

Chapter 6 — Genotoxicity, Immunotoxicity, and Genetic Susceptibility to Pesticide Health Effects Mediated by Genetic Polymorphisms

Genotoxicity is of interest as a pesticide health effect because most human carcinogens are genotoxic, and genotoxicity may be a useful biomarker as an intermediate endpoint in carcinogenesis (32, 34). Immunotoxicity refers to the effect of pesticides on immune system components such as cytokines. Genetic polymorphisms are metabolizing systems such as cytochromes that may be favourable (fast) or unfavourable (slow) in their capacity to detoxify pesticides.

Three distinct types of studies were assessed by reviewers in this area:

- ① Genotoxicity studies testing for clastogenic or mutagenic effects of pesticides on genetic material in cells taken from pesticide-exposed subjects and controls (22 papers)
- ② Immunotoxicity studies examining immune system parameters or clinical immune function as the health effects of concern (2 papers)
- ③ Genetic polymorphism studies examine the mediating effects of “poor metabolizer” genetic polymorphisms on health effects of pesticides (3 papers)

Details on the findings of these studies appear in the tables for this chapter.

① Genotoxicity

In the Group 1 studies of genotoxic effects of pesticides, the most frequently used test for genetic damage is classical chromosome aberration (CA) analysis of peripheral blood lymphocytes (PBLs). The lymphocytes are collected from single or multiple timed blood samples and prepared by a standard technique to undergo mitosis. Usually 100 or 200 metaphases per exposed and control subject are examined and the results are reported in terms of the percentage of cells affected. An inexpensive and simpler test looks for micronuclei (MN), whole or fragmented extra chromosomes resulting from abnormal mitosis. Some studies use newer tests to assess DNA damage (1, 34); these tests include sister chromatid exchange (SCE), Comet assay, FISH assay, and challenge dicentrics. However, the mechanisms that produce these forms of genetic damage are not as well understood as those revealed by the classical chromosome aberration test, and their clinical significance is not established. While the newer tests may be more sensitive and better suited to field testing, there is currently no longitudinal validation study to show that they predict increased cancer risk. Of all the tests for genetic damage used in the Group 1 studies, only CA frequency has been shown by a long-term prospective study to be predictive of an increased cancer rate in a pooled Nordic and Italian prospective cohort ($n = 5271$, follow-up 13–23 years) (35). Further longitudinal studies are required to assess the clinical predictive validity of other types of pesticide-induced genetic damage.

Methodological Issues

Important confounders for the genotoxic effect of pesticides are gender, smoking, diet, caffeine, and alcohol consumption. Alcohol induction of liver enzymes may alter pesticide metabolism, and smoking induces chromosome damage as well as increasing oral exposure if

workers smoke while handling the pesticides (1). Results are mixed as to whether the number of chromosomal aberrations increases with age. Women consistently experience greater genetic damage, with a higher percentage of chromosomal aberrations, than men on a population basis. Few studies analyzed the effects of all five potential confounders.

Other causes of chromosomal aberrations in populations include diagnostic and therapeutic radiation, and the use of mutagenic drugs such as chemotherapy agents. The latter has become a more important confounder in more recent studies since more chemotherapy drugs are now used for common non-malignant medical conditions including rheumatoid arthritis, inflammatory bowel disease, and psoriasis. Many studies excluded subjects who had x-rays or took mutagenic drugs in the year prior to cytogenetic testing.

The control groups used in some of the genotoxic studies involved participants living in the same geographic area as the exposed subjects. This may confound results, as background pesticide exposure from an area's diet, drinking water, air, and soil may cause an elevated baseline rate of cytogenetic damage among control subjects. The problem of contaminated controls could occur both in agricultural areas and highly industrialized sites. Examples of three studies in which this may be a problem are Hoyos (13) and Bolognesi (6) (agricultural), and Kaioumova (15) (industrial). This may result in a bias toward not finding genotoxic pesticide health effects.

