COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT.

GENOTOXICITY IN PESTICIDE APPLICATORS: FURTHER INFORMATION FOLLOW-UP OF OCTOBER 2004 INITIAL REVIEW

1. The Medical and Toxicology panel of the Advisory Committee on Pesticides has asked the Com for advice on the genotoxicity in pesticide applicators. The referral statement is given below.

"*To review investigations of mutagenicity and DNA adducts in pesticide applicators and workers exposed to pesticides (e.g. handling cut flowers) and factory (manufacturing) staff engaged in pesticide manufacture/formulation and produce a statement for the Advisory Committee on Pesticides and its Medical and Toxicology Panel. The review should include all studies identified by the MTP and any other relevant studies published, particularly those originating from the UK.*

The review should consult COC epidemiologists with regard to the rigour of studies evaluated. this should include design, selection of controls, bias, confounding and use of multiple statistical comparisons.

The review should be initiated at the October 2004 COM meeting."

2. The DH Toxicology Unit in collaboration with the secretariat drafted an initial overview of studies from the EU. (MUT/04/19). A further review paper has now been drafted which updates members on progress of the review since October 2004.

3. The primary objective for the February 2005 COM meeting is to consider the selection criteria identified by COM members in the light of comments from the independent epidemiologist and to reach conclusions on these criteria.

4. The paper also outlines the characteristics of the included studies. Some additional work was undertaken on magnitude of response. Members are asked for their comments on the outcome of these analyses.

4. The Committee is also asked to consider publications from the rest of the world which have not been considered in full so far and to provide comments (MUT/05/6).

5. A number of suggested actions are outlined in the appended review. Members comments are sought

Secretariat January 2005

Cytogenetic effects on pesticide workers – review approach

Introduction

1. This review follows on from the report presented at the 7th October 2004 COM meeting, namely 'Biomonitoring studies from EU of genotoxicity in pesticides applicators', MUT/04/19, carried out upon request of the Advisory Committee on Pesticides (ACP) and its Medical and Toxicology Panel (MTP).

2. Members will recall that the data from individual studies from EU were discussed and it was suggested that some studies should be excluded from further analyses. The ACP had requested that an independent epidemiologist should also be consulted in addition to advice from COM.

3. The present report outlines the review approach used to exclude or indeed select studies, from EU and rest of the world, for further evaluation and summarises the meeting held between the DH toxicology unit, the COM chair and secretariat and Dr. Lesley Rushton (COT member and epidemiologist). In addition, overviews of the outcome of analyses and characteristics of selected studies used for further analyses are given.

Review approach

4. In total, 48 studies were retrieved regarding cytogenetic effects in pesticide workers. Due to time constraints, only those from EU were presented at the COM meeting in October 2004.

5. All 48 studies were evaluated for further evaluation, according to a number of criteria suggested by COM members. Based on such criteria, 33 of the studies were initially excluded for further consideration. The exclusion strategy and criteria were presented to the epidemiologist from IEH Following the meeting and upon advice from COM, criteria and studies were re-evaluated, resulting in 23 studies being excluded.

Meeting with IEH

6. The Department of Health Toxicology Unit, COM chair and COM secretariat met with an epidemiologist from IEH on 29th November 2004.

7. It was emphasised that it was important to avoid introducing bias when identifying selection criteria and asked the COM for clear guidance on criteria used. Thus, for example, did excluding MN studies on the basis of extended incubation (i.e. 72 hours), a criterion set for false negative studies, result in the exclusion of potentially adequate studies giving positive results?

8. It was agreed recommended that studies should not be specifically excluded when controls were not matched to subjects, according to age, sex and smoking habits, as information regarding the impact of such effect modifiers could be appropriately modelled.

9. It was agreed to present exclusion criteria in a more hierarchical format and to consult COM members prior to the February 2005 meeting.

Exclusion criteria (annexes I & II)

10. Studies using sister chromatic exchange as the endpoint were excluded from further consideration in view of the uncertainty of the biological relevance of this end point to mutagenicity. Three studies were excluded, all reporting negative data.

11. Studies were excluded due to the incubation time used in the micronucleus assay. Those using incubation periods of 72 hours were excluded, as many of the cells would have been through three cell divisions post-sampling. The extent of DNA repair and cell loss could complicate the evaluation of such data, possibly leading to false negative data. Twelve studies reported the use a 72 hour incubation period, 11 of which reported negative data, and which were excluded from further analysis. The study that reported positive data was included for further analysis.

12. The time of blood sampling relative to pesticide exposure was considered to be of concern as false negative data may be obtained if sampling was carried out too long after exposure. Two studies were excluded, both of which reported negative data.

13. Several studies were excluded as it was considered that inappropriate protocols were used. For example, the protocols used for the comet assay and the subjective grading of comets was regarded as inappropriate in some of the studies, hence three were excluded from further analysis, all reporting positive data. One study was excluded due to an inappropriate protocol used for CA assay, although positive data were reported.

14. Three studies were excluded due to reporting duplicated data previously presented in other studies. All three studies reported positive data.

Included studies (annexes III & IV)

15. Twenty-five studies were considered to have met the above-mentioned criteria. Twenty-one of the studies included reported cytogenetic effects following pesticide exposure, whereas four reported negative data. Of these studies, chromosome aberrations were measured in 18 studies; the micronuclei assay was

carried out in four studies; two studies measured DNA adducts and two studies used the comet assay to assess DNA damage.

