17

Agrochemicals: Horticulture Use Conditions Determine Genotoxic Effects and Oxidative Damage in Rural Populations in Santa Fe, Argentina

Marta Ana Carballo[1], María Fernanda Simoniello[1,2] and Elisa Carlotta Kleinsorge[2]

[1]*Cátedra de Toxicología, Farmacología y Bioquímica Legal. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Santa Fe.*

[2]*CIGETOX - Citogenética Humana y Genética Toxicológica – INFIBIOC, Departamento Bioquímica Clínica - Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Argentina*

1. Introduction

The horticultural productivity in the subtropical regions of the world is severely limited by the pests and diseases affecting crops. The losses in the field and the reduction of the commercial values of the products caused by pests and diseases make the horticultural business less profitable than expected. The fact that the quality of the products has become a priority worldwide has led to the generation of a group of quality standards in response to the demands of the consuming market. The main criterion used regarding this issue is related to the visual aspect related to the shape, the color and absence of damages. The use of agrochemicals is the most common method used for the control of pests and diseases but also one of the most important factors affecting natural resources as well as the health of the rural workers and potential consumers.

Horticultural activity in Santa Fe Province, Argentina, has low participation respect other crops: 4.9 % to field and 1.2 % in crops under cover, being leafy vegetables, cruciferous crops and other crops such as tomato and pepper some of the most relevant (Giunta et al., 2004; 2005).

The so-called "Cinturón hortícola Santafesino" (horticultural belt of Santa Fe) contributes 1.2% to the national market of products. This productive zone is in The Capital Department of Santa Fe Province (Argentina), an area constituted of approximately 3100 Hectares (ha), 1200 ha of which are cultivated in intensive form, and where 2000 people are employed as temporary workers, according to the season. Historically, the horticulture has been an agricultural activity of great economic importance for the region. Nevertheless, throughout time, and considering the low productivity, the technical lag and climatic phenomena such as floods and hail, the activity has modified its principal parameters.

The number of producers decreased from 350 in 1980 to about 150 in 2008. Since 2006, the establishments of productive activity (EAP) with larger surface (more than 50 ha) have

tended to replace the horticultural activity by the cultivation of soybean, maize, alfalfa and wheat.
At present, the horticultural production is focused on leaf-vegetable crops, mainly lettuce, chicory and arugula (22%), spinach beet and spinach (13%), cabbage, broccoli and cauliflower (13%), tomato and pepper (15%), zucchini, gourd, and cucumber (10%) and other vegetables such as beet, radish, aubergine, leek and parsley in a much smaller percentage.

2. Use of pesticides

Horticulture is characterized by a more intensive use of agrochemicals by unit of area or surface than other types of agricultural production. A high-risk crop, such as tomato, receives nearly 40 treatments with insecticides and fungicides along its process of development. The application often includes three active simultaneous or sequenced ingredients (Castignani et al., 2004).
Tomato productivity is severely limited by plagues and diseases. Performance losses have been detected in tomato attacked by the red mite, *Aculops licopersisci* (Lin et al., 1999), the white fly, *Trialeurodes vaporariorum* (Lei et al., 1998), and the tomato moth (Miranda et al., 1998). The most known diseases are those caused by Verticillium and Fusarium. In addition, there are viruses that can cause plant death.
The most common pesticides used in tomato crops are Mancozeb, Cypermethrin, Deltamethrin, Buprofezin, Imidacloprid, Chlorpyrifos, Methamidophos and Copper oxychloride. By analyzing the results of our cross-country research through a survey of producers, we determined that most of the EAP use different types of biocides alone and that the application of fertilizers is scanty. This agrees with that found in other researches carried out in the same area, which show that 57% of the producers use only pesticides, and that the remaining 43% applies credit foliate, urea, adherents, and so on (Rodriguez & Lenardon, 2007).
The most frequently used biocides are insecticides, nematicides and acaricides (57%; Methamidophos, Chlorpyrifos, Cypermethrin, Lambdacialotrin, Chlorfenapyr, Carbofuran, Imidacloprid), herbicides (35%; Glyphosate, Trifluralin, Linuron), and fungicides (8%; Mancozeb, Zineb, Copper oxychloride) (Castignani et al., 2004; Rodriguez & Lenardon, 2007; Simoniello et al., 2008). Many of these agrochemicals are prohibited in developed countries such as the United Kingdom, the U.S.A. and China.

3. Pesticide mixture conflicts

The current social conditions of agrochemical use are far from laboratory conditions which determine their safety. In general, agrochemicals are rarely applied with suitable protection equipment. As a consequence, subjects who work and/or live near vegetable crops usually suffer from pesticide-induced illnesses, generally considered as conditions typical of their daily lives, since they do not lead to a significant incapacity for work.
Toxicity of pesticides, expressed by the LD50, is reported only for individual products and not for mixtures. It is widely known that active ingredients, when combined, can increase their individual ability to cause damage or generate new kinds of damages.
The vast majority of toxicological studies of chemicals have focused on the evaluation of exposures to single compounds. Humans are exposed to complex and variable mixtures of

chemicals, which may act independently as in a single exposure, but may also interact to modulate the effects of the mixture as a whole and the components therein. The risk assessment of real-life exposures is thus much more difficult than that of exposure to single agents. In assessing such risks from a public health perspective, it is necessary to assess whether the chemicals in a mixture interact to cause either an increased or a different overall response as compared with the sum of the responses of the individual chemicals present in the mixture, or whether the overall effect is simply a summation of the expected effect of each chemical (Hughes & Wood, 2002).

Two basic methodological strategies exist to study the toxicology of mixtures: component interaction analysis and whole mixture analysis. Component interaction analysis (bottom-up approach) can be applied to the analysis of simple mixtures with a small number of constituents and where the composition is clearly known. In the absence of specific knowledge of the composition of a mixture, or where there are numerous components (a complex mixture), whole mixture analysis may be more appropriate. However, such studies cannot define the extent of true interactions between components of the complex mixture without data on the fractions of the mixture (Carpenter et al., 2002).

When using a metabolite as a quantitative indicator of exposure it is important to be aware that various factors can affect the proportion of compound being metabolised by a particular route and therefore the amount of metabolite appearing in blood or urine. For example, chemicals other than the compound in question may either induce or inhibit cytochrome P450, which is involved in the metabolism of many chemicals including organophosphorus (OP) pesticides. Furthermore, if the hydrolytic pathways, important in the detoxification of OP pesticides, are inhibited, this can increase the toxicity although the excretion of dialkylphosphate metabolites (often used as biomarkers of exposure) may be lower. Also, genetic factors and age may influence metabolism. For example, the elimination of drugs may be lower in neonates and young children than in adults. The enzymes which can hydrolyse OPs (esterases) show a ten-fold variation in activity in humans and the main enzyme involved exhibits a genetic polymorphism. Thus, although measurement of a single metabolite may indicate that exposure has occurred, using it for precise exposure quantification may not always be appropriate or possible.

Biological monitoring and biological effect monitoring have been little used to study combined effects of exposures to pesticide mixtures. Measurement of pesticides or their metabolites in asymptomatic populations provides no information on the combined effects of pesticides, even if parent compounds or specific metabolites are measured in biological fluids. Group-specific metabolites are measured, as with OPs, much more frequently, and these are difficult to relate to the toxicity of specific pesticides. On the other hand, studies in human milk and fat surveys (Lenardon et al., 2000; Trossero et al., 2009) show that simultaneous exposure to more than one pesticide clearly occurs. A further limitation of biomonitoring is that strategies for biomonitoring the exposure are still strongly influenced by the availability of suitable biomarkers. In fact, for many pesticides, there are none. The alternative of biological effect monitoring may be more promising for the study of combined effects, when new techniques become more widely available. The present methods of biological effect monitoring are rather insensitive.

Humans are often exposed to different pesticides or pesticide mixtures, either simultaneously or in series, making it difficult to identify the effects of each one separately. Chronic exposure to pesticides involves exposure to complex mixtures of different types of chemicals, active ingredients and by-products, such as impurities, solvents and other

compounds produced during the storage procedure, present in technical formulations. Moreover, although inert ingredients have no pesticide activity, they may be biologically active and sometimes the most toxic component of a pesticide formulation.

It is important to consider that each active ingredient has a specific mode of action for controlling a pest, and has its own possible side effects on the wild-life and humans exposed to it. Dangerous effects of pesticides in the environment have been documented in many investigations, on soil microorganisms and aquatic flora and fauna. Occupational exposure to pesticides may increase the risk for adverse reproductive outcomes, brain and nervous system disturbances, may cause immunodepression and lead to cancer in later life and can also induce heritable changes. Three million cases of pesticide poisoning, about 220,000 of which are fatal, occur world-wide every year (Raipulis et al., 2009). The activities or circumstances in horticultural work that cause the greatest number of accidents are those that involve the preparation or application of agrochemicals (48%), and those that take place when using tractors and agricultural machinery, carrying boxes, and/or repairing greenhouses (13% each). Of those interviewed in a survey argentina, 13% did not provide an answer in relation with this issue. With regards to the place where the accidents took place, they found that 50% were in the greenhouse, 25% in the open field, and 25% in the shed. As to the body areas affected, in 52% of the cases, intoxication with agrochemicals affected the body in general, whereas in 13% of the cases, lesions were produced in the eyes, and 7% in other body areas (Paunero et al., 2009).

Genotoxicological biomonitoring of human populations is a useful tool to estimate the genetic risk posed by an integrated exposure to complex mixtures of chemicals. Cytogenetic studies refer to different typology of exposure and provide different information about the genetic risk associated with pesticide exposure. Few studies are available on acute pesticide exposure in poisoned subjects. The large majority of cytogenetic monitoring studies in human populations exposed to pesticides concern the genotoxic effects of chronic low doses of a single compound or of a complex mixture of chemicals (Bolognesi et al., 2003).

4. Biomonitoring and biomarkers

Human biomonitoring depends on the use of biomarkers, defined as quantitative indicators of molecular and cellular events in biological systems, relevant to human health, development and aging. Biomarkers are measured in biological material (generally blood or urine) collected from patients or volunteer subjects in observational or intervention studies (Collins & Dusinska, 2009). The molecular epidemiological approach, which measures molecular or cellular biomarkers as indicators of disease risk or of exposure to causative or preventive factors, has applications in studies of environmental and occupational exposure, disease etiology, nutrition, lifestyle, and so on. It is a valuable adjunct to conventional epidemiology, and has the advantage that it requires far fewer subjects and much less time (and is therefore more economical) than the conventional approach. In addition, the biomarkers, if carefully chosen, can give useful information about the molecular mechanisms involved in disease etiology, for example if they reflect an early stage in the progression of the disease (Collins & Dusinska, 2009).

To help planning a biomonitoring study, it is important to consider the following issues:

- it is always necessary to obtain an ethical approval;
- the sampling of subjects should be performed in the same way throughout the study;

- it is fundamental to be aware of the possibility of 'seasonal effects' and thus collect samples from both the controls and the exposed/treated subjects at the same time, rather than in consecutive phases;
- it is necessary to carry out a pilot study for every critical aspect in order to check for unforeseen problems and to assess experimental variation (and, if possible, control each biomarker evaluated);
- it is essential to use the same protocols and chemicals from the same company and avoid making any change in procedure, however slight it may seem, while carrying out a particular study;
- it is required to follow the principles of Good Laboratory Practice, as far as possible (Dusinska & Collins, 2008).

A working group formed by IARC (1997) has defined biomarkers as ''any substance, structure or process that can be measured in the body or its products and may influence or predict the incidence or outcome of disease''. This definition is further extended by the definition of WHO-ICPS (1993).

The primary purpose of using biomarkers of effect is surveillance, i.e., the identification of individuals or a population at risk of adverse health effects, so that preventive measures can be taken. Although a biomarker of effect is usually also related to exposure to a specific chemical, it is generally more closely related to the occurrence of an adverse health effect (De Zwart et al., 1999).

The selection of appropriate biomarkers is of critical importance because of the opportunity for greater precision in the assessment of risk in individuals or population sub-groups, with the consequent implications for mitigation and health protection. However, this selection will depend upon the state of scientific knowledge and be influenced by social, ethical and economic factors. The process of selection and validation requires careful consideration of the specificity and sensitivity of the biomarker as a measure of the contribution of the exposure to an observed adverse health outcome. A similar process must also be applied to establish the accuracy, precision and quality assurance of the analytical procedure for measuring the selected biomarker, evaluating the intra- and inter-individual variation for a non-exposed population, and reviewing ethical and social considerations. Subject to ethical considerations, the use of validated biomarkers to monitor exposed populations may provide the basis for early, health-protective intervention (EHC 155, 1993).

Sampling both exposed (treated) and control (reference) individuals on the same day reduce the likelihood of day-to-day experimental variation influencing results. This may not be feasible; but what should be definitely avoided is collecting samples from all exposed subjects and then from all controls (or vice versa), over different time frames (Dusinska & Collins, 2008).

A number of factors have been used to describe pesticide exposure in cytogenetic studies: pesticide consumption (kg per year), amount of toxic chemicals used, total number of pesticide formulations used, extension of the areas of pesticide application, and working conditions (greenhouse versus open field), exposure magnitude, the use of protective measures and the specific genotoxic potential of the pesticides used. In addition, the crop type and the environmental factors can influence the kind of pesticide formulations used as well as the chemical absorption. Also, the complex combination of formulations used depending on the region and season, the sample size, the exposure times and intervals after the exposure mainly in relation to the samplings, represents major factors of uncertainty in the comparison of results from different studies (Bolognesi, 2003).

A biomarker of effect can be objectively measured and evaluated as an indicator of normal biological or pathological processes, or toxicological responses to a chemical exposure. The most reliable biomarkers of effect are mechanistically based. The measurement of such biomarkers forms the basis of biological effect monitoring. Some biomarkers can be used as surrogate endpoints. These can substitute for a clinical endpoint, and should be able to predict clinical outcome.

Sensitive biomarkers of effect offer considerable potential for use in studies of individuals exposed to low levels of pesticides and may be invaluable as a bridge between studies in experimental animals and studies in humans and between those in cultured cells and in the intact organism. Much effort is now being devoted to the application of modern biological methods, including transcriptomics, proteomics, metabonomics and non- or minimally-invasive imaging, to identify and develop effective biomarkers of effect. Examples of the application of this approach have been published recently (Petricoin et al., 2002; Issaq et al., 2002). Any accessible biofluid or tissue can be used for biomarker assessment. Techniques now available offer high sensitivity and are applicable to a broad range of endpoints. However, for use in studies of pesticide interactions, it will be important to establish the mechanistic relationship between biomarkers of effect identified in this way and biological responses of concern. Adequate validation, demonstrating their reproducibility and reliability, will be necessary before adopting their widespread use in the study of the toxicology of mixtures.

5. Acetylcholinesterase and Butyrylcholinesterase

The existence of two types of cholinesterases has been proved: acetylcholinesterase (AChE), or 'true cholinesterase', which is found in erythrocytes and in cholinergic nerve terminals; and butyrylcholinesterase (BChE), or pseudocholinesterase, found in plasma, liver, smooth muscle and fat cells. It is well known that AChE can be an effect biomarker of organophosphorous (OP) and methyl-carbamic (MC) compounds. Also, there is evidence that AChE inhibition correlates with OP-induced symptoms of toxicity (Ranjbar et al., 2002).

The inhibition resulting in the accumulation of endogenous acetylcholine responsible for toxicity in the nervous system presents a dose–response pattern of relatively mild symptoms at a 50–60% inhibition of AChE, with weakness, headache, dizziness, nausea and salivation and a convalescence of 1–3 days. Moderate symptoms at 60–90% inhibition are reversed within periods of a few weeks and are characterized by sweating, vomiting, diarrhea, tremors, disturbed gait, pain in the chest and cyanosis of the mucous membranes. At 90–100% inhibition, the prognosis is death from respiratory or cardiac failure.

Biological effect monitoring could be an important component to study the interactive effects of pesticides and related compounds. To be effective, the biomarker should reflect a response (e.g. inhibition of AChE) that is common to several components of a mixture (e.g. OPs). This may be a more meaningful parameter than the measurement of a metabolite common to compounds of differing potencies. Biomarkers of effect in current use lack sensitivity. For example, alkyl phosphates can be detected in the urine of individuals after exposure to amounts of OP well below those causing depression of AChE activity, although this may also reflect the concentration-effect relationship that exists for such compounds (Moretto & Lotti, 1998).

Measurements of AChE activity in red blood cells have routinely been performed to survey exposures to OPs in exposed environments. It has also been established that if AChE activity

(based on individual pre-exposure level – baseline) decreases by 25%, a second measurement has to be carried out, and that if a decrease in AChE activity is confirmed, exposure has to be avoided for 14 days (Knudsen & Hansen 2007). Historically, the measurement of both cholinesterases in plasma and erythrocytes, which reflected the influence of absorbed OPs on the inhibition of these blood enzymes as surrogates of AChE in neural tissue and neuromuscular junctions, was carried out (Cocker et al., 2002). However, it is well recognized that this is a relatively insensitive indicator of an absorbed dose of OP (Reid & Watts, 1981; Drevenkar et al., 1991; Nutley & Cocker, 1993; Hardt & Angerer, 2000). Blood cholinesterase activity needs at least 15% depression from an individual's normal level of plasma or erythrocyte enzyme activity to be considered indicative of pesticide over-exposure.

Furthermore, due to the large inter-individual variability in cholinesterase activity, this approach requires the collection of both baseline and post-exposure samples from an individual and long-term precision of the methods as this directly influences the level of BChE or AChE depression that can be considered significant (Mason & Lewis, 1989).

In addition, the collection of blood samples is sometimes considered invasive and, in some occupational settings, logistically difficult. BChE and AChE measurements have been used for a number of years in cases of clinical poisoning and accidental OP exposure, and in monitoring workers with high risk of exposure. Depression of the plasma BChE enzyme activity is not necessarily associated with symptoms of anti-cholinergic toxicity and large depressions in BChE have been noted in the absence of any effect on erythrocyte AChE. Decreases in the red cell enzyme activity have been suggested to have closer relations to these symptoms. Therefore, in both clinical toxicology and monitoring high-risk occupational activities, the measurement of both enzymes has been recommended (HSE, 2000; Heath & Vale, 1992).

6. Oxidative status

Oxidative stress is a mechanism that could link pesticide exposures to a number of health outcomes observed in epidemiological studies. In blood, normal erythrocyte function depends on the intactness of cell membrane, which is the target for many toxic factors including pesticides (Banerjee et al., 1999).

Free radicals are generally very reactive molecules possessing an unpaired electron. They are produced continuously in cells either as by-products of metabolism, or for example, by leakage from mitochondrial respiration. The most important reactions of free radicals in aerobic cells involve molecular oxygen and its radical derivatives (superoxide anion and hydroxyl radicals), peroxides and transition metals. Cells have developed a comprehensive set of antioxidant defense mechanisms to prevent free radical formation and to limit their damaging effects. These mechanisms include enzymes that inactivate peroxides, proteins that sequester transition metals and a range of compounds that scavenge free radicals. Reactive free radicals formed within cells can oxidize biomolecules and this may lead to cell death and tissue injury (De Zwart et al., 1999).

Continuous exposure of aerobic organisms to prooxidant challenges has endowed living cells with efficient and sophisticated antioxidant systems. These can be divided into enzymatic antioxidant and non-enzymatic antioxidant systems. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHpx) have been distinguished as the most important members of the enzymatic defense systems against oxygen radicals.

Obviously, assaying these enzymes can offer an indication of the antioxidant status of an individual. Besides measuring the enzymatic antioxidant systems in blood samples, non-enzymatic antioxidants, such as vitamin E and C, b-carotene, urate, retinyl esters and GSH, can be monitored as well (Jaeschke, 1995). The available data on experimental animals and humans, obtained both from *in vitro* and *in vivo* studies, indicate that the enzymes associated with antioxidant defense mechanisms are altered under the influence of pesticides (Barerjee et al., 1999, Ranjar et al., 2002, Gultekin et al., 2001).

Oxidative stress plays an important role in the toxicity of various xenobiotics, including pesticide mixtures. Lipid peroxidation is probably the most extensively investigated process induced by free radicals. The abundant presence of membrane phospholipids at sites where radicals in general and, more specifically, reactive oxygen species are formed, render them easily accessible endogenous targets rapidly affected by free radicals. The extent of lipid peroxidation in whole blood was evaluated by measuring the formation of thiobarbituric acid reactive substances (TBARS). The higher oxidative stress in pesticide sprayers is evidenced by an increased concentration of TBARS in plasma and red blood cells, changes in antioxidant status, and altered activities of cellular enzymes. The increased concentration of TBARS observed could be due to the increased peroxidation of membranes. However, oxidative stress is a balance between free radical production and antioxidant activity, and it is possible that the increased TBARS are due to a decreased antioxidant activity (Prakasam et al., 2001).

7. Comet assay

The Single Cell Gel Electrophoresis (SCGE) or Comet assay is a very sensitive method for measuring DNA strand breaks in individual cells. The assay is now a well-established, simple, versatile, rapid, visual, sensitive, and extensively used tool to assess DNA damage and repair, both quantitatively and qualitatively in individual cell populations (Dusinska & Collins, 2008).

The version of the Comet assay developed by Singh et al. 1988, electrophoresis under highly alkaline conditions (pH>13), has been found to be up to two orders of magnitude more sensitive than neutral version (Ostling & Johanson 1984). This enables the DNA supercoils to relax and unwind and allows the detection of alkali-labile sites and single-strand breaks in DNA during electrophoresis. This method measures low levels of strand breaks with high sensitivity.

The simplest types of DNA damage detected by the Comet assay are double-strand breaks (DSBs). DSBs result in DNA fragments and can be detected by merely subjecting them to electrophoretic mobility at neutral pH. Single-strand breaks (SSBs) do not produce DNA fragments unless the two strands of the DNA are separated / denatured. This is accomplished by unwinding the DNA at pH 12.1. It is also possible that single-strand breaks can relax the DNA and hence can also be detected with the Comet assay at neutral pH. Other types of DNA damage broadly termed alkali-labile sites (ALS) are expressed when the DNA is treated with alkali at a pH greater than 13. Breaks can also be introduced at the sites of DNA base modifications by treating the DNA with lesion-specific glycosylases / endonucleases and the fragments thus produced can also be detected by the Comet assay.

At the same time, by controlling the conditions that produce nicks at the sites of specific DNA lesions, the Comet assay can be used to detect various classes of DNA damage. While breaks increase DNA migration, DNA binding and crosslinks can retard DNA migration

and can also be detected by the Comet assay. Therefore, increased migration in the Comet assay can be attributed to strand breaks, alkali-labile sites and incomplete excision repair sites, while decreased DNA migration could be attributed to crosslinks, DNA–DNA or DNA–protein interactions. Some other lesions of DNA damage such as DNA cross-linking (e.g. thymidine dimers) and oxidative DNA damage may also be assessed using lesion-specific antibodies or specific DNA repair enzymes in the Comet assay.

The assay can be performed both *in vivo* and *in vitro* in a variety of samples. Peripheral blood lymphocytes, nasal and buccal epithelial cells have extensively been used to assess human genotoxicity in clinically or occupationally exposed population (Valverde et al., 1997). Also, *in vitro* studies have been conducted in cell lines and primary cell cultures for environmental biomonitoring using fish, earthworms and molluscs (Akcha et al., 2003). The *in vivo* assay with different tissues and organs from mice has also been used (Sasaki et al., 2000) for both DNA damage and repair and widely used in genetic toxicology (Dhawan et al., 2002), human epidemiology (Dhawan et al., 2001; Bajpayee et al., 2002), monitoring of human genotoxicity (Kassie et al., 2000; Palus et al., 2003; Basaran et al., 2003; Piperakis et al., 2003), patients undergoing radio/chemotherapy (Vaghef et al., 1997), and aging (Piperakis et al., 1998; Singh et al., 2003). Also, the *in vivo* assay has been used to monitor the dietary factors in various diseases such as diabetes (Raslova et al., 2000; Pitozzi et al., 2003) and thalassemia (Anderson et al., 2001; Ruf et al., 2003).

Single Cell Gel Electrophoresis has gained wide acceptance as a valuable tool in fundamental DNA damage and repair studies, genotoxicity testing and human biomonitoring. Human blood cells are particularly useful for biomonitoring purposes as they are easily acquired (Dusinska & Collins, 2008).

The biochemical changes induced after exposure to pesticides or their active metabolites include target cell/receptor binding, protein and DNA adduct formation, and induction or inhibition of enzymes (Lopez et al., 2007). DNA damage and oxidative stress have been proposed as mechanisms that could mechanistically link pesticide exposures with a number of health outcomes observed in epidemiological studies (Muñiz et al., 2008).

8. Biomonitoring of pesticide-exposed workers: DNA Damage (Part A)

Study population.

The Regional Ethical Committee established the regulations for the development of the study and informed consent was given by each individual prior to the beginning of the study. A face-to-face questionnaire was completed to obtain information on a) standard demographic data (age, gender, etc), b) individual lifestyle (diet, smoking habit, alcohol and medicine consumption), c) occupational aspects (working hours/days, years of exposure to pesticides, use of protective measures, etc), and d) pesticides used.

The study involved 84 subjects divided into three groups. The first group consisted of 27 pesticide sprayers and applicators and the second group of 27 agricultural workers and farmers. The control group consisted of 30 workers, from the same area, with no history of occupational exposure to pesticides or any potential genotoxic agent. Peripheral blood heparinized samples were obtained from all the subjects involved in the study.

Cell Viability using Fluorescent Dyes.

A cell suspension was mixed with fluorescent DNA-binding dyes an examined by fluorescent microscopy to visualize and count cells with aberrant chromatin organization

(Mercille & Massie, 1994). This mixture was examined with a 40 x objective using a fluorescent microscope. A minimum of 200 total cells was counted, recording the number of viable cells (V) and nonviable cells (NV). The percentages of each of these cellular states in relation to the total number of cells were obtained (Simoniello et al., 2008).

Alkaline Comet Assay.

The standard procedure originally described by Singh et al. (1988) was used with minor modifications. Two slides were processed for each sample, including negative and positive (H_2O_2 50 μM) controls. DNA strand breaks were measured with the Comet assay. One hundred randomly selected Comet assays from each of two duplicate gels were analysed visually on a scale of 0–4 (categories depending on DNA damage level). The overall score, between 100 and 400 arbitrary units, is related to the DNA break frequency and a comet-like image indicates the presence of DNA breaks (Simoniello et al. 2008). The Damage Index Comet Assay (DICA) was calculated.

DNA repair assay.

We tested lymphocytes for their resistance to oxidative DNA damage using Bowden et al. (2003), with modifications. Briefly, aliquots of cells were resuspended in RPMI 1640 medium. A cell suspension were mixed with hydrogen peroxide solution and and was generated oxidative damage. The reaction was quenched using DMSO solution in PBS. Each cell sample was centrifuged, washed again, and resuspended in RPMI 1640 medium supplemented with fetal bovine serum at 37 ºC for 30 min. The remainder of the alkaline Comet assay procedure was performed as previously reported. The Damage Index Repair Assay (DIRA) was calculated (Simoniello et al., 2008).

Part A, results.

The demographic characteristics were similar in the three groups evaluated, except for the occupational exposure (Table 1).

Parameter	Controls (n=30)	Pesticide Applicator Workers (n=27)	Non-pesticide applicator Workers (n=27)
Age (X±S.D.)	37.70±14.07	40,20±11,44	34,06±12,60
Gender (n)(%)			
Female	14 (47)	8 (30)	15 (44)
Male	16 (53)	19 (70)	12 (56)
Smoking (n)(%)			
Yes	7 (23)	3 (11)	6 (22)
No	23 (77)	24 (83)	21 (78)
Alcohol (n)(%)			
Yes	15 (50)	16 (59)	14 (52)
No	15 (50)	11 (41)	13 (48)

Table 1. Demographic characteristics of controls and exposed workers.

A summary of the pesticides most commonly used in horticulture zone, the CAS number, the IARC classification and the US EPA and WHO hazard classification is presented in Table 2.

Pesticides	Compound	CAS Number	Chemical Class	IARC	US EPA	WHO
Fungicide	Captan	133-06-2	Thiophthalimide	3	NL	U
	Copper	7440-50-8	Inorganic-Copper	NL	D	NL
	Mancozeb	8018 01 7	Dithiocarbamate-Inorganic Zinc	NL	B2	U
Insecticide-Nematicide	Chlorpyrifos	2921-88-2	Organophosphorus	NL	E	II
Insecticide	Cypermethrin	67375-30-8	Pyrethroid	NL	NL	II
	Dimethoate	60-51-5	Organophosphorus	NL	C	II
	Endosulfan	115-29-7	Organochlorine	NL	NL	II
	Imidacloprid	105827-78-9	Chloro-nicotinyl	NL	NL	II
	Malathion	121-75-5	Organophosphorus	3	Suggestive	III
	Methamidophos	10265-92-6	Organophosphorus	NL	E	Ib
	Parathion	56-38-2	Organophosphorus	3	C	Ia
	Permethrin	54774-45-7 51877-74-8	Pyrethroid	3	Suggestive	II
Herbicide	Glyphosate	1071-83-6	Phosphonoglycine	NL	NL	III

Table 2. List of pesticides most commonly used (questionnaire answers) by the exposed subjects, CAS number, IARC classification, US EPA classification, and WHO hazard classification. **IARC Classification**: 3: Not classifiable as to carcinogenicity to humans; NL: Not Listed. **US EPA Classification: Group B:** Probable human carcinogen; **B2**: Sufficient evidence of carcinogenicity from animal studies; **Group C**: Possible human carcinogen; **Group D**: Not classifiable as to human carcinogenicity; **Group E**: Evidence of non-carcinogenicity to humans. **WHO hazard classification: Ia:** Extremely hazardous; **Ib**: Highly hazardous; **II**: Moderately hazardous; **III**: Slightly hazardous; **U**: Unlikel to pose an acute hazard in normal use.

Both exposed groups revealed a significant increase in DICA when compared to controls ($P < 0.0001$; Table 3). Sensitivity of lymphocytes to oxidative damage by H_2O_2 *in vitro*, indicated by strand breakage evaluated by the repair assay, showed significant increases in DIRA in both cases when compared to controls ($P < 0.0001$; Table 3).

Individuals	Comet Assay (DICA)	Repair Assay (DIRA)
Control (n=30)	113.20±13.68	116.24±12.49
Pesticide applicators workers (n=27)	215.29±15.06*	218.22±20.89*
Pesticide non-applicators workers (n=27)	221.66±19.95*	224.14±19.99*

Table 3. Damage Index in Comet Assay and Repair Assay in control and exposed workers. Values are mean ±S.D. DICA: Damage Index Comet Assay, DIRA: Damage Index Repair Assay, P< 0.05. *t*-Test

When the Damage Index was analyzed with the Comet assay and the Repair assay in pesticide sprayers, no statistically significant differences were observed in relation to confounding factors such as age, gender, smoking and alcohol consumption. However, this

group exhibited a marginally significant difference in DICA when the years of exposure were analysed by the Mann Whitney's test (P = 0.05). At the same time, a significant difference (P < 0.05) was detected when the individual's protection was used as a comparison factor.

Also, in relation to the Damage Index obtained by the Comet assay and Repair assay for the non-exposed workers, no statistically significant differences (P > 0.05) were detected in relation to confounding factors such as age, gender, smoking, alcohol consumption, working years.

Figure 1 shows the behaviour of individual groups when analyzed before and after repair treatment. We observed an important difference between the exposed groups (both) and the control (P < 0.0001). The efficiency of leukocytes in repairing damaged DNA was assessed together with the evaluation of DNA damage, using the Comet assay. Moreover, using paired samples t-Test, the DNA repair efficiency for each group was estimated as the number of people in whom the damaged DNA was not reduced during incubation at 37 ^{o}C for 30 minutes (P > 0.05).

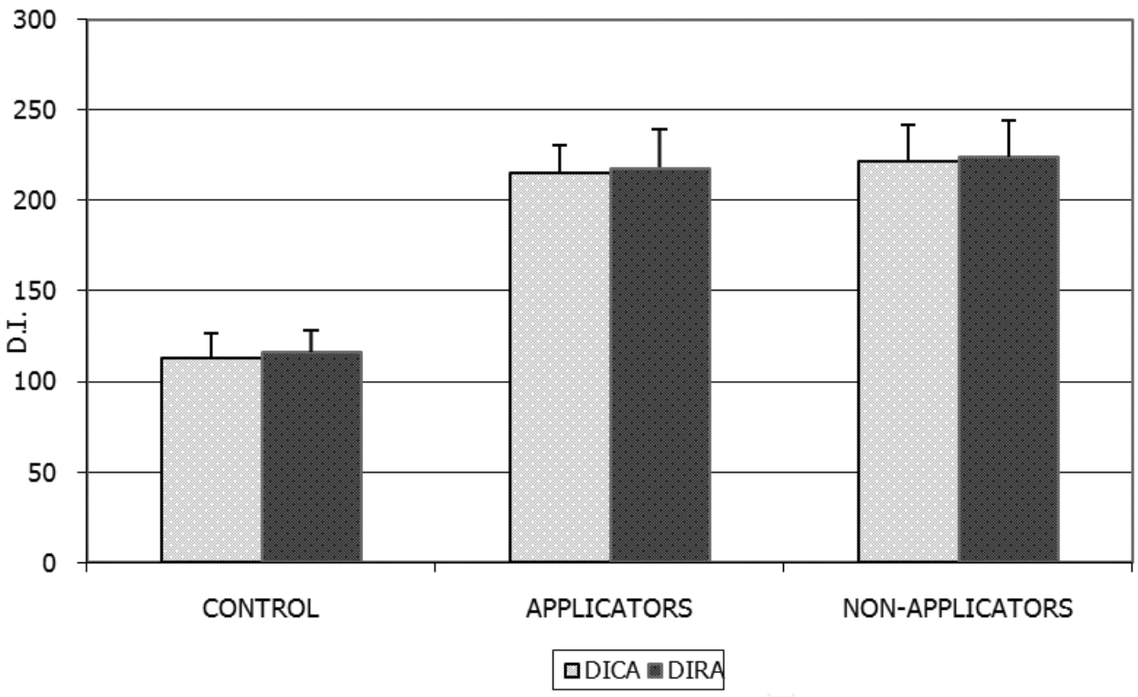


Fig. 1. Damage Index in Exposed (applicators and non-applicators) and control people using the Comet Assay and Repair Assay. DICA: Damage Index Comet Assay, P<0.0001 (ANOVA). DIRA: Damage Index Repair Assay, P<0.0001 (ANOVA)

Taking these results into account, we decided to consider both exposed populations as one. Figure 2 exhibits box-plots showing DICA (Damage Index in Comet Assay) and DIRA (Damage Index in Repair Assay) in control and all pesticide-exposed workers. In both cases, we observed significant differences (P < 0.0001) when compared to controls (analysed with t-Test).

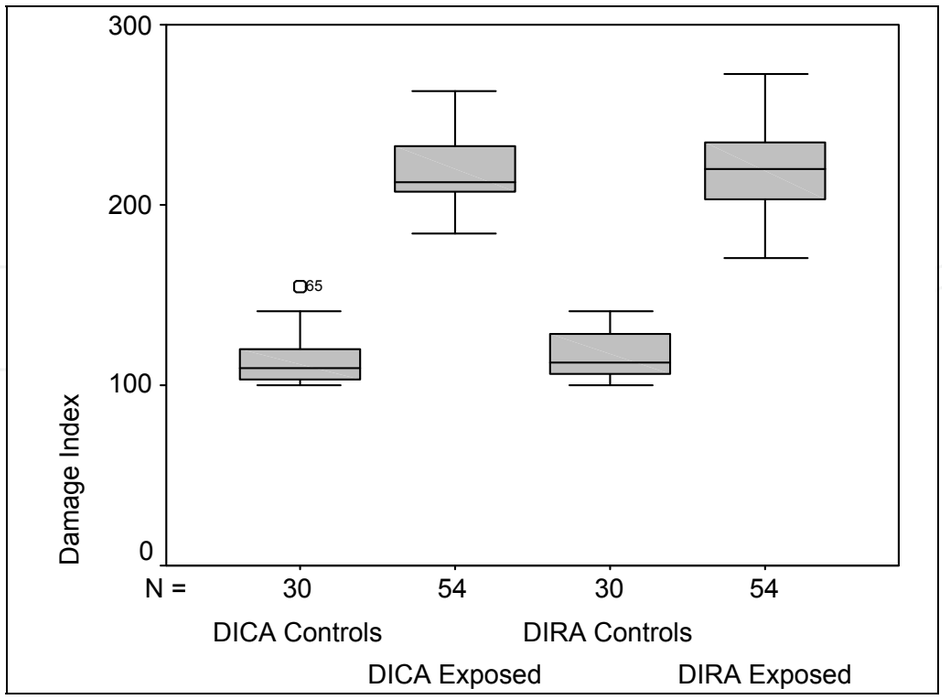


Fig. 2. Boxplots showing the Damage Index Comet Assay (DICA) and Damage Index Repair Assay (DIRA) in control and pesticide-exposed workers. Boxes are limited by 1st and 3rd quartiles divided by the median; thin vertical lines represent minimum and maximum values except when outliers (o) are present.

9. Biomonitoring of pesticide exposed workers: Markers of oxidative stress and genotoxicity (Part B)

Study subjects.

The Provincial Hospital Ethical Committee established the regulations for the development of the study. A face-to-face questionnaire was completed to obtain information on: standard demographic data, individual lifestyles, occupational aspects and pesticides used. The study involved 124 subjects divided into three groups: a group consisting of 18 pesticide sprayer workers directly exposed to pesticides, a group consisting of 23 non-applicator agricultural workers and farmers indirectly exposed to pesticides, and a control group consisting of 82 people from the same area without current or previous exposure to pesticides in their workplace (two unexposed were sought for each exposed subject). In addition, 41 horticultural workers between 18 and 65 years of age, who had been occupational exposed to pesticide mixtures in the previous month, with a minimum work history of 1 year and a maximum of 25 years, were included in the study.

Acetylcholinesterase activity in erythrocytes.

An aliquot of washed erythrocytes were haemolyzed by adding demineralized water at a 1:10 dilution. The hydrolysis rate of acetylthiocholine iodide (substrate) in erythrocyte dilution was measured at 405 nm with spectrophotometer by the reaction with DTNB to give the yellow 5-thio-2-nitrobenzoate anion. Enzyme activity was expressed as U/L of Red Blood Cells (RBC) (Ellman et al., 1961)

Plasmatic Butyrilcolinesterase.

The hydrolysis rate of butyrilthiocholine (substrate) in plasma was measured at 405 nm with spectrophotometer by the reaction of thiocholine iodide with DTNB, to give the yellow 5-thio-2-nitrobenzoate anion. Enzyme activity was expressed as U/L. (Ellman et al., 1961.)

Catalase activity in erythrocytes.

Erythrocytes were haemolyzed by adding ice-cold demineralized ultrapure water at a 1:100 dilution. CAT activity in haemolysate erythrocytes was measured by monitoring the decrease in H_2O_2 concentration spectrophotometrically over time (Aebi, 1984). The specific activity of each sample was calculated on the basis that one unit of enzyme activity was defined as the activity required to degrades 1 mole hydrogen peroxide during 60 s/g Hb.

Lipid peroxidation in erythrocytes.

MDA as a marker for lipid peroxidation in red blood cells (dilution 1:4 with ice-cold) was determined by measuring the formation of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA (TBARS Assay) according to a modification of the method of Buege & Aust (1978). The sample absorbance was determined at 535 nm and the TBARS concentration was calculated using the extinction coefficient 1.56×10^5 M^{-1} cm^{-1}. MDA concentration in erythrocytes was expressed as nmol/g Hb.

Alkaline Comet Assay.

The standard procedure originally described by Singh et al. (1988) with modifications was used. Damage Index Comet Assay (DICA) was calculated for each sample (Simoniello et al., 2010).

Cell Viability using Fluorescent Dyes.

The same cell suspension used in the comet assay, was mixed with fluorescent DNA-binding dyes and examined by fluorescent microscopy to visualize and count cells with aberrant chromatin organization. The percentages of each of these cellular states in relation to the total cells were obtained (Simoniello et al 2010).

Part B, results.

Demographic features of both exposed groups and controls were analyzed; groups were similar regarding age and smoking habits (Table 4).

Parameter	Controls (n=82)	Pesticide Applicator Workers (n=18)	Non-pesticide applicator Workers (n=23)
Age (X±S.D.)	37.70±14.07	40,66±11,44	33,78±11,26
Gender (n)(%)			
Female	37 (45)	6 (33)	13 (56)
Male	45 (55)	12 (67)	10 (44)
Smoking (n)(%)			
Yes	20 (25)	3 (17)	3 (13)
No	62 (75)	15 (83)	20 (87)
Alcohol (n)(%)			
Yes	41 (50)	13 (72)	13 (56)
No	41 (50)	5 (28)	10 (44)

Table 4. Demographic characteristics of controls and exposed workers.

The levels (mean ± SD) of Comet Assay, BChE and AChE assays, CAT activity and TBARS assay, in control and exposed workers are shown in detail in Table 5.

Parameter	Controls (n=82)	Pesticide Applicator Workers (n=18)	Non-pesticide applicator Workers (n=23)
Comet Assay	113.56±16.01	212.94±14.79**	224.73±20.56**
BChE Assay	6993.31±1131.92	6777.77±1281.84	6313.86±1268.26
AChE Assay	9045.54±2191.56	6740.33±5.19**	7651.52±2062.07**
CAT Activity	187.12 ±23.71	72.60±30.48**	106.12±37.15**
TBARS Assay	151.14±30.26	192.74±42.13*	138.90±31.89

Table 5. Comet Assay, BChE and AChE assays, CAT Activity and TBARS assay, in control and exposed workers. Values are presented as mean ± S.D. Comet Assay (DICA); BChE Assay (U/L); AChE Assay (U/L RBC); CAT Activity (kU/g Hb); TBARS Assay (nmol/g Hb). *P: <0.05, **P: <0.001 (Mann Whitney's Test).

Statistical evaluations of the two exposed groups were contrasted in all cases with the control population. AChE decrease was significant ($P < 0.01$), showing an inhibition of 25 and 15 % in the directly and indirectly exposed group, respectively. BChE decrease was not significant ($P > 0.05$), showing an inhibition of 4 and 10 % in the directly and indirectly exposed group, respectively.

A significant increase (51 %) in the levels of TBARS was found in pesticide sprayers ($P < 0.001$), but no differences were observed in the indirectly exposed group ($P > 0.05$).

CAT activity decreased in the whole pesticide-exposed population (applicators and non-applicators). The decrease in CAT activity was 61 % in the directly ($P < 0.0001$) and 43 % in the indirectly exposed group ($P < 0.05$). The analysis of the Comet assay values (mean±SD) indicated a significant increase in DICA (approximately 50%) in both the directly and indirectly exposed groups ($P < 0.001$; Mann–Whitney's U-test). Cell viability (> 85%) was evaluated and expressed as a proportion of living cells.

The Spearman correlation analysis showed a significant inverse correlation between erythrocyte TBARS and AChE in both exposed groups. On the other hand, the Comet assay showed a positive correlation with TBARS in the indirectly exposed group. Figure 3 shows the significant linear regression between AChE and TBARS in both exposed groups: Pesticide Applicators ($r = - 0.33$, $P < 0.05$) and Non-pesticide Applicators ($r = - 0.1747$, $P < 0.05$).

We considered using Personal Protective Equipment (PPE) when at least two items/types of protection (gloves, breathing masks, glasses, impermeable boots, etc.) had been used, since 93% of the pesticide-exposed workers reported using only one kind of protection during the preparation and application of pesticides. None of them reported using the full protective equipment.

A significant correlation was found between age and CAT in agricultural and farmer workers (indirectly exposed), but no significant difference was obtained for other confounding factors.

According to the answers to our questionnaire, the great majority of the subjects in the exposed group were in contact with many pesticides, including Captan, Copper, Mancozeb Chlorpyrifos, Carbofuran, Cypermethrin, Dimethoate, Endosulfan, Imidacloprid, Malathion, Methamidophos, Parathion, Permethrin and Glyphosate. These pesticides were

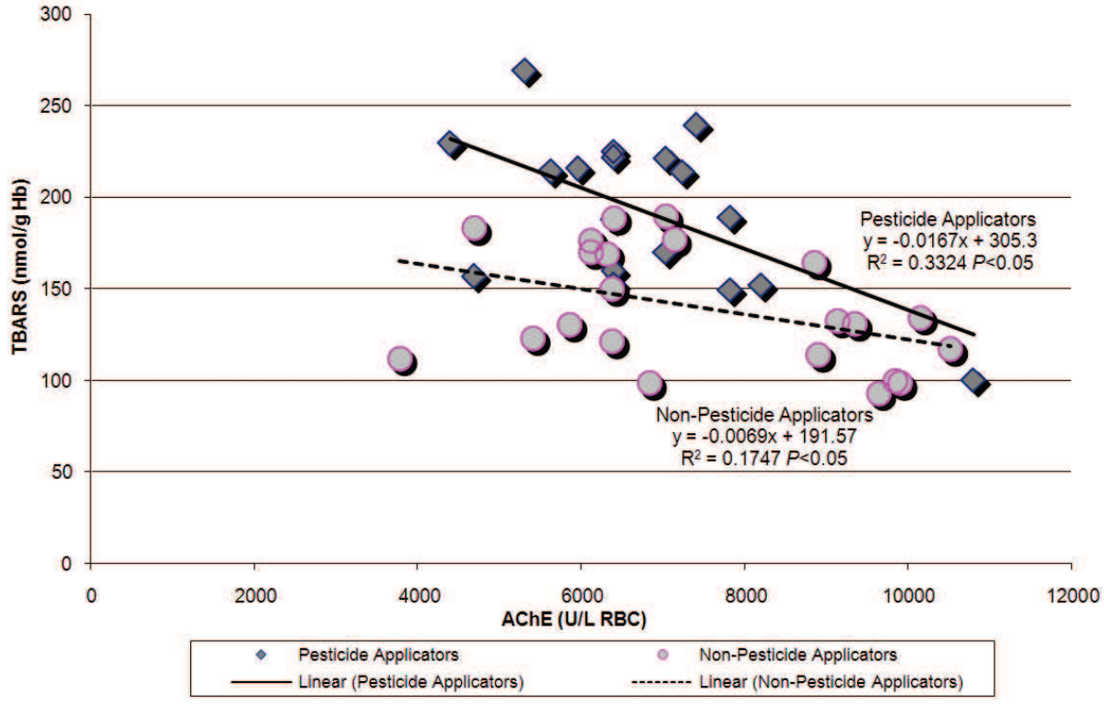


Fig. 3. Correlation of biochemical parameters (TBARS vs. AChE) in blood samples.

similar to those found in part A, but different pesticide mixtures were used in each treatment. For this reason, we could not associate the observed biochemical changes and DNA damage with a specific product or chemical class. In addition, no detailed data were available on the quantities of these pesticides used by the individuals.

