GENOTOXIC EFFECTS OF PESTICIDE EXPOSURE ON FARMWORKERS IN THE FRASER VALLEY OF BRITISH COLUMBIA

by

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ABSTRACT

This study measured micronuclei in peripheral blood lymphocytes from British Columbia seasonal farmworkers and controls. The farmworkers were employed to harvest berry crops and were occupationally exposed to pesticides. In British Columbia, seasonal farmworkers are predominately of East Indian ancestry and the majority are female. Cytogenetic analysis was performed on 39 female subjects of East Indian descent; 18 farmworkers employed during 1993 season and 21 age-matched controls. The mean age was 55.9±9.8 years. An average of 2000 binucleated lymphocytes were scored per individual, and micronuclei were also scored for the presence of kinetochores using CREST anti-bodies. There was no significant difference between the farmworker group (21.68 ± 10.42 micronuclei per 1000 binucleates), and the control group (23.60 ± 10.87 micronuclei per 1000 binucleates). However, among the farmworkers employed during 1993, there was a weak positive association between micronucleated cell frequency and weeks worked: 18.46 ± 8.50 micronuclei per 1000 binucleates in those working less than to 20 weeks to 27.87 ± 15.33 micronuclei per 1000 binucleates in those working more than 23 weeks. In those who had ever been employed as farmworkers, there was an elevated frequency of micronucleated cells in the group with the longest history of employment as a farmworker (26.58 ± 10.53 micronuclei per 1000 binucleates) vs. those with the shortest employment history (17.60 ± 9.04 micronuclei per 1000 binucleates). This trend remained evident after adjusting for age, RBC folate, meat and coffee consumption, recent vaccination, and socio-economic status. A positive association between the consumption of meat and micronucleus frequency was also noted. Non-meat eaters were likely life-long vegetarians. This study indicates that seasonal farmworkers may have an elevated risk of genetic damage, and steps should be taken to mitigate their exposure to pesticides. Further studies employing more subjects, internal controls and utilizing a larger battery of genotoxicity assays are required. Studies on farm sites also need to be undertaken to develop adequate analyses of farmworker tasks and exposure potential. Use of biological monitoring for pesticide metabolites or exposure modeling using dermal transfer coefficients may provide quantitative exposure data.

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DEDICATION

With love, to Lillian.

INTRODUCTION

There is increasing evidence of elevated cancer morbidity in individuals exposed to pesticides as demonstrated by several recent reviews (Devesa, 1995, Blair and Zahm, 1991, Blair *et al*., 1992). Pesticide involvement in other diseases with genetic etiology is also suspected, and there is considerable experimental evidence demonstrating the mutagenic potential of pesticides, as well as their carcinogenic effects in animals (Weisenburger, 1993).

Zahm and Blair (1993) noted that although many epidemiologic studies have investigated the association between pesticides and cancer they have largely focused on farm owner/operators. Little work has been done with seasonal and migrant farmworkers. Yet farmworkers appear quite distinct from other groups occupationally exposed to pesticides. They are primarily employed to do manual field labour, and are consequently exposed to pesticide residues on foliage and fruits. They are not exposed to many additional potential carcinogens as farmers might be, and epidemiologic studies suggest that they are at elevated risk of different neoplastic diseases to those of farmers (Zahm and Blair, 1993).

For seasonal farmworkers, dermal exposure is considered the primary contributor to dose. The primary safeguard for their protection from pesticide residues is the re-entry interval, but there is evidence that its current use does not provide adequate protection from long-term low-level exposures (Kreiger *et al*., 1992). In British Columbia, farmworkers may be continually exposed to the fungicide, captan, which is prophylactically applied to guard against endemic fruit rot disease (Peters, 1994), and to many other pesticides (Seehra, 1988, Oja *et al*., 1990). Inadequate regulation, poor health and safety attitude, widespread ignorance of proper pesticide handling practices among farmers, and improper pest management techniques may also have contributed to excessive exposures to pesticides in the past (Seehra, 1988, Delloite, Haskins and Sells, 1988).

In BC the farmworker population is largely seasonal. Farmworkers tend to be older, female, and to be recent immigrants. Many reside in Vancouver and surrounding cities, however, and are therefore easily accessible. They have some knowledge of the health problems associated with pesticides, and concern over

pesticide exposure is a community issue. It was decided to work with this community interest to undertake a study to look at the genotoxic effects of pesticide exposure on farmworkers. The micronucleus assay was chosen to investigate the early, pre-clinical effects of putative genotoxic exposure to pesticides.

Biomonitoring techniques for genetic damage, such as the micronucleus assay, measure early biological markers that are associated with long-term health effects such as cancer and birth defects. Although the data is not available to determine the predictive value of these markers, they are a surrogate index for events that are believed to be causative in the onset of disease. Early detection allows cause and effect relationships to be more easily observed (Perera, 1987). This has obvious benefits when studying a group such as the farmworkers, where follow up may be difficult, or in studying diseases which have a long latency. Assays such as the micronucleus assay have demonstrated positive genotoxic effects in occupational groups exposed to ethylene oxide, and cytotoxic drugs (Anwar *et al.*, 1994, Ribeiro *et al.*, 1994). Bolognesi *et al.* (1993) found increased genetic damage using the micronucleus assay in a study of floriculturists occupationally exposed to pesticides in Italy. The micronucleus assay is technically straightforward, and has objective scoring criteria, which makes it relatively inexpensive and easy to learn.

The aims of this study were to identify and recruit a study population of farmworkers who were working in the fruit berry industry in the Fraser Valley in the summer of 1993, and a suitable control group of matched, unexposed individuals. Biological samples were to be obtained and analyzed for micronucleus frequency in the Occupational Hygiene Programme facility at UBC. Data regarding employment history, and various health and diet factors were to be collected by questionnaire. Following analysis, results would be reported back to the study participants.

This thesis is in 9 chapters. Chapter 1 covers pesticide exposure in the Fraser Valley area, the exposure potential for farmworkers, and various factors which influence exposure. Because no exposure assessment was made as part of this study, an effort was made to describe the exposure situation as fully as possible. Chapter 2 discusses genotoxicity and the health outcomes associated with genotoxicity. It looks at the evidence which links pesticides with genotoxicity, and one agent in particular, captan. Because of the frequency of its application, and the persistence of its residues, this pesticide is perhaps the greatest

exposure threat to farmworkers, and there is considerable evidence that it may be genotoxic to humans. Chapter 3 introduces the micronucleus assay and discusses the meaning of the micronucleus frequency.

Chapter 4 presents the study hypothesis, and the specific goals of the study. It discusses details of the study design. Chapter 5 presents the methods of the study with respect to contacting and recruiting the study population, and obtaining biological samples and administering the questionnaire. Chapter 6 deals with the methods of the micronucleus assay itself, and the scoring and analytical processes.

Chapter 7 presents the results of the study. Chapter 8 discusses the results, and the findings with respect to other published work. Chapter 9 contains the conclusions and recommendations.

1. PESTICIDE EXPOSURE

1.1 Pesticides, and Pesticide Formulations

"Pesticide" is a general term given to any substance, or mixture of substances, which are designed to prevent, destroy, repel or mitigate any undesirable, harmful or destructive animals, plants and microorganisms (Ecobichon, 1991). Pesticides form an extremely large and chemically diverse group. There are at least 35 major chemical classes of pesticides (Briggs, 1992), and since 1945 it is estimated that over 35,000 pesticide products have come into use around the world (Forget, 1991). Pesticides are categorized by their target organism, for example insecticides, herbicides, fungicides and miticides, or by their chemical group, for example organophosphates, organochlorines, carbamates, and pyrethroids. Within these categories, toxicity to humans varies widely. This diversity is a problem for the study of pesticide exposure, because it is hard to find large groups of individuals with similar exposures. This difficulty is compounded in the case of seasonal and migrant farmworkers, whose transient work practices involve work at multiple farms and with multiple crops, further complicating their chemical exposure profile.

Pesticides are rarely simple compounds. They are generally formulated using an active ingredient (the component primarily responsible for the controlling effect of the product) and various other agents designed to improve the product's physical characteristics (e.g. solvents, propellants, surfactants and emulsifiers), termed "formulants" or "inerts". These inert ingredients, although not toxic to the target species, can still be biologically active and may occasionally be the most toxic part of the formulation to humans (Briggs, 1992). Considered trade secrets by their producers, inerts have not had to be declared on the product label. Some of these ingredients are known carcinogens and mutagens (IARC, 1991). In a review of over 8000 pesticides registered for use in Italy, 71 different solvents were identified in various technical formulations, including chlorinated organic compounds, 8 of which had been classified by IARC as having sufficient evidence of carcinogenicity in either humans or animals (Petrelli *et al*., 1993).

Pesticide formulations often contain impurities introduced during the manufacturing process, or due to degradation and reaction during storage. Technical grades of a pesticide, (grades used for application) are often more toxic than the pure active ingredient (Flessel *et al.*, 1993). Farmworkers who are exposed to pesticides and their residues while performing manual labour in fields may be exposed to higher levels of these degradation products than are applicators.

Pesticides may be formulated or applied as a combination of products. The toxicity of these combinations have not been widely studied. One particularly worrisome combination is that of pesticides and nitrogen-based fertilizers (Zahm and Blair, 1993). Pesticides that are N-nitrosatable (especially those containing a secondary amine), can react with nitrites to form N-nitroso compounds, some of which are carcinogenic (IARC, 1983).

It can be seen that pesticides are an extremely diverse group of chemicals which are further complicated by trade formulations, contaminants and mixtures. This makes the task of exposure assessment extremely difficult. Probably the greatest weakness of epidemiological studies investigating pesticide-related health effects is the inability to accurately determine exposure, either qualitatively or quantitatively. Often, for community-based studies such as the one undertaken for this thesis, the most that can be accomplished is to describe the patterns of pesticide usage in the study group region. Unfortunately, this results in a large degree of misclassification of exposure, probably resulting in an underestimate of the true risk.

1.2 Pesticide Use In The Fraser Valley Berry Industry

In British Columbia, pesticides are widely utilized in agriculture. In 1991, over 688, 000 Kg of active ingredient was applied by farmers (Norelco, 1993). Herbicides were most heavily used, followed by insecticides and then fungicides, and this pattern has remained similar since at least 1983. In that year, there were approximately 150 active ingredients in use in British Columbia (Eaton, 1983). By 1991, the number of active ingredients in use had risen to 274. The total number of formulations of course, is greater still.

Data on pesticide usage within the British Columbia berry industry itself is not collected. However, Statistics Canada reported in 1991 that agricultural pesticide expenditures in the Fraser Valley, where the berries are a key crop, had risen 169% between 1971 and 1985 (Statistic Canada, 1991) to over $2,000,000 in 1971 dollars.

Despite the large number of pesticides available, only a small subset accounts for the majority of the applications in the province. Eight active ingredients account for over 50% of all agricultural pesticide use in British Columbia, and just 23 account for 75.5% of the total agricultural use. The list can also be narrowed by considering specific crops, because pesticides are registered for use by crop, and beyond that their price, efficacy, and other considerations further limit the number of pesticides actually used. There is still, however, considerable variety in the pesticides being used. A 1989 survey of 89 Fraser Valley berry growers found 33 different pesticides being used (Oja *et al.*, 1990). Forty percent of applications were fungicides, 30% were herbicides, and 26% insecticides. This elevated use of fungicides compared to the rest of the province is probably due to berry fruit's susceptibility to fungal disease (e.g. *Botrytis*), which is worsened by the damp climate found in the Fraser Valley (Peters, 1994).

