FLUORIDE-INDUCED THYROID PROLIFERATIVE CHANGES AND THEIR REVERSAL IN FEMALE MICE AND THEIR PUPS

Hanen Bouaziz,[a] Lotfi Soussia,[a] Fadhel Guermazi,[b] Najiba Zeghal[a]

Sfax, Tunisia

SUMMARY: To determine effects of fluoride on thyroid function in suckling mice, adult female mice were given 500 ppm NaF (226 ppm F^-) in their drinking water from the 15th day of pregnancy until either the 4th or 14th day after parturition. On day 14 of full-period treatment, pups and their mothers were sacrificed. In the F^-exposed group the body weight of the 14-day-old pups was 15% less than the controls. This reduction in pup body weight is attributed to a defect in plasma thyroid hormone levels: free thyroxine (FT_4: –15%) and free triiodothyronine (FT_3: –6%). These reductions also correlate with a decrease in thyroid iodine content of the experimental pups (–55%) and their mothers (–68%), along with hypertrophy of their thyroid glands (+29% in the pups and +41% in the mothers), probably caused by a 1.74- and 3.15-fold increase of plasma thyroid stimulating hormone (TSH) in the pups and their mothers, respectively. These biochemical modifications also correspond histologically with an increase in thyroid follicular number and a reduction in colloid volume. When fluoride ion was eliminated from the mothers' drinking water four days following parturition, a partial recovery occurred after ten days in body weight, thyroid gland weight, and plasma TSH level in both pups and their mothers. However, essentially complete recovery was observed in plasma levels of FT_4 and FT_3 and in thyroid iodine content. Compared with NaF-treated mice, significant amelioration in thyroid histological aspects was also observed after withdrawal of NaF treatment.

Keywords: Iodine in thyroid; Mice; Offspring mice; Pregnant mice; Sodium fluoride; Thyroid function; Thyroid hormones (FT_3; FT_4); TSH.

INTRODUCTION

Various factors such as iodine deficiency, dietary goitrigens, malnutrition, heredity, ionizing radiation, and pollution can induce non-toxic goiter.[1,2] Although there is evidence to the contrary,[3] it is generally believed that fluorine as used to prevent dental caries at the level of 1 ppm F^- in drinking water does not affect either thyroid function or its structure.[4] On the other hand, if fluorine intake is extremely high as in an endemic fluorosis area, several parameters of thyroid activity are reported to be disturbed.[5] In our previous studies,[6,7] 500 ppm of NaF (226 ppm F^-) given to pregnant mice in their drinking water for 20 days, decreased plasma thyroxine (FT_4) and triiodothyronine (FT_3) levels in their 14-day-old pups.

Some years ago fluoride compounds were used to treat hyperthyroidism and depress thyroid function.[8] Numerous studies on the effects of fluoride on thyroid function have been conducted on humans,[9,10] cattle,[11] calves,[12] and adult rats.[13-15] Both adult[5,16,17] and young mice[6,7] have been shown to

[a]For correspondence: Prof Dr N Zeghal, Animal Physiology Laboratory, Sciences Life Department, Faculté des Sciences de Sfax-Route de la Soukra-Km 3.5, 3018 Sfax BP 802, Tunisia. E-mail: najiba.zeghal@tunet.tn [b]Nuclear Medicine Service, CHU Habib Bourguiba-Sfax.

develop hypothyroidism after ingestion of fluoride. However, reports remain scarce or appear to be absent in mice after withdrawal of this pollutant.

The purpose of this study was to investigate induction and reversibility of thyroid proliferative changes in suckling mice and their mothers administered sodium fluoride in their drinking water.

MATERIALS AND METHODS

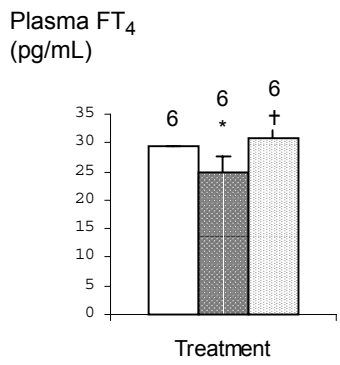
The same protocol was used as our previous papers.[6,7,18] Thirty pregnant mice were separated into three groups of ten animals in each group. The first group (A) of animals did not receive supplemental fluoride and was considered the untreated or control group. The other two groups received sodium fluoride in their drinking water at a concentration 0.5 g/L of water (226 ppm F^-) from the 15^{th} day of pregnancy until either the 14^{th} day after parturition for the second group (B) or the 4^{th} day for the third group (C). Mice of the last group were allowed free access for the next ten days to tap water without added fluoride. This group was added in the current study in contrast to our relevant previous reports.[6,7,18]

