NTP Supplemental 2-Year Study of Sodium Fluoride in Male F344 Rats (CAS No. 7681-49-4)

Abstract

In an effort to examine the effect of exposure to sodium fluoride on the incidence of bone tumors induced by ionizing radiation, the femoral-tibial joint of the left hind limb of 100 male F344 rats was irradiated with 3000 R from a ^{137}Cs source and the animals divided into two groups of 50. One group was then administered drinking water containing 250 ppm sodium fluoride for two years while the other group received plain deionized water. Two additional groups of 50 male F344 rats (not exposed to radiation) received drinking water containing 250 ppm sodium fluoride (113 ppm fluoride ion), or plain deionized water for two years. Survival, mean body weights, food and water consumption of all groups was comparable throughout the two year study duration. Bone fluoride analysis conducted at the end of the study revealed significant accumulation of fluoride in the bones of groups of rats exposed to sodium fluoride. Exposure to irradiation, sodium fluoride, or both irradiation and sodium fluoride was not associated with an increase in bone tumors or other neoplastic lesions. Non-neoplastic lesions of incisor teeth including ameloblast and odontoblast degeneration and malformation of enamel and dentine, were increased in groups exposed to sodium fluoride.

Introduction

The ability of fluoride to modify the crystalline state of bone mineral and stimulate bone formation in vivo is well documented and has led to a number of clinical trials that have examined the use of sodium fluoride as a treatment for osteoporosis (Pak et al, 1994; Riggs et al, 1994). Morphometrc studies of fluoride treated bone suggest that these changes are accompanied by increased numbers of osteoblasts (Braincon and Meunier, 1981), and studies in vitro have demonstrated that fluoride stimulates the proliferation and differentiation of cultured osteoblasts (Farley et al, 1983) and osteoblast precursors (Kassem et al, 1994) at concentrations approximating serum fluoride concentrations in patients receiving fluoride treatment.

The anabolic action of fluoride on bone suggests the possibility that fluoride treatment might have some impact on the incidence of osteosarcoma associated with external beam radiation therapy. Although uncommon, osteosarcoma associated with radiation therapy has been well documented and occurs in bones present in the therapeutic radiation field. For example among breast cancer patients that had received radiation therapy, osteosarcoma occurred most frequently in the scapula (Pendlebury et al, 1995).

Previous studies in rats indicated that osteosarcomas can be successfully induced by irradiating the hind limb with x- or gamma radiation. Since the rest of the animal can be shielded this provides an opportunity to restrict the radiation field to only one limb. Cater et al (1959; see also Baserga et al, 1961) induced bone osteosarcomas by irradiating both knee joints of female Wistar rats. Benstead et al (1965) exposed one entire hindlimb of groups of male rats to 140 kvp x-rays. Besides bone tumors, fibrosarcomas and mammary tumors were observed in soft tissues within the radiation field in a few animals. Tinkey et al (1998) irradiated the entire right rear leg of groups of male Sprague-Dawley rats with cobalt 60 gamma rays. In this study all observed osteosarcomas were extraskeletal and occurred within soft tissue.

The goal of the present study was to examine the potential impact of long term exposure to NaF on the induction of bone tumors by external beam irradiation. To accomplish this we irradiated a section of the left hindlimb of male F344 rats with ^{137}Cs gamma radiation and then exposed the animals to 250 ppm NaF in the drinking water for two years. The radiation field was restricted to a narrow band centered on the femoral-tibial joint of the left hindlimb to minimize the amount of overlying soft tissue and to include an actively growing skeletal region within the radiation field. We selected a dose of 250 ppm sodium fluoride based on the study reported by the National Toxicology Program (1990). In this latter study the highest dose of sodium fluoride administered to rats was 175 ppm, which produced only an uncertain response, and therefore a secondary goal of this study was to evaluate a higher dose of sodium fluoride.

Materials and Methods

Animals: Weanling male F344 rats were purchased from Charles River Laboratories (Raleigh NC) and were quarantined and acclimated to laboratory conditions for 14 days prior to study start. One week prior to start an individual identification number was tattooed on the tail each male rat, the body weight of each animal recorded and the animals randomized by weight into treatment and control groups consisting of 50 animals each. Within each group animals were randomly assigned to cages, 3 per cage. Cages were polycarbonate shoe box type (Lab Products) and were lined with heat treated hardwood chips and covered with non-woven fiber filter cage covers (Snow Filtration). Cages were maintained in stainless steel racks and were rotated within each rack every two weeks; racks were rotated within the animal room once per month. Feed (NIH-07 pellets) and water were available ad libitum. Water was administered in 500 ml polycarbonate bottles with double ball-bearing sipper tubes and rubber stoppers; water consumption for each cage was measured for one week each month. Animals were checked twice per day (morning and afternoon); body weights and formal clinical examinations were recorded once per month. The animal room was maintained at 74±1° F with a 12-hour on 12-hour off light cycle.

