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Pesticides: Genotoxic Risk of Occupational Exposure

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1. Introduction

Exposure to pesticides remains a major environmental health problem. Pesticides are one of the most extensively used chemical products to control agricultural pests. The progress of agrochemical industries in the 20th century originated a great number of highly aggressive compounds against humans and altered the equilibrium in the ecosystems. To a high or low degree, human populations are unavoidably exposed to environmental pollution in physical, chemical or biological forms through products degraded in the air, water, soil or food and their inclusion in the alimentary chain.

With the objective of increasing and preserving crops, chemicals have been used to control and eliminate a wide variety of insects and other noxious organisms in agricultural production causing illnesses that affect plants and decrease the amount of food products obtained; therefore, it is important to protect the crops during the periods of sowing, harvest, storage and distribution of the products. The increase in the world population and the need to produce more food are major factors that stimulate the industry to produce new and more effective pesticides. DDT used to be effective for controlling insects, but later the synthesis of new substances became the main source to fight pests, and the world production of crops duplicated between 1970 and 1985.

It has been estimated that 2.5 million tons of pesticides are applied worldwide each year and the amount continues increasing with the passage of time. At the end of the 20th century, sales reached 40 thousand million dollars annually in the world, which corresponds to 2,800 million kilograms of active ingredients and more than 50 thousand commercial formulations. Developing countries use around 40% of this total. More than one thousand formulations or commercial names are used throughout the world as insecticides, fungicides, herbicides, rodenticides and antimicrobians (WHO/UNEP, 1990; PNUMA/OMS, 1992; OPS/OMS, 1993; OPS, 2002).

As to pesticide categories, herbicides represent 49% of the world sales, followed by insecticides 27%, fungicides 20% and other uses 4%. Hundreds of active ingredients and thousands of formulations are available in an uncontrolled fashion and they are promoted by both manufacturer and distributor as being essential for crop production (Eddleston et al., 2002).

The leading producers and exporters of pesticides in the world are Germany, the USA, England, Switzerland, France, Japan and Italy, countries that export the major part of their production to the Third World; regulation agencies consider that around 30% of such production are pesticides designated to agriculture and public health with a value of 900 million dollars, substances that do not meet the quality norms accepted internationally. These pesticides frequently contain compounds or impurities that are restricted in many countries because they constitute a risk for human health and the environment (OMS, 1990). Occupational exposure may occur through the pesticide formulation, manufacture and application phases which involve the exposure to complex mixtures of different types of chemicals, active ingredients and other substances included in the technical formulations such as impurities, solvents and other compounds produced during the storage procedure. Moreover, although inert ingredients do not have pesticidal activity, they may be biologically active and sometimes the most toxic component of a pesticide formulation (Bolognesi, 2003).

Pesticides are substances with very different characteristics and they are designed to kill a great variety of undesirable organisms for humans. They are toxic substances whose application should be controlled because their indiscriminate use and abuse constitute a risk to human health. Due to the large amount and variety of pesticides used at present to protect crops, great controversy has been generated about their use because of the adverse effects for humans, the environment and other organisms, although there is clear evidence of the benefits obtained by humans with the application of control vectors that have transmitted endemic diseases mainly in tropical countries, for example in Pakistan to control the dengue virus with sprayed deltamethrine or the malaria control program with malathion (Tariq et al., 2007), or to increase the daily production of food for the world population.

In several countries, the cotton plant still represents the most important crop and the main element for the national economy, as in the case of Egypt. Here the pests infesting cotton affect the quality and quantity of the yield, and thus pesticides are considered essential to protect this crop, with a high number of workers spraying three to five times each season (Mansour, 2004). The same problem occurred in Pakistan where about 80% of total pesticides used in this country are applied to the cotton plants (Tariq et al., 2007). The continuous shifting from one compound to another has been mainly attributed to the development of resistance of the cotton leaf worm (Mansour, 2004).

One of the main risks of pesticides for humans is occupational exposure, which occurs with agricultural workers in open fields, greenhouse workers, with individuals involved in the production of pesticides and exterminators of house pests, as well as with sanitation workers, workers packing pesticides, and other similar cases.

The World Health Organization estimates that every year between 500,000 and 1 million individuals suffer pesticide intoxications, and between 5,000 and 20,000 die. At least half of those intoxicated and 75% of those who die are agricultural workers, while the others die because of poisoning from contaminated food. Totally mortality for both groups was 220,000 annual deaths (OMS, 1990; Eddleston et al., 2002).

In accordance with the International Agency for Cancer Research (IARC) 56 pesticides have been classified as carcinogens in laboratory animals. The pesticide association for cancer in humans reported the use of 2,4,5-trichlorophenoxyacetic acid, lindane, methoxychlor, toxaphene and several organophosphates (IARC, 2002).

The International Network Against the Use of Pesticides has informed that developing countries account for the 5th part of the world consumption of these compounds, that the number of intoxications with such substances increased to 25 million cases and that 99% of the deaths are attributable to pesticides (PAN International, 1990).

The wide spectrum of effects on health produced by pesticides includes acute and persistent damage in the nervous system (Ecobichon et al., 1990; Kamel et al., 2005, 2007), lung and respiratory disorders (Barthel, 1981; Blair et al., 1983; Hoppin et al., 2008), alterations in the reproductive organs (Hileman, 1994) as well as in the immunological (Turner, 1994) and endocrine systems, in addition to birth defects (Gray, 1992; Rojas et al., 2000). Other causes of worry are the carcinogenic and genotoxic effects, considered as being among the most important of the effects, are possibly side effects associated with agricultural chemicals (Anwar, 1997).

In a review, Mansour (2004) concludes that there is strong scientific evidence that pesticides, as a whole, can induce severe effects to human health ranging from myelotoxicity to cytogenetic damage and carcinogenicity. The developed countries have already addressed the pesticide problem, but are still facing some problems in certain locations, whereas in the Third World countries pesticides should be used carefully since toxic outbreaks are often attributed to misuse of these substances.

2. Pesticides in Mexico

Pesticides are one of major sources of pollution derived from synthetic products and generated as a result of agricultural activity. Some are forbidden or restricted in many countries because they are toxic for human health and they affect natural resources, yet in Mexico there is an indiscriminate use which increases the risk of exposure to them on account of their genotoxic action.

Although there is wide-spread usage of pesticides in Mexico to control pests, as in other countries, this has caused environmental and human health problems. In accordance with the Mexican Association of Pesticide and Fertilizer Industrials (Asociación Mexicana de la Industria de Pesticidas y Fertilizantes), the pesticide volume used in Mexico in 1995 was 54,678.96 tons, 47% of which corresponded to insecticides, 29% to herbicides, 17% to fungicides and 7% to other uses. According to Cofepris (2010), the use of pesticides has increased and herbicides are the most utilized chemicals, followed by insecticides and fungicides.

In Mexico, the land available for agriculture is around 23 million hectares, which is 12% of the total surface of the country. The most important crops and the ones that are sprayed with the greatest volume of these chemicals are corn, bean, sorghum, wheat, potato, cotton, chilli, tomato, avocado, coffee, tobacco, pot-herb in amounts ranging from 395 to 13,163 pesticide tons per year (AMIPFAC, 1995). The official data for 2001 show that the population working in agriculture was around 7 million persons; however, this number does not include the rural population that was also exposed to pesticides and was calculated to be 25.4% of the total population in Mexico (AMIPFAC, 2001; Martínez Guerrero, 2001). Sixty percent of the 22 pesticides classified as dangerous to health and the environment are

commonly used in the Mexican Republic, and 42% are made in the country; 30 to 90% have been restricted or cancelled (INEGI, 1998). Pesticide handling and use in Mexico is regulated by different federal agencies: their transport by the Ministry of Communications and Transport (Secretaría de Comunicaciones y Transportes), their environmental impact by the Ministry of Environment and Natural Resources (Secretaria del Medio Ambiente y Recursos Naturales), their biological effectiveness for agricultural application by the Ministry of Agriculture, Livestock and Fisheries (Secretaría de Agricultura, Ganadería y Pesca), and the sanitary aspects by the Ministry of Health (Secretaría de Salud) (SEMARNAP, 1996; Rosales Castillo, 2001).

Besides the large amounts of pesticides imported by Mexico, there are industrial plants located in several states of the republic as Coahuila, Chihuahua, Guanajuato, Estado de México, Querétaro, Tlaxcala and Veracruz. The products for marketing are classified according to their toxicity: 57% slightly toxic, 25% moderately toxic, 9% highly toxic and 9% extremely toxic (Perea, 2006).

The Health Ministry considered that intoxications from pesticides registered every year in the world had occurred in developing countries. In Mexico 260 pesticide trademarks are used, 24 of these are prohibited and 3 restricted; in summary, the main cases of intoxication are due to lack of control and to deficient prevention. In agreement with the epidemiological norms of this Ministry, the number of intoxications caused by the use of pesticides decreased significantly from 8,000 to 2,532, between the years 1995 to 2001. In 2002 the number increased slightly to 2,802, in 2003 it increased again to 3,849 and in 2005 it was 3,898. However, the authority itself recognized the presence of a sub-register or "black data" as to the number of intoxications caused by the use of agrochemicals. The indiscriminate and exhaustive use of pesticides has originated very serious problems for the environment as well as for non-target organisms and humans (CICOPLAFEST, 1998). The states with highest use of pesticide are Sinaloa, Veracruz, Jalisco, Nayarit, Colima, Sonora, Baja California, Tamaulipas, Michoacán, Tabasco, Estado de México, Puebla and Oaxaca. Approximately 80% of all pesticides are applied in these regions (Grammont & Lara Flores, 2004; Albert, 2005).

