Hydrogen fluoride and sodium fluoride

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Hydrogen fluoride and sodium fluoride*
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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van waterstof- en natriumfluoride op de vruchtbaarheid en het nageslacht; het betreft ook effecten die optreden na blootstelling via de borstvoeding. Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroeps-uitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie Reproductietoxische stoffen. Het is vervolgens getoetst door de Beraadsgroep Gezondheid en Omgeving van de raad.

Ik heb dit advies vandaag ook ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieu.

Met vriendelijke groet,

prof. dr. J.A. Knottnerus

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 70 17
E-mail: a.vd.burght@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

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Subcommittee on the Classification of Reproduction Toxic Substances
A Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2009/04OSH, The Hague, December 17, 2009

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The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, Agriculture, Nature & Food Quality, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie Reproductietoxische Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de raad, hierna aangeduid als de commissie, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie waterstoffluoride en natriumfluoride onder de loep genomen.

De aanbevelingen van de commissie zijn:

- voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens zijn, maar dat voldoende diergegevens laten zien dat waterstof- en natriumfluoride de fertiliteit niet schaden. De commissie adviseert daarom waterstoffluoride en natriumfluoride niet te classificeren.
- voor effecten op de ontwikkeling meent de commissie dat er onvoldoende geschikte humane gegevens zijn, maar dat voldoende diergegevens laten zien dat waterstoffluoride en natriumfluoride de ontwikkeling van het nageslacht niet schaden. De commissie adviseert daarom waterstoffluoride en natriumfluoride niet te classificeren.
- voor effecten tijdens lactatie is de commissie van mening dat er voldoende humane gegevens zijn om waterstoffluoride en natriumfluoride niet te labellen.

Executive summary

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The evaluation and subsequent classification, according to the Directive 93/21/EEC of the European Union, are performed by the Subcommittee on Classification of Reproduction toxic substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the committee. In the present report, the committee has reviewed hydrogen fluoride and sodium fluoride.

The committee's recommendations are:

- for effects on fertility, the committee recommends not classifying hydrogen and sodium fluoride on the basis of a lack of appropriate human data and sufficient animal data which show that classification is not indicated.
- for effects on development of the progeny, the committee recommends not classifying hydrogen and sodium fluoride on the basis of a lack of appropriate human data and sufficient animal data which show that classification is not indicated.
- the committee is of the opinion that sufficient human data regarding effects of hydrogen and sodium fluoride on lactation show that a label is not indicated.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Subcommittee on Classification of Reproduction toxic substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council, hereafter called the committee, according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1, 2 or 3) or labelled as 'may cause harm to breastfed babies' (R64).

1.2 Committee and procedure

The present document contains the classification of hydrogen- and sodium fluoride by the Health Council's Subcommittee on Classification of Reproduction toxic substances. The members of the committee are listed in Annex A. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development and lactation of the above mentioned compound.

Classification and labelling was performed according to the guidelines of the European Union listed in Annex B.

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In 2009, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, the committee has agreed upon a number of additional considerations.

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex B, 4.2.3.1 category 1).
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification)

in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex B, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labeling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of the dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration is above an exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Current Contents and Medline, starting from 1966 up to 2007 and by searches on internet. An additional search was performed for the period 2007 up to October 2009. Literature was selected primarily on the basis of the text of the abstracts. Publications cited

* Organisation for Economic Cooperation and Development.

in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted, but not cited. Data are described in the text and animal studies with respect to fertility and development are summarised in Annex D. For each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

1.6 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- Lack of appropriate data precludes assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxic to reproduction is indicated.

1.7 Final remark

The classification of compounds is based on hazard evaluation[1] only, which is one of a series of elements guiding the risk evaluation process. The committee emphasizes that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organisations.

Chapter 2

Hydrogen fluoride and sodium fluoride

2.1 Introduction

Name	:	*Hydrogen fluoride (HF)*
CAS-no	:	7664-39-3
Synonyms	:	Hydrofluoric acid, anhydrous hydrofluoric acid
Use	:	HF is a naturally occurring gas and is used for the production of organofluor compounds, and inorganic fluorides. It is a catalyst in alkylation reactions in the petrochemical industry, and used for etching of glass and pickling of stainless steel
Mol weight	:	20.006
Chem formula	:	HF
Conversion factor	:	1 mg/m^3 = 1.223 ppm, 1 ppm = 0.82 mg/m^3
General toxicity	:	In a human case study, accidental HF contact (dermal and inhalatory after explosion of a bottle with HF) caused severe dermal lesions and damage to the respiratory tract and lungs and even death.[2] Respiratory contact of HF (122 ppm (100 mg/m^3) for 1 minute) by volunteers showed eye and nose irritation, and pain in exposed skin and respiratory irritation.[3] In animal studies, eye and mucous membrane irritation, corneal opacity, skin erythema, respiratory distress, pulmonary oedema, haemorrhage, and weight loss were observed. Also in rats, dermal contact (0.5 ml of a 50% HF solution on shaved skin for 5 minutes) resulted in decreases in spontaneous ventilation and movement, tremor, loss of co-ordination, loss of righting reflex, and even death[4]
Occupational exposure limit	:	2.5 mg/m^3 (ATSDR)[3]
Max. amount allowed in drinking water	:	4.0 mg fluoride/l (ATSDR)[3]
Upper tolerable intake	:	For infant < 8 yr: 0.1 mg F/kg bw/ day (IOM)

Kinetics	:	Absorption of inorganic fluoride is thought to be a passive process. Inorganic fluoride of any source is thought to be transported across biological membranes primarily as molecular (non-ionic thus uncharged) HF. At physiological pH (in blood, intercellular fluid, mucus), HF dissociates into free fluoride (thus not associated with e.g. proteins or lipids) and exists primarily as fluoride ion (F^-); only 0.01% of the total free fluoride concentrations exists as molecular HF in equilibrium with the ionic form. The fate and effects of absorbed inorganic fluoride are independent of the fluoride source[5]
Name	*:*	*Sodium fluoride (NaF)*
CAS-no	:	7681-49-4
Synonyms	:	Floridine; sodium monofluoride; disodium difluoride; sodium fluoride; Florocid
Use	:	NaF's principal use is for the prevention of dental carries. NaF is widely used to strengthen the teeth by formation of fluoroapatite; therefore, toothpaste often contains NaF. In the USA, water used to be fluoridated by NaF. NaF has also been used as an antibiotic and a rat poison, and is used in ceramics.
Mol weight	:	42.00
Chem formula	:	NaF
Conversion factor	:	Not Applicable[6]
General toxicity	:	Toxicological signs of short-term high exposure to sodium fluoride in humans are nausea, dizziness, headache, vomiting, fever, and respiratory difficulties. Skin and eye contact with sodium fluoride cause irritation. Inhalation can cause respiratory irritation. An intake of more than 4 grams can be fatal. Long-term exposure to sodium fluoride can cause lung damage and calcification of bones, ligaments, and tendons[3]
Occupational exposure limit	:	2.5 mg/m^3 (ATSDR)[3]
Max. amount allowed in drinking water	:	4.0 mg fluoride/l (ATSDR)[3]
Upper tolerable intake	:	For infant < 8 yr: 0.1 mg F/kg bw/ day (IOM)
Kinetics	:	Absorption of any inorganic fluoride is thought to be a passive process. Inorganic fluoride of any source is thought to be transported across biological membranes primarily as molecular (non-ionic thus uncharged) NaF. At physiological pH (in blood, intercellular fluid, mucus), NaF dissociates into free fluoride (thus not associated with e.g. proteins or lipids) and exists primarily as fluoride ion (F^-); only 0.01% of the total free fluoride concentrations exists as molecular NaF in equilibrium with the ionic form. The fate or effects of absorbed inorganic fluoride are independent of the fluoride source[5]

No reproductive toxicity studies with HF are available. However, effects on reproduction are systemic and HF occurs in the systemic circulation only as free ionic or as organically bound fluoride rather than as HF, NaF or any other form (see also 2.1 kinetics in table above). Because many data on the reproductive toxicity of NaF are available in the literature, NaF can also provide insight in the reproductive toxicity of HF. In humans, exposure to fluoride mainly occurs via drinking water.

2.2 Human studies

Fertility

Freni[7] described an ecologic study into the impact of fluoride in drinking water on human fertility. A decrease in total fertility rate (determined as the number of births per 1000 women) was associated with increasing fluoride drinking water concentrations. However, the many assumptions, potential biases, and confounding factors may have invalidated this study.

Susheela *et al*.[8] compared serum levels of testosterone in patients afflicted with skeletal fluorosis (n=30) and healthy males consuming water containing less than 1 ppm fluoride (control 1, n=26), and a second category of controls with males (n=16) living in the same house as the patients and consuming the same highly fluoriated water (1.5-14.5 ppm) as patients, but not exhibiting clinical manifestations of skeletal fluorosis. Circulating serum testosterone in skeletal fluorosis patients were significantly lower than those of control 1 ($p<0.01$). Testosterone concentrations of the second controlgroep were also lower than those of first control group ($p<0.05$), but higher than those of the patient group (p=unknown).

Ortiz-Pérez *et al*.[9] studied reproductive parameters in a population exposed to fluoride at doses of 3-27 mg/day (high fluoride exposed group (HFEG), exposure via drinking water and occupationally). Urinary fluoride levels, semen parameters, and reproductive hormones in serum (luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, prolactin, inhibin-β, free and total testosterone) were measured. Results were compared with a group of individuals exposed to fluoride at lower doses: 2-13 mg/day (low fluoride exposed Group (LFEG), exposure only via drinking water)). A statistically significant increase in FSH ($p<0.05$) and a reduction of inhibin-β, free testosterone and prolactin in serum ($p<0.05$) were observed in the HFEG. A decreased sensitivity was found in the FSH response to inhibin-β ($p<0.05$) and a negative partial correlation was observed between urinary concentrations of fluoride and serum levels of inhibin-β ($r = -0.333$, $p = 0.028$) in LFEG. Furthermore, a partial correlation was observed between a chronic exposure index for fluoride and the serum concentrations of inhibin-β ($r = -0.163$, $p = 0.037$). No abnormalities were found in the semen.

