to cyanobacterial toxins through the consumption of certain algal food tablets. An additional, minor route of exposure is through inhalation while taking showers; microcystin-LR, however, is very water soluble and non-volatile, so inhalation and absorption through the lungs are unlikely, unless the toxin is inhaled as an aqueous aerosol in air (Lambert et al., 1994b). The extent to which cyanobacterial toxins move up the food-chain (e.g. freshwater mussels and fish) has been investigated recently (Falconer et al., 1992b; Negri & Jones, 1995). The duration of toxin exposure would generally be shorter in colder countries than in those with milder climates.

The levels of microcystin-LR in the lakes and dugout ponds of Alberta, Canada, ranged from 4 to 605 µg/g dry weight of biomass (Kotak et al., 1993) or up to 1500 µg/g (Hrudey et al., 1994). More than 70% of over 380 bloom biomass samples from 19 lakes in Alberta between 1990 and 1992 showed detectable levels (>1 µg of microcystin-LR per g dry weight of biomass) of toxin (Hrudey et al., 1994). Similarly, levels of microcystin-LR from natural blooms of *Microcystis* in Japan, between 1989 and 1991, ranged from 27 to 622 µg/g dry weight of biomass (Park et al., 1993). In the same blooms, the levels of microcystin-RR and microcystin-YR ranged from 11 to 979 µg/g dry weight of biomass and from 9 to 356 µg/g dry weight of biomass, respectively, with a total maximum level of microcystins of 1732 µg/g dry weight of biomass (Park et al., 1993).

For two Alberta drinking-water supplies, the raw water intake levels of microcystin ranged from 0.15 to 4.3 µg/litre, with a large coefficient of variation of 59% for hourly fluctuations over an 11.5-hour period. In treated water, levels ranged from 0.09 to 0.64 µg/litre, with a small coefficient of variation of 10%. Over a 5-week period, similar coefficients of variation were obtained in the two types of samples (Hrudey et al., 1994).

In the summer of 1993, microcystin-LR was detected (>0.5 µg/litre) in water samples collected from Shoal Lake, Manitoba, Canada, and from within the drinking-water distribution system following the presence of *M. aeruginosa* blooms in Shoal Lake (Jones, 1996). Following this, in 1995, 160 surface water supplies, located mainly in southwestern Manitoba, were chosen for algal study. Treated water samples were analysed only for those sites in which raw water supplies were found to have detectable levels of toxins (detection limit 0.1 µg/litre). Toxin was present in 68% of the treated water samples collected from both the municipal water supply and dugouts used for domestic and livestock consumption. Thus, it appears that conventional treatment methods may be only partially successful in removing the toxins. Toxin concentrations ranged from <0.1 to 1.0 µg/litre in raw water samples and from <0.1 to 0.6 µg/litre in treated water samples.

Fastner et al. (1995) detected seven different microcystins in 9 of 12 eutrophic water bodies in Germany in 1993. The microcystin concentration was up to 800 µg/g dry weight for a single microcystin. Cytototoxins were also observed in six field samples.

With appropriate water treatment, maximum exposure to total microcystins is probably less than 1 µg/litre, based on the above data. Average exposure generally would probably be well below this level. Not all water supplies, however, are treated by filtration or adsorption; many are untreated or simply chlorinated.

Cylindrospermopsis cultured from a drinking-water supply reservoir (Hawkins et al., 1985) has been shown to contain the toxic alkaloid cylindrospermopsin at 0.5% dry weight of algae (Ohtani et al., 1992).

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

The most likely route of exposure to cyanobacterial toxins is via oral ingestion. However, there have been no pharmacokinetic studies with orally administered microcystins. After intravenous or intraperitoneal injection of sublethal doses of variously radiolabelled toxins in mice and rats, microcystin appears to be transported by bile acids transporter in both the intestine (Falconer et al., 1992a) and the liver (Runnegar et al., 1991). About 70% of the toxin is rapidly localized in the liver (Brooks & Codd, 1987; Meriluoto et al., 1990; Lin & Chu, 1994). The kidney and intestine also accumulate significant amounts of microcystin-LR (Meriluoto et al., 1990; Robinson et al., 1991a). Plasma half-lives of microcystin-LR, after intravenous administration, were 0.8 and 6.9 minutes for the alpha and beta phases of elimination, but the concentration of radioactive (^{3}H-microcystin-LR) label in the liver did not change throughout a 6-day study period (Robinson et al., 1991a). Microcystin-LR was excreted rapidly, with 75% of the total excretion occurring within 12 hours. The remaining 24% of the administered dose was excreted after 6 days, about 9% via the urinary route and 15% slowly (1% per day) via the faecal route.

Microcystin-LR does not readily cross cell membranes and does not enter most tissues. It crosses the ileum through the multispecific organic ion transport system (Runnegar et al., 1991) and mainly enters hepatocytes, where it is covalently bound to a 40 000-dalton protein (protein phosphatase 2A and possibly protein phosphatase 1) in the cytosol (Robinson et al., 1991b).

The liver plays a large role in the detoxification of microcystins (Brooks & Codd, 1987; Robinson et al., 1991b). Detoxification products were seen in urine, faeces, and liver cytosolic fractions (Robinson et al., 1991a), but these products have not been structurally identified. The detoxification products of microcystin-LR are more water soluble than the parent toxin.

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO SYSTEMS

5.1 Acute exposure

Fatalities in animals have been reported following the consumption of water containing large numbers (>10^6/ml) of cyanobacterial cells (Beasley et al., 1989; Carmichael, 1992).

Microcystin-LR is an extremely acute toxin. The LD_{50} by the intraperitoneal route is approximately 25-150 µg/kg of body weight in mice; the oral (by gavage) LD_{50} is 5000 µg/kg of body weight in mice, and higher in rats (Fawell et al., 1994). The intraperitoneal LD_{50}s of several of the commonly occurring microcystins (microcystin-LA, -YR, and -YM) are similar to that of microcystin-LR, but the intraperitoneal LD_{50} for microcystin-RR is about 10-fold higher (Kotak et al., 1993; Rinehart et al., 1994).

The microcystins are primarily hepatotoxins. After acute exposure to microcystin by intravenous or intraperitoneal injection, severe liver damage occurs, characterized by a disruption of liver cell structure, a loss of sinusoidal structure, increases in liver weight due to intrahepatic haemorrhage, haemodynamic shock, heart failure, and death. Other organs affected include the kidney and lungs (Hooser et al., 1990).

Intestinal damage is a consequence of the transport of microcystins through the lining cells, which are damaged in a similar manner to hepatocytes (Falconer et al., 1992a).

5.2 Short-term exposure

Microcystin-LR was administered orally by gavage to groups of 15 male and 15 female mice at 0, 40, 200, or 1000 µg/kg of body weight per day for 13 weeks. No definite treatment-related changes were noted at the lowest dose. At 200 µg/kg of body weight per day, there was slight liver pathology in some male and female mice. At the highest dose level, all male and most female mice showed liver changes, which included chronic inflammation, focal degeneration of

hepatocytes, and haemosiderin deposits. In male mice at the two highest dose levels, serum transaminases were significantly elevated, serum gamma glutamyl transferase was significantly reduced, and there were small but significant reductions in total serum protein and serum albumin. In female mice, changes in transaminases were observed, but only at the highest dose level. Also at the highest dose level, food consumption in male and female mice was increased by 14 and 20%, respectively, but body weight was 7% lower in both sexes compared with control mice. The NOAEL for microcystin-LR was considered to be 40 µg/kg of body weight per day (Fawell et al., 1994).

In another study, extract from *M. aeruginosa* was given to groups of five pigs in their drinking-water for 44 days at dose levels calculated to be equivalent to microcystin doses of 280, 800, or 1310 µg/kg of body weight per day (Falconer et al., 1994). The extract contained at least seven microcystin variants, with microcystin-YR tentatively identified as the major constituent. A no-adverse-effect level (NAEL) for microcystins of 280 µg/kg of body weight per day was reported by the authors, with liver injury (evident from histopathology and changes in serum enzymes) observed at the two highest dose levels. However, one pig was also affected at the lowest dose level, and it is appropriate to consider the 280 µg/kg of body weight per day dose level as a LOAEL.

5.3 Long-term exposure

An oral repeated-dose study was conducted with *M. aeruginosa* extract supplied to mice at five concentrations (equivalent to 750-12 000 µg of microcystin-YM per kg of body weight per day) in their drinking-water for up to 1 year. At the higher concentrations, increased mortality, increased incidences of bronchopneumonia, and chronic liver injury were noted. No liver cancer was seen, but the authors indicated that there may have been some evidence of tumour promotion. No clear NOAEL was established in this study (Falconer et al., 1988).

5.4 Reproductive and developmental toxicity

To investigate the effects of microcystin-LR on the embryonic and fetal development of the mouse, four groups of 26 time-mated female mice of the Cr1:CD-1 (ICR) BR strain were dosed once daily by oral gavage with aqueous solutions of microcystin-LR from days 6 to 15 of pregnancy, inclusive. The dose levels were 0, 200, 600, or 2000 µg/kg of body weight per day. Maternal clinical signs, body weights, and food consumption were recorded. On day 18 of pregnancy, the females were killed, a necropsy was performed, and the fetuses were examined for abnormalities. Only treatment at 2000 µg/kg of body weight per day was associated with maternal toxicity and mortality. Nine of the 26 females died or were sacrificed prematurely during the dosing period. At necropsy, a number of females had abnormal livers, and retardation of fetal weight and skeletal ossification were observed at the maximum dose. There was no evidence of embryolethality, teratogenicity, or embryonic growth retardation at all dose levels. There was no apparent effect of treatment at any dose level on litter size, post-implantation loss, or the sex distribution of the live fetuses. The NOAEL for any aspect of developmental toxicity was 600 µg/kg of body weight per day (Fawell et al., 1994). These data are in agreement with those of Falconer et al. (1988), which similarly provided no evidence of teratogenicity, embryonic mortality, or reduction in fertility in mice exposed to microcystin at 750 µg/kg of body weight per day in drinking-water from weaning (for 17 weeks prior to mating) through mating.

5.5 Mutagenicity and related end-points

No mutagenic response was observed for purified toxins derived from *Microcystis* in the Ames *Salmonella* assay (strains TA98, TA100, and TA102) with or without S9 activation. The *Bacillus subtilis* multigene sporulation test was also negative with regard to mutagenicity using both the 168 and hcr-9 strains (Repavich et al., 1990). In contrast, results of a study in which the purified toxins were tested against human lymphocytes suggested that the toxins may be clastogenic, as indicated by dose-related increases in chromosomal breakage (Repavich et al., 1990).

5.6 Carcinogenicity

There has been some evidence of tumour promotion in animal studies. In a modified two-stage carcinogenesis mouse skin bioassay, dimethylbenzanthracene (DMBA) (500 µg) in acetone was applied to the skin of four out of six groups of 20 3-month-old Swiss female mice. After 1 week, the DMBA-treated mice received drinking-water, *Microcystis* extract in drinking-water (actual microcystin-YM dose not provided), croton oil (as a positive control) applied to the skin (0.5% in 0.1 ml acetone twice a week) plus drinking-water, or croton oil plus *Microcystin* extract; the control mice received drinking-water or *Microcystis* extract in drinking-water. After 52 days from initiation, substantial skin tumours and ulcers were visible on the DMBA-treated mice consuming *Microcystis* extract. There was a significant increase in the mean weight of skin tumours per mouse in treated mice given the *Microcystis* extract compared with water. The actual number of tumours per mouse and the weights of the tumours in relation to the weights of the animals were not provided. It was concluded by the authors that oral consumption of *Microcystis* extract in drinking-water may act as a promoter. However, the mechanism of action is not clear, as microcystins have difficulty penetrating epidermal cells. The tumour weight per mouse in DMBA-treated mice given both croton oil and the algal extract was slightly lower than in those given croton oil and drinking-water. These latter findings could not be explained by the author (Falconer, 1991).

In a two-stage carcinogenicity bioassay, groups of 10-19 7-week-old male Fischer 344 rats were initiated by intraperitoneal injection with 200 mg of diethylnitrosamine (DEN) per kg of body weight, followed by partial hepatectomy at the end of the third week. Tumour promotion was assessed by intraperitoneal injection of microcystin-LR at 1 or 10 µg/kg of body weight from the third week of the experiment, 3 or 5 times per week. Tumour promotion, as indicated by an increase in glutathione *S*-transferase placental form (GST-P) positive liver foci, was seen after 8 weeks in animals dosed with 10 µg of microcystin-LR per kg of body weight (Nishiwaki-Matsushima et al., 1992). In a second similar experiment, the same authors used dose levels of 10 µg/kg of body weight per day before, and 10, 25, or 50 µg/kg of body weight per day after, partial hepatectomy. It was found that the increase in GST-positive foci was dose related. However, these results should be interpreted with caution, because the doses used were very high and could have caused significant damage to hepatocytes. Microcystin-LR had no effect when given to non-initiated rats or to rats that had received partial hepatectomy but no promotion dose of microcystin-LR.

