GENOTOXIC EFFECTS OF BORAX ON CULTURED LYMPHOCYTES

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Abstract. The effect of borax on human chromosomes was analyzed in this study. Venous blood from 30 male students at Thammasat University, Thailand (age 18-25 years) was collected for lymphocyte cell cultures. This experiment was divided into two groups: the first group was the control group and the second group was the experimental group. The lymphocyte cells in the control group were cultured without borax. The experimental group was divided into four subgroups. The lymphocyte cells in each experimental subgroup were cultured with different concentrations of borax (0.1 mg/ml, 0.15 mg/ml, 0.2 mg/ml and 0.3 mg/ml). Human chromosomes were studied for abnormalities through Giemsa-staining and G-banding. The results show that the numbers of metaphase plates (the metaphase plate which contained 46 chromosomes; 46, XY) and metaphase chromosomes were reduced when lymphocyte cells were cultured with 0.15 mg/ml (57.2%), 0.2 mg/ml (50.8%) and 0.3 mg/ml (42.3%) concentrations of borax. There was a statistically significant difference between the control and experimental subgroups ($p<0.05$). Sister chromatid separation was found in the 0.3 mg/ml borax concentration experimental subgroup. This shows that borax (at 0.15, 0.2 and 0.3 mg/ml concentrations) affects the cell and human chromosomes (both numerical and structural abnormalities). Borax may cause human chromosome abnormalities and lead to genetic defects.

INTRODUCTION

Food additives are substances added to food to preserve flavor or improve its taste and appearance. Borax is a substance used in food preparation as a firming agent, meat rub and tenderizer. Borax ($Na_2B_4O_2.(H_2O)_{10}$) is a borate compound. It is a low toxicity mineral with insecticidal, fungicidal and herbicidal properties. The basic structure of borax contains chains of interlocking $BO_2(OH)$ triangles and $BO_3(OH)$ tetrahedrons bonded to chains of sodium and water octahedrons. It is a white powder consisting of soft colorless crystals that dissolves easily in water. Borax is used in detergents and cosmetics, as an ingredient in enamel glazes, glass and ceramics, as an insecticide to kill ants and fleas and as a precursor for sodium perborate monohydrate used in detergents. Borax is widely used in manufacturing, fertilizers, pesticides and pharmaceuticals (NCAMP, 2001). Borax can be produced synthetically from other boron compounds. Borax is used as a food additive in some countries with an E number of *E285*. Its use is similar to salt, and it appears nota-

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bly in French and Iranian caviar.

The symptoms of borax toxicity include headache, fever, nausea, vomiting, and erythematous eyes. Symptoms appear 2 to 4 hours after ingesting borax. Borax is slowly excreted by the kidney. Nephrotoxicity is the most common organotoxicity, followed by fatty liver degeneration, cerebral edema and gastroenteritis (Weir and Fisher, 1972). There have been many studies regarding the toxicity of borax (Landolph, 1985; Sylvain *et al*, 1998; Wester *et al*, 1998). In reproductive and developmental toxicity studies using rats, mice and rabbits, there were maternal hepatic and renal effects, decreased weight gain during pregnancy, and decreased fetal body weights. Borax has toxicity to humans, including reproductive and developmental toxicity, neurotoxicity, and acute toxicity (Moseman, 1994). Borax causes irritation of the skin and respiratory tract. The gastrointestinal tract, skin, vascular system and central nervous system are the principal systems and organs affected. It can cause nausea, vomiting, abdominal pain, diarrhea, erythematous and exfoliative rashes, unconsciousness, depression and renal failure. Borax has been shown to adversely affect male fertility in animal models. Testicular toxicity and decreases in body weight result at extreme doses. Chunks of borax, used to control pH in drilled mud, were found on the ground at an abandoned oil well drilling site and consumed by cattle. The cattle appeared depressed, developed hemorrhagic gastroenteritis and dehydration. The exposure to the borax was presumed to be of short duration and probably consisted of ingestion on only one occasion (Brockman *et al*, 1985).