Development

Zierler *et al*.[10] studied the effect of chemicals in drinking water (including fluoride) on congenital heart disease in a case-control study. A total of 270 affected children and 665 healthy children were enrolled in the study. Mothers provided information during a telephone interview on their health, pregnancy management and demographic characteristics. Information on water contaminants was provided by public water services. Crude estimates of prevalence odds ratios of any congenital heart disease showed no association with fluoride in drinking water.

The relationship between community drinking water and the occurrence of adverse pregnancy outcomes was investigated in a case-control study among women who delivered infants during August 1977 through March 1980.[11] Water quality indices were compared among 1039 congenital anomaly cases, 77 stillbirths, 55 neonatal death cases and 1177 controls. Trace element levels were gathered from routine analyses of public water supplies of the communities in which the women resided during pregnancy. Multivariable analyses revealed slight, but not statistically significant, inverse associations between fluoride levels and the frequency of neonatal deaths (adjusted OR 0.4, 95% CI 0.2-1.0), major malformations (adjusted OR 0.7, 95 CI 0.5-1.2), and the occurrence of organ system defects (adjusted ORs ranged from 0.5 to 0.8).

A retrospective comparative study was conducted by Gupta *et al*.[12] on 100 children in two areas with a high and low fluoride concentrations in drinking water. Group A (n=50) consisted of children living in regions with 4.5 or 8.5 ppm fluoride in drinking water, who showed clinical, dental and/or skeletal fluorosis. Group B (control, n=50) consisted of children who received less than 1.5 ppm fluoride from drinking water and did not show evidence of fluoride toxicity. All children were evaluated by maternal history during pregnancy, general clinical examination, evidence of fluoride toxicity, and radiological evaluation of skull, spine, lumbosacral region and forearm. In group A, 44% of the children showed signs of spina bifida *occulta** in the lumbosacral region, whereas in group B, 12% of the children revealed spina bifida *occulta*, which falls within the normal prevalence range of 5-20% according to literature. It is difficult to draw conclusions from this study as the groups were selected on the occurrence of fluorosis.

* Spina bifida occulta: This is the mildest form of spina bifida. Although the vertebral arches failed to unite, there is no protrusion of cord or membrane. This condition may be found in about 20% of all spines examined roentgenographically.

Down's Syndrome

In a French article, Rapaport[13] described a 2-3 times increased incidence in Down's syndrome in a region with 1 ppm fluoride added to drinking water in a poorly described, and often criticized, study. Many studies have been conducted to verify Rapaport's findings since. Many of these studies were also designed poorly.[14,15] A few, however, are worth mentioning:

Data of a total of 2,469 cases of children with Down's syndrome born alive in the state of Massachusetts were used for a study performed by Needleman *et al.*[16] For each subject, information on date of birth, age of mother, and town of residence was available. Thirty communities had fluoridated water (approximately 1 ppm) and 321 communities had water fluoridated below 0.3 ppm. Each subject was classified either a fluoride or non-fluoride case, determined by the fluoridation status of the town of residence nine months before the date of birth. Prevalence rates at birth for Down's syndrome (when corrected for confounding by maternal age) were 1.53 per 1000 births for the fluoride group and 1.46 per 1000 for the non-fluoride group.

The occurrence of congenital malformations by water fluoridation was studied by Erickson *et al.*[17] During the period 1961 through 1966 in one area and 1967 through 1973 in another area, 1,387,027 births were examined for congenital malformations. In these areas, pregnant women exposed and not exposed to fluoridation of water were compared for congenital malformations. No statistically significant differences were found between the exposed and non exposed groups for any of the selected malformations (anencephaly, cardiac and other circulatory system defects, cleft lip with/without palate, cleft palate, clubfoot, Down's syndrome, hydrocephalus, hypospadias, reduction deformities of the extremities and spina bifida).

Erickson *et al.*[18] also analyzed data obtained from birth certificates from large US cities and studied the effect of fluoride on down syndrome, corrected for maternal age. A total of 636,765 white live births between 1973 and 1975 were analyzed. Among these children, 268 cases of Down syndrome were reported. Maternal age-specific rates in the two fluoridation categories were similar, being 4.1 and 4.5 per 10,000 live births in the fluoridated and non-fluoridated categories, respectively.

A systematic review by Whiting *et al.*[19] concerning six epidemiological papers [13-16] confirmed that only Rapaport found an association between water fluoride levels and the prevalence of Down's syndrome, but this was not observed in the other studies. Therefore, the committee is of the opinion that the overall evidence concerning the prevalence of Down's syndrome is inconclusive.

Lactation

Ekstrand *et al*.[20] studied the transfer of fluoride from blood plasma to breast milk in five mothers, aged 27-36. An oral dose of 1.5 mg NaF (in aqueous solution) was administered after 10 hours of fasting. Blood and breast milk were sampled simultaneously at 30, 60, 90 and 120 minutes after treatment. The plasma fluoride concentration in all subjects rapidly increased 10 fold 30 minutes after fluoride intake and decreased steadily at the following time points. There was no corresponding increase in fluoride concentrations in breast milk. The highest fluoride concentrations in breast milk varied between 0.1 and 0.4 µmol/l (~ 0.002 and 0.008 mg/l).

Fluoride content in human milk after intake of different levels of fluoride was determined by Spak *et al.*[21] The fluoride concentration in breast milk of women living in areas with high fluoride in drinking water (1.0 ppm = 1.0 mg/l) and low fluoride in drinking water (0.2 ppm = 0.2 mg/l) had a milk fluoride concentration of 0.36±0.02 µmol/l (0.007 mg/l) and 0.28±0.02 µmol/l (0.005 mg/l), respectively. Within the 1.0 ppm fluoride area, the intra- and inter-individual differences in fluoride concentration were very small.

A case study, in which a 33 year old woman received two daily doses of 25 mg NaF for 1 month, was described by Ekstrand *et al.*[22] The experiment took place 7 months after delivery, and a normal breast milk production was recorded. The patient fasted for 8 hours and had not received any fluoride for 48 hours. Following an oral dose of 25 mg NaF, venous blood was collected at 0, 2, 4, 6, and 8 hours. Breast milk was sampled 0.25 hours before and 0.5, 1, 1.5, 2, 4, 6 and 8 hours after NaF intake. Plasma fluoride reached a maximum two hours after starting the treatment (C_{max}~ 15 µM = 0.29 mg F^-/l), declining at the following time points. A small increase in fluoride concentration in breast milk was also found (C_{max}~ 3 µM = 0.057 mg F^-/l).

Fluoride concentrations were determined in 210 samples of human milk by Dabeka *et al.*[23] Geometric mean and median levels were 7.08 and 12 ng/g, in a

range from < 2 to 97 ng/g. Of mothers taking no fluoride supplements and living in communities with fluoride (1 µg/g) in drinking water, the mean fluoride in milk was 9.8 ng/g. Where no fluoride was present in drinking water, the mean level was 4.4 ng/g.

In 27 nursing mothers, breast milk fluoride levels and 24 hour intake of fluoride through foods and beverages were determined. The daily fluoride intake averaged 22.1 mg (9.5-37.2 mg). The breast milk fluoride concentration averaged 0.033 mg/l (0.011-0.073 mg/l). No significant correlation between milk fluoride and intake were established. Milk fluoride level was, however, correlated positively to mothers' age and negatively to mothers' weight.[24]
The committee concludes that the above mentioned studies show that fluoride levels up to 0.033 mg/l could be detected in human breast milk.

Based on a tolerable upper intake level established by the Institute of Medicine, the committee is of the opinion that a maximal level of 0.5 mg fluoride per liter breast milk is unlikely to cause health effects in breast fed children (see Annex E for calculations).

2.3 Animal studies

In tables 1 and 2 (Annex D) fertility and developmental studies performed in animals are summarised.

Male fertility

Male rats

The effect of NaF ingestion on male rat (strain and number unknown) spermatozoa was studied by Chinoy *et al*.[25] Adult male rats were orally administered with 0, 5 or 10 mg NaF/kg body weight/day for 30 days. Epididymal sperm motility was significantly decreased in the 10 mg/kg body weight group and epididymal sperm count was significantly decreased in both treatment groups. Furthermore, the fertility rate was significantly lower in both treatment groups when compared to the control group. Body weight of the treated groups was not significantly different from the control group in the 5 mg/kg body weight group and decreased, but not significantly in the 10 mg/kg body weight group.

Krasowska *et al.*[26] treated male Wistar rats with 0, 100 or 200 ppm NaF (= 0, 100 or 200 mg/l) for 6 and 16 weeks (all groups n=7, administration in drinking water). Both groups showed a significant increase in the concentration of NaF in the testes, but no dose response or time response correlation was found. Both treated groups had a significant decrease in zinc (Zn) concentrations in plasma and testes after 16 weeks. Fifty percent of the treated rats exhibited histopathological changes in the germinal epithelium of the testes after 16 weeks. The authors suggested that a deprivation of testicular Zn, due to a high NaF intake, might be responsible for the injury of testicular tubules. No data on general toxicity was provided.

Narayana *et al.*[27] studied the effects of NaF on rat testicular steroidogenesis. Male Charles Foster albino rats were treated with 0 or 10 mg NaF/kg body weight/day for 50 days (n=10, administration procedure unknown). After 50 days, testosterone levels were significantly reduced. In addition, Leydig cell diameter and Leydig cell nuclear diameter were both significantly reduced. Activities of intermediary enzymes in androgenesis were lowered, but not significantly different. No data on general toxicity was provided.

Narayana *et al.*[28] also studied the effects of sodium fluoride ingestion on spermatozoa of the rat. Male Charles Foster albino rats were orally treated with a daily dose of 0 or 10 mg NaF/kg body weight/day for 50 days (n=25). A third group of rats with treated similar with 10 mg Naf/kg body weight per day for 50 days but the rats maintained on a standard diet for 70 days (n=30). Sperm acrosomal hyaluronidase and acrosin were significantly reduced. Silver nitrate staining revealed acrosomal damage and deflagellation. Cauda epididymal sperm count was significantly decreased, as was sperm motility, which contributed to the significant reduction in fertility by NaF treatment. Withdrawal of NaF treatment for 70 days after the 50 days treatment period produced incomplete recovery. No data on general toxicity was provided.