Microcystin-LR was found to be a potent inhibitor of eukaryotic protein serine/threonine phosphatases 1 and 2A both *in vitro* (Honkanen et al., 1990; Mackintosh et al., 1990) and *in vivo* (Runnegar et al., 1993). Such substances are considered to be non-phorbol ester (TPA) type tumour promoters. The inhibition of protein phosphatase 2A by microcystin-LR can be effectively reversed in the presence of polyclonal antibodies against microcystin-LR (Lin & Chu, 1994). The inhibition of protein phosphatase type 1 and type 2 activities by microcystin-LR is similar to that of the known protein phosphatase inhibitor and tumour promoter okadaic acid (Nishiwaki-Matsushima et al., 1992). The implications for low-level chronic exposure to microcystins are not known.

6. EFFECTS ON HUMANS

Blue-green algae have been known to cause animal and human poisoning in lakes, ponds, and dugouts in various parts of the world for over 100 years.

Through the recreational use of contaminated water, cyanobacterial blooms of *Microcystis*, *Anabaena*, and others have been linked to incidence of human illness in many countries, but no fatalities have been reported (Lambert et al., 1994b). In Canada, human illnesses have been reported in Saskatchewan, with symptoms including stomach cramps, vomiting, diarrhoea, fever, headache, pains in muscles and joints, and weakness (Dillenberg & Dehnel, 1960). Similar

symptoms as well as skin, eye, and throat irritation and allergic responses to cyanobacterial toxins in water have also been reported in other countries (Ressom et al., 1994). The reported instances of illnesses are few, but, because they are difficult to diagnose, such illnesses may in fact be more common than has been reported (Carmichael & Falconer, 1993).

In Saskatchewan, Canada, 10 children became sick with diarrhoea after swimming in a lake covered with cyanobacteria. *Anabaena* cells, which produce microcystin-LR, were found in the stools of one child (Dillenberg & Dehnel, 1960). In the United Kingdom, 10 of 18 army recruits on a military exercise in a reservoir with a bloom of *M. aeruginosa* suffered abdominal pain, nausea, vomiting, diarrhoea, sore throat, dry cough, blistering at the mouth, and headache. Two were hospitalized and developed an atypical pneumonia. Serum enzymes indicating liver damage were elevated. Microcystin-LR was identified in the bloom material (Pearson et al., 1990). Nevertheless, substances other than microcystin-LR may have been present, and some of the observed effects were probably due to other materials in the water.

In the USA and Australia, several different cyanobacterial toxins have been implicated in human illness from certain municipal water supplies, often after algal blooms had been treated with copper sulfate (Bourke et al., 1983; Falconer, 1989; Ressom et al., 1994). In most cases, the cyanobacteria and sometimes the toxins involved have been identified, but the levels of toxin associated with illness have not been established in any of the outbreaks.

The Palm Island mystery disease, affecting about 140 people, largely children, in Australia, occurred after a dense cyanobacterial bloom on a water supply was treated with copper sulfate. Within a week, severe illness was seen, characterized by vomiting, hepatomegaly, and kidney dysfunction, with loss of electrolytes, glucose, and plasma protein; recovery took 1-3 weeks (Byth, 1980). The cause of illness was not identified until a subsequent cyanobacterial bloom in the same water supply reservoir was shown to be highly toxic (Hawkins et al., 1985). The causative organism was *C. raciborskii*, containing the cytotoxic alkaloid cylindrospermopsin, a powerful inhibitor of protein synthesis (Terao et al., 1994).

Possible liver damage, as evidenced by significant increases in gamma glutamyl transferase, was seen in persons drinking water from supplies containing blooms of *Microcystis* after treatment with copper sulfate (Malpus Dam, Armidale, Australia) compared with persons drinking uncontaminated water (Falconer, 1989).

In Salisbury, Rhodesia, seasonal acute childhood gastroenteritis during the years 1960-1965 was linked to annual blooms of *Microcystis* in the lake serving as the water supply. An adjacent water supply was not similarly affected and was not associated with this disease (Zilberg, 1966).

El Saadi & Cameron (1993) reported on 26 cases (aged 1-64 years) with skin diseases and multiple systemic symptoms associated with exposure (some via drinking-water) to river water or rainwater in Australia during 1991-1992. The water was stored in open tanks and contained *Anabaena* blooms. Further case-control studies in the same area are ongoing.

Recently, an epidemiological survey in Haimen city (Jian-Su province) and Fusui county (Guangxi province) in China found a close relationship between the incidence of primary liver cancer and the use of drinking-water from ponds and ditches (Ueno et al., 1996). In 1993 and 1994, microcystin concentrations ranged from 0.058 to 0.460 µg/litre; the highest concentrations occurred from June to September. Microcystin was not detected in deep well-water. A similar survey on 26 drinking-water samples in the Guangxi province showed a high frequency of microcystins in the water of ponds/ditches and rivers, but no microcystins were found in shallow and deep wells. According to Ueno et al. (1996), the combined effect of microcystin toxin from the drinking-water of ponds/ditches and rivers or both and other carcinogens such as aflatoxin B1 found in food may be the cause of the high incidence of primary liver cancer in Haimen city and other areas in China.

Cyanobacterial blooms tend to occur repeatedly in the same water supply. Therefore, some human populations are at risk of repeated ingestion of cyanobacterial toxins. However, the available data are not sufficient to allow a quantitative assessment of human exposure.

7. PROVISIONAL GUIDELINE VALUE

There are insufficient data to derive a guideline value for cyanobacterial toxins other than microcystin-LR. Only a guideline value for this compound is derived.

A 13-week study in mice with microcystin-LR (Fawell et al., 1994) is considered the most suitable for the derivation of a guideline value. In this study, a NOAEL of 40 µg/kg of body weight per day was determined for liver pathology. A TDI of 0.04 µg/kg of body weight per day can be calculated by applying an uncertainty factor of 1000 (100 for intra- and interspecies variation, 10 for limitations in the database, in particular lack of data on chronic toxicity and carcinogenicity) to the NOAEL. An allocation factor of 0.80 is used for the proportion of daily exposure arising from drinking-water, because there is little exposure from any other source and route. The resulting guideline value for total microcystin-LR (free plus cell-bound) is 1 µg/litre (rounded figure) in drinking-water.

The guideline value thus calculated is supported by a 44-day study in which pigs were exposed, in their drinking-water, to an extract from *M. aeruginosa* containing microcystin-LR.

The guideline value of 1 µg/litre for microcystin-LR is provisional, as the database is limited, new data for the toxicity of cyanobacterial toxins are being generated, and the guideline value covers only microcystin-LR.

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Edetic acid (EDTA)

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In view of the possibility of zinc complexation, a provisional guideline value for edetic acid (EDTA) of 200 µg/litre was derived in the 1993 *Guidelines for drinking-water quality*, assuming consumption of 1 litre of drinking-water by a 10-kg child and allocating 10% of the TDI (190 µg/kg of body weight) to drinking-water. This TDI was derived from the 1973 JECFA ADI of 2.5 mg/kg of body weight for calcium disodium edetate as a food additive (1.9 mg/kg of body weight as the free acid). An extra uncertainty factor of 10 was introduced to reflect the fact that the JECFA ADI had not been considered since 1973, as well as concern over zinc complexation.

The guideline value was considered to be provisional because it was based on a rather old

(1973) evaluation by JECFA. As JECFA evaluated the toxicological studies available on sodium iron EDTA and the calcium/sodium salts of EDTA in 1993, the Coordinating Committee for the Updating of WHO *Guidelines for drinking-water quality* recommended that EDTA be re-evaluated in the 1998 Addendum.

1. GENERAL DESCRIPTION

1.1 Identity

CAS no.: 60-00-4
Molecular formula: $C_{10}H_{16}N_2O_8$

Edetic acid (ethylenediaminetetraacetic acid) and its salts are commonly referred to as EDTA. Other names include *N*,*N*'-1,2-ethanediylbis[*N*-(carboxymethyl)glycine], Versene acid, and (ethylenedinitrilo)tetraacetic acid.

1.2 Physicochemical properties

Property	*Value*
Physical appearance	Colourless crystals
Solubility in water	0.5 g/litre at 25°C

1.3 Organoleptic properties

EDTA has a slightly salty taste.

1.4 Major uses

EDTA has been used extensively in medicine as a chelating agent for the removal of toxic heavy metals. The disodium salt of EDTA is a common component in many eye drops and contact lens wetting and cleansing solutions. EDTA is also used in a number of personal care and hygiene products, such as shampoos, liquid soaps, creams, and lotions.

Household disinfectants often contain EDTA, especially if fatty acid soaps are used in the disinfectant formulation. These soaps are sensitive to calcium and magnesium, and the chelating agent prevents the formation of hard-water soap curds (Hart, 1984).

EDTA is also used as a food additive in a range of products, including canned shrimp and prawns, canned mushrooms, and frozen french fries. It is added to salad dressings to prevent rancidity.

EDTA is used in many industrial processes, in agriculture, in photochemicals, pharmaceuticals, and textiles, and in galvanizing and paper manufacturing. The usage of EDTA in West Germany in 1986 by industry was: metal processing and galvanizing technology, 30%; detergents, 20%; photographic industry, 20%; textiles, 10%; paper, 5%; and miscellaneous (antioxidants in soaps and cosmetics, pharmaceuticals, and foodstuffs), 15%. The total use over the year was about 15 000 t (Brauch & Schullerer, 1987).

1.5 Environmental fate

Once EDTA is present in the aquatic environment, its speciation will depend on the water quality and the presence of trace metals with which it can combine. The fate and behaviour of the different complexes may vary considerably. The iron(III) EDTA complex is the most labile because it is very photo-active. Svenson et al. (1989) calculated a half-life of 11 minutes for the photolysis of iron(III) EDTA dissolved in water and irradiated with sunshine equivalent to the annual maximum intensity. The photo-oxidation of free EDTA in water at pH 9.0 has a measured

half-life of 36 years for an initial hydroxyl radical concentration of 5×10^{-9} mol/litre and a 12-hour daylight duration. The iron(III) EDTA will exchange slowly with other trace metals after discharge to the environment, a process that will be dependent upon the pH of the water, as each trace metal has an optimum pH for chelation. Other metal complexes of EDTA are much more persistent and are not readily biodegraded in the aquatic environment. In soil-water systems, degradation has been observed, but the extent varies with soil type and length of exposure. The removal of EDTA from communal wastewater by biodegradation in sewage purification plants is very limited. A limited removal takes place by adsorption on sludge. Similarly, elimination of EDTA by different treatment methods of drinking-water is negligible, including filtration on activated carbon. The most effective elimination is by ozonation (Gilbert & Beyerle, 1992).

There has been concern that EDTA mobilizes heavy metals in the environment. However, based on stoichiometry, 40 µg of EDTA per litre (the maximum concentration observed in the Rhine and Meuse rivers) would complex 4-15 µg of metals per litre at most, and this would be likely to pose problems for drinking-water only with regard to cadmium. A further modifying factor is that the effect on cadmium leaching will be limited because the EDTA is primarily bound to other metals at these concentrations (van Dijk-Looyard et al., 1990). For the majority of fresh waters, EDTA will be associated largely with calcium, provided that the EDTA is not present in stoichiometric excess. For waters of pH lower than 6.0, however, competition from hydrogen ions for available ligand assumes greater importance.

2. ANALYTICAL METHODS

EDTA can be analysed by potentiometric stripping analysis (Fayyad et al., 1988). This method, which has been used to detect EDTA in a wide variety of wastewater and natural water samples, has a detection limit of 1 µg/litre.

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Water

Most of EDTA's uses will result in its release to the aquatic environment. It has been estimated that concentrations of 50-500 µg/litre are present in wastewaters. Annual average concentrations of EDTA in European surface waters ranged between >1 and >60 µg/litre, and a concentration of 900 µg/litre was found in the Zerka River in Jordan (van Dijk-Looyard et al., 1990). Measured concentrations in natural waters were also reported to range from 10 to 70 µg/litre, with a median value of 23 µg/litre (Frank & Rau, 1990). Mean EDTA concentrations at 45 different sampling points on 29 different rivers of Germany in 1993 ranged between almost 50 µg/litre (Lippe River at Wesel) and a few µg/litre, with most annual mean values being between 5 and 15 µg/litre (EFA-Germany, 1995). EDTA has also been detected in surface waters and in drinking-water prepared from surface waters at concentrations of 10-30 µg/litre (van Dijk-Looyard et al., 1990).

3.2 Food

EDTA's use as a food additive has been limited. The maximum levels of EDTA in canned shrimp and prawns, canned mushrooms, and frozen french fries are 250, 200, and 100 mg/kg, respectively (Smith, 1990).

