Cytotechnology (2016) 68:821–827
DOI 10.1007/s10616-014-9833-x

ORIGINAL RESEARCH

Cytogenetic and oxidative alterations after exposure of cultured human whole blood cells to lithium metaborate dehydrate

Fatih Çağlar Çelikezen · Başak Toğar · Fatma Betül Özgeriş · Mehmet Sait İzgi · Hasan Türkez

Received: 9 July 2014 / Accepted: 1 December 2014 / Published online: 14 February 2015

Abstract Boron compounds have an ability of supporting antioxidant properties in human and animal tissues. Lithium metaborate dihydrate ($LiBO_2$-$2H_2O$; LMD) is commonly used in nonlinear optic materials, cellular phones and pagers. But, there are limited data on the genotoxic and antioxidant effects of LMD in cultured human whole blood cells. The aim of this study was to evaluate for the genotoxicity and antioxidant/oxidant activity of LMD on human whole blood lymphocytes ($n = 5$). LMD was applied at various concentrations (0–1,280 μg/ml) to cultured blood samples. Antioxidant/oxidant activity was evaluated by measuring the total oxidant status (TOS) and total antioxidant capacity levels. Micronuclei and chromosomal aberration tests were used in genotoxicity studies. Our results clearly revealed that all tested concentrations of LMD were found to be non-genotoxic when compared to that of the control group. In addition, LMD exhibited antioxidant activities at low concentrations. In addition the TOS levels were not changed at all concentrations of LMD. Consequently, our results clearly demonstrated that LMD is non-genotoxic and it has an important antioxidant potential in vitro.

Keywords Lithium metaborate dihydrate · Total antioxidant capacity · Total oxidant status · Micronuclei · Chromosomal aberration

F. Ç. Çelikezen (✉) · M. S. İzgi
Department of Chemistry, Science and Letter Faculty, Bitlis Eren University, 1300 Bitlis, Turkey
e-mail: celikezen@hotmail.com

B. Toğar
Department of Biology, Faculty of Science, Atatürk University, Erzurum, Turkey

F. B. Özgeriş
Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, Turkey

H. Türkez
Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Turkey

Introduction

Boron, the fifth element in the periodic table, is a naturally occurring element widely distributed in the earth's crust. It is found in the environment primarily combined with oxygen to form compounds called borates. They are commonly found in nature, and are present in oceans, sedimentary rocks, coal, shale, and some soils (Argust 1998; Turkez et al. 2012a). Boron compounds are used in borosilicate glass products, but are also used in agriculture, in fire retardants, and in soaps and detergents (Richold 1998). In recent years, there is a growing interest in the biological activities of boron compounds. New studies demonstrated that boron compounds strengthen the antioxidant defense mechanism (Pawa and Ali 2006; Geyikoglu and

Turkez 2008; Celikezen et al. 2014). In addition, it has been reported that boron compounds (such as borax, boric acid) are non-genotoxic agents on human blood cells (Turkez et al. 2007, 2010; Geyikoglu and Turkez 2008; Turkez 2008). Furthermore, boron compounds have attracted much interest with respect to their protective effect against free radical damage that may be the cause of many diseases including cancer (Korkmaz et al. 2007; Mahabir et al. 2008).

The exploring of novel natural or synthetic antioxidants becomes a very popular research area in the World since the last 20 years (Geyikoglu et al. 2005; Geyikoglu and Turkez 2005); in addition, many efforts have been performed to explore new antioxidant-featured compounds (Cingolani et al. 2000; Cacciatore et al. 2003, 2005; Rispoli et al. 2004; Turkez and Sisman 2007; Heuking et al. 2009; Di Stefano et al. 2009a, b; Sozio et al. 2010; Turkez et al. 2012b). Newly, scientists and researchers are interested in the medical application of boron compounds. But the data on their physiological effects and risk potentials in animals and human is very limited (Geyikoglu and Turkez 2007; Colak et al. 2011). On the other hand, lithium metaborate dihydrate (LMD) is used as nonlinear optic material, in cell phones and pagers (Schubert and Brotherton 2006). As much as we know, there is no study about cytogenetic and oxidative potentials of LMD. Therefore, the aim of the present study was to firstly evaluate the cytogenetic (MN and CA assays) and oxidative effects (TAC and TOS analysis) of LMD on human peripheral blood cultures for its possible and safe use in several industries including chemical, glass, electronic, cosmetic and pharmaceutic industry.

