Arch Toxicol (2002) 76: 430–436
DOI 10.1007/s00204-002-0355-5

GENOTOXICITY

Ülkü Ündeğer · Nurşen Başaran

Assessment of DNA damage in workers occupationally exposed to pesticide mixtures by the alkaline comet assay

Received: 8 January 2002 / Accepted: 8 April 2002 / Published online: 24 May 2002

Abstract The potential genetic hazard of pesticides to human beings is of great concern in occupational and environmental settings because of the widespread use of these chemicals for domestic and industrial applications. Various studies have revealed a significantly elevated risk for particular tumours in humans exposed to some pesticides. Results from the biological monitoring or cytogenetic methods for the detection of health risks to pesticides have given both positive and negative results of mutagenicity. In this study DNA damage in peripheral lymphocytes of 33 pesticide-exposed workers employed in the municipality of Ankara (Turkey) for at least 1 year was examined by alkaline single-cell gel electrophoresis, the 'comet' technique. Results were compared with those from 33 controls of comparable age, sex and smoking habits, which were not occupationally exposed to pesticides. Work characteristics of the exposed workers and the use of personnel protective measures were also investigated. The DNA damage observed in lymphocytes of the workers was significantly higher than that in the controls ($P < 0.001$). The observed DNA damage was found to be significantly lower ($P < 0.001$) in workers applying some of the necessary individual safety protections during their work. Cigarette smoking was not related to increases in DNA damage; also, no significant association was found between the duration of occupational exposure to pesticides and the degree of DNA damage.

Keywords Pesticides · Single-cell gel electrophoresis · Comet assay · DNA damage

Ü. Ündeğer · N. Başaran (✉)
Hacettepe University,
Department of Pharmaceutical Toxicology,
Faculty of Pharmacy, Ankara, 06100, Turkey
E-mail: nbasaran@hacettepe.edu.tr
Tel.: +90-312-3092958
Fax: +90-312-3114777

Introduction

Pesticides are some of the chemicals that are most frequently released into the environment because of their widespread use in public health and agriculture. Despite the beneficial effects associated with the use of pesticides, many of these chemicals may pose potential hazards to humans and to nature. The use of these agricultural chemicals without necessary protection may lead to alterations in the genetic material and the possible development of some types of tumours. Toxicological evidence of the carcinogenicity of several pesticides has attracted the attention in various studies, which have revealed a significantly increased incidence of cancer (e.g. leukaemia, non-Hodgkin lymphoma and multiple myeloma) in farmers relative to non-farmers, although overall mortality from cancer was lower in the rural population (Blair et al. 1985; Morrison et al. 1992; Daniels et al. 1997; Zahm and Blair 1997).

Several pesticides have recently been reviewed as mutagenic compounds in both in vitro and in vivo test systems. In vitro studies on human and Chinese hamster cell lines showed positive evidence for mutagenicity when treated with different pesticides (Nicholas et al. 1979; Sobti et al. 1982; Wang et al.1987; Rupa et al. 1988). Some human biomonitoring studies on populations occupationally exposed to such agrochemicals have shown that this exposure induces a significant increase in cytogenetic damage. Increased frequencies of sister chromatid exchanges (SCEs) (Crossen et al. 1978; Dulout et al. 1985; Rupa et al. 1989), chromosomal aberrations (CAs) (Kourakis et al. 1992; Anwar 1994; Carbonell et al. 1995; Au et al. 1999) and micronuclei (MN) (Bolognesi et al. 1993; Falck et al. 1999) were found in human pesticide applicators, although some of these positive findings have not been substantiated by other investigators (Hoyos et al. 1996; Scarpato et al. 1996, 1997; Titenko-Holland et al. 1997; D'Arce and Colus 2000). Human population studies have generally

focused on workers and farmers who are exposed chronically to pesticide mixtures. Variations in the degree of exposure or the use of different pesticides may explain the controversies among the findings; alternatively differences in personnel working habits and hygiene may affect the results.

In the last decade, single-cell gel electrophoresis or the 'comet' assay has become established as a sensitive and rapid method for the detection of DNA single-strand breaks and alkali-labile sites, and incomplete excision repair events in individual cells. In this technique, a suspension of cells is embedded in agarose on microscope slides, lysed, and subjected to electrophoresis before being stained with a fluorescent DNA-binding dye. During alkali gel electrophoresis, the broken DNA strands move towards the anode forming a comet (Singh et al. 1988; McKelvey-Martin et al. 1993; Fairbain et al. 1995). The electric current draws the charged DNA from the nucleus such that relaxed and broken DNA fragments migrate further. Up to now, the comet assay has been shown to be a promising tool for measuring the genetic damage induced in vitro by different genotoxic agents as well as for examining DNA repair under a variety of experimental conditions.

