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## Annex I: Cytogenetic changes in workers following pesticide exposure in EU Covering discussion paper

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## Introduction

1. The objective of this report is to review investigations of mutagenicity and DNA damage in pesticide applicators, workers exposed to pesticides during manufacture, formulation and during and after use, and in occupants of treated premises, in order to aid the COM in producing a statement for the Advisory Committee on Pesticides (ACP) and its Medical and Toxicology Panel (MTP).

2. Due to the large volume of published data available, the review focuses on studies carried out in countries within the European Committee. The report is divided according to occupation, although it is recognised that some of the occupational categories may overlap. Furthermore, it is subdivided into mutagenicity/DNA damage endpoint used.

3. As part of this study, individual studies from the EC were assessed and the adequacy of the reports overviewed in the different occupational categories. In addition, the review focuses on the potential for confounding factors, the statistical approach used in the studies and the power of the studies in order to detect an effect, the guidelines proposed by the International Programme on Chemical Safety (IPCS) working group (Albertini *et al.*, 2000) being used as a framework.

4. In the UK, the mutagenicity data on pesticide active ingredients are thoroughly reviewed by the independent Advisory Committee on Pesticides. *In vivo* mutagens are not generally recommended for approval. However, certain pesticides such as benomyl (benzimidazoles) induce mutations via a mechanism that has a definable threshold (spindle disruption). It is therefore possible to undertake a quantitative risk assessment for such compounds and products for use in agriculture/horticulture are currently approved.

### *Structure of the report*

This report comprises several annexes to the COM covering paper:

Annex I – covering paper

Annex II – summaries of reports

Annex III – table outlining data according to occupational category

Annex IV – table outlining statistical methods used in each report

Annex V – table outlining pesticides used.

### **Application of genetic endpoints in human biomonitoring studies**

5. A number of cytogenetic studies have been used to identify possible genetic damage following pesticide exposure. The assessment of cytogenetic effects in exposed subjects has been suggested as an early indicator of increased risk of cancer (Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; Falck *et al.*, 1999). Hence,

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several studies have been carried out which focussed on genetic modifications in workers occupationally exposed to pesticides (Bolognesi *et al.*, 2004). A number of biomarkers are available to assess both transient DNA damage and more permanent genotoxic effects e.g. clastogenicity. Most studies carried out on populations occupationally exposed to pesticides largely focus on cytogenetic endpoints such as micronuclei (MN) frequency, sister chromatid exchanges (SCE), chromosome aberrations (CA), and on DNA damage (comet assay) and the formation of DNA adducts.

6. The IPCS working group (Albertini *et al.*, 2000) proposed a set of guidelines for monitoring genotoxic effects, and these guidelines have been used to help assess the adequacy of the study. The approaches used are summarised in Table 1. For all assays, it was recommended that samples from exposed and control populations should be collected concurrently. Blood samples should be mixed with heparin to avoid clotting, and processed preferably within 24 hours. In cases where this is not possible, samples should be maintained at 8°C. The collection, storage and transportation of all samples should be recorded. Although not often used in human population studies, a quality control parameter should be included in the assays to demonstrate their adequacy to detect positive samples and the ability of the scorers to identify DNA damage.

7. In terms of statistical analyses, the guidelines suggest that data from the exposed group and the controls should be compared, and whenever possible, exposure-response or time response associations should be considered. Moreover, statistical analysis should be carried out using the hypothesis testing approach, or an estimation approach.

8. Many statistical methods are available for the analysis of genotoxicity endpoints and there is no agreed method that should be used. Depending on the shape of distribution, data should be presented as mean and standard deviations (Grover *et al.*) or medians and percentile distributions. The choice of statistical methods used is largely dependent on the distribution of data. If not normally distributed, methods based on Poisson, binomial distributions can be used or non-parametric methods. Data may be also transformed to allow parametric analysis. For the estimation approach, the relative and absolute effects of exposure and confidence intervals (CI) should be calculated to estimate the precision of the effects. Stratified analyses can be used to study the effect of confounders. Analysis should be based on individual exposure information. Consideration for the effect of multiple comparisons on false positive rate may be included.

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**Table 1. Recommendations for carrying out genotoxicity assays**

Recommendations taken from the IPCS guidelines for the monitoring of genotoxic effects of carcinogens (Albertini *et al.*, 2000)

Method	Cells	Subject information and confounders	Experimental procedure	Optimal sampling time	No. cells scored	Results	Statistical analysis
MN/CBMN	Lymphocytes Buccal epithelial cells	Smoking, drug use, health status, age, and sex. Confounders: sex and age.	Cyt- B added to cultures to limit analysis to cells dividing once in vitro. For CBMN method, only binucleated cells scored.	Lymphocytes : During long-term chronic exposure or within 2 days of an acute exposure or termination of chronic exposure. Epithelial cells: The correct sampling time is difficult to estimate.	1000-2000 lymphocytes or 3000-5000 epithelial cells scored	Significantly higher frequency of MN cells in exposed population compared to controls considered a positive result.	The frequency of micronucleated cells or the frequency of MN per 1000 cells should be calculated. If the whole chromosome content of MN is determined, then parameters should be calculated separately for centromere/kinetochore positive and negative MN. The unit of exposure is the individual and not the cell, hence pooling data from individuals in the same groups masks variability.

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CA	Lymphocytes	Smoking, drug use, health status, age, sex, and genetic polymorphisms	Cells cultured with phytohaemagglutinin (PHA). BrdU ensures first generation metaphases and colchicine added 1-2 hr prior to harvesting.	During long-term chronic exposure or within 2 days of an acute exposure or termination of chronic exposure.	>200 metaphases containing 45-47 centromeres scored. Gaps recorded separately.	For each damaged cell, record the number and type of structural or numerical chromosome damage.	For each subject, the total aberration frequency and the percentage of damaged cells should be calculated, including and excluding gaps. The unit of exposure is the subject not the cell, hence pooling data masks variability.
SCE	Lymphocytes	Smoking, drug use, health status, age, sex, and genetic polymorphism.	Cells cultured with PHA and BrdU. Colchicine added 1-2 hr prior to harvesting.	During long-term chronic exposure or within 2 days of an acute exposure or termination of chronic exposure.	30-50 second-division metaphase cells scored	At least 80 second division metaphases scored. Every switch of staining between sister chromatids is scored as a SCE.	The SCE frequency per cell should be calculated for each individual. The unit of exposure is the subject and not the cell, hence pooling data within groups masks variability. The dispersion test may be used to compare the distribution of SCE data amongst individuals.

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Comet	Whole blood leukocytes or subsets (granulocytes, lymphocytes, T-cells, gastric or nasal epithelial cells.	Smoking, drug use, health status, age, sex, genetic polymorphism, and recent anaerobic exercise.	Assess viability. DNA unwinding conditions pH>13 and >60 min. Same electrophoretic conditions used in all studies to eliminate intra- and inter-run differences in DNA migration.	During long-term chronic exposure or within hours of an acute exposure or termination of chronic exposure.	At least 100 cells scored (50 per replicate).	DNA migration measured as tail length,, migrated DNA or tail moment by visual, photographi c or image analysis methods.	<p>The measure of DNA migration, mean + SD/SE, median + CI, range, n and, where possible, distribution of migration among cell, should be presented. When tail moment is used, individual data on the % of migrated DNA and tail length should be given also.</p> <p>The unit of exposure is the individual, not the cell, hence pooling data across individuals within a study group masks sources of variability.</p> <p>Analysis based on the distribution of comets may be carried out by analysing the effect of exposure on the dispersion coefficient.</p>
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32P- postlabelling	Lymphocytes , whole blood, tissues.	Smoking, drug use, health status, age, sex, and genetic polymorphism.	Variation arises due to: selection of digestion enzymes for DNA; enrichment methods for adducts; labelling conditions; separation of adducts.	During long- term chronic exposure or within hours to days of an acute exposure or termination of chronic exposure.	Criteria for identifying positive results should be stated prior to the study.	Data should be presented as mole adduct per mole normal bases.
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### *Micronuclei frequency*

10. Micronuclei are small, extranuclear bodies that arise from acentric chromosome fragments or whole chromosomes lagging behind during mitotic cellular division as a result from direct DNA breakage, replication on a damaged DNA template or inhibition of DNA synthesis, failure of any of the mitotic apparatus or alterations in cellular physiology and mechanical disruption (Albertini *et al.*, 2000; Lucero *et al.*, 2000). In most cases, the cytokinesis-block MN method is used, in which scoring only takes place in cells that have only divided once in culture (Falck *et al.*, 1999). Micronuclei analysis can be used for a number of cells, both *in vitro* and *in vivo*, including lymphocytes (Bolognesi, 2003; Bolognesi *et al.*, 2004; Bolognesi *et al.*, 2002) and buccal epithelial cells (Pastor *et al.*, 2002a; Pastor *et al.*, 2001a; Pastor *et al.*, 2001b; Pastor *et al.*, 2002b). It is unclear however, whether MN formation has a specific role in carcinogenesis (Albertini *et al.*, 2000).