10. Discussion

New, more selective and efficient pesticides, possibly ''safer'' for non-target organisms, have been produced in the past few years. So far, they have coexisted in the farming practice with agents in which one or more active principles have been found to be genotoxic and cytotoxic to various systems (De Marco et al., 2000). The primary objective of mutagen testing in genetic toxicology is to determine whether a chemical has the potential to cause genetic alterations in humans. The fundamental concern is the risk to future generations.

The association of mutagenesis with other endpoints such as carcinogenesis, teratogenesis, and aging has been noted. Hence, it is necessary to obtain quantitative data from clinical observations and epidemiological studies in order to predict virtually safe or tolerable levels of exposure (Bajpayee et al., 2005). Genotoxic monitoring in farming population could be a useful tool to estimate genetic risk from exposure to complex pesticide mixtures over extended lengths of time.

The agricultural workers included in this study were also exposed to a great number of pesticides (all of the subjects were exposed to more than two different pesticides), some of which are classified as being carcinogenic by the US Environmental Protection Agency (US-EPA) and hazardous by the World Health Organization (WHO), although not yet listed by the IARC (Table 2). In our study, the recommendation for the use of some agrochemicals to the producers come from technical advice in 35% and the rest consults different sources,

principally to sellers of these inputs, a situation already distinguished by Ringuelet & Laguens (2000) in Great La Plata, Buenos Aires Province, and by Bulacio & Panelo (2000) in the Horticultural Belt of Rosario, Santa Fe Province. Considering the chemicals used, it is important to note that some of these, such as metamidophos, have been banned in other countries because of their high toxicity, while in developing countries such a prohibition is limited (Castillo-Cadena et al., 2006). Assessment of the associations with individual pesticide exposure is very difficult because most occupations involve the regular use of a large number of different pesticides, together with other chemicals such as co-formulants, which vary greatly in their potential toxicities and potencies. Furthermore, measurements of systemic exposure to pesticides were not taken and therefore correlations between increased genotoxicity biomarkers and the degree of exposure were not possible to obtain (Bull et al., 2006).

Pesticides act selectively against certain organisms without adversely affecting others. However, absolute selectivity is difficult to achieve and most pesticides are a toxic risk also to humans. Pesticide application is still the most important method in self-poisoning in the developing world. The International Agency for Cancer Research (IARC) has reviewed the potential carcinogenicity of a wide range of insecticides, fungicides, herbicides and other similar compounds and classified several of them as carcinogenic to laboratory animals; in addition, the IARC has reported the association of different chemical agents, such as phenoxy acid herbicides, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), lindane, methoxychlor, toxaphene and several organophosphates, with cancer in human studies (Bolognesi, 2003).

The mixtures of pesticides used included some that have proved to have genotoxic effects *in vivo* in human biomonitoring. Considering pesticide information and taking previous reports into account, we found a partial coincidence in the mixtures used in this study and those that had been used in other researches (Dulout et al., 1985; Carbonell et al,. 1995; Peluso et al., 1996; Gómez-Arroyo et al., 2000; Grover et al., 2003).

Multiple chemical exposures are of great interest in Toxicology and Public Health (US-EPA, 1998). Most of the research has been performed on individual chemical agents (Groten, 2000) without considering that the effect of a chemical mixture could be either more or less powerful than the exposure to the individual compounds. Multiple exposures are a rule and not an exception in agricultural practice: pesticide applicators spray large amounts of agrochemical mixtures including a significant number of genotoxic compounds. The pesticides most often used are chlororganics and, more recently, carbamates, organophosphates and pyrethroids, which have been reported to be positive for genotoxic effects in experimental studies in bacterial and in mammalian systems (Bolognesi, 2003).

Although several studies have reported that pesticide sprayers (applicators) represent the most exposed group of agricultural workers (Bolognesi, 2003), in our study, non-applicators were included in the exposed group since they were present during all working activities, including pesticide applications. This can be due to the misconception that non-applicators are not as exposed as applicators. Our study revealed similar frequencies in the Comet assay considering applicators and non-applicators in agreement with previous research (Costa et al., 2006). Therefore, occupational exposure to pesticides can fluctuate in time and the skin constitutes a significant exposure route of absorption mainly in agriculture (Jakubowski & Trzcinka-Ochocka, 2005). On the other hand, some reports consider para-occupational or take-home exposure although agricultural chemicals move from the work place to residential environments through the activities of farm workers (Curl et al., 2002). All farm

workers live close to the fields where chemicals are applied and these are taken home on workers´ bodies, clothing, and shoes, accumulating in the home environment.

Different researches have recognized the invaluable role of AChE monitoring in rural workers at high risk of exposure to OPs and MC pesticides (Mc Cauley et al., 2006). In our work, in agreement with different reports (Ranjbar et al., 2002; Singh et al., 2007), AChE showed a significant decrease in directly and indirectly pesticide-exposed workers. Measuring BChE activities is a frequent marker of exposure in pesticide sprayers, is easier to assay and is more widely available; in our case, BChE activity inhibition was not significant. This may be related to the differential profiles of cholinesterase inhibition that can be observed depending on the particular OP compound; for example, chlorpyrifos and malathion are preferential inhibitors of BChE whereas dimethoate is a preferential inhibitor of AChE. In the interpretation of cholinesterase monitoring results, we may consider that both groups had been exposed to different pesticide mixtures and take into account inter-individual variation and confounding factors in enzymatic activity.

Oxidative damage is thought to be an important mechanism of several pesticides (Banerjee et al., 1999, Prakasam et al., 2001). In blood, normal erythrocyte function depends on an intact cell membrane, which is the target for many toxics, including pesticides. The results of the present study indicate that CAT activity decreased significantly in both pesticide applicators and non-pesticide applicators ($P < 0.001$).

The available data on experimental animals (Seth et al., 2001), *in vitro* studies (Gultekin et al., 2000; Prasanthi et al., 2005) and *in vivo* studies (Ranjbar et al., 2002; Lopez et al., 2007) indicate that the enzymes associated with the antioxidant defence mechanism change under the influence of pesticides. These enzymes efficiently scavenge toxic free radicals and are partly responsible for protection against lipid peroxidation due to pesticide exposure (Banerjee et al., 1999). Hence, the increased level of TBARS observed in this work could be due to increased peroxidation of membranes and/or to decreased antioxidant activity, caused by exposure to pesticide mixtures.

Different OPs, such as phosalone, chlorpyrifos ethyl, and diazinon, have been reported to induce oxidative stress as shown by enhancement of MDA production (Gultekin et al., 2000; Prakasam et al., 2001; Altuntas et al., 2003; Catalgol et al., 2007). Carbamate pesticides may induce oxidative stress, which leads to the generation of free radicals and an alteration in antioxidant enzymes or OFR scavenging enzymes (Seth et al., 2001; Dettbarn et al., 2006). Some pyrethroids affect the flow of erythrocyte membrane due to increased lipid peroxidation (Kale et al., 1999, Gabbianelli et al., 2002; Nasuti et al, 2003). It is likely that the production of O_2^- or the direct action of pyrethroid on the production of GPx could be the cause of oxidative damage (Prasanthi et al., 2005; El Demerdash, 2007). The correlation between TBARS and AChE activity found in the present study (Figure 3) is similar that obtained by other authors (Ranjbar et al., 2002; Akhgari et al., 2003; Singh et al., 2007).

Several different pathways by which oxidative DNA damage occurs have been proposed. These include chemical modification of nucleotides (Cicchetti & Argentin, 2003), direct action of ROS on DNA, or indirect lipid peroxidation degradation products (Collins, 1999). The Comet assay has been used to determine the extent of DNA damage in leukocytes from rural workers occupationally exposed to a variety of pesticides (Garaj-Vrhovac & Zeljezic, 2000; Shadnia et al., 2005; Remor et al., 2008). Our results show that pesticide-spraying workers and farmers presented a significant increase in DICA as well as in DIRA when compared to controls ($P < 0.0001$ in both cases). However, the spraying group exhibited a marginally significant difference in DICA when years of exposure were considered

($P = 0.05$), and a significant difference ($P < 0.05$ in Part A, $P = 0.05$ in Part B) when we used the personal protective equipment (PPE) worn by individuals as a comparison factor.

The positive genotoxicity observed in the exposed workers of this study may be due to the lack of protective measures or protective clothing, gloves or boots in a few cases. In other works carried out in Argentina, 86% of the workers interviewed declared to use PPE, but the authors commented that only 20% had the complete equipment, existing cases in which they wore gloves only. In the present work, 35% of the workers interviewed admitted not using PPE (Panero et al., 2009); this finding agrees with the results indicated by Souza Casadinho (2003) with regard to the unawareness in relation to the danger of using agrochemicals, although it is widely admitted that horticultural activity is risky. In agreement with this, when asking about the aspects which they considered that should be improved, only 5 % of the workers interviewed mentioned aspects of hygiene and safety in the work.

An increase in micronuclei was seen in pesticide-exposed people who did not wear protective gloves (Bolognesi et al., 2002). At the same time, increases in the frequencies of chromosomal aberrations and micronuclei have been found in some studies where the population exposed to pesticides wore no protection during work activities (Costa et al., 2006) or little or no protective clothes (Dulout et al., 1985). Interestingly, several studies that reported a majority of workers using protective measures (>60%) concluded that the results were negative (Bolognesi et al., 2004; Pastor et al., 2001 and 2002; Piperakis et al., 2003 and 2006), suggesting the importance of PPE for preventing exposure. Therefore, field workers may be affected by a lack of available work-site laundering facilities, prolonging their exposure to pesticides and other farm chemicals.

DMSO inhibited H_2O_2-induced DNA damage (Klein et al., 1981). Oxidative DNA damage, as measured in lymphocytes, is maintained in a dynamic steady-state by antioxidant defences, which control input of damage, together with cellular DNA repair, which removes the damage that occurs in spite of the antioxidant protection. In the present work, neither of the exposed groups showed statistically significant differences in DNA damage before and after the repair process, when compared to controls (Figure 1). Palus et al. (1999) reported a significant reduction in the number of cells with DNA damage after a 1-hour repair process in workers of a wooden furniture plant, whereas Piperakis et al. (2003; 2006) observed that the repair efficiency was similar in the studied groups, in agreement with our findings.

Results from *in vitro* and *in vivo* studies can be influenced by individual sensitivity, status of the immune system, genetic predisposition, metabolic or DNA repair differences and simultaneous exposure to other environmental toxicants. These factors may contribute to the variation of the individual response in each subject (Islas-Gonzalez et al., 2005). The individual genetic variability in the enzymes that metabolize agricultural chemicals may also be involved in this process. When these enzymes are not efficient in detoxification, metabolic products accumulate, contributing to a carcinogenic process.

The influence of confounding factors, such as age, gender, smoking and alcohol consumption, on the genotoxic effects of occupational exposure to pesticides was investigated and no significant differences were observed in relation to DICA and DIRA ($P > 0.05$). Other authors have reported similar results when evaluating micronucleus frequency in pesticide-exposed workers (Sailaja et al., 2006). Likewise, smoking failed to have a significant influence on the number of CA (Zeljezic & Garaj-Vrhovac, 2001), level of MN (Bolognesi et al., 2002) and increase in comet tail-length values (Garaj-Vrhovac & Zeljezic, 2000; Liu et al., 2006). However, the discrepancy in some reports is not surprising since the failure to show an effect of smoking could be due to the kind of exposure, target

tissue, and individual susceptibility of subjects in the population. When individuals are exposed to mixtures, it is difficult to predict the final genotoxic effect because of the interaction that could occur between the agents involved, either maximizing or antagonizing the effect (Castillo-Cadena et al., 2006).
A significant correlation was found between age and CAT in indirectly exposed workers, which could be due to random deleterious effects of free radicals produced during aerobic metabolism causing DNA, lipid and protein damage and accumulation over time (Valko et al., 2007).

11. Conclusion

Our study shows that, under the conditions of this experimental work, subjects directly and indirectly exposed to pesticides have enzymatic alterations, modifications in oxidative balance and genotoxic damage when compared to controls. Further studies should be carried out enlarging the sample size and conducting a serial and routine monitoring of populations exposed to pesticide mixtures, using effect and exposure biomarkers.

12. Future perspectives

Cells are continuously exposed to endogenous and exogenous agents that damage DNA. One of the most common kinds of damage is oxidation. A great variety of oxidized bases have been identified in nuclear DNA but 8-oxo-7,8-dihydroguanine (8-oxo-G) is one of the most abundant and readily formed oxidized DNA lesions (Azqueta et al., 2009). Comet assay was adapted to measure oxidized purines and oxidized pyrimidines by the incubation of the nucleoids with bacterial DNA repair enzymes. Formamidopyrimidine glycosylase (FPG) is used to detect oxidized purines, mostly 8-oxo-G, and endonuclease III (EndoIII) is used to detect oxidized pyrimidines. FPG and EndoIII will be employed to investigate environmental or occupational exposure of humans to pesticides associated with oxidative stress.
On the other hand, oxidative DNA damage, as measured in lymphocytes, is maintained in a dynamic steady-state by antioxidant defences, which control input of damage, together with cellular DNA repair, which removes the damage that occurs in spite of antioxidant protection. It would be important to assess the extent of inter-individual variation in repair capacity, or the susceptibility of repair in humans pesticide exposed. The most obvious new direction for molecular epidemiological studies is in dealing with the availability of new technologies (Bonassi & Au, 2002). New information coming from the genome-derived methods can be of paramount importance in human studies. Since the role of susceptibility genes can be investigated more efficiently, their influence on specific effects of exposures and response to genotoxic agents will be elucidated.
Also, diet plays an important role in preventing cancer, but the mechanisms still not clear. Convincing epidemiological evidence links consumption of fruits and/or vegetables with decreased risk of cancer of the lung, mouth, pharynx, oesophagus, stomach, colon and rectum (Collins et al., 2003). These foods are rich in antioxidants such as vitamins C and E, carotenoids and flavonoids, which are capable of decreasing oxidative damage to DNA and thus might prevent mutation and cancer. Knowledge of specific responses and how these responses are affected by nutrimental status, in each region, will facilitate the development of new clinical strategies for the prevention of cancer and other pathological conditions.

13. References

Aebi, H. (1984). Catalase in vitro, *Method. Enzymol*., 105, 121-6, ISSN 0076-6879.

Akcha, F.; Leday, G. & Pfohl-Leszkowicz, A. (2003). Potential value of the Comet assay and DNA adduct measurement in dab (Limanda limanda) for assessment of in situ exposure to genotoxic compounds, *Mutat. Res*., 534, 21–32 ISSN 0921-8262.

Akhgari, M. (2003). Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats, *Hum. Exp. Toxicol*., 22, 4, 205-211, ISSN 0960-3271.

Altuntas, I.; Delibas, N.; Doguc, D.K.; Ozmen, S. & Gultekin, F. (2003). Role of reactive oxygen species in organophosphate insecticide phosalone toxicity in erythrocytes in vitro, *Toxicol. in Vitro,* 17, 153–157, ISSN 0887-2333

Anderson, D. (2001) Factors that contribute to biomarker responses in humans including a study in individuals taking vitamin C supplementation, *Mutat. Res*., 480–481, 337–47, ISSN 0921-8262.

Azqueta, A.; Shaposhnikov, S. & Collins, A.R. (2009). Detection of Oxidised DNA Using DNA Repair Enzymes, In: *The Comet Assay in Toxicology,* Dhawan, A. & Anderson (Ed.), D., 57-78, RSC Publishing, ISBN 978-0-85404-199-2, Cambridge, U.K.

Bajpayee, M.; Pandey, A. K.; Parmar, D. & Dhawan, A. (2005) Current Status of Short-Term Tests for Evaluation of Genotoxicity, Mutagenicity, and Carcinogenicity of Environmental Chemicals and NCEs. (Review), *Toxicology Mechanisms and Methods*, 15: 155–180, ISSN 1537-6516

Banerjee, B.D.; Seth, V.; Bhattacharya, A.; Pasha, S.T. & Chakraborty A.K. (1999). Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers, *Toxicol. Lett*, 107, 33-47, ISSN 0378-4274.

Basaran, N.; Shubair, M.; Undeger, U. & Kars, A. (2003). Monitoring of DNA damage in foundry and pottery workers exposed to silica by the alkaline Comet assay, *Am. J. Ind. Med*., 43, 6, 602–10, ISSN 0271-3586.

Beuge, J.A. & Aust SD. (1978). Microsomal lipid peroxidation. *Method. Enzymol*. 52, 302–10, ISSN 0076-6879.

Bolognesi, C. (2003). Genotoxicity of pesticides: a review of human biomonitoring studies, *Mutat. Res*., 543, 251–272, ISSN 0921-8262.

Bolognesi, C.; Landini, E.; Perrone, E. & Roggieri P. (2004). Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe, *Mutat. Res.,* 557, 109–117, ISSN 0921-8262.

Bolognesi, C.; Perrone, E. & Landini, E. (2002). Micronucleus monitoring of a floriculturist population from western Liguria, Italy, Mutagenesis, 17, 391–397, ISSN 0267-8357.

Bonassi, S. & Au, W. (2002). Biomarkers in molecular epidemiology studies for health risk prediction. Review, *Mutat. Res.,* 511, 73–86, ISSN 0921-8262.

Bowden, R.D.; Buckwalter, M.R.; McBride, J.F.; Johnson, D.A.; Murray, B.K. & O´Neill, K.L. (2003). Tail profile: a more accurate system for analyzing DNA damage using the comet assay, *Mutat. Res.,* 537, 1-9, ISSN 0921-8262.

Bulacio, L.G. & Panelo, M.S. (2000). Cinturón Hortícola de Rosario: Situación actual en el manejo de fitosanitarios, *Horticultura Argentina*, 19, 46, 5-14, ISSN 0327-3431.

Bull, S.; Fletcher, K.; Boobis, A.R. & Battershill, J.M. (2006). Evidence for genotoxicity of pesticides in pesticide applicators: a review, *Mutagenesis,* 21, 93-103, ISSN 0267-8357.

Carbonell, E.; Valbuena, A.; Xamena, N.; Creus, A. & Marcos, R. (1995). Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides, *Mutat. Res*. 344, 127–134, ISSN 0921-8262.

Carpenter, D.O.; Arcaro, K. & Spink, D.C. (2002). Understanding the Human Health Effects of Chemical Mixtures, *Environ Health Perspect* 110, 1, 25–42 ISSN 0091-6765.

Castignani, M.I. ; Arregui, M.C.; Pelatti, N.S. (2004). Estimación de contaminación por plaguicidas con indicadores ambientales y económicos en huertas de tomate en Santa Fe, *Revista FAVE Ciencias Agrarias*, 3 ,1-2, ISSN 1669-1555.

Castillo-Cadena, J.; Tenorio-Vieyra, L.E.; Quintana-Carabia, A.I.; Garcia-Fabila, M.M.; Ramirez-San Juan, E. & Madrigal-Bujaidar, E. (2006). Determination of DNA Damage in Floriculturists Exposed to Mixtures of Pesticides, *Jour. Biomedicine and Biotechnology*, 1–12, ISSN 1110-7251.

Catalgol, B.K.; Ozden, S. & Alpertunga, B. (2007). Effects of trichlorfon on malondialdehyde and antioxidant system in human erythrocytes, *Toxicol. in Vitro*, 21, 1538–1544, ISSN 0887-2333.

Cicchetti, R. & Argentin, G. (2003). The role of oxidative stress in the in vitro induction of micronuclei by pesticides in mouse lung fibroblasts, *Mutagenesis,* 18, 127-32, ISSN 0267-8357.

Cocker, J.; Mason, H.J.; Garfitt, S.J. & Jones K. (2002). Biological monitoring of exposure to organophosphate pesticides, *Toxicology Letters*, 134, 97–103, ISSN 0378-4274

Collins, A.R. & Dusinska, M. (2009) Applications of the Comet Assay in Human Biomonitoring, In: *The Comet Assay in Toxicology,* Dhawan, A. & Anderson, D. (Ed.), 201-227, RSC Publishing, ISBN 978-0-85404-199-2, Cambridge, U.K.

Collins, A.R. (1999). Oxidative DNA damage, antioxidants, and cancer, *Bioassays*. 21, 238-46.

Collins, A.R.; Harrington, V.; Drew, J. & Melvin, R. (2003). Nutritional modulation of DNA repair in a human intervention study, *Carcinogenesis,* 24, 3, 511–515, ISSN 0143-3334.

Costa, C.; Teixeira, J.; Silva, S.; Roma-Torres, J.; Coelho, P.; Gaspar, J.; Alves, M.; Laffon, B.; Rueff, J. & Mayan, O. (2006). Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticides, *Mutagenesis,* 21, 343-350, ISSN 0267-8357.

Curl, C.; Fenske, R.; Kissel, J.; Shirai, J.; Moate, T.; Griffith, W.; Coronado, G. & Thompson, B. (2002). Evaluation of Take-Home organophosphorus pesticide exposure among agricultural workers and their children, *Environ Health Perspect*, 110, 787-792, ISSN 0091-6765.

De Marco, A.; De Salvia, R.; Polani, S.; Ricordy, R.; Sorrenti, F.; Perticone, P.; Cozzi, R.; D'Ambrosio, C.; De Simone, C.; Guidotti, M.; Albanesi, T.; Duranti, G.; Festa, F.; Gensabella, G. & Owczarek, M. (2000). Evaluation of Genotoxic and Cytotoxic Properties of Pesticides Employed in Italian Agricultural Practices, *Environ. Res*. Section A., 83, 311-321, ISSN 0013-9351.

De Zwart, L.; Meerman, J.H.N.; Commandeur, J.N.M. & Vermeulen, N.P.E. (1999). Biomarkers of free radical damage applications in experimental animals and in humans, *Free Radical Biology & Medicine,* 26, 1, 2, 202–226, ISSN 0891-5849.

