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Annex IV: Statistical analysis used to detect cytogenetic changes in workers following pesticide exposure in EU

Author	Subjects	Demographic differences between groups	Information collected	Type of study	End-point	Outliers identified	One or two sided analysis	Statistical analysis
Jablonicka, <i>et al.</i> , 1989	44 production workers and 30 controls, employed in the same plant with no exposure to pesticides, selected due to having similar habits and age.	No data given.		Cross sectional study.	CA and SCE	NA	NS	Student's t-test to identify differences between the exposed and control groups, using the test of significance of the differences in 2 relative values, by inverse trigonometric functions.

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Joksic, <i>et al.</i> , 1997	27 male vine growers; 15 controls - male school teachers from same area; 20 controls - volunteers outside agricultural area. All subjects selected due to non-smoking status. Controls matched according to sex, age and smoking habit.	No data given.	Diseases, drugs taken, exposure to ionising, radiation or compounds with genotoxic effects.	Longitudinal study	CA, SCE and MN freq.	NA	NS	Wilcoxon rank-sum test to investigate differences between mean values of CA and MN. Analysis of variance (F test) to evaluate SCE data.
Lander and Ronne, 1995	118 male and 16 female sprayers; 137 male and 20 female controls, randomly selected from bank and clerical workers.	No significant differences between the two groups.	All subjects: Age, smoking, alcohol. exposed: application of anti-cholinesterase agents and pesticides, protective clothing.	Cross-sectional study	SCE freq.	NA	NS	Stepwise multiple regression analysis, ANOVA and Student's t-test for independent samples used to analyse data.
Paldy, <i>et al.</i> , 1987	80 male chemical plant protection mixers and sprayers 24 male controls	No data given.	Smoking, alcohol, drugs.	Cross-sectional study	CA freq.	NA	NS	Fischer test and the χ^2 test to evaluate significance.
Kourakis, <i>et al.</i> , 1992	29 sprayers; 14 controls living in the same area.	No data given.	Age, smoking, duration of exposure	Cross-sectional study	CA freq.	NA	NS	χ^2 test to assess significance

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Kourakis, <i>et al.</i> , 1996	29 sprayers in greenhouses; 27 sprayers in open fields; 30 age and sex matched controls living in same area.	No data given.	Smoking, alcohol, drug consumption, X-rays.	Cross-sectional study	CA and SCE freq.	NA	NS	χ^2 test to assess significance
Undeger and Basaran, 2002	33 male applicators; 33 male controls selected according to age, sex, socio-economic status and smoking.	No data given.	All subjects: Age, occupational history, health, smoking, dietary habits, exposure to drugs and chemicals. Workers: Duration of exposure, protective clothing.	Cross-sectional study	Comet assay	NA	NS	Chi square test to compare exposed and controls groups. Linear regression analysis to evaluate duration of exposure on DNA damage.

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Bolognesi, <i>et al.</i> , 2004	51 floriculturists; 24 controls, selected from blood donors living in the same area.	No significant differences between the two groups.	All subjects: Personal data, Smoking habit, Medical history and treatment Workers: Kind of crops, Pesticide use, Duration of exposure, Working activity, Protective clothing.	Cross-sectional study	MN freq.	NA	NS	Student's t-test to detect differences in BNMN between exposed workers and controls. Mann-Whitney U-test for C+MN and C-MN data. Linear regression to test correlation between age and C+MN.
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Bolognesi, <i>et al.</i> , 2002	107 floriculturists; 61 controls selected from blood donors living in the same area.	Workers had more males (85%) compared to controls (68%). Workers had fewer smokers (21.5%) than controls (32.8%) but more non-smokers (44.8% vs 36.1%).	All subjects: Personal data, Smoking habits, Medical history and treatment. Workers: Kind of crops, pesticide use, duration of exposure, cultivation conditions, protective clothing.	Cross-sectional study	MN freq.	NA	NS	Student's t-test to detect differences between workers and controls. Mann-Whitney U-test to evaluate differences between group means. Regression analysis to assess relationship between BNMN and protective clothing.
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Bolognesi, <i>et al.</i> , 1993a	71 floriculturists; 75 controls living in the same area. Floriculturists were randomly selected from applications for authorisation for pesticide use	No significant differences between the two groups.	Smoking habits, Medical history, Pesticide use.	Cross-sectional study	MN freq.	No outliers reported by author. Two outliers were found in exposed workers using Grubbs test	NS	Data underwent square-root transformation. Cochran-Armitage test for trend on medians used to test for a dose-relationship. Poisson regression analysis to test the effect of pesticide exposure. Model adjusted for age, sex, smoking and duration of exposure. The regression coefficient to estimate the rate ratio for exposed workers.
Bolognesi, <i>et al.</i> , 1993b	56 male and 15 female floriculturists; 75 controls living in the same area, selected from blood donor registry.	No significant differences between the two groups.	Working environment, pesticide use, smoking habits, medical history and treatment.	Cross-sectional study	MN freq.	NA	NS	Data underwent square root transformation. Mean values of exposed workers and controls compared. Poisson regression analysis to estimate relationship between MN frequency and pesticide exposure. Model adjusted for age, gender and smoking.