Irradiations: Irradiation was performed with a Shepard Model 431 Irradiator which contains a ^{137}Cs source. The irradiator is equipped with a collimator that provides a column of radiation 1 cm wide. During irradiation the animals were restrained in a clear plastic cylindrical rat restraint which had a slot cut in one side to allow the left hind limb to be exposed. Prior to being placed in the restraint the animals were lightly anesthetized with ketamine and the position of the femoral-tibial joint on the left hind limb marked with a fine line black magic marker. The animal was then placed in the restraint with the left hind limb protruding from the slot, fully extended to the rear and taped to the restraint for immobilization during irradiation. The restraint was then positioned to center the femoral-tibial joint of the left hind limb in the 1 cm wide beam of the collimator. In this configuration the only part of the rat exposed to the radiation column was a 1 cm wide band on the left hind limb; the remainder of the animal was completely shielded. The exposure rate from the source at the position of the femoral-tibial joint was 1000 R/min.; since a total exposure of 3000R was desired, the animals were irradiated for 3 min. After recovery from anesthesia animals were returned to their cages.

Chemical: Sodium fluoride was obtained form Apache Chemical Inc. (Seward IL.).Identity and purity were verified by elemental analysis, Karl Fischer water analysis, spark source mass spectrometry, and titration of acid components. The purity was found to be at least 99%. Dose formulations were prepared by mixing the appropriate amount of NaF with deionized water; new formulations were prepared every two weeks and stored in a cold room (40^{o}F) in the dark. Dose formulations were stable for at least 3 weeks when stored in the dark at room temperature. The fluoride concentration of dosing solutions was determined potentiometrically with an Orion fluoride ion electrode.

Study design: The study included four groups of 50 male rats each; 1) an unirradiated control group not exposed to NaF; 2.) An irradiated control group not exposed to NaF; 3.) An unirradiated group that received 250 ppm NaF in the drinking water for two years, and 4.) An irradiated group that received 250 ppm NaF in the drinking water for two years. Irradiations were performed over a two day period; on the day following the last day of irradiations, the designated groups were started either on deionized drinking water or drinking water containing 250 ppm NaF. Twenty four months later the surviving animals were necropsied.

Histopathology: All animals received a complete necropsy which included examination of organs and tissues for gross lesions and collection of the following: gross lesions, adrenal gland, bone, bone marrow, brain, clitoral gland, epididymis, esophagus, gall bladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas and islets, parathyroid gland, pituitary gland, preputial gland, prostate, salivary gland, seminal vesicles, skin, small intestine (duodenum, jejunum, ileum), stomach (forestomach, glandular), testes, thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues were fixed and preserved in 10% neutral buffered formalin. To aid in the identification of bone lesions radiographs were made of the fixed intact carcass. The following bones were then collected; right tibia, right humerus, right femur, thoracic vertebra 7, 8, and 9, maxilla, and the left femoral-tibial joint. Bones were decalcified, trimmed, embedded in paraplast, sectioned at 5 um, and stained with H&E for microscopic examination.

Analysis of Bone Fluoride: The Bone sample was freed of all excess tissue and marrow and dried in an oven for 1 hour at 90^{0} C. After drying the bone was ground to a fine powder with a mortor and pestel and a 25 mg sample accurately weighed into a small porcelain crucible and ashed overnight in a muffle furnace at 550^{0} C. After cooling to room temperature, the sample was reweighed, and 1.5 ml of 0.25M HCL was added and the mixture stirred with a magnetic stirring bar for 30 min. The sample was then quantitatively transferred to a 10 ml polyethylene beaker, washing with 1 ml Milli-Q water and 3 ml of 0.125M NaOH; 0.5 ml of TISAB III (total ionic strength adjustment buffer) was added and the electrode potential was measured with an Orion fluoride selective electrode before and after the addition of a known concentration of a fluoride standard solution. The concentration of fluoride was calculated form the difference in electrode potential before and after the addition of the known standard. Prior to analyzing bone samples from the study, the method was validated with matrix standards prepared from control bone spiked with known concentrations of fluoride.

