NATIONAL INSTITUTE OF PUBLIC HEALTH AND ENVIRONMENTAL PROTECTION

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THE NETHERLANDS

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INTEGRATED CRITERIA DOCUMENT ARSENICUM

EFFECTS

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CONTENTS

INTRODUCTION

Data in the present Appendix are underlying those in the chapter on "effects" (chapter 5) of the "Integrated Criteria Document Arsenic". The Criteria Document, prepared by the National Institute of Public Health and Environmental Protection in The Netherlands, comprises a systematical survey and a critical evaluation of the most important data on the "priority substance" arsenicum, as much as possible with regard to the specific situation in The Netherlands. The information in the Criteria Document will serve as a scientific basis for an "effect oriented policy" in The Netherlands, especially with regard to the general population and aquatic and terrestrial ecosystems.
The Criteria Document, including the present Appendix, has been written on behalf of the Ministry for Housing, Physical Planning and Environment, Directorate Substances and Risk-management. By order of this principal in the present Appendix considerable reference has been made to previous reviews; these reviews are marked in the text by "R". However, in many cases data presented in reviews are too limited for evaluation. Therefore the original publications have also been studied whenever it appeared necessary; this applies especially with regard to data which are used in the risk assessments.
Extensive reviews on chemobiokinetics and metabolism in mammals and on effects on mammals have been published by the World Health Organization (IPCS, 1981-R) and the U.S. Environmental Protection Agency (EPA, 1983-R, 1987-R). Reviews on more specific items are mentioned in the sections in question.

The data which are considered to be necessary for a risk assessment for the general population, are described in chapter 1. Data on the impact of arsenic on aquatic and terrestrial organisms are described in chapter 2 and chapter 3, respectively. In chapter 4 data on agricultural crops and livestock are described. Chapter 5 contains the risk assessment for man and the environment.

An online literature search has been conducted in september 1988, especially in order to retrieve recent publications.

1. HUMAN TOXICITY

1.1. KINETICS AND METABOLISM

Essentiality

At present it is not clear if arsenic has to be considered as an essential element to humans. Studies on chickens and goats suggest that arsenic deficiency in the diet may contribute to adverse growth and reproductive effects. If arsenic is essential to animals it could be essential to humans too. There are, however, no comparable data on humans and the biological role of arsenic is not known. Therefore arsenic will be considered as a non essential element (IPCS, 1981-R, EPA, 1983-R).

With regard to kinetics and toxicity arsenic compounds can be divided into three groups; water soluble inorganic compounds, insoluble arsenic compounds and organic compounds (Wibowo et al., 1982-R).

1.1.1. Absorption

- Gastro-intestinal absorption

animal studies

From experiments in various species (rats, pigs, monkeys and dogs) it appears that the absorption from the gastro-intestinal tract of soluble arsenic compounds after oral administration is almost complete. Data on the amounts excreted in the faeces indicate that between 85% and 98% of the administered dose was absorpted. When arsenic trioxide was given in suspension, the amount excreted in the faeces was markedly higher (59% and 69% for rats and rabbits, respectively). In hamsters 70% of the administered dose of arsenic acid was found in the faeces.

In experimental animals organic arsenic from seafood is also almost completely absorbed. For organic arsenic compounds of other sources the amount taken up differs over a wide range, depending on chemical properties of the compound. An agent of low lipid solubility was shown to be absorbed poorly, whereas other agents (such as sodium methane arsenate and dimethyl arsinic acid) were absorbed rapidly, most likely by diffusion (IPCS, 1981-R).

human studies

After oral exposure, as in animals, soluble arsenic compounds are almost completely absorbed in humans. From data on urinary and faecal excretion, absorption was estimated to be 80-90%. From an experiment with finely powdered arsenic selenide it appears that undissolved particles are poorly absorbed. In general the absorption of trivalent arsenic is greater than that of pentavalent arsenic.

The absorption of "seafood arsenic" (mainly arsenobetaïne) is also fast and almost complete (80%-95%). Arsenic from meat of animals fed additives containing arsenic, for example arsanilic acid, is absorbed for about 40% (IPCS, 1981-R, EPA, 1983-R).

- Respiratory absorption

animal studies

Two studies indicate that arsenic in the form of an aerosol is absorbed by experimental animals. In hairless mice (used to minimize oral intake of arsenic deposited on the fur) exposed for several weeks to fly ash with a particle size < 10 μm containing 0.18 mg $As.m^{-3}$ increased tissue levels of arsenic were demonstrated. In rats exposed to condensation areosols containing 0.001, 0.004 or 0.046 mg $As.m^{-3}$ from arsenic trioxide for three months increased tissue levels were found in the two highest exposure groups. In both studies it was not possible to differentiate between inhaled and ingested arsenic (IPCS, 1981-R).

About 60% of arsinegas is absorbed by mice exposed by inhalation (IARC, 1980-R). After intratracheal administration of a solution of ^{74}As-labelled sodium arsenate (0.1-4 mg $As.kg^{-1}$ bw) to rats a rapid and complete absorption was reported. Dimethyl arsinic acid is absorbed for more than 95% within 15 min. after intratracheal instillation in rats (IPCS, 1981-R).

Human studies

With regard to the absorption of arsenic after inhalation by humans data are available from terminally ill patients or occupationally exposed persons.

In one test terminal lung cancer patients volunteered to smoked cigarettes impregnated with ^{74}As-labelled arsenite. Of the arsenic originally present in the cigarettes between 5-8% was deposited in the thoracic region. When two other lung cancer patients inhaled a nebulized solution of ^{74}As-labelled arsenite (particle size < 5 μm) 32% and 62% appeared to be deposited in the thoracic region. Described studies were performed on lung cancer patients, so extrapolation from these data to healthy subjects is bedatable. In exposed smelter workers a correlation was found between urinary levels of arsenic and average airborne arsenic concentrations, indicating a significant absorption of inhaled arsenic (IPCS, 1981-R).
Assuming that after deposition in the lung soluble arsenic compounds are nearly completely absorbed in humans, as in animals, the net absorption may be calculated at 30-60%. The absorption of insoluble arsenic is lower. In one report an average of 20% absorption for total (soluble and insoluble) inorganic arsenic was estimated (Wibowo et al., 1982-R). No data were found concerning the respiratory absorption of organic arsenic in man.

- Dermal absorption

animal studies

In only one experiment dermal absorption of inorganic arsenic in animals has been described. This study, in which rat tails were immersed in solutions containing 750, 7,500 and 15,000 mg As.l^{-1} as labelled sodium arsenate, showed that arsenic can be absorbed through the intact skin. The amount absorbed was not given (IPCS, 1981-R).

human studies

Human data on dermal absorption are also very limited and quantitative data are not available. In one case a patient developed an arsenic intoxication with peripheral neuropathy after accidental dermal contact with arsenic acid (Wibowo et al., 1982-R). No data are available on the absorption of organic arsenic through the skin of humans.

1.1.2. Transport and distribution

- animal studies

After absorption arsenic compounds are mainly transported via the blood, from which it is cleared rapidly to tissues. In most animal species one or two days after administration of inorganic arsenic less than about 6% of the dose remains in the blood. The distribution pattern of the rat, however, differs from that of other species (including humans). The haemoglobin of the rat has a strong affinity for arsenic, about 50% of the dose accumulating in erythrocytes. For this reason the half-life of inorganic arsenic (both trivalent and pentavalent) in the blood of rats is relatively long, namely 60 to 90 days (i.e. the life-span of erythrocytes) (IPCS, 1981-R, EPA, 1983-R).
There are no data on blood arsenic levels of organic arsenic from seafood. With regard to the clearance of dimethyl arsinic acid (DMAA) the same difference is seen between the rat and other species. After three months about 10% of the dose was still present in the blood of rats, whereas DMAA, as major metabolite of inorganic arsenic, might be expected to be cleared from the blood fairly rapidly. Less than 6% of doses of 4 different organo-arsenic drugs, given intravenously to rabbits, remained in the blood 2 h after administration (IPCS, 1981-R).

After oral exposure of mice to arsenite or arsenate (single doses of 0.4 or 4 mg $As.kg^{-1}$ bw) highest arsenic levels were found in kidneys, liver and bile. After exposure to arsenite most tissues contained higher amounts of arsenic than after exposure to arsenate, especially the liver and the biliary gland. After intraperitoneal injections of lower doses (up to 25 μg $As.kg^{-1}$ bw) in rabbits these differences were not seen. After parenteral exposure of several animal species to inorganic arsenic compounds an initial acumulation in liver, kidney, spleen, skin and lung was found. In most organs the concentration declined rapidly, with the exception of the skin. This could be explaned by the reaction of trivalent arsenic with sulphydryl groups of proteins, which are abundant in the skin. Arsenite and arsenic trioxide were found to pass the blood-brain barrier in many species. The concentration in the brain, however, was lower than in the

other organs. Placental transfer of inorganic arsenic has been demonstrated in hamsters, mice and monkeys. Arsenic levels in embryo's of hamsters 24 hours after exposure to arsenate (20 mg As.kg^{-1} bw) were comparable to that of the mother (0.05 mg As.kg^{-1} tissue). Data on tissue distribution or placental transfer of "seafood arsenic" (mainly arsenobetaine) were not found. Administration of a diet containing 50 mg.kg^{-1} monosodium acid methanearsenate for 52 weeks caused a rapid increase in the arsenic contents in the liver and kidney of rabbits during the first 2 weeks. Dimethyl arsinic acid passes the placental barrier of rats, with fetal blood levels being comparable to those of the mother (IPCS, 1981-R, EPA, 1983-R).

There seems to be an adaptation or tolerance towards arsenic in animals. During continous exposure of mice to arsenic trioxide (32 days 250 mg As.l^{-1}, and 256 days 50 mg As.l^{-1}) via drinking water a maximum concentration in skin and liver was reached at day 16. A decline of concentrations was seen during the rest of the experiment. Similar results were obtained with dogs orally exposed and with mice and rabbits exposed by inhalation. It is not clear whether this phenomenon is caused by a decreased absorption or by an increase in excretion (IPCS, 1981-R).

- <u>human studies</u>

After intravenous injections of ^{74}As arsenite (for locating tumours in man) the arsenic concentrations in plasma and erythrocytes were measured. After 10 h the erythrocytes contained 3 times more arsenic than the plasma. The decline rate was comparable. The decrease from the plasma follows three phase kinetics, the first half-time being very short. Less than 0.1% of the initial dose remained in the plasma after 24 h. The second and third phase showed half-times of 10 and 300 h, respectively (IPCS, 1981-R).

In patients (terminally ill) receiving labelled arsenite intravenously, a distribution of arsenic through the whole body was seen, with highest concentrations in liver and kidneys. Arsenic can cross the placenta; levels in the blood of newborn baby's were found to be comparable to those of the mothers. The concentration of arsenic in fetal tissue inceases with age (4 to 7 months). No data are available on the distribution of organic arsenic compounds (IPCS, 1981-R).

In humans arsenic is usually found in higher concentrations in hair and nails than in other parts of the body. These two matrices contain high amounts of keratin with SH-groups to which arsenic binds (IPCS, 1981-R). In populations exposed via drinking water mean arsenic levels of hair and urine showed increasing concentrations with increasing exposure level. The blood arsenic levels did not increase until the water contained about 400 $\mu g\ As.l^{-1}$ (Valentine et al., 1979).

1.1.3. Elimination

- animal studies

In all species (except the rat) absorbed inorganic arsenic is excreted rapidly, mainly in the urine. Experiments with arsenic trioxide in various species show that between 70 and 85% of the dose (0.1-1 mg $As.kg^{-1}$ bw) , administered by various routes, was excreted in the urine. Elimination in the rat occurs very slowly because of the accumulation in erythrocytes mentioned earlier. The elimination in dogs follows a two phase model, with halflife-times of 6 hours and 2.4 days. The amount of arsenic excreted into the bile differs between animal species. Trivalent arsenic is excreted to a lesser extent than pentavalent arsenic. Elimination via the lungs is of minor or no importance.
Arsenobetaine administered to animals is also eliminated rapidly. In general between 50%-90% of the given dose will be excreted in the urine within three days. In contrast, arsanilic acid is eliminated predominantly in the faeces. In rats and guinea pigs the influence of the molecular structure of some arsenic-containing drugs on the elimination kinetics was demonstrated. Drugs with functional hydrophilic groups, that facilitates elimination without biotransformation, are eliminated rapidly (65-90% in 24 h). Hydrophobic drugs were excreted slowly (20-50% in 24 h), predominantly into the bile (IPCS, 1981-R).

- human studies

In humans urine is also the predominant excretion route. The excretion of inorganic arsenic after administration of a single dose amounts to 30%

within 24 h and approximately to 80% after a few days. After repeated administration there seems to develop a balance with 70% of the dose excreted daily. The excretion follows a three-compartment model; 66% of the dose is eliminated with a half life of 2.1 days, 30% of the dose with 9.4 days and 4% with a half life greater than 38.4 days.
As in animals organic arsenic from seafood has been shown to be eliminated for >70% in urine within three days. For arsanilic acid 20% of the ingested dose was found in the urine and 64-74% in the faeces within 6 days.
A small amount of the absorbed arsenic is removed by other routes. Under hot and humid conditions the sweat of two persons contained a mean of 1.5 $\mu g.l^{-1}$. Arsenic levels in sweat under normal conditions were not reported. Desquamation of the skin will result in removal of arsenic, since arsenic has a high affinity for skin. Some data also indicate a loss of arsenic through hair. From arsenic levels in hair of people, who were poisoned, it was estimated that via this route at most 0.6% of the ingested amount will be eliminated (IPCS, 1981-R, EPA, 1983-R).

1.1.4. Biotransformation

- animal studies

In every animal species studied methylation of inorganic arsenic to mono- and dimethyl arsenic has been reported. Methylation of inorganic arsenic represents a route of detoxification, methylated forms being less toxic and easier excretable than inorganic forms (IPCS, 1981-R, EPA, 1983-R). Among various tissues (red blood cells, brain, lung, intestine and kidney homogenates) the liver appeared to have the highest methylating capacity (Buchet and Lauwerys, 1985).
Quantitative features of biotransformation may vary among species, but, in general, dimethyl arsinic acid (DMAA) is the major transformation product. It is relative rapidly formed and excreted. Methylation shows saturation characteristics. The degree of methylation seems to be dose-dependent (in mice), decreasing in relative percentage with increasing dose levels. Experiments also show biomethylation of trivalent arsenic to a greater extent than pentavalent.

In a few reports an interconversion of the two valency forms of arsenic was suggested. Methylated forms of arsenic, which could have influenced the results, were not taken into account, so no conclusions can be made sofar. When DMAA was given orally to rats it appeared to be mainly unchanged. Some demethylation seems to occur. Organic arsenic compounds used as feed additives and as drugs are converted to more easily excretable compounds (IPCS, 1981-R).

- human studies

Following ingestion or inhalation of inorganic arsenic between 70% and 85% is excreted as methylated arsenic. The major excretion products are dimethyl arsinic acid and methyl arsonic acid accounting for about 65% and 20%, respectively, of the total quantity excreted. A minor part of inorganic arsenic is excreted unchanged; after drinking arsenite-rich wine this appears to be 10% of the dose. The pattern of biotransformation was reported to be independent of route of uptake and probably takes place in the liver. However, the intestinal flora could also play a role in this process (Wibowo et al., 1982-R).
Several studies indicate that organic arsenic compounds, mainly from seafood, are excreted without prior biotransformation. Arsenobetaine, which accounts for 70% of the arsenic in seafood, was demonstrated in the urine after consuming of rock lobster tails (IPCS, 1981-R).

Summary and conclusions "kinetics and metabolism"

In the present report arsenic is considered to be nonessential.
After oral exposure the absorption percentage of water soluble inorganic arsenic compounds is circa 80% to 100% in animals and 80% to 90% in humans. The absorption of "seafood arsenic" (mainly arsenobetaine) is nearly complete. From experimental studies and occupational data it was estimated that the absorption of water soluble inorganic arsenic compounds after inhalation is 30% to 60%. The absorption percentage of insoluble compounds is lower after both exposure routes.
In most species, including man, the absorbed arsenic is rapidly cleared from the blood to various tissues, with initial accumulation in liver,

kidneys, spleen, skin and lung. In most organs the arsenic concentration declines rapidly, except for those which contain large amounts of proteins with SH-groups (to which trivalent arsenic binds), for example the skin. The distribution pattern in the rat is different. The heamoglobin of the rat has a strong affinity for arsenic, therefore the disappearance from the blood is relatively slow compared with other species. Arsenic can cross the placenta.

Excretion in the urine is the predominant elimination route for both inorganic and organic compounds; within a few days 60% to 90% of the arsenic is excreted by the kidneys. Organic arsenic compounds are excreted unchanged. Inorganic arsenic compounds are methylated in the liver and this proces shows saturation caracteristics. The major excretion products are dimethyl arsinic acid and methyl arsonic acid, accounting for about 65% and 20%, respectively, of the total quantity excreted. A minor part of inorganic arsenic is excreted unchanged.

1.2. TOXICITY

1.2.1. Short-term exposure (acute and subacute toxicity)

- Animal studies

The toxicity and LD50-values observed for inorganic arsenic compounds vary greatly depending on the chemical form and oxidation state (IARC, 1980-R). The oral LD50 for inorganic arsenic ranges from 15 to 293 mg As.kg^{-1} bw in rats and from 10 to 150 mg As.kg^{-1} bw in other species (EPA, 1983-R, IARC, 1980-R). Trivalent compounds are more toxic than pentavalent ones. Soluble compounds generally have lower LD50-values than poorly soluble ones. The observed effects in acutely intoxicated animals include gastroenteritis, diarrhoea, lowered blood pressure and ECG-changes.

No data were available on the subacute toxicity of "seafood arsenic". Some data were found concerning organic arsenic compounds used as pesticides or industrial biocides. The oral LD50-values for methanearsonic acid disodium salt and methanearsonic monosodium salt in rats were reported as 700 and 2,800 mg.kg^{-1} bw, respectively. The oral LD50-values for phenoxarsine oxide

in guinea pigs and rats were 24 and 40 mg.kg^{-1} bw (7 and 12 mg As.kg^{-1} bw), respectively. Those for phenarzarsine oxide were 77 and 83 mg.kg^{-1} bw (23 and 25 mg As.kg^{-1}bw), respectively (IPCS, 1981-R). After a single oral dose of PXO (10 mg As.kg^{-1} bw) to rats hepatotoxic effects were observed. When rats were given a similar dose of PZO no toxic effects were seen.
In a subacute study rats were given arsenic trioxide at doses of 1.5 or 7.6 mg As.kg^{-1} bw per day by gavage for 40 days. Rats receiving the higher dose showed hairloss and eczema, hyperplasia and hyperkeratosis of the skin. Bleeding, ulceration and crustforming also occurred. No adverse effects occurred in the animals from the dose lower group. Dysfunction of the bloodbrain barrier was indicated in rats fed 290 mg As.kg^{-1}bw as arsenite for 35 days. In rats given 40, 85 or 125 mg As.l^{-1} in drinking water for 6 weeks an increase in relative kidney weights was found (IPCS, 1981-R, IARC, 1980-R).
An impaired resistance to viral infections has been reported in mice subacutely exposed to inorganic arsenic by different routes; subcutaneously (2-4 mg As.kg^{-1} bw), in drinking water (75-150 mg.kg^{-1} bw) or via intraperitoneal injection (1.8 mg As.kg^{-1}bw). Impaired kidney function was also described in rabbits intravenously exposed to 0.6 mg As.kg^{-1} for 2-12 weeks three times a week (IPCS, 1981-R).

The most toxic form of arsenic seems to be arsine gas (Coddington, 1986-R). The LC50 for arsine in mice by inhalation has been estimated to be 500 mg.m^{-3} after 2.4 min. Exposure to 75 mg.m^{-3} arsine during 30 min may be lethal for mice. In a subacute study rats were exposed to aerosols containing dimethylarsinic acid. It was estimated that the concentration in the aerosols was approximately 840 mg.m^{-3}. No increased mortality occurred. In another study rats were inhalatory exposed to PXO and PZO (0.3-0.6 mg As.m^{-3}) 5 h a day during 7 or 8 weeks. No toxic effects were seen except for cellular infiltration of the portal tracts of the liver (IPCS, 1981-R).

Intraperitoneal injection of arsine in mice resulted in a LD50-value of 2.5 mg As.kg^{-1}bw (IARC, 1980-R). The intraperitoneal LD50-values of methanearsonic acid disodium salt and dimethylarsinic acid in mice were about 550 mg.kg^{-1}bw. Guinea pigs given 0.07 mg As.kg^{-1}bw as sodium arsenate intraperitoneally for 2 months showed effects on the ear (leading

to deafness), diminished acetylcholinesterase activity in the brain and decreased blood cholinesterase levels (IPCS, 1981-R).

- Human studies

Acute effects after large oral doses of inorganic arsenic are in general the same as in animals. The major lesion is gastrointestinal damage, resulting in vomiting and diarrhoea. Other acute symptoms are muscular cramps, facial oedema and (reversible) cardiac abnormalities. Shock can develop as a result of dehydration. Lethal doses of 70 to 180 mg arsenic trioxide (0.8 - 2.3 mg As.kg^{-1} bw) have been reported (IPCS, 1981-R, IARC, 1980-R). The lethal dose for elemental arsenic is 120 mg (2 mg As.kg^{-1} bw) (Fourtes, 1988-R).

Subacute effects mainly involve the respiratory, gastrointestinal, cardiovascular, nervous and haematopoietic systems. In Japan a group of nearly 12,000 infants were exposed to pentavalent inorganic arsenic via milk powder for two to three weeks. The total intake per infant was estimated to be 1.3-3.6 mg arsenic per day. Symptoms included fever, insomnia and anorexia. From a small group examined in a hospital nearly all children showed swollen livers. Autopsies of fatal cases showed hemorrhagic necrosis and fatty degeneration of the liver. Liver function tests in children who survived were normal and the liver size returned to normal. Severe hearing loss was observed in 18% of the children. In general, less than 1% of the children in the same age-group have hearing defects. The percentage of EEG-abnormalities in exposed children (14%) was twice as high as would be expected. In another study on the same population a significant increase in EEG-abnormalities was found in infants fed contaminated milk compared with breastfed infants.

More than 400 people were poisoned through soy sauce contaminated with an inorganic arsenic compound (probably calcium arsenate). The daily intake was estimated to be approximately 3 mg arsenic per day for two to three weeks. in this group facial oedema, anorexia, upper respiratory tract symptoms, skin lesions, peripheral neuropathy and effects on the liver occurred (IARC, 1980-R, IPCS, 1981-R). In individuals surviving acute poisoning, encephalitis, myelitis, nephritis or dermatitis may develop

during the convalescence. Dermatitis occurred in some patients treated with arsobal (organic arsenic compound) for trypanosomiasis (IPCS, 1981-R).
Inhalation of arsine concentrations of 25 $mg.m^{-3}$ for 30 min. can be fatal. Exposure to airborn arsenic compounds (mainly arsenic trioxide) in a smelter caused irritation of the respiratory tract; conjunctivitis and dermatitis (IARC, 1980-R).

1.2.2. Long-term exposure (subchronic and chronic toxicity, exclusive of carcinogenicity)

- Animal studies

In rats fed arsenate at a concentration of 50 mg $As.kg^{-1}$ bw for 10 weeks a decrease in haematocrit and haemoglobin was found. Liver cirrhosis and necrosis and bile duct proliferation were seen in rabbits fed daily doses of 0.7-4.7 mg $As.kg^{-1}$ bw as lead-, copper- or sodium arsenate for 50-250 days (IPCS, 1981-R). In rats given drinking water containing arsenic trioxide at levels of 0, 0.125, 12.5 or 62.5 mg $As.l^{-1}$ for 7 months liver injury and a dose-dependent proliferation of the bile duct was found (Ishinishi et al., 1980). In another study (performed by the same group), however, in which rats were exposed to arsenous acid in drinking water at concentrations up to 62.5 $mg.l^{-1}$ (corresponding to a maximum dose of 6.1 mg $As.kg^{-1}$ bw per day) for 54 weeks no toxic effects were found as related to growth, physical appearance or haematology. Slight temporary biochemical changes related to liver changes were observed at the 15th week, but no differences with the controls were found thereafter. It was suggested that: "an adaptation to the toxic effects of arsenic does seem to occur, at least, in rats" (Hisanaga, 1982).
In an oral study in which rats were fed a "shrimp diet" containing about 14 mg $As.kg^{-1}$ (0.7 mg $As.kg^{-1}$ bw) for 12 months no toxic effects were seen. On the basis of this study the toxicity of "seafood arsenic" seems to be relatively low.
Minor changes in kidney function and histology were found in rats orally exposed to calcium- and leadarsenate at doses of 1.5 and 1.25 mg $As.kg^{-1}$ bw, respectively, for two years. In a chronic feeding study, which was

lacking a control group, cats were given arsenite or arsenate at concentrations of 1.5 mg $As.kg^{-1}$ bw. Changes in electrocardiograms were observed (in 9 out of 12 animals) as well as a decrease in haematocrit and haemoglobin (IPCS, 1981-R). In rats fed arsenite at concentrations of 250 mg $As.kg^{-1}$ diet (about 12.5 $mg.kg^{-1}$ bw) during 2 years a decrease in haematocrit and haemoglobin was also found (Byron et al., 1967).

Inhibition of cholinesterase activity was observed in rats exposed to condensation aerosols containing 46 $\mu g\ As.m^{-3}$ as arsenic trioxide for three months. Disturbances in the functional state of the central nervous system (for example a change in conditioned reflexes) were seen. In a group rats exposed to aerosols containing 3.7 $\mu g\ As.m^{-3}$ the same effects were seen although less severe (IPCS, 1981-R). In a group of rats continuosly exposed to arsenic trioxide aerosols (submicron <0.3 μm MMAD) containing 0, 60 or 120 $\mu g\ As.m^{-3}$ no increased mortality was found after one year of observation (Glaser et al., 1986).