Cancer is a leading cause of death in humans. The causes of cancer include cigarette smoking, dietary factors, infection, chronic inflammation, and food additives. There are many kinds of food additives associated with cancer (Sugimura, 2002). Mutagenic food additives are carcinogenic and therefore their use is banned (Nicklin and Miller, 1989; Tobacman *et al*, 2001; Sugimura, 2002). The Food and Drug Administration, Ministry of Public Health, Thailand (1999) has declared borax as a prohibited substance in Thai food because of its toxicity to humans, including reproductive and developmental toxicity, neurotoxicity, and nephrotoxicity. Borax toxicity effects human cellular development and function.

In Thailand, though prohibited, many merchants add borax to food as a firming agent, preservative and tenderizer. In 1995, the prevalence of borax abuse in food in Bangkok was 7.2% with a risk of 49.3% in minced meat, pork, chicken and fish; 20% in meat, pork, chicken and Thai sweets made from flour; and 10.1% in preserved fruits ($p=0.00001$) (Flanagan, 1995). In 1999, the prevalence of borax in food increased to 29.6% in Bangkok (Food and Drug Administration, 1999). We studied the effect of borax on human cells and chromosomes (*in vitro*) to verify borax toxicity at the cytogenetic level.

MATERIALS AND METHODS

Blood samples

Heparinized blood from 30 consenting healthy Thai males was collected for chromosomal abnormalities research.

Cell culture and harvesting

Human lymphocytes were cultured in the thirty heparinized blood samples. The heparinized blood was cultured in RPMI medium (Gibco BRL Life Technologies, San Diego, CA), 10% fetal calf serum (Invitrogen, Carlsbad, CA) and penicillin and streptomycin. Phytohemagglutinin (0.08 ml) was added to 5 ml of the RPMI medium as a mitogen. The cultures were divided into 5 groups. Borax solution at concentrations of

0.1, 0.15, 0.2, 0.3 mg/ml were added to 4 of these groups and the fifth group had no borax solution added, and was used as a control. After 72 hours incubation, human chromosomes were harvested and stained with Giemsa.

G-banding technique

A G-banding technique was used to analyze the chromosomes in all the groups. The effect of the various concentrations of borax on cell growth was also studied. Cells were treated with the various concentrations of borax for 72 hours. They were then stained with 0.4% trypan blue dye and the viable cells were then counted using a hemocytometer.

Cell growth, cell survival, the numbers of metaphase plates and chromosome abnormalities in the control group and the experimental groups were observed under a microscope.

Statistical analysis

Statistical analysis was done by ANOVA test.

RESULTS

Effect of borax on cell growth and survival

The data regarding the effect of borax on cell growth and survival are shown in Table 1 and Figs 1- 5. Fig 1 shows the effect of various concentrations of borax on cell growth and cell survival in the 30 samples. This data includes the cell counts (mean ± SD) at each concentration of borax. The lymphocytes grew well in the control group and in the experimental subgroup at a borax concentration of 0.1 mg/ml. Many viable cells and metaphase plates were observed in the control group and the 0.1 mg/ml borax concentration subgroup (Figs 2 and 3). Lymphocytes did not grow well and many dead cells were found in the 0.2 and 0.3 mg/ml borax concentration subgroups. The numbers of metaphase plates in the 0.2 and 0.3 mg/ml borax concentration subgroups were less than the numbers of metaphase plates in the 0.1 mg/ml borax concentration subgroup and the control group (Figs 4 and 5).

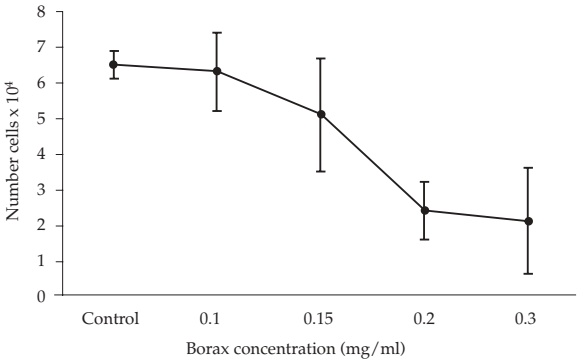

Fig 1–Effect of various borax concentrations on cell growth. Data represent the numbers of cells counted (mean ± SD) at each borax concentration. Borax concentrations of 0.15, 0.2 and 0.3 mg/ml affected lymphocyte growth but the concentration of 0.1 mg/ml did not affect cell growth.