Statistical Analysis: The prevalence of neoplastic and non-neoplastic lesions was analyzed using the Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1999; Piegorsch and Bailer, 1997) using a value of k=3 for the shape parameter (Bailer and Portier, 1988). Tests of significance included pairwise comparisons of chemical exposed or irradiated groups with the appropriate controls and a test for overall dose related trend.

Results

Survival was comparable among treatment and control groups (Figure 1) and overall survival for each group was over 60% at week 100. Mean body weights of all groups were also similar throughout the study with the difference between any treatment groups and the control never exceeding 5% (Figure 2).

Figure 1

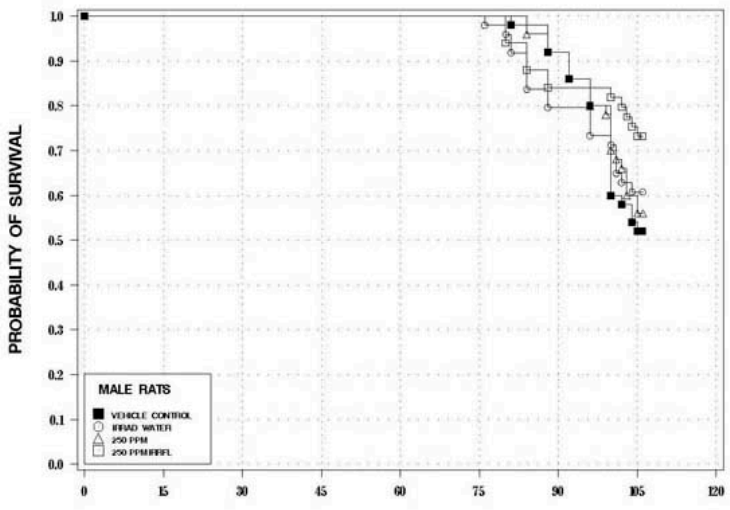
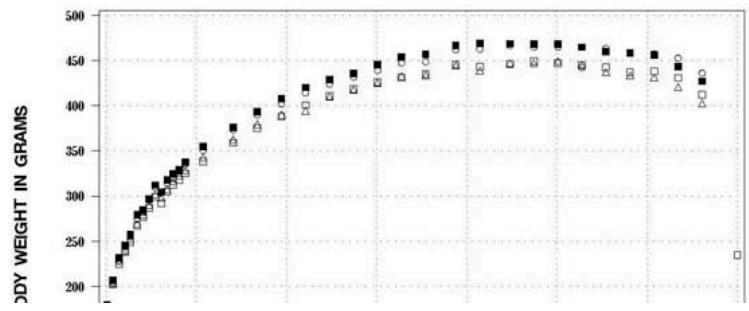
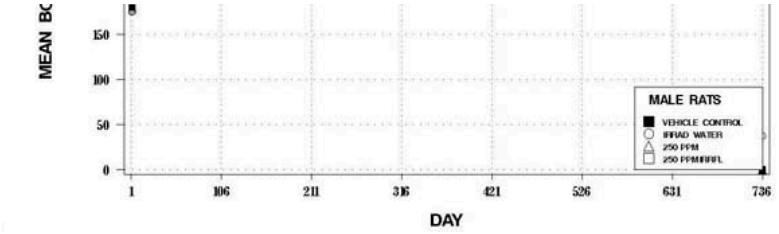


Figure 2





Feed and water consumption were similar among all groups throughout the study (Table 1). Analysis of ten randomly selected left humeri from groups that received NaF and a group that received no NaF revealed significant accumulation of fluoride in the bone of exposed animals over the two year duration of this study (Table 1).

Table 1. Feed and Water Consumption and Bone Fluoride Analysis

	Control	NaF	NaF + Irrad.	Irrad.
mean feed consumption (g/day)	10.1	10.1	9.8	9.6
mean water consumption (g/day)	17.1	17.2	17.1	16.6
Bone Fluoride[a] (ug/g dry bone)	ND	3610 ± 260	3370 ± 280	93 ± 18
Bone Fluoride (ug/g ash = ppm)	ND	5172.7 ± 354	4818 ± 388	134 ± 26

ND = not done

a. Bone fluoride was determined at the end of the study.

