

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

MUT/05/11

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

BIOLOGICAL MONITORING STUDIES OF GENOTOXICITY IN PESTICIDE APPLICATORS

EPIDEMIOLOGICAL OVERVIEW

1. The attached paper has been prepared by Dr Rushton.

**Secretariat
May 2005**

This is a draft paper for discussion. It should not be quoted, cited or reproduced.



**Institute for Environment
and Health**

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

The Institute for Environment and Health was established by the Medical Research Council at the University of Leicester in 1993. The Institute is principally funded by UK Government Departments and Agencies by way of specific research and consultancy contracts.

The views expressed here do not necessarily represent those of any Government Department or Agency

This document is a report by the Institute for Environment and Health for the Department of Health and has not been prepared for general circulation.

Written by Dr Lesley Rushton

©Institute for Environment and Health, 2005

MRC Institute for Environment and Health
University of Leicester
94 Regent Road
Leicester
LE1 7DD
UK

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Contents

Contents.....	4
1. Introduction.....	5
2. Review of individual studies.....	5
Bolognesi <i>et al.</i> 1993. Frequency of micronuclei in lymphocytes from a group of floriculturists exposed to pesticides.....	5
Bolognesi <i>et al.</i> 2004 Cytogenetic biomonitoring of a floriculturist population in Itlay: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe.....	6
Carbonell <i>et al.</i> Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides.....	6
Ferrari <i>et al.</i> Cytogenetic biomonitoring of an Italian population exposed to pesticides: Chromosome aberration and sister-chromatid exchange analysis in peripheral blood lymphocytes.....	7
Dulout <i>et al</i> 1985 Sister-chromatid exchanges and chromosomal aberrations in a population exposed to pesticides.....	7
Falck <i>et al</i> 1999 Micronuclei in blood lymphocytes and genetic polymorphism for GSTM1, GSTT1 and NAT2 in pesticide-exposed greenhouse workers.....	8
Garry <i>et al</i> 1996 Pesticide applicers with Mixed Pesticide Exposure: G-banded Analysis and Possible Relationship to Non-Hodgkin's Lymphoma.....	9
Garry <i>et al</i> 2001 Biomarker Correlations of Urinary 2,4-D Levels in Foresters: Genomic Instability and Endocrine Disruption.....	9
Summary.....	10
Gomez-Arroyo <i>et al</i> 2000 Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides.....	10
Grover <i>et al</i> 2003 Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay.....	11
Hogstedt <i>et al</i> 1980 Cytogenetic study of pesticides in agricultural work.....	11
Kourakis <i>et al</i> 1992 Frequencies of chromosomal aberrations in pesticide sprayers working in plastic green houses.....	11
Lander <i>et al</i> 2000 Chromosome aberrations in pesticide-exposed greenhouse workers.....	12
Lebailly <i>et al</i> 1998 DNA Damage in Mononuclear Leukocytes of Farmers Measured Using the Alkaline Comet Assay: Discussion of Critical Parameters and Evaluation of Seasonal Variations in Relation to Pesticide Exposure.....	12
Lebailly <i>et al</i> 1998 DNA Damage in Mononuclear Leukocytes of Farmers Measured Using the Alkaline Comet Assay: Modifications of DNA Damage Levels after a One-Day Field Spraying Period with Selected Pesticides.....	13
Lebailly <i>et al</i> 2003 Urine mutagenicity and lymphocyte DNA damage in fruit growers occupationally exposed to the fungicide captan.....	14
Munnia <i>et al</i> 1999 Exposure to Agrochemicals and DNA Adducts in Western Liguria, Italy.....	14
Mustonen <i>et al</i> 1986 Effects of phenoxyacetic acids on the induction of chromosome aberrations <i>in vitro</i> and <i>in vivo</i>	15
Paldy <i>et al</i> 1987 Cytogenetic studies on rural populations exposed to pesticides.....	15
Pasquini <i>et al</i> 1996 Cytogenetic Biomonitoring of Pesticide-Exposed Farmers in Central Italy.....	16
Pastor <i>et al</i> 2001 Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells.....	16
Pastor <i>et al</i> 2002 Occupational Exposure to Pesticides and Cytogenetic Damage: Results of a Hungarian Population Study Using the Micronucleus Assay in Lymphocytes and Buccal Cells.....	17
Peluso <i>et al</i> 1996 P-Postlabeling Detection of DNA Adducts in Peripheral White Blood Cells of Greenhouse Floriculturists from Western Liguria, Italy.....	17
Piperakis <i>et al</i> 2003 Biomonitoring with the Comet Assay of Greek Greenhouse Workers Exposed to Pesticides.....	18
3. Overall summary.....	19

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

1. Introduction

The Committee on Mutagenicity (COM) are currently reviewing the literature reporting studies of mutagenicity and DNA damage in workers exposed to a range of pesticides. From the large body of literature 24 studies were selected for review regarding issues of epidemiological design and analysis. This report summarises and critiques these aspects for each of the 24 studies. An overall summary is then given.