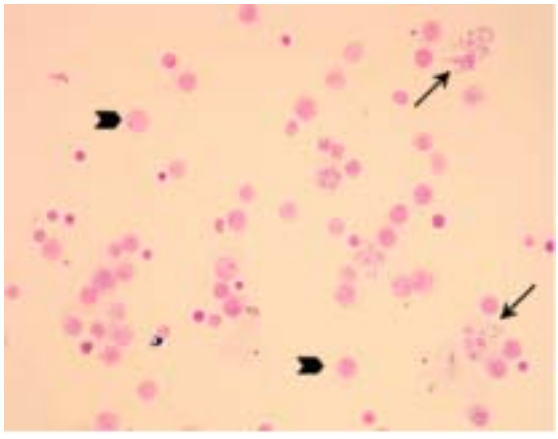

Fig 2–Cells and metaphase plates in the control group. The symbol (→) indicates the human metaphase plate. The symbol (➤) indicates the viable lymphocytes. There were many metaphase plates and viable lymphocytes found in the control group.

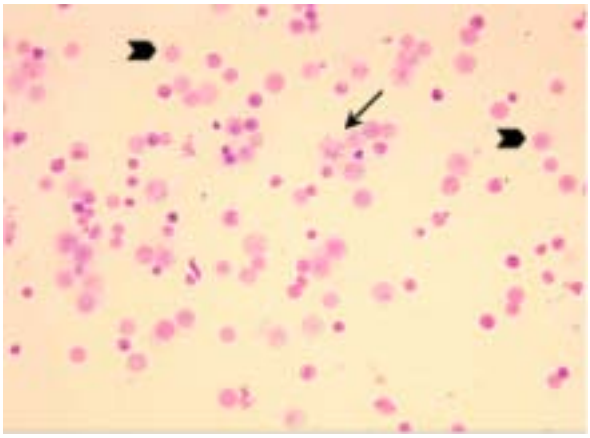

Fig 3–The cells and metaphase plates in the 0.1 mg/ml borax concentration subgroup. The symbol (→) indicates a human metaphase plate. The symbol (➤) indicates a viable lymphocyte. There were many metaphase plates and viable lymphocytes found in the 0.1 mg/ml borax concentration subgroup.

Effect of borax on chromosomes

Numerical effect of borax on chromosomes. Borax affected the numbers of metaphase plates and metaphase chromosomes. The numbers of metaphase plates (the metaphase plates which contained 46 chromosomes; 46, XY) among the control group and the experimental groups were different. Comparing the percentage of the numbers of metaphase plates among these groups showed that the numbers of metaphase plates were reduced when the concentration of borax increased. The number of metaphase plates in the control group was 62.8% and the numbers of metaphase plates in the 0.1, 0.15, 0.2 and 0.3 mg/ml borax concentration subgroups were 62.0%, 57.2%, 54.1%, and 42.3%, respectively (Table 2). The number of metaphase chromosomes also

Table 1
Effect of borax on cell growth and survival.

Borax concentration (mg/ml)	Cell growth and survival
Non-borax	Cells grew well and survived
0.1	Cells grew well and survived
0.15	Cells did not grow well and some dead cells were found
0.2	Cells did not grow well and at this concentration more dead cells were found than at the 0.15 mg/ml borax concentration
0.3	Cells did not grow well and many dead cells were found

Table 2
Comparison of means and percentages of numbers of metaphase plates (the metaphase plate which contained 46 chromosomes) between the control group and the borax containing subgroups.

