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Research Article

Genotoxic Effect of Atrazine, Arsenic, Cadmium and Nitrate, Individually and in Mixtures at Maximum Contaminant Levels on mammalian Breast Cell Lines

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ABSTRACT

There is strong evidence that hormonally active agents (HAAs) such as Atrazine (ATZ), Cadmium (Cd), Arsenic (As) and Nitrate (NO_3) have both estrogenic activity and carcinogenic potential. Atrazine has clastogenic effects and may also act as tumor promoter as it induces the aromatase enzyme. Arsenic and Cadmium have been implicated in the etiology of skin, lung, prostate and liver cancers. Nitrate in drinking water has been found to increase the risk of bladder cancer.

This study examined the genotoxicity of the aforementioned HAAs alone and in mixtures using mammalian breast cell lines, MCF-7 and MCF-10A, which are estrogen receptor-positive (ER+) and estrogen receptor-negative (ER-), respectively. To study the clastogenic potential by whole cell and flow karyotype damage, cells were exposed to environmentally relevant concentrations of ATZ, Cd, As and NO_3 for 4 and 7 days.

Results indicated that all treatments induced whole cell clastogenicity in MCF-7 cells; except Cd and NO_3 after 4 and 7 days as well as the 10% quaternary As mixture after 1 week. In MCF-10A cells, all treatments except the 10% mixture induced whole cell clastogenicity after 4 days, where flow karyotype damage was detected in all treatments except for the 10% mixture after 1 week. Estrogen caused whole cell damage but not flow karyotype damage in MCF-7. On the other hand, estrogen caused flow karyotype damage and not whole cell damage in MCF-10A cells, suggesting that estrogen receptor modulated the genotoxicity of estrogen. Cd caused flow karyotype damage but not whole cell damage in MCF-7 indicating that Cdôs gentoxicity is not related to its estrogenic activity.

INTRODUCTION

Breast cancer is a complex multifactorial disease. It is the most common cancer affecting women worldwide, comprising 23% of the estimated annual female cancer diagnoses (Coughlin and Ekwueme, 2009). Breast cancer rates have increased in both developing and non-developing countries. For instance, in Japan and Korea breast cancer rates have doubled in the past 40 years (Porter, 2008). While, in the past decade, Chinaôs urban cancer registries have reported an increase in breast cancer incidence rates between 20% and 30% (Porter, 2008). In the United States, breast cancer is the most frequently diagnosed cancer in women and the second leading cause of death after lung cancer (Khan and Bui, 2010); and the state of Maryland was listed in 2010 among the states with the highest breast cancer incidence rates (125.1 to 142.9) in the U.S. (CDC, 2014).

The etiology of breast cancer has been the subject of numerous studies since the pioneer investigation of

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Lane Claypon in 1926. Risk factors belong to different domains such as reproductive life, hormones, diet, genetics and exposure to radiation and selected chemicals. Yet, breast cancer remains unexplained and new etiological links must be sought, including occupational factors and exposure to hazardous chemicals such as pesticides and other endocrine disruptors (Guillette, 2006).

Environmental data has documented widespread exposure to Atrazine, arsenic (As), cadmium (Cd) and nitrate in the Eastern Shore of Maryland. Atrazine, one of the most commonly used herbicides in the United States is persistent in the environment and mobile in surface and ground water (Sass and Colangelo, 2006). Thus it is not surprising that Atrazine is the most commonly detected herbicide in groundwater across the U.S and, in particular, on the Delmarva Peninsula (Denver et al., 2004). Furthermore, Atrazine is most commonly detected pesticide in Maryland's Eastern Shore in surficial groundwater where concentrations used for domestic supplies exceeded 0.007 ug /L (Denver et al., 2004). Local concentrations of atrazine in Maryland for runoff water range between 0.2-180 ppm (Eisler, 1985).

Atrazine has been extensively studied to determine possible adverse effects to human health. Both animal and human studies have suggested that Atrazine is a carcinogen and an endocrine disruptor with the ability to interfere with reproduction and development (Sass and Colangelo, 2006). In addition, several studies have reported that Atrazine at agricultural concentrations can induce genotoxicity in plants (Plewa et al., 1984). A flow cytometric analysis of effects of atrazine, simazine and bentazon on Chinese Hamster Ovary (CHO) cells demonstrated that atrazine can cause whole cell clastogenicity in mammalian systems (Biranda and Rayburn, 1995a). Whole cell clastogenesis may occur because of structural chromosomal aberrations, amplifications, deletions or changes in chromatin structure. Further studies were conducted by Biradar and Rayburn (1995b) to ascertain whether chromosomal aberrations are the cause for the whole cell clastogenicity observed in CHO cells exposed to different herbicides. They demonstrated that chromosomal breakage was associated with whole cell clastogenicity in Atrazine treated cells. An increase in chromosomal aberrations in lymphocyte cultures of farm workers exposed to pesticides, including the herbicide Atrazine, was reported by Yoder et al. (1973). Chromosomal aberrations have also been detected in bone marrow cells of mice exposed to a single dose of Atrazine (Loprieno et al., 1980). Another study conducted on human lymphocyte cultures exposed to a low level of Atrazine (0.1 pg/ml) showed a significant percentage of cells exhibited chromosome aberrations (Meisner and Roloff, 1993). Arsenic, a paradoxical human carcinogen, has affected millions of people all over the world either through contaminated drinking water or through occupational exposure. Concentrations of arsenic in surface and groundwater are usually less than10 ug/L, but in some areas, levels exceeding 1000 ug /L have been reported (Pershagen, and Vahter, 1999).

