Fluorides

MAK value (2005)	**1 mg/m³ I (inhalable fraction) as fluoride**
Peak limitation (2005)	**Category II, excursion factor 4**
Absorption through the skin (2005)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2005)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value (1983)	**7 mg fluoride/g creatinine at the end of the shift**
4 mg fluoride/g creatinine at the beginning of the next shift	
Chemical name (CAS)	fluoride
CAS number	16984-48-8

Substance name	CAS number	Molecular formula	Molecular weight	Melting point (°C)[a]	Solubility in water g/l[a]
Ammonium fluoride	12125-01-8	NH_4F	37.0	S	820 (20°C)
Ammonium hydrogen fluoride	1314-49-7	NH_4HF_2	57.0	125.6	–
Barium fluoride	7787-32-8	BaF_2	175.32	1386	1.7 (23°C)
Calcium fluoride	7789-75-5	CaF_2	78.1	1418	0.017 (20°C)
Calcium phosphate fluoride		$Ca_5(PO_4)_3F$	504.2	–	insoluble
Caesium fluoride	13400-13-0	CsF	151.9	682	

The MAK-Collection Part I, MAK Value Documentations 2015
DFG, Deutsche Forschungsgemeinschaft

Substance name	CAS number	Molecular formula	Molecular weight	Melting point (°C)[a]	Solubility in water g/l[a]
Potassium fluoride	7789-23-3	KF	58.1	858	920 (20°C)
Potassium hydrogen fluoride	7789-29-9	KHF_2	78.1	D	–
Lithium fluoride	7789-24-4	LiF	25.9	848	2.7 (18°C)
Magnesium fluoride	7783-40-6	MgF_2	62.3	1263	< 0.002 (0–60°C)
Sodium fluoride	7681-49-4	NaF	42.0	993	42.2 (18°C)
Sodium hydrogen fluoride	1333-83-1	$NaHF_2$	62.0	–	–
Rubidium fluoride	13446-74-7	RbF	104.5	775	–
Strontium fluoride	7783-48-4	SrF_2	125.6	1473	0.11 (0°C)
Zinc fluoride (rutile)	7783-49-5	ZnF_2	193.4	872	–

S: sublimation; D: decomposition
[a] Details from ECB (2000 a, b, c, d, e), Korth Kristalle GmbH (2005), Sigma-Aldrich (2005)

Fluorides comprise simple to complex compounds of fluorine with other chemical elements. This evaluation is based on reviews of the toxicological data for fluorides (ATSDR 2003; WHO 2002). Hydrogen fluoride was dealt with in a separate document (see Fluorwasserstoff "Hydrogen fluoride" 2001, only available in German). However, the data for the systemic effects of hydrogen fluoride are also used in this assessment.

1 Toxic Effects and Mode of Action

Fluorides in gaseous and particle form are absorbed completely by the respiratory tract. After ingestion, the amount absorbed depends on the pH and the calcium, magnesium or aluminium concentrations in the gastrointestinal tract, which form poorly soluble complexes with fluoride ions. About 50% of serum fluoride is bound

to organic molecules (50% in the bones and 50% eliminated within 24 hours). Elimination in humans and animals mainly takes place via the urine within 24 hours. Elimination from the blood in humans lasts 2 to 9 hours, elimination from the bones takes 8 to 20 years. Sodium fluoride, calcium fluoride and hydrogen fluoride have irritative effects on the skin and eyes. Short-term inhalation of hydrogen fluoride produces irritation in the upper respiratory tract and in the lungs.

After short-term oral administration, diarrhoea, vomiting, and respiratory or cardiac arrest occur. The kidneys and the stomach are other target organs.

The most important systemic effect in humans and animals is the occurrence of skeletal fluorosis. The preclinical symptom of skeletal fluorosis is increased bone density, which can be determined by means of radiography. As fluorosis is based on the effect of the fluoride ion, all substances that form fluoride ions can contribute to the degree of fluorosis. This is of particular importance with exposure to a combination of different fluorides, as frequently occurs in practice. Initial evidence of skeletal fluorosis is found in workers who were exposed to fluoride concentrations in the air of 4 ml/m^3 and above for more than 10 years. Determination of fluoride ions in the urine is used to monitor fluoride exposure (Henschler and Lehnert 1983). In rats, a decrease in the strength and mineralization of the bones is found after fluoride doses of 3.2 mg/kg body weight and above for 16 weeks.

After fluoride doses of 12.5 mg/kg body weight and above, skeletal variations accompanied by maternal toxicity were observed in rats, and after 13.8 mg/kg body weight and above in rabbits.

Fluorides were not found to be mutagenic in *Salmonella* mutagenicity tests and it seems that in vitro and depending on the test protocol they indirectly produce clastogenicity as a result of a delay in the cell cycle. In vivo, the picture is very inconsistent: in vivo chromosomal aberration tests and micronucleus tests with comparable test protocols yielded both positive and negative results. The reasons for this are not clear.

The data available for carcinogenicity in humans after oral intake of fluorides do not allow any assessment to be made. Two carcinogenicity studies with rats and mice in which sodium fluoride was administered with the drinking water do not provide any clear evidence that sodium fluoride has carcinogenic effects. However, there is evidence of the occurrence of osteosarcomas in male rats given fluoride doses of 2.4 mg/kg body weight and of osteomas in mice after fluoride doses of 1.8 mg/kg body weight and above. The presence of C type virus particles in most of the osteomas in mice limits the validity of the findings.

2 Mechanism of Action

Skeletal fluorosis is the most sensitive parameter for the systemic effects of exposure to fluorides. It is manifest as an accumulation of fluoride in the bones with resultant brittleness.

Around half of the absorbed fluoride is incorporated into the mineral structure of the bones and teeth by exchange with hydroxyl groups (Dinman et al. 1976 b; WHO 1984). The fluoride concentration in the bones therefore increases with age. It is assumed that fluoride is not evenly distributed, but is deposited in two specific regions of the bones. According to this hypothesis, all cells that resorb bones are exposed to fluoride. This applies to osteoclasts and particularly to osteocytes. As a result of impaired osteocytic osteolysis, a change in the resorption of bones and thus lesions in the bones can occur (DECOS 1989; WHO 2002).

Fluoride ions can both activate and inhibit enzymes in vitro and in vivo by forming complexes with the metal ions of the enzymes. There is evidence that low fluoride concentrations in serum (0.18 mg/l) stabilize and activate enzymes, whereas at higher concentrations (> 0.3 mg/l) enzymes are inhibited (superoxide dismutase, catalase) or activated (glutathione peroxidase, alanine aminotransferase, alkaline phosphatase) (Shanthakumari et al. 2004; WHO 2002). After short-term ingestion of high fluoride doses, also enzymes responsible for vital processes such as the transmission of nerve impulses are inhibited. The impairment of those body functions regulated by calcium is of particular importance. On account of the high affinity of fluoride to calcium, the formation of fluorapatite and its precipitation can occur (WHO 2002).

3 Toxicokinetics and Metabolism

3.1 Ingestion, distribution, elimination

Ingested fluorides are rapidly absorbed via the gastrointestinal tract. This absorption can be influenced by the presence of various minerals such as calcium, magnesium and aluminium, which bind to fluorides to form poorly soluble complexes (ATSDR 2003). Gaseous and soluble fluorides are absorbed almost completely via the respiratory tract; the more soluble the fluoride compounds and the smaller the particles, the more fluoride is absorbed. By contrast, fluoride compounds with low solubility such as barium, calcium, magnesium, lithium und strontium fluoride are absorbed much less readily (WHO 2002).

Sodium fluoride is rapidly absorbed by the stomach, and the absorption rate decreases with increasing pH of the stomach content (WHO 2002). After rats were given oral doses of sodium fluoride of 300 mg/kg body weight (corresponding to fluoride doses of 138 mg/kg body weight), the concentration of fluoride in the plasma reached its highest value after 10 to 15 minutes, while in rabbits given fluoride doses of 40 mg/kg body weight as sodium fluoride the maximum was reached after 53 minutes (ATSDR 2003). Smaller quantities of fluoride are absorbed also via the oral cavity (WHO 2002).

Fluorides are distributed with the blood throughout the entire body. 75% of the fluoride in blood is present in the serum, 25% in the erythrocytes (WHO 1984, 2002).

Fluoride crosses the placental barrier in humans and animals (WHO 2002). In humans and animals, around 99% of the fluoride found in the body is stored in the bones and teeth, the remainder is distributed between soft tissues and blood (WHO 2002).

A physiologically based, pharmacokinetic model (PBPK model) of the uptake of fluoride in the bones has been described. With this model, the fluoride concentration in the bones can be estimated and related to the chronic toxicity of the fluorides. Furthermore, the PBPK model can provide a basis for across-species extrapolation of fluoride doses (Rao et al. 1995).

In humans, fluorides are eliminated mainly with the urine. In addition, smaller quantities are eliminated with breast milk, exhaled air and the faeces. Elimination with the urine is a function of the physiological balance. This is dependent on former and recent exposures to fluorides, the fluoride content in bones and teeth, the fluoride released from calcified tissue, age, urine flow, the pH of the urine and renal parameters (Ekstrand 1978; Ekstrand et al. 1982; Schiffl and Binswanger 1980; Whitford 1996). The fluoride concentration in the urine of healthy individuals is between 0.2 and 1 mg/l and depends on the quantity of fluoride absorbed (WHO 2002). Increased fluoride values are found in the urine of individuals subjected to increased exposure via inhalation or who live in regions with endemic skeletal fluorosis (WHO 2002).

Half-times for the elimination of fluoride from plasma in humans are given as 2 to 9 hours, the half-times for the elimination from bones as 8 to 20 years (Dinman et al. 1976 b; WHO 1984, 2002).

3.2 Metabolism

About 50% of the serum fluoride is bound to organic molecules, mainly fatty acids. About half of the absorbed fluoride accumulates in the mineral structure of the bones and teeth in exchange with hydroxyl groups, and the other half is excreted with the urine within 24 hours (Dinman et al. 1976 b; WHO 1984).

4 Effects in Humans

4.1 Single exposures

Case reports

Accidental ingestion of sodium fluoride in high doses immediately produces symptoms such as nausea and vomiting, accompanied by burning, cramp-like abdominal

pains and diarrhoea. Clonic convulsions and pulmonary oedema were reported in some cases; the latter may, however, have been caused by the aspiration of vomit. While some of these deaths were suicides, most resulted from accidents in which the contents of containers were mistaken. On the basis of these accidents, the lethal dose for adults was estimated to be 5 to 10 g fluoride (fluoride doses of 32–64 mg/kg body weight) (ATSDR 2003).

A 3-year-old boy died after swallowing 200 sodium fluoride tablets (1 mg fluoride per tablet). This corresponds to a lethal fluoride dose of 16 mg/kg body weight. Immediately after ingestion, vomiting and slight recovery occurred, but circulatory collapse 4 hours later resulted in death. Autopsy revealed haemorrhagic oedema in the lungs, haemorrhagic gastritis and severe cerebral oedema. The effects in the lungs were probably produced by aspiration of the stomach contents (Eichler et al. 1982).

A child aged 27 months died 5 days after ingesting about 100 fluoride tablets (no other details), corresponding to a fluoride dose of approximately 8 mg/kg body weight. On the basis of this case, an approximate toxic dose of about 5 mg/kg body weight was calculated (ATSDR 2003).

Cardiovascular effects and cardiac arrest are induced by exposure to high doses of fluoride only (> 5 mg/kg body weight). The binding of serum calcium to fluoride leads to hypocalcaemia, which reduces myocardial contractility and can result in cardiovascular collapse. It has been suggested that hyperkalaemia, which can cause repeated episodes of ventricular fibrillation and even death, is often associated with high-level exposure to fluorides or fluoride poisoning (ATSDR 2003; Augenstein et al. 1991; Baltazar et al. 1980; Bayless and Tinanoff 1985; WHO 2002).

4.2 Repeated exposures

Epidemiological studies

Most studies investigate whether there is a connection between the fluoride concentration in drinking water and adverse effects on health, particularly with skeletal effects and cancer. Most of the studies quoted are population-based and provide no data concerning individual exposure or any particular fluoride compound (ATSDR 2003). The most important studies are described below.

Case–control studies

A case–control study investigated 914 cases of hip fracture and 1196 control persons for a correlation between fluoride uptake and the occurrence of hip fracture. No increase in hip fractures was found at fluoride concentrations in drinking water of above 1 mg/l, even when such confounding factors as age, sex, body mass index, physical activity, the age at which the menopause occurred, alcohol consumption, smoking habits, treatment with corticosteroids and the intake of calcium were taken into account (Hillier et al. 2000). Similar results were obtained in other investi-

gations (ATSDR 2003). Assuming an intake of 2 to 3 litres drinking water for an adult per day, the concentration of 1 mg/l corresponds to fluoride concentrations in air of 0.2 to 0.3 mg/m^3 .

Cohort studies

In the Chinese province Qianan with a high fluoride content in the drinking water (3 mg/l), morbidity was 90% for dental fluorosis and 20.9% for skeletal fluorosis. The fluoride concentrations in blood and urine were increased in fluorosis patients (0.326 and 2.36 mg/l, respectively) in comparison with those in the controls (0.075 and 1.16 mg/l, respectively) (Zhang et al. 2003). As there are no other details regarding the study size, investigation methods, etc., this study cannot be used for evaluation.