### *Chromosome aberrations*

11. Structural chromosome aberrations arise from direct DNA breakage, replication on a damaged DNA template or inhibition of DNA synthesis and may involve both chromatids of the chromosome (chromosome-type CA), or only one chromatid of the chromosome (chromatid-type CA) (Albertini *et al.*, 2000; Hagmar *et al.*, 2004). Chromosome aberration analysis has been commonly performed on human peripheral blood lymphocytes to assess DNA damage (Carbonell *et al.*, 1995; De Ferrari *et al.*, 1991; Falck *et al.*, 1999; Kourakis *et al.*, 1996; Nehez *et al.*, 1988; Paldy *et al.*, 1987). To ensure that only first-generation metaphase cells are scored for CA, bromodeoxyuridine is commonly added to the culture medium prior to DNA replication *in vitro* (Kourakis *et al.*, 1996). Both structural and numerical chromosome aberrations may cause alterations of oncogene and tumour suppressor genes of somatic cells, and hence are involved in the induction of cancer in humans (Mitelman, 1994).

### *Sister chromatid exchange*

12. Sister chromatid exchanges arise from exchange of DNA replication products between two identical loci of sister chromatids of a duplicated chromosome (Tucker *et al.*, 1993), due to errors in DNA replication on a damaged DNA template, possibly at the replication fork (Tucker *et al.*, 1993). In the most commonly used method of SCE analysis, DNA replication is required for two consecutive cell cycles, hence bromodeoxyuridine is added to the culture medium and cells are scored in the second division metaphase (De Ferrari *et al.*, 1991; Scarpatto *et al.*, 1996). Although the induction of SCE has been widely used as an indicator of DNA damage following exposure to pesticides (Carbonell *et al.*, 1993; Falck *et al.*, 1999; Lander and Ronne, 1995; Linnainmaa, 1983; Pasquini *et al.*, 1996;

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Scarpato *et al.*, 1996), the mechanism of formation and biological significance of SCEs are still unknown(Tucker *et al.*, 1993).

### *Comet assay*

13. The comet assay, or single cell gel electrophoresis technique is a more recent technique established as a sensitive method for detecting DNA single strand and double strand breaks, alkali-labile sites, DNA cross linking and incomplete excision repair events (Daza *et al.*, 2004; Faust *et al.*, 2004; Lebailly *et al.*, 1998a; Undeger and Basaran, 2002). The comet assay can be carried out with a number of cells, both *in vitro* and *in vivo*, including peripheral blood leukocytes, bladder, liver, buccal, gastric and sperm cells. To date, peripheral blood lymphocytes are mainly used for human biomonitoring studies following occupational exposure to an array of chemicals (Faust *et al.*, 2004; Lebailly *et al.*, 1998a; Piperakis *et al.*, 2003; Undeger and Basaran, 2002). However, the relevance of the endpoint measured in the comet assay has yet to be established, as it is a temporary strand breakage, which, gets repaired within a few hours under normal circumstances prior to becoming fixed as a mutation (Faust *et al.*, 2004).

### *DNA adducts*

14. A DNA adduct is a chemical entity covalently bound to DNA (Albertini *et al.*, 2000), and is formed following the interaction of an electrophilic molecule with a nucleophilic site of DNA (Farmer, 2004). They are involved in the mode of action of genotoxic carcinogens, and this may lead to a mutation and altered gene function if not repaired. In epidemiological studies DNA adducts are particularly useful as they provide information on the exact chemical exposure of the individual (Farmer, 2004). <sup>32</sup>P-DNA postlabelling technique has been widely used to measure non-radioactive carcinogenic DNA adducts in humans, due to it being a highly sensitive technique (Farmer, 2004).

15. The COM guidance on a strategy for testing chemicals for mutagenicity recognised that artifactual positives may be obtained in the cell assays that do not reflect intrinsic mutagenic activity. Factors such as hyperthermia, hypothermia or induction of erythropoiesis may produce MN or CA (Asanami *et al.*, 2001) or exercise immediately prior to sampling may lead to increased DNA damage measured by the comet assay (Ohkuwa *et al.*, 2004).

### **Occupational exposure to pesticides**

16. Several groups of workers are exposed to pesticides in their occupational setting. This report focuses on the genetic damage following pesticide exposure in floriculturists and greenhouse workers, agricultural workers and farmers, pesticide sprayers and applicators, production workers and forestry workers, working within Europe.

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17. Conflicting data have been presented regarding the genotoxic effects of pesticides following short term or chronic occupational exposure in agricultural workers, floriculturists, pesticide sprayers, greenhouse workers etc. (Lander and Ronne, 1995). Some studies have shown an increase in cytogenetic damage following occupational pesticide exposure (Bolognesi, 2003; Bolognesi *et al.*, 2004; Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; De Ferrari *et al.*, 1991; Kourakis *et al.*, 1992; Peluso *et al.*, 1996), whereas others failed to show any detectable damage (Lander *et al.*, 2000; Lucero *et al.*, 2000; Nehez *et al.*, 1988; Pastor *et al.*, 2002a; Pastor *et al.*, 2001a; Pastor *et al.*, 2001b). Figure 1 shows that some occupational categories have a higher percentage of studies in which workers displayed genetic damage, as measured by a number of different endpoints.

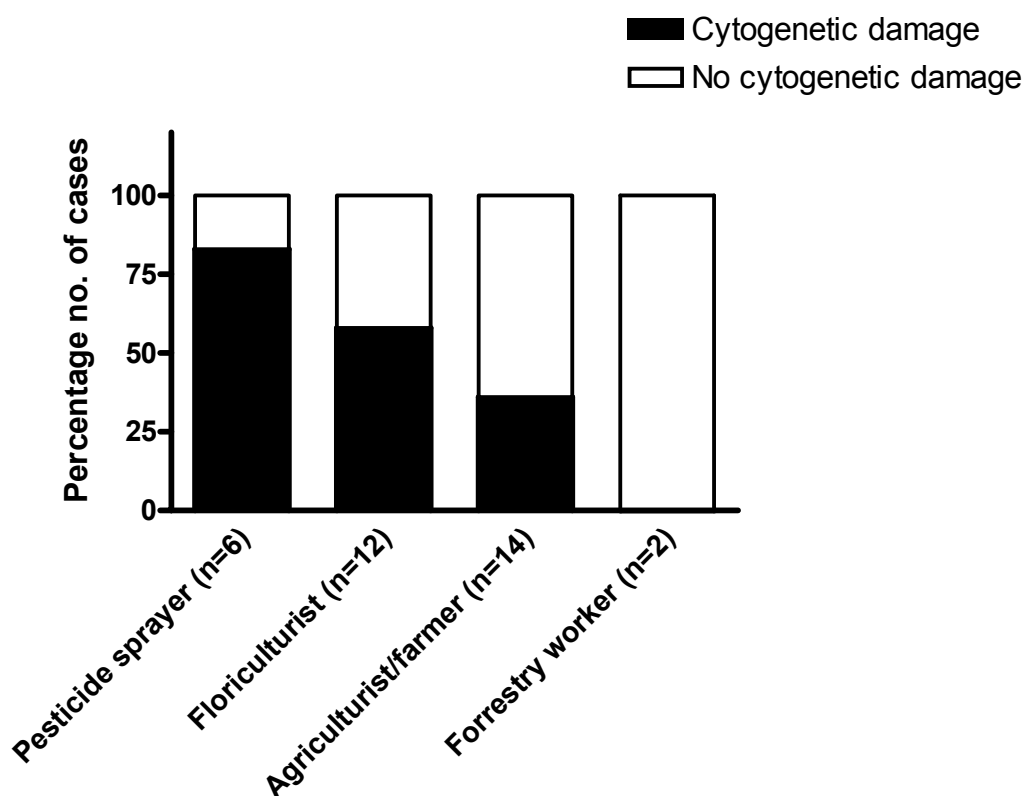


Figure 1 Number of studies showing genetic damage in different occupational categories

#### *Production workers*

18. The production of pesticides is undertaken throughout the year, as opposed to pesticide application, which occurs seasonally. Hence production workers are constantly exposed to pesticides, as well as to raw materials such as formaldehyde, acrylonitrile, toluene and benzene (Bolognesi, 2003). One cross-

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sectional study was retrieved regarding cytogenetic damage in pesticide production workers (Table 2).

