Chapter-5

Discussion
5.0 DISCUSSION

Pesticides are high-volume, widely used, environmental chemicals and there is continuous debate concerning their possible role in many chronic human health effects. Chronic effects thought to involve pesticides include carcinogenesis, neurotoxicity, and reproductive and development effects. Occupational exposure occurring at all stages of pesticide formulation, manufacture and application involves exposure to complex mixtures of different types of chemicals, active ingredients and by-products present in technical formulations such as impurities, solvents and other compounds produced during the storage procedure. Epidemiological data on cancer risk in farmers are conflicting. Meta-analyses showed that farmers were at risk for specific tumors including leukaemia (Daniels et al., 1997; Zahm et al., 1998) and multiple myeloma (Khuder et al., 1997).

In our study, we made an attempt to elucidate the antigenotoxic effect of curcumin and carvacrol against the genotoxic damage induced by malathion and parathion using SCE and comet assay as a biomarkers of genotoxicity. We used sister chromatid exchange (SCE) and comet assay to evaluate the genotoxicity of malathion and parathion as well as to elucidate the antigenotoxicity of curcumin and carvacrol. As biomarker of susceptibility, we studied the effect of GSTTI and GSTMI (glutathione S-transferase) polymorphism on genotoxicity of malathion and parathion as well as on the antigenotoxic effect of curcumin and carvacrol.

5.1. Genotoxicity of pesticides (malathion and parathion)

In order to elucidate the antigenotoxic potential of curcumin and carvacrol, we first observed the genotoxic potential of malathion and parathion using SCE and comet assay as a biomarkers of genotoxicity. SCE is a more sensitive indicator of genotoxic effects (Neuss and Speit, 2008). Similarly single cell gel electrophoresis (SCGE) technique, also known as the comet assay, is a sensitive, simple and rapid technique for detecting DNA single and double strand breaks, alkali labile sites, incomplete excision repair sites, and genomic structural discontinuities (Singh et al., 1988).
We observed that the peripheral blood lymphocytes (PBL) exposed to 10-30µg/ml of malathion and 0.5-2.5µg/ml of parathion had shown dose dependent increase in frequency of SCEs as well as in tail moment (TM). Malathion and parathion both induced maximum significant DNA damage to PBL at the concentrations of 30 and 2.5µg/ml respectively. While their higher concentrations were lethal to PBL. Pesticides are well reported in literature for their genotoxic nature. In our study, malathion and parathion were found to be genotoxic to PBL in both the assays i.e. SCE and comet. Similar to our findings, several reports are available in literature supporting the genotoxicity of malathion and parathion and other pesticides using these assays. Rupa et al. (1990) reported that methyl parathion was genotoxic to PBL. They evaluated the cytogenetic effect of four different concentrations of quinalphos and methyl parathion on human PBL over different time periods, by using chromosomal aberrations (CA) and SCE assays. A significant increase in CA was observed when cells were treated with quinalphos for 48 and 72 hours (h) and a significant increase in SCE was observed at all the concentrations and over all the time periods. Methyl-parathion did not induce CA but it significantly induced SCE over all time periods.

Balaji and Sasikala (1993) had found malathion to be genotoxic to cultured human PBL. They treated the leukocytes with four different concentrations of malathion (0.02, 0.2, 2 and 20µg/ml), added to the culture medium at 0, 24 and 48 h after culture initiation. They observed the dose-dependent increase in the frequency of CA as well as SCE as the concentration of malathion increases from 0.02 to 20µg/ml. There was a significant decrease in mitotic index at all concentrations of malathion.