In six regional communities of China with different natural fluoride concentrations in the drinking water, 8266 persons over 50 years old were investigated with regard to the prevalence of bone fractures over the preceding 20 years. The parameters evaluated included fluoride exposure, the prevalence of bone fractures, demographic details, medical history, physical activity, cigarette smoking and alcohol consumption. The fluoride content in the food of a random selection of 10% of the persons was determined. The 3-day analysis of the food revealed no differences between the regional communities as regards nutrition. 99.1% of the reported bone fractures were confirmed by previous or recent x-ray films. Drinking water was found to be the main source of fluoride exposure. The fluoride concentrations in the drinking water of the individual communities were 0.25 to 0.34, 0.58 to 0.73, 1.00 to 1.06, 1.45 to 2.19, 2.62 to 3.56 and 4.32 to 7.97 mg/l. This corresponds to a calculated daily fluoride uptake of 0.73, 1.62, 3.37, 6.54, 7.85 or 14.13 mg for each person (assuming an average body weight of 55 kg for an Asian population). The relationship between the fluoride concentration in the drinking water and the prevalence of bone fractures followed a U-shaped pattern. The prevalence of bone fractures was significantly increased both in communities with a very low fluoride level of 0.25 to 0.34 mg/l drinking water (daily fluoride uptake of 0.73 mg) as well as in communities with a very high fluoride level of 4.32 to 798 mg/l drinking water (daily fluoride uptake of 14.13 mg) compared with in communities with a medium fluoride level of 1 to 1.06 mg fluoride/l drinking water (daily fluoride uptake of 3.37 mg) and taking age and sex into consideration. No increase in the prevalence of bone fractures was observed in the remaining communities with fluoride concentrations in the drinking water of between 0.58 and 3.56 mg/l (daily fluoride uptake between 1.62 and 7.85 mg). When only the hip fractures over the preceding 20 years were considered (after adjustment for age and body mass index), the prevalence of such fractures was significantly increased only in the group with a very high fluoride level (4.32–7.97 mg/l) compared with the prevalence of hip fractures in the group with a medium fluoride level (1–1.06 mg/l) (Li et al. 2001). According to this study, the daily uptake of fluoride levels of between 1.62 and 7.85 mg does not result in an increase in bone fractures. Assuming an average body weight of

55 kg for the Asian population, the quantities of fluoride absorbed correspond to 0.03 to 0.15 mg/kg body weight and day. As with all ecological studies, this study determines the fluoride exposure only in populations occupying different geographical areas, but not that in individuals, and thus provides only a limited insight into the true fluoride exposure. This is because of the impossibility of accounting for the confounding factors, and distortion resulting from the non-randomized selection of the populations in the different geographical areas. Therefore, this study is not suitable for deriving a MAK value, although it provides information on the exposure levels at which an increase in bone fractures or no bone fractures at all may be expected.

Studies at the workplace

As a rule, workers are exposed to a mixture of fluoride compounds; for this reason, often the concentration of total fluorides is given in studies of workers. The results of studies of exposure to a mixture of fluorides and hydrogen fluoride are summarized in Table 1.

Table 1 Effects in workers after exposure to a mixture of fluorides and hydrogen fluoride (see also Fluorwasserstoff "Hydrogen fluoride" 2001 "Hydrogen fluorides", only available in German)

Fluoride concentration		NOAEL/ LOAEL	Effects (number of persons investigated)	References
in mg/m³ air	in urine or serum			
0.48 (total F^-) 0.20 (as gas)	2.7 ml/l urine (at end of shift)	NOAEL	no signs of skeletal fluorosis in workers with exposure for more than 10 years; increased bone density in some cases; previous level of exposure not indicated; no effects on haematopoietic system, liver, kidneys; slight change in lung function parameters of unknown origin (n = 157)	Chan-Yeung et al. 1983 a, b
0.85	3.4 mg/l urine (at end of shift) 1.9 mg/l urine (at beginning of next shift)	NOAEL	no skeletal fluorosis (n = 305)	Hodge and Smith 1977
0.48–0.89	"normal"	NOAEL	no skeletal fluorosis (n = 350)	Hodge and Smith 1977
0.06–0.94	4.8 mg/l urine (at end of shift) 2.4 mg/l urine (at beginning of next shift)	NOAEL	no skeletal fluorosis (n = 200)	Hodge and Smith 1977

Table 1 (Continued)

Fluoride concentration in mg/m³ air	Fluoride concentration in urine or serum	NOAEL/ LOAEL	Effects (number of persons investigated)	References
0.3–1.4	1.1 mg/l urine	NOAEL	no skeletal fluorosis (n = 61)	Hodge and Smith 1977
< 2.5	3.0 mg/l urine	NOAEL	no skeletal fluorosis (factory at Massena) (n = 231)	Kaltreider et al. 1972
< 2.5	2.8 mg/l urine (at beginning of next shift) 7.7 mg/l urine (at end of shift)	NOAEL	skeletal fluorosis (n = 56)	Dinman et al. 1976 a
about 1.88 (extrapolated)	about 8 mg/l urine (extrapolated; at end of shift)	NOAEL	no skeletal fluorosis	Dinman et al. 1976 b
2.65	4.53 mg/l urine (50.9% > 4 mg/l)	NOAEL	no skeletal fluorosis (n = 57)	Derryberry et al. 1963
3.4	5.18 mg/l urine (70.6% > 4 mg/l)	LOAEL	minimum increase in skeletal fluorosis (n = 17)	Derryberry et al. 1963
0.14–3.43; 15%–74% as gas; 16%–85% in particle form	full-time employees (♂): > 9 mg/24-hour (urine) part-time employees (♂): about 3–4 mg/24-hour (urine)	LOAEL	full-time employees (n = 232 ♂): increased coughing: 12.83% (controls 3.9%); skeletal fluorosis (n = 189): 25.4% (controls 4%); no significant difference between fluoride concentration in urine of workers with and without skeletal fluorosis	Hodge and Smith 1977
0.5–3.7	–	LOAEL	skeletal fluorosis (n = 18 of 20)	Hodge and Smith 1977
2.4–6 (total fluoride F^-) 0.87–3 (as gas)	8.7 mg/l urine	LOAEL	pronounced skeletal fluorosis without physiological effects (factory at Niagara) (n = 76 of 79)	Kaltreider et al. 1972
0.06–8.2	4.25 mg/l urine (at end of shift) 2.6 mg/l urine (at beginning of next shift)	LOAEL	skeletal fluorosis (n = 1 of 4)	Hodge and Smith 1977
15–20	about 16 mg/l urine	LOAEL	skeletal fluorosis (n = 57 of 68)	Hodge and Smith 1977

NOAEL: no observed adverse effect level; LOAEL: lowest observed adverse effect level

Earlier studies of workers occupationally exposed to fluorides, mostly for several years, were reviewed in Hodge and Smith (1977) and Hodge et al. (1970) (Table 1). Evidence of an increase in the occurrence of fluoride-induced osteosclerosis was found in workers exposed to fluoride concentrations of 3.4 mg/m^3 and above for an average of 14 years (Derryberry et al. 1963). Pronounced skeletal fluorosis without physiological effects occurred in workers exposed to fluoride concentrations of 2.4 to 6.0 mg/m^3 for more than 10 years (Kaltreider et al. 1972). Workers with no signs of skeletal fluorosis had been exposed to average concentrations of 2.65 mg/m^3 (Derryberry et al. 1963) and below for 14 years (Dinman et al. 1976 a, b; Kaltreider et al. 1972).

In an epidemiological study of 2066 workers at an aluminium smelter, the influence of working in the potroom on the musculoskeletal system, the haematopoietic system, the liver and the kidneys (Chan-Yeung et al. 1983 b) and on lung function (Chan-Yeung et al. 1983 a) was investigated. In the potroom, the workers were exposed to aluminium (concentration not specified), total fluoride concentrations of 0.48 mg/m^3 or fluoride concentrations of 0.28 mg/m^3 and benzo[*a*]pyrene concentrations of 3.5 mg/m^3, sulfur dioxide concentrations of 0.75 ml/m^3 and carbon monoxide concentrations of 10 ml/m^3. No details of previous exposure concentrations were available to the authors. For the workers in the potroom, fluoride concentrations in the urine of 1.9 mg/l were found before the beginning of the shift, 2.7 mg/l after the shift and 3.0 mg/l at the end of the working week. Effects on the haematopoietic system, liver and kidneys were not observed. No definitive cases of skeletal fluorosis were observed, although radiographic investigations showed there to be increased bone density, calcification of ligaments and periosteal changes in a number of workers who had been exposed for more than 10 years. Evaluation of the findings by two radiologists produced conflicting opinions, however (Chan-Yeung et al. 1983 b; Table 1). A statistically significant reduction in lung function parameters was described in the workers who spent more than 50% of their working day in the potroom ($FEV_1FEF_{25-75\%}$). The authors point out that the role played by hydrogen fluoride in the changes in lung function parameters is not known (Chan-Yeung et al. 1983 a; Table 1).

Use as a therapeutic

The topical application of fluorides in the treatment of Basedow's disease has been reported. Here, both diluted hydrogen fluoride solution and 10% sodium fluoride in ointment are said to have been effective (Litzka 1936).

4.3 Local effects on skin and mucous membranes

Calcium fluoride was found to be slightly irritating (no other details; ECB 2000 b). No investigations are available for other fluorides.

4.4 Allergenic effects

There are no studies available of the allergenic effects of fluorides in humans.

4.5 Reproductive and developmental toxicity

Fertility

In a study of the impairment of fertility resulting from the intake of fluoride via drinking water, a decreasing number of births was correlated with the increasing fluoride concentration in drinking water (Freni 1994). Because of the large number of confounding factors not accounted for, however, a definitive statement cannot be made on the basis of this study.

Significantly decreased serum testosterone concentrations were described in 30 men with skeletal fluorosis and in 16 men related to those with skeletal fluorosis living in the same household. No correlation was found between the serum testosterone concentration and the fluoride concentration in urine or serum (Susheela and Jethanandani 1996). The restricted scope of the study and the confounding factors not accounted for limit the usefulness of the study. It can therefore not be used in the evaluation.

Developmental toxicity

Fluoride is able to pass the placental barrier and is detectable in foetal and placental tissue. Analysis of the births in regions with fluoridated drinking water in comparison with those in regions with low fluoride concentrations in the drinking water revealed no differences in the incidence of birth defects (ATSDR 2003).

In three studies, a decrease in intelligence quotients was found in Chinese children living in regions with endemically high fluoride concentrations in the water (Li et al. 1995 a; Lu et al. 2000; Zhao et al. 1996). All studies had shortcomings as regards their design: potential confounding factors were not taken into account and the group sizes were too small. The validity of the studies is therefore greatly limited, and they are not suitable for inclusion in the evaluation.

No change was found in the intelligence quotient of Mexican children aged 6 to 8 years who had been exposed to fluoride concentrations in drinking water of 1.2 to 3 mg/l. However, changes in reaction time were discovered in tests of visuospatial organization which correlated with the fluoride concentration in urine (Calderon et al. 2000).

4.6 Genotoxicity

No change in sister chromatid exchange in lymphocytes was found in a Chinese population with a high fluoride concentration in the drinking water (n = 120; up to 4.0 mg/l drinking water; fluorides not further specified) (Li et al. 1995 b).

4.7 Carcinogenicity

The database for the carcinogenicity of ingested fluorides for humans has been assessed by the International Agency for Research on Cancer to be "inadequate" (IARC 1982). No more recent investigations are available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

For short-term inhalation exposure to fluorides there are only data for hydrogen fluoride available. The LC_{50} after 1 hour was about 1000 to 2000 ml/m^3 in rats, and about 280 to 420 mg/m^3 in mice, corresponding to 336 to 504 ml/m^3. The local irritative effects are the main effects of this substance (see Fluorwasserstoff "Hydrogen fluoride" 2001 "Hydrogen fluoride", only available in German).

5.1.2 Ingestion

The data for the oral LD_{50} values are summarized in the ATSDR (2003) report.

After administration of sodium fluoride, the LD_{50} in the rat corresponded to fluoride doses of 31 to 126.3 mg/kg body weight (De Lopez et al. 1976; Lim et al. 1978; Skare et al. 1986 a, b; Whitford 1990). Differences in strain, weight and housing conditions seem to have contributed to the wide scatter of the LD_{50} values in the rat. The LD_{50} values in young female rats (52–54 mg/kg body weight) were higher than in older female rats (31 mg/kg body weight) (De Lopez et al. 1976).

The LD_{50} in the mouse was 44.3 mg/kg body weight (Lim et al. 1978).

Short-term oral exposure to fluorides produced salivation, vomiting, diarrhoea and respiratory or cardiac arrest. In addition, effects on the kidneys and the stomach were observed (WHO 2002).

5.1.3 Dermal absorption

The dermal LD_{50} for sodium fluoride (as ointment, no details of irritative effects) was about 330 mg/kg body weight in the mouse. Subcutaneous injection was described as necrotizing (no other details; Litzka 1936).

