Bull. Environ. Contam. Toxicol. (2005) 74:566–572

DOI: 10.1007/s00128-005-0621-3

Bulletin of Environmental Contamination and Toxicology

Genotoxic Evaluation of Sodium Fluoride and Sodium Perborate in Mouse Bone Marrow Cells

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Received: 20 February 2004/Accepted: 4 December 2004

Sodium fluoride is a constituent of different products used for human comfort; for example, it is a supplement for potable water, a component of insecticides, disinfectants, wood conservatives, and it is used in paper manufacturing (Zeiger et al. 1993; Budavari et al. 1996). However, its main application is in dentistry, where it is used in a variety of products to prevent caries, in amounts which may reach 2 % of a topical solution of sodium fluoride (Wefel 1985; Brambilla 2001).

Human exposure to boron and its salts is also high, as suggested by the more than three million tons of the chemical produced annually worldwide, and by its consumption through food and water in amounts ranging from 1-7 mg/day (Richold 1998). Sodium perborate in particular, is well known for its bleaching and bactericidal properties, and therefore, it is used as a component of numerous detergents and oral hygienic products (Seiler 1989; Chng et al. 2002).

A large number of reports have been published with respect to the genotoxic potential of sodium fluoride. The studies have been made in procaryotes and eucaryotes, including mammalian cell culture assays (Aardema et al. 1989; Slamenova et al. 1992; Zeiger et al. 1993); however, heterogeneous results have been obtained from such research showing both positive and negative data with respect to the induction of revertant bacteria, chromosomal aberrations, sister chromatid exchanges (SCE), and micronuclei; thus, no clear conclusion concerning the genotoxicity of sodium fluoride has been obtained as yet. In regard to sodium perborate, the information on the matter is limited. A report using the *Salmonella typhimurium* assay (strains TA100 and TA102, without S9) showed a positive increase of revertant bacteria, as well as chromosomal damage in Chinese hamster ovary cells (Seiler 1989); however, no studies in other systems have been found in the literature.

The widespread human exposure to sodium fluoride and sodium perborate, and the uncertainty about their genotoxic potential are matters of concern. The purpose of the present study was to contribute to understand the *in vivo* effect of the compounds, by determining their potential for increasing the rate of SCE in mouse bone marrow cells.

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MATERIALS AND METHODS

Sodium fluoride (NaF, 99% pure), bromodeoxyuridine (BrdU), colchicine, and Hoescht 33258 were obtained from Sigma Chemicals St Louis Mo, USA., sodium perborate ($NaBO_3 \cdot 4H_2O$, 97% pure) was obtained from Fluka St Louis Mo, USA. Mytomicin C and the Giemsa stain were purchased from Merck, Mexico City. Sodium chloride, potassium chloride, sodium hydroxide, sodium phosphate, and potassium phosphate were obtained from J.T. Baker, Mexico City.

Tablets of BrdU (50 mg) were made and 70% coated with paraffin. Colchicine was dissolved in distilled water (0.5 mg/mL); sodium fluoride, sodium perborate, as well as mytomicin C were also dissolved in distilled water and intraperitoneally (ip) administered to mice.

Eight-week-old male mice (NIH), weighing 30 g were obtained from the Mexican National Institute of Health. The animals were maintained in metallic cages, where they consumed food (Nutricubos, Purina, Mexico City) and tap water, *ad libitum*. The animal room was set at 24°C and a 12 h dark-light cycle.

To determine the LD_{50}, we followed a two step assay that calls for few animals (Lorke 1983). Initially, three mice *per group* received 10, 100, and 1000 mg/kg, respectively; then depending on the observed lethality, we also used three other mice *per group* to test four new doses for each compound. In the case of sodium fluoride, we tested 60, 40, 25 and 15 mg/kg, and for sodium perborate, we applied 2900, 1600, 1000 and 600 mg/kg.

For the genotoxicity assay, a BrdU tablet of 50 mg was subcutaneously implanted to each animal (five mice *per dose*); one hour later the tested compounds were ip administered. In the case of sodium fluoride 2.0, 4.0, 8.0, 16.0, and 24.0 mg/kg were applied; with respect to sodium perborate, 3.0, 12.0, 48.0, and 193 mg/kg were administered. The assay also included a negative control group (0.3 mL of distilled water) and a positive control group administered with mytomicin C (2 mg/kg). Twenty one hours after inoculation with these chemicals, the mice were injected with colchicine (5 mg/kg); three hours later their femurs were dissected and the bone marrow dispersed in a tube containing 7 mL of KCl 0.075 M at 37°C. The cells were then centrifuged at 400 g for 10 min and the sediment was fixed in methanol-acetic acid (3:1).This procedure was repeated at least twice. A small amount of the cell suspension was dropped onto ethanol cleaned slides and the procedure to differentially stain the sister chromatids was applied to the chromosomes (Madrigal-Bujaidar et al. 1998).

