Cytogenetic effects of a mixture of selected metals following subchronic exposure through drinking water in male rats

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Received 1 May 2006; revised 11 September 2006

The current study examines the genotoxic effects of subchronic exposure via drinking water to a mixture of eight metals (arsenic, cadmium, lead, mercury, chromium, nickel, manganese and iron) found as contaminants of water sources in different parts of India and its possible association with oxidative stress. Male rats were exposed to the mixture at 0, 1, 10 and 100 times the mode concentration of each metal daily for 90 days. Another dose group at concentration equivalent to maximum permissible limit (MPL) for each metal and a reference group given ip cyclophosphamide were incorporated. The mixture at 100× level significantly increased chromosomal aberrations and micronuclei induction (2.4 folds) in bone marrow cells and reduced the ratio of polychromatic to normochromatic erythrocytes by 25%. The mixture significantly increased sister chromatid exchange in bone marrow (1.67 and 2.3 folds) and spleen (1.57 and 1.98 folds) cells with both 10× and 100× doses. Cyclophosphamide was more potent than the mixture in causing cytogenetic damage in these parameters. In rat spleen, the mixture at 10× and 100× doses caused dose-dependent increase in lipid peroxidation (25.95 and 52.71%) and decrease in the activities of superoxide dismutase (20.36 and 40.62%), catalase (18.24 and 35.50%), glutathione peroxidase (22.33 and 36.12%) and glutathione reductase (19.22 and 31.35%) and in the level of GSH (19.76 and 35.15%). The results suggest that the mixture induced genotoxicity in rat bone marrow and spleen cells at concentrations relatively higher than that found in groundwater sources and the genotoxic effect could relate to induction of oxidative stress. However, observations with lower doses indicate that additive or synergistic interactions following exposure to metal components at MPL levels or at mode concentrations of contemporary groundwater levels in India may not result in clastogenicity in male rats.

Keywords: Genotoxicity, Groundwater contaminants, Metal mixture, Oxidative stress, Rat

Exposure to toxic metals has become an increasingly recognized source of illness worldwide. Heavy metals are ubiquitous in the environment and found in hazardous concentrations in air, food and water. Exposure through food, water and occupational sources can contribute to a spectrum of diseases. Groundwater contamination is a very important source of environmental exposure to toxic metals. It’s a global public health problem, affecting both the industrialized and developing nations. Metal contamination reflects natural as well as anthropogenic sources in which many metals occur in association with one another. Contamination of water resources with various metals, including arsenic, cadmium, lead, mercury, chromium, nickel, manganese and iron, is a widespread problem in India. Metals like arsenic, chromium, cadmium, nickel, iron and lead are carcinogenic in human and/or animal studies. A number of experimental and epidemiological studies on the genotoxic effects of heavy metals and their compounds have been published. These demonstrate that arsenic, cadmium, mercury, lead, chromium, nickel, manganese and iron are clastogens inducing chromosomal aberrations, micronuclei induction, sister chromatid exchange (SCE) and chromosomal loss in human and animal cells.

Metal-induced oxidative stress has been shown to cause DNA damage through the production of reactive oxygen species (ROS) such as superoxide anion radical (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻). There is considerable evidence that ROS-mediated oxidative damage induced by several metals is an underlying basis of their genotoxicity. Another important
The mechanism of metal-induced genotoxicity has been attributed to their ability to react with the sulphydryl (-SH) groups²⁴, ²⁵. It is known that cellular nonprotein -SHs consist essentially of reduced glutathione (GSH, ≈95%) and other low-molecular-weight aminothiols such as cysteine and cysteamine. The metal binding to -SH groups of glutathione blocks its function as a free radical scavenger. Thus, free radicals become available to cause DNA damage. These mechanisms can lead to double-strand breaks that can be visualized as the chromatid gaps and/or give rise to chromosomal alterations such as breaks, fragments, exchange and rearrangements²⁶.