2. Review of individual studies

Bolognesi *et. al.* 1993. Frequency of micronuclei in lymphocytes from a group of floriculturists exposed to pesticides

This study is a cross-sectional study comparing MN levels of 67 floriculturists (56, male, 15 female) with those of a control group of 75 blood donors in Italy. There is no indication in the paper as to how the exposed group and controls were selected and recruited and whether there are potential issues of non-response and selection bias. The sample size is small and there is no assessment of the adequacy of the sample size. It is not clear whether the results showing that exposed subjects had significantly higher MN levels than controls are adjusted for smoking (or other confounders), although the paper states that a slightly higher proportion of those exposed were smokers (figures not given). Duration of exposure is analysed categorically but the categories used are 'non-standard', being 0, 1-18, 19-30, >30 years. The results show a significant dose-response using these categories but this could be just an artefact of the categorisation cut offs and could have been chosen because they give significant results. Many of the results are presented narratively and the figures and risk estimates on which these statements are made are not given. For example, the paper states that Poisson regression confirmed the role played by exposure to pesticides, gender and age in excess MN but the relative risks are not given for the last two, nor for smoking. Mean frequencies and standard errors of MN are given but not the distributions so it is not possible to judge whether these are skewed or normal and thus whether the statistical tests used are appropriate. No exposure data were collected other than work in greenhouses and/or open field but the sample size would be inadequate to allow for exploration of the influence of different pesticides or groups of pesticides.

Summary

Design: Inadequate reporting of sampling strategy. No individual exposure assessment.

Analysis: No description of MN distribution; unclear whether results adjusted for confounders; categorisation of variables doubtful; lack of presentation of actual results.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Bolognesi *et. al.* 2004 Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe

This is a similar study to Bolognesi 1993. A small cross sectional study of 51 floriculturalists doing mainly work in greenhouses (20% also worked in open fields) were compared with 24 controls from health blood donors. Most of the limitations of Bolognesi 1993 apply to this paper, i.e. no details on subject selection and recruitment, and no sample size considerations. A major problem for this paper is that many means and standard deviations are given (once again no distributions are described) with a complete lack of any results of statistical tests, which are merely described in the text. Not only are there no probability values, but no confidence intervals are given either. However, the authors must have carried out hundreds of paired comparisons and some significant results would therefore have occurred by chance. The pattern of results is unclear in this paper, particularly the differences between the exposed and control groups. In particular the pattern of results for the controls appears more variable. No adjustment appears to have been carried out for differences in the distribution of confounding factors. Once again choice of cut off point for categorisation of variables seems rather arbitrary.

Summary

Design: Inadequate reporting of sampling strategy. No individual exposure assessment.

Analysis: No description of MN distribution; unclear whether results adjusted for confounders; categorisation of variables doubtful; lack of presentation of actual results. Complete lack of results of statistical tests which are hundreds of paired comparisons.

Carbonell *et. al.* Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides

This is Spanish cross-sectional study of 29 men working in flower and/or fruit cultivation who were attending a course in the correct handling of methyl bromide and other pesticides. Two control groups matched on age and 'habits' of 29 and 24 health men who were not directly exposed to 'any environmental agent' were used. Two blood samples were taken, the first in a period of 'high' exposure (spring-summer), and the second in a period of lower exposure (autumn-winter). No detail is given on whether all those attending the course were included in the study nor how typical these workers in general. It is not clear why 2 control groups were chosen and no details are given at all of how they were selected, nor how 'healthy' is defined. It is not clear whether the samples from the controls were taken in summer or winter. Sample size is not considered. The results are presented as means and standard errors with no indication of the distributions, and show seasonal differences in CA percentages. No results of statistical tests are given. The authors comment on differences between the exposed group and the pooled results from the two control groups. However, the results for the two control groups differ and this is not discussed by the authors. The workers were exposed to a wide range of pesticides but there is no analysis by pesticide type, nor are details given on whether pesticide use varied by season. However, the authors do report a general lack of use or incorrect use of protective measures whilst spraying. A wide range in the number of hours spent spraying is also reported. A large amount of data, including on potential confounders, was collected by self-reported questionnaire but is not used in the

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

analysis at all, including adjustment, nor is any reference made to it in the paper. Multiple comparisons are probably a potential problem in this paper.

Summary

Design: Use of two control groups unclear; Inadequate reporting of sampling strategy.

Analysis: No indication of CA distributions; Results of statistical tests not given; Pooling of results for the control groups; No adjustment for confounders; Multiple comparisons.

Ferrari *et. al.* Cytogenetic biomonitoring of an Italian population exposed to pesticides: Chromosome aberration and sister-chromatid exchange analysis in peripheral blood lymphocytes

This is a cross-sectional study of two groups of workers in the Italian flower industry with and without bladder cancer and a control group of individuals living in the same area with no history of occupational exposure to pesticides. The bladder cancer group were chosen apparently because there is a high incidence of the disease in the area. There is no indication of how the sample sizes were chosen nor how the subjects were selected. The paper gives no information on the gender of the subjects. The results did not vary substantially between the 2 exposed groups but CA and SCE incidence was increased compared to controls.

The distribution of subjects by age, sex and smoking habit was not homogeneous. Univariate stratified analyses tended to show similar results for CA and SCE incidence i.e. greater for exposed subjects.

Summary

Design: Inadequate reporting of sampling strategy; two “exposed” groups.

Analysis: Stratified analysis carried out but heterogeneous distribution by confounders.