3.3 Estimated total exposure and relative contribution of drinking-water

Human exposure to EDTA arises directly from its use in food additives, disinfectants, medicines, and personal care and hygiene products. Exposure to EDTA from drinking-water is probably very small in comparison to exposure from other sources.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

4.1 Metabolism in laboratory animals and humans

Foreman et al. (1953) examined the metabolism of ^{14}C-labelled calcium disodium EDTA in the rat in two series of experiments involving the administration of doses of 50 mg/kg of body weight, intraperitoneally, intravenously, intramuscularly, or orally by intubation, to Sprague-Dawley rats. The studies indicated that calcium disodium EDTA was poorly absorbed from the gastrointestinal tract. About 80-95% of the dose appeared in the faeces after 24 hours. The amount absorbed in 24 hours, determined from the quantity found in the tissues and urine, ranged from 2 to 18%, with most of the values being between 2 and 4%. At the low pH of the stomach, the calcium chelate is dissociated with subsequent precipitation of the free acid, and this is only slowly redissolved as it passes through the alimentary tract. Increasing the dose is not necessarily a good way of increasing the amount absorbed, as the rats exhibited diarrhoea at higher doses. Srbrova & Teisinger (1957) confirmed the dissociation of the calcium chelate in the stomach. When a dose of 200 mg of calcium disodium EDTA was introduced into the duodenum of rats, the authors found absorption to be 6.5-26%.

Experiments in humans also revealed poor absorption; only 2.5% of a 3-g dose of calcium disodium EDTA was excreted in the urine (Srbrova & Teisinger, 1957). Only 5% of a dose of 1.5 mg of ^{14}C-labelled calcium disodium EDTA given in a gelatin capsule to normal healthy men was absorbed (Foreman & Trujillo, 1954). EDTA has also been shown to be rapidly excreted from the body. Intravenous doses of 3 g of radiolabelled calcium disodium EDTA, given to two subjects, were almost entirely excreted within 12-16 hours (Srbrova & Teisinger, 1957).

A summary of a 1956 Ph.D. thesis by Chan in Anonymous (1964) reported biochemical studies with disodium EDTA. In a study in rats, 32 hours following administration of a single oral dose of 95 mg of disodium EDTA per rat, 93% of the dose was recovered from the colon. After doses of 47.5, 95, and 142.5 mg of disodium EDTA, the amount of EDTA recovered in the urine was directly proportional to the dose given, suggesting that EDTA was absorbed from the gastrointestinal tract by passive diffusion.

4.2 Metal complexation with EDTA

EDTA is a hexadentate chelator capable of combining stoichiometrically with virtually every metal in the periodic table (Chaberck & Martell, 1959). With divalent or trivalent metal ions, a neutral or anionic metal chelate results. The metal is largely prevented from reacting with competing anions, and its solubility is greatly increased. The effectiveness of EDTA as a chelate for a particular metal ion is given by its stability constant with the metal ion. Chelation potential is affected by pH, the molar ratio of chelate to metal ion, and the presence of competing metal ions capable of forming complexes with EDTA (Plumb et al., 1950; Martell, 1960; Hart, 1984). The stability constants for different metal-EDTA complexes vary considerably, and any metal that is capable of forming a strong complex with EDTA will at least partially displace another metal.

Of the nutritionally important metals, Fe^{3+} has the highest stability constant (log k = 25.1), followed by Cu^{2+} with 18.4, Zn^{2+} with 16.1, Fe^{2+} with 14.6, Ca^{2+} with 10.6, Mg^{2+} with 8.7, and Na^{+} with 1.7 (West & Sykes, 1960). The situation is somewhat complicated by each metal having an optimum pH for chelate formation, ranging from pH 1 for Fe^{3+} to pH 3 for Cu^{2+}, pH 4 for Zn^{2+}, pH 5 for Fe^{2+}, pH 7.5 for Ca^{2+}, and pH 10 for Mg^{2+} (West & Sykes, 1960). When sodium iron EDTA is ingested with foods, the Fe^{3+} ion would be expected to remain firmly bound to the EDTA moiety during passage through the gastric juice, but it could be exchanged for Cu^{2+}, Zn^{2+}, Fe^{2+}, or Ca^{2+} in the duodenum (WHO, 1993).

In biological systems, Ca^{2+} will usually be most accessible to EDTA. In general, zinc seems to be the next most accessible. About 80% of the zinc in liver is freely available to EDTA. The overall availability of the other physiologically important metals is probably in the order copper > iron > manganese > cobalt (Chenoweth, 1961). EDTA removes about 1.4% of the total iron from ferritin at pH 7.4 to form an iron chelate (Westerfield, 1961).

Perry & Perry (1959) investigated the changes in normal concentrations of trace metals in human urine following administration of EDTA. Calcium disodium EDTA had been observed to lower the level of cholesterol in human plasma, and therefore this study investigated metal concentrations in consecutive 24-hour urine samples from hypercholesterolaemic patients before, during, and after the intravenous administration of calcium disodium EDTA. The results indicated a 10-fold increase in urinary excretion of zinc during the administration of calcium disodium EDTA. A smaller effect on cadmium and manganese may have occurred, but the results were not clear, because some of the control concentrations were too low to quantify. There were also suggestive increases in the excretion of lead and vanadium. Foreman (1961) reported that EDTA enhanced the excretion of cobalt, mercury, manganese, nickel, lead, thallium, and tungsten.

When EDTA is present in food, iron (primarily Fe^{3+}) remains complexed with EDTA under the acidic conditions prevailing in the stomach. The chelate holds the iron in solution as the pH rises in the upper small intestine, but the strength of the complex is progressively reduced, allowing at least partial exchange with other metals and the release of some of the iron for absorption. There is convincing evidence that iron chelated by EDTA (sodium iron EDTA) is available for absorption via the physiologically regulated pathways responsible for iron uptake (Candela et al., 1984). The results of the absorption studies with sodium iron EDTA indicate that iron is dissociated from the EDTA moiety prior to absorption.

Hurrell et al. (1994) examined the influence of sodium iron(III) EDTA, used as a food fortificant, on the metabolism of calcium, zinc, and copper in the rat. The results showed that changing the iron fortificant from iron sulfate to sodium iron(III) EDTA increased the apparent zinc absorption, retention, and excretion in the rats receiving the zinc-deficient diets. The authors suggest that the study appears to indicate a beneficial effect on zinc nutritional status following addition of sodium iron(III) EDTA as an iron food fortificant. However, the results are complicated by the fact that iron status is an important factor in zinc metabolism and a high level of iron inhibits the availability of zinc. In addition, the diets contained soybean protein, which is high in phytate, an inhibitor of both iron and zinc absorption. These factors make it difficult to draw any definite conclusions from this study.

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Acute toxicity studies have been carried out with disodium EDTA and calcium disodium EDTA in laboratory animals. LD_{50} values (mg/kg of body weight) reported in these studies are summarized below:

Rat	Oral: 2000-2200, Na_2EDTA	(Yang, 1952)
	Oral: 10 000 ± 740, $CaNa_2EDTA$	(Oser et al., 1963)
Rabbit	Oral: 2300, Na_2EDTA	(Shibata, 1956)
	Oral: ~7000, $CaNa_2EDTA$	(Oser et al., 1963)
	Intraperitoneal: ~500, $CaNa_2EDTA$	(Bauer et al., 1952)
Dog	Oral: ~12 000, $CaNa_2EDTA$	(Oser et al., 1963)

Oser et al. (1963) reported that the oral LD_{50} in rats was not affected by the presence of food in the stomach or by pre-existing deficiency in calcium, iron, copper, or manganese.

5.2 Short-term exposure

In a study involving the intraperitoneal administration of 250 or 500 mg of calcium disodium EDTA per kg of body weight per day to groups of five male rats for 3-21 days, it was reported that

weight gain was satisfactory and that the histology of the lung, thymus, liver, spleen, adrenal, small gut, and heart was normal; there was a mild to moderate effect on the kidney (Reuber & Schmieller, 1962).

Groups of five male rats were given 250, 400, or 500 mg of disodium EDTA per kg of body weight per day intraperitoneally for 3-31 days; some groups were observed for an additional 2 weeks. At 500 mg/kg of body weight per day, all rats became lethargic and died within 9 days; the kidneys were pale and swollen, and there was moderate dilation of the bowel and subserosal haemorrhages. Histological examination of a number of organs showed lesions only in the kidneys. Animals at the 400 mg/kg of body weight per day dose level died within 14 days; kidney and bowel symptoms were similar to those seen at the high dose. One rat in the 250 mg/kg of body weight per day dose group showed haemorrhage of the thymus. All three groups exhibited varying degrees of damage to the kidney, with recovery occurring on withdrawal of the disodium EDTA (Reuber & Schmieller, 1962).

Four groups of one male and three female mongrel dogs were fed diets containing 0, 50, 100, or 200 mg of calcium disodium EDTA per kg of body weight per day for 12 months. At the end of the study, all dogs appeared to be well, and there were no significant changes in blood or urine analysis. Gross and microscopic examinations of the major organs were normal (Oser et al., 1963).

5.3 Long-term exposure

The long-term toxicity of EDTA is complicated by its ability to chelate essential and toxic metals, both in water and in animals. Toxicity data are therefore equivocal and difficult to interpret.

An early study by Krum (1948) demonstrated no adverse effect on weight gain, appetite, activity, and appearance in rats fed for 44-52 weeks on a diet containing 0.5% disodium EDTA.

A 2-year study was carried out in which groups of rats were fed 0, 0.5, 1, or 5% disodium EDTA. The highest dose group showed a reduced food intake compared with the other groups and also suffered diarrhoea. No significant effects on weight gain were noted, nor were blood coagulation time, red blood cell counts, or bone ash adversely affected. Mortality of the animals could not be correlated with the level of disodium EDTA, as the highest mortality was observed in the control group and was due to pneumonia. Gross and microscopic examinations of the major organs did not reveal any significant differences between the groups (Yang, 1952).

In another study, groups of 25 male and 25 female rats were fed diets containing 0, 50, 125, or 250 mg of calcium disodium EDTA per kg of body weight per day for 2 years, and the study was carried on through four successive generations. The rats were mated after 12 weeks of feeding and were allowed to lactate for 3 weeks, with 1 week's rest before producing a second litter. Ten male and 10 female rats from the F_1 generation and similar F_2 and F_3 generations were allowed to produce two litters. With the second litter in the F_1, F_2, and F_3 generations, only the control and the 250 mg/kg of body weight per day dose groups were kept until the end of the 2-year study on the F_0 generation. No significant abnormalities in appearance or behaviour were noted during the 12 weeks of the post-weaning period in all generations. The experiments showed no statistically significant differences in weight gain, food efficiency, haematopoiesis, blood sugar, non-protein nitrogen, serum calcium, urine, organ weights, and histopathology of the liver, kidney, spleen, heart, adrenals, thyroid, and gonads (Oser et al., 1963).

Fifty weanling albino rats were fed a low-mineral diet (0.54% calcium and 0.013% iron) with the addition of 0, 0.5, or 1% disodium EDTA or 0.5 or 1% calcium disodium EDTA for 205 days. Diarrhoea was observed in the 1% disodium EDTA group, along with other abnormalities: growth retardation of the males, lowered erythrocyte and leukocyte counts, a prolonged blood coagulation time, slightly but significantly raised blood calcium level, a significantly lower ash content of the bone, and considerable erosion of the molars. Gross and histological examinations

of the major organs revealed nothing abnormal. Rats fed for 220 days on an adequate mineral diet containing 1% disodium EDTA showed no evidence of dental erosion (Chan, 1956).

Groups of 50 male and 50 female $B6C3F_1$ mice received trisodium EDTA in the diet at concentrations of 3, 750, or 7500 mg/kg of feed for 103 weeks, followed by 1 week during which the mice were fed standard diet without EDTA. The animals were examined twice per day for signs of toxicity. Gross and histopathological examinations of major organs and tissues were performed on animals found dead or moribund and on those sacrificed at the end of the study. Body-weight gain was decreased in high-dose males during the second year of the study, although no statistical analysis was presented. No treatment-related tumours or non-neoplastic lesions were observed in this study (NCI, 1977).

5.4 Reproductive and developmental toxicity

Groups of six rats were maintained on diets containing 0.5, 1, or 5% disodium EDTA for 12 weeks. The only toxic symptoms observed were diarrhoea and a reduction in food consumption at the 5% level. When the animals were 100 days old, mating was carried out and was repeated 10 days after weaning of the first litters. The animals given 5% disodium EDTA did not produce any litters. The other dose groups produced normal first and second litters (Yang, 1952).

Oser et al. (1963) carried out a four-generation study in which groups of rats received calcium disodium EDTA at doses of 50, 125, or 250 mg/kg of body weight per day via the diet. There were no reproductive or teratogenic effects noted in any of the three generations of offspring.