All mice were sacrificed by intra-abdominal administration of chloral hydrate, body weights were measured, and blood was collected from the brachial artery of each mouse and centrifuged at 2200 g. Plasma samples were withdrawn and kept at –20°C until analysis. Plasma FT_3, FT_4, and TSH levels were determined by commercially available radio-immunoassay kits (Immunotech, ref: 1363, 1579 and Biocode Hycel, ref: AH R001, respectively), according to the manufacturers' instructions. Some thyroid glands were removed from pups and dams. They were weighed and preserved at –20°C until analysis for their iodine content after being made alkaline by the method of Sandell and Kolthoff.[19] Other thyroid samples, destined for light microscopy, were dissected with a piece of trachea and submitted to the same protocol as reported earlier.[7]

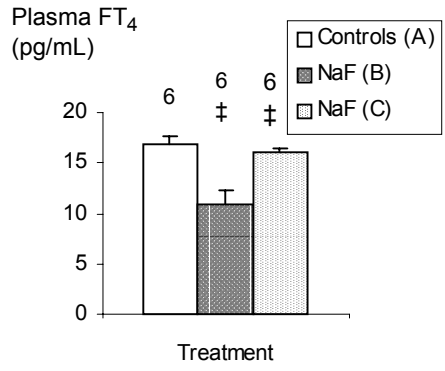
Numerical results are expressed as arithmetical means ± SEM. Statistical significance among groups was determined by using Student's t test[20] with a p value of less than 0.05.

RESULTS

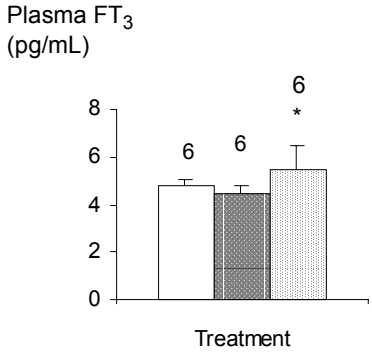
Compared to the control group, fourteen day-old mice, whose mothers had been administered 500 ppm of NaF in their drinking water, showed a decrease in body weight (Table) and in plasma levels of FT_3 and FT_4 (Figure 1) as demonstrated in our earlier paper.[6] Thyroid iodine content was also decreased by 68% in dams and by 55% in their offspring (Figure 2). At the same time, plasma TSH levels were increased 3.15- and 1.74-fold, respectively (Figure 1). The weights of thyroid glands were also increased by (+41% and +29%) (Table). Withdrawal of NaF treatment for 10 days resulted in a partial recovery in dams and their offspring in body weight, thyroid gland weight (Table), and plasma TSH levels (Figure 1). However, complete recovery occurred in plasma levels of FT_4 and FT_3 and in thyroid iodine content of pups. In dams complete recovery occurred in plasma levels of FT_4 and FT_3 in spite of only a partial recovery of thyroid iodine content when compared to controls (Figures 1 and 2).