Sprando *et al.*[29] studied the effect of NaF on spermatogenesis in a reproduction rat study. Male and female parental (n=12-14 / dose group) Sprague-Dawley rats received <0.2 (control), 25, 100, 175 or 250 ppm NaF (=<0.2, 25, 100, 175 or 250 mg/l) in their drinking water *ad libitum* for 14 weeks: 10 weeks before mating, 3 weeks during mating and 1 week after mating. However, in an earlier study by Collins *et al.*[30] (same research group, see *rat development*), in the highest doses, rats consumed less water than in other groups, reducing the dose of NaF to 0, 3.9, 15.6, 24.7 and 25.1 mg NaF/kg body weight/day, respectively. Testicular

tissues were collected from the parental (P) males 1 week after mating. Male and female rats within a treatment group were mated and pregnant females continued to be exposed to fluoride from day 0 of gestation until the end of lactation. On post natal day 21, male and female rats were randomly selected for the F_1 generation. The weanlings remained in the same treatment groups as their parents and were exposed to NaF for 14 weeks (10 weeks pretreatment, 3 weeks mating and 1 week after mating) at which the testicular tissues were collected from male F_1 (n=12 for each dose group). No significant differences in body weights of the animals of the treatment groups were observed when compared to the control group. No dose related effects were observed within the P and F_1 treatment groups in testis, prostate and seminal vesicle weight, testicular spermatid counts, sperm production per gram testis per day, sperm production per gram testis or LH, FSH and serum testosterone concentrations. Additionally, no histological changes were observed in testicular tissues for both P and F_1 treated groups.

Sprando *et al.*[31] also studied the effect of NaF on testis morphology in a one-generation rat study using the same study design as described above. In the F1 male group, only a significant decrease in the absolute volume and volume percent of the lymphatic endothelium was observed in the 175 and 250 ppm NaF-treated groups and in the absolute volume of the testicular capsule in the 100 ppm NaF-treated group were found. No significant differences were observed with respect to absolute volume of the seminiferous tubules, interstitial space, Leydig cells, lymphatic space, tubular lumen or absolute tubular surface area, mean Sertoli cell nucleoli, mean seminiferous tubule diameter or mean height of the seminiferous epithelium. A non-dose related decrease in body weight was found in the 100, 175 and 250 ppm treated groups when compared to the control.

Collins *et al.*[32] performed a multigenerational evaluation of sodium fluoride in CD CRL:CD-BR rats (240 males and 240 females). Throughout the study, rats were fed a low fluoride diet (7.95 ppm fluoride) and were given 0, 25, 100, 175 or 250 ppm (= 0, 25, 100, 175 or 250 mg/l) NaF in drinking water. Feed and fluid consumption, body weights and clinical signs were recorded at regular intervals. Decreased fluid consumption observed at 175 and 250 ppm NaF was attributed to decreased palatability and did not affect reproduction. No cumulative effects were observed in the three generations. Mating, fertility and survival indices were not affected. Organ-to-body-weight ratios and organ-to-brain weight ratios were not affected. Sodium fluoride up to 250 ppm did not affect reproduction in rats.

The effects of ingestion of NaF at 0, 100 or 300 ppm (= 0, 100 or 300 mg/l) in drinking water during 12 weeks by adult male Sprague-Dawley rats was investigated for effects on sexual behavior and fertility by Bataineh *et al.*[33]. Body weight and relative testes weight were not affected, but the average weights of epididymis, ventral prostate, seminal vesicles and preputial glands decreased significantly in both treatment groups. Except for four unaccountable deaths in the highest treatment group, are animals were healthy. A significant decline in spermatogenesis in testes due to a decrease in the number of spermatocytes and spermatids in the treatment group was found. In addition, sperm motility and density were significantly decreased in the cauda epididymis and in testes in both treatment groups. Testosterone and FSH levels were also significantly decreased. Furthermore, treatment diminished sexual behavioral parameters. Time to first mount was prolonged, intromission latency was increased, number of intromissions was decreased, post-ejaculatory interval was prolonged, number of pregnant females was decreased and number of fetal resorptions in female rats was increased.

Sixty four male Wistar rats were given normal distilled water as a control (n=32) or fluoride rich water (150 mg NaF/l, 68 mg F^-/l, n=32) by Wan *et al.*[34] After 50, 80, 100 and 120 days of treatment, eight rats from each group were randomly selected and weighted. In addition testes and epididymis tissues were removed. Sperm viability was calculated and morphology of testis was examined. Treated rats exhibited a decline in sperm viability and a significant increase of sperm abnormalities after 50, 80, 100 and 120 days of administration. Sperm density declined markedly at day 80 and day 120. The number of seminiferous epithelium cell layers, the thickness of the seminiferous tubule and the diameter of the seminiferous tubule in the testis all decreased at day 50, 100 and 120.

In the same study design as described before, Wan *et al.*[35] treated rats for 10 days with NaF. After this, rats were examined for expression of epidermal growth factor. A decreased epidermal growth factor was found in Leydig cells, spermatogonia and spermatocytes, along with diminished epidermal growth factor receptors in NaF treated rats, when compared to controls.

Zhang *et al.*[36] studied the effects of NaF and SO_2 on sperm motility and testosterone levels in male Wistar rats. Rats were treated with 45 ppm (=45 mg/l) NaF in drinking water or <0.6 ppm NaF in drinking water (control) and received 23.39±1.04 ppm NaF in diet (both control and treated). Both control and treatment groups consisted of 24 animals. Animals were treated for 2, 4, 6 or 8 weeks

after which the rats were sacrificed (6 animals per sacrifice per group) and sperm motility and testosterone levels were determined. No significant changes were found in testosterone levels. A significant change in sperm motility was found after 6 weeks of treatment, but no differences were found for the other treatment periods. No significant changes were found on body weight and testis weight. Other clinical signs were not studied.

Gupta *et al.*[37] described the effects of NaF in mature male Wistar rats. After exposure to 0, 2, 4, and 6 mg NaF/L in their drinking water, sperm motility and density were assessed. Fluoride treatment decreased the weight of testis, epididymis and ventral prostate. In addition, sperm motility and density were reduced. There was a reduction in the number of spermatocytes and spermatids as well. No data on general toxicity were given.

Male Mice

Kour *et al.*[38] treated adult male albino mice (strain unknown) with 0, 10, 500 or 1000 ppm NaF (0, 10, 500 or 1000 mg/l) in drinking water for 1, 2 and 3 months, respectively (all groups N=25). The control group (N=25) received water without fluoride. The high dosage groups showed a lack of maturation and differentiation of spermatocytes. In the highest dose group, spermatogenesis had stopped and the seminiferous tubules had become necrotic. No data on general toxicity was provided.

Li *et al.*[39] studied the genotoxic effects of NaF on sperm. Male mice of the genotype $B_6C_3F_1$ were treated via the drinking water with the Maximum Tolerated Dose (MTD) of NaF (70 mg NaF/kg body weight/day) or one of the following doses: 0.1, 1.0, 10, 20 or 35 mg/kg body weight (all groups N=9). A positive (cyclophosphamide 20 mg/kg body weight/day) and a negative (distilled water) control were included. The counts of abnormal sperm and the weights of the testes for mice exposed to NaF doses up to the MTD were not significantly different from those of the negative control. No data on general toxicity was provided.

Another study by this group using the same study design but a different dose range 1, 10, 50 or 75 ppm NaF (=1, 10, 50 or 75 mg/l), also showed no significant effect on morphology or testis weight. No data on general toxicity was provided.[40]

Chinoy *et al*.[41] investigated effects of NaF fed in the diet on histology and histocytometry of reproductive organs of the adult male Swiss mice (n=40/group) in two doses (10 and 20 mg NaF/kg body weight/day equivalent to 230 and 400 ppm/animal/day, water used as a vehicle) for 30 days. In two extra groups, treatment of 10 mg/kg body weight for 30 days was followed by a withdrawal period of for a period of 30 and 60 days, respectively. Histoarchitecture of the testis was altered after treatment with 10 and 20 mg/kg body weight NaF. Disorganisation and denudation of germinal epithelial cells of seminiferous tubules with absence of sperm in the lumina was observed. In addition, the epithelial cell height was significantly reduced. Leydig cell and nucleus diameters were not affected. In the caput epididymis, epithelial cell nuclear pyknosis and absence of luminal sperm were observed. In the cauda epididymis, for both treatment doses, a significant reduction in epithelial cell height, nuclear pyknosis, denudation of cells and absence of sperm occurred. In the vas deferens, nuclear pyknosis, clumped stereocilia and cell debris was shown, but no spermatozoa. Withdrawal of treatment for 30 or 60 days after treatment with 10 mg NaF/kg body weight/day caused recovery in the architecture in all described organs. No effects on other organs or body weight were described.

Using the same study design as described above, Chinoy *et al*.[42] observed biochemical changes in reproductive organs of male mice. In this study, in both treatment doses, weight of the seminal vesicles and prostrate were increased significantly ($p<0.001$). Testis succinic dehydrogenase was significantly decreased and prostatic protein and acid prosphatase were significantly increased. In the vas deferens, glycogen concentrations were increased significantly. Recovery was noted after withdrawal of treatment for 60 days for all substances. Body weights after treatment were decreased when compared to control, but returned to normal after withdrawal.

Using the same study design, Chinoy *et al*.[43] observed a decrease in cauda epididymis sperm motility after 30 days of treatment with both doses. Also, epididymal sperm count was decreased for both doses, but this was not supported by a statistical analysis. Scanning electron microscopy showed abnormalities in head, midpiece and tail sections of spermatozoa from cauda epididymis in treated mice, when compared to control. Silver nitrate staining of cauda epididymal spermatozoa showed a diffused staining of the acrosomal, post acrosomal and midpiece regions after treatment with NaF. In the uteri of females mated with treated males (on the first day after treatment stopped) the implantation sites were absent. Withdrawal of treatment for a period of two months resulted in a

recovery in sperm count, sperm motility and fertility rate, however, the dose of the treatment before withdrawal was not reported.

Adult male Swiss albino mice were treated via drinking water with 10 mg NaF/kg body weight/day for 30, 60 or 90 days, the control group received water without NaF (each group, N=20) by Bano *et al.*[44]. Biochemical estimations of total soluble protein (TSP) acid phosphatase (AcPase) and alkaline phosphatase (AlkPase) were made in the testicular homogenates of experimental and control mice. Total protein decreased significantly after 30 (45%), 60 (25%) and 90 (10%) days. AcPase levels significantly decreased after 30 (63%), and 90 (90%) days, but was elevated after 60 days (153%). The concentration of AlkPase was increased on days 30 (15%, $p<0.01$) and day 60 (26%), but declined at day 90 (19%), when compared to control. Lactate dehydrogenase (LDH) levels decreased by 25% on day 30 and on day 60 and 90 with 12% and 9%, respectively. These data suggest that fluoride interferes with metabolic status of testicular cells and is detrimental to spermatogenesis and androgenesis. No data on general toxicity was provided.