3. Biomarkers used in cytogenetic biomonotoring studies in populations exposed to pesticides

Biomarkers are the measure of biochemical, physiological or morphological changes produced in a biological system and they are interpreted as a reflex or marker of a toxic agent (Garte & Bonassi, 2005). The studies of cytogenetic biomonitoring in human populations exposed to pesticides show different results because diverse biomarkers have been widely utilized in heterogeneous populations (Paldy et al., 1987; Rupa et al., 1989a,b; De Ferrari et al., 1991; Carbonell et al., 1993; Bolognesi et al., 2002, 2004). In studies on the exposure to pesticides the genotoxic effects of such biomarkers should be considered; the research should also take into account the damage resulting from the exposure, the robustness of the studies, the similarity of the control groups and the protocols used to determine the genotoxicity (Bull et al., 2006). Most of the adverse effects to health are the result of the genetic damage induced by genotoxic agents in somatic as well as in germinal cells. If this damage occurs, the condition can, among other effects, derive into cancer and contribute to premature aging, cause vascular illness and other similar ailments (Norppa, 2004). A large percentage of the chemical agents delivered to the environment has not been

assessed adequately in relation with genotoxic activity and it is essential to identify the subjects so as to determine the genetic risk to live organisms, including humans (Jamil et al., 2005). The word biomarker has been used very frequently in the last decade. Currently a great amount of research is being done with the objective of finding toxicological biomarkers that will detect different substances because persons are more exposed now than in past decades (Ríos & Solaris, 2010). Cytogenetic damage has been evaluated through biomarkers as chromosomal aberrations (AC), micronuclei (MN), sister chromatid exchange (SCE) and recently the unicellular alkaline electroforesis or comet assay (CoA).

3.1 Chromosomal aberrations

This assay can be used as a reliable biomarker of cellular damage whose increment in lymphocytes can predict cancer risk in humans (Hagmar et al., 1998; Bonassi et al., 2008). Several studies on the chromosomal effect of pesticides have been made using CA; the positive results obtained showed a correlation with the exposure time (Dulout et al., 1985; Paldy et al., 1987; Rupa et al., 1989a; Carbonell et al., 1993; Joksić et al., 1997; Kaioumova & Khabutdinova, 1998; Cuenca & Ramírez, 2004; Zeljezic et al., 2009). Other positive results did not find correlation between the exposure time and the CA induction (Rita et al., 1987; Rupa et al., 1988, 1989b, 1991a, El-Ghazali et al., 1990; Kourakis et al., 1992; Scarpato et al., 1996; Amr, 1999; Au, 1999; Antonucci & de Syllos Colus, 2000; Lander et al., 2000; Paz-y-Miño et al., 2002; Sailaja et al., 2006); meanwhile others authors although also found positive frequency of CA but did not determined this correlation (Nehéz et al., 1988; Jabloniká et al., 1989; De Ferrari et al., 1991; Carbonell et al., 1995; Mohammad et al., 1995; Kourakis et al., 1996; Lander et al., 2000; Garaj-Vrhovac & Zeljezic, 2001, 2002; Ascarrunz et al., 2006; Ergene et al., 2007; Mañas et al., 2009). As well negative results have been obtained (Mustonen et al., 1986; Steenland et al., 1986; Hoyos et al., 1996; D'Arce & de Syllos Colus, 2000; Costa et al., 2006).

3.2 Micronucleus

This assay is a genotoxic biomonitoring method widely used for evaluating exposure risk to pesticides. The micronuclei originate from acentric fragments or whole chromosomes that were not included in either of the daughter nuclei remaining in the cytoplasm, and in the interphase they are observed as small nuclei. The MN showed signs of chromosomal damage and afforded a marker of an early-stage of chronic diseases as cancer; they also revealed an increase in micronuclei frequency predicting cancer risk in humans (Bonassi et al., 2005, 2007). The use of the micronucleus assay in peripheral blood lymphocytes is useful for detecting clastogenic and aneuploidogenic effects together. Bolognesi et al. (1993a) suggested that micronuclei analysis in peripheral blood lymphocytes could be considered a good biomarker of genotoxic exposure to detect early biological effects in individuals having occupational contact with pesticides. The cytokinesis-block micronucleus technique supplied a robust methodology for monitoring human populations (Fenech & Morley, 1985); the studies in populations exposed to pesticides showed positive results with a correlation between the micronuclei frequencies and the years of exposure (Bolognesi et al., 1993a,b, 2002; Pasquini et al., 1996; Joksić et al., 1997; Falck et al., 1999; Bhalli et al. 2006; Costa et al., 2006); positive results without relation to the exposure time (Márquez et al., 2005; Kehdy et al., 2007; Da Silva et al., 2008); positive but not determining this correlation (Garaj-Vrhovac & Zeljezic, 2002; Vlastos et al., 2004; Ascarrunz et al., 2006; Tope et al., 2006; Bolognesi et al.,

2009; Rohr et al., 2010); and negative (Barbosa & Bonin, 1994; Scarpato et al., 1996; Titenko-Holland et al., 1997; Calvert et al., 1998; Venegas et al., 1998; Windham et al., 1998; Holland et al., 2002; Pastor et al., 2002a; Bolognesi et al., 2004; Vlastos et al., 2006).

The MN assay has also been performed in exfoliated buccal cells, which constitutes a minimally invasive method for monitoring populations exposed to pesticides. The assay in exfoliated cells was initially applied at the beginning of the 1980s, using cells of the buccal mucosa to evaluate the genotoxic effect of tobacco (Stich et al., 1982; Stich & Rosin, 1983). Micronuclei are formed by chromosomal damage in the basal cells of the epithelium; when these cells divide themselves, chromosomal fragments or entire chromosomes that lack an attachment to the spindle apparatus are excluded from the main nuclei in the daughter cells and they appear as Feulgen-specific bodies called micronuclei in the cytoplasm. Later, these cells mature and then exfoliate (Rosin, 1992). Other potential sites for MN studies included nasal cavity, bronchi, esophagus, cervix, bladder and urinary tract (Stich et al., 1983; Reali et al., 1987). The analysis of MN in exfoliated buccal cells is relevant because about 92% of cancer cases have an epithelial origin (Rosin & Gilbert, 1990) and recently has been considered as a tool for biomonitoring DNA damage (Holland et al., 2008). Likewise, other nuclear anomalies have been observed: for example, binucleate cells (presence of two nuclei within a cell), condensed chromatin (aggregated chromatin), broken eggs (cinched nuclei with a Feulgen-negative band), pycnosis (shrunken nuclei), karyorrhexis (disintegrated nuclei) and karyolysis (nuclear dissolution, with a Feulgen-negative ghost-like image of the nucleus remaining); all were classified according to Tolbert et al. (1992). This MN assay has been recently used to estimate exposure risk to pesticides. The studies realized have reveled positive results (Gómez-Arroyo et al., 2000; Sailaja et al., 2006; Ergene et al., 2007; Bortoli et al., 2009; Martínez-Valenzuela et al., 2009; Remor et al., 2009). In not any case, correlation between the micronuclei frequency and the exposure time was observed or determined. Other authors have described negative results for the analysis of MN in both peripheral blood lymphocytes and exfoliated buccal cells realized at the same time (Lucero et al., 2000; Pastor et al., 2001a,b, 2002b, 2003). Negative results were also found in peripheral blood lymphocytes, in oropharyngeal cells (Calvert et al., 1998) and in umbilical cord blood cells (Levario-Carrillo et al., 2005).

3.3 SCE

The SCE assay events produced in S-phase (Wolff et al., 1974), is a sensitive biomarker to detect DNA damage (Alptekin et al., 2006). It represents the symmetric interchange between homologous loci of replication products (Wolff, 1982). SCE occur without loss of either DNA or of changes in the chromosomal morphology, and it is possible detect them in metaphase. The assay was based on the incorporation of the thymidine DNA base analog 5-bromodeoxyuridine (BrdU) inside the DNA cells that replicated twice (Latt, 1979; Latt et al., 1981). In addition to SCE analysis, the BrdU differential staining technique can be used to assess the effects of pesticides in cell replication through the cell proliferation kinetics (CPK) (Gómez-Arroyo et al., 2000).

In studies in which SCE has been used to detect exposure risk to pesticides, results have varied: they were positive with correlation between the frequency and the exposure time (Rupa et al., 1991b; Padmavathi et al., 2000; Shaham et al., 2001; Martínez-Valenzuela et al., 2009); they were positive without correlation (Rupa et al., 1988, 1991a; Lander & Rønne, 1995; Scarpato et al., 1996); they were not determined (Jabloniká et al., 1989; De Ferrari et al., 1991; Dulout et al., 1992; Zeljezic & Garaj-Vrhovac, 2002; Ascarrunz et al., 2006), and they

were negative (Steenland et al., 1986; Carbonell et al., 1990; 1993; El-Ghazali et al., 1990; Gómez-Arroyo et al., 1992; Hoyos et al., 1996; Kourakis et al., 1996; Pasquini et al., 1996; Joksić et al., 1997).

3.4 Comet assay

The alkaline single cell gel electrophoresis assay or comet assay has constituted a useful tool for human biomonitoring studies in the detection of DNA single-strand breaks, alkali-labile sites, and incomplete excision repair events. It is a rapid and sensitive assay to demonstrate the damaging effect of several agents on DNA at the individual cell level. Cells in which DNA is damaged display an increased migration of DNA fragments from the nucleus originating a comet shape, given that during alkali gel electrophoresis the broken DNA strands move towards the anode forming a comet (Singh et al., 1988; Fairbairn et al., 1995).

The capacity of DNA to migrate depends upon the size as well as the number of breaks produced by the agent (Garaj-Vrhovac y Zeljezic, 2001). Each damaged cell has the appearance of a comet with head and tail bright; the undamaged cells appear intact or with complete nuclei and no tail (Möller, 2006).

The application of the comet assay to evaluate the DNA damage, biomontoring in populations occupationally exposed to pesticides has demonstrated positive results (Lebailly et al., 1998a,b; Garaj-Vrhovac & Zeljezic, 2000, 2001; Zeljezic & Garaj-Vrhovac, 2001; Ündeğer & Başaran, 2002; Grover et al., 2003; Ascarrunz et al., 2006; Castillo-Cadena et al., 2006; Remor et al., 2009; Rohr et al., 2010). Besides, correlation between the CoA and exposure time was not observed or determined in any of these cases. However, in other studies negative results have been found (Lebailly et al., 2003; Piperakis et al., 2003).

4. Cytogenetic biomonitoring studies

Several groups of workers are exposed to pesticides and the genotoxic effect has focused on evaluating the cytogenetic damage in those who work in open fields and greenhouses, with pesticide sprayers and applicators, as well as in industrial workers and individuals working in sanitation and pest eradication.