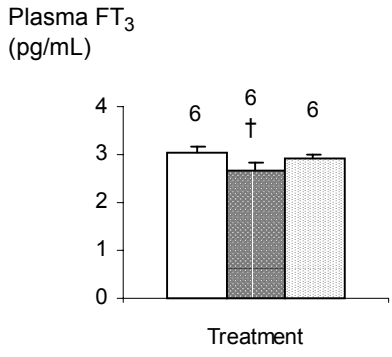
Plasma FT_4 in offspring



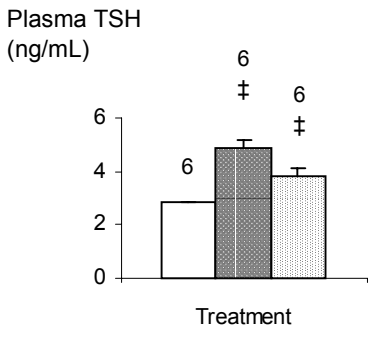
Plasma FT_4 in mothers



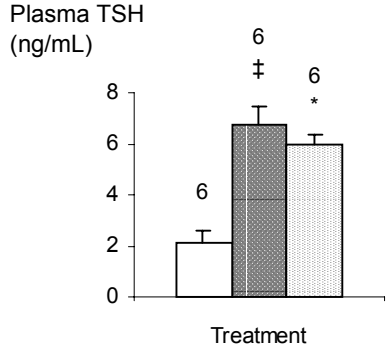
Plasma FT_3 in offspring



Plasma FT_3 in mothers



Plasma TSH in offspring



Plasma TSH in mothers

Figure 1. Plasma FT_4, FT_3, and TSH levels of 14 day-old mice and their mothers: controls (A) and mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the 14th day (B) or the 4th day (C) after parturition.
Significant differences: Treated (B) vs Controls (A): $^{*}p \le 0.05$; $^{\dagger}p \le 0.01$; $^{\ddagger}p \le 0.001$.
Treated (C) vs Treated (B): $^{*}p \le 0.05$; $^{\dagger}p \le 0.01$; $^{\ddagger}p \le 0.001$.

Table. Body and thyroid weights of 14-day-old mice and of their mothers: controls (A) and mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the 14th day (B) or the 4th day (C) after parturition

Parameter	Group	Treatment		
		Controls (A) (Mean±SEM)	Treated (B) (Mean±SEM)	Treated (C) (Mean±SEM)
Body weight (g)	Offspring (n = 40)	8.99±0.44	7.55±10.39†	8.75±0.14†
	Mothers (n = 10)	41.44±1.68	33.52±1.86†	35.33±1.13
Thyroid weight (mg)	Offspring (n = 40)	1.04±0.18	1.33±0.14*	1.14±0.22*
	Mothers (n = 10)	3.67±0.41	5.16±0.98*	3.98±0.31

Significant differences: Treated (B) vs. Controls (A): *p ≤0.05; †p≤ 0.001.
Treated (C) vs. Treated (B): *p ≤ 0.05; †p ≤ 0.001.

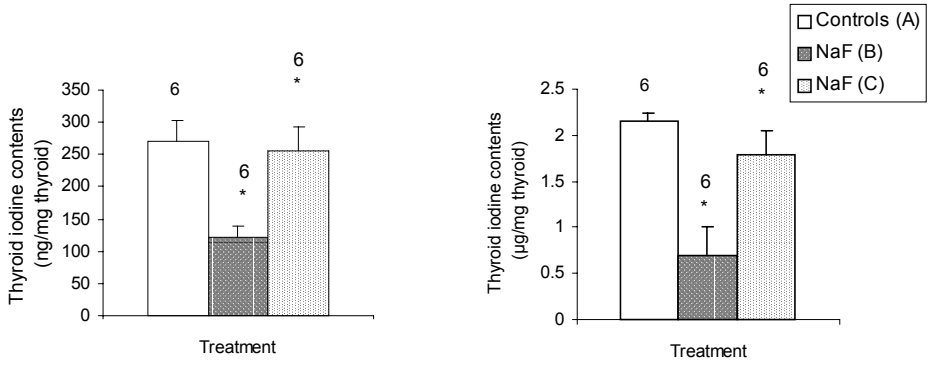


Figure 2. Thyroid content of 14 day-old mice and their mothers: controls (A) and mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the 14th day (B) or the 4th day (C) after parturition.
Significant differences: Treated (B) vs Controls (A): *p ≤ 0.001.
Treated (C) vs Treated (B): *p ≤ 0.001.

Light microscopy revealed changes in the thyroid glands of pups of NaF-treated mice (group B) compared to untreated animals (group A) (Figures 3B and 3A). Ten days after discontinuing treatment in group C, a partial recovery of thyroid histological aspect was obtained (Figure 3C).