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Bolognesi, <i>et al.</i> , 1993c	56 male and 15 female floriculturists and 75 controls selected from blood donor registry.	No significant differences between the two groups.	Working environment, pesticide use, smoking habit, medical history.	Cross-sectional study	MN freq.	NA	NS	Data underwent square root transformation and mean values of exposed and controls compared. Multivariate Poisson regression analysis to estimate the relationship between MN frequency and pesticide exposure. Model adjusted for age, sex, smoking.
De Ferrari, <i>et al.</i> , 1991	32 floriculturists; 32 exposed subjects with bladder cancer (not receiving radio- or chemotherapy); 31 controls living in the same area.	Distribution of subjects within the groups by age, sex and smoking habits not homogeneous (data not given)	Working activity, Type and duration of exposure to pesticides, medical history, X-rays, drugs, caffeine, alcohol, smoking.	Cross-sectional study	CA and SCE freq.	NA	NS	Mann-Whitney U test for differences between groups.
Scarpato, <i>et al.</i> , 1996	43 floriculturists; 42 matched controls working as bank clerks in the same area. Subjects selected and matched according to sex, age and smoking habit.	No significant differences between the two groups.	Sex, Age, Smoking, X-rays, Other chemicals, Viral infections, Drug use.	Cross-sectional study	CA, SCE and MN freq.	NA	NS	Multiple linear regression for effect of sex, smoking and pesticide exposure on SCE and MN. Data were log-transformed. Poisson regression for CA data. Rate ratio calculated by exponentiation of the coefficient of each variable, after adjusting for other variables.

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Falck, <i>et al.</i> , 1999	20 male and 14 female floriculturists; 17 male and 16 female controls working as bank clerk in the same area.	No significant differences between the two groups.	Drug use, smoking X-ray exposure, viral infections, vaccinations, diet, alcohol consumption.	Cross-sectional study	CA, SCE and MN freq.	NA	NS	Multiple linear regression to assess effect of pesticide exposure. Model adjusted for age, sex, smoking, duration of exposure, chemical exposure and genotype. Data log transformed. RR calculated by exponentiation of the regression coefficient of each variable after adjusting for other factors.
Lander, <i>et al.</i> , 2000	116 male greenhouse workers; 29 male controls, members of an organic association in the same area.	Controls were significantly older (38) than the workers (28) All controls were non-smokers whereas 38% of workers were smokers.	Medical history, occupational history, life style history.	Longitudinal study	CA freq.	NA	NS	Poisson distribution for numbers of cells with CA. Multiple log-linear Poisson to control for spraying season, protective clothing, time of sampling, use of gloves, smoking, age and caffeine intake.

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Peluso, <i>et al.</i> , 1996	19 male and 7 female floriculturists; 13 male and 9 females controls from the same area. All subjects selected due to being non-smokers.	No data given	All subjects: Recent illness, hospitalisation, X-rays, occupational exposure to solvents, dyes, asbestos, ionising radiation and pesticides Workers: Pesticides sold in area, pesticide use, type of cultivation, pesticide handling, protective clothing.	Cross-sectional study	³² P DNA post-labelling	NA	Two sided	Multiple logistic regression analysis to test effect of pesticide exposure on DNA adducts, adjusting for age and gender. Mann-Whitney rank sum test to compare level of DNA adducts in exposed and control groups.
Munnia, <i>et al.</i> , 1999	40 male and 17 female greenhouse workers; 22 male and 11 female age-matched controls from same area. Non-smoking subjects used.	No data given on two groups.	Illness, exposure to solvents, tar, products, dyes, hospitalisation, occupational use of pesticides 12 months prior to study, cultivation.	Cross-sectional study	³² P DNA post-labelling	NA	NS	ANOVA using log-transformed data.