Further steps

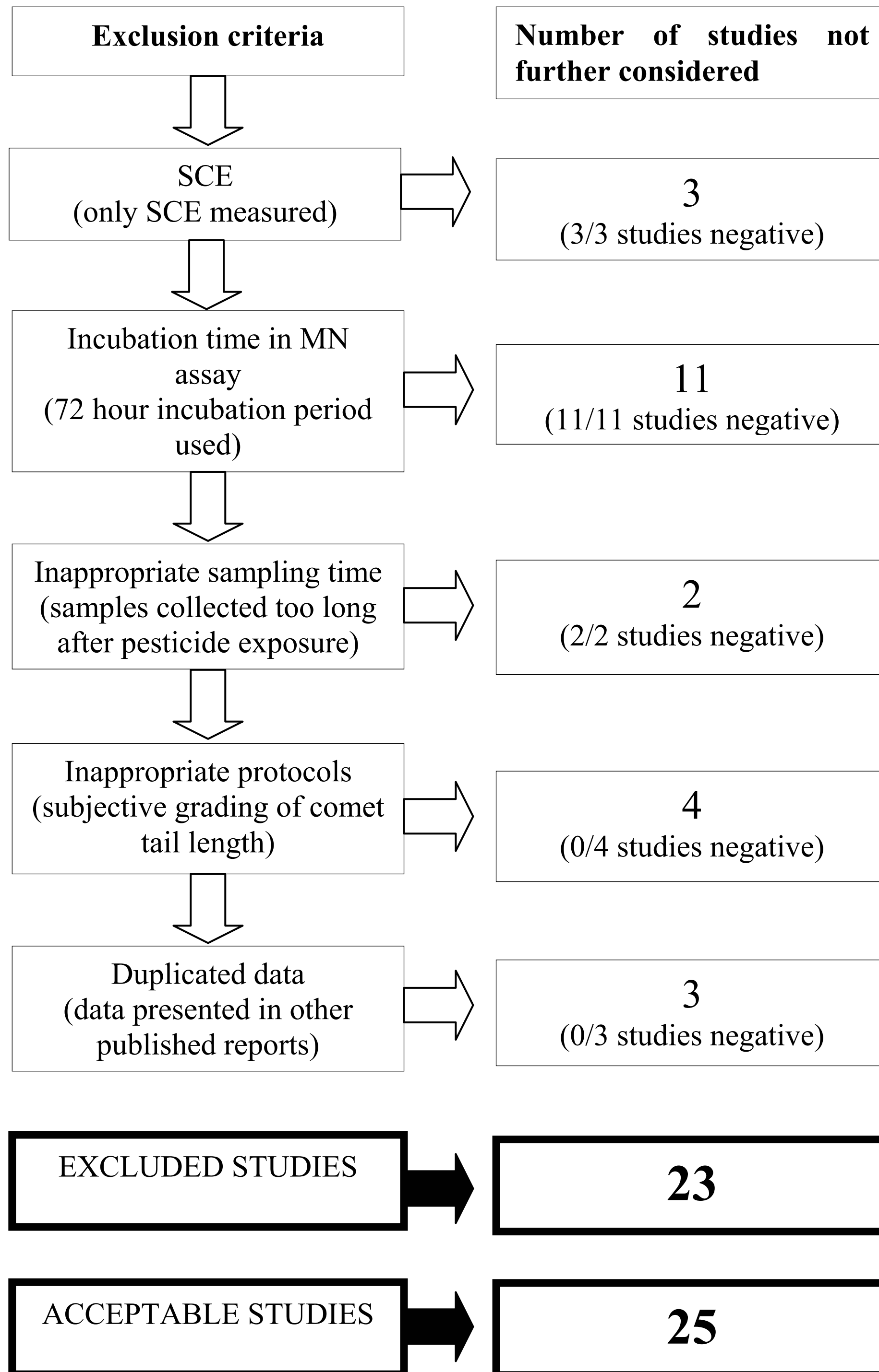
16. The key objective is to identify genuine positive studies. In this respect the COM is asked to consider the new tabulation of magnitude of response (annexes V & VI).

17. The second objective is to identify exposure patterns of pesticides in positive selected studies compared to equivalent application operations in the UK. A brief tabulation of EU studies was provided to members at the October 2004 meeting. A further tabulation (annex VII) covering all positive studies is appended. Members previously considered that no clear exposure patters could be discerned for the available data. [The secretariat is discussing the evaluation of exposure with PSD. Appropriate expertise for exposure evaluation can be sought from PSD and ACP].

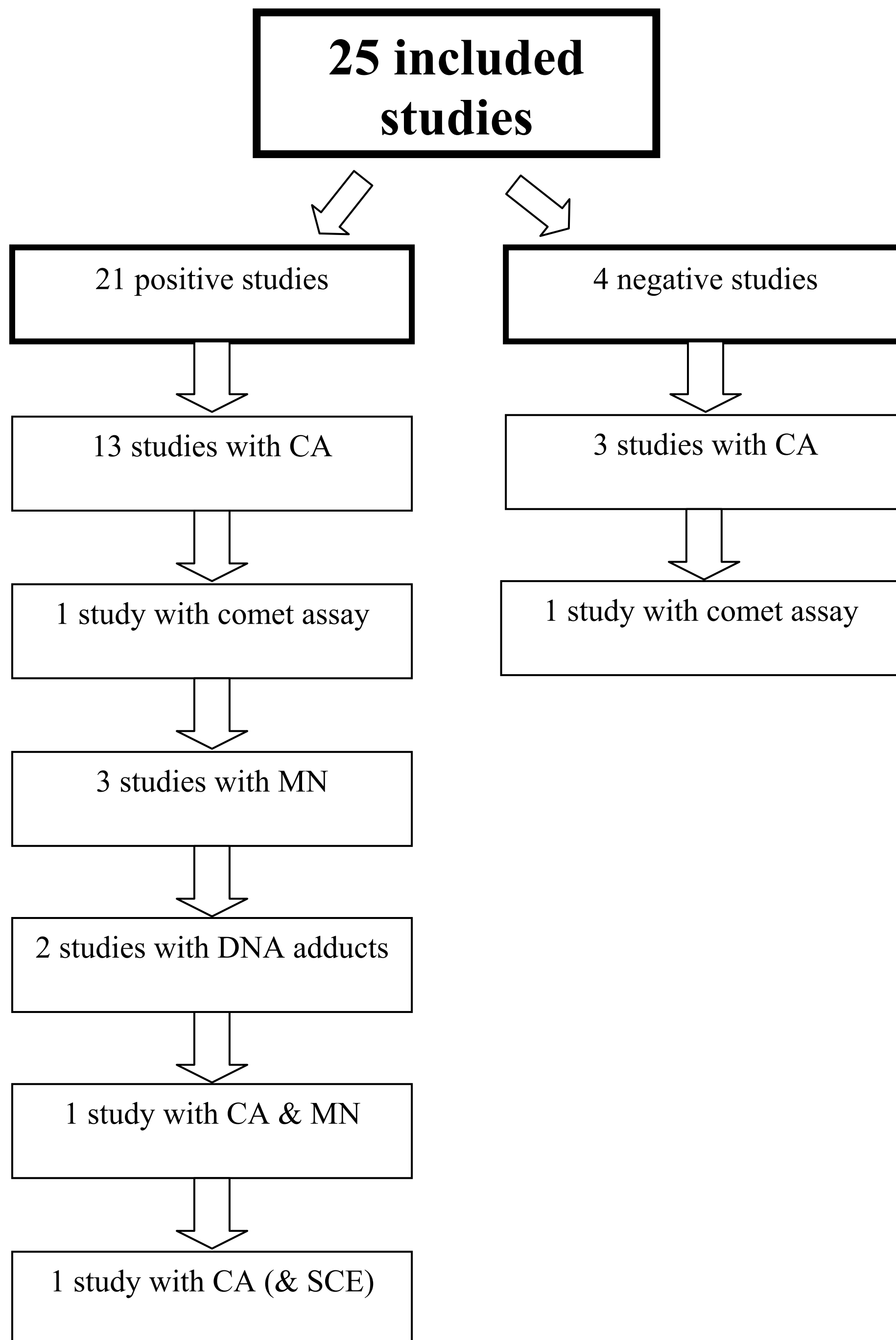
18. Epidemiology overview will be presented in the May 2005 COM meeting.

Annex II: Flow chart of excluded studies.

The flow chart demonstrates how many studies were excluded from further analysis due to various selection criteria, and the data reported in such studies.