Arsenic is known to induce DNA damage in mammalian cells. As has been reported to increase the rate of chromosome aberration and sister chromatin exchanges (Lee et al., 1988). Chromosomal aberrations were also induced by Arsenite in human fibroblasts and Chinese hamster ovary cells at high concentrations. In contrast, arsenic induced sister chromatin exchange at lower concentrations (Huang et al, 1995; Kochhar et al., 1996). Exposure to low concentrations of arsenic for prolonged periods was found to be associated with genetic instability and loss of chromosome 9q and gain of chromosome 4q in human HaCaT, and the exposure could result in the transformation of nontumorigenic human keratinocytes to cells that were tumorigenic in nude mice; these chromosomal abnormalities are closely related to arsenic induced oxidative DNA damage (Chien et al., 2004).

Exposure to cadmium (Cd) is ubiquitous; high levels of this metal found in certain occupational exposures have been associated with renal effects. Cadmium levels in water vary over time and between sites, and usually ranges from 0.5 to 1.5 ppb (Eisler, 1985). Although cadmiumôs genotoxic mechanism is still obscure, the International Agency for Research on Cancer (IARC) has classified cadmium as a category 1 carcinogen (human carcinogen), because of its impact as a lung carcinogen (Kochhar et al., 1996), In vitro studies using different types of mammalian cells have demonstrated that Cd could induce various types of genotoxic effects (Chien et al., 2004). It is evident that cadmium could produce direct DNA damage, such as strand breaks or oxidative DNA base mutations by induced oxidative stress (Dally and Hartwig, 1997). Human studies data have been conflicting; for example, whereas no increased frequency of chromosomal aberrations in Cd exposed individuals was reported by Fleig et al., (1983), another study (Fu et al., 1995) showed that the incidence of lymphocytes cells with structural aberrations was significantly elevated in Cd-exposed individuals compared with controls. In fact, Verougstarete et al., (2002 had reported that no definite conclusions can be

drawn from existing human studies. It had become apparent in recent years that Cd targets a host of DNA repair mechanisms, including base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) (Wieland et al., 2009).

Recent research suggested that the nitrate in manure and fertilizer runoff can act as an endocrine disrupter (Guillette and Edward, 2005). It has been shown that mitochondria in cells can reduce nitrate from food and water to nitric oxide. Nitric oxide is a potent inhibitor of cytochrome P-450, which is a group of enzymes that is involved in the synthesis of steroids (Jensen, 2003). The dietary intake of nitrate is responsible for large amounts of nitrate, nitrite and many N-nitroso compounds that are excreted by the kidneys via the urinary tract. These compounds are known to have a carcinogenic effect on urothelial cells (Pelley, 2003).

The objective of this study was to examine the individual and interactive DNA damaging effects of four chemicals (Atrazine, As, Cd and NO_3) known to be present in the drinking water of residents of Marylandôs Eastern Shore.

MATERIALS AND METHODS

Cell Culture

Human breast tumorigenic cells, MCF-7 (ER +), and non-tumorigenic human breast cells, MCF-10A (ER-), were cultured in 75 cm^2 tissue culture flasks. MCF-7 cells were grown in minimum essential medium (MEM) supplemented with 10% heat deactivated fetal bovine serum (FBS) and 1% penicillin and streptomycin. Cells and chemicals were purchased from American Type Tissue Culture Collection (ATCC) (Manassas, VA). MCF-10A cells were grown in DMEM/F12 media containing 5% horse serum and the following supplements: 10 ug/ml insulin, 20 ng/ml hEGF, 100 ng/ml cholera toxin, 0.5 ug/ml hydrocortisone and 2 mM L-glutamine. Media and supplements were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD) . Both MCF-7 and MCF-10A cell lines were incubated at 37°C in 5% CO_2 and humidified atmosphere. When MCF-7 cells reached 75-80 % confluency, they were washed with phosphate buffer saline (PBS), trypsinized with 3 ml of 0.25% w/v trypsin, 0.53 mM EDTA and incubated at 37C for 5 minutes. For MCF-10A cells, 3.0 ml of a 0.05% trypsin, 0.53 mM EDTA solution was added and cells were incubated at 37C for 15 minutes. At the end of the incubation period, 3 ml of a solution of 0.1% soybean trypsin inhibitor was added to the MCF10 A to neutralize the trypsin. Both cells types were centrifuged at 3000 rpm for 2 minutes at 4°C. Cells were washed with saline, re-suspended in MEM, and counted with a Beckman coulter counter in preparation for the chemical exposure studies.