The application of the comet assay for human biomonitoring in occupational situations is quite scarce. To provide further data on the possible applicability of this assay in human biomonitoring studies and also to investigate the potential hazards associated with the occupational chronic exposure to pesticide mixtures, we have evaluated by use of the alkaline comet assay the eventual genetic damage in the peripheral lymphocytes of the pesticide applicators who were working in the municipality of Ankara.

Materials and methods

Chemicals

The chemicals used in these experiments were purchased from the following suppliers: normal-melting agarose (NMA) and low-melting agarose (LMA) were obtained from Boehringer-Mannheim (Mannheim, Germany); sodium chloride (NaCl) and sodium hydroxide (NaOH) were from Merck Chemicals (Darmstadt, Germany); dimethylsulfoxide (DMSO), ethidium bromide, Triton X-100 and phosphate-buffered saline (PBS) tablets were obtained from Sigma Chemical Co. Ltd. (St. Louis, Mo., USA); ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), *N*-lauroylsarcosinate and Tris were from ICN Biomedicals Inc. (Aurora, Ohio, USA).

Subjects

The exposed group consisted of 33 pesticide applicators, currently employed in the municipality of Ankara (Turkey), who had been continuously involved in the preparation and the application of pesticides for a period of 1–23 years with a mean duration of 10 ± 6 years. As the control group, 33 healthy subjects of comparable age, sex, socio-economic life style and smoking habits, and with no history of pesticide exposure, were chosen from university staff. Both the exposed and the control groups consisted of 33 male subjects ranging in age from 28 to 61 years (mean 43 ± 7 years). All subjects participated in the study voluntarily and all of them provided oral consent (in Turkish) before blood samples were withdrawn from them. A questionnaire designed to yield information on sex, age, occupational history, general health status, smoking and dietary habits, and exposure to drugs and chemicals was used for each exposed and control subject. Duration of occupational exposure and work characteristics of the exposed workers, such as the use of protective equipment (overalls, masks, gloves, eyeglasses), were also investigated.

Blood sampling and lymphocyte preparations

A 5 ml heparinized (50 U/ml sodium heparin) whole blood sample was taken by venepuncture from each individual at the end of the working week. Lymphocytes were isolated by Ficoll-Hypaque density gradient (Boyum 1976) and washed in PBS. Cell concentrations were adjusted to approximately 2×10^5 per ml in the buffer. Aliquots of 5–10 µl of the cells were suspended in 75 µl of 0.5% LMA for embedding on slides. Cells were checked for viability by Trypan blue exclusion.

Slide preparation

The basic alkaline technique of Singh et al. (1988), as further described by Anderson et al. (1994), was followed. Fully frosted microscope slides (Surgipath, Winnipeg, Manitoba, Canada) were each covered with 110 µl of 0.5% NMA at about 45°C in Ca^{2+}- and Mg^{2+}-free PBS. They were immediately covered with a large no. 1 coverslip and kept at room temperature for about 5 min to allow the agarose to solidify. This layer was used to promote the attachment of the second layer of 0.5% LMA. Approximately 10,000 cells were mixed with 75 µl of 0.5% LMA to form a cell suspension. After gently removing the coverslip, the cell suspension was rapidly pipetted onto the first agarose layer, spread using a coverslip, and maintained on an ice-cold flat tray for 5 min to solidify. After removal of the coverslip, the third layer of 0.5% LMA (75 µl) at 37°C was added, spread using a coverslip, and again allowed to solidify on ice for 5 min. After removal of the coverslip the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, pH 10, with 1% Triton-X100 and 10% DMSO added just before use) for a minimum of 1 h at 4°C.