The cytogenetic biomonitoring in human populations is a useful tool to estimate the genetic risk from the exposure to complex mixtures of pesticides. Our analysis was based on the review of 88 cytogenetic biomonitoring studies done in the past 25 years (1985 to 2010). Table 1 shows that 64 results were positive and 34 negative; the total is primarily due to the fact that several of those studies included two or more biomarkers that indicated positive results in some and negative ones in others. In creating this table no exclusion criteria was applied, as suggested by Bull et al. (2006). In our case all the studies related with occupational exposure to pesticides were included. Biomonitoring was done using chromosomal aberrations (CA), micronucleus (MN), sister chromatid exchange (SCE) and comet assay (CoA). The studies mentioned above were carried out in different continents of the world as America (Argentina, Brazil, Bolivia, Chile, Colombia, Costa Rica, Ecuador, Mexico and the USA); Europe (Croatia, ex Czechoslavakia, ex Yugoslavia, Denmark, Finland, France, Greece, Hungary, Italy, Poland, Portugal, Spain, Russia and Turkey); Africa (Egypt and Syria); Asia (India, Pakistan and Turkey); the Middle East (Israel) and Australia.

Of all the studies shown in Table 1, 62 were made using only one biomarker: twenty with CA, twenty-five with MN (seventeen of these in peripheral blood lymphocytes, one in exfoliated buccal cells, five using both, one in peripheral blood lymphocytes and

oropharyngeal cells and one in peripheral blood lymphocytes of mothers exposed to pesticides and in the umbilical cord of her newborns); eight with SCE and five with CoA. In 18 studies two biomarkers were used: two with CA and MN (one in peripheral blood lymphocytes and one in exfoliated buccal cells); three with SCE and MN (one in peripheral blood lymphocytes and two in exfoliated buccal cells); two with CoA and MN (one in peripheral blood lymphocytes and one in exfoliated buccal cells); nine with CA and SCE and one with CA and CoA. In 5 studies that were carried out, the three biomarkers used were: CA, SCE and MN (two in peripheral blood lymphocytes; two with exfoliated buccal cells) and 2 with four biomarkers: CA, SCE, CoA and MN (in peripheral blood lymphocytes).

Duration of time exposure has been used as a substitute of exposure in a great number of studies due to the difficulty in making a quantitative evaluation of the exposure. The incidence of CA, MN, SCE and CoA correlated with duration exposure in many of these investigations has been included. The data in Table 1 show that of the 61 positive results only 17 of them established correlation between the time of exposure to pesticides and the cytogenetic effect, since in 20 there was no such correlation. In some cases the authors did not include evidence of exposure as they were considered not determined in this review; in 24 cases these data were not mentioned.

In the same table one observes that of the 85 cases of occupational exposure to pesticides, 67 of them correspond to agricultural workers (mainly greenhouse and open field workers, sprayers, pesticide applicators, mixture preparing workers, etc.), 12 to pesticide production (industrial plant workers), 2 to packing, 2 to agricultural pest eradication, 1 to sanitation programs, and 1 to mothers exposed to pesticides with their newborns. It is important to mention that the occupational groups with highest risk are the sprayers and the greenhouse workers as has been mentioned previously by other authors (Bolognesi, 2003; Bull et al., 2006).

In about 88% of the studies shown in Table 1 the workers had been exposed to pesticide mixtures, and therefore it is very difficult to know what to attribute their effect to. This constitutes a complicated factor for comparing the different studies due to the high number and variety of chemicals used. In several cases the pesticides applied are classified as carcinogenic by the U.S. Environmental Protection Agency (2005) and hazardous by the World Health Organization (WHO, 2004), or they are mentioned as carcinogens by the International Agency for Research on Cancer (IARC, 1991, 2002).

For validation of the studies, the robustness of the biomarkers employed must be known. The data given in Table 1 allow one to calculate that in 84% of the studies analyzing CA, the results are positive and that in 21% the correlation with the time of exposure was established. In relation with micronuclei, 56% of the studies done revealed positive results and 31% showed a correlation between their frequency and the exposure time. The SCE in 66% of the studies was positive and in 50% there was correlation with the exposure time. As to the CoA, 87% of the studies presented positive results, but correlation with the pesticide time exposure was not established. Several studies that have examined biomarkers have found that the micronuclei frequency is less sensitive than CA and SCE (Tates et al., 1994; Van Hummelen et al., 1994). The results obtained of the studies done in human populations exposed to pesticides CA, MN and SCE show these are adequate assays with a good percentage of positive results; besides, the studies carried out on CA and MN have been correlated as predictors of future cancer risk (Bonassi et al., 1995; Smerhovsky et al., 2001). Since in the case of the SCE their biological significance is unknown, their use has progressively disappeared from the scientific literature while new methods have become

available, as is the case of MN (Bonassi et al., 2005). In a review of evidence for the genotoxicity of pesticides Bull et al. (2006) excluded the SCE as endpoint, because the true biological relevance for mutagenecity or carcinogenicity risk were questionable and therefore not useful (Tucker et al., 1993). With respect to CoA , this is an assay that has been used recently to biomonitor populations exposed to pesticides and has had a good percentage of positive results, although correlation was not found with the time of exposure to pesticides.

5. Methods used in the studies on occupational exposure to pesticides in Mexican populations

5.1 Sister chromatid exchanges (SCE) (Fig. 1)

Venous samples were taken with heparinized syringes and transferred to the laboratory within a few hours. Eight drops of blood were added to 3 ml of RPMI medium 1640 with L-glutamine (Gibco) plus 0.2 ml of phytohemagglutinin (Gibco). The cultures were incubated at 37 ºC for 72 h. Twenty-four hours later, 5-bromodeoxyuridine (BrdU, Sigma) was added to the culture medium to obtain a final concentration of 5 μg/ml. Afterwards, colchicine (100 μl) was added 2 h prior to the harvest.

Metaphase cells were harvested by centrifugation, treated with 0.075 M KCl and fixed in methanol-acetic acid (3:1). Slides were stained by the fluorescence-plus-Giemsa technique (FPG) (Perry & Wolff, 1974). Fifty second-division metaphases were scored for each sample. Besides SCE examination, the BrdU differential staining technique can be used to assess the effects of pesticides on cell replication. The cell proliferation kinetics (CPK), which is the proportion of first, second and third metaphases, was scored through the analysis of 100 consecutive mitoses for each individual (Fig. 2). The RI is the average number of replications completed by metaphase cells; it was obtained considering the CPK proportion and was
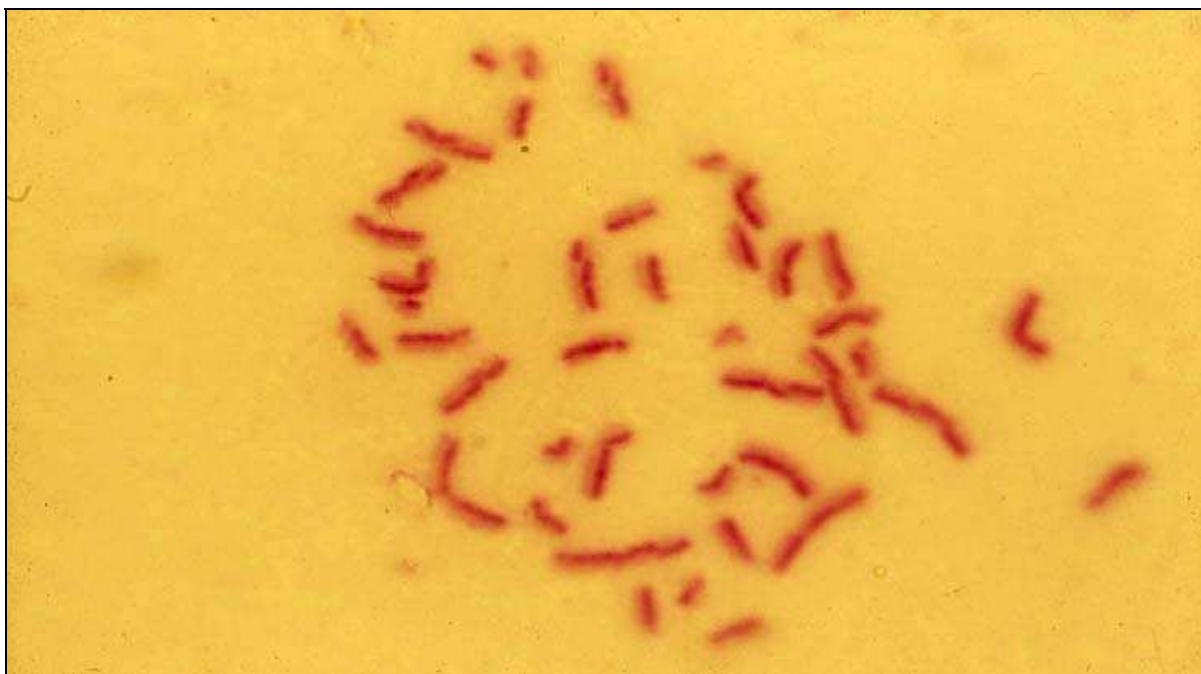

Fig. 1. Metaphase of human peripheral blood lymphocyte after differential staining of sister chromatid with FPG technique
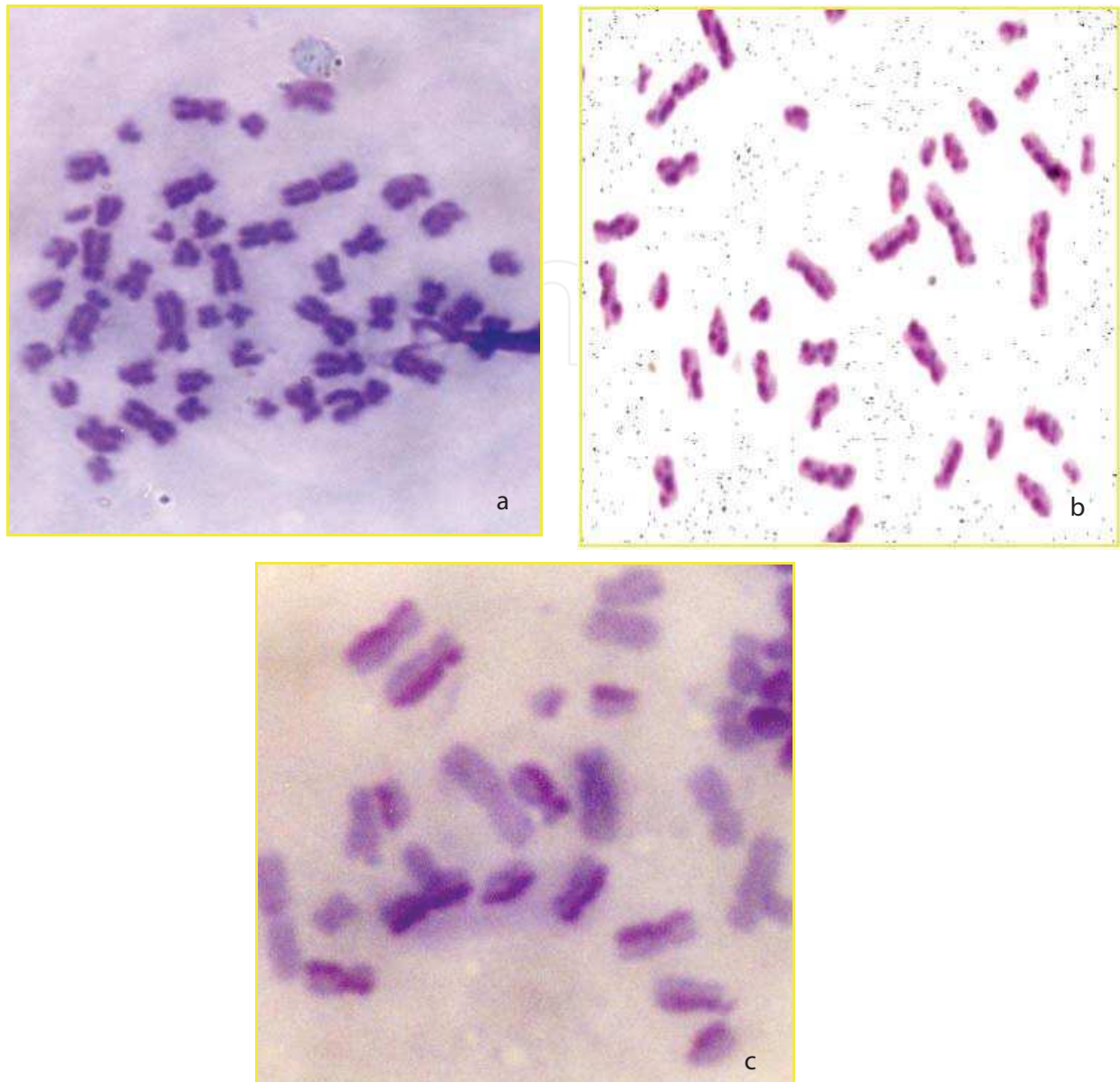
Fig. 2. Metaphases in first (a), second (b) and third (c) division in presence of BrdU

calculated following the formula RI = $1M_1 + 2M_2 + 3M_3/100$. The mitotic index (MI), considered as a measure of the proliferation status of a cell population, was determined in 3000 cells of each donor in order to ascertain the cytotoxic action of pesticides. The slides were handled by code in order to keep their origin unknown and avoid bias.

5.2 Micronucleus test in buccal exfoliated cells (Fig. 3)

The subjects were asked to rinse their mouth with water, and a wooden spatula was used to obtain the sample cells from the buccal mucosa. The sample was then applied to a clean microscope slide. Smears were air dried and fixed in methanol-acetic acid (3:1). The cell smears were stained using the Feulgen reaction technique described by Stich & Rosin (1984) and Stich (1987); it was modified as follows: smears were pretreated with 1 N HCl for 10 min at room temperature, placed for 10 min into 1 N HCl at 60 ᵒC, rinsed in distilled water, put into Schiff's reagent for 90 min and washed with running tap water. The criteria

followed for estimating the frequency of micronucleated cells were according to Stich & Rosin (1984). Three thousand epithelial cells were screened for each individual to determine the micronucleus (MN) frequency, and other nuclear anomalies as broken eggs (BE), karyolysis (KL), karyorrhexis (KR), and binucleate cells (BN), which were classified according to Tolbert et al. (1992). All the slides were also coded before scoring so as to avoid bias.

Fig. 3. Micronucleus of exfoliated buccal cells

5.3 Comet assay in buccal exfoliated cells (Fig. 4)

The comet assay was carried out in the buccal epithelial cells. Alkaline comet assay was performed according to the procedure described previously (Singh et al., 1988; Tice et al., 2000; Speit & Hartmann, 2006) with some modifications. The buccal cells were collected with a small sterile spoon, rinsed three times and resuspended in 50 µl of physiological solution at 37 ºC, then added to 50 µl agarose with low melting point (0.75% in phosphate buffer). The sample was carefully stirred, dropped on a coverslide and put on a microscope slide, precoated with normal agarose (1% in phosphate buffer) and kept on ice during the polymerization of each gel-layer. Two slides were made per donor. Slides were then immersed in a tank filled with a freshly made lysis solution (2.5 M NaCl, 100 mM EDTA, 1 mM Tris, 10% DMSO, and 1% Triton X-100, adjusted at pH 10) for 24 h. All the process was done under minimal illumination at room temperature.

To allow DNA unwinding, we incubated slides in a freshly made electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13) for 20 min. The slides were then placed in a horizontal electrophoresis chamber, immersed in fresh electrophoresis buffer, and exposed to 25 volts for 20 min at 300 mA. After electrophoresis, slides were washed twice in freshly prepared
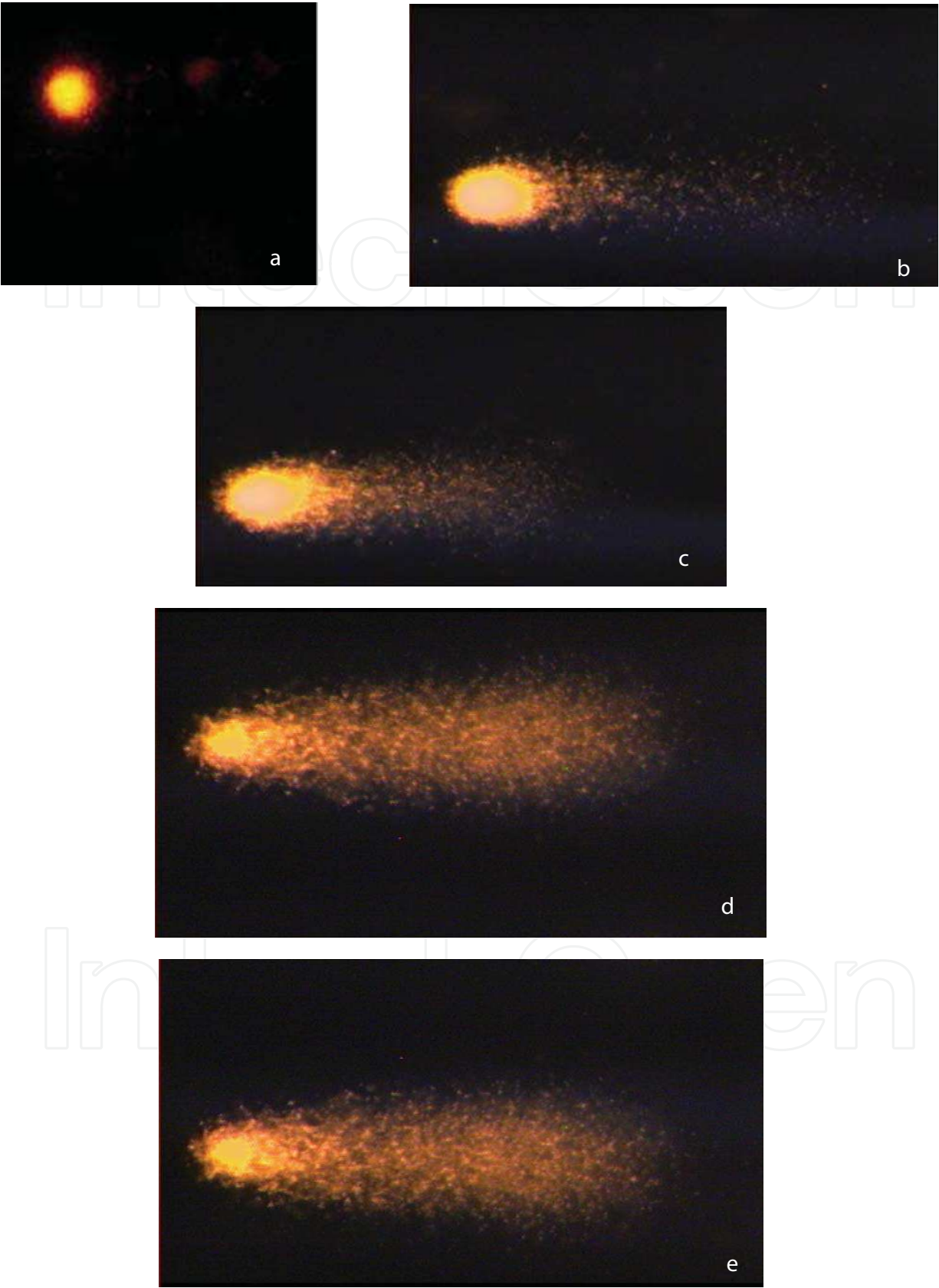
Fig. 4. Exfoliated buccal cells without (a) and with (b, c, d and e) comets having differential length

neutralization buffer (0.4 M Tris, pH 7.5) and fixed in absolute methanol for 5 min and stained with 10% ethidium bromide for 10 min. For each slide 50 cells were analyzed in an epifluorescence Axiostar Plus Zeiss microscope. All the slides were coded before scoring so as to avoid bias.

The statistical analysis was carried out through the Student's t-test, applied to the results of SCE and RI, and the Mann-Whitney U-test was used for MI, MN and other nuclear anomalies. The analysis of variance (ANOVA) was used to determine the effect on cell kinetics (M1, M2 and M3 cells) and the influence of smoking habits, alcohol consumption, age and gender; when significant values were found ($p < 0.001$), the Tukey-Kramer multiple comparison test was used to identify groups showing significant differences at $p < 0.001$. The analysis of correlation was applied between exposure time to pesticides and the frequencies of both SCE and micronuclei and comet assay.