Dulout *et al* 1985 Sister-chromatid exchanges and chromosomal aberrations in a population exposed to pesticides

This 1985 paper reports a study of SCE frequency in a small community of 154 people involved in floriculture (greenhouse production) in Argentina. An epidemiological questionnaire was first administered, presumably to all individuals although this is not stated in the paper. Samples of 20 individuals showing chronic intoxication symptoms (the most frequent symptoms described in the paper are fatigue, pain and numbness in higher and lower limbs, muscle weakness in legs and arms, leg cramps and abdominal pain) and 20 individuals without symptoms were then selected, stratified by sex and age according to the total population structure. Sample selection was presumably random within the strata, although this is not specifically stated. Sample size considerations are not discussed. Blood samples were taken however, from only 36 individuals. No details are given of why this occurred. At the time of sampling some of the asymptomatic group were reclassified as symptomatic giving 21 symptomatic and 15 asymptomatic. SCE analysis was only performed on 14 and 13 samples from these groups respectively. The authors do not give any details of any differences in characteristics between the original 40 and the 27 finally obtained. They also

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

do not discuss the impact this might have had on the representativeness and generalisability of their results. In addition a control group of 15 “healthy” (not defined) donors was used for comparison of structural chromosome aberrations. No details are given of how these donors were selected and recruited, nor of their characteristics.

The paper uses the terms case-control and cohort in reference to the statistical methodology (Wilcoxon on matched pairs and Mann Whitney U test respectively) but this is basically a cross-sectional study. Mann Whitney is used to compare the mean SCE frequencies between symptomatic and asymptomatic (6.45 (1.19) versus 5.47 (1.03) respectively) ($p < 0.05$). However, the authors then create 9 pairs matched on age and sex (details of age matching not given) and carry out a Wilcoxon matched pairs test. This is inappropriate as the pairs are created post hoc.

The results of the statistical test comparing the structural chromosome aberrations between symptomatic and asymptomatic groups are not given although are stated to show no difference. A significant difference was found for these two groups together compared with the healthy donor group for the frequency of dicentric chromosomes but it is not clear from the paper how much smoking might have influenced this result.

The paper gives details of the general use of pesticides but there is no exposure assessment for individuals and no analysis of the SCE results by pesticide use. Other than smoking, no other potential confounders are considered.

Summary

Design: Stratified sampling; Definition of symptomatic given. Reduced numbers of blood samples and SCE analyses; Reclassification as symptomatic.

Analysis: Post-hoc creation of matched pairs; Actual results not always given. Unclear influence of smoking. No analysis by pesticides.

Falck *et al* 1999 Micronuclei in blood lymphocytes and genetic polymorphism for GSTM1, GSTT1 and NAT2 in pesticide-exposed greenhouse workers

This paper reports a cross-sectional study of MN frequency of 34 pesticide-exposed greenhouse flower production workers (20 men, 14 women) in Italy compared to 33 healthy unexposed bank clerks (17 men, 16 women) from the same region, matched for age and smoking. No details are given on recruitment and selection procedures, including non response, refusal etc, and sample size is not discussed. A detailed questionnaire was used to collect information on confounding factors, including drug use, smoking, x-ray exposure, viral infections, vaccinations, dietary habits and alcohol consumption. Some exposure assessment has been carried out in this study including years of exposure, yearly pesticide treatments and hectares treated. This was used to form three exposure groups: extensive contact (e.g. mean year 22 (10)), less contact-sprayers and non sprayers. Some account appears to have been taken of the use of personal protective equipment. Stratified univariate analyses (exposure, smoking) have been reported. Log MN frequencies (presumably transformed because the distribution was skewed) were reported on pesticide exposure adjusted for confounders and show a statistically significant increase in MNCs among extensive sprayers. The authors also comment on the other significant influences of raised MNC frequencies i.e. age > 43 (NB the cut offs for categories are rather odd) and positive GSTM1, the latter particularly seen in smokers. In the discussion the authors comment that

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

the blood samples were taken in during a period of extensive pesticide application. The authors also draw attention to the fact that their analysis of smoking does not show lower MN levels in moderate smokers compared with non-smokers.

Summary

Design: Inadequate details of sampling strategy; Individual exposure assessment.

Analysis: One of the authors must be a statistician; Has taken account of the distribution of MN; Analysis adjusts for confounders and is appropriate.

Garry *et al* 1996 Pesticide applicers with Mixed Pesticide Exposure: G-banded Analysis and Possible Relationship to Non-Hodgkin's Lymphoma

This paper reports a US cross-sectional study of chromosome rearrangements in male licensed pesticide applicers. The subjects were selected from 719 responders to a survey of 1000 applicers according to exposure to fumigant use, insecticide or herbicide application. It is not stated in the paper how many responders were in each of these groups and how many were contacted about participating i.e. details on non-response rates and characteristics cannot be ascertained. Wilcoxon rank sum test was used to compare aberration rates with results of the Wilcoxon test for chromosome rearrangement rates being based on individual rearrangement rates/100 cells for all subjects within a group, not the pooled rearrangement rate. Two-sided values are reported.

All 3 applicers groups had higher frequency of chromosome rearrangements compared to a control group of 33 subjects, frequency matched on age and smoking status selected from volunteers who did not apply pesticides. (It is not clear whether these "volunteers" are part of the 719 survey respondents). There appear to have been a considerable number of analyses comparing the proportion of breaks occurring at fragile sites and to identify hot spots for chromosome breakage. It is not clear from the paper how many actual statistical tests were carried out or whether this analysis was intended to evaluate patterns. The results tend to be reported narratively rather than as formal tests.