Borax concentration (mg/ml)	Mean number of metaphase plates (containing 46 chromosomes; 46, XY) ± SD	Mean number of metaphase plates ± SD	% of metaphase plates (containing 46 chromosomes; 46, XY)	*p*-value
Non-borax	279 ± 0.1	444 ± 0.1	62.8	
0.1	274 ± 0.2	442 ± 0.1	62.0	
0.15	202 ± 0.1	353 ± 0.3	57.2	<0.05
0.2	151 ± 0.2	279 ± 0.1	54.1	<0.05
0.3	104 ± 0.1	246 ± 0.1	42.3	<0.05

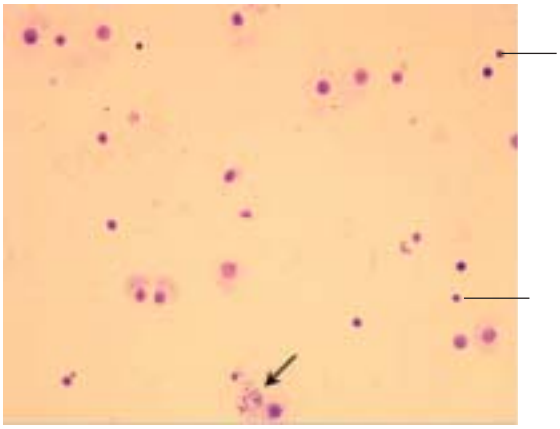

Fig 4–The cells and metaphase plates in the 0.2 mg/ml borax concentration subgroup. The symbol (→) indicates a human metaphase plate. The symbol (——) indicates a dead lymphocyte. Many dead lymphocytes were found in the 0.2 mg/ml borax concentration subgroup but many viable cells were found in the control group and the 0.1 mg/ml borax concentration subgroup.

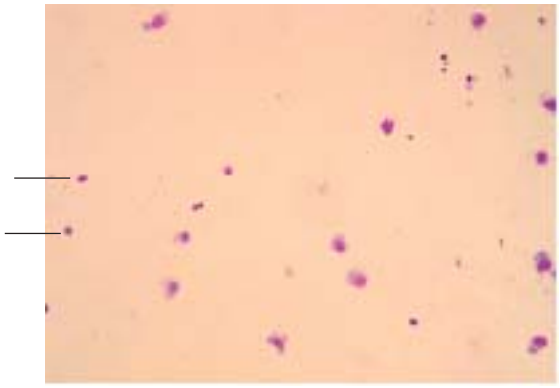

Fig 5–Dead lymphocytes in the 0.3 mg/ml borax concentration subgroup. The symbol (——) indicates dead lymphocytes. Many dead lymphocytes were found in the 0.3 mg/ml borax concentration subgroup. The number of metaphase plates in this subgroup was less than the numbers of metaphase plates in the control group and in the 0.1 mg/ml borax concentration subgroup.

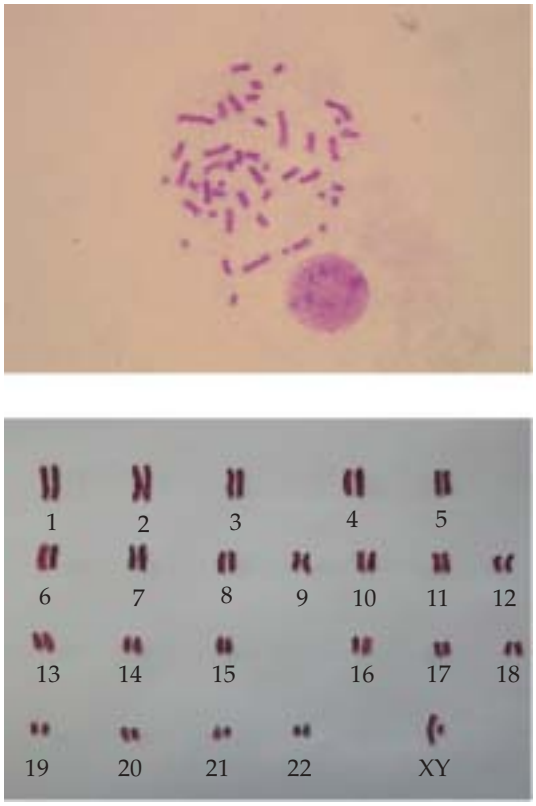

Fig 6–The metaphase plate and karyotype of the control group. The metaphase plate in the control group contains 46 chromosomes (46, XY).

decreased when the concentration of borax increased. The effect of borax on the number of metaphase plates in the control group and each borax concentration experimental subgroups and the number of living lymphocytes in each group are shown in Figs 2-5.