Analysis of Whole Cell Clastogenicity

Both normal breast cells MCF-10A and breast cancer cells MCF-7 were exposed to environmentally relevant concentrations [EPA's maximum contaminant level (MCL)] of Atrazine, As, Cd and nitrate at 3 ppb, 10 ppb, 5 ppb and 10000 ppb respectively. The cell lines were also exposed to a mixture of the four chemicals at 10%, 100% and 1000% of their MCL concentrations. Seventy five pico-gram/ml of estrogen was used as a positive control for measuring estrogenic activity of the cell lines. After 96 h and one week exposure periods, nuclei were isolated from exposed and control cells and stained with propidium iodide (PI) using a hypotonic lysis solution (Ghosh et al., 2007). Briefly, cells were washed with sterile 1% PBS, followed by the addition of 1.5 ml of the PI solution (0.05mg/ml, 0.1 Triton X 100, 0.1 % sodium citrate, 7 unites /ml RNAse). Plates were refrigerated and tilted every 3-5 minutes to release the nuclei from the lysed cells. The samples were filtered through a 53-um mesh filter and kept on ice until analysis. The refrigeration time differed for each cell line. MCF-7 cells were refrigerated for one hour and MCF-10A cells were refrigerated for 6 hours as nuclei from MCF10A takes longer time to be collected than does MCF-7 cells. The isolated nuclei were then analyzed using a BD FACSCalibur equipped with 4-Color filters; 530 nm (FITC), 585 nm (PE/PI), 670nm (PerCP) and 661 nm (APC), (San Diego, LA, USA). The excitation wavelength (488 nm) was provided by a 5 W argon ion laser. Approximately 20,000 nuclei per sample were analyzed for each exposure concentration. The histograms obtained in each analysis were analyzed manually and the coefficient of variation (CV) of the G1 and G2 peaks were recorded. A representative DNA histogram is shown in Figure 1. An analysis of variance (ANOVA) was performed on the CVs of the various treatments. A least significant difference (LSD) analysis was performed on the mean CVs of the various treatments to obtain the statistical differences (Birandar and Rayburn, 1995b).

RESULTS

Flow karyotype Analysis

Chromosomes were isolated from actively growing cells for analysis of chromosomal aberrations by the method reported by Biradar and Rayburn (1995b). Cell lines were exposed to 3ppb atrazine, 5 ppb cadmium, 10ppb arsenic, 10, 000ppb nitrate and a mixture of

these chemicals at 10%,100%, 1000% MCL for 96 h and one week. As a positive control, cells were exposed to estrogen which is a mitogen in MCF 7 and MCF 10A cells. At the end of the exposure period, media was replaced with 10 ml complete media containing colcemid (1.0 ug/ml) and the flasks were incubated for 3-5 hours.

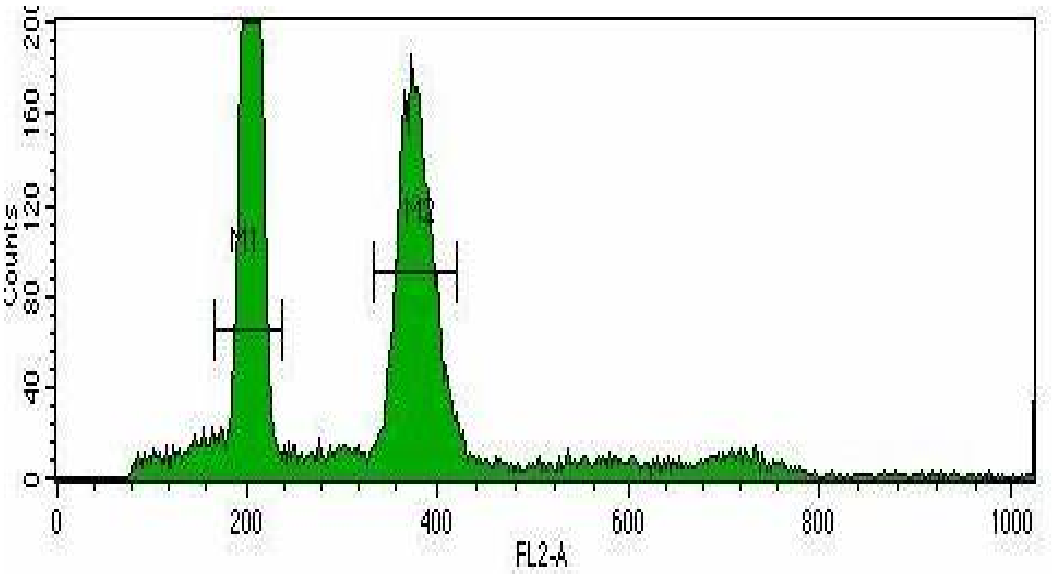

Fig. 1. Representative DNA histogram for nuclei of MCF-10A breast cells whole cell analysis

Table 1: Statistical analysis of whole cell Clastogenicity in MCF-7 and MCF-10A cells after 96 hours of exposure. Results are shown as Coefficient of variation (CV) and percent CV relative to control

Treatment	MCF-7		MCF-10A	
	Mean CV of G0/G1 peak*	% CV to Control	Mean CV of G0/G1 peak*	% CV to Control
Control	6.0 de	100	9.4 c	100
Estrogen	6.6 ab	110.5	9.4 c	100.5
Atrazine 3 ppb	6.6 abc	109.3	8.8 d	94.0
Arsenic 10 ppb	6.8 a	113.0	10.0a	106.8
Cadmium5 ppb	5.9 ef	97.5	9.9 a	105.8
Nitrate10000ppb	6.3 cd	104.2	10.1a	107.6
10% MCL mixture	5.6 f	92.6	9.3 c	99.1
100% MCL mixture	6.4 bc	107.2	8.9 d	94.8
1000% MCL mixture	6.4 c	105.99	8.8 d	93.6

*Means within a column lacking a common letter are significantly different (pÒ0.001)

Table 2: Statistical analysis of whole cell clastogenicity in MCF-7 and MCF-10A cells after one week of exposure. Results are shown as Coefficient of variation (CV) and percent CV relative to control