Summary

Design: Lack of complete information on sampling strategy but data on pesticides used to obtain a stratified sample; Controls frequency matched on age and smoking status.

Analysis: Complex and specialised; Tendency to report results descriptively; Success (or not) of frequency matching not reported.

Garry *et al* 2001 Biomarker Correlations of Urinary 2,4-D Levels in Foresters: Genomic Instability and Endocrine Disruption

This is a US cross-sectional study of licensed forest/roadside pesticide applicators who applied dichlorophenoxyacetic acid (2, 4-D) more than 5 days per year exposed compared to a control group who did not apply pesticides in the past year. Both groups were identified from responses to a survey on health and pesticide use (86.3% response rate). 60 potentially exposed or clearly not exposed individuals were identified from the survey and 39 volunteered to participate (24 exposed, 15 controls). Of the remainder 14 failed to meet health or herbicide use criteria and 7 refused. Exposure assessment was carried out using

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

each participant's pesticide application records. Urine and blood specimens were taken twice, at the end of the peak of the 2, 4-D application season and within 6 weeks of the beginning of the following season's work.

A number of statistical methods were used to carry out comparisons (including Poisson regression) to compare changes in log hormone levels, evaluate associations between 2, 4-D levels and hormone level changes, variation in CA frequencies and frequency of V(D)J arrangements. The results show a ranking in urine 2, 4-D levels by expected ranking of increasing exposure. The largest changes in hormone levels over the season occurred in those with the expected highest exposure and highest 2, 4-D levels as did chromosome damage and frequency of two or more rearrangements.

Summary

Design: Clear design; response rates given.

Analysis: Various statistical techniques used but probably appropriate; study collects both reported exposure and markers of exposure and effect.

Gomez-Arroyo *et al* 2000 Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides

This is a cross-sectional Mexican study of 30 floriculturists (22 females with 10 years (average?) exposure, 8 males with 15 years exposure) working in four plastic greenhouses. High amounts of a range of pesticides were used, some simultaneously and no protective equipment was used by the workers. When the blood samples were taken the workers had not been in contact with pesticides for 3 months. The controls were from the local community and had similar socioeconomic conditions as those exposed, but no contact with pesticides. However, no details are given of how the participants were selected and recruited.

A questionnaire was used to collect data on pesticide use, medical history and lifestyle information. Student t-tests and chi-square tests were used. The paper states that none of the exposed workers smoke or drank but this information is not given for the controls. SCE means for exposed and referent groups were 7.1 (0.17) and 4.0 (0.1) respectively ($p < 0.001$). No significant difference was found between males and females. Similar results were found for both the mitotic index and MN frequencies. The presentation of the results is fairly simple and no attempt appears to have been made to examine the results in terms of other variables in spite of the large amount of individual data collected. However, there are some comments in the discussion on acute exposure and skin and nasal irritation in female floriculturists, differences in the hours exposed each day between men and women and a lack of correlation between exposure time and SCE frequencies. Results for the latter are not given.

Summary

Design: Lack of detailed data on selection and recruitment; Lot of data collected on individual histories and exposure.

Analysis: Simplistic in analysis and presentation; May have carried out other analyses but results not given explicitly; no analysis by nature of pesticide use.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Grover *et al* 2003 Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay

This paper reports a cross-sectional study of 54 workers employed for at least 3 years in the pesticide production unit of a pesticide manufacturing unit in India. The exposed group were identified by a medical doctor after completing a questionnaire including occupational exposure from an initial 160 workers. It is not clear whether those selected were a sample of those eligible or all those eligible. The control group (54) were “selected from the general population with no history of occupational exposure to pesticides or any particular environmental agent”. No further details are given. Untransformed data on DNA damage (the authors tested for normality) were analysed using t tests and ANCOVA. Mean Comet tail length was significantly different in pesticide workers between smokers and non-smokers, and < 10 years exposure compared with > 10 years exposure. Stratified analyses for smoking were carried out comparing controls and exposed groups. However, four separate t tests are carried out for the four potential comparisons although the adjusted results given by the ANCOVA analysis is more appropriate.

Summary

Design: No detail on selection of controls

Analysis: Normality of data tested; Overuse of t-tests. Adjustment for smoking, age and gender.

Hogstedt *et al* 1980 Cytogenetic study of pesticides in agricultural work

This brief report give results of a study of 10 male Swedish pesticide workers (5 smokers) with between 3 and 20 weeks work with a variety of pesticides during the preceding season. They were compared with 7 farm workers (4 smokers) who had never worked with any form of pesticides. No details are given on the selections and recruitment of either group. Number of aberrations per 100 cells and percentage of cells with aberrations were compared. Differences were non-significant although it is generally not clear exactly what comparisons were made nor which statistical tests were used.

Summary

Design: Very small study; No details on sampling strategy.

Analysis: Suspect several tests carried out but not clearly stated and results not explicitly given.