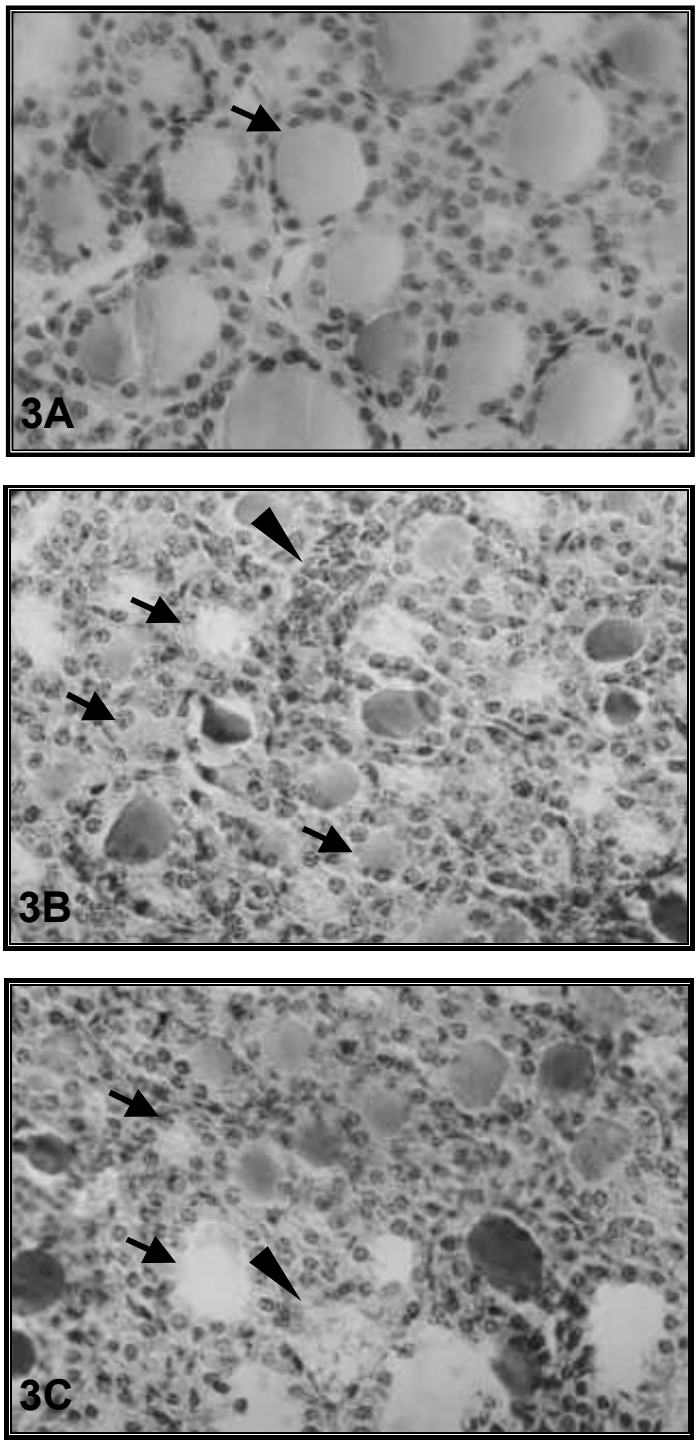


Figure 3. Histology of thyroid sections of 14-day-old mice: controls (A) and pups of mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the14th day (B) or the 4th day (C) after parturition. Optical microscopy HE (x 400).

Arrows: → indicate thyroid follicles; ► indicate blood vessels.

DISCUSSION

Our results showed, after NaF treatment of mothers, a decrease in plasma thyroid hormone levels of nursing pups as also reported in our earlier paper.[6] This may be explained either by transplacental transfer of fluoride from the mothers to their fetuses as demonstrated by Shen and Taves,[21] or by inhibition of sodium iodide coupled symport transport abolishing prolactin effects on iodide accumulation and incorporation in the mammary gland.[22] A low total iodide pool may impose a reduction of thyroid iodine content and a critical limitation on hormonal synthesis,[8] since the iodine is necessary for thyroid hormone biosynthesis. This last hypothesis is confirmed by data of Jentzer,[23] who found that rabbits fed with 0.05 mg F^- per day had their thyroid iodine content reduced by 25%. Other previous findings of Galletti and Joyet[8] also suggested that fluoride inhibits thyroid iodide concentration. Likewise, Kahl and Bobek[24] found a significant reduction of protein-bound iodine as well as an overall reduction of iodide and a reduction of iodine uptake by the thyroid gland in fluoride-treated rats. Other reports in the literature suggest that tyrosine and its metabolite levels are also influenced by fluoride. In fact, increased urinary loss of tyrosine is known to occur in men living in high-fluoride areas[25] and in monkeys receiving low daily doses of fluoride.[26]

Still other concepts of fluoride interference with thyroid hormone synthesis have been proposed and documented. Thus, Tezelman et al.[27] reported that fluoride, by increasing the intracellular cAMP concentration, causes desensitization of the thyroid stimulating hormone receptor. On the other hand, Willems et al.[28] demonstrated that NaF depresses the endocytosis of colloid and thyroid secretion by inhibiting aerobic glycolysis in the follicular cells. We therefore suggest that administration of this pollutant provokes thyroid hyperplasia in mice. Indeed, the weight of the thyroid gland was increased. The mechanism for this response is an increase in TSH levels as seen in the present study due to a decrease in production of thyroid hormones as reported in our previous papers.[6,7] The changes in the thyroid weight in our study are also similar to those described in other studies. Several investigators found that fluoride administration to experimental rats may cause an enlargement of the thyroid gland.[15] Similarly, a close relationship was found between fluoride intake and the incidence of goiter in areas containing high levels of fluoride in water.[29-32] Histological changes seen in the thyroid gland of mice treated with NaF are characterized by follicular cell hyperplasia, an increase of follicular number and vascularity, and a decrease of colloid.

In our study, when fluoride ion was removed from the dams' drinking water on day four after delivery, body growth rate increased and thyroid weight decreased, but they did not return to control values after ten days. Reversibility of sodium fluoride effects was thus observed with a return of thyroid hormone production and of thyroid iodine content, although plasma TSH level was only partially restored. Restoring iodide, by removing sodium fluoride from the dams' drinking water on day four after delivery, reduced the hypertrophy of follicle epithelial

cells and increased the colloidal volume. Some thyroid follicles became larger than those of treated mice in group B. In human pathologies, goiter size has been found to be reduced either by application of an iodide treatment,[33] which results in a decrease in the size of cells and follicles[34] or in a reduction of plasma TSH levels as demonstrated by our results. In fact, Li et al.[33] reported that iodide uptake by the thyroid gland is competitively inhibited by fluoride, especially in high-fluoride areas and is alleviated by the provision of iodized salt causing a decrease in the occurrence of goiter. This reversibility evidently involves the return to normal aspect of thyroid gland in which the phenomena of proliferation and cell death must be involved.

In conclusion, this study has shown that NaF administered in drinking water (0.5 g/L) provoked hypothyroidism in female mice and in their nursing pups, which could be largely reversed by withdrawing NaF treatment for a period of ten days.

ACKNOWLEDGEMENTS

The authors thank to Mrs Nabiha Mezghanni for her skillful technical assistance in radio-immunoassay determinations of FT_3, FT_4, and TSH plasma levels.

REFERENCES

1 Beckers C. Non toxic goiter. In: De Visscher M. Comprehensive endocrinology: the thyroid gland. New York: Raven Press; 1982. p. 257-78.
2 Hall R, Köbberling J. Thyroid disorders associated with iodine deficiency and excess. New York: Raven Press; 1985.
3 Hara K. Studies on fluorosis, especially effects of fluoride on thyroid metabolism. Shikoku Eisei Gakkai Zasshi 1980;30:42-57.
4 Buergi H, Siebenhuner L, Miloni E. Fluorine and thyroid gland function: a review of literature. Klinische Wochenschrift 1984;62:564-9.
5 Zhao W, Zhu H, Yu Z, Aoki K, Misumi J, Zhang X. Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. Endocr Regul 1998;32:63-70.
6 Bouaziz H, Ammar E, Ghorbel H, Ketata S, Jamoussi K, Ayadi F, et al. Effect of fluoride ingested by lactating mice on the thyroid function and bone maturation of their suckling pups. Fluoride 2004;37(2):133-42.
7 Trabelsi M, Guermazi F, Zeghal N. Effect of fluoride on thyroid function and cerebellar development in mice. Fluoride 2001;34(3):165-73.
8 Galletti PM, Joyet G. Effect of fluorine on thyroidal iodine metabolism in hyperthyroidism. J Clin Endocr 1958;18:1102-10.
9 Yu YN. Effects of chronic fluorosis in the thyroid gland. Chinese Med J 1985;65(12):747-9.
10 Bachinskii PP, Gutsalenko OA, Naryzhniuk ND, Sidora VD, Shliakhta AI. Action of fluoride on the function of pituitary-thyroid system of healthy persons and patients with thyroid disorders. Probl Endokrinol 1985;31(6):25-9.
11 Hillman D, Bolenbaugh DL, Convey EM. Hypothyroidism and anemia related to fluoride in dairy cattle. J Dairy Sci 1979;62:416-23.
12 Kapoor V, Prasad T, Paliwal VK. Blood biochemical constituents in calves following subclinical levels of fluoride toxicosis. Fluoride 2001;34(2):126-31.
13 Guan ZZ, Zhuang ZJ, Yang PS, Pan S. Synergistic action of iodine deficiency and fluorine intoxication on rat thyroid. Chin Med J 1988;101(9):679-84.
14 Bobek S, Kahl S, Ewy Z. Effect of long-term fluoride administration on thyroid hormone levels in rats. Endocrinol Exp 1976;10:289-95.