Treatment	MCF-7		MCF-10A	
	Mean CV of G0/G1 Phase*	% CV to Control	Mean CV of G0/G1 Phase*	% CV to Control
Control	4.6 ef	100	10.3 d	100.0
Estrogen	4.9 cd	106.4	10.4 cd	100.9
Atrazine (3 ppb)	5.1 abc	112.2	10.5 c	101.8
Arsenic (10 ppb)	4.5 f	99.4	10.7 a	104.1
Cadmium (5 ppb)	4.8 def	104.8	9.9 e	96.1
Nitrate(10000 ppb)	4.8 cde	106.1	10.5 bc	102.4
10% MCL mixture	4.9 bce	108.0	10.7 ab	104.0
100% MCL mixture	5.2 ab	113.1	10.6 ab	102.8
1000% MCL mixture	5.2 a	114.9	8.7 f	85.0

*Means within a column lacking a common letter are significantly difference (pÒ0.001)

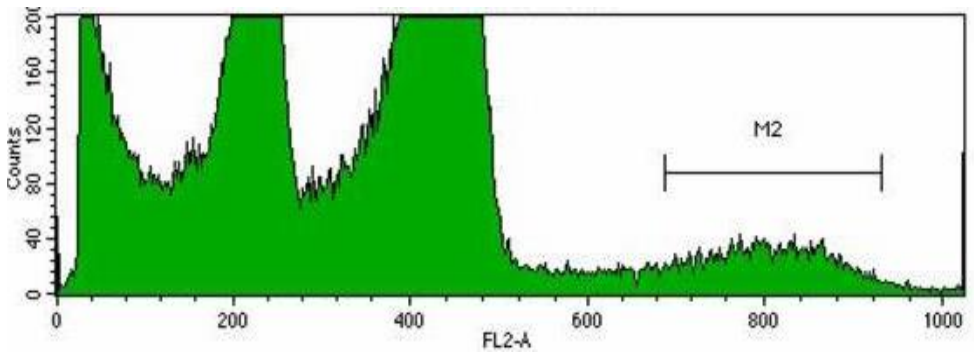

Fig. 2. Representative Flow karyotype histogram of MCF-10A breast cells

Table 3: Statistical analysis of chromosomal damage in MCF-7 and MCF-10A cells after 96 hr exposures. Results are presented as Coefficient of variation (CV) and percent CV relative to control

Treatment	MCF-7		MCF10A	
	Mean CV of the Largest Chromosome*	% CV to Control	Mean CV of the Largest Chromosome*	% CV to Control
CTR	12.1 c	100	6.7 f	100
EST	12.0 c	99.1	8.3 cde	123.3
ATZ	10.4 f	85.5	7.6 e	113.6
As	11.3 de	92.8	12.2a	181.8
Cd	12.8 ab	105.6	11.4b	169.3
NO3	12.3 bc	101.5	8.2 de	121.4
10% MCL mixture	10.7 e	88.4	8.8 cd	131.4
100% MCL mixture	11.5 d	95.0	11.5ab	171.5
1000% MCL mixture	13.4 a	110.2	9.0 c	133.8

*Means within a column lacking a common letter are significantly difference (pÒ0.001)

Cells were then collected, re-suspended in 0.5 ml propidium iodide staining solution (75 mM KCL and 50 ug/ml PI), and incubated for 10 minutes at 37 C. 0.25 ml of a staining/cell lysis solution (75 mM KCL, 50ug/ml PI, and 1% Triton) was added and chromosome separation was achieved by pushing samples through a 22 gauge needle. The chromosomes were analyzed with the FACSCalibur. Flow karyotype histograms were analyzed by taking the CV of the largest chromosome at a count of 100,000 chromosome/sample. The borders around the largest chromosome were set manually on the control histogram, and all treatments were compared to control sample manually. A representative histogram from the flow karyotype analysis is shown in Figure 2. Analysis of variance (ANOVA) was performed on the CVs of the various treatments. A least significant difference (LSD) analysis was performed on the mean CVs of the treatments.

Results

Whole Cell Clastogenicity

The result of cell cultures of MCF-7 and MCF-10A exposed to four chemicals (Atrazine, arsenic, cadmium and nitrate) individually at each of their EPA maximum contamination levels (MCLs) and to different concentrations of their mixture, for 96 hours and one week (168 hours) are shown in Tables 1 and 2, while Figures 3 and 4 show the whole cell clastogenicity analysis results for both breast cell lines exposed for 96 hours and one week, respectively.

Within the 96hours exposure period, estrogen, Atrazine, As, 10%, 100% and 1000% of the mixture