In order to elucidate the genotoxic potency of a compound present in commercial preparations of malathion, the DNA damaging effect of the insecticide and its isomer isomalathion was investigated using alkaline single cell gel electrophoresis (Blasiak et al., 1999). They incubated freshly isolated human PBL with 25, 75 and 200 mM of the chemicals for 1 h at 37°C. Malaoxon and isomalathion introduced damage to DNA in a dose-dependent manner. The effect induced by malaoxon was more pronounced than that caused by isomalathion. Treated cells were able to recover within 60 minutes incubation in insecticide-free medium at 37°C except the PBL exposed to malaoxon at 200mM, which did not show measurable DNA repair. The latter result suggests a considerable
cytotoxic effect (cell death) of malaoxon at the highest concentration used. The reported genotoxicity of malathion might, therefore, be consequence of its metabolic biotransformation to malaoxon or the presence of malaoxon and/or isomalathion as well as other unspecified impurities in commercial formulations of malathion. This clearly indicates that malathion used as commercial product, i.e., containing malaoxon and isomalathion, can be considered as a genotoxic substance in vitro.

Blasiak and Kowalik (1999) demonstrated the genotoxic effect of the commonly used organophosphorus insecticide (Parathion-methyl and its immediate metabolite paraoxon-methyl) in human PBL using the comet assay. They treated the human PBL with 25, 75 and 200 µM of both the chemicals and observed the tail moment trend. Parathion-methyl at 25 and 75µM did not cause any significant changes but at 200µM, a significant increase in the tail moment was observed as compared with the control. Paraoxon-methyl at 25, 75 and 200µM evoked dose-dependent DNA damage measured as a significant increase in comet tail moment of PBL. The change evoked by paraoxon-methyl at 200µM was much more pronounced than that by parathion-methyl at the same concentration. The results indicated that the reported genotoxic effects of parathion-methyl could be mainly attributed to its metabolite paraoxon-methyl.

Metabolite of malathion i.e. malaoxon was reported to be genotoxic by Blasiak and Stankowska (2001). They investigated the action of malaoxon on DNA in PBL pretreated with a potent antioxidant, α-tocopherol (vitamin E), using the comet assay. Human PBL were treated with malaoxon at 25, 75, or 200 µM and DNA damage was measured. They reported the dose dependent increase in the comet tail moment of the PBL as the concentration of malaoxon increases. Addition of α-tocopherol to a final concentration of 20 and 80 µM reduced a dose dependent DNA damaging effect of a 1 h incubation of human PBL at 37°C with malaoxon at 25, 75, or 200 mM, measured as the decrease in the comet tail moment of the PBL.

Malathion was also reported to be genotoxic under in vivo conditions by Giri et al. (2002). They demonstrated the genotoxic effect of malathion using CA, SCE and sperm abnormality assays in mice. They tested the three acute doses (2.5, 5 and 10 mg/kg) of malathion and reported the significant dose-dependent increase in the
frequency of chromosome aberrations and sperm abnormalities, but did not observe any effect on total sperm count. The highest acute dose i.e. 10 mg/kg induced a >12-fold increase in the frequency of chromosome aberrations, two-fold increase in the frequency of SCE and four-fold increase in the frequency of sperms with abnormal head morphology following intraperitoneal (i.p.) exposure. Further, a significant increase in the frequency of SCE was observed, but the increase was not dose-dependent.

Undeger and Basaran (2005) evaluated the genotoxic potential of commonly used pesticides (i.e., dimethoate and methyl parathion from the organophosphate class, propoxur and pirimicarb from carbamates, and cypermethrin and permethrin from pyrethroids) by using the single cell gel electrophoresis or comet assay in freshly isolated human PBL. The cells were incubated with 10, 50, 100 and 200 µg/ml concentrations of the test substances for 0.5 h at 37ºC and DNA damage was compared with that obtained in PBL from the same donor not treated with substances. Dimethoate and methyl parathion at 100 and 200 µg/ml; propoxur at 50, 100 and 200 µg/ml, and pirimicarb, cypermethrin and permethrin at 200 µg/ml significantly increased the DNA damage (measured as Tail length, Tail intensity and Tail moment) in human PBL.