15 Narbutt B, Romer TE, Grabski J, Szymik N. Effect of sodium fluoride on thyroid structure in rats. Endokrynol Pol 1971;22(5):445-51.
16 Kendall-Taylor P. Comparison of the effects of various agents on thyroid adenyl cyclase activity with their effects on thyroid hormone release. J Endocr 1972;54:137-45.
17 McLaren JR. Possible effects of fluoride on the thyroid. Fluoride 1976;9(2):105-11.
18 Bouaziz H, Ghorbel H, Ketata S, Guermazi F, Zeghal N. Toxic effects of fluoride by maternal ingestion on kidney function of adult mice and their suckling pups. Fluoride 2005;38(1):23-31.
19 Sandell EB, Kolthoff IM. Microdetermination of iodine by a catalytic method. Microchem Acta 1937;1:9-25.
20 Schwartz D. Méthodes Statistiques à l'usage des Médecins et des Biologistes [Statistical methods for Doctors and Biologists's use]. Paris: Flammarion Publisher; 1963.
21 Shen YW, Taves DR. Fluoride concentrations in the human placenta and maternal and cord blood. Am J Obstet Gynecol 1974;119(2):205-7.
22 Rillema JA, Yu TX, Jhiang SM. Effect of prolactin on sodium iodide symporter expression in mouse mammary gland explants. Ann J Physiol Endocrinol Metab 2000;279:E769-72.
23 Jentzer A. Effet du fluor et du fluor-iod sur la teneur en iode de la thyroïde de lapin [Fluoride and fluoro-iodine effects on thyroid iodine content in rabbits]. Bull Schweiz Akad Med Wiss 1959;15:412-22.
24 Kahl S, Bobek S. Effect of fluoride administration on radiothyroxine turnover in rats. Endokr Pol 1975;26(4):391-6.
25 Murray MM, Wilson DC, Exley D, Hasan S. Investigation of an epidemic of goitre in West Pakistan. Trans R Soc Trop Med Hyg 1958;52(3):223-9.
26 Singh A, Vazirani SJ, Jolly SS, Bansal BC. Endemic fluorosis with particular reference to dental and systemic intoxication. Postgrad Med J 1962;38:150-6.
27 Tezelman S, Shaver JK, Grossman RF, Liang W, Siperstein AE, Duh QY et al. Desensitization of adenylate cyclase in Chinese hamster ovary cells transfected with human thyroid-stimulating hormone receptor. Endocrinology 1994;134(3):1561-9.
28 Willems C, Berberof-Van Sande J, Dumont JE. Inhibition of thyroid secretion by sodium fluoride *in vitro*. Biochim Biophys Acta 1972;264:197-204.
29 Day TK, Powell-Jackson PR. Fluoride, water hardness and endemic goitre. Lancet 1972;27:1135-8.
30 Desai VK, Solanki DM, Bansal RK. Epidemiological study of goitre in endemic fluorosis district of Gujarat. Fluoride 1993;26:187-90.
31 Jooste PL, Weight MJ, Kriek JA, Louw AJ. Endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province of South Africa. Eur J Clin Nutr 1999;53(1):8-12.
32 Latham MC, Grech P. The effects of excessive fluoride intake. Am J Public Health Nations Health 1967;57:651-60.
33 Li Y, Zhang J, Li Z. Prevention of iodine deficiency in high fluoride areas in Tianjin City, China. Fluoride 1998;31(3):S18.
34 Gartner R. Thyroxine treatment of benign goiter. Acta Med Austriaca 1994;21(2):44-7.

Published by the International Society for Fluoride Research
http://homepages.ihug.co.nz/~spittle/fluoride-journal.htm
Editorial Office: 727 Brighton Road, Ocean View, Dunedin 9051, New Zealand