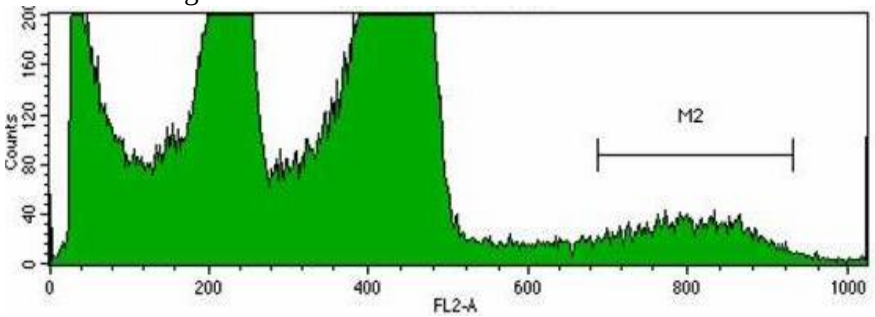

Fig. 2. Representative Flow karyotype histogram of MCF-10A breast cells

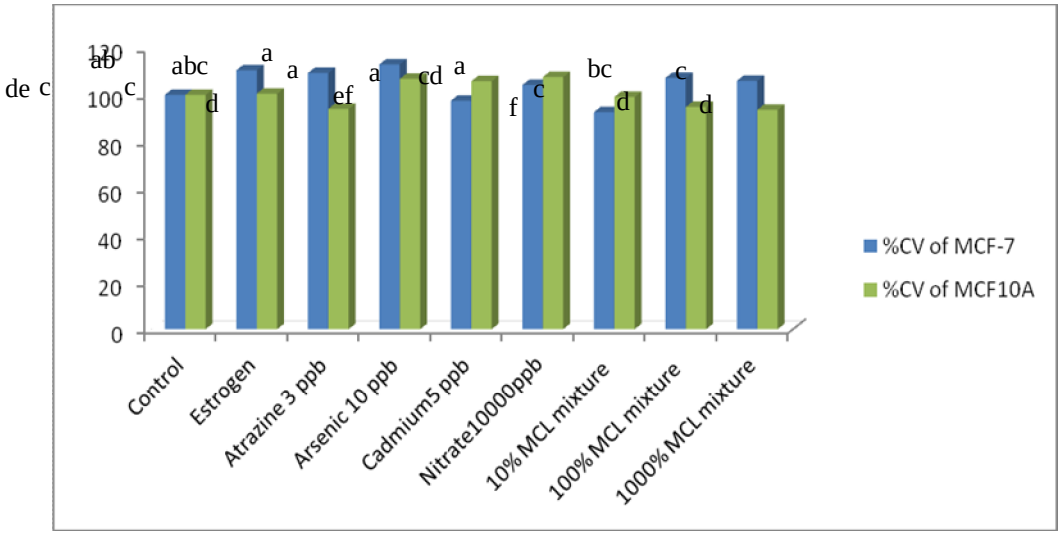

Fig. 3. Percentage CV of G0/G1 phase versus treatments for MCF-7 and MCF10A breast cell lines after 96 h exposure

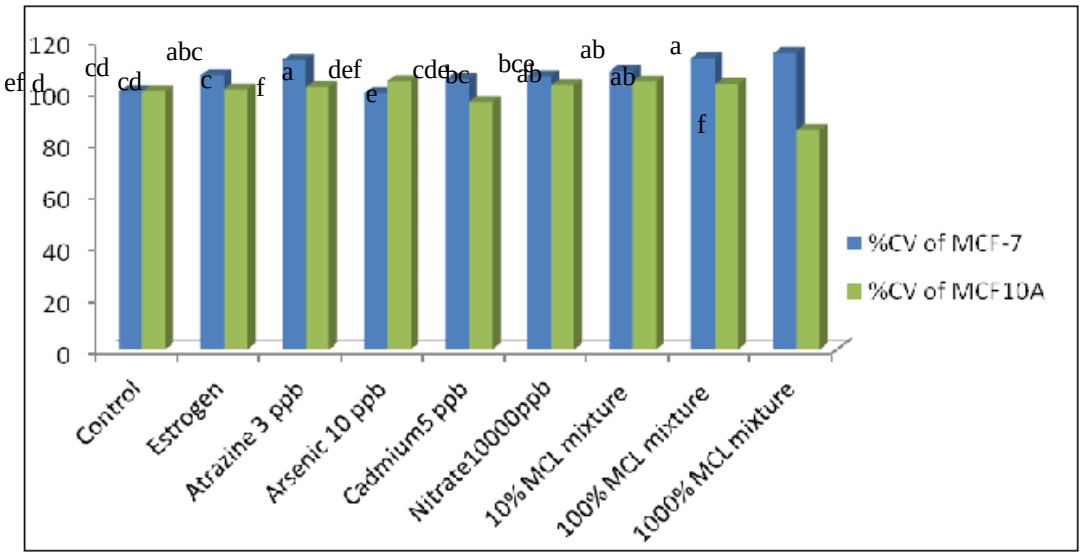

Fig. 4. Percentage CV of G0/G1 phase versus treatments for MCF-7 and MCF10A breast cell lines after one week of exposure

induced significant (pÒ0.001) whole cell clastogenicity in MCF-7 cell line while in MCF-10A cells, all treatments induced whole cell clastogenicity with the exception of estrogen and the 10% mixture. During the one week exposure duration, estrogen, Atrazine, 100% and 1000% mixture induced significant whole cell clastogenic effects in MCF-7 cells and in MCF-10A all but estrogen induced whole cell clastogenicity.

Tables 3 and 4 and Figures 5 and 6 show the results of the flow karyotype measurements of chromosomal damage observed in MCF-7 and MCF-10A cells exposed to chemicals for 96 h and one week, respectively. Both breast cells were exposed to Atrazine, arsenic, cadmium, nitrate at their maximum contamination levels and a quaternary mixture of these chemicals at 10 %, 100% and 1000% of the MCL for each chemical.