Statistical analysis (ANOVA) showed that the numbers of metaphase plates in the control group and the 0.15, 0.2 and 0.3 mg/ml borax concentration subgroups were significantly different ($p<0.05$). The numbers of metaphase plates in the control and 0.1 mg/ml borax concentration subgroup were not significantly different ($p>0.05$). The numbers of metaphase plates in the 0.1 mg/ml borax

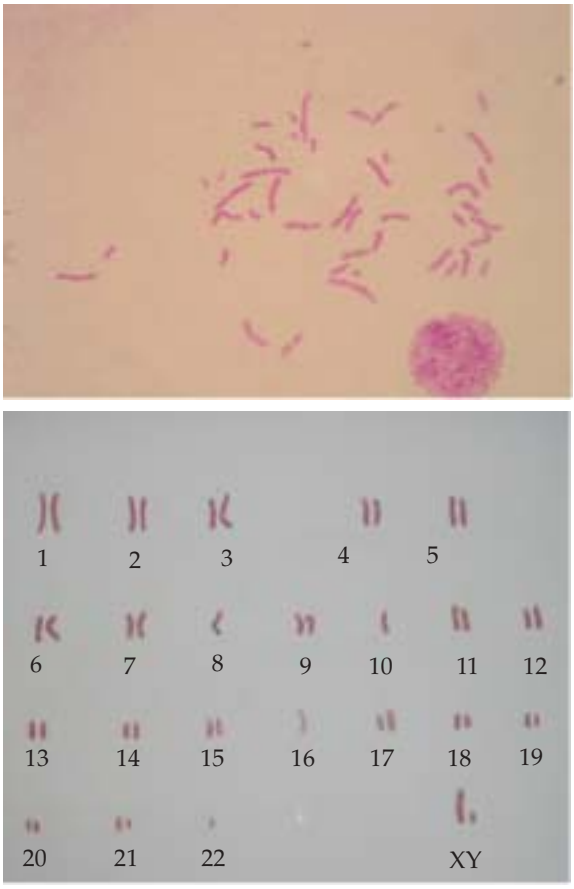

Fig 7–The metaphase plate and karyotype of the 0.2 mg/ml borax concentration subgroup. The metaphase plate in this figure contains 42 chromosomes. Loss of chromosomes 8, 10, 16 and 22 were detected in this metaphase plate of the 0.2 mg/ml borax concentration subgroup.

concentration subgroup and the 0.3 mg/ml borax concentration subgroup were significantly different ($p<0.05$).

The metaphase plate and the karyotype of the control group are shown in Fig 6. Loss of chromosomes in many metaphase plates was detected in the 0.2 and 0.3 mg/ml borax concentration experimental subgroups. The metaphase plate and the karyotype of the 0.2 mg/ml borax concentration subgroup are shown in Fig 7. Loss of chromosomes 8, 10, 16, and 22 was detected in the metaphase plate of the 0.2 mg/ml borax concentration subgroup as seen in Fig 7. Loss of metaphase chromosomes increased when the borax concentration increased.

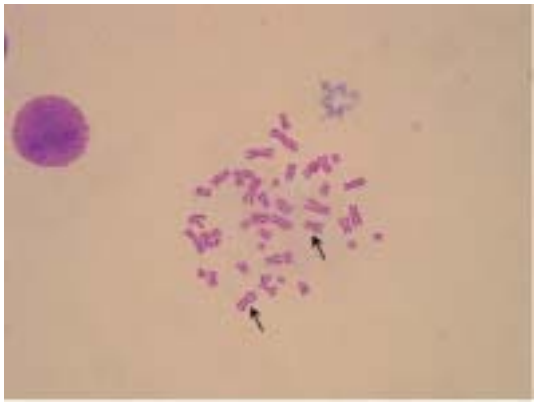

Fig 8–Sister chromatid separation was detected in the chromosomes of the 0.3 mg/ml borax concentration subgroup. The symbol (→) indicates sister chromatid separation.

Effect of borax on the structure of chromosomes. Borax affected the structure of chromosomes. Sister chromatid separation was detected in many metaphase chromosomes in the 0.3 mg/ml borax concentration subgroup (Fig 8).

DISCUSSION

Borax is a chemical that many Thai merchants add to food. Many additives, such as carrageenan, increase the risk of cancer. Carrageenan, a naturally occurring gum derived from red seaweed, has been widely used as a thickener, emulsifier, and stabilizer in chocolate milk, ice cream and low fat processed meat. It increases the incidence of breast carcinoma (Tobacman *et al*, 2001). Saccharin induces bladder cancer in rats when fed at high doses (Weihrauch and Diehl, 2004). In Japan, a high intake of salt-preserved food is associated risk for gastric

cancer (Tsugane, 2005). The combination of quinoline yellow and aspartame food additives cause cellular cytotoxicity (Lau *et al*, 2006).

Borax can result in reproductive and developmental toxicity, neurotoxicity and acute toxicity (Landolph, 1985; Wester *et al*, 1998). There are many reports regarding the cytotoxicity of borax. The cytotoxicity of borax was studied in human fibroblasts and C3H/10T1/2 mouse embryo fibroblasts by Landolph (1985). A 0.8 mg/ml borax concentration reduced cell growth and caused cytotoxicity (Landolph, 1985). Apoptotic lesions in rat thymocytes were detected when rats received 2000 ppm of borax in their food for 16 days. Numerous macrophages containing apoptotic cells were present in the thymus (Sylvain *et al*, 1998). The route of borax absorption is important for human health risk assessment. The degree of borax toxicity depends on the doses or concentration that the human body receives. In an *in vivo* study of borax absorption, percutaneous absorption through intact human skin was low and was significantly less than the average daily dietary intake (Wester *et al*, 1998). In one study borax caused testicular degeneration in dogs, including spermatogenic arrest and atrophy of the seminiferous epithelium of the tubules. They also found that borax affected human cell growth. Developmental toxicity was found to be more likely than other toxicity due to borax (Murray, 1995).

On studying the effects of borax (at concentrations of 0.1, 0.15, 0.2 and 0.3 mg/ml) on human chromosomes, 0.15, 0.2 and 0.3 mg/ml borax concentrations affected cell survival, cell growth and chromosomes. Increasing the borax concentration reduced the growth of cells. Compared with the control group, the number of metaphase plates (the metaphase plate which contained 46 chromosomes; 46, XY) were reduced as the borax concentration increased ($p<0.05$). The 0.3 mg/ml borax concentration had the greatest effect on cell survival, cell growth and chromosomes. The 0.3 mg/ml borax concentration affected the number of metaphase plates significantly compared to the 0.1 mg/ml borax concentration ($p<0.05$). Loss of chromosomes was observed at the 0.2 and 0.3 mg/ml borax concentrations and sister chromatid separation was detected in the 0.3 mg/ml borax concentration subgroup.

Borax is a food additive that affects human cell growth, cell survival and human chromosomes. Data regarding viable cell counts (Table 1 and Figs 1-5) suggest the cells are under stress at borax concentrations of 0.15, 0.2 and 0.3 mg/ml. The data related to chromosome abnormalities (Figs 7 and 8) also suggests borax causes genetic toxicity at these doses. The toxicity of borax affects the chromosomes and leads to genetic defects. *In vivo* study of high borax concentrations is needed to investigate the effect of borax to human chromosomes.

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