Groups of pregnant Sprague-Dawley rats were given diets containing 2 or 3% disodium EDTA from day 1 through day 21 of gestation. A further group of pregnant rats was fed diets containing 3% disodium EDTA from day 6 to day 14 of gestation, whereas a third group was given diets containing 3% disodium EDTA and 1000 mg of zinc per kg of feed from day 6 to day 21 of gestation. The control animals received a standard diet that contained 100 mg of zinc per kg of feed. In rats fed the 2% disodium EDTA, litter size was normal and fetuses were alive. Gross abnormalities were evident in 7% of the treated fetuses, compared with 0% in the control group. In rats fed 3% disodium EDTA, almost half of the implantation sites had dead fetuses or resorptions. Full-term young were significantly smaller than controls, and 100% of them were malformed. Maternal toxicity was indicated by diarrhoea and was observed in both the 2% and 3% dose groups. The malformations included severe brain malformations, cleft palate, malformed digits, clubbed legs, and malformed tails. Supplementation of the diet with 1000 mg of zinc per kg of feed prevented these detrimental effects, and it was suggested that the teratogenic effects of EDTA given to rats at very high levels were due to zinc deficiency (Swenerton & Hurley, 1971).

Schardein et al. (1981) performed teratogenesis studies with EDTA and its salts in rats. EDTA (967 mg/kg of body weight), disodium EDTA (1243 mg/kg of body weight), trisodium EDTA (1245 mg/kg of body weight), tetrasodium EDTA (1374 mg/kg of body weight), and calcium disodium EDTA (1340 mg/kg of body weight) were administered orally (by intubation) to groups of 20 inseminated female rats during organogenesis. The dosing regimen was twice daily on days 7-14 of gestation. There were two control groups. One group received 1.0 ml of phosphate buffer per kg of body weight twice daily to serve as a vehicle control group, and the other remained untreated and served as an untreated control group. The dams were killed on day 21 of gestation, and litter data for each dam were collected. The fetuses were then examined for gross external anomalies, visceral abnormalities, and skeletal anomalies. Diarrhoea was apparent in all the treated groups, and reduced activity was observed in a few of the dams. The diarrhoea generally occurred following treatment and disappeared on the last day of dosing or the day after. Three dams died during treatment with disodium EDTA. Examination of these did not indicate any gross abnormalities. Food intake was slightly reduced compared with controls during the treatment period of days 7-14 but was comparable to the controls during the pre-treatment period (gestation days 0-7) and post-treatment period (gestation days 14-21). None of the test compounds significantly affected litter size at term when compared with either control group. The mortality

index of the offspring in all treated groups as measured by post-implantation loss was also comparable to both control groups. A total of 24 pups from the treated groups had abnormalities, including bifid vertebrae, agenesis of the ribs, inhibition of osteogenesis of the skull or ribs, and malformed ribs. There was, however, no pattern between treatment with any particular compound and the appearance of anomalies. In addition, the untreated control group had eight pups with some major defect. The results of these studies demonstrated that there was an absence of teratogenic effects even at doses that were maternally toxic.

An important study was carried out by Brownie et al. (1986), who investigated the teratogenic effect of calcium EDTA in rats and the protective effect of zinc. Pregnant Long-Evans rats were randomly assigned to 11 treatment groups corresponding to differing doses of calcium EDTA (2, 4, 6, or 8 mmol/m^2 per day), zinc EDTA (8 or 20 mmol/m^2 per day), and zinc calcium EDTA (8 or 20 mmol/m^2 per day), plus controls. Each group contained 20 rats, except for the control group receiving 0.9% NaCl, which contained 30 rats. A further 12 animals per group were used for maternal plasma and liver and fetal zinc analysis. The rats were treated by subcutaneous injection of the chelating agent or saline solution on days 11 through 15 of gestation. Results showed increases in several abnormalities (e.g. submucous cleft, cleft palate, curly tail, abnormal rib and vertebrae) with increasing doses of calcium EDTA. No malformations were seen with zinc EDTA at either dose or with zinc calcium EDTA at the lower dose. However, submucous cleft was seen in 6 of 20 litters from the dams receiving the higher dose of zinc calcium EDTA. It was concluded that calcium EDTA is teratogenic in rats at concentrations that, except for decreased weight gain, produce no discernible toxicity to the dam, and that protection is afforded by incorporating zinc in the chelate.

A study was carried out by Kimmel (1977) in which groups of pregnant CD rats were treated with disodium EDTA via a number of different routes of exposure. Forty-two rats received a dose of 954 mg/kg of body weight per day via the diet. Twenty-two received a dose of 1250 mg/kg of body weight per day, which was split into a dose of 625 mg/kg of body weight twice per day by gastric intubation. Eight rats received 1500 mg/kg of body weight per day as a split dose of 750 mg/kg of body weight twice per day by gastric intubation. Twenty-five rats received a dose of 375 mg/kg of body weight per day by subcutaneous injection. All the animals were dosed on gestation days 7-14. Fetuses were removed on day 21 of gestation and examined for gross and visceral abnormalities. The results showed that for the dietary group, there were no maternal deaths but a significant increase in fetal death compared with the controls, and 71% of the fetuses were malformed. In the group that was administered 625 mg/kg of body weight per day by gavage, only 64% of the dams survived treatment. Those that survived exhibited fetal resorptions comparable to controls, and 20.5% of the fetuses were malformed. In the group administered 750 mg/kg of body weight per day, seven of the eight dams died. The group receiving subcutaneous injection of disodium EDTA showed a 76% survival of the dams. However, there was a significant increase in fetal resorptions compared with controls, although the proportion of malformed fetuses was similar to the controls. This study demonstrates that the route of exposure to EDTA is an important factor in the determination of the toxicity and teratogenic potential of EDTA.

5.5 Mutagenicity and related end-points

Trisodium EDTA was tested for its mutagenic potential in the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay. Two experiments were performed with S9 metabolic activation system and three without S9 at concentrations of EDTA up to 5000 mg/litre. No mutagenicity was observed either with or without the S9 (McGregor et al., 1988).

Trisodium EDTA was also tested in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 and in *Escherichia coli* WP uvrA in the presence and absence of the S9 metabolic activation system. There was no evidence of any mutagenic potential in any of these bacterial systems (Dunkel et al., 1985).

5.6 Carcinogenicity

In an experiment in which groups of 50 male and 50 female $B6C3F_1$ mice received trisodium EDTA in the diet at concentrations of 3, 750, or 7500 mg/kg of feed for 103 weeks, followed by 1 week during which the mice were fed standard diet without EDTA, no treatment-related tumours were observed (NCI, 1977).

6. GUIDELINE VALUE

JECFA evaluated the toxicological studies available on sodium iron EDTA in 1993 (WHO, 1993), and there was no further information, compared with its 1973 evaluation (WHO, 1974), of noteworthy importance regarding the toxicity of EDTA and its calcium/sodium salts. Concern has been expressed over the ability of EDTA to complex zinc and therefore reduce its availability. However, this is only of significance at elevated doses substantially in excess of those encountered in the environment. The use of an additional uncertainty factor and the assumption of a 10-kg child were therefore considered inappropriate.

A guideline value for EDTA in drinking-water can be derived by allocating 1% of the JECFA ADI (1.9 mg/kg of body weight as the free acid) to drinking-water (because of the potential for significant exposure from food owing to its use as a food additive). Therefore, assuming a 60-kg adult ingesting 2 litres of drinking-water per day, the guideline value for EDTA (free acid) is 600 µg/litre (rounded figure). This value is no longer considered to be provisional.

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Polynuclear aromatic hydrocarbons

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The 1993 WHO *Guidelines for drinking-water quality* recommended a guideline value for benzo[*a*]pyrene (BaP) in drinking-water of 0.7 µg/litre, corresponding to an excess lifetime cancer risk of 10^{-5}. This guideline value was based on an increased incidence of forestomach tumours in mice fed BaP in the diet, quantified using the two-stage birth-death mutation model. There were insufficient data available to allow the derivation of drinking-water guideline values for other polynuclear aromatic hydrocarbons (PAHs).

The 1992 Final Task Group Meeting recommended that PAHs other than BaP be evaluated in the

future, and a draft IPCS Environmental Health Criteria monograph on PAHs was finalized in 1995. The Coordinating Committee for the updating of the WHO *Guidelines* therefore recommended that a group of representative PAHs be selected for evaluation in the 1998 Addendum.

1. GENERAL DESCRIPTION

PAHs are a class of diverse organic compounds containing two or more fused aromatic rings of carbon and hydrogen atoms. They are ubiquitous pollutants formed from the combustion of fossil fuels and are always found as a mixture of individual compounds. Owing to their low solubility and high affinity for particulate matter, PAHs are not usually found in water in notable concentrations. Their presence in surface water or groundwater is an indication of a source of pollution. PAHs are only slowly biodegradable under aerobic conditions and are stable to hydrolysis. The relative concentrations of PAHs in air, water, and food are usually the same, although this can change depending on certain sources of pollution. In drinking-water, the PAHs detected in the highest concentrations are fluoranthene (FA), phenanthrene, pyrene (PY), and anthracene. Of the six PAHs usually measured in water for regulatory purposes, FA is the only PAH that is detected to any significant extent. Some PAHs are known carcinogens, but many of these have not been measured in drinking-water, have not been detected in drinking-water (e.g. dibenzo[*a*,*e*]pyrene, dibenzo[*a*,*h*]pyrene, chrysene), or have been found in relatively low concentrations in drinking-water. BaP, a carcinogen, is the most extensively studied PAH (WHO, 1996).

Chlorinated PAHs may be formed where residual chlorine is present in drinking-water (Shiraishi et al., 1985). Little is known about the toxicity of these compounds compared with that of their parent compounds (Bhatia et al., 1987).

The PAHs discussed in this document were chosen on consideration of their solubility, their presence in surface water and drinking-water from water survey data, and their classification as known or suspected carcinogens.

1.1 Identity

Compound	*CAS no.*	*Molecular formula*	*No. of aromatic rings*	*Abbreviation used*
Fluoranthene	206-44-0	$C_{16}H_{10}$	4	FA
Pyrene	129-00-0	$C_{16}H_{10}$	4	PY
Benz[*a*]anthracene	56-55-3	$C_{18}H_{12}$	4	BaA
Benzo[*b*]fluoranthene	205-99-2	$C_{20}H_{12}$	5	BbFA
Benzo[*j*]fluoranthene	205-82-3	$C_{20}H_{12}$	5	BjFA
Benzo[*k*]fluoranthene	207-08-9	$C_{20}H_{12}$	5	BkFA
Benzo[*a*]pyrene	50-32-8	$C_{20}H_{12}$	5	BaP
Dibenz[*a*,*h*]anthracene	53-70-3	$C_{22}H_{14}$	5	DBahA
Benzo[*ghi*]perylene	191-24-2	$C_{22}H_{12}$	6	BghiP
Indeno[1,2,3-*cd*]pyrene	193-39-5	$C_{22}H_{12}$	6	IP

1.2 Physicochemical properties (WHO, 1997)

At ambient temperatures, PAHs are colourless to yellow solids. The general characteristics common to the class are high melting and boiling points, low vapour pressures, and low water solubilities; the latter tend to decrease with increasing molecular mass. PAHs are highly lipophilic.

PAH	*Melting point (°C)*	*Boiling point (°C)*	*Vapour pressure at 25°C (kPa)*	*Water solubility at 25°C (µg/litre)*	*Henry's law constant at 25°C (kPa m^3/mol)*	*n-Octanol-water partition coefficient (log K_{ow})*
FA	108.8	375	1.2×10^{-6}	260	6.5×10^{-4}	5.22
PY	150.4	393	6.0×10^{-7}	135	1.1×10^{-3}	5.18
BaA	160.7	400	2.8×10^{-8}	14	n.g.[d]	5.61

BbFA	168.3	481	6.7×10^{-8}	1.2[b]	5.1×10^{-5}	6.12
BjFA	165.4	480	2.0×10^{-9}[a]	2.5[b]	$4.4\times10^{!5}$	6.12[a]
BkFA	215.7	480	1.3×10^{-11}	0.76	n.g.	6.84[a]
BaP	178.1	496	7.3×10^{-10}	3.8	3.4×10^{-5}	6.50
DBahA	266.6	524	1.3×10^{-11}	0.5 (27°C)	7×10^{-6}[a]	6.50
BghiP	278.3[a]	545	1.4×10^{-11}	0.26	2.7×10^{-5}	7.10
IP	163.6	536	1.3×10^{-11}	62[b]	2.9×10^{-5}	6.58

[a] Calculated.
[b] Temperature not given.
[c] Range of measured data for sediments and soils.
[d] Not given.

1.3 Major uses

Only a small number of PAHs are produced commercially, including FA and PY, which are used mainly as intermediates in the production of fluorescent dyes (FA) and perinon pigments (PY) (Franck & Stadelhofer, 1987; Griesbaum et al., 1989). In 1993, one of the greatest producers worldwide manufactured <50 t of FA and <500 t of PY (WHO, 1997).

Coal and crude oils contain PAHs in considerable concentrations owing to diagenetic formation in fossil fuels (IARC, 1985, 1989). As a consequence, the compounds are also found in coal and mineral oil products such as coke, bitumen, coal tar (and creosote), heating oils, vehicle fuels, lubricating and cutting oils, and printing colour oils (Grimmer et al., 1981; IARC, 1984; Tetzen, 1989; Menichini et al., 1990).

