CHAPTER IV

DISCUSSION

The purpose of human biomonitoring is to provide precise information on exposure of genotoxic agents and health risk assessment for effective prevention of the health problems in population that are potentially exposed to environmental genotoxic agents. Various genetic endpoints have been used for monitoring human populations exposed to environmental mutagens.

Petrol pump workers were chosen for the evaluation of genetic damages in their peripheral blood lymphocytes. People with occupationally exposed to benzene have been studied for evaluation of genetic changes by researchers various countries.

Tunca and Egeli (1996) found significant increased structural chromosome aberrations in shoe workers who had been occupationally exposed to benzene. Similar results reported by Kasuba et al. (2000) that they studied a group of workers in the same field and found a significant increase in the frequency of sister chromatid exchanges.

A relationship between benzene exposure and numerical chromosomal aberration was observed. Zhang et al. (1999) applied metaphase FISH for the detection of aneuploidy for chromosome 7 and 8 in human subjects exposed to benzene. They found that the incidence of aneuploidy in the occupationally exposed group were significantly higher than in the control group. Corresponding the study of Chung and Kim (2002) detected the specific chromosomes 5, 7, 8 and 21 changes in vitro induced by benzene.

Carere and coworkers (1998) applied the FISH technique to evaluate the aneuploidy of chromosomes 7, 11, 18, and X in peripheral blood lymphocytes of the gasoline station attendants in Rome, Italy. They found that the incidence of aneuploidy in the occupationally exposed group was significantly higher than control group. Yadav and Seth (2001) found a significant elevation of chromosome

aberrations and frequency of sister chromatid exchanges in petrol pump workers in India. They concluded that these workers are at risk to cytogenetic damage at their workplaces.

Recently, Celik and Akbas (2005) investigated peripheral blood lymphocytes in 30 gasoline station attendants in the city of Mersin, Turkey. They found significant differences in the frequencies of sister chromatid exchanges and chromosome aberrations in exposed workers compared to the controls. Tompa and coworkers (2005) found a significantly higher frequencies of sister chromatid exchanges and chromosome aberrations in benzene-exposed oil refinery workers.

Most of the biomonitoring studies on the genetic effects of gasoline exposure showed a significant increase in the frequencies of aneuploidy, sister chromatid exchanges and structural chromosome aberrations in the occupationally exposed persons.

Andreoli and coworkers (1997) were able to demonstrate genotoxic effects of gasoline exposure using the comet assay. They performed the comet assay on peripheral blood lymphocytes of gasoline station attendants who were exposed to benzene. The result revealed a significant excess of DNA damage in lymphocytes of exposed workers compared to matched unexposed controls. They also discussed in relation to the role of peripheral blood lymphocytes as target tissue in the biomonitoring of human exposure to genotoxic agents and concluded that the comet assay is a sensitive technique for detecting DNA damage at the single cell level.

The results of the chromosomal aberration analysis and the comet assay presented in this study show that occupational exposure to air pollutants in the working place could cause a significant increase in the level of DNA damage with a significant increase in the number of cells with aberrations and comet parameters found in the workers. This finding is supported by the appearance of dicentric chromosome as a complex aberration that was only detected in the exposed workers corresponding to the previous report of Zeljezic and Garaj-Vrhovac (2001) which found dicentric chromosomes in the workers but did not find any in control subjects. In contrast, Kasuba et al. (2000) detected dicentric chromosomes in both control and exposed subjects but the incidence of dicentric chromosome was significantly higher in exposed workers than in controls.

With regard to the types of aberrations, this study found the incidence of chromatid type aberrations more frequent than chromosome type aberrations. Similar results were reported by other researchers (Yadav and Seth, 2001; Zeljezic and Garaj-Vrhovac, 2001; Celik and Akbas, 2005). The literature review in Chapter 1 described the types of aberrations that arise from DNA damage in different stages of the cell cycle.

The chromatid type aberration formed in in vivo study by cultivation of lymphocytes could have originated from lesions on a single strand of DNA in the G_0 phase. When the lymphocyte was stimulated by phytohaemagglutinin, the cell entered G_1, S and G_2 phases. After the S phase a single strand break became a double strand break, and at metaphase, a chromatid type aberration was found (Fig. 16.). A similar event could be demonstrated for chromosome type aberrations, a double strand break on DNA present in G_0 phase of circulating lymphocytes. When they were stimulated and entered mitosis, a chromosome type aberration will be observe at metaphase.