Materials and methods

Production of lithium metaborate dihydrate

Lithium metaborate dihydrate (LMD) was produced according to the following equation;

$$Li(OH)aq + H_3BO_3(aq) \rightarrow LiBO_2 \cdot 2H_2O$$

The produced LMD was analyzed by using the titrimetric method to determine the B_2O_3 content of the sample (Snell and Hilton 1968). It was found that the produced LMD content of B_2O_3 was about 40.56 % (Fig. 1).

Experimental design

Whole heparinized human blood from five healthy non-smoking male donors between the ages of 24 and 27 with no history of exposure to any genotoxic agent was used in our experiments. Questionnaires were obtained for each blood donor to evaluate exposure history, and in addition, each donor signed informed consent forms. For all volunteers involved in this study, hematological and biochemical parameters were analyzed and no pathology was detected (Evans and O'Riordan 1975). 10 ml of peripheral blood was collected from each donor aseptically in a sodium-heparinized syringe. We added 6 ml of PB-MAX Karyotyping Medium (Gibco, Carlsbad, CA, USA) to each culture tube to be used for the assay. Then we added 0.5 ml heparinized blood to each tube. Various concentrations (0, 1.25, 2.5, 5, 10, 20, 40, 80, 160, 320, 640 and 1,280 mg/l) of LMD were tested in blood cultures. The concentrations were selected according to previous studies (Turkez et al. 2007; Arslan et al. 2008; Kahraman et al. 2013). MN and CA rates were assessed in peripheral lymphocytes and the method that was used for the preparation of the peripheral lymphocytes is explained in sections below. Experiments were conformed according to the guidelines of the World Medical Assembly (Declaration of Helsinki). The cultures without LMD were studied as control$^-$ group. Mitomycin C (10 μM, Sigma-Aldrich, Steinheim, Germany; Vijayalaxmi et al. 1996) was used as the positive control in MN and CA assays. Ascorbic acid (10 μM, Sigma, St. Louis, MO, USA; Turkez 2011) and hydrogen peroxide (25 μM, Sigma; Benhusein et al. 2010) were also used as positive controls in total antioxidant capacity

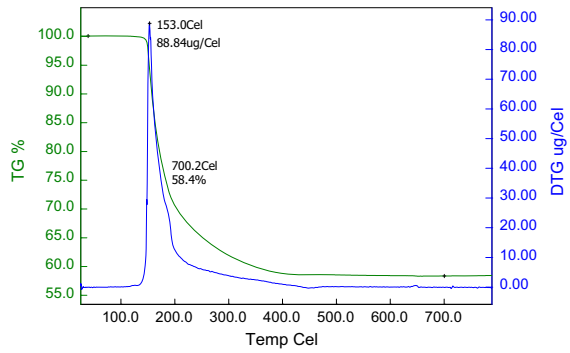

Fig. 1 Lithium meta borate dehydrate TG/DTA decay curve

(TAC) and total oxidant status (TOS) analysis, respectively.

TAC and TOS analysis

The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just in the antioxidant capacity of a single compound (Kusano and Ferrari 2008). Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the TOS of a sample is measured and this is named total peroxide, serum oxidation activity, with reactive oxygen metabolites or with some other synonyms. The automated Trolox equivalent TAC and TOS assays were carried out in plasma samples obtained from blood cultures for 24 h by commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey).

