Review

Genotoxicity of pesticides: a review of human biomonitoring studies

Claudia Bolognesi*

Toxicological Evaluation Unit, National Cancer Research Institute—Largo Rosanna Benzi 10, 16132 Genova, Italy

Abstract

Pesticides constitute a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases. Pesticides have been considered potential chemical mutagens: experimental data revealed that various agrochemical ingredients possess mutagenic properties inducing mutations, chromosomal alterations or DNA damage. Biological monitoring provides a useful tool to estimate the genetic risk deriving from an integrated exposure to a complex mixture of chemicals. Studies available in scientific literature have essentially focused on cytogenetic end-points to evaluate the potential genotoxicity of pesticides in occupationally exposed populations, including pesticide manufacturing workers, pesticide applicators, floriculturists and farm workers. A positive association between occupational exposure to complex pesticide mixtures and the presence of chromosomal aberrations (CA), sister-chromatid exchanges (SCE) and micronuclei (MN) has been detected in the majority of the studies, although a number of these failed to detect cytogenetic damage. Conflicting results from cytogenetic studies reflect the heterogeneity of the groups studied with regard to chemicals used and exposure conditions. Genetic damage associated with pesticides occurs in human populations subject to high exposure levels due to intensive use, misuse or failure of control measures. The majority of studies on cytogenetic biomarkers in pesticide-exposed workers have indicated some dose-dependent effects, with increasing duration or intensity of exposure.

Chromosomal damage induced by pesticides appears to have been transient in acute or discontinuous exposure, but cumulative in continuous exposure to complex agrochemical mixtures.

Data available at present on the effect of genetic polymorphism on susceptibility to pesticides does not allow any conclusion. © 2003 Elsevier Science B.V. All rights reserved.

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1. Introduction

Pesticides constitute a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases. Their application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhanced agricultural productivity and crop yields.

A total of about 890 active ingredients are registered as pesticides in USA and currently marketed in some 20,700 pesticide products [1].

Many of these compounds, because of their environmental persistence, will linger in our environment for many years to come.

All people are inevitably exposed to pesticides, through environmental contamination or occupational use. The general population is exposed to the residues

* Tel.: +39-010-5600215.
E-mail address: claudia.bolognesi@istge.it (C. Bolognesi).

of pesticides, including physical and biological degradation products in air, water and food.

Occupational exposure occurring at all stages of pesticide formulation, manufacture and application involves exposure to complex mixtures of different types of chemicals, active ingredients and by-products present in technical formulations such as impurities, solvents and other compounds produced during the storage procedure. Moreover, although inert ingredients have no pesticidal activity, they may be biologically active and sometimes the most toxic component of a pesticide formulation.

Pesticides act selectively against certain organisms without adversely affecting others. Absolute selectivity, however, is difficult to achieve and most pesticides are a toxic risk also to humans.

Pesticides are the most important method in self-poisoning in the developing world. Three million cases of pesticide poisoning, nearly 220,000 fatal, occur world-wide every year [2].

While data on the acute toxicity of many of these chemicals is plentiful, knowledge on their delayed effects is much more limited. The International Agency for Cancer Research (IARC) has reviewed the potential carcinogenicity of a wide range of insecticides, fungicides, herbicides and other similar compounds. Fifty-six pesticides have been classified as carcinogenic to laboratory animals. Associations with cancer have been reported in human studies for chemicals such as phenoxy acid herbicides, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), lindane, methoxychlor, toxaphene and several organophosphates [3].

Epidemiological data on cancer risk in farmers are conflicting. Meta-analyses showed that farmers were at risk for specific tumours including leukaemia [4–6] and multiple myeloma [7]. For most other cancer sites, farmers were found to have lower rates than other people, probably due to healthy worker effect.

Exposure to pesticides has also been the subject of great concern in view of its possible role in the induction of congenital malformations. The incidence of congenital malformations and parent's exposure to pesticides have been covered by a number of studies, the results of which have been conflicting and sometimes inconclusive [8–10]. Recent findings suggest that female workers in flower greenhouses may

have reduced fertility, and that exposure to pesticides may be part of the causal chain [11].

Genotoxic potential is a primary risk factor for long-term effects such as carcinogenic and reproductive toxicology. The majority of pesticides have been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage [12–16]. Pesticides have been considered potential chemical mutagens: experimental data revealed that various agrochemical ingredients possess mutagenic properties. The genotoxic potential for agrochemical ingredients is generally low, as they yield positive results in few genotoxicity tests. The lowest effective dose in single test is generally very high. As most occupational and environmental exposures to pesticides are to mixtures, the genotoxic potential evaluated on single compounds could not be extrapolated to humans.

The genotoxicological biomonitoring in human populations is a useful tool to estimate the genetic risk from an integrated exposure to complex mixtures of chemicals.

Although a number of biomarkers are available to assess transient and permanent genotoxic responses, biomonitoring studies on human populations exposed to pesticides have essentially focused on cytogenetic end-points, namely chromosomal aberrations (CA), micronuclei (MN) frequency and sister-chromatid exchanges (SCE).

2. Cytogenetic biomonitoring studies

Genetic damage at the chromosomal level entails an alteration in either chromosome number or chromosome structure, and such alterations can be measured as CA or MN frequency. Conventional techniques for measuring chromosomal changes require proliferating cells so that chromosomes can be seen at mitosis.

Micronuclei are acentric chromosomal fragments or whole chromosomes left behind during mitotic cellular division and appear in the cytoplasm of interphase cells as small additional nuclei. In contrast to the CA evaluation the scoring of micronuclei in lymphocytes is simple and fast.

The SCE analysis was also adopted as an indicator of genotoxicity, although the exact mechanism that

leads to an increased exchange of segments between sister chromatids is not known in detail at present.

Recent studies revealed the nucleotide pool imbalance can have severe consequences on DNA metabolism and it is critical in SCE formation. The modulation of SCE by DNA precursors raises the possibility that DNA changes are responsible for the induction of SCE and mutations in mammalian cells [17,18].

While increased levels of CA have been associated with increased cancer risk [19,20], a similar conclusion has not been reached for SCE or MN. However, high levels of SCE and MN frequency have been observed in persons at higher cancer risk due to occupational or environmental exposure to a wide variety of carcinogens [21–25].

A review of the literature dealing with genotoxicity in human groups exposed to pesticides showed a large number of studies employing CA test, SCE analysis, or MN assay. Their findings are reported in Tables 1–4, and so far are not conclusive.

Evidence of CA increases, mainly as structural chromosomal aberrations in occupationally exposed populations, was demonstrated in the vast majority of available studies. The sensitivity of SCE is lower than that of the CA test in detecting genotoxic effects related to pesticide exposure and fewer data are

therefore, available for MN than for the other cytogenetic endpoints. The negative studies outnumber the positive ones [26–31].

Cytogenetic studies in the scientific literature, 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 studies concern groups of people involved in pesticide production or use generally exposed to moderate levels of complex mixtures of genotoxic chemicals.

3. Acute exposure

3.1. Poisoned subjects

Poisoned subjects who suffered severe acute intoxication by attempting to commit suicide, accidentally or by violating labour safety measures, represent the most interesting human model to study the genotoxicity of these compounds in man.

Although millions of cases of pesticide poisonings were documented every year around the world [32,33], only few data on cytogenetic analysis in these subjects are available.

Table 1
Cytogenetic effects in human populations exposed to pesticides—chemical plant workers

Study subjects (exposed/controls)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
44/30	Novozir Mn80 (mancozeb-contained fungicide)	Up to 2	CA SCE	Pos (+1.83) Pos (+1.17)	Jablonika et al. [37]
14/50, nine formulators, five packers	Azynphos methyl, dimethoate, malathion, methyl parathion	N.D.	SCE	Pos (+1.21)	Laurent et al. [40]
19/36	2,4,5-T, 2,4-D	10–30	CA	Pos (+2.05)	Kaioumova and Khabutdinova [38]
20/20	Pesticide mixture; most commonly used pesticides: 2,4-D, atrazine, alachlor, cyanazine, malathion	4–30 (sampling carried out after 8 months high exposure period)	CA MN	Pos (+6.10) Pos (+3.63)	Garaj-Vrhovac and Zeljezic [30,31], Zeljezic and co-workers [43,45]
20/20			SCE	Pos (+2.23)	Zeljezic and Garaj-Vrhovac [44]
135/111	Organophosphates	1–24	SCE	Pos (+1.85 smokers) (+1.63 non-smokers)	Padmavathi et al. [39]

[a] Chromosomal aberrations, SCE and micronuclei in peripheral blood lymphocytes.

Table 2
Cytogenetic effects in human populations exposed to pesticides—pesticide sprayers

Study subjects (exposed/control)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
(a) Exposure to single pesticide					
35/15	*Forestry workers*: 2,4-D, MCPA	N.D.	SCE		Linnainmaa [56]
	Before spraying			Neg	
	During spraying			Neg	
	After spraying			Neg	
19/15	*Forestry workers*: 2,4-D, MCPA; after spraying season	6–28 (days)	CA	Neg	Mustonen et al. [57]
60/42	*Papaya workers*: ethylene dibromide	5 (average)	CA	Neg	Steenland et al. [63]
			SCE	Neg	
24/24	*Fumigant appliers (open-field)*: phosphine and other pesticides	Discontinuous use of phosphine, at least 8 months	CA	Pos (+3.58)	Garry et al. [52]
18/26	*Fumigant appliers (open-field)*: phosphine and other pesticides	Discontinuous use of phosphine, at least 8 months	CA	Pos. (+3.4)	Garry et al. [53]
31/21	*Fumigators*: phosphine	N.D.	MN	Neg	Barbosa and Bonin [54]
38/16	*Medfly eradication program*: malathion	After spraying season	MN	Neg	Titenko-Holland et al. [62]
31/30	Ethylenbis (dithiocarbamate)				
	Fungicide sprayers	N.D.	CA	Pos (+1.32)	Steenland et al. [61]
13/30	*Tomato farmers*	N.D.	CA	Neg	
31/30	*Fungicide sprayers*	N.D.	SCE	Pos (+1.12)	Steenland et al. [61]
31/27	*Fumigant appliers*: methylbromide	0.3–22	MN[b]	Neg	Calvert et al. [60]
12/9	*Pesticide applicators*: 2,4-D	Discontinuous use	MN	Neg	Figgs et al. [59]
(b) Exposure to pesticide mixture					
109/57	No data	2–20	CA	Pos (+1.68)	Nehez et al. [69]
80/24	Pesticide mixture (80 formulations): carbamates, dithiocarbamates, heterocyclic compounds, nitro-compounds, organochlorines, phenoxy-acetic acids, phthalimides, pyrethroids, sulphur and copper containing chemicals	1 to >15	CA	Pos (+2.69 to +3.89)	Paldy et al. [71]
15/10	*Vineyard workers*: copper sulfate, DDT, dichlorvos, dieldrin, dithane, lindane, metasystox, parathion, quinalfos	5–12	CA	Pos (+4.16)	Rita et al. [70]
55/60	*Greenhouse workers*: pesticide mixture; insecticides (carbamates, organophosphates); pyrethroid fungicides, acaricides	2–15	CA	Pos. (+1.18–1.52)	Nehez et al. [72]
25/30 (male smokers)	*Vegetable garden workers*: BHC, DDT, dimethoate, fenitrothion, gromor, malathion, parathion, urea	5–38	CA	Pos (+1.72–2.08)	Rupa et al. [65]
			SCE	Pos (+1.43–1.64)	

Table 2 (*Continued*)

Study subjects (exposed/control)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
52/25 (male non-smokers)	*Cotton field workers*: BHC, DDT, cypermethrin, dimethoate, endosulfan, fenvalerate, malathion, methyl parathion, monocrotophos, phosphamidon, quinolphos	1–25	CA	Pos (+3.32–6.51)	Rupa et al. [73]
50/47 (male smokers)	*Cotton field workers*: BHC, DDT, cypermethrin, dimethoate, endosulfan, fenvalerate, malathion, methyl parathion, monocrotophos, phosphamidon, quinolphos	1–25	CA	Pos (+2.01–2.12)	Rupa et al. [74]
			SCE	Pos (+1.32–1.88)	Rupa et al. [64]
26/26 (male non-smokers)	*Cotton agriculturists*: cypermethrin, dimethoate, endosulfan, fenvalerate, malathion, methyl parathion, monocrotophos, phosphamidon, quinolphos	2–18	CA	Pos (+3.61)	Rupa et al. [75]
61/45 (male non-smokers)		2 to >20	SCE	Pos (+2.36)	Rupa et al. [67]
29/14	*Greenhouse workers*: carbamates, dithiocarbamates, organochlorines, organophosphates	4–30	CA	Pos (+4.42)	Kourakis et al. [66]
56/30	*Tomato*, *cucumber cultivation*: organophosphates, carbamates, dithiocarbamates, organochlorines	6	CA	Pos (+5.01)	Kourakis et al. [80]
29/30	Greenhouse			Pos (+6.35)	
27/30	Open-field			Pos (+3.54)	
			SCE	Neg	
7/6	*Open-field*: pesticide mixture, cypermethrin, deltametrin	3–38	CA	Pos (+2.81)	Mohammad et al. [76]
134/157	*Greenhouse floriculturists*: pesticide mixture—benzimidazolics, carbamates, organophosphates, polychlorinated, pyrethroids, thiophthalimides	1–50	SCE		Lander and Ronne [68]
	Smokers			Neg	
	Non-smokers			Pos (+1.12)	
48/50	*Farmers* (*cereals*, *fruits*, *vegetables*): pesticide mixture; most commonly used pesticides: alachlor, atrazine,benomyl, carbaryl, deltamethrin, dinocap, linuron, mancozeb, MCPA, metobromuron, metolachlor, oxadixyl, oxyfluorfen, propineb, triadimenol	4–50	SCE	Neg	Pasquini et al. [78]
			MN	Pos (+1.20)	
27/20	*Vineyard workers*: pesticide mixture; most commonly used pesticides: 2,4-D, desmedipham, diazinon, dithiocarbamate, ethofumesate, metalaxyl + Cu, phenmedifan, propiconazole, triadimefon, vinclozolin	12, 1; end of spraying, season	CA	Pos (+15.8)	Joksic et al. [26]

Table 2 (*Continued*)

Study subjects (exposed/control)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
			SCE	Neg	
			MN	Pos (+7.67)	
22/16	Pesticide mixture: captan, cyfluthrin, cypermethrin, deltamethrin, dichlorvos, diazinon, endosulfan, fenitrothion, fenvalerate, linuron, magnesium–aluminium phosphide, methamidophos, methomyl, methyl bromide, parathion pentachlorphenol, propoxur	7	MN	Neg	Venegas et al. [79]
	Chlorinated hydrocarbons, carbamates (propoxur), organophosphates (dichlorvos, dimethoate, malathion), pyrethroids (cypermethrin, D-allethrin, deltamethrin, sumithrin)		CA		Amr [77]
39/20	*Formulators*	5–25		Pos (+1.61)	
32/20	*Applicators*	5–15		Pos (+2.38)	

[a] Chromosomal aberrations, SCE and micronuclei in peripheral blood lymphocytes.

[b] The MN frequency was also evaluated in buccal mucosa cells.

A study carried out in 31 patients acutely intoxicated by organophosphorous insecticides [34] represents the only evidence for an increase in chromosomal damage.

These patients suffering acute organophosphate insecticide intoxication evidenced a temporary but significant increase in the frequency of chromatid breaks and stable chromosome-type aberrations, deletions and translocations. The frequency of CA was significantly elevated immediately after intoxication and 1 month later, but the normal frequency was restored 6 months after the acute exposure. A chromosomal damage was also observed in mildly intoxicated persons in this study but the frequency of numerical and chromatid aberrations was not significant [34].

A systematic study in self-poisoning individuals over 20 years in Hungary shows an increase in chromosomal damage and aneuploidy in small groups of subjects intoxicated by malathion or trichlorfon. A possible dose–effect relationship was hypothesised, but the limited number of cases and the uncertainty of chemical exposure data did not allow a clear conclusion [35].

A further study carried out in a small group of firemen (20 exposed subjects/20 controls) accidentally intoxicated by dimethoate, a organophosphorous insecticide, reports a statistically significant increase of SCE 2 months after the accident when the compound was still present in the biological fluids of a number of subjects [36].

4. Occupational exposure

4.1. Chemical plant workers

All the available cytogenetic studies on chemical plant workers yielded positive results: a significant difference was observed in exposed subjects with respect to controls with 1.17–6.10 increment folds (Table 1). The production varies in the pesticide industry by the seasons in association with the market trend, characterising on intermittent exposure of the employers. These factors could be responsible for the large range in cytogenetic responses observed in the studies.

Significant simultaneous increase in CA and SCE was observed in workers involved in the production of mancozeb formulation containing fungicide [37] and in CA in herbicide production workers exposed to 2,4, 5-T and 2,4-dichlorophenoxy acetic acid (2,4-D) [38].

Organophosphate exposure was also associated with an increase in cytogenetic damage as SCE frequency:

Table 3
Cytogenetic effects in human populations exposed to pesticides—floriculturists

Study subjects (exposed/control)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
36/15 sprayers and not sprayers	*Greenhouse workers*: pesticide mixture—carbamates, organochlorines, organophosphates	At least 10	CA	Pos (+1.02) (+4.3 exchange type aberrations)	Dulout et al. [81], Dulout et al. [86]
14/13	Symptomatic group/asymptomatic group	At least 10	SCE	Pos (+1.18)	Dulout et al. [81], Dulout et al. [86]
38/32	*Plant breeders*: pesticide mixture—organophosphates, carbamates, organochlorines	At least 10	CA	Neg	Dulout et al. [92]
	Greenhouse and open-field: chloroganics, hydrocarbon derivatives, organotin compounds, nitroorganics, organophosphates, pyrethorids, thio-organics	N.D.	CA		De Ferrari et al. [82]
32/31	Healthy people			Pos (+1.86)	
32/31	Cancer patients			Pos (+1.45)	
28/15	Healthy people	N.D.	SCE	Pos (+1.40)	De Ferrari et al. [82]
14/15	Cancer patients	N.D.	SCE	Pos (+1.50)	De Ferrari et al. [82]
71/75	*Floriculturists and horticulturists*: pesticide mixture; most commonly used pesticides: benzimidazoles, carbamates, chloroorganics, dithiocarbamates, morpholines, nitroorganics, organophosphates, organotins, phthalimides, pyrethroids	2/55	MN	Pos (+1.28)	Bolognesi et al. [28,89]
27/28	*Floriculturists and horticulturists*: pesticide mixture; most commonly used pesticides: benomyl, captan, deltamethrin, fenvalerate, methomyl, paraquat	>10	SCE	Neg	Carbonell et al. [91]
61/60	*Floriculturists and horticulturists*: pesticide mixture; most commonly used pesticides: amides, carbamates, diazines, organochlorines, organophosphates, pyrethroids, thiocarbamates, triazines	5–29	CA	Pos (+1.39)	Carbonell et al. [83]
67/67			SCE	Neg	Carbonell et al. [83]
29/53	*Floriculturists and horticulturists*: pesticide mixture; most commonly used pesticides: abamectine, acephate, benomyl, buprofecin, captan, chlorpyrifos, cypermethrin, cyromazine, deltamethrin, diquat, endosulfan, fenitrothion, folpet, methomyl, ofurace, paraquat, procymidone	N.D.	CA	Pos (+1.55)	Carbonell et al. [84]
43/41	*Greenhouse workers*: >100 agrochemical formulations	N.D.	CA	Neg	Scarpato et al. [90]
			SCE	Neg	
			MN	Neg	

Table 3 (*Continued*)

Study subjects (exposed/control)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
34/33	*Greenhouse workers*: pesticide mixture; most commonly used pesticides: acephate, azocyclotin, benfuracarb, captan, chlorothalonil, dichlorvos, dimethoate, dicofol, endosulfan, fenpropathrin, iprodione, mancozeb, metiram, methomyl, procymidone, propineb, toclofos-methyl, trichlorfon, vinclozolin	7/41	MN	Neg	Falck et al. [27]
17/33 pesticide sprayers highly exposed			MN	Pos (+1.22)	Falck et al. [27]
110/29	*Greenhouse workers*: pesticide mixture; most commonly used pesticides: amitraz, alfacypermethrin, benomyl, buprofezin, carbendazim, chlorpyrifos, chlorothalonil, chlormequatchlorid, deltamethrin, daminozid, dienochlor, endosulfan, fenpropathrin, iprodion, pirimicarb, methomyl, praclobutrazol, thiram, vinclozolin	N.D.	CA		Lander et al. [85]
68/29	*Preseason*			Pos (+1.18)	Lander et al. [85]
58/29	*Postseason*			Pos (+1.38)	Lander et al. [85]
30/30	*Greenhouse workers*: pesticide mixture—carbamates, organochlorines, organophosphates	1.5–10	SCE	Pos (+1.77)	Gomez-Arroyo et al. [88]
			MN in buccal mucosa cells	Pos (+2.63)	
104/44	*Greenhouse workers*: pesticide mixture—carbamates, organochlorines, organophosphates	2.5–55.5	SCE	Pos (+1.26)	Shaham et al. [87]
107/61	*Greenhouse and open-field workers*: pesticide mixture—organophosphates, carbamates, benzimidazoles, pyrethroids, tiophthalimides, pyrimidinol compounds, organochlorines, bypirydilics, amides, morpholinics	2–70	MN	Pos (+1.45)	Bolognesi et al. [29]

[a] Chromosomal aberrations, SCE and micronuclei in peripheral blood lymphocytes.

positive results were reported in workers employed in insecticide production plants [39,40].

A further study (not reported in the table) [41] failed to detect an increase in CA in a group of workers chronically exposed to the organophosphate insecticide methyl parathion. A number of factors do not allow to evaluate the exposure level in this study. First the length of exposure to methyl parathion was extremely variable from 1 week to 7 years with intermittent period of no-exposure. In addition, the mean blood cholinesterase level less than 75% as the main criterion used to select the exposed subjects [42], could not be considered as a useful index for a specific exposure.

Finally, in very recent studies, simultaneous increases in CA, SCE and MN frequency occurred in workers exposed to a complex mixture of compounds including atrazine, malathion, cyanazine, and 2,4-dichlorophenoxy acetic acid in a pesticide plant in

Table 4

Cytogenetic effects in human populations exposed to pesticides—agricultural workers

Study subjects (exposed/ control)	Exposure	Duration (years)	Analysed biomarker[a]	Result	Reference
10/7	Pesticide mixture; most commonly used pesticides: 2,4-D, diquat, MCPA, MCPP, dithiocarbamates	2–29	CA	Neg	Hogstedt et al. [58]
94/76	*Horticulturists*: pesticide mixture; most commonly used pesticides: carbamates, organophosphates, organochlorines, triazines, thiocarbamates, ureics	1–35	SCE	Neg	Gomez Arroyo et al. [95]
71/29	*Open-field and greenhouse workers*: pesticide mixture; most commonly used pesticides: benzimidazoles, carbamates, dithiocarbamates, morpholines, nitroorganics, organochlorines, organophosphates, phthalimides, pyrethroids	2–52	MN	Neg	Bolognesi et al. [96]
30/30	*Potato cultivation*: pesticide mixture; most commonly used pesticides: carbamates, dithiocarbamates, organophosphates	5	CA	Neg	Hoyos et al. [98]
			SCE	Neg	
18/21	*Berry pickers*: pesticide mixture; most commonly used pesticides: captan, carbofuran, diazinon, endosulfan, malathion	1–24	MN	Neg	Davies et al. [97]
20/20	*Banana workers*: pesticide mixture—chlorpyrifos, dibromochloropropene, fenamiphos, gramoxone, imalzabile, terbufos, thiabendazole	N.D.	CA	Pos (+1.26)	Au et al. [93]
23/23	Pesticide mixture; most commonly used pesticides: carbamates, organophosphates	0–16	CA	Pos (+3.25)	Antonucci and Colus [94]
20/16 male	Pesticide mixture; most commonly used pesticides: agrimycin, benlate, cercobin, curzate, dacostar, endosulfan, folidol, folicur, lannate, manzate, methamidophos, microshield, nuvacron, orthene, pyrimicin, recop, roundup, sencor, vertimec	10–40	CA	Neg	D'Arce and Colus [99]
64/50	*Greenhouse workers*: pesticide mixture; most commonly used pesticides: abamectine, acrinathrin, cymoxanil, cyromazyne, endosulfan, imidacloprid, malathion, mancozeb, methamidophos, methomyl, oxamyl, piryproxifen, procymidone, tralomethrin	9.82 ± 1.01	MN[b]	Neg	Lucero et al. [100]
50/66	Pesticide mixture; most commonly used pesticides: cymoxanil, cyromazine, endosulfan, imidacloprid, mancozeb, methomyl, methamidophos, oxamyl, permethrin, procymidone, pyriproxifen, tralomethrin	8.62 ± 1.13	MN[b]	Neg	Pastor et al. [101]
49/50	Pesticide mixture; most commonly used pesticides: cafenvalerate, carbosulfan, chlorothalonil, deltamethrin, dimethoate, iprodione, lambda-cyhalothrin, methomyl, propanocarb, vinclozolin	16.28 ± 1.10	MN[b]	Neg	Pastor et al. [102]
39/22	*Greenhouse workers*: pesticide mixture; most commonly used pesticides: abamectine, acrinathrin, cymoxanil, cyromazyne, endosulfan, imidacloprid, malathion, mancozeb, methamidophos, methomyl, oxamyl, piryproxifen, procymidone, tralomethrin	8.31 ± 1.12	MN	Neg	Pastor et al. [103]
84/65	*Greenhouse workers*: pesticide mixture; N.D.	18.75 ± 0.89	MN[b]	Neg	Pastor et al. [104]

[a] Chromosomal aberrations, SCE and micronuclei in peripheral blood lymphocytes.

[b] The MN frequency was also evaluated in buccal mucosa cells.

Croatia [30,31,43–45]. A statistically significant increased number of aberrant cells, chromatid and chromosome breaks, acentric fragments, dicentric chromosomes, MN frequency and SCE were found in exposed subjects compared with controls. Different samplings in the same group of workers revealed an increase in cytogenetic parameters regardless of the period of sampling, before or after the end of the production season.

The heterogeneity of exposure in pesticide production plants does not allow ascertaining the causal agents. Workers employed in the pesticide industry are generally occupationally exposed not only to the final products, but also to a wide range of toxic chemicals identified as clastogenic agents that are used as raw materials including formaldehyde [46], acrylonitrile [47] and organic solvents such as toluene [48,49] and benzene [50,51].

4.2. Pesticide users

The large majority of cytogenetic monitoring studies in human populations exposed to pesticides concerns the genotoxic effects of chronic low doses of a single compound or of a complex mixture of chemicals. A number of studies have reported a significant incidence of cytogenetic damage such as CA, SCE and MN frequency in agricultural workers, forestry workers, floriculturists, vineyard cultivators, cotton field workers and others. However, these positive findings have not been substantiated by all investigators.

The inconsistent responses among studies could reflect different exposure conditions, such as the 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.

A number of factors have been used to describe pesticide exposure in cytogenetic studies: pesticide consumption (kg per year), amount of genotoxic chemicals used, total number of pesticide formulations used, extension of the areas of pesticide application, and working conditions (greenhouse versus open-field).

However the lack of homogeneity in the exposure description prevents the use of all these data in the comparison of the different studies.

In addition 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 represent major factors of uncertainty in the comparison of results from different studies.

Our analysis was based on a classification of the available cytogenetic biomonitoring studies according to the major determinants of the exposure such as agricultural task and crop grown.

Agricultural tasks greatly influence the extent of exposure independently from the grown crops. People involving in preparing and spraying pesticide mixture could be identified as the most exposed groups of farmers.

Types of crops characterise the pesticide use and the average frequency of application. Ornamental crops are the most intensively treated crops. A large consumption of a wide variety of compounds belonging to different chemical classes is common in producing ornamental crops highly susceptible to pests, considering also the low health hazard for consumers of flowers marketed exclusively for aesthetic appeal.

High exposure was reported to be associated with intensive activity or with work in greenhouses in producing flowers or ornamental plants.

The environmental conditions in the greenhouses, such as enclosed spaces, high temperature, and high humidity favour the pesticide exposure. Greenhouse workers are exposed to pesticides mainly during the preparation, mixing and application stages, but may also come into contact with the agents during re-entry activities such as the cutting and potting of recently treated cultures. Re-entering intervals may not adequately protect against chronic low level exposure, above all to persistent chemicals with a long half-life. Pesticide residues on fruit and foliage could be absorbed mainly through the skin of the hands and the forearms.

4.3. Pesticide sprayers

Pesticide sprayers represent the most exposed group of agricultural workers, positive findings being obtained in 18 out of 27 biomonitoring studies (Table 2a and b). Negative results were obtained in 7 out of 10 studies concerning exposure to single compounds and

only in 2 out of 17 studies for exposure to pesticide mixture.

4.4. Exposure to single pesticide

The studies that examined cytogenetic effects in workers exposed to a single pesticide (Table 2a) are much more conclusive as regards the genotoxic effects of specific compounds.

Fumigators exposed to highly toxic phosphine gas, at concentrations exceeding the accepted standard (0.3 ppm), were shown to have more than two times higher frequencies of gaps, breaks, deletions and total CA than control subjects or grain workers, who may be only incidentally exposed to phosphine and other pesticides [52,53].

A more recent study evaluating MN frequency in phosphine fumigators after the improvement in technology of storage and fumigation did not show any significant increase in chromosomal damage associated with occupational exposure at concentrations very close to the time weighted accepted standard. Measurement of urine mutagenicity in this study did not show any significant difference between fumigators and controls [54]. Phosphine proved to be a weak genotoxic agent in experimental in vivo studies, with positive results only at very high concentrations [55]. The improvement in practices of use of a genotoxic compound keeps exposure at levels that do not present detectable genotoxic health risks, as it has been demonstrated by the use of cytogenetic biomarkers, micronucleus frequency in peripheral blood lymphocytes and urine mutagenicity [54].

No genotoxic effects, measured as SCE, associated with exposure to phenoxy acid herbicides, such as 2,4-dichlorophenoxy acetic acid and MCPA, were observed in workers populations engaged in the defoliation of a Finnish forest [56,57]; likewise, no significant increase of CA was revealed in a study carried out in agricultural workers [58].

A further study [59] did not find any relationship between MN frequency in human lymphocytes and the occupational exposure to 2,4-D measured as urinary concentrations. The data of these biomonitoring studies support the experimental results, which indicate the phenoxy acid herbicides are not direct DNA-damaging agents [13].

Exposure to methyl bromide has been associated with an increased risk of CA. A recent study of lymphocytes and oro-pharyngeal cells in humans confirmed the experimental evidence of the genotoxicity of the fumigant although no consistent differences between workers and referents were observed for frequencies of kinetochore-negative or kinetochore-positive lymphocyte MN [60]. This study is limited by the small sample and by the absence of data about the exposure levels.

Increase in chromosomal aberrations and SCEs was observed in a population of backpack sprayers applying ethylenebis dithiocarbamate (EDC) fungicides to tomato cultures compared to a group of less exposed landowners and to non-exposed referents. The urinary ETU, as a measure of the exposure revealed higher levels of the metabolite in applicators not using protective devices, than in landowners while all non-exposed had urinary ETU levels below the limit of detection [61]. EDC fungicides, such as maneb and mancozeb have been considered weakly genotoxic agents [12]. The positive result obtained in this cytogenetic study carried out in Mexico could be attributed to the level of exposure to the fungicides higher than the available data for applicators in Europe and in United States [61].

The potential risk in humans for chromosomal damage stemming from exposure to malathion can be considered relatively low, as suggested by a biomonitoring study in intermittently exposed workers involved in a fruit fly eradication program [62].

Finally, a negative result, in terms of CA and SCE, was also obtained for low exposure to ethylene dibromide also for long periods in a cytogenetic study on papaya workers [63]. Ethylene dibromide is a carcinogenic compound and showed positive results in a number of mutagenic assays [3,13].

4.5. Exposure to pesticide mixtures

Multiple exposures are a rule and not an exception in agricultural practice: pesticide applicators spray large amount of agrochemical mixtures including a significant number of genotoxic compounds.

Cytogenetic studies on pesticide sprayers cover a broad range of populations employed in the cultivation of different crops and in different settings: grapes,

vegetables, cotton, flowers, and tomatoes, grown in greenhouses and in the open-field.

The pesticides most often used were 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 [12–16].

The occupational exposure to pesticide mixtures in sprayers is associated with a genetic risk, as it has been demonstrated by the use of cytogenetic biomarkers. Fifteen out of 17 studies give positive results inducing an increase of a cytogenetic parameter CA, SCE or MN frequency with a range of 1.12–15.8 increment folds (Table 2b).

The effects of pesticide exposure during the spraying season was primarily observed for those workers who had not used protective clothing or devices [64–67] mainly during re-entry activities [68].

An increase of CA, chiefly chromatid gaps and breaks, was observed in sprayers from the beginning to the end of the spraying season [26,69–77]. A number of studies also provides evidence of a pesticide induced frequency of SCE, with significantly higher values among the pesticide sprayers during the entire duration of exposure [64,65,67,68].

Two out [26,78] of three studies [26,78,79] on the MN frequency in pesticide sprayers exposed to complex mixtures of agrochemical formulations report positive results. Evidence of a significant increase in MN frequency related to a heightened incidence of CA without any increase in SCE frequency was observed in vineyard workers heavily exposed during the spraying season. [26].

A biomonitoring study on pesticide sprayers in central Italy [78] exposed to several insecticides, fungicides and herbicides showed slight evidence of chromosomal damage, detected as an increase in MN frequency, only in subjects exposed for more than 18 years without corresponding effects on SCE induction.

A similar result was obtained in a population of pesticide sprayers working in selected tomato and cucumber farms in the province of Thessaloniki, Greece. A significant increase in chromosomal and chromatid-type aberrations was observed in greenhouse as well as in open-field workers, without any indication of an increase in their basal frequency of SCE [66,80].

An increase of micronuclei frequency but not statistically significant was obtained in a study carried out on pesticide sprayers in Chile, where the small size of groups (22 sprayers/18 controls) reduced the statistical power of the study [79].

4.6. Floriculturists

Investigations on the cytogenetic effects in populations of floriculturists mainly exposed in greenhouses reported a significant increase in the incidence of CA in five [81–85] out of seven studies, SCE in four [81,82,86–88] out of seven, and MN frequency in three [27–29,89] out of four (Table 3). The extensive use of a wide variety of compounds belonging to different chemical classes is commonplace in the cultivation of ornamental plants, given that these are highly susceptible to pests, and that the use of pesticides for flowers marketed exclusively for decorative purposes entails few health risks for consumers.

In addition, greenhouse work represents a potential genotoxic risk due to the environmental conditions, the enclosed spaces, and high temperatures and humidity that favour exposure to pesticides. Continuous exposure could also be fostered by re-entry activities, such as cutting and potting.

The negative results described in a number of studies [90–92] were related to low exposure associated with a limited and most adequate use of pesticides [92] or to short periods of pesticide contact [90].

A significant increase of cytogenetic effects as CA or SCE was reported in Buenos Aires Province (Argentina) in a population involved in intensive production of flowers in greenhouses where considerable amounts of pesticides were applied with little or no protection devices [81,86]. A second cytogenetic analysis, carried out during the winter period in a subgroup of plant breeders from the same population, failed to reveal a cytogenetic damage as a consequence of a lower exposure due to less use of pesticides [92].

4.7. Agriculturists

The last section includes the studies on farmers indirectly exposed to pesticides during agricultural practices and involved in the production of different kinds of crops, chiefly vegetables and fruits.

Positive results were seen in two out of six [93,94] studies on the CA test, while all the studies on SCE and MN gave rise to negative results (Table 4).

Positive results in CA tests from occupational exposure to a mixture of pesticides were reported in recent studies. The first-one, carried out in a population of agricultural workers employed in an agronomic institute in Brazil, showed a significant increase of CA frequencies despite the adoption of protective/preventive measures [94]. The second, conducted on unprotected banana plantation workers in Costa Rica who were exposed on a year-round basis to the pesticides dibromochloropropene, chlorpyrifos, thiobendazole, gramoxone, and terbufos, revealed a substantial increase in chromosomal abnormalities measured by the standard CA assay and an abnormal DNA repair response using the challenge assay [93].

The lack of genetic damage observed in a large number of studies concerning farm workers [58,95–104] reflects a lesser use of pesticides in the production of food crops intended for human consumption and a different kind of exposure mainly through contact with foliar and fruit residues during the agricultural practices. In addition, improvements in working habits and conditions, namely the use of gloves and protective clothing, and more appropriate techniques for the application of agrochemicals could explain the negative results described in more recent studies.

5. Specific cytogenetic effects

A number of recent papers appearing in the scientific literature have focused on the investigation of the cytogenetic effects in pesticide-exposed populations and on the role that these effects play in the evolution of specific tumours.

A pilot study was carried out in phosphine fumigators to investigate the chromosome bands involved in increased breakage and the related frequency of rearrangements. Seasonal exposure to the fumigant phosphine induced a significant increase in chromosome rearrangements in exposed subjects compared to controls. A drop in the frequency of rearrangements was reported within 1 year's time. Four specific bands (1p13, 2p23, 14q32 and 21q22) were shown to account for a significant excess of breaks in exposed subjects and for no breaks in the control groups. These

bands are localised in the known proto-oncogene regions NRAS, NMYC, ELK2 and ETS2, respectively.

A possible role of these specific cytogenetic alterations in the excess of non-Hodgkin's lymphoma detected amongst workers in the grain industry was suggested, as rearrangements or deletions involving bands 1p13, 2p23 and 14q32 were associated with this tumour [53].

Fragile sites (FS) in human chromosomes are specific bands that exhibit non-random gaps or breaks when the cells are exposed to specific agents [105]. Most of the common fragile sites are induced by aphidicolin, a specific inhibitor of DNA polymerase. Fragile sites were thought to be implicated in the chromosomal rearrangements in many cancers. It has been suggested that FS could be the target of the clastogenic action of many genotoxic agents [106,107].

The expression of aphidicolin-sensitive common fragile sites, i.e. breakage-prone regions resulting from exposure to specific agents, was studied in greenhouse floriculturists potentially exposed to mutagenic agents. Two different investigations [108,109] revealed reproducible enhanced expression of FS, following pesticide exposure, in specific chromosomal bands where oncogenes or tumour suppressor genes are localised. The involvement of these bands in chromosomal rearrangements found in haematological malignancies suggests that these cytogenetic alterations may contribute to the initiation of the carcinogenic process.

A very recent study revealed the analysis of aphidicolin-induced-fragile sites appeared a very sensitive biomarker of chromosomal damage resulting from the low level of exposure to organophosphate-based pesticides [110].

6. Effects of genotypes on cytogenetic damage

Genotypes responsible for interindividual differences in the ability to activate or detoxify genotoxic substances are recognised as biomarkers of susceptibility to mutations, cancer and other diseases.

Many enzymatic isoforms have been suggested to contribute to individual cancer susceptibility as genetic modifiers of cancer risk after exposure to genotoxic agent [111,112].

The role of specific polymorphisms of cytochrome P450 (*CYP*) genes involved in the activation and detoxification of xenobiotics, namely cytochrome P450 2E1 (CYP2E1), glutathione *S*-transferase M1 (GSTM1), glutathione *S*-transferase theta 1 (GSTT1), *N*-acetyltransferase 2 (NAT2) and paraoxonase 1 (PON1), in modulating cytogenetic effects was studied in pesticide-exposed populations. The selection of these polymorphic genes was related to their role in the metabolism of pesticides [109,113,114].

CYP2E1, one of the most complex cases in polymorphism nomenclature, belongs to the family of CYP enzymes involved in the typical activation reaction (phase 1) which converts indirect carcinogens to active electrophiles capable of interacting with the biological macromolecules DNA, RNA and proteins.

The CYP2E1 is a cytochrome P450 superfamily involved in the metabolism of many indirect carcinogens such as nitrosamines [115] some components of tobacco smoke [116] and many organic chlorided and non-chlorided solvents [115]. More than 70 different substrates are specifically metabolised by this enzyme [117,118]. This enzyme may be induced by ethanol [119] and thus alcohol intake could modulate oncogenetic process by exposure to carcinogens activated by CYP2E1. In addition, a number of environmental factors, including pesticides, may modify the cancer risk through the altered CYP2E1 enzyme activity. The *CYP2E1* gene is implicated also in the generation of reactive oxygen radicals and the induction of this enzyme is expected to increase oxygen radical generation [120]. Important interindividual differences in the expression of human hepatic CYP2E1 have been demonstrated [121,122].

CYP2E1 is polymorphically distributed in human populations: the estimated frequency of rapid metabolisers is around 10 and 26% in caucasians and oriental populations [123–125]. Significant associations between CYP2E1 polymorphism and cancer risk was reported in a number of studies [121–123].

Glutathione *S*-transferases (GSTs) are the most important group of detoxifying enzymes. This family of enzymes presents genetic polymorphisms in human populations responsible for the glutathione conjugation of various reactive species of many chemicals including pesticides [126]. Null genotypes for the *GSTT1* and *GSTM1* genes have been identified to be associated with an increase of cancer risk [127,128].

No studies have yet determined the relative activities of human GST polymorphism toward specific pesticides or class of pesticides: however numerous studies have demonstrated that the resistance of a variety of insects to several different insecticides could be attributed to the overexpression of theta-class GSTs [129].

N-Acetyltransferases (NATs) catalyse reactions of both activation (*O*-acetylation) and detoxification (*N*-acetylation). Two *NAT* genes are expressed in humans, NAT1 and NAT2 [130]; the latter is better known, and it characterises rapid and slow acetylators with an increased risk for different cancers [131].

Paraoxonases (PONs) are responsible for metabolism of organophosphate-based insecticides [114,132, 133].

Serum paraoxonase (PON1) activity plays a major role in the metabolism of organophosphates. Individuals with low PON1 activity are more susceptible to parathion poisoning than individuals with higher PON1 activity [132]. Isoforms of serum paraoxonase exhibit a substrate dependent polymorphism characterised by a different efficiency in metabolising different chemical compounds belonging to organophosphate class [133].

Unfavourable versions of the different polymorphic genes have been associated with an increased activation and decreased detoxification of hazardous compounds, and could entail an increased genetic susceptibility to pesticides. Positive effects on indicator genotype interaction are reported for cytogenetic biomarkers, such as SCE, CA or MN, although the large majority of studies in the scientific literature failed to reveal any clear indication [134,135].

Five studies on the association between metabolic genotypes and early biomarkers of genotoxic exposure CA, MN and SCE in pesticide-exposed populations are available in the scientific literature.

Three studies on pesticide-exposed greenhouse workers [27,136,137] showed genotype effects not depending on pesticide exposure.

Despite the limited number of subjects Scarpato et al. [137] observed a higher chromatid type CA frequency in smokers, exposed and controls with the GSTM1 and also with GSTT null genotypes. In a further study [136], slight and not significative associations were observed between baseline SCE and

GSTT1 positive genotype and between CA frequency and GSTM1 genotypes in smokers.

Falck et al. [27] neither found any genotype effect exclusively in the pesticide-exposed subjects. The GSTM1 positive genotype was associated with an increased MN frequency irrespective of exposure. The NAT2 fast acetylator genotype was associated with an increased MN frequency in all smokers including exposed and controls.

In a population of banana workers [93] the association between CYP2E1, GSTM1 and PON1 genotypes and the increase of cytogenetic outcomes, was studied considering the traditional CA, the challenge assay to evaluate abnormal DNA repair response, and the tandem probe FISH assay in interphase cells, to evaluate breaks in chromosome 1. The results evidenced an underrepresentation of the unfavourable version of the polymorphic genes in the group of farmers and no statistically significant differences were observed when comparing different types of CAs between each unfavourable genotype and favourable genotype.

Statistically significant differences were seen in the challenge assay for dicentrics between farmers with the PON A/A (6 subjects) and PON AB-BB (14 subjects) genotypes but no comparison in the control group was reported.

In the challenge assay, comparison of farmers and controls with each unfavourable genotype revealed statistically significant effects for GSTM1 null on CAs, CYP2E1 m* on breaks and PON A/A on dicentrics. It is difficult to interpret these results because no comparison of the respective favourable genotypes were reported. The observed effect in the challenge test could be interpreted as differential pesticide exposure related response to an in vitro treatment with ionising radiation.

Finally no significant association between GSTM1 and GSTT1 genotypes on the micronuclei frequency was found in a group of Spanish greenhouse workers exposed to pesticides [100].

Although different isozymic forms of human serum enzymes such as paraoxonase (PON1) have been associated with increased toxic effects such as delayed neurotoxicity or reproductive outcome related to the exposure to certain organophosphate compounds [138–141], the data available at present on the influence of genetic polymorphism on pesticide induced cytogenetic damage do not allow any clear conclusion.

The role of polymorphic variations in biotrasformation enzymes on cytogenetic effects induced by pesticide exposure has to be investigated in larger population groups due to the low genotoxic potential of the large majority of pesticides.

7. Dose-dependence of cytogenetic damage

A dose–effect relationship was observed for cytogenetic damage in pesticide-exposed populations.

The increase seen in cytogenetic damage was related to the extent of exposure, with cytogenetic parameters increasing as a result of heavy pesticide exposure. Positive findings were even reported when blood samples were obtained from people suffering from severe pesticide intoxication resulting from violation of occupational safety measures or attempted suicides [50,51]. Significant differences in cytogenetic damage were detected in individuals with symptoms of chronic intoxication with respect to those without [87,92].

In agricultural workers an increase in chromosomal damage was observed during the spraying season when pesticides were used intensively, mainly in workers who had not used protecting clothing and gloves [27,81,84,85,91]. By contrast, negative results were obtained in agricultural workers who had documented low levels of exposure [54,92,95]. The condition of exposure was also associated with an increase of cytogenetic damage. It was observed that individuals working exclusively in greenhouses showed higher levels of chromosomal damage as CA [80] or MN [26,89] than subjects working in open-fields. A significant increase of cytogenetic effects was observed regarding individual protection. The use of mask and gloves seems to protect the workers by reducing incidence of cytogenetic outcomes [68,86,87].

Finally, smoking may potentiate the genotoxic effects of pesticides due to an increase of oral exposure during the agricultural practices. A high frequency of chromosomal damage was detected in smoking greenhouse workers who had not used protective gloves [85].

An additive effect of smoking in inducing a chromosomal damage was also demonstrated. A significant increase in CA [65,74] and SCE [39,69] was observed in smokers compared with non-smokers from pesticide-exposed groups.

Table 5
Cytogenetic effects in human populations exposed to pesticide mixture (summary of the results)

	Analysed biomarker	Number of studies (positive/total)	Results (range of effects)
Pesticide sprayers	CA	13/13	1.18–15.8
	MN	2/3	1.20–7.67
	SCE	4/7	1.12–2.36
Floriculturists	CA	5/7	1.02–1.86
	MN	3/4	1.22–1.45
	SCE	4/7	1.18–1.77
Agricultural workers	CA	2/5	1.26–3.25
	MN	0/7	–
	SCE	0/2	–

Table 5 summarises the results concerning the exposure to pesticide mixture. An increase in the cytogenetic effects is evident with increased exposure as number of positive studies/total and as extent of cytogenetic effect. CA seems the most effective assay in detecting genotoxic damage associated with pesticide exposure although a direct comparison of the results is not possible.

A limited (14/59) number of studies reports results for different biomarkers on the same population. Contrasting results were reported only in four studies with no significant increase in SCE with respect to CA [26,83,80] or MN [26,78].

Micronucleus test was also applied in buccal mucosa cells in a number of studies [88,100–102,104]. Data indicated positive results only in one study [88].

8. Time-dependence of cytogenetic damage

Duration of employment was used as a surrogate of exposure in a number of studies where a quantitative evaluation of the exposure is usually difficult. The incidence of CA, MN and SCE positively correlated with duration of exposure in many of these investigations [26,28,29,71,73,87,89,94]. On the contrary few studies described an increase of chromosomal damage in pesticide-exposed subjects irrespective of the duration of exposure [64,66,67].

The persistence of chromosomal damage was shown to be short-lived for acute or discontinuous exposure. In poisoned patients [50], a temporary increase in the frequency of stable aberrations was found and the usual frequency was restored after approximately 6 months.

The same kinetics for CA were described in seasonal workers, where a drop in the number of CA was observed during the period of low exposure [72,90].

The frequency of chromosomal damage in terms of CA and MN frequency in exposed sprayers was significantly higher during the heavy spraying season compared to the pre-spraying period.

As an example, cessation of exposure to phosphine was accompanied by a significant decline in the chromosome rearrangements frequency within 1 year time [53].

The reversion of chromosomal damage fit with the information about the normal turnover of lymphocyte populations. Lymphocyte survival cannot be considered a passive phenomenon, but it is rather a continuous and active process in which each lymphocyte must compete with other lymphocytes [142]. The majority of lymphocytes in peripheral blood has an half-life of less than 2 weeks: new lymphocytes are continuously produced.

However a subset of around 10% of all circulating lymphocytes may live for almost 9 months or more [143,144].

The clastogenic effects seem to be cumulative for continuous exposure to pesticide mixtures. People chronically exposed are more susceptible to the clastogenic action of pesticides.

Increased chromosomal damage, measured as CA or MN frequency, associated with years of employment has been demonstrated in farmer populations as a result of a continuous exposure to a complex mixture

of pesticides and mainly in floriculturists generally exposed year-round [13,89,90,95,98,100].

9. Conclusions

Occupational exposure to mixtures of pesticides has been associated with an increase in genotoxic damage.

The cytogenetic damage induced by pesticides appears to depend on the degree of exposure. A dose–response relationship can be hypothesised. Negative results have been associated with low levels of exposure. By contrast, clearly positive results were reported in populations subject to high exposure levels, namely people suffering from severe intoxication as a result of attempted suicide or workers neglecting occupational safety measures during the spraying season.

A dose–effect increase of cytogenetic damage was also revealed in a number of field studies where the extent of exposure was described as quantity of pesticide used, extension of area of pesticide application and inadequate working conditions. The time-dependence of the chromosomal damage could be related to the kind of contact, given that acute or discontinuous exposure gives rise to short-lived damage.

Chronic exposure to low doses of complex mixtures of pesticides induces cumulative cytogenetic effects. This situation is similar to what occurs with other genotoxic chemicals. Long-lasting cumulative damage was demonstrated in patients treated with chemotherapy or with combined chemotherapy and radiation therapy for period of up to 12 months [115,116].

Genotoxic damage by chemical compounds could also be influenced by the individual inheritance of variant polymorphic genes involved in the metabolism of chemical compounds and in DNA repair mechanisms. Although the available data on farmer populations suggest that subjects with unfavourable metabolising alleles are more susceptible to genotoxic effects than those with favourable alleles, there are no conclusive findings on whether metabolic polymorphisms affect the chromosomal damage induced by pesticides.

Since workers are frequently exposed to complex mixtures of pesticides, it is difficult to attribute the genotoxic damage to any particular chemical class or compound. The organochlorine compounds used in

the past have been replaced by organophosphates and carbamates, and more recently by pyrethroids, which represent the chemical classes of pesticides most often used nowadays. The experimental evidence shows that a wide range of these compounds induce genotoxic effects on different genetic end-points in bacterial as well as in mammalian systems [13–17].

Although the significance of increased genotoxic effects is difficult to predict for individual subjects the positive findings ensuing from biomonitoring studies suggest a genotoxic hazard at the group level.

The evidence of a genetic hazard related to exposure resulting from the intensive use of pesticides stresses the needs for educational programmes for farmers in order to reduce the use of chemicals in agriculture and to implement protection measures.

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