Kourakis *et al* 1992 Frequencies of chromosomal aberrations in pesticide sprayers working in plastic green houses

This paper reports a cross-sectional study of chromosome aberrations in 29 pesticide sprayers occupationally exposed to a mixture of pesticides in plastic green houses in Greece apparently in tomato and cucumber culture. The paper gives details of the frequency and nature of handling pesticides during September to November. Protective clothing appears not to have been used. Most had worked at least 4 years. No details are given on selection and recruitment of exposed workers nor for the 14 “healthy persons” who were the controls. The frequency of lymphocytes with aberrant cells was compared between controls and exposed using chi-squared tests. The pattern by duration of exposure showed an increase in the 11-12 year group compared 4-10 but a reduction in the 18-30 year group. The duration categories

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

chosen are unusual. No clear patterns were shown by 10 year age category although there was a tendency for both mean chromatid-type aberrations (gaps and breaks) to decrease as age increased. The sample size in each category was small however. Smokers in both the exposed and control groups tended also to have higher means.

Summary

Design: No information on sampling strategy.

Analysis: Chi-squared used in some comparisons; Tests not stated for others and results not given; sample size very small in some categories; category cut offs non-standard.

Lander *et al* 2000 Chromosome aberrations in pesticide-exposed greenhouse workers

This paper reports the results of a study of structural chromosome aberrations in 116 healthy male Danish greenhouse workers engaged in floriculture. A reference group of 29 men (all nonsmokers) who were members of an organic association was used. No details are given of how either group were selected and recruited. Both groups completed a questionnaire or medical, occupational and life-style. Paired samples taken pre-season (March 1994) and post-season (October 1994) were obtained for 83 of the greenhouse workers. The controls were sampled in March 1995. The characteristics of the study population regarding caffeine and alcohol intake, X-ray exposure and smoking habits were given. A large number of insecticides, fungicides and growth regulators were used and a graph of number of applications over the year is given in the paper. Poisson regression models were used and the paper states which variables were included in the models. Two tables give mean (sd) frequencies of cells with CAs in various subgroupings (non-smoking referents, greenhouse workers by smoking, pre & post season, glove use). It is not easy to tease out patterns from these tables and the authors report various statistically significant comparisons. The results from the models are reported in the methods and results sections of the text, sometimes narratively and sometimes giving risk ratios. The presentation of these analysis is inadequate and could have provided useful information on the risk ratios of the individual variables/confounders and how these influenced the risk ratio for pesticide exposure. This is a recent paper and COM might consider asking the authors for these results.

Summary

Design: No information on sampling strategy; Data collected on many confounders.

Analysis: Modelling used with more confounders included than many studies. Univariate analyses difficult to interpret. Models inadequately presented.

Lebailly *et al* 1998 DNA Damage in Mononuclear Leukocytes of Farmers Measured Using the Alkaline Comet Assay: Discussion of Critical Parameters and Evaluation of Seasonal Variations in Relation to Pesticide Exposure

This paper reports a study of haematological parameters of 29 French farmers with 41 samples collected for four groups (proxy for exposure) defined by time of collection (summer 1995 (10), January 1996 (13), April 1996 (15), June 1996 (17)). Blood samples were collected on two consecutive mornings before (S0) and after a day of spraying (S1). A questionnaire collected data on diet, smoking, alcohol and medicine consumption and sum of areas sprayed with pesticides. This paper reports the results from the S0 samples. T-tests and

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

ANOVA were performed. The 29 participating farmers were obtained from 55 initially enrolled. No details are given on the enrolment strategy or why only 29 participated. 18 had only 1 sample, 10 had 2 and 1 had 3. There was a wide range of farm area, total area sprayed and number of days without pesticide use before sampling (0-34). Mean (\pm SE) of leukocytes, other WBCs, RBCs, and platelets are given for each of the samples by group and smoking status. There were only 9 borderline significant differences overall (presumably from ANOVA) for two of the results with means tending to increase from groups 1 to 4 i.e. as the spraying season advanced. Values were higher among current smokers than among non- and ex- smoker. This is reflected in similar results from the scores of smoking. An overall between group difference in DNA damage level was found with mean scores being highest in groups 1 and 3. An analysis including only one sample per farmer showed an interaction between smoking and group. The authors appear to have carried out many different analyses and comparisons with a focus on differences between the groups. However, group is a crude surrogate for exposure and thus the unclear patterns sometimes found may reflect this. In addition the authors do not appear to have considered that the repeated samples cannot really be treated as truly independent in that they are from the same individual. A repeated measures ANOVA could have been considered as an alternative. The authors comment that DNA damage appears to be transient but this is not always obvious.

Summary

Design: No information on enrolment strategy; Non-response rates reported but no detail on difference between responders and non-responders; complex design with four periods of data collection but not all participants had multiple samples; Period is a crude proxy for exposure; No control group.

Analysis: A lot of data and therefore statistical analysis and comparisons; often rather muddled in presentation and reporting.