The timing of blood sampling in relation to the spraying season is not considered important by some authors who believe that the genotoxic effects are long lasting. The three reviewed studies that found no association between pesticide exposure and chromosome aberrations (11, 13, 25) share the potential problem that blood sampling was not timed to occur during the season of peak exposure. Some studies found significant differences in the percentage of chromosomal aberrations pre- and post-spraying season, suggesting that pesticide-induced abnormalities can normalize rapidly after cessation of exposure (14, 20). A similar pre-post season trend was also found for micronuclei frequency (6). Using Comet tail assay, Lebailly (19) found increases in DNA damage after only one day of pesticide spraying, and concluded that this test was very sensitive for measuring current rather than cumulative exposure effects. An excellent discussion of the time-dependence of cytogenetic damage is found in a new review (1). Future studies of genotoxic pesticide effects will be more useful if the biomarker chosen is appropriate to the exposure condition, especially the timing of exposure with respect to the blood sampling.

An interesting problem raised by Scarpato (25) is that highly damaged cells may fail to undergo mitosis, the first step in growing cells to metaphase for laboratory analysis of cytogenetic damage. In other words, cytogenetic damage severe enough to be fatal to cells cannot be assessed by any of these methods. For example, Pastor (21), in a study of pesticide-exposed men, found a large increase in miscarriage among their partners, but no cytogenetic effects. A possible explanation would be that the pesticides killed cells (which were then unable to undergo mitosis), and caused significant reproductive effects. A study of exposed female banana farm workers and non-exposed local controls (24) found no significant differences in MN frequency between groups, but exposed women who had stillbirths or spontaneous abortions were 1.45 times more likely to have increased MN frequency than co-workers who did not experience these adverse reproductive outcomes. Beyond these two examples, few studies combine a search for cytogenetic abnormalities with measurement of relevant clinical endpoints such as reproductive outcomes. In the future, studies that combine cytogenetic and physiologic assessments with discussion of clinically relevant short-term and long-term health effects will be extremely useful.

Conclusions—Genotoxicity Studies

As in Bolognesi's (1) review, positive associations between pesticide exposure and chromosome aberrations were found in the majority of studies. Of the 22 Group 1 studies (4–26) assessed acceptable by reviewers (see Chapter 2—Methods for a description of the assessment tool), 11 find a statistically significant increase in frequency of chromosome aberrations (CAs) in the pesticide-exposed group. One study finds a significant dose–response relationship between micronuclei (MN) frequency and years of exposure (6), and three other studies show significant pesticide effects on MN frequency (9, 14, 26). Two studies find no pesticide effects on MN frequency, but one of these is positive for effect of male pesticide exposure on miscarriage (21) and the other is positive for increased rate of female miscarriage in the exposed group (23). Five studies show statistically significant associations between pesticide exposure and abnormal Comet tail assay; one is positive for DNA damage in exposed subjects (24) and one is positive for pesticide effects for both DNA repair response of cells, and challenge dicentric (5). Two studies showing no pesticide effects on CAs and one showing no effect on micronuclei share the problem of blood sampling not being timed to pesticide exposure (11, 13, 25).

The use of a reproducible laboratory measurement (chromosomal aberrations per 100 metaphases) to assess a pesticide health effect allows for aggregation of results across studies. For percent chromosome aberrations, the *n*-weighted average across 15 positive and negative studies is 2.36% for controls (*n* = 500) and 5.25% for pesticide-exposed subjects (*n* = 529) (Figure 1). It has been proposed that the “normal” rate of CAs in the population is 1%. The aggregated results thus suggest substantial exposure effects in the aggregated control group, and a significant pesticide-related increase in CAs in the exposed group. Chromosome aberrations have been proven to be a biomarker for cancer risk by a large, long-term prospective study (35), so it can be clearly stated that at least some pesticides are carcinogens.

② Immunotoxicity

An excellent literature review (3) on immunotoxicity summarized the research to date on pesticide effects on the immune systems of laboratory animals, wildlife, and humans. Three important concepts were highlighted by the review:

- (i) The three components of the immune system—humoral, cell-mediated and non-specific immunity—work in an interregulating way, so that an alteration in one part of the system may cause a compensatory change in another. Thus pesticide-induced, immune-mediated disease may result from either direct immunotoxicity or a compensatory response.
- (ii) The immune system can be stimulated or suppressed by pesticides; the same pesticide (e.g., malathion) can have either of these effects depending on the dose.
- (iii) Acute toxicity is not directly related to the immunomodulating properties of pesticides. For example, the carbamate aldicarb is the most acutely toxic in its group, but is the least potent inhibitor of T-cell proliferation through the mechanism of reduced production of interleukin-1 (IL-1).

Two studies published since Voccia's 1999 review (3) were found. Both studied organochlorines, but were included because the pesticides studied have been in wide use, are in the process of being phased out, and are very persistent.