Georgia et al. (2007) assessed the genotoxicity of imidacloprid and metalaxyl (insecticides) in cultured human PBL using MN and SCE as a biomarkers of genotoxicity. They treated the human PBL with imidacloprid and metalaxyl at final concentrations of 0.1, 1, 5, 10, 50 and 100µg/ml in separate treatments and of 10, 25, 50, 100 and 200µg/ml in mixture treatments. The results of the SCE analysis showed that SCE frequencies after treatment with imidacloprid did not differ significantly from those in the controls. A statistically significant increase ($p < 0.05$) in SCE frequency resulted from treatments with metalaxyl at 5, 10 and 100µg/ml and with the combination of imidacloprid and metalaxyl at 100 and 200µg/ml.

Malathion had shown genotoxic effect in experiment performed by Pamela et al. (2010). They examined the role of oxidative stress in malathion-induced cytotoxicity and genotoxicity. To achieve this goal, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], lipid peroxidation, and single cell gel electrophoresis (Comet) assays were performed, respectively, to evaluate the levels of cell viability,
malondialdehyde (MDA) production, and DNA damage in human liver carcinoma (HepG2) cells. HepG2 cells were treated with malathion (6, 12, 18, and 24 mM) and comet assay was performed. Study results indicated that malathion is mitogenic at lower levels of exposure, and cytotoxic at higher levels of exposure. Upon 48 h of exposure with 24 mM malathion, the comet assay showed a significant increase in percentage of DNA damage and comet tail length.

Chlorpyrifos (an organophosphate pesticide) was found to be genotoxic to human PBL by Khanna et al. (2011) using CA and comet assay as a biomarker of DNA damage. They treated the human lymphocyte cultures with 75μg/ml chlorpyrifos and observed the DNA damage. The results showed that PBL treated with the pesticide exhibited increased DNA damage but the increase was statistically insignificant (P>0.05). However a significant (P<0.05) increase in CA was observed in cultures treated with 75μg/ml chlorpyrifos as compared to controls.

Xian et al. (2012) evaluated the cytotoxic and genotoxic effect of five organophosphates (OPs) or metabolites, Acephate (ACE), Methamidophos (MET), Chloramidophos (CHL), Malathion (MAT) and Malaoxon (MAO), and clarified the role of oxidative stress, using PC12 cells (Cell line derived from rat adrenal medulla). DNA damage studies were carried out using comet assay and measured as an increase in tail moments. Their results demonstrated that MET, MAT and MAO caused significant inhibition of cell viability and increased DNA damage in PC12 cells at 40mg/l.

Role of oxidative stress in methyl parathion and parathion induced toxicity was investigated by Falcicia et al. (2013) in HepG2 cells. To achieve this goal, they performed the MTT assay for cell viability, lipid peroxidation assay for MDA production, and comet assay for DNA damage, respectively. Results from MTT assay indicated that methyl parathion and parathion gradually reduces the viability of HepG2 cells in a dose-dependent manner, showing 48 hours LD₅₀ values of 26.20 mM and 23.58 mM, respectively. Lipid peroxidation assay resulted in a significant increase (p<0.05) of MDA level in methyl parathion and parathion treated HepG2 cells compared to controls, suggesting that oxidative stress plays a key role in organophosphate insecticides (OPI) induced toxicity. Comet assay results indicated a significant increase in genotoxicity (as
evidenced from increase in the percentage of DNA damage and the length of comet tail) at higher concentrations of OPI exposure. Overall, the results indicated that methylparathion is slightly less toxic than parathion to HepG2 cells.

Rozenn et al. (2014) studied the cytotoxicity and genotoxicity of malathion and isomalathion in human HepaRG (human cholangiohepatocarcinoma) cells. They observed that isomalathion reduced cell viability starting at a 100μM concentration after a 24 h exposure. It also significantly induced caspase-3 activity in a dose-dependent manner starting at 5 μM. Malathion and isomalathion either separately or in combination, slightly induced MN formation at low concentrations or had additive genotoxic effect when combined at 25μM. Individually or combined isomalathion directly inhibited activity of carboxyesterases which are involved in detoxication of malathion. Hence their findings suggest that isomalathion was much more cytotoxic than malathion while both compounds had comparable genotoxic effect in HepaRG hepatocytes at low concentrations.