Using the same study design as described above by Bano *et al.*[44], Sahdev *et al.*[45] studied, in addition to the enzymes studied by Bano *et al.*, the effect of NaF treatment on adenosine triphosphatase (ATPase) (an energy generator for spermatozoa, through degradation of ATP), succinic dehydrogenase (SDH), which stimulates growth and maturation of germ cells and lactate dehydrogenase (LDH), which also plays a role in the spermatogenesis. Sahdev *et al.* observed a decrease in SDH and LDH levels after 30 and 60 days and no further decrease after 90 days, but no significance was reported. ATPase increased significantly at day 30 and was elevated on day 60 with no increase on day 90. No data on general toxicity was provided.

Male Swiss mice were exposed to 0, 100, 200 and 300 ppm NaF (= 0, 100, 200 or 300 mg/l) in their drinking water (ad libitum) for 4 or 10 weeks (all groups n=10) by Elbetieha *et al.*[46] Due to differences in water consumption the NaF intake of the animals was 0, 12, 22 and 39 mg NaF/kg body weight/day, respectively for the 4 week groups and 0, 9, 16 and 27 mg NaF/kg body weight/day, respectively for the 10 week groups. After the exposure period each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for ten days during which two estrus cycles should have elapsed. Males were removed and 10 days later females were sacrificed, after which number of pregnant females, viable fetuses and embryo resorptions were

assessed. No clinical signs of toxicity with 4 week exposure was observed, however, in the 10 week exposed groups, 2 animals out of 10 and 3 animals out of 10 died during the last week of the exposure to 100 and 300 ppm NaF, respectively. Preputial gland weights were significantly decreased in the 200 ppm and 300 ppm after 4 weeks of treatment. Animals exposed to NaF for 10 weeks showed no significant effects on relative weights of any reproductive organ. No effects on male fertility (number of pregnant females) was found after 4 week exposure, but the number of resorptions was significantly increased in females mated with males exposed to NaF at 100 ppm, 200 ppm and 300 ppm. The number of females mated after 10 weeks was significantly reduced when placed together with males exposed with 100 ppm, 200 ppm and 300 ppm NaF. The number of implantations was significantly lower in the 200 ppm group. Also, the number of viable fetuses was reduced in the 200 ppm group.

Six-week-old male Kunming mice were given 0, 50, 100, 200 and 300 mg NaF/L in their drinking water (20 animals per group) for 8 weeks. At the end of the exposure period, effects on sperm quality (sperm count, sperm motility, sperm survival and sperm abnormality) and oxidative stress were observed at 200 and 300 mg/L. Data on general toxicity were not provided.[47]

Male offspring of female BALB/c mice (6 to 10 per group) were exposed to fluoride via lactation starting at prenatal day 1. Female mice were exposed to NaF, resulting in 0, 1, 10, 100 mg F^-/L in drinking water (equivalent to 2.2, 22, 220 mg NaF/L). After 4 weeks, male offspring was also exposed to the same drinking water for an additional 2 months. No mortality, clinical signs of discomfort or body weight loss was observed in the male offspring. Exposure to 10 and 100 mg/L, resulted in a decreased level of spermatogenesis. Furthermore, the number of sperm capable of undergoing head tyrosine phosphorylation and actin polymerization in the cortical acrosome region decreased during capacitation *in vitro*.[48]

Male Rabbit

Male rabbits were orally administered with 10 mg NaF/kg body weight/day and sacrificed after 18 months (n=7) or 29 months (n=3) by Susheela *et al.*[49] The control group (n=10) was held under the same laboratory conditions. Feeding and water supply was *ad libitum*. After sacrifice, testis, epididymis and vas deferens were studied under light and scanning microscopy. After 29 months of treatment, spermatogenic cells in the seminiferous tubules were disrupted,

degenerated and devoid of spermatozoa. In both groups, loss of cilia was observed on the epithelial cells lining the lumen of the ductuli efferentes of the caput epididymidis and loss of stereocilia was observed on the epithelial cells lining the lumen of the vas deferens. Mucus droplets were found in the vas deferens of the control group, but were absent in both treated groups. Spermatogenesis ceased in the 29 months treatment group. No data on general toxicity was provided.

Chinoy *et al*.[50] studied the effects of NaF ingestion (route of administration not described) on alterations in spermatozoa in adult male rabbits (strain unknown). Rabbits were administered 20 or 40 mg NaF/kg body weight/day for 30 days (all groups n=5). In addition, one group was withdrawn from the diet for 30 days after treatment with 40 mg/kg body weight NaF. A significant decrease in cauda epididymal sperm motility and cauda epididymal sperm count was observed in both 20 and 40 mg/kg body weight treated groups. Fertility rate was 95% in control rabbits, but after administration of 20 or 40 mg NaF/kg body weight, fertility was reduced to 33% and 0% respectively. Withdrawal for 30 days of the 40 mg/kg body weight group showed no significant recovery of sperm motility, sperm count or fertility rate. However, when rabbits treated with 40 mg NaF/kg body weight/day were administered with ascorbic acid (100 mg NaF/kg body weight/day) and 125 mg calcium during the withdrawal period, no significant differences were found in sperm motility, sperm count and fertility rate when compared to the control animals. Body weight decreased after NaF treatment compared to controls.

Shashi *et al*.[51] studied the effect of NaF on testicular proteins and DNA. Young male albino rabbits (strain unknown) were injected with NaF subcutaneously in doses of 0 (control), 5, 10 , 20 and 50 mg NaF/kg body weight/day (each group n=12). After 3½ months, rabbits were sacrificed and the percentage of acidic, basic, total proteins and DNA were determined. Acidic, basic and total testicular proteins were dose-related and significantly decreased in all treated groups when compared to control. A dose response was also found for total DNA, where a significant decrease in DNA was found in all groups, when compared to the control. No data on general toxicity was provided.

Male rabbits (strain unknown) were treated for 18 months with 10 mg NaF/kg body weight/day, administered orally (n=8) by Kumar *et al*.[52] Testis and epididymis were investigated for ultra structural details of spermatids and spermatozoa. A wide variety of structural defects were observed in the flagellum,

acrosome and the nucleus of the spermatids and epididymal spermatozoa of fluoride-treated rabbits. Abnormalities included absence of outer micro-tubules, complete absence of axonemes, structural and numeric aberrations of outer dense fibers, breakdown of the fibrous sheath and structural defects in the mitochondria of the middle piece of the flagellum. The described abnormalities rendered the sperm nonfunctional and ineffective. No data on general toxicity was provided.

Male rabbits (strain unknown) were treated for 20 months (n=6) or 23 months (n=6) with 10 mg NaF/kg body weight/day by Kumar *et al.*[53] Treated and control animals were sacrificed after 20 months (n=6) and after 23 months (n=6). Blood fluoride levels in both treatment groups were significantly higher when compared to controls. Loss of stereocilla, significant decrease in the height of the pseudostratified columnar epithelium, and an increase in the diameter of both the caput and cauda ductus epididymis were observed in the 23 month treated animals. Decrease in epithelial cell height and tubular diameter of the testis was significant in the 23 month treated group. The weights of caput and cauda epididymis were significantly reduced in the 23 month treatment group. No data on general toxicity was provided.[53]

Male albino rabbits (strain unknown) were treated subcutaneously with 5, 10, 20 or 50 mg NaF/kg bw/day for 100 days (n=6/group) by Shashi *et al.*[54] Rabbits had free access to food and water. Controls were administered with distilled water. After 100 days, rabbits were sacrificed and testes were removed for histopathological study. In the 5 mg/kg group, no macroscopical or microscopical differences were observed. In the 10 and 20 mg/kg groups, foci of necrosis in seminiferous tubules, deficient differentiation and maturation of spermatocytes and an increase in number of interstitial cells were observed. In the highest dose group, seminiferous tubules was degenerated, infiltration of cells in the interstitial zone of the testicular tubules was found and deficient differentiation and maturation of the spermatocytes was found.

Male Guinea-Pig

The effect of NaF ingestion on male guinea-pig fertility was studied by Chinoy *et al.*[55] Adult male guinea-pigs (strain unknown) were administered with 30 mg NaF/kg body weight/day in drinking water for 30 days, after which they were sacrificed. Both control and treated group consisted of 10 animals. After treatment, cauda epididymal spermatozoa structural and metabolic alterations led to decreases in motility ($p<0.05$), live: dead ratio ($p<0.001$) and sperm mitochon-

drial activity index ($p<0.05$). Increases were found in sperm abnormalities ($p<0.05$) and alterations in sperm membrane phospholipids ($p<0.05$). No data on general toxicity was reported.

Female Fertility

Female Rat

Al-Hiyasat *et al.*[56] investigated the toxic effects of NaF on the reproductive system of female Sprague-Dawley rats. Rats (n=10/group) were treated with 0.5 (control), 200, 400 or 600 ppm NaF in their drinking water for 30 days. Rats in the two highest dose groups showed clinical signs of toxicity (were dehydrated, lethargic and showed a hunched posture) and death, unlike those exposed to NaF at a concentration of 200 ppm. All animals in the 600 ppm group died before the end of the experiment and only three out of ten animals of the 400 ppm group survived the thirty days exposure period. 200 ppm NaF had no effect on the pregnancy rate of the rats nor on number of implantations, but a significant reduction in the number of viable fetuses and number of resorptions and the number of implantations was found when compared to the control group. Furthermore, a significant increase in maternal organ weights (ovary weight and for uterus weight), embryo weights and kidney weights was observed.