Micronucleus (MN) assay

Human lymphocytes were stimulated by LMD and cultured in a 37 °C incubator with a humidified atmosphere of 5 % CO_2 for about 72 h. After 44 h LMD stimulation, cytochalasin B (Sigma, MO, USA; final concentration of 6 mg/ml) was added. Whole blood cells were harvested by centrifugation, treated with a hypotonic solution [0.075 M KCl (Merck, Darmstadt, Germany) at 37.4 °C]. Then the culture tubes were centrifuged at 2000 rpm for 5 min, the supernatant was discarded, and the pellet was resuspended using 10 mL of fresh fixative solution (methanol and acetic acid, 3:1 (Merck, Darmstadt, Germany)). The tubes were centrifuged at 2000 rpm for 5 min and the supernatant was discarded. This procedure was repeated 3 times. The resulting cells were re-suspended and dropped onto clean slides. To prepare the slides, 3–5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained with Giemsa (Sigma, St Louis, MO, USA) in phosphate buffer (pH 6.8) and scored. MN was scored in 1,000 binucleated cells and the frequency of cells with micronuclei was determined (Fenech and Morley 1985).

Chromosomal aberration (CA) assay

CA tests were performed not only to study the cytotoxicity of the material on cells but also to determine the aberrations induced by the particular material on chromosomes of the human lymphocytes cell line. Human lymphocytes were stimulated by LMD and cultured for about 72 h in a 37 °C incubator with a humidified atmosphere of 5 % CO_2. Two hours prior to harvesting, 0.1 ml of colchicine (0.2 mg/ml, Sigma, St Louis, MO, USA) was added to the culture flask. Cells were harvested by centrifugation, treated with a hypotonic solution [0.075 M KCl (Merck, Darmstadt, Germany), at 37.4 °C]. Again, the culture tubes were centrifuged at 2000 rpm for 5 min, the supernatant was discarded, and the pellet was resuspended using 10 mL of fresh fixative solution (methanol and acetic acid, 3:1 (Merck, Darmstadt, Germany)). The tubes were centrifuged at 2000 rpm for 5 min and the supernatant was discarded. This procedure was repeated 3 times. The resulting cells were resuspended and dropped onto clean slides. To prepare the slides, 3–5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained with Giemsa (Sigma, MO, USA) in phosphate buffer (pH 6.8). For each treatment, 30 well-spread metaphases were analyzed to detect the presence of chromosomal aberrations. Criteria to classify the different types of aberrations (chromatid or chromosome gap and chromatid or chromosome break) were in accordance with the recommendation of EHC (Environmental Health Criteria) 46 for environmental monitoring of human populations (IPCS 1985).

Statistical analysis

Statistical analysis was performed using SPSS software (version 16.0, SPSS, Chicago, IL, USA). The Duncan's test was used to determine whether any treatment significantly differed from controls or each other. Statistical decisions were made with a significance level of 0.05.

Results

The serum TAC and the TOS were evaluated by using an automated colorimetric measurement method. As shown from the results presented in Table 1, four concentrations of LMD (5, 10, 20 and 40 mg/l) resulted a significant increase of TAC levels in cultured human blood cells compared with the controls. On the other hand, LMD did not change the TOS levels in cultured lymphocytes at all the concentrations.

Table 1 The level of total antioxidant capacity (TAC) and total oxidant status (TOS) in human blood cultures treated with LMD for 24 h

Concentrations (mg/L)	TAC (mmol Trolox Equiv. L)	TOS (mmol H_2O_2 Equiv. L)
Control$^-$	6.12 ± 0.54^a	11.56 ± 2.84^a
Control$^+$	13.26 ± 0.96^c	38.15 ± 4.61^b
1.25	6.18 ± 0.61^a	11.48 ± 2.58^a
2.5	6.27 ± 0.58^a	10.94 ± 2.97^a
5	7.61 ± 0.54^b	10.87 ± 3.01^a
10	8.10 ± 0.64^b	11.25 ± 3.17^a
20	8.19 ± 0.61^b	11.37 ± 2.98^a
40	8.11 ± 0.74^b	11.89 ± 2.67^a
80	6.84 ± 0.78^a	11.97 ± 2.75^a
160	6.15 ± 0.85^a	10.95 ± 3.16^a
320	6.54 ± 0.69^a	10.67 ± 3.28^a
640	6.27 ± 0.64^a	11.48 ± 3.14^a
1,280	6.15 ± 0.75^a	11.66 ± 2.91^a

Positive control: ascorbic acid (10 μM) and hydrogen peroxide (25 μM) in TAC and TOS analysis, respectively. The bars shown by different letters are significantly different from each other at a level of 5 %