This study concerned itself with workers in the Fraser Valley berry fruit industry, which included strawberries, raspberries and blueberries (blackberries and cranberries are also grown, but no workers from these crops were included in this study). Until the introduction of the regulations covering agricultural operations by the British Columbia Workers Compensation Board in 1993, there was no requirement for pesticide application records. Thus it has been difficult to obtain a complete picture of the pesticide use on berries. Table 1-1, however, lists pesticides which have been reported used by various previous studies, and also those currently recommended by the British Columbia Ministry of Agriculture's berry industry specialists.

In this table, the chemical name is given, along with the local common name, and the chemical class to which the pesticide belongs. The fourth and fifth column show the results of two recent farm surveys (Oja *et al.*, 1990, Seehra, 1988). The sixth column lists the pesticides recommended for use on strawberries, raspberries and blueberries by the "Berry Production Guide" (BCMAFF, 1994). This is an important source

of information for the berry farmers - Oja *et al.* (1990) reported that about 1/3 of the berry farmers they interviewed used the Guide as their primary source of pesticide handling information. The final column

TABLE 1 - 1. LIST OF PESTICIDES COMMONLY USED IN BERRY PRODUCTION, FRASER VALLEY, BRITISH COLUMBIA

Chemical Name	Common Name	Chemical Class[a]	% Farmers Reporting Use in 1984[b]	Percent of Applications in 1989[c]	1994/95 BCMAFF Berry Guide[d]	Common usage in 1993[e]
HERBICIDES						
Simazine	Princep	triazine	67	24	√	√
Dinoseb	Dinitro General[f]	phenol	34	20		
Paraquat	Gramoxone	bypiridyl	10	25	√	√
Chloroxuron	Tenoram	urea	21	5		
Napropamide	Devrinol	misc.	7	5	√	√
Glyphosphate	Roundup	OP	2	9	√	√
Dichlobenil	Casaron	benzonitrile	3	8	√	√
Trifuralin	Rival, Treflan	dinitro-aniline	7	-	√	
Propyzamide	Kerb	misc.	5	1		
Ferrous sulfate		metal	-	1		
Fluazifop-p-butyl	Fusilade	phenoxy	-	1	√	
Amitrole	Amizol	triazole	-	1		
Dalapon	Dowpon	misc.	-	1		
Stoddard Solvents		petroleum	-	1		
Terbacil	Sinbar	uracil	-	-	√	
Oxyfluorfen	Goal	misc.	-	-	√	√
Clopyralid	Lontrel	misc.	-	-	√	
Diphenamide	Enide	amide	-	-	√	
Unknown			-	2		
FUNGICIDES						
Captan	Captan	pthalate	60	64	√	√
Triforine	Funginex	misc.	-	24	√	√
Metalaxyl	Ridomil	methyl ester	12	4	√	√
Captafol	Difolitan	pthalate	-	3		
Folpet	Folpan	pthalate	-	2		
Lime/sulfur			-	1		
Benomyl	Benlate	benzimidazole	2	-	√	
Chlorothalonil	Bravo	benzonitrile	2	-		
Anilazine	Dyrene	triazine	-	-	√	
Iprodione	Rovral	amide	-	-	√	
Unknown			-	2		
INSECTICIDES						
Diazanon	Basudin	OP	67	40	√	√
Malathion	Cythion	OP	26	33	√	√
Carbofuran	Furadan	carbamate	40	4	√	√
Endosulphan	Thiodan	OC	9	3	√	√
Parathion	Parathion	OP	5	5	√	
Azinphosmethyl	Guthion	OP	4	7	√	√

TABLE 1 - 1. Continued

Chemical Name	Common Name	Chemical Class[a]	% Farmers Reporting Use in 1984[b]	Percent of Applications in 1989[c]	1994/95 BCMAFF Berry Guide[d]	Common usage in 1993[e]
INSECTICIDES CONT.						
Oil spray			5	-		
Fensulfothion	Dasanit	OP	-	5		
Mevinphos	Phosdrin	OP	-	3		
Dimethoate	Cygon	OP	2	-		
Carbaryl	Sevin	carbamate	1	-	√	
Deltamethrin	Decis	pyrethroid	1	-	√	√
Bacillus thuringiensis	Dipel	organic	1	-	√	√
Lime/sulfur			1	-		
Oxydemeton-methyl	Metasystox	OP	-	-	√	√
cypermethrin	Cymbush	pyrethroid	-	-	√	

[a] OP - organophosphate; OC - organochlorine (Briggs, 1992), misc. - miscellaneous.

[b] 90 farmers interviewed (Seehra, 1988).

[c] 89 Farms surveyed, total of 637 pesticide applications (Oja *et al.*, 1990).

[d] Recommended pesticides for use on strawberries, raspberries, and blueberries (BCMAFF, 1994). Does not include molluscicides and miticides (Lannate, omite and Kelthane).

[e] Peters, 1994, Hornage, 1994.

[f] Dinoseb deregistered in 1992

indicates those pesticides which were most likely to have been in use during the 1993 berry season, and takes into account first hand knowledge of farmer preference due to reasons of economic and efficacy (Peters, 1994, Hornage 1994).

The table shows trends in pesticide use, and identifies several pesticides that pose high potential exposure problems due to their common usage. In particular, the following have been widely used historically, and remain in common use: the herbicides simazine, paraquat, napropamide, and glyphosphate; the fungicides captan and triforine; and the insecticides diazinon, malathion, carbofuran, and endosulfan.

1.3 Seasonal Farmworkers in the British Columbia Berry Industry

Exposure to pesticides and pesticide residues is an occupational hazard experienced by several groups of agricultural workers including those mixing and applying pesticides, pilots of aerial application aircraft, veterinarians, and farmworkers. While exposed farmworkers may have a variety of duties, this study concerns itself with a particular subset of farm employees who are seasonally employed to perform simple

manual field labour, primarily harvesting, weeding and thinning; they generally do not mix or apply pesticides. This group is usually referred to as "harvesters", "berry pickers", "pickers" or "farmworkers". I shall refer to this group as "farmworkers" throughout this document.

Farmworkers are utilized heavily in the berry crop industry in British Columbia. In 1983, there were approximately 28,000 person-months of hired labour in the three berry crops, strawberries, raspberries and blueberries (Eaton, 1983). This was the second most labour intensive crop in the province with 5 person-months/hectare worked. In comparison, mushrooms, a highly intensive crop utilized 200 person-months/hectare, while tree fruits and grains utilized 3 and 0.03 person months/hectare, respectively. It has been observed that labour-intensive crops such as fruits receive the highest concentration of pesticides of all crop types (Mentzer and Villalba, 1988, Mobed, *et al.*, 1992), and when pesticide volume per hectare is taken into account, the lower mainland including the Fraser Valley has the highest potential for worker exposure to pesticides in the province (Eaton, 1983).

The precise number of individuals working as seasonal farmworkers in the berry industry is difficult to determine, but it is estimated that between 3000 and 8000 individuals each year since 1980 have been employed as seasonal farmworkers in the lower mainland/Fraser Valley region. Sources of these data do not distinguish berry pickers from other types of farmworker (e.g. dairy hand) but it is assumed that the majority of these seasonal farmworkers work in the berry industry which is the major employer of seasonal farm labour in the Fraser Valley (Eaton, 1983). Table 1-2 shows estimates of the worker number from 1980 to 1993.

Despite their work being seasonal, many farmworkers work extremely long hours, which over one summer can approach the total hours normally worked in a whole year of permanent employment. A report of farmworker health (MACS, 1982) found that 71.7% worked over 20 weeks per year, and that the average number of hours worked per day at peak season was 10. Assuming the farmworkers work seven days a week, these individuals worked an average of 1400 hours a year. In comparison, the standard working year is approximately 1950 hours.

TABLE 1 - 2. SEASONAL FARMWORKER NUMBER ESTIMATES, 1980 -1993.

Year	Number	Source	Comment
1993	3000	Sidhu, 1994	Fraser Valley, Bush Berries, working greater than 16 weeks per year.
1993	8000	Sidhu, 1994	Fraser Valley, Bush Berries, all workers.
1992	4500	Thompson, 1994	Fraser Valley and Okanagon regions
1990	8012[a]	Statistics Canada, 1990	Mainland
1986	8166[a]	BCMAFF, 1988	Lower Mainland
1980-81	5741[a]	Eaton, 1983	Mainland

[a]Estimated by dividing reported number of weeks of paid labour by 1993 average weeks worked.

Farmworkers may be employed directly by farm operators, and may reside on the farm site during the work season, if the farm is sufficiently large. Many farmworkers, however, are employed by farm labour contractors who organize a labour pool, and then contract to supply this labour to farms. The three main crops - strawberries, raspberries and blueberries - have different monthly requirements for labour (see Table 1-3), and so the labour pool permits the same farmworkers to work on a number of farms during the season, moving from one site to another as their labour is required.

TABLE 1 - 3. SEASONAL FARMWORKER REQUIREMENTS BY CROP TYPE (MAN-MONTH IN 1981)

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Strawberries	0	0	0	250	1500	6000	200	700	700	0	0	0
Raspberries	500	700	400	400	1500	1500	6000	1500	500	100	0	0
Blueberries	50	50	50	200	200	200	500	4500	1000	50	50	50

From Eaton, 1983.

As can be seen from Table 1 -3, the bulk of the seasonal farm work is done between June and August, which are the months of the berry harvest. Some farmworkers continue to work through the fall months, employed harvesting vegetable crops such as potatoes, onions and beans, etc.. Outside of the harvest period, farmworkers are also employed doing tasks such as weeding, training raspberry canes, and thinning (Peters, 1994). The movement of workers through many different crops and farms complicates their pesticide exposure profile greatly.

1.4 Pesticide Application and Pesticide Residues

The availability of pesticide residues to farmworkers is primarily dependent on two things: first, the scheduling of applications and their coincidence with farmworker activity, and second, the persistence of

the chemical agent on the foliage, fruit, and in the soil. Many pesticides are considerably persistent, and present potential exposures well beyond the period that may be associated with acute toxic effects (Kreiger *et al.*, 1990, Giles and Blewett, 1991). Fungicides and insecticides which are applied immediately before or during the harvest season represent the greatest potential exposure hazard to farmworkers. Herbicides, generally applied earlier in the growing season, may still be an exposure hazard if they are particularly persistent, or if there is concurrent manual labour with their application, such as pruning or weeding. The following sections give more detail regarding the scheduling of pesticide application, and the coincidence of applications with farmworker activities.

1.4.1 Strawberries

Manual labour in strawberry crops generally occurs between late March and the end of September. Handweeding is done between March and June, and again in August and September. Harvesting begins around June 15, and lasts until mid-July. Pesticides commonly used by strawberry growers include: simazine and napropamide (herbicides); oxydemeton-methyl, endosulfan and carbofuran (insecticides); and captan (a fungicide). Other chemicals listed in the Berry Production Guide are used infrequently. This is for a variety of reasons, including lower effectiveness, higher cost, potential damage to crop, toxicity to beneficial insects (especially bees and predacious insects), or unusual requirements [e.g. clearing new ground for strawberry production, (Peters, 1994)].