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Piperakis, <i>et al.</i> , 2003	30 male and 20 female greenhouse workers; 41 male and 25 female controls working in clerical jobs in the same area. All subjects selected due to being non- or ex-smokers	Controls consumed significantly more wine (4.04 ± 0.77 glasses/week) and spirits (2.42 ± 0.77) than workers (2.00 ± 0.41 and 0.98 ± 0.43, respectively).	All subjects: Age, smoking, diet alcohol, medical history, Workers: Pesticide application, work activity, protective clothing.	Cross-sectional study	Comet assay	NA	NS	Kruskal-Wallis test and multivariate analysis of variance (GLM-repeated measures) to estimate differences in the distribution of DNA damage Mann-Whitney U-test to identify differences between groups, with regard to mean value of confounding factors.
Pastor, <i>et al.</i> , 2002b	39 agricultural workers; 22 male controls working as clerks in the same area.	Controls (37.55 ± 2.18) were significantly older than workers (31.00 ± 1.37). Controls (1.79 ± 0.32 cups per day) consumed significantly more coffee than workers (0.69 ± 0.15).	All subjects: Age, smoking. Workers: Duration of exposure	Longitudinal study	MN freq.	NA	NS	Mann-Whitney U-test to test differences between exposed and control groups regarding age, smoking, coffee and alcohol consumption. ANCOVA of repeated measures to detect differences in BNMN and CBPI over time, using log-transformed BNMN data. ANCOVA to evaluate effect of age, coffee and alcohol.