PAHs in the environment are almost always derived from anthropogenic activities. The largest amount of PAHs enters the environment via the atmosphere from incomplete combustion processes, such as processing of coal and crude oil (e.g. refining, coal gasification, coking), industrial use of coal and mineral oil products (aluminium production, iron and steel production, foundries), heating (power plants and residential heating using wood, coal, and mineral oil), fires (e.g. forest, straw, agriculture, cooking), incineration of refuse, vehicle traffic, tobacco smoking, and volcanic activities (for quantitative data on the release of PAHs in the environment, see WHO, 1997).

1.4 Environmental fate

PAHs are emitted mainly into the atmosphere and have been detected long distances from their source (Bjørseth & Sortland, 1983; McVeety & Hites, 1988). Because of their low vapour pressures, compounds with five or more aromatic rings will exist mainly adsorbed to airborne particulate matter, such as fly ash and soot. Those with four or fewer rings will occur both in the vapour phase and adsorbed to particles (Hoff & Chan, 1987; Baker & Eisenreich, 1990).

PAHs reach the hydrosphere mainly by dry and wet deposition and road runoff but additionally from industrial wastes containing PAHs and leaching from creosote-impregnated wood. PAHs are adsorbed strongly to the organic fraction of sediments and soils. Leaching of PAHs from the soil surface layer to groundwater is assumed to be negligible owing to the adsorption and to biodegradation in the aerobic soil surface layer, although their presence in groundwater has been reported, mainly at contaminated sites. The volatility of the compounds from water phases is low, with half-lives of 500 and 1550 hours for BaA and BaP, respectively (Southworth, 1979).

The compounds are very slowly biodegradable under aerobic conditions in the aqueous compartment. The biodegradation rates decrease drastically with increasing number of aromatic rings. In laboratory experiments with soil samples, the calculated half-lives for the selected compounds vary widely, from about 100 days to a couple of years (Bossert & Bartha, 1986; Coover & Sims, 1987; Park et al., 1990; Wild et al., 1991). PAHs are stable towards hydrolysis.

The most important degradation process for PAHs in air and water is indirect photolysis under the influence of hydroxyl radicals. Under laboratory conditions, the reaction of the compounds with airborne hydroxyl radicals shows maximum half-lives between about 3 and 11 hours (Atkinson, 1987). For pure water, the photodegradation half-lives appear to be in the range of hours (Mill et al., 1981; Mill & Mabey, 1985), whereas the half-lives increase drastically when sediment/water partitioning is taken into account (Zepp & Schlotzhauer, 1979).

Measured bioconcentration factors (BCFs) for the compounds in the aquatic environment vary widely owing to different measurement techniques and are especially high for some algae (BCF = 2398-55 800), crustaceans (BCF = 180-63 000), and molluscs (BCF = 58-8297). Bioconcentration factors in fish appear to be much lower than in these organisms because of rapid biotransformation processes (BCF = 10-4700) (WHO, 1997).

In summary, it can be concluded that sediments and soils are the main sinks for PAHs in the environment and that PAHs with four or more aromatic rings are persistent in the environment (Mackay et al., 1992).

2. ANALYTICAL METHODS

A preconcentration step for sample enrichment may be necessary for the analysis of PAH levels in uncontaminated aqueous samples. Further, considerable adsorption losses during collection and storage of samples have to be taken into account. Apart from liquid/liquid extraction procedures (e.g. with dichloromethane), various solids have been successfully used for the preconcentration: Tenax-GC, XAD resins, open-pore polyurethane foam (PUF), and bonded-phase silica gel (van Noort & Wondergem, 1985; Basu et al., 1987). Detection is carried out by gas chromatography with a flame ionization or a mass selective detector and by high-performance liquid chromatography with an ultraviolet or a fluorescence detector. The detection limits are between 0.01 and 200 ng/litre (Basu & Saxena, 1978a; Desideri et al., 1984).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Numerous studies refer to the occurrence of PAHs in the environment. The compounds were detected in all environmental compartments. The concentrations differ widely owing to the widely differing sampling locations and conditions.

In the matrices air, water, and food, the relative concentrations of the selected PAHs appear to be in the order of FA ≅ PY > BghiP ≅ IP > BbFA ≅ BkFA > BaA ≅ BaP > DBahA. Very few data were available for BjFA; its concentration is probably in about the same range as those of BbFA and BkFA.

3.1 Air

In the absence of industrial or other point sources of pollution, PAHs in the atmosphere are mainly from residential heating and vehicle traffic. The levels of individual substances vary over several orders of magnitude and are generally in the range between <0.1 and 100 ng/m^3 (WHO, 1997). PAHs are mainly adsorbed to airborne particulate matter.

3.2 Water

Apart from highly industrially polluted rivers, the concentrations of individual PAHs in surface and coastal waters are generally ≤50 ng/litre (WHO, 1997). Concentrations above this level (sometimes into the 10 µg/litre range) indicate contamination by PAHs mainly through industrial point sources and shipyards, atmospheric deposition, and urban runoff. Ships for inland navigation are periodically treated with coal tar to prevent corrosive damage. The leaching/abrasion of this coating is a source of PAHs (Berbee, 1992). In addition, wood preserved

with creosote can leach PAHs into the environment, especially into waters where wood is used for bank protection or harbours and in the disposal of creosote-impregnated railway ties (Berbee, 1992; Sandell & Tuominen, 1996).

PAH levels in uncontaminated groundwater are usually in the range of 0-5 ng/litre. Leaching of PAHs from soils into groundwater is negligible, as the compounds tend to adsorb strongly to the soil organic matter (Woidich et al., 1976; Stuermer et al., 1982). Only at heavily contaminated sites do the PAHs reach the groundwater, giving concentrations above 10 µg/litre (Environment Canada, 1994).

Elevated concentrations of PAHs (predominantly FA, BbFA, PY, IP, phenanthrene) were observed in rainwater and especially in snow and fog (WHO, 1997). This is probably a result of the adsorption of the compounds to air particulate matter, which is finely dispersed into the water during wet deposition.

The typical concentration range for the sum of the selected PAHs in drinking-water is from about 1 ng/litre to worst cases of 11 µg/litre (see Table 1 [after the references]). Many individual PAHs are at concentrations below the detection limit. As an example, in 1988-1989, the sum of the six Borneff PAHs was below the detection limit of 5 ng/litre in 88% (5287 of 5975) of drinking-water samples from waterworks in Germany; the concentrations were below 40 ng/litre in 10% (588 samples); and concentrations above 200 ng/litre were detected in 0.08% (5 samples) (Dieter, 1994).

The main source of PAH contamination in drinking-water is usually not the raw water sources but the coating of the drinking-water distribution pipes. At least in the past, coal tar was a common coating material for water pipes, used to give effective protection against corrosion. After the passage of drinking-water through those pipes or after repair work, significantly increased PAH levels have been detected in the water (Vu Duc & Huynh, 1981; Basu et al., 1987; Davi et al., 1994); for example, a concentration of 2.7 µg of Borneff PAHs per litre was detected in one sample of such water (State Chemical Analysis Institute, 1995). Although WHO has called for a cessation of this practice (WHO, 1996), many countries still have a large amount of pipes lined with coal tar coating. If BaP is present at elevated concentrations in drinking-water, this is indicative of the presence of particulate matter (e.g. from the deterioration of the coal tar coating).

Table 1. Concentrations of the selected PAHs in drinking-water (ng/litre)

Location, year [reference]	Source of water	FA[b]	PY	BaA	BbFA[b]
Austria, 1976 [1]	Spring water and well- water	3.5-6.5	1.6-3.5	n.d.-1.9	0.2-0.8
USA, 1976- 1977 [2,3]	Treated water from polluted source	2.4-24			
Norway, 1978- 1981 [4,5]	Tap- water	0.58-24	<0.3-15	0.1-5.5	0.05-4.0
Canada, 1987-1990 [6]	Treatment plant water	<5-623	40		<5-40
Poland, 1984 [7]	Spa water	4-21			4-29
Switzerland, 1981 [8]	Reservoir[a]	150-3400			9-14
	Tap-water	3.3			0.4-0.6
Italy, 1991-1993 [9]	Treatment plant	<20	<10	<10	<20
	Fountain; new coal tar lining	<20	max. 30	max. 20	<20
England & Wales, 1996 [10]	Tap-water (hard water)	585			20
	Tap-water (soft water)	6520			1600

n.d. = not detected; tr = traces.

[a] The authors attribute these high levels to the use of coal tar distribution pipes.
[b] PAHs measured for regulation purposes in the EEC (Borneff PAHs).

References:

[1] Woidich et al. (1976)
[2] Thruston (1978)
[3] Basu & Saxena (1978b)
[4] Kveseth et al. (1982)
[5] Berglind (1982)
[6] Environment Canada (1994)
[7] Babelek & Ciezkowski (1989)
[8] Vu Duc & Huynh (1981)
[9] Davi et al. (1994)
[10] Drinking Water Inspectorate, personal communication (1997)

In Canada, significantly increased levels of PAHs in drinking-water were reported for which the reason is not known (Environment Canada, 1994). Also, the PAH concentrations in spa waters from 10 different spas in the Sudetes region (Poland) are surprisingly high (Babelek & Ciezkowski, 1989). In most of the PAH-contaminated spas, groundwater, presumably polluted, also contributes to the spa water.

In the majority of drinking-water samples taken in England and Wales, PAHs are not detected above the standard (EEC, 1980; CEC, 1995) for PAHs of 0.2 µg/litre. Only 5% of the reported samples fail to meet the standard. In practically every case where the PAH standard has been exceeded, the only PAH detected to any significant extent is FA. This is indicative of a coal tar pitch lining in good condition where the hard groundwater very slowly dissolves the lining. There are very few cases where other PAHs have been detected in significant concentrations, and these occur mainly where soft corrosive water is derived from surface water sources. This is probably indicative of physical deterioration of the lining, releasing particulate containing PAHs into the water supply (Drinking Water Inspectorate, personal communication, 1997).

3.3 Food

PAHs have been detected in fresh vegetables, fruits, and cereals as a result of the deposition of airborne PAHs, particularly near industrial sources or in areas with high traffic (Tuominen et al., 1988; de Vos et al., 1990; Dennis et al., 1991). PAHs have also been found in mussels, snails, and fish from contaminated waters (Sirota & Uthe, 1981; Rostad & Pereira, 1987; Speer et al., 1990). PAHs are also present at elevated levels in some vegetable oils and margarine (Dennis et al., 1991; Thomson et al., 1996), probably formed during processing. PAHs are also formed during some methods of food preparation, such as char-broiling, grilling, roasting, frying, or baking. The highest levels were detected in smoked and grilled meat and fish samples (up to about 200 µg/kg) (WHO, 1997).

3.4 Estimated total exposure and relative contribution of drinking-water

For the general population, the major routes of exposure to PAHs are from inhalation via ambient and indoor air and ingestion via food.

For ambient air, residential heating and vehicle traffic appear to be the main sources of exposure. In the direct vicinity of an emission source, a maximum intake of 1 µg of BaP per day may be reached (WHO, 1987; LAI, 1992). For the other selected compounds, maximum intakes of between 0.004 (DBahA) and 0.06 (BbFA) µg/day were estimated (Chen et al., 1980; Guicherit & Schulting, 1985). For indoor air, an important contribution is from smoking. In this case, the BaP intake may almost reach that for polluted ambient air. Especially in developing countries, the use of open fires for heating and cooking may further increase PAH exposure (Mumford et al., 1987; Raiyani et al., 1993).

The main contributors of PAHs to the total dietary intake appear to be cereals, oils, and fats. The

oil and fat group has high individual PAH levels, whereas the cereal group, although never containing high individual PAH concentrations, is a main contributor by weight to total intake in the diet. Smoked meat and fish products, although containing the highest PAH levels, appear to be low to modest contributors, as they are minor components of the usual diet (Larsson, 1982, 1986; Dennis et al., 1983, 1991; Maga, 1986). However, it should be noted that various countries and cultures have very different diets and methods of cooking, which may result in exposure to very different amounts of PAHs.

There are a few studies on daily intake of individual PAHs from food from western Europe (Dennis et al., 1983; Vaessen et al., 1984; de Vos et al., 1990; Pfannhauser, 1991; Turrio-Baldassarri et al., 1996) and Canada (WHO, 1996). The results for the individual PAHs were in the same range. BaP, BghiP, PY, and FA can each reach a maximum daily intake of ≤10 µg per person; for each of DBahA, IP, BkFA, and BaA, the maximum daily intake is ≤0.5 µg per person. The maximum/median intake levels for the PAHs selected in this guideline, in µg/day per person, have been estimated to be as follows: FA (4.3/0.6); PY (4.0/0.6); BaA (0.14/0.02); BbFA (1.0/0.005); BjFA (0.9/0.03); BkFA (0.3/0.04); BaP (0.36/0.05); DBahA (0.10/0.015); BghiP (7.6/0.12); and IP (0.31/0.025) (Pfannhauser, 1991).