The fungicide captan has probably the highest exposure potential for farmworkers. It may be applied from the first blossom on, throughout the harvest season, as protection against *Botrytis* (fruit rot). The Berry Production Guide (BCMAFF, 1994) warns of endemic *Botrytis*, and recommends prophylactic treatment of the crop every 7 days. The number of applications may be reduced if the weather is dry, or if a farmer is concerned about damaging the low-lying fruit with his spray rig, but up to 8 applications may be required according to the guide. A potential for exposure thus occurs when captan application is required before, or during, the harvesting season (Peters, 1994).

During the pre- and post-harvest hand weeding periods, several herbicide applications are made. Napropamide may be applied anytime from the end of harvest until first bloom the following year.

Simazine may be applied in the months immediately following harvest, but usually is the last operation of the Fall. Application of an herbicide is normally made after manual work is complete so that the workers will not disturb the herbicide. Exposure potential to herbicides is therefore lower, but may occur where herbicide application precedes or coincides with hand weeding (Peters, 1994).

Insecticides oxydemeton-methyl, endosulfan and carbofuran may be used between April and harvest, and then oxydemeton-methyl and carbofuran only, immediately following harvest. Normally, applications are not made after there are blossoms on the crop, because of the pesticides' toxicity to bees, and lack of access to crop due to developing fruit. However, applications of insecticides may rarely be made if required during the harvest season and exposure may occur in this situation. Omite (a miticide) is applied following harvest. Exposure may occur if this application precedes hand weeding (Peters, 1994).

1.4.2 Raspberries

The two major manual tasks in the raspberry crop are the pruning and training of canes, and harvesting. Pruning and training may occur anytime following harvest through to the beginning of March. Harvesting begins between June 20-25 and lasts until August 10-15. Pesticides commonly used by raspberry growers include: simazine, napropamide, paraquat, dichlobenil and oxyfluorfen (herbicides); diazinon, azinphos-methyl and *Bacillus thuringiensis* (insecticides); and captan and metalaxyl (fungicides). Other chemicals listed in the Berry Production Guide are used infrequently, for the same reasons indicated for strawberries (Peters, 1994).

Simazine, napropamide and paraquat are used in March and April to control weeds. Dichlobenil is used only for spot applications in November and December to treat hard-to-control weeds. Oxyfluorfen is utilized in April only, to remove early shoots. Usually, pruning and training would be complete prior to application of herbicides. Exposure potential is low due to timing of sprays, and because of the use of tools (hoes) for weeding (Peters, 1994).

Diazanon, azinphos-methyl and *Bacillus thuringiensis* are directed against early season pests and applied in the early spring through to first blossom. This would occur after pruning and training was completed.

Diazanon is used in February or March against the crown borer, but again, following pruning. Diazanon is not used after the start of flowering. Azinphos-methyl, *Bacillus thuringiensis* and other insecticides may be applied as required during the harvest season, and thus exposure is possible (Peters, 1994).

Captan is applied after first blossom to protect against fruit rot (*Botrytis)*. Farmers are warned that "losses may be assumed" without prophylactic treatment with fungicides (BCMAFF, 1994). At least 3 applications are recommended , every 7 - 10 days. Exposure is possible where captan is applied before or during harvesting period.

1.4.3 Blueberries

The two major manual tasks in the blueberry crop are the pruning and harvesting. Pruning occurs between December and February. Harvesting begins in July (as early as the first week) and can continue through to late August, depending on the cultivar grown. Pesticides commonly used by blueberry growers include: simazine, paraquat, dichlobenil and glyphospate (herbicides); malathion and deltamethrin (insecticides); and captan and triforine (fungicides). Other chemicals listed in the Berry Production Guide (BCMAFF, 1994) are used infrequently, as explained for strawberries (Hornage, 1994).

Pruning begins in December or January. The last pesticide application before this would probably have been captan in July or August (to protect fruit from *Botrytis)* or malathion during same period. Captan may have been applied as late as October if spraying was done for canker (Hornage, 1994).

Pruning continues into February, and is normally be completed by March 1. Dichlobenil may be applied during February, but generally after workers have finished in the field so that the herbicide is not disturbed. Captan may also be applied to protect against canker during February. Exposure is therefore possible if pruning is not complete before the dichlobenil or captan applications (Hornage, 1994).

There are few if any workers in the blueberry fields during March, April, May and June. However, several applications are made during this time, and residues could persist (see section 1.5). Triforine, a fungicide used against mummyberry infection, may be applied four or five times beginning in March through to late May (every 10-14 days). Also in March, Insecticides may be used against bud-eating insects. Simazine and

paraquat can be applied in April, and insecticides (e.g. *Bacillus thuringiensis*) are applied against for leafrollers, spanworms and winter moths. Also in April, captan sprays begin against *Botrytis* if the weather has been wet during blossom period. In May, there may be a need to spray for aphids and caterpillars with malathion. In June, captan is applied if weather has been wet during pre-ripening period, and through the ripening period every 7 to 10 days. Insecticides and herbicides are used as required (Hornage, 1994).

Captan applications continue in July if the weather has been wet. Malathion is used for aphids and scale insects. Harvesting begins, and lasts until August. There is a potential for exposure from recently applied fungicides, herbicides and insecticides. Exposure potential increases if captan or insecticide treatments are required during harvesting (Hornage, 1994). The Berry Production Guide (BCMAFF, 1994) again warns of losses due to *Botrytis* if prophylactic applications of fungicides are not made.

1.4.4 Summary of Pesticide Exposure Potential in Berry Crops

While there appears to be a potential for exposure to a wide number of pesticides, the occurrence of exposure appears to be modulated by a number of factor which limit applications around the time of worker activity. Captan is an important exception, however, because of its intensive application through the blossom and fruit development period, and during harvest, when the greatest number of workers are in the fields.

It is also important to note that these observations related by government agricultural experts represent a view of current, and perhaps ideal, practices. There is evidence to suggest, however, that past experience has been quite different, and that many berry farmers lacked adequate pesticide management skills to ensure worker safety (see section 1.8.1).

1.5 Persistence of Pesticide Residues, Re-entry Intervals

Modern pesticides are considerably less persistent in the environment than the older classes of chemical, particularly the organochlorines such as DDT, dieldrin, and aldrin. However, persistence of commonly used pesticides still ranges from days to weeks, and the degradation rate of the agent is highly variable, influenced by many factors including temperature, humidity, rainfall, light, pH, and microbial action

(Zweig *et al.*, 1985). The degradation products of the original pesticide formulation are referred to as the pesticide residue, and contain reduced portions of the original substance, metabolic products, chemical derivatives, and surviving portions of formulants such as diluents and solvents (Briggs, 1992).

To protect field workers from high levels of pesticide residues, "re-entry intervals" have been established by many jurisdictions. These are periods of time following application of a pesticide during which workers are prohibited from entering the treated area. These re-entry intervals or re-entry periods have been primarily designed to prevent acute intoxication by the pesticide. In British Columbia re-entry intervals are based on a three level acute toxicity scheme (Table 1 - 4, WCB, 1994). In the United States, however, studies have demonstrated that even following the Environmental Protection Agency (EPA) approved re-entry interval, toxic levels of residues may remain on foliage. US federal standards have a similar basis to the British Columbia standard, but episodes of illness and poisonings in California resulted in that state introducing stricter crop and chemical specific intervals (Krieger *et al.*, 1992). An EPA study in 1983 followed workers for several months, and found that while their exposure was always within the EPA's acceptable range, all individuals who had worked consistently showed significant cholinesterase suppression, which indicated organophosphate intoxication. Kidney, liver, respiratory and dermal problems were also reported (referenced in Mentzer and Villalba, 1988). It is thought that many workers, including those in British Columbia, suffer from the effects of pesticide intoxication, but that they are not diagnosed because they do not recognize the symptoms themselves, or their physicians do not recognize them (BCMA, 1982, Mobed *et al.*, 1992).

TABLE 1- 4. RE-ENTRY INTERVALS TO TREATED AREAS IN BRITISH COLUMBIA

Toxicity	Dermal LD_{50} (mg/Kg)	Oral LD_{50} (mg/Kg)	Re-entry interval (hours)[a]
Slightly toxic	> 1000	> 500	24
Moderately Toxic	200 - 1000	50 - 500	48
Very Toxic	0 - 200	0 - 50	48

[a]Unless superseded by pesticide label

As the re-entry interval is intended only to prevent acute intoxication, workers may still be continually exposed at sub-acute toxic levels for long periods. In fact, some of the low acute toxicity agents have long residual lives, and therefore exposure can be considerable. An example of this is the fungicide captan.

Captan is applied prophylactically on berry crops, and technical recommendations call for application every 7 -14 days (BCMAFF, 1994, Giles and Blewett, 1991). Yet captan has a demonstrated half-life (the point at which the original amount applied is reduced by one-half) of 13 days (Zweig *et al.*, 1985). Because of its low acute toxicity, farmworkers are permitted in to treated fields 24 hours after its application, and this means that these farmworkers may be continually exposed to low levels of captan residues.

Table 1-5 lists the pesticides identified as high exposure risks due to common use in the Fraser Valley berry industry, and their respective persistence and re-entry times. It can be seen that in virtually all cases persistence of the agent far exceeds the re-entry period. Thus, even if the risk of acute poisoning is diminished, there is potential for continuing contamination from remaining residues.

TABLE 1-5. PERSISTENCE OF HIGHER RISK PESTICIDES AND THEIR CORRESPONDING RE-ENTRY INTERVALS

Toxicity	Persistence (days)[a]	Re-entry interval (days)[b]
Simazine	60	1
Paraquat	>365[c]	2
Napropamide	70	1
Captan	13[d]	1
Triforine	21	1
Diazanon	40	2
Malathion	1	1
Endosulfan	50	2
Carbofuran	50	2

[a] Half lives; from Wauchope, *et al.*, 1993
[b]Unless superseded by pesticide label
[c]Paraquat binds tightly to the soil
[d]Zweig *et al.*, (1985)

1.6 Bioavailability

The primary route of pesticide residue exposure in farmworkers is dermal (Fenske and van Hemmen, 1994). Pesticide residues on the foliage, fruit, and in the surrounding soil are transferred to the skin or clothes of the worker as they touch or brush against the plants while performing manual tasks. The extent of contact with the treated surfaces is an important factor in determining the amount of residue which is transferred to the skin. This in turn is affected by the tasks being performed and the size and height of the foliage canopy.