Female Mouse

The effect of NaF in drinking water on female mice fertility was studied by Darmani *et al.*[57] Female Swiss mice (60 days old) were given food and water ad libitum. NaF was added to water in concentrations of 0, 100, 200 and 300 ppm (= 0, 100, 200 or 300 mg/l) (n=10/group) for 4 or 12 weeks. Water consumption and body weight was measured every week. After treatment, female mice were housed with a sexually mature untreated male of proven fertility for 10 days. One week after removal from the males, females were sacrificed. No differences in water consumption were found in any group, when compared to the control group. No differences in pregnancies, implantations, viable fetuses or resorptions/implantations were found after 4 weeks of treatment. However, after 12 weeks of treatment, the number of pregnant females was significantly lower in all dose groups, the number of viable fetuses was significantly lower in the 200 ppm and 300 ppm groups and the number of implantations significantly lower in the 300 ppm group. Furthermore, ovary weights were significantly increased in the 300 ppm group after 4 weeks and after 12 weeks in both 200 ppm and 300

ppm group. Embryo weights were significantly increased after four weeks in the 200 and 300 ppm groups and after 12 weeks in the 300 ppm group. No signs of maternal toxicity were reported. However, in the 12 wks group, one animal of 100 ppm, two animals of 200 ppm and two animals of 300 ppm died in the last week.

Female Rabbit

White albino rabbits (strain unknown) were injected subcutaneously with NaF for 100 days in the concentration of 5, 10, 20 and 50 mg NaF/kg body weight/day (all n=6).[54] The ovaries were examined for histopathological changes. Control and 5 mg NaF/kg body weight/day treated groups displayed normal follicles with oocytes and interstitial tissue in ovaries. In animals treated with 10 or 20 mg NaF/kg body weight/day, the ovaries exhibited congested oocytes in the follicles, necrosis of follicles, necrosis of follicle cells and interstitial oedema. Degenerative changes were most abundant in animals treated with 50 mg NaF/kg body weight/day, showing complete atrophy of follicles along with oocyte disintegration and marked necrosis of cells accompanied by infiltration of monocytes, lymphocytes and histocytes in interstitial tissue. No data on the general toxicity were available.

Development

Female rats (Sprague Dawley rats) were given 150 ppm (=150 mg/l) (n=6/group) fluoride in drinking water for 10 weeks prior to breeding and during three successive pregnancy and lactation periods. Rebreeding periods commenced immediately following a 3-week lactation period. At 3 weeks of age, all third pregnancy pups from both groups were then sacrificed and bones were fixed in 10% buffered formalin. Femurs were examined by light and scanning electron microscopy. No pathological changes were observed in the femurs as a result of maternal ingestion of fluoride.[58]

Mated CD-CRL:CD-BR, VAF+ rats were given 0, 10, 25, 100, 175 or 250 ppm (= 0, 10, 25, 100 or 250 mg/l) NaF daily in drinking water throughout gestation by Collins *et al*.[30] Water consumption by females in the 175 and 250 ppm groups was significantly ($p<0.001$) decreased when compared to the control females, making the daily amount of NaF ingested 0, 1.4, 3.9, 15.6, 24.7 and 25.1 mg NaF/kg body weight/day, respectively. No dose-related behavioral changes or maternal clinical signs were noted. The mean number of viable fetuses per

female in all treated groups was similar to the control group. A significant ($p<0.01$) decrease in the mean number of implantations per litter in the 250-ppm group was found, which is probably linked to the significantly lower mean number of *corpora lutea* in this group ($p<0.01$). The occurrence of *in utero* deaths was similar in the control and treated groups. Fetal growth was not affected by treatment, despite that food and water consumption of the dams in the 250 ppm group was significantly less. No dose-related increase in number of external anomalies in fetuses was found after NaF treatment. However, an increase was observed in the average number of fetuses with three or more skeletal variations (not specified) in the 250 ppm group. No dose-related effect of NaF on soft tissue variations was observed.

NaF was administered *ad libitum* in de-ionized/filtered drinking water to Sprague-Dawley derived rats (n=26/group) on gestation days 6 through 15 at l evels of 0, 50, 150 or 300 ppm (= 0, 50, 150 or 300 mg/l) and New Zealand White rabbits (n=26/group) on gestation days 6 through 19 at levels of 0, 100, 200 or 400 ppm (= 0, 100, 200 or 400 mg/l). Drinking water contained less than 0.6 ppm NaF and the NaF content of the feed was 12.4 ppm fluoride for rats and 15.6 ppm fluoride for rabbits. Rats were sacrificed on gestation day 20 and rabbits on gestation day 30 and examined for implantations, fetal weight, sex and morphological development. In the high dose group of both species, an initial decrease of maternal body weight gain, which recovered over time, and decreased water consumption were observed. No clear clinical signs of toxicity were observed. Maternal exposure to NaF during gestation did not significantly affect the frequency of post-implantation loss, mean fetal body weight or external, visceral or skeletal malformations in either rat or rabbit.[59]

A two generation study receiving 0-250 ppm NaF was performed by Collins *et al.*[32,60] CRL:CD-BR rats were given 0, 25, 100, 175 or 250 ppm NaF (=0, 25, 100, 175 or 250 mg/l) in drinking water. Rats were fed a low fluoride diet (7.95 mg/l NaF). The effects of NaF on developmental toxicity measured in the parental (F0) generation and two filial (F1 and F2) generations were studied. Rats were acclimatized for 1 week and each animal was assigned to either control or one of four treatment groups. Each F0 group contained 48 males and 48 females. F0 animals were treated for 10 weeks and then mated 1:1 within each group. Presence of sperm in the vaginal lavage was designated as gestation day 0. At gestation day 20, a caesarean section was performed and 8 of the F0 females were euthanized and anomalies in the maternal animal were recorded as were anomalies in the F1 fetuses. Remaining F0 females were allowed to litter

and wean their pups. On postnatal day 21, 36 F1 males and 36 F1 females were randomly selected for the next generation. F1 animals were treated as described above. At gestation day 20, mated F1 females were euthanized, caesarean sections were performed and the viable fetuses were euthanized and macroscopically examined. No remarkable clinical signs were found in F0 and F1 females, and morphological development of F1 and F2 fetuses was similar in all groups. Water consumption showed a dose-related decrease at 100, 175 and 250 ppm (only 250 ppm ($p<0.01$) decrease significantly different in F0 and 175 ($p<0.0001$) and 250 ppm ($p<0.0001$) significantly different in F1). NaF did not affect reproduction parameters of either F0 or F1 dams. Sex distribution of F1 and F2 fetuses showed no dose-related response. No significant differences were found in F1 and F2 fetus development. On skeletal development of the F1 and F2 fetuses, only hyoid ossification at 250 ppm NaF was significantly decreased ($p<0.05$). Also, no dose-related changes were noted in the development of fetal soft tissues.

Female Wistar strain rats were orally treated with 40 mg/kg NaF in drinking water from day 6 through 19 of gestation (n=10/group).[61] Also the effect of vitamin D on NaF treatment was studied. On day 20 of gestation rats were sacrificed and the uterus was removed by caesarian section. The number of live and dead fetuses, resorptions, individual fetus weight and sex of fetuses were recorded. Furthermore, fetuses were observed for external malformations. Oral administration of NaF caused significantly decreased body weight ($p<0.05$), feed consumption ($p<0.05$), absolute uterine weight ($p<0.05$) and number of implantations ($p<0.05$). As compared with the control, higher incidence of skeletal and visceral abnormalities was recorded in the fetuses of fluoride treated pregnant rats. In the NaF and vitamin D treated group, fluoride induced reductions in body weight, feed consumption and absolute uterine weight were ameliorated.

Lactation

No studies on animal lactation were found.

2.4 Conclusion

Mainly because of its high local toxicity, no reproductive toxicity data of HF are available. Since fluoride compounds of any source occurs in the systemic circulation only as free ionic or as organically bound fluoride and because of all fluoride compounds, most information is available about the reproductive effects of

NaF, in this evaluation the effects of NaF are described to give insight into the reproductive effects of fluoride compounds.

Exposure to fluoride compounds mainly occurs via the drinking water. In human studies, the source of fluoride exposure is generally not specified. Three studies are available concerning the effects of fluoride compounds on human fertility.[7-9] These studies are, however, of limited quality and the committee is of the opinion that they are not suitable for evaluating the reproductive toxic effects.

The effects of NaF on male fertility have frequently been studied in mice[38-46], rats[25-29,31-36,62], rabbits[49-54] and guinea-pigs.[55]

A number of studies describe effects on motility, count and morphology of sperm cells, histopathology of male reproductive organs, biochemical parameters in reproductive organs, sex hormones and reproductive performance[25,27,28,41-43,52,53,55,62], where other studies did not find any effects on fertility after treatment with fluorides.[29,31,32,39,40]

The committee is of the opinion that the animal studies of Sprando *et al.*[29,31] and Collins *et al.*[32], and two earlier studies[39,40] are well-performed and well-reported studies, in which no effects are observed. On the other hand, the committee questions the findings in the other studies as the effects were predominantly found in the presence of general toxicity, or general toxicity was not reported. Moreover, the committee cannot exclude that the observed effects are the result of an unknown contaminant (e.g. aluminium) as the effects were not observed in the well-performed studies.

Only a few publications are available on the effects of NaF on female fertility of rat[56], mice[57] and rabbits.[54] Al-Hiyasat *et al.*[56] did not show an effect on pregnancy rate and the number implantations in rats. In a study of Darmani *et al.*[57] prolonged exposure to NaF (12 weeks) induced fertility effects in mice and Shashi *et al.*[54] showed degenerative changes in the ovaries of rabbits after subcutaneous exposure. However, the effects were observed in the presence of (severe) general toxicity.

Therefore, based on the animal studies with NaF the committee is of the opinion that sufficient animal data show that classifying HF and NaF for effects on fertility is not indicated.

Human studies on the developmental effects are inconsistent. Gupta *et al.*[12] found an increased prevalence of spina bifida *occulta* in children with fluorosis

living in a area with high fluoride concentration (4.5-8.5 ppm fluoride) in drinking water when compared to children showing no fluoride toxicity living in an area with low fluoride concentrations (1.5 ppm fluoride) in drinking water. Zierler *et al.*[10] studied the effect of fluoride on congenital heart disease, but found no correlation. Achengrau *et al.*[11] showed in a case control study, that detectable fluoride levels were associated with decreases in the occurrence of congenital anomalies, stillbirths and neonatal deaths. These studies are, however, of limited quality and the committee is of the opinion that they are not suitable for evaluating the reproductive toxic effects.

Heindel *et al.*[59] and Ream *et al.*[58] found no effects of NaF on development (fetal body weight or external, visceral or skeletal malformations) of rat fetuses. In contrast, Collins *et al.*[32,60], Sherlin *et al.*[61] and Devoto *et al.*[63] observed a higher incidence of dead fetuses and skeletal and visceral abnormalities in rats. However, these effects are found in the presence of maternal toxicity.