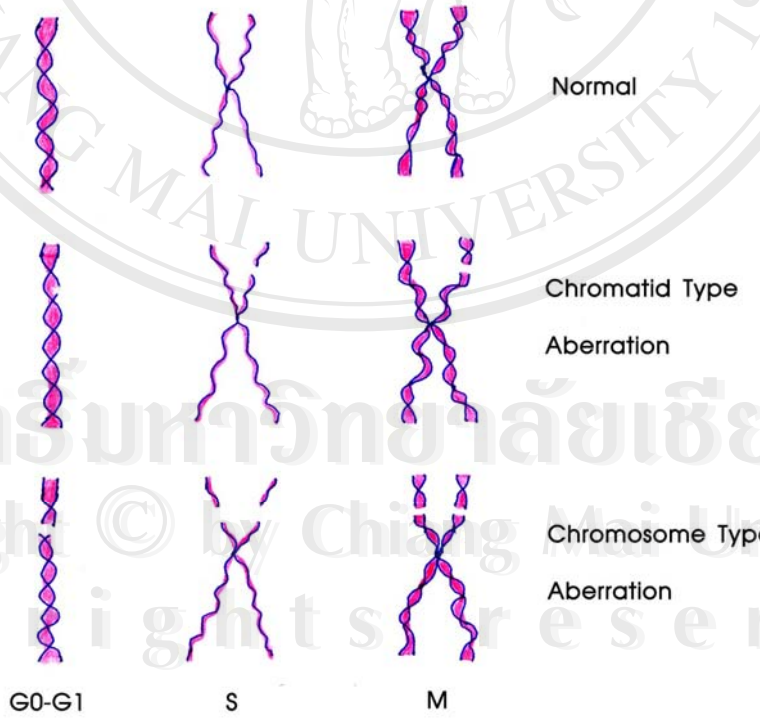

Figure 16 Mechanisms of the formation of chromatid and chromosome types aberration (Mevatee, 2005).

Although this chromosome aberration test seems to be sensitive in indicating DNA damage caused by chemical agents and is usually used as a biomonitoring tool in human occupational or environmental genotoxic exposure it is a limited method. It could detect double strand break lesion only. Other types of DNA lesions such as, single-strand breaks, base damage, DNA-DNA or DNA-protein crosslinks could not detected with this method because these lesions were not visible under the light microscope. The comet assay is a method of choice for the detection of single and double strand breaks.

The results of the comet assay presented in this study confirm the result of chromosome aberration tests by showing clearly the correlation between the comet parameters and the chromosome aberration test indicating that the exposed workers are at higher a risk for having DNA damage. The results confirm the ability of gasoline and air pollutant exposures to cause a significant increase the level of DNA damage in the petrol pump workers.

In view of the exposure of these workers, it should also be emphasized that petrol pump workers are exposed not only to benzene present in gasoline, but also at the same time exposed to other fuel components such as, butadiene, toluene, hydrocarbons that are well known mutagenic and carcinogenic agents. In addition, they are exposed to several genotoxic substances such as, airborne pollution, dust, traffic fumes, lead acetate, carbon monoxide that are emitted from motor vehicles, in to the ambient air of petrol pump stations. Therefore, it is hard be prove benzene is the single causative agent of DNA damage in these workers. Additionally, exposure to these agents is mainly by inhalation.

The chromosome aberration frequencies and comet parameters between non-smokers and smokers among the petrol pump workers were not significantly different. There were significantly greater in the comet parameters and chromosome aberration frequencies found in non-smokers of petrol pump workers than in the controls. This evidence stressed a high risk for petrol pump workers having DNA damage. An evidence of chromosome damage was not detected in the workers whose working period was not longer than 6 months. But the elevation of DNA damage in this subgroup was detected using the comet assay. This finding supports previous studies that the comet assay is more sensitive than chromosome aberration assay (Anderson et

al., 1997; Andreoli et al., 1997; Puaninta, 2000; Tice et al., 2000; Zhu et al., 1999; Faust et al., 2004). Among the workers with a working period of 6 months to 1 year, a significant increase DNA damage was detected using both the chromosome aberration and comet assays.

The pollution in the service station is composed not only of gasoline and their derivatives but also exhaust from motor vehicles and dust particle from the road and those are carried by motor vehicles into the petrol pump station.

Dust particles from the road in Chiang Mai City were investigated by Vinitketkumnuen et al. (2002). They collected PM10 and PM 2.5 in four parts of Chiang Mai City. The particulate matters were extracted using dichloromethane. The extracts were mutagenic to *Salmonella typhimurium* strain TA100 without metabolic activation. Mokmued et al. (2004) evaluated the toxicity of total suspended particles collected from heavy traffic areas in Chiang Mai. The toxicity to human lymphocytes of dichloromethane extracts of the particles collected from Warorod market was evaluated using comet assay. The tail moment of lymphocytes exposed to the extracts was significantly greater than in the control samples. The toxicity of the extract from the total suspended particles collected from Warorod market was also evaluated using the micronucleus test. The extracts induced a significant increase in the frequency of micronucleus formation in human lymphocytes (Thongnoon, 2004). These data demonstrated that the dust particles from traffic areas are genotoxic on human lymphocytes.

Since an increase of chromosome aberrations in circulating lymphocytes is a risk factor for having cancer. Forni (1996) reported that long term effects of benzene exposure on chromosome aberrations were present up to 30 years, and the high frequency of chromosome aberrations in peripheral blood lymphocytes was related to the high risk of having neoplasia. Hagmar and coworkers (2004) performed a cohorts study in Nordic and Italian subjects , using the chromosome aberrations test in peripheral blood lymphocytes with a median follow-up of 17 years. They found that the high level of chromosome aberrations in test were clearly associated with an increased total incidence of cancer in the Nordic cohorts and an increased total cancer mortality in the Italian cohort. The results showed that both double strand breaks and

other initial DNA lesions responsible for chromosome type and chromatid type aberrations are associated with cancer risk.

It could be emphasized that petrol pump workers are people at a higher risk for having DNA damage and health problems. These people should take prevention measures for avoiding inhalation of gasoline, its derivatives and dust particles. Those prevention measures should be applied to all the petrol pump workers elsewhere.