The flow chart demonstrates the results and endpoints used in the included studies.



Annex IV: Overview of discussion with COM committee members (8-9th December 2004).

1. In the COM meeting on 7th October 2004, committee members suggested a number of criteria that should be used to evaluate the adequacy of each study retrieved from EU and rest of the world, regarding cytogenetic effects on pesticide workers. Members agreed that studies should be excluded based on six criteria:

a. Use of SCE exchange as the cytogenetic endpoint
   Members were unsure of the biological relevance of this end point to mutagenicity.
b. Use of a 72 hour incubation period in the micronucleus assay
   Members considered this too long and may result in false negatives.
c. Use of inappropriate protocols
   i. Subjective grading of comet tail length in the comet assay Members considered the subjective grading of comet tail length inaccurate and could introduce false positives
   ii. DNA adduct protocols
       Members were concerned about the lack of butanol extraction
   iii. Isolation of lymphocytes for use in the micronucleus assay
       Members thought this might result in false positives as the isolation procedure may render the lymphocytes more susceptible to damage
   iv. Shipping samples to another country or by air
       Members considered the long period of time between sampling and processing to be inappropriate and were concerned about the transport conditions of the cells eg. temperature. Moreover, X-ray scanning, if transported by air, may result in additional cell damage
d. Use of un-matched controls
   Members agreed that subjects and controls should be matched according to age, sex and smoking habits.
e. Sampling time of blood samples relative to pesticide exposure
   Members thought that sampling blood long after pesticide exposure would result in false negative data. The IPCS guidelines recommend samples be taken within 48-72 hours after cessation of exposure.
f. Duplication of data
   Members agreed that studies presenting duplicated data were inappropriate to include

2. Initially, studies were excluded on the above criteria. However, after consultation with Dr. Lesley Ruston, the list of exclusion criteria was amended. Based on such recommendations, all studies were re-evaluated and advice was sought from Prof. David Philips (COC and COM member and expert on, Dr. Gillian Clare (COM member and expert on), Dr. Elizabeth Parry (COM member and expert

on) and Dr. Julie Clements (COM member and expert on) regarding specific exclusion criteria and studies.

72 hour incubation period in MN assay

3. Advice was sought from Dr. Gillian Clare, Dr. Elizabeth Parry and Dr. Julie Clements. All were in agreement that studies using a 72 hour incubation period could be excluded as cells would undergo several cell cycles and hence damaged cells could potentially be 'lost' or diluted, resulting in false negative data. The importance of this point was reinforced by the fact that most of the studies using a longer incubation period did report negative results.

4. All studies reporting negative data that used a 72 hour incubation period were excluded. Pasquini et al., 1996 reported positive effects and hence were included for further analysis.

Inappropriate protocols

^{32}P-DNA post-labelling

5. Advice was sought from Prof. David Philips regarding the study carried out by Munnia et al., 1999, in which butanol extraction in the ^{32}P-DNA post-labelling assay was not performed. Prof. Phillips stated that lack of butanol extraction may lead to false negatives as DNA adducts may be missed, however recommended that the study could be included as the study reported positive data.

Isolation of lymphocytes for use in the MN assay

6. Advice was sought from Dr. Gillian Clare, Dr. Elizabeth Parry and Dr. Julie Clements regarding the use of lymphocytes isolated for use in the MN assay. Both were in agreement that although such lymphocytes would be more sensitive to an in vitro treatment, possibly resulting in more positive data, their use in studies with *in vivo* exposure is less of a concern, as long as controls were treated in the same manner. Hence both agreed that studies should not be excluded due to using isolated lymphocytes alone. On their recommendation, the study carried out by De Ferrari *et al*., 1991 was included for further analysis.

Shipping samples

7. Advice was sought from Dr. Gillian Clare and Dr. Elizabeth Parry regarding the shipping of samples to other laboratories for processing. Dr. Clare discussed that X-raying could potentially damage cells hence such studies should be viewed with caution. However, seeing as both studies previously excluded due to shipping

the samples reported negative data, this is less of a concern. Dr. Parry stated that the time between sampling and processing was of slight concern, as were the transportation conditions eg. temperature, but agreed that studies previously excluded on such grounds could be included in further analyses. On their recommendation, the studies carried out by Lander *et al*., 2000 and Au *et al*., 1999 were included for further analysis.

Sampling time

8. Advice was sought from Dr. Gillian Clare and Dr. Elizabeth Parry regarding the time between blood sampling relative to pesticide exposure. Both thought that studies should be excluded if sampling took place long after pesticide exposure, as the half-life of lymphocytes, repair mechanisms and the elimination of damaged cells may result in false negative data being obtained. All studies that did not collect blood samples 48-72 hours after exposure were excluded from further analysis, with the exception of those carrying out longitudinal studies in which multiple sampling times were used.

Annex I: Characteristics of excluded studies

Author	Results	Protocols	Sampling time and exposure time	Duplicated data
[a]Lander and Ronne, 1995	+ SCE	Only SCE measured	Blood samples collected at end of spraying season.	
[a]Carbonell *et al.*, 1990	- SCE	Only SCE measured	Not stated	
[a]Linainmaa, 1983	- SCE	Only SCE measured	Blood samples taken before, during and after spraying.	
[a]Falck *et al.*, 1999	- MN	72 hour incubation time used for MN assay.	Blood samples collected during pesticide application.	
[a]Pastor *et al.*, 2001b	- MN	72 hour incubation time used for MN assay.	Not stated	
[a]Pastor *et al.*, 2003	- MN	72 hour incubation time used for MN assay. Disparate groups used.	Not stated	
[a]Pastor *et al.*, 2002a	- MN	72 hour incubation time used for MN assay.	Not stated	
[a]Pastor *et al.*, 2002b	- MN	72 hour incubation time used for MN assay.	Blood samples collected during a period of high and low exposure.	

[a]Pastor *et al*., 2001a	- MN	72 hour incubation time used for MN assay.	Not stated
[a]Lucero *et al*., 2000	- MN	72 hour incubation time used for MN assay.	Not stated
[a]Bolognesi *et al*., 2002	- MN	72 hour incubation time used for MN assay.	Not stated
[a]Piperakis *et al*., 2003	- comet	Subjective scoring of comet tails.	Blood samples collected late winter/early spring. Exposure time
[a]Scarpato *et al*., 1996	- MN - SCE - CA	72 hour incubation time used for MN assay.	Blood samples taken 1 month after exposure.
[a]Undeger and Basaran, 2002	+ comet	Subjective scoring of comets	Not stated
[a]Lebailly *et al*., 1998a	+ comet	Inappropriate study design and subjective grading of comets.	Four groups used; workers in summer, January, April and June.
[a]Lebailly *et al*., 1998b	+ comet	Inappropriate study design and subjective grading of comets.	Not stated
[b]Nehez *et al*., 1988	+ CA	Low cell number scored and that the karyotype was not reported.	Blood samples collected from different workers at different times points.