In conclusion, in several studies no effects are found on the development of experimental animals or the described effects of NaF on animal development are observed in the presence of maternal toixicity. Therefore, the committee is of the opinion that sufficient data show that classifying HF and NaF for effects on development is not indicated.

Ekstrand *et al.*[20,22], Dabeka *et al.*[23] and Opinya *et al.*[24] all showed that plasma fluoride is poorly transferred to breastmilk. When maternal fluoride plasma levels rise, only a small increase in fluoride concentration was found in breast milk and no significant correlation between milk fluoride and intake were established. The highest reported fluoride concentration in human breast milk, 0.057 mg fluoride/liter[22] is lower than the calculated tolarable level of fluoride in human breast milk of 0.5 mg fluoride/liter breast milk (see Annex E). No studies concerning the excretion of fluoride in animal milk were available.

Proposed classification for fertility

Based on animal studies with sodium fluoride, the committee is of the opinion that sufficient animal data show that classifying hydrogen fluoride and sodium fluoride for effects on fertility is not indicated.

Proposed classification for developmental toxicity

Based on the animal studies with sodium fluoride, the committee is of the opinion that sufficient animal data show that classifying hydrogen fluoride and sodium fluoride for effects on development is not indicated.

Proposed labelling for effects during lactation

The committee is of the opinion that sufficient human data regarding effects of hydrogen fluoride and sodium fluoride on lactation show that no classification is indicated.

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Annexes

Annex **A**

The committee

- A.H. Piersma, *chairman*
  Professor in reproductive toxicology, University of Utrecht / National Institute of Public Health and the Environment, Bilthoven
- H.F.P. Joosten
  Toxicologist, formerly NV Organon, Department of Toxicology and Drug Disposition, Oss
- D. Lindhout
  Professor of Medical Genetics, paediatrician, clinical geneticist, University Medical Centre, Utrecht
- N. Roeleveld
  Reproductive epidemiologist, Radboud University Nijmegen Medical Centre, Nijmegen
- J.G. van Vliet
  Reproductive toxicologist, Schering Plough, Oss
- D.H. Waalkens-Berendsen
  Reproductive toxicologist, TNO Quality of Life, Zeist
- P.J.J.M. Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- A.S.A.M. van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag
- J.T.J. Stouten, *scientific secretary*
  Health Council of the Netherlands, Den Haag

A first draft of this report was prepared by P.T. Theunissen, MSc and A.P.M. Wolterbeek, PhD at the Toxicology and Applied Pharmacology department of TNO Quality of Life, Zeist, The Netherlands, by contract with the Dutch Health Council.

The Health Council and interests

Members of Health Council Committees – which also include the members of the Advisory Council on Health Research (RGO) since 1 February 2008 – are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

Directive (93/21/EEC) of the European Community

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific conse-

quence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.

- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:
T; R60: May impair fertility

For substances that cause developmental toxicity:
T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:
T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:
T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:
Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:
Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1 *Effects on male or female fertility,* includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2 *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri-postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity.

The placing of a compound in Category 1 for effects on Fertility and/or Developmental Toxicity is done on the basis of epidemiological data. Placing into Categories 2 or 3 is done primarily on the basis of animal data. Data from *in vitro* studies, or studies on avian eggs, are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

In common with most other types of toxic effect, substances demonstrating reproductive toxicity will be expected to have a threshold below which adverse effects would not be demonstrated. Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administrated, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in Category 3, or even no classification, will be warranted.

Annex V of the Directive specifies a limit test in the case of substances of low toxicity. If a dose level of at least 1000 mg/kg orally produces no evidence of effects toxic to reproduction, studies at other dose levels may not be considered necessary. If data are available from studies carried out with doses higher than the above limit dose, this data must be evaluated together with other relevant data. Under normal circumstances it is considered that effects seen only at doses in excess of the limit dose would not necessarily lead to classification as Toxic to Reproduction.

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. Where there are

studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification into Category 2 should only be made where there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it was demonstrated that impaired fertility in animal studies was due to failure to mate, then for classification into Category 2, it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposure is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as 'Toxic to Reproduction', unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

a) toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or
b) on the basis of results of one or two generation studies in animals which indicate the presence of adverse effects on the offspring due to transfer in the milk, and/or
c) on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **C**

Comments on the public draft

A draft of the present report was released in 2009. The following persons or organisations have commented on the draft document:

- R.D. Zumwalde, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA.

Annex **D**

Fertility and developmental toxicity studies

Table 1.1 Fertility studies in male rats with sodium fluoride.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Chinoy (1992)	Male rats Unknown strain (n=unknown)	Treatment for 30 days Administration with gavage	0, 5 and 10 mg NaF/kg bw/d	No significant body weight differences in both treatment groups, although lowered in 10 mg/kg group (not significant)	10 mg NaF/kg bw/d decreased epididymal sperm motility. 5 and 10 decreased epididymal sperm count and fertility rate.
Krasowska (1992)	Male Wistar rat (n=7/group)	Treatment for 6 and 16 wks, administration route unknown	0, 100 or 200 ppm NaF (=0, 100 or 200 mg/l)	Not reported	Significant increase in NaF in testes, but no dose response. Significant decrease in zinc levels in plasma and testes and 50% showed histopathological changes in germinal epithelium of testes after 16 wks.
Narayana (1994a)	Male Charles Foster albino rats (n=10/ group)	Treatment for 50 days, by gavage	0 or 10 mg NaF/ kg bw/d	Not reported	Testosterone levels, Leydig cell diameter and nuclear diameter were significantly reduced. A non-significant decrease in intermediary enzyme activity in androgenesis was observed.

Narayana (1994b)	Male Charles Foster albino rats (n= 25 control and 50 days, n=30 70 days)	Treatment for 50 or 70 days, by gavage	0 or 10 mg NaF/kg bw/d	Not reported	In both treatment groups, sperm acrosomal hyaluronidase and acrosin were significantly reduced. Silver nitrate staining showed acrosomal damage and deflagellation. Sperm count, motility, and fertility were significantly reduced. Withdrawal after 70 days showed incomplete recovery.
Sprando (1997)	Male and Female Sprague Dawley Rats (P, n=12-14; F1,n=12)	Treatment parental total 14 wks: 10 wks before mating, 3 wks during mating, 1 wk after mating for males and for females during gestation and lactation. F1-generation: continued exposure during lactation, then same as parental.	<0.2, 25, 100, 175 or 250 ppm NaF (= <0.2, 25, 100, 175 or 250 mg/l) *ad libitum* in drinking water (= 0, 3.9, 15.6, 24.7, 25.1 mg/kg bw/d)	No significant body weight differences in any treatment groups	No dose related effects in both P and F1 group were found for testis, prostate and seminal vesicle weight. Testicular spermatid count, sperm production, LH, FSH and serum testosterone concentrations. Furthermore, no histological changes were found.
Sprando (1998)	Male and Female Sprague Dawley Rats (P, n=12-14; F1, n=12)	Treatment parental total 14 wks: 10 wks before mating, 3 wks during mating, 1 wk after mating for males and for females during gestation and lactation. F1-generation: continued exposure during lactation, then same as parental.	>0.2, 25, 100, 175 or 250 ppm NaF (=>0.2, 25, 100, 175 or 250 mg/l) *ad libitum* in drinking water	No significant body weight differences in any treatment groups	Statistical significant decrease in absolute volume of lym-phatic endothelium in 175 and 250 ppm NaF groups. No differences for volume of semi-niferous tubules, interstitial sace, Leydig cells, lymphatic space, tubular lumen, Sertoli cell nucleoli or height of sem-iniferous endothelium.
Collins (2001)	CRL:CD-BR rats. (F0 n= 48 males, 48 female/grp; F1 n=36 male, 36 female)	Two generation study. F0: Treatment 10 wks, than mating 1:1. 20 days after gestation 8 females sacrificed. F1 generation same treatment as F0 and provide F2 generation. Performed under GLP.	0, 25, 100, 175 or 200 ppm NaF (= 0, 25, 100, 175 or 200 mg/l) in drinking water *ad libitum*. Rats were fed a low fluoride diet (7.95 ppm NaF)	No remarkable clini-cal signs were found in F0 and F1 females. A dose-related decrease in drinking water consumption was found at 100, 175 and 250 ppm	Morphological development of F1 and F2 fetuses was similar in all groups. Reproduction parameters were not affected in F0 or F1 dams.

Pushpalatha (2005)	Male Wistar rats (n=unknown)	Treatment 75 days	0, 4.5 or 9.0 ppm NaF (= 0, 4.5 or 9.0 mg/l) *ad libitum* in drinking water	Body weights were significantly reduced in the 9.0 ppm group. Also a not further specified significantly dose related reduced brain index was found in this group.	Significant and dose related decreases in testicular index, sperm count, sperm viability, sperm motility and sperm physiological response in both groups. Non significant rise in sperm abnormalities.
Bataineh (2006)	Male Sprague Dawley rats (n=20 (control), n=20 (10 ppm) and n=16 (300 ppm)	Treatment 12 wks.	0, 100 or 300 ppm NaF (= 0, 100 or 300 mg/l) *ad libitum* in drinking water	No significant differences on body weight were found. Four unaccountable deaths in the 300 ppm group	Significant changes were found for both treatment groups for: epididymis, prostate, seminal vesicles and preputial gland weights. Spermatogenesis in testes was decreased as was, motility and sperm density. Testosterone and FSH levels were also decreased. A diminished sexual behavior was found.
Wan (2006a)	Male Wistar rats (n=32 treatment group, n=32 in control group; 8 rats/time point)	Treatment 50, 80, 100 or 120 days. In drinking water	0 or 150 mg/ NaF/L *ad libitum* in drinking water	No significant differences on body weight were found	A significant increase of sperm abnormalities and a decreased viability was found at all time points. Number of seminiferous epithelium cell layers, thickness and diameter of seminiferous tubule were reduced significantly at day 50, 100 and 120.
Wan (2006b)	Male Wistar rats (n=8, treatment group, n=8 in control group.	Treatment 10 days. In drinking water	0 or 150 mg/ NaF/L *ad libitum* in drinking water	No significant differences on body weight were found	Decreased epidermal growth factor was found in Leydig cell, spermatogonia and spermatocytes.
Gupta 2007	Male wistar rats (n=10 per group)		0, 2, 4 and 6 ppm NaF in drinking water at libitum for 6 months	No effect on body weight	Decreased testis weight and decreased epididymis and ventral prostate. Reduction in number of primary and secondary spermatocyte, sertoli cell counts

n = number of animals; d = day; wk = week; bw = body weight; ppm = parts per million.