5.1.4 Intraperitoneal, subcutaneous and intravenous injection

After intraperitoneal injection of sodium fluoride the LD_{50} in the rat was found to be 11 to 24 mg/kg body weight and that in the mouse to be 38 mg/kg body weight (ECB 2000 a).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Fluorides

Groups of 6 mice were exposed to aerosol concentrations of fluoride (derived from sodium fluoride solution) of 0, 2, 5 or 10 mg/m^3 for 4 hours per day over 14 days. More than 98% of the fluoride particles were inhalable with a diameter of less than 10 µm; of these, 65% were l than 2 µm. The fluoride concentrations in urine were determined on the third, seventh and fourteenth day; the body and lung weights, the number of macrophages, the total protein in the bronchoalveolar lavage fluid and the values for albumin and lactate dehydrogenase were determined on day 14. The fluoride concentration in urine increased linearly with the fluoride concentration in the inhalation chamber. In the bronchoalveolar lavage fluid there was a significant increase in polymorphic nuclear neutrophils and lymphocytes and a significant increase in total protein and albumin at fluoride concentrations of 5 mg/m^3 and above. A significant reduction in the number of alveolar macrophages and a significant increase in lactate dehydrogenase activity were found at concentrations of 10 mg/m^3 and above. At the highest concentration, body weights and relative lung weights decreased significantly, and histological examination revealed oedema in the lungs (Yamamoto et al. 2001).

In two groups of 6 mice exposed to fluoride concentrations of 13.3 mg/m^3 as sodium fluoride aerosol (particle size < 10 µm > 99.7%) for 4 hours a day for 20 or 30 days, there was a significant increase in relative lung weights. The fluoride concentration in the bones of the mice increased significantly with time, while the concentrations of the trace elements copper, zinc and iron, but not of calcium and magnesium, decreased. The relative liver and kidney weights remained unaffected (Chen et al. 1999). As no histopathological investigation was carried out, the relevance of the findings is unclear.

Hydrogen fluoride

The studies conducted with hydrogen fluoride are of relevance for the systemic effects of fluorides because the substance acts as fluoride in the body. The local irritation caused by hydrogen fluoride is not of relevance here (for further information see Fluorwasserstoff "Hydrogen fluoride" 2001 and documentation Fluorwasserstoff 2006, both only available in German).

In a 14-day inhalation study with F344 rats exposed to hydrogen fluoride for 6 hours a day on 5 days a week, body weights were markedly reduced, and thus also the absolute weights of several organs (no other details), at the lowest concentration of 10 ml/m^3 (corresponding to fluoride concentrations of 7.9 mg/m^3). Concentrations of 25 ml/m^3 (corresponding to fluoride concentrations of 19.8 mg/m^3) and above were lethal for the females and concentrations of 65 or 100 ml/m^3 (fluoride concentrations of 51.4 or 79 mg/m^3) lethal for the males (ECB 2000c).

In the subsequent 13-week inhalation study, 20 F344 rats per group and sex were exposed to hydrogen fluoride concentrations of 0, 0.1, 1 or 10 ml/m^3 (corresponding to fluoride concentrations of 0, 0.08, 0.79 and 7.9 mg/m^3) for 6 hours a day on 5 days a week. No relevant changes were found at the two lowest concentrations. The high concentration was lethal for five males and one female. Clinical changes (red discharge from eyes and nose, rough coat, alopecia, hunched posture) and dental changes were observed only in this exposure group. In addition, the body weights were noticeably reduced and the relative weights of the kidneys, liver, lungs, testes, spleen, brain, heart and adrenal glands were increased in this exposure group. No histological changes were observed in the nasal cavity, nasopharynx, larynx, oesophagus or lungs. Histological changes were found especially in the dead animals in the form of lymphocyte depletion in the lymph nodes of the bronchi and thymus and in the thymus and spleen. Degeneration of the germinal epithelium in the testes was observed in one animal of the medium-exposure group and one in the high-exposure group. Among the five dead males of the high-exposure group, also chronic inflammation of the preputial gland (n = 2), prostate, Harderian gland and the skin (n = 1) were found. Clinico-chemical, haematological and urine investigations revealed changes which were mainly observed in the high-exposure group. The number of segmented lymphocytes and platelets was increased, while the number of leukocytes and erythrocytes, the albumin/globulin ratio in serum, serum glucose and serum albumin were decreased; corresponding investigations in the dead animals are not available. Changes in the leukocyte count and the albumin/globulin ratio in serum were sporadically observed also in the medium-exposure group, although these were slight and within the range of the laboratory reference values (EPA 1991). In this report, no NOAEL (no observed adverse effect level) was derived, and the food intake was presumably not determined. It is thus not permissible to correlate the decrease in serum glucose and serum albumin with reduced food intake. Despite this criticism of the report, the study is well performed, and a NOAEL for hydrogen fluoride of 1 ml/m^3 (corresponding to fluoride concentrations of 0.79 mg/m^3) for systemic effects can be justifiably derived from it.

In addition, a large number of inhalation studies with rats, mice, guinea pigs, rabbits, dogs and rhesus monkeys are available. In these studies, either only relatively high concentrations of hydrogen fluoride were used, for which reason the studies are not suitable for the derivation of NOAELs, or the studies have serious methodological shortcomings. There are also studies with guinea pigs which are not relevant for humans because of species-specific effects (see Fluorwasserstoff "Hydrogen fluoride" 2001, only available in German).

5.2.2 Ingestion

For sodium fluoride, there are numerous drinking water and feeding studies available. These are shown in Table 2.

Table 2 Toxicity of fluorides after administration of sodium fluoride in the drinking water or with the diet

Species, strain, number of animals per sex and dose	Duration, dose[a]	Dose (mg fluoride/kg body weight and day)[a]: findings	References
rat, F334/N, groups of 5 ♂ and 5 ♀	**14 days,** 0, 50, 100, 200, 400, 800 mg sodium fluoride/l drinking water (about 0, 1.4, 2.8, 5.6, 11.25, 22.5 mg fluoride/kg body weight and day)	**up to 5.6 mg:** no effects; **11.25 mg and above:** body weights decreased; mortality increased in ♀; dehydration, lethargy, water consumption decreased **22.5 mg:** mortality increased; no microscopic investigation	NTP 1990
rat, Wistar, groups of 6 ♀	**5 weeks,** 0, 70, 140, 350 mg sodium fluoride/l drinking water (about 0, 2, 4, 9.8 mg fluoride/kg body weight and day); with 0.5% or 2.0% calcium	**up to 4 mg:** no effects **9.8 mg:** bone and body weight gains decreased with 0.5% calcium; reduction in the effects with 2.0% calcium as a result of reduced fluoride intake	Harrison et al. 1984
rat, SD, groups of 7 animals; no other details	**5 weeks,** 0, 75, 100, 150 mg fluoride/l drinking water (about 0, 4.7, 6.25, 9.4 mg/kg body weight and day); no other details	**4.7 mg: LOAEL** **4.7 mg and above:** mineralization decreased; proline content in protein and fluoride content in dental enamel increased; fluoride content in serum increased **6.25 mg and above:** total protein content in dental enamel increased **9.4 mg:** body weights decreased	Den Besten and Crenshaw 1984
rat, Wistar, groups of 6 ♂	**8 or 16 weeks,** 0.25 mg sodium fluoride/l drinking water (about 0.7 mg fluoride/kg body weight and day)	**0.7 mg:** activity of ALT, AST, alkaline phosphatase in serum increased; activity of superoxide dismutase, glutathione peroxidase and catalase decreased in the liver and kidneys; histopathology: inflammation and infiltration in hepatocytes and cohesive focal necrosis in the liver after 8 weeks and enlarged hepatocytes after 16 weeks; necrosis of vascular glomeruli and tubular changes in the kidneys	Shanthakumari et al. 2004

Table 2 (Continued)

Species, strain, number of animals per sex and dose	Duration, dose[a]	Dose (mg fluoride/kg body weight and day)[a]: findings	References
rat, CD-CRL:CD-BR**,** groups of 48 ♂ and 48 ♀ in the F_0 generation and groups of 36 ♂ and 36 ♀ in the F_1 generation	**generation study; 16 to 19 weeks** 0, 25, 100, 175, 250 mg sodium fluoride/l drinking water (for ♂: 0, 1.4, 5.1, 8.3, 10.7 mg fluoride/kg body weight and day; for ♀: 0, 1.6, 6.2, 9.7, 12.5 mg fluoride/kg body weight and day)	**1.4 mg ♂ and 1.6 mg ♀: NOAEL** **5.1 mg ♂ and 6.2 mg ♀ and above:** loss of pigmentation of the teeth as a sign of dental fluorosis; hyperplasia in the stomach increased **10.7 mg ♂ and 12.5 mg ♀:** water consumption decreased; no other histological changes	Collins et al. 2001 a, b; Sprando et al. 1997, 1998
rat, SD, groups of 16 ♂	**16 or 48 weeks** 0, 5, 15, 50 mg fluoride/l (0, 0.32, 0.94, 3.2 mg fluoride/kg body weight and day) combined with different daily calcium intake levels (25%, 50% or 100%)	**0.94 mg: NOAEL** for changes in the bones **3.2 mg and above:** after 16 weeks strength and mineralization of the bones decreased; after 48 weeks significant increase in osteoid surfaces anddiameters in %; no effect of calcium on effects produced by fluoride	Turner et al. 2001
rat, F334/N, groups of 10 ♂ and 10 ♀	**6 months,** 0, 10, 30, 100, 300 mg sodium fluoride/l drinking water (about 0, 0.28, 0.84, 2.8, 8.4 mg fluoride/kg body weight and day)	**0.84 mg: NOAEL** **2.8 mg and above:** hyperplasia in the stomach; dose-dependent increase in the fluoride concentration in urine and bones **8.4 mg:** body weights decreased; food and water consumption decreased; dental fluorosis, hyperplasia and necrosis in the stomach increased; no effects on the liver	NTP 1990
rat, Wistar, groups of 7 ♂ F_2-offspring	**6 months** 1 (controls), 10, 50, 100 mg sodium fluoride/l drinking water (about 0.028 (controls), 0.28, 1.4, 2.8 mg fluoride/kg body weight and day)	**0.28 mg:** no effects on the lungs, dose-dependent increase in the serum fluoride concentration **1.4 mg and above:** body weights and relative lung weights decreased; brown spots on the lungs; histopathology: intraparenchymal hyperaemic vessels, inflammatory reactions, respiratory epithelial proliferation, bronchiolitis, enzyme activities of superoxide dismutase, reduced	Aydin et al. 2003

Table 2 (Continued)

Species, strain, number of animals per sex and dose	Duration, dose[a]	Dose (mg fluoride/kg body weight and day)[a]: findings	References
		glutathione peroxidase and catalase decreased, lipid peroxidation increased **2.8 mg:** loss of alveolar architecture, changed lung parenchyma, thick walled vessels, pneumonia, parenchymal fibrosis, hyperplasia of alveolar cells, macrophages in the alveolar lumen, fragmentation of the cell nucleus of the alveolar macrophages, emphysematous regions	
rat, Wistar, groups of 6 ♂ and 6 ♀	**7 months,** 0, 30, 100 mg sodium fluoride/l drinking water (about 0, 0.84, 2.8 mg fluoride/kg body weight and day)	**0.84 mg and above:** phospholipids in the liver decreased, unsaturated fatty acids decreased, saturated fatty acids increased, ubiquinone level decreased; only the liver was investigated	Wang et al. 2000
rat, F334/N, groups of 70–100 ♂ and 70–100 ♀	**2 years,** 0, 25, 100, 175 mg sodium fluoride/l drinking water (for ♂: 0, 0.6, 2.3, 3.9 mg fluoride/kg body weight and day; for ♀: 0, 0.6, 2.5, 4.3 mg fluoride/kg body weight and day)	**0.6 mg: NOAEL** dose-dependent increase in the fluoride concentration in urine and bones **2.3 mg ♂ or 2.5 mg ♀ and above:** dental fluorosis; in 1/50 ♂ osteosarcoma **3.9 mg ♂ or 4.3 mg ♀:** ♀: osteosclerosis; 3/80 ♂ (4%): osteosarcomas (incidence in historical controls 6%); no effects on the liver	Bucher et al. 1991; NTP 1990
rat, Sprague Dawley, groups of 70 ♂ and 70 ♀	**2 years,** 0, 4, 10, 25 mg sodium fluoride/kg body weight and day (about 0, 1.8, 4.5, 11 mg fluoride/kg body weight and day); with the diet	**1.8 mg and above:** thickening of the teeth with white spots from week 26 **4.5 mg and above:** thickening of the bones, hyperplasia in the stomach; chronic microscopic inflammation of the stomach from week 53; increased relative stomach weights **11 mg:** body weights decreased; increased relative femur weights from week 25; no preneoplastic and neoplastic lesions	Maurer et al. 1990

Table 2 (Continued)