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Pastor, <i>et al.</i> , 2002a	58 male and 26 female agricultural workers; 53 male and 12 female controls, 2/3 from the same area, 1/3 from capital city.	Controls (45.05 ± 0.96) were significantly older than workers (41.98 ± 0.73). Exposed groups had significantly more smokers and smoked more cigarettes (35.7 %, 18.67 ± 1.87 cigarettes) than controls (20 %, 14.15 ± 1.88). Workers consumed significantly more coffee (2.55 ± 0.24) than controls (1.86 ± 0.18).	Age, gender, smoking diet, medication.	Cross-sectional study	MN freq.	NA	Two-sided	Student's t-test to detect differences between groups, adjusted for age, smoking and alcohol. Multiple linear regression to evaluate BNMN, MNL and CBPI, including variables exposure, sex, age and no. cigarettes. MNL and BNMN data were square root transformed. Multiple linear regression with different variable selections (forward and backward) to compare the exposed and control groups. All significant variables were subjected to a multiple regression analysis. Models were checked for adequacy of fit by analysis of residuals, tolerance limits and homogeneity of variances. A general model compared differences between exposed workers, adjusted for variables age, work, alcohol consumption and exposure (moderate or high exposure). Poisson regression followed by a binomial regression analysis (backward selection method) was used to analyse BCMN and MNBC data.
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Pastor, <i>et al.</i> , 2003	247 agricultural workers; 231 controls; Greece: 50 exposed and 66 controls, all subjects were non-smokers; Spain: 63 exposed and 51 male controls; Poland: 50 exposed and 49 male controls; Hungary: 84 exposed and 65 controls.	Controls were significantly older (41.91 ± 0.64) than workers (39.34 ± 0.63). Spanish workers were youngest. Mediterranean workers were exposed for shorter durations than middle European workers. Greek farmers drank significantly more, and Hungarian farmers drank significantly less alcohol, males drank significantly more alcohol than females. Significantly more males smoked than females. Middle Europeans ate significantly more red meat and less fish.	All subjects: Age, gender, smoking, alcohol consumption, vaccinations, medication, X-rays, diet. Workers: occupational activity, duration of exposure, protective clothing used, pesticides used crops grown.	Cross-sectional study	MN freq.	NA	Two sided	Student's t-test, ANOVA and χ^2 test to compare means, for demographic factors, diet and smoking between populations, pesticide exposure and sex. A generalised linear model followed by a post hoc Tukey's test for BNMN and CBPI data. Model adjusted for age, sex, exposure, country, diet and smoking. BMNM data were square root transformed. A negative binomial regression for the BCMN data, using the backward selection method. A type III sum of squares method used
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Pastor, <i>et al.</i> , 2002b	39 agricultural workers; 22 male controls working as clerks in the same area.	Controls (37.55 ± 2.18) were significantly older than workers (31.00 ± 1.37). Controls (1.79 ± 0.32 cups per day) consumed significantly more coffee than workers (0.69 ± 0.15).	All subjects: Age, smoking. Workers: Duration of exposure	Longitudinal study	MN freq.	NA	NS	Mann-Whitney U-test to test differences between exposed and control groups regarding age, smoking, coffee and alcohol consumption. ANCOVA of repeated measures to detect differences in BNMN and CBPI over time, using log-transformed BNMN data. ANCOVA to evaluate effect of age, coffee and alcohol.
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Pastor, <i>et al.</i> , 2001a	49 male agricultural workers; 50 controls from the same area.	No significant differences between the two groups.	All subjects: Diet, drugs, exercise, smoking, alcohol, occupational history, medical history, family history. Workers: Working activities, pesticides used, protective clothing.	Cross-sectional study	MN freq.	NA	NS	Multiple linear regression to test the association between all predictor continuous and categorised variables and cytogenic endpoints (BNMN, MNL, CBPI) using variable selection stepwise, backward and forward with the 'pairwise' option for missing data. Multiple regression analysis to further test any significant variables. Data for MNL and BNMN were square root transformed. Analyses of residuals, tolerance limits and homogeneity of variances were checked to evaluate the adequacy of fit for each multiple regression model. A negative binomial regression by the backward method to test MNBC and BCMN between subjects. Student's t-test to detect differences between groups with regard to mean values of confounding factors such as age, smoking and alcohol.
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Pastor, <i>et al.</i> , 2001b	30 male and 20 female agricultural workers; 41 male and 25 female controls, working as clerical workers in the same village.	Controls consumed more wine (4.64 ± 0.77) than workers (2.00 ± 0.41).	All subjects: Age, gender, health, genetic disorders, vaccinations, medication, X-rays, smoking, diet, coffee, alcohol. Workers: Duration of employment, crops farmed, pesticide application, protective clothing used.	Cross-sectional study	MN freq.	NA	Two-sided	Mann-Whitney U-test to detect differences between the exposed and control groups with regard to mean value of confounding factors. Continuous and dichotomised variables were taken into account for the models for main variable adjustment, and interactions between the most important variables studied. Multiple linear regression analysis to test the BNMN, MNL and CBPI in lymphocytes. MNL data underwent square-root transformation. Box-Cox transformation was used for BNMN. Different methods for variable selection (stepwise, forward, backward) used. Final multiple regression analysis for all relevant variables. Each model was checked for adequacy of fit by the analysis of residuals, tolerance limits and homogeneity of variances. Poisson regression to initially study BCMN and MNBC, followed by a binomial regression analysis.
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Carbonell, <i>et al.</i> , 1990	27 agricultural workers; 28 controls selected due to age, sex and smoking habits and living in the same area.	No data given.	Personal history, habits and pesticide exposure.	Cross-sectional study	SCE freq.	No outliers reported by author. Two outliers in exposed workers were identified by Dixon's test.	NS	Student's t-test to compare SCE data in the exposed and control groups.
Carbonell, <i>et al.</i> , 1995	29 male agricultural workers; 2 matched control groups according to age and habits; 1 st , 29 males; 2 nd , 24 males.	No significant differences between the two groups.	All subjects: Smoking, alcohol, diet, drugs and medication, health status and medical history, exposure to genotoxic agents. Workers: Working activity, duration of exposure, pesticides used, protective clothing.	Longitudinal study	CA freq.	NA	NS	Mann-Whitney U-test to compare exposed workers and controls. Wilcoxon matched pairs test to compare data before and after the high exposure.

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Carbonell, <i>et al.</i> , 1993	70 male agricultural workers divided into floriculturists, horticulturists and combined workers. 69 males matched controls, according to age, smoking and drinking habits, living in the same area.	Horticulturists were significantly older than controls (43.3 ± 11.5 vs 35.3 ± 9.9). Workers consumed significantly more alcohol (187.3 ± 204.3 mg/day) compared to controls (77.8 ± 107.9). Workers consumed significantly less coffee (1.1 ± 1.9 cups/day) compared to controls (1.9 ± 1.8). Horticulturists (11.1 ± 11.4 years) smoked for significantly longer than controls (6.1 ± 8.6 years) but floriculturists smoked for significantly less time (1.3 ± 2.9 years).	All subjects: Smoking, alcohol, diet, drugs and medication, health status and medical history, exposure to genotoxic agents. Workers: Working activity, duration of exposure. pesticides used Protective clothing	Cross-sectional study	CA and SCE freq.	NA	NS	Student's t-test to compare SCE data in the exposed and control groups. Mann-Whitney U-test to compare CA data in the two groups.
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Nehez, <i>et al.</i> , 1988	55 male agricultural workers; 60 male controls selected from the blood donor registry.	No significant differences between the two groups.	Personal history, family history, occupation, social background, personal habits.	Cross-sectional study	CA freq.	NA	NS	Fisher probe
Pasquini, <i>et al.</i> , 1996	48 male farmers; 50 males controls living in the same area.	No significant differences between the two groups.	Personal data, working activity, X-rays, drug use, smoking, caffeine alcohol.	Cross-sectional study	SCE and MN freq.	No outliers reported by author. No outliers were identified using Grubb's or Dixon's test.	NS	Man-Whitney U-test to compare exposed group and controls. Variance analysis to compare mean values of SCE, PRI and MN in exposed and control groups. Two-way ANOVA fitted, including variable factors, age as covariate and each variable of biological effects as a response variable.