Flow karyotype Analysis

Table 3: Statistical analysis of chromosomal damage in MCF-7 and MCF-10A cells after 96 hr exposures. Results are presented as Coefficient of variation (CV) and percent CV relative to control

Treatment	MCF-7		MCF10A	
	Mean CV of the Largest Chromosome*	% CV to Control	Mean CV of the Largest Chromosome*	% CV to Control
CTR	12.1 c	100	6.7 f	100
EST	12.0 c	99.1	8.3 cde	123.3
ATZ	10.4 f	85.5	7.6 e	113.6
As	11.3 de	92.8	12.2a	181.8
Cd	12.8 ab	105.6	11.4b	169.3
NO3	12.3 bc	101.5	8.2 de	121.4
10% MCL mixture	10.7 e	88.4	8.8 cd	131.4
100% MCL mixture	11.5 d	95.0	11.5ab	171.5
1000% MCL mixture	13.4 a	110.2	9.0 c	133.8

*Means within a column lacking a common letter are significantly difference (pÒ0.001)

Table 4: Statistical analysis of chromosomal damage in MCF-7 and MCF-10A cells after one week of exposure. Results are presented as Coefficient of variation (CV) and percent CV relative to control

Treatment	MCF-7		MCF10A	
	Mean CV of the Largest Chromosome*	%CV to control	Mean CV of the Largest Chromosome*	%CV to control
CTR	8.7 e	100.0	6.6 d	100.0
EST	8.8 e	100.4	8.3 a	126.6
ATZ	10.2 c	116.7	7.5 bc	114.8
As	10.0 c	115.2	8.1 ab	122.5
Cd	10.8 a	123.4	7.2 c	108.8
NO3	10.2 c	117.0	7.3 c	111.2
10% MCL mixture	9.5 d	109.0	7.0 cd	106.8
100% MCL mixture	10.4 b	119.7	8.3 a	126.7
1000% MCL mixture	10.7 a	122.8	7.4 c	112.3

*Means within a column lacking a common letter are significantly difference (pÒ0.001)

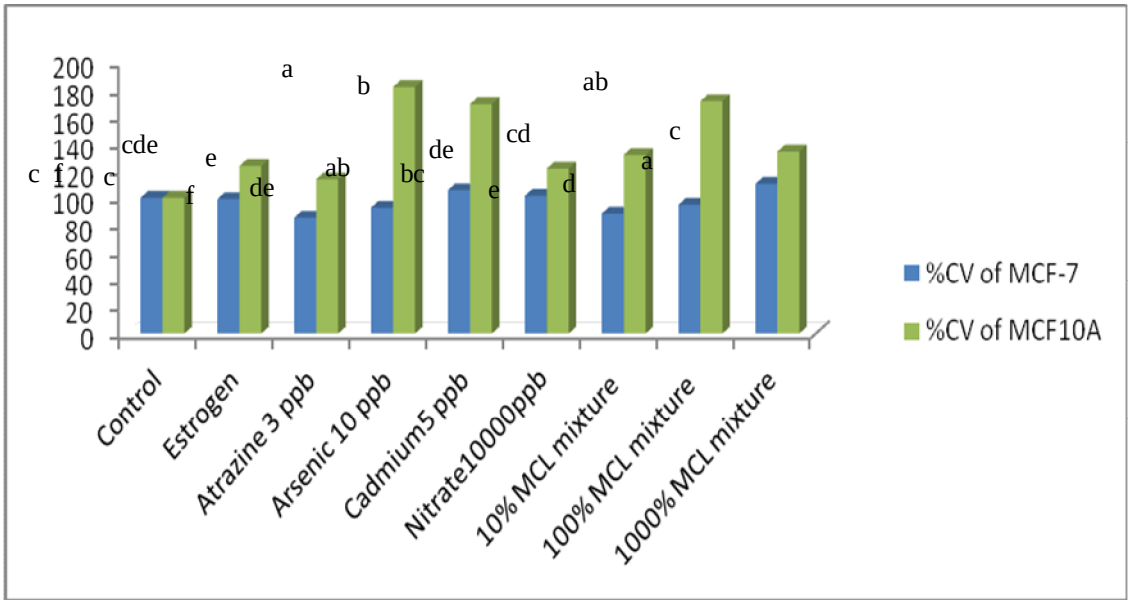

Fig. 5. Percentage CV of largest chromosome versus treatments for MCF-7 and MCF-10A breast cell lines after 96 h of exposure.

Flow karyotype damage observed was detected in all treated cells in the MCF-10A cells following 96 hours of exposure, including the estrogen positive control. Similar results were detected in MCF-7 cells except for the estrogen and nitrate treatments. The one week exposure regimen produced flow karyotype damage in all treatments with MCF-7 cells with the exception of estrogen, and all MCF10A exposures except for the 10% mixture induced flow karyotype damage.

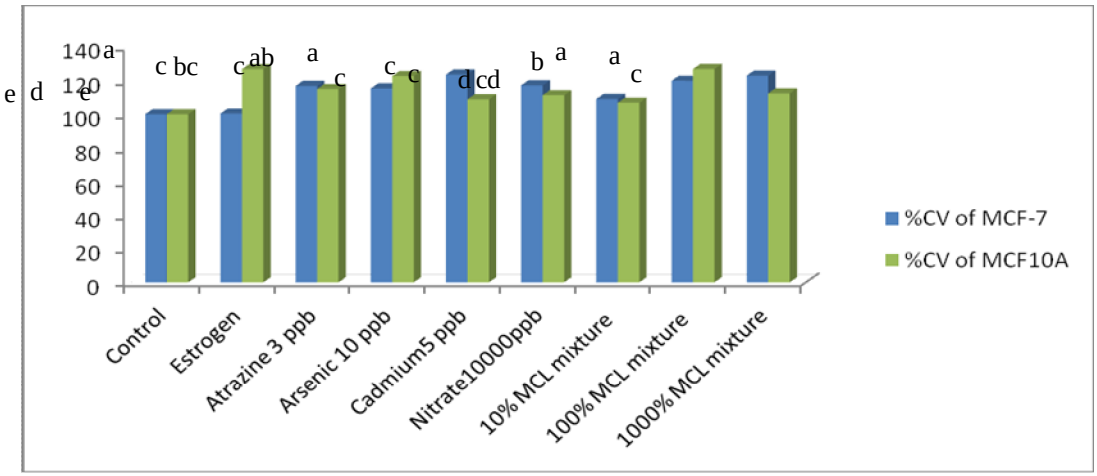

Fig.6. Percentage CV of largest chromosome versus treatments for MCF-7 and MCF-10A breast cell lines after one week of exposure