Species, strain, number of animals per sex and dose	Duration, dose[a]	Dose (mg fluoride/kg body weight and day)[a]: findings	References
mouse, $B6C3F_1$, groups of 5 ♂ and 5 ♀	**14 days,** 0, 50, 100, 200, 400, 800 mg sodium fluoride/l drinking water (about 0, 7.9, 15.8, 31.5, 63, 126 mg fluoride/kg body weight and day)	**63 mg:** body weights and water consumption decreased; **Above 126 mg**: ♂: mortality increased; no microscopic investigation	NTP 1990
mouse, Kunmin, groups of 12–15 ♂	**100 or 150 days,** 0, 0.6, 30 mg fluoride/l drinking water (about 0, 0.21, 10.5 mg fluoride/kg body weight and day); combined with 0, 20, or 2500 µg iodine/l	significant and dose-dependent increase in the fluoride content in the pelvic bones; no other details	Zhao et al. 1998
mouse, $B6C3F_1$, groups of 8–12 ♂ and 8–12 ♀	**6 months,** 0, 10, 50, 100, 200, 300, 600 mg sodium fluoride/l drinking water; (about 0, 1.6, 8, 15.8, 31.5, 47.3, 59.5 mg fluoride/kg body weight and day)	**8 mg and above:** minimal changes in bone growth; dose-dependent increase in the fluoride concentration in urine and bones **31.5 mg and above:** body weights decreased; white discolouration and spots on the teeth **47.3 mg and above:** 1/8 ♂ and 9/11 ♀ died; acute nephrosis, lesions in the liver and myocardium, necrosis or degeneration of seminiferous tubules	NTP 1990
mouse, $B6C3F_1$, groups of 70–100 ♂ and 70–100 ♀	**2 years,** 0, 25, 100, 175 mg sodium fluoride/l drinking water (♂: 1.1, 4.3, 7.5 mg fluoride/kg body weight and day; ♀: 1.3, 5.1, 8.5 mg fluoride/kg body weight and day)	**1.1 mg ♂ or 1.3 mg ♀: NOAEL** dose-dependent increase in the fluoride concentration in urine and bones **4.3 mg ♂ or 5.1 mg ♀ and above:** white discoloration of the teeth	Bucher et al. 1991; NTP 1990

Table 2 (Continued)

Species, strain, number of animals per sex and dose	Duration, dose[a]	Dose (mg fluoride/kg body weight and day)[a]: findings	References
mouse, CD-1, groups of 60 ♂ and 60 ♀	**2 years,** 0, 4, 10, 25 mg sodium fluoride/kg body weight and day (about 0, 1.8, 4.5, 11 mg fluoride/kg body weight and day); with the diet	**1.8 mg and above:** dose-dependent increase in the fluoride concentration in teeth, bones, stomach **1.8 mg and above and in the controls:** osteomas; only of limited validity (see Section 5.2.2 and Section 5.7)	Maurer et al. 1993
rabbits, albino, groups of 5 ♂ and 5 ♀	**6 months**, 0, 10, 20 mg sodium fluoride/kg body weight and day (about 0, 4.5, 9 mg fluoride/kg body weight and day); gavage	**4.5 mg: LOAEL** **4.5 mg and above:** fluoride in the lungs increased, pale areas on the surface of the lungs and dark brown areas in sections of the lungs; histopathology: congestion, oedema, desquamation of the respiratory epithelium	Purohit et al. 1999

[a] assumed values: water consumption: rat: 25 ml/day, mouse: 7 ml/day; body weight: rat: 400 g, mouse: 20 g.

Rat

In a 14-day study with F344 rats, survival was reduced after fluoride doses of 11.25 mg/kg body weight and day administered as sodium fluoride in the drinking water (NTP 1990). In Wistar rats, this was observed after fluoride doses of 8.4 mg/kg body weight and day and above (Harrison et al. 1984).

Fluoride induced in particular effects in the skeleton. In rats, bone mineralization was reduced after fluoride doses of 4.7 mg/kg body weight and day (Den Besten and Crenshaw 1984) or 9.8 mg/kg body weight and day (Harrison et al. 1984) in the drinking water for 5 weeks.

In an 8 and 16-week study, sodium fluoride concentrations in drinking water of 25 mg/l (fluoride doses of about 0.7 mg/kg body weight and day) were given daily to male Wistar rats. The activity of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in the serum increased, that of superoxide dismutase, glutathione peroxidase and catalase in the liver and kidneys decreased. Inflammation and necrosis in the hepatocytes and tubular changes in the kidneys were observed (Shanthakumari et al. 2004).

In a 16 and 48-week study, groups of 16 male SD rats were given daily fluoride concentrations of 0, 5, 15 or 50 mg/l (fluoride doses of 0, 0.32, 0.94 and 3.2 mg/kg body weight and day) in the drinking water. After fluoride doses of 3.2 mg/kg body weight and day, reduced strength and mineralization of the bones was observed after 16 weeks, and a significant increase in osteoid surfaces and osteoid diameters

after 48 weeks. The doses were selected to produce plasma fluoride levels in the rat corresponding to those resulting in humans consuming drinking water with fluoride concentrations of 0, 1, 3 or 10 mg/l (Turner et al. 2001). A NOAEL corresponding to fluoride doses of 0.94 mg/kg body weight and day was thus obtained for the change in bone strength in rats. This means that the derived NOAEL of 0.94 mg/kg body weight and day in rats (corresponding to fluoride concentrations in drinking water of 15 mg/l) corresponds to 3 mg/l drinking water for humans.

In a 6-month study, a loss of pigmentation in the teeth and hyperplasia of the stomach a was found after 16 or 19 weeks in CD rats given fluoride in the drinking water in doses of 5.1 or 6.2 mg/kg body weight and day and above (males and females, respectively); after fluoride doses of 10.7 or 12.5 mg/kg body weight and day (males and females, respectively) water consumption was reduced (Collins et al. 2001 a, b; Sprando et al. 1997, 1998). The NOAEL for dental fluorosis in rats in this study was therefore 1.4 or 1.6 mg/kg body weight and day (males and females, respectively).

In a 6-month study with Wistar rats, above all the lungs were investigated after the administration of sodium fluoride in the drinking water. Body weights and the relative lung weights were reduced after fluoride doses of 1.4 mg/kg body weight and day, and there were brownish areas on the lungs. Histopathological investigations revealed inflammatory reactions in the lungs, bronchiolitis, and changes in the enzyme activity of superoxide dismutase, glutathione peroxidase and lipid peroxidation. After fluoride doses of 2.8 mg/kg body weight and day, also hyperplasia of the alveolar cells, fragmentation of the cell nucleus of the alveolar macrophages, parenchymal fibrosis and changed lung parenchyma were found (Aydin et al. 2003). Other organs, in particular the bones and the teeth as the most sensitive end points, were not examined. In other studies in which the lungs were investigated, no effects on the lungs were found, (Maurer et al. 1990; NTP 1990). It is thus difficult to assess the relevance of the results described.

In groups of 6 male and 6 female Wistar rats given sodium fluoride concentrations in drinking water of 0, 30 or 100 mg/l (fluoride doses of about 0, 0.84 and 2.8 mg/kg body weight and day) for 7 months, the levels of phospholipids, ubiquinone and unsaturated fatty acids in the liver decreased after doses of 0.84 mg/kg body weight and day and above, whereas the level of saturated fatty acids increased (Wang et al. 2000).

In a 2-year study sodium fluoride concentrations in drinking water of 0, 25, 100 or 175 mg/l (♂: fluoride doses of 0, 0.6, 2.3, 3.9 mg/kg body weight and day; ♀: 0, 0.6, 2.5, 4.3 mg/kg body weight and day) led in F334 rats to dental fluorosis and a dose-dependent increase in the fluoride concentration in bones and urine after fluoride doses of 2.3 or 2.5 mg/kg body weight and day and above (males and females, respectively) (Bucher et al. 1991; NTP 1990). A NOAEL corresponding to fluoride doses of 0.6 mg/kg body weight and day was thus derived for the rat. In another 2-year feeding study with sodium fluoride doses of 0, 4, 10 or 25 mg/kg body weight and day (fluoride doses of about 0, 1.8, 4.5, 11 mg/kg body weight and day), dental changes were found in SD rats at fluoride doses of 1.8 mg/kg body

weight and day and above, and thickening of the bones, hyperplasia of the stomach from week 26 and increased relative stomach weights at 4.5 mg/kg body weight and day and above. Body weight gains were reduced and the relative femur weights increased from week 26 after fluoride doses of 11 mg/kg body weight and day (Maurer et al. 1990). No NOAEL can be derived.

Mouse

In a 14-day study, survival was reduced in male B6C3F_1 mice after fluoride doses of 63 mg/kg body weight and day administered in the form of sodium fluoride with the drinking water (NTP 1990). In B6C3F_1 mice, changes in bone growth were apparent after 6 months exposure to sodium fluoride corresponding to fluoride doses of 8 mg/kg body weight and day. Body weight gains were reduced and the teeth were white and spotty after fluoride doses of 31.5 mg/kg body weight and day and above. After fluoride doses of 47.3 mg/kg body weight and day and above, increased mortality, acute nephrosis, lesions in the liver and myocardium and necrosis or degeneration of the seminiferous tubules were found (NTP 1990). A NOAEL corresponding to fluoride doses of 1.6 mg/kg body weight and day was obtained for systemic toxicity in mice.

In a 2-year study with B6C3F_1 mice given sodium fluoride doses of 0, 25, 100 or 175 mg/kg body weight and day (♂: fluoride doses of 0, 1.1, 4.3, 7.5 mg/kg body weight and day; ♀: 0, 1.3, 5.1, 8.5 mg/kg body weight and day) with the drinking water, dental fluorosis and a dose-dependent increase in the fluoride concentration in bones and urine were observed after fluoride doses of 4.3 or 5.1 mg/kg body weight and day and above (males and females, respectively) (Bucher et al. 1991; NTP 1990). A NOAEL corresponding to fluoride doses of 1.1 mg/kg body weight and day was obtained for the mouse.

In the CD-1 mice of a 2-year feeding study given sodium fluoride doses of 0, 4, 10 or 25 mg/kg body weight and day (fluoride doses of about 0, 1.8, 4.5, 11 mg/kg body weight and day), the fluoride concentration in teeth, bones and stomach increased in a dose-dependent manner (see also Section 5.7). Osteomas were found in all treated groups and in the controls. In addition, mostly C type virus particles were found in the osteomas. In view of this, the authors conclude that induction of the osteomas was virus-based (Maurer et al. 1993). However, the C-type virus particles were not found in all osteomas. Direct proof that the C-type virus particles induced the osteomas was not provided. This 2-year study is therefore of limited relevance (see Section 5.7).

Rabbit

In a 6-month study in which rabbits were given daily gavage doses of sodium fluoride of 0, 10 or 20 mg/kg body weight (fluoride doses of about 0, 4.5 and 9 mg/kg body weight), effects in the lungs were found similar to those in Wistar rats (see above, Aydin et al. 2003). From this study a LOAEL (lowest observed adverse effect

level) for the lungs of rabbits was derived corresponding to fluoride doses of 4.5 mg/kg body weight and day (Purohit et al. 1999).

5.2.3 Subcutaneous injection

In rabbits given subcutaneous injections of sodium fluoride in doses of 0, 5, 10, 20 or 50 mg/kg body weight and day for 100 days, alveolar haemorrhages, necrosis of the alveolar epithelium and bronchiolitis were observed after doses of 10 mg/kg body weight and day (fluoride doses of 4.5 mg/kg body weight and day) and above. At the high dose of 50 mg/kg body weight and day, corresponding to fluoride doses of 22.5 mg/kg body weight and day, the lung parenchyma appeared distorted, accompanied by a loss of alveolar architecture. The effects increased with the fluoride dose (Shashi et al. 1988).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

After application of sodium fluoride in the form of a 0.5% or 1.0% solution to the shaved intact or abraded skin of rats, about 5 cm skin was removed, the tissue homogenized and centrifuged and the fluoride content analyzed with a spectrofluorometric method dating from 1959. Sodium fluoride produced no microscopically visible effects on the shaved intact skin. Inflammation, oedema and superficial necrosis were observed on the abraded skin (Essman et al. 1981). For practical purposes, only the results with the non-abraded epidermis of the rat skin have any relevance. After 24-hour application of the 1% sodium fluoride solution, about 0.1% sodium fluoride remained in the skin. The authors report a mean fluoride concentration of 1.48 µg/mg the tissue. This indicates a very low level of dermal penetration. From an analytical point of view, the method used is very problematical and does not adhere to present-day analytical procedures. Effects such as necrosis/oedema are described only for the abraded skin.

Sodium fluoride was not irritating in one test (no other details; Marhold 1972) and very irritating to the skin in another (no other details; ECB 2000 a). No investigations are available for other fluoride compounds.

5.3.2 Eyes

In the eyes, the irritative effects of sodium fluoride were moderate (no other details; Marhold 1972) or pronounced (no other details; ECB 2000 a). No investigations of other fluorides are available.

5.4 Allergenic effects

There are no studies available of the allergenic effects of fluorides in animals.

5.5 Reproductive toxicity

5.5.1 Fertility

There are numerous studies available of the effects of sodium fluoride on male fertility. Sodium fluoride doses of 0, 10 or 20 mg/kg body weight and day (corresponding to fluoride doses of 0, 4.5 or 9 mg/kg body weight and day) for 30 days led in male mice to histological changes in the epithelial cells of the seminiferous tubules, an absence of sperms in the lumen, reduced sperm counts and sperm motility and a loss of fertility (Chinoy and Sequeira 1989, 1992). Reduced sperm counts and sperm motility, and reduced fertility were likewise found in male rats after sodium fluoride doses of 5 mg/kg body weight and day (corresponding to fluoride doses of 2.25 mg/kg body weight and day) and above (Chinoy und Sequeira 1992).