**Table 2 Genetic damage in production workers exposed to pesticides**

Author	Exposure	Pesticide use and sampling time	Protective clothing used	Environment	Result
Jablonicka <i>et al.</i> , 1989	< 2 years	NS	NS	Production plant	+ CA + SCE

19. Jablonicka and coworkers reported a significant increase in CA and SCE in Czech workers employed in the production of mancozeb (Jablonicka *et al.*, 1989). Very few details concerning the exposures that occurred in the manufacturing plants were presented, and no quantitative data on mancozeb exposure was reported.

### *Pesticide sprayers and applicators*

20. Pesticide sprayers and applicators potentially represent the most exposed group of workers as most are directly exposed to pesticides during mixing and spraying practices (Bolognesi, 2003). Two longitudinal studies and four cross-sectional studies were retrieved in which cytogenetic damage in pesticide sprayers was investigated, using mainly CAs and SCE as an endpoint for genotoxicity.

21. Five out of the six studies reported positive results for at least one endpoint, in pesticide applicators compared to controls (Joksic *et al.*, 1997; Kourakis *et al.*, 1996; Kourakis *et al.*, 1992; Paldy *et al.*, 1987; Undeger and Basaran, 2002), or after spraying (Joksic *et al.*, 1997; Paldy *et al.*, 1987), whereas one study showed no increase in cytogenetic damage in exposed workers (Lander and Ronne, 1995) (Table 3).

22. Joksic and coworkers carried out a longitudinal study in which pesticide sprayers applying pesticides in open situations, controls living in nearby villages and references living outside the region were tested for the frequency of CA, MN and SCE at three time points, pre-spraying, one month after spraying and at the end of the spraying season.

23. Several significant differences were reported in such subjects. During the pre-spraying period, exposed workers had a higher frequency of CA compared to controls and references, although MN and SCE frequency remained unchanged. Similarly, the number of CA in workers after the spraying season appeared higher than controls or references, although authors did not make this correlation. Both

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CA and MN frequency in the exposed workers was increased throughout the spraying season. Interestingly, the MN frequency in samples taken after spraying was significantly higher in the control group (persons living near the spraying area) compared to samples taken during the pre-spraying period and when compared to the references, living outside the region (Joksic *et al.*, 1997). Although several interesting findings were reported, there were several anomalies in the presentation of the data in the published paper that severely limited any conclusions from being drawn.

**Table 3 Genetic damage in pesticide sprayers and applicators exposed to pesticides**

Author	Exposure	Pesticide use and sampling time	Protective measured used	Environment	Result
Lander and Ronne, 1995	17 years (range 1-50)	Spraying: 5 months preceding sampling.	Groups assigned according to protection worn.	Greenhouses	- SCE
Kourakis <i>et al.</i> , 1996	6 years	Sampling: spring.	No	Greenhouses Open fields	+ CA - SCE
Kourakis <i>et al.</i> , 1992	4 years	Spraying: September-November. Sampling: Autumn.	No	Greenhouses	+ CA
Underger and Basaran, 2002	10 years ± 6 (range 1-23)	Sampling: End of working week.	64% overalls + masks w/o filters. 27% no protection. No worker wore gloves or glasses	Outdoors	+ COMET
Joksic <i>et al.</i> , 1997	12.1 years ± 6.02	Spraying: 3-7 days/week, 6 months. Sampling: prespraying period, a month after spraying and at end of spraying period.	NS	Outdoors	+ CA - SCE + MN
Paldy <i>et al.</i> , 1987	1- >15 years	Spraying: March – October. Sampling: November and following April.	NS	Outdoors	+ CA

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24. Paldy and colleagues carried out a second longitudinal study in which samples were collected from outside workers, having different durations of pesticide exposure, and controls, in November and the following April, relative to the pesticide spraying taking place in March to October (Paldy *et al.*, 1987). However, authors presented only one set of data and it was unclear whether it related to the November or April sampling. A duration-dependent increase was evident as there was a significant increase in CA in workers employed for longer periods, despite the sampling taking place 1-6 months after cessation of spraying.

25. In most of the cross-sectional studies carried out cytogenetic damage was reported, with the exception of the study by Lander and Ronne (1995). Exposed workers spraying pesticides both in greenhouses (Kourakis *et al.*, 1996; Kourakis *et al.*, 1992) and in open fields (Undeger and Basaran, 2002) displayed a significant increase in DNA damage compared to controls, measured by CA and the comet assay, respectively, although SCE frequency remained unchanged in the greenhouse workers (Kourakis *et al.*, 1996; Lander and Ronne, 1995). In all cases, sampling took place during or shortly after pesticide spraying, hence complying with the IPCS guidelines.

26. Very few details concerning the exposures that occurred whilst spraying pesticides were presented. This aspect is further considered below.

### *Floriculturists and greenhouse workers*

27. Floriculturists and greenhouse workers are involved in the production of flowers and ornamental plants, which are commonly sprayed with pesticides, as they are susceptible to pests (Bolognesi, 2003).

28. Twelve studies were retrieved, in which the cytogenetic effects of pesticides in floriculturists were investigated. One longitudinal study, in which samples were collected pre-seasonally, in March, and post-seasonally in October (Lander *et al.*, 2000) and 11 cross-sectional studies were carried out (Bolognesi *et al.*, 1993b; De Ferrari *et al.*, 1991; Munnia *et al.*, 1999; Scarpato *et al.*, 1996). The majority of studies (83%) were carried out in Italian floriculturists or greenhouse workers (Table 4).

29. Micronuclei analysis was carried out in seven of the 11 studies, mainly carried out by Bolognesi and colleagues in Italian floriculturists, using lymphocytes. Chromosome aberrations (De Ferrari *et al.*, 1991; Lander *et al.*, 2000; Scarpato *et al.*, 1996), comet assay (Piperakis *et al.*, 2003) and SCE (De Ferrari *et al.*, 1991; Scarpato *et al.*, 1996) were also used to determine genetic damage as well as <sup>32</sup>P DNA postlabelling (Munnia *et al.*, 1999; Peluso *et al.*, 1996).

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30. Overall, seven out of the 12 studies reported positive data regarding genetic damage in floriculturists. Two out of seven studies reported a significant increase in MN frequency in exposed workers, CA were significantly increased in two out of three studies and SCE were elevated in one out of two studies. One study failed to detect DNA damage, when using the comet assay, whereas positive results were reported in both studies measuring DNA adducts.

**Table 4 Genetic damage in pesticide-exposed agricultural workers and farmers**

Author	Exposure	Pesticide use and sampling time	Protective measures used	Environment	Result
Lander <i>et al.</i> , 2000	Not stated	Sampling: pre-season (March); post-season (October).	Gloves worn by some workers.	Greenhouses	+ CA
Bolognesi <i>et al.</i> , 2004	26.35 years ± 14.46 (range 2-10)	NS	88%	80 % Greenhouses	- MN
Bolognesi <i>et al.</i> , 2002	27.8 years ± 15.5 (range 2-70)	NS	84%	56% open fields	- MN
Bolognesi <i>et al.</i> , 1993a	25.3 years ± 1.57 (range 2-55)	NS	NS	39% open fields 55% fields/greenhouses 6% greenhouses	+ MN
Bolognesi <i>et al.</i> , 1993b	1 - >30 years	NS	NS	39% open fields 55% fields/greenhouses 6% greenhouses	+ MN
Bolognesi <i>et al.</i> , 1993c	1 - >30 years	NS	NS	39% open fields 55% fields/greenhouses 6% greenhouses	+ MN

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De Ferrari <i>et al.</i> , 1991	Not stated	NS	NS	NS	+ CA + SCE
Scarpato <i>et al.</i> , 1996	Not stated	Spraying: September- November. Sampling: 1 month after spraying.	NS	Greenhouses	- MN - CA - SCE
Falck <i>et al.</i> , 1999	7-41 years	Sampling: during pesticide application	1 group full protection 1 group gloves and boots.	Greenhouses	+ MN
Peluso <i>et al.</i> , 1996	22 years ± 12	Spraying: NS Sampling: 9- 10am	NS	NS	+ adducts
Munnia <i>et al.</i> , 1999	Not stated	Spraying: NS Sampling: 9- 10am	NS	Greenhouses	+ adducts
Piperakis <i>et al.</i> , 2003	8.62 years ± 1.13 (range 5- 15)	NS	62%	Greenhouses	- comet

31. Bolognesi and co-workers presented inconsistent data, as an increase in MN frequency was reported in only three out of their five studies (Bolognesi *et al.*, 1993a; Bolognesi *et al.*, 1993b; Bolognesi *et al.*, 1993c). Other studies failed to demonstrate a significant increase in MN, SCE and CA frequencies in Italian floriculturists (Falck *et al.*, 1999; Scarpato *et al.*, 1996) although increases in CA and DNA adducts were reported in other studies (Lander *et al.*, 2000; Munnia *et al.*, 1999; Peluso *et al.*, 1996).

32. Few studies carried out in floriculturists and greenhouse workers reported sampling time following pesticide exposure. One study collected blood one month after pesticide spraying (Scarpato *et al.*, 1996), which may account for the negative data presented, as, according to the IPCS guidelines, sampling should take place within 2 days of exposure in order to optimise the chance of identifying genetic damage. Only one study was carried out that stated that blood was collected during pesticide spraying, although two studies reported collecting blood in the morning. These studies reported an increase MN frequency and DNA adducts, respectively, in the greenhouse workers compared to controls (Falck *et al.*, 1999; Munnia *et al.*, 1999; Peluso *et al.*, 1996).

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33. Floriculturists may potentially have an increased risk of cytogenetic damage due to working in smaller areas, humid conditions and a potential continuous exposure through re-entry activities such as cutting and potting, for which protective clothing is seldom used (Bolognesi, 2003; Scarpato *et al.*, 1996).

34. Floriculturists may be relatively highly exposed to pesticides during loading, mixing and applying pesticides as well as during manual activities following continuous contact with flowers and ornamental plants (Munnia *et al.*, 1999).

35. No individual pesticide active ingredient was identified consistently in exposed groups, although positive studies reported the majority of pesticides used were organophosphorus compounds, pyrethroids, carbamates, benzimidazoles, dithiocarbamates and thiocarbamates, inorganics, amides and substituted urea, and miscellaneous pesticides. This aspect is further discussed below.

### *Agricultural workers and farmers*

36. Agricultural workers and farmers are involved in the production of crops, fruit and vegetables and hence are indirectly exposed to pesticides.

37. Fourteen studies were retrieved concerning cytogenetic damage in agricultural workers or farmers. Seven longitudinal studies were carried out in which samples were collected during and after extended exposure (Carbonell *et al.*, 1990; Nehez *et al.*, 1988; Pasquini *et al.*, 1996; Pastor *et al.*, 2002b) or acute exposure (Lebailly *et al.*, 2003; Lebailly *et al.*, 1998a; Lebailly *et al.*, 1998b) and seven cross-sectional studies (Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; Pastor *et al.*, 2003; Pastor *et al.*, 2002a) (Table 5).

38. Micronuclei analysis was carried out in seven of the 14 studies, mainly carried out by Pastor and colleagues in Hungarian, Greek, Polish and Spanish agricultural workers, using both lymphocytes and buccal epithelial cells (Pasquini *et al.*, 1996; Pastor *et al.*, 2003; Pastor *et al.*, 2002a; Pastor *et al.*, 2001a; Pastor *et al.*, 2001b; Pastor *et al.*, 2002b). Chromosome aberrations and SCE were determined by Carbonell and coworkers, Nehez, and Pasquini to detect DNA damage in Spanish, Hungarian and Italian workers, respectively (Carbonell *et al.*, 1990; Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; Nehez *et al.*, 1988; Pasquini *et al.*, 1996), whereas Lebailly carried out the comet assay using lymphocytes obtained from French farmers (Lebailly *et al.*, 2003; Lebailly *et al.*, 1998a; Lebailly *et al.*, 1998b).

39. Overall, five of the 14 studies reported positive data regarding cytogenetic damage. None of the studies carried out by Pastor and co-workers reported an

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increased MN frequency, in either lymphocyte or buccal cells, in exposed workers employed in both greenhouses and open situations. In contrast, Pasquini and co-workers reported a significantly increase frequency of MN in agricultural workers (Pasquini *et al.*, 1996). Lebailly presented inconsistent data, as an increase in DNA damage in pesticide sprayers applying pesticides in open situations was reported in only two out of the three studies. In addition, increases in CAs, but not SCE, were demonstrated in exposed farmers working predominantly in open situations (Carbonell *et al.*, 1990; Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; Nehez *et al.*, 1988; Pasquini *et al.*, 1996). Pasquini and colleagues attributed this lack of genetic damage in agricultural workers/farmers to the pesticide exposure levels being too low to initiate a detectable increase in SCE (Pasquini *et al.*, 1996).

40. According to the IPCS guidelines, blood sampling should be carried out during chronic exposure or immediately after acute exposure to pesticides. In some studies carried out in which negative results were obtained, the sampling time was not reported (Pastor *et al.*, 2003; Pastor *et al.*, 2002a), or took place in late winter or early spring (Pastor *et al.*, 2001b), although one study did collect blood in March-April following pesticide spraying (Lucero *et al.*, 2000). Moreover, some of the longitudinal studies only present data without referring to the sampling time (Pasquini *et al.*, 1996).

**Table 5 Genetic damage in floriculturists and greenhouse workers exposed to pesticides**

Author	Exposure	Pesticide use and sampling time	Protective measured used	Environment	Result
Pastor <i>et al.</i> , 2003	9-18 years	NS	80%	Greenhouses and open fields	- MN
Pastor <i>et al.</i> , 2002a	18.75 years ± 0.89	NS	85 %	Greenhouses and open fields.	- MN
Pastor <i>et al.</i> , 2002b	8.31 years ± 1.12	Spraying: spring. Sampling: March-April; November-December.	93%	Greenhouses and open fields.	- MN
Pastor <i>et al.</i> , 2001a	8.62 years ± 1.13	Spraying: summer>spring>autumn>winter Sampling: NS	78 %	Greenhouses and open fields.	- MN
Pastor <i>et al.</i> , 2001b	16.28 years ± 1.10	Sampling: late winter-early spring.	62 %	Greenhouses	- MN
Lucero <i>et al.</i> , 2000	9.82 years ± 1.01	Spraying: preceding	~80 %	Greenhouses	- MN

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		sampling. Sampling: March-April.			
Carbonell <i>et al.</i> , 1995	>10 years	NS	3% full protection. 59% wore masks incorrectly.	Open fields > greenhouses	+ CA
Carbonell <i>et al.</i> , 1993	<5 - >29 years	NS	NS	Open fields > greenhouses	- SCE + CA
Carbonell <i>et al.</i> , 1990	> 10 years	Spraying: spring-summer; autumn-winter. Sampling: spring-summer; autumn-winter.	NS	Open fields	- SCE
Nehez <i>et al.</i> , 1988	<1 - >15 years	Spraying: 1-6 days or 1-2 months after spraying.	100%	Greenhouses and open fields.	+ CA
Pasquini <i>et al.</i> , 1996	18.35 years ± 12.42 (range 3-50)	Spraying: March – July. Sampling: May – September and 3 years later.	29%	Open fields	- SCE + MN
Lebailly <i>et al.</i> , 1998a	Not stated	Spraying: January, Spring, June-Autumn. Sampling: before and after day of spraying.	NS	Open fields	+ comet
Lebailly <i>et al.</i> , 1998b	1 day	Spraying: 1 day. Sampling: morning before spraying and morning after spraying.	34% wore gloves. 15% wore gloves + masks.	Open fields	+ comet
Lebailly <i>et al.</i> , 2003	Not stated	Spraying: 1 day. Sampling: morning before and evening after spraying and following morning.	14% no protection, 14% masks/protective clothes, 17% gloves, 41% full protection.	Open fields	- comet

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41. No individual pesticide active ingredient was identified consistently in exposed groups, although positive studies reported the majority of pesticides used were organophosphorus compounds, pyrethroids, carbamates, benzimidazoles, dithiocarbamates and thiocarbamates, inorganics, amides and substituted urea, and miscellaneous pesticides. This aspect is further discussed below.

### *Forestry workers*

42. Two studies were retrieved that measured cytogenetic damage in forestry workers. Mustonen and co-workers carried out a cross-sectional study (Mustonen *et al.*, 1986), whereas a longitudinal study was carried out by Linnainmaa (Linnainmaa, 1983). Both studies reported negative results, as measured by CA and SCE (Table 6). Blood was collected in accordance with the IPCS guidelines, as samples were obtained after pesticide spraying (Mustonen *et al.*, 1986) or before, during and after the spraying season (Linnainmaa, 1983).

43. Very few details concerning the exposures that occurred whilst spraying pesticides were presented. This aspect is further discussed below.