From the intake data for food and the drinking-water levels (see Table 1 [after the references]), it can be estimated that about 1% of the total dietary intake of PAHs is from drinking-water, assuming a consumption of 2 litres/day. Where there are elevated PAH levels from contamination by coal tar coatings, which would occur mainly during and after repair work, PAH intake from drinking-water could be equal to or even exceed other dietary intakes.

Exposure via the oral and inhalation pathways varies considerably depending on diet and lifestyle, with inhalation exposure being of greater importance where indoor levels of PAHs are high because of smoking (Greenberg, 1996; Ihme & Wichmann, 1996; Jansen et al., 1996).

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

4.1 Absorption

PAHs are absorbed in experimental animals and humans through the pulmonary tract, the gastrointestinal tract, and the skin. Absorption of BaP, DBahA, and PY was high (30-90%) following low and high oral doses in rats (Chang, 1943; Foth et al., 1988; Withey et al., 1991). Absorption from the gastrointestinal tract occurs rapidly. Oral administration of FA, PY, and BaA to rats caused peak concentrations of these compounds in the blood after 1-2 hours (Lipniak & Brandys, 1993). The intestinal absorption of the individual PAHs is highly dependent on their solubility, their lipidity, the presence of bile (Rahman et al., 1986), and the lipidity of the various PAH-containing foods ingested. Whereas oils enhanced the absorption of BaP, water and solid food suppressed the absorption (Kawamura et al., 1988).

4.2 Distribution

In laboratory animals, PAHs become widely distributed in the body following administration by any one of a variety of routes and are found in almost all internal organs, particularly those rich in lipid (WHO, 1997). Maximum concentrations of BaA in perfused tissues (e.g. liver, blood, brain) were achieved within 1-2 hours after administration of high oral doses (76 and 152 mg/kg of body weight). In lesser perfused tissues (e.g. adipose and mammary tissue), maximum levels of this compound were reached in 3-4 hours (Bartosek et al., 1984). In male Wistar rats receiving a gavage dose of 2-15 mg of [^{14}C]-pyrene per kg of body weight, the fat had the highest levels of radioactivity, followed by the kidney, liver, and lungs (Withey et al., 1991). Orally absorbed DBahA in rats was also widely distributed to several tissues. After continuous oral administration of 0.5 µg of [^{3}H]BaP daily to male rats for up to 7 days, the radioactivity persisted in liver, kidney, lung, and testis (Yamazaki & Kakiuchi, 1989). Orally administered BaP (200 mg/kg of body weight) has been shown to cross the placental barrier and has been detected in fetal tissues

(2.77 µg/g) (Shendrikova & Aleksandrov, 1974). Using ^{14}C-tagged BaP, a BaP concentration 1-2 orders of magnitude lower in embryonic than in maternal tissues was determined after oral administration in mice (Neubert & Tapken, 1988). Differences in concentrations in the fetus among the various PAHs appeared to be highly dependent on the gastrointestinal absorption of the compound.

4.3 Metabolism

The metabolism of PAHs is complex. Generally, the process involves epoxidation of double bonds, a reaction catalysed by the cytochrome P-450-dependent monooxygenase, the rearrangement or hydration of such epoxides to yield phenols or diols, respectively, and the conjugation of the hydroxylated derivatives. Reaction rates vary widely, and interindividual variations of up to 75-fold have been observed, for example, with human macrophages, mammary epithelial cells, and bronchial explants from different donors. Most metabolism results in detoxification, but some PAHs in some situations become activated to DNA-binding species, principally diol-epoxides, that can initiate tumours (WHO, 1997).

Although the PAHs are similar, they have structural differences that are the basis for differences in metabolism and relative carcinogenicity. The metabolism of the more carcinogenic, alternant (equally distributed electron density) PAHs, such as BaP, BaA, and DBahA, seems to differ in some ways from that of non-alternant (uneven electron density distribution) PAHs, such as FA, BbFA, BkFA, BjFA, IP, BghiP, and PY (Phillips & Grover, 1994; ATSDR, 1995).

In general, little is known about the metabolism of most PAHs, particularly in non-rodent species. It should be noted that there appear to be species differences in the enzymes that activate PAHs (Michel et al., 1995) and in the formation of DNA adducts (Kulkarni et al., 1986).

4.4 Excretion

PAH metabolites and their conjugates are excreted predominantly via the faeces and to a lesser extent in the urine. Conjugates excreted in the bile can be hydrolysed by enzymes of the gut flora and reabsorbed. It can be inferred from available data on total body burdens in humans that PAHs do not persist for long periods in the body and that turnover is rapid. This excludes those PAH moieties that become covalently bound to tissue constituents, in particular to nucleic acids, and are not removed by repair (WHO, 1997). The excretion of urinary metabolites is a method used to assess internal human exposure of PAHs.

5. EFFECTS ON LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

The toxicological effects of the PAHs are summarized individually in order of molecular weight, with emphasis on oral studies where available. The toxicology of FA is described in the most detail because this is the PAH found in notable quantities in tap-water where there is contamination by coal tar coatings and because of the uncertain classification of this PAH.

Research on the toxicological effects of PAHs has been focused on the carcinogenicity of some selected compounds, but usually employing dermal, inhalation, or subcutaneous rather than oral exposure. The carcinogenic classification of the various PAHs is given in Table 2. There are only limited studies on non-carcinogenic end-points.

5.1.1 Fluoranthene (FA)

5.1.1 Acute and short-term exposure

The oral LD_{50} for FA in the rat is about 2000 mg/kg of body weight (range 1270-3130 mg/kg of body weight) (Smyth et al., 1962).

Male and female CD-1 mice (20 per sex per group; 30 per sex for controls) were given FA by gavage for 13 weeks at 0, 125, 250, or 500 mg/kg of body weight per day and then sacrificed and autopsied (US EPA, 1988). All treated mice exhibited nephropathy, increased salivation, and increased liver enzyme levels in a dose-dependent manner. Mice given 500 mg/kg of body weight per day had increased food consumption and increased body weight. At doses of 250 and 500 mg/kg of body weight per day, statistically increased serum glutamate-pyruvate transaminase (SGPT) levels and increased absolute and relative liver weights were noted, as well as compound-related microscopic liver lesions (indicated by pigmentation) in 65 and 87.5% of the mice, respectively. Based on these increased SGPT levels, kidney and liver pathology, and clinical and haematological changes, the NOAEL is 125 mg/kg of body weight per day.[1]

[1] Source: Integrated Risk Information System (IRIS). Online. Cincinnati, OH, US Environmental Protection Agency, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment.

Table 2. Evaluation of individual PAHs for carcinogenicity in animals and humans

PAH	WHO, 1997[a]	IARC, 1987[b]
BaA	positive	2A
BbFA	positive	2B
BjFA	positive	2B
BkFA	positive	2B
BghiP	negative	3
BaP	positive	2A
DBahA	positive	
FA	(positive)[c]	3
IP	positive	
PY	(questionable)	

[a] Based on animal carcinogenicity studies only.

[b] 2A - probably carcinogenic to humans; 2B - possibly carcinogenic to humans; 3 - not classifiable as to human carcinogenicity.

[c] Recent data on FA since this meeting could change the FA rating to questionable.

5.1.1.2 Mutagenicity and related end-points

In vitro genotoxicity studies for FA are mostly positive, but *in vivo* genotoxicity studies are mostly negative (IARC, 1983; US EPA, 1992; ATSDR, 1995; WHO, 1997).

FA tested positive with metabolic activation for gene mutation in *Salmonella typhimurium*, in the *Escherichia coli* SOS chromotest for DNA damage, and in *in vitro* tests in mammalian cells for DNA damage, mutation, and chromosomal effects.

After oral administration of FA at 750 mg/kg of body weight, *in vivo* tests for sister chromatid exchange in mouse bone marrow were negative (Palitti et al., 1986). FA did not show any evidence of genotoxicity in the mouse bone marrow micronucleus or rat liver unscheduled DNA synthesis test systems following acute oral administration at levels of up to 2000 mg/kg of body weight (Stocker et al., 1996).

A major FA-DNA adduct has been identified in most of the tissues examined (including liver, lung, heart, kidney, spleen, and thymus) in Sprague-Dawley rats chronically fed FA in the diet (Gorelick et al., 1989). In BLU:Ha and CD-1 mice treated intraperitoneally with tumorigenic doses of FA (total of 3.5 mg over 2 weeks), highest levels of FA-DNA adduct were found in the lung (Wang et

al., 1995a,b).

5.1.1.3 Dermal carcinogenicity studies

Dermal application of 1% FA 3 times a week for 1 year to the backs of 20 female Swiss-Albino Ha/ICD/Mill mice did not induce skin tumours (Hoffmann et al., 1972), nor did 250 µg of FA applied to 15 male C3H mice for 82 weeks (Horton & Christian, 1974).

Application of 40 µg of FA alone caused no tumours in 50 female Swiss mice treated for 440 days, but FA was a co-carcinogen in a study in which the same dose of FA in combination with BaP induced a 2-fold increase in mouse skin tumours compared with BaP alone (van Duuren & Goldschmidt, 1976).

FA did not exhibit tumour-initiating activity after 24 weeks in 30 female Swiss mice topically administered 10 doses (0.1 mg per animal) followed by promotion with croton oil for 20 weeks (Hoffmann et al., 1972).

5.1.2 Pyrene (PY)

Male and female CD-1 mice (20 per sex per group) given PY by gavage at doses of 0, 75, 125, or 250 mg/kg of body weight per day in corn oil for 13 weeks exhibited kidney effects (renal tubular pathology, decreased kidney weights) (US EPA, 1989). The low dose (75 mg/kg of body weight per day) was considered the NOAEL for nephropathy and decreased kidney weights. [2]

[2] Source: Integrated Risk Information System (IRIS). Online. Cincinnati, OH, US Environmental Protection Agency, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment.

Mutagenicity and related studies gave negative or equivocal results. There were no oral carcinogenicity studies. Skin painting assays in mice for complete carcinogenesis or initiating capacity have been negative or inconclusive. Mice injected intraperitoneally did not show significant elevated tumour rates. A ^{32}P-postlabelling test for covalent DNA binding of PY to mouse skin *in vivo* gave negative results. PY was co-carcinogenic with BaP in a mouse skin assay (WHO, 1997).

5.1.3 Benz[a]anthracene (BaA)

Male B6AF1/J newborn mice (40 per group) were administered 1.5 mg of BaA per day by oral gavage twice over 3 days (Klein, 1963). After 568 days of observation, increased incidences of hepatomas and pulmonary adenomas (80% and 85%, respectively) were noted, compared with the controls with solvent only (10% and 30%). No malignant tumours were observed. In a parallel study with the same dose 3 times weekly over 5 weeks with sacrifice at 547-600 days, 100% hepatomas and 95% pulmonary adenomas were noted (controls: 10% and 35%).

C57BL mice receiving a total dose of 0.5, 4, or 8 mg of BaA by gavage showed forestomach papillomas (0/13, 1/10, and 1/8, respectively) after 16 months (Bock & King, 1959).

BaA is genotoxic. It produces tumours in most assays in mice treated dermally, intraperitoneally, and subcutaneously. There are indications of immunotoxicity and fetotoxicity in subcutaneous studies. DNA adducts were detected in mouse skin after dermal application of BaA (US EPA, 1992; ATSDR, 1995; WHO, 1997).

5.1.4 Benzo[b]fluoranthene (BbFA)

BbFA is genotoxic. Exposure of rats to BbFA by lung implantation resulted in tumour formation, as did intraperitoneal exposure of newborn mice. Skin painting and initiation/promotion studies in

mice were positive. DNA adducts were detected *in vitro* and *in vivo* (US EPA, 1992; ATSDR, 1995; WHO, 1997).

5.1.5 Benzo[j]fluoranthene (BjFA)

From limited studies, there is evidence that BjFA is genotoxic and carcinogenic. BjFA showed tumorigenic activity in one skin painting assay, in initiation/promotion studies, and in the newborn mouse intraperitoneal bioassay. Exposure of rats by lung implantation did not result in tumour formation. DNA adducts were detected *in vitro* and in mouse skin *in vivo* after topical application of BjFA (US EPA, 1992; ATSDR, 1995; WHO, 1997).

5.1.6 Benzo[k]fluoranthene (BkFA)

From the available evidence, BkFA is genotoxic and carcinogenic. Skin painting assays were not positive, but initiation/promotion studies resulted in increased tumour incidence. No significant tumorigenic activity was found in a lung adenoma bioassay in newborn mice. Lung implantation of BkFA produced tumours in rats. DNA adducts have been detected *in vitro* and *in vivo* (US EPA, 1992; ATSDR, 1995; WHO, 1997).

5.1.7 Benzo[a]pyrene (BaP)

BaP is genotoxic in a variety of *in vitro* tests with metabolic activation and in *in vivo* studies. In mice, oral administration of BaP induces tumours of the forestomach. It induces mammary gland tumours after oral administration in rats. BaP has produced skin tumours after dermal application in mice, rats, rabbits, and guinea-pigs. It produced lung and respiratory tumours when administered intratracheally to rats and hamsters. Lung implantation of BaP in rats caused pulmonary tumours. BaP administered intraperitoneally induced lung and hepatic tumours in mice. It was carcinogenic after subcutaneous administration to mice, rats, hamsters, guinea-pigs, and some primates. BaP binds to DNA and forms DNA adducts in target organs.