Hence above discussed studies supports the genotoxic nature of various pesticides like malathion and parathion by employing various assays such as SCE, comet assay, MN etc. which is in accordance with our findings.

5.2. Antigenotoxicity of curcumin and carvacrol against malathion and parathion induced genotoxicity

Curcumin, the active principle of turmeric is well-known for its antioxidant property. The antioxidant mechanism of curcumin is due to its conjugated structure, having two methoxylated phenols (Masuda et al., 2001). Its free radical scavenging antioxidant property helps it in reducing genotoxic damage. Carvacrol is a predominant aromatic compound in oil of oregano. It reduces the DNA damage by scavenging free radicals generated by xenobiotic compounds, supported its antioxidant property because of its phenolic groups (Ozkan and Erdogan, 2011). We observed that 25 and 50μg/ml of curcumin had significantly reduced the genotoxic damage caused by malathion while in case of parathion 10 and 15μg/ml had shown antigenotoxic effect. We also observed that carvacrol had antigenotoxic effect against malathion and parathion at the concentrations of 2.5 and 5.0 μg/ml, supporting its antigenotoxic activities. Antimutagenic activity of carvacrol against malathion might be due to change in membrane lipids and permeability.
of ion channels (Ultee et al., 2002) and thus inhibiting the uptake of malathion into the cells. SCE induced by Mitomycin are greatly reduced by carvacrol supplementation to cultured PBL suggested its antigenotoxic effect (Ipek et al., 2003).

Antigenotoxic effect of curcumin have been reported in many investigations which supports our findings. Curcumin has been shown to inhibit lipid peroxidation using linoleate, a polyunsaturated fatty acid that is able to be oxidized and form a fatty acid radical. It has been demonstrated that curcumin acts as a chain-breaking antioxidant at the 3’ position, resulting in an intramolecular Diels-Alder reaction and neutralization of the lipid radicals (Masuda et al., 2001).

Joe et al. (2004) demonstrated the free radical-scavenging activity of curcumin. It has been shown to scavenge various reactive oxygen species produced by macrophages (including superoxide anions, hydrogen peroxide and nitrite radicals) both in vitro as well as in vivo using rat peritoneal macrophages as a model. Oxidative stimulation of G-proteins in human brain membranes by metabolic prooxidants, homocysteine, and hydrogen peroxide was shown to be significantly depressed by the Curcumin (Jefremov et al., 2007). Cyclophosphamide-induced lung injury caused by antioxidative defense mechanisms was increased by administration of curcumin. Later also inhibited lipid peroxidation in liver microsomes as well as in brain homogenates of laboratory rats (Hatcher et al., 2008). Curcumin is reported to reduce the clastogenic effects of gamma-radiation in human PBL culture under in vitro conditions. Exposure of PBL pretreated with curcumin at 1 and 2 Gy (SI unit of absorbed dose of radiations) resulted in decrease in SCE as compared to untreated PBL (AlSuhaibani and Entissar, 2009).

Chlormadinone acetate (CMA) is a synthetic progestin. The prolonged use has been reported to be carcinogenic in various experimental animal models. Siddique et al. (2010) studied the effect of CMA along with curcumin at various doses in cultured human PBL using cell viability, lipid peroxidation and DNA damage assay as a parameters. The treatment of curcumin results in a significant reduction of the toxic effects induced by CMA alone. The treatment of 30μM of CMA along with 5, 10 and 15μM of curcumin results in a dose dependent significant increase in cell viability i.e. 74% and 79% respectively. Similarly, the treatments of 30 and 40μM of CMA were
associated with 53% and 62% of DNA damage respectively while the treatment of 30μM of CMA along with 5, 10 and 15μM of curcumin resulted in a significant decrease in DNA damage i.e. 42%, 35% and 30% respectively.