In male CD rats given sodium fluoride with the diet in concentrations of 100 or 200 mg/kg (fluoride doses of about 4.5 and 9 mg/kg body weight and day) for 60 days, histological changes in the seminiferous tubules and at the high dose reduced testosterone levels in the serum were observed (Araibi et al. 1989). However, these findings could not be confirmed in other well-performed studies. Intratesticular injection of high doses of sodium fluoride did not produce substance-related testicular effects (Sprando et al. 1996). Also the administration of sodium fluoride in the drinking water in concentrations of 0, 25, 100, 175 or 250 mg/l (fluoride doses of about 0, 0.7, 2.8, 5 or 7 mg/kg body weight and day, assuming water consumption to be 25 ml per day and body weights of 400 g) to male Sprague-Dawley rats over two generations did not lead to detectable impairment of male fertility. Detailed histopathological examination of the reproductive organs likewise revealed no signs of damage (Sprando et al. 1997, 1998). A NOAEL for male fertility of 250 mg/l (corresponding to fluoride doses of about 7 mg/kg body weight and day) can be derived from this study.

Prior to mating, groups of 48 male and 48 female rats were given sodium fluoride in the drinking water daily for 10 weeks in concentrations of 0, 25, 100, 175 or 250 mg/l. In both generations, the daily fluoride dose (calculated from the water consumption) was 0, 1.6, 6.2, 9.7 and 12.5 mg/kg body weight for the female rats and 0, 1.4, 5.1, 8.3 and 10.7 mg/kg body weight for the male rats. Ten male and 10 female rats of the parent generation or of the F_1 offspring were subjected at the age of 25 to 27 days and 90 days to complete histopathological examination. Eight pregnant rats per group were subjected to caesarean section and the offspring were examined (see Section 5.5.2). The offspring of the other parent animals were reared and 36 rats per sex and group were treated for 10 weeks with water containing

fluoride and then mated 1:1 within the group. The dams and their offspring were investigated after caesarean section on day 20 of gestation (see Section 5.5.2). Water consumption was significantly reduced after fluoride doses of 9.7 mg/kg body weight and day and above in the parent generation and in the female F_1 animals and after fluoride doses of as little as 5.1 mg/kg body weight and day in the male rats of the F_1 generation. No effects on reproduction were found. The hyperkeratosis that occurred between the glandular stomach and the forestomach in some parent animals (1 ♂, 3 ♀ at fluoride doses of 5.1 and 6.2 mg/kg body weight, respectively, 1 ♂ at 8.3 mg/kg body weight and 1 ♀ at 12.5 mg/kg body weight) was attributed to the toxic effects of sodium fluoride but did not affect food consumption, growth or reproduction (Collins et al. 2001 a).

5.5.2 Developmental toxicity

Groups of 33 to 37 pregnant rats were given sodium fluoride in the drinking water in concentrations of 0, 10, 25, 100, 175 or 250 mg/l daily throughout the entire gestation period. The daily doses of sodium fluoride were reported to be 0, 1.4, 3.9, 15.6, 24.7 or 25.1 mg/kg body weight and day. This corresponds to fluoride doses of 0, 0.63, 1.8, 7, 11.1 and 11.3 mg/kg body weight and day. The drinking water consumption was reduced in the two high-dose groups. Food consumption and body weight gains were reduced in the dams of the high-dose group. In this group, a slight but statistically significant increase in foetuses with skeletal variations (0.4 affected foetuses per litter compared with 0.1 in the controls) was observed; the number of affected litters was increased (21.6% compared with 8.8% in the controls) but the increase was not statistically significant (Collins et al. 1995). No other toxic effects on development were found. The NOAEL for developmental toxicity and maternal toxicity thus corresponds to fluoride doses of 11.1 mg/kg body weight.

Groups of 48 male and female rats were given sodium fluoride in the drinking water in concentrations of 0, 25, 100, 175 or 250 mg/l daily for 10 weeks prior to mating and then the animals were mated as described in Section 5.5.1. The daily fluoride doses (calculated from the water consumption) were, in both generations, 0, 1.6, 6.2, 9.7 or 12.5 mg/kg body weight for the female rats and 0, 1.4, 5.1, 8.3 or 10.7 mg/kg body weight for the male rats. No maternal or embryotoxic abnormalities were found in either the parent generation or the F_1 generation. In the F_2 generation, the incidence of rib variations per litter was significantly increased in the foetuses of the group given fluoride doses of 9.7 mg/kg body weight and day, as was the incidence of specific skeletal variations in the foetuses of the group given fluoride doses of 12.5 mg/kg body weight and day; these effects were accompanied by a significant decrease in maternal body weights and food and water consumption (Collins et al. 2001 b). The dose of 9.7 mg/kg body weight and day was regarded as the NOAEL for developmental toxicity and maternal toxicity in the rat.

A significant increase in foetuses with skeletal and visceral anomalies was observed also in rats given gavage doses of sodium fluoride corresponding to fluoride doses of 18 mg/kg body weight and day on days 6 to 19 of gestation. More foetuses with wavy ribs, with a 14th rib, with altered 5th or 2nd or absent 5th sternebrae, with incomplete ossification of the skull and with thickened tibiae were observed. The body weights and body weight gains of the dams and food consumption were significantly reduced. The number of living foetuses was not significantly changed (Guna Sherlin and Verma 2001).

Groups of 26 pregnant rats were given sodium fluoride in deionized water on days 6 to 15 of gestation in concentrations of 0, 50, 150 or 300 mg/l corresponding to sodium fluoride doses of 0, 6.6, 18.3 or 27.1 mg/kg body weight and day. The total fluoride intake via drinking water and feed was reported to be 0, 1.0, 4.0, 9.3 or 13.2 mg/kg body weight and day. In the high-dose group, drinking water consumption was reduced as a result of bad palatability. No other significant effects on the dams or offspring were found. Therefore, the NOAEL for maternal toxicity corresponds to fluoride doses of 9.3 mg/kg body weight and day, and the NOAEL for developmental toxicity to doses of 13.2 mg/kg body weight and day (Heindel et al. 1996).

Sodium fluoride was administered via the drinking water to pregnant rabbits in concentrations of 0, 100, 200 or 400 mg/l, corresponding to sodium fluoride doses of 0, 10.3, 18.1 or 29.1 mg/kg body weight and day on days 6 to 19 of gestation. This corresponds to total fluoride doses of 0.8, 5.8, 8.8 or 13.8 mg/kg body weight and day. In the animals of the high-dose group, water intake was reduced. Also food consumption and body weights were reduced on days 6 to 8 of gestation. No other significant maternal or embryotoxic effects were observed. The NOAEL for maternal toxicity was stated to be fluoride doses of 8.8 mg/kg body weight and day, and the NOAEL for developmental toxicity to be 13.8 mg/kg body weight and day (Heindel et al. 1996).

5.6 Genotoxicity

A review of the genotoxic effects of fluorides can be found in the ATSDR report (ATSDR 2003) and the WHO report (WHO 2002) as well as in Zeiger et al. (1993). The available studies were carried out with sodium fluoride, potassium fluoride and hydrogen fluoride.

5.6.1 In vitro

No mutations were induced in *Salmonella* mutagenicity tests with either sodium fluoride or hydrogen fluoride (ATSDR 2003; Bayer AG 1987; NTP 1990, 2005; WHO 2002). The *Salmonella typhimurium* strain TA98 is reported not to take up fluorides in relevant amounts (Ahn and Jeffery 1994).

Sodium fluoride was not found to induce DNA damage in UDS tests (for unscheduled DNA synthesis) with primary rat hepatocytes (Skare et al. 1986 a; Tong et al. 1988) nor in the human diploid fibroblast cell line Wi-38 (Skare et al. 1986 a). The results of other UDS tests are not included in the evaluation because hydroxyurea, a DNA repair inhibitor, was used (Tsutsui et al. 1984 a, b, c), and therefore no clear evidence of repair can be obtained.

Sodium fluoride did not induce an increase in SCE (sister chromatid exchange) in human peripheral lymphocytes after concentrations of 4 to 420 µg/ml or 2 to 160 µg/ml or 10 to 30 µg/ml (fluoride concentrations of about 1.8–189 µg/ml and 0.9–72 µg/ml and 4.5–13.5 µg/ml) (Gadhia and Surat 1997; Thomson et al. 1985; Tong et al. 1988) or after sodium fluoride concentrations of 2 to 160 µg/ml (fluoride concentrations of 0.9–72 µg/ml) in CHO (Chinese hamster ovary) cells (Li et al. 1987 b; NTP 1990, 2005; Tong et al. 1988) or after sodium fluoride concentrations of 0.04 to 4.2 µg/ml or potassium fluoride concentrations of 0.06 to 5.81 µg/ml (fluoride concentrations of about 0.02–1.9 µg/ml) in bone marrow cells of the rat (Khalil and Da'dara 1994). On the other hand, an increase in SCE in SHE (Syrian hamster embryo) cells was observed after sodium fluoride concentrations of 20 µg/ml (fluoride concentrations of 9 µg/ml) after incubation for 24 hours (Tsutsui et al. 1984 c) and in CHO cells after sodium fluoride concentrations of 66.7 µg/ml (fluoride concentrations of about 30 µg/ml) without metabolic activation, or after sodium fluoride concentrations of 1200 µg/ml (fluoride concentrations of about 540 µg/ml) with metabolic activation (NTP 1990, 2005). The differences in preparation time required to compensate for the delay in the cell cycle, and the differences in sensitivity to sodium fluoride of individual cell cycle stages can, in part, explain the inconsistency in the results from the SCE tests.

In L5178YTK$^{+/-}$ mouse lymphoma cells, gene mutation was induced at the TK locus by sodium fluoride concentrations of 62.5 µg/ml (fluoride concentrations of about 28 µg/ml) after incubation for 16 hours in the presence and absence of metabolic activation (Caspary et al. 1987; Cole et al. 1986; NTP 1990, 2005). The authors suspect that the small mutated colonies induced were caused by chromosomal damage and not by point mutations. This is supported by the absence of induced ouabain-resistant mutants in the same cells. Likewise, gene mutations were found at the TK locus or at the HPRT locus in human lymphoblastoid cells after exposure to sodium fluoride concentrations of 200 to 600 µg/ml (fluoride concentrations of about 90–270 µg/ml) for 20 hours (Caspary et al. 1987; NTP 1990, 2005), and to concentrations of 200 to 600 µg/ml or 600 µg/ml (fluoride concentrations of about 90–270 µg/ml) for 28 hours (Crespi et al. 1990). After exposure to minimally toxic sodium fluoride concentrations of 65 µg/ml (fluoride concentrations of about 29 µg/ml) and above for 20 days, mutations were observed at the TK locus. At the HPRT locus, however, no induction of mutations was observed (Crespi et al. 1990). Likewise, no mutagenicity was found at the HPRT locus in an epithelial liver cell line of the rat up to sodium fluoride concentrations of 160 µg/ml for 72 hours (fluoride concentrations of about 72 µg/ml) (Tong et al. 1988) or at the TK locus in V79 cells after exposure to sodium fluoride concentrations of 10 to 400 µg/ml

(fluoride concentrations of about 4.5–180 µg/ml) for 24 hours (Slamenova et al. 1992).

The induction of chromosomal aberrations, primarily of gaps and chromatid breaks, has been demonstrated with various mammalian cells. These observations appear to depend greatly on the study protocol used (Aardema et al. 1989; Li et al. 1988). Chromosomal aberrations were induced mainly in the form of gaps and chromatid breaks in CHO cells at sodium fluoride concentrations of 25 to 100 µg/ml (fluoride concentrations of about 11–45 µg/ml) (Aardema et al. 1989) and in SHE cells at sodium fluoride concentrations of 50 and 100 µg/ml (fluoride concentrations of about 22.5 and 45 µg/ml) (Tsutsui et al. 1984 c). In CHO cells sodium fluoride induced most of the chromosomal aberrations in the G2 phase of the cell cycle, that is about 8 to 12 hours after exposure. After 20 hours, corresponding to exposure in the G1/S phase, only a slight increase in aberrant cells was found. This means that, in the case of exposure to sodium fluoride, the G2 phase is the most sensitive stage in the cell cycle. Further investigations of the sensitivity of the G2 phase cells to sodium fluoride produced an increase in the number of aberrant cells at low concentrations. After exposure for 3 hours to sodium fluoride concentrations of 10 µg/ml (fluoride concentrations of 4.5 µg/ml), no increase in the number of aberrant cells was detectable (Aardema et al. 1989). In an NTP study (1990, 2005) with CHO cells, sodium fluoride concentrations of up to 200 µg/ml (fluoride concentrations of about 90 µg/ml) were not found by the first laboratory to induce chromosomal aberrations without metabolic activation and a preparation time of 20.5 hours. However, the second laboratory found chromosomal aberrations at higher concentrations of 400 to 600 µg/ml (fluoride concentrations of about 180–270 µg/ml) without metabolic activation and a shorter preparation time of 13 hours. With metabolic activation chromosomal aberrations were not induced at concentrations up to 1600 µg/ml (fluoride concentrations of about 720 µg/ml) irrespective of the preparation time (NTP 1990, 2005). In cultured human lymphocytes and fibroblasts, chromosomal aberrations were reported after exposure to sodium fluoride concentrations in the range of 20 to 40 µg/ml (fluoride concentrations of about 9–18 µg/ml) (Albanese 1987; Scott and Roberts 1987; Tsutsui et al. 1984 a). The variance of sodium fluoride-induced chromosomal aberrations in individual donors of oral keratinocytes was compared. Despite interindividual variations, an increase in chromosomal aberrations was found in all donors at concentrations of 952 and 1429 µM sodium fluoride (sodium fluoride concentrations of 40 and 60 µg/ml; fluoride concentrations of about 18 or 27 µg/ml) (Tsutsui et al. 1991).