Table 2 shows the incidence and location of neoplastic lesions that occurred in the bone, teeth, and oral mucosa of the control and all treatment groups. There is no apparent association between the pattern of neoplastic response and treatment with either NaF or irradiation. The most frequently observed neoplams were nonosseous squamous cell carcinomas of the hard palate (oral mucosa). These neoplasms appeared to originate from the squamous epithelium near the root of molar teeth. Of the seven animals with sarcoma or osteosarcoma, the site of the neoplasm was different in five and the site could not be determined unambiguously in one (NOS).

Table 2. Neoplastic Lesions of the Bone, Teeth, and Oral Mucosa.

	Control	NaF	NaF + Irrad.	Irrad.
	(49)*	(49)*	(50)*	(49)*
Bone				
Osteosarcoma				
mandible	1	0	1	0
pelvis	1	0	0	0
Sarcoma				
joint	0	0	0	1
maxilla	1	0	0	0
vertebra	0	1	0	0
Tissue; NOS**				
Osteosarcoma	0	1	0	0
Osteosarcoma combined	2	1	1	0
Sarcoma or Osteosarcoma	3	2	1	1
Tooth				
Odontoma	1	0	0	1
Oral Mucosa				
squamous cell carcinoma	5	5	3	2

* Number of animals examined.
** NOS not otherwise specified

Fibrous osteodystrophy and hyperostosis, non nenoplastic bone lesions associated with normal aging in rats, occurred in a few animals (Table 3). Non-neoplastic lesions of incisor teeth (Table 4) were more prevalent in groups that received NaF than in groups not exposed to fluoride. Non-neoplastic lesions of incisor teeth were increased in groups exposed to NaF. The lesions included ameloblast and odontoblast degeneration as well as malformation of enamel and dentine. A dose related increase in similar lesions in rats occurred in the NaF drinking water studies reported by the NTP (1990) and in the study by Maurer et al.

Table 3. Non-neoplastic Lesions of Bone

	Control	NaF	NaF + Irrad.	Irrad.
	(49)*	(49)*	(50)*	(49)*
Tibia				

Tibia				
fibrous osteodystrophy				1
hyperostosis	1		1	2
Femur				
fibrous osteodystrophy				1
hyperostosis				1
Vertebra				
fibrous osteodystrophy				1
hyperostosis			1	1
Humerus				
fibrous osteodystrophy		1		1
hyperostosis			1	

* Number of animals examined

Table 4. Non-neoplastic Lesions of Incisor Tooth.

	Control	NaF	NaF + Irrad.	Irrad.
	(49)*	(49)*	(50)*	(49)*
dysplasia	1	0	0	1
ameloblast, degeneration	0	6[a]	8[e]	2
ameloblast, degeneration, focal	0	2	3	0
odontoblast, degeneration	10	21[b]	15	15
enamel, malformation	0	6[c]	9[f]	0
dentine, malformation	0	9[d]	7	2

* Number examined

Control vrs. NaF
a. p=0.018; b. p=0.024; c. p=0.018; d. p=0.002

Irrad. vrs NaF + Irrad
e. p=0.045; f. p=0.002

Discussion

Osteosacroma is an uncommon but well documented sequel of external beam irradiation associated with radiation therapy in humans. The induction of osteosarcoma by external beam irradiation has also been the subject of several studies in animals. Cater et al (1959; see also Baserga et al, 1961) irradiated both knee joints of 136 female Wistar rats with 3000 R of gamma radiation from an iridium 192 source; 34 rats served as unirradiated controls. The radiation field included the knee joint as well as the lower half of the femur and upper half of the tibia. Both the control and irradiated animals were randomized in to 4 groups that received one of the following treatments: injection of saline, of growth hormone, of thyroxine, or of growth hormone and thyroxine. Of 31 irradiated animals injected with saline that survived to the end of the study (mean survival, 494 days), bone osteosarcomas were found in seven. A similar incidence was found in irradiated animals that received growth hormone (12/30), thyroxine (8/29) or both growth hormone and thyroxine (7/31). The overall combined incidence of osteosarcoma was 34/116. No osteosarcomas occurred in unirradiated controls.