For example, Kreiger *et al.* (1992), noted that there may be as much as a 60-fold difference in amount of residue transferred when comparing a simple "reach and pick" operation, such as harvesting strawberries, and the "search/reach/pick" tasks required in harvesting a tree fruit. The primary areas of the dermal contact are also be different in the two cases, with only the hand and arm affected in the reach/pick operations, but the whole upper body involved in the "search/reach/pick" operations. This was also demonstrated by Zweig *et al.* (1983), who demonstrated the difference in distribution of dermal exposure between strawberry harvesters and blueberry harvesters. Strawberry harvesters received on average 75% of their exposure on the hands, and a further 12% on the lower arms. The next most heavily exposed region was the lower leg which the authors presumed was as a result of walking through treated crops. Blueberry harvesters picking from bushes approximately 4 to 6 feet in height received a more homogeneous exposure over the whole body. Their exposure on the hands was 53.9%, the lower arms 16.8%, the lower legs 6.0%, chest and stomach 6.0%, upper arms 5.8%, back and shoulders 3.4%, and head and neck, 7.4%. This difference in distribution is important because dermal penetration rates vary over the skin surface (Grandjean, 1990). Generally, penetration rates can be ranked as follows: plantar pads > scrotal region > palmar pads > dorsum of hand > forehead and scalp > arms, legs and trunk. Therefore different picking styles, and foliage volume and height can influence the ultimate dose received by the worker.

A number of field studies have quantitatively investigated dermal exposures to farm workers (Wolfe and Durham, 1967, Zweig *et al.*, 1983, 1985, and Winterlin *et al., 1984*,). In a series of experiments conducted in California and Oregon between 1981 and 1983, Zweig *et al.* (1985) investigated dermal exposure among strawberry harvesters to captan. They found a range of dermal exposure between 4.7 mg/hr to 17.41 mg/hr . Table 1 - 6 summarizes their results.

High dermal exposure suffered by weeders (Table 1 - 6, experiment 3) was assumed to result from the large amounts of contaminated soil dust which was generated. Generally the dermal exposure appears to decrease with time following application, but worker contamination is still apparent several weeks after application.

No studies of this type have been conducted in British Columbia, even though differences in climate, environment and crop type might be expected to influence to dermal exposure rate of the workers.

TABLE 1 - 6. DERMAL CAPTAN EXPOSURE TO STRAWBERRY HARVESTERS AND WEEDERS

Experiment	Application rate (lb/acre)	Days post application	Subject activity	Number of subjects	Mean Dermal exposure (mg/hr)
1	2.5	3	Harvesters	6	16.37
2	2.5	4	Harvesters	15	17.41
3	2.5	4	Weeders	4	94.13
4	2.8	13	Harvesters	20	6.50
5	4.8	26	Harvesters	23	4.70
6	1.3	48	Harvesters	10	5.88

Adapted from Zweig *et al.*, (1983, 1985)

1.7 Existing Controls

Currently, farmworkers rely primarily on the farmer's implementation of a re-entry interval to protect them from exposure to pesticide residues. Re-entry intervals in British Columbia are regulated by the Workers Compensation Board. It is questionable, however, whether re-entry intervals are correctly implemented by local berry farmers. Twenty seven percent of berry farmers questioned in one survey did not know how to calculate a re-entry interval (Oja *et al.*, 1990), and in another survey of 90 berry farmers in 1984, the researcher noted that he had not seen a re-entry sign posted (a regulatory requirement) in any of his farm visits (Seehra, 1988). There has been little regulatory presence on farms to ensure that the proper procedures are followed; Oja *et al.*, reported only 25% of farmers reported having ever been inspected by the responsible authorities.

Distinct from the re-entry interval is the recommended "pre-harvest interval" (PHI). This interval is established to ensure that pesticide residues on fruit fall below a maximum residual limit, set by the Food and Drugs Act. These intervals are often longer than the re-entry interval, and thus may provide some coincidental protection to farmworkers. Captan, for example, has a PHI of 2 days on all crops, and diazinon 5 days on strawberries. These intervals would not afford protection to workers outside of harvest time.

There is no regulatory requirement for the use of personal protective equipment in pesticide-treated areas after the re-entry interval has elapsed. Most farmworkers work in street clothes, though some wear gloves. East Asian workers, especially females, are likely to keep their arms and legs covered, but even so, bare

hands and forearms are common. Clothing that is worn may not afford full or even partial protection (Grandjean, 1990). Clothing can actually enhance permeation of the chemical through the skin because it occludes the skin, preventing evaporation, and rubs the contaminant against the skin. Loose fitting clothing can behave as bellows, and cause air-borne contamination to be drawn inside clothing (van Hemmen, 1993). Studies have also noted that normal laundering of pesticide contaminated clothing may not be sufficient to remove the chemicals from the garments, and so low-level contamination may be prolonged. Gloves also cause occlusion which may lead to increased rates of absorption. Because material selection is critical to effective protection, it is unlikely anyway that a single clothing material will be an effective barrier in all exposure situations. Insufficient personal bathing may also increase ingestion and inhalation of residues.

1.8 Other Determinants Of Exposure

1.8.1 Physical Factors

Although the primary exposure route is through dermal contact with residues on foliage, fruit or soil, farmworkers may also be exposed by other modes, including: accidental direct spraying during pesticide application, pesticide drift from adjacent fields being sprayed, eating in fields with contaminated hands, eating recently picked fruits and vegetables, drinking water from pesticide containers, smoking with contaminated hands, and cross-contaminating the genital area with contaminated hands after elimination (Mentzer and Villalba, 1988). Lack of adequate washing facilities in the field would exacerbate these cross contamination problems, and potentially result in longer periods of absorption (Zahm and Blair, 1993).

Climate and local environmental conditions also influence exposure. Greater quantities of fungicides may be applied if the climate is wet and favours fungal growth. Similarly, hot, dry conditions may decrease fungicide requirements, but cause increases in insect pests and consequently in the amount of insecticides used. Rainfall can also modify exposure potential by washing residues from the foliage and fruit (Zweig *et al.*, 1985).

1.8.2 Farmworker Knowledge, Attitude

Farmworkers are often unaware of the risk of pesticides to their health. In one survey of farmworkers, 44% did not know how to get information on pesticides and 45% said their primary source of information was the farmer, who is often ill-equipped to give accurate information (Oja *et al.*, 1990). In general, the farmworkers language and literacy skills are poor. Oja *et al.* found 54% did not read English, and 22% were illiterate in both English and their native language. The authors noted that even these numbers may have been biased towards literacy because of undersampling of females, who are less likely to be literate than males.

Many recent immigrants to Canada turn to farmwork because they are unable to find other work. Older immigrants are ineligible for a Canadian pension until they have lived in Canada for 10 years, and with poor language skills, preventing them finding better work, many pick berries to support themselves (Parton, 1994). The farm-labour contract system in which they work has been widely criticized for its unfair treatment of farmworker employees. Farmworkers are paid on a piece rate system that often is below the province's minimum wage. From that, the labour contractor may take a cut, and may apply charges for transportation. Some workers have complained of not being paid until the end of a season, and others had not been paid at all, instead being offered a "job letter", which qualifies them for unemployment insurance, and which is necessary to sponsor a relative to come to Canada (Parton, 1994). Few farmworkers belong to unions, and are unlikely to complain individually of their working conditions for fear of losing their jobs.

This socio-economic background means that many farmworkers are ill-equipped to educate themselves about the hazards of pesticides, and ways of protecting themselves. Their low status, poor job prospects, high job insecurity, and their lack of power in labour relations, are vital factors in their willingness to accept risks (Seehra, 1988). Farmworkers in this position are less likely to report unsafe working conditions, and unlikely to refuse unsafe work if it occurred, even though that is a right they have under the Workers Compensation Act of British Columbia.

1.8.3 Farmer Knowledge and Attitudes

Farmers' knowledge and attitudes toward pesticide handling are important factors in the maintenance of a safe and healthy workplace. New Workers Compensation Board regulations (WCB, 1993) require all farmers applying moderately-toxic or very-toxic pesticides (see Table 1-4) to be certified in safe pesticide handling, but there are indications that historically many farmers have had a casual attitude toward pesticide use, and lacked training in the efficient and safe use of pesticides (Seehra, 1988). Seehra looked specifically at the East Asian growers who made up 47% of berry farmers in Fraser Valley area. He estimated that 60% had low functional literacy in English. A combination of lack of training and illiteracy contributed to problems such as incorrect use of equipment, and the application of improper volumes of pesticides, which could have lead to excessive pesticide residues and to environmental contamination. Seehra also observed that pest control failures which occurred for technical reasons were often attributed to ineffective pesticides. Growers would frequently then change their pest control practices, potentially resulting in the gross overdosing of crops.

The British Columbia Federation of Agriculture (BCFA), which was supposed to oversee the health and safety education of its member farmers, was chastised in a 1988 report to the WCB by independent consultants who found that the BCFA safety programs were "low key and without focus; [lacking] coordination, urgency, influence, commitment and proper funding" (Delloite, Haskins and Sells, 1988).

Sixty percent of farmers interviewed by Seehra (1988) considered pesticide use entailed no risk to themselves. Twenty five percent relied on chemical companies as their primary source of information on pesticides, rather than government fruit industry specialists, or the Berry Production Guide. The objectivity of the chemical companies' advice was questioned by Seehra. He also found widespread use of illegal pesticides, with 34% of surveyed growers using pesticides no longer registered in Canada (Seehra, 1988),

2. PESTICIDES AND GENOTOXICITY

2.1 Background

Pesticides are by definition toxic, designed to interfere with biological systems. Although most pesticides are developed to act specifically against a particular target species, all organisms share common physiologic, metabolic and structural processes. Given this, it is perhaps not surprising that many pesticides have been found to cause adverse health effects in humans.

The acute effects of pesticide intoxication are well documented, and generally understood. These include the inhibition of acetylcholinesterases by organophosphate and carbamate pesticides, which leads to the familiar neurotoxic symptoms produced by the over stimulation of cholinergic neurons. Clorophenoxy herbicides and pyrethroid insecticides have been associated with a temporary peripheral neuritis, or cutaneous parasthesia, and paraquat causes severe and progressive lung damage, and possibly renal failure and liver dysfunction (Lang, 1993).

The chronic effects of pesticides are much less well understood. The long latency period between exposure and health outcome makes it more difficult to identify cause and effect relationships. However neurologic, immunologic, reproductive, developmental and carcinogenic effects have all been identified (Lang, 1993). These last three all share at least one common etiology - genotoxicity. The remainder of the chapter will be devoted to genotoxicity.

2.2 Definitions and Mechanisms

The term 'genotoxic' describes agents which alter, or 'mutate', the base-pair (nucleotide) sequence of the genetic material (DNA) in cells, as well as agents which change the chromosome number. Genotoxins may act directly upon DNA by substituting, adding or deleting nucleotides. A genotoxin can modify the DNA in such a way that normal cell maintenance such as DNA replication and repair is upset, and damaged DNA results. Further, genotoxins can modify the cellular mechanisms responsible for normal cell division, resulting in unbalanced DNA segregation to progeny cells (Brusick, 1987).

Mutations are categorized into three types. "Gene mutations" occur at the nucleotide level, and consist of basepair substitutions and frameshift mutations. A basepair substitution, such as a G-C pair replacing an A-T pair, can lead to truncated or modified messenger RNA and subsequent protein abnormalities. They can also lead to changes in the regulation of gene activity. Loss or gain of one or more basepairs causes the tri-nucleotide transcription sequence, or "reading frame" to become out of register (a frameshift). Gene mutations can only be detected by molecular techniques (e.g. restriction length fragment polymorphism analysis), or by classical genetic analysis identifying mutant phenotypes.