The genotoxic/cytotoxic evaluation for each mouse were made as follows: thirty second division mitosis were analyzed to determine the rate of SCE. One thousand cells were scored to establish the mitotic index (MI), and one hundred cells were scored to determine the cellular proliferation kinetics. To carry out this evaluation, the number of cells in first (M1), second (M2), and third (M3) cellular division was determined; then with this data the average generation time (AGT) was established using the formula AGT=21/(M1+2M2+3M3) 100. Finally, the SCE

data were statistically analyzed with the ANOVA and the Student t tests; the MI and the AGT were analyzed with the Chi square test.

RESULTS AND DISCUSSION

The LD_{50} was obtained as the geometric mean of the determined experimental data on mice lethality. The value for sodium fluoride was 32 mg/kg and for sodium perborate the result was 775 mg/kg. The results concerning the SCE rate induced by sodium fluoride are shown in Table 1. Although no significant increase was observed with the two low doses tested (from 2 to 4 mg/kg), a significant SCE increase was found with the three highest doses. The cumulative frequency of these data reveals about 70% of cells with four SCE in the group treated with the high dose, a value which is twice the level of the negative control (Figure 1).

Table 1. Sister chromatid exchanges (SCE), average generation time (AGT), and mitotic index (MI) induced by sodium fluoride in mouse bone marrow.

Agent	Dose (mg/kg)	SCE $\bar{x}$ ± ED	CPK (%) M1	M2	M3	AGT h	MI (%)
Control	-	1.15±0.11	52.4	36.8	10.8	13.25	5.0
Sodium fluoride	2	1.38±0.16	48.4	37.8	13.8	12.69	**3.9
Sodium fluoride	4	1.45±0.12	50.4	39.0	10.6	13.10	**3.8
Sodium fluoride	8	*1.79±0.08	38.6	53.2	8.2	12.38	4.7
Sodium fluoride	16	*1.84±0.38	53.4	38.2	8.4	13.54	4.2
Sodium fluoride	24	*2.83±0.37	44.6	48.2	7.2	12.91	4.8
Mytomicin C	2	*4.07±0.28	57.0	34.8	8.2	13.88	**3.7

CPK = Cellular proliferation kinetics; M1, M2, and M3 = cells in first, second, and third cellular division.
* Statistically significant difference with respect to control. ANOVA and Student t tests ($p \leq 0.05$).** Statistically significant difference with respect to control. Chi square test ($p \leq 0.05$).

Also, a tendency for an SCE increase relative to the used doses is observed in the figure. The cellular proliferation kinetics and the MI are indicated in Table 1. With respect to the first parameter, the distribution of the evaluated cell divisions in the experimental groups gave rise to no significant variations of the AGT; in regard to the MI, the variability among the studied groups made it difficult to reach a conclusion.

The results obtained in the animals treated with sodium perborate are presented in Table 2. In this case, a statistical difference of the tested compound in comparison with the control level was observed in all four tested doses, although no dose response was detected. The SCE cumulative curve shows a homogeneous distribution of the groups administered with the chemical, inducing about 50% of

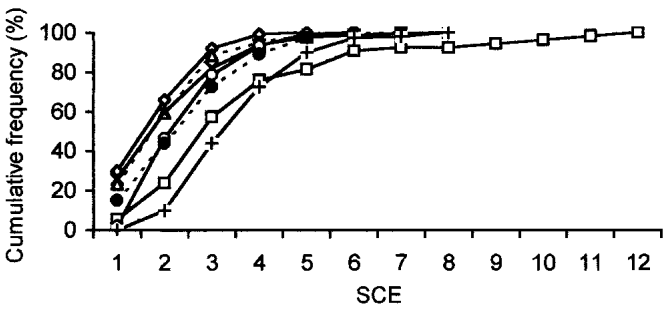

Figure 1. Cumulative frequency of sister chromatid exchanges (SCE) induced by sodium fluoride in mouse bone marrow cells. (◊) distilled water, (□) 2 mg/kg of mytomicin C, (-Δ-) 2, (×) 4, (○) 8, (-●-) 16 and (+) 24 mg/kg of sodium fluoride.

cells with two SCE, while control treated mice have only one SCE (Figure 2). With respect to the AGT, we found no alterations induced by the treatments, and no tendency for a significant MI decrease.