[a]Kourakis *et al.*, 1996	+ CA - SCE	Adequate protocol	Not stated	Duplicated data from previous paper (Kourakis *et al.*, 1992).
[a]Bolognesi et al., 1993a	+ MN	72 hour incubation time used for MN assay.	Not stated	Subjects and data are replicated from previous papers (Bolognesi *et al.*, 1993b).
[a]Bolognesi *et al.*, 1993c	+ MN	72 hour incubation time used for MN assay.	Not stated	Subjects and data are replicated from previous papers (Bolognesi *et al.*, 1993b).
[b]Holland et al., 2002	- MN	72 hour incubation times were used in the MN assay. Isolated lymphocytes from peripheral blood were used.	Blood samples collected before and after exposure.	
[b]Titenko-Holland et al., 1997	- MN	72 hour incubation times were used in the MN assay. Isolated lymphocytes from peripheral blood were used.	Blood samples collected September and December.	
[b]Davies et al., 1998	- MN	72 hour incubation times were used in the MN assay.	Blood samples collected in October-November at end of season.	

[a] Studies from EU

[b] Studies from rest of world

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Annex III: Characteristics of selected studies

Author	Results	Coded samples	Appropriate protocols	Sampling time and exposure time	Pesticide exposure	Effect of confounders	Controls
[b]Antonucci and de Syllos Colus, 2000	+ CA	Yes	Few details given	Not stated	Mixtures including orgaonophosphates and carbamates	No effect of age, smoking or duration of exposure	Matched on age
[b]Au *et al*., 1999	- CA	Yes	Adequate	All year round exposure	Previous exposure to dibromochloropropane, current exposure to mixtures of pesticides	Not stated	Matched on age and smoking
[a]Bolognesi *et al*., 1993b	+ MN	Yes	Adequate	Not stated	Mixtures including orgaonophosphates, pyrethroids and carbamates	MN increased with age, sex or duration of exposure	Not stated
[a]Bolognesi *et al*., 2004	+ MN	Yes	Long incubation time. FISH used.	Not stated	Mixtures including orgaonophosphates, pyrethroids, carbamates and benzimidazoles	No effect of age, sex, smoking or duration of exposure on MN. C+MN increased with age and sex	Matched on age and smoking. More females in exposed group
[a]Carbonell *et al*., 1993	+ CA	Yes	Adequate	May-June Period of pesticide exposure not stated	Mixtures including orgaonophosphates, pyrethroids and carbamates	No effect of age or sex. CA increased with duration of exposure	Matched on age and smoking

[a]Carbonell *et al*., 1995	+ CA	Yes	Adequate	Spring/summer and autumn/winter High pesticide exposure during spring/summer	Mixtures of insecticides, fungicides and herbicides	Not stated	Matched on age and habits
[a]De Ferrari *et al*., 1991	+ CA + SCE	Yes	Adequate	Not stated	Mixtures including organophosphates, organochlorinates, carbamates and triazines	No effect of age or smoking	Controls with bladder cancer with same exposure or controls with no exposure
[b]Dulout *et al*., 1985	+ CA	Yes	Adequate	Spring/summer High pesticide exposure during spring/summer	Mixtures including organophosphates, organochlorides and carbamates	CA increased with smoking	Not stated
[b]Hoyos *et al*., 1996	- CA	Yes	Adequate	Not stated	Mixtures of pesticides and fungicides	No effect of age or smoking	Matched on age, smoking and sex
[b]Garry *et al*., 1996	+ CA	Not stated	CA scored according to ISCN	Middle of the season of pesticide use	Mixtures of insecticides and herbicides	Not stated	Matched on age and smoking
[b]Garry *et al*., 2001	+ CA	Yes	Adequate	Spring/summer in middle of pesticide spraying season	2,4-D	No effect of smoking	Matched on age

[b]Grover, *et al.*, 2003	+ comet	Yes	Adequate	October – December. Period of pesticide exposure not stated	Mixture of pesticides	DNA tail length increased with smoking, age and in males.	Not stated
[a]Jablonicka *et al.*, 1989	+ CA	Not stated	Adequate	Not stated	Mancozeb	No effect of smoking	Matched on habits and social position
[a]Joksic *et al.*, 1997	+ CA + MN	Yes	CA scored according to IAEA	Before/after spraying	Mixtures of insecticides, fungicides and herbicides	Not stated	Matched on age, sex and smoking
[a]Kourakis *et al.*, 1992	+ CA	Yes	Adequate	Autumn during spraying season	Mixtures including orgaonophosphates, carbamates, dithiocarbamates and organochlorides	No effect of age or smoking	Matched on age and sex
[a]Lander *et al.*, 2000	+ CA	Yes	Adequate	Pre-season and post-season sampling. Control samples collected pre-season of the following year	Mixtures of insecticides, fungicides and growth regulators	CA decreased with protective gloves and increased with smoking	Non-smoking controls compared with non-smoking and smoking subjects

[a]Lebailly *et al*., 2003	- comet	Not stated	Adequate	Before/after 1 day spraying	Captan	Not stated	No controls due to longitudinal study
[b]Mohammed *et al*., 1995	+ CA	Yes	Adequate	April, July and October - during spraying season	Deltamethrin and cypermethrin	Not stated	Not stated
[a]Munnia *et al*., 1999	+ DNA adducts	Yes	Did not carry out butanol extraction	Sampled in the morning. Pesticide exposure not stated	Mixtures of insecticides, fungicides and herbicides. Aromatic amines and/or nitroaromatic compounds not used	Effect of age and sex	Matched on age and smoking
[a]Mustonen *et al*., 1986	- CA	Yes	Adequate	July-October after spraying season	2,4-D and MCPA	No effect of duration of exposure	Matched on age, sex and smoking
[b]Paldy *et al*., 1987	+ CA	Not stated	Adequate	November and April after spraying March to October	Mixture of pesticides	Effect of duration of exposure. No effect of age. Unequivocal effect of smoking and alcohol	Matched on sex
[a]Pasquini *et al*., 1996	+ MN	Yes	72 hour incubation time	Exposure March to July. Sampling from May to September 1992	Mixture of pesticides	Effect of duration of exposure. No effect of age.	Not stated

[b]Paz-y-Mino *et al*., 2002	+ CA	Not stated	CA scored according to ISCN.	Not stated	27 different pesticides	No effect of duration of exposure	Matched on age and sex
[a]Peluso *et al*., 1996	+ DNA adducts	Yes	Nuclease P1 procedure and butanol extraction carried out	Sampled in the morning. Continuous pesticide exposure	Mixtures of insecticides, fungicides and herbicides	No effect of age or gender.	Matched on age, sex and smoking
[b]Rupa, *et al*., 1991	+ CA	Yes	Adequate	Samples collect in January. Exposure to pesticides in spring and winter.	Mixtures of pesticides	CA increased with duration of exposure.	Matched on age, smoking and socio-economic class

[a] Studies from EU

[b] Studies from rest of world

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Annex V: Characteristics of selected studies

Numbers in subscript refer to data on the figure 1.

Author	Results	Data for controls	Data for exposed subjects	Fold increase over controls
[b]Antonucci and de Syllos Colus, 2000	+ CA	Cells with CA=4 (2 gaps 3 breaks 2 aces total CA = 7)	Cells with CA=13 (0 gaps 10 breaks 3 aces total CA = 13) [1]	**1.9-fold increase**
[b]Au *et al.*, 1999	- CA	Aberrant cells=1.9 ± 1.34 Chromatid breaks=1.8 ± 1.34	Aberrant cells=2.4 ± 1.34 [2] Chromatid breaks=2.2 ± 1.78 (mean ± SD)	1.3-fold increase 1.2-fold increase
[a]Bolognesi *et al.*, 1993b	+ MN	MN frequency = 6.67 ± 3.12	MN frequency = 8.57 ± 5.02 [3] (mean x 1000 cells ± SD) RR = 1.25; 95 % CI = 1.11 – 1.41	**1.3-fold increase**
[a]Bolognesi *et al.*, 2004	- MN	Total MN (C+MN) = 2.18 ± 6.31 Total MN (C-MN) = 1.32 ± 3.38	Total MN (C+MN) = 2.79 ± 12.21[4] Total MN (C-MN) = 1.56 ± 6.00 [5] (mean x 1000 cells ± SD)	1.2-fold increase **1.3-fold increase** **1.2-fold increase**

[a]Carbonell *et al*., 1993	+ CA	Cells with aberrations = 4.20 Chromatid-type aberrations = 3.22 % Chromosome-type aberrations =1.55% Total aberrations = 4.77%	Cells with aberrations = 5.93 Chromatid-type aberrations = 4.74 % Chromosome-type aberrations = 1.88% Total aberrations = 6.62 % [6]	1.4-fold increase 1.5-fold increase 1.2-fold increase **1.4-fold increase**
[a]Carbonell *et al*., 1995	+ CA	Spring/summer Cells with aberrations = 4.56 ± 2.53 % Chromatid-type aberrations = 3.14 ± 2.76 % Chromosome-type aberrations = 1.90 ± 1.51 % Total aberrations = 5.04 ± 2.85 % Autumn/winter Cells with aberrations = 3.39 ± 2.4 % chromatid-type aberrations = 2.57 ± 2.0 % Chromosome-type aberrations = 1.32 ± 1.96 % Total aberrations = 3.90 ± 3.23 %	Spring/summer Cells with aberrations = 6.27 ± 2.96% Chromatid-type aberrations = 5.31 ± 3.12 % Chromosome-type aberrations = 1.63 ± 1.56 % Total aberrations = 6.93 ± 3.5 % [7] Autumn/winter Cells with aberrations = 3.69 ± 2.14 % chromatid-type aberrations = 2.49 ± 0.56 % Chromosome-type aberrations = 1.21 ± 1.83 % Total aberrations = 3.70 ± 2.15 % [8] (mean ± SD)	 1.4-fold increase 1.7-fold increase 0.9-fold increase **1.4-fold increase** 1.1-fold increase 1.0-fold increase 0.9-fold increase **1.0-fold increase**
[a]De Ferrari *et al*., 1991	+ CA + SCE	 Chromatid-type aberrations = 4.44 ± 3.06 Chromosome-type aberrations = 1.08 ± 1.28 Complex rearrangements = <0.02	Exposed subjects Chromatid-type aberrations = 7.46 ± 6.22 Chromosome-type aberrations = 2.72 ± 1.58 Complex rearrangements = 0.12 ± 0.12	 1.7-fold increase 2.5-fold increase 6.0-fold increase

		Total aberrations = 5.52 ± 4.12		**1.9-fold increase**
			Total aberrations = 10.30 ± 7.18 [9]	
			Exposed subjects with bladder cancer;	
			Chromatid-type aberrations = 5.07± 3.90	1.1-fold increase
			Chromosome-type aberrations = 2.65 ± 02.26	2.5-fold increase
			Complex rearrangements = 0.30 ± 0.62	15.0-fold increase
			Total aberrations = 8.02 ± 4.98 [10]	**1.5-fold increase**
			(Mean per 100 metaphases ± SD)	
[b]Dulout *et al.*, 1985	+ CA	Abnormal cells = 2.65 ± 1.01 %	Abnormal cells = 2.71 ± 0.36 %	1.0-fold increase
		Gaps = 1.56 ± 2.09	Gaps = 2.43 ± 1.62	1.6-fold increase
		chromatid breaks = 1.70 ± 0.74	chromatid breaks = 1.51 ± 1.26 [11]	0.9-fold increase
		Chromosome breaks = 0.54 ± 0.62	Chromosome breaks = 0.95 ± 1.08 [12]	1.8-fold increase
		dicentric chromosome and ring chromosome = 0.10 ± 0.21	dicentric chromosome and ring chromosome = 0.43 ± 0.84 [13]	4.3-fold increase
		(CA per 100 cells gaps ± SD)	(CA per 100 cells gaps ± SD)	
[b]Garry *et al.*, 1996	+ CA	Rearrangement frequency = 0.4 ± 0.57	Rearrangement frequency	
			Fumigant = 1.4 ± 1.44[14]	3.5-fold increase
			Insecticide = 1.4 ± 1.28 [15]	3.5-fold increase
			Herbicide = 1.0 ± 1.34 [16]	2.5-fold increase
			(mean ± SD)	
[b]Garry *et al.*, 2001	+ CA	Translocations/inversions/deletions = 0.65 ± 1.12	Translocations/inversions/deletions	
			Low volume (1-100 gall) = 1.20 ± 1.13 [17]	1.9-fold increase
			Mid-range (100-1000 gall) = 1.00 ± 1.13 [18]	1.5-fold increase
			Heavy (>1000 gall) = 2.22 ± 1.14 [19]	3.4-fold increase

[b]Grover, *et al.*, 2003	+ comet	Smokers Comet tail length = 7.03 ± 11.46 Non-smokers Comet tail length = 10.34 ± 13.25	Smokers Comet tail length = 18.26 ± 9.76 [20] Non-smokers Comet tail length = 19.75 ± 14.48 [21]	 2.6-fold increase 1.9-fold increase
[b]Hoyos *et al.*, 1996	- CA	Total chromatid breaks = 2.1 ± 1.30	Total chromatid breaks = 1.67 ± 1.44 [22] (Mean ± SD)	0.8-fold increase
[a]Jablonicka *et al.*, 1989	+ CA	Breaks per cell = 0.012; Percentage aberrant cells = 1.13 %	Breaks per cell = 0.027 Percentage aberrant cells = 2.07 % [23]	2.3-fold increase **1.8-fold increase**
[a]Joksic *et al.*, 1997	+ CA + MN	CA; Control group Percentage unstable CA Before spraying = 0.067 During spraying = not stated After spraying = 0.064 Reference group CA; Control group Percentage unstable CA Before spraying = 0.05 During spraying = not stated After spraying = 0.055 MN; Control group Before spraying = 5.90 ± 3.44	CA Percentage unstable CA Before spraying = 0.13 During spraying = 0.22 After spraying = 0.79 [24, 25, 26] MN Before spraying = 5.41 ± 3.67	 1.9-fold increase - 12.3-fold increase 2.6-fold increase - 14.4-fold increase 0.9-fold increase

		During spraying = not stated After spraying = 9.63 ± 5.69	During spraying = 17.78 ± 5.68 After spraying = 39.92 ± 12.31 [27, 28, 29] (mean MN/1000 cells ± SD)	- 4.1-fold increase
		MN; reference group Before spraying = 5.09 ± 2.53 During spraying = not stated After spraying = 5.20 ± 2.01		1.1-fold increase - 7.7-fold increase
[a]Kourakis *et al.*, 1992	+ CA	Chromosome-type aberrations = 0.2 ± 0.37 chromatid-type aberrations = 0.34 ± 0.60 Total aberrations = 0.54 ± 0.90 %	Chromosome-type aberrations = 1.34 ± 1.62 chromatid-type aberrations = 0.80 ± 0.81 Total aberrations = 2.14 ± 1.62 % [30] (mean per 100 metaphases ± SD)	4.6-fold increase 6.1-fold increase **5.0-fold increase**
[a]Lander *et al.*, 2000	+ CA	Pre-season Chromatid-type aberrations-gaps = 1.34 ± 1.14 Chromosome-type aberrations-gaps = 0.28 ± 0.45 Total aberrations-gaps = 1.62 ± 1.15 %	Pre-season; Chromatid-type aberrations-gaps = 1.39 ± 1.10 Chromosome-type aberrations-gaps = 0.49 ± 0.74 Total aberrations-gaps = 1.87 ± 1.31 % [31]	1.0-fold increase 1.8-fold increase **1.2-fold increase**
			Post-season Chromatid-type aberrations-gaps = 1.99 ± 1.18 Chromosome-type aberrations-gaps = 0.39 ± 0.60 Total aberrations-gaps = 2.34 ± 1.45 %	1.4 (1.5) -fold increase 0.8 (1.4) -fold increase **1.3 (1.4)** -fold increase

			[32, 33] (Mean per 100 metaphases ± SD)	compared with pre-season (compared with controls)
[a]Lebailly *et al.*, 2003	- comet		DNA damage Morning before pesticide use = 10 % (2-21 %) Evening after pesticide use = not measured Following morning = 13 % (5-49%) [34] Tail moment Morning before pesticide use = 4.35 ± 1.11 (2.16-5.85) Evening after pesticide use = not measured Following morning = 4.80 ± 2.57 (3.18-12.76) [35] (Mean ± SD)	1.3 -fold increase (compared to before pesticide use) 1.1-fold increase (compared to before pesticide use)
[b]Mohammed *et al.*, 1995	+ CA	Breaks = 4.9 ± 1.71 %; Total CA = 4.3 ± 1.39 %	Dealer and controller group Breaks = 15.28 ± 3.1 % Total CA = 12.14 ± 3.84 % [36] Sprayer group Total CA Before = 7 % [37] During = 10.1 %[38] After exposure = 13.77 % [39] (Mean ± SD)?	 11.0-fold increase **2.8-fold increase** 1.6-fold increase 1.4 (2.3) -fold increase 2.0 (3.1) -fold increase compared with before

				spraying (compared with controls)
[a]Munnia *et al*., 1999	+ DNA adducts	DNA adducts = 2.17 x 10^9 ± 5.75 RAL	DNA adducts = 8.50 x 10^9 ± 14.95 RAL [40] (Mean ± SD)	**3.9-fold increase**
[a]Mustonen *et al*., 1986	- CA	Aberrant metaphases-gaps Non-smokers; 1.5 ± 0.73 Smokers; 1.9 ± 1.2	Aberrant metaphases-gaps Non-smokers; 1.2 ± 1.5 [41] Smokers; 1.8 ± 1.26 [42] (Mean ± SD)	 0.8-fold increase 1.0-fold increase
[b]Paldy *et al*., 1987	+ CA	Chromosome aberrations = 1.1 ± 0.36	Years of exposure Chromosome aberrations 0-5 years = 2.96 ± 0.36 [43] 6-10 years = 3.55 ± 0.75 [44] 11-15 years = 4.28 ± 0.76 [45] (Sum of aberrations per 100 cells without gaps ± SD)?	 2.7-fold increase 3.2-fold increase 3.9-fold increase
[a]Pasquini *et al*., 1996	+ MN	MN frequency = 13.30 ± 5.35 Overall MN frequency = 13.30 ± 5.35	MN frequency (>19 year) = 18.30 ± 7.22[46] Overall MN frequency = 15.98 ± 7.65 [47] (Mean x 1000 cells ± SD)	**1.2-fold increase**
[b]Paz-y-Mino *et al*., 2002	+ CA	Total structural CA = 109 Total numerical CA = 3 Total CA = 112	Total structural CA = 750 Total numerical CA = 94 Total CA = 844 [48] (Mean)	6.9-fold increase 31.3-fold increase 7.5-fold increase

[a]Peluso *et al.*, 1996	+ DNA adducts	Percentage DNA adducts = 9%	Percentage DNA adducts = 42% [49]	4.7-fold increase
		DNA adduct level (RAL x 10^{-9})	DNA adduct level (RAL x 10^{-9})	
		Negative = 91 %	Negative = 57.7 %	0.6-fold increase
		<10 = 4.5 %	<10 = 19.3 %	4.3-fold increase
		10-19.99 = 4.5 %	10-19.99 = 15.4 %	3.4-fold increase
		20-29.99 = 0	20-29.99 = 3.8 %	-
		>30 = 0	>30 = 3.8 %	-
[b]Rupa, *et al.*, 1991	+ CA	48 hr; Total CA – gaps = 1.69	48 hr; Total CA – gaps = 6.10 [50]	3.6-fold increase
		72 hr; total CA – gaps = 1.54	72 hr; total CA – gaps = 4.87 [51]	3.2-fold increase

[a] Studies from EU [b] Studies from rest of world

Mean fold increase of positive studies over controls ± SD (SE) = 3.21 ± 4.24 (0.47)

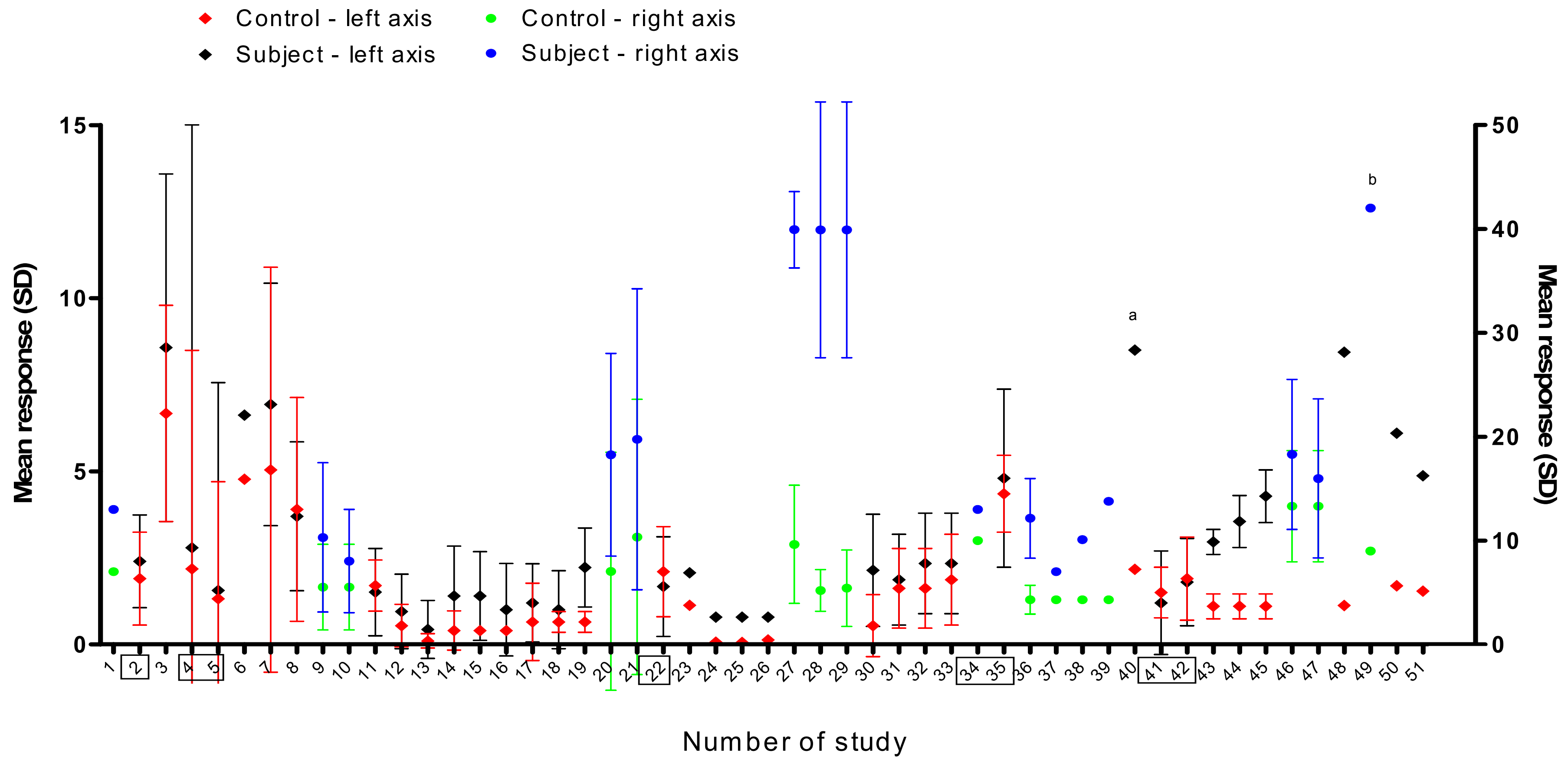

Figure 1. Graphical presentation of data from all selected studies.

For details about each study, refer to the table. Data are presented as mean ± SD. [a] mean x 10^9 [b] mean x 10^2

Studies in boxes presented negative data.

Some data sets on the graph represent data that appear to be significantly different.
Garry *et al*., 2001: Workers carrying out heavy pesticide use (> 1000 gallons)
Joksic *et al*., 1997: Workers after spraying compared to control and reference group, and workers compared to before spraying.
Mohammed *et al*., 1995: Dealer and controller groups exposed to pesticides.
Paldy *et al*., 1987: Workers exposed to pesticides for 0-5, 6-10 or 11-15 years.

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Cytogenetic changes in workers following pesticide exposure:

Selected studies from EU and world

Subjects (exposed/ controls)	End-point	Duration of exposure	Protective clothing	Results	Comments	Author
Production workers (23/23)	**CA**	**4-17 years**	**Used all protective measures available**	Positive **Significantly increased total CA and number of cells with CA in workers compared to controls.**	**100 metaphases counted per subject**	**Antonucci and de Syllos Colus, 2000**
Agricultural worker (20/20)	**CA Tandem probe FISH assay**	**Continuous pesticide use**	**None used**	Negative **No significant differences in CA between exposed workers and controls**	**Samples transported by air. 150 cells scored per subject.**	**Au, *et al.*, 1999**

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Italian floriculturist (71/75)	**MN**	**1 - >30**	**Not stated**	Positive. **Significantly higher MN in exposed subjects and in females.** **MN frequency increased with age and duration of employment and with smoking. Significantly higher MN frequency in greenhouse workers or those working both inside and outside.**	**Subjects worked in open fields (39.4%) both in fields and greenhouses (55%) or greenhouses (5.6%).**	**Bolognesi, *et al.*, 1993b**
Italian floriculturist (51/24)	**MN**	**26.35 ± 14.46 (range 2-10)**	**Most (86%) wore protection**	Negative **Non-significant increase in MN in subjects exposed to pesticides, in those not using protective clothing, in females. Non-significant relationship with age and duration of exposure. Non-significant decrease in MN frequency in smoking workers.**	**Subjects (80%) worked mainly in greenhouses.**	**Bolognesi, *et al.*, 2004**
Spanish agricultural worker (70/69)	**CA and SCE**	**<5 - >29**	**Not stated.**	Negative SCE **No significant difference in SCE frequency in exposed workers and no dose-relationship. Significant increase in SCE in smokers, related to time smoked.** Positive CA **Significant increase in CA frequency in total workers. Horticulturists, but not floriculturists had significant higher CA. Frequency of CA related to duration of exposure but not age.**	**Groups differed in terms of alcohol and coffee consumption and smoking. Subjects worked in open fields for more time than greenhouses. Blood samples were collected during a high exposure period.**	**Carbonell, *et al.*, 1993**

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Spanish agricultural worker (29/53)	**CA**	**> 10 years**	**3% wore protection. 59% wore masks incorrectly.**	Positive. **Significant increase in CA during high exposure period to pesticides. During low exposure period, CA not significantly different to controls.**	**Exposed subjects worked in open fields more than greenhouses. Blood was collected during a high and low exposure period.**	**Carbonell, *et al.*, 1995**
Italian floriculturist (32/32)	**CA and SCE**	**Not stated**	**Not stated**	Positive CA **Significantly increased total CA in exposed workers, but non-significant increase in chromatid-type aberrations.** Positive SCE **SCE frequency significantly increased in exposed workers. Smoking significantly increased SCE frequency**	**Little demographic data on the groups. Three groups were studied; healthy workers and bladder cancer patients, both exposed to pesticides and the control group. The groups were different in terms of age, sex, smoking.**	**De Ferrari, *et al.*, 1991**
Floriculturist (36/15)	**CA**	**> 10 years**	**Little or no protection used**	Positive **Significantly higher number of exhange-type aberrations in expoased workers compared to controls.** **No difference in CA between workers with or without chronic intoxication.**	**Samples collected during spring/summer.**	**Dulout, *et al.*, 1985**
Pesticide sprayers (23/18/20/33)	**Not stated**	**Not stated**	**Not stated**	Positive **Significantly increased chromosome rearrangements in fumigant applicators and insecticide appliers but not herbicide appliers compared to controls. No significant difference in the number of breaks occurring at fragile sites between all groups and controls.**	**Workers groups; used fumigants; insecticides; herbicides; controls. Blood samples were collected from intoxicated subjects two weeks after healthy subjects. 100 cells scored**	**Garry, *et al.*, 1996**

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					per subject.	
Forester (25/15)	**CA**	**> 5 days/year**	**Not stated**	Positive **Significantly higher chromosome deletions, gaps and breaks in workers spraying > 1000 gallons of herbicides compared to those spraying smaller volumes or controls. No correlation between urinary 2,4-D concentrations and CAs.**	**Pesticides applied by back pack, boom spray, aerial or skidder. Samples collected within 24 hours of end of peak 2,4-D application season. 2,4-D measured in urine.**	Garry, *et al.*, 2001
Production workers (54/54)	**Comet assay**	**8.57 years (range 3-13 years)**	**Not reported**	Positive **Significantly greater tail length in exposed workers compared to controls. Slight increase seen correlated with duration of exposure.**	**Samples collected between October and December. 50 cells scored per subject.**	Grover, *et al.*, 2003
Agricultural worker (30/	**CA**	**> 5 years**	**Not stated**	Negative **No significant differences in chromatid breaks or total chromatid breasks between workers and controls.**	**100 cells counted**	Hoyos, *et al.*, 1996

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Czech production workers (44/30)	**CA and SCE**	**< 2 years**	**Not stated**	Positive CA **Both males and females had a significantly higher percentage of CA** Positive SCE **Only male workers had a significantly higher SCE frequency.**	**Workers were exposed to Mancozeb.**	**Jablonicka, *et al.*, 1989**
Yugoslavian pesticide sprayer (27/15/20)	**CA, SCE and MN**	**12.1 ± 6.02**	**Not stated**	Positive CA **Significant higher CA in workers compared to controls and references in prespraying period. CA in exposed workers increased at 3rd sampling time.** Positive MN **No significant difference in MN frequency between workers, controls and references. Significant increase in MN in exposed workers at 2nd and 3rd sampling time.** Negative SCE **No significant variation in SCE frequency in exposed workers.**	**Three groups used; exposed workers; controls and references. All subjects were non-smokers. Air samples taken showed the presence of diazinon. Blood was collected during the pre-spraying period, a month after spraying and at the end of the spraying season.**	**Joksic, *et al.*, 1997**
Greek pesticide sprayer (29/14)	**CA**	**4**	**None of the workers used protective clothing.**	Positive **Increase in CA in exposed workers. No correlation between CA and duration of exposure.**	**Blood samples during autumn, after spraying from September to November.**	**Kourakis, *et al.*, 1992**

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Finnish greenhouse worker (116/29)	**CA**	**Not stated**	**Gloves worn by some workers.**	Positive. **CA significantly higher in workers after spraying compared to before spraying. Non-significant increase in CA in non-smoking workers, pre-seasonally and a significant increase post-seasonally, compared to controls. Increased RR for workers not wearing gloves.**	**Blood samples collected before and after the spraying season.**	**Lander, *et al.*, 2000**
French fruit farmer (19)	**Comet assay**	**Not stated**	**14% wore no protection, 14% wore masks/protective clothes, 17% wore only gloves, 41% wore all protection.**	Negative. **Overall, no significant differences before and after spraying. Five out of twelve had a significantly increase in DNA damage.**	**Mean predictive dose of Captan calculated as 14.8 mg and 14.0 mg for each season.**	**Lebailly, *et al.*, 2003**
Pesticide sprayers (9/7/6)	**CA**	**3 years**	**Not stated**	Positive **Significant increase in breaks and total CAs in exposed workers compared to controls at beginning, middle and end of spraying season. Significant increase in breaks and total CAs in dealer/controllers compared to controls.**	**Samples collected between April and October. 100 cells scored per subject. Groups of exposed workers, dealer/controllers and controls**	Mohammad, *et al.*, 1995
Italian greenhouse workers (57/33)	**^{32}P DNA post-labelling**	**Not stated**	**Not stated**	Positive. **Six DNA adducts observed in some workers. Significant increased levels of DNA adducts in workers.**	**All subjects were non-smokers. Little demographic data presented.**	**Munnia, *et al.*, 1999**

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Finnish forestry workers (19/15)	**CA**	**6-28 days**	**Not stated**	Negative. **No significant increase in CA in exposed workers.**	**Blood collected after spraying season. Low 2,4-D and MCPA concentrations measured in breathing zone of workers**	**Mustonen, *et al.*, 1986**
Hungarian pesticide sprayer (80/24)	**CA**	**1- >15**	**Not stated.**	Positive. **Significant increase in CA in exposed workers. Age not associated with CA induction.**	**Blood samples collected before and after pesticide application.**	**Paldy, *et al.*, 1987**
Italian farmer (48/50)	**SCE and MN**	**18.35 ± 12.42 (range 3-50)**	**Few workers (29%) wore protective clothing.**	Negative SCE **No difference in SCE frequency following pesticide exposure. SCE frequency not affected by age or duration of exposure. Smoking significantly increased SCE frequency in workers and controls.** Positive MN **Significant increase in MN frequency in workers. MN frequency increased in smokers in both groups. MN not affected by age, but significant associated with duration of exposure.**	**Blood was collected during pesticide application periods during two seasons (1992 and 1995).**	**Pasquini, *et al.*, 1996**
Floriculturist (41/41)	**CA**	**39.49 ± 20.25 (range 6-66 months)**	**Not stated**	Positive **Significant increase in CAs in exposed group compared to controls. No correlation between CA and duration of exposure.**	**100 cells scored per subject.**	**Paz-y-Mino, *et al.*, 2002**

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Italian greenhouse floriculturist (26/22)	**^{32}P DNA post-labelling**	**22 ± 12**	**Not stated**	Positive**.** **DNA adduct rate higher in exposed workers and females. No significant associations with age or gender.**	**All subjects were non-smokers. Little demographic data presented**	**Peluso, *et al.*, 1996**
Pesticide applicators (26/26)	**Not stated**	**8 hr/day in spring and winter**	**Not stated**	Positive **Total CA significantly increased in workers compared to controls.**	**Samples collected in January.**	**Rupa, *et al.*, 1991**