BaP was discussed in the 1993 WHO *Guidelines for drinking-water quality* (WHO, 1996). Only the oral studies on BaP and coal tar published since then are given here. It should be noted that further long-term oral studies are in progress.

The tumorigenic activity of BaP (and coal tar; see section 5.3) after ingestion was investigated (Weyand et al., 1995). BaP (16 or 98 mg/kg of feed, equal to 41 and 257 µg/day, respectively) in a basal gel diet was fed to female A/J mice (30 per group) for 260 days. After sacrifice, forestomach tumours and pulmonary adenomas were diagnosed and counted. The incidence of forestomach tumours after oral administration was 20% and 100%, respectively; tumour multiplicity was 0.24 and 4.22. The incidence of forestomach carcinomas in mice with forestomach tumours was 8% and 52%, respectively. Controls ingesting basal gel diet only showed no forestomach tumours. Whereas 16 mg/kg of feed did not induce a significant level of lung tumours (36%; 25 mice; 0.48 tumours per mouse), ingestion of 98 mg/kg of feed induced lung tumours in 52% of the mice (27 mice; 0.59 tumours per mouse). Nineteen per cent of the controls on basal diet showed pulmonary adenomas (21 mice; 0.19 tumours per mouse). It should be noted that A/J mice are susceptible to pulmonary tumours.

In a 2-year carcinogenicity bioassay, female B6C3F$_1$ mice were fed BaP at 0, 5, 25, or 100 mg/kg of feed (see also study with coal tar, section 5.3) (Culp et al., 1996). Forestomach tumours were induced in all groups of mice fed BaP. A 6% incidence was observed at the 5 mg/kg of feed dose (18.5 µg/day), with the incidence increasing sharply to 78% at the 25 mg/kg of feed dose (90 µg/day) and then to 98% at 100 mg/kg of feed (350 µg/day). All of the mice fed BaP at 100 mg/kg of feed were removed by 80 weeks because of morbidity or death. A linear dose-response was observed between BaP dose and adduct levels in the forestomach of mice fed the same doses in a 4-week study.

5.1.8 Dibenz[a,h]anthracene (DBahA)

There are only limited studies on oral exposure to DBahA, but they do provide some evidence for carcinogenicity through this route.

In a lifetime study, mammary carcinomas were observed in 1/20 female and 13/24 pseudo-pregnant BALB/c mice dosed with 0.5% DBahA after 15 weeks of dosing (Biancifiori & Caschera, 1962). No control group was included.

DBA/2 mice (21 per sex) were given DBahA in an aqueous olive oil emulsion (0.8 mg of DBahA per day) for 200 days (Snell & Stewart, 1962). In general, the animals did not tolerate the vehicle well, and extensive dehydration and emaciation led to early death. At the end of the exposure, almost all surviving treated mice (14 males and 13 females), but only one of the control mice, showed pulmonary adenomatosis, alveologenic carcinoma, mammary carcinoma, and haemangioendotheliomas.

DBahA is genotoxic *in vitro* and *in vivo*. It causes tumours in various organs in mice after oral administration and is a potent carcinogen in several species after various routes of administration. It forms DNA adducts in mouse skin *in vitro* and *in vivo* (US EPA, 1992; ATSDR, 1995; WHO, 1997).

5.1.9 Benzo[ghi]perylene (BghiP)

There are insufficient data on the genotoxic potential of BghiP, although the existing evidence is positive. BghiP tested negative for carcinogenicity activity and tumour-initiating activity in mouse skin. It was negative in the rat lung implantation assay. It has shown some co-carcinogenicity with BaP in mouse skin. BghiP binds to DNA *in vitro* and *in vivo* (US EPA, 1992; ATSDR, 1995; WHO, 1996, 1997).

5.1.10 Indeno[1,2,3-cd]pyrene (IP)

The limited data on the genotoxicity of IP are generally positive. IP has tumour-initiating activity in mouse skin and is carcinogenic in rat lungs. It bound to mouse skin with the formation of DNA adducts (US EPA, 1992; ATSDR, 1995; WHO, 1997).

5.2 Comparative studies

The following is a summary of the comparative studies on tumorigenic activity of individual PAHs, which have been used as the basis for comparative potency factors (see section 7). Full details and discussion of the adequacy of the databases are given in the original references and elsewhere (Clement Associates, 1988; US EPA, 1992). In general, it can be said that data from the skin painting and lung implantation studies have been used preferentially to those of initiation/promotion experiments and intraperitoneal studies for estimating comparative potencies. There are no comparative studies on oral administration.

Exact comparative data are given only where this is possible (e.g. where single PAHs were tested in the same experiment at the same dose).

5.2.1 Carcinogenicity studies

5.2.1.1 Dermal

Skin-painting studies

Solutions of 0.5% BaP, BbFA, BjFA, or BkFA were applied dermally 3 times weekly to female Swiss Millerton mice (20 per group) through their lifetime, and the number of skin tumours was

determined (Wynder & Hoffmann, 1959b). The percentages of papillomas/carcinomas for these compounds after 4 months were 70/20, 95/10, 40/5, and 0/0, respectively. Minimal activity (10 papillomas) was found with BkFA after 11 months. BaP > BbFA > BjFA > BkFA.

In a similar study regime, 0.01% solutions of BaP or DBahA applied dermally to mice (20 per group) showed 10%/10% and 15%/5% papillomas/carcinomas after 6 months. A 0.1% solution of FA and 10% solution of PY showed no activity (Wynder & Hoffmann, 1959a). BaP = DBahA >> FA; PY.

A further study compared the carcinogenicity of BaP, BghiP, and IP applied dermally to mice 3 times weekly as above (Hoffmann & Wynder, 1966). A dose of 0.05% of BaP, BghiP, or IP produced 17/20, 0/20, and 0/20 tumour-bearing mice, showing that BaP is more potent than either of the other PAHs. There were no controls. BaP >> BghiP; IP.

In a lifetime skin painting assay with female NMRI mice, BaP and BbFA were carcinogenic, BjFA was weakly carcinogenic, and BkFA and IP had no cancer-inducing effects (Habs et al., 1980). BaP >> BbFA > BjFA > BkFA; IP.

Initiation/promotion assay

Ten doses of BaP, BghiP, or IP at a total dose of 0.25 mg per mouse were applied every second day to the backs of Swiss Millerton mice followed by promotion with 2.5% croton oil in acetone. Tumour-bearing animals were reported as 24/30, 2/27, and 5/30, respectively (Hoffmann & Wynder, 1966). BaP >> IP > BghiP.

In an initiation/promotion assay in CD-1 mice, four PAHs (BaP, BbFA, BjFA, and BkFA) were each applied at a total dose of 30 µg in 10 subdoses over 20 days to the shaved backs of 20 mice per group (LaVoie et al., 1982). Ten days after completion of the initiation, promotion was begun by thrice-weekly application of 12-*O*-tetradecanoylphorbol-13-acetate in 0.1 ml of acetone. The skin tumours were predominantly squamous cell papillomas. After 20 weeks (10 weeks for BaP), the percentage of skin tumour-bearing animals was 85, 45, 30, and 5, respectively. The vehicle controls had no tumours. BaP > BbFA > BjFA > BkFA.

5.2.1.2 Other routes

Intraperitoneal injection in newborn mice

The tumorigenic activity of the non-alternant PAHs (BbFA, BjFA, BkFA, and IP) as well as BaP was evaluated by injecting intraperitoneally a total of 0.5, 1.1, 2.1, 2.1, or 0.5 µmol of each compound, respectively, in dimethyl sulfoxide in aliquots of 5, 10, or 20 µl on days 1, 8, and 15 of life, respectively, to CD-1 mice (LaVoie et al., 1987). A direct comparison was not possible owing to differences in the total amount injected; however, both BbFA and BjFA exhibited significant tumorigenic activity, whereas neither BkFA nor IP was tumorigenic under these conditions. There were problems with the solubility of IP. BaP > BbFA = BjFA > BkFA; IP.

Lung implantation

Deutsch-Wenzel et al. (1983) and Wenzel-Hartung (1990) investigated the carcinogenic effects of PAHs after intrapulmonary injection and assessed the relative potencies with respect to epidermoid carcinomas and pleomorphic sarcomas. A rank order was based on BaP as reference substance: DBahA (1.91) - BaP (1.00) - BbFA (0.11) - IP (0.08) - BkFA (0.03) - BjFA (0.03). BghiP showed no tumour-producing effects.

Subcutaneous injection

Dose-response curves for BaP and DBahA were established following a single subcutaneous

injection of the PAHs in tricaprylin into the right axilla of male C3H mice (Bryan & Shimkin, 1943). Ninety-nine per cent of the tumours detected were spindle-cell sarcomas. Vehicle control response levels were not included. Under the conditions in this experiment, the potency of DBahA was estimated to be 4.5 times that of BaP. DBahA >> BaP.

In a study with C57 black mice, 8/10 males and 6/10 females had injection-site tumours 60-80 weeks after 10 weekly subcutaneous injections of 1 mg of BaA (Boyland & Sims, 1967). After a dose of 1 mg of DBahA, 20/20 males and 17/20 females had tumours. DBahA > BaA.

5.2.1.3 Further evidence

Sebaceous gland assay

Application of carcinogenic PAHs to mouse skin leads to the destruction of sebaceous glands, hyperplasia, hyperkeratosis, and even ulceration (Bock, 1964). A sebaceous gland assay has been used as a screening method for the tumorigenic potential of PAHs. Acute topical application of BaP, BaA, or DBahA was reported to suppress sebaceous glands (Bock & Mund, 1958). BaP = DBahA > BaA. In a further sebaceous gland assay using other PAHs, it was found that, compared with BaP, the activity was BaP > BbFA = BjFA = BkFA = IP (Habs et al., 1980).

DNA adduct formation

In a ^{32}P-postlabelling test for covalent DNA binding of PAHs to mouse skin *in vivo* following a single topical application, relative DNA adduct levels were BaP > BaA = DBahA = BghiP (Reddy et al., 1984). DNA adducts were not detected with PY. In a similar study, the relative covalent binding of PAHs to DNA was BbFA > BjFA > BkFA > IP (Weyand et al., 1987). In an *in vitro* study, the relative covalent binding of PAHs to DNA was reported as BaP > DBahA > BaA > PY (Grover & Sims, 1968).

5.2.2 Summary

The results of these carcinogenicity and other studies, although not always giving the identical order, can be summarized as follows: BaP = DBahP > BaA > BbFA > BjFA > BkFA > IP > FA > BghiP > PY.

FA has been included in very few comparative studies, but the above placing is probably correct (see recent studies in section 5.1.1).

A quantitative evaluation of comparative studies in this section has been attempted by several authors, leading to near agreement on values of relative potencies using BaP as 1 (see Table 3).

Table 3. Relative potencies of PAHs considered in this evaluation

Compound	Ref. 1	Ref. 2	Ref. 3	Ref. 4	Ref. 5	Ref. 6	Summary[a]
Benz[*a*]anthracene	0.145	0.1	0.1	0.1	0.1	0.1	**0.1**
Benzo[*a*]pyrene	1.0	1.0	1.0	1.0	1.0	1.0	**1.0**
Benzo[*b*]fluoranthene	0.141	0.1	0.1	0.1	0.1	0.1	**0.1**
Benzo[*ghi*]perylene	0.022	0.01	0.01	0.01			
Benzo[*j*]fluoranthene			0.1			0.1	**0.1**
Benzo[*k*]fluoranthene	0.061	0.1	0.1	0.1	0.01	0.1	**0.1**
Dibenz[*a,h*]anthracene	1.11	5	1.0	1.0	1.0	1.0	**1.0**
Fluoranthene		0.001	0.001	0.01			
Indeno[1,2,3-*cd*]pyrene	0.232	0.1	0.1	0.1	0.1	0.1	**0.1**
Pyrene	0.81	0.001	0.001	0.001			

[a] BghiP, FA, and PY are not included owing to their negative or uncertain rating as carcinogens.

References:

1. Krewski et al. (1989)
2. Nisbet & LaGoy (1992)
3. Malcolm & Dobson (1994)
4. Kalberlah et al. (1995)
5. US EPA (1993)
6. McClure & Schoeny (1995)

5.3 Studies with coal tar

Contamination of drinking-water with PAHs occurs mostly from the leaching of these compounds from coal tar coated distribution pipes. Some studies relevant to toxicity resulting from the presence of coal tar in drinking-water are therefore mentioned here, although they are not directly applicable. It should be remembered that the relative amounts of PAHs (and other compounds) in drinking-water depend on their solubility in water (e.g. FA is very soluble), and the chemical profile and concentrations will be different from that of coal tar itself.

Coal tars, also known as manufactured gas plant residue (MGP), are complex mixtures containing over 1000 compounds, of which at least 30 are PAHs. The chemical composition varies with changes in feedstocks and processing temperatures. Coal tars are known skin carcinogens when applied topically to experimental animals, and this carcinogenicity correlates with their high PAH content (Wallcave et al., 1971). There are comparatively few studies on the carcinogenic potential of coal tars after chronic ingestion. Only those studies relevant to oral toxicity are mentioned here.

5.3.1 Mutagenicity and related end-points

Coal tar paints (CTP) used in potable supply systems have been found to be mutagenic in the Ames test with metabolic activation (Robinson et al., 1984; Silvano & Meier, 1984). In a mutagenicity study on water from water distribution pipes before and after the water treatment process, the mutagenic activity did not correlate with the levels of PAHs in the water (Basu et al., 1987).

5.3.2 Carcinogenicity

CTP was positive in a dermal initiation/promotion assay with SENCAR mice, and one coal tar product was positive when tested as a complete carcinogen in the mouse at 2 µl per dermal application once weekly for 30 weeks (Robinson et al., 1984). The biological responses to the products were greater than expected from their PAH content.

In a further study, a suspension of CTP particulate was administered to groups of 40 female A/J mice by gavage over 8 weeks (Robinson et al., 1987). Total doses of 24, 240, or 1320 mg of CTP particulate resulted in 35%, 97%, and 72% of the mice developing lung tumours (tumour multiplicity: 0.46, 4.27, 4.33). Twenty-nine per cent of the control mice had lung tumours (tumour multiplicity: 0.32). Forestomach tumours were induced only at the highest dose of 1320 mg of CTP particulate. Controls had no forestomach tumours.

Female A/J mice (30 per group) were fed a basal gel diet for 260 days with 0.1% or 0.25% coal tar (MGP; 7 and 16.3 µg of BaP per day, as MGP contained 2.76 mg of BaP per g) (Weyand et al., 1995). Seventy per cent and 100% of the mice developed lung tumours, with a multiplicity of 1.19 and 12.17 tumours per mouse, respectively. Nineteen per cent of the controls had tumours, with a tumour multiplicity of 0.19 tumours per mouse. No forestomach tumours were found.

Comparing these results with those reported in the same study with pure BaP (41 and 257 µg/day; see section 5.1.7), MGP produced a considerably higher lung tumour rate than would be expected from its BaP content. In contrast, pure BaP produced forestomach tumours, which was not the case with MGP at the given concentrations.

In a follow-up study using the same dose and administration regimen (i.e. basal gel diet) in female A/J mice for 2 weeks, DNA adducts induced by MGP and BaP in mouse lung and forestomach were characterized (Weyand & Wu, 1995). The major adduct in forestomach was attributable to BaP. Three adducts were detected in mouse lung, two of which could be contributed by BbFA and BaP, respectively, but the major DNA adduct could not be attributed to any of the PAHs identified as constituents of MGP.

In a 2-year carcinogenicity bioassay, female B6C3F$_1$ mice were fed 0, 0.01, 0.03, 0.1, 0.3, 0.6, or 1.0% coal tar containing 2.24 mg of BaP per g (Culp & Beland, 1994; Culp et al., 1996). Forestomach tumours were found in each dose group, with the incidence increasing sharply from 6% in mice fed 0.1% coal tar to 30% at the 0.3% coal tar dose (equivalent to 8.4 µg and 19.1 µg of BaP per day, respectively). The incidence of forestomach tumours was approximately the same at 0.3% and 0.6% coal tar but declined at the 1.0% dose, apparently as a result of mortality from a high incidence of small intestinal adenocarcinomas in mice fed 0.6% or 1.0% coal tar. Lung tumour incidence was not reported. A parallel study with 18.5, 90, or 350 µg of BaP per day resulted in a tumour incidence of 6%, 78%, and 98%, respectively. In BaP-treated mice, one major DNA adduct was observed; this adduct accounted for 7-15% of the forestomach adducts in mice fed coal tar. A dose-related increase was observed in adduct levels in the forestomachs of BaP- and coal tar-fed mice.

From the above study, it can be seen for comparison that the same (6%) forestomach tumour incidence was noted at an oral dose of 18.5 µg of BaP per day and a 0.1% dose of coal tar containing 8.4 µg of BaP per day (i.e. coal tar has more than twice the tumorigenic potency as BaP).

Therefore, it seems that the effects of complex mixtures may be different from those of the PAHs alone. Interaction of PAHs and other compounds in coal tar may cause higher or lower tumour rates than can be expected from their content of known carcinogenic PAHs.

6. EFFECTS ON HUMANS

Human exposure to PAHs is not to individual compounds but to a mixture of these compounds in either occupational or environmental situations. There are no reports on the effects of oral ingestion by humans of the PAHs selected for evaluation, although people who consume grilled or smoked food do ingest these compounds.

A high lung cancer mortality in Xuan Wei, China, has been linked to PAH exposure from unvented coal combustion (Mumford et al., 1987; Lewtas et al., 1993). PAHs present in tobacco smoke (mainstream and sidestream) are implicated as contributing to lung and other cancers (IARC, 1986; Grimmer et al., 1987, 1988).

Most available human data are from inhalation and percutaneous absorption of PAHs from a large range of occupational exposures. In earlier times, following high dermal exposure, chimney sweeps developed skin cancers, especially scrotal cancer. Epidemiological studies are available for workers exposed at coke ovens in coal coking and coal gasification, in asphalt works, in foundries, in aluminium production plants, and from diesel exhaust (Verma et al., 1992; Armstrong et al., 1994; Partanen & Boffetta, 1994; Costantino et al., 1995). In all these occupations, there is also exposure to other chemicals, making a direct correlation of cause to increased levels in lung cancer more problematic. There is additionally the confounding factor of smoking. Evaluation of these studies shows, however, that it is plausible that the increased risk of lung cancer occurring in several of these occupations can be attributed at least in part to PAHs

(WHO, 1997).

Biomarkers have been developed to assess internal PAH exposure (WHO, 1997). Most studies focus on measurement of PAH metabolites in urine, of which 1-hydroxypyrene is the most widely used (Levin, 1995). Pyrene is normally abundant in environmental PAH mixtures. Increased urinary levels of 1-hydroxypyrene have been found, for example, in patients cutaneously treated with coal tar, in workers exposed to creosote oil, in coal tar distillery workers, in road paving workers, in coke oven workers, and in workers exposed to bitumen fumes (Jongeneelen et al., 1986, 1988a,b; Clonfero et al., 1989; Burgaz et al., 1992; Jongeneelen, 1992; Ny et al., 1993; Levin et al., 1995). Significant correlations were obtained between urinary 1-hydroxypyrene of coke oven workers or city residents and levels of PY or BaP in the ambient air (Zhao et al., 1990, 1992; Sherson et al., 1992). A controlled human exposure study showed that a 100- to 250-fold increase in a dietary dose paralleled a 4- to 12-fold increase in urinary 1-hydroxypyrene elimination (Buckley & Lioy, 1992). Trial studies suggest that urinary 1-hydroxypyrene may be a useful marker of PAH pollution in the environment (Kanoh et al., 1993). Background levels amount to 0.06-0.23 µmol/mol of creatine in non-smokers. Smokers have about double that level (WHO, 1997).

7. GUIDELINE VALUES

Evidence that mixtures of PAHs are carcinogenic in humans comes primarily from occupational studies of workers. Cancer associated with exposure to PAH-containing mixtures in humans occurs predominantly in the lung and skin following inhalation and dermal exposure, respectively. There are no data available for humans for the oral route.

There are only a few animal carcinogenicity studies on oral administration of PAHs. BaA, BaP, DBahA, and mixtures of PAHs (coal tar) were tested orally and were carcinogenic. Most studies found forestomach tumours in rodents. The best data are from the BaP study by Neal & Rigdon (1967), described in WHO (1996), although this study is inadequate in many respects (Rugen et al., 1989; Collins et al., 1991). Results from recent studies with BaP by Weyand et al. (1995) and Culp et al. (1996), although limited, are in agreement with risk calculations based on this older study.

Further information is available on the carcinogenicity of single PAHs from experiments with dermal application. Following dermal exposure, BaA, BaP, BbFA, BjFA, BkFA, DBahA, and IP are tumorigenic in mice. FA was not positive in the mouse skin assay but was found to be tumorigenic in the intraperitoneal lung adenoma assay in newborn mice. The relevance of these types of short-term cancer bioassays is under discussion. From the limited data available, BghiP and PY are not carcinogenic. With the exception of PY (equivocal results), all PAHs discussed here are genotoxic at least *in vitro* (ATSDR, 1995; WHO, 1997). Table 2 compares evaluations of individual PAHs for carcinogenicity in animals and humans.

It is not possible to assess directly the risk of PAHs to humans for the oral route owing to a lack of human data. One must rely on animal data to estimate the risk of exposure to individual PAHs, not forgetting that humans are exposed to mixtures of PAHs and not to pure individual PAHs. The extrapolation of risk to humans from animal data is complicated: the relevance of forestomach tumours in rodents when considering extrapolation to humans is not clear. There is some indication that there are interspecies differences in the enzymes that activate PAHs (Michel et al., 1995); further, intraspecies differences in susceptibility in humans may be due to differences in cytochrome P-450 enzymes (Guengerich & Shimada, 1991).

7.1 Guideline value for BaP

The guideline value for BaP, one of the most carcinogenic PAHs, in drinking-water corresponding to an excess lifetime cancer risk of 10^{-5} was estimated as 0.7 µg/litre (WHO, 1996). This is based on the oral carcinogenicity study of Neal & Rigdon (1967) and calculated using a two-stage birth-

death mutation model, which incorporates variable dosing patterns and time of sacrifice (Thorslund & Farrar, 1990). The data of Weyand et al. (1995) and Culp et al. (1996) on forestomach tumour incidence in mice give nearly identical results, giving support for the validity of the Neal & Rigdon (1967) study.

If BaP is present in drinking-water at significant concentrations, this indicates the presence of coal tar particles, which may arise from seriously deteriorating coal tar linings.

7.2 Guideline value for FA

FA is the PAH most commonly detected in drinking-water, primarily in association with coal tar linings of cast or ductile iron distribution pipes. A guideline value for this PAH was estimated from a 13-week oral gavage study in mice with a NOAEL of 125 mg/kg of body weight per day, based on increased SGPT levels, kidney and liver pathology, and clinical and haematological changes. An uncertainty factor of 10 000 (100 for inter- and intraspecies variation, 10 for the use of a subchronic study and inadequate database, and 10 because of clear evidence of co-carcinogenicity with BaP in mouse skin painting studies) gives a TDI of 0.0125 mg/kg of body weight per day. Assuming a 60-kg adult drinking 2 litres of water per day with an allocation of 1% of the TDI to water, because there is significant exposure from food, a health-based value of 4 µg/litre (rounded figure) can be calculated.

This health-based value is significantly above the concentrations normally found in drinking-water. Under usual conditions, therefore, the presence of FA in drinking-water does not represent a hazard to human health. For this reason, the establishment of a numerical guideline value for FA is not deemed necessary.

7.3 Relative potency of other PAHs compared with BaP

Attempts have been made to compare the carcinogenicity of individual PAHs using BaP as a standard. Although there are no comparative studies on the oral toxicity of PAHs, there are several comparative studies based on mouse skin carcinogenesis, initiation/promotion on mouse skin, intrapulmonary administration to rats, subcutaneous injection in mice, and intraperitoneal injection in newborn mice. There have been various attempts to rank selected PAHs in order of potential potencies based on these studies (see Table 3). Owing to the fact that the relative potencies of the individual PAHs were comparable in these studies, although the route of application was different, it is assumed that the relative carcinogenicity of these compounds is also similar for the oral and other routes of application.

7.4 Complex mixtures

It cannot be assumed that the carcinogenic effects of individual PAHs are additive or that PAHs present in a mixture (e.g. coal tar) act in the same way as each PAH individually. There is ample evidence for enhancement or inhibition of carcinogenicity by other PAHs (see Warshawsky et al., 1993; ATSDR, 1995).

7.5 Recommendations

Although WHO (1996) called for the use of coal-tar-based pipe linings to be discontinued, it is apparent from reports in the recent literature that coal tar linings are still being used in new as well as in existing pipes. Furthermore, monitoring studies in areas where these coal tar linings are still in existence show that, depending on the conditions (particularly where soft corrosive water is being carried), the linings seem to be deteriorating, releasing particulate matter containing PAHs into the water supply. Such particulate matter is also released during repair work on water pipes with coal-tar-based linings. This particulate matter is likely to contain the more carcinogenic PAHs (e.g. BaP).

It is recommended, as before, that:

- the use of coal-tar-based and similar materials for pipe linings and coatings on storage tanks be discontinued; and

- the monitoring of levels of individual indicator PAHs (including FA and BaP) and not just total PAHs in drinking-water continue, with the objective of detecting where coal-tar-based linings are deteriorating, so that they can be replaced in a timely manner by new pipes.

8. REFERENCES

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