The results of both Group 2 studies are positive for measurable effects of pesticides on immune system function. Daniel (27) studied 190 patients with high pentachlorophenol (PCP) exposure and found that increased blood levels of this commonly used wood preservative were significantly associated with impaired responses to humoral and cellular immune stimulation tests. Phillips (28) found that children exposed to chlordane and/or heptachlor had more cytokine panel abnormalities than matched controls.

Summary—Genotoxicity and Immunotoxicity

The Group 1 and 2 studies do not answer the question as to which pesticides are most likely to cause chromosome aberrations. One study found a strong effect for synthetic pyrethrins on CAs (20), and one study where 88% of exposed subjects had depressed RBC cholinesterase levels and very high CA frequencies (20.6%) suggests an important organophosphate effect (22). Garry (10) found significant increases in the frequency of CAs in fumigant and insecticide applicators compared with controls. The fumigant and herbicide applicator groups also showed increased frequency of the most common chromosome rearrangements observed in non-Hodgkin's lymphoma patients (10).

Subjects in the majority of studies assessed were repeatedly exposed to several classes and types of pesticides including fungicides, phenoxy herbicides, and the insecticide classes pyrethroids, carbamates, and organophosphates. Further *in vitro* studies, such as Whalen (40), which showed cytotoxic effects of triazine herbicides and carbamates on human natural killer cells, are needed to determine whether some classes of pesticides are more genotoxic and immunotoxic in humans, and to which cells and components of the human immune system. There is also important information in animal studies about the relative immunotoxicity of specific pesticides; this is reviewed by Voccia (3) but was beyond the scope of this review.

③ Genetic Susceptibility to Pesticide Health Effects Mediated by Genetic Polymorphisms

The Human Genome Project has created a new and potentially powerful method for studying the mechanisms of individual susceptibility to pesticide health effects. Gene mapping has allowed the identification of genetic polymorphisms in individuals that are associated with impaired metabolism of xenobiotics, including drugs and pesticides. Although all the pathways for pesticide detoxification are not fully understood, the process involves three main systems: the cytochrome P450 enzymes, glutathione S-transferases, and the paraoxanase system that metabolizes organophosphate insecticides. Inheritance of the unfavourable “poor metabolizer” versions of these genes has been shown to cause increased activation or reduced detoxification and elimination of environmental mutagens such as pesticides (5). It is hypothesized that, subject to comparable pesticide exposures, poor metabolizers would develop higher rates of cancer and other health effects than would normal metabolizers. Bolognesi's recent study (1) contains an excellent review and discussion of genetic polymorphism studies. Three examples of papers investigating the relationship of genetic polymorphisms and pesticide susceptibility follow:

1. Infante-Rivard (29) studied children with acute lymphocytic leukemia (ALL) who had been genotyped at birth. Children with poor metabolizer polymorphisms, representing just over 40% of the Montreal study group, had overall interaction odds ratios (ORs) of 1.05 to 5.55 for ALL if exposed to pesticides during pregnancy or childhood. The highest

ORs (4.33–5.55) were for exposure to repellants and sprays for outdoor insects during pregnancy, and exposure to mite and spider killers during pregnancy or between birth and leukemia diagnosis. Herbicide use (mainly 2,4-D) both during pregnancy and childhood, showed a consistent interaction with poor metabolizer genes and was associated with a 2-fold increase in leukemia incidence.

2. Hubble et al. (30) compared patients who had the dementia subtype of Parkinson's disease with a control group of Parkinson's disease patients without dementia. Genetic markers for poor metabolism were measured in both groups and found to occur in equal frequency. However, occurrence of the poor metabolizer gene combined with pesticide exposure was more common in the dementia group (12%) than in the Parkinson's patients without dementia (2%). This study suggests a possible mechanism for the consistent findings of epidemiological studies that pesticide exposure is a risk factor for the development of Parkinson's disease (35).
3. Au (5) studied Costa Rican banana farmers who had been previously exposed to dibromochloropropane (DBCP), a now-banned pesticide known to cause reduced sperm counts, and who were currently heavily exposed to agricultural pesticides in their work. The workers with poor metabolizer polymorphisms had an impaired DNA repair response, compared with co-workers who were normal metabolizers. The study also found that poor metabolizers were significantly underrepresented in the farmers' group compared with controls. The author speculated that poor metabolizers might have selected themselves out of farming because of their increased susceptibility to adverse health effects from high pesticide exposure.

Summary—Genetic Polymorphisms

Genetic susceptibility to the adverse effects of pesticide exposure is an important new concept for further study and application, and may represent the cutting edge of pesticide health effects research. It may be a mediating factor to explain mechanisms for “healthy worker” effects, variations in results between racial groups, and mixed results in epidemiological studies. In other areas of environmental health risk assessment, genetic polymorphisms have been investigated as a predictor of lung cancer risk in smokers (33), and lead poisoning severity in children (36). Screening for poor metabolizer genetic susceptibility certainly offers hope of preventing cancer in the future by educating those who are most susceptible to pesticide health effects to limit their pesticide exposures. However, until such techniques become clinically available, the presence of increased susceptibility to pesticide health effects in about 40% of Canadians, as suggested by the Montreal leukemia study (29), makes a strong argument for a general reduction of pesticide use and human exposure.

Chapter 6—Systematic Review of Pesticide Health Effects

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① Genotoxicity

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③ Genetic Polymorphisms

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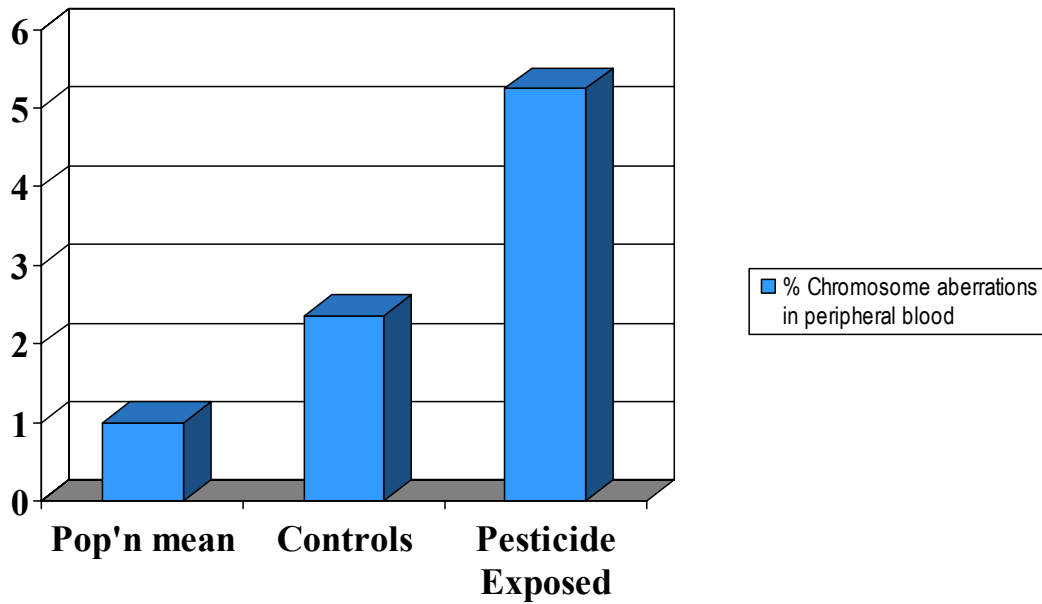
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Figure 1: N-weighted frequency of chromosome aberrations in exposed and non-exposed subjects

N-weighted mean frequency of chromosome aberrations for 500 control and 529 exposed subjects



Chapter 6—Systematic Review of Pesticide Health Effects

Tables

Table 1 Genotoxicity

<u>Reference</u>	<u>Population Description</u>	<u>Pesticides Type and Exposure Assessment</u>	<u>Covariates</u>	<u>Health Outcomes and Measurement (for non-cancer papers)</u>	<u>Statistical Analysis</u>	<u>Measures of Association and Values</u>	<u>Global Rating</u>
Antonucci 2000	23 E sprayers 23 NE controls- Brazil	carbamates, organophosphates +others mean= 11 yr. employed Used protective equip.	age, smoking, exposure time all non-smokers	CA's lymphocytes	r McNemar Test- matched pairs	Exposed vs controls 13% vs 4% (p<.05)	5,5
Au 1999	20 Costa Rican banana farmers, 20 controls	ALL PREV DBCP EXP – 6 currently used- named	all non-smokers, genotype for detoxifying polymorphisms	CA's,DNA repair response,FISH assay	1-way ANOVA, indep t + fischer's exact for genotype	CA's NSE1.6%, NE 1.3%- Repair rspnse Poor detox>normal>control for challenge dicentrics Poor detoxifiers under- rep. in farmers	6,6
Carbonell 1995	29n Spanish flower/friut farmers taking pesticide applicator course; 2 control groups 29 + 24	17 pest used by 10% or more; # hrs, spray setting(indoor/outdoor), pers. protection	age,smoking, alcohol NS 	CA's, LFT's, hematologic	Mann-Whitney U	E vs NE 6.93 vs 4.52 (p<.05)	5,4

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Carbonell 1993	70 Spanish E farmers and 69 C office workers	# hrs/yr spraying,type, work activity, protective gear #hrs/yr spraying,type, work activity, protective gear	age,smokng NS Signif. more alcohol in E group-not analyzed	CA's, SCE's	CA's-Mann-Whitney U SCE's-t-test	CA's:E 5.93% vs C 4.2%(p<.001) SCE's higher smokers	5,5
Garaj-Vrhovac 2002	10 pesticide workers Croatia,20 contols	x 22.25 yr. producing pesticides-2.,4D,atrazine, alachlor,cyanazine, malathion	Smoking-asked not analyzed Smoking higher in E	CA's in PBL,MNA, Comet tail assay	Chi sq. t-test.Comet	CA 1.02% C 7.8% E MNA 3.85 C 30.5 E Comet More DNA migration E (p<.001)	4,5
Garry 1996	61 male pesticide applicators 33 occupationally unexposed male controls Minnesota	herbicides n=20 insecticides n=18 fumigants n=23	controls matched for age and smoking status Current health status,medication use	Chromosome rearrangement (translocation or inversion).	wicoxon rank sum, 2-sided P values	1.Chromosome rearrangements significantly increased in fumigant and insecticide appliers, both 1.4% (p<.05) 2.Band 14q32 excess and band 18q21 excess in fumigant and herbicide appliers respectively-the 2 most common rearrangements observed in non-Hodgkin's lymphoma	6, Good exposure assessment. Pesticide-specific results. Suggests possible mechanism for excess NHL in pesticide appliers.

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Gregio D'Arce 2000	36 Brazilians 20 E, 16 C	19 listed pest's used, 2-5 app's/wk., blood sampling NOT timed to high exposure	age, smoking, GSTM1	CA's, mitotic index	t-test, ANOVA	patients No diff CA's E vs C (1%, .9%) Higher MI controls	4,4
Grover 2003	54 Indian pest workers, 53 controls	carbamates, organophosphates, pyrethroids	Age, sex NS Smoking p<.02 , yrs exp. p<.003	Comet tail assay	t-test, Chi sq., ANCOVA	Non-smokers C 7.03, 18.26 Smokers C 10.34, E19.75	6,5
Hoyos 1996	Colombia-30 E potato farmers, 30 C living in same area	Carbamates, organophosphates, dithiocarbamate fungicides, >5 yr, mean=16.5 yr.	smoking, alcohol, age all NS diet not assessed -? control group exp. by potato-eating	CA's, SCE's	CA- Chi sq SCE's-t-test	CA's: 1.7% E 2.1% NE SCE's 5% E 4.8% NE Both NS	5,5 no info re: timing bloodwork vs spraying
Joksic 1997	27 Yugoslav vineyard workers, 15 controls - local, 20 controls city	9 pesticides, active substances listed 3 herb's, 1 organophos., 5 fungicides, hr' sprayed, pre+post, airpack measures	age, sex, smoking matched	CA's, SCE's, MN measured pre, post, end of spray season	Wilcoxon rank sum, ANOVA	CA's ^ .79% E, .067% and .055% C; MN^ pre-post 5.41 vs 39.92, control 0; SCE NS	5,5
Kaioumova 1998	19 Russian pest workers, 36 NE controls, 21 local controls	workers prev. made 2,4,5-T switched to 2,4-D 1987	none	CA's in PBL	t-test	CA's 4.47% workers, 2.18% and 2.29% controls	4,3 all groups CA's higher than

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Kourakis 1996	Exposed 56 Controls 30	Organophos Carbamates Dithiocarbamates Organochlorine No protective gear Indoor + outdoor used same pest's	Age, sex Smoking – NS	CA's in PBL	Chi Sq.	E xp. 2.66% Cont .53% (.001 Indoor/outdoor 3.37% 1.88% indoor-industrial 3.37 non-industrial 2.14%	normal (1%)- ?diet 4,4 Lack of exposure measurement
Lander 2000	116E greenhouse floriculturists 29 NE non- smokers	# sprays/mo., what pesticide, use of protective gear 50 pest's – 10 insect., 6 fungicide, 3 growth reg (most used)	Age, smoking, caffeine use, use of gloves for re- entry work after spraying	CA's	Log-linear regression	CA's pre/post 1.87% vs. 2.34% and 1.62% for controls .02, .05 RR=2.88 – not wearing gloves during re-entry	6,6
Lebailly 1998a	French farmers with pesticide exposures; control slides for assessment of Comet tail assay reliability	Timing and cumulative area sprayed; no info on type	Age, smoking, alcohol, medications, passive smoking	Hematologic parameters; comet tail assay for DNA damage	Anove, t-test	DNA damage –ve correlated with number of days since spraying; DNA damage higher in mid and end-spray season. Interaction for DNA damage between smoking and exposure (p<.0001)	4,5 Lab methods and controls good; exposure assessment weak.
Lebailly 1998b	55 enrolled	1 day of spraying-4 groups each using a	Use of protective	comet tail assay, cell viability,	Paired sample t- test pre-post 1	Increases in DNA damage with	4,4

<u>Reference</u>	<u>Population Description</u>	<u>Pesticides Type and Exposure Assessment</u>	<u>Covariates</u>	<u>Health Outcomes and Measurement (for non-cancer papers)</u>	<u>Statistical Analysis</u>	<u>Measures of Association and Values</u>	<u>Global Rating</u>
	41 blood sampled Volunteer French farmers	different pesticide combination	equipment	hematologic parameters	day exposure: Student's t and Wilcoxon	fungicide-insecticide mixtures but not with triazine herbicide. Signif. trend (p<.01) between area sprayed and increased DNA damage.	
Mohammad 1995	22 Syrians: 9 sprayers(acute) 7dealers(chronic) 6 controls	-deltamethrin cepermethrin 3 hr./day sprayers -mixed yr.-round exp. dealers	controls non-smokers Acute vs chronic	CA's in PBL -spray group tested 3 X	Chi sq.	contols 4.9% dealers 15.28% Sprayers B 7.5% M 11.9% E 15.3%	5,4
Pastor 2001	49 Polish men occup exp 50 controls NE	% using each of 30 pest's deltamethrin 38% Duration, use of protective gear	age,smoking,diet , coffee alcohol	MN in PBL and buccal cells Miscarriage rate	t-test confounders linear regression	E = NE for MN in PBL and buccal cells Miscarriage C 2/49 E 11/50	4,4
Paz-Y-Mino 2002	41E men and women Ecuador 41 age+sex-matched NE living in same area	Type, duration , type of work activity	age and sex-matched; smoking not asked -duration of work -place of work	CA's in PBL, RBC cholinesterase	Chi sq for E vs NE; r for RBC chol./ CA's	88% E had reduced RBC chol. CA's 20.6% E and 2.7% NE (p<.001) r= -0.416 RBC chol vs CA's	6,5
Ramírez 2001	Exposed; 32 Women working in banana farms .	Mainly exposed to Imazalile and Thiabendazole and	Personal habits, exposure to chemical	Genotoxic effect measure through the frequency of	Man-Whitney Wilcoxon test. Logistic	There was no differences in micronuclei frequency	4, 4 Low power but it is a interesting

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	Unexposed: 37 women from the same region without occupational exposure to pesticides.	chloropyriphol (Organophosphorus insecticide and fungicides). Questionnaire and Records (work process). Grouping by months of exposition.	substances, medication, radiation and others.	micronuclei in cultured peripheral lymphocyt. _ Dichotomous: Present or absent.	regression. Stratified analysis by months of exposure.	between both groups. However, women who worked at the packagin plant and had still birth or spontaneous abortions were 1.45 times more likely to have an increased micronuclei frequency that their coworkers who lacked those disorders. Genetic susceptibility?	and useful study. Mixed methods.
Ramírez 2002	58. 30 banana workers and 28 unexposed select "voluntarily"	Fungicide Imazalil and Thiabendazole and Insecticide OP Chlorpirifos. Questionnaire and data related with job.	Age, smoking, infectious diseases and X-Ray exposure.	DNA ruptures measure through comet assay. Double blind and laboratory quality control.	Only crude analyses. T-student, ANOVA and linear regression (simple)	Damage to single stranded DNA after working 5-15 years ($R^2=0.12$), it means lineal relation between exposure time and AND damage, but is crude.	4,4 Low power, mixed methods and crude analyses but is useful as a pilot study.
Varona 2003	31 exposed 30 unexposed (administrative) Women worker in flowers industry	Mixed. Insecticides 70.6%, fungicides 17.6% and herbicides 5.9%. Questionnaire and information related with	Demographic, clinics, socioeconomic, but they only used them for description, not	Cytogenetic Alterations (micronuclei and chromosome aberrations), DNA repair	Only crude analysis. Proportions and differences of proportions.	Exposed group had a significantly higher frequency of cells with chromosome aberrations and micronuclei ($p<0.05$).	4,4 Low power and poor statistical analyses, but good design and

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		work. Acetyl cholinesterase (AC).	for adjustment.	deficiencies. Laboratory. Quality control		AC among normal values and no differences between both groups.	quality control in the laboratory analyses. Useful for demonstrate that AC doesn't discriminate sub chronic exposure.

Table 2 Immunotoxic

<u>Reference</u>	<u>Population Description</u>	<u>Pesticides Type and Exposure Assessment</u>	<u>Covariates</u>	<u>Health Outcomes and Measurement (for non-cancer papers)</u>	<u>Statistical Analysis</u>	<u>Measures of Association and Values</u>	<u>Global Rating</u>
Daniels 2001	190 PCP-exposed Germans,95 men,95 women - study done over 3-9 yr period after PCP banned in Germany	At least 6 mo. exp. to PCP-containing pesticide	None	28 measures of immune response-counts+stimulation assays	Spearman's rank,Wilcoxon's, Fischer's exact	More abnormalities if PCP blood > 10ug/l Blood PCP-ve assoc. with counts and +ve assoc. with # impaired stimulation tests	5,5
Phillips 2000	25 exp.children aged 4-7;25 age-matched controls	12-14 mo exp. to chlordane and /or heptachlor	sick vs 'well' exposed children Age-matched	8 cytokine profiles corresponding to 8 immune parameters tested 1 mo. post-exp.	t-test	Exp- cytokine abn vs controls signif .05 on 3/8 panels. Doctor visits over 1 yr. NS	6,5

Table 3 Genetic Polymorphisms

<u>Reference</u>	<u>Population Description</u>	<u>Pesticides Type and Exposure Assessment</u>	<u>Covariates</u>	<u>Health Outcomes and Measurement (for non-cancer papers)</u>	<u>Statistical Analysis</u>	<u>Measures of Association and Values</u>	<u>Global Rating</u>
Au 1999	20 Costa Rican banana plantation workers,20 controls	6 currently used + all prev exposed to DBCP	genotype for detoxifying polymorphisms	CA's, DNA repair response,FISH assay	1-way ANOVA,t-test;Fischer's exact for genotype	CA's NS: E 1.6%,NE 1.3%. Slower repair response assoc. with poor detoxifying polymorphisms.	6,6

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Hubble, 1998	246 patients with Parkinson's screened; 43 met dx. criteria for Parkinson's disease with dementia (PD+D)	Unspecified-questionnaire+ structured interview	Age, gender, education, pesticide exposure, family Hx PD, poor metabolizer genetic polymorphism	Parkinson's disease with Dementia	Chi Square, t-test, multiple regression	Poor detoxifiers underrepresented in farmers Pesticide exp. in combination with poor metabolizer genetic polymorphism signif. asoc. with PD+D (p<.032)	5,5
Infante-Rivard 1999	491 leukemia(ALL) cases and 491 controls ages 0-9 yr.-Quebec. Case-only genotyping study N=123	Mothers filled out questionnaire on pesticide exposures from 1 month preconception to birth, and birth to dx. Includes home and garden use, use on pets.	Maternal age, age, sex	OR, regression analysis	OR for ALL increases with # of pesticides used, and maternal frequency of use(OR's 3.0-4.2 for >5 times used) Case-only: Increases in interaction OR's with poor metabolizer genes and increased pesticide exposure		5,5