Table 1.2 Fertility studies in male mice with sodium fluoride.

Authors	Species	Experimental period/ design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Kour (1980)	Male albino mice (strain unknown) (all groups (n=25/group)	Treatment for 1 (10 ppm), 2 (500 ppm) or 3 (1000 ppm) months	0, 10, 500 or 1000 ppm NaF (= 0, 10, 500 or 1000 mg/l) *ad libitum* in drinkingwater	Not reported	500 and 1000 ppm groups showed lack of maturation and differentiation of spermatocytes In 1000 ppm, spermatogenesis had stopped and seminiferous tubes were necrotic.
Li (1987)	Male mice, genotype $B_6C_3F_1$ (n=9 per group)	Treatment for 5 days, sacrificed after 35 days	0.1, 1.0, 10, 20, 35 or 70 (=MTD) NaF mg/kg bw/d, *ad libitum* in drinking water. Positive control: cyclophosphamide 20 mg/kg bw/d	Not reported	No significant differences on abnormal sperm counts or testes weights were found in any treatment group.
Chinoy (1989)a	Male Swiss mice (n=40/group, 5 treatment groups)	Treatment for 30 days. Sacrifice (for both doses) or sacrifice after 30 or 60 days withdrawal of 10 mg/kgbw	0, 10 mg/kg bw/d (230 ppm) and 20 mg/kg bw/d (400 ppm) *ad libitum* in drinking water	Not reported	For 10 and 20 mg/kg/d, histoarchitecture of testis was altered: disorganization and denudation of germinal epithelial cells of seminiferous tubules, with absence of sperm in lumina. In Cauda epididymis epithelial cell nuclear pyknosis and absence of luminal sperm were observed. Withdrawal of treatment for 30 or 60 days (10 mg/kg) caused recovery in the architecture.
Chinoy (1989)b	Male Swiss mice (n=40/group, 5 treatment groups)	Treatment for 30 days. Than sacrifice (for both doses) or sacrifice after 30 or 60 days withdrawal of 10 mg/kgbw	0, 10 mg/kg bw/d (230 ppm) and 20 mg/kg bw/d (400 ppm), *ad libitum* in drinking water	Body weights were not significantly decreased after treatment. Withdrawal returned body weight to normal	For 10 and 20 mg/kg/d, weight of seminal vesicles and prostrate were increased significantly. Testis succinic dehydrogenase was decreased and prostatic protein, acid prophatase, and glycogen concentrations were increased. Recovery was observed after withdrawal for 60 days.

Chinoy, (1992)	Male Swiss mice (n=40/group, 5 treatment groups)	Treatment for 30 days. Than sacrifice (for both doses) or sacrifice after 30 or 60 days withdrawal of 10 mg/kgbw	0, 10 mg/kg bw/d (230 ppm) and 20 mg/kg bw/d (400 ppm), *ad libitum* in drinking water	Not reported	Cauda epididymis sperm motility decreased for both 10 and 20 mg/kg/d after 30 treatment. Abnormalities in head midpiece and tail sections were found and acrosomal, post acrosomal and midpiece regions showed diffused silver nitrate staining. When treated males were mated one day after stop of treatment, implantation sites were absent. Withdrawal after 2 months resulted in full recovery (treatment before withdrawal not stated).
Dunipace (1989)	Male mice, genotype $B_6C_3F_1$ (n=9 per group)	Treatment for 5 days, sacrificed after 35 days	1, 10, 50, 75 ppm (=1, 10, 50 or 75 mg/l) NaF, *ad libitum* in drinking water	Not reported	No significant differences on abnormal sperm counts or testes weights were found in any treatment group.
Bano (1996)	Male Swiss albino mice (n=20/group)	Treatment for 30, 60 or 90 days	0, or 10 mg NaF /kg bw/d *ad libitum* in drinking water	Not reported	Total protein testes decreased in all treated groups. AcPase levels were decreased after 30 and 90 days, but were elevated after 60 days. AlkPase was increased on day 30 and 60 and decreased on day 90. LDH levels were decreased in all treated groups.
Sahdev (1996)	Male Swiss albino mice (n=20/group)	Treatment for 30, 60 or 90 days	0, or 10 mg NaF /kg bw/d *ad libitum* in drinking water	Not reported	Decrease in SDH and LDH levels after 30 and 60 days, no further increase after 90 days (no significance reported). ATPase increased at day 30 and increased further at day 60 and stayed elevated at day 90.

Elbe-thieha (2000)	Male Swiss mice (n=10 / group). Female Swiss mice (2 per male)	Treatment for 4 or 10 wks. After treatment, 2 females per 1 male for mating for 10 days. 10 days after mating, females were sacrificed. Males sacrificed 10 days after end of treatment	0, 100, 200 or 300 ppm NaF (=0, 100, 200 or 300 mg/l), *ad libitum* in drinking water(due to less water consumption NaF intake was, 0, 12, 22 and 39 mg NaF/kg bw/day for the 4 wk group and 0, 9, 16, 27 mg/kg bw.)	In 10 week groups, 2 animals died in 100 ppm group and 3 animals died in 300 ppm group, during the last week. Reduced water intake in the treatment groups when compared to control	Preputial gland weights were significantly reduced after 4wks in 200 and 300 ppm groups. No effect on male fertility after 4wks. After 10wks, females mated with 100, 200 and 300 ppm treated males, showed increase in number of resorptions. Reduction of mated females was found in 200 and 300 ppm group and number of viable fetuses was reduced in 200 ppm group.
Huang (2007)	Kunming mice (20 per group)	Treatment for 8 weeks	0, 50, 100, 200, 300 mg NaF/L in drinking water	Not provided	At 100, 200, 300 mg/L NaF: lower levels of serum and testicular testosterone. At 200 and 300 mg/L NaF: effects on sperm quality (sperm count, sperm motility, sperm survival and sperm abnormality) and oxidative stress.
Dvorak-ová (2008)	BALB/c mice (6-10 per group)	Treatment starting at day 1 of their postnatal development via their lactating mother for 4 weeks, thereafter via the same drinking water for an additional 2 months	0, 1, 10, and 100 ppm F^- in drinking water (4 ml /day)	No mortality, clinical signs of discomfort or body weight loss	At 10 and 100 ppm: abnormalities of spermatogenises and the ability of epididymal spermatozoa to capacitate in vitro. Decreased sperm head tyrosine phosphorylation and actin polymerization.

n = number of animals; h = hour; d = day; wk = week; bw = body weight; ppm = parts per million.

Table 1. 3 Fertility toxicity studies rabbits and guinea-pigs with sodium fluoride.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Shashi (1990)	Male albino rabbit (strain unknown) (n=6/group)	Treatment for 100 days	0, 5, 10, 20 or 50 mg NaF/kg bw/d, subcutaneously	Not reported	5 mg: no macroscopical of microscopical changes 10 and 20 mg: foci of necrosis in seminiferous tubules and deficient differentiation and maturation of spermatocytes. 50 mg: Degeneration of seminiferous tubes, tubular atrophy and necrosis.
Susheela (1991)	Male rabbit (strain unknown) (n=7 18 months group, n=3 29 months group, n=10 control)	Treatment for 18 or 29 months	0 or 10 mg NaF/kg bw/d by gavage	Not reported	In both groups: loss of cilia on epithelial cells of the lumen, ductuli efferentes, and caput epididymis. 29 months group: spermatogenic cells in seminiferous tubules were disrupted, degenerated and devoid of spermatozoa.
Chinoy (1991)	Male rabbit (strain unknown) (n=5/group)	Treatment for 30 days. In addition one group treated for 40 mg/kg bw was withdrawn from treatment for an additional 30 days	0, 20 or 40 mg NaF/kg bw/d	Decreased bw after treatment with NaF	In both treatment groups, a decrease in epididymal sperm motility and cauda sperm count was observed. Fertility rate in 20 mg NaF/ kg bw/d group was 33% and 0% in 40 mg NaF/kg bw/d group (control = 95%). Withdrawal for 30 days at 40 mg NaF/kg bw/d showed no significant recovery of sperm motility count or fertility rate.
Shashi (1992)	Male albino rabbits (strain unknown) (n=12/group)	Treatment for 3.5 months	0, 5, 10, 20 and 50 mg NaF /kg bw/d injected subcutaneously	Not specified fluoride intoxica-tion. No further data on general toxicity provided	Acidic basic and total testicular proteins were significantly decreased in all treated groups. A dose response for DNA decrease was found.

Kumar (1994)	Male rabbits (strain unkown) (n=8/group)	Treatment for 18 months	0 or 10 mg NaF/kg bw/d by gavage	Not reported	Structural defects were observed in flagellum, acrosome and nucleus of spermatids and spermatozoa in treated rabbits. In addition absence of outer micro-tubules complete breakdown of the fibrous sheath and structural defects in mitochondria of the middle piece of the flagellum were observed.
Kumar (1995)	Male rabbits (strain unkown) (n=6/group)	Treatments for 20 or 23 months	0 or 10 mg NaF /kg bw/d by gavage	Not reported	In both treatment groups, loss of stereocilla, decreased height of pseudostratified columnar epithelium were found. An increase in diameter of both caput and cauda ductus epididymis was observed in the 23m group. Cauda epididymis weight was significantly reduced in the 20m treatment group
Chinoy (1997)	Male guinea-pigs (strain unknown) (n=10/group)	Treatment for 30 days	0 or 30 mg NaF /kg.bw/d in drinking water *ad libitum*	Not reported	After treatment, cauda epididymal sperm structural and metabolic alterations led to decreases in motility, live/ dead ratio and sperm mitochondrial activity. Increases were found in sperm abnormalities and alterations in sperm membrane phospholipids.

n = number of animals; h = hour; d = day; wk = week; m = month; bw= body weight; ppm = parts per million; i.p.= intraperitoneally.