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Lebailly, <i>et al.</i> , 1998a	29 male farmers subdivided into groups: 8 farmers working in summer; 11 farmers working in January; 14 farmers working in April; 8 farmers working in June. One male control.	Farmers working in January sprayed pesticides for approximately 100 hours compared to 30 and 50 hours for those working in April and June, respectively. Little demographic data given.	Diet, smoking, alcohol consumption, drugs taken, healthy status and medical history, areas sprayed and date of last pesticide use prior to the study.	Longitudinal study	Comet assay	No outliers reported by author. No outliers were identified using Grubb's or Dixon's test.	NS	ANOVA to compare DNA damage between the groups. The influence of confounding factors was estimated by second ANOVA, limited to one sample per farmer. Selection criteria: if the farmer was included in group 1 (worked in summer) and another group, the sample from the other group was used; if the farmer was included in group 2 (worked in January) and in another group, the sample from group 2 was used.
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Lebailly, <i>et al.</i> , 1998b	29 male farmers subdivided into groups: 8 farmers-chlorothalonil; 11 farmers-isoproturon + herbicides; 14 farmers-triazoles +/-herbicides; 8 farmers-chlorothalonil + insecticides.	Little demographic data given.	Diet, smoking, alcohol consumption, drugs taken, healthy status and medical history, areas sprayed and date of last pesticide use prior to the study.	Longitudinal study	Comet assay	No outliers reported by author. Five outliers were identified in workers by Grubb's test.	NS	A paired sample test was used as each farmer acted as his own control. The Student's t-test to identify differences in DNA damage before (S1) and after (S0) pesticide spraying. The Wilcoxon test to compare changes in tail moment distributions before and after exposure and after excluding highly damaged cells.
Lebailly, <i>et al.</i> , 2003	19 fruit growers	Only one group.	Age, health status medical history, diet, smoking, alcohol, pesticides used in crop treatment, chemicals used for cattle care, welding, mechanical repairs, pesticide exposure, dose, sprayed areas, crop sprayer used, protective clothing, commercial formulations used.	Longitudinal study	Comet assay and Ames test	NA	NS	Student's t-test after exclusion of highly damaged cells. Wilcoxon test to compare tail moments before and after exposure.
Mustonen, <i>et al.</i> , 1986	19 male pesticide sprayers; 15 male	Little demographic data	Smoking, exposure period.	Cross-sectional	CA freq.	No outliers reported by	NS	Fischer's exact probability test.

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	controls selected according to sex, age and smoking habit.	given, although groups were matched.		study		author. No outliers were identified using Grubb's or Dixon's test.		
Linnainmaa, 1983	50 pesticide sprayers; 15 unexposed controls.	Little demographic data given.	Occupational history, health status, viral infections, vaccinations, smoking, alcohol consumption.	Longitudinal study	SCE freq.	No outliers reported by author. Two outliers were observed in non-smoking workers before (Grubb's) and after (Dixon's test). One outlier was identified by Dixon's test in smoking workers.	NS	Cochrane's approximate t-test to compare the means of SCE frequencies.

Outliers were calculated from individual data, by using Grubb's test and Dixon's test.

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Abbreviations

ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BNMN	Binucleated cells with micronuclei
BCMNI	Mononucleated buccal cells with micronuclei
C+MN	Centromere positive micronuclei
C-MN	Centromere negative micronuclei
CA	Chromosome aberrations
CBPI	Cytokinesis block proliferation index
MNBC	Total number of micronuclei in buccal cells
MNL	Total number of micronuclei in lymphocytes
NA	Not applicable as individual data were not presented in the report
NS	Not stated
PRI	Proliferative rate index
RR	Rate ratio
SCE	Sister chromatid exchange

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