Current understanding of genotoxicity of these metals is primarily based on genotoxicity studies performed on laboratory animals exposed to a single agent. The reported effects are seen mostly in high levels of exposure. The human and animal populations are ubiquitously exposed to complex mixtures of these metals generally at much lower levels than those routinely examined in animal studies and the effects of any interactions between such metals on mammalian cytogenetics is virtually unknown. Groundwater meets 80% of India’s drinking water requirement²⁷. Since each hazardous agent. The reported effects are seen mostly in high doses generally used in animal toxicity experiments, and to assess whether the genotoxic effect could be linked to cellular oxidative stress. The genotoxic potential of the mixture was also examined at a dose level, where each metal was incorporated in the mixture at the drinking water maximum permissible limit (MPL) as determined by the World Health Organization (WHO).

Materials and Methods

Selection of metals — The mixture was formulated to reflect the types of frequently occurring metals to which general populations of India could conceivably be exposed through drinking water at concentrations above the maximum permissible limit (MPL). Data on contamination of water resources in different parts of India with various metals were collected through literature survey. From the reported concentrations of each metal, the mode concentrations of these metals were derived by group frequency distribution. The metals were selected on the basis of two criteria: (1) frequency of occurrence, and (2) contamination level above MPL in drinking water. A minimum of 15 to a maximum of 30 reports were considered for selection of an individual metal.

Chemicals—Cadmium chloride, mercuric chloride, chromium trioxide, lead acetate and nickel chloride were procured from E. Merck (India) Ltd., Mumbai. Sodium arsenite, manganese chloride and ferric chloride were purchased from Sigma Chemicals, USA; S. D. Fine Chemicals, Mumbai and Himedia, Mumbai, respectively. All other chemicals used in the study were of analytical grade from Sigma Chemicals, USA; E. Merck, Germany/India; SRL Chemicals, India.

Mixture formulation — The mixture was prepared following the protocol reported by Wade et al²⁸. All the metal salts, which are soluble in water, were weighed into a glass bottle in amounts appropriate to make the 100× stock. Lower doses (1× and 10×) of the dosing solution were prepared by 10-fold serial dilution of the 100× stock. Dosing solution for the concentrations equivalent to the MPL (WHO) was prepared separately. Dosing solutions were prepared daily.

Animals and experimental protocol — Male Wistar rats (100-120 g) procured from the Laboratory Animal Resources Section of the Institute were acclimatized to holding facilities for one week prior to commencement of dosing. They were caged in pairs in clean plastic cages containing wheat straw chips for bedding and maintained under standard management conditions. All animals were given standard pellet diet (Amrut Inbred Rat and Mice Feed, Pranav Agro Industries, Delhi) and deionized water ad libitum. Animal care and handling were in accordance with the Institute Animal Ethics Guidelines. Rats were exposed daily to the mixture of metal components through drinking water (deionized) for 90 days. Six groups of rats comprising 15 animals each were used. The mixture of metals and its dose levels are presented in Table 1. Group I was given only deionized water and served as control. Group II, the baseline dose group (1×), was given mode concentrations of individual metals. Groups III and IV were, respectively, set at 10- and 100-fold dilution of the 100× stock. Lower doses (1× and 10×) of the dosing solution were prepared by 10-fold serial dilution of the 100× stock. Dosing solution for the concentrations equivalent to the MPL (WHO) was prepared separately. Dosing solutions were prepared daily.
concentrations of those of the baseline group. Rats of Group V received metals at concentrations equivalent to the MPL (WHO) in drinking water (Table 1), while rats of Group VI (Positive control) were injected i.p. with cyclophosphamide at 20 mg/kg b. wt. in normal sterile saline. During the course of experiment, 2 to 3 rats died in each group, including control. None of the animals died showed any visible signs of toxicity. However, to maintain the uniformity of sample size of all groups, six of the surviving animals were used for chromosomal aberration assay, while another six were used for assessment of micronuclei induction, SCE and oxidative stress. Rats were killed by cervical dislocation after 24 h of the last exposure.