Electrophoresis

The slides were removed from the lysing solution, drained and placed in a horizontal gel electrophoresis tank side by side, avoiding spaces and with the agarose ends facing each other, nearest the anode. The tank was filled with fresh electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. Before electrophoresis, the slides were left in the solution for 20 min to allow the unwinding of the DNA and expression of alkali-labile damage. Electrophoresis was conducted at a low temperature (4°C) for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level and using a compact power supply (Power Pack P25, Biometra Analytik GmbH, Göttingen Germany). All of these steps were conducted under dimmed light (the tank was covered with a black cloth) to prevent additional DNA damage occurring. After electrophoresis, the slides were taken out of the tank. Tris buffer (0.4 M Tris, pH 7.5) was gently added dropwise to neutralize the excess alkali and the slides were left undisturbed for 5 min. The neutralizing procedure was repeated three times.

Staining

To each slide, 65 µl ethidium bromide(20 µg/ml) was added. The slides were covered with a coverslip, placed in a humidified

air-tight container to prevent drying of the gel, and analysed within 3–4 h.

Scoring of DNA damage

For visualization of DNA damage, slides were examined at ×1000 magnification, using a ×100 objective (oil immersion), on a fluorescence microscope (Zeiss, Oberkochen, Germany). Images of 200 randomly selected lymphocytes, i.e. 100 cells from each of two replicate slides, were analysed from each sample and the DNA damage was scored visually as described by Anderson et al. (1994). Comets are formed by the principle of releasing damaged DNA from the core of the nucleus with electrophoresis; at low damage levels, stretching of attached strands of DNA is likely occur, and with increasing numbers of breaks, DNA fragments migrate freely into the tail of the comet. In the experiments, the cells were graded by eye into four categories on the basis of damage extent, i.e. undamaged, slightly damaged, damaged and highly damaged. Analysis was performed by one slide reader, thus minimising the possibility of variability due to subjective scoring.

Statistical analysis

Results are expressed as mean ± SD, and the statistical comparison of results from exposed and non-exposed individuals, was performed using the chi-square test. The chi-square test was also used to compare the grades of DNA damage in workers and controls according to smoking habits, and in workers according to their individual protection. A linear regression analysis was used to estimate the effects of the duration of occupational exposure to pesticides on the DNA damage (Snedecor and Cohran 1988).

Results

The workers investigated in this study had handled, and thus been exposed to, a great diversity of chemicals such as pyrethroids, carbamates, heterocycles and organophosphates, and consequently they were presented with multiple pesticide exposure during the mixing, loading, and application. According to the information from the workers, the most frequently used pesticides in the last three years were cypermethrin, permethrin, deltamethrin, tetramethrin, cyphenotrin, D-phenotrin, propoxur, pirimicarb, diazinon, chlorpyrifos-ethyl, and azinphos-methyl. The work characteristics and the precautions taken during the exposure period and the number of cigarettes smoked by the workers are shown in Table 1. There were 10 non-smokers and 23 smokers in each of the exposed and the control groups. The average cigarette consumption of smokers in both groups was nearly 22 cigarettes per day. From the questionnaire on protective measures we found that 21 of the pesticide-exposed workers wore overalls and masks, 3 of the workers wore only overalls but the other 9 workers had not taken individual safety precautions and did not even wear masks. On the other hand, the masks that the workers had been using were ordinary surgical masks without any filter, and none of the workers wore gloves

Table 1. The work characteristics and the cigarette consumption of the pesticide application workers (*SM* surgical mask, *O* overalls, *N* no protection)

Subject	Age (years)	Cigarette consumption	Duration of exposure to pesticides (years)	Type of protection
SD	42	20/day	18.0	SM, O
MK	35	Non-smoker	10.0	SM, O
AA	47	20/day	6.0	SM, O
CM	28	20/day	1.0	SM, O
RM	43	Non-smoker	18.0	SM, O
DA	56	20/day	1.0	SM, O
HY	48	Non-smoker	17.0	SM, O
AB	45	Non-smoker	23.0	SM, O
ŞS	47	40/day	4.5	SM, O
SÖ	40	20/day	13.0	SM, O
ÖÖ	61	20/day	2.0	SM, O
Fİ	37	20/day	6.0	SM, O
HS	50	Non-smoker	18.0	SM, O
MD	50	Non-smoker	17.0	SM, O
MY	50	Non-smoker	17.0	SM, O
CB	42	20/day	7.0	SM, O
FA	37	20/day	13.0	SM, O
KŞ	40	20/day	10.0	SM, O
AA	50	20/day	17.0	SM, O
HF	40	Non-smoker	17.0	SM, O
HC	44	20/day	8.0	SM, O
EA	36	30/day	5.0	N
TY	33	20/day	4.0	O
Vİ	36	30/day	5.0	N
AK	44	20/day	9.0	N
EG	43	20/day	7.0	N
FE	35	20/day	5.0	O
MY	47	Non-smoker	18.0	N
TS	44	20/day	8.0	N
SO	40	20/day	6.0	N
HT	43	20/day	8.0	N
AE	47	Non-smoker	18.0	O
SS	35	20/day	5.0	N