Benstead et al (1965) exposed one entire hindlimb of groups of male rats (Marshall X August: F1) to single exposures of 3000, 1500, 1000, or 500 R of 140 kvp x-rays (filtered through 0.3mm of copper) as well as a split exposure of 3 X 1000 R. An additional group received split exposures of 6 X 500 R of 230 kvp x-rays. The highest incidence of bone tumors occurred in the group receiving a single exposure of 3000R; of 31 animals alive seven months after irrradiation, 12 (40%) had bone tumors in the irradiated leg. No bone tumors were observed in the group that received a single exposure of 1500 R, and seven months after irradiation bone tumors occurred in 1/21 animals exposed to 1000R and 1/43 animals exposed to 500 R. Besides bone tumors, fibrosarcomas and mammary tumors were observed in soft tissues within the radiation field in a few animals.

In a more recent study Tinkey et al (1998) irradiated the right rear leg of groups of male Sprague-Dawley rats with doses of cobalt 60 gamma rays ranging from 0 to 106 Gy. Animals were irradiated 5 days a week in 2-Gy fractions at a dose rate of approximately 73 cGy/minute. A final dose of 16 Gy was given to bring the total cumulative dose to 46, 66, 86, or 106 Gy, depending on the group. Osteosarcomas occurred in 1/27 (3.7%) rats that received a total dose of 66 Gy, in 2/20 (10%) rats that received 86 Gy, and in 7/41 (17%) rats that received 106 Gy. Interestingly, all osteosarcomas were extraskeletal and occurred within soft tissue around the stifle joint. In addition to osteosarcomas, a malignant fibrous histiocytoma occurred in the irradiated limb of one animal that received 66 Gy, and a fibrosarcoma occurred in the irradiated limb of one animal that received 106 Gy.

In this study a 1 cm wide area encompassing the femoral-tibial (knee) joint of young F344 rats was irradiated with a single exposure of 3000 R of gamma radiation from a ^{137}Cs source. After two years post irradiation no osteosarcomas or other lesions of the femoral-tibial joint occurred in any of the animals. In view of the previous results this was somewhat surprising. Although actual dose was not determined in this study, the exposure rate and total exposure was sufficient to produce doses comparable to or greater than those reported in the previous work. Therefore the major factor that could potentially account for the lack of response is the smaller size of the radiation field.

In the present study only a small area of the leg around the knee joint was irradiated; the rest of the leg and the entire body of the animal was totally shielded. Thus only the joint itself and the ends of the femur and tibia were in the radiation field. By contrast in the study by Cater et al (1959) at least half of the femur and tibia, as well as the joint, were in the radiation field, and in the studies by Benstead et al (1965) and Tinkey et al (1998) the entire hind limb was irradiated. None of the studies reported neoplasms arising in cartilage. Therefore the amount of bone in the radiation field as well as the type of bone may be a factor, especially for radiation characterized by low linear energy transfer (LET).

Survival, mean body weights, and feed and water consumption of groups that received 250 ppm NaF for two years were comparable to control. This is in agreement with the results of two previous chronic studies of NaF in rats. In a study conducted by the National Toxicology Program (NTP) male and

female F344 rats received 0, 25, 100, or 175 ppm NaF in drinking water for 2 years. Survival, mean body weights, and feed and water consumption of groups of rats exposed to NaF were comparable to those of the controls throughout the two year study.

In another study (Maurer et al, 1990), groups of male and female Sprague-Dawley rats received diets containing NaF for 2 years with dietary concentrations adjusted to administer 0, 4, 10 or 25 mg/kg/day (For comparison, the average dose of NaF in the present study was 10mg/kg, and for male rats in the NTP study 8.6 mg/kg). Mean body weights of the groups receiving 4 or 10 mg/kg were comparable to control but body weights of the groups receiving 25 mg/kg were significantly lower than control throughout most of the study. Survival of all groups receiving NaF was comparable to control. Therefore doses of NaF up to at least 10mg/kg administered either in drinking water or in the diet appear to be reasonably well tolerated by rats.

Exposure to 250 ppm NaF for two years was not associated with an increase in osteosarcomas or other neoplasms of the bone, teeth, or oral mucosa, in either the irradiated or unirradiated groups. Osteosarcomas of the bone occurred in 2/49 controls and 1/49 rats exposed to both NaF and irradiation. An extra skeletal osteosarcoma occurred in 1/49 rats that received NaF only. The incidence of osteosarcomas as well as the combined incidence of bone tumors was similar in the control group and groups exposed to NaF.

In the NTP study osteosarcomas of the bone were found in 1/50 male rats that received 100 ppm NaF and in 3/80 male rats that received 170 ppm. No osteosarcomas occurred in dosed female rats or in dosed mice. Although the incidence of bone osteosarcomas was not significantly increased in any of the NaF exposed groups, this response was considered uncertain rather than negative because bone is a target organ for fluoride and the incidence of osteosarcomas in male rats followed a dose related trend that was statistically significant.

In the study by Maurer, a total of five bone tumors were observed: an osteosarcoma in 1/52 females that received 10 mg/kg, a sarcoma in 1/70 males that received 125 mg/kg, a chordoma in 1/70 males that received 10 mg/kg, and a chondroma in 1/52 females that received 10 mg/kg and in 1/70 males that received 4 mg/kg. The incidence of bone tumors in this study was not significantly increased (relative to control) in any group exposed NaF and did not follow a dose response; the authors concluded that NaF was not carcinogenic in Sprague-Dawley rats.

The major goal of this study was to examine the interaction between sodium fluoride exposure and the development of osteosarcomas resulting from exposure to ionizing radiation. Unfortunately under the conditions of irradiation employed no osteosarcoma response was observed. Although the reason for a lack of response is uncertain, only the joint itself and the ends of the femur and tibia were in the radiation field, whereas in previous studies either the whole hind limb or a significant portion of the hind limb were included in the radiation field. Therefore the amount and type of bone irradiated may be a factor in the tumor response to low LET irradiation.

Bibliography

Bailer, A.J., Portier, C.J. Effects of treatment induced mortality and tumor induced mortality on tests for carcinogenicity in small samples. Biometrics, 44: 417-431, 1988.

Bensted, J.P.M., Chir, B., Couotenay, V.D. Histological changes in the rat bone after varying doses of X-rays with particular reference to bone tumor production. British Journal of radiology, 38, 261-270, 1965.

Briancon, D., Meunier, P.J. Treatment of osteoporosis with fluoride, calcium, and vitamin D. In: Frost, H.M ed. The orthopedic clinics of North America. Philadelphia, Saunders. vol 12: 629-648, 1981.

Cater, D.B., Baserga, R., Lisco, H. Studies on the induction of bone and soft tissue tumors in rats by gamma radiation and the effect of growth hormone and thyroxine. British Journal of cancer 13: 214-227, 1959.

Farley, J.R., Wergedal, J.E., Baylink. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone forming cells. Science, 222: 330-332, 1983.

Kassem, M., Mosekilde, Eriksen, E.F. Effects of fluoride on human bone cells in vitro: differences in responsiveness between stromal osteoblast presursors and mature osteoblasts. European journal of Endocrinology. 130: 381-386, 1994.

Maurer, J.K., Cheng, M.C., Boysen, B.G., Anderson, R.L. Two-Year carcinogenicity study of sodium fluoride in rats. J. Natl. Cancer Inst., 82:1118-1126, 1990.

Pak, C.Y.C., Sakhaee, K., Piziak, V., Peterson,R., Breslau, N.A., Boyd, P., Poindexter, J.R., Herzog, J.,Heard-Sakhaee, A., Haynes, S., Adams-Huet, B., Reisch, J.S. Slow release sodium fluoride in the management of postmenopausal osteoporosis. Annals of Internal medicine, 120, 625-632, 1994.

Pendlebury, S.C., Bilous, M., Langlands, A.O. Sarcomas following radiation therapy for breast cancer: a report of three cases and a review of the literature. Int. J. Radiation Oncology Biol. Phys. 31(2): 405-410, 1995.

Piegorsch, W.W, Bailer, A.J., Stastics for environmental biology and toxicology, section 6.3.2 Chapman and Hall, london, 1997.

Portier, C.J., Bailer, A.J., Testing for increased carcinogenicity using a survival adjusted quantal response test. Fundam. Appl. Toxicol. 12: 731-737, 1989

Riggs, B.L., O'Fallon, W.M., Lane, A., Hodgson, S.F., Wahner, H.W., Muhs, J., Chao, E., Melton, L. J. Clinical trial of fluoride therapy in postmenopausal osteoporotic women: extended observations and additional analysis. Jouranl of Bone and Mineral Research, 9(2): 1994.

Tinkey, P.T., Lembo, T.M., Evans, G.R.D., Cundif, J.H., Gray, K.N., Postirradiation sarcomas in sprague-dawley rats. Radiation Research, 149: 401-404, 1998.

Toxicology and Carcinogenesis studies of sodium fluoride in F344 rats and B6C3F1 mice. National Toxicology Program Technical report # 393, 1990.

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