Siddique et al. (2010) also suggested the protective role of curcumin against the genotoxic damage caused by tinidazole (A drug used for treatment of trichomoniasis, giardiasis and amebiasis) in cultured human PBL. Curcumin at the doses of 5, 10 and 15μM had shown the dose dependent decrease in SCE/cell against 10 μg/ml concentration of tinidazole. Tiwari and Rao (2010) evaluated the role of curcumin as a potential natural antioxidant to mitigate the genotoxic effects of arsenic (As) and fluoride (F) in human PBL. The study was divided into nine groups consisting of negative control, positive control treated with ethyl methane sulphonate (EMS; 1.93mM) and curcumin control with only curcumin (1.7μM) in blood culture. As (1.4μM) and F (34μM) were added alone as well as in combination, to the cultures, with and without curcumin. Cultures were analyzed for CA (both structural and numerical) and primary DNA damage via comet assay as the genotoxic parameters after an exposure duration of 24 hours. Results revealed that curcumin efficiently ameliorates the toxic effect of As and F by reducing the frequency of structural aberrations (>60%), hypoploidy (>50%) and primary DNA damage.

Mahmaud et al. (2010) reported the protective role of curcumin against oxidative stress and cytotoxic damage caused by lead exposure. Comet assay was performed to assess the DNA damage induced by lead acetate and curcumin along with other assays to assess oxidative stress effects. The results of the study showed that curcumin had antioxidant effect and reduced the DNA damage in lead exposed rats. So the study concluded that curcumin had protective effect against oxidative stress and cytotoxic damage caused by lead exposure. In our study, carvacrol had shown protective effect against malathion and parathion which is supported by a study conducted by Balakrishnan et al. (2010). They observed the protective effect of carvacrol against oxidative stress and DNA damage by U.V. radiations in cultured PBL. They used the series of in vitro assays (hydroxyl radical, superoxide, nitric oxide, DPPH (2, 2-Diphenyl-1-picryl hydrazyl), ABTS (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay and comet assay to demonstrate the antioxidant property.
of carvacrol. UVB exposure significantly increased thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LHPs), % tail DNA and tail moment; decreased % cell viability and antioxidant status in UVB-irradiated PBL. Treatment with carvacrol 30min prior to UVB-exposure resulted in a significant decline of TBARS, LHP, % tail DNA and tail moment and increased % cell viability as carvacrol concentration was increased. On the basis of their results, they concluded that carvacrol had protective effect against UVB radiations.

Ahmed et al. (2011) examined the role of oxidative stress in genotoxicity following pesticide exposure using peripheral blood mononuclear cells (PBMC) under in vitro conditions. Further possible attenuation of genotoxicity was studied using N-acetylcysteine (NAC) and curcumin as known modulators of oxidative stress. Cultured mononuclear cells was isolated from peripheral blood of healthy volunteers, and exposed to varying concentrations of different pesticides: endosulfan, malathion, and phosphamidon for 6, 12, and 24 h. Lipid peroxidation was assessed by cellular MDA level and DNA damage was quantified by measuring 8-hydroxy-2'-deoxyguanosine (8-OH-dG) using ELISA. Both MDA and 8-OH-dG were significantly increased in a dose-dependent manner following treatment with these pesticides. There was a significant decrease in MDA and 8-OH-dG levels in PBMC when co-treated with NAC or/and curcumin as compared to pesticide alone. These results indicate that pesticide-induced oxidative stress is probably responsible for the DNA damage, and NAC or curcumin attenuate this effect by counteracting the oxidative stress.

Ozkan and Erdogan (2012) studied the antioxidant/prooxidant effects of carvacrol and thymol at various concentrations on membrane and DNA of parental and drug resistant H1299 cells. They reported that carvacrol and thymol protect the cells against H$_2$O$_2$-induced cytotoxicity, and membrane and DNA damage when the cells were preincubated with these two compounds at lower concentration (< IC50) before H$_2$O$_2$ incubation. Hashemipour et al. (2013) studied the effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. This trial was conducted to evaluate the effects of dietary supplementation of phytogenic product containing an equal mixture of thymol and carvacrol at 4 levels (0, 60, 100, and 200
mg/kg of diet) on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. The phytogenic product linearly increased (P < 0.05) superoxide dismutase and GSH peroxidase activities and decreased (P < 0.05) MDA level in thigh muscle at day 42 and serum and liver at day 24 and 42. Thus, feed supplementation with thymol along with carvacrol enhanced performance, increased antioxidant enzyme activities, retarded lipid oxidation, enhanced digestive enzyme activities, and improved immune response of broilers.