**Table 6 Genetic damage in forestry workers exposed to pesticides**

Author	Exposure	Pesticide use and sampling time	Protective clothing used	Environment	Result
Mustonen <i>et al.</i> , 1986	6-28 days	Sampling: after spraying season.	NS	Forest	- CA
Linnainmaa <i>et al.</i> , 1983	Not stated	Sampling: before, during and after season.	NS	Forest	- SCE

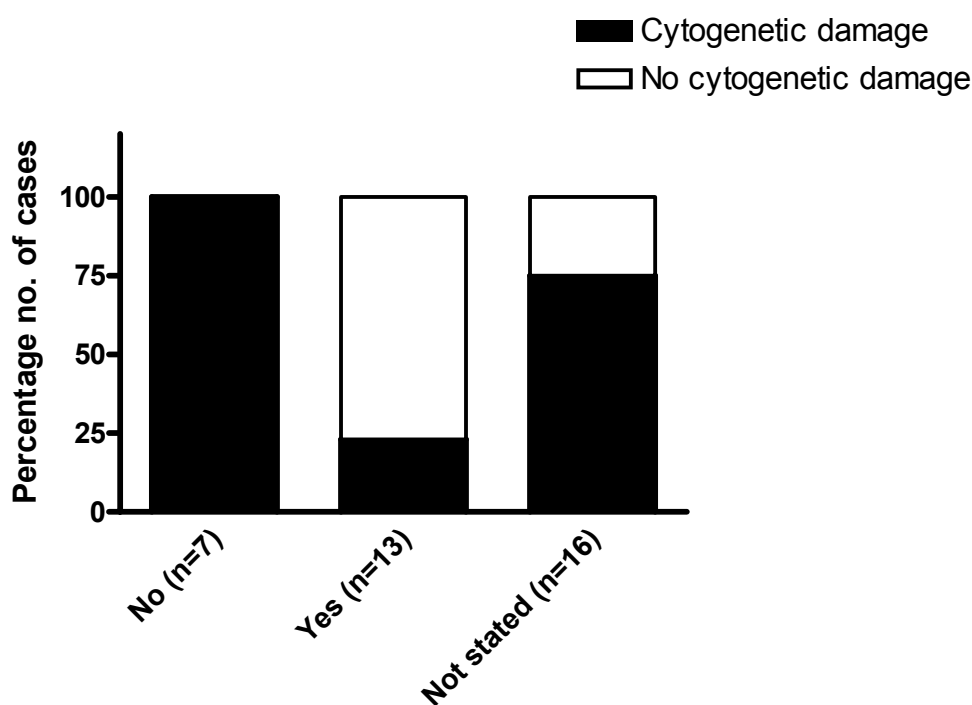
### **Confounding factors**

#### *Use of protective clothing*

44. Prominent differences in the use of protective clothing were noted between studies, and it was also reported that some pesticide sprayers wore protective clothing to various degrees (Lander and Ronne, 1995; Undeger and Basaran, 2002), whereas others mixed pesticides using bare hands and sprayed using no protection (Kourakis *et al.*, 1996; Kourakis *et al.*, 1992). Cytogenetic damage in pesticide workers was reported in all seven studies in which workers wore little or no protective clothing, or in cases where faulty equipment was used. In contrast, in cases where the majority of workers took protective measures, 11 out of 13 studies reported negative results. Sixteen studies did not state if workers used protective clothes, and in such studies, 12 reported an increase in genetic damage in exposed workers (Figure 2).

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45. Lander and Ronner suggested that the degree of protection used during spraying displayed a strong association with the amount of pesticide uptake, resulting in an increased frequency of cytogenetic effects (Lander and Ronne, 1995). Several studies have reported a significant increase in the SCE, CA and MN frequency and have correlated this to the lack of protective measures during pesticide use (Carbonell *et al.*, 1995; Crossen and Morgan, 1978; Dulout *et al.*, 1985; Lander *et al.*, 2000; Pasquini *et al.*, 1996).



**Figure 2 Cytogenetic damage in workers with or without protective clothing.**

46. Although many floriculturists reportedly used protective measures, in some cases, authors stated that due to humid conditions within the greenhouses, full protective clothing was not always used (Pastor *et al.*, 2002b). Similarly, agricultural workers may potentially be exposed to pesticides through the handling of crops. Pesticide sprayers also have a potential for pesticide exposure, as they handle pesticides throughout the year and those spraying chemicals from above head height are at risk of oral and percutaneous exposure if adequate measures are not taken.

47. Overall, the use of protective clothing, including gloves, masks, glasses and boots appears to protect workers from the genotoxic effects in studies.

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### *Working in greenhouses*

48. Several studies reported an increase in genetic damage in greenhouse workers, compared to those spraying pesticides in open situations (Bolognesi *et al.*, 1993a; Bolognesi *et al.*, 1993b; Bolognesi *et al.*, 1993c). The environmental conditions inside the greenhouses such as small, enclosed spaces, poor ventilation and high temperature and humidity are all conducive to high pesticide exposure. Furthermore, the climatic conditions within greenhouses allow for a continuous production of fruit, vegetables and flowers, requiring a constant application of pesticides throughout the year, resulting in a potentially continuous exposure (Lucero *et al.*, 2000; Pastor *et al.*, 2002b).

### *Sampling time*

49. The IPCS guidelines recommend that sampling for all endpoints be carried out during chronic exposure when damage and repair mechanisms are at steady state, or within 2 days after cessation of chronic or acute exposure (Albertini *et al.*, 2000), as the persistence of some genetic damage, i.e. DNA adducts, CAs, are generally removed within hours to months after the chemical exposure (Peluso *et al.*, 1996). However, other studies have reported that CAs reached baseline values after six months, although lymphocytes displaying genetic damage were eliminated or diluted into peripheral blood during this time (Carbonell *et al.*, 1995). This maybe suggests that in those studies measuring CAs and possibly SCE, there is a longer period of time in which blood samples may be collected after exposure and hence genetic damage can be identified.

### *Age*

50. Several reports have demonstrated that age significantly modifies frequency of MNs (Barale *et al.*, 1998; Bolognesi *et al.*, 1997; Bukvic *et al.*, 2001; de Arce, 1981; Norpa, 2004; Sari-Minodier *et al.*, 2002; Soper *et al.*, 1984), SCE (Bolognesi *et al.*, 1997; Bonassi *et al.*, 1995; Norpa, 2004) and CAs (Norpa, 2004). In contrast, other studies failed to show an association between age and frequency of MN (Sukas *et al.*, 2004), SCE (Bender *et al.*, 1988; Bolognesi *et al.*, 1997) and CA (Bender *et al.*, 1988; Bolognesi *et al.*, 1997).

51. Age was significantly associated with an increase in MN occurrence and CAs in floriculturists in some studies used in this report (Bolognesi *et al.*, 1993a; Bolognesi *et al.*, 1993b; Falck *et al.*, 1999). In contrast, other studies reported that age was not associated with the increase in CAs (Lander *et al.*, 2000; Scarpato *et al.*, 1996). In general, age appears to have little effect on the induction of CAs or SCE in controls or pesticide-exposed workers (Carbonell *et al.*, 1993; Paldy *et al.*, 1987), whereas MN frequencies are more significantly affected in some subjects (Scarpato *et al.*, 1996).

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### *Sex*

52. The effect of sex on cytogenetic damage has been widely investigated and has been repeatedly shown to be significantly associated with an increase in MN and SCE (Barale *et al.*, 1998) frequencies, as females subjects exhibited higher values in comparison to males. However, other studies reported no difference in SCE level between the sexes (Bender *et al.*, 1988).

53. A significantly higher MN frequency (Bolognesi *et al.*, 1993a) and CA (Scarpato *et al.*, 1996) percentage was reported in females compared to males, following pesticide exposure. Similarly, Bolognesi showed that females had a significantly higher MN frequency compared to males, independent of exposure (Bolognesi *et al.*, 2002) and associated the effect with high micronucleation of the X chromosome. In contrast, sex did not significantly affect CA and SCE in controls or pesticide-exposed workers (Carbonell *et al.*, 1993; Paldy *et al.*, 1987).

### *Smoking*

54. Smoking is known to increase the level of SCE and CA (de Arce, 1981; Lazutka *et al.*, 1994; Norpa, 2004; Sarto *et al.*, 1985; Soper *et al.*, 1984), although its effect on MN is unclear (Bolognesi *et al.*, 1993b; Norpa, 2004).

55. Conflicting data have been presented by studies used in this report regarding the influence of smoking on cytogenetic damage. Various studies presented data showing that both CA and SCE were significantly increased due to smoking (Scarpato *et al.*, 1996). These data correlated to those presented in other studies, which also showed a significant difference between smoking and non smoking workers (Linnainmaa, 1983) or controls (Jablonicka *et al.*, 1989). However, in other groups of subjects occupationally exposed to pesticides, no significant increase was found in smokers compared to non-smokers (Carbonell *et al.*, 1993; De Ferrari *et al.*, 1991; Falck *et al.*, 1999; Kourakis *et al.*, 1992). Several authors suggest that smoking may potentiate the genotoxicity of pesticides, either by its own genotoxicity or due to an increase in oral exposure to pesticides (Lander *et al.*, 2000).

### *Selection of subjects*

56. The selection of subjects and controls should be taken into account in the study design. In a number of studies, healthy controls were selected from a population living in the same region as the exposed workers (Bolognesi *et al.*, 1993a; De Ferrari *et al.*, 1991; Falck *et al.*, 1999; Lander *et al.*, 2000; Lucero *et al.*, 2000; Nehez *et al.*, 1988; Pasquini *et al.*, 1996; Pastor *et al.*, 2002a), although not being occupationally exposed to pesticides. Several studies selected sex and age matched controls (Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; Kourakis *et al.*, 1996;

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Mustonen *et al.*, 1986; Scarpato *et al.*, 1996; Undeger and Basaran, 2002) or chose controls on the basis of smoking habit (Pastor *et al.*, 2003; Peluso *et al.*, 1996; Piperakis *et al.*, 2003). Joksic and co-workers used two different control groups comprising subjects from the agricultural area, or from outside the region (Joksic *et al.*, 1997). Other occupational factors, not only pesticide exposure, may differ between workers and controls, such as exposure to sunlight, exercise, should be considered as they may affect the outcome of the studies.

### *Cytogenetic endpoint*

57. From the reports retrieved, 15 studies used MN frequency to detect genetic damage following pesticide exposure, 12 used CA and ten used SCE. The comet assay and <sup>32</sup>P postlabelling were used to a lesser extent, being reported in five and two studies, respectively. Chromosome aberration analysis gave the highest number of positive results, as ten out of the 12 studies reported an increase in CA following pesticide exposure. Positive results were reported in three out of five studies using the comet assay. Similarly, two out of ten studies showed an increase in SCE, four out of 15 studies reported an increase in MN in exposed workers, and both studies that used <sup>32</sup>P postlabelling showed the presence of DNA adducts (Figure 3).

58. The discordance in results between MN frequency and CAs may suggest artefactual positives being detected in CA, or a difference in relative sensitivity of the end-points. Tates and colleagues reported a higher sensitivity for CA compared to MN in biomonitoring studies of workers exposed to styrene and dichloromethane (Tates *et al.*, 1994), although this may also reflect the mechanism of genetic damage.

59. Several studies were carried out that measured more than one endpoint. In some cases, similar results were reported for different assays. Scarpato and co-workers reported that the same results were obtained for MN, CA and SCE in greenhouse floriculturists, as none were increased following pesticide exposure (Scarpato *et al.*, 1996). Similarly, both CA and SCE gave the same results in floriculturists, although both were significantly increased following pesticide exposure (De Ferrari *et al.*, 1991).

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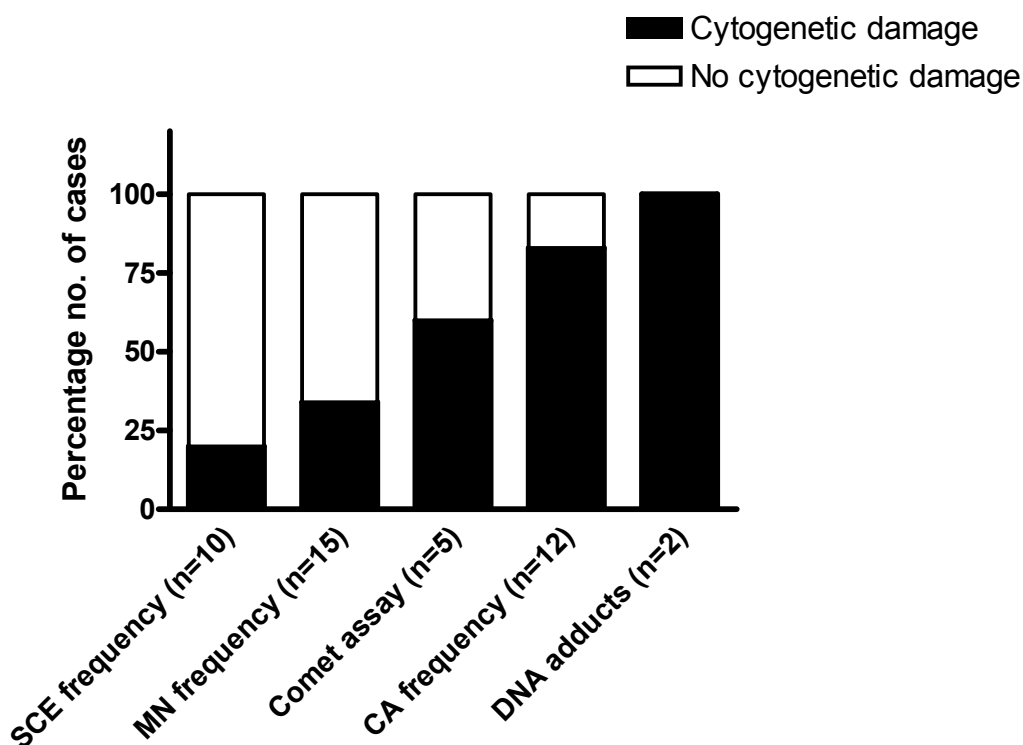


Figure 3 Cytogenetic effects measured by different endpoints

60. Joblonicka also reported that both CAs and SCE were significantly higher in workers following pesticide exposure (Jablonicka *et al.*, 1989). However, a number of studies reported different results for the different assays carried out. Although a significant increase in CAs was observed in Spanish agricultural workers compared to controls, the frequency of SCE remained unchanged (Carbonell *et al.*, 1993). Similarly, CAs were increased in pesticide sprayers although MN and SCE assays gave negative results (Joksic *et al.*, 1997). Pasquini reported an increase in the frequency of MN in Italian farmers, although SCE was again not significantly increased (Pasquini *et al.*, 1996).

### Statistical issues

61. In general, a variety of statistical methods has been used in the studies used for this report. The IPCS guidelines did not specify a precise statistical method that should be used for each genotoxicity endpoint. However, it is generally accepted that if the data are not normally distributed, but following Poisson, binomial or negative binomial distribution etc, then data may be analysed by using non-parametric methods, based on these distributions or alternatively, carry out data transformation in order to use parametric methods.

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62. Some of the studies used report normally distributed data and hence used parametric statistical methods (Bolognesi *et al.*, 2004; Bolognesi *et al.*, 2002; Carbonell *et al.*, 1990; Jablonicka *et al.*, 1989; Lander and Ronne, 1995; Lebailly *et al.*, 1998a; Lebailly *et al.*, 1998b; Pastor *et al.*, 2003) amongst others, although the majority of studies reported data that were not normally distributed. In such cases, many studies transformed data, in most cases by log transformation (Falck *et al.*, 1999; Scarpato *et al.*, 1996) or square root transformation (Bolognesi *et al.*, 1993a; Bolognesi *et al.*, 1993b; Bolognesi *et al.*, 1993c; Pastor *et al.*, 2002a) or carried out Poisson regression analysis (Bolognesi *et al.*, 1993a; Bolognesi *et al.*, 1993b; Bolognesi *et al.*, 1993c; Lander *et al.*, 2000; Pastor *et al.*, 2002a) or multiple linear regression (Falck *et al.*, 1999; Pastor *et al.*, 2001a; Peluso *et al.*, 1996; Scarpato *et al.*, 1996).

63. In the guidelines it was stated that the individual is considered the unit of exposure rather the cell and most authors carried out statistical analysis accordingly. By pooling data, as was the case in most studies, a source of variability was eliminated.

### *Multiple comparisons*

64. Few of the papers included in this review corrected data for multiple comparisons, which could be potentially misleading, especially when more than one endpoint is used. This may result in false positives being reported. Appropriate calculations have not been carried out in this report.

### *One or two tailed tests*

65. With the exception of studies carried out by Pastor and co-workers (Pastor *et al.*, 2003; Pastor *et al.*, 2002a; Pastor *et al.*, 2001a; Pastor *et al.*, 2001b), who used two-sided analyses, none of the other studies used in the review reported whether one- or two-sided analyses were used. As the p value used in the one-sided test is half that used in the two-sided test,  $p=0.05$  two-sided being equivalent to  $p=0.025$  one-sided, it is important to state which method was used. Moreover, in most cases, authors do not justify the methodology chosen for statistical analysis.

### *Outliers*

66. Seven of the studies retrieved presented individual data for each subject, as well as present data as the mean and SD or SE of the different groups i.e. exposed worker or control (Bolognesi *et al.*, 1993a; Carbonell *et al.*, 1990; Lebailly *et al.*, 1998a; Lebailly *et al.*, 1998b; Linnainmaa, 1983; Mustonen *et al.*, 1986; Pasquini *et al.*, 1996). Lebailly and co-workers presented data from workers, categorised into different groups according to pesticide exposure, before and after spraying and

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showed the modification of DNA damage between the two time points of each individual (Lebailly *et al.*, 1998b).