6. Studies of occupational exposure carried out in Mexico

In Mexico human pesticide exposure occurs in workers in open fields and greenhouses, when the mixtures are prepared, and during the spraying; in all cases the individuals are in constant risk of suffering accidents related with these substances. Exposure is increased in tropical regions due the high level of humidity and environmental temperatures which cause these substances to remain in the air movement associated with water molecules; the winds then allow them to reach urban zones, as occurred in the states of Morelos, Sinaloa, Guerrero and other agricultural areas in Mexico. This is why we evaluated the genotoxic effect produced by pesticide mixtures, using SCE in peripheral blood lymphocytes as well as the micronucleus test and the comet assay in exfoliated buccal cells of workers occupationally exposed in four states of the Mexican Republic: Tlaxcala, Morelos, Sinaloa and Guerrero.

A study done in Tlaxcala, Mexico on a rural population exposed to pesticides (Gómez-Arroyo et al., 1992) produced the following results: of 170 men 94 were exposed, with age range under sixteen and older than sixty-five, and duration of pesticide range from one to thirty-five years; 76 were non-exposed showing SCE negative results. This lack of effect could possibly be due to the fact that people were exposed to the pesticides chronically, but for short periods each year and they work on very small parcels of land where the level of exposure was not enough to produce SCE.

The study in Morelos state (Gómez-Arroyo et al., 2000) was made in 30 floriculturists -22 women and 8 men- who worked in greenhouses, and who had ten and one and a half years of pesticide exposure, respectively. The data obtained of the questionnaire filled out by the exposed individuals showed that they did not have smoking or drinking habits. The medical examination revealed that the pesticide exposed workers did not show health problems as cancer or respiratory and digestive disturbances. However, the 22 female floriculturists presented acute intoxication, occasional cephalea, skin and nasal mucosa irritations, and nausea when they were in contact with the pesticides.

The 30 non-exposed individuals with an age average of forty years showed SCE mean $\pm$ S.E. of 4.0 ± 0.1 in a range of 3 to 5 SCE per cell; in the exposed group the age average was 35.5 ± 2.22 and the SCE mean $\pm$ S.E. 7.1 ± 0.17 in a range of 5.5 to 10.7. A Student's t-test showed a significant difference of $p < 0.001$ when these data were compared. When SCE frequencies

were compared between males (7.28 $\pm$ 0.27) and females (7.02 $\pm$ 0.27) no significant differences were observed, probably due to the different exposure duration among men and woman. Although men had been in contact with the pesticides for only one and a half years and women for ten years, men had to work 10 to 12 h a day, while woman were only exposed for 6 h. The exposure condition for both was in plastic greenhouses with poor ventilation, but men were in charge of spraying the pesticides once or twice every day while the women went out of the greenhouses and returned after the pesticide application; then both continued working in the greenhouses. The lack of correlation between exposure time to pesticides and SCE frequencies might be related to the fact that the group with lower time of exposure has the greatest pesticide exposure.

The cell proliferation kinetics (CPK) was also determined. The controls were 28.78 M1, 41.70 M2 and 29.21 M3, while for the exposed group they were 25.25 M1, 36.46 M2 and 39.23 M3 in which the M2 cells decreased and M3 cells increased significantly meaning that the pesticide exposure induced acceleration of the cell cycle, and the mitotic index also increased.

The micronucleus frequency in epithelial cells of the buccal mucosa of the workers exposed to pesticides was 1.01 $\pm$ 0.03 and in the non-exposed individuals it was 0.038 $\pm$ 0.021 (p < 0.001), a result which allowed concluding that pesticide exposure significantly increases cytogenetic damage in this population exposed to pesticide mixtures. According to Tolbert et al. (1992) the analysis of exfoliated cells of buccal mucosa also provides evidence of other nuclear anomalies as binuclated cells, condensed chromatin, broken egg, karyolysis, pycnosis and karyorrhexis; only in the last three, the results were significant between the individuals exposed to pesticides and the non-exposed.

The occupational exposure of the floriculture workers is intense and acute in closed plastic greenhouses without ventilation. Such workers are considered to have high risk exposure, which is worsened because not only do they not use protective clothing when working in the greenhouses but wear clothes impregnated with the pesticide outside of the work area.

In the study carried out in Sinaloa state (Martínez-Valenzuela et al., 2009) genotoxic damage was evaluated in 70 agricultural workers, 25 women and 45 men, exposed to pesticides in Las Grullas, Ahome, Sinaloa, Mexico, with an average of 7 years of exposure. The effect was detected through the sister chromatid exchanges (SCE) in lymphocytes of peripheral blood and micronuclei (MN) and other nuclear anomalies (NA) in buccal exfoliated cells. Also, the influence on (CPK) was studied by means of the replication index (RI) and the cytotoxic effect was examined with the mitotic index (MI). The non-exposed group consisted of 70 individuals, 21 women and 49 men from the city of Los Mochis, Sinaloa, Mexico. The SCE mean $\pm$ S.E. in the exposed group was 6.36 $\pm$ 0.22 and in the non-exposed 3.71 $\pm$ 0.11, significant differences were obtained between them p < 0.001. The analysis of correlation between the average values of SCE and exposure time to pesticides evidenced a significant correlation (p < 0.001). In the non-exposed group CPK had a parametric distribution with 30.7 M1, 45.9 M2 and 23.2 M3 cells, while in the exposed group the M1 was 24.3, M2 decreased significantly to 33.8 and M3 increased significantly to 41.9 when we applied the ANOVA and the Tukey-Kramer multiple comparisons test. These results mainly showed that in the pesticide exposed group there were induced alterations in the CPK due to the fact that M2 cells decreased but M3 cells increased significantly, suggesting that the pesticide exposure had induced an acceleration of the cell cycle; the same behavior was observed in the floriculture workers of Morelos (Gómez-Arroyo et al., 2000). No significant differences

were found regarding CPK between alcohol consumers and smokers in either the exposed group or the non-exposed group. Age, gender and time of exposure did not correlate with CPK.

MN frequencies in the exposed group were 2.83 $\%_o$ and 0.37 $\%_o$ in the non-exposed group; the Mann-Whitney U-test was significant ($p < 0.0001$). The MN frequency was not correlated with age, gender and exposure time to pesticides. When we compared MN frequency between exposed smokers and alcohol consumers, and between exposed non-smokers and non-alcohol consumers, no statistical differences were found. We did not find correlation between exposure time and MN frequency.

The agricultural workers referred to in the present study were exposed to complex mixtures of pesticides that had different active ingredients, mainly organophosphorus and carbamates. Some of those active ingredients include two compounds which according to WHO (2004) are "extremely hazardous" (parathion-methyl and aldicarb) and five that are "highly hazardous" (azinphos-methyl, monocrotophos, gusathion, lannate and vydate).

In three locations of Guerrero state (Carbajal-López et al., unpublished data), the study was made in 111 agricultural workers exposed to pesticide mixtures; the individuals were from the towns of Arcelia (62), Ajuchitlan (13) and Tlapehuala (36). Their exposure ranged from 1 to 57 years, and ages extended from 13 to 83 years; the non-exposed group constituted 50 individuals whose ages went from 15 to 66 years. All the participants were males working in open fields; they used no protective measures, but they mentioned that they did use clean cloths after handling the pesticides and washed their hands before eating. Cells of the buccal ephitelium were sampled and the comet assay was used as biomarker to know the DNA damage. The average of the comet tail was screened in 100 cells of each individual: in the exposed group, the mean $\pm$ S.D. of cells with comet was 81.11 $\pm$ 12.75 and the tail length was 190.33 $\pm$ 43.26 μm; in the non-exposed group it was 8.72 $\pm$ 3.85 and 106.08 $\pm$ 20.04 μm, respectively. The micronuclei test was carried out in 3000 epithelial cells for each participant: the mean $\pm$ S.D. in the exposed group was 2.33 $\pm$ 1.16 and in the non-exposed 0.88 $\pm$ 0.56; other nuclear anomalies as broken eggs, karyolysis, karyorrhexis and binucleate cells were also evaluated. The results revealed that in the exposed group of the three areas studied the frequency of cells with comet increased significantly in relation with the non-exposed group. The same behavior was observed in the tail migration of DNA. Micronuclei exhibited significant differences between the exposed and the non-exposed groups, and they showed nuclear anomalies associated with a cytotoxic or genotoxic effect. No positive correlation was noted between exposure time and comet tail length, nor between cells with comet and with micronuclei frequency. No significant effect on genetic damage was observed as a result of smoking and alcohol consumption. This study afforded valuable data for establishing the possible risk to human health associated with pesticide exposure.

Due to the fact that the smoking and alcohol drinking habits have been considered confounding factors that could influence the frequency of genetic damage, we evaluated these risk factors. In none of the four studies carried out we did find statistical differences between exposed smokers and alcohol consumers in relation with non-smoker and non-alcohol consumer (Gómez-Arroyo et al., 1992, 2000; Martínez-Valenzuela et al., 2009; Carbajal-López et al., unpublished). No significant difference was observed in SCE and micronuclei frequency in either gender or age (Gómez-Arroyo et al., 2000; Martínez-Valenzuela et al., 2009).

7. Relevance of our studies

Mexico can be considered as a "mega-diverse" country belonging to the group of countries that have a great diversity of animals and plants, and which have almost 70% of the world diversity of species. This group of twelve countries comprises Mexico, Colombia, Ecuador, Peru, Zaire, Madagascar, China, India, Malacca, Indonesia and Australia. However, Mexico is characterized with having regions for agriculture development mainly in the Northwest of the country, in addition to using mixtures of pesticides that affect not only the health of the persons involved but also the environment.

The population in the rural environment suffers various cultural conditions such as a high level of illiteracy and low education. Such factors prevent the agricultural workers from knowing and developing an awareness of the risk involved in working in direct and indirect contact with these compounds; for example, in most cases they handle pesticides without any type of protection.

Rural workers lack social support from the landowners who make labor arrangements verbally or in most cases through intermediaries. Besides, the workers do not have medical insurance (Gómez & García, 2002). In the Northwest of the Mexican Republic, the agricultural activity is outstanding but climatic conditions have favored the development of pests and plant diseases, creating a culture related with the use of pesticides. In the fields, children between the ages of 6 and 14 years very often collaborate in agricultural activities and are exposed to pesticides, as are scarcely newly born infants being carried by their mothers during the long work day in the crops. Likewise, persons living on or near treated croplands can be exposed through agricultural application, as in most parts of Mexico where huge amounts of pesticides are sprayed in the crop fields. The problem is more critical when the pesticide mixtures are applied aerially with the use of small planes, which is a method that contaminates more since only part of the pesticide mixtures reach the crops and the rest are distributed on other places.

In 2008 the Ministry of Health reported death in females older than 40 years produced by cancer in the breast (15%), in the uterus (14%), as well as in liver and bile ducts (9.2%). In men, deaths resulting from tumors were due to prostate (17.1%), and to lung, trachea and bronchi (16%). The register of the Ministry of Health showed that agricultural areas of Mexico present a high incidence of cancer. This is why we must mention the enormous lack of information related with scientific investigation, which evidences the effects generated by the use of pesticides and their mixtures, not only in the occupationally exposed workers but also in families that live on or near the crop lands and in the general population. Therefore, we carried out our studies in several states of the Mexican Republic which have been pioneers in the use of biomarkers as SCE, MN and CoA. They constitute evidence that supports the fact that pesticide exposure caused genotoxic damage, and they afford the scientific bases for the authorities to take the corresponding decisions.

Considering the abovementioned information, the introduction of agricultural practices to reduce the use of pesticides is important; furthermore, the utilization of measurements for biological control as well as the integration of pest management is relevant. Also important are efforts to intensify and permanently train the workers in agricultural practices so as to increase prevention activities and improve education in the community.

Cytogenetic biomonitoring is very important because it is the basis for integrating correct medical watchfulness; it allows evaluating the potential risk of occupational exposure and helps take the right steps to identify genetic risks earlier.

No. of individuals and exposure type	Biomarker	Country	Exposure time (years) [1]range [2]average N.D. not determined	Result [a]with or [b]without time exposure correlation [c]not determined	Reference
36 floriculturist (21 with chronic intoxication symptoms, 9 female and 11 male and 15 without intoxication symptoms 7 female and 9 male) exposed to mixtures of organophosphorus, carbamate and organochlorine pesticides; and 15 healthy donors	CA, SCE	Argentina	At least 10	Positive[a]	Dulout et al., 1985
19 male pesticide sprayers exposed to phenoxyacetic acids, and 15 male controls	CA	Finland	N.D.	Negative	Mustonen et al., 1986
60 male working in papaya-packing plants exposed to ethylene dibromide, and 42 male controls	CA, SCE	USA (Hawaii)	5[2]	Negative	Steenland et al., 1986
80 male workers exposed to complex mixtures of pesticides organophosphates, dithiocarbamates, nitro compounds, triazines, ureas, phtalamides, organochlorines, phenoxy-acetic acids, pyrethroids, cabamates, heterocyclic compounds, among others, and 24 male controls	CA	Hungary	1 to >15[1]	Positive[a]	Paldy et al., 1987
15 workers of grape gardens exposed to pesticides DDT, lindane, quinalphos, diethane M_{45}, metasystox, parathion, cooper sulfate, dichlorvos and dieldrin, and 10 controls	CA	India	5 to 15[1]	Positive[b]	Rita et al., 1987
55 male working with pesticides in open fields (14) or in closed space (41) exposed to mixtures of organophosphates, carbamates, pyrethroids, fungicides and acaricides, and 60 male controls	CA	Hungary	1 to 15[1]	Positive (in open fields) [c] Negative (in closed space)	Nehéz et al., 1988
25 male workers exposed to DDT, BHC, malathion, parathion, dimethoate, fenitrothion, urea and gromor, and 30 male controls	CA, SCE	India	5 to 38[1]	Positive[b]	Rupa et al., 1988
44 workers (30 male and 14 female) exposed to mancozeb during the production of the pesticide novozir Mn80 and 30 control (18 male and 12 female)	CA, SCE	Ex Czech oslavakia	Up to 2	Positive[c]	Jabloniká et al., 1989
50 smokers exposed to insecticides DDT, BHC, endosulfan, malathion, methyl parathion, monocrotophos, quinolphos, dimethoate, phosphamidon, cypermethrin, and fenvelrate, and 47 controls (30 non-smokers and 27 smokers)	CA	India	5 to 25[1]	Positive[a]	Rupa et al., 1989a
52 male pesticide sprayers exposed mainly to DDT, BHC, endosulfan, malathion, methyl parathion, monocrotophos, quinolphos,	CA	India	1 to 25[1]	Positive[b]	Rupa et al., 1989b

dimethoate, phosphamidon, cypermethrin, and fenvelrate, and 25 male controls					
50 smoking pesticide sprayers exposed to DDT, BHC, endosulfan, malathion, methyl parathion, dimethoate, monocrotophos, phosphamidon, quinolphos, fenvelrate and cypermethrin, and 47 controls (20 non-smokers and 27 smokers)	SCE	India	1 to 25[1]	Positive[a]	Rupa et al., 1989c
27 workers exposed to pesticide mixture mainly benomyl, captan, deltamethrin, fenvelrate, methomyl and paraquat, and 28 controls	SCE	Spain	10[2]	Negative	Carbonell et al., 1990
28 workers packing pesticides and 20 controls	CA, SCE	Egypt	12.9 ± 6.2 [2]	Positive (CA) [b] Negative SCE	El-Ghazali et al., 1990
32 healthy floricultors and 32 individuals hospitalized for bladder cancer (without radio- or chemo-therapy before blood sampling) exposed to pesticide mixtures as nitro-organic herbicides and fungicides, nitrothiorganics, organophosphates, organothiophosphates, organochlorines, pyrethroids, among others, and 31 controls	CA, SCE	Italy	N.D.	Positive[c]	De Ferrari et al., 1991
26 male pesticide applicators exposed to endosulfan, malathion, methyl parathion, dimethoate, phosphamidon, monocrotophos, quinalphos, cypermethrin and fenvelrate, and 26 male controls	CA	India	2 to 18[1]	Positive[b]	Rupa et al., 1991a
61 male pesticide applicators who sprayed DDT, BHC, endosulfan, malathion, methyl parathion, phosphamidon, dimethoate, monocrotophos, quinalphos, fenvelrate and cypermethrin, and 45 male controls	SCE	India	1 to 20 [1]	Positive[a]	Rupa et al., 1991b
27 floriculturist exposed to pesticide mixture 14 with chronic intoxication symptoms and 13 without chronic intoxication symptoms, and 32 non-exposed	SCE	Argentina	About 10	Positive[c] in both groups (the median value is higher in floriculturist with chronic intoxication symptoms)	Dulout et al., 1992
94 male rural workers exposed to mixtures of insecticides organophosphates, organochlorines and carbamates, fungicides as manzate, mancozeb, benomyl and carbendazin, herbicides mainly triazines, hormones, thiocarbamics, and ureics, and 70 male controls	SCE	Mexico	1 to 35[1]	Negative	Gomez–Arroyo et al., 1992

29 pesticide greenhouses sprayers exposed at the same mixtures of organophosphates, carbamates, dithiocarbamates, and organochlorines, and 14 controls	CA	Greece	4 to 30[1]	Positive[b]	Kourakis et al., 1992
71 floriculturists (57 male and 14 female) in open fields or in greenhouses exposed to pesticide mixtures as dithiocarbamates, organophosphates, and organochlorines, and 75 control (66 male and 9 female)	MN (peripheral blood lymphocytes)	Italy	2 to 55[1]	Positive[a]	Bolognesi et al., 1993a,b
70 male working in flower and fruit cultivation exposed to pesticides organochlorines, organophosphorus, carbamates, and pyretroids, fungicides as cooper compounds, thiocarbamates, heterocycles, and antibiotics, and 69 male controls	CA, SCE	Spain	5 to 29[1]	Positive (CA)[a] Negative (SCE)	Carbonell et al., 1993
31 fumigators exposed to phosphine during the high fumigation season, and 21 controls	MN (peripheral blood lymphocytes)	Australia	1.5 to 32[1] 11.6[2]	Negative	Barbosa & Bonin, 1994
9 male sprayers exposed to deltamethrin and cypermethrin and 7 agricultural workers exposed to pesticide mixture, and 6 male controls	CA	Syria	3 to 38[1]	Positive[c]	Mohammad et al., 1995
134 greenhouse sprayers (118 male and 16 female) exposed to pesticide complex mixture of almost 50 insecticides, fungicides and growth regulators, and 157 referents (137 male and 20 female)	SCE	Denmark	1 to 50[1] 17[2]	Positive[b]	Lander & Rønne 1995
29 male agricultural workers exposed to pesticide mixtures manly carbamates, heterocycles, organochlorines, organophosphorus, and pyrethroids, among others, and 29 and 24 male controls	CA	Spain	N.D.	Positive (in the period of major exposure) [c] Negative (in the period of minor exposure)	Carbonell et al., 1995
30 workers (26 male and 4 female) exposed to mixtures of insecticides as carbamates and orgaphosphates, fungicides as dithiocarbamates and carbamates, and 30 controls (26 male and 4 female)	CA, SCE	Colombia	16.5±8.8[1]	Negative	Hoyos et al., 1996
56 (29 indoor and 27 outdoor) agricultural workers exposed to mixture of orgaonophosphorus, carbamates, dithiocarbamates, and organochlorine, and 30 controls	CA, SCE	Greece	At least 6	Positive (CA)[c] Negative (SCE)	Kourakis et al., 1996
48 male agricultural workers exposed to pesticide mixtures of carbaryl, delthametrin, benomyl, dinocap, oxadixyl, propineb, mancozeb, triadimenol, alachlor, atrazine, linuron, MCPA, metobromuron, metalachlor and oxyfluorfen, and 50 male controls	MN (peripheral blood lymphocytes), SCE	Italy	4 to 50[1]	Positive (MN) [a] Negative (SCE)	Pasquini et al., 1996