Dettbarn, W.D.; Milatovic, D. & Gupta, R. (2006). Oxidative Stress in Anticholinesterase-induced Excitotoxicity, In: Toxicology of Organophospahate and Carbamate Compounds, Gupta, R. (Ed), Elsevier: 511-532, ISBN 0-12-088523-9, Academic Press, Hopkinsville, KY, USA.

Dhawan, A.; Mathur, N. & Seth, P. K. (2001). The effect of smoking and eating habits on DNA damage in Indian population as measured in the Comet assay, *Mutat. Res.*, 474,1, 2, 121–8, ISSN 0921-8262.

Dhawan, A.; Anderson, D.; de Pascual-Teresa, S.; Santos-Buelga, C.; Clifford, M.N. & Ioannides, C. (2002). Evaluation of the antigenotoxic potential of monomeric and dimeric flavanols, and black tea polyphenols against heterocyclic amine-induced DNA damage in human lymphocytes using the Comet assay, *Mutat. Res.*, 515, 1,2, 39–56, ISSN 0921-8262.

Drevenkar, V.; Radic, Z.; Vasilic, Z. & Reiner, E. (1991). Dialkylphosphate metabolites in the urine and activities of esterases in the serum as biochemical indices for human absorption of organophosphorus pesticides, *Arch. Environ. Contamin. Toxicol.,* 20, 417–422, ISSN 0090-4341.

Dulout, F.N.; Pastori, M.C.; Olivero, O.A.; Gonzalez Cid, M.; Loria, D.; Matos, E.; Sobel, N.; de Bujan, E.C. & Albiano, N. (1985). Sisterchromatid exchanges and chromosomal aberrations in a population exposed to pesticides, *Mutat. Res.,* 143, 237–244, ISSN 0921-8262.

Dusinska, M. & Collins, A. R. (2008). The Comet assay in human biomonitoring: gene–environment interactions, *Mutagenesis,* 23, 191–205, ISSN 0267-8357.

El-Demerdash, F.M. (2007). Lambda-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants, *Toxicol. in Vitro*, 21, 392–397, ISSN 0887-2333.

Ellman, G.L.; Curtney, K.D.; Andrews, V. & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetyl cholinesterase activity, *Biochem. Pharmacol.*, 7, 88–95, ISSN 0006-2952

Environmental Health Criteria 155. (1993) International programme on chemical safety. Biomarkers and Risk Assessment. Concepts and Principles. (http://www.inchem.org/pages/ehc.html)

Environmental Protection Agency (EPA). (2006). Integrated Risk Information (IRIS). Washington, DC (http://www.epa.gov/iris).

Gabbianelli, R.; Falcioni, G.; Nasuti, C. & Cantalamessa, F. (2002). Cypermethrin-induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity, *Toxicology,* 175, 91-101, ISSN 0300-483X

Garaj-Vrhovac V. & Zeljezic D. (2001). Cytogenetic monitoring of croatian population occupationally exposed to a complex mixture of pesticides, *Toxicology,* 165: 153–162, ISSN 0300-483X

Giunta, C.R. (2004) La Provincia de Santa Fe en el total agropecuario nacional. Una aplicación del Censo Nacional Agropecuario 2002. Dirección Gral. de Programación y Coordinación Económica Financiera, Ministerio de la Producción. (www.santafe.gov.ar/index.php/web/)

Giunta, C.R.; Zalesky, M. & Sona G. (2005) Cambios registrados en el sector agropecuario santafesino entre 1988 y 2002.Análisis de las variables más significativas. (www.santafe.gov.ar/index.php/web/)

Gomez-Arroyo, S.; Noriega-Aldana, N.; Osorio, A.; Galicia, F.; Ling, S. & Villalobos-Pietrini, R. (1992). Sister-chromatid exchange analysis in a rural population of Mexico exposed to pesticides, *Mutat. Res.,* 281,173–179, ISSN 0921-8262.

Groten, J.P. (2000). Mixtures and interactions. *Food Chem. Toxicol*. 38, 1, S65–S71, ISSN 0278-6915.

Grover, P; Danadevi, K.; Mahbood, M.; Rozati, R.; Saleha Banu, B. & Rahman, M.F. (2003). Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet Assay, *Mutagenesis,* 18, 201-205, ISSN 0267-8357.

Gultekin, F.; Ozturk, M. & Akdogan, M. (2000). The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro), *Arch. Toxicol*, 74, 9, 533-8, ISSN 0090-4341.

Hardt, J. & Angerer, J. (2000). Determination of dialkyl phosphates in human urine using gas chromatography-mass spectrometry, *J. Anal. Toxicol*., 24, 678–684, ISSN 0146-4760.

Heath, A. & Vale, J. (1992). Clinical presentation and diagnosis of acute orgnophosphate insecticide and carbamate poisoning. In: *Clinical and Experimental Toxicology of Organophosphates and Carbamates,* Ballantyne, B. & Marrs, T. (Ed.), 513–519, ISBN 0750602716, Butterworth, Oxford.

HSE (2000). Medical Aspects of work-related exposure to organophosphates Guidance note MS17: Health and Safety Executive, HSE Books, ISBN 0 7176 1977 X, Sudbury Suffolk.

Hughes, I. & Wood, H.F. (2002). Risk Assessment of Mixtures of Pesticides and Similar Substances. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Hughes, I. & Wood, H.F. (Ed.), 1-298, Food Standards Agency.

IARC. International Agency for Research on Cancer, 2008. Agents reviewed by the IARC monographs. Vols 1–88, IARC, Lyon. (http://monographs.iarc.fr).

Islas-Gonzalez, K.; Gonzalez-Horta, C.; Sánchez-Ramirez, B.; Reyes-Aragon, E. & Levario-Carrillo M. (2005). In vitro assessment of the genotoxicity of ethyl paraoxon in newborns and adults, *Human Experim. Toxicology.* 24, 319-324, ISSN: 0960-3271.

Issaq, H.J.; Veenstra, T.D.; Conrads, T.P. & Felschow, D. (2002) The SELDI-TOF MS approach to proteomics: proteinprofiling and biomarker identification, *Biochem Biophys Res Commun*, 292, 587-592, ISSN 0006-291X.

Jaeschke, H. (1995) Mechanisms of oxidant stress-induced acute tissue injury, *Proc. Soc. Exp. Biol. Med.* 209,104–111, ISSN 0037-9727.

Jakubowski, M. & Trzcinka-Ochocka M. (2005). Biological Monitoring of exposure: Trends and key developments. *J. Occup. Health.* 47, 22-48, ISSN 1341-9145.

Kale, M.; Rathore, N.; John, S. & Bhatnagar, D. (1999). Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species, *Toxicol. Lett*., 105, 197–205, ISSN 0378-4274.

Kassie, F.; Parzefall W. & Knasmuller, S. (2000). Single-cell gel electrophoresis assay: a new technique for human biomonitoring studies, *Mutat. Res*., 463, 1, 13–31, ISSN 0921-8262.

Klein, S.M.; Cohen, G. & Cederbaum, A. (1981). Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating system, *Biochemistry*, 20, 6006-6012, ISSN 0302-4369.

Knudsen, L.E. & Hansen, A.M. (2007) Biomarkers of intermediate endpoints in environmental and occupational health, Int. J. Hyg. Environ. Health, 210, 461–470, ISSN 1438-4639.

Lei, H.; Tjallingii, W. F. & Van Lenteren, J. C. (1998). Probing and feeding characteristics of the greenhouse whitefy in association with host-plant acceptance and whitefy strains, *Entomologia Experimentalis et Applicata*, 88, 73-80, ISSN 0013-8703.

Lenardon, A.; Maitre, M.I.; Lorenzatti, E. & Enrique, S. (2000). Plaguicidas organoclorados en leche materna en Santa Fe, *Acta Toxicologica Argentina,* 8, 1, 2-4, ISSN 1851-3743.

Lin, T.; Schwartz, A. & Saranga, Y. (1999). Non-stomatal factors limit cotton photo-synthesis under silverleaf whitefy stress, *Physiologia Plantarum*, 107, 303-31, ISSN 0031-9317.

Liu, Y.J.; Huang, P.L.; Chang, Y.F.; Chen, Y.H.; Chiou, Y.H.; Xu, Z.L. & Wong, R.H. (2006). GSTP1 Genetic Polymorphism Is Associated with a Higher Risk of DNA Damage in Pesticide-Exposed Fruit Growers, *Cancer Epidemiol Biomarkers Prev*., 15 ,4, 659–66. ISSN 1055-9965.

Lopez, O.; Hernandez, A.F.; Rodrigo, L.; Gil, F.; Pena, G.; Serrano, J. L.; Parron, T.; Villanueva, E. & Pla, A. (2007). Changes in antioxidant enzymes in humans with long-term exposure to pesticides, *Toxicol. Lett.*, 171, 146-153, ISSN 0378-4274.

Mason, H.J. & Lewis, P. (1989). Intra-individual variation in plasma and erythrocyte cholinesterase activities and the monitoring of uptake of organophosphorus pesticides, *J.Soc. Occup. Med.,* 39, 121–124, ISSN 0301-0023.

McCauley, L.A.; Anger, W.K.; Keifer, M.; Langley, R.; Robson, M.G. & Rohlman, D. (2006). Studying Health Outcomes in Farmworker Populations Exposed to Pesticides, *Environ. Health Perspect*., 114, 953–960, ISSN 0091-6765.

Mercille, S. & Massie, B. (1994). Induction of apoptosis in nutrient-deprived cultures of hybridoma and myeloma cells, *Biotechnology and Bioengineering,* 44, 1140-1154, ISSN 0006-3592.

Miranda, M. M. M.; Picanco, M.; Zanuncio, J. C. & Guedes R. N. C. (1998). Ecological life table of Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), *Bio-control Sci,. & Technol*., 8, 597-606, ISSN 0958-3157.

Moretto, A. & Lotti, M. (1998) Poisoning by organophosphorus insecticides and sensory neuropathy, *J Neurol Neurosurg Psychiatry,* 64, 463-468, ISSN 00223050.

Muniz, J.F.; McCauley, L; Scherer, J.; Lasarev, M.; Koshy, M.; Kow, Y.W.; Nazar-Stewart, V. & Kisby, G.E. (2008). Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study, Toxicology and Applied Pharmacology, 227, 97–107, ISSN 0041-008X.

Nasuti, C.; Cantalamessa, F.; Falcioni, G. & Gabbianelli, R. (2003). Different effects of Type I and Type II pyrethroids on erythrocyte plasma membrane properties and enzymatic activity in rats, *Toxicology,* 191, 233-244, ISSN 0300-483X.

Nutley, B. & Cocker, J. (1993). Biological monitoring of workers occupationally exposed to organophosphorus pesticides, *Pesticide Sci,*. 38, 315–322, ISSN 1096-9063.

Ostling, O. & Johanson, K.J. (1984). Microelectrophoretic study of radiation induced DNA damages in individual mammalian cells, *Biochem. Biophys. Res. Commun.*, 123, 1, 291–8, ISSN 0006-291X.

Palus, J.; Dziubaltowska, E. & Rydzynski, K. (1999). DNA damage detected by the comet assay in the white blood cells of workers furniture plant, *Mutat. Res*., 444, 61-74, ISSN 0921-8262.

Palus, J.; Rydzynski, K.; Dziubaltowska, E.; Wyszynska, K.; Natarajan, A.T. & Nilsson, R. (2003). Genotoxic effects of occupational exposure to lead and cadmium, *Mutat. Res*., 540, 1, 19–28, ISSN 0921-8262.