Table 1.4 Fertility toxicity studies in female animals with sodium fluoride.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Al-Hiyasat (2000)	Female Sprague-Dawley rats (n=10/ group)	Treatment for 30 days	0.5, 200, 400 or 600 ppm NaF (= 0.5, 200, 400 or 600 mg/l) in drinking water, *ad libitum*	Rats in all treated groups showed clinical signs of toxicity, death (all of 600 ppm group and 7 in 400 ppm group), reduced body weight and decreased water consumption	200 ppm group: Reduction in number of viable fetuses and number of resorptions and implantations was found. Significant increase of maternal organ weights (ovary, uterus and kidney) and embryo weight.
Darmani (2001)	Female Swiss mice. (n=10/ group)	Treatment for 4 or 12 wks	0, 100, 200, 300 ppm NaF (=0, 100, 200 or 300 mg/l), in water *ad libitum*. Actual intake = 9.52-27.70 mg NaF/kg bw/d for 4 wks and 5.82-18.85 mg NaF/kg bw/d for 12 wks group	No clinical signs of toxicity were observed, however in the 12 wks group, one animal of 100 ppm, two animals of 200 ppm and two animals of 300 ppm died in the last week.	After 12 wks treatment, number of pregnancies was lower in all treated groups, number viable foetuses was declined in 200 and 300 ppm group and implantations was lower in 300 ppm group, also ovary weights were increased in the 200 and 300 ppm groups.
Shashi (1990)	Female white albino rabbits (strain unknown) (n=6/group)	Treatment for 100 days	Distilled water (0), 5, 10, 20 or 50 mg NaF/kg bw/d injected subcutaneously	Not reported	In 10 and 20 mg/kg treated groups, ovary had congested oocytes in follicles, necrosis of follicles and cells and interstitial oedema. The 50 mg NaF/kgbw group showed complete atrophy of follicles along with oocyte disintegration and marked necrosis of cells accompanied by infiltration of monocytes, lymphocytes and histocytes in interstitial tissue.

n = number of animals; h = hour; d = day; wk = week; m=month; bw = body weight; ppm = parts per million.

Table 2.1 Developmental toxicity studies in animals with sodium fluoride.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Devoto (1972)	Female rats (strain unkown) n=6/ treaetment group, control: n=20	Rats were injected i.p. or subcutaneously from day 10-18 of gestation.	1, 5, 10, 15 or 20 mg NaF/kg bw/d.	No reported	Percentage of dead fetuses was higher in NaF treated groups. Differences in percentage of dead fetuses and necrotic placenta between treated and control were significant. i.p treated rats showed significant higher percentages of dead fetuses when compared to subcutaneously treated rats. No maxillofacial malformations were found in viable fetuses.
Ream (1983)	Female Sprague Dawley rats (n=6/ group)	Treatment 10 weeks prior to breeding and during 3 successive pregnancy and lactation periods. Pups of third pregnancy sacrificed at 3 weeks.	0 or 150 ppm NaF (=0 or 150 mg/l) in drinking water, *ad libitum*	Not reported	Femurs of third pregnancy pups showed no pathological changes.
Collins (1995)	Female CD-CRL:CD-BR VAF^{+} rats (n=unknown)	Treatment for 20 days (during gestation).	0, 10, 25 100, 175 or 250 ppm NaF (=0, 10, 25, 100, 175 or 250 mg/l) in drinking water *ad libitum*. Lowered drinking water consumption resulted in 0, 1.4, 3.9, 15.6 24.7 and 25.1 mg/kg bw/d NaF consumption. Low fluoride food (7.95 ppm) was administered	No dose-related behavioral changes or clinical maternal signs were observed.	A decrease in the number of implants per litter and lower number of corpora lutea in 250 ppm group was found. A decrease in number of fetuses was found in all groups. *In utero* deaths was in all treatment groups same as control. Fetal growth was not affected by treatment, despite less feeding and drinking in 250 ppm group. An increase in average number of fetuses with three or more external anomalies was found in 250 ppm group.

Heindel (1996)	Female Sprague Dawley derived rats (n=26/group) Female New Zealand White rabbits (n=26/group)	Rat treatment: on gestation days 6-15 Rabbit treatment: on gestation days 6-19	Rat: 0, 50, 150 or 300 ppm NaF. Rabbit: 0, 100, 200 or 400 ppm NaF (= 0, 100, 200 or 400 mg/l). *Ad libitum* in drinking water. Drinking water contained less than 0.6 ppm NaF and feed 12.4 ppm for rats, 15.6 for rabbits	No clear clinical signs of toxicity were observed. Body weight and water consumption were constant in all groups.	For both rat and rabbit: exposure to NaF during gestation did not affect frequency of post-implantation loss, mean fetal body weight or external visceral or skeletal malformations.
Collins (2001a)	CRL:CD-BR rats. (F0 n= 48 males, 48 female/grp; F1 n=36 male, 36 female)	Two generation study F0: Treatment 10 wks, than mating 1:1. 20 days after gestation 8 females sacrificed. F1 generation same treatment as F0 and provide F2 generation. Performed under GLP	0, 25, 100, 175 or 200 ppm NaF (= 0, 25, 100, 175 or 200 mg/l) in drinking water *ad libitum*. Rats were fed a low fluoride diet (7.95 ppm NaF)	No remarkable clinical signs were found in F0 and F1 females. A dose-related decrease in drinking water consumption was found at 100, 175 and 250 ppm.	Morphological development of F1 and F2 fetuses was similar in all groups. Reproduction parameters were not affected in F0 or F1 dams. No change in sex distribution, fetus development or soft tissue development in F1 or F2 generation. Hyoid ossification development was significantly decreased at 250 ppm in both F1 and F2 generation.
Collins (2001b)	CRL:CD-BR rats. (F0 n= 48 males, 48 female/grp; F1 n=36 male, 36 female)	Two generation study. F0: Treatment 10 wks, than mating 1:1. 20 days after gestation 8 females sacrificed. F1 generation same treatment as F0 and provide F2 generation. Performed under GLP	0, 25, 100, 175 or 200 ppm NaF in drinking water *ad libitum*. Rats were fed a low fluoride diet (7.95 ppm NaF)	No remarkable clinical signs were found in F0 and F1 females. A dose-related decrease in drinking water consumption was found at 100, 175 and 250 ppm.	No significant changes were found on male or female reproduction data. No significant changes found on pup survival or weights.
Sherlin (2001)	Female Wistar strain rats (n=10/group)	Treatment from day 6 trhough day 19 of the gestation period	0 or 40 mg NaF/kg bw/d in water, *ad libitum*	Body weight and feed consumption were lower in treated groups.	In the treated group, absolute uterine weight and number of implantations were decreased. Higher incidence of skeletal and visceral abnormalities were observed. When NaF and vitamin D were administered, fluoride induced reduction in body weight feed consumption and absolute uterine weight were ameliorated.

n = number of animals; h = hour; d = day; wk = week; bw= body weight; ppm = parts per million; i.p.= intraperitoneally.

Table 2.2 Developmental toxicity studies in animals with sodium fluoride, placental transfer.

Authors	Species	Experimental period/ design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Hudson (1967)	Female guinea pigs (n=7/group) strain unknown. Second part, n=4/group	Females were treated and at time of birth and amount of fluoride in femurs of offspring was analyzed. Second part: Previously low treated (1 ppm) females were mated for a second time.	NaF free water, 1, 5, 10, 25 or 50 ppm NaF (= 1, 5, 10, 25 or 50 mg/l) in drinking water, *ad libitum*. Animals received a fluoride low diet. Animals in the second part of the study received 5, 10, 25 or 50 ppm NaF 5, 10, 25 or 50 mg/l) in drinking water.	Not reported	In the first generation, a significant dose related increase of fluoride in both femur as carcass was found when compared to control. In the second-generation study, a significant increase in concentration of fluoride was found, when compared to control of the same generation.
Parker (1986)	Female Hartley guinea pigs (n=35 females, randomly divided in dose groups, n=not stated)	Treatment for 55 days, at which maternal and fetal plasma was collected.	Pregnant sows were treated with control, 5 or 20 ppm NaF (= 5 or 20 mg/l) in drinking water, *ad libitum*. Food contained 35 ppm for all groups.	Not reported	An increase in mean fluoride concentration for maternal plasma in 20 ppm group was found. Mean fetal plasma fluoride was lower at baseline and increased much less than did maternal plasma levels.

n = number of animals; h = hour; d = day; wk = week; bw = body weight; ppm = parts per million.

Annex **E**

Calculation safe level of fluoride in human breast milk

In order to protect up to 6-month-old breastfed children from the effects of HF and NaF through intake of breast milk, the committee uses the following default values:

- Body weight infant: 4.5 kg
- Intake human breast milk per infant per day: 900 mL
- An infant is as sensitive for the effects of fluoride as an adult

These assumption are used for the calculation of a tolerable level of fluoride in human breast milk. These values are conservative figures estimated from growth curves published for the Netherlands[1,2] and by the WHO[3] and breast milk intake.[4]

The Institute of Medicine (IOM) established a Tolerable Upper Intake Level (TUIL) of 0.1 mg fluoride/kg/day for infants, toddlers and children through the age of 8 years of age, based on the Lowest Observed Adverse Effect Level (LOAEL) for moderate fluorosis, using dietary fluoride intake data.[5]
This corresponds to:

- A tolerable intake of 0.10 mg/kg body weight/day
- A tolerable intake of 0.45 mg/infant/day
- A tolerable intake of 0.50 mg/l breast milk

In conclusion, the committee considers 0.5 mg fluoride / liter breast milk as a tolarable level

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Annex **F**

Abbreviations

AcPase	acid phosphatase
AlkPase	alkaline phosphatase
ATPase	adenosine triphosphatase
bw	body weight
d	day
EPA	Environmental Protection Agency
FSH	follicle stimulating hormone
h	hours
HF	hydrogen fluoride
HFEG	high fluoride exposed group
IOM	Institute of Medicine
i.p.	intraperitoneal
LDH	lactate dehydrogenase
LFEG	low fluoride exposed group
LH	luteinizing hormone
LOAEL	Lowest Observed Adverse Effect Level
MTD	maximum tolerated dose
n	number
NaF	sodium fluoride
OECD	Organisation for Economic Cooperation and Development
OR	odds ratio

AcPase	acid phosphatase
AlkPase	alkaline phosphatase
ppm	parts per million
SDH	succinic dehydrogenase
TFR	total fertility rate
TSP	total soluble protein
TUIL	tolerable upper intake level
wk	weeks
Zn	zinc