43 greenhouse floriculturist (24 male and 19 female) exposed to pesticide mixtures of more than 100 of active ingredients, and 42 controls (22 male and 20 female)	CA, MN (peripheral blood lymphocytes), SCE	Italy	N.D.	Positive (SCE and CA in smokers)[b] Negative (MN)	Scarpato et al., 1996
27 male vineyard growers exposed to pesticide mixtures of the insecticide diazinon and fungicides dithiocarbamates being the most commonly used, and 35 male controls	CA, MN (peripheral blood lymphocytes), SCE	Ex Yugoslavia	12.1 ± 6.02^2	Positive (CA and MN)[a] Negative (SCE)	Joksić et al., 1997
38 malathion exposed workers (29 male and 9 female) involved in the Mediterranean Fruit Fly Eradication Program, and 16 unexposed (9 male and 7 female)	MN (peripheral blood lymphocytes)	USA	At least 6 months	Negative	Titenko-Holland et al., 1997
32 male methyl bromide-exposed fumigation workers, and 28 male referents	MN (peripheral blood lymphocytes and oropharyngeal cells)	USA	0.3 to 22^1 3^2	Negative	Calvert et al., 1998
19 herbicide plant workers (17 male and 2 female) exposed to 2,4,5-trichlorphenol (2,4,5-T) and 2,4-dichlorophenoxiacetic acid (2,4-D) (dioxin-containing products), and 36 controls	CA	Russia	10 to 30^1	Positive[a]	Kaioumova & Khabutdinova, 1998
29 male farmers (14 non-smokers, 7 ex-smokers and 8 smokers) exposed to pesticides	CoA	France	N.D.	Positive[c]	Lebailly et al., 1998a
29 male farmers (8 smokers) exposed to mixtures of dimethoate, ethephon, omethoate, oxydemeton-methyl, thiometon, befenthrin, b-cyfluthrin, deltamethrin, mancozeb, carbendazin, endosufan, chlorothalanil, iprodine, diflufenicanil, l-cyhalothrin, pymethanil, fluroxypyr, cyproconazole, epoxyconazole, flutriafol, tebucanazole, atrazine, MCPA, isoproturon, 2,4-D, amidosulfuron, bentazon, bifenox, bromoxynil, clopyralid, fenopropadin, imazamethabenz-methyl, ioxynil, mecoprob and sethoxydim a longitudinal study on the same individuals (each farmer was his own control)	CoA	France	N.D.	Positive[c]	Lebailly et al., 1998b
22 pesticide sprayers exposed to mixture of the insecticides deltamethrin, dichlorvos, diazinon, methamidophos, cyfluthrin, propoxur, cypermethrin, endosulfan, parathion, among others, herbicides and fungicides as linuron, captan pentachlorophenol, methyl bromide,	MN (peripheral blood lymphocytes)	Chile	About 7	Negative	Venegas et al., 1998

among others, and the raticides bromadialone, brodifacoum, coumatetralyl, and diphacinone, and 16 controls					
53 workers exposed to malathion in Medfly eradication (40 male and 13 female), and 4 male controls	MN (peripheral blood lymphocytes)	USA	N.D.	Negative	Windham et al., 1998
39 male formulators and 32 male applicators exposed to insecticides organochlorine, carbamates as propoxur, organophosphates as dichlorvos, dimethoate and malathion and pyrethroids as cypermethrin, D-allethrin, deltamethrin, and sumithrin, and 40 male controls (20 for each group)	CA	Egypt	5 to 15[1] 5 to 25[1]	Positive[c]	Amr, 1999
20 male workers exposed to pesticide mixture of chlorpyriphos, dibromochloropropene, fenamiphos, gramoxone, imalzabile, terbufos, and thiabendazole, and 20 male controls	CA	Costa Rica	N.D.	Positive[c]	Au et al., 1999
34 greenhouse workers (20 male and 14 female) exposed mainly to acephate, azocyclotin, benfuracarb, captan, chlorothalonil, dichlorvos, dicofol, dimethoate, endosulfan, fenopropathrin, iprodione, mancozeb, mathalaxyl, methiocarb, metiram, methomyl, procymidone, propineb, toclofos, methyl trichlorfon, and vinclozin, and 33 controls (17 male and 16 female)	MN (peripheral blood lymphocytes)	Italy	7 to 41[1] in sprayers with extensive contact, 8 to 27[1] and 2 to 26[1] in sprayers and others with less contact	Positive[a]	Falck et al., 1999
23 workers exposed to pesticide mixtures of carbamates and organophosphates, and 23 controls	CA	Brazil	0 to 16[1]	Positive[b]	Antonucci & de Syllos Colus, 2000
20 male workers exposed to the insecticides tamaron, orthene, nuvacron, folidol, endosulfan, lannate and vertimec, the bactericides agrimicin, primycin, microshield and recop; the fungicides manzate, benlate, dacosar, cercobin, folicur and curzate and the herbicides roundup, and sencor, and 16 male controls	CA	Brazil	10 to 40[1]	Negative	D'Arce & de Syllos Colus, 2000
10 workers (7 male and 3 female) employed in pesticide production simultaneously exposed to atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion, and 10 controls (7 male and 3 female)	CoA	Croatia	4 to 30[1] 22.2[2]	Positive (after period of high pesticide exposure)[c] Positive (but significantly decreased 6 months out of the pesticide exposure)[c]	Garaj-Vrhovac & Zeljezic, 2000

30 greenhouse floriculturist (22 female and 8 male) exposed to pesticide mixtures meanly organochlorine, organophosphates, and carbamates, and 30 controls (28 female and 2 male)	MN (exfoliated buccal cells), SCE	Mexico	1.5 to 10[1]	Positive[b]	Gómez-Arroyo et al., 2000
116 greenhouse workers exposed to a complex mixture of almost 50 insecticides, fungicides and growth regulators, and 29 non-pesticide exposed	CA	Denmark	N.D.	Negative (in preseason) Positive (after summer season)[c]	Lander et al., 2000
64 male greenhouse workers exposed to insecticides as abamectine, acrinathrin, buprofesin cyromazine, dichlorvos, endosufan, formetanate, midacloprid, malathion, methamidophos, methomyl, oxamyl, permethrin, pyriproxyfen, tebufenozide and tralomethrin, bactericides as kasugamycin, fungicides as carbendazim, cymoxanil, diethofencarb, mancozeb, nuarimol, fosetyl-aluminium, procymidone, propamocarb, and propineb, and 50 male controls	MN (peripheral blood lymphocytes and exfoliated buccal cells)	Spain	9.82±1.01[2]	Negative	Lucero et al., 2000
135 workers (83 non-smokers and 52 smokers) from organophosphorus pesticide industry and 111 control (65 non-smokers and 46 smokers)	SCE	India	1 to 24[1]	Positive[a]	Padmavathi et al., 2000
20 workers (17 male and 3 female) of pesticide production of atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion, and 20 controls (12 male and 8 female)	CA, MN (peripheral blood lymphocytes), SCE, CoA	Croatia	4 to 30[1]	Positive (after period of high pesticide exposure and 8 months out of the pesticide exposure)[c]	Garaj-Vrhovac & Zeljezic, 2001
49 male workers exposed to pesticides as the insecticides deltamethrin, dimethoate, methomyl, carbosulfan, lamba-cyhalothrin, cafenvalerate, pirimicarb, acetamiprid, diazinon, and the fungicides chlorothalonil, propamocarb, vinclozolin, iprodione, triforine, thiophanate, bupirimate, captan, among others, and 50 male controls	MN (peripheral blood lymphocytes and exfoliated buccal cells)	Poland	16.28±1.1[2]	Negative	Pastor et al., 2001a
50 agricultural workers (30 male and 20 female) exposed to complex mixtures, and 66 non-exposed (41 male and 25 female)	MN (peripheral blood lymphocytes and exfoliated buccal cells)	Greece	8.62±1.13[2]	Negative	Pastor et al., 2001b
104 greenhouse farmers exposed to pesticide mixture mainly organophosphates, carbamates, glyphosate, pyrethroids, triazoles, phthalamides, organochlonines, and phenoxyacetic acid, and 44 unexposed individuals	SCE	Israel	2.5 to 55.5[1]	Positive[a]	Shaham et al., 2001

20 workers (17 male and 3 female) of pesticide production simultaneously exposed to complex mixture of atrazine, alachor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion, and 20 controls (12 male and 8 female)	CA, CoA	Croatia	4 to 30[1] 22.5[2]	Positive (after period of high pesticide exposure and 8 months out of the pesticide exposure)[c]	Zeljezic & Garaj-Vrhovac, 2001
107 floriculturist (92 male and 15 female) of greenhouses and open field exposed to mixture mainly of organophosphates and carbamates, and 61 control subjects (42 male and 19 female)	MN (peripheral blood lymphocytes)	Italy	2 to 70[1] 27.8$\pm$15.5[2]	Positive[a]	Bolognesi et al., 2002
10 workers (7 male and 3 female) of pesticide production simultaneously exposed to complex mixture of atrazine, alachor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion, and 20 controls (12 male and 8 female).	CA, MN, (peripheral blood lymphocytes), CoA	Croatia	4 to 30[1] 22.5[2]	Positive[c]	Garaj-Vrhovac & Zeljezic, 2002
12 male applicators exposed to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), and 9 male controls	MN (peripheral blood lymphocytes)	USA	N.D.	Negative	Holland et al., 2002
39 male greenhouse workers exposed to pesticide mixture mainly carbamates, organophosphorus, and pyrethroids, and 22 male non-exposed	MN (peripheral blood lymphocytes)	Spain	8.31$\pm$1.12[2]	Negative	Pastor et al., 2002a
84 workers (58 male and 26 female) exposed in greenhouses and open fields to mixtures of insecticides, fungicides, and herbicides, and 65 controls (53 male and 12 female)	MN (peripheral blood lymphocytes and exfoliated buccal cells)	Hungary	18.75$\pm$0.89[2]	Negative	Pastor et al., 2002b
41 workers (28 male and 13 female) exposed to pesticides as aldicarb, fenamiphos, benomyl, captan, carbofuran, cypermethrin, deltamethrin, endosulfan, methyl bromide, among others, and 41 non-exposed (28 male and 13 female)	CA	Ecuador	6 to 66[1] 39.49[2]	Positive[b]	Paz-y-Miño et al., 2002
33 male workers exposed to pesticide mixtures such as pyrethroids, carbamates, heterocycles, and organophosphates, and 33 male controls	CoA	Turkey	1 to 23[1] 10[2]	Positive [b]	Ündeğer & Başaran, 2002
20 workers (17 male and 3 female) of pesticide production simultaneously exposed to complex mixture of atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion, and 20 (12 male and 8 female) controls	SCE	Croatia	4 to 30[1]	Positive (after period of high pesticide exposure and 8 months out of the pesticide exposure)[c]	Zeljezic & Garaj-Vrhovac, 2002
54 pesticide workers (42 male and 12 female) employed in a pesticide manufacturing mainly acephate, chlorpyriphos, phorate, fenvalerate, cypermethrin, monocrotophos, and dimethoate, and 54 controls (43 male and 11 female)	CoA	India	3 to 13[1] 8.57[2]	Positive[b]	Grover et al., 2003

19 male fruit growers exposed to captan mixed with other pesticides as fungicides, insecticides, and herbicides, and 1 male control	CoA	France	N.D.	Negative	Lebailly et al., 2003
247 agricultural workers (201 male and 46 female) exposed to complex mixtures of pesticides mainly carbamates, organophosphates, and pyrethroids, and 231 unexposed controls (194 male and 37 female)	MN (peripheral blood lymphocytes and exfoliated buccal cells)	Greece Spain Poland Hungary	8.62 ± 1.3^2 9.82 ± 1.03 16.28 ± 1.1 18.75 ± 0.89	Negative	Pastor et al., 2003
50 workers of greenhouse (30 male and 20 female) exposed to pesticide mixture mainly insecticides as buprofezin, cyromazine, dichlorvos, endosulfan, imidacloprid, malathion, mathamidophos, methomyl, oxamyl, permetryn, pyriproxyfen and tralometrin; bacetericides as kasugamycin and fungicides as carbendazin, cymoxalin, diethofencard, mancozeb, fosetyl-aluminium, procymidone, propamocard, and propined, and 66 controls (41 male and 25 female)	CoA	Greece	8.62 ± 1.13^2	Negative	Piperakis et al., 2003
52 floriculturists in greenhouses (86.2% male and 7% female) exposed to pesticide mixture mainly organophosphates, carbamates benzimidazoles, pyrethroids, tiophtalamides, pyrimidinol compounds, organochlorines, bypiridylics, amides, and morpholinics, and 24 controls (62.50% male and 9% female)	MN (peripheral blood lymphocytes)	Italy	26.35 ± 14.46^2	Negative	Bolognesi et al., 2004
10 female workers directly exposed to the fungicides imazalil and tiabendazol, and the insecticide chlorpyriphos, and 10 female controls	AC	Costa Rica	14^2	Positive[a]	Cuenca & Ramírez, 2004
11 farmers exposed to a mixture of metalaxyl and imidacloprid before and after spaying, and 11 controls	MN (peripheral blood lymphocytes)	Greece	$23.64. \pm 4.13^2$	Positive[c] (before and after spraying)	Vlastos et al., 2004
21 healthy newborns who preganacies developed without complications of urban areas; 12 healthy newborns from Mexico City whose pregnancies were without complications and the mothers no reported occupational exposure to toxic compounds, 16 whose mothers lived in agricultural areas, and 15 with mothers with high risk pregnancies	MN (umbilical cord blood and peripheral blood from the mothers)	Mexico	N.D.	Negative	Levario-Carrillo et al., 2005
64 female agricultural workers exposed to pesticide mixtures in thinning and pruning fruit trees in harvesting and packing fruits the pesticides most often used were carbamates, organophosphates, and pyrethroids, and 30 female controls	MN (peripheral blood lymphocytes)	Chile	8.0 ± 4.8^2	Positive[b]	Márquez et al., 2005

259 individuals were studied: 131 agricultural workers exposed to pesticides, 77 controls and 51 pesticide manager (30% female and 70% male)	CA, SCE, MN (peripheral blood lymphocytes), CoA	Bolivia	At least 5	Positive[c]	Ascarrunz et al., 2006
29 male workers involved in the pesticide manufacturing industry exposed to mixtures specifically organophosphates and pyrethroids, and 35 male controls	MN (peripheral blood lymphocytes), SCE	Pakistan	13.48 ± 3.48[2]	Positive[a]	Bhalli et al., 2006
52 floriculturist (37 male and 15 female) in greenhouse exposed to pesticide mixture mainly organophosphates, organochlorines, carbamates, and pyrethroids, and 38 controls (22 male and 16 female)	CoA	Mexico	2 to 48[1]	Positive[b]	Castillo-Cadena et al., 2006
33 farmers (17 male and 16 female) exposed to fungicides as benzimidazoles, azoles, pyrimidines, dithiocarbamates, triazines, insecticides mainly organophosphates, pyrethroids, carbamates and organochlorine, the rodenticide acrinathrin, acaricides as N-methyl carbamates and herbicides as phosphoglycines and ureas, among others, and 33 controls (17 male and 16 female)	CA, MN (peripheral blood lymphocytes), SCE	Portugal	0.5 to 48[1]	Positive (MN and SCE)[a] Negative (CA)	Costa et al., 2006
54 pesticide workers (12 female and 42 male) employed in a pesticide-manufacturing exposed simultaneously to a complex mixture of organophosphates, carbamates, and pyrethroids, and 54 controls (11 female and 43 male)	CA, MN (bucal epithelial cells)	India	3 to 13[1] 8.57[2]	Positive[b]	Sailaja et al., 2006
15 farm workers (3 female and 12 male) exposed to pesticides as endosulfan, chlorpyriphos, dimethoate, diazinon, and maleic hydrazide, and 10 controls (4 female and 6 male)	MN (peripheral blood lymphocytes)	USA	18.2 ± 1.3[2]	Positive[c]	Tope et al., 2006
11 farmers exposed to pesticide mixtures as abamectin, cypermethrin deltamethrin, dimethoate, fenthion, methamidophos, mathidathion, parathion, nenomyl, among others, and 11 controls	MN (peripheral blood lymphocytes)	Greece	25 to 60[1] 26.45 ± 3.38[2]	Negative	Vlastos et al., 2006
32 male exposed to pesticide mixture mainly to organoclhlorine, organophosphates, carbamates, pyrethroids, benzol ureas, among others, and 32 male controls	CA, MN (buccal epithelial cells), SCE	Turkey	34.5 ± 10.47[2]	Positive[c]	Ergene et al., 2007
29 male sanitation workers exposed to the insecticides a-cypermethrin, cypermethrin, deltamethin, temephos, malathion, fenitrothion, and the rodenticides brodifacum, coumachlor, coumafuryl, coumatetralyl, difethialone,	MN (peripheral blood lymphocytes)	Brazil	1.5 to 18[1] 5.28 ± 0.60[2]	Positive[b]	Kehdy et al., 2007

flocoumafen, difenacoum, bromadiolone, diphacinone, and pindone, and 30 male controls					
108 male vineyard workers exposed to pesticides mainly carbamates and organophosphates, and 65 male controls	MN (peripheral blood lymphocytes), CoA	Brazil	29.8±14.2[2]	Positive[b]	Da Silva et al., 2008
14 rural workers (12 male and 2 female) exposed mainly to glyphosate, cypermetrhin, and atrazine, and 12 controls (10 male and 2 female)	CA	Argentina	8 to 35[1]	Positive[c]	Mañas et al., 2009
29 male exposed to a complex mixture mainly organophosphates and pyrethroids, and 37 male non-exposed	MN (buccal epithelial cells)	Brazil	16.3±10.0[2]	Positive[b]	Bortoli et al., 2009
137 female and 137 male exposed to glyphosate	MN (peripheral blood lymphocytes)	Colombia	N.D.	Positive[c]	Bolognesi et al., 2009
70 agricultural workers (45 male and 25 female) exposed to pesticide mixtures mainly organophosphopus, carbamates and pyretroids and 70 non exposed (49 male and 21 female)	MN (buccal epithelial cells), SCE	Mexico	7[2]	Positive (SCE)[a] (MN)[b]	Martínez-Valenzuela et al., 2009
37 male pesticide appliers exposed to insecticides organophosphorus, carbamates, and pyrethroids, fungicides as cooper compounds, dithiocarbamates and azoles, and herbicides triazine, ureas, phosphanoglycine, bypyridilium, imidazolidones, chloronicotinyls, and 20 male controls	MN (buccal epithelial cells), CoA	Brazil	25.29±10.1 4[2]	Positive[b] (CoA) Negative (MN)	Remor et al., 2009
32 pesticide plant workers (18 male and 14 female) exposed to carbofuran, matalaxyl, and dodine, and 32 controls (18 male and 14 female)	CA	Croatia	1 to 36[1] 16.2±10.9[2]	Positive[a]	Zeljezic et al., 2009
108 vineyard male workers exposed to pesticides mainly bipyridyls, organophosphates, cooper sufates, carbamates, among others, and 65 male non-exposed	MN (peripheral blood lymphocytes), CoA	Brazil	More than 10	Positive[c]	Rohr et al., 2010
111 male workers exposed to methamidophos, malathion, methyl parathion, methomyl, propoxur, cypermethrin, atrazine, compounds bypiridylics, 2,4-dichlorophenoxyacetic acid, paraquat, glyphosate, among others, and 50 male controls	MN, CoA (buccal epithelial cells)	Mexico	1 to 57[1]	Positive[b]	Carbajal et al., Unpublished

Table 1. Cytogenetic biomonitoring studies by the use of chromosomal aberrations (CA), micronucleus (MN), sister chromatid exchanges (SCE) and comet assay (CoA) in human populations exposed to pesticides in different countries

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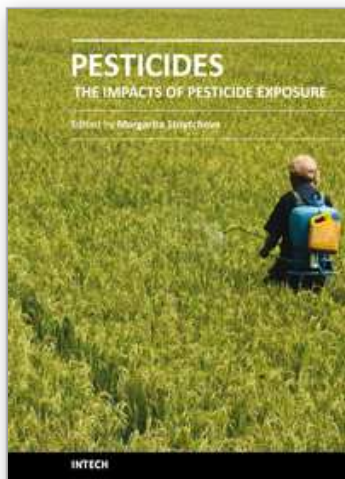
Pesticides - The Impacts of Pesticides Exposure

Edited by Prof. Margarita Stoytcheva

Pesticides are supposed to complete their intended function without â€œany unreasonable risk to man or the environmentâ€. Pesticides approval and registration are performed â€œtaking into account the economic, social and environmental costs and benefits of the use of any pesticideâ€. The present book documents the various adverse impacts of pesticides usage: pollution, dietary intake and health effects such as birth defects, neurological disorders, cancer and hormone disruption. Risk assessment methods and the involvement of molecular modeling to the knowledge of pesticides are highlighted, too. The volume summarizes the expertise of leading specialists from all over the world.

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