5.2.1. Combinatorial antigenotoxic effect of curcumin and carvacrol against malathion and parathion

Curcumin is a multi-target natural chemopreventive compound by itself. In addition to its high chemopreventive potential, this natural compound can act synergistically with other natural compounds or other kinds of therapy (radiotherapy, chemotherapy and hormonotherapy). Both positive and negative interactions of curcumin with other phytochemicals such as genistein (Verma, et al., 1997), epigallocatechin-3 gallate (Khafif et al., 1998), omega-3 fatty acids (Swamy et al., 2008), resveratrol (Majumdar, et al., 2009), and piperine (Kakarala et al., 2009) have been reported in different cell lines. In our study, PBL treated with combination of curcumin and carvacrol had shown small increase or decrease in SCE frequency and TM value as compared to malathion and parathion treated PBL. However, this small increase or decrease in SCE frequency and TM value was not found to be significant (P>0.05) as compared to their separate treatments.

5.3. Effect of GSTT1 and GSTM1 polymorphism on genotoxicity of malathion and parathion.

Individuals have different responses to environmental chemicals due to their different genotypes. In present study, we studied the effect of GSTT1 and GSTM1 polymorphism on genotoxicity of malathion/parathion and the antigenotoxic effect of curcumin and carvacrol. We found no significant effect of GSTM1 and GSTT1 polymorphism on malathion/parathion induced genotoxicity and antigenotoxicity of curcumin and carvacrol under in vitro conditions. The few studies available on genetic polymorphism and the rate of cytogenetic alterations in humans in vivo have almost exclusively been performed using PBL. Thus far, the studies have addressed induced or
baseline cytogenetic damage in association with polymorphisms of \textit{GSTMI} and \textit{GSTTI}. Positive effects on indicator genotype interaction are reported for cytogenetic biomarkers, such as SCE, CA or MN, although the large majority of studies in the scientific literature failed to reveal any clear indication.

Regarding the effect of \textit{GSTM1} and \textit{GSTT1} polymorphism on pesticides induced genotoxicity, few \textit{in vivo} studies are available which supports our findings. Falck \textit{et al.} (1999) did not find any genotypic effect exclusively in the pesticide-exposed subjects. The \textit{GSTM1} positive genotype was associated with an increased MN frequency irrespective of exposure. Similarly in another study, no significant association between \textit{GSTM1} and \textit{GSTT1} genotypes on the MN frequency was found in a group of Spanish greenhouse workers exposed to pesticides (Lucero \textit{et al.}, 2000).

However, contrary to our study, few workers have found positive interactions of \textit{GSTM1} and \textit{GSTT1} genotypes with biomarkers of genotoxicity in pesticides exposed populations. Carla \textit{et al.} (2006) studied the cytogenetic damage in PBL from 33 farmers of Oporto district (Portugal) exposed to pesticides by means of MN, SCE and CA. In addition, effect of polymorphic genes of xenobiotic metabolizing enzymes (\textit{GSTM1}, \textit{GSTT1}, \textit{GSTP1}, \textit{CYP2E1} and \textit{EPHX1}) was also evaluated. A significant increase in SCE frequency was observed in \textit{GSTT1} positive individuals of control group (P < 0.05) while no association was found between \textit{GSTM1}, \textit{GSTP1} or \textit{CYP2E1} and cytogenetic damage.