Lebailly *et al* 1998 DNA Damage in Mononuclear Leukocytes of Farmers Measured Using the Alkaline Comet Assay: Modifications of DNA Damage Levels after a One-Day Field Spraying Period with Selected Pesticides

This paper used the same study populations as the previous paper but presents the results of samples taken after one day of spraying. The pesticides used are given for each farmer in each of the four groups. Paired sample t-tests compare mean levels from samples S0 and S1 for 8 different leukocyte, WBC, RBC, and platelets for each group and pooled results (40 tests). Means for leukocytes and neutrophils are lower from S1 for all samples and significantly for some groups, with very slight increases for mean eosinophil in number (note pooled means are 0.24 (0.13) and 0.25 (0.12) for S0 and S1 respectively ($p < 0.05$)). The authors have used Wilcoxon tests to compare changes in tail moment distribution between S0 and S1 for each farmer separately and within each group (Note some of their farmers appear in more than one group). Although not given in detail the authors appear to have used t-tests as well to compare S0 and S1 for each farmer but it is not clear why this was done. The advantage of this study seems to be that some indication of the effect of different pesticides can be obtained, although most used multiple pesticides. However the samples are small in each case, the effect of the use of gloves unclear and the analyses subject to problems of multiple comparisons and data dredging.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Summary

Design: No information on enrolment strategy; Non-response rates reported but no detail on difference between responders and non-responders; complex design with four periods of data collection but not all participants had multiple samples; Period is a crude proxy for exposure; No control group.

Analysis: A lot of data and therefore statistical analysis and comparisons; often rather muddled in presentation and reporting.

Lebailly *et al* 2003 Urine mutagenicity and lymphocyte DNA damage in fruit growers occupationally exposed to the fungicide captan

This paper reports haematological parameters and DNA damage resulting from a spraying period with captan by 19 fruit growers in France. Participants were identified through a fruit grower organisation but no other details are given on selection and recruitment. Data on individual characteristics and lifestyle were collected by questionnaire a few days before the first sampling. Three urine samples were collected in the morning (S1), evening (S2) and the morning after (S3) of the day of spraying. Blood was collected at S1 and S3. The study was collected over two spraying seasons. Data on occupational exposure related to agricultural activities were collected at S1 and on pesticide exposure during the one-day spraying at S3. The predicted absorbed dose of captan was estimated using a UK model. Various parametric and non-parametric tests were carried out. The distributions of the measurements were tested for normality. 19 farmers provide samples for at least one of the two seasons. Although the focus was on one day of spraying with captan a variety of other pesticides were used on the same day and the day before by some farmers. All participants had an absorbed dose of captan predicted from the exposure model many times greater than the ADI. The results are mainly presented in the text. A large number of comparisons appear to have been made between sample times for haematological and mutagenicity parameters and for exposure and other confounding factors (many seem to have been considered). The authors state that generally no relationships were found with exposure to captan.

Summary

Design: No information on selection and recruitment; A lot of data collected and measured for such a small sample.

Analysis: Sample size probably a serious problem for so many comparisons and subgroups; Results generally presented in text format but multiple comparisons a potential issue.

Munnia *et al* 1999 Exposure to Agrochemicals and DNA Adducts in Western Liguria, Italy

This study reports a cross-sectional study of 57 non-smoking floriculturists (40 males, 17 females) and 33 non smokers age-matched referents (22 males, 11 females) in Italy. No detail is given of sample selection and recruitment procedures. A questionnaire was used to collect data on previous illness, occupational exposure to solvents, for products and dyes and use of pesticides during the previous year. DNA adduct patterns are illustrated for 4 “representative” floriculturists. Mean values were significantly higher in floriculturists than in controls after adjustment for age and gender using ANOVA (results for separate variables not given). No analysis appears to have been carried out by all the other variables collected including other occupational exposures.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Mustonen *et al* 1986 Effects of phenoxyacetic acids on the induction of chromosome aberrations *in vitro* and *in vivo*

This Finish paper reports the results from both *in vitro* and *in vivo* studies of chromosomal aberrations due to exposure 2, 4-D). The latter included male workers employed by a forest industrial company. Exposed workers sprayed phenoxy-acetic acid herbicides (10 smokers and 9 non-smokers). The controls did not work with herbicides (9 smokers and 6 non-smokers). Age was also used to match the groups. No other details are given on sample selection and recruitment. Blood and urine samples were taken after the spraying season but no detail is given as to exactly when. Spraying exposure varied. 18 air samples were collected from the breathing zone of 9 sprayers during spraying giving very low values compared to the Finnish TLV. Results for aberrations are either presented for each individual or as grouped means (\pm SE). Percentage of aberrant metaphases were higher for smokers than non-smokers for both controls and exposed subjects but there was no obvious difference between exposed and controls. No formal statistical testing appears to have been carried out.

Summary

Design: Restricted mainly to phenoxyherbicides; Time of sampling vague; some exposure sampling but not clear if concurrent with biological sampling; little information on participant recruitment and selection.

Analysis: Descriptive only, no statistical testing; only smoking taken into account.

Paldy *et al* 1987 Cytogenetic studies on rural populations exposed to pesticides

This Hungarian paper reports the results of chromosome aberration analysis from blood samples of 80 male workers exposed to pesticides and 24 male controls. The study was part of a much larger epidemiological study to assess the health risk of pesticides on a rural population, although no details of this study are given. The exposed workers were members of brigades of chemical plant protection mixers and field sprayers (more than 600 hours per year). No further details are given on selection of the exposed or referent subjects. About 80 different pesticides are used. Data were collected (details not given) on personal history, from medical examination and on smoking, alcohol and drug consumption. Fishers exact test and chi-squared tests were carried out. Statistical tests have been carried out comparing each of 4 categories of duration of exposure separately with the control group (all highly significant). An overall test of trend would have been preferable. A similar analysis is presented comparing age categories with the controls. No account is taken of age categories within the controls. The effects of alcohol and smoking could not be evaluated because of small numbers of non smokers and non-drinkers (not stated). Morbidity is compared between the two groups but it is not clear whether this is current incidence or total incidence over a particular period.