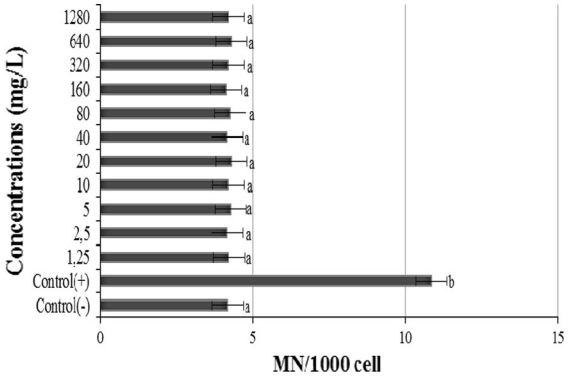

Fig. 2 Micronucleus (MN) rates in human lymphocytes after treatment with LMD in vitro. (Positive control: Mitomycin C (10^{-7} M). Values are expressed as mean ± SD for five cultures in each group. The *bars* marked by *different letters* are significantly different from each other at a level of 5 %.)

Results obtained from the analysis of MNs and CAs in human lymphocytes were cultured with no treatment (Control$^-$), were treated with MMC (Control$^+$) or with different LMD concentrations are shown in Figs. 2 and 3, respectively. LMD at the tested concentrations did not induce significant ($P > 0.05$) number of MNs and CAs frequencies. However, the MMC applied culture (as positive control) showed

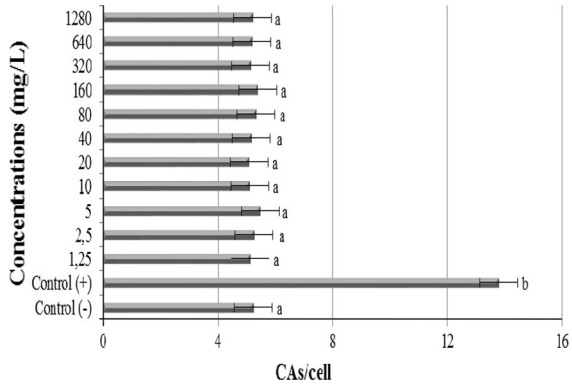

Fig. 3 Chromosome aberration (CA) frequencies in human lymphocytes after treatment with LMD in vitro. Abbreviations are as in Fig. 2

about three fold increases of both parameters as compared to the control$^-$ group.

Discussion

The alterations in enzyme activities and DNA damage are extensively used as significant biomarkers to assess the genotoxicity and oxidative stress of natural or man-made chemical materials (Turkez et al. 2005; Turkez and Togar 2010; Sisman and Turkez 2010; Dong et al. 2012). In the present study, for assessing the antioxidant/oxidant effects of LMD, TAC and TOS assays were performed for the first time. In fact, total antioxidant capacity is one of the suitable biochemical parameter to evaluate antioxidant status of body fluids. And the capacity in plasma is related to production or intake of antioxidants. Hence, it monitors the action potential to increased or normal status of reactive oxygen species (Miller et al. 1993; Lantos et al. 1997; Ghiselli et al. 2000; Balogh et al. 2001). This study reported that LMD treatments at low concentrations (5, 10, 20 and 40 μg/ml) caused significant increase of TAC levels, while TOS levels were not affected. To our knowledge, there is no report in literature about the antioxidant/oxidant effect of LMD on cultured human lymphocytes cells or any other cell lines. Therefore, its antioxidant/oxidant effect could not be compared with other boron compounds. According to the literature, Hunt and Idso (1999) reported that boron prevents oxidative damage by increasing of glutathione and its analogs or by supporting other neutralizing agents. Pawa and Ali

(2006) administrated borax to rats as a boron source followed by administration of thioacetamide. As a result they reported that boron might have positive effects on the oxidant/antioxidant balance. Boron was shown to exhibit ameliorative effects against cyclophosphamide induced lipid peroxidation and genotoxicity by enhancing antioxidant defense mechanisms in rats (Ince et al. 2014). Besides, Ince et al. (2010) found that boron supplementation decreases lipid peroxidation and enhances antioxidant defense mechanism in vivo. Again, administration of boron in a dose-dependent manner reversed malathion-induced oxidative stress, lipid peroxidation and antioxidant enzyme activity in rats (Coban et al. 2014). Likewise, Turkez et al. (2012a) explored that different borates, including borax, colemanite, boric acid and ulexite, exhibited antioxidant features by using TAC assay in vitro. Similar to the present results, a very recent study indicated that potassium tetraborate showed antioxidant properties in vitro (Celikezen et al. 2014). In addition, our results revealed that 1.25 and 2.5 mg/l of LMD did not exhibit antioxidant features. Because, the observed slight increases of TAC values, were not statistically different from the control group at these two concentrations. The reason could be that the lower doses (<5 g/ml) used are close to the physiological limits. In fact, the normal value of BA in the blood has been reported as 3 mg/l (EVM, 2002).

In this study, the genotoxic potential of LMD was investigated by using the MN and CA assays. This study established that LMD was non-genotoxic because results did not indicate any significant increase in the ratios of the CAs and MNs in lymphocytes exposed to LMD as compared to control values. These findings are in agreement with previous assessments. Landolph (1985) assessed mutagenic effect of crude borax in V79 Chinese hamster cells, C3H10T ½ mouse embryo fibroblasts and diploid foreskin fibroblasts of human. At the end of the study, borax did not show genotoxic effect. Similarly, boric acid did not affect DNA synthesis in male F344 rat hepatocytes (Bakke 1991) and it was assessed for mutagenic potential in the L5178Y tk± mouse lymphoma cell and result of the study was negative (Mc Gregor et al. 1988). In addition, Türkez and Geyikoglu (2006) studied the mutagenic properties of some boronated compounds in cultured human lymphocytes. They reported that the used boronated compounds did not change the sister-chromatid exchange (SCE) frequencies or MN formations. Again, Turkez et al. (2007) evaluated the genotoxic potential of boric acid, borax, colemanite and ulexite by SCE, MN and CA assays in human blood cell cultures and found that no tested boron compounds led to genotoxicity.

As a conclusion, the present study clearly indicated for the first time that LMD has antioxidant ability with its non-genotoxic nature. However, further studies are needed to understand biological activity of LMD in different in vivo and in vitro cell models.

Conflict of interest The authors declare that there are no conflicts of interest.

References

Argust P (1998) Distribution of boron in the environment. Biolog Trace Elem Res 66:131–143

Arslan M, Topaktas M, Rencuzogullari E (2008) The effects of boric acid on sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes. Cytotechnology 56:91–96

Bakke JP (1991) Evaluation of the potential of boric acid to induce unscheduled DNA synthesis, in the in vitro hepatocyte DNA repair assay using the male F-344 rat. US Borax Corp. MRID No. 42038903

Balogh N, Gaal T, Ribiczeyene PS, Petri A (2001) Biochemical and antioxidant changes in plasma and erythrocytes of pentathlon horses before and after exercise. Vet Clin Pathol 30:214–218

Benhusein GM, Mutch E, Aburawi S, Williams FM (2010) Genotoxic effect induced by hydrogen peroxide in human hepatoma cells using comet assay. Libyan J Med 13:5–10

Cacciatore I, Caccuri AM, Di Stefano A, Luisi G, Nalli M, Pinnen F, Ricci G, Sozio P (2003) Synthesis and activity of novel glutathione analogues containing an urethane backbone linkage. Farmaco 58:787–793

Cacciatore I, Caccuri AM, Cocco A, De Maria F, Di Stefano A, Luisi G, Pinnen F, Ricci G, Sozio P, Turella P (2005) Potent isozyme-selective inhibition of human glutathione S-transferase A1-1 by a novel glutathione S-conjugate. Amino Acids 29:255–261

Celikezen FÇ, Türkez H, Togar B, İzgi MS (2014) DNA damaging and biochemical effects of potassium tetraborate. Excli J 13:446–450

Cingolani GM, Di Stefano A, Mosciatti B, Napolitani F, Giorgioni G, Ricciutelli M, Claudi F (2000) Synthesis of L-(+)-3-(3-hydroxy-4-pivaloyloxybenzyl)-2,5-diketomorpholine as potential prodrug of L-dopa. Bioorg Med Chem Lett 10:1385–1388

Coban FK, Ince S, Kucukkurt I, Demirel HH, Hazman O (2014) Boron attenuates malathion-induced oxidative stress and acetylcholinesterase inhibition in rats. Drug Chem Toxicol 24:1–9

Colak S, Geyikoglu F, Keles ON, Turkez H, Topal A, Unal B (2011) The neuroprotective role of boric acid on aluminum

chloride-induced neurotoxicity. Toxicol Ind Health 27:700–710

Di Stefano A, Sozio P, Iannitelli A, Cerasa LS (2009a) New drug delivery strategies for improved Parkinson's disease therapy. Expert Opin Drug Deliv 6:389–404

Di Stefano A, D'Aurizio E, Trubiani O, Grande R, Di Campli E, Di Giulio M, Di Bartolomeo S, Sozio P, Iannitelli A, Nostro A, Cellini L (2009b) Viscoelastic properties of *Staphylococcus aureus* and *Staphylococcus epidermidis* mono-microbial biofilms. Microbial Biotechnol 2:634–641

Dong L, Gao J, Xie X, Zhou Q (2012) DNA damage and biochemical toxicity of antibiotics in soil on the earthworm *Eisenia fetida*. Chemosphere 89:44–51

Evans HJ, O'Riordan ML (1975) Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat Res 31:135–148

EVM (2002) Expert Group on Vitamins and Minerals. Revised review of boron. EVM/99/23/P.REVISEDAUG2002

Fenech M, Morley AA (1985) Measurement of micronuclei in lymphocytes. Mutat Res 147:29–36

Geyikoglu F, Turkez H (2005) Protective effect of sodium selenite on genotoxicity to human whole blood cultures induced by aflatoxin B1. Brazil Arch Biol Technol 48:905–910

Geyikoglu F, Turkez H (2007) Acute toxicity of boric acid on energy metabolism of the breast muscle in broiler chickens. Biologia 62:112–117

Geyikoglu F, Turkez H (2008) Boron compounds reduce vanadium tetraoxide genotoxicity in human lymphocytes. Environ Toxicol Pharmacol 26:342–347

Geyikoglu F, Turkez H, Keles SM (2005) The role of fruit juices in the prevention of aluminum sulphate toxicity in human blood in vitro. Fresen Environ Bull 14:878–883

Ghiselli A, Serafini M, Natella F, Scaccini C (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med 29:1106–1114

Heuking S, Iannitelli A, Di Stefano A, Borchard G (2009) Toll-like receptor-2 agonist functionalized biopolymer for mucosal vaccination. Int J Pharm 381:97–105

Hunt CD, Idso JP (1999) Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research progress. J Trace Elem Exp Med 12:221–233

Ince S, Kucukkurt I, Cigerci IH, Fidan AF, Eryavuz A (2010) The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol 24:161–164

Ince S, Kucukkurt I, Demirel HH, Acaroz DA, Akbel E, Cigerci IH (2014) Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. Chemosphere 108:197–204

IPCS (International Program on Chemical Safety) (1985) American Oil Environmental Health Criteria 46. Guidelines for the study of genetic effects in human populations. WHO, Geneva, 45–54

Kahraman E, Gürhan İD, Korkmaz M (2013) Investigation of possible genotoxic and cytotoxic effects of differential boron compounds in ccl 62 (hela contaminant) human amniotic ephitelial cell line. Med Sci 2:454–468

Korkmaz M, Uzgören E, Bakırdere S, Aydın F, Ataman OY (2007) Effects of dietary boron on cervical cytopathology and on micronucleus frequency in exfoliated buccal cells. Environ Toxicol 22:17–25

Kusano C, Ferrari B (2008) Total antioxidant capacity, a biomarker in biomedical and nutritional studies. J Cell Mol Biol 7:1–15

Landolph JR (1985) Cytotoxicity and negligible genotoxicity of borax and borax ores to cultures mammalian cells. Am J Ind Med 7:31–43

Lantos J, Roth E, Czopf L, Nemes J, Gal I (1997) Monitoring of plasma antioxidant status in different diseases. Acta Chir Hung 36:188–189

Mahabir S, Spitz MR, Barrera SL, Dong YQ, Eastham C, Forman MR (2008) Dietary boron and hormone replacement therapy as risk factors for lung cancer in women. Am J Epidemiol 167:1070–1080

Mc Gregor D, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspari W (1988) Responses of the L5178Y tk-/tk- Mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environ Mol Mutagen 12:85–154

Miller NJ, Rice-Evans CA, Davies MJ, Gopinathan V, Milner A (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci 84:407–412

Pawa S, Ali S (2006) Boron ameliorates fulminant hepatic failure by counteracting the changes associated with the oxidative stress. Chem Biol Interact 160:89–98

Richold M (1998) Boron exposure from consumer products. Biol Trace Elem Res 66:121–129

Rispoli V, Rotiroti D, Carelli V, Liberatore F, Scipione L, Marra R, Giorgioni G, Di Stefano A (2004) Choline pivaloyl esters improve in rats cognitive and memory performances impaired by scopolamine treatment or lesions of the nucleus basalis of Meynert. Neurosci Lett 356:199–202

Schubert DM, Brotherton RJ (2006) Boron: inorganic chemistry. Encyclopedia of Inorganic Chemistry. 2nd Ed. ISBN: 978-0-470-86078-6

Sisman T, Turkez H (2010) Toxicologic evaluation of imazalil with particular reference to genotoxic and teratogenic potentials. Toxicol Ind Health 26:641–648

Snell DF, Hilton CL (1968) Encyclopaedia of industrial chemical analysis. Wiley, New York

Sozio P, D'Aurizio E, Iannitelli A, Cataldi A, Zara S, Cantalamessa F, Nasuti C, Di Stefano A (2010) Ibuprofen and lipoic acid diamides as potential codrugs with neuroprotective activity. Arch Pharm 343:133–142

Turkez H (2008) Effects of boric acid and borax on titanium dioxide genotoxicity. J Appl Toxicol 28:658–664

Turkez H (2011) The role of ascorbic acid on titanium dioxide-induced genetic damage assessed by the comet assay and cytogenetic tests. Exp Toxicol Pathol 63:453–457

Türkez H, Geyikoglu F (2006) Protective effects of kernite and probertite against titanium dioxide genotoxicity in vitro. 3rd International Boron Symposium, Ankara, Turkey, 475–479

Turkez H, Sisman T (2007) Anti-genotoxic effect of hydrated sodium calcium aluminosilicate on genotoxicity to human lymphocytes induced by aflatoxin B1. Toxicol Ind Health 23:83–89

Turkez H, Togar B (2010) The genotoxic and oxidative damage potential of olanzapine in vitro. Toxicol Ind Health 26:583–588

Turkez H, Geyikoglu F, Keles MS (2005) Biochemical response to colloidal bismuth subcitrate: dose-time effect. Biol Trace Elem Res 105:151–158

Turkez H, Geyikoglu F, Tatar A, Keleş S, Özkan A (2007) Effects of some boron compounds on peripheral human blood. Z Naturforsch 62:889–896

Turkez H, Tatar A, Hacimuftuoglu A, Ozdemir E (2010) Boric acid as a protector against paclitaxel genotoxicity. Acta Biochim Pol 57:95–97

Turkez H, Geyikoglu F, Mokhtar YI, Togar B (2012a) Eicosapentaenoic acid protects against 2,3,7,8-tetrachlorodibenzo-p-dioxin- induced hepatic toxicity in cultured rat hepatocytes. Cytotechnology 64:15–25

Turkez H, Geyikoglu F, Tatar A, Keles MS, Kaplan I (2012b) The effects of some boron compounds against heavy metal toxicity in human blood. Exp Toxicol Pathol 64:93–101

Vijayalaxmi Reiter RJ, Leal BZ, Meltz ML (1996) Effect of melatonin on mitotic and proliferation indices, and sister chromatid exchange in human blood lymphocytes. Mutat Res 351:187–192