Sharma \textit{et al.} (2010) evaluated the genotoxic effects of pesticide in association with \textit{GST} polymorphism. They studied the DNA damage and \textit{GSTM1} and \textit{GSTT1} genotypes from PBL of pesticide-exposed and unexposed (control) agricultural workers of the Punjab region (India). DNA damage was evaluated by using an alkaline comet assay. They found that all the pesticide-exposed subjects have higher Damage index (DI), Damage frequency (DF) and \% DNA in tail in comparison to the controls. In addition, the \textit{GSTT1} gene deletion and simultaneous deletions of \textit{GSTM1} and \textit{GSTT1} genes resulted in increased DNA damage in the exposed group.

To the best of our knowledge, there are no \textit{in vitro} studies available regarding effect of \textit{GSTM1} and \textit{GSTT1} polymorphism on pesticide induced genotoxicity. However
few in vitro studies in other genotoxicants reported both positive and negative results. As in our study, Park et al. (2002) studied the association of GSTM1 and GSTT1 polymorphism on benzopyrene induced genotoxicity in cultured PBL under in vitro conditions. They did not find any correlation between GSTM1 and GSTT1 polymorphism and genotoxicity induced by the Benzopyrene. Contrary to our study, Bernardini et al. (2002) investigated the influence of GSTM1 and GSTT1 genotypes on SCE induction by styrene in cultured human PBL. Their results suggest that the lack of the GSTM1 and GSTT1 genes increases the genotoxic effects of styrene in human cells.

Silva et al. (2004) evaluated the effect of GSTM1, GSTT1, and GSTP1 polymorphisms on the frequency of MN and SCE induced by hydroquinonone in cultured human PBL. Treatment of PBL with hydroquinone significantly increased the overall frequencies of MN and SCE (P<0.0001). Individuals with the GSTM1 null genotype had a significantly higher frequency of MN compared with GSTM1 non-null individuals (P=0.013) while the GSTM1 non-null genotype had no effect on hydroquinone-induced SCE frequency. The other polymorphisms did not significantly affect the frequencies of MN or SCE. These results suggest that GSTM1 is involved in the metabolic fate of hydroquinone and that polymorphisms in GSTM1 could be related to inter-individual differences in DNA damage arising from the exposure to this compound.

Angelinia et al. (2008) investigated the association between genetic polymorphisms in the GSTT1, GSTM1, XPD, XRCC1, and XRCC3 genes and the levels of spontaneous and Bleomycin (BLM)-induced DNA damage in PBL from 200 healthy, unexposed individuals. The MN assay was used to detect the spontaneous and BLM-induced genetic damage whereas, genotype analysis was carried out using methods based on polymerase chain reaction. Genotype analysis revealed a clear association between GSTT1-null and XPD polymorphisms and both spontaneous and BLM-induced MN frequencies, whereas the effect of the XRCCI polymorphism was marginally significant only with regard to spontaneous MN frequency. Genotype analysis did not reveal a clear association between the other studied SNPs (GSTM1 and XRCC3) and MN frequencies. Poisson regression analysis revealed no association between the score of protective alleles and the frequency of spontaneous MN. However, an increased number of protective alleles was significantly associated with a lower frequency of BLM-induced
MN (P = 0.0003). These finding highlights the genetic basis for BLM sensitivity, which could be a valid and useful surrogate for identifying genotypes that might increase susceptibility in population exposed to carcinogens.

Kumar et al. (2009) studied the GSTM1, GSTT1, and GSTP1 polymorphism in north Indian population and its influence on the hydroquinone (HQ)-induced in vitro genotoxicity. They treated the PBL with/without GSTM1, GSTT1, and GSTP1 (ile/ile or val/val) to HQ (20, 40, or 80µM) and examined CA or cytokinesis-block MN assays. They observed that individuals with null GSTM1, GSTT1, and GSTP1 (val/val) showed inhibition of mitotic index (MI) and significant (p < 0.01) induction of CA as compared to individuals with GSTM1, GSTT1, and GSTP1 (ile/ile). MN formation was found to be significant (p < 0.05 or 0.01) in both the genotypes. Their results indicate that GSTM1, GSTT1 (null), and GSTP1 (val/val) individuals are sensitive to HQ genotoxicity.