The determination of the genotoxic potential of compounds that are present in the environment is necessary before taking the appropriate and timely preventive measures. The present report includes two examples of chemicals involved in various human activities, which have not clearly shown their mutagenic potential.

Table 2. Sister chromatid exchanges (SCE), average generation time (AGT), and mitotic index (MI) induced by sodium perborate in mouse bone marrow.

Agent	Dose (mg/kg)	SCE $\bar{x}$ ± ED	CPK (%) M1	M2	M3	AGT h	MI (%)
Control	-	1.89±0.10	24.4	48.6	27.0	10.36	5.04
Sodium perborate	3	*2.59±0.42	24.2	45.6	30.2	10.19	3.58
Sodium perborate	12	*2.39±0.35	23.0	45.4	31.6	10.06	3.76
Sodium perborate	48	*2.25±0.22	27.0	48.6	24.4	10.63	4.84
Sodium perborate	193	*2.49±0.46	25.4	44.0	30.6	10.23	3.88
Mytomicin C	2	*3.55±0.19	26.4	40.6	33.0	10.16	3.90

CPK = Cellular proliferation kinetics; M1, M2, and M3 = cells in first, second, and third cellular division.

* Statistically significant difference with respect to control. ANOVA and Student t tests ($p \leq 0.05$).

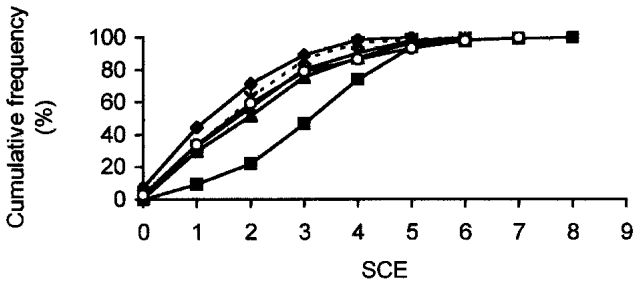

Figure 2. Cumulative frequency of sister chromatid exchanges (SCE) induced by sodium perborate in mouse bone marrow cells. (◆) distilled water, (■) 2 mg/kg of mytomicin C, (▲) 3, (×) 12, (−∗−) 48 and (○) 193 mg/kg of sodium perborate.

In the case of sodium fluoride numerous *in vitro* studies have produced contradictory information. The mammalian *in vivo* approach is usually considered the final step for determining genotoxicity and defining risk assessment, before regulatory measures may be taken. About fifteen *in vivo* studies using different research models were made between 1970 and 1998 (Kram et al. 1978; Mohamed and Chandler 1982; Li et al. 1987; van Asten et al. 1998). Most of these reports reveal negative results; in fact, only one of them is clearly positive showing increases of chromosomal aberrations in somatic and germ cells of mice that had drunk fluoridated water. The concern regarding the sodium fluoride hazard is understandable because of the direct exposure of humans to the chemical for more than 50 years; however, most *in vivo* reports, suggest that only high doses of the chemical may pose a danger for the genetic material and that the usual low amounts to which humans are exposed in the environment are harmless. Our data agree with this assumption, moreover, if we consider that the highest daily intake of the chemical in fluoridated water is about 6 mg/kg (Zeiger et al. 1993). In our case, we determined a genotoxic effect starting with 8 mg/kg, and only 24 mg/kg (75% of the LD_{50}) induced a duplication of the basal level.

With respect to sodium perborate, the almost lack of genotoxic reports is surprising because humans are exposed to the salt contained in diverse products, and its use has been related with probable cytotoxicity in long-term oftalmic therapy, as well as with cytotoxic damage in aquatic organisms (Warne et al. 1999; Noecker 2001). However, our present data also suggest that the genotoxic damage *in vivo* is low, and that a biologically significant effect may require doses higher than the usual level of the human exposure. On the other hand, it is known that sodium fluoride and sodium perborate are free radical inducers mainly in long term exposures (Venditti et al. 1995; Chlubek et al. 2003), and that these molecules are highly reactive and may damage DNA. In view of this, our *in vivo* genotoxic results, as well as those of others, such as Li et al. (1989), suggest efficient repair and detoxification processes in mammals. Nevertheless, it seems

pertinent to carry out well controlled chronic studies that measure various endpoints to reach a final conclusion.

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