Sodium fluoride did not induce chromosomal aberrations in CHO cells exposed for 3 hours to low concentrations of 1 to 10 µg/ml (fluoride concentrations of about 0.45–4.5 µg/ml) (Aardema et al. 1989) or in human fibroblasts exposed for 1 to 3 weeks (Tsutsui et al. 1995). No significant increase in gene mutations or chromosomal aberrations was reported in other investigations (Khalil 1995; NTP 1990, 2005; WHO 2002). The pattern of chromosomal aberrations, the increased induction of endoreduplicated cells, the increase in cell cycle duration and the increased sensitivity of the cells to sodium fluoride in the G2 phase indicate that sodium fluoride

has an indirect clastogenic effect via inhibition of DNA synthesis or repair (Aardema et al. 1989). However, no proof of this has yet been provided.

To summarize, it may be stated that fluorides are not mutagenic in *Salmonella* mutagenicity tests, but are able to produce clastogenic effects in vitro probably indirectly depending on the test protocol used as a result of the delays in cell cycle.

5.6.2 In vivo

Fluorides

Sodium fluoride induces recessive lethal mutations in *Drosophila* (Dominok and Miller 1990).

No DNA strand breaks were found after 2, 6 or 24 hours by means of alkaline elution in the testicular cells of Sprague-Dawley rats given single gavage doses of sodium fluoride of 0, 8.4, 28 or 84 mg/kg body weight (fluoride doses of about 0, 3.8, 13, 38 mg/kg body weight) or sodium fluoride doses of 0, 4.2, 14 or 42 mg/kg body weight (fluoride doses of about 0, 1.9, 6.3, 19 mg/kg body weight) for 5 days. Whether sodium fluoride really does not produce DNA strand breaks or whether this is the case only because the fluoride levels reached in the testes are low, still needs to be clarified (Skare et al. 1986 b).

No increase in the incidence of SCE was observed in the bone marrow of Chinese hamsters given single gavage doses of sodium fluoride of 0.1 to 130 mg/kg body weight (fluoride doses of about 0.05–58.5 mg/kg body weight) for 18 hours, or sodium fluoride concentrations of 0, 1, 10, 50 or 75 mg/l (fluoride doses of about 0, 0.02, 0.18, 0.9, 1.4 mg/kg body weight) with the drinking water for 24 weeks (Li et al. 1987 b, 1989). Also in the bone marrow of Swiss-Webster mice no SCE was induced over 7 generations after concentrations of 50 mg/l in the drinking water (fluoride doses of about 7.9 mg/kg body weight) (Kram et al. 1978).

Chromosomal aberrations mostly in the form of gaps were observed in the bone marrow of Swiss mice 6, 24, or 30 hours after oral, intraperitoneal and subcutaneous sodium fluoride doses of 10 to 40 mg/kg body weight (fluoride doses of about 4.5–18 mg/kg body weight) and sodium fluoride doses of 40 mg/kg body weight (fluoride doses of about 18 mg/kg body weight) and above (Pati and Bhunya 1987). The inclusion of gaps in the analysis of chromosomal aberrations is unusual, as it is very subjective. In addition, no concurrent positive controls were used. The relevance of this study is thus limited.

In Balb/c mice given sodium fluoride in drinking water in concentrations of 1 to 200 mg/l (fluoride doses of about 0.16–32 mg/kg body weight) for 3 or 6 weeks, a dose-dependent increase in chromosomal aberrations was observed in bone marrow or testicular cells after concentrations of 1 mg/l (fluoride doses of about 0.16 mg/kg body weight) and above. In this study, cells in the anaphase, metaphase and early telophase were evaluated and assessed together (Mohamed and Chandler 1982). The combined assessment of the data and the unusually high number of aberrant cells in the controls make interpretation of the results very difficult (Zeiger

et al. 1993). In a study with similar treatment for 6 weeks and concentrations in drinking water of 1 to 100 mg/l (fluoride doses of about 0.16–16 mg/kg body weight), no chromosomal aberrations were found in metaphase cells of the bone marrow and testes of the same strain of mice. The fluoride concentration, which increased in a dose-dependent manner, was determined in bone (Martin et al. 1979). Studies with Swiss-Webster mice over 5 generations and concentrations in drinking water of 50 mg/l (fluoride doses of about 7.9 mg/kg body weight) yielded negative results both in the bone marrow and the testes (Martin et al. 1979).

In another study, B6C3F_1 mice were given sodium fluoride in the drinking water for up to 6 weeks in concentrations corresponding to fluoride concentrations of 0, 100, 200 or 400 mg/l. This corresponds to fluoride doses of 0, 25, 50 or 75 mg/kg body weight and day. A reduction in body weight gains was observed in the groups given 50 or 75 mg/kg body weight and day. In the high-dose group, a number of animals died. The fluoride levels in bone were determined and found to increase with the dose. In bone marrow cells no increase in the formation of chromosomal aberrations was observed, either in anaphase or metaphase cells. Likewise, no increase in the formation of micronuclei was observed in peripheral erythrocytes (Zeiger et al. 1994).

No micronuclei were found in B6C3F_1 mice given gavage doses of sodium fluoride of 0.1 to 80 (♂) or 115 (♀) mg/kg body weight (fluoride doses of about 0.05–36 (♂) or 52 (♀) mg/kg body weight) for 30, 48 or 72 hours. Doses up to the maximum tolerable dose were tested (Li et al. 1987 a).

No micronuclei were found in the bone marrow of the Alpk rat 24 or 48 hours after single gavage doses of sodium fluoride of 0, 500 or 1000 mg/kg body weight (fluoride doses of about 0, 225 or 450 mg/kg body weight). The sodium fluoride dose of 1000 mg/kg body weight was cytotoxic (Albanese 1987). Intraperitoneal administration of sodium fluoride doses of 0, 10, 20, 30 or 40 mg/kg body weight (fluoride doses of about 0, 4.5, 9, 12.5, 16 mg/kg body weight) twice within 24 hours led, on the other hand, after 30 hours to an increase in the frequency of micronuclei in polychromatic erythrocytes of the bone marrow of Swiss mice after sodium fluoride doses of 10 mg/kg body weight and above (Pati and Bhunya 1987). Unlike in the other studies of micronucleus induction described above, no positive control was included.

Hydrogen fluoride

A number of earlier investigations of the genotoxicity of hydrogen fluoride in vivo are available (ECB 2000 c). As a result of methodological shortcomings (for example, only one concentration group; inadequate documentation of the methods and results) it is not possible, however, to make a conclusive evaluation from these investigations. In *Drosophila melanogaster*, hydrogen fluoride induced X-chromosome-bound recessive lethal mutations (WHO 1984).

In rats exposed to hydrogen fluoride concentrations of 1 mg/m³ (1.2 ml/m³) for 6 hours a day for one month, an increase in the incidence of chromosomal aberrations was found in bone marrow cells, especially in old animals (Voroshilin et al. 1975). After short-term or long-term exposure of rats, impairment of the chromosomal apparatus in bone marrow cells was reported (Tazhibaev et al. 1987). In a cytogenetic investigation in mice exposed to hydrogen fluoride concentrations of 0.1 mg/m³ (hydrogen fluoride concentrations of 0.12 ml/m³; corresponding to fluoride concentrations of 0.95 mg/m³) for 2 months, no increase in the incidence of cells with translocations was found in the testes (Voroshilin et al. 1973). In dominant lethal tests, male mice were exposed to hydrogen fluoride concentrations of 1 mg/m³ (hydrogen fluoride concentrations of 0.12 ml/m³; corresponding to fluoride concentrations of 0.95 mg/m³) for 2 or 4 weeks. No dominant lethal mutations were observed (Voroshilin et al. 1975).

The overall picture presented by the in vivo results regarding the genotoxicity of fluorides is inconsistent. A number of experiments yielded positive results after toxic doses only, others produced positive results even after fluoride concentrations as low as 1 to 5 mg/l—concentrations which are close to the dose ingested by humans via drinking water and food that leads to effects. Chromosomal aberrations were observed in anaphase cells which in other experiments using the same dose and protocol were not detectable, however, in metaphase cells. This is very difficult to explain without any further data. Contradictory results were obtained also in various experiments investigating the induction of micronuclei. At present, it is not possible to clarify whether fluorides cause damage to chromosomes in vivo or not.

5.7 Carcinogenicity

Only investigations of sodium fluoride are available.

In a carcinogenicity study of the NTP, sodium fluoride was administered to at least 50 F344 rats and B6C3F$_1$ mice per group and sex with the drinking water in concentrations of 0, 25, 100 or 175 mg/l. The doses corresponded to fluoride doses of about 0, 0.6, 2.3 and 3.9 mg/kg body weight for male rats and 0, 0.6, 2.5 and 4.3 mg/kg body weight for female rats or 0, 1.1, 4.3 and 7.5 mg/kg body weight for male mice and 0, 1.3, 5.1 and 8.5 mg/kg body weight for female mice. Survival and body weights were not affected in either rats or mice. Only in male rats was there an increase in the incidence of osteosarcomas (controls: 0/80; low dose: 0/51; medium dose: 1/50; high dose: 3/80). The authors pointed out that osteosarcomas occurred in historical controls with an incidence of 0.5% (range 0% to 6%). However, the historical controls are not directly comparable, because examination of bones was more comprehensive in this study than in previous studies, and the fluoride content of the feed was not investigated previously. The NTP regards the results for male rats as “equivocal evidence of carcinogenic activity”. For female rats and for mice, the results are described as “no evidence of carcinogenic activity” (Bucher

et al. 1991; NTP 1990). The medium and high doses in the male rats correspond to fluoride doses of about 2.3 and 3.9 mg/kg body weight and day. For a worker with a body weight of 70 kg and a respiratory volume of 10 m^3 per 8-hour shift, this is equivalent to an air concentration of about 16 or 27 mg/m^3.

In a combined toxicity/carcinogenicity study with Sprague-Dawley rats (Maurer et al. 1990) and CD mice (Maurer et al. 1993), sodium fluoride was administered to the animals in the feed for almost 2 years (99 or 97 weeks) in doses of 0, 4, 10 or 25 mg/kg body weight and day. This corresponds to fluoride doses of 0, 1.8, 4.5 and 11 mg/kg body weight and day. Seventy rats and 60 mice were used per group and sex. In rats and mice, changes in teeth and bones occurred in all dose groups with increasing incidence and intensity. Reduced body weight gains were observed in rats of the high-dose group, but not in mice. In rats, the incidence of preneoplastic or neoplastic changes was not increased. In mice of the high-dose group, the number of animals with multiple osteomas was, however, increased (controls: 3; diet control 3; low dose: 4; medium dose: 4; high dose: 26). The authors present a number of arguments in favour of a viral cause of the osteomas. The incidence of osteomas in the control groups is higher than in historical controls, retroviral particles were detected in the osteomas and the osteomas were of periosteal origin. On the other hand, in one animal osteomas were described in which no virus particles were found. Direct proof that the virus particles induced the osteomas was not provided (for example, by means of antisera and the inoculation of other animals with tumour material containing viruses). At the high dose, the number of osteomas increased markedly. The mechanism by which sodium fluoride in combination with C type virus particles produces osteomas is not known. Osteomas are rare tumours in most strains of laboratory animal, including the CD-1 strain used. The OF-1 strain is one of the exceptions, but the tumours are mainly distributed over other bone regions (Wilson et al. 1985). There is still a suspicion that sodium fluoride in high doses is able to produce osteomas also without the influence of C type virus particles. This should be clarified.

6 Manifesto (MAK value, classification)

The data available for the carcinogenicity of fluorides in humans after ingestion do not allow any conclusive assessment to be made. No clear statement on the carcinogenic effects of sodium fluoride can be derived from two carcinogenicity studies with rats and mice given sodium fluoride with the drinking water. There is, however, evidence for the occurrence of osteosarcomas in male rats at high sodium fluoride doses which needs further clarification. In another study, osteomas were observed in mice. However, virus particles were detected by electron microscope in all mice with osteomas with one exception. The data available at present do not justify classification in a carcinogenicity category; the suspected carcinogenicity should, however, be investigated. Fluorides have clastogenic effects in vitro, but the

mechanism of action has not been clarified. As regards genotoxicity, the picture in vivo is very inconsistent: chromosomal aberration tests and micronucleus tests in vivo yielded conflicting results with comparable test protocols. For this reason, it cannot be decided at present whether fluorides produce chromosomal damage in vivo or not.

The induction of skeletal fluorosis is the most important effect of all fluoride compounds as regards systemic toxicity. The previous MAK value of 2.5 mg/m^3 (inhalable fraction) was established on the basis of the systemic effects of fluorides on the human skeletal system. Fluoride concentrations in the air of more than 2.4 to 6 mg/m^3 or 3.4 mg/m^3 (8.7 mg/l urine or 5.18 mg/l urine) resulted in skeletal fluorosis in workers exposed for 10 years and more (Derryberry et al. 1963; Kaltreider et al. 1972). In contrast, no effects on the skeleton were found in workers exposed for 10 years to average fluoride concentrations of 2.4 or 2.65 mg/m^3, that is at the level of the previous MAK value (Derryberry et al. 1963; Kaltreider et al. 1972). However, more recent data from persons exposed to fluoride and animal experiments indicate that adherence to this value is possibly not sufficient protection against effects on the bones. Clinical stage III skeletal fluorosis may be reached after 20 years of exposure to doses of 20 mg per day and above (US DHHS 1991 a, b; WHO 2002). Accordingly, the MAK value of 2.5 mg/m^3, which would correspond to 25 mg per day in the case of complete absorption, is too high. In Germany, an adult absorbs 0.7 to 1.2 mg fluoride daily via water and food if fluoridated salt is taken into account (Bergmann and Bergmann 1995). The Environmental Protection Agency (EPA 1985) prescribes an upper limit of 10 mg per day. This value is based on the observation that no skeletal fluorosis has occurred in the USA at a fluoride concentration in drinking water of 4 mg/l, which corresponds to a dose of 8 mg with an intake of 2 l drinking water per day. From this upper limit, a MAK value of 1 mg/m^3 is obtained (assuming 10 m^3 air is inhaled per 8 hour shift).

This MAK value finds support from a Chinese study indicating that the intake of 14 mg fluoride per day (fluoride doses of about 0.25 mg/kg body weight; corresponding to 1.5 mg/m^3 assuming body weight to be 70 kg and the volume of air inhaled in 8 hours to be 10 m^3) over 20 years results in a greater number of bone fractures, which was not found at doses of 7.85 mg and day (about 0.15 mg/kg body weight; corresponding to 1 mg/m^3 with a body weight of 70 kg and 10 m^3 air inhaled in 8 hours). The study of Hillier et al. (2000), in which no increase in hip fractures was found below 0.2 to 0.3 mg per day, does not contradict this assessment.

Furthermore, a study with rats showed bone strength to be reduced after fluoride doses of 3.2 mg/kg body weight and day and above (Turner et al. 2001) and gave a NOAEL of 0.94 mg/kg body weight and day (fluoride concentration in drinking water of 15 mg/l). As the doses in the rats were selected to correspond to fluoride concentrations in the drinking water of humans of 0, 1, 3 or 10 mg/l, the NOAEL for the rat corresponds to concentrations in the drinking water of humans of 3 mg/l (Turner et al. 2001), or concentrations in air of 0.9 mg/m^3 (assuming humans to inhale 10 m^3 air and consume 3 litres of drinking water per day).

To monitor the internal exposure to fluorides, the BAT value must be considered.

To avoid the effects of hydrogen fluoride on the respiratory tract, a MAK value for hydrogen fluoride of 2 ml/m^3 (1.66 mg/m^3) was established. However, at this value fluorides cause systemic effects (see above). Therefore, the MAK value for hydrogen fluoride has correspondingly been reduced to 1 ml/m^3 (0.83 mg/m^3) (see Supplement "Fluorwasserstoff" 2006, "Hydrogen fluoride" only available in German). As the irritative effects of hydrogen fluoride are stronger than those of calcium fluoride and sodium fluoride, it can be assumed that the MAK value of 1 mg/m^3 will also protect against the local irritative effects of these fluorides in the respiratory tract.

As systemic toxicity is the main effect of the fluorides, classification in Peak Limitation Category II has been confirmed. In view of the half-life of fluorides of 2 to 9 hours in human blood, short-term exposure peaks do not alter blood fluoride levels to any notable extent. In addition, fluoride accumulates in bone, and peaks in the blood fluoride level are not of decisive importance. Therefore, the excursion factor has been changed from 2 to 4.

On account of the low dermal LD_{50} in the mouse, absorption through the skin must be assumed to take place in relevant amounts. As the MAK value is very low, and as it was established on the basis of systemic toxicity, notable absorption through the skin cannot be excluded; the water-soluble fluorides are therefore provisionally designated with an "H".

Studies are available of the developmental toxicity of sodium fluoride in rats and rabbits. The NOAEL for developmental toxicity in the rat corresponds to fluoride doses of 9.7 mg/kg body weight and day, and in rabbits to doses of 13.8 mg/kg body weight and day. These values are higher by a factor of 10 than the NOAEL for systemic toxicity in rats. Fluorides have therefore been assigned to Pregnancy Risk Group C.

No data are available which would justify designation with "Sh" or "Sa" or classification in a germ cell mutagen category.

7 References

Aardema MJ, Gibson DP, LeBoeuf RA (1989) Sodium fluoride-induced chromosome aberrations in different stages of the cell cycle: a proposed mechanism. *Mutat Res* 223: 191–203

Ahn HW, Jeffery EH (1994) Effect of aluminium on fluoride uptake by Salmonella typhimurium TA98; implications for the AMES mutagenicity assay. *J Toxicol Environ Health* 41: 357–368

Albanese R (1987) Sodium fluoride and chromosome damage (in vitro human lymphocyte and in vivo micronucleus assays). *Mutagenesis* 2: 497–499

Araibi AAA, Yousif WH, Al-Dewachi OS (1989) Effect of high fluoride on the reproductive performance of the male rat. *J Biol Sci Res* 20: 19–29

ATSDR (Agency for Toxic Substances and Disease Registry) (2003) Fluorides, hydrogen fluoride, fluorine report,
www.atsdr.cdc.gov/tfacts11.html

Augenstein WL, Spoerke DG, Kulig KW, Hall AH, Hall PK, Riggs BS, El Saadi M, Rumack BH (1991) Fluoride ingestion in children: a review of 87 cases. *Pediatrics* 88: 907–912

Aydin G, Cicek E, Akdogan M, Gökalp O (2003) Histopathological and biochemical changes in the lung tissues of rats following administration of fluoride over several generations. *J Appl Toxicol* 23: 437–446

Baltazar RF, Mower MM, Reider R, Funk M, Salomon J (1980) Acute fluoride poisoning leading to fatal hyperkalemia. *Chest* 78: 660–663

Bayer AG (1987) Hydrogen fluoride 71/75% Salmonella/microsome test to evaluate for point-mutagenic effects. Report No. 16300, Study No. T 4025962, Bayer AG, Wuppertal

Bayless JM, Tinanoff N (1985) Diagnosis and treatment of acute fluoride toxicity. *J Am Dental Assoc* 110: 209–211

Bergmann KE, Bergmann RL (1995) Salt fluoridation and general health. *Adv Dent Res* 9: 138–143

Bucher JR, Hejtmancik MR, Toft II JD, Persing RL, Eustis SL, Haseman JK (1991) Results and conclusions of the National Toxicology Program's rodent carcinogenicity studies with sodium fluoride. *Int J Cancer* 48: 733–737

Calderon J, Machado BM, Navarro M-E, Carrizales L, Ortiz MD, Diaz-Barriga F (2000) Influence of fluoride exposure on reaction time and visuospatial organization in children (Abstract). *Epidemiology* 11: S153

Caspary WJ, Myhr B, Bowers L, McGregor D, Riach C, Brown A (1987) Mutagenic activity of fluorides in mouse lymphoma cells. *Mutat Res* 187: 165–180

Chan-Yeung M, Wong R, MacLean L, Tan F, Schultzer M, Enarson D, Martin A, Dennis R, Grzybowski S (1983 a) Epidemiologic health study of workers in an aluminium smelter in Kitimat, British Columbia. Effects on the respiratory system. *Am Rev Respir Dis* 127: 465–469

Chan-Yeung M, Wong R, Tan F, Enarson D, Schulzer M, Subbarao K, Knickerbocker J, Grzybowski S (1983 b) Epidemiologic health study of workers in an aluminium smelter in Kitimat, British Columbia II. Effects on musculoskeletal and other systems. *Arch Environ Health* 38: 34–40

Chen X, Machida K, Ando M (1999) Effects of fluoride aerosol inhalation on mice. *Fluoride* 32: 153–161

Chinoy NJ, Sequeira E (1989) Effects of fluoride on histoarchitecture of reproductive organs of male mouse. *Reprod Toxicol* 3: 261–267

Chinoy NJ, Sequeira E (1992) Reversible fluoride induced fertility impairment in male mice. *Fluoride* 25: 71–76

Chinoy NJ, Pradeep PK, Sequeira E (1992) Effect of fluoride ingestion on the physiology of reproductive organs of male rats. *J Environ Biol* 13: 55–61

Cole J, Muriel WJ, Bridges BA (1986) The mutagenicity of sodium fluoride to L5178Y [wild-type and $TK^{+/-}$ (3.7.2c)] mouse lymphoma cells. *Mutagenesis* 1: 157–167

Collins TFX, Sprando RL, Shackelford ME, Black TN, Ames MJ, Welsh JJ, Balmer MF, Olejnik N, Ruggles DI (1995) Developmental toxicity of sodium fluoride in rats. *Food Chem Toxicol* 33: 951–960

Collins TFX, Sprando RL, Black TN, Shackelford ME, Bryant MA, Olejnik N, Ames MJ, Rorie JI, Ruggles DI (2001 a) Multigenerational evaluation of sodium fluoride in rats. *Food Chem Toxicol* 39: 601–613

Collins TFX, Sprando RL, Black TN, Shackelford ME, Bryant MA, Olejnik N, Ames MJ, Rorie JI, Ruggles DI (2001 b) Developmental toxicity of sodium fluoride measured during multiple generations. *Food Chem Toxicol* 39: 867–876

Crespi CL, Seixas GM, Turner T, Penman BW (1990) Sodium fluoride is a less efficient human cell mutagen at low concentrations. *Environ Mol Mutagen* 15: 71–77

DECOS (Dutch Expert Committee on Occupational Standards) (1989) Fluorine, hydrogenfluoride and inorganic fluoride compounds. Health-based recommended occupational exposure limit, RA 1/89 Sdu Uitgeverij, Den Haag, Netherlands

De Lopez OH, Smith FA, Hodge HC (1976) Plasma fluoride concentrations in rats acutely poisoned with sodium fluoride. *Toxicol Appl Pharmacol* 37: 75–83

Den Besten PK, Crenshaw MA (1984) The effects of chronic high fluoride levels on forming enamel in the rat. *Arch Oral Biol* 29: 675–679

Derryberry OM, Bartholomew MD, Fleming RBL (1963) Fluoride exposure and worker health. The health status of workers in a fertilizer manufacturing plant in relation to fluoride exposure. *Arch Environ Health* 6: 503–514

Dinman BD, Elder MJ, Bonney TB, Bovard PG, Colwell MO (1976a) Prevention of bony fluorosis in aluminum smelter workers. A 15-year retrospective study of fluoride excretion and bony radiopacity among aluminum smelter workers—Pt 4. *J Occup Med* 18: 21–23

Dinman BD, Bovard WJ, Bonney TB, Cohen JM, Colwell MO (1976 b) Prevention of bony fluorosis in aluminum smelter workers. Absorption and excretion of fluoride immediately after exposure—Pt 1. *J Occup Med* 18: 7–13

Dominok B, Miller G (1990) Effects of fluoride on Drosophila melanogaster in relation to survival and mutagenicity. *Fluoride* 23: 83–91

ECB (European Chemicals Bureau) (2000 a) Natrium (Sodium) Fluoride, IUCLID Data Sheet, 19.2.2000, ECB, Ispra, Italy

ECB (European Chemicals Bureau) (2000 b) Calcium Fluoride, IUCLID Data Sheet, 19.2.2000, ECB, Ispra, Italy

ECB (European Chemicals Bureau) (2000 c) Hydrogen Fluoride, IUCLID Data Sheet, 19.2.2000, ECB, Ispra, Italy

ECB (European Chemicals Bureau) (2000 d) Potassium Fluoride, IUCLID Data Sheet, 19.2.2000, ECB, Ispra, Italy

ECB (European Chemicals Bureau) (2000 e) Calcium Fluoride Phosphate, IUCLID Data Sheet, 19.2.2000, ECB, Ispra, Italy

Eichler HG, Lenz K, Fuhrmann M, Hruby K (1982) Accidental ingestion of NaF tablets by children. *Int J Clin Pharmacol Ther Toxicol* 20: 334–338

Ekstrand J (1978) Relationship between fluoride in the drinking water and the plasma fluoride concentration in man. *Caries Res* 12: 123–127

Ekstrand J, Spak CJ, Ehrnebo M (1982) Renal clearance of fluoride in a steady state condition in man: influence of urinary flow and pH changes by diet. *Acta Pharmacol Toxicol* 50: 321–325

EPA (Environmental Protection Agency) (1985) Drinking water criteria document on fluoride. Environmental Protection Agency, Office of Drinking Water (Contract 68–03–3279), Cincinnati, Ohio, USA

EPA (Environmental Protection Agency) (1991) Subchronic inhalation exposure study of hydrogen fluoride in rats. Environmental Protection Agency, Washington DC, USA

Essman EJ, Essman WB, Valderrama E (1981) Histaminergic mediation of the response of rat skin to topical fluorides. *Arch Dermatol Res* 271: 325–340

Freni SC (1994) Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. *J Toxicol Environ Health* 42: 109–121

Gadhia PK, Surat SJ (1997) Sodium fluoride induced chromosome aberrations and sister chromatid exchange in cultured human lymphocytes. *Fluoride* 30: 153–156

Guna Sherlin DM, Verma RJ (2001) Vitamin D ameliorates fluoride-induced embryotoxicity in pregnant rats. *Neurotoxicol Teratol* 23: 197–201

Harrison JE, Hitchman JW, Hasany SA, Hitchman A, Tam CS (1984) The effect of diet calcium on fluoride toxicity in growing rats. *Can J Physiol Pharmacol* 62: 259–265

Heindel JJ, Bates HK, Price CJ, Marr MC, Myers CB, Schwetz BA (1996) Developmental toxicity evaluation of sodium fluoride in rats and rabbits in drinking water. *Fundam Appl Toxicol* 30: 162–177

Henschler D, Lehnert G (Eds) (1983) Hydrogen fluoride and inorganic fluorine compounds (fluorides). In Biological Exposure Values for Occupational Toxicants and Carcinogens, Volume 1, 1994, VCH Verlagsges, Weinheim, Germany, 89–97

Hillier S, Cooper C, Kellingray S, Russell G, Hughes H, Coggon D (2000) Fluoride in drinking water and risk of hip fracture in the UK: a case-control study. *Lancet* 355: 265–269

Hodge HC, Smith FA (1977) Air quality criteria for the effects of fluorides on man. *J Air Pollut Control Assoc* 20: 226–232

Hodge HC, Smith FA, Gedalia I (1970) Excretion of fluorides. In: Fluorides and human health. WHO Monograph Series No 59, Geneva WHO, 158–159

IARC (International Agency for Research on Cancer) (1982) Inorganic fluorides used in drinking water and dental preparations. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol 27, IARC, Lyon, 237–303

Kaltreider NL, Elder MJ, Cralley LV, Colwell MO (1972) Health surveillance of aluminum workers with special reference to fluoride exposure. *J Occup Med* 14: 531–541

Khalil (1995) Chromosome aberrations in cultured rat bone marrow cells treated with inorganic fluorides. *Mutat Res* 343: 67–74

Khalil AM, Da'dara AA (1994) The genotoxic and cytotoxic activities of inorganic fluoride in cultured rat bone marrow cells. *Arch Environ Contam Toxicol* 26: 60–63

Korth Kristalle GmbH (2005) Löslichkeit der Fluoride (The solubility of fluorides) (German). www.korth.de

Kram D, Schneider EL, Singer L, Martin GR (1978) The effects of high and low fluoride diets on the frequencies of sister chromatid exchanges. *Mutat Res* 57: 51–55

Li Y, Dunipace AJ, Stookey GK (1987 a) Lack of genotoxic effects of fluoride in the mouse bone-marrow micronucleus test. *J Dent Res* 66: 1678–1690

Li YM, Heerema NA, Dunipace AJ, Stookey GK (1987 b) Genotoxic effects of fluoride evaluated by sister-chromatid exchange. *Mutat Res* 192: 191–201

Li Y, Dunipace AJ, Stookey GK (1988) Genotoxic effects of fluoride: a controversial issue. *Mutat Res* 195: 127–136

Li YM, Zhang W, Noblitt TW, Dunipace AJ, Stookey GK (1989) Genotoxic evaluation of chronic fluoride exposure: sister chromatid exchange study. *Mutat Res* 227: 159–165

Li XS, Zhi JL, Gao RO (1995 a) Effect of fluoride exposure on intelligence in children. *Fluoride* 28: 189–192

Li Y, Liang CK, Katz BP, Brizendine EJ, Stookey GK (1995 b) Long-term exposure to fluoride in drinking water and sister chromatid exchange frequency in human blood lymphocytes. *J Dent Res* 74: 1468–1474

Li Y, Liang C, Slemenda CW, Ji R, Sun S, Cao J, Emsley CL, Ma F, Wu Y, Ying P, Zhang Y, Gao S, Hang W, Katz BP, Niu S, Cao S, Johnston Jr CC (2001) Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. *J Bone Miner Res* 16: 932–939

Lim JK, Renaldo GJ, Chapman P (1978) LD_{50} of stannous fluoride, sodium fluoride and sodium monofluorophosphate in the mouse compared to the rat. *Caries Res* 12: 177–179

Litzka G (1936) Allgemeine biologische Wirkungen einer kernfluorierten Aminosäure (Fluortyrosin) (General biological effects of fluorotyrosin, an amino acid fluorinated in the nucleus) (German). *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol* 183: 427–435

Liu M-W, Tang Y-W, Huang C-H, Fang R-H (1997) Hydrofluoric acid burns of the hands. *J Surg Assoc Repub China* 30: 26–34

Lu Y, Sun ZR, Wu LN, Wang X, Lu W, Liu SS (2000) Effect of high-fluoride water on intelligence in children. *Fluoride* 33: 74–78

Marhold JV (1972) Sborník výsledků toxikologického vyšetření látek a přípravků (Collected results of toxicological investigations on materials and preparations) (Czech), Institut pro výchover vedoucích pracovníků chemického průmyslu (Prague), Czech Republic), 20

Martin GR, Brown KS, Matheson DW, Lebowitz H, Singer L, Ophaug R (1979) Lack of cytogenetic effects in mice or mutations in Salmonella receiving sodium fluoride. *Mutat Res* 66: 159–167

Maurer JK, Cheng MC, Boysen BG, Anderson RL (1990) Two-year carcinogenicity study of sodium fluoride in rats. *J Nat Cancer Inst* 82: 1118–1126

Maurer JK, Cheng MC, Boysen BG, Strandberg JD, Weisbrode SE, Seymour JL, Anderson RL (1993) Confounded carcinogenicity study with sodium fluoride in CD-1 mice. *Regul Toxicol Phamacol* 18: 154–168

Mohamed AH, Chandler ME (1982) Cytological effects of sodium fluoride in mice. *Fluoride* 15: 110–118

Nedeljković M, Matović V (1991) The effect of dose on maternal-foetal transfer of fluoride in rabbits. *Arh Hig Rada Toksikol* 42: 43–46

NTP (National Toxicology Program) (1990) Toxicology and carcinogenesis studies of sodium fluoride (CAS No. 7681-49-4) in F344/N rats and B6C3F1 mice (drinking water study). National Toxicology Program, Technical Report 393, US Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, USA

NTP (National Toxicology Program) (2005) Genotoxicity of sodium fluoride.
http://ntp-apps.niehs.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=7681% 2D49%2D4

Pati P, Bhunya S (1987) Genotoxic effect of an environmental pollutant, sodium fluoride, in mammalian in vivo test system. *Caryologia* 40: 79–87

Purohit SD, Gupta RC, Mathur AK, Gupta N, Jeswani ID, Choudhary VK, Purohit SK (1999) Experimental pulmonary fluorosis. *Indian J Chest Dis Allied Sci* 41: 27–34

Rao HV, Beliles RP, Whitford GM, Turner CH (1995) A physiologically based pharmacokinetic model for fluoride uptake by bone. *Regul Toxicol Pharmacol* 22: 30–42

Schiffl H, Binswanger U (1980) Renal handling of fluoride in healthy man. *Renal Physiol* 5: 192–196

Scott D, Roberts SA (1987) Extrapolation from in vitro tests to human risk: experience with sodium fluoride clastogenicity. *Mutat Res* 189: 47–58

Shanthakumari D, Srinivasalu S, Subramanian S (2004) Effect of fluoride intoxication on lipid peroxidation and antioxidant status in experimental rats. *Toxicology* 204: 219–228

Shashi A, Thapar SP, Singh JP (1988) Pulmonary damage caused by fluoride in rabbits during experimental fluorosis. *Acta Pathol Micrbiol Immunol Scand* 96: 333–336

Sigma-Aldrich (2005) Fluoride. www.sigma-aldrich.com

Skare J, Wong T, Evans L, Cody D (1986 a) DNA repair studies with sodium fluoride: comparative evaluation using density gradient ultracentrifugation and autoradiography. *Mutat Res* 172: 77–87

Skare J, Schrotel K, Nixon G (1986 b) Lack of DNA strand breaks in rat testicular cells after in vivo treatment with sodium fluoride. *Mutat Res* 170: 85–92

Slamenova D, Gabelova A, Ruppova K (1992) Cytotoxicity and genotoxicity testing of sodium fluoride on Chinese hamster V79 cells and human EUE cells. *Mutat Res* 279: 109–115

Sprando RL, Black TN, Ames MJ, Rorie JI, Collins TFX (1996) Effects of intratesticular injection of sodium fluoride on spermatogenesis. *Food Chem Toxicol* 34: 377–384

Sprando RL, Collins TFX, Black TN, Rorie J, O'Donnell M (1997) Testing the potential of sodium fluoride to affect spermatogenesis in the rat. *Food Chem Toxicol* 35: 881–890

Sprando RL, Collins TFX, Black TN, Olejnik N, Rorie J (1998) Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study. *Food Chem Toxicol* 36: 1117–1124

Susheela AK, Jethanandani P (1996) Circulating testosterone levels in skeletal fluorosis patients. *Clin Toxicol* 34: 183–189

Tazhibaev SS, Kozhakhmetova EB, Aldargenova KU, Mamyrbaev AA (1987) Modifying influence of nutrition on the mutagenic effect of phosphorus and fluorine compounds (Russian). *Vorp Pitan* 4: 63–66

Thomson EJ, Kilanowski FM, Perry PE (1985) The effect of fluoride on chromosome aberration and sister-chromatid exchange frequencies in cultured human lymphocytes. *Mutat Res* 144: 89–92

Tong CC, McQueen CA, Brat SV, Williams GM (1988) The lack of genotoxicity of sodium fluoride in a battery of cellular tests. *Cell Biol Toxicol* 4: 173–186

Tsutsui T, Suzuki N, Ohmori M, Maizumi H (1984 a) Cytotoxicity, chromosome aberrations and unscheduled DNA synthesis in cultured human diploid fibroblasts induced by sodium fluoride. *Mutat Res* 139: 193–198

Tsutsui T, Ide K, Maizumi H (1984 b) Induction of unscheduled DNA synthesis in cultured human oral keratinocytes by sodium fluoride. *Mutat Res* 140: 43–48

Tsutsui T, Suzuki N, Ohomori M (1984 c) Sodium fluoride-induced morphological and neoplastic transformation, chromosome aberrations, sister chromatid exchanges and unscheduled DNA synthesis in cultured Syrian hamster embryo cells. *Cancer Res* 44: 936–941

Tsutsui T, Kawamoto Y, Suzuki N (1991) Cytotoxicity and chromosome aberrations in normal human oral keratinocytes induced by chemical carcinogens: comparison of inter-individual variations. *Toxicol In Vitro* 5: 353–361

Tsutsui T, Tanaka Y, Matsudo Y, Uehama A, Someya T, Hamaguchi F, Yamamoto H, Takahashi M (1995) No increase in chromosome aberrations in human diploid fibroblasts following exposure to low concentrations of sodium fluoride for long times. *Mutat Res* 335: 15–20

Turner CH, Hinckley WR, Wilson ME, Zhang W, Dunipace AJ (2001) Combined effects of diets with reduced calcium and phosphate and increased fluoride intake on vertebral bone strength and histology in rats. *Calcif Tissue Int* 69: 51–57

US DHHS (United States Department of Health and Human Services) (1991 a) Public health service report on fluoride benefits and risks. Ad Hoc Subcommittee on Fluoride, Committee to Coordinate Environmental Health and Related Programs, Washington DC, USA, www.cdc.org/public/pubhsrvc.html

US DHHS (United States Department of Health and Human Services) (1991 b) Public health service report on fluoride benefits and risks. *J Am Med Assoc* 266: 1061–1067

Voroshilin SI, Plotko EG, Gatiyatullina EZ, Gileva EA (1973) Cytogenetic effect of inorganic fluorine compounds on human and animal cells in vivo and in vitro (Russian). *Genetika* 9: 115–120

Voroshilin SI, Plotko EG, Nikiforova VY (1975) Mutagenic effect of hydrogen chloride on animals (Russian). *Cytology Genetics* 9: 42–44

Wang Y-N, Xiao K-Q, Liu J-L, Dallner G, Guan Z-Z (2000) Effect of long term fluoride exposure on lipid composition in rat liver. *Toxicology* 146: 161–169

Whitford G (1990) The physiological and toxicological characteristics of fluoride 1993–1995. *Fluoride* 29: 82–88

Whitford G (1996) The metabolism and toxicity of fluoride. in: Meyers HM (Ed.), Monographs in Oral Science, 2nd rev. ed., Karger, Basel, Vol. 16, 46–58

WHO (World Health Organization) (1984) Fluorine and Fluorides, Environmental Health Criteria 36, WHO, Geneva

WHO (World Health Organization) (2002) Fluorides, Environmental Health Criteria 227, WHO, Geneva

Wilson JT, Hauser RE, Ryffel B (1985) Osteomas in OF-1 mice: no alteration in biologic behaviour during long-term treatment with cyclosporine. *J Natl Cancer Inst* 75: 897–903

Yamamoto S, Katagiri K, Ando M, Chen X (2001) Suppression of pulmonary antibacterial defenses mechanisms and lung damage in mice exposed to fluoride aerosol. *J Toxicol Environ Health A* 62: 485–494

Zeiger E, Shelby MD, Witt KL (1993) Genetic toxicity of fluoride. *Environ Mol Mutagen* 21: 309–318

Zeiger E, Gulati DK, Kaur P, Mohamed AH, Revazova J, Deaton TG (1994) Cytogenetic studies of sodium fluoride in mice. *Mutagenesis* 9: 467–471

Zhang B, Hong M, Zhao Y, Lin X, Zhang X, Dong J (2003) Distribution and risk assessment of fluoride in drinking water in the west plain region of Jilin province, China. *Environ Geochem Health* 25: 421–431

Zhao ZP, Yaun MB, Liu GF (1996) X-ray analysis of 80 patients with severe endemic fluorosis caused by coal burning. *Fluoride* 29: 79–81

Zhao W, Zhu H, Aoki K, Misumi J, Zhang X (1998) Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. *Endocr Regul* 32: 63–70

completed 4.4.2005