Discussion

Whole cell clastogenicity is a quick, reliable technique to efficiently screen for potential genomic damage in cells at interphase. Parameters identified that may be associated with changes in the coefficient of variation (CV) include chromosome structure changes, modifications in chromatin composition, apoptosis and cell cycle changes (Birandar and Rayburn, 1995a). While each of these mechanisms is different, they do indicate genome alterations that could have negative consequences. A study compared three genotoxicity assays (comet, whole cell clastogenicity, and forward mutation rate) using various known clastogenic agents (Birandar and Rayburn, 1995b); although the endpoints of each of these assays were different, comet and whole cell clastogenicity gave comparable results, thereby confirming that the whole cell clastogenicity method reflected DNA damage. Thus, whole cell clastogenicity experiments when properly designed and executed, are able to detect genotoxic effects of chemicals.

Results from this study indicated that the 100% and 1000% mixtures of the chemicals along with the individual metals (Az, As, Cd, and nitrate) at their MCLs caused whole cell clastogenicity in both cell lines after exposure for both 4 and 7 days. This results is in agreement with data previously presented by Biradar and Rayburn (1995a); these authors reported that cells exposed to Atrazine at the 3ppb had significantly higher CVs than control cells. In their work, they ruled out the possibility that the increase in CV could be due to the physical presence of Atrazine in the nuclei. Furthermore, studies by Gosh et al., (2007) have shown that both high and low levels (Arsenite) can result in cell death and the induction of micro nuclei (MN), however the kinetics of cell death and MN induction versus arsenite accumulation were different. Results in our study showed that arsenic causes whole cell clastogenicity in both cell lines after 96 hours of exposure but only in the MCF-10A cell line after 1 week of exposure. The mechanism behind this difference might be that MCF7 cells were able to repair the DNA damage during a longer exposure time but MCF-10A could not, which could be attributed to tumorigenic differences between cell lines. MCF-7 is a tumorigenic cell line and ER (+), while MCF-10A cells are non-tumorigenic and ER

The DNA damage caused by arsenic exposure at EPAôs MCL of 10 ppb could be due to an aneugenic effect on breast cells. It was found that arsenite treatment resulted in disturbance of mitosis, alteration of chromosome segregation, and chromosome loss in human skin fibroblasts (HFW) cells at low dose levels. A proposed mechanism for the cryptogenic toxicity of arsenic is the inhibition of various enzymes involved in DNA repair and possibly by induction of reactive oxygen species (ROS) capable of causing DNA damage or the induction of genes that result in altered DNA repair. It has been reported that the total number of chromosomal alterations was higher in a cohort of individuals exposed to arsenic in their drinking water (Taets and Rayburn, 1998). This further suggests that at the population level, an increased frequency of chromosomal aberrations in peripheral lymphocytes is predictive of increased risk of cancer. In arsenic exposed population, chromosome type aberration (CSAs) is found to be significantly higher in cases compared to control group while chromatid type aberration (CTAs) is similar in both groups. These

findings indicate that it is the contribution of the CSA type aberrations that is responsible for the observed difference in the overall increased CA frequency in the case group compared to the control group. It is known that, CSA type damage has an important function in activation of proto-oncogenes and their interaction plays a direct role in the formation of malignancies (Schoen, A., et al., 2004). Other chromosomal aberrations such as dicentrics normally observed with radiation have also been noted after As exposure (Yu and Chai, 2006). Nagymajtenyi et al (1985) observed a significant increase in CA in the fetal chromosomes of mice after inhalation exposure of pregnant females to 28.3 mg/m^3 of As_2O_3 for 4 h per day on the 9th, 10th, 11th and 12th days of gestation. Another study has reported structural chromosome aberrations in bone-marrow cells of male Sprague-Dawley rats after injection intra-peritoneal, once a day for 5 days with doses of 5, 10, 15 and 20 mg/kg body weight (BW) of arsenic trioxide dissolved in distilled water. In this study, a significantly higher incidence of aberrant cells with a mean of 15.6% versus 2.4% in control was reported. Micronuclei induction in rat bone-marrow cells exposed to As_2O_3 has also been reported and most studies of micronuclei induction by As exposure were found to be dose dependent Patlolla, and Tchounwou, 2005).

Nitrate and Cd exposure induced whole cell clastogenicity in this study in MCF-10A cells but not in MCF-7 cells. Again, differences could be attributable to differences in cell type characteristics with respect to tumorigenicity and the presence or absence of the estrogen receptor (ER). Nitrate has been showed to have genotoxic potential. In a study involving nitrate in the drinking water, 70 children of 12-15 years old were exposed to a range of concentrations of nitrate. Results of this study showed a significant increase in the mean number of chromosome breaks in the children exposed to nitrate concentrations that exceeded 70.5 mg/l, but there was no significant increase in the mean number of sister chromatid exchanges per cell (Tsezou, 1996). This result is consistent with our study with MCF-10A cells, which showed a 20% increase in chromosomal aberration than control after 96 h exposure to 10mg/l. The CV of largest chromosome was increased in MCF-7 as well as MCF-10A after one week of exposure.