and safety glasses and boots. The duration of pesticide application varied from 1 to 23 years and the exposed group handled pesticides throughout the whole year in which on average the number of hours that the worker had been directly involved in handling these chemicals was a minimum of 6 h.

The grades of DNA damage in peripheral lymphocytes of the workers and the controls are summarized in Table 2. A significant increase ($\chi^2 = 97.566$, $P < 0.001$) in the DNA damage was observed in the workers who routinely applied mixtures of pesticides. The number of lymphocytes without DNA damage was significantly higher in the unexposed controls than in the workers. The correlations between the duration of exposure and the grades of DNA damage, i.e. undamaged, slightly damaged, damaged and highly damaged were $r = -0.021$ ($P > 0.05$), 0.153 ($P > 0.05$), 0.040 ($P > 0.05$) and –0.002 ($P > 0.05$), respectively. A significant association could not be found between the DNA damage and the length of exposure.

Cigarette smoking was also not related to DNA damage observed in the lymphocytes of the workers since no additional DNA damage was observed when the smoking and the non-smoking workers were compared ($\chi^2 = 5.25$, $P > 0.05$). However, the number of lymphocytes without DNA damage was found to be significantly higher in the non-smoker controls than in the smoking controls (Table 3).

As shown in Table 4, workers who had taken the necessary individual safety precautions have less DNA damage than the 9 who had taken no precautions. The increases in the number of damaged and highly damaged cells were significant in workers without protection, whereas the number of undamaged and slightly damaged cells were increased in workers with protection ($\chi^2 = 44.266$, $P < 0.001$).

Discussion

Because of the potential environmental impact connected with the heavy use and the large population exposed, determination of the effects and the risks of chronic exposure to pesticides are needed. Pesticide formulations are complex mixtures, which contain in addition to the active ingredients, several other components, such as solvents, wetting and emulsifying agents, and additives. Since workers are exposed differently to several classes of pesticides, toxicity information concerning active ingredients and/or formulations alone are not sufficient to evaluate the risk of adverse health effects. In general, exposure level measurements such as analysis of air samples or determination of blood and urine pesticide concentrations have been performed under conditions of numerically limited exposure such as manufacturing or spraying of no more than one class of pesticide. However there are some studies indicating that no correlation was found between these parameters and the induction of risks (Rabello et al. 1975; Linnainmaa 1983; Desi et al. 1986). In our study, due to the elevated number of active ingredients used and the lack of accurate information on the quantity (kg/year) of compounds sprayed, it was not possible to define an exposure unit for the population. However, on the basis of the response to the questionnaire submitted to each worker, we attempted to obtain an indirect measure of the intensity of exposure and found that the workers may have high occupational exposure. They handled various groups pesticides throughout the year without adequate conditions and protective measures. The workers who had taken the necessary protective measures claimed that they neglected to use personal protection from time to time. Although the DNA damage

Table 2. Grades of DNA damage in peripheral lymphocytes of the pesticide workers and the controls

Groups	Grades of DNA damage								Total no. of samples	Statistics	
	Undamaged		Slightly damaged		Damaged		Highly damaged			χ^2	P
	No.	%	No.	%	No.	%	No.	%			
Workers	5027	76.2	729	11.0	498	7.5	346	5.2	6600	97.566	< 0.001
Controls	5347	81.0	707	10.7	248	3.8	298	4.5	6600		

Table 3. Grades of DNA damage in pesticide workers and controls according to smoking habits

Groups	n	Grades of Damage								Total no. of samples	Statistics	
		Undamaged		Slightly damaged		Damaged		Highly damaged			χ^2	P
		No.	%	No.	%	No.	%	No.	%			
Workers												
Smokers	23	3480	75.7	512	11.1	369	8.0	239	5.2	4600	5.254	0.154
Non-smokers	10	1547	77.4	217	10.9	129	6.5	107	5.4	2000		
Controls												
Smokers	23	3652	79.4	553	12.0	195	4.2	200	4.3	4600	39.555	< 0.001
Non-smokers	10	1695	84.8	154	7.7	53	2.7	98	4.9	2000		

Table 4. DNA damage in pesticide workers according to their individual protection

Workers	*n*	Grades of DNA Damage								Total no. of samples	Statistics	
		Undamaged		Slightly damaged		Damaged		Highly damaged			χ^2	*P*
		No.	%	No.	%	No.	%	No.	%			
Without safety protection	9	1348	74.9	166	9.2	142	7.9	144	8.0	1800	44.266	<0.001
With safety protection	24	3679	76.6	563	11.7	356	7.4	202	4.2	4800		

observed in the pesticide-exposed workers was significantly higher ($P<0.001$) than in the controls, the damage in 24 workers who used overalls and simple cloth masks was less than that in 9 workers who did not protect themselves. From the workers' protection point of view, it is important to monitor the possible risk.

Cytogenetic changes associated with pesticide exposure have been reported in many studies after exposure to specific compounds or to complex mixtures. An increased incidence of CAs and SCEs that is related to pesticide exposure has been obtained in different populations (Yoder et al. 1973; Rabello et al. 1975; Dulout et al. 1985; Paldy et al. 1987; Jablonicka et al. 1989; Rupa et al. 1989; De Ferrari et al. 1991; Kourakis et al. 1992; Carbonell et al. 1995). From the results of Carbonell et al. (1995), it appears that individuals exposed to pesticides showed evidence of chromosomal damage, mainly chromatid-type aberrations, during the period of major exposure. Subjects mixing or spraying pesticides without using any protection showed higher CA levels than those of the control group (Kourakis et al. 1992). However, these positive findings have not been confirmed in other investigations (Högstedt et al. 1980; Nehez et al. 1988; Hoyos et al. 1996). Scarpato et al. (1997) found that pesticide exposure was not associated with elevated frequencies of CAs, SCE or MN in a group of Italian floriculturists exposed to a mixture of pesticides. Also, there was no detectable differences in induction of CAs in Brazilian farm workers (D'Arce and Colus 2000). Conflicting results from cytogenetic monitoring on pesticide-exposed populations seem to be due to multiple and different exposure types or to the heterogenous composition of the various study populations rather than to limited sensitivity of the cytogenetic assays. The exposure generally involves complex mixtures of many kinds of compounds. In addition, there is a high interindividual variability in the degree of exposure, including differences in personal protection and hygiene. The present study is one of the very few studies by the alkaline comet assay showing that workers who are exposed to pesticide mixtures have an increase in DNA damage related to their work. The results were compared with those from a group of technicians and secretaries who did not have a history of occupational exposure to pesticides. Matched control groups were used because of possible confounders like age, sex, smoking habits and exposure to chemicals. Garaj-Vrhovac and Zeljezic (2000) found increased DNA damage in the peripheral lymphocytes of workers who were employed for at least 8 months in the production of pesticides, mainly atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, chlorophenoxy acetic acid and malathion. After 8 months of non-exposure the workers showed a significantly decreased DNA migration compared with the results of the first sampling, but it was still significantly higher than that of controls. Moretti et al. (2000) examined the DNA damage in the peripheral blood leukocytes of 17 male farm workers and of 17 age-matched controls using the comet assay and showed a statistically significant increase in the number of damaged cells in the farmers compared with controls. Lebailly et al. (1998) also found an increased DNA damage in 29 Italian farmers who were occupationally exposed to pesticides. To date, a few authors have also used the comet assay to measure in vitro pesticide-induced DNA damage (Pool-Zobel et al. 1993; Ribas et al. 1995; Lebailly et al. 1997; Scassellati et al. 1997; Speit et al. 1998; De Marco et al. 2000). The DNA-damaging activity has seemed to depend on the chemical and the cell type studied. It was observed that chlorothalonil induced DNA damage in human lymphocytes and in Chinese hamster ovary (CHOC1) cell line, as detected with alkali comet assay, whereas no DNA damage was observed with carbendazim and isoproturon either in human lymphocytes or in the CHOK1 cell line (Lebailly et al. 1997; Vigreux et al. 1998). Paraquat also did not significantly induce DNA lesions with V79 Chinese hamster cells (Speit et al. 1998). The DNA-damaging activity of lindane, depends on cell type and species (Pool-Zobel et al. 1993), whereas positive results have been provided with some pesticides examined by comet assay (Ribas et al. 1995). From our results it seems that the comet assay can be considered a very promising tool in biomonitoring studies on occupationally exposed human populations. In our workers occupationally exposed to pesticides, no significant difference was found in smokers compared to non-smokers. Moretti et al. (2000) also did not observe any statistically significant effect of cigarette smoking on the extent of DNA damage assessed in the comet assay but Lebailly et al. (1998) found that smoking significantly influenced DNA damage levels. These data are also consistent with the studies that have found no significant increase in CA frequencies in smokers compared to non-

smoker farmers (Dolout et al. 1987; Jablonicka et al. 1989; De Ferrari et al. 1991; Kourakis et al. 1992; Carbonell et al. 1995). No relationship between the induced DNA damage and the duration of exposure was found in our study. There are also some reports suggesting that genetic changes in farmers and floriculturists were not dependent on duration of pesticide exposure (Kourakis et al. 1992; Anwar 1994; Hoyos et al. 1996), even though individuals exposed for a longer period were found to present an increase in cytogenetic alterations (Rabello et al. 1975; Paldy et al. 1987).

Identification, characterization and control of pesticides that pose potential hazards to humans and to nature continue to be key public health goals. Results of experimental animal studies and human epidemiological studies suggest an association between certain pesticides and cancer. Organochlorine compounds have been associated with lymphoma, leukaemia, soft tissue sarcoma, neuroblastoma, and cancers of the pancreas, breast and lung. Organophosphate pesticides have been reported to increase the risk of lymphoma and leukaemia (Pope and Rall 1995; Zahm and Devesa 1995). Most of the human data come from studies of farmers, pesticide applicators and other agricultural workers, but the clear identification of the pesticides as carcinogens with appropriate consideration of the mechanisms by which the tumours occur, or their relevance to humans has not been elucidated yet. Variations in drug-metabolising enzymes as genetic susceptibility factors, and in growth factor regulators and homeobox genes may underlie susceptibility to cancer (Faustman et al. 2000). The DNA damage observed in pesticide-exposed populations also plays a role in susceptibility. Au et al. (1999) found significantly abnormal DNA repair responses, as determined by challenge assay, in a group of farmers and they observed that increased abnormalities were frequently associated with farmers who had inherited 'unfavourable' versions of the monitored polymorphic metabolising genes compared with farmers who had inherited 'favourable' genes and with controls.

Conclusion

In conclusion, the finding of increased DNA damage in the peripheral lymphocytes of pesticides applicators indicates the potential genetic hazards posed by pesticides and emphasises the necessity and the importance of protective measures and safety regulations to minimize or prevent further exposure. Our results also show that the single-cell gel electrophoresis or comet assay seems to be a reliable, rapid, and highly sensitive method of detecting DNA damage in individual cells and fulfils the requirements of a biological marker for pesticide exposure.

Acknowledgements This study could not been carried out without the collaboration with the workers of municipality of Ankara.

References

Anderson D, Yu T-W, Philips BJ, Schmezer P (1994) The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. Mutat Res 307:261–271

Anwar WA (1994) Assessment of cytogenetic changes in human populations at risk in Egypt. Mutat Res 313:183–191

Au WW, Sierra-Torres CH, Cajas-Salazar N, Shipp BK, Legator MS (1999) Cytogenetic effects from exposure to mixed pesticides and the influence from genetic susceptibility. Environ Health Perspect 107:501–505

Blair A, Malker H, Cantor KP, Burmeister LF, Wiklund K (1985) Cancer among farmers. Scand J Work Environ Health 11:397–407

Bolognesi C, Parrini M, Bonassi S, Ianello G, Salanitto A (1993) Cytogenetic analysis of a human population occupationally exposed to pesticides. Mutat Res 285:239–249

Boyum A (1976) Isolation of lymphocytes, granulocytes, and macrophages. Scand J Immunol 5:9–15

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

Crossen PE, Morgan WF, Horan JJ (1978) Cytogenetic studies of pesticide and herbicide sprayers. N Z Med J 88:192–195

Daniels JL, Olshan AF, Savitz DA (1997) Pesticides and childhood cancers. Environ Health Perspect 105:1068–1077

D'Arce LPG, Colus IMS (2000) Cytogenetic and molecular biomonitoring of agricultural workers exposed to pesticides in Brazil. Teratog Carcinog Mutagen 20:161–170

De Ferrari M, Artuso M, Bonassi S, Bonatti S, Cavalieri Z, Pescatore D, Marchini E, Pisano V, Abbondandolo A (1991) Cytogenetic biomonitoring of an Italian population exposed to pesticides: chromosome aberration and sister-chromatid exchange analysis in peripheral blood lymphocytes. Mutat Res 260:105–113

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 83:311–321

Desi I, Palotas M, Vetro G, Cosolle I, Nehez M, Zimanyi M, Ferke A, Huszta E, Nagymajtenyi L (1986) Biological monitoring and health surveillance of a group of greenhouse pesticide sprayers. Toxicol Lett 33:91–105

Dolout FN, Pastori MC, Gonzales Cid M, Matos E, von Guradze HN, Maderna CR, Loria D, Sainz L, Albiano N, Sobel N (1987) Cytogenetic analysis in plant breeders. Mutat Res 189: 381–386

Dulout FN, Pastori MC, Olivero OA, Gonzalez Cid M, Loria D, Matos E, Sobel N, de Bujan EC, Albiano N (1985) Sister chromatid exchanges and chromosomal aberrations in population exposed to pesticides. Mutat Res 143:237–244

Fairbain DW, Olive PL, O'Neill KL (1995) The COMET assay: a comprehensive review. Mutat Res 339:37–59

Falck GCM, Hirvonen A, Scarpato R, Saarikoski ST, Migliore L, Norppa H (1999) Micronuclei in blood lymphocytes and genetic polymorphism for GSTM1, GSTT1 and NAT2 in pesticide-exposed greenhouse workers. Mutat Res 441:225–237

Faustman EM, Silbernagel SM, Fenske RA, Burbacher TM, Ponce RA (2000) Mechanisms underlying children's susceptibility to environmental toxicants. Environ Health Perspect 108:13–21

Garaj-Vrhovac V, Zeljezic D (2000) Evaluation of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis (SCGE) assay. Pesticide genotoxicity revealed by comet assay. Mutat Res 469:279–285

Högstedt B, Kolnig AM, Mitelman F, Skerfying S (1980) Cytogenetic study of pesticides in agricultural work. Hereditas 92:177–178

Hoyos LS, Carvajal S, Solano L, Rodriguez J, Orozco L, Lopez Y, Au W (1996) Cytogenetic monitoring of farmers exposed to pesticides in Colombia. Environ Health Perspect 104:535–538

Jablonicka A, Polakova H, Karelova J, Vargova M (1989) Analysis of chromosome aberrations and sister-chromatid exchanges in peripheral blood lymphocytes of workers with occupational exposure to the mancozeb-containing fungucide Novozir Mn 80. Mutat Res 224:143–146

Kourakis A, Mouratidou M, Kokkinos G, Barbouti A, Kotsis A, Mourelatos D, Dozi-Vassiliades J (1992) Frequencies of chromosomal aberrations in pesticide sprayers working in plastic green houses. Mutat Res 279:145–148

Lebailly P, Vigreux C, Godard T, Sichel F, Bar E, LeTalaer JY, Henry-Amar M, Gauduchon P (1997) Assessment of DNA damage induced in vitro by etoposide and two fungusides (carbendazim and chlorothalonil) in human lymphocytes with the comet assay. Mutat Res 375:205–217

Lebailly P, Vigreux C, Lechevrel C, Ledemeney D, Godard T, Sichel F, Letalaer JY, Henry-Amar M, Gauduchon P (1998) DNA damage in mononuclear leukocytes of farmers measured using the alkaline comet assay: discussion of critical parameters and evaluation of seasonal variations in relation to pesticide exposure. Cancer Epidemiol Biomarkers Prev 7:917–927

Linnainmaa K (1983) Sister chromatid exchanges among workers occupationally exposed to phenoxy acid herbicides 2,4-D and MCPA. Teratog Carcinog Mutagen 3:269–279

McKelvey-Martin VJ, Green MHL, Schmezer P, Pool-Zobel BL, De Meo MP, Collins A (1993) The single cell gel electrophoresis assay (comet assay): a European review. Mutat Res 288:47–63

Moretti M, Villarini M, Sforzolini GS, Pasquini R (2000) Pesticide-induced primary DNA damage in peripheral blood leukocytes of farm workers evaluated by the computerized 'comet' assay. Biomarkers 5:192–204

Morrison HI, Wilkins K, Semenciw R, Mao Y, Wigle D (1992) Herbicides and cancer: a review. J Natl Cancer Inst 84:1866–1874

Nehez M, Boros P, Ferke A, Mohos J, Palotas M, Vetro G, Zimanyi M, Desi I (1988) Cytogenetic examination of people working with agrochemicals in the Southern region of Hungary. Regul Toxicol Pharmacol 8: 37–44

Nicholas HA, Vienne M, Berghe H (1979) Induction of sister chromatid exchanges in cultured human cells by an organophosphorus insecticide: malathion. Mutat Res 67:167–172

Paldy A, Puskas N, Vincze K, Hadhazi M (1987) Cytogenetic studies on rural populations exposed to pesticides. Mutat Res 187:127–132

Pool-Zobel BL, Guigas C, Klein R, Kuchenmeister F, Neudecker C, Renner HW, Schmezer P (1993) Assessment of genotoxic effects by lindane. Food Chem Toxicol 31:271–283

Pope AM, Rall DP (1995) Environmental Medicine. Integrating a missing element into medical education. Institute of Medicine, National Academy Press, Washington DC

Rabello MN, Becak W, De Almeida WF, Pigati P, Ungaro MT, Murata T, Pereira CAB (1975) Cytogenetic study on individuals occupationally exposed to DDT. Mutat Res 28:449–454

Ribas G, Frenzilli G, Barale R, Marcos R (1995) Herbicide induced DNA damage in human lymphocytes evaluated by the single-cell gel electrophoresis (SCGE) assay. Mutat Res 344:41–54

Rupa DS, Rao PVL, Reddy PP, Reddi OS (1988) In vitro effect of monocrotophos on human lymphocytes. Bull Environ Contam Toxicol 41:737–741

Rupa DS, Reddy PP, Reddi OS (1989) Analysis of sister-chromatid exchanges, cell kinetics and mitotic index in lymphocytes of smoking pesticide sprayers. Mutat Res 223:253–258

Scarpato R, Migliore L, Angotzi G, Fedi A, Miligi L, Loprieno N (1996) Cytogenetic monitoring of a group of Italian floriculturists: no evidence of DNA damage related to pesticide exposure. Mutat Res 367:73–82

Scarpato R, Hirvonen A, Migliore L, Falck G, Norppa H (1997) Influence of *GSTM1* and *GSTT1* polymorphisms on the frequency of chromosome aberrations in lymphocytes of smokers and pesticide-exposed greenhouse workers. Mutat Res 389:227–235

Scassellati-Sforzolini G, Pasquini R, Moretti M, Villarini M, Fatigoni C, Dolara P, Manarca S, Caderni G, Kuchenmeister F, Schmezer P, Pool-Zobel BL (1997) In vivo studies on genotoxicity of pure and commercial linuron. Mutat Res 390:207–221

Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191

Snedecor G, Cochran W (1988) Statistical Methods. Iowa State University Press, Iowa

Sobti RC, Krishan A, Pfaffenberger CD (1982) Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutat Res 102:89–102

Speit G, Haupter S, Hartmann A (1998) Evaluation of the genotoxic properties of paraquat in V79 Chinese hamster cells. Mutat Res 412:187–193

Titenko-Holland N, Windham G, Kolachana P, Reinish F, Parvatham S, Osorio AM, Smith MT (1997) Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: A study of malathion-exposed workers. Mutat Res 388:85–95

Vigreux C, Poul JM, Deslandes E, Lebailly P, Godard T, Sichel F, Henry-Amar M, Gauduchon P (1998) DNA damaging effects of pesticides measured by the single cell gel electrophoresis assay (comet assay) and the chromosomal aberration test, in CHOK1 cells. Mutat Res 419:79–90

Wang TC, Lee TC, Lin MF, Lin SY (1987) Induction of sister-chromatid exchanges by pesticides in primary rat tracheal epithelial cells and Chinese hamster ovary cells. Mutat Res 188:311–321

Yoder J, Watson M, Benson WW (1973) Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutat Res 21:335–340

Zahm SH, Blair A (1992) Pesticides and non-Hodkin's lymphoma. Cancer Res 52: 5485–5488

Zahm SH, Devesa SS (1995) Childhood cancer: Overview of incidence trends and environmental carcinogens. Environ Health Perspect 103:177–184