Assessment of chromosomal aberration — Preparation of bone marrow and assessment of chromosomal aberration were carried out as described by Malhi and Grover and Chauhan. Briefly, 24 hr prior to their sacrifice, colchicine (4 mg/kg in water) was injected ip to arrest mitosis. After sacrifice, both femurs were immediately dissected out and cut at the both ends with bone snips. The marrow was aspirated from the body of the femurs in Hank’s balance salt solution (HBSS, pH 7.2) and cell suspension was centrifuged for 10 min at 1000 rpm. The cells were centrifuged and the pellets were resuspended in chilled Carnoy’s 3:1 methanol and glacial acetic acid fixative. After 2 hr of fixation, the cells were centrifuged and resuspended in the same fixative for 24 hr. The fixed cells were then dropped on chilled and labelled slides, air dried for 24 hr, stained with 2% Geimsa for 15-25 min, dehydrated in 1:1 acetone and xylene mixture, pure xylene and mounted with DPX. Fifty well spread metaphase plates per rat were examined. Cells with severe fragmentation of chromosomes (≥10 aberrations) were classified as pulverized and scored singly for expressing the frequency of aberrations and aberrant cells. Chromatid/isochromatid gaps and endomitotic reduplication were recorded but not included in the total of aberrations or aberrant cells.

Assessment of micronucleus induction — Isolation of bone marrow cells and preparation of slides for micronucleus assay were carried out following the methods of Hayashi and Chauhan. Briefly, marrow from femur was aspirated in the HBSS (pH 7.2), containing EDTA and bovine serum albumin and cell suspension was centrifuged for 10 min at 1000 rpm. The cells in the sediment were mixed carefully. A drop of cell suspension was smeared on clean slide, fixed with methanol for 5 min and stained with acridine orange for 3 min at room temperature. The slides were rinsed thrice with the Sorensens’s buffer (pH 6.8) for 1-3 min each time, mounted with the same buffer and sealed with Balsam paraffin. Observations were made within a day. Prior to scoring, slides were randomly coded so that the scorer was unaware of the treatment group from which each slide originated. The frequencies of micronucleated cells were determined by scoring a minimum of 2000 polychromatic erythrocytes (PCE) per animal, while 200 bone marrow erythrocytes per animal were examined to derive the ratio of PCE to normochromatic erythrocytes (NCE).

Sister chromatid exchange (SCE) in bone marrow and spleen cells — SCE assay was performed in bone marrow cells and splenocytes as described by Perry and Wolf and Krishna. Briefly, after sacrifice of rats, spleen and both the femurs were excised. The
marrow was flushed out with Ham’s F 10 (HF-10) medium in 5 ml sterilized vials containing HF-10 complete culture medium. Spleen was smashed with HF-10 medium and the debris was removed. The cells were washed with PBS containing 2% heat activated fetal bovine serum and centrifuged at 250 × g for 6 min. The final cell suspension was cultured in 5 ml tissue culture sterilized vials. Bone marrow and splenocyte culture vials were incubated at 37°C in a BOD incubator for 30 min and 40 hr, respectively. After incubation, colchicine (30 µM) was added to both the cultures. The cells were harvested by decanting the contents of the vials into 15 ml centrifuge tubes. Each vial was rinsed with 3 ml HBSS, which was also transferred to the centrifuge tube. The tubes were centrifuged at 285 × g for 6 min and cells were processed for slide preparation as described elsewhere for chromosomal aberration assay. Staining was done according to the modified technique of Perry and Wolf32. Slides were stained for 15 min with Hoechst 33258 (Sigma, 5 µg/ml) and rinsed briefly in deionized water. The slides were then mounted temporarily in citrate buffer (pH 6.8). The cover slips were sealed with molten wax to prevent evaporation. The same slides were then exposed to ultraviolet light for 10 to 24 hr and examined intermittently for differential fluorescence of the chromatids. After removing the cover slips, slides were incubated in distilled water for 2 hr (60°C) and stained for 30 min in 2% Giemsa solution. Scoring was done in 30 well differentially stained metaphases per rat and the results were expressed as SCE/cell.

**Preparation of spleen homogenate** — After sacrifice of rats, spleen was removed, cleaned and a piece of adequate amount was stored at −80°C. Frozen spleen samples were partially thawed and 200 mg of each sample was taken in 2 ml ice-cold normal saline. Another 200 mg was taken in 2 ml of 0.02 M EDTA for GSH estimation. Tissue homogenate (10%) was prepared in ice-cold normal saline and centrifuged for 10 min at 3000 rpm. The supernatant was used for estimation of the following biochemical attributes for assessment of induction of oxidative stress in spleen.

**Assay of lipid peroxidation (LPO)** — LPO was assayed by the method of Shafiq-U-Rehman34 and expressed as nmol malondialdehyde/g wet tissue. In brief, 1 ml of homogenate was incubated at 37°C±0.5°C for 2 hr. To each sample, 1 ml of 10% w/v trichloroacetic acid was added. After thorough mixing, the mixture was centrifuged at 2000 rpm for 10 min. To 1 ml of supernatant, an equal volume of 0.67% thiobarbituric acid was added and kept in boiling water bath for 10 min. After cooling, it was diluted with 1 ml of distilled water (DW). The absorbance was read at 535 nm.

**Determination of GSH level** — GSH content was determined by estimating free –SH groups, using 5,5’-dithiobis-2-nitrobenzoic (DTNB) acid method of Sedlak and Lindsay35 and expressed as mmol GSH/g wet tissue. Briefly, to 1 ml supernatant, 0.8 ml of DW and 0.2 ml of 50% trichloroacetic acid were added and incubated at room temperature for 15 min; centrifuged at 3000 rpm for 15 min. From this, 0.4 ml supernatant was added to 0.8 ml of 1 M Tris buffer (pH 8.9) followed by 0.2 ml of DTNB (0.01 M) and absorbance was read at 412 nm within 5 min.

**Determination of superoxide dismutase (SOD) activity** — SOD activity was measured by the procedure of Madesh and Balasubramanian36. In brief, the reaction mixture contained 0.65 ml PBS saline (pH 7.4), 30 µl 3-(4-5-dimethylthiazol 2-xl) 2,5-diphenyltetrazolium bromide (MTT; 1.25 mM), 75 µl pyrogallol (100 µM) and 10 µl homogenate. The mixture was incubated at room temperature for 5 min and the reaction was stopped by adding 0.75 ml of dimethyl sulfoxide. The absorbance was read at 570 nm against DW and the activity has been expressed as Unit. One unit is the µg of protein required to inhibit MTT reduction by 50%.

**Determination of catalase activity** — Catalase activity was assayed by the method of Aebi37 and expressed as k/g wet tissue. One k stands for nmol H2O2 utilized/min. Briefly, 2 ml PBS (pH 7.4) and 10 µl homogenate were taken in a cuvette. Reaction was started by adding 1 ml H2O2 (20 mM) and the absorbance was recorded at every 10 sec for 1 min at 240 nm against water blank.

**Determination of glutathione peroxidase (GPx) activity** — GPx activity was assayed by the method of Paglia and Valentine38 and expressed as unit/mg protein. One unit (U) is the nmol NADPH utilized/min/mg protein at 25°C. The reaction mixture contained 2.48 ml PBS (pH 7, containing 5 mM EDTA), 0.1 ml NADPH (8.4 mM), 0.1 ml GSH (150 mM), 0.01 ml sodium azide (112.5 mM), 4.6 U glutathione reductase (Type III; Sigma Chemicals, USA) and 10 µl of homogenate. The reaction was initiated by adding 0.1 ml H2O2 (2.2 mM) to the
mixture containing 500-1000 μg protein. The change in absorbance was read at 540 nm for 4 min.

**Determination of glutathione reductase (GR) activity** — GR activity was measured by the method of Goldberg and Spooner and expressed as unit/mg protein. One unit is the mmol NADPH utilized/min/mg protein at 25°C. The 3 ml of reaction mixture contained 2.6 ml PBS (0.12 M, pH 7.2), 0.1 ml EDTA (15 mM), 0.1 ml GSSG (65.3 mM). To this 10 μl of homogenate was added and the volume was made up to 2.95 ml with DW. After incubation at room temperature for 5 min, 0.05 ml of NADPH (9.6 mM) was added. Decrease in absorbance/min was recorded immediately at 340 nm for 3 min. Control was run without GSSG.

**Estimation of tissue protein** — Protein level in the homogenate was measured by the method of Lowry et al., using bovine serum albumin as the standard.

**Statistical analysis** — Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s t-test to compare the cytogenetic effects and biochemical changes induced by different doses of the metal mixture in bone marrow and spleen. Differences were considered significant at the probability level of \( P < 0.05 \).

**Results**

**Chromosomal aberrations** — Chromosomal aberrations, viz., gaps, breaks, exchange, pulverization and endoreduplication induced with the different doses of the metal mixture in bone marrow and spleen. Differences were considered significant at the probability level of \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells/Rat</th>
<th>Gaps*</th>
<th>Breaks</th>
<th>Fragment</th>
<th>Exchange</th>
<th>Pulveri-</th>
<th>Endore-</th>
<th>Total</th>
<th>Frequency of aberrations (%)</th>
<th>Frequency of aberrant cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>320/6</td>
<td>6 (2)</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>4</td>
<td>2</td>
<td>1.28±0.62ab</td>
</tr>
<tr>
<td>MPL</td>
<td>308/6</td>
<td>9 (4)</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>1.92±0.69ab</td>
</tr>
<tr>
<td>1×</td>
<td>314/6</td>
<td>10 (3)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>2.19±0.56ab</td>
</tr>
<tr>
<td>10×</td>
<td>318/6</td>
<td>7 (2)</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>2.82±0.42ab</td>
</tr>
<tr>
<td>100×</td>
<td>325/6</td>
<td>8 (3)</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>14</td>
<td>9</td>
<td>4.31±0.62b</td>
</tr>
<tr>
<td>Positive Control</td>
<td>310/6</td>
<td>12 (1)</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>21</td>
<td>15</td>
<td>6.77±0.42c</td>
</tr>
</tbody>
</table>

MPL: Maximum permissible limit in drinking water (WHO). Positive control: Rats were given ip cyclophosphamide at 20 mg/kg. PCE: Polychromatic erythrocytes. Values in the same column bearing no superscript common vary significantly (\( P < 0.05 \)).
Sister chromatid exchange (SCE) — Effects of the mixture of metals on SCE in bone marrow and spleen cells of rats are presented in Table 4. In both types of the cells, effect of the mixture on SCE was dose-dependent. SCEs produced by the lower exposure levels (MPL and 1×) were not significantly different from the control rats. But, it was significantly increased with 10× and 100× exposure levels by 1.67 and 2.3 folds in bone marrow cells and by 1.57 and 1.98 folds in splenic cells, respectively. Cyclophosphamide was significantly more potent than the metal mixture in escalating the SCE in bone marrow cells (7.41 fold) and spleen cells (6.63 fold).

Oxidative stress in spleen — Effects of the metals mixture on oxidative stress-related indices in spleen are presented in Table 5. The mixture at MPL and 1× dose levels did not significantly alter any of the spleen biochemical attributes examined. Compared to control rats, LPO was significantly increased with 10× (25.95%) and 100× (52.71%) doses. These two higher doses significantly decreased all the antioxidant parameters assayed. With these doses, the activity of SOD was decreased by 20.36 and 40.62%, catalase by 18.24 and 35.50%, GPx by 22.33 and 36.12% and GR by 19.22 and 31.35%, while the level of GSH by 19.76 and 35.15%, respectively.

Discussion

While considerable amount of research has been devoted to the genotoxicity of single exposures to various metals and their compounds, very less emphasis has been given to evaluation of the genotoxic potential of mixtures of metals. The present results demonstrate that subchronic exposure to the mixture of metals, found as contaminants in various water sources of India, produces genotoxicity in rat bone marrow and spleen cells in relatively high doses (10× and 100×) and the cytogenetic effects were associated with dose-dependent increase in LPO and decrease in the enzymatic and nonenzymatic antioxidative systems in spleen. It is interesting to note that, in quantitative terms (% change), the impact of the mixture on all the antioxidative attributes was almost identical. However, no significant impact of the MPL and 1× dose levels on cytogenetic tests and oxidative stress paradigm suggests that the interactions among the coexposed mixture components in these exposure levels were not enough to induce any chromosomal damage in bone marrow and spleen cells, and oxidative stress in spleen. This, in spite of the known genotoxic and oxidative stress-inducing effects of these metals in various cells, offers some support for the assumption that derivation of safe levels of exposure from single agent toxicity studies provides reasonable protection from adverse effects in male animals.

Cytogenetic effects induced by the mixture in bone marrow cells indicate its toxic implications on bone marrow. This is also evident by the significant reduction in PCE/erythrocytes ratio in bone marrow. This, while considerable amount of research has been devoted to the genotoxicity of single exposures to various metals and their compounds, very less emphasis has been given to evaluation of the genotoxic potential of mixtures of metals. The present results demonstrate that subchronic exposure to the mixture of metals, found as contaminants in various water sources of India, produces genotoxicity in rat bone marrow and spleen cells in relatively high doses (10× and 100×) and the cytogenetic effects were associated with dose-dependent increase in LPO and decrease in the enzymatic and nonenzymatic antioxidative systems in spleen. It is interesting to note that, in quantitative terms (% change), the impact of the mixture on all the antioxidative attributes was almost identical. However, no significant impact of the MPL and 1× dose levels on cytogenetic tests and oxidative stress paradigm suggests that the interactions among the coexposed mixture components in these exposure levels were not enough to induce any chromosomal damage in bone marrow and spleen cells, and oxidative stress in spleen. This, in spite of the known genotoxic and oxidative stress-inducing effects of these metals in various cells, offers some support for the assumption that derivation of safe levels of exposure from single agent toxicity studies provides reasonable protection from adverse effects in male animals.

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Cytogenetic effects induced by the mixture in bone marrow cells indicate its toxic implications on bone marrow. This is also evident by the significant reduction in PCE/erythrocytes ratio in bone marrow. This, while considerable amount of research has been devoted to the genotoxicity of single exposures to various metals and their compounds, very less emphasis has been given to evaluation of the genotoxic potential of mixtures of metals. The present results demonstrate that subchronic exposure to the mixture of metals, found as contaminants in various water sources of India, produces genotoxicity in rat bone marrow and spleen cells in relatively high doses (10× and 100×) and the cytogenetic effects were associated with dose-dependent increase in LPO and decrease in the enzymatic and nonenzymatic antioxidative systems in spleen. It is interesting to note that, in quantitative terms (% change), the impact of the mixture on all the antioxidative attributes was almost identical. However, no significant impact of the MPL and 1× dose levels on cytogenetic tests and oxidative stress paradigm suggests that the interactions among the coexposed mixture components in these exposure levels were not enough to induce any chromosomal damage in bone marrow and spleen cells, and oxidative stress in spleen. This, in spite of the known genotoxic and oxidative stress-inducing effects of these metals in various cells, offers some support for the assumption that derivation of safe levels of exposure from single agent toxicity studies provides reasonable protection from adverse effects in male animals.
nickel\textsuperscript{18, 19}, manganese\textsuperscript{20, 21} and iron\textsuperscript{22, 23} have been reported to produce cytogenetic damage, including chromosomal aberrations, micronuclei induction and SCE in bone marrow and other cells. The SCE assay is recognized as a good index of DNA damage and can be detected at lower doses of the compounds than those required to induce chromosomal aberrations\textsuperscript{42}. In the present study, the mixture of metals caused significant increase in SCE with 10× and 100× doses in both bone marrow and spleen cells. While chromosomal aberrations and micronuclei induction were observed with the highest dose only (100×). This shows that the present results are in agreement with the observation of Latt et al.\textsuperscript{42} and the SCE was more sensitive than others assays in the current study for detection of cytogenetic effect of the metal mixture.

Genotoxic metals can directly or indirectly damage DNA; it means an increased risk of cancer. The toxic metals may lower the genetic stability predominantly by two modes of action; the induction of oxidative DNA damage and the interaction with DNA repair processes\textsuperscript{19, 41}. Experimental studies in mammalian cells have demonstrated that active oxygen radicals may contribute to clastogenesis directly\textsuperscript{43} and indirectly through the production of lipid peroxides\textsuperscript{44}. Increased production of lipid peroxide, malondialdehyde, and decrease in antioxidative systems, as observed in the present study, suggest that the mixture caused generation of ROS and oxidative stress in rat spleen. This indicates that free radical-induced oxidative damage to DNA could be a factor in mediating the cytogenetic changes in spleen cells with the metal mixture. Malondialdehyde, a major end product and biomarker of LPO, reacts with deoxyribonucleosides to produce DNA adducts and is documented as mutagenic in mammalian cells\textsuperscript{34, 45}. Decrease in the activities of SOD, catalase and GPx may lead to accumulation of ’O\textsubscript{2}− and H\textsubscript{2}O\textsubscript{2} in the cell. H\textsubscript{2}O\textsubscript{2} and ’O\textsubscript{2}− themselves can cause DNA damage in the form of chromosome breakage, rearrangement and SCE\textsuperscript{10, 26, 46}. ROS, particularly OH“, play an important role in the genotoxicity of metals, including arsenic and nickel in mammalian cells\textsuperscript{10, 18}. SOD acts as an antiproliferative agent, anticarcinogen and inhibitor at initiation and promotion/transformation stage in carcinogenesis\textsuperscript{46}. Addition of the ’O\textsubscript{2}− scavenging enzyme, SOD, reduces the frequency of arsenite-induced SCEs\textsuperscript{47}. GPx and catalase are important in the inactivation of many environmental mutagens. Catalase and GPx have been reported to reduce the arsenite-induced micronuclei in Xrs-5 cells\textsuperscript{48}. Catalase reduces the SCE levels and chromosomal aberrations\textsuperscript{46}. These enzymatic antioxidants can prevent genetic changes by inhibiting DNA damage induced by the reactive oxygen molecules\textsuperscript{46}. Hartwig\textsuperscript{41} reported that metals such as arsenic, cadmium, lead, nickel and cobalt cause inhibition of DNA repair processes at low, non-cytotoxic concentrations of the respective metal compounds, and concluded that even though different steps in DNA repair are affected by the diverse metals, one common mechanism may be the competition with essential metal ions. Therefore, in the present study, the observed cytogenetic effects of the metal mixture may relate to oxidative stress-induced damage to DNA, interference with the DNA repair process and substitution of cellular essential metal ions.

It may be concluded that the mixture of eight heavy metals induced genotoxicity in rat bone marrow and spleen cells and oxidative stress in spleen at concentrations relatively higher than that found in groundwater sources. The oxidative stress could be the underlying basis of the metal mixture-induced cytogenetic effects. Results with lower doses suggest that additive or synergistic effects of exposure to metal contaminants at MPL levels derived by the WHO or at concentrations representative of contemporary Indian groundwater levels are unlikely to induce oxidative stress and result in adverse effects on the cytogenetics of male rats. However, it would be important to examine the degree to which changes in these sensitive cytogenetic end points in male rats can predict carcinogenic potential of the mixture in order to determine the significance of genotoxicity of this mixture.

Acknowledgement

The Senior Research Fellowship awarded to the first author by the Institute is gratefully acknowledged. The authors are grateful to Dr. S. M. Deb, Division of Animal Genetics, for assistance in cytogenetic assays.

References

2. CPCB, Groundwater Quality Series: GW9/1-4/1995-96 (Central Pollution Control Board, New Delhi, India) 1995-96.
3. Acharyya S K, Arsenic contamination in groundwater affecting major parts of southern West Bengal and parts of
Kawanishi S, Inoue S, Oikawa S, Yamashita N, Toyokuni S, Calcium, lymphocytes chromosomal and nuclear chromatin in human blood

M'Bemba-Meka P, Lemieux N & Chakrabarti S K, Nickel in chromium-exposed workers,


Paglia D E & Valentine W N, Studies on the quantitative and qualitative characterization of erythrocyte glutathione