Summary

Design: Inadequate information of sample selection and recruitment. Multiple exposures.

Analysis: Statistical analyses dubious.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Pasquini *et al* 1996 Cytogenetic Biomonitoring of Pesticide-Exposed Farmers in Central Italy

This Italian paper reports a cross-sectional study of SCE and MN frequencies for a group of 48 male pesticide-exposed farmers and a control group of 50 male subjects living in the same area with no history of occupational exposure to pesticides or other occupational chemical components. The farmers grew a variety of crops and used a range of pesticides in Spring and Summer. No details are given on selection and recruitment of either farmers or controls. Data on personal information, work activity, type and duration of pesticide contact, X-rays, use of drugs, alcohol, coffee and smoking habits were collected. The authors tested the distribution of the biomarkers for normality and used a square-root transformation. Mann-Whitney U tests and ANOVA were used. SCE frequencies were higher for smokers than non-smokers in both exposed and control groups (Note: there were only 9 current smokers in the exposed group). PRI values were lower in the exposed group and in older ages and longer duration of exposure groups. The authors overdo the univariate analyses by combining exposed and control groups in addition to separate analyses. Two ANOVA analysis examine occupational exposure adjusted for (i) smoking and (ii) years of exposure. It is unclear why a model including both of these was not carried out unless small numbers prevented this. Interaction effects have, however, been examined. Annoyingly p values are only given for $p < 0.05$ so the pattern of results cannot be fully interpreted. There appear to be interactions between pesticide exposure and both smoking and years of exposure for PRI but not for SCE and MN. No analyses were carried out for any of the data on type of pesticides used.

Summary

Design: Inadequate information on sample selection and recruitment; multiple exposures.

Analysis: Many univariate comparisons; ANOVA carried out to take out account of confounding but separate models used.

Pastor *et al* 2001 Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells

This Greek paper reported a cross-sectional study of micronuclei of 116 individuals (50 exposed to pesticides, 66 controls). The exposed group (30 men, 20 women) were village farmers regularly exposed to a range of pesticides in fields and greenhouses. Controls (41 men, 25 women) who carried out clerical jobs were selected from the same village. No further details were given on selection and recruitment. A questionnaire was used to collect information on medical history lifestyle and occupation from all participants. For the exposed groups a further questionnaire collected data on type of crops, pesticide use and use of protective measure etc. All individuals were non- or ex-smokers. The authors examined the data for normality and thus used Mann-Whitney U tests. Multiple linear regression models were used on transformed MNL and BNMN values. Stepwise, backward, and forward methods were used for variable selection. (It is not clear why all these methods were used). Goodness of fit was examined. Two-sided p values were used. The section on statistical methods is very detailed. However both the descriptive results and the results from all the modelling are sparse and very selective, with patchy reporting of significance testing for the former and a focus on just a few significant results from the modelling. A general lack of an association with exposure was found inspite of the large number of analyses obviously carried out. The effect of confounders are not reported in any detail with the exception of age.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Summary

Design: Exposed and controls from same village; Lack of information on selection and recruitment; Exposure data on specific exposures not used.

Analysis: Theoretically fine but reporting of results disappointing; suspicion of rather too many analyses and very selective reporting.

Pastor *et al* 2002 Occupational Exposure to Pesticides and Cytogenetic Damage: Results of a Hungarian Population Study Using the Micronucleus Assay in Lymphocytes and Buccal Cells

This reports a similar study to that reported in Pastor *et al* 2001. The 149 participants were from a group of 155 Hungarians surveyed regarding personal and lifestyle factors and medical history. The exposed groups of 84 agricultural workers (58 men, 26 women) was exposed to many different pesticides and working in greenhouses and outdoors as sprayers, harvesters and related jobs. They were divided into 65 moderately exposed workers and 19 (all men) highly exposed (based on recent pesticide exposure and symptoms such as tiredness, weakness, dizziness etc). The controls worked in clerical jobs, health care and manufacturing with about two thirds from the same rural area and one third from Budapest. No further details are given on selection and recruitment. Very similar statistical methods were used to Pastor *et al* 2001. There were differences in characteristics between the exposed and control groups e.g. younger age, more smokers and more coffee drinking in the former. The reporting of modelling is again limited with only a few significant results reported and no detail on the results for exposure and how the various cofounder influenced this. The general conclusion is again a general negative relationship with exposure. However, all the values for the highly exposed men are higher than the moderately exposed men (table VI) but those of the moderately exposed are not higher than the values of the control men (table II). ANOVA analysis indicates a positive relationship of age and pesticide application activity with increased MNL and BNMN.

Peluso *et al* 1996 P-Postlabeling Detection of DNA Adducts in Peripheral White Blood Cells of Greenhouse Floriculturists from Western Liguria, Italy

This Italian paper reports on study of ³²P-postlabelling DNA adduct formation in 26 floriculturists (19 male, 7 female) compared with 22 population age-gender matched referents (13 males, 9 female)(all non-smokers). Participants were part of a larger investigation of biomarkers (68, 55 respectively). No other details are given on selection and recruitment although these may be available in another paper. A questionnaire was used to collect information on medical history, occupational exposure to solvents, for product, dyes, asbestos and ionizing radiation and use of pesticides. More information on type and use of pesticides was also collected from the floriculturalists. Multiple logistic regression was used to compare rates of DNA adduct-positive samples in exposed and referent subjects adjusting for age and gender. The results are presented in a very straightforward and informative way. The cut off value for age categorisation is stated i.e. the median. Numbers of adduct-negative and adduct-positive samples are given for referents and exposed, by age and sex, together with rates, crude ORs and ORs adjusted for age and sex. Older ages and being female increase the risk of being DNA adduct-positive, although not significantly, and this results in a reduced, but still significant OR for exposure when adjusted for these confounder. The paper states that no relationship was found with the use of specific pesticides or amount of agrochemicals used although the data are not given. There was a statistically significant increase in the level

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

of DNA adducts in floriculturists compared with referents. There appears to have been careful investigation, with appropriate statistical analysis, of the reproducibility of some of the results.

Summary

Design: Very small; Little detail on selection and recruitment of subjects; large amount of data on each individual collected.

Analysis: Carefully and appropriately carried out; well presented in figures, tables and text

Discussion: Thorough.

Piperakis *et al* 2003 Biomonitoring with the Comet Assay of Greek Greenhouse Workers Exposed to Pesticides

This Greek paper reports a study related to that reported by Pastor *et al* (2001). The same study populations appear to have been used. More detail is given on the type of work, however. 78% appear to have been involved in growing ornamental plants, 8% vegetables and 8% both. The pesticide applicator was carried above the head. Some form of protection was used by 62%. Table 1 in both papers is identical and gives the pesticides used. Tables II & III are also almost identical. The paper by Pastor *et al* (2001) reported MN frequencies. Piperakis *et al* (2003) report DNA damage. For each subject 300 comets were used (100 comets/slide, triplicate slides/data point) from which mean scores were calculated. The authors used Kruskal Wallis tests and repeated measures generalised linear models to evaluate differences in the distribution of DNA damage and repair. Mann-Whitney U tests were used to compare groups with regard to the mean value of confounding factors.

The DNA damage by degree of comet migration (3 groups) is given in graphs and tables for males/females non-smokers/ex-smokers, exposed/not exposed i.e. the authors then seemed to have separately statistically tested various pairs within each comet group e.g. non-smoking controls versus non-smoking exposed, men versus women etc. and then compared the migration groups. (There is a horrible three dimensional graph illustrating some of this). In spite of all these comparisons no damage appears to be related to pesticides.

Summary

Design: Exposed and controls from same village; lack of information on selection and recruitment; more information given on work practices.

Analysis: Tables I – III identical to those in paper by Pastor *et al* (2001); Statistical methods fine but graphs not ideal.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

3. Overall summary

In general all of the studies are very small and none of them discuss sample size considerations such as power. Although a small amount of information on the exposed and control groups are usually given, very few give sufficient details to enable an assessment of selection and recruitment biases to be carried out. (Garry et al 2001 is a good example of the sort of detail needed). Issues of non-response and generalisability of results are not discussed. Most of the studies use “standardised” questionnaires to collect information on demographics, medical history, lifestyle factors such as smoking, alcohol consumption etc and sometimes other potential occupational exposures of concern such as to solvents, radiation etc. Often detailed data are also collected on work with pesticides including type of pesticides, duration and frequency of exposure and use of protective measures. However, generally these data are not used in the analyses nor even reported (Lander et al is a paper that reports several of the characteristics). The majority of studies do not attempt to analyse the data for specific pesticides or groups of pesticides with most using the dichotomous grouping of exposed or not exposed in general to pesticides. Multiple exposures, including to non-pesticide exposure are not generally considered. An important point to note, however, is that although stratified analyses by e.g. age, gender, smoking are carried out in some studies most do not have a sufficiently large sample to explore the influence of all the variables collected.

The study designs are generally cross-sectional with most studies collecting data at a single point in time. A few have taken multiple samples, for example, in low and high exposure seasons or before and after a day spraying. The time between exposure and sampling thus varies considerably between studies. The issue of what should be the critical window of exposure for the outcomes under investigation is important and it is not clear whether any of the authors have explicitly addressed this when designing their studies. This has implications for evaluating the persistence of the effects and whether different exposures might lead to transient or permanent effects.

The methods of statistical analysis vary between the studies with many carrying out simple paired comparisons using both parametric and non-parametric tests. A few of the studies test their data for normality and either carry out transformations to normalise the data or use an appropriate non-parametric test. Many do not consider normality. Some have used statistical modelling to investigate the effect of confounders.

The reporting of the results in many papers is patchy with some results reported narratively without the actual figures. The reporting of the results of modelling is particularly poor with the results from only selected models given (usually significant) and a lack of information on the goodness of fit and the influence of inclusion and exclusion of variables (see Peluso et al for good reporting of models). As expected all the papers focus on results that are statistically significant even when the absolute difference between groups is tiny (Lebailly 1998 0.24 versus 0.25).

In summary, all the studies have their limitations. Direct comparisons between them is problematical because of differences in the biomarkers measured, the exposure (pesticide/chemical type, timing of exposure to samples, intensity, duration, frequency, use of protective equipment etc.), and confounders considered. All have potential problems related to small sample size.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.