67. None of the authors in the review reported the presence of outliers, representing a potential sensitive sub-population. However, calculations undertaken for the purposes of this review using Grubb's test and Dixon's test highlighted outliers in four studies. Two outliers were found in exposed workers in the study carried out by Bolognesi and colleagues, identified using Grubbs test (Bolognesi *et al.*, 1993a). Two outliers were observed in exposed, non-smoking workers, during (Grubb's test) and after (Dixon's test) spraying, and one outlier was identified by Dixon's test in smoking workers during pesticide spraying (Linnainmaa, 1983). Similarly, in data presented by Lebailly and co-workers, 2 outliers were identified, by Grubb's test, in workers in group 1 before spraying pesticides, 1 worker in group 3 before pesticide spraying and 2 in workers in group 2 after pesticide spraying. In contrast, in the study carried out by Carbonell and co-workers, two outliers were identified by Dixon's test in exposed workers. One showed significantly higher levels of DNA damage whereas the other had lower levels of SCE (Carbonell *et al.*, 1990).

68. Despite outliers being identified, the impact of them being removed from the data set for statistical analysis is minimal in all studies. Data presented by two of the studies reported no significant differences in DNA damage between exposed workers and controls (Carbonell *et al.*, 1990; Lebailly *et al.*, 1998b; Linnainmaa, 1983), whereas a significant increase in MN frequency was reported by Bolognesi and co-workers (Bolognesi *et al.*, 1993a). In the latter study, elimination of the outliers or the use of non-parametric methods still resulted in a significantly higher MN frequency in exposed workers compared to the controls.

69. In the study carried out by Lebailly, 14 of the workers had significantly increased DNA damage, measured in terms of mean tail moment (highly damaged cells excluded), after one-day spraying pesticide, whereas 16 subjects showed a decrease in DNA damage, and 13 showed no change.

### *Historical control data*

70. Few of the studies using in the review report the use of historical data to either check that the reference values are within expected ranges, or to help in assessing the magnitude of increase that may be deemed biologically significant. Moreover, historical data are needed in order to prospectively calculate the number of subjects needed in order to give a defined power.

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### *Sensitivity analysis*

71. The IPCS guidelines recommend that during study design, investigators establish that studies are adequately powered to detect a difference between control and exposed groups of the magnitude of concern, i.e. that an appropriate number of subjects are included. This rather begs the question as to the magnitude of change in a genotoxic endpoint that might be considered of concern, and by inference that changes below this are not of concern. However, discussion of this issue is beyond the scope of this review, and a pragmatic difference of 1.5 or 2-fold has been assumed.

72. None of the published investigations cited in this review have reported information on study power. It is possible that uncertainty as to what constitutes a biologically significant increase in genetic damage militate against calculation of power, but this was never stated.

73. In this review, rather than assuming a specific value for a biological significant difference, as would be necessary for a power calculation, retrospective sensitivity calculations have been undertaken (using the Statgraphics Plus 5 statistical software), for those studies in which no significant change was reported. It has been assumed that data were normally distributed. In the absence of the raw data, this assumption could not be tested, but it was noted that authors had generally used parametric methods in the analysis of their data. The upper and lower 95% CIs of the mean difference between exposed and control groups were then calculated, based on the respective SDs for the groups. Thus, for example, in Lander and Ronne, 1995 (the first negative study summarised in this review), the mean frequency of SCE in lymphocytes was  $6.8 \pm 2.3$  in non-exposed and  $7.2 \pm 3.5$  in exposed subjects (not significantly different). The mean difference  $\pm$  95% CI between the means was  $0.4 \pm 0.67$ . These data enable the sensitivity of the study, for the number of subjects included, to be determined. In this case, it should have been possible to detect any change of 10% or more, with 80% power,  $\alpha = 0.05$  (2-tailed). In order to obtain some indication of the general level of sensitivity of the studies, the minimum effect that could have been detected (i.e. mean of control group plus 95% CI for the difference in the means of the control and exposed groups) was compared to a pragmatic value of 50% for an increase in the genetic damage indicated, relative to the control group. Almost all of the studies reviewed here would have been sufficiently sensitive to detect such a difference (i.e. across several genetic end points including frequency of total chromosomal aberrations, incidence of MN or SCE; overall range 10-40% except for several studies using CAs). It was noted that in many screening mutagenicity assays, an increase of 1.5X or 2X is used as part of the assessment of a positive response for genetic damage. As most of the studies should have detected a minimum increase of less

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than 50% of the mean of the control group, on the basis of these criteria the studies were adequately powered.

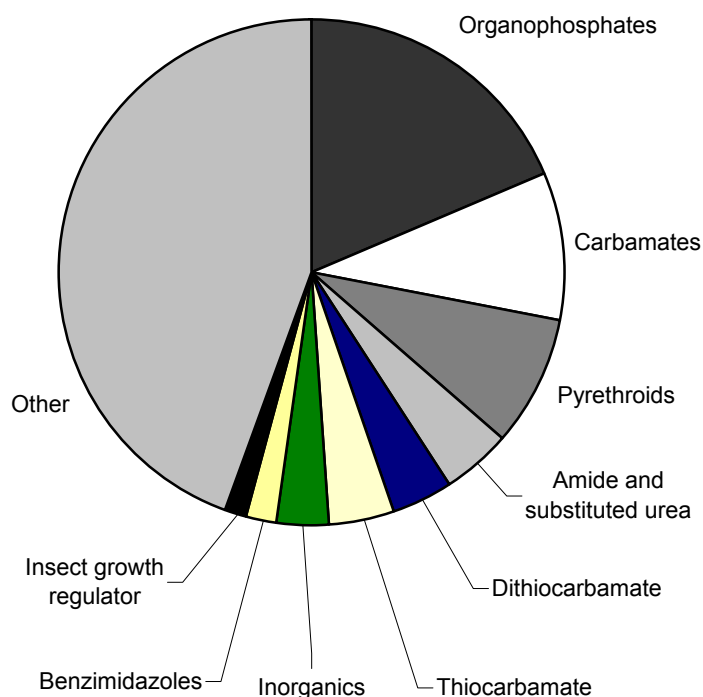
### Assessment of exposure to pesticides

74. The majority of pesticide exposures are to complex mixtures of compounds containing the active ingredient with pesticide activity and other inert chemicals such as additives, co-formulants, surfactants, dyes, stabilisers etc (Munnia *et al.*, 1999). Most people are exposed to pesticides through environmental contamination by means of degradation products in air, water and food. However, occupational exposure to pesticides during their manufacture and application can represent a different mode of exposure (Bolognesi, 2003), as not only exposure to pesticide active ingredients occurs, but also exposure to 'inert' compounds (Munnia *et al.*, 1999). In many instances, several pesticide products may be used together or sequentially, which further complicates exposure assessment.

75. All occupational groups are exposed to a wide range of pesticides from different chemical classes (Figure 4). Information from studies, where there are detailed chemical analyses, is presented in Annex V.

76. Little individual pesticide exposure data were retrieved from studies carried out in EC, with the exception of data following carbamate (mancozeb) (Jablonicka *et al.*, 1989), chlorothalonil and isoproturon (Lebailly *et al.*, 1998b) and captan (Lebailly *et al.*, 2003) exposure. This latter study assessed pesticide exposure by using the United Kingdom Predictive Operator Exposure Model (UK-POEM) to calculate the predicted absorbed dose of captan, but did not correlate the increased pesticide exposure with DNA damage. The authors comment that a lack of correlation between pesticide exposure and DNA damage could be due to several reasons; a lack of genotoxic effect of captan *in vivo* in humans at this exposure level or the genotoxic effect was not adequate to effect the endpoint of the assays used (Lebailly *et al.*, 2003).

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**Figure 4 Main classed of pesticides used by pesticide applicators**

77. DNA damage was also measured in farmers following exposure to chlorothalonil, isoproturon or triazole fungicides (Lebailly *et al.*, 1998b). Overall, authors report a significant increase in DNA damage after spraying chlorothalonil, although only three out of eight workers showed such an increase. In contrast, DNA damage was observed in only five out of 11 (three out of 11 excluding highly damaged cells) workers using isoproturon, the authors concluding that DNA damage was significantly lower after pesticide spraying. Following spraying of triazole fungicides, seven farmers (six when highly damaged cells were excluded) were reported to have more DNA damage, whilst seven presented less damage. Overall, authors reported that no significant modification of DNA damage was detected after triazole fungicide use.

78. Cytogenetic analysis was carried out on workers engaged in the production of Novozir Mn80 in Czechoslovakia. The exposed workers had clearly higher percentages of aberrant cells, breaks per cell and SCEs compared to the control group. Women had a higher rate of CAs (2.64%) compared to men (1.80%) (although both were significantly higher than controls) and authors suggest it could reflect their exposure level. Women commonly work in packing and final product dispatch departments, hence being exposed to fine particles that are easily dispersed in the air (Jablonicka *et al.*, 1989). The exposure, as measured by dust concentrations, was highest in these departments in this study. Although

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mancozeb was not directly measured in the study, its exposure is assumed from available data.

79. Overall, few biomonitoring data were available from the studies presented. From the limited number of studies that were retrieved, there is some limited evidence to suggest that mancozeb and chlorothalonil may potentially induce genotoxicity and DNA damage following occupational exposure, although no definite conclusions can be drawn. A review of mutagenicity data on these individual pesticide active ingredients is beyond the scope of this paper.

80. As part of the review there has been an attempt to identify pesticides used in studies that report DNA modification in exposed workers. This difficult task, as evidenced by table X in annex V, highlights the observation that it is not possible to draw conclusions regarding individual active ingredients in the studies reviewed. No individual pesticide active ingredient was identified consistently in exposed groups.

81. Studies that report genotoxicity following pesticide exposure also report the majority of pesticides used were organophosphorus compounds, pyrethroids, carbamates, benzimidazoles, dithiocarbamates and thiocarbamates, inorganics, amides and substituted urea, and miscellaneous pesticides. In the case of organophosphorus compounds, benzimidazoles, thiocarbamates and inorganic pesticides, the mean number used in studies showing positive genotoxic effects was higher than those showing no genotoxicity (Figure 5). In contrast, pyrethroids, carbamates, and amides and substituted urea compounds were used more in studies reporting negative effects. Although it is recognised that this is a crude estimation, this may highlight the potential risk of DNA damage following exposure to organophosphorus compounds, benzimidazoles, thiocarbamates and inorganics pesticides.

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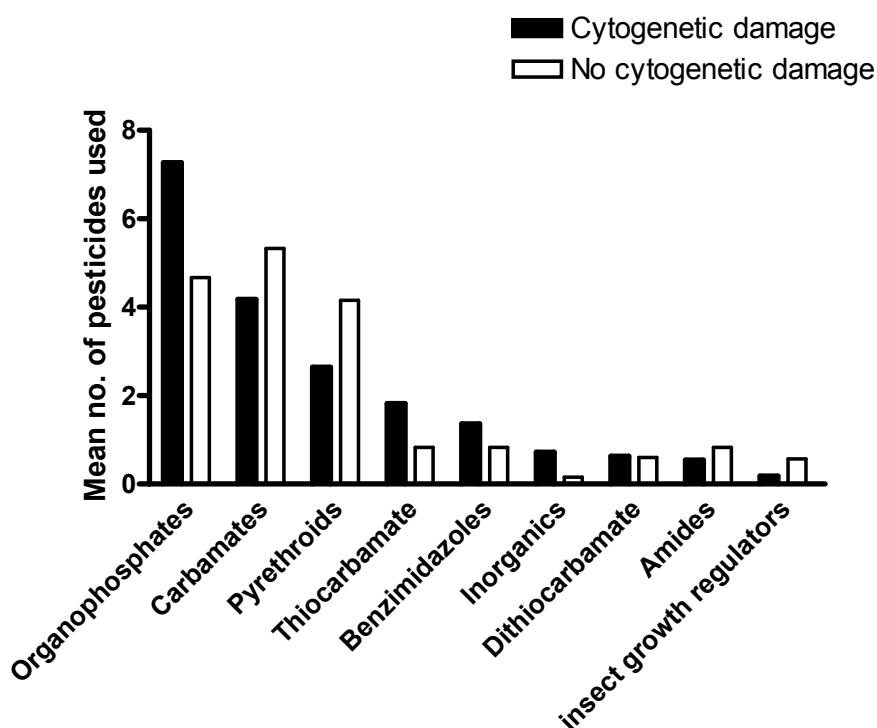


Figure 5. Mean number of pesticides used in retrieved studies

#### *Benzimidazole exposure*

82. The approval status of benzimidazoles was reported in the introduction of this paper. A further evaluation of this group is presented here, as it is reasonable to hypothesise that exposures above the threshold for spindle disruption could lead to genotoxicity. In the studies retrieved for this report, nine out of 11 (data across all endpoints) of those reporting positive effects following pesticide exposure used at least one benzimidazole (Carbonell *et al.*, 1995; Carbonell *et al.*, 1993; De Ferrari *et al.*, 1991; Lander and Ronne, 1995; Pasquini *et al.*, 1996), four of which reported the use of 2-3 different benzimidazole compounds (Bolognesi *et al.*, 2004; Bolognesi *et al.*, 2002; Lander *et al.*, 2000; Peluso *et al.*, 1996). Although six out of the seven studies reporting negative effects also used benzimidazole compounds (Lebailly *et al.*, 2003; Lucero *et al.*, 2000; Nehez *et al.*, 1988; Pastor *et al.*, 2003; Pastor *et al.*, 2001a; Piperakis *et al.*, 2003), all used only one benzimidazole product, rather than multiple products, as in those studies where genotoxic effects were documented. In most studies, benomyl was the predominant benzimidazole used. Carbendazim was used in four studies reporting positive effects but only two reporting negative data, whereas thiabendazole and tiofanate methyl were used only by workers exhibiting genetic damage. Overall, no clear consistent picture of exposure to benzimidazoles in positive studies is apparent.

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### Discussion

83. A detailed evaluation was carried out to explore the relationship between exposure to pesticides associated and genetic damage. Table 7 shows a summary of data obtained from workers in different occupational settings, and by using different cytogenetic endpoints.

**Table 7 Cytogenetic effects in workers occupationally exposed to pesticides – summary of results**

Occupation	Endpoint	Number of studies (positive/total)
Production worker	MN	-
	CA	1/1
	SCE	1/1
	Adducts	-
	Comet	-
Sprayer	MN	-
	CA	4/4
	SCE	0/3
	Adducts	-
	Comet	1/1
Agriculturalist	MN	1/7
	CA	3/3
	SCE	0/2
	Adducts	-
	Comet	2/3
Floriculturist	MN	4/7
	CA	2/3
	SCE	1/2
	Adducts	2/2
	Comet	0/1
Forestry worker	MN	-
	CA	0/1
	SCE	0/1
	Adducts	-
	Comet	-

84. The report has attempted to consider all studies using the IPCS guidelines as a reference. A number of confounding factors were considered, including the use of protective clothing, working environment eg. greenhouses, open situations, sampling time, age, smoking, sex and genetic endpoint used. It is noteworthy that there is no UK study investigating cytogenetic damage following pesticide exposure in an occupational environment. A comparison of exposures in the

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studies reported and equivalent practices in the UK is outside the scope of this report.

85. The majority of positive findings from many studies suggest a correlation between pesticide exposure and cytogenetic effect, although exposure to the pesticides was often poorly documented and no individual pesticide could be identified that could be responsible for such genotoxicity. In such studies where exposure was well documented, genotoxicity could not be demonstrated following captan (Lebailly *et al.*, 2003) or isoproturon exposure (Lebailly *et al.*, 1998a), although there was some evidence to suggest that mancozeb (Jablonicka *et al.*, 1989) and chlorothalonil (Lebailly *et al.*, 1998a) could potentially induce genotoxic effects.

86. A number of general conclusions are suggested below:

1. Overall, there is some evidence for increased mutagenicity (cytogenetic damage) in pesticide applicators/floriculturists.
2. There are a number of confounding factors that complicate the assessment of the cytogenetic data.
3. It is not possible to draw conclusions regarding the exposures to pesticides and/or other chemicals in many of the studies that may have been causative in pesticide exposure.
4. The available information suggests that studies of cytogenetics and micronuclei formation in peripheral blood lymphocytes have not provided consistent evidence for positive effects.
5. There is comparatively little evidence for other endpoints such as DNA damage (comet assay) and DNA adducts. There is preliminary evidence for positive results in floriculturists (DNA adducts) and agricultural workers (comet assay).
6. It is not possible to directly extrapolate the results from EU countries to the UK (a comparative review of exposure is beyond the scope of this review).
7. Some information was retrieved regarding mancozeb, captan, isoproturon and chlorothalanil. No definite conclusions can be drawn from this review with regard to mutagenicity of individual pesticide active ingredients.
8. All the available evidence suggests that appropriate biomonitoring studies in the UK would be required to draw conclusions pertaining to UK use of pesticides in floriculturists and greenhouse workers.

### Abbreviations

ACP	Advisory Committee on Pesticides
CA	Chromosome aberrations

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CI	Confidence interval
IPCS	International Programme on Chemical Safety
MTP	Medical and Toxicology Panel
NA	Not applicable as individual data were not presented in the report
NS	Not stated
PHA	Phytohaemagglutinin
SCE	Sister chromatid exchange
SD	Standard deviation
SE	Standard error of the mean
UK-POEM	United Kingdom Predictive Operator Exposure Model

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