Cadmium effects on genome stability appear to be indirect, mainly via an increase in oxidative stress which can lead to DNA damage (Nagymajtenyi et al., 1985). Cd-induced oxidative stress might be due to decreases in cellular antioxidants, oxidative active metallothionen and/or enzymes that mitigate reactive oxygen species (ROS) (Newman and Unger, 2003). It has been reported that Cd-induced oxidative stress causes the production of typical oxidative generated mutagenic lesions in DNA such as 8-oxo-7, 8- dihydro-2-deoxyguanosine (8-oxodG) adducts in human lymphoblastoid cells after exposure to 5-35 uM of cadmium (Mikhailova et al., 1997). Hence the genome instability found in our study after Cd exposure might be due to the creation of ROS. A number of in vitro studies using different types of mammalian cells have demonstrated that Cd induces various genotoxic effects. In a study designed to assess genotoxic damage in somatic cells of workers in a Polish battery plant after high-level occupational exposure to cadmium (Cd).A Cd-exposed group showed a significant increase in MN frequency a consequence of chromosomal aberrations and spindle disturbances, SCE frequency and percentage of cells with damaged DNA (Rozgaj and Fucic, 2002).

Interactive effects between chemicals were also observed in this study, although differences between metals alone and the 100% mixture exposure were not large. It is well known that the protein metallothionen plays a very important role in cadmium toxicity (Mikhailova et al., 1997), and it has been shown that Cd at non-cytotoxic concentrations in combination with other DNA damaging agents such as UV light, gamma-radiation or alkylating chemicals like methyl methanesulfonate, N-methyl-N-nitrosourea, N-nitro-N-nitrosoguanidine showed enhanced genotoxic effects. A similar effect was detected in MCF-7 cells. Cadmium exposure at its MCL concentration of 5 ppb did not cause significant DNA damage in MCF-7, but in a mixture of the chemicals all present at 10 %, 100% and 1000% of their MCL concentrations, whole cell clastogenicity was significantly increased above controls after 96 h and one week.

The 10% mixture which contained Atrazine (3ppb), As (10 ppb), Cd (5 ppb) and nitrate (10,000 ppb) induced whole cell clastogenicity in MCF-7 cells during 96 hours of exposure but no significant DNA damage was detected in cells exposed for one week. The explanation might again be that during the 96 hours of exposure the cells did not have sufficient time to repair the damage caused by the chemicals, but were able to do so during one week exposure. On the contrary, the 10% mixture exposure induced whole cell clastogenicity in MCF-10A cells during one week exposure but not during 96 hours exposure. This suggests that MCF 10A cell defense mechanisms were saturated during the longer exposure time. This consistent difference in results obtained with the MCF-7 versus MCF-10A cells again

indicates important differences in sensitivity to genotoxic chemicals in tumorigenic versus non-tumorigenic cell lines.

Differences were observed between the two measures of DNA damage used in this study. With the exception of nitrate exposure of MCF-7 cells for 96 hours and the10% mixture exposure of MCF-10A for one week, all the exposure regimes caused flow karyotype damage. Estrogen caused whole cell damage but not flow karyotype damage in MCF-7 cells. On the contrary estrogen caused flow karyotype damage and not whole cell damage in MCF-10A cells. These observed differences between estrogen exposure and clastogenicity might be due to differences in the generation of reactive oxygen species (ROS) between these cell lines. ROS generation is known to cause DNA damage (Felty, 2006). It was reported that physiological concentrations of estrogen (367 fmol and 3.67 pmol) triggered a rapid 2-fold increase in intracellular oxidants in endothelial cells. It has also been reported that oxidative stress affects the expression of estrogen receptors alpha and beta (ERalpha and ERbeta) differently, demonstrating cell-specific response (Tamur et al., 2002). Hence the existence of estrogen receptors which can be regulated by ROS in MCF-7 cells and the lack thereof in MCF-10A cells might lead to differences in clastogenicity between these cell lines during estrogen exposure. Taets et al., (1999) reported that simazine (a pesticide similar to Atrazine) caused whole cell clastogenesis in Chinese hamster ovary cells but not flow karyotype damage. Cd caused flow karyotype damage but not whole cell damage in MCF-7 but not in MCF 10A, these differences are consistent with other evidence that the ER and oxidative stress may be involved in Cd and atrazineôs mechanism of DNA damage.

Conclusion

The 100 and 1000% quaternary mixtures were observed to induce both whole cell clastogenicity and flow karyotype damage, which suggests that MCLs set for these chemicals may not be protective when they are present together in a mixture. Even the 10% mixture exposure to MCF-7 cells caused both whole cell clastogenicity and flow karyotype damage during 96 hours exposure, although it caused only flow karyotype damage during one week of exposure. Similarly, the 10% mixture exposure to MCF-10 cells caused flow karyotype damage and not whole cell clastogenicity during 96 hours exposure and caused only whole cell clastogenicity during one week exposure. Studies of the mechanisms involved in the DNA damage and repair in these two breast cell lines that differ in their tumorigenicity and ER expression will provide a better understanding of the role of chemical genotoxicity in the development of breast cancer.

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