Pastor, S.; Creus, A.; Xamena, N.; Siffel, C. & Marcos R. (2002). Occupational exposure to pesticides and cytogenetic damage: results of a Hungarian population study using the micronucleus assay in lymphocytes and buccal cells, *Environ. Mol. Mutagen*., 40, 101–109, ISSN 0893-6692.

Pastor, S.; Gutierrez, S.; Creus, A.; Xamena, N.; Piperakis, S. & Marcos R. (2001). Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells, *Mutagenesis*, 16, 539–545, ISSN 0267-8357.

Paunero, I.E.; Mitidieri, M.; Ferratto, J.; Giuliani, S.; Bulacio, L.; Panelo, M.; Amoia, P.; Strassera, M.E.; Granitto, G.; del Pino, M., Martínez, S., Fortunato, N.; Tangorra, M.; Andreau, R.; Garbi, M. & Martínez-Quintana, O. (2009). Identifying the primary types of accidents that occur to horticultural workers in Argentina, *Agricultura, Sociedad y Desarrollo*, 6, 2, 177-182, ISSN 1870-5472.

Peluso, M.; Merlo, F.; Munnia, A.; Bolognesi, C.; Puntoni, R. & Parodi, S. (1996). (32)P-postlabeling detection of DNA adducts in peripheral white blood cells of greenhouse floriculturists from western Liguria, Italy, *Cancer Epidemiol. Biomarkers Prev*., 5, 361–369, ISSN 1055-9965.

Petricoin, E.F.; Ardekani, A.M.; Hitt, B.A.; Levine, P.J.; Fusaro, V.A.; Steinberg, S.M.; Mills, G.B.; Simone, C.; Fishman, D.; Kohn, E.C. & Liotta, L.A. (2002) Use of proteomic patterns in serum to identify ovarian cancer, *Lancet*, 35, 9, 572-577, ISSN 0140-6736.

Piperakis, S. M.; Visvardis, E. E.; Sagnou, M. & Tassiou, A. M. (1998). Effects of smoking and aging on oxidative DNA damage of human lymphocytes, *Carcinogenesis,* 19, 4, 695–698, ISSN 0143-3334.

Piperakis, S.M.; Kontogianni, K.; Piperakis, M.M. & Tsilimigaki, S. (2006). Effects of Pesticides on Occupationally Exposed Humans, *The Scientific World Journal*, 6, 1211-1220, ISSN 1537-744X.

Piperakis, S.M.; Petrakou, E.; Tsilimigaki, S.; Sagnou, M.; Monogiudis, E.; Haniotakis, G.; Karkaseli, H. & Sarikaki, E. (2003). Biomonitoring with the comet assay of Greek greenhouse workers exposed to pesticides, *Environ. Mol. Mutagen*., 41, 104–110, ISSN 0893-6692.

Pitozzi, V.; Giovannelli, L.; Bardini, G.;. Rotella, C. M & Dolara, P. (2003). Oxidative DNA damage in peripheral blood cells in type 2 diabetes 42 Chapter 1mellitus: higher vulnerability of polymorphonuclear leukocytes, *Mutat. Res*, 529(1–2), 129–33, ISSN 0921-8262.

Prakasam, A.S.; Sethupathy, S. & Lalitha, S. (2001). Plasma and RBCs antioxidant status in occupational male pesticide sprayers, *Clin. Chim. Acta*, 310, 107–112, ISSN 0009-8981.

Prasanthi, K.; Muralidhara & Rajini, P.S. (2005). Morphological and biochemical perturbations in rat erythrocytes following in vitro exposure to Fenvalerate and its metabolite, *Toxicol. in Vitro,* 19, 449–456, ISSN 0887-2333.

Raipulis, J.; Maija, M. & Balode M. (2009). Toxicity and Genotoxicity Testing of Roundup. *Proceedings of the latvian academy of sciences Section B*, 63, 29–32, ISSN 1407-009X.

Ranjbar, A.; Pasalar, P. & Abdollahi, M. (2002). Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers. *Hum. Exp. Toxicol*. 21, 179-82, ISSN 0960-3271.

Raslova, K.; Nagyova, A.; Dobiasova, M.; Ptackova, K. & Dusinska, M. (2000). Effect of ciprofibrate on lipoprotien metabolism and oxidative stress parameters in patients with type 2 diabetes mellitus and atherogenic lipoprotein phenotype. *Acta. Diabetol*. 37, 3, 131–134, ISSN 0940-5429.

Reid, S.J. & Watts, R.R. (1981). A method for the determinationof dialkyl phosphate residues in urine. *J. Anal. Chem*. 5,126–132, ISSN 0937-0633.

Remor, A.P.; Totti, C.C.; Moreira, D.A.; Dutra, G.P.; Heuser, V.D. & Boeira, J.M. (2008). Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation. *Environ. Int*. 35, 2, February 2009, 273-278, ISSN 0160-4120.

Ringuelet, R. & Laguens, J. (2000). Notas sobre el uso de agroquímicos. Espacio tecnológico, población y reproducción social en el sector hortícola de La Plata. Universidad Nacional de La Plata. Biblioteca del Congreso de la Nación, (http://consulta.bcn.gov.ar/bcn/Catalogo)

Rodriguez, A.R. & Lenardon, A.L. (2007). Provincia Santa Fe Sur, In: *La problemática de los agroquímicos y sus envases, su incidencia en la salud de los trabajadores, la población expuesta y el ambiente. Estudio colaborativo multicéntrico.* Ministerio de Salud. Secretaría de Ambiente y Desarrollo Sustentable, Secretaría de Ambiente y Desarrollo Sustentable: OPS: AAMMA, 247-268, ISBN 978-987-96256-7-5, Buenos Aires.

Ruf, A.A.; Jerwood, D.; Webb, J. & Anderson, D. (2003). Sensitivity of different thalassaemia genotypes to food mutagens in the Comet assay. *Teratog. Carcinog. Mutagen*. Suppl 2, 83–91, ISSN 0270-3211.

Sasaki, Y.F.; Sekihashi, K.; Izumiyama, F.; Nishidate, E.; Saga, A.; Ishida, K. & Tsuda, S. (2000) The Comet assay with multiple mouse organs: comparison of Comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and US NTP Carcinogenicity Database, *Crit. Rev. Toxicol.*, 30, 629, ISSN 1040-8444.

Seth, V.; Banerjee, B.D. & Chakravorty, A.K. (2001). Lipid peroxidation, free radical scavenging enzymes, and glutathione redox system in blood of rats exposed to Propoxur, *Pest. Biochem. Phys*, 71, 133-139, ISSN 0048-3575.

Shadnia, S.; Azizi, E.; Hosseinil, R.; Khoei, S.; Fouladdel, S.; Pajoumand, A.; Jalali, N. & Abdollahi, M. (2005) Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. *Hum.Exp. Toxicol*. 24, 439- 445, ISSN 0960-3271.

Simoniello, M.F.; Kleinsorge, E.C.; Scagnetti, J.A.; Grigolato, R.A.; Poletta, G.L. & Carballo, M.A. (2008) DNA damage in workers occupationally exposed to pesticide mixtures. *J. Appl. Toxicol*. 28, 8, 957-965, ISSN 0260-437X.

Simoniello, M.F.; Kleinsorge, E.C.; Scagnetti, J.A.; Mastandrea, C.; Grigolato, R.A.; Paonessa, A.M. & Carballo, M.A. (2010). Biomarkers of cellular reaction to pesticide exposure in a rural population, *Biomarkers*, 15, 1, 52-60, ISSN 1354-750X.

Singh, N.P.; McCoy, M.T.; Tice, R.R. & Schneider, E.L. (1988). A simple technique for quantitation of low levels of DNA damage in individuals cells, *Exp. Cell. Res,* 175, 1, 184-191, ISSN 0014-4827.

Singh, N.P.; Muller, C.H. & Berger, R.E. (2003) Effects of age on DNA double-strand breaks and apoptosis in human sperm, *Ferti. Steril*., 0, 6, 1420–30, ISSN 0015-0282.

Singh, V.K.; Jyoti; Krishna Reddy, M.M.; Kesavachandran, C.; Rastogi, S.K. & Siddiqui, M.K.J. (2007). Biomonitoring of organochlorines, glutathione, lipid peroxidation and cholinesterase activity among pesticide sprayers in mango orchards, *Clin. Chim. Acta*, 377, 268–272, ISSN 0009-8981.

Souza Casadinho, O. & Bocero, S. (2008). Agrotóxicos: Condiciones de utilización en la horticultura de la Provincia de Buenos Aires (Argentina), *Revista Iberoamericana de Economía Ecológica*, 9, 87-101, ISSN 1390-2776.

Trossero, S.M; Trossero, N.; Scagnetti, J.; Portillo, P. & Kleinsorge E. (2009). Plaguicidas en leche materna y su potencial efecto disruptor endocrino en gestantes del cordón hortícola de Santa Fe (Argentina) (Informe Preliminar), *Revista FABICIB*, 13, 97-103, ISSN 0329-5559.

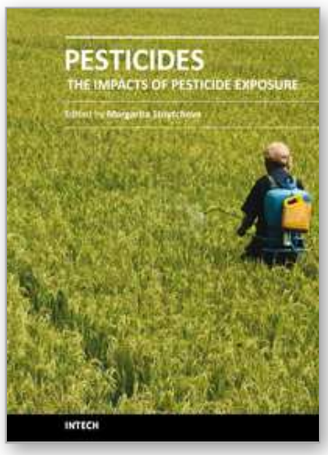
Vaghef, H.; Nygren, P.; Edling, C.; Bergh, J. & Hellman, B. (1997). Alkaline single-cell gel electrophoresis and human biomonitoring for genotoxicity: a pilot study on breast cancer patients undergoing chemotherapy including cyclophosphamide, *Mutat. Res*, 395, 2–3, 127–138, ISSN 0921-8262.

Valko, M.; Leibfritz, D.; Moncola, J.; Cronin, M.T.D.; Mazura, M. & Telser, J. (2007). Review Free radicals and antioxidants in normal physiological functions and human disease, *Intern. J. Biochem. Cell Biology,* 39, 44–84. ISSN 1357-2725.

Valverde, M.; Lopez, M.; Lopez, I.; Sanchez, I.; Fortoul, T.; Ostrosky-Wegman P. & Rojas, E. (1997). DNA damage in leukocytes and buccal and nasal epithelial cells of individuals exposed to air pollution in Mexico City, *Environ. Mol. Mutagen*., 30, 147–152, ISSN 0893-6692.

WHO, World Health Organization, 2009. The WHO Recommended classification of pesticides by hazard and guidelines to classification. World Health Organization, Geneva. ISSN: 1684-1042.
http//www.who.int/ipcs/publications/pesticide_hazard_2009

Zeljezic, D. & Garaj-Vrhovac, V. (2001). Chromosomal aberration and single cell gel electrophoresis (comet) assay in the longitudinal risk assessment of occupational exposure to pesticides, *Mutagenesis*, 16, 4, 359–363, ISSN 0267-835.



Pesticides - The Impacts of Pesticides Exposure
Edited by Prof. Margarita Stoytcheva

ISBN 978-953-307-531-0
Hard cover, 446 pages
Publisher InTech
Published online 21, January, 2011
Published in print edition January, 2011

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.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Marta Ana Carballo, María Fernanda Simoniello and Elisa Carlotta Kleinsorge (2011). Agrochemicals: Horticulture Use Conditions Determine Genotoxic Effects and Oxidative Damage in Rural Populations in Santa Fe, Argentina, Pesticides - The Impacts of Pesticides Exposure, Prof. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-531-0, InTech, Available from: http://www.intechopen.com/books/pesticides-the-impacts-of-pesticides-exposure/agrochemicals-horticulture-use-conditions-determine-genotoxic-effects-and-oxidative-damage-in-rural-

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen