CHAPTER-1

Introduction

Pesticides, the heterogeneous mixture of compounds, are globally used to keep pests under control and to increase the crop productivity. Due to their toxicity, persistency and biomagnifications the usage of pesticides in agriculture or domestic purpose is a pivotal health concern of each country. Because of poor working conditions and lack of knowledge about potential hazards associated with the manufacturing and application of chemical pesticides, generally users in developing countries are at high risk of chronic exposure to pesticides (Baker et al., 1978; Brunetti et al., 1988; Benedetti et al., 2013; Kausar et al., 2014). Apart from target organisms, pesticides also exert their effects on the environment as well as non-target organisms including humans (Panemangalore et al., 1999; Prakasam et al., 2001; Hernández et al., 2005; Thetkathuek et al., 2005). Besides being the largest producer of pesticides in Asia, India is also the third largest consumer of pesticides in the world (Kumari et al., 2003) and so about 70% of her population depending on agriculture for their livelihood as cultivators, farm owners and laborers (Chakraborty et al., 2009) are at risk of exposure to the toxic pesticides. Workers get exposed to hazardous substances in the form of gases, vapours, fumes or particles that are present in the occupational environment (Çelik et al., 2003; Benites et al., 2006). Though inhalation is the primary route of exposure to these substances, exposure may also take place through oral ingestion or dermal absorption (Benites et al., 2006). Different health hazards which includes a wide range of subclinical and clinical effects (cancer, adverse reproductive outcomes and other chronic illness) are associated with occupational exposure to pesticides (IARC 1991; Arbuckle and Sever, 1998; Lander et al., 2000; Meinert et al., 2000; Priyadarshi et al., 2000; Hagmar et al., 2001; Jenner, 2001; Ji et al., 2001; Alavanja et al., 2004; Hanke and Jurewicz, 2004; Kamel and Hoppin, 2004). Therefore, the evaluation of the toxic effect of the pesticides on the non-target organisms including human beings is the need of the hour.

By using suitable biomarkers the effect of the pesticides can be assessed. A biomarker is a characteristic that can be objectively measured as well as evaluated as an indicator of normal biological or pathogenic processes and pharmacologic responses to therapeutic or other health care interventions (Biomarker Definitions Working Group, 2001). They serve as useful indicators of molecular and cellular events occurring in biological system which may illuminate relationships between hazards, human health and the disease processes (Dusinska and Collins, 2008). Unintended environmental exposure, such as, to chemicals or nutrient can be determined with the help of biomarkers. The response that is measured may be functional, physiological and biochemical at the cellular level or molecular interaction (WHO, 1993). Biomarkers are the internal indicators of environmental or occupational exposures which have the potential to prevent the effects of exposure to carcinogen by early detection (Smith et al., 1993). The growing interest in the use of biomarkers in occupational and environmental medicine parallels the development of human biomonitoring which can be defined as repeated and controlled measurement of chemical(s) or biomarkers in fluids, tissues or other accessible samples from those subjects that are currently exposed or had been exposed in the past or are to be exposed to chemical, physical or biological risk factors in the work place and/or general environment (Manno et al., 2010).

Biomarkers that are used to monitor environment and human health can be divided into three classes: biomarkers of exposure, effect and susceptibility (Knudsen and Hansen, 2007). Biomarker of exposure has been defined as an exogenous substance, its metabolite or the product of interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism, while the biomarkers of effect are any biochemical, physiological, behavioral or other alteration within an organism that can be measured and depending on the magnitude can be recognized as associated with an

established or possible health impairment of diseases (WHO, 1993). Biomarker of susceptibility is an indicator of inherent or acquired ability of an organism towards exposure to xenobiotics (WHO, 1993). Human biomonitoring can serve as useful tool in estimation of exposure of selected populations and is currently in use for surveillance programme across the world (Lionetto et al., 2013).

Organophosphates (OPs) inhibit acetylcholinesterase (AChE) in synapses and neuromuscular junctions causing acetylcholine accumulation which results in overstimulation of nervous systems (Kwong, 2002). Butyrylcholinesterase (BuChE) also bind covalently to OPs (Worek et al., 2005). AChE and BuChE are preferentially inhibited by certain OPs, for example, dimethoate predominantly inhibits AChE, while chlorpyrifos and malathion preferentially inhibit BuChE (Simoniello et al., 2008). Both AChE and BuChE can be used as an effective biomarker for exposure to certain pesticides (OPs and carbamates) (Chakraborty et al., 2009; Simoniello et al., 2010; Kapka-Skrzypczak et al., 2011).

Micronuclei are chromosomal fragments or whole chromosome which are not included into daughter nuclei during cell division and are incorporated as small nuclei (Schmid, 1975). Micronucleus (MN) is formed from induction of substances that cause chromosomal breakage (clastogens) and by agents that affect the spindle apparatus (aneugens) (Ghosh et al., 2008). Micronucleus assay is widely used to test the adverse effects of mutagens and carcinogens. It is a commonly used short-term assay in cultured mammalian cells, primary mitogen stimulated lymphocytes (Fenech and Morley, 1985) and in exfoliated epithelial cells like oral, urothelial, nasal etc. (Holland et al., 2008). MN assay is a non-invasive method to study DNA damage, chromosomal instability, cell death and the regenerative potential of buccal mucosal tissue (Çelik et al., 2003; Bonassi

et al., 2011; Thomas and Fenech, 2011). Cytokinesis-block micronucleus (CBMN) technique was developed, which stop the dividing cells from performing cytokinesis by using cytochalasin B. This allows cells which have completed one nuclear division to be recognized as they are binucleate in appearance (Fenech and Morley, 1985, 1986). Umegaki and Fenech (2000) proposed that nucleoplasmic bridges (NPBs) in between the nuclei of binucleated cells should also be scored in CBMN assay since they offer a good measurement of chromosomal rearrangement. NPBs are observed with binucleated cells because cytokinesis is blocked and nuclear membrane is formed around the chromosome. Micronucleus also forms by a unique mechanism called nuclear budding. It can be observed under selective conditions in culture (Toledo et al., 1992; Ma et al., 1993; Shimura et al., 1999) where gene amplification is induced. The amplified DNA is selectively located at the periphery of nucleus. MN forms during the S-phase of cell cycle via elimination of nuclear budding (Shimizu et al., 1998, 2000). **Figure 1.1** explains the various possible fates of cultured cells blocked in cytokinesis following exposure to cytotoxic/genotoxic agents as proposed by Fenech and Crott (2002). Human buccal cell is a valuable source to biomonitor DNA damage by determining frequency of micronuclei (Holland et al., 2008). The oral epithelium maintains itself by continuous cell renewal. New cells that are produced by mitoses in the basal layer migrates to the surface, replacing the ones that are shed (Ten Cate et al., 1998). The basal layer contains stem cells which may express genetic damage as chromosomal breakage or loss in the form of

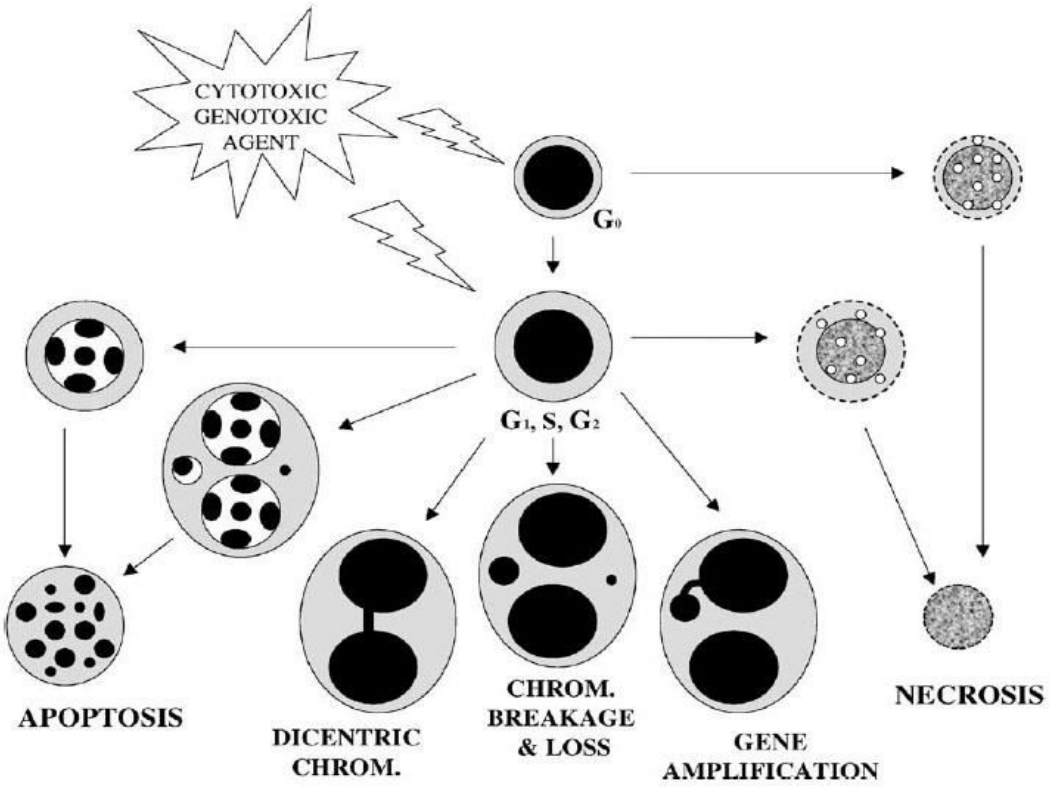

Figure 1.1: Various possible fates of cultured cells blocked in cytokinesis following exposure to cytotoxic/genotoxic agents as proposed by Fenech and Crott (2002).

MN during nuclear division (Rosin, 1992). The daughter cell may or may not contain MN, differentiate into the stratum spinosum cell layer and keratinized superficial layer, and finally exfoliate into the buccal cavity. Some of the cells may degenerate into condensed chromatin, fragmented nuclei, pyknotic nuclei or karyolytic cells which do not have nuclear material. In some cases, cells may be blocked in a binucleated stage or may also exhibit nuclear bud, which is a biomarker of gene amplification, also known as broken eggs (Suspiro and Prista, 2011; Kashyap and Reddy, 2012).

DNA damage and repair can also be detected in individual cells by comet assay, also known as single cell gel electrophoresis (SCGE), which is a rapid and a very sensitive method. Comet assay has important applications in toxicology, which ranges from clinical investigations and mechanistic studies to molecular epidemiology and biomonitoring (Collins, 2002). It was first introduced by Ostling and Johanson (1984) and later modified by Singh et al. (1988). The alkaline version of Comet assay is a useful method to quantify DNA damage (Tice et al., 2000). Comet assay is less time-consuming and can be performed with any type of eukaryotic cells, so is very popular (Hartmann et al., 2003). A number of studies have shown its potential to detect the extent of DNA damage in workers employed in pesticide manufacturing industry (Zeljezic and Garaj-Vrhovac, 2001; Grover et al., 2003; Bhalli et al., 2006; Sailaja et al., 2006; Naravaneni and Jamil, 2007).

The enzymes that belong to the families CYP1, CYP2 and CYP3 are found to catalyze the oxidative biotransformation of exogenous compounds (drugs, pro-carcinogens and alcohols). Metabolism of endogenous compounds (fatty acids, prostaglandins and steroids) is mediated by other CYP450 enzymes (van der and Steijns, 1999). Xenobiotic metabolism is mainly carried out by CYP2C9 and CYP2C19 and genes encoding these

enzymes are polymorphically expressed in the population (Goldstein, 2001; Daly, 2003). Human cytochrome CYP2C9 gene is located on chromosome 10q24 and has an approximate length of 55 kb (Lee et al., 2002). This major enzyme of the CYP2C subfamily in humans constitutes approximately 20% of the hepatic cytochrome P450 enzymes (Takahashi and Echizen, 2001). The biotransformation of a huge variety of xenobiotics including the organophosphates, chlorpyrifos and parathion are found to be associated with CYP2C9 (Foxenberg et al., 2007). Decreased metabolism of substrates have been shown to be associated with CYP2C9*2 and CYP2C9*3 allelic variants (Aynacioglu et al., 1999). Genetic polymorphism can be defined as a mutation which occurs in DNA sequence and is present in at least 1% of the population. The polymorphic alleles contains single nucleotide polymorphisms (SNPs) which results in inversions, deletions or base substitutions. The SNPs may result in a change of amino acid sequence, premature stop codon or a splicing error. The resulting enzyme may have increased, decreased or zero activity as reviewed by Rosemary and Adithan (2007).

Tea is cultivated as a major agro industry in Terai and the Dooars regions of northern part of West Bengal, India. Tea planters of this region use a large number of pesticides to control arthropod pests (Gurusubramanian et al., 2008). This results in the exposure of non-target organisms including humans to pesticides. The female workers of this region are mainly involved in the tea leaf plucking and males are engaged in pesticide spraying and mixing and therefore get occupationally exposed to pesticides (Dutta and Bahadur, 2016). Singh et al. (2015) have reported residual pesticide contamination in water, sediment and fish from the rivers flowing through the tea gardens of Terai region of West Bengal. Residues of ethion, chlorpyrifos, heptachlor, dicofol, alpha-endosulfan, beta-endosulfan, endosulfan sulfate, cypermethrin and deltamethrin have been detected in made tea, fresh tea leaves, soil and water bodies from certain tea gardens in the Dooars

and the hill regions of West Bengal (Bishnu et al., 2009). Owing to increasing pest problem as a result of resistance to pesticides (Sarkar and Mukhopadhyay, 2003, 2006), the planters apply a mixture of pesticides to combat the mixed pest infestation in the tea gardens of this region (Roy et al. 2008; Bishnu et al. 2009; Singh et al. 2015). A number of studies have been conducted related to the pesticide contamination, presence of residues in the water, sediment, fish and development of resistance/tolerance against the pesticides, but studies related to the effect of pesticides on the worker population using biomarkers are lacking. Therefore, to evaluate the effect of pesticides on the occupationally exposed tea-garden workers, the AChE and BuChE activity in the blood, micronuclei frequency, DNA damage by Comet assay and genetic polymorphism of CYP2C9 gene have been attempted. The data generated can be used for proper health risk assessment and effective health care strategy in future.

1.1 Objectives

In the light of the above, following objectives were undertaken for the present study:

1. A survey will be carried out to know the socioeconomic status and living condition of the workers in tea gardens of North Bengal region.
2. To determine the level of acetylcholinesterase and butyrylcholinesterase in pesticide-exposed tea garden workers and control.
3. To study the extent of nuclear DNA damage in the pesticide-exposed tea garden workers.
4. Genetic polymorphism of Cytochrome P450 gene involved in the detoxification of pesticides.
5. Data will be subjected to statistical analysis for association of genomic damages/genotoxicity with appropriate software packages.