Embryotoxic and teratogenic effects have been reported in rodents after high oral and parenteral doses. After a single oral dose of 120 $mg.kg^{-1}$ bw of sodium arsenate (48 mg $As.kg^{-1}$ bw), given to mice on one of days 7 to 15 of gestation, embryotoxic effects (decreased weight and increased mortality rates) were found. An increase in skeletal malformations was seen after treatment on the 9th day. Incidences of other malformations did not show a significant increase. Maternal toxicity was also evident. After single intraperitoneal injection of 40 $mg.kg^{-1}$ bw sodium arsenate (16 mg $As.kg^{-1}$ bw), which also caused maternal toxicity, both embryotoxic and teratogenic effects were found. It appears that the mode of exposure to arsenate influences its teratogenic effects (Hood et al., 1978). In hamsters orally dosed with 20 or 25 $mg.kg^{-1}$ bw sodium arsenite (12 or 15 mg $As.kg^{-1}$ bw) on one of days 8 to 12 of gestation no teratogenic effects were observed, whereas these doses were toxic to the mothers. After intraperitoneal injections of 2.5 or 5 $mg.kg^{-1}$ bw sodium arsenite (1.4 or 3 mg $As.kg^{-1}$ bw) to hamsters during the same period of gestation embryotoxic effects and some malformations (which were not statistically significant) occurred. The doses caused maternal toxicity (Hood and Harrison, 1982). In an oral study pregenant mice were given 20, 40 or 45 $mg.kg^{-1}$ sodium

arsenite (12, 23 or 26 mg As.kg^{-1} bw) on one of days 8 to 15 of gestation. The lowest dose did not cause embryotoxic or teratogenic effects and no maternal toxicity was observed. At the higher doses both embryotoxic effect and maternal toxicity was found. Low incidences of malformations (exencephaly and open eyes) occurred in these groups (Baxley et al., 1981). Teratogenic effects were seen after intraperitoneal injections of 45 mg.kg^{-1} bw sodium arsenate in mice (11 mg As.kg^{-1} bw, based on hydrated sodium arsenate) (Hood and Bishop, 1972). In rats given arsenate (5-12 mg As.kg^{-1} bw) teratogenic effects included eye-defects (IPCS, 1981-R). Intravenous injections of 3 mg As.kg^{-1} bw or more as arsenate to hamsters on the 8th (or 9th) day of gestation caused several teratogenic effects. An increased incidence of exencephaly, anencephaly, renal agenesis, rib and genito-urinary abnormalities was found. Both resorption and malformation rates in the fetus increased with increasing doses of arsenate (IPCS, 1981-R, Friberg, 1986-R).
In a three-generation experiment mice were exposed to 5 mg As.kg^{-1} diet (about 0.5 mg As.kg^{-1} bw) in the form of arsenite. Except for a reduced litter size no abnormalities were found. In a 7-generation study rats were fed diets containing 0.01, 0.02, or 0.05% arsanilic acid (3.5, 7, or 17.5 mg As.kg^{-1} diet). No teratogenic effects were seen. The litter sizes and the survival of the pups were found to increase significantly (IPCS, 1981-R).

The embryotoxic effects of inhaled arsenic were studied in mice. Pregnant mice were exposed to 0.21, 2.3 or 22 mg As. m^{-3} as arsenic trioxide for 4 h per day during days 9 through 12 of gestation. At the highest concentration a significant increase in the number of fetuses with retar-ded growth was observed. The number of fetuses with skeletal malformations was also significantly increased at the highest concentration. The average fetal weight decreased at all concentrations compared to the controls (Nagymajtenyi et al., 1985).

- Human studies

Chronic effects of arsenic may involve many organs or organ systems, including the skin, the liver, the respiratory and gastrointestinal tract,

and the cardiovascular, nervous, and haematopoietic system. In the following section the effects will be described per organ (system).
Data in this section are mainly derived from studies on populations exposed to high arsenic levels in drinking water or from studies among occupationally exposed populations. It is believed that the arsenic in the drinking water was mainly in its inorganic form (valence often not given). With regard to the occupational studies it is considered that the smelter workers are exposed to trivalent inorganic arsenic, while workers handling pesticides are mainly exposed to pentavalent inorganic arsenic.
No data were available considering the toxicity of organic arsenic.

effects on the skin

Long-term oral intake of inorganic arsenic causes a number of caracteristic skin lesions. Hyperpigmentation (melanosis), often associated with pale spots, and hyperkeratosis of the soles and palms are often seen in populations which have ingested large amounts of arsenic via drinking water or via drugs. In Mexico a study was conducted to compare the prevalence of signs and symptoms of chronic arsenic poisoning in two rural populations. The arsenic concentration in the water of the exposed persons was 0.41 mg $As.l^{-1}$, that of the control population was 0.007 mg $As.l^{-1}$. It was reported that arsenic was for 70% in its pentavalent form. The calculated relative risk of the development of several different skin disorders varied from 3.6 (ulcerative zones) to 36 (palmoplantar keratosis) (Cebrian et al., 1983).
On the basis of a number of epidemiological studies, in which populations were exposed through drinking water, the "Joint Committe on Food Additives" (JECFA) concluded that after long-term intake of water containing 1 mg $As.l^{-1}$ the appearance of toxic effects will be most likely. After long-term intake of water containing 0,1 mg $As.l^{-1}$ the chance that toxic effects will occur in "sensitive" persons is not negligible. Taking into account a daily consumption of 2 l water and a mean body weight of 70 kg this corresponds to a daily intake of 2 $\mu g\ As.kg^{-1}$ bw (JECFA, 1983).
Among a group of 262 patients orally treated with large doses arsenite (sometimes more than 10 mg arsenic per day for several years) for skin disorders the same symptoms were seen. A dose-response relationship was observed between the total intake of arsenic and the incidence of

hyperkeratosis. Multiple keratosis can occur and develop into precancerous states (IPCS, 1981-R).
Hyperkeratosis and hyperpigmentation are rarely seen among smelter workers exposed to inorganic arsenic by inhalation, but have been reported in other occupational situations. In smelter workers dermatosis due to local irritation may occur (IPCS, 1981-R).

effects on the respiratory tract

Effects of arsenic on the respiratory system have been reported primarily as a result of occupational exposure. A few reports describe respiratory effects after oral exposure. In one study, for example, a high frequency of chronic cough and a history of bronchopulmonary disease were reported among inhibitants of Chili exposed to arsenic via drinking water. Because arsenic can suppress the immune response (see "other effects") these effects may be due to an impaired resistence to infections (IPCS, 1981-R, EPA, 1983-R).
After inhalatory exposure to relative high arsenic concentrations in smelters, lesions of the mucous membranes in the respiratory tract and perforation of the nasal septum were reported. Among copper smelter workers in Sweden, exposed to arsenic levels up to 7 mg $As.m^{-3}$ in some workplaces, but generally not to a higher level than 0.5 mg $As.m^{-3}$, two types of respiratory syndroms were found. Symptoms of the upper respiratory tract (septum perforation, laryngitis and changes in nasal mucosa) were mainly found in workers exposed to arsenic in the "crude or refined form". The other syndrom, which included tracheobronchitis and signs of pulmonary insufficiency, was found among workers with mixed exposure to arsenic and sulfur dioxide. No control group was used and smoking habits were not included (IPCS, 1981-R, EPA, 1983-R).

effects on the liver

Chronic oral intake of inorganic arsenic has been associated with the development of portal hypertension, malignant liver disease and cirrhosis. Long-term intake of drugs containing inorganic arsenic (mainly Fowler's solution containing arsenite) resulted in portal hypertension and sometimes in liver cirrhosis. In patients treated with organic arsenic (arsobal for treatment of trypanosomiasis) liverdamage can occur as side-effect.

A large number of German vintners, exposed to arsenic-containing pesticides and contaminated wine, developed liver cirrhosis. The consumption of excessive amounts of wine (3-4 litres daily) by these people played a role in this as well. In serum of smelter workers exposed to moderate levels of arsenic (less than 13 μg $As.m^{-3}$, as a 6-h average) no significant increase in liver enzymes could be demonstrated. Two studies on smelter workers showed an tendency towards increased mortality from liver cirrhosis (IPCS, 1981-R).

effects on the cardiovascular system

A specific cardiovascular effect associated with chronic arsenic exposure is the Blackfoot disease (BFD), a peripheral vascular disease leading to gangrene of the toes, feet, legs and fingers. A high prevalence of BFD (and other symptoms associated with chronic oral intake) was found in Taiwan, where arsenic levels in drinking water ranged from 0.01-1.82 mg $As.l^{-1}$ (mainly between 0.4-0.6 mg $As.l^{-1}$, mean 0.5 mg $As.l^{-1}$). A roughly positive linear relationship existed between prevalence of BFD and total ingested dose of arsenic or duration of water intake. A rise in prevalence can be expected with increasing age, but the differences between these groups could not be explained by the age-factor alone. A control group was not included, but a group of people exposed to arsenic levels between 0.001 and 0.2 mg $As.l^{-1}$ showed non of these lesions. No new cases occurred since arsenic levels in the water had been reduced (concentration not given) (Tseng, 1977, IPCS, 1981-R, EPA, 1983-R).

The role of arsenic and a certain fluorescent compound (FC) in the water of this area in the development of BFD and chronic arsenism was studied. It was suggested that FC is the primary substance affecting the endothelial cells and that it has an important effect in BFD. When these effects were combined with arsenic, chronic arsenism may develop (Yu et al., 1984). The role of various FCs in the development of BFD is suggested by others as well (IPCS, 1981-R; EPA, 1983-R; Chen, 1986). Some of the fluorescent substances were found to have vasoactive actions (EPA, 1983-R).

Among inhabitants of the Antofagosta region of Chili supplied with water containing 0.6 mg $As.l^{-1}$ peripheral vascular diseases were reported. Most common were Raynaud's syndrom and acryanosis. Children seemed to be more susceptible than adults.

Peripheral vascular diseases have rarely been reported among occupationally exposed populations or among patients receiving arsenic medications. In one study, however, some changes in peripheral circulation were found among copper smelter workers with long-term exposure to arsenic dust (mainly containing arsenic trioxide). An increase in vasospastic reactivity as well as phenomenon of Raynaud were found, which could be due to functional alternations in the vessels of the hand. It was estimated that at time of study the maximal daily absorption did not exceed 300 μg. It was not possible to determine if the increased reactivity was due to past (significantly higher) or present exposure (Lagerkvist et al., 1986). A few studies indicated an increased mortality from cardiovascular diseases among smelter workers (Lee and Fraumeni, 1969; Lee-Feldstein, 1983), but in other studies no excess in cardiovascular mortality was found (EPA, 1983-R).

effects on the nervous system

Peripheral and central nervous system (CNS) injury are important effects after inorganic arsenic exposure. Neurological involvement is demonstrated by several sensory changes and considerable muscle tenderness. Varying degrees of motor weakness, more severe at proximal parts are seen, which can develop into paralysis of affected muscle groups or extremities. Other symptoms of arsenic intoxication are headache, sleepiness, and in severe cases loss of memory and confusion. Changes in personality are also reported (EPA, 1983-R; IARC, 1980-R).

A high percentage of electromyographic (EMG) abnormalities was reported among persons using drinking water containing more than 0.05 mg $As.l^{-1}$. Non of the controls showed these abnormalities. Several factors that would predispose people to have EMG abnormalities were not taken into account (IPCS, 1981-R). From a neurological examination of a population in Alaska it appeared that a daily ingestion between 1 and 4521 μg arsenic from well water did not result in clinical or subclinical neuropathy (Kreiss et al., 1983). Among patients having taken anti-asthmatic herbal preparations containing inorganic arsenic, more than 50% had neurological complications, mostly sensimotor polyneuropathy. The recommended daily dose for these patients contained several mg arsenic trioxide or arsenic sulfide. Other symptoms of arsenic poisoning were also seen in this group. In patients treated with arsobal (organic arsenic) for trypanosomiasis (dose not given)

1.5% of 1066 individuals developed encephalopathy. From these patients 3-4% had visual effects. Two women who had been on food supplements prepared from kelp (duration and dose not mentioned) developed neuropathy. It is not clear if organic arsenic had attributed to this (IPCS, 1981-R).
Cases of neuropathy have also been reported after occupational exposure to arsenate spray. Workers of a copper smelter with long-term (11-32 years) exposure to inorganic arsenic developed symptoms and signs of sensimotor polyneuropathy. After cessation of the exposure some recovery was seen. A slightly increased prevalence of subclinical neuropathy, manifested as low conduction velocities in peripheral nerves in smelter workers with long-term exposure to airborn inorganic arsenic. The level of arsenic in the air before 1975 was estimated to be < 500 $\mu g.m^{-3}$ and approximately 50 $\mu g.m^{-3}$ thereafter. Daily absorption was estimated to be less than 300 μg. There was no significant difference between the prevalence of electromyographic abnormalities in the exposed group compared to a control group. It was concluded that when the exposure is kept below 50 $\mu g.m^{-3}$ the risk on clinical neuropathy will be very low (Blom et al., 1985). Among children living near a arsenic emitting copper smelter hearing loss has been reported. Two cases of peripheral neuropathy were decribed after cutaneous exposure to arsenic acid and to a paste containing arsenic (form not known) (IPCS, 1981-R).

other effects, including teratogenicity
Exposure to inorganic arsenic resulted in disturbances of the haematopoietic system. Inorganic arsenic compounds cause bone marrow depression (Winship, 1984) and disturbed erythropoiesis. Anaemia and leucopenia are seen in nearly all cases, frequently accompanied by trombocytopenia. Severe granulocytopenia can have effects on resistance to bacterial infections. Disturbance of the haematopoietic system occurs as side effect of treatment with arsobal (IPCS, 1981-R).
Teratogenic effects of inorganic arsenic were studied several times in a population living near a Swedish smelter, emitting lead, arsenic and sulphur dioxide. Exposure concentrations were not given. The frequency of congenital malformations in the offspring of female employees was compared to that of the population living nearby the smelter. The frequency of (multiple) malformations was significantly higher in women who worked at

the smelter during pregnancy compared to the population living nearby. (In this population there was no variation in total or specific frequencies). Frequencies of spontaneous abortion were studied in populations located at different distances from the smelter. In the population located near the smelter a significant increase in abortion (11% of the pregnancies) was found compared to the population living further away (7.6%). It must be kept in mind that the population living closest to the factory includes many employees of the smelter. A decreased birthweight in the population living near the smelter was also reported. The studies indicate that workers at the smelter and probably people living close by the smelter have an increased risk of genetic damage (Nordström, 1978, 1979). Ingestion of 30 ml of a rat poison containing 1.32% arsenic as arsenic trioxide by a woman in the 30 th week of pregnancy caused death of the infant (Lugo et al., 1969).

Summary and conclusions "short- and long-term toxicity"

The toxicity of arsenic compounds depends on the chemical form and the oxidation state. In general, the inorganic arsenic compounds appear to be more toxic than organic compounds and trivalent inorganic compounds more toxic than the pentavalent ones. Oral LD50-values range from 10 to 130 mg $As.kg^{-1}$ bw for inorganic arsenic compounds and from 700 to 2,800 $mg.kg^{-1}$ bw for organic compounds. The lethal oral dose for humans ranges from 70 to 180 mg arsenic trioxide (0.8-2.3 mg $As.kg^{-1}$ bw). Inhalation of 25 $mg.m^{-3}$ arinegas may be lethal.

After long-term exposure to inorganic arsenic in both animals and humans effects are seen on several organs or organ systems. In a subchronic study on rabbits fed 0.7 to 4.7 mg $As.kg^{-1}$ per day as arsenate liver cirrhosis and (a dose-dependent) proliferation of the bile duct was found. Subchronic exposure to arsenic trioxide via drinking water (doses up to about 6 mg $As.kg^{-1}$ bw) caused the same symptoms. In a chronic study (rats exposed through drinking water to same doses) with arsenous acid no toxic effects were appearent. In rats orally exposed to 1.5 mg $As.kg^{-1}$ bw per day as arsenate for 2 years minor changes in kidney function were found. In a chronic feeding study in which cats were given 1.5 mg $As.kg^{-1}$ bw as

arsenite changes in electrocardiogram as well as decreases in haematocrit and haemoglobin concentrations were found. Exposure to organic arsenic from seafood (0.7 mg $As.kg^{-1}$ bw) caused no toxic effects. Disturbances of the central nervous system were observed in rats exposed to aerosols containing 3.7 $\mu g\ As.m^{-3}$ as arsenic trioxide during three months.
High oral and parenteral doses of arsenite and arsenate cause embryotoxic and teratogenic effcets in experimental animals. After a single oral dose of 48 mg $As.kg^{-1}$ bw as arsenate embryotoxic and teratogenic effects occurred in mice. This dose also caused maternal toxicity. Lower oral doses (up to 25 mg $As.kg^{-1}$ bw), which were still toxic to the mothers, did not cause teratogenic effects. When administered parenterally significant lower doses (3 mg $As.kg^{-1}$ bw) are able to produce teratogenic effects. Inhalatory exposure to 22 mg $As.m^{-3}$ had no teratogenic effects in mice.

Long-term oral intake by humans caused caracteristic skin lesions; hyperpigmentation (melanosis) and hyperkeratosis. These lesions were found among people exposed through drinking water or through medication. From a drinking water study it was calculated that the relative risks for developing skin disorders varied between 3.6 (ulcerative zones) and 36 (palmoplantar keratosis). High arsenic intake may also affect the cardiovascular system: blackfoot disease (BFD) (one of the peripheral vascular diseases that may occur). A high prevalence rate was found in areas with naturally high arsenic concentrations (up to 1.8 mg $As.l^{-1}$) in drinking water. The role of other substances in the water which also could have had effects on the vascular system can not be ruled out. Neurological effects were reported among patients and people using drinking water containing 50 $\mu g\ As.l^{-1}$ and more, but in another study no (sub) clinical neuropathy was found after an intake of up to 4500 μg per day. Effects were also reported on liver and respiratory tract.
From studies among occupationally exposed groups it appeared that chronic exposure to arsenic by inhalation leads to effects on the respiratory system, the cardiovascular system, the liver and the nervous system. Among copper smelter workers exposed to 7000 $\mu g\ As.m^{-3}$ (mostly up to 500 $\mu g\ As.m^{-3}$) several lesions of the upper respiratory tract were found. Long-term inhalation of dust containing arsenic trioxide caused changes in peripheral blood circulation among smelter workers. In the same group

slight subclinical neuropathy was found. It was estimated that at time of study the concentration was about 50 $\mu g\ As.m^{-3}$ and that maximal daily absorption did not exceed 300 μg. It was, however, not possible to determine if the effects were due to present or past exposure, when it was significantly higher (up to 500 $\mu g\ As.m^{-3}$). The risk on clinical neuropathy is considered to be very low when exposure is kept below 50 $\mu g\ As.m^{-3}$. In workers exposed to levels below 13 $\mu g\ As.m^{-3}$ (as a 6-h average) no significant increase in liver enzymes was found.
One study on the offspring of women working at a smelter indicates an increased frequency of spontaneous abortions and malformations in the offspring. The women were also exposed to other toxic substances, so no conclusion can be drawn on the role of arsenic.

1.3. GENOTOXICITY

- Results from tests:

Various inorganic arsenic compounds have been tested for genotoxicity in a variety of test systems. Data on the genotoxicity have been reviewed among others by Jacobson-Kram and Montalbano (1985), IARC (1980, 1987) and IPCS (1981). According to Jacobson-Kram and Montalbano the following conclusions can de drawn ;

1. Arsenic is either inactive or extremely weak for the induction of gene mutations *in vitro*.
2. Arsenic induces chromosome aberrations and sister chromatid exchanges (SCE's) in a variety of cell types, including human cells, *in vitro;* trivalent arsenic is approximately an order of magnitude more potent than pentavalent arsenic.
3. Arsenic does not appear to induce chromosome aberrations *in vivo* in experimental animals.
4. Several studies suggest that humans among others exposed to arsenic demonstrate higher frequencies of SCE's and chromosomal aberrations in peripheral lymphocytes.

- Remarks

Ad 1. Arsenic has been found to be negative in a range of microbial gene mutation tests (Baker, 1985), except with arsenite in an *E. coli* reverse mutation test (Nishioka, 1975). This positive study was criticized by Rossman et al. (1980); according to these latter investigators the method used by Nishioka to calculate mutation frequencies was inappropriate. In addition, the positive conclusion was based on a difference between 8 mutants per plate in the control and 37.7 mutants per plate in the arsenite treated sample, whereas values of 10-30 mutants were usually found in control-plates by Rossman et al.

Ad 2. In a number of studies no increase in chromosome aberrations was found after exposure of lymphocytes to arsenic in the nonstimulated (G_0) phase. This indicates that arsenic must be present during DNA synthesis (S-phase) to have an effect (Crossen, 1983, Nordenson et al., 1981).

Ad 3. Only one *in vivo* experiment was conducted; no chromosome aberrations were found in mouse bone marrow cells and spermatogonia after single intraperitoneal injections of arsenic trioxide (up to 12 mg $As.kg^{-1}$ bw) (Poma et al., 1981).

Ad 4. These persons were either workers from copper smelters or patients and in both situations simultaneously exposure to other substances occurred. Therefore, it can not be excluded that the effects observed are due to other factors than exposure to arsenic.

The genotoxicity of arsenobetaine (organic arsenic from seafood) was investigated in various *in vitro* short-term test systems. At concentrations up to 5 $mg.plate^{-1}$ no mutagenic effects in three different *Salmonella typhimurium* strains were seen either with or without metabolizing systems. There was no increase in frequency of SCE's in chinese hamster cells *in vitro* (Jongen et al., 1985).

- Mechanisms of action:

A number of studies indicate that the increased frequency of SCE's and chromosome aberrations found *in vitro* only occur if arsenic is present during the S-phase of the cell-cyclus. Besides arsenic has been shown to be incorporated into both nuclear and cytoplasmic RNA (and probably also into

DNA) in place of posphorus (Kay, 1965). Inorganic arsenic has also been reported to inhibit DNA repair enzymes (Rossman et al., 1977, Jung and Trachsel, 1970).
The unusual genotoxic profile (i.e. the induction of chromosome aberrations *in vitro* but not of gene mutations) might be due to the strong interaction of arsenic with sulfhydryl groups of enzymes.

Summary and conclusions "genotoxicity"

From *in vitro* genotoxicity tests it appeared that inorganic arsenic compounds do not induce gene mutations. But on the other hand in both animal and human cells increased frequencies of SCE's and chromosome aberrations were found. However, in the only *in vivo* experiment (mice) no induction of chromosome aberrations could be found in bone marrow cells or in spermatogonia. Organic arsenic compounds did not induce gene mutations nor a higher frequency of SCE's *in vitro*.
In peripheral lymphocytes from people exposed to inorganic arsenic higher frequencies of chromosome aberrations and SCE's were demonstrated. However, because of the fact that these persons were simultaneously exposed to other substances, it can not be ruled out that the positive results must be ascribed to other factors than arsenic exposure.
Possible explanations for the unusual genotoxic profile of inorganic arsenic are the inhibition of DNA repair enzymes, the substitution of arsenic instead of phosphorus in the DNA-backbone, or the interaction of arsenic with sulfhydryl groups of enzymes.

1.4. CARCINOGENICITY

1.4.1 Animal studies

Several arsenic compounds have been tested for carcinogenicity in experimental studies. The studies, which were evaluated by the IARC (1980) and the IPCS (1981), are summarized in table 1.1. Additionally, some recent studies are summarized (of which references are stated in table 1.1).

No increased tumorincidences were found after long-term oral exposure to inorganic arsenic in rats and mice. In the only long-term inhalation study performed, in which rats were exposed to arsenite-aerosols, no increase in the number of tumours was observed. Subchronic dermal and intratracheal administration did not result in an increase in the number of tumours in rats and mice. In most subchronic studies it was not clear if the animals were observed for lifetime. In two studies it appeared that hamsters develop lung adenomas upon intratracheal administration. In one of these experiments the concentrations used were found to be toxic to the lung-cells; an excessive protein production was observed.
In a number of studies the development of maligne tumours was reported upon treatment with inorganic arsenic. After repeated intravenous or subcutaneous administration in mice lymphocytic leucaemia as well as lymphomas were found. After intratracheal application of arsenic-containing "mixtures" to experimental animals malignant tumours were observed. When rats were given intratracheally a mixture containing calcium arsenate, copper sulphate and calcium hydroxide several adenocarcinomas or cell-carcinomas of the respiratory tract were found. The oral administration of a "drug" containing inorganic arsenic caused malignant tumours in mice. In another experiment, in which rats were given intratracheal injections of tin oxide ore dust containing arsenic, the incidence of precancerous and malignant changes at autopsy were very high in the treated group. A relationship between the arsenic content of the dust and these incidences was found.
No long-term studies with organic arsenic compounds were conducted.

On the basis of the data above mentioned it is concluded that, as far as exposition-routes which are relevant for humans are concerned, there is inadequate evidence for the carcinogenicity of arsenic in experimental animals. The "International Agency for Research on Cancer" (IARC, 1980, 1987) arrived at the same conclusion.

1.4.2 Human studies

Data on the carcinogenicity of inorganic arsenic in humans have been reviewed by the IPCS (1981), the EPA (1983), the IARC (1980, 1987), Pershagen (1981) and the WGD (1984). With regard to organic arsenic compounds no data could be found.

- Cancer of the skin

Chronic oral exposure to inorganic arsenic has been associated several times with an increased incidence of skin cancers. This relationship was found in several studies on populations living in different parts of the world which were exposed through naturally contaminated drinking water. Long-term ingestion of drugs containing inorganic arsenic was associated with an increased incidence of skin cancer as well. The medicines contained mainly arsenite. It is assumed that the arsenic present in drinking water is of inorganic nature, but its valence is often not known. A short overview of epidemiologic studies on orally exposed populations is given in table 1.2. A number of studies will be described below.

A general survey was carried out among more than 40,000 inhabitants from an area of Taiwan with high concentrations of arsenic in well water. The arsenic level of water was reported to range from 0.01 to 1.8 mg $As.l^{-1}$ (mainly from 0.4 to 0.6 mg $As.l^{-1}$, mean 0.5 mg $As.l^{-1}$). Skin cancers were found in 428 of 40,421 examined inhabitants. The most common type was intra-epidermal carcinoma and the body areas most frequently involved were unexposed surfaces. Nearly all patients had multiple skin cancer. A positive dose-response relationship was demonstrated between intake and the prevalence of skin cancer (Tseng, 1968, 1977). A control-group was not included in this study. In another study cancer mortality rates in the same

area were studied. The total daily intake of arsenic was estimated to be as high as 1 mg. The standardized mortality ratio's (SMR's) for cancers of bladder, kidney, skin, lung, liver and colon were found to be elevated compared to the general population in Taiwan (Chen et al., 1985). In addition a case-control study was conducted (same area). The odds ratio's (OR's) for developing bladder, lung and liver cancer for inhabitants who used well water for 40 years or more were 3.9, 3.4, and 2.7, respectively, compared to those who never used this water. A positive dose-response was also reported (Chen et al., 1986). As was mentioned in section 1.2.2. besides arsenic other substances (including organochlorides and ergot alkaloïds) were identified in the drinkingwater (Yu et al., 1984, Chen et al., 1985). A population of a county in the US where arsenic levels in drinking water ranged from 0.0 to 2100 μg As.l^{-1} (mean 16.5 μg As.l^{-1}) was studied. No excess of skin cancer was found in this population and the incidence of lung cancer did not correlate with arsenic levels in the county (Morton et al., 1976). It was noted that these results were not necessarily contradictory to the study of Tseng in Taiwan, because average arsenic concentration in the described study were about 30 times lower than in Taiwan (IARC, 1980-R, IPCS, 1981-R).

A group of 262 patients treated with arsenite (mainly Fowlers' solution) for chronic skin disorders for up to 26 years was studied. In 8% of the patients various types of skin cancer were found, the most common being multiple basal cell carcinoma. The prevalence of skin cancer increased as the total dose ingested increased. No conclusions can, however, be drawn regarding dose-response relationships because of the fact that only 18% of the patients intended for the study responded and participated. Moreover, a control group was not included. A clear increase of morbidity (prevalence rate > 20%) did occur among patients who ingested more than 7.6 g arsenic (Fierz, 1965). A mortality analysis was carried out on a cohort of patients (also treated for chronic skin disorders) given arsenite for periods ranging from 2 weeeks to 12 years. The median dose level was 448 mg arsenic (about 7.5 mg As.kg^{-1} bw), but some had doses up to 10 g ($\pm$170 mg As.kg^{-1} bw). There was no overall increased mortality from cancer, but an incresae in both fatal and non-fatal skin cancers was found (Cuzick et al., 1982).

On the basis of the epidemiological studies, in which populations were exposed through drinking water, the "Joint Expert Committe on Food

Additives" (JECFA) concluded that "there is epidemiological evidence of an association between the overexposure of humans to inorganic arsenic from drinking water and increased cancer risk". Additionally, the JECFA stated: "Human exposure to levels of arsenic below those which cause arsenicism do not appear to carry a carcinogenic risk" (JECFA, 1983).

- Cancer of the respiratory tract

There is substantial evidence of increased incidence of cancer of the respiratory tract in association with exposure to inorganic arsenic (and other substances) by inhalation. Epidemiological studies on smelter workers demonstrate in most cases a positive relation between exposure to inorganic arsenic and respiratory cancer. Similar results were found in groups handling pesticides containing inorganic arsenic. A short overview of epidemiologic data is given in table 1.3. and a number of studies will be described below.

exposure to pesticides

The mortality of workers exposed to arsenic was compared to that of workers of the same factory who had no exposure to arsenic. Several pesticides containing arsenic were produced. Airborn arsenic levels were reported up to 40 mg $As.m^{-3}$. A positive dose-response relationship was found between the total dose of arsenic and lung cancer mortality. In the exposed group 16 of the 28 deaths due to respiratory cancer occurred in workers exposed less than one year. An increase in cancers of the lymphatic system and haematopoietic system, other than leukaemia was also found (Ott et al., 1974). It should be noted that only those workers who were employed until death or who retired were used. This means a loss of data especially on those exposed to low concentrations (IPCS, 1981-R). Corresponding to the exposure categories made by Ott et al. 48-h time- weighted averages (TWA) were calculated over 40 years of exposure. If workers exposed for less than one year were excluded, no clear dose-response relationship was observed below a TWA of 90 $\mu g\ As.m^{-3}$ daily (Blejer and Wagner, 1976, evaluated in reviews). A retrospective cohort analysis on the same population resulted in a relative risk (RR = 3.5) of lung cancer in the exposed group compared to U.S. mortality rates. The relative risk of lymphatic and haematopoietic

cancer (except leukaemia) was also increased (RR = 3.9). According to the authors no differences in smoking habits in the exposed and control group existed (IARC, 1980-R). The mortality of this cohort was updated through 1982 and it appeared that, with these additional data, the RR of lung cancer was no longer significant. The same applies to the risk of lymphatic and haematopoeitic cancer. A dose-response relationship between lung cancer risk and exposure to arsenic could be demonstrated. The effects of smoking alone or in interaction with arsenic could not be assessed (Sobel et al., 1988). In a pesticide-manufacturing factory a significant increase in mortality rates of respiratory cancer, anaemias and lymphomas was found among exposed workers compared to non exposed workers. A dose-response relationship existed between lung cancer risk and exposure to arsenicals. However, simultaneous exposure to other compounds occurrred and no data on smoking habits were given (Mabuchi et al., 1979, evaluated in reviews). A higher mortality rate of cancer was found among workers exposed to arsenite (in producing sheep dip powder) compared to non exposed workers. This excess was due to higher percentages of respiratory and skin cancer. Median arsenic levels ranged from 70 to 700 $\mu g\ As.m^{-3}$ during sampling periods of 10 minutes "or more". The excess cancer deaths were limited to workers "heavily" exposed (Hill and Faning, 1948, evaluated in reviews).

Studies concerning the relation between the spraying of pesticides containing inorganic arsenic compounds (mostly of low solubility) and lung cancer were contradictory. Several case reports described skin and lung cancers in vineyard workers suffering from chronic arsenic intoxication. In epidemiological studies, however, no increase in these cancers has been found. A mortality study among orchadists exposed to lead arsenate did not show an excess in mortality of all cancers nor of lung cancer (Nelson et al., 1973, evaluated in reviews). This study was critized; the cancer mortality of exposed persons would not have been predicted accurately (IPCS, 1981-R). To evaluate the hypothesis that past exposure to the pesticide lead arsenate leads to an excess mortality from lung cancer a case-control study was conducted among male orchadists. There appeared to be no difference between the two groups according to presence, intensity or duration of lead arsenate exposure. Information on occupation and on smoking were obtained by interviewing persons who were considered to be

well informed. The authors concluded that the specific cause of increased mortality remains to be identified because there was also no difference in smoking habits (Wicklund et al., 1988). The negative finding of this study was consistent with a study in which no relationship could be found between exposure to arsenates (either lead or calcium arsenate) and lung cancer in agricultural setting (Barthel et al., 1981, cited by Wicklund, 1988).

exposure in smelters

Among copper smelter workers exposed to high levels of arsenic an increased risk of lung cancer has been reported many times (see table 1.3).

In one study the mortality rates among more than 8,000 white male smelter workers in the USA were compared with that of the white population of the same states. A positive dose-reponse relationship was found between SMR and estimated degree of arsenic exposure, with the SMR in the highest exposure group being 800. The results are, however, difficult to interpret because of the fact that a positive dose-response relationship was also found between exposure to sulphur dioxide and respiratory cancer risk. Data on smoking habits and age-distribution in the groups were not given (Lee and Fraumeni, 1969). The mortality of the same cohort was studied again with a broader follow-up period (1938-1977). Relative exposure categories were made. The mortality due to lung cancer increased with an increasing degree of arsenic trioxide exposure and with an earlier period of first employment (Lee-Feldstein, 1983). The risk of lung cancer also increased (lineair) with cumulative exposure indices (Lee-Feldstein, 1986). In another study a sample of 1,800 men of the cohort originally studied by Lee and Fraumeni was examined. Three indices of arsenic exposure were developed; TWA [<100 $\mu g\ As.m^{-3}$, 100-499 $\mu g\ As.m^{-3}$, 500-4999 $\mu g\ As.m^{-3}$ and >5000 $\mu g\ As.m^{-3}$], 30-day ceiling and cumulative. A dose-response relationship existed between arsenic exposure and respiratory cancer. Ceiling arsenic exposure seemed to be more important than TWA exposure. It appeared that workers whose exposure was kept below 500 $\mu g\ As.m^{-3}$ demonstrated no excess in lung cancer mortality. Smoking habits, although contributing to the respiratory cancer incidence did not appear to be as important as arsenic. Sulphur dioxide and asbestos (indirect estimated) did not appear to be important in the excess of respiratory cancer, although sulphur dioxide and arsenic exposures could not be separated completely (Welch et al., 1982, Lamm and Lederer, 1984).

In another study a positive dose-response relationship was described between respiratory cancer deaths and both lenght of employment and estimated level of exposure (Tokudome and Kuratsune, 1976, evaluated in reviews). Essential data on smoking habits, exposure concentrations and age distribution were lacking in this study (IPCS, 1981-R). A case-control study resulted in an overall OR of 5 to lung cancer for exposed versus nonexposed workers. Using a composite score (of dose level and duration of employment) OR's were 2.1, 5.9 and 8.8 for 'low', 'medium' and 'high' exposures (Axelson et al., 1978, evaluated in reviews). A mortality study was conducted among employees of a copper corporation who died between 1959 and 1969. It appears that 7% of the deaths in copper smelters were due to respiratory cancer compared to nearly 3% for men in the whole state (Rencher et al., 1977). The cases of lung cancer occurred in men who had considerably higher exposure indices than persons who died of other cancers or nonmalignant respiratory disease (IARC, 1980-R).
A linear dose-response relationship was also found between respiratory cancer mortality and TWA-exposure to arsenic trioxide among men who retired from a copper smelter. From urinary arsenic levels it was estimated that the airborn concentration was about 50 $\mu g\ As.m^{-3}$. For respiratory cancer a SMR = 305 was found. Data on retired workers showed a decreasing risk with increasing age, suggesting that the effect of past arsenic exposure disappears. Because other contaminants were also present, the mortality may not completely be attributed to arsenic. According to the authors the data suggested the existence of a threshold for arsenic (Pinto et al., 1977, 1978).

Using a "non-threshold" extrapolation method and the data from four smelter studies, the "unit risk" [defined as the additional lifetime cancer risk due to continuous exposure of 1 $\mu g\ As.m^{-3}$] was estimated to range from 1.25 to 7.6×10^{-3}, with a geometric mean of 4.3×10 (EPA, 1983). In the Air Quality Guidelines (WHO, 1987) a "unit risk" of 3.0×10^{-3} has been proposed as "conservative" estimate of the lung cancer risk.

- Mechanisms of action

The absence of carcinogenicity in experimental animals treated with individual arsenic compounds suggests that carcinogenicity in humans is due to a combination of arsenic with other materials (Leonard, 1984). In

several reports it was suggested that inorganic arsenic compounds should be considered as co-carcinogens (Ishinishi et al., 1977; Rossman, 1981; Lee et al., 1988). In one study the tumor promoting potential of arsenite, arsenate and dimethyl arsinic acid in the kidneys of rats was determined. It was found that arsenite and arsenate did not have any initiator activity in "intact" male Wistar rats, but significantly promoted diethylnitrosamine (DENA)-initiated renal tumors in these animals. Dimethyl arsinic acid had neither initiator nor promotor activity. A dose dependent effect (not significantly) for arsenite promotion in partially hepatectomized DENA-initiated rats was found at concentrations of 40, 80 and 160 mg $As.l^{-1}$ in the drinking water (EPA, 1987b).

Summary and conclusions "carcinogenicity"

From experimental studies it appeared that long-term oral or inhalatory exposure to inorganic compounds did not result in increased cancer incidences. After repeated intratracheal administration the development of benign lung tumours were reported. An increase in number of malign tumours were observed after intravenous or subcutanous administration as well as after exposure to arsenic containing mixtures. It was concluded that there is inadequate evidence for carcinogenicity in animals for exposure-routes relevant for humans. The IARC (1980, 1987) arrived at the same conclusion. A mechanistic study indicated that both tri- and pentavalent inorganic arsenic have "tumor promoting" activities. In some other studies it was suggested that inorganic arsenic should be considered as a co-carcinogen.

In epidemiological studies an association was found between long-term oral exposure to inorganic arsenic and skin cancer. Several studies on populations exposed through drinking water or medication showed increased skin cancer incidences. Most studies also showed positive dose-response relationships; for example the "drinkingwater" study from Taiwan [among more than 40,000 people using water containing up to 1,820 $\mu g\ As.l^{-1}$ (mean 500 $\mu g\ As.l^{-1}$)]. In one study conducted in Oregon, however, no increased skin cancer incidences were found. Mean arsenic levels in this study were much lower (mean 16 $\mu g\ As.l^{-1}$) than in the other ones. It is assumed that

the arsenic in medicines and drinking water was present as arsenite and "inorganic arsenic" (valence not known), respectively.
There is substantial evidence of an increased incidence of cancer of the respiratory tract in association with exposure to inorganic arsenic (and other substances) by inhalation. Epidemiological studies among copper smelter workers demonstrated in most cases a positive dose-response relationship between exposure to inorganic arsenic and respiratory cancer. In one study ceiling exposure seemed to be more important than TWA-exposure in the increased incidence of lung cancer. It was suggested that when exposure is kept below 500 $\mu g\ As.m^{-3}$ the risk on lung cancer will be very low. Increased incidences of cancer of the respiratory tract were also reported among workers handling pesticides containing inorganic arsenic.

In most of the epidemiological studies mentioned above simultaneous exposure to other substances occurred. In the "drinkingwater" studies besides arsenic other substances (including organochlorides and ergot alkaloïds) were identified in the water. Exposure to other substances than arsenic was reported in studies on copper smelter workers (sulphur dioxide) as well as in studies on workers handling pesticides (other pesticides). It can therefore not be excluded that the observed effects can be ascribed to other causes than exposure to arsenic.
In spite of these facts it is concluded that inorganic arsenic compounds are skin and lung carcinogens in humans. Data suggesting an increased risk for cancer at other sides are considered to be inadequate for evaluation. No data could be found regarding the carcinogenicity of organic arsenic compounds.
The IARC (1980, 1987) considered the evidence, that inorganic arsenic compounds are skin and lung carcinogens in humans, to be "sufficient". With regard to oral exposure the JECFA concluded that there is no carcinogenic risk without toxic effects. With regard to the lung cancer risk, on the basis of four smelter studies (and making use of a "non-threshold extrapolation method) the EPA calculated "unit risks" in the range from 1.25 to 7.6×10^{-3} (mean 4.3×10^{-3}). A "unit risk" of 3.0×10^{-3} was proposed by the WHO as a "conservative" estimate of the lung cancer risk.

Table 1.1 Short summary of experimental carcinogenicity studies. The data are mainly derived from the IARC (1980) and the IPCS (1981). Some recent studies were also summarized, from these studies the references are given at the foot of the table (see "notes").

Species	compound(s) tested	dose/duration	effect	note
Oral exposure ;				
exposure through drinkingwater				
mouse and rat	As_2O_3	within 15 months arsenic level increased from 3 to 26 mg As/l estimated daily intake was between 0.08 and 0.6 mg per animal	-	
mouse	As_2O_3	76 mg As/l for lifespan	-	
mouse and rat	$NaAsO_2$	5 mg As/l for lifespan	-	
mouse	$NaAsO_2$	5.8 mg As/l for 60 weeks	-	
mouse	As_2O_3	15 mg As/l for lifespan, or in addition after 6 months painting with DMBA or croton oil.	-	
mouse	As_2O_3	76 mg As/l for 3-14 weeks did not enhance carcinogenesis induced by 3-methylcholanthrene.	-	
mouse	As_2O_3	76 mg As/l for 40-60 weeks in combination with treatment with crotonoil, DMBA or urethane.	-	
exposure through the diet				
mouse	DMAA	46 mg/kg bw. per day at an age of 7-28 days and than for 18 months 121 mg/kg diet	-	
mouse	AA	500 then 250 mg/kg diet AA or 338 then 169 mg/kg diet potassium arsenite and two skin applications of DMBA and crotonoil	-	
rat	$Pb_3(AsO_4)_2$ $Ca_3(AsO_4)_2$	daily doses of about 2 mg As for 2 years	-	
rat	$NaAsO_2$ or Na_3AsO_4	up to 250 mg As/kg diet as sodium arsenite (25 mg As/kg bw) or up to 400 mg As/kg diet as sodium arsenate (40 mg As/kg bw) for two years.	-	
rat	$Pb_3(AsO_4)_2$ Na_3AsO_4	up to 400 mg As/kg diet as lead arsenate or 100 mg As/kg diet as sodium arsenite (40 or 10 mg As/kg bw, respectively) for 29 months either with or without additional administration of NDEA 5 days a week for 29 months.	-	
dog	$NaAsO_2$ or Na_3AsO_4	up to 125 mg As/kg diet (3.1 mg As/kg bw) for 2 years.	-	
Exposure by gavage				
mouse	As_2O_3 or	weekly doses of drugs containing arsenic trioxide for 5 months total dose ± 220 mg As/kg bw.	m	(1)
Dermal exposure				
mouse	$KH_2(AsO_2)_2$	10 weekly administrations, total dose 8.7 mg As after 25 days once weekly applications of croton oil.	-	(2)
mouse	$KH_2(AsO_2)_2$	8 applications of arsenite over 5 days (total dose 1.2 mg arsenite per animal), followed by 2 weekly applications of croton oil.	-	
		1 application of DMBA, followed by applications of arsenite (total dose 64 mg arsenite per animal per week).	-	
mouse	Na_3AsO_4	2 weekly application of arsenate for 60 weeks with or without additional treatment with crotonoil, DMBA or urethane.	-	
Exposure by inhalation				
mouse	$NaAsO_2$	1% (w/w) aqueous solution of sodium arsenite 20-40 min/day 5 days a week for 55 weeks	-	(3)

Table 1.1 (continued)

Species	compound(s) tested	dose/duration	effect	note
Intratracheal instillations				
rat	As_2O_3 copper ore flue dust	15 doses of 0.26 mg As_2O_3, 2.5 mg copper ore (3.95% As) or 2 mg flue dust (10.6% As) alone or in combination with benzo[a]pyrene	-	
hamster	As_2S_3 or $Ca_3(AsO_4)_2$	15 weekly administrations with a total of ± 3 mg As/kg bw as arsenic trioxide or calcium arsenate	b	(4)
hamster	As_2O_3	15 weekly administrations with a total dose of 5.25 or 3.75 mg As	b	(5)
rat	mixture	1 injection of a mixture containing calcium arsenate, copper sulphate and calcium hydroxide (total of 0.07 mg As)	m	(6)
rat	mixture	2 weekly injections of 6 or 10 mg of dust of tin oxide ore containing about 2% to 30% arsenic, total dose 90 to 150 mg.	m	(7)
Other exposure routes				
mouse	Na_3AsO_4 sc	20 daily injections of 0.5 mg/kg bw throughout pregnancy	m	(8)
		Progeny of treated mothers were either injected sc with with 0.5 mg/kg bw (20 times) or left untreated.	m	(9)
mouse	DMAA sc	single injection of 464 mg/kg bw at 28 days of age.	-	
rat	$Ca_3(AsO_4)_2$ sc	administration of a pellet containing 75 mg $Ca_3(AsO_4)_2$ or injection of 100 mg $Ca_3(AsO_4)_2$ in solution.	-	
mouse	Na_3AsO_4 iv	20 weekly injections of 0.5 mg As/kg bw	m	(10)

Abbreviations:
AA = arsanilic acid; DMAA = dimethylarsinic acid; DMBA = 7,12-dimethylbenz[a]anthracene;
NDEA = N-nitrosodiethylamine.
m = maligne; b = benigne.
iv = intravenous administration; sc = subcutaneous administration

Notes:
(1) Higher incidences of adenocarcinoma of the skin, lung, peritoneum and lymph nodes were seen. No tumours occurred in the controls. Very brief and incomplete description of the study.
(2) in both the treated group as the control group (treated only with croton oil) some skin papillomas were seen.
(3) (Glaser et al., 1986)
(4) After treatment with arsenic trisulphide one lung adenoma appeared in 28 animals, after treatment with calcium arsenate 4 lung adenomas appeared in 35 animals. No tumours were found in the controls. The concentrations used were toxic to the lungcells; excessive production of protein was observed. (Pershagen and Björklund, 1985)
(5) After a total dose of 3.75 mg arsenic 3/10 animals developed lung adenomas, after a total dose of 5.25 mg arsenic 2/20 developed lung adenomas. No lung tumours were seen in the control group. (Ishinishi, 1983).
(6) During the first week of treatment 10 out of 25 animals died. Of the surviving 9 developed lung tumours (7 bronchogenic adenocarcinomas and 2 bronchiolar-alveolar cell-carcinomas).
(7) As the arsenic content in dust increased from about 2% to 30% the incidence of malignancies increased form 15% to 23% (the incidence of precancerous lesions increased from 5% to 45%).
(8) 11 out of 24 animals developed lymphocytic leukaemia or lymphomas within 24 months after start of the experiment.
(9) Treated and untreated progeny responded similar in developing lymphomas or lymphocytic leukaemia. A number of animals were still alive at the end of the experiment, so the results are difficult to interpret.
(10) 11 out of 20 treated animals developed lymphomas. It is not clear if a control group was used.

Tabel 1.2 Overview of epidemiological studies concerning long-term oral exposure to inorganic arsenic (through drinking water) and skin cancer risk.

Studied population (reference)	Chemical info/ source	Dose/duration	Effects / dose-response relationship
A group of skin cancer patients, Oregon case-control study (Morton et al., 1976)	drinking water from different sources	mean: 16 $\mu g\ As.l^{-1}$, range: 0-2150 $\mu g\ As.l^{-1}$	No positive relationship was found between arsenic levels in drinking water and skin cancer incidence.
general survey of more than 40000 inhabitants from south-west Taiwan (Tseng, 1977)	artesian well water	mean: 500 $\mu g\ As.l^{-1}$, range: 0-1,800 $\mu g\ As.l^{-1}$	A positive dose-response relationship was found between arsenic levels in drinking water and incidences of skin cancer.
998 exposed vs. 1488 non exposed persons from North Mexico (Cebrian et al., 1983)	well water, for 70% pentavalent arsenic	exposed: 0.41 mg $As.l^{-1}$ control: 0.007 mg $As.l^{-1}$	21.6% of the exposed population showed at least one of the cutaneous signs of chronic arsenic poisoning against 2.2% of the control population. RR's were 3.6 to 36 for developing cutaneous lesions in the exposed group compared to the control group.
residents of a BFD-endemic area in Taiwan different villages (Chen et al. 1985)	high arsenic artesian well water, valence state unknown	0.35-1.14 mg $As.l^{-1}$ estimated total daily intake as high as 1 mg	SMR's for cancers of bladder, kidney, skin, lung, liver and colon were 1100, 772, 543, 320, 170, 160 resp for males and 2009, 1119, 652, 413, 229 and 168 resp. for females. A dose-response relationship was observed between SMR's of the cancers and BFD-prevalence rate of the villages.
204 cancer patients 368 controls, Taiwan, case-control study (Chen et al., 1986)	either artesian well water or shallow well water was used, valence state unknown	artesian: 0.35-1.14 mg $As.l^{-1}$; shallow: 0-0.04 mg $As.l^{-1}$	A positive dose-response relation was observed between exposure to artesian well water and cancers of bladder, lung and liver. O.R.'s were 3.90, 3.39 and 2.67 for those who used well water for $\geq$ 40 yr compared to those who never used well water.
67 exposed persons vs. 96 non exposed persons rural West-Bengal India (Guha Mazumder et al., 1988)	tubewell water, valence state unknown	exposed group: 0.2-2 mg $As.l^{-1}$ c-group: $\leq$ 0.5 mg $As.l^{-1}$	exposed group: 93% showed evidence of chronic dermatosis and hepatomegaly control group: 6.3% had non-specific hepatomegaly, no skin lesions.

Tabel 1.3 Overview of epidemiological studies concerning long-term inhalatory exposure to inorganic arsenic and respiratory cancer risk.

studied population (reference)	exposition to and concentration(s)	results	remarks
Exposition to pesticides			
workers producing sheep dip powder vs. non exposed workers (Hill and Faning, 1948, evaluated in reviews)	sodium arsenite median conc. 70-700 $\mu g\ As.m^{-3}$	32% of the exposed workers died from cancer of the respiratory tract compared to 16% of the deaths from other occupational groups	no smoking habits were recorded
1231 orchard workers Washington (Nelson, 1973, evaluated in reviews)	lead arsenate classified into groups based on urinary As levels (measured in 1938)	no differences was found between the cohort and State of Washington for overall cancer mortality or for lung cancer mortality	No detailed data on exposure
173 workers handling insecticides vs. 1809 non exposed workers follow-up 1940-1972 (Ott et al., 1974)	lead arsenate, copper arsenate, acetoarsenite, magnesium arsenite and calcium arsenate. up to 40 mg $As.m^{-3}$	16% of the deaths in the exposed group compared to 6% of non exposed workers were due to respiratory cancers. A positive dose-response relationship between the degree of arsenic exposure and lung cancer mortality was indicated. SMR's ranged from 0.6 in the lowest exposure category to 7.0 in the highest. No differences in smoking habits between exposed and control group were suggested.	16 of the 28 deaths in the exposed group occurred in individuals who were less than 1 year exposed
idem (Blejer & Wagner, 1976, evaluated in reviews)	idem, 48-h TWA were made, varying between 1 and 740 $\mu g\ As.m^{-3}$	No clear dose-response relationship was observed below daily TWA-doses of 90 $\mu g\ As.m^{-3}$.	
idem follow-up 1940-1982 (Sobel et al., 1988)	idem	9 additional lung cancer cases were observed, whereas 7.8 were expected; risk ratio no longer significant. No new cases of lymphatic or haematopoeitic cancer occurred.	
1393 workers handling pesticides follow-up 1946-1974 (Mabuchi et al., 1979, evaluated in reviews)	arsenic compounds, copper sulphate, chlorinated hydrocarbons, organophosphates, carcarbamate and other organic herbicides. 500 $\mu g\ As.m^{-3}$	overall SMR nearly 100, sign. increases for lung cancer (SMR = 168), anaemias in males (SMR = 1000), and lymphomas in males (SMR = 190) (based on death rates for the general population) Positive dose-response relationship was observed between lung cancer risk and both duration of employment and duration of exposure.	No data on smoking habits
a group of orchadists case-control study follow-up 1968-1980 (Wicklund et al., 1988)	past exposure to lead arsenate	no association was found between lung cancer and presence, intensity or duration of lead exposure. There were no differences in smoking habits between the 2 groups	

Table 1.3 (continued)

studied population (reference)	exposition to and concentration(s)	results	remarks
Exposition in smelters			
8000 copper smelter workers, Montana follow-up 1938-1963 (Lee & Fraumeni, 1969)	arsenic trioxide and sulphur dioxide, relative exposure categories	smelterworkers had an excess mortality from respiratory cancer (observed 147 versus 44.7 expected; SMR=329, p<0.01). The risk increased in proportion to the degree of exposure to arsenic and sulphur dioxide. SMR's were 239, 478 and 667 in those with heavy, medium and light exposure. The excess was 8-fold for workers who were for more than 15 years heavily exposed to arsenic.	no data on smoking habits and actual exposure levels. effects of arsenic or sulphur dioxide difficult to seperate.
idem follow-up 1938-1977 (Lee-Feldstein, 1983, 1986)	idem	a dose-response relationship was found beteen degree of exposure to arsenic trioxide and risk of lung cancer. Highest SMR was found for men heavily exposed and employed before 1925 (SMR=800), from another analysis by the same author it appears that the cancer mortality increases linear with cumulative exposure index, with SMR from 200 to 900.	
idem (a sample of 1800 workers) follow-up 1938-1977 (Welch et al., 1982)	idem TWA-categories were made; <100; 100-499; 500-4999 and >5000 $\mu g\ As.m^{-3}$	A dose-response relationship was found between exposure categorie and respiratory cancer risk. Ceiling exposure seemed to be more important than TWA-catergories. It was suggested that exposure $<500\ \mu g\ As.m^{-3}$ did not increase lung cancer risk.	
copper smelter workers Tacoma Washington, follow-up 1950-1971 (Milham & Strong, 1971, evaluated in reviews)	arsenic, among others	there were 40 observed lung cancer deaths versus 18 expected, SMR=222, p<0.001	methodof calculating expected deaths inadequately described (IARC, 1980).
metal workers Japan, follow-up 1949-1971 (Tokudome & Kuratsume, 1976, evaluated in reviews)	arsenic, among others	an sign. increased mortality for lung cancer(trachea, lung and bronchus) was found (29 observed vs. 2.44 expected; SMR=1189, p,0.01). A positive dose-response relationship was found between SMRs for lung cancer and duration of employment as well as between estimated levels of exposure and SMR. There was also an increase in mortality from coloc cancer (3 vs. 0.6) and in mortality from liver cancer (11 vs. 3.26).	no detailed information about smoking habits, age distribution and exposure (IPCS, 1981).
residents living near a copper smelter, Sweden case-control study (Axelson et al., 1978, evaluated in reviews)	arsenic, among others	an overall OR of 4.6 was found for lung cancer mortality among smelters. Using a composite score OR's were 2.1, 5.9 and 8.8 for 'low', 'medium' and 'high' exposures. There was also a significant increased risk of leucemia and myeloma among smelters.	Excess lung cancer did not correlate with exposure to Ni, Pb, Cu, Bi, An, Se or sulphur dioxide.

Table 1.3 (continued)

studied population (reference)	exposition to and concentration(s)	results	remarks
copper smelter workers Magna (Utah), follow-up 1959 -1969 (Rencher et al., 1977)	arsenic among others	7% of the deaths of workers were due to lung cancer compared to 2.2% for the other factory workers and 2.7% for the males in the state. No indication of a smoking synergism could be found. Estimated cumulative exposure indices for arsenic, sulphur dioxide and lead were higher among those dying from lung cancer than for other causes.	
retired copper smelter workers, Washington (Tacoma), follow-up 1949-1973 (Pinto et al., 1977, 1978)	mainly to arsenic trioxide; $\pm$ 50 μg As.m^{-3}	A highly sign. increase in mortality from cancer of the respiratory system was found for the whole group former workers. SMR=305 A linear relationship between lung cancer mortality and estimated time-weighted index of total lifetime exposure was found.	the existence of a threshold suggested
copper smelter workers Sweden, (Pershagen, 1978)	500 μg As.m^{-3}	an OR of about 5 for lung cancer mortality was observed for workers exposed to arsenic compared to those who were not exposed. A positive dose-response relationship was indicated.	

BFD = blackfoot disease; RR = relative risk; OR = odds ratio; SMR = standard mortality ratio; TWA = time weighted average.

2 ECOTOXICITY - AQUATIC ORGANISMS

2.1 BIOTRANSFORMATION AND BIOAVAILABILITY

The role of algae in arsenic biochemistry is significant. Arsenate (the predominant form of arsenic in the marine environment) can be taken up by algae because of its similarity to essential phosphate. Several studies indicate that inorganic arsenic is reduced and methylated by phytoplankton. The algal turnover reduces the toxicity of arsenic by producing non-reactive, stable and methylated compounds. Organic arsenic compounds are less toxic to algae and the other organisms to which arsenic can transferred via the foodchain. (Sanders, 1979a, Edmonds and Francesconi, 1987, Lunde, 1972). In higher organisms (invertebrates and fish) arsenic is predominantly present in the organic form. The major compound found in especially marine organisms was arsenobetaïne. Two arsenical compounds [2-hydroxy-3-sulphopropyl-5-deoxy-5-(dimethylarsenoso)furanoside and 2,3-dihydroxypropyl-5-deoxy-5-dimethyl-arsenoso)furanoside] which could be intermediates in the chain from arsenate to arsenobetaïne were identified in the seaweed *Ecklonia radiata* (Edmonds and Francesconi, 1981). The arsenic concentrations of marine macroalgae vary significantly. This is probably due to metabolic differences and not to variations in the total mount of available arsenic in the surrounding water. Considerable quantities of the arsenic in the algae were in the inorganic form (Sanders, 1979a). The seaweeds *Fucus spiralis* and *Ascophyllum nodosum* were found to accumulate more arsenate than arsenite. An increase in temperature resulted in an increased arsenic uptake, whereas variation in salinity or pH had no effects. The uptake of arsenate was reduced by phosphate (Klumpp, 1980a).

The availability of arsenic from estuarine sediments, with total arsenic concentrations ranging from 2 to 2,800 mg $As.kg^{-1}$, to several benthic organisms was studied. In general, the arsenic levels in the deposit feeder *Scrobicularia plana* (bivalve), the polychaete worm *Nereis diversicolor* and the seaweed *Fucus vesiculosus* correlated with the arsenic concentration in the sediment. In the case of *S. plana* the iron/arsenic ratio in the sediment correlated more significant with arsenic levels in the organism than did the arsenic concentration alone. With a decreasing iron concentration in the

sediment the availability of arsenic to this bivalve increased. The iron / arsenic ratio did not correlate with the arsenic levels in the other organisms. The concentrations of arsenic in *S. plana* were higher than those for most other species and ranged from 5 to 190 mg $As.kg^{-1}$ dw (Langston et al., 1980). Arsenic levels in several plants, invertebrates and fish from a Bay with contaminated sediment (up to 715 mg $As.kg^{-1}$ wet weight) showed that little arsenic had been transformed into the biota. The arsenic levels were either not detectable or very low; the mullet *Mugil cephalus* , the gastropod *Thoedoxus vespertinus* , the fish *Lutjanus fulvus* and *Eleotris sandwicensis* contained 1.3, trace, 0.2 and 0.2 mg $As.kg^{-1}$ wet weight, respectively (Hallacher et al., 1985). Transfer-experiments showed that *Scrobicularia plana* can be used as an indicator of available sediment-bound arsenic. To the filter-feeder *Ceratoderma edule* the major source of arsenic seemed to be suspended matter. The seaweed *Fucus vesiculosus* can be used as an indicator of dissolved arsenic because of its rapid respons to changes in water concentration. Responses in *Littorine* species were thought to result from dietary intake (they graze on *F. vesiculosus)* and not from changes in dissolved arsenic (Langston et al., 1984). A positive correlation has been found between arsenic levels in tubificides (worms) and the arsenic concentration in pore-water form sediments from the Rhine and the Maas. This correlation was not found between arsenic levels in the worm and the arsenic concentration in the sediments (Hueck-vd Plas, 1984).

2.2 BIOCONCENTRATION AND BIOMAGNIFICATION

Arsenic is a natural component of the aquatic systems and is bioaccumulated by many components in the aquatic environment. Seawater organisms contain more arsenic than do most freshwater organisms and aquatic plants generally contain more arsenic than do higher members of the foodchain (Woolson, 1975). Arsenic does not appear to accumulate to as high a degree as some other elements, which may be due to the complex transformations of arsenic resulting in loss of the compound by reduction to highly volatilized forms (Spehar et al., 1980). From two deposit feeding molluscs and a polychaete worm especially *Scrobicularia* was found to reflect contamination of the sediment. *Scrobicularia* from contaminated waters contained about 20 times the concentration in

animals from uncontaminated water (Bryan et al., 1980). Bioconcentration factors (BCF's) were generally higher in organisms of lower trophic levels than in organisms of higher trophic levels. BCF' (based on wet weight) of phytoplankton, invertebrates and fish were reported up to 400, 700 and 150, respectively (Mance et al., 1984).

Laboratory experiments indicated that arsenic is bioconcentrated by aquatic organisms but not biomagnificated. In one experiment stoneflies, snails, amphipods and trout were (separately) exposed to about 100 and 1,000 μg $As.l^{-1}$ from arsenic trioxide, arsenic pentoxide, sodium dimethyl arsenate (SDMA) and disodium methylarsenate (DSMA). BCF's were calculated by dividing the residue tissue concentration (dry weight) by the corresponding exposure concentration. The highest accumulation (BCF=131) in the stonefly *Pteronarys dorsata* was found after 28 days exposure to 100 μg $As(III).l^{-1}$.In the snails *Helisoma campanulata* and *Stagnicola emarginata* BCF's of 99 and 92, respectively, were found after 28 days exposure to 89 μg $As(V).l^{-1}$. A BCF=219 was found in *Daphnia magna* after exposure to 96 μg $As(III).l^{-1}$ for 21 days. The concentration in amphipods and trout were not elevated compared to controls. It can, however, be doubted if the exposure time was long enough to equilibrate the uptake (Spehar et al., 1980). The uptake and accumulation of several arsenic compounds from the water by *Poecilia reticulata* was studied by keeping the fish in labelled solutions of arsenic acid, methylarsonic acid, dimethylarsinic acid, arsenic trioxide, methylarsonous acid, dimethylarsinious acid and trimethylarsine. It was found that only trimethylarsine can be readily taken up by fish. An exact value could not be given because methylarsine is volatilized and oxidized in aqueous solutions and because trimethylarsines are sorpted by the skin of fish (Stary et al., 1982). Marine bivalve molluscs *Argopecten irradians* accumulated arsenic resulting in a BCF of about 10 (based on wet weight) for concentrations up to 3.0 mg $As.l^{-1}$ (just not toxic) (Nelson et al., 1976). Highest concentrations of arsenic in the green sunfish *Lepomis cyanellus* were found in the gallbladder and bile. Levels increased from about 35 to 78 to 159 mg $As.kg^{-1}$ fresh weight during 2.4- and 6-day exposure to 60 mg $As.l^{-1}$ as arsenate (Sorensen et al., 1979). Data on polychaete worms living in sediments of estuarines, indicated that some species can accumulate arsenic to levels around 200 $mg.kg^{-1}$ dry weight, even under low arsenic concentrations. A very remarkable worm is the

cirratulid *Tharyx marioni* , in which whole body content of arsenic exceeded 2,000 mg.kg^{-1} dry weight, irrespectable of the environmental arsenic levels. A concentration factor of 40 (calculated as As in dm/ As in the sediment) was measured. Much of the arsenic appeared to be organically bound. The exact role of arsenic in this worm is unknown (Gibbs et al., 1983).

Bioconcentration of arsenic was studied in several organisms comprising (limited) microcosms in two Bays. The sediment (2.40 mg As.kg^{-1} wet weight) contained significant more arsenic than did the water (0.005 mg As.l^{-1}). BCF's (based on wet weight) for the barnacle, crab, oyster, clam and polycaete were 400, 14, 114, 478 and 224, respectively (Guthrie et al., 1979). In an environmental survey the arsenic concentration was measured in various species of marine fish and invertebrates. Crabs were found to contain the highest level of arsenic (up to 37.8 mg As.kg^{-1} wet weight) of all organisms tested (LeBlanc and Jackson, 1973). From an extensive field study it appeared that the ability of the organisms to concentrate arsenic decreased with increasing arsenic concentration in the environment. Arsenic concentrations were measured in sediments, macrophytes and several invertebrates in arsenic-contaminated lakes (with 0.70-5.5 mg As.l^{-1} total dissolved arsenic) and in 'normal' lakes (<0.01-0.07 mg As.l^{-1}) in Canada. Concentration factors (CF) were calculated as the concentration in organism (mg As.l^{-1} wet weight) divided by the concentration in water (mg As.l^{-1}). The data provided no evidence for arsenic biomagnification (see table 2.1.) (Wagemann et al., 1978). In organisms living in the river bassins of the Rhine and Maas higher arsenic levels were found in zooplankton than in fish (Hueck-vd Plas, 1983).

Table 2.1. Mean concentration factors (wet weight) for different trophic levels of littoral fauna from the contaminated and uncontaminated lakes (Wagemann et al., 1978).

	Mean CF's (range CF-values)		
Lake	*herbivores*	*carnivores*	*omnivores*
contaminated	*20 (0.44-80)*	*14 (0.35-44)*	*63 (1.7-400)*
uncontaminated	*179 (21-590)*	*100 (7.3-700)*	*116 (14-400)*

Accumulation (and elimination) of arsenic was studied in a foodchain consisting of the macroalga *Fucus spiralis*, the snail *Littorina littoralis* and the predatory snail *Nucella lapillus*. The diet appeared to be the main source of arsenic in this foodchain. The results indicated that arsenic is not biomagnificated. The arsenic taken up was easily eliminated by the organisms. (Klumpp, 1980b). In an experimental food chain radioarsenic was used to identify various chemical forms of arsenic accumulated from food or water. The chain consisted of phytoplankton *Dunaliella marina*, zooplankton *Artemia salina* and a shrimp *Lysmata seticaudata*. In *D. marina* only organic arsenic could be detected after exposure both via water and via food. The arsenic taken up from the water by *L. seticaudata* was largely converted to arsenite, after uptake via food arsenic was mainly found in the organic form. The shrimp contained both organic and inorganic arsenic after exposure by water. It was suggested that arsenic was converted by the intestinal microflora (Wrench et al., 1979). The accumulation and biomagnification of the organic arsenic compounds cacodylic acid (CA) and dimethylarsine (DMA) was studied in a model ecosystem consisting of fish, daphnids, snails and algae. The organisms were exposed for 3, 29, 32 and 32 days, respectively. It appeared that lower food chain organisms (algae and daphnids) bioaccumulated more CA and DMA than did higher food chain organisms (snails and fish) and that both CA and DMA did not biomagnify (Isensee, 1973). It should be noted that in this experiment the exposure time for fish may not have been enough to establish an equilibrium.

2.3 TOXICITY

2.3.1 Freshwater organisms short-term

The relevant short-term toxicity tests with freshwater organisms resulting in L(E)C50-values are summarized in table 2.2.

- Inorganic arsenic

Short-term tests with freshwater organisms have resulted in L(E)C50-values between 206 μg As.l^{-1} for the crustacean *Bosmina longirostris* and 82,500 μg As.l^{-1} for the fish *Pimephales promelas* for inorganic arsenic compounds.

The compounds most often used in the tests were sodium arsenate, sodium arsenite and arsenic trioxide. For the fish *Esox masquinongy* the life stage seemed to be important for the toxicity; the 96-h LC50-value for fry of *E. masquinongy* in the swim-up stage was 1,100 $\mu g\ As.l^{-1}$, whereas the LC50-value for 5 week old organisms was 16,000 $\mu g\ As.l^{-1}$ (Spotila and Paladino, 1979).
The data from the table do not indicate a difference between toxicity of tri- or pentavalent arsenic compounds. Based on the available tests crustaceans seem to be the most sensitive organisms, whereas freshwater fish are relatively unsensitive. According to the review by Mance (1987) water hardness has no effect on the toxicity of arsenic to fish.

Additional information
Using a conditional avoidance technique 100 $\mu g\ As.l^{-1}$ was found to be the lowest concentration of arsenic which gave a significant impairment in the goldfish *Carassius auratus* after 48 h exposure. The 48-h LC50 for *C. auratus* was determined at 32.0 mg $As.l^{-1}$ (Weir and Hine, 1970).

- Organic arsenic

Very few data are available on the toxicity of organic compounds. The only compound tested was monosodium salt methane arsonic acid. The reported LC50-values were relatively low; ranging from 417 to 509 $\mu g\ As.l^{-1}$.

2.3.2 Freshwater organisms long-term

Data on long-term toxicity tests with freshwater organisms are summarized in table 2.3.

- Inorganic arsenic

Long-term toxicity tests resulted in NOL(E)C-values ranging from 10 $\mu g\ As.l^{-1}$ for the alga *Ankistrodesmus falcatus* to 10,000 $\mu g.l^{-1}$ for the alga *Selenastrum capricornutum* for inorganic arsenic compounds. The highest and lowest NOEC-values were found in the same study in which four freshwater algae were tested in the same medium. One of the species tested, *Scenedesmus obliquus*, was found to be extremely sensitive to arsenate. At the lowest concentration tested (10 $\mu g\ As.l^{-1}$) a significant growth inhibition was found.

The growth of the alga *Micocoleus vaginatus* was not affected at the highest concentration tested (100,000 μg As.l^{-1}) (Vocke et al., 1980).
The chronic toxicity of several inorganic (and organic arsenic) compounds was tested in organisms from various taxonomic groups; molluscs, crustaceans, insects and fish. No effects were found at the highest tested concentrations of 1,000 μg As.l^{-1} from arsenic trioxide, arsenic pentoxide or sodium arsenite (Spehar et al., 1980). The parr-smolt transformation appeared to be the most sensitive life stage of the Coho salmon *Oncorhynchus kisutch* . Based on biochemistry and development the NOEC-value was reported to be 76 μg As.l^{-1} from arsenic trioxide (Nichols et al., 1984).

Additional information
The wide range in sensitivity of algae to inorganic arsenic had been found in several other studies as well. A concentration of 75 μg As.l^{-1} as arsenate in the medium significantly inhibited the growth rates of the algae *Melosira granulata* and *Ochromonas vallesiaca* . The alga *Chlamydomonas reinhardtii* required 750 μg As.l^{-1} as arsenate for the same degree of depression, whereas *Cryptomonas erosa* and *Anabaena variabilis* were unaffected up to 7,500 μg As.l^{-1} as arsenate. It was shown that depletion of phosphate increased the toxicity of arsenate (Planas and Healey, 1978). The primary productivity of *Skeletonema costatum* was inhibited by arsenate at 67 nM when the concentration of phosphate was kept low. A phosphate enrichment > 0.3 μM decreased the toxicity of arsenate. Dimethylarsinic acid (organic arsenic) was less toxic than the inorganic compounds (Sanders, 1979b). The effect of arsenic trioxide on the ovarian function and spermatogenesis of the freshwater fish *Colisa fasciatus* was studied. Exposure to 1.5 mg As.l^{-1} for 15 and 30 days and exposure to 10.6 mg As.l^{-1} for 15 days showed no effect on the development of oocytes, whereas exposure to 10.6 mg As.l^{-1} for 30 days decreased the development of oocytes. With regard to spermatogenesis similar results were obtained, with the same test concentrations and exposure times. Only after 30 days exposure to 10.6 As.l^{-1} an effect was seen (Shukla and Pandey, 1984a, 1984b). Arsenic had a significant effect on the temperature tolerance and survival of newly hatched fry *Esox masquinongy* .Fry in the swim-up phase (8-14 days post hatch) were exposed to 0-5.0 mg As.l^{-1} as arsenite. In all tests, including the control, a significant decline in the Critical Thermal Maxima (CTM) occurred during this period. The control group

was the only group to survive the experiment and recover from this drop (Paladino and Spotila, 1978).
The effect of arsenic on the growth and mortality of a natural assemblage of copepods using natural food and water was determined. The NOLC-values for arsenate and arsenite were $\geq$10.0 and 1.6 mg As.l^{-1}, respectively (see table 6.2). To express the effects on growth of the copepods the term SC20 was introduced. The SC20 can be defined as the sublethal concentration resulting in a drop in biomass growth rate equal to 20% of the control growth rate. The SC20 causes in the final biomass a decrease to about half that of the control, which is comparable to an EC50 based on growth. The SC20's of arsenate and arsenite were determined at 1,380 and 320 μg As.l^{-1} (Borgmann et al., 1980).

Arsenic fed to *Salmo gairdneri* significantly impaired growth at levels of 20 and 30 mg As.kg^{-1} diet (probably inorganic arsenic). The growth of fish exposed to 10 mg As.kg^{-1} diet was not different from that of the control (Oladimeji et al., 1984). The toxicity of several dietary arsenic compounds in juvenile *S. gairdneri* was tested. Reduced growth and feed consumption and altered feeding behaviour were seen at all levels of supplied inorganic arsenic. The NOEC-values were reported to be $\leq$137 mg As.kg^{-1} diet and $\leq$180 mg As.kg^{-1} diet for disodium arsenate heptahydrate and arsenic trioxide, respectively. Feed refusal was seen in the inorganic arsenic test. It is uncertain whether the reduction in growth is due to a reduction in food intake or to the toxicity of arsenic (Cockell and Hilton, 1988).

In outdoor pools the effect of various concentrations and treatment frequencies of sodium arsenite on the fish *Lepomis macrochirus* and several invertebrates (rotifers, cladocerans, copepods and microcrustaceans) was investigated. From the organisms tested the cladocerans were found to be the most sensitive. They were only abundant in the control pool and in the pool with the lowest total treatment (0.23 mg As.l^{-1}) (Gilderhus, 1966).

- Organic arsenic

Data on long-term toxicity of organic compounds are limited. In one study the chronic toxicity of two organic (and inorganic) arsenic compounds had been tested in organisms from various taxonomic groups; molluscs, crustaceans, insects and fish. No effects were found at concentrations up to 1,000 μg

$As.l^{-1}$ from disodiummethylarsenate and sodium dimethylarsenate (Spehar et al., 1980). The toxicity of several dietary arsenic compounds in juvenile *S. gairdneri* was tested. Even the highest doses organic arsenic did not cause any effects. Dimethylarsinic acid and arsanilic acid provided NOEC-values of $\geq$1497 and $\geq$1503 mg $As.kg^{-1}$ diet, respectively (Cockell and Hilton, 1988).

2.3.3 Freshwater sediment systems

The presence of sediments significantly decreased the toxicity of arsenite to *Daphnia magna*. In two series consecutive tests with several arsenite concentrations a difference was seen in the 48-h survival of neonate *D. magna* (< 24-h in age) tested in beakers with or without sediment. Survival in the beakers with sediment increased over time. Arsenite in the water column apparently became adsorbed to sediment through time, becoming less available to *D. magna*. For example, 47 mg $As.l^{-1}$ as arsenite caused 100% mortality in repetitive tests until day 20. This percentage decreases to 37% at day 28. Alkaline phosphatase, a principle enzym released by *D. magna*, was shown to decrease in activity (Burton et al., 1987).

2.3.4. Marine organisms short-term

Data on relevant short-term toxicity tests with marine organisms resulting in L(E)C50-values are presented in table 2.4.

- Inorganic arsenic

The short-term tests with marine organisms have resulted in L(E)C50-values between 232 μg $As.l^{-1}$ (48-h) for larval stages of the crustacean *Cancer magister* to 28,500 μg $As.l^{-1}$ (96-h) for the adult fish *Limanda limanda* .
For adult crustaceans a 96-h LC50-value of 17,000 μg $As.l^{-1}$ was reported.

Additional information

The effects of arsenate and arsenite on the filtering rate of the mussel *Perna perna* was examined after one hour of exposure. A difference in toxicity between tri- and pentavalent arsenic was found. Sodium arsenate did not affect the filtering rate significantly at levels up to 150 mg $As.l^{-1}$, whereas a

concentration of 4 mg $As.l^{-1}$ as sodium arsenite caused a 50% reduction in the filtering rate. The authors mentioned that these concentrations are too high to have relevance for the marine environment (Watling and Watling, 1982). Arsenic was found to in duce developmental defects and mitotic abnormalitis in the sea urchins *Paracentrotus lividus* and *Sphaerechinus granularis*. Exposure to 750 µg As (III).l^{-1} and 3,750 µg As(V).l^{-1} for 48 h caused in about 60% of the embryos gut abnormalities. An effect on the mitotic action was observed after pretreatment of eggs and sperm (Pagano et al., 1982). The acute toxicity of arsenate to three estuarine invertebrates has been investigated at three temperatures (5, 10 and 15 °C) and a range of salinities (5 to 35 o/oo) in static tests. The organisms used are the amphipod *Corophium volutator* , the bivalve *Macoma balthica* and the annelid *Tubifex costatus* . 96-h LC50-values for *C. volutator* ranged from 6 to 60 mg $As.l^{-1}$ depending on temperature. For *M. balthica* LC50-values ranged from 140 to 800 mg $As.l^{-1}$ also depending on temperature. After 96-h no mortality was observed for *T. costatus*. No significant effect of salinity on LC50-values could be found for all three species (Bryant et al., 1985).

- Organic arsenic

Only one test (partial) on toxicity of organic arsenic was available. The effect of monosodium methanearsonate (MSMA) on the filtering rate of the mussel *P. perna* was examined after one hour of exposure. A 50% reduction in the filtering rate was found at a concentration of 25 mg $As.l^{-1}$ as MSMA (Watling and Watling, 1982).

2.3.5 Marine organisms long-term

The limited relevant data on long-term effects of arsenic on marine organisms resulting in NOL(E)C50-values are presented in table 2.5.

- Inorganic arsenic

The 14-d NOEC-value of 34.6 µg $As.l^{-1}$ for the macrophyte *Champia parvula* was the only available NOEC-value. One study on the crustacean *Eurytemora affinis* produced a 16-d NOLC-value of $\geq$ 18 or $\geq$36 µg $As.l^{-1}$ for juvenile and adult organisms, respectively. In a life history test with the same organism a NOEC-value (26-33 d) of $\geq$9.0 µg $As.l^{-1}$ was found.

Additional information

The toxicity of arsenic to marine algae has been studied several times. Marine algae show, just like freshwater algae, wide ranges of sensitivity to arsenic. Single species tests were conducted to study the effect of up to 100 μg $As.l^{-1}$ as arsenate on the growth of seven species of marine phytoplankton. *Cylindrotheca closterium* was most resistance (NOEC $\geq$ 100 μg $As.l^{-1}$). *Thalassiosira pseudonana* and *Tetraselmis contracta* were slightly affected (growth depression 27 and 19%, respectively). *Skeletonema costatum* was greatly affected (growth depression 60%). The other species *Isochrysis galbana*, *Amphidinium carterae* and *chaetoceros pseudocrinitum* were most affected; their growth was terminated by arsenate (Sanders and Vermersch, 1982). An arsenate concentration of 75 μg is inhibitory to the growth of the green alga *Chlamydomonas* (Christensen and Zielski, 1980).

In a number of studies it was found that phosphate enrichment reduced the uptake of arsenate by algae. Under low phosphate conditions the growth of algae was affected at lower doses. Studies indicated that both arsenite and arsenate inhibit cellular functions at low levels, but in different ways. Arsenite is thought to react with SH-groups of proteins. Arsenate competes with phosphate for transport into the cell. It inactivates the phosphate active transport mechanism and may inhibit glucose metabolism. In addition it competes with phosphate in the oxidative phosphorylation. Therefore the extracellular concentration of phosphate may be important in the toxic effect of arsenate (Sanders, 1979b). The influence of phosphate on the toxicity of arsenate and arsenite on the macroalga *Champia parvula* was also investigated in the following study. Phosphate had no effect on the toxicity of arsenite. At a concentration of 212 μg $As.l^{-1}$ the growth of *C. parvula* was significantly less than that for the control, but the effect was independent of phosphate concentrations. The toxicity of arsenate was inhibited by phosphate. When no phosphate was added all plants died at 1,290-9,330 μg $As(V).l^{-1}$, whereas with an addition of 4,5 μM phosphate the growth in all treatments was equal to that in the control (Thursby and Steele, 1984).

The effect of arsenate on the growth rate of a natural phytoplankton assemblage was tested at concentrations of 5, 15 and 25 μg $As.l^{-1}$. The NOEC-value based on growth was <5 μg $As.l^{-1}$ after 72 h. The species composition was

altered by the arsenate addition. The growth rates of *Skeletonema costatum* were greatly inhibited. *Chaetoceros* spp. dominated the assemblage and the microflagellates were largely unaffected. Natural phytoplankton assemblages cultivated outdoors in large-volume cultures were exposed to 7.7 and 20 $\mu g\ As.l^{-1}$ for 15 days. As in laboratory cultures arsenate significantly affected growth rate, measured by total cell number (NOEC $<7.7\ \mu g\ As.l^{-1}$). The relative importance of different morphologic groups of phytoplankton changed greatly due to arsenate addition (Sanders and Vermersch, 1982). The survival of zooplankton on natural phytoplankton assemblages in which the composition was altered by arsenate dosing has been studied. Natural phytoplankton and zooplankton communities were used, with continuous, flow-through phytoplankton in the tanks. Two tanks were daily dosed with 15 $\mu g.l^{-1}$ arsenate to alter the phytoplankton community. By day 10 the arsenate treated phytoplankton assemblage was strongly dominated by *T. pseudonana*, whereas flagellates became increasingly important in control assemblages. After day 13 densities of nauplii and copepodites were slightly elevated. By days 16-24 zooplankton densities in control tanks were 2-3 times those in the arsenate dosed tanks (Sanders, 1986).

- Organic arsenic

No data could be found on the long-term toxicity of organic compounds to marine organisms.

Summary and conclusions "aquatic organisms"

- Biotransformation and bioavailability

The role of algae in the biotransformation is significant. Several studies indicate that arsenic can be taken up by the phosphate uptake system, because of its similarity to essential phosphate. In the presence of phosphate less arsenate is taken up. Algae are thought to convert inorganic arsenic into organic compounds which are less toxic. In higher trophic levels arsenic is mainly present in the organic form, predominantly (especially in marine organisms) arsenobetaïne.

From sediments relatively little arsenic is taken up into biota. The iron/arsenic ratio in sediments correlated more significantly with the arsenic levels in the organisms than did the arsenic concentration in the sediment alone.

- Bioconcentration/biomagnification

Arsenic is a natural component of aquatic systems and is bioaccumulated in small to moderate amounts. In organisms of lower trophic levels higher bioconcentration factors (BCF = concentration in organisms/ concentration in water) are found than in organisms of higher trophic levels. BCFs up to 400, 700 and 150 are reported for algae, invertebrates and fish, respectively. In one study the BCF was found to increase with a decreasing concentration of arsenic in the water. There are indications that the organic arsenic in higher trophic levels are taken up from by food. Biomagnification does not seem to occur.

- Toxicity

Freshwater organisms

Short-term toxicity tests with inorganic arsenic compounds have resulted in L(E)C50-values ranging from 206 μg As.l^{-1} for the crustacean *Bosmina longirostris* to 82,500 μg As.l^{-1} for the fish *Pimephales promelas*. The relevant long-term toxicity tests with freshwater organisms have resulted in NOE(L)C-values between 10 and 10,000 μg As.l^{-1}. The lowest and highest NOEC-values were found for the algae *Ankistrodesmus falcatus* and *Selenastrum capricornutum* in the same study, using identical testconditions. This wide variance in sensitivity of algae to arsenic has been found in several other studies as well. The toxicity of arsenate to algae decreases in the presence of phosphate, whereas the toxicity of arsenite seems to be independent of phosphate. Chronic exposure to about 1,000 μg As.l^{-1} as arsenic trioxide, arsenic pentoxide, sodium arsenite, disodium methylarsenate and sodium dimethylarsenate did not cause toxic effects in molluscs, crustaceans, insects and fish. In the presence of sediment the acute toxicity of arsenite to *Daphnia magna* decreased.

Based on both short- and long-term tests which were considered relevant a difference in toxicity between tri- and pentavalent inorganic arsenic could not be demonstrated. In addition the data on the toxicity of organic arsenic compounds are so limited that a comparison between toxicity of inorganic and organic arsenic is not possible. The data suggest that organic compounds, when expressed as the element arsenic, are more toxic in short-term tests.

Marine organisms

Data on the toxicity of arsenic to marine organisms are very limited and concern mostly inorganic arsenic compounds. The relevant short-term tests have resulted in L(E)C50-values ranging from 232 μg $As.l^{-1}$ for larval stages of the crustacean *Cancer magister* to 28,500 μg $As.l^{-1}$ for the adult fish *Limanda limanda*. In one study the effect of arsenate, arsenite and monosodium methanearsonate on the filtering rate of the mussel *Perna perna* was investigated; arsenite was found to be the most toxic compound to this mussel, arsenate the least. Effects of arsenic on the development of the sea urchin (at circa 1 mg $As.l^{-1}$) and on the mortality of several estuarine organisms (> 1 mg $As.l^{-1}$) were also reported.

Only one long-term study resulted in a NOEC-value; in a 14-d study a NOEC-value of 34.6 μg $As.l^{-1}$ was found for the macrophyte *Champia parvula*. Marine algae show, like freshwater algae, wide differences in sensitivity to arsenic. An effect of phosphate on the toxicity of arsenate (a decrease) was also found. The composition of natural phytoplankton assemblages (studied both in laboratory and outdoor) was found to be altered at 5 μg $As.l^{-1}$ from arsenate.

Table 2.2. Short-term "single species" toxicity tests - freshwater organisms

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result $\mu g\ As.l^{-1}$		Reference
Inorganic arsenic compounds											
Aschelminths											
Philodina roseola		S	$NaAsO_4 \times 7H_2O$	n.m.	-	-	96-h	LC50	**13,000**	[1]	Schaefer & Pipes '73
Molluscs											
Aplexa hypnorum	-	S	As_2O_3	lake	7.4/ 7.7	56	96-h	LC50	**18,560**		Holcombe et al. '83
Crustaceans											
Bosmina longirostris (24-h)	+	S	Na_2HAsO_4	s.w.w.	6.8	120	96-h	$EC50_i$	**206**	[2]	Passino & Novak '84
Ceriodaphnia dubia < 24-h old	+	R	$Na_2AsO_4 \times 7H_2O$	r.h.w.	8.1	165	48-h	$LC50^i$	**1,450**		Spehar & Fiandt '86
Daphnia magna	-	S	$NaAsO_2$	tap	8.1	40	26-h	$EC50_i$	3,000		Crosby & Tucker '66
Daphnia magna	-	R	Na_3AsO_4	lake	7.7	45	48-h	$LC50^i$	**7,400**		Biesinger & Christensen '72
Daphnia magna	+	S	$NaAsO_2$	a.w.w.	7.2/ 8.1	48	48-h	$EC50_i$	**1,500**	[3]	Lima et al. '84
Daphnia pulex (24-h)	+	S	Na_2HAsO_4	s.w.w.	6.8	120	48-h	$EC50_i$	**12,040**		Passino & Novak '84
Gammarus pseudolimnaeus	+	CF	$NaAsO_2$	a.w.w.	7.2/ 8.1	48	96-h	$EC50_i$	**874**		Lima et al. '84
Insects											
Tanytarsus dissimilis	-	S	As_2O_3	lake	7.2/ 7.7	37	48-h	LC50	**73,485**		Holcombe et al. '83
Fish											
Channa punctatus fingerlings	-	S	As_2O_3	tap	7.2	124	96-h	LC50	**10,830**	[4]	Shukla et al. '87
Colisa fasciatus fingerlings	-	S	As_2O_3	tap	7.1	-	96-h	LC50	**6,100**	[4]	Pandey & Shukla '82
Coregonus hoyi fry 15-19 d	+	S	As_2O_3	r.s.w	-	40-48	96-h	LC50	19,700	[5]	Passino & Kramer '80
Coregonus hoyi fry 22-d	+	S	As_2O_3	r.s.w.	-	40-48	96-h	LC50	**12,900**		Passino & Kramer '80
Esox masquinongy fry swim-up stage	+	S	$NaAsO_2$	tap	7.2/ 7.7	-	96-h	LC50	**1,100**		Spotila & Paladino '79
Esox masquinongy 5-w old	+	S	$NaAsO_2$	tap	7.2/ 7.7	-	96-h	LC50	**16,000**		Spotila & Paladino '79
Jordanella floridae fry	+	CF	$NaAsO_2$	a.w.w	7.2/ 8.1	48	96-h	LC50	**14,400**	[6]	lima et al. '84
Lepomis cyanellus	-	S	Na_3AsO_4	well	-	-	48-h	LC50	**54,000**		Sorensen '76
Pimephales promelas	+	S	As_2S_3	r.w	7.2/ 7.9	40-48	96-h	LC50	82,400	[7]	Curtis et al. '79
Pimephales promelas	+	CF	$NaAsO_2$	a.w.w.	7.2/ 8.1	48	96-h	LC50	**14,100**	[8]	Lima et al. '84
Pimephales promelas 30-d old	+	CF	$Na_2AsO_4 \times 7H_2O$	l.s.w.	7.4	44	96-h	LC50	**12,600**		Spehar & Fiandt '86
Organic arsenic compounds											
Ictalurus lacustris	-	S	MSMA	tap	-	-	96-h	LC50	1,412		Anderson et al. '75
Micropterus dolomieu fingerlings	-	S	MSMA	tap	-	-	96-h	LC50	417		Anderson et al. '75
Procumbaru spp.	-	S	MSMA	tap	-	-	96-h	LC50	509		Anderson et al. '75

i = immobility
n.m. = nutrient medium
r.h.w. = reconstituted hard water
l.s.w. = lake superior water
s.w.w. = softened well water
r.s.w. = reconstituted soft water
a.w.w. = adapted well water

[1] Test conducted at 20 ^{0}C. At temperatures of 15 and 25 ^{0}C the LC50's were 18,000 and 13,000 $\mu g.l^{-1}$, respectively.
[2] In the 96-h test with B. longirostris the organisms were once fed at 48-h
[3] EC50 for daphnia's fed during the 48-h test is 4,630 $\mu g.l^{-1}$.
[4] Test conducted following the procedures outlined in the Standard methods by the APHA.
[5] Concentrations tested were 0, 10 and 25 μg arsenate.l^{-1} (0, 3.6 and 9.0 μg As.l^{-1}) based on the salt.
[6] The $EC50_i$ was estimated to be 200 $\mu g.l^{-1}$ less than the LC50 for J. floridae.
[7] Arsenic trisulphide is unsoluble. It was administered as a non-wettable powder, which took about 48h to disperse.
[8] The $EC50_i$ was estimated to be the same as the LC50 for P promelas.

List of abbreviations tables 2.2. to 2.5.

A	+ Test substance analysed in test solution
	- Test substance not analysed in solution
> and ≥	Value indicated is highest concentration used in the test.
<	Value indicated is lowest concentration used in the test.
Test type	S: static; R: renewal: CF: continuous flow
Test time	h: hour(s); d:day(s); wk: week(s); m: month(s)
Criterion	LC50: Lethal concentration for 50% of the organisms exposed
	EC50: Effect concentration for 50% of the organisms exposed
	NOLC: No-observed-lethal-concentration
	NOEC: No-observed-effect-concentration

Table 2.3. Long-term "single species" toxicity tests with As - freshwater organisms

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result $\mu g\ As.l^{-1}$		Reference
Inorganic arsenic compounds											
Bacteria											
Pseudomonas putida		S	$Na_2HAsO_4 \times 7\ H_2O$	n.m	-	-	16-h	$NOEC_g$	**4,860**	[9]	Bringmann & Kuhn '80
Algae											
Ankistrodesmus falcatus		S	Na_2HAsO_4	n.m.	7.0	-	14-d	$NOEC_{g[c]}$	**10**	[10]	Vocke et al. '80
Asterionella formosa	+	CF	AsO_4	s.l.w.	8.2	-	24-d	$NOEC_g$	**86**	[11]	Conway '78
Micocoleus vaginatus		S	Na_2HAsO_4	n.m.	7.0	-	14-d	$NOEC_{g[c]}$	>100,000	[12]	Vocke et al. '80
Scenedesmus obliquus		S	Na_2HAsO_4	n.m.	7.0	-	14-d	$NOEC_{g[c]}$	<10		Vocke et al. '80
Scenedesmus quadricauda		S	Na_2HAsO_4	n.m.	-	-	7-d	$NOEC_{g[c]}$	**2,350**	[9]	Bringmann & Kuhn '80
Selenastrum capricornutum		S	Na_2HAsO_4	n.m.	7.0	-	14-d	$NOEC_{g[c]}$	**10,000**		Vocke et al. '80
Protozoa											
Entosiphon sulcatum	-	S	Na_2HAsO_4	n.m.	-	-	72-h	$NOEC_g$	**2,400**	[9]	Bringmann & Kuhn '80
Macrophytes											
Lemna paucicostata (6746)	-	S	As_2O_3	n.m.	4-7	-	7-d	$NOEC_g$	<1,000	[13]	Nasu & Kugimoto '81
Molluscs											
Helisoma campanulata and Stagnicola emarginata	+	CF	As_2O_3	lake	±7.0	42-45	28-d	NOLC	≥961	[14]	Spehar et al. '80
Helisoma campanulata and Stagnicola emarginata	+	CF	As_2O_5	lake	±7.0	42-45	28-d	NOLC	≥973		Spehar et al. '80
Crustaceans											
Cerodaphnia dubia < 24-d old	+	CF	$Na_2AsO_4 \times 7H_2O$	l.r.w.	8.2	100	7-d	$NOEC_{r,m,g}$	<1,140		Spehar & Fiandt '86
Cyclops & Diaptomus spp. naplii	-	S	$NaAsO_2$	n.m.	7.6/ 8.8	139	14-d	NOLC	920		Borgmann et al. '80
Daphnia magna	+	S	As_2O_3	lake	±7.0	42-45	14-d	$NOEC_{m,r}$	≥955		Spehar et al. '80
Daphnia magna	+	S	As_2O_5	lake	±7.0	42-45	14-d	$NOEC_{m,r}$	≥931		Spehar et al. '80
Daphnia magna – P≤1d -> F[lc]	-	R	$NaAsO_4$	lake	7.7	45	3-wk	$NOEC_{r,g,b}$	**520**	[15]	Biesinger & Christensen '72
Daphnia magna <24-h old	+	R	$NaAsO_2$	a.w.w.	7.2/ 8.1	48	28-d	$NOEC_{r,g}$	**633**		Lima et al. '84
Gammarus pseudolimnaeus	+	CF	As_2O_3	lake	±7.0	42-45	14-d	NOLC	≥88	[16]	Spehar et al. '80
Insects											
Pteronarcys dorsata larvae	+	CF	As_2O_3	lake	±7.0	42-45	28-d	NOLC	≥961		Spehar et al. '80
Pteronarcys dorsata larvae	+	CF	As_2O_5	lake	±7.0	42-45	28-d	NOLC	≥973		Spehar et al. '80
Fish											
Jordanella floridae egg/fry	+	CF	$NaAsO_2$	a.w.w.	7.2/ 8.1	48	31-d	$NOEC_{h,m,g}$	**2,130**	[17]	Lima et al. '84
Oncorhynchus kisutch parr-smolt transformation	-	CF	As_2O_3	n.m	8.2	69	6-m	$NOEC_{b,d}$	**76**	[18]	Nichols et al. '84
Pimephales promelas egg/fry	+	CF	$NaAsO_2$	a.w.w.	7.2-8.1	48	29-d	$NOEC_{h,m,g}$	**2,130**	[17]	Lima et al. '84
Pimehales promelas	+	CF	$Na_2AsO_4 \times 7H_2O$	l.s.w.	7.4	44	32-d	$NOEC_{g,r,m}$	<3,330		Spehar & Fiandt '86

Table 2.3. (continued)

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result µg As.l^{-1}		Reference
Salmo gairdneri juv. hatchery-reared	-	CF	As_2O_3	-	-	-	21-d	$NOEC_g$	<3,000	[19]	Speyer & Leduc '75
Salmo gairdneri	+	CF	As_2O_3	lake	±7.0	42-45	28-d	NOLC	≥961		Spehar et al. '80
Salmo gairdneri	+	CF	As_2O_3	lake	±7.0	42-45	28-d	NOLC	≥973		Spehar et al. '80
Organic arsenic compounds											
Molluscs											
Helisoma campanulata and Stagnicola emarginata	+	CF	DSMA	lake	±7.0	42-45	28-d	NOLC	≥970		Spehar et al. '80
Helisoma campanulata and Stagnicola emarginata	+	CF	SDMA	lake	±7.0	42-45	28-d	NOLC	≥846		Spehar et al. '80
Crustaceans											
Daphnia magna	+	S	DSMA	lake	±7.0	42-45	14-d	$NOEC_{m,r}$	≥833		Spehar et al. '80
Daphnia magna	+	S	SDMA	lake	±7.0	42-45	14-d	$NOEC_{m,r}$	≥1,112		Spehar et al. '80
Insects											
Pteronarcys dorsata larvae	+	CF	DSMA	lake	±7.0	42-45	28-d	NOLC	≥970		Spehar et al. '80
Pteronarcys dorsata larvae	+	CF	SDMA	lake	±7.0	42-45	28-d	NOLC	≥846		Spehar et al. '80
Fish											
Salmo gairdneri	+	CF	DSMA	lake	±7.0	42-45	28-d	NOLC	≥970		Spehar et al. '80
Salmo gairdneri	+	CF	SDMA	lake	±7.0	42-45	28-d	NOLC	≥846		Spehar et al. '80

b = biochemistry; d = development; g = growth (c: chlorophyll content of cells); m = mortality; r = reproduction; e = loss of equilibrium

n.m. = nutrient medium
l.r.w. = Lester river water
s.l.w. = synthethic lake water
DSMA = disodium methyl arsenate
SDMA = sodium dimethyl arsenate

List of abbreviations on p. 55

[9] Based on toxicity tresholds (NOEC = TGK/2)
[10] A modification of the EPA Algal Assay Procedure Bottle Test was used. 16:8 light:dark regime
[11] phosphate had no effect on toxicity of As(III) light:dark 16:8
[12] a significant stimulating respons was seen at 75 mg.l^{-1}.
[13] Different media were used with pH-values ranging from 4.1-5.1-6.1 and 7.1
[14] untreated lake Superior water was heated and than used
[15] The value given represents the 16%-reproductive impairment concentration. It is the minimal reproducible value below which the variability in reproduction could not be detected from controls.
[16] two concentrations tested; 88 and 961 ug As(III).l^{-1}. At the lowest concentration no decrease in survival was found. At 961 µg As(III).l^{-1} after 7 days a significant decreased survival to 20% was seen and by 14 days all amphipods were killed.
[17] given concentration has no effect on hatching, % hatch development, hatch survival, lenght and weight.
[18] concentration which is not affecting parr-smolt transformation of juvenile coho salmon. Migration is inhibited at this concentration (must be successful in order to survive and grow).
[19] based on wet and dry weight changes

Table 2.4. Short-term "single species" toxicity tests - marine organisms

Organism	A	Test-type	Test-subst.	Remark medium	Salinity o/oo	Exp.-time	Crite-rion	Result $\mu g\ As.l^{-1}$	Reference
Inorganic arsenic compounds									
Macroalgae									
Plumaria elegans sporelings	-	S	$NaAsO_2$	s.w	-	18-h	LC50	>580	[20] Boney et al. '59
Molluscs									
Crassostrea gigas embryo	+	S	As_2O_3	n.s.w.	34	48-h	$EC50_d$	**326**	Martin et al. '81
Crassostrea virginica embryo	-	S	$NaAsO_2$	s.s.w.	25	48-h	LC50	**7,500**	Calabrese et al. '73
Mytilus edulis embryo	+	S	As_2O_3	n.s.w.	34	48-h	$EC50_d$	**3,000**	Martin et al. '81
Crustaceans									
Argopecten irradians	-	R	$NaAsO_2$	n.s.w.	25	96-h	LC50	**3,490**	Nelson et al. '76
Cancer magister zoea I stage larvae	+	S	As_2O_3	n.s.w.	34	48-h	$EC50_d$	**232**	Martin et al. '81
Scylla serrata		S	As_2O_3	s.s.w.	-	96-h	LC50	**17,000**	Krishnaja et al. '87
Fish									
Chelon labrosus	+	CF	As_2O_3	d.s.w.	34.5	96-h	LC50	**27,300**	Taylor et al. '85
Limanda limanda	+	CF	As_2O_3	d.s.w.	34.5	96-h	LC50	**28,500**	Taylor et al. '85

d = development

n.m. = nutrient medium
d.s.w. = diluted seawater
s.s.w. = synthetic seawater

List of abbreviations on p. 55

[20] at 577 $\mu g.l^{-1}$ 0% of the sporelings died.

Table 2.5. Long-term "single species" toxicity tests with As - marine organisms

Organism	A	Test-type	Test-subst.	Test-water.	pH	Salinity o/oo	Exp.-time	Crite-rion	Result $\mu g\ As.l^{-1}$		Reference
Inorganic arsenic compounds											
Algae											
Chlamydomonas	-	S	Na_2HAsO_4	n.m.	-	-	25-d	NOEC	<75	[21]	Christensen & Zielski '80
Skeletonema costatum		S	AsO_4^{-3}	s.w.	-	-	8-d	$NOEC_g$	≥12.5		Sanders '79b
Skeletonema costatum		S	AsO_2^{-1}	s.w.	-	-	8-d	$NOEC_g$	≥20.3		Sanders '79b
Macrophytes											
Champia parvula		R	As(III)	n.m.	-	-	14-d	$NOEC_{g,r}$	34.6	[22]	Thursby & Steele '84
Champia parvula		R	As(V)	n.m.	-	-	14-d	$NOEC_g$	≥3,600	[23]	Thursby & Steele '84
Crustaceans											
Eurytemora affinis juvenile	-	R	AsO_4^{-3}	n.e.w	-	11	16-d	NOLC	≥18	[24]	Sanders '86
adult									≥36		
Eurytemora affinis (<2-d old naplii) life history test		R	AsO_4^{-3}	n.e.w.	-	-	26-33-d	$NOEC_{m,g,r}$	≥9.0	[25]	Sanders '86

g = growth, r = reproduction, m = mortality

n.m. = nutrient medium
n.e.w. = natural estuarine water
s.s.w. = synthetic seawater
d.s.w. = diluted seawater

List of abbreviations on p. 55

[21] Algal Assays Procedure Bottle Test (U.S. EPA, 1978) was used.
[22] phosphate had no effect on toxicity of As(III) light:dark 16:8
[23] arsenate toxicity was inhibited in the presence of phosphate light:dark 16:8
[24] concentration arsenic calculated on basis of $Na_3AsO_4.\ l^{-1}$
[25] concentrations tested were 0, 10 and 25 μg arsenate.l^{-1} (0, 3.6 and 9.0 $\mu g\ As.l^{-1}$), based on the salt.

3 ECOTOXICITY - TERRESTRIAL ORGANISMS

3.1 ACCUMULATION

The correlation between arsenic concentration in earthworm species and that in soil from 20 different, contaminated and uncontaminated, sites was determined. The arsenic concentrations ranged from 0.77 to 3.5 mg $As.kg^{-1}$ dry weight (dw) in the uncontaminated soils and from 1.4 to 33 mg $As.kg^{-1}$ dw in the contaminated soils. In general, arsenic levels in the earthworms from the uncontaminated soils were not elevated compared to those from contaminated soils. Earthworms collected from uncontaminated sites contained trace amount to 1.5 mg $As.kg^{-1}$ dw. The concentrations in earthworms from contaminated soils ranged from trace amounts to 0.81 mg $As.kg^{-1}$ dw. The correlation coefficient between As levels in worms and those in soils was found to be +0.45 ($p<0.05$). This value could have been strongly influenced by one high value; *Pheretima* sp. contained arsenic levels of 10 mg $As.kg^{-1}$ dw with a corresponding soil level of 20 mg $As.kg^{-1}$ dw. Only a third of the variation in the arsenic concentration in earthworms could be explained by the concentrations in the soil (Beyer and Cromartie, 1985).

3.2 TOXICITY

- Microbe-mediated processes

Soil acidification seemed to have an effect on the availability of metals and on the biological activity of the soil. A combination of a low pH with a heavy metal pollution increased the toxicity of the heavy metals, as measured by decomposition and mineralisation processes (Tyler, 1983).
The effect of several heavy metals (Pb, As, Cd and Cu) on micro-organisms in soils (not specified) near a secundary lead smelter was studied. Analyses showed that with a decreasing concentration of the heavy metals in the soil (samples to a depth of 10 cm) the population counts of bacteria, actinomyces, fungi, nematodes and earthworms increased. The arsenic concentration in the soil decreased from 972 to 163 mg $As.kg^{-1}$ at an increasing distance of 15 to 180 m from the smelter. The correlation

coefficients between bacteria, actinomyces, fungi, nematodes and soil levels of arsenic were significant (also for Pb and Cd) (Bisessar, 1982). The influence of various heavy metals, including arsenic, on the development and activity of soil micro-organisms were investigated (separately). The investigations were carried out in a sandy soil (pH=6.9, total C-content=1.8%) and an alluvial soil (pH=7.1, total C-content=1.1 %). Arsenic was applied to the soil at concentrations of 1,000, 5,000 and 10,000 As(V).kg^{-1} dm as $Na_2HAsO_4.7H_2O$ and at concentrations of 500, 1,500 and 5,000 mg As(V).kg^{-1} dm as As_2O_3. After treatment the soils were incubated for 6 months and then analysed. The dehydrogenase activity was inhibited at 500 mg As (III).kg^{-1} dm in both soils. The activity was inhibited 1,000 and 3,000 mg As(V).kg^{-1} dm in the alluvial and sandy soil, respectively. After 6 months of incubation the number of nitrifiers in the sandy soil was decreased at 500 mg As(III).kg^{-1} dm and at 5,000 mg As(V).kg^{-1} dm. In the alluvial soil this parameter was not affected after 6 months. However, after 2 months As(V) seemed to stimulate and As(III) seemed to inhibit the development of the nitrifiers in the alluvial soil. The development of bacteria was inhibited at 500 and 1,500 mg As(III).kg^{-1} dm in the sandy and alluvial soil, respectively. At 3,000 mg As(III).kg^{-1} dm the growth was greatly stimulated (in alluvial soil). The growth was stimulated at 5,000 and 10,000 mg As(V).kg^{-1} dm in the sandy and alluvial soil, respectively. The growth of actinomycetes was stimulated at all concentrations tested by both compounds. The growth of fungi was inhibited at all concentrations tested. Azotobacter were inhibited at all concentrations tested of As(III) and As(V). From this study it appears that for microbe-mediated processes the NOEC-values for As(III) and As(V) are below 500 and 1,000 mg As.kg^{-1} dm, respectively (Maliszewska et al., 1985).

- Other organisms

The influence of several inorganic compounds, including sodium arsenite and sodium arsenate, in solution on the hatching of eggs of several *Heterodera* species was studied. The hatching, measured by mean cumulative hatches per batch of cysts, was found to be inhibited by 300 mg As.l^{-1} as arsenite and 225 mg As.l^{-1} as arsenate. According to the classification of the authors the two substances were very active inhibitors (Clarke and Shepherd, 1966).

In one study the influence of spray programs on beneficial anthropods of apple orchards has been investigated. The sensitivity to lead arsenate varied widely among different predator and parasite species. Exposition in this study was variable for the different species (Macphee and Sanford, 1954).
In a field experiment the arsenic content of vegetables was examined 16 years after application of lead arsenate. From 1949 to 1953 lead arsenate was added to a sandy loam soil. In 1969 an arsenic concentration of 275.5 kg $As.ha^{-1}$ dm was measured (0-15 cm depth). Assuming a specific gravity of 1.5 $kg.l^{-1}$ soil, this corresponds to a concentration of 122 mg $As.kg^{-1}$ dm. The number of mites and collembolans were counted in soil samples of 0-12.7 cm depth. The number of collembolans was found to be greater in the treated soil than in the control soil. The number of mites was not changed (Chisholm and Macphee, 1972).

Summary and conclusions "terrestrial organisms"

- Accumulation

With regard to the accumulation of arsenic the data are limited to one study on earthworms. This study indicated that arsenic levels in several earthworm species from contaminated soils were not elevated compared to those in earthworms from uncontaminated soils. Based on these data the accumulation of arsenic in earthworms does not seem to be a problem.

- Toxicity

Data on the toxicity of arsenic to terrestrial organisms or processes are limited and mostly concern microbe-mediated processes. In one study the influence of As(III) and As(V) on the number of micro-organisms and on the activity of microbe-mediated processes was investigated in a sandy and in an alluvial soil. At the lowest concentrations tested (500 mg $As(III).kg^{-1}$ from arsenic trioxide and 1,000 mg $As(V).kg^{-1}$ from arsenate) effects were still seen. Therefore the NOEC-values for As(III) and As(V) were below 500 and 1,000 mg $As.kg^{-1}$ dm, respectively. Round a smelter the populations of

bacteria, actinomycetes, fungi and nematodes increased significant at an increasing distance from the smelter. The arsenic concentration in the soil decreased from 972-163 mg $As.kg^{-1}$. The concentration of several other metals in this soil decreased as well. The hatching of *Heteroderma* species was strongly inhibited in solutions containing 225 mg $As.l^{-1}$ as arsenite and 300 mg $As.l^{-1}$ as arsenate.

4 TOXICITY TO AGRICULTURAL CROPS AND LIVESTOCK

4.1 AGRICULTURAL CROPS

4.1.1 Accumulation

Arsenic levels in cereals, potatoes, vegetables, fruits, and the major fodder crops (grass, silage maize, and crowns and leaves of sugar beet) grown in The Netherlands and the arsenic concentration in the corresponding "normal" soils were measured. Levels in cereals (0.045-0.189 mg As.kg^{-1} fw), especially oats (0.189 mg As.kg^{-1} fw), were high compared with those of the other crops. Arsenic concentration (in mg As.kg^{-1} fw) were 0.013 in potatoes, 0.001-0.022 in vegetables and 0.004-0.014 in apples. The (mean) arsenic contents of grass, silage maize and (the crowns and leaves of) sugar beet were found to be 0.28, 0.17, and 0.51 mg As.kg^{-1} dm, respectively. The corresponding soils contained 12, 10, and 14 mg As.kg^{-1} dm, respectively (Wiersma et al., 1986). Arsenic levels in potatoes grown under "normal" conditions and in potatoes grown on (soils mixed with) municipal waste compost (MWC), sewage sludge or dregded materials from contaminated harbours were studied. Based on own experiments and data from literature potatoe (tuber) levels of 0.013 and 0.10 mg As.kg^{-1} fresh weight (fw) were considered normal and (too) high, respectively. A maximum permissible level for arsenic in potatoes of 0.10 mg As.kg^{-1} fw was proposed (see Criteria Document). Potatoes grown on a light sandy soil and a heavy fluvial clay soil containing 2 and 12 mg As.kg^{-1} dry matter (dm), respectively, contained about 0.004 mg As.kg^{-1} fw. Potatoes grown on mixtures of these soils with MWC in a soil/MWC ratio of 90/10 or on 100% MWC (containing 4 mg As.kg^{-1} dm) did not result in higher levels in the tubers. Arsenic levels in potatoe tubers grown on mixtures of the sandy and clay soil (with 2 and 12 mg As.kg^{-1} dm, respectively) with sewage sludge in a ratio soil/sludge: 90/10 (by volume) or on 100% sewage sludge (46 mg As.kg^{-1} dm) were 0.012 and 0.004 mg As.kg^{-1} fw for mixtures with the sandy and the clay soil, respectively, and 0.014 mg As.kg^{-1} fw for the sewage sludge. In potatoe tubers grown on sewage sludges from different sites (6-46 mg As.kg^{-1} dm) levels stayed below the (proposed maximum level of) 0.10 mg As.kg^{-1} fw. In an experiment using the same sandy soil mixed with (another) liquid sewage sludge (5 annual

applications of 22.5 t dm .ha^{-1}) the arsenic levels in tubers were 0.002-0.007 mg As.kg^{-1} fw. When contaminated fluvial river sediments were used with 35-69 mg As.kg^{-1} dm the potatoe tuber levels stayed also below the permissible level whereas the leaves of these potatoes contained about 0.16 mg As.kg^{-1} fw (De Haan and Lubbers, 1983). Several vegetable crops (broccoli, beet, cabbage, corn, green been, lettuce, potato, swiss chard and tomato) were grown in Matapeake silt loam soil treated with 100 mg As.kg^{-1} as arsenic acid. The compounds of arsenic in the vegetables were identified by different extraction methods and the amounts measured. The total arsenic contents of the edible parts of the plants ranged from trace amounts for cabbage and corn to 3 mg As.kg^{-1} dm for peeled potatoe tubers. Levels in lettuce, potatoe flesh and swiss chard were slightly higher than 'normal' levels (Pyles and Woolson, 1982).

In a field experiment (described in 3.2) the arsenic content of vegetables was examined 16 years after application of 122 mg As.kg^{-1} dm of lead-arsenate. In general, arsenic concentrations in crops grown on treated soil were higher than those in control crops, but the distribution pattern was similar. Highest levels (in mg As.kg^{-1} dm) were found in certain plant parts; the leaves of beans (4.58 treated versus 1.57 control), whole earial of hay (2.65 vs 1.31), the tops of onions (4.28 vs 0.08) and the roots of carrots (1.47 vs 0.29). Arsenic levels in grass, green beans and potatoes were a factor 2 to 3 higher in the treated crops compared to the control crops (Chisholm and Macphee, 1972).
In a historical mining area in Cornwall a study was carried out to a) establish the degree of contamination of the soil with arsenic and associated metals and to b) examine how much of the arsenic is taken up into edible parts of some commonly grown garden crops (lettuce, onion, beetroot, carrot, pea and bean). Total arsenic amounts in surface garden soils (0-15 cm depth) were found to be high; 144 to 892 mg As.kg^{-1}. Water soluble arsenic constitutes 0.1 to 0.5% of the total and acid fluoride extractable arsenic less than 5% of the total amount in these soils, both are significantly correlated with the total arsenic content. The soils studied were similar in texture; a sandy silt loam, a sandy loam and a silt loam (mean pH=7.4). Mean total arsenic concentrations in the crops (without lettuce) were found to range from 0.01 to 0.93 mg As.kg^{-1} dm. Lettuce

contained concentrations up to 3.88 mg As.kg^{-1} dm (mean 0.85). A positive relationship was found between arsenic levels of the edible parts of beetroot, lettuce, onion and pea and both extractable and total arsenic in soil. Arsenic in carrots was not significantly related to soil content. In the case of lettuce soil iron was demonstrated to reduce arsenic uptake by giving relatively low solubility products and by adsorption of arsenic to iron hydroxides). The effect of phosphorus (less arsenic taken up) may be due to the fact that phosphorus is preferred to arsenate by the phosphate uptake system. In spite of the large arsenic amounts in these soils all the vegetables examined were below the level of 1 mg.kg^{-1} fw. The authors conclude that plants act as 'geochemical barriers' in the environment and are only making a small contribution to man's exposure (Xu and Thornton, 1985).

To evaluate the effect of residual arsenic in soils on the yield, quality, and arsenic uptake by vegetable crops a field trial was carried out on Plainfield sand. The caracteristics of the soil used were; 0.7% carbon; 4% silt; 7% clay; pH 5.5 and 4.0 meq.100 g^{-1} cation exchange capacity. Sodium arsenite was added to the soil at amounts of 0, 45, 90, 180 and 720 kg.ha^{-1}. This equals concentrations of 0, 20, 40, 80 and 320 mg As.kg^{-1} dm (assuming s.g.=1.5 kg.l^{-1} soil). Arsenic was below detection limits (<0.02 mg As.kg^{-1} fw) in the edible portions of peas and sweet corns. Levels of the above-ground portions of potatoes showed no correlation with the arsenic content in the soil. External contamination by wind blown sand was probably the principal source of contamination for the above-ground portions. The concentrations in potatoe tubers and in snap beans increased with increasing concentrations in the soil. Snap bean (seeds and pods) contained up to 1.5 mg As.kg^{-1} fw. In potatoe tubers the peelings contained up to 83 mg As.kg^{-1} fw, whereas the peeled potatoe tubers contained only (maximal) 0.5 mg As.kg^{-1} fw (Jacobs et al., 1970).

Data on wild plants

In one study the uptake of arsenic by vegetation of the former Rhine estuary, containing 10 to 200 mg As.kg^{-1} dm, was studied. The roots of *Phragmites australis* and *Urtica dioica* contained 0.08-20 and 0.1-4.7 mg As.kg^{-1} dw, respectively. The root/soil accumulation factor was calculated to be 0.006-0.23 and 0.008-0.12 for *P. australis* and *U. dioica* ,

respectively (Otte et al., 1988). The mean concentrations of arsenic in the sporophores of the litter-inhabiting *Collybia peronata* from 30 beech woodland sites were compared with mean arsenic levels in litter. Mean arsenic concentration were 1.10 $mg.kg^{-1}$ dw in *C. peronata* and 0.19 $mg.kg^{-1}$ dw in beech leaf litter. The accumulation ratio was 5.8 (Tyler, 1982).

4.1.2. Toxicity

In a pot experiment the differences in phytotoxicity of several arsenic compounds to corn *Zea mays L.* were determined. Fe-, Al-, Na-, and $Ca(H_2AsO_4)$ were added to 300 g of Lakeland loamy sand at concentrations of 0.01, 0.1, 1.0, 10, or 100 mg $As.kg^{-1}$ dm. The soil contained 8 mg $As.kg^{-1}$ dm. Three corn plants were grown for four weeks. Adding up to 10 mg $As.kg^{-1}$ (total of 18 mg $As.kg^{-1}$) of all compounds caused no effects on the growth. An addition of 100 mg $As.kg^{-1}$ from NaH_2AsO_4, $Al(H_2AsO_4)_3$, and $Ca(H_2AsO_4)_2$ caused >50% growth reduction, whereas 100 mg $As.kg^{-1}$ from $Fe(H_2AsO_4)_3$ caused no (or only minor) growth reduction. The authors concluded that the toxicity of the arsenic compound differed with the availability of arsenic (or with the solubility of the compound). Low arsenic levels (5 mg $As.kg^{-1}$ extractable) were observed to have a positive effect on the growth (Woolson et al., 1971). Lettuce *(Lactuca sativa)* was used to evaluate two root elongation test methods with six substances, including monosodium methanearsonate. The chemical concentration which produced root lenght at 50% of the mean lenght of control for lettuce was about 60 $mg.l^{-1}$ in both test methods. A detailed description of the methods used is described by the authors (Ratsch and Johndro, 1986). In a pot experiment the toxicity levels of arsenic for soybeans were determined in two different soils. Vegetative soybean yields were significantly decreased at applied rates of 28 kg $As.ha^{-1}$ (corresponds to 12.4 mg $As.kg^{-1}$, assuming sg=1.5 $kg.l^{-1}$) in a fine sandy loam and 186 kg $As.ha^{-1}$ (82.8 mg $As.kg^{-1}$) in Houston Black clay. The water soluble As levels detrimental to soybeans were 3 and 12 mg $As.kg^{-1}$ in the sandy loam and the clay soil, respectively (Deuel and Swoboda, 1972).

In a field experiment (mentioned in 3.2) the effects of lead arsenate on crops grown on it were examined 16 years after application. A concentration

of 12.2 mg $As.kg^{-1}$ had no significant effect on crop yields during the period covered by this study (1959-1969). In one year (1966) a detrimental effect on beans was found but this was not repeated (Chisholm and Macphee, 1972). In a field study (mentioned in 4.1.1) the arsenic residue toxicity to vegetable crops was investigated. Sodium arsenite was added to the soil up to 320 mg $As.kg^{-1}$. Yields from the 80 and 320 mg $As.kg^{-1}$ treatments were significantly lower than check yields. The yields of potatoes and peas decreased lineair with increasing arsenic concentration in the soil. A small increase in yield was observed at the lowest concentration (20 mg $As.kg^{-1}$), which could be due to a greater availability of phosphate in the soil. The data indicate that with this soil marked yield reductions of peas, snap beans and sweet corn occur at 1 and 10 $mg.kg^{-1}$ NH_4OAc or Bray P-1 extractable (0.025 N HCl; 0.03 N H_4F) arsenic-values, respectively (Jacobs et al., 1970).

Data on wild plants

Among plants differences in sensitivity to arsenic have been observed. One study provided some evidence that arsenic tolerance in the plant *Holcus lanatus* is caused by an altered phosphate uptake system. The tolerance of six clones of *H. lanatus*, measured as root lenght, was determined. The differences in root lenght between tolerant, partially tolerant and non-tolerant were all statistically significant. At low levels of arsenate the high phosphate treatment reduced toxicity of arsenate in non-tolerance but not in tolerant ones. Tolerant plants took up less arsenate than nontolerant ones. The effect of phosphate on the toxicity of arsenate was only clear in nontolerant plants. The results suggest that arsenate is taken up by the phosphate uptake system in nontolerant plants, and that in tolerant plants this system had changed (Macnair and Cumbes, 1987).

In an outdoor experiment two plants *Phragmites autralis* and *Urtica dioica*, were grown on soil (80% garden soil, 20% sand) artificially contaminated with 0, 1, 5 or 30 mg $As.kg^{-1}$ dm as lead arsenate or sodium cacodylate $[(CH_3)_2AsO(ONa)]$. The experiment with *P. australis* lasted two months, that with *U. dioica* one month. The growth of *U. dioica* was significantly inhibited at 30 mg $As.kg^{-1}$ dw from both compounds. The growth of *P. australis* was not affected significantly. At added concentrations of 5 mg $As.kg^{-1}$ dm from both compounds no effects were seen. The difference could be

explained by interactions with the uptake of phosphate. *U. dioica* may be more sensitive to differences in phosphate availability (Otte et al., 1988).

Summary and conclusions "agricultural crops"

- Accumulation

Potatoes, vegetables and apples grown in the Netherlands on "normal" soils, containing an average of 10 mg $As.kg^{-1}$ dm, had arsenic levels varying between 0.001 and 0.014 mg $As.kg^{-1}$ fresh weight (fw). Cereals (especially oats) contained relative large amounts of arsenic (0.045-0.189 mg $As.kg^{-1}$ fw). The arsenic levels in the major fodder crops varied between 0.17 and 0.51 mg $As.kg^{-1}$ dm. In potatoes grown on (soils mixed with) various contaminated materials with arsenic concentrations up to 69 mg $As.kg^{-1}$ dm arsenic levels were found not to be elevated compared to levels which were considered normal (0.013 mg $As.kg^{-1}$ fw). The arsenic levels of crops grown on soil containing up to 892 mg $As.kg^{-1}$ varied from 0.01 to 0.93 mg $As.kg^{-1}$ dm. A positive relationship was found between the edible parts of beetroot, lettuce, onion and pea and arsenic levels in the soil. On the basis of these data it is concluded that the accumulation in agricultural crops is relatively low.

- Toxicity

From pot experiments it can be concluded that the chemical form of arsenic in the soil is essential to its toxicity. Adding 10 mg $As.kg^{-1}$ dm as Fe-, Al-, Na-, or Ca-arsenate to a sandy soil (containing 8 mg $As.kg^{-1}$ dm) caused no effects on the growth of corn. Adding 100 mg $As.kg^{-1}$ dm as Al-, Na- and Ca-arsenate caused more than a 50% growth reduction, whereas at 100 mg $As.kg^{-1}$ dm as $Fe(H_2AsO_4)_3$ no effects were seen. In another experiment an (calculated) added concentration of 12.4 mg $As.kg^{-1}$ caused a significant reduction in the growth of soybeans (arsenic content of the soil used not given). Added concentrations of 80 and 320 mg $As.kg^{-1}$ dm as lead arsenate and 122 mg $As.kg^{-1}$ as arsenite produced a significant reduction in yields.

In a field experiment with the wild plant *Urtica dioica* a significant growth inhibition was found at an added concentration of 30 mg As.kg^{-1} dw as lead arsenate or sodium cacodylate [$(CH_3)_2AsO(ONa)$]. The soil used consisted of 80% garden soil and 20% sand. The backgroundconcentration of arsenic of this soil was not given. At added concentrations of 5 mg As.kg^{-1} dw from both compounds no effects were seen.
Among plants some differences with regard to sensitivity to arsenic were observed. One study with a wild plant indicated that the phosphate uptake system of "tolerant" plants is altered (arsenate can no longer be taken up by this system).
In these experiments the highest concentration without effect, taking into account the backgroundconcentration of arsenic in the soil used, was circa 20 mg As.kg^{-1} dw. The lowest concentration with effect was reported to be 30 mg As.kg^{-1} (exclusive of the backgroundconcentration).

4.2 **LIVESTOCK**

4.2.1 Accumulation

Experiments were carried out to study the transfer of arsenic from feed into muscle, brain, liver and kidney tissues of fattening lambs. Ten week old lambs were fed arsenic as a soluble compound or such as present in sewage or harbour sludge for 3 months. Part of the lambs (32 out of 48) were kept indoors. The diets contained 1.6 and 2.6 mg As.kg^{-1} dry matter (dm) from arsenic trioxide (for lambs kept indoors and outdoors, respectively), 2.5 mg As.kg^{-1} dm from harbour sludge and 0.9 mg As.kg^{-1} dm from sewage sludge. Control diets contained 0.3 mg As.kg^{-1} dm. Arsenic levels in the various tissues were analysed after three months. Only the soluble arsenic compound and the arsenic from harbour sludge appeared to give some accumulation in muscle, liver and kidney tissues of the animals kept indoors. The tissues contained about 13, 24 and 50 μg As.kg^{-1} fw, compared to 2, 2 and 5 μg As.kg^{-1} fw in tissues of controls. For liver and kidney tissues a dose-dependent accumulation was found. No accumulation was found the brain (only one sample from each group) (Van der Veen and Vreman, 1986). A similar study was carried out to examine the transfer of arsenic

into milk and edible tissues of dairy cows. A group of grazing cows were given arsenic pentaoxide by wafers of concentrates for 3 months, with a total daily intake of 33 mg As. A group of cows kept indoors received arsenic trioxide, or concentrates of harbour or sewage sludge. The daily intake of these cows was 33, 21 and 6.8 mg As, respectively. The controls had an intake of 3.4 mg As. Levels of arsenic in milk and blood were not elevated. The highest arsenic levels in muscle, liver and kidney were found after administration of the soluble arsenic compounds (arsenic trioxide or arsenic pentaoxide); concentrations (in $\mu g\ As.kg^{-1}$ fw) in these tisues were 30, 100 and 160 compared to 5, 10 and 35 in control animals. Intermediate levels were found for administrations of harbour and sewage sludge (Vreman et al., 1986). The finding that arsenic is apparantly not excreted into cows milk is confirmed in other studies for both inorganic and organic arsenic compounds. Daily doses of 0.03-0.66 mg $As.kg^{-1}$ bw as arsenic acid given to cows for 8 weeks did not result in increased arsenic levels in the milk. Levels in milk did also not increase when the cows were fed methylarsonic acid and dimethylarinic acid (no quantitative data) (IPCS, 1981). Arsenic levels in milk from two cows fed arsanilic acid or 3-nitro-4-hydroxyphenylarsonic acid at concentrations of 1.6-3.2 mg $As.kg^{-1}$ bw for both compounds showed a slight increase (from 0.015 to 0.026 mg $As.kg^{-1}$) (NRC, 1980). In cattle with chronic arsenic poisoning the arsenic content in the milk ranges from 0.07 to 1.5 mg $As.kg^{-1}$, that in the liver from 7.0 to 70 $mg.kg^{-1}$ fw, and that in the kidney from 5 to 53 $mg.kg^{-1}$ (Vreman et al., 1986).

Cattle exposed to dietary monosodium dimethylarsinic acid or dimethyl arsinic acid showed increased arsenic levels in hair. Arsanilic acid concentrates in the liver and kidney of castrated rams and pigs. Injections of trivalent organoarsenic drug resulted in 50-100 times higher arsenic levels than after the same injections with pentavalent drug.

4.2.2. Toxicity

Arsenic has been described many times as a source of acute poisoning in domestic animals. Arsenic compounds were used (in the past) as pesticides, herbicides etc. The misuse of these compounds or the accidental exposure of animals to the compounds resulted frequently in toxicity (Ammerman, 1973).

The organic arsenic compounds which have been used as growth promotors, for example arsanilic acid, seem to be less toxic than inorganic arsenic (NRC, 1980).

A group of nearly 6,000 cattle fed for 1-2 days with a mixture containing arsenic trioxide at concentrations between 490 and 2,900 mg $As.kg^{-1}$ was studied. Of this group 1,464 animals died (50% within one week). Acute symptoms were drastic reduction in milk (85%), diarrhoea, dyspnoea, cyanosis, abortion and effects on the nervous system. Chronic exposure resulted in effects on skin and joints, blindness and pathological changes in internal organs (IPCS, 1981). Two adult cows died after ingestion of about 20 g of wood ashes, which contained high amounts of arsenic (780 mg $As.kg^{-1}$ wet weight), chromium and copper (Thatcher, 1985).

Sheep fed lakeweed with a daily dosis of 1.4 mg $As.kg^{-1}$ bw for 3 weeks did not show any adverse effects (IPSC, 1981). Horses and cattle could ingest arsenic up to concentrations of 2.66 and 4 mg $As.kg^{-1}$ bw daily, respectively, without any signs of toxicity. Lead arsenate at a level of 4.68 mg $As.kg^{-1}$ bw in cattle did not result in adverse effects. Potassium arsenite and arsanilic acid fed to sheep for 56 days at concentrations up to 285 mg $As.kg^{-1}$ diet (22.8 mg $As.kg^{-1}$ bw) produced no toxic effects, whereas a concentration of 570 mg $As.kg^{-1}$ diet (45.6 mg $As.kg^{-1}$ bw) resulted in effects as convulsions, weight loss and decreased feed consumption . Arsonic acid fed to sheep for 56 days in concentrations of 1,139 mg $As.kg^{-1}$ diet (91 mg $As.kg^{-1}$ bw) gave no toxic effects. In chickens a dose of 10 mg $As.kg^{-1}$ diet (1.8 mg $As.kg^{-1}$ bw) from arsenic(V)oxide for 56 days did not cause adverse effects. At 100 mg $As.kg^{-1}$ diet (18 mg $As.kg^{-1}$ bw) a decrease in body weight, feed intake and egg production was seen (NRC, 1980). Administration of 350 mg $As.kg^{-1}$ as arsanilic acid to pigs caused various toxic effects (IPCS, 1981). Administration of 1,000 mg $As.kg^{-1}$ diet to swines produced toxic effects after 18 days (NRC, 1980).

Data on chronic toxicity could not be found, but chronic administration of low arsenic levels seemed to have a positive effect on the hair of cattle. After withdrawal of the arsenic this improvement was lost, the animals lost weight and had inflamed eyes. The growth promotant level of arsanilic acid for swines is 100 mg $As.kg^{-1}$ diet (NRC, 1980).

Summary and conclusions "livestock"

- Accumulation

From one study, in which the transfer of several inorganic arsenic compounds from food into organs and tissues of cattle was examined, it appeared that only the water soluble compounds gave some accumulation. In lambs fed for 3 months a diet containing about 2 mg $As.kg^{-1}$ dm the arsenic levels in muscle-, liver- and kidney-tissues were elevated (with a factor 10) to 13, 24 and 50 μg $As.kg^{-1}$ fw, respectively. These tissues contained also the highest concentrations in cows given 33 mg As from arsenic trioxide daily for three months.
Several studies indicated that both inorganic and organic arsenic compounds were not excreted into cows milk. A daily intake of up to 33 mg As as arsenic trioxide did not cause elevated arsenic levels in the milk of cows. A slight elevation of arsenic levels occured after exposure to 1.6-3.2 mg $As.kg^{-1}$ bw from arsanilic acid or arsonic acid.

- Toxicity

Pesticides containing arsenic were frequently reported to be the cause of acute poisoning in domestic animals. Organic arsenicals, used as growth promotor seemed to be less toxic than inorganic arsenic compounds. Exposure to 490-2,000 mg $As.kg^{-1}$ diet from arsenic trioxide for 1-2 days caused more than 50% mortality in a group of more than 6,000 cattle. Toxic effects were similar to that found in experimental animals. After subacute exposure to 22.8 mg $As.kg^{-1}$ bw as arsenite and arsanilic acid no toxic effects were found in sheep, whereas 45.6 mg $As.kg^{-1}$ bw resulted in effects. After a daily intake of 5 mg $As.kg^{-1}$ bw during 3 weeks no adverse effects were seen in horses and cattle. In chickens toxic effects were observed after exposure to 18 mg $As.kg^{-1}$ bw for 60 days.
No data on chronic toxicity were available. Low levels of arsenic had a beneficial effect on the haircoat of animals.

5 RISK ASSESSMENT

5.1 RISK ASSESSMENT FOR MAN

Arsenic is considered to be a non essential element, and is therefore a contaminant.

Epidemiological studies have demonstrated positive dose-response relationships between exposure to inorganic arsenic compounds and cancer risk. Long-term oral intake of inorganic arsenic (through drinking water or medication) has been associated with an increased skin cancer risk. An increased risk on cancer of the respiratory tract has been found in many studies among populations exposed by inhalation. Although in most of these studies simultaneously exposure to other substances occurred, so it can not be excluded that the effects were due to other factors than arsenic exposure, it is concluded that inorganic arsenic can cause tumours in humans.

From experimental studies it appeared that inorganic arsenic is not carcinogenic in animals as far as exposure routes relavant for humans are concerned. A mechanistic study showed that both tri- and pentavalent arsenic have tumorpromoting activities.

Arsenic has an unusual genotoxic profile; in *in vitro* tests a clear induction of chromosome aberrations was observed, but not of gene mutations. Other mechanisms than direct DNA damage could be involved.

From metabolism studies it appeared that in both animals and humans inorganic arsenic is methylated to organic arsenic compounds; this process mainly takes place in the liver. At higher exposure levels this metabolism becomes saturated and this results in higher blood arsenic levels. Epidemiological studies demonstrated that the lung cancer risk correlated stronger with peak exposure than with cumulative exposure.

On the basis of all results (epidemiological studies, experimental studies, genotoxicity tests, metabolism studies) it is concluded that, with regard to the carcinogenic effect of arsenic, there are insufficient arguments to use a "non-threshold" extrapolation method for risk assessment.

It must be noted that the EPA, in contrast with the point of view that is formulated in this document, for risk assessment did apply a "non-threshold" extrapolation for both oral (EPA, 1987) and inhalatory (EPA, 1983) exposure. The EPA noticed that a linear extrapolation may overestimate the risks from low-level arsenic exposure. For oral exposure the WHO assumed the existence of a threshold (JECFA, 1983), but not for inhalatory exposure (WHO, 1987). For comparison the values that were calculated using a "non-threshold" extrapolation are also given.

In experimental studies no embryotoxic or teratogenic effects are reported without the occurrence of maternal toxicity. One study on the offspring of women working at a smelter indicates an increased frequency of spontaneous abortion and malformations in the offspring. The women were also exposed to other toxic substances, so no conclusions can be drawn on the role of arsenic.

The assessment of a toxicological limit value for arsenic will be based on epidemiological data because of the fact that appropriate long-term experimental studies are lacking for both oral and inhalatory exposure.

- Oral exposure

To assess a toxicological limit value the epidemiological studies, in which exposure took place through drinking water, are considered to be most relevant. On the basis of the effects observed in these studies it was, however, not possible to establish a dose without effect as a basis for a limit value.

On the basis of these drinking water studies (among others of Tseng, 1977 and Cebrian et al., 1983) the WHO (JECFA, 1983) concluded that "Human exposure to levels of arsenic below those which cause arsenicism do not appear do not appear to carry a carcinogenic risk". It has been derived that after long-term intake of drinking water containing 1 mg $As.l^{-1}$ toxic effects will be very likely to occur. After long-term consumption of water containing 0,1 mg $As.l^{-1}$ effects in groups "at risk" can not be ruled out. Taking into account a daily consumption of 2 l water and a mean body weight

of 70 kg* this corresponds with an intake of 2,0 μ As.kg^{-1} bw a day (JECFA, 1983).
In the present document the point of view of the WHO is adopted. However, it must be emphasized that an intake of 2,0 μg As.kg^{-1} bw per day may not be considered as an "acceptable daily intake" (as defined by the WHO) because effects can not be ruled out after long-term daily intake of this amount.

The WHO did not establish a limit value for organic arsenic compounds. Assuming that these compounds are at least a factor 10 less toxic than the inorganic ones, it is concluded that effects are unlikely after long-term daily intake of 20 μg As.kg^{-1} bw.

Using a "non-threshold" extrapolation and the data from the study of Tseng (1977) the "unit risk" [the lifetime risk due to 1 μg As.kg^{-1} bw of arsenic intake from water] was estimated to be circa 1,5 x 10^{-3} (1 and 2 x 10^{-3} for males and female, respectively) (EPA, 1987).
Assuming an acceptable risk of one extra case of skin cancer per million persons exposed lifetime, this risk corresponds with a daily intake of 6,7 x 10^{-4} μg As.kg^{-1} bw.

- Inhalatory exposure

On the basis of the occupational studies it was not possible to establish a usable dose-response relationship. In a number of studies "threshold values" of 90 or 500 μg As.m^{-3} are described. Exposure to peak concentrations also appeared to be of importance.
Toxic effects described among occupationally exposed persons include effects on peripheral bloodvessels and nervous system; slight toxic effects occurred at a concentration of about 50 μg As.m^{-3} (Lagerkvist et al., 1986, Blom et al., 1985). In these two studies it was, however, not possible to determine if the effects had to be described to present or past exposure, when it was significantly higher; up to 500 μg as.m^{-3}.

* The RIVM normally assumes a daily consumption of 1,5 l water and a mean body weight of 60 kg; this corresponds then to 2,5 μg As.kg^{-1} bw.

Assuming that at 5 μg As.m^{-3} these effects will not occur on the workplace and taking into account an extrapolationfactor of 10 (conform Gezondheidsraad, 1985) a concentration of 0.5 μg As.m^{-3} is proposed as toxicological "limit" value for the general population.

In the Air Quality Guidelines (WHO, 1987) a "unit risk" of 3.0 x 10^{-3} is proposed as "conservative" estimate of the lung cancer risk. Assuming an acceptable risk of one extra lung cancer case on million persons exposed for lifetime, this risk corresponds to an airborn concentration of 3.3 x 10^{-4} μg As.m^{-3} (0.3 ng As.m^{-3}).

5.2 RISK ASSESSMENT FOR THE ENVIRONMENT

5.2.1 Aquatic organisms

- Introduction

At present there are no generally accepted methods for extrapolation of the results of laboratory "single species" toxicity studies to natural ecosystems. Therefore different theoretical methods (see below) are used provisionally to calculate acceptable concentrations in fresh and sea water, at long-term exposure. In this risk assessment the use of these methods is in accordance with a proposal of a Dutch advisory board (Gezondheidsraad, 1988).
In the method according to Slooff et al. (1986), calculated concentrations are based on one toxicity value, either an L(E)C50 from a short-term test or an NOE(L)C from a long-term test. In both cases the value used in the calculation is the lowest value that is considered reliable. Using an L(E)C50 two different concentrations can be calculated, namely an NOEC for single species ($NOEC_{ss}$) and an NOEC for ecosystems ($NOEC_{eco}$). Using an NOEC, only an $NOEC_{eco}$ can be calculated. The values calculated must be divided bij an "uncertainty factor" which depends on the formula used. The final results are considered to be "safe" concentrations.
In the method according to Kooijman (1987) also different toxicity values can be used, namely short-term L(E)C50-values, long-term L(E)C50-values or

long-term NOEC-values. In this method all available data of a kind are used, for example all long-term NOEC-values. Using short-term L(E)C50-values or long-term L(E)C50-values, a HCS ("hazardous concentration for sensitive species") is calculated; at these concentrations there is a probability (for example 10%, arbitrary) that up to 50% of individuals of the most sensitive species will die at short-term and long-term exposure, respectively. At the HCS calculated from long-term NOEC-values, there is a probability that adverse non-lethal effects will occur in up to 50% of individuals of the most sensitive species.

The method according to van Straalen (1987) is similar to that according to Kooijman. In the method of van Straalen all long-term NOEC-values are used, resulting in an HC5 ("hazardous concentration for 5% of the species"). At the HC5 there is a probability of 5% that adverse non-lethal effects will occur in up to 5% of the species exposed. The values of 5% also are arbitrary chosen. The HC5 is considered to be a "threshold" value ("limit" value).

The results of these methods of extrapolation are presented in table 5.1. for both fresh water and seawater. The L(E)C50-values and NOEC-values used in the methods according to van Straalen (1987) and Kooijman (1987) are printed **bold** in the tables 2.2, 2.3, 2.4 and 2.5 in chapter 2 of the present document. In cases of the presence of two or more NOEC-values for one single species, only one value has been used. The "lowest" L(E)C50-values and NOEC-values used in the method according to Slooff et al. (1986) are underlined in these tables. The values used were selected on the basis of both test procedure (reliability, test medium, exposure time, effect-parameters) and representative value for the species involved. Therefore, in all cases only values from primary literature sources have been used in the methods of extrapolation. The distribution of NOEC-values for freshwater organisms is shown in figure 5.1. The only NOEC-value for seawater organisms has been marked by a cross.

Data for the organic arsenic compounds were much too limited to be used in assessing "limit" values.

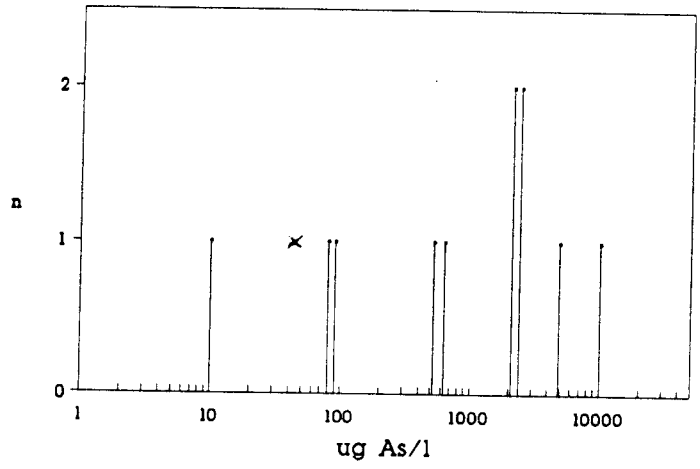

Figure 5.1. Distribution of NOEC-values of inorganic arsenic in fresh water.

Fresh water

Based on the data available it is concluded that there is no relevant difference in toxicity between tri- and pentavalent inorganic arsenic to freshwater organisms. Therefore one "limit" value for arsenic is proposed, which applies to the sum of tri- and pentavalent inorganic arsenic.
Because sufficient long-term NOEC-values for arsenic are available the "limit" value will be based on these data. Using the method according to van Straalen (1987) a "limit" value of 6 $\mu g\ As.l^{-1}$ has been calculated for fresh water.
Using the method according to Slooff et al. (1986) on the basis of the lowest NOEC-value of 10 $\mu g\ As.l^{-1}$ an $NOEC_{eco}$ of 30,2 $\mu g\ As.l^{-1}$ has been calculated. If this value is divided by the UF of 33,5 a "safe" concentration of 0,9 $\mu g\ As.l^{-1}$ is obtained for fresh water.
The difference between the values calculated using the methods according to van Straalen and Slooff et al. is smaller than a factor 10. Therefore, in accordance with the proposal of the "Gezondheidsraad" (1988), the value calculated with the method according to van Straalen is recommended as toxicological "limit" value. This value is based on "total"-arsenic concentrations ("dissolved"- plus "particulate"-arsenic) in the test waters used. However, because of the differences between test waters used

and surface waters (especially with regard to the difference in particulate matter content), it is assumed that in most tests arsenic was present as "dissolved"-arsenic. For this reason the concentration of 6 μg As.l^{-1} is considered to be "dissolved"-arsenic (< 0.45 μm).

Based on L(E)C50-values from short-term tests a HCS of 0.5 μg As.l^{-1} has been calculated using the method according to Kooyman (1987). Using the method according to Slooff et al. (1986) on the basis of the lowest L(E)C50-value an $NOEC_{ss}$ and $NOEC_{eco}$ of 32.7 and 68.0 μg As.l^{-1}, respectively, have been calculated. Dividing these values by the UF results in "safe" concentrations of 1.3 and 0.8 μg As.l^{-1}, respectively.

Seawater

Data on the toxicity of inorganic arsenic to seawater organisms are limited, especially with regard to chronic toxicity. Because only one NOEC-value from long-term tests is available, the method according to van Straalen can not be used for risk assessment. Using the method according to Slooff et al., based on the lowest L(E)C50-value from short-term tests, a $NOEC_{ss}$ and $NOEC_{eco}$ of 9.3 and 23.3 μg As.l^{-1} , respectively, have been calculated. By dividing these values by the UF's the "safe" concentrations of 0.36 and 0.27 μg As.l^{-1} for single species and ecosystems, respectively, were obtained. Using the method according to Kooyman (1987) on the available L(E)C50-values results in a HCS of 0.0095 μg As.l^{-1}. On the basis of the lowest NOEC-value a $NOEC_{eco}$ of 86.7 μg As.l^{-1} has been calculated, using the method according to Slooff et al. The "safe" value for seawater organisms is found when this value is divided by the UF; this results in a concentration of 2.6 μg As.l^{-1}.

It is not possible to compare the data on seawater organisms with those on freshwater organisms, because the dataset on seawater is too limited. However, there does not seem to be a consistent difference: the lowest relevant L(E)C50-value for freshwater organisms is higher (factor 3) than for seawater organisms, whereas the lowest NOEC-value for fresh water is lower (factor 3) than for seawater. Therefore, the "limit" value proposed for fresh water is provisionally recommended for seawater as well; 6 μg As.l^{-1} "dissolved"-inorganic arsenic.

Tabel 5.1. Calculated concentrations (in µg As.l^{-1}) of inorganic arsenic in fresh water and seawater based on the methods of extrapolation according to Slooff et al. (1986), Van Straalen (1987) en Kooijman (1987)

		fresh water	seawater
Lowest relavant L(E)C50 *(short-term tests)*		*874*	*232*
Slooff et al.:	$NOEC_{ss}$: UF	*32,7 : 25,6 =* ***1,3***	*9,3 : 25,6 =* ***0,36***
	$NOEC_{eco}$: UF	*68,0 : 85,7 =* ***0,8***	*23,3 : 85,7 =* ***0,27***
L(E)C50-values *(short-term tests)*		+	+
Kooijman (1)	*HCS*	*0,5*	*0,0095*
Lowest relevant NOEC *(long-term tests)*		*10*	*34,6*
Slooff et al.:	$NOEC_{eco}$: UF	*30,2 : 33,5 =* ***0,9***	*86,7 : 33,5 =* ***2,6***
NOEC-values *(long-term tests)*		+	-
Van Straalen (2) :	*HC5*	***6***	-

+/- : sufficient or insufficient data, respectively
UF :"Uncertainty factor"
[1] Dm from table 1 in Kooijman (1987) at d = 0.1; theorethical number of species in the ecosystem ("n") is 1000.
[2] Dm from table 1 in Kooijman (1987) at d = 0.05.

5.2.2 Terrestrial organisms

The dataset on accumulation in, and toxicity to terrestrial organisms is too limited to be used in assessing a "limit" value.
With regard to the toxicity of arsenic to plants two experiments, in which arsenic was added as water soluble compounds, were considered to be usable. In the first experiment an added concentration of 30 mg As.kg^{-1} dm as lead arsenate or sodium cacodylate [$(CH_3)_2AsO(ONa)$] caused a significant reduction in growth of the wild plant *Urtica dioica* (Otte et al., 1988). In this experiment the backgroundconcentration of the soil used was not given. In the second study no effects were found at circa 20 mg As.kg^{-1} dm, consisting of a backgroundconcentration of 10 mg As.kg^{-1} dm and an added concentration of 8 mg As.kg^{-1} dm from Fe-, Al-, Na-, and Ca-arsenate. At an added concentration of 100 mg As.kg^{-1} dm from Al-, Na-, and Ca-arsenate more than 50% growth inhibition was found. When arsenic was added as Fe-arsenate, at an added concentration of 100 mg As.kg^{-1} dm no or only a slight effect was observed (Woolson et al., 1971). These effect concentrations are significant lower than the lowest tested concentrations in terrestrial organisms.
Only one study was available on the toxicity of arsenic to terrestrial organisms and this study was limited to microbe-mediated processes. At the lowest concentration tested, which were 500 mg As.kg^{-1} from arsenic trioxide and 1000 mg As.kg^{-1} from arsenate, effects were still seen on the number of micro-organisms and on the activity of microbe-mediated processes in a sandy and an alluvial soil (Maliszewski et al., 1985).
On the basis of these data higher plants seem to be the most sensitive to arsenic. It appears that the highest concentration without effect is 20 mg As.kg^{-1} dm (including the backgroundconcentration) and the lowest concentration with effect is 30 mg As.kg^{-1} dm, exclusive of backgroudconcentration.

Because of the fact that accumulation of arsenic in agricultural crops is relatively low and the fact that the toxicity of arsenic to livestock is also low, it is concluded that exposure to a concentration which causes no effect in higher plants will not cause adverse effects on humans and animals.

REFERENCES

REFERENCES CHAPTER 1 : HUMAN TOXICITY

Baker, R.S.U. (1985)
Evaluation of metals in *in vitro* assays, interpretation of data and possible mechanisms of action
In: E. Merian et al. (eds.), Carcinogenic and Mutagenic Metal Compounds, Environmental and Analytical Chemistry and Biological Effects, 186-206, Gordon and Breach Science Publishers, New York

Baxley, M.N. et al. (1981)
Prenatal toxicity of orally administered sodium arsenite in mice
Bull. Environ. Contam. Toxicol. 26, 749-756

Blom, S. et al. (1985)
Arsenic Exposure to Smelter Workers
Scand. J. Work Environ. Health 11, 265-269

Buchet, J.P. and R. Lauwerys (1985)
Study of inorganic arsenic methylation by rat liver in vitro: Relevance for the interpretation of observations in man
Arch. Toxicol. 57, 125-129

Byron, W.R. et al. (1967)
pathologic changes in rats and dogs from two-year feeding of sodium arsenite or sodium arsenate
Toxicol. Appl. Pharmacol. 10, 132-147

Cebrian, M.E. et al. (1983)
Chronic Arsenic Poisoning in the North of Mexico
Human Toxicol. 2, 121-133

Chen, C.-J. et al. (1985)
Malignant neoplasms among residents of a blackfoot disease-endemic area in Taiwan: High-arsenic artesian well water and cancers
Cancer Res. 45, 5895-5899

Chen, C.-J. et al. (1986)
A retrospective study on malignant neoplasms of bladder, lung and liver in blackfoot disease endemic area in Taiwan
Br. J. Cancer 53, 399-405

Coddington, K. (1986)
A review of arsenicals in biology
Toxicol. Environ. Chem. 2, 281-290

Crossen, P.E. (1983)
Arsenic and SCE in human lymphocytes
Mut. res. 119, 415-419

Cuzick, J. et al. (1982)
Medicinal arsenic and internal malignancies
Br. J. Cancer 45, 904-911

EPA (1983)
Health assessment document for inorganic arsenic - Review draft
United States Environmental Protection Agency, EPA 600/8-83/021 A
Environmetal Criteria and Assessment Office, Research Triangle Park
N.C. 27711

EPA (1987)
Special Report on ingested inorganic arsenic: Skin cancer; nutritional essentiality - SAB Review draft
United States Environmental Protection Agency, EPA 625/3-87/013 A,
Prepared for the Risk Assessment Forum U.S. EPA, Washington, D.C.

EPA (1987b)
Carcinogenic effects of arsenic in drinking water
EPA/600/1-87/007, U.S. EPA, National Technical Information
Service (NTIS)

Fierz, U. (1965)
Katamnestische Untersuchungen über die Nebenwirkungen der Therapie mit anorganischem Arsen bei Hautkrankheiten
Dermatologica 131, 41-58

Friberg, L. et al. (1986)
Handbook on the toxicology of metals. Second edition, vol II:
Specific Metals, Elsevier, New York - Oxford

Fuortes, L. (1988)
Arsenic poisoning, ongoing diagnostic and social proplem
Postgrad. Med. 83 (1), 233-244

Glaser, U. et al. (1986)
Long-term inhalation studies with NiO and As_2O_3 aerosols in Wistar rats
Int. Congress Ser. Exerpta Med., 325-328

Guha Mazumder, D.N. et al. (1988)
Chronic arsenic toxicity from drinking tubewell water in rural West Bengal
Bull. of the WHO 66 (4), 499-506

Hisanaga, A. (1982)
Chronic toxicity of arsenous acid in rats with special reference to the dose-response
Fukuoka Acta Med. 73 (1), 46-63

Hood, R.D. et al. (1978)
Prenatal effects of oral versus intraperitoneal sodium arsenate in mice
J. Environ. Pathol. Toxicol. 1, 857-864

Hood, R.D. and S.L. Bishop (1972)
Teratogenic effects of sodium arsenate in mice
Arch. Environ. Health 24, 62-65

Hood, R.D. and W.P. Harrison (1982)
Effects of prenatal arsenite exposure in the Hamster
Bull. Environ. Contam. Toxicol. 29, 671-678

IARC (1980)
IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Volume 23: Some metals and metallic compounds, 39-141, International Agency for Research on Cancer, WHO, Lyon

IARC (1987)
IARC Monographs, Overall evaluations of the carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42.
Supplement 7, WHO, Lyon

IPCS (1981)
Environmental Health Criteria 18: Arsenic
IPCS International Programme on Chemical Safety, World Health Organization, Geneva

Ishinishi, N. et al. (1977)
Preliminary experimental study on carcinogenicity of arsenic trioxide in rat lung
Environ. Health Perspect. 19, 191-196

Ishinishi, N., T. Tomita and A. Hisanaga (1980)
Study on chronic toxicity of arsenic trioxide in rats with special reference to the liver damages
Fukuoka Acta Med. 71 (1), 27-40

Ishinishi, N. et al. (1983)
Tumorigenicity of arsenic trioxide of the lung in syrian golden hamsters by intermittent Instillations
Cancer Lett. 21, 141-147

Jacobson-Kram, D. and D. Montalbano (1985)
The Reproductive Effects Assessment Group's Report on the Mutagenicity of Inorganic Arsenic
Environ. Mutagen. 7, 787-804

JECFA (1983)
Toxicological evaluation of certain food additives and food contaminants, twenty-seventh report of the Joint FAO/WHO expert Committee on Food Additives, WHO Food Additives Series 18, 176-202, Geneva (Wld Hlth Org. techn. Rep. Ser., 696)

Jongen, W.M.F. et al. (1985)
Genotoxicity testing of arsenobetaine, the predominant form of arsenic in marine fishery products
Fd. Chem. Toxicol. 23 (7), 669-673

Jung, E.G. and B. Trachsel (1970)
Molekularbiologische Untersuchungen zur Arsencarcinogenese
Arch. kli. exp. derm. 237, 819-826

Kay, E.R.M. (1965)
Incorporation of radioarsenate into proteins and nucleic acids of the Ehrlich lettre ascites carcinoma in nitro
Nature 206, 371-373

Kreiss, K. et al. (1983)
Neurological evaluation of a population exposed to arsenic in Alaskan well water
Arch. Environm. Health 38 (2), 116-121

Lagerkvist, B. et al. (1984)
Vasospastic tendency and Raynaud's phenomenon in smelter workers exposed to arsenic
Environ. Res. 39, 465-474

Lamm, S.H. and W.H. Lederer (1984)
A possible carcinogenic threshold for arsenic exposure
Third Conference on Epidemiology in Occupational Health, Singapore, sept. 28-30, 1983.
Scand. J. Work Environ. Health 10 (2), 128 (abstract)

Lee, T.-C. et al. (1988)
Induction of gene amplification by arsenic
Science 241, 79-81

Lee, A.M. and J.F. Fraumeni (1969)
Arsenic and respiratory cancer in man : an occupational study
J. Natl. Cancer Inst. 42, 1045-1052

Lee-Feldstein, A. (1983)
Arsenic and respiratory cancer in humans: Follow-up of copper smelter employees in Montana
JNCI 70 (5), 601-610

Lee-Feldstein, A. (1986)
Cumulative exposure to arsenic and its relationship to respiratory cancer among copper smelter employees
J. Occup. Med. 28 (4), 296-302

Leonard, A. (1984)
Recent advances in arsenic mutagenesis and carcinogenesis
Toxicol. Environ. Chem. 7, 241-250

Lugo, G. et al. (1969)
Acute maternal arsenic intoxication with neonatal death
Am. J. Dis. Child 117, 328-330

Morton, W. et al. (1976)
Skin cancer and water arsenic in Lane County, Oregon
Cancer 37, 2523-2532

Nagymajtenyi, L. et al. (1985)
Chromosomal aberrations and fetotoxic effects of atmospheric arsenic exposure in mice
J. Appl. Toxicol. 5 (2), 61-63

Nishioka, H. (1975)
Mutagenic activities of metal compounds in bacteria
Mut. Res. 31, 185-189

Nordenson, I., A. sweins and L. Beckman (1981)
Chromosome aberrations in cultured human lymphocytes exposed to trivalent and pentavalent asenic
Scand. J. Work Environ. Health 7, 277-281

Nordström, S. et al. (1978)
Occupational and environmental risks in and around a smelter in northern Sweden, III. Frequencies of spontaneous abortion
Hereditas 88, 51-54

Nordström, S. et al. (1979)
Occupational and Environmental Risks in and around a Smelter in Northern Sweden, VI. Congenital Malformtions
Hereditas 90, 297-302

Ott, M.G. et al. (1974)
Respiratory cancer and occupational exposure to arsenicals
Arch. Environ. Health 29, 250-255

Pershagen, P. (1981)
The carcinogenicity of arsenic
Environ. Health Perspect. 40, 93-100

Pershagen, P. and N.-E. Bjorklund (1985)
On the pulmonary Tumorigenicity of Arsenic Trisulfide and Calcium Arsenate in Hamsters
Cancer Lett. 27, 99-104

Pinto, S.S. et al. (1977)
Mortality experience in relation to a measured arsenic trioxide Exposure
Environ. Health Perspect. 19, 127-130

Pinto, S.S. et al. (1978)
Mortality experience of arsenic-exposed workers
Arch. Environ. Health 33, 325-332

Poma, K. et al. (1981)
Cytogenetic analysis of bone marrow cells and spermatogonia of male mice after in vivo treatment with arsenic
Experienta 37, 129-130

Rossman, T.G. (1981)
Enhancement of UV-mutagenesis by low concentrations of arsenite in E. coli
Mutation Res. 91, 207-211

Rossman, T.G. et al. (1980)
Absence of arsenite mutagenicity in E. coli and chinese hamster cells
Environm. Mut. 2, 371-379

Rossman, T.G., M.S. Meyn and W. Troll (1977)
Effects of arsenite on DNA Repair in Escherichia coli
Environ. Health Perspect. 19, 229-233

Sobel, W. et al. (1988)
An update of respiratory cancer and occupational exposure to arsenicals
Am. J. Ind. Med. 13, 263-270

Tseng, W.-P. (1977)
Effects and dose - response relationships of skin cancer and Blackfoot disease with arsenic
Environ. Health Perspect. 19, 109-119

Valentine, J.L., H.K. Kang and G. Spivey (1979)
Arsenic levels in human blood, urine, and hair in response to exposure via drinking water
Environ. Res. 20, 24-32

Welch, K. et al. (1982)
Arsenic exposure, smoking, and respiratory cancer in copper smelter workers.
Arch. Environm. Health 37 (6), 325-335.

WGD (1984)
Rapport inzake grenswaarde Anorganische arseenverbindingen (exclusief arseenwaterstof)
Werkgroep van deskundigen van de Nationale MAC-Commmissie, No. 2/84
Voorburg

WHO (1987)
Air Quality Guidelines for Europe
WHO Regional Publications, European Series No. 23, 171-181
World Health Organization, Regional office for Europe, Copenhagen

Wibowo, A.A.E. et al. (1982)
De inzichtelijke opbouw van advieswaarden voor de niet-mutagene, niet-carcinogene en niet-immunotoxische stoffen: Arsenicum, Werkstuk t.b.v. Commissie van de Gezondheidsraad inzake uitgangspunten voor normstelling. Amsterdam.

Wicklund, K.G. et al. (1988)
Respiratory cancer among orchadists in Washington State, 1968 to 1980
J. Occup. Med. 30 (7), 561-564

Winship, K.A. (1984)
Toxicity of inorganic arsenic salts
Adv.Drug React. Ad. Pois. Rev. 3, 129-160

Yu, H.-S. et al. (1984)
Studies on Blackfoot disease and chronic arsenism in Southern Taiwan, with special reference to skin lesions and fluorescent substances
J. Dermatol. 11, 361-370

REFERENCES CHAPTER 2, 3 AND 4 : ECOTOXICOLOGY AND TOXICITY TO AGRICULTURAL CROPS AND LIVESTOCK

Ammerman, C.B. et al. (1973)
Toxicity of certain minerals to domestid animals: A review
Animal Science Research report AL73-6, 17-18

Anderson, A.C. et al. (1975)
The acute toxicity of MSMA to black bass *(Micropterus dolomieu)*, crayfish *(Procambarua sp.)* andchannel catfish *(Ictalurus lacustris)* .
Bull. Environm. Contam. Toxicol. 14 (3), 330-333.

Beyer, W.N. and J. Cromartie (1987)
A survey of Pb, Cu, Zn, Cd, Cr, As, and Se in earthworms and soil from diverse sites
Environ. Monit. Assess. 8, 27-36

Biesinger, K.E. and G.M. Christensen (1972)
Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*.
J. Fish. Res. Board Can. 29 (12), 1691-1700.

Bisessar, S. (1982)
Effect of heavy metals on microorganisms in soils near a secondary lead smelter
water, Air and soil pollut. 17, 305-308

Boney, A.D. et al. (1959)
The effects of various poisons on the growth and variability of sporelings of the red alga *Plumaria elegans* (Bonnem.) Schm.
Biochem. Pharmacol. 2, 37-49.

Borgmann, U. et al. (1980)
Effect of metals on the biomass production kinetics of freshwater copepods.
Can. J. Fish. Aquat. Sci. 37, 567-575.

Bringmann, G. and R. Kuhn (1980)
Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test.
Water Res. 14, 231-241.

Bryan, G.W., W.J. Langston and L.G. Hummerstone (1980)
The use of biological indicators of heavy metal contamination in estuaries, with special reference to an assessment of the biological availability of metals in estuarine sediments from south-west Britain.
Marine Biological Association of the U.K., Occasional Publ. no 1.

Bryant, V. et al. (1985)
Effect of temperature and salinity on the toxicity of arsenic to three estuarine invertebrates *(Corophium volutator, Macoma balthica, Tubifex costatus)*.
Mar. Ecol. Prog. Ser. 24, 129-137.

Burton, G.A. et al. (1987)
Arsenic toxicity changes in the presence of sediment.
Bull. Environm. Contam. Toxicol. 38, 491-499.

Calabrese, A. et al. (1973)
The toxicity of heavy metals to embryos of the american oyster *Crassostrea virginica*
Marine Biology 18, 162-166.

Canton, J.H. et al. (1987)
Onderzoek naar de modelijke effecten van de in het IMP-gestelde Basiskwaliteit op een tweetal zoetwaterorganismen,
Rapportnr. 718622 001, RIVM

Chisholm, D. and A.W. MacPhee (1972)
Persistence and Effects of Some Pesticides in Soil
J. Econom. Entol. 65 (4), 1010-1013

Christensen, E.R. and P.A. Zielski (1980)
Toxicity of arsenic and PCB to a green alga *(Chlamydomonas)*.
Bull. Environm. Contam. Toxicol. 25, 43-48.

Clarke, A.J. and A.M. Sheperd (1966)
Inorganic ions and the hatching of Heterodera spp.
Ann. appl. Biol. 58, 497-508

Cockell, K.A. and J.W. Hilton (1988)
Preliminary investigations on the comparative chronic toxicity of four dietary arsenicals to juvenile rainbow trout *(Salmo gairdneri* R.).
Aquat. Toxicol. 12, 73-82.

Conway, H.L. (1978)
Sorption of arsenic and cadmium and their effect on the grwoth, micronutrient utilization, and photosynthetic pigment composition of *Asterionella formosa*.
J. Fish. Res. Board. Can. 35, 286-294.

Crosby, D.G. and R.K. Thucker (1966)
Toxicity of aquatic herbicides to Daphnia magna.
Science 154, 289-291.

Curtis, M.W. et al. (1979)
Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms.
Water Res. 13, 137-141.

Dabrowski, K.R. (1976)
The effect of arsenic on embrional development of rainbow trout *(Salmo gairdneri)*, RICH.).
Water Res. 10, 793-796.

De Haan, J. and J. Lubbers (1983)
Microelements in potatoes under 'normal' conditions, and as affected by microelements in municipal waste compost, sewage sludge, and dredged materials from harbours, Rapport 3-83, Instituut voor bodemvruchtbaarheid, Haren (Gr), The Netherlands

Deuel, L.E. and A.R. Swoboda (1972)
Arsenic toxicity to cotton and soybeans
J. Environ. Quality 1 (3), 317-320

Edmonds, J.S. and K.A. Francesconi (1987)
Transformations of arsenic in the marine environment
Experienta 43, 553-557.

Edmonds, J.S. and K.A. Francesconi (1981)
Arseno-sugars from brown kelp *(Ecklonia radiata)* as intermadiates in cycling of arsenic in marine ecosystem.
Nature 289, 602-604.

Gibbs, P.E. et al. (19830
Tharyx marioni (Polychaeta): A remarkable accumulator of arsenic.
J. Mar. Niol. Ass. U.K. 63, 313-325.

Gilderhus, P.A. (1966)
Some effects of sublethal concentrations of sodium arsenite on bluegills and the aquatic environment.
Trans. Am. Fish. Soc. 95, 289-296.

Guthrie, R.F. et al. (1979)
Biomagnificarion of heavy metals by organisms in a marine microcosm.
Bull. Environm. Contam. Toxicol. 21, 53-61.

Hallacher, L.E. et al. (1985)
Distribution of arsenic in the sediments and biota of Hilo Bay, Hawaii.
Pacific Science 39 (3), 266-273.

Holcombe, G.W. et al. (1983)
Toxicity of selected priority pollutants to various aquatic organisms.
Ecitox. Environm. Safety 7, 400-409.

Hueck- van der Plas, E.H. (ed.) (1984)
Zware metalen in Aquatische systemen: geochemisch en biologisch onderzoek in Rijn en Maas en daardoor gevoede bekkens,
Studie en informatiecentrum TNO voor Milieu-onderzoek, Delft

IPCS (1981)
Environmental Health Criteria 18: Arsenic
WHO, Geneva

Isensee, A.R. et al. (1973)
Distribution of alkyl arsenicals in model ecosystem
Environ. Sci. Techn. 7 (9), 841-845.

Jacobs, L.W., D.R. Keeney and L.M. Walsh (1970)
Arsenic Residue Toxicity to Vegetable Crops Grown on Plainfield Sand
Agronomy Journal 62, 588-591

Klumpp, D.W. (1980a)
Characteristics of arsenic accumulation by the seaweeds *Fucus spiralis* and *Asophyllum nodosum* .
Marine Biology 58, 257-264.

Klumpp, D.W. (1980b)
Accumulation of arsenic from water and food by *Littorine littoralis* and *Nucella lapillus* .
Marine Biology 58, 265-274.

Krishnaja, A.P. et al. (1987)

Toxic effects of certain heavy metals (Hg, Cd, Pb, As and Se) on the intertidal crab *Scylla serrata*.

Mar. Environm. Res. 21, 109-119.

Langston, W.J. (1980)

Arsenic in U.K. estuarine sediments and its availability to benthic organisms.

J. Mar. Biol. Ass. U.K. 60, 869-881.

Langston, W.J. (1984)

Availability of arsenic to estuarine and marine organisms: a field and laboratory evaluation.

Mar. Biol. 80, 43-154.

LeBlanc, P.J. and A.L. Jackson (1973)

Arsenic in marine fish and invertebrates.

Mar. Pol. Bull. 4, 88-90.

Leeuwen, K. van, G. Niebeek and W. Luttmer (1987)

Basiskwaliteitsnormen voor zware metalen: een effectgerichte evaluatie

H_2O 20 (9), 200-206

Lima, A.R. et al. (1984)

Acute and chronic toxicities of arsenic(III) to fathead minnows, flagfish, daphnids, and an amphipod.

Arch. Environ. Contam. Toxicol. 13, 595-601.

Lunde, G. (1972)

The analysis of arsenic in the lipid phase from marine and limnetic algae.

Acta Chem. Scand. 26 (7), 2642-2644.

Macnair, M.R. and Q. Cumbes (1987)

Evidence that arsenic tolerance in Holcus lanatus L. is caused by an altered phosphate uptake system

New Phytol. 107 (2), 387-394

MacPhee, A.W. and K. Sanford (1954)

The influence of spray programs on the fauna of apple orchards in Nova Scotia. VII. Effects on some beneficial Arthropods

Can. Entomol. 86, 128-135

Maliszewska, W. et al. (1985)
The influence of various heavy metal compounds on the development and activity of soil micro-organisms
Environ. Pollut. (Series A) 37, 195-215

Mance, G. et al. (1984)
Proposed environmental quality standards for list II substances in water: Arsenic, Water Research Centre, Medmenham, Technical Report TR212,

Mance, G. (ed.) (1987)
Polution threat of heavy metals in aquatic environments, Elsevier Applied Science Publishers LTD, London and new York

Martin, M. et al. (1981)
Toxicities of ten metals to *Crassostrea gigas* and *Mytillus edulis* embryos and *Cancer magister* larvae.
Mar. Poll. Bull. 12 (9), 305-308.

Nasu, Y. and M. Kugimoto (1981)
Lemna (Duckweed) as an indicator of water pollution, I, the sensitivity of Lemna paucicostata to heavy metals
Arch. Environ. Cotam. Toxicol. 10, 159-169

Nelson, D.A. et al. (1976)
Biological effects of heavy metals on juvenile bay scallops, *argopecten irradians* , in short-term exposures.
Bull. Environm. Contam. Toxicol. 16 (3), 275-282.

Nichols, J.W. et al. (1984)
Effects of freshwater exposure to arsenic trioxide on the parr-smolt transformation of Coho salmon (Oncorhynchus kisutch)
Environ. Toxicol. Chem. 3, 143-149

NRC (1980)
Mineral Tolerance of Domestic Animals, Subcommittee on mineral toxicity in animals. National Research Council, National Academy of Sciences, 40-53, Washington, D.C.

Oladimeji, A.A. et al. (1984)
Long-term effect of arsenic accumulation in rainbow trout *Salmo gairdneri*.
Bull. Environm. Contam. Toxicol. 32, 732-741.

Otte, M.L. et al. (1988)
Uptake of Arsenic by Vegetation of the former Rhine estuary
Proc. Third Intern. Conference Environmental Contamination, sept 1988, Venice, 529-531

Pagano, G. et al. (1982)
Arsenic-induced developmental defects and mitotic abnormalities in sea-urchin developments.
Mut. Res. 104, 351-354.

Paladino, F.V. and J.R. Spotila (1978)
The effect of arsenic on the thermal tolerance of newly hatched muskellunge fry *(Esox masquinongy)*
J. Thermal. Biology 33, 223-227.

Pandey, K. and J. Pd. Shukla (1982)
Deleterious effects of arsenic on the grwoth of fingerlings of a freshwater fish, *Colisa fasciatus* (Bl. & Sch.).
Acta Pharmacol. et Toxicol. (Letter) 50, 398-400.

Passino, D.R.M. and J.M. Kramer (1980)
Toxicity of arsenic and PCBs to fry of deepwater ciscoes *(Coregonus)*.
Bull. Environm. Contam. Toxicol. 24, 527-534.

Passino, D.R.M. and A.J. Novak (1984)
Toxicity of arsenate and DDT to the cladoceran *bosmina longirostris* .
Bull. Environm. Contam. Toxicol. 33, 325-329.

Planas, D. and F.P. Healey (1978)
Effects of arsenate an growth and phosphorus metabolism of phytoplankton
J. Phycol. 14, 337-341

Pyles, R.A. and E.A. Woolson (1982)
Quantitation and characterization of arsenic compounds in vegetables grown in arsenic acid treated soil
J. Agric. Food Chem. 30, 866-870

Ratsch, H.C. and D. Johndro (1986)
Comparative toxicity of six test chemicals to lettuce using two root elongation test methods
Environ. Monit. Assess. 6, 267-276

Sanders, J.G. (1979a)
The concentration and speciation of arsenic in marine macro-algae.
Estuarine Coastal Mar. Sci. 9, 95-99.

Sanders, J.G. (1979)
Effects of arsenic speciation and phosphate concentration on arsenic inhibition of Skeletonema costatum (Bacillariophyceae)
J. Phycol. 15, 424-428

Sanders, J.G. and P.S. Vermersch (1982)
Respons of marine phytoplankton to low levels of arsenate.
J. Plankton Res. 4 (4), 881-893.

Sanders, J.G. (1986)
Direct and indirect effects of arsenic on the survival and fecundity of estuarine zooplankton.
Can. J. Fish. aquat. Sci. 43, 684-699.

Schaefer, E.D. and W.O. Pipes (1973)
Temperature and the toxicity of chromate and arsenate to the rotifer *Philodina roseola* .
Water Res. 7, 1781-1790.

Shukla, J.P. and K. Pandey (1984a)
Impaired ovarian functions in arsenic treated freshwater fish, *(Colisa fasciatus* (Bl. and Sch.).
Toxicol. Letters 20, 1-3.

Shukla, J.P. and K. Pandey (1984b)
Impaired spermatogenesis in arsenic treated freshwater fish, *Colisa fasciatus* (Bl. and Sch.).
Toxicol. Letters 21, 191-195.

Shukla, J.P. et al. (1987)
Survival and impaired growth in arsenic treated fingerlings of *Channa punctatus* , a fresh water murrel.
Acta hdrochim. hydrobiol. 15, 307-311.

Sorensen, E.M.B. (1976)
Toxicity and accumulation of arsenic in green sunfish *Lepomis cyanellus* , exposed to arsenate in water.
Bull. Environm. Contam. Toxicol. 15 (6), 756-761.

Sorensen, E.M.B. et al. (1979)
Arsenic accumulation, tissue distribution and cytotoxicity in teleosts following indirect aquous exposures.
Bull. Environm. Contam. Toxicol. 21, 162-169.

Spehar, R.L. et al. (1980)
Comparative toxicity of arsenic compounds and their accumulation in invertebrates and fish.
Arch. Environm. Contam. Toxicol. 9, 53-63.

Spehar, R.L. and J.T. Fiandt (1986)
Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species.
Environm. Toxicol. Chem. 5, 917-931.

Speyer, M.R. and G. Leduc (1975)
Effects of arsenic trioxide on growth of rainbow trout.
Proc. Int. Conf. Heavy metals in the environment (Toronto), C17-C19.

Spotila, J.R. and F.V. Paladino (1979)
Toxicity of arsenic to developing muskellunge fry *(Esox Masquinongy)*.
Comp. Biochem. Physiol. 62C, 67-69.

Stary, J. et al. (1982)
The cumulation of chromium and arsenic species in fish *(Poecilia reticulata)*.
Intern. J. Environ. Anal. Chem. 12, 253-257.

Taylor, D. et al. (1985)
The acute toxicity of nine 'grey list' metals (arsenic, baron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species: dab *(Limanda limanda)* and grey mullet *(Chelon labrosus)*.
Aquat. Toxicol. 7, 135-144.

Thatcher, C.D. et al. (1985)
Arsenic toxicosis and suspected chromium toxicosis in a herd of cattle
JAVMA 187 (2), 179-182

Thursby, G.B. and R.L. Steele (1984)
Toxicity of arsenite and arsenate to the marine macroalga *Champia parvula* (Rhodophyta).
Environm. Toxicol. Chem. 3, 391-397.

Tyler, G. (1982)
Accumulation and exclusion of metals in Collybia peronata and Amanita rubescens
Trans. Br. mycol. Soc. 79, 239-245

Tyler, G. (1983)
Does acidification increase metal availability and thereby inhibit decomposition and mineralisation processes in forest soils?
In: Ecological Effects of acid deposition, National Swedish Environment Protection Board, Report PM 1636, 245-256

Veen, N.G. van der and K. Vreman (1986)
Transfer of cadmium, lead, mercury and arsenic from feed into various organs and tissues of fattening lambs
Neth. J. Agric. Sci. 34, 145-153

Vocke, R.W. et al. (1980)
Growth responses of selected freshwater algae to trace elements and scrubber ash slurry generated by coal-fired power plants
Water Res. 14, 141-150

Vreman, K. et al. (1986)
Transfer of cadmium, lead, mercury and arsenic from feed into milk and various tissues of dairy cows: chemical and pathological data
Neth. J. Agric. Sci. 34, 129-144

Wagemann, R. et al. (1978)
Arsenic in sediments, water and aquatic biota from lakes in the vicinity of Yellowknife, Northwest territories, Canada.
Arch. Environm. Contam. Toxicol. 7, 169-191.

Watling H.R. and R.J. Watling (1982)
Comparative effects of metals on the filtering rate of the brown mussel *(Perna perna)*
Bull. Environm. CONTAM. Toxicol. 29, 651-657.

Weir, P.A. and C.H. Hine (1970)
Effects of various metals on behaviour of conditioned goldfish.
Arch. Environ. Health 20, 45-51.

Wiersma, D., B.J. van Goor and G. van der Veen (1986)
Cadmium, lead, mercury, and arsenic concentrations in crops and corresponding soils in the Netherlands
J. Agric. Food Chem. 34, 1067-1074

Wong, P.T.S. et al. (1978)
Toxicity of a mixture of metals on freshwater algae
J. Fish. Res. Board Can. 35, 479-481

Woolson, E.A., J.H. Axley and P.C. Kearney (1971)
Correlation between available soil arsenic, estimated by six methods, and response of corn (Zea mays L.)
Soil Sci. Soc. Amer. Proc. 35, 101-105

Woolson, E.A. (1975)
Bioaccumulation of arsenicals
In: E.A. Woolson (ed.) Arsenical pesticides, American Chemical Society Symposium, Series no 7, 97-107, American Chemical Society, Washington, D.C.

Wrench, J. et al. (1979)
Arsenic metabolism in a marine foodchain.
Mar. Poll. Bull. 10, 18-20.

Xu, J. and I. Thornton (1985)
Arsenic in garden soils and vegetable crops in Cornwall, England: Implications for human health
Environ. Geochem. Health 7 (4), 131-133

REFERENCES OF CHAPTER 5 : RISK ASSESSMENT

Gezondheidsraad (1985)
Advies inzake uitgangspunten voor normstelling, de inzichtelijke opbouw van advieswaarden voor niet-mutagene, niet-carcinogene en niet-immunotoxische stoffen
Gezondheidsraad, Rapport No. 31, 's Gravenhage

Gezondheidsraad (1988)
Ecotoxicologische risico-evaluatie van stoffen
Gezondheidsraad, Rapport 1988/28, 's Gravenhage, The Netherlands
(An english translation of this report: "Analysing the risk of toxic chemicals for ecosystem's is available; Report 1988/28, Health Council of the Netherlands)

Kooijman, S.A.L.M. (1987)
A safety factor for LC50-values allowing for differences in sensitivity among species
Wat. Res. 21, 269-276

Slooff, W. et al. (1986)
Margins of uncertainty in ecotoxicological hazard assessment
Environ. Toxicol. Chem. 5, 841-852

Straalen, N.M. van (1987)
Stofgehalten in de bodem - (Geen) Effecten op bodemdieren
In: Symposium Bodemkwaliteit, 10 december 1986, Ede, 75-84
VTCB M86/44 (Voorlopige Technische Commissie Bodembescherming)
Ministerie van Ruimtelijke Ordening, Volkshuisvesting en Milieubeheer, Leidschendam

WHO (1987)
Air Quality Guidelines for Europe
WHO Regional Publications, European Series No. 23, 171-181
World Health Organization, regional office for Europe Copenhagen