The second type of mutation, "chromosomal mutations", are gross changes to the structure or organization of a chromosome. This includes deletions of large fragments of DNA, fragment inversions, or translocations, where DNA is exchanged between two non-homologous chromosomes. Chromosome mutations may result in the loss of genetic material, or abnormal juxtaposition of genes, such that inappropriate genetic control may be established causing the abnormal activation or inactivation of genes. Genotoxins which cause DNA breakage of the kind associated with chromosomal rearrangement are called "clastogens". This type of mutation may be visible under a light microscope, and is therefore amenable to cytogenetic assay.

A third type of mutation, the "numerical chromosomal mutation", results in the gain or loss of whole chromosomes. Gain or loss of individual chromosomes is called "aneuploidy". Gain of a single chromosome results in a "trisomic" cell; loss of a chromosome results in a "monosomic" cell. Aneuploidogens are thought to act upon the protein components of the mitotic spindle, which normally perform the segregation of chromosomes during cell division. It is possible that mutations in the genes coding for the mitotic apparatus could also result in aneuploidy. This kind of mutation is also visible under the light microscope, and amenable to cytogenetic study.

2.3 Health Outcomes Associated With Genetic Toxicity

Mutations have been associated with various adverse health effects, including heritable mutations, birth defects, reproductive disorders, and cancer (Brusick, 1987). In addition, certain non-neoplastic diseases have also been linked to somatic mutation (Benditt, 1977).

When a molecule of DNA is damaged, there is a high probability that the mutation will occur in an unused portion of DNA, as over 97% of the human genome is not transcribed into RNA and protein. Even if the mutation does occur in an active gene, then disruption of the gene product will probably lead to cell death, and the loss of the mutation, because it will not be passed to progeny cells. In some cases, however, the mutation is compatible with cell life and division, and if so the location of the affected cell in the body in part determines the final outcome.

If the affected cell is a germ cell, then the outcome may be a heritable mutation, which can affect future generations, or may be a reproductive disorder in the exposed individual, resulting in sterility, or loss of a fetus. If the mutation is in a somatic cell (a somatic mutation), then only the individual exposed to the genotoxin is affected. In cases where the cell survives the mutation there may be resulting loss of growth regulation, and uncontrolled cell proliferation leading to a neoplasm.

2.3.1 Reproductive Outcomes

Heritable mutations may be expressed immediately in off-spring if they are dominant, or remain concealed until future generations when a homozygous (both copies of gene identical) recessive child is born of two heterozygous (each copy of gene different) parents. For example, achondroplasia, a form of dwarfism, is a dominant trait which is a new mutation in 80% of cases. Sickle cell anemia, on the other hand, is caused by a protein deficiency which is clinically expressed only in homozygotes. Therefore introduction of a new mutation may not be detected until there are homozygous recessive offspring of two heterozygotes. Recessive mutations are difficult to detect except in sex-linked recessives, which behave like dominant mutations.

Loss of a fetus, and developmental abnormalities, are often due to chromosomal aberrations. Chromosomal and numerical mutations are associated with over 60 identifiable syndromes in humans, and are present in 0.7% of all live births, 2.0% of pregnancies in women over 35 years of age, and 50% of all first trimester abortions (Thompson *et al.*, 1991).

The most common clinically important mutation is aneuploidy, and perhaps the most familiar example is trisomy 21, or Down syndrome. Trisomy 13 and 18 also occur, but are very rare. They are compatible with life, but have very poor survival rates. Almost without exception, other trisomies are lethal to the fetus. Trisomy for chromosome 1 is not even seen in abortuses, indicating the severity the condition. Some teratogens are genotoxins which cause somatic mutations in the embryo, although the majority of teratogens are non-genotoxic (Brusick, 1987).

Clastogenic breakage resulting in loss of genetic material also causes a variety of syndromes. Examples include cri-du-chat (CDC) syndrome, which results in mental retardation, hypotonia, microcephaly, hypertelorism, low-set or poorly developed ears and heart abnormalities. CDC is thought to have an incidence as high as 1.5 per 1000 among the mentally retarded. CDC is caused by a partial deletion of the small arm of chromosome 5. Prader-Willi syndrome, which is characterized by mental retardation, obesity, short stature, polyphagia, small hands and feet, and hypogonadism, is caused by a large deletion in the long arm of chromosome 15 (Thompson *et al*., 1991). Other abnormalities, such as insertions, inversions, duplications and ring chromosomes are also associated with various congenital malformations (Therman, 1986).

2.3.2 Neoplastic Outcomes

Compton *et al.*, (1991) described several lines of evidence that associate mutation with cancer: (a) stable, consistent DNA alterations are associated with certain cancers. For example in chronic myelogenous leukemia (CML), 95% of adult cases present a supernumerary chromosome which is a translocation product of chromosomes 9 and 22 (Yunis, 1983). (b) Syndromes involving deficiencies in DNA replication or repair, such as ataxia telangiectasia, Bloom syndrome and xeroderma pigmentosum, are all characterized by defects in the DNA repair process, and all have an associated elevated risk of cancer. (c) Tumours are clonal; that is, all tumour cells are progeny of a single transformed cell. (d) Most carcinogens have been demonstrated to be mutagens. (e) DNA from tumour cells can be introduced to normal cells, which are then transformed (i.e. they begin to exhibit the characteristics of the tumour cell). (f) Cancers often arise in

rapidly dividing tissues, where there may be insufficient time to adequately repair DNA damage before cell division, so the mutation becomes fixed in the progeny cells.

Chromosomal rearrangements are believed to play a central role in the development of many neoplasms. In addition to the CML example given above, 85 - 95 % of follicular lymphomas have the same chromosomal translocation involving chromosomes 14 and 18, and fully 100% of Burkitt lymphoma patients present with a translocation between chromosome 8 and either chromosome 14 (80%), chromosome 22 (15%), or chromosome 2(5%). There are many other consistent chromosomal rearrangements associated with particular cancers (Croce, 1987).

In all of these examples, the translocation results in the juxtaposition of a proto-oncogene with an actively transcribed region of DNA, which in these examples is an immunoglobulin gene. A proto-oncogene is a gene whose normal function is to regulate cell growth and differentiation. A mutation in the proto-oncogene, in its regulatory region, or as in this example, a rearrangement which places the gene under the control of another genetic region, can lead to inappropriate proto-oncogene expression and to abnormal growth and differentiation (Weinstein, 1988). Interestingly, in lymphoid neoplasias, many of these rearrangements involve regions of the immunoglobulin genes which undergo intrachromosomal rearrangement as part of the normal generation of the unique variable regions of immunoglobulins and T-cell receptors. Some researchers have proposed that the lymphocyte recombination mechanism responsible for the normal immunoglobulin gene rearrangement could be solely responsible for these interchromosomal rearrangements. However, it is also considered likely that random breakage and reunion - as expected with random mutation events - may be sufficient to cause the clustering of breakpoints and rearrangements in one particular region, providing the rearrangement gave some growth advantage to the progeny cells of the tumour cell (Tycko and Sklar, 1990).

2.4 Evidence of Genotoxicity in Pesticides

Evidence for the genotoxicity of pesticides come from both epidemiological and experimental studies. Despite the large amount of work done, however, the overall evidence remains equivocal, and few pesticides have been identified as confirmed human carcinogens. Epidemiological evidence shows some

consistencies, but is generally weak. Experimental studies in animals and lower organisms is stronger, but suffers from problems of extrapolating risk to humans. A summary of both avenues of research follows.

2.4.1 Epidemiological Evidence of Pesticide Genotoxicity

Maroni and Fait (1993) reviewed over 440 papers published between 1975 and 1991 which dealt with health effects associated with pesticides. Despite this volume of research, the evidence for the carcinogenicity of any pesticide or pesticide class remains equivocal. Methodologic problems have weakened these studies, including uncertainty regarding the reliability and validity of exposure assessments, size of exposed groups, and the effects of unknown confounders (Blair and Zahm, 1990). Farmers are geographically widely dispersed, and have used combinations of agents which changed over the years; because of the latencies of most cancers, exposures have to be recalled from 3 or 4 decades previously. There are many confounding exposures - including other agricultural chemicals, zoonotic viruses, organic dusts, etc.. There can also problems with misclassification of disease, as diagnosis in rural areas is not as likely to be as accurate as in urban (Blair *et al*., 1985).

TABLE 2 - 1. HIGH-RISK CANCERS IN MORTALITY SURVEYS OF FARMERS

	No. of studies	No. of studies with RR > 1.0	No. of significant studies	Range of RR's
Lip	9	9	5	1.3 - 3.1
Melanoma	12	8	3	0.5 - 6.3
Leukemia	21	12	3	0.3 - 2.0
Non-Hodgkin's Lymphoma	21	11	3	0.6 - 2.6
Multiple Myeloma	16	12	4	0.4 - 3.1
Hodgkin's disease	13	10	1	0.6 - 1.5
Soft Tissue Sarcoma	9	6	0	0.9 - 1.5
Brain	20	15	2	0.7 -6.5
Stomach	23	12	8	0.5 - 1.7
Prostate	24	17	10	0.9 - 2.0

In general, epidemiological studies have revealed only small increases in relative risks, but for certain cancers the evidence is consistent, although the degree of risk associated may be variable. Generally, farmers are considered remarkably healthy, and Blair and Zahm (1991) reported significant deficits in farmers for all causes of death combined, heart disease and cancer. This is probably due largely to low smoking rates among farmers, and perhaps physical activity, which is thought to be inversely related to

some cancers. Other factors such as diet and a rural environment may also contribute to this good health. Relative risks for cancer of the esophagus, colon, bladder, lung, rectum, liver, pancreas, testis, kidney, nose and nasal cavities are generally lower than one, or if greater than one, rarely statistically significant. However, several cancers are consistently elevated among farmers, including leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and cancers of the skin, lip, brain, prostate, stomach and connective tissues. A review of these findings, as summarized by Weisenburger (1993), is presented in Table 2-1.

It has been difficult in most studies to investigate risks associated with specific pesticides, because of mixed exposures within the exposed group, fluctuation of the chemicals and chemical classes used by farmers over time, and problems of accurate recall. However, some studies have taken advantage of situations where more stable or better exposure information was available to gauge the risks associated with classes of pesticides, or specific chemicals. Most studies have been conducted in grain and fiber producers, which are non-labour intensive, and use larger quantities of herbicides than insecticides and fungicides. Following, however, is a review of studies which have included pesticide specific investigations of some of the agents used commonly in the Fraser Valley.

Insecticides have been associated with non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), soft-tissue sarcoma (STS), and leukemia. In Nebraska, the use of organophosphate (OP) insecticides by farmers resulted in a 2.4 fold increase in risk for NHL, which increased to 3.0 fold in those using for more than 21 days of the year (Weisenburger, 1993). The same class of insecticide (OP) was associated with a odds ratio (OR) of 1.9 for NHL in Eastern Nebraska, and an OR of 1.8 for carbamate insecticides (Weisenburger, 1990). In a 1990 study, Brown *et al*. found elevated relative risks (RR) for leukemia associated with use of several insecticides, including the organophosphates crotoxyphos (RR=11.1), dichlorvos (RR=2.0), famphur (RR=2.2), and pyrethrins (RR=3.7). Kansas farmers exposed to insecticides showed a slightly elevated risk of STS (RR=1.3) which increased in those exposed to animal insecticides (RR=1.6). The risk continued to increase with increasing time since first use, rising to a relative risk of 4.9 in individuals who used animal insecticides prior to 1945 (Blair and Zahm, 1991).

Hoar *et al.*, (1986) found an elevated risk of NHL (OR=2.1) in individuals who had treated seeds with fungicides. Brown *et al.* (1990), found risks for acute non-lymphocytic leukemia were elevated (OR=2.3) with use of any fungicide. They also noted an increase in all leukemia (OR=1.5) for users of the fungicide captan, although the increase was not statistically significant (95% CI 0.7 - 3.3).

The triazine herbicides atrazine and simazine have been associated with NHL, ovarian cancer and multiple myeloma. Women exposed to these herbicides which were at a 2.7 - fold risk of developing ovarian cancer, and the risk was found to be dose-related to duration and probability of exposure (Donna *et al.*, 1989). Eastern Nebraska farmers exposed to atrazine were at an elevated risk of NHL (OR 1.4) which was also duration-related, rising to 2.0 with 21+ years of exposure. NHL risk was also elevated among Kansas farmers who had ever used triazine herbicides (OR=2.5, Hoar, *et al.*, 1986). Among licensed pesticide applicators in the Netherlands, an elevated risk for multiple myeloma was found (SMR = 815, 95% CI 164-2382). From municipal records, the researchers determined that in 1980, 82.6% of pesticides applied were herbicides, and 29% of those were triazine herbicides, simazine and atrazine (Swaen *et al.*, 1992).

2.5 Seasonal Farmwork and Cancer

Zahm and Blair have recently reviewed the research into cancer among seasonal and migrant farmworkers (Zahm and Blair, 1993). They noted that farmworkers had a different exposure profile to that of farmers, who have been the subject of the majority of epidemiological cancer studies to date. Farmers were more likely to have additional exposures to fuels, solvents, welding fume, fumigants, and to animal confinement facilities; the majority of their pesticide exposure was probably while mixing and applying. Farmworkers on the other hand got most of their exposure from contact with foliage while doing manual labour in farm fields. While there were similarities in the cancer risks experienced by the farmworkers and the farmers, there were also differences. Farmworkers experienced similar elevations in multiple myeloma, and cancer of the stomach, prostate and testis. However, farmworkers differed by experiencing excesses for cancers of the buccal cavity, pharynx, lung and liver. Non-significant excesses were also reported for cancers of the bone, connective tissue, skin, larynx, esophagus and rectum. Cancer of the cervix was elevated in one study (Stubbs *et al.*, 1984). Epidemiological studies of farmworkers suffer from many of the same problems as

those of farmers, but in addition, farmworkers are probably harder to follow-up, because there are few large enough employers, and few records kept. Farmworkers are also highly likely to be misclassified with respect to occupation on a death certificate, as there is no accepted definition of farmworker; in the US many get coded as farmers.

2.6 Farmwork and Cancer in British Columbia

British Columbia farmers and farm managers have been shown to have similar excesses of leukemia, and cancers of the stomach, lip, and prostate, as have been demonstrated in other populations of farmers (Gallagher *et al.*, 1989). In the same proportionate mortality study, farm labourers did not show any elevated risk of cancer. The authors believed elevated levels may have been suppressed by a high PMR for accidental death. Farm labourers also showed excesses in death from tuberculosis, pneumonia, burns and drowning. The authors concluded that the overall pattern of mortality was typical of low socio-economic status occupations. This study is not sufficient to conclude an absence of risk in this population, however. First, the data presented was for males only, and currently, females make up the majority of the farmworker work force. Secondly, many farmworkers, particularly the younger ones, have other work or move on to better employment, and therefore it is unlikely that their death certificate would record "farmworker" as their usual occupation. Seasonal employment in an individual's past might not be considered pertinent for inclusion on a death certificate.

2.7 Epidemiological Evidence Of Other Genotoxic Outcomes

Several attempts have been made to assess the effect of exposure to pesticides on reproductive outcomes (Nurminen *et al.*, 1995, Restrepo *et al.*, 1990, Schwartz *et al.*, 1986, and Gordon and Shy, 1981).

Nurminen *et al.* (1995), examined over 1,300 cases of congenital malformations, and interviewed cases and controls with respect to pesticide exposure and potential confounders. They found an odds ratio of 1.4 (CI 95% 0.9-2.0) for structural defects in the offspring of women working in agricultural work, and an odds ratio (OR) of 1.9 (CI 95% 1.1-3.5) for orofacial clefts. This supported an earlier finding of Gordon and Shy (1981) who reported an OR of 1.7 - 2.9 for orofacial clefts among offspring of exposed individuals. This

earlier study used a surrogate exposure measure (area of residence, comparing areas of high and low chemical usage), and a surrogate source of outcome data (national health statistics).

Schwartz *et al.* (1986), reported on the outcome of 2,523 births at a hospital serving an agricultural region in California, situated in a county with the highest use of restricted pesticides in the state. They reported an excess of limb reduction deficits in children of agricultural workers (5.07/1000 live births) when compared to the offspring of non-agricultural workers (2.22/1000 live births). However, the study suffered from small number of cases, and a large number of outcomes variables, raising the possibility that the association was one of chance. There also was confounding by age, ethnicity, and socio-economic status, which meant the association could also have been explained by economic or medical factors. Restrepo *et al.*, (1990) interviewed 8,867 workers in the Colombian floriculture industry and obtained detailed reproductive histories. They compared pregnancy outcomes before and after exposure, and found elevations of spontaneous abortions, premature deliveries and malformations following exposure. Interestingly, captan was the most heavily used pesticide in the workplaces. However the authors reported several problems with study design, including serious misclassification of exposure and outcome.

2.8 Experimental Evidence

There is considerable experimental evidence in animals and microbial systems linking pesticides to genotoxicity. Garrett *et al.* (1986) found that 54% of the 65 agents he tested were positive for mutation in bacterial assays. Börzsönyi *et al.* (1984) reviewed data on 83 pesticides, and concluded that 20% of them were genotoxic based on their positive results in short term tests. Three more were concluded to be suspect genotoxins. In a survey of 228 pesticides, Moriya *et al.* (1983), found 50 to be mutagenic, including 25 out of 88 insecticides, 20 out of 60 fungicides, and 3 out of 62 herbicides.

Since the 1950's animal studies have also been used widely to evaluate the safety of chemicals. Carcinogenicity tests in a minimum of 2 species (both sexes) are a requirement of pesticide registration, and in addition, independent laboratories study pesticides for non-registration purposes. In a collection of 51 pesticides examined by the US National Toxicology Program, 22% were positive in at least one species (Blair *et al.*, 1990). Positive results are found in chemical in every class of pesticide, including

organophosphate, organochlorine, pyrethroid, carbamate, and many other miscellaneous compounds (Weisenburger, 1993).

Of the pesticides identified in chapter one as having the highest potential for farmworker exposure, several have been demonstrated to be genotoxic. Malathion, for example, was the subject of a recent genotoxicity review by Flessel *et al.*, (1993). The review concluded that both malathion and its major metabolite malaoxon were capable of producing chromosomal changes, although genotoxic effects in humans were insufficiently studied. Carbofuran was found to be mutagenic in a bacterial mutation study, and in a *in vitro* mammalian cell assay, though not in other tests (Gupta, 1994). The nitroso-derivative of carbofuran, nitroso-carbofuran, was also shown to be capable of producing chromosomal damage in mammalian cells *in vitro*. The insecticide endosulfan has been shown to cause elevations in the frequency of chromosomal aberrations in mouse bone marrow cells when fed in an *in vivo* experiment (Khan and Sinha, 1993), and the organophosphate diazinon has been shown to cause significant increases in mutation frequency in another mammalian assay (McGregor *et al.*, 1988).

Captan has been the subject of many studies of mutagenic activity. Because it probably represents the greatest exposure hazard to farmworkers in the Fraser Valley, evidence of its genotoxicity will be reviewed in more detail.

2.8.1 Genotoxicity of Captan

Captan has proven highly mutagenic in bacterial test systems. Of the 228 pesticides reviewed by Moriya *et al.*, (1983) in a panel of bacterial mutation assays, captan was found to be the most mutagenic, being positive in all 6 tests of their battery. Legator *et al.*, (1969) demonstrated a dose-dependent increase in mutants in a bacterial reversion assay. Börzsönyi *et al.*, (1984) reviewed the various test data for captan and noted that it was positive for DNA damage, positive in repair and recombination testing, equivocally positive for gene mutation, but equivocally negative for chromosomal mutation. They concluded, however, that captan was definitely a genotoxic carcinogen.

In a 1975 summary of its genotoxic effects, Bridges concluded that captan is a "potent mutagen in both prokaryotic and eukaryotic cells, that it produces dominant lethal mutations in mice and rats, and heritable second generation effects in mice (probably due to polygenic point mutations)"; and that it also produced chromosomal aberrations in cultured mammalian cells. He went on to note that captan had been shown to form DNA adducts both *in vitro* and *in vivo* (the primary adduct is 7-trichloromethylsulphenyl guanine), which perhaps gives a indication of its mechanism of action.

Bridges also noted that captan appears to have two mutagenic components: a volatile mutagen which is active in petri-plate experiments, and thought probably to be thiocarbonyl chloride; and a non-volatile mutagen. Only the non-volatile component is thought to be active in an *in vivo* genotoxic situation, because the volatile component is highly unstable in aqueous solutions.

In a review of published animal carcinogenicity studies of captan, Reuber (1989), concluded that captan is an unequivocal carcinogen in mice and rats. He found increased malignant neoplasms in both low and high-dosed male and female rats. He also found elevated levels of duodenal neoplasms in male mice; these are neoplasms which are normally extremely rare in animals. Hasegawa *et al.* (1993) utilized a medium term carcinogenic assay which looked for early neoplastic lesions to evaluate the carcinogenic effects of captan. The assay was conducted in multiple organs in rats. They concluded that captan induced cancer of the kidney, stomach and thyroid gland.

IARC (1991) classified captan as a group 3 carcinogen indicating that it is not classifiable as a human carcinogen, but they noted limited evidence of carcinogenicity available for animals. Quest *et al.* (1993), however, noted that IARC had access only to published rodent data. His group re-evaluated captan, and two structurally related fungicides, folpet and captafol, using US Environmental Protection Agency (EPA) data from three unpublished studies and using EPA guidelines for risk assessment. Their conclusion was that captan (and the other two agents) should be categorized as probable human carcinogens (EPA class B2), based on their ability to produce malignant tumours in multiple studies. Captan was found to induce neoplasms of the intestinal tract in mice, and to induce renal carcinomas and adenomas and uterine tumours in rats. The investigators also noted structure-activity relationship (SAR) between the three related

fungicides: captan and folpet, which share a trichloromethylthio moiety, both produced tumours of the gastrointestinal tract; and captan and captafol, which share a partially saturated tetrahydropthalimide ring and were both associated with renal tumour.

Tezuka *et al.* (1980) studied pesticide-induced chromosomal damage in mammalian cells *in vitro*. They considered several pesticides, including captan, which they considered to be potent mutagens based on prior microbial reversion tests. They found that captan induce both chromosomal aberrations and sister chromatid exchanges, but not polyploidy, in hamster cells. The sister chromatid exchanges were induced in a dose-dependent fashion. Vigfusson and Vyse (1980) investigated captan's ability to induce sister chromatid exchange in human lymphocytes *in vitro*. They were only able to test low concentrations, because higher concentrations were toxic to the cell, but significant elevations over controls were observed.

The ability of captan to produce micronuclei in mammals has also been studied. Mice treated with captan showed dose-dependent increases in both micronuclei and chromosome aberrations in bone marrow cells (Feng and Lin, 1987). Captan also induced chromosomal aberrations in the spermatagonia and primary spermatocytes of the mice.

Finally, captan has been demonstrated to have teratogenic effects in animals. Non-viable embryos and hatched chicks from chicken eggs treated with captan were examined for skeletal abnormalities (Verrett *et al.*, 1969). Captan was found to have high teratogenic activity in the embryos, and was specifically associated with amelia (missing limb) and phocomelia (shortened limb). This is interesting because captan is structurally related to the drug thalidomide which was shown to be a powerful teratogen in humans, and was also strongly phocomelic (Sutton, 1988).

2.9 Evidence From In-Vivo Studies In Pesticide Workers

Early evidence of the genotoxic potential of pesticides in human studies was published by Yoder *et al.* in 1973, who studied chromosome aberrations (gaps and breaks) in a group of 42 pesticide applicators, and a comparison group of 16 matched unexposed individuals. They obtained two blood samples from each participant, one prior to the start of the pesticide application season, and another at its peak. The exposed

group showed an elevation in the frequency of chromosome damage which was not apparent in the controls. Several of the pesticides used by the exposed group are also widely used in the berry industry in British Columbia including diazinon, malathion and endosulfan.

Rupa *et al.* (1988), reported on 25 male pesticide applicators working in vegetable gardens who routinely sprayed pesticides, including malathion, for 8 hours a day without precautionary measures. They noted significant elevations in both chromosome aberrations and sister chromatid exchanges compared to the non-exposed control groups. In 1991, The same authors (Rupa *et al.*, 1991) demonstrated significantly elevated levels of chromosome aberrations in a group of 26 pesticide applicators working in cotton fields, when compared to a matched control group.

Lipkowitz *et al.* (1992), recently demonstrated an increase in the frequency of abnormal antigen-receptor gene recombinations in agricultural workers. The antigen-receptor gene normally undergoes rearrangement in all lymphocytes as part of the basis of antibody diversity. Abnormal rearrangements between non-homologous chromosomes occur at a low background level, but this level was found to be increased 50 to 100-fold in patients with ataxia telangiectasia who are known to be at elevated risk of cancer. The researchers found that agricultural workers exposed to a variety of pesticides, including captan and malathion, had a 10 - 20-fold increase in these abnormal rearrangements in peripheral blood lymphocytes. This is especially interesting because abnormal rearrangement of these immune receptor genes is thought to be associated with various lymphoid malignancies, and these are also cancers seen in excess in agricultural populations.

Finally Cuneo *et al.* (1992), reported that individuals with acute myeloid leukemia (AML) who have a history of exposure to pesticides or organic solvents, may have a distinct cytogenetic and clinicopathological presentation. Seventy patients diagnosed with AML were categorized into three exposure groups: pesticide exposed, organic solvent exposed, and un-exposed. Cuneo *et al.* found that the three groups varied significantly with respect to morphologic, immunologic and cytogenetic findings. The exposed groups had a greater occurence of chromosome abnormalities involving chromosome 5 and/or 7 than the unexposed group, which tended to have a greater occurence of the chromosome aberrations usually

associated with AML (translocations involving chromosomes 8, 9, 11, 15, 17, 21). An immunological marker (CD34) was expressed at a much higher frequency in cells of the exposed group (80%) than the unexposed group (22%). Finally, the remission rate in the exposed group was much lower than in the unexposed group, with mean survival times of 2 and 9 months, respectively, although prognostic indicators other than chemical exposure were not different among the three groups. The authors noted that the chromosome abnormalities in the exposed group were similar to those seen in secondary leukemia, a condition brought about by the action of cytotoxic drugs and radiation used in tumour therapy, which also suggests a distinct disease type for leukemia's of a chemical-exposure origin.

3. THE MICRONUCLEUS ASSAY

3.1 Background

The use of biological markers as indices of pre-clinical effects is increasing in epidemiological studies. In particular, genetic markers are being used to link carcinogen exposure to initiating events in the formation of tumours, by the detection of early biological responses (Wogan, 1992). Techniques such as the micronucleus assay permit "the precision of laboratory methods to quantify carcinogenic dose or pre-clinical response in humans with the relevance and rigour of analytic epidemiology" (Perera, 1987).

Traditional epidemiologic methods for studying cancer causation have been hampered by the long latency of most neoplastic diseases. This intervening period complicates follow up of individuals, makes accurate exposure assessment difficult, and does not permit intervention before disease is established. Monitoring an early, pre-clinical effect can help overcome these problems. Because the monitoring is concurrent with, or soon after exposure, this can improve the accuracy of exposure assessment, and increase the power of the study to determine causal relationships (Perera, 1987). The much reduced follow-up time also means that it can be easier to locate exposed individuals. This is particularly important in the study of seasonal or migrant workers, such as farmworkers, because of the transient and temporary nature of their work. Detection of subclinical disease permits early intervention, and may actually lead to disease prevention. Other potential benefits include the identification of susceptible individuals in the presence of adverse exposure, more homogeneous classification of disease, and increased knowledge of disease pathogenesis (Wogan, 1992).

Other health effects which do not necessarily have long latencies, but which have traditionally been hard to study include infertility, spontaneous abortion, developmental disorders, and teratogenesis. Spontaneous abortion, for example, is difficult to quantify because many spontaneous abortions occur before the affected individuals realize that they are pregnant. Markers of genetic damage may be used in investigations of these adverse health effects which all share at least one common etiology (Perera, 1987). In such studies, it is not necessary to obtain germline tissue, surrogate tissue can be used, usually peripheral blood lymphocytes, which are used as an index of the genotoxic effect at the true receptor (Yager, 1992).

The study of pre-clinical lesions may also increase a study's statistical power. Because the progression from pre-clinical lesion to tumour has a very low probability, lesions are far more common than tumours; studying a more frequent outcome allows the detection of relationships with fewer subjects (Perera, 1987).

Biomonitoring studies of genotoxic endpoints in humans also offer improvements in risk assessment. Studies of genotoxicity in humans reflect the influence of the body's metabolic activation and detoxification systems and have more relevance than *in vitro* and *in vivo* animal studies, which suffer from the problems of extrapolation to the human, insensitivity to small increases in dose, and incomplete simulation of the human response (Vine, 1990, Perera, 1987).

A weakness of many biomonitoring studies is that they are cross-sectional in design, and therefore it can be difficult to establish a cause-effect relationship. As well, although a large number of studies have already been conducted, many of them have concentrated on the molecular aspects of the assay, and have neglected fundamental aspects of epidemiological studies which would improve their design, power, and usefulness. These deficiencies have included inadequate sample sizes, inappropriate control populations, and inattention to potential confounding variables (Perera, 1987).

With respect to the study of genotoxic effects of pesticides, however, the study of biomarkers of early effect has several benefits. First, the exposure is generally a complex mixture of multiple chemicals, primarily pesticides, but also including pesticide derivatives, metabolites, contaminants, and formulants. The study of a pre-clinical effect integrates all of these exposures, while other genotoxic assays such as DNA adducts are highly specific to a single mutagen. As well, although dermal exposure predominates, other routes of exposure still contribute to the total dose, and the study of a biomarker integrates all of these exposures. The benefits of immediate feedback in a population such as the farmworkers who would otherwise be very difficult to follow are obvious.

3.2 Genotoxicity Assays

There are two categories of human genotoxicity assays: those that measure change at the gross chromosomal structure level, and those that measure change at the level of the gene (Compton *et al.*, 1991).

Gene mutation assays must be able to identify cells containing a mutant gene against a background of normal cells. Such cells are rare; to find them, assays use mutations in genes which are easily recognized because of the absence or change in their protein product. For example, the Hb-S assay uses fluorescently-labeled antibodies which react with mutated hemoglobin. When a treated blood sample is viewed under a microscope, only those rare cells which have undergone a mutation in the Hb gene will fluoresce. Other gene mutation assays include the *hprt* assay, the glycophorin-A assay, and the HLA-A assay. None of these assays has been widely exploited in population monitoring.

Assays which detect change at the chromosome level include the chromosome aberration (CA) assay, the sister-chromatid exchange (SCE) assay, and the micronucleus (MN) assay. The CA assay enumerates chromosome damage in metaphase cells using light microscopy. Damage is classified by the type of aberration, whether overt breakage, rearrangement, or missing chromosome (Sorsa and Yager, 1987). CA's are most sensitive to ionizing radiation, and to other agents which break the DNA strand directly. The CA assay is technically demanding, and therefore expensive, time-consuming and difficult to learn (Yager, 1992). SCE's are thought to be more sensitive to chemical mutagens, as they are more efficiently induced by substances that interfere with the DNA structure by alkylating bases, or by intercalating between the bases in the double helix. SCE's are formed by the exchange of both DNA strands between sister chromatids, and are thought to be caused by errors in replication (Wolff, 1982). The biological significance of SCE's is not understood, however.

MN arise from chromosomes - either whole or fragments - which lag behind at the metaphase plate and are not incorporated into daughter nuclei during anaphase. They are therefore the result of either breaks in chromosomes or damage to the mitotic mechanism. The MN assay, unlike CA's and SCE's, does not require metaphase spreads for analysis, and is therefore simpler. In addition, scoring is more objective than the scoring of CA's and so it can be undertaken by less experienced scorers, and is relatively fast. The biological significance of MN is more readily apparent than for SCE's. See section 3.4.

3.3 Principles of the Micronucleus Assay

3.3.1 Micronuclei Formation

Normally when a cell divides, DNA which replicated at an earlier stage of the cell cycle is equally divided between the two daughter cells. During mitosis, the replicated chromosomes, each consisting of two identical "sister" chromatids, align at the metaphase plate. Each chromosome is attached to the mitotic spindle apparatus at its centromere. The spindle apparatus pulls one of each pair of the duplicate sister chromatids to opposite poles of the dividing cells, thus ensuring both daughter cells receives an exact duplicate of the genetic material. During subsequent phases of mitosis, each daughter cell will normally form an identical nucleus by enclosing the chromatids (now distinct chromosomes) in a nuclear membrane.

Micronuclei are formed when acentric chromatid or chromosomal fragments (fragments lacking a centromere), fail to migrate to the spindle poles, and are not incorporated into the daughter nuclei. Whole chromosomes may also lag, and be excluded. In either case, this extra-nuclear DNA forms an accessory nuclear body which is surrounded by a separate nuclear membrane, and which is visually distinct from the main nucleus. Figure 3-1 illustrates a typical micronucleated cell. Because micronuclei contain a fraction of the DNA of the main nucleus, they are a fraction of the size (generally between 1/16 and 1/3 diameter), but are otherwise morphologically identical to the main nucleus.

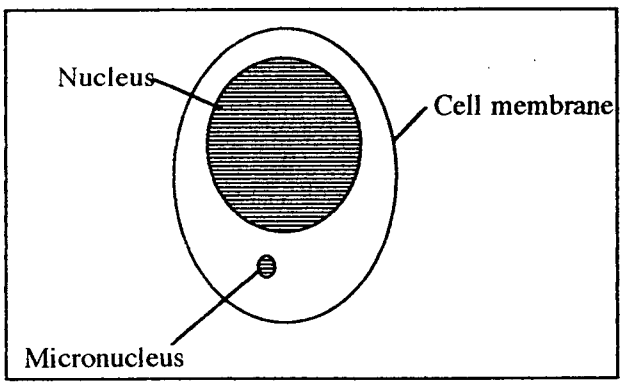

FIGURE 3 - 1. ILLUSTRATION OF A MICRONUCLEATED CELL

Figure 3-2 illustrates this process of micronucleus formation (adapted from Eastmond and Tucker, 1989). The upper row of cells demonstrates how a MN is formed when an aneuploidogen interferes with the mitotic spindle. The lower row demonstrates the effects of a clastogen. Note that the formation of micronuclei requires a dividing cell population; lymphocytes, which are commonly used in the MN assay, are normally non-dividing, but latent lesions in the DNA are expressed when the cells are artificially stimulated to divide using a chemical mitogen.

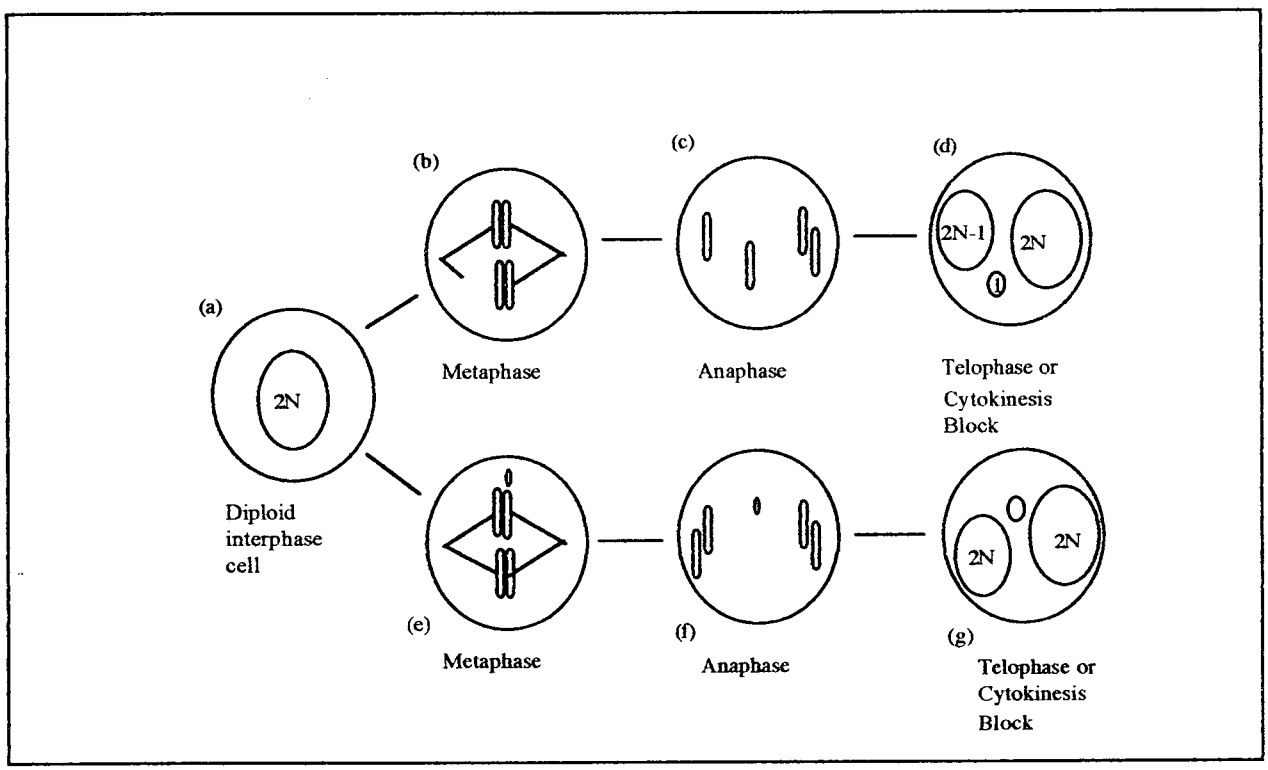

FIGURE 3 - 2. PROCESS OF MICRONUCLEUS FORMATION: Treatment of a normal diploid cell (a) with an aneuploidogen interferes with the mitotic spindle (b) resulting in a lagging chromosome at the metaphase plate at anaphase (c); when nuclei re-form, lagging chromosome is encapsulated separately, forming a micronucleus (d). Treatment of normal cell with a clastogen causes chromosome fragmentation (e), and an acentric fragment which does not migrate with the rest of the chromosomes at anaphase (f); when nuclei re-form, acentric fragment forms a micronucleus.

As an example of pesticide-induced micronucleus formation, one may consider the fungicide captan. It forms the DNA adduct 7-trichloromethylsulphenyl-guanine. An error in DNA replication or DNA repair at the adducted base may cause a break in the DNA strand, resulting in a fragmentation of the chromosome.

The resulting acentric fragment will not migrate with the other chromosomes at anaphase, and will result in a micronucleus.

3.3.2 Damage Which Causes Micronuclei

Micronuclei are formed by two fundamental types of damage: clastogenesis, which is the breakage of DNA and the formation of acentric chromatid or chromosomal fragments; and aneuploidogenesis, which is the imbalance of genetic material as a result of the loss or gain of whole chromosomes. In healthy human subjects, both types of damage are thought to occur at approximately the same frequency in peripheral blood lymphocytes (Fenech, 1993).

Clastogenic action causes breaks in either one or both chromatids, depending on the point in the cell cycle when damage occurs. The cell cycle can be divided into four major stages: G_1, S, G_2, and mitosis, or cell division. During G_1 (also called G_0 in cells which are non-dividing) the cell is producing proteins, etc.; in the S phase, DNA is replicated in preparation for cell division; during G_2, protein production continues and the cell readies for division; during mitosis the DNA content of the cell, and the cytoplasm is equally distributed to two identical daughter cells. Damage is measured at metaphase or following cell division. Damage which occurs during G_0 is replicated to both chromatids during the S-phase, resulting in chromosomal aberrations; this is typical of damage by ionizing radiation, or radiomimetic chemicals. Damage which occurs in S or G_2 stage results in chromatid aberrations (Sorsa *et al.*, 1992). This is more typical for chemically induced aberrations, due to misreplication or mis-repair of an adducted base. Chromosome-type aberrations are rarely found as a result of chemical clastogens, and are probably formed by a different mechanism, perhaps an apurinic or apyrimidinic site, converted to a strand-break and then misrepaired during G_0 (Natarajan, 1993).

Micronuclei may involve whole chromosomes if they fail to migrate properly during anaphase. Usually, aneuploidogens, such as spindle poisons, are considered effective only in actively dividing tissues (Norrpa *et al.*, 1993). Exposure to chemicals in the G_0 stage may result in later aneuploidogenesis if a) the aneuploidogens act upon the G_0 precursors of the mitotic apparatus, b) there is sufficient aneuploidogen remaining during the first *in vitro* divisions, c) there are high levels of MN from *in vivo* divisions, or d) if

mutations occur in the kinetochore proteins, centromere or spindle apparatus leading to unequal chromosome distribution at anaphase (Fenech, 1993).

3.3.3 MN Assay Does Not Detect All Mutations Events

The micronucleus assay does not detect all forms of aneuploidy, nor all clastogenic events. Some mechanisms of aneuploidy (e.g. non-disjunction of chromatids) would not result in micronuclei formation. Normal repair mechanisms also accurately repair latent DNA damage prior to the damage being made permanent by DNA replication. Experiments with c-arabinoside, a chemical which interferes with normal DNA repair, have shown that if the repair process is damaged, then the frequency of micronuclei is greatly increased following a genotoxic insult (Preston, 1985). However, peripheral blood lymphocytes are long-lived, and have a slow repair rate compared to other cells, and therefore in lymphocytes, there is an improved probability of detecting DNA damage.

3.4 Significance Of Increased Micronuclei Frequency

3.4.1 Association Of Micronucleus Frequency To Disease

Micronuclei (MN) are the product of chromosomal mutations, which in somatic cells are considered an important aspect in the initiation of a tumour, and the association between chromosomal mutation and neoplasms has already been discussed in chapter 2. However there is still no documented evidence that the development of genetic disease such as cancer is associated with elevated chromosomal damage, such as micronuclei (Vine, 1990). To unequivocally link increased MN frequency to disease would require a prospective study to follow individuals who have previously demonstrated elevated MN levels. Such a prospective study is underway in Scandinavia, which is following a cohort of nearly 3000 individuals who were examined cytogenetically between 1970 and 1988. To date, a non-significant positive association has been observed between pooled cytogenetic markers (including CA, SCE and MN) and cancer mortality, however in the subcohort which was examined for micronuclei only, there were only two deaths in the period of interest, which was insufficient to draw conclusions (Brøgger *et al.*, 1992).

Other pieces of evidence suggest a link between elevated MN levels and disease. The frequency of MN in patients with ataxia telangiectasia (AT), who are an elevated risk of cancer, has been found to be approximately three times higher than in controls (Tomanin *et al.*, 1990). AT cells treated with hydrogen peroxide, or with extract from areca nuts (oxidative and exogenous stressors) were found to respond with greater frequencies of MN than did normal controls (Yi *et al.*, 1990). Cells from individuals with Down's syndrome and Fanconi's anemia, again conditions with an associated elevated cancer risk, show an increased incidence of radiation-induced micronuclei (Huber *et al.*, 1989). Populations known to be at high risk of cancer and exposed to inhaled carcinogens had higher frequencies of micronucleus in their buccal mucosal cells (Stich and Rosin, 1984). The same studies also demonstrated that within a particular tissue, regions of elevated MN frequency could be detected which topographically mapped to regions with higher risk of tumour development.

Because a direct link between markers of genotoxicity such as micronuclei and genetic disease has not been demonstrated (Vine, 1990), only group results and not individual results are normally released to participants in genotoxicity studies. This is further indicated by the overlap of levels of other markers such as sister chromatid exchanges and DNA adducts between exposed and unexposed groups, and between high and low risk groups (Perera, 1987, Sorsa and Yager, 1987).

3.4.2 Association of Micronuclei to Exposure

Many *in vitro* and *in vivo* studies in animal and humans have demonstrated elevations of MN in response to exposure to known and suspected carcinogens (Vine, 1990, Titenko-Holland, 1994, Lee, 1994). The most detailed work has been accomplished with ionizing radiation, which is highly clastogenic (Huber and Bauchinger, 1987), and can produce high yields of micronuclei. Micronucleus frequency has been shown to be a "sensitive biological indicator of chromosomal damage due to ionizing radiation" , and a number of authors have reported a quadratic dose-response function in micronuclei induced by X-ray radiation (Thierens *et al.*, 1991). From observed micronucleus frequencies, the authors believe that exposures as low as 0.2 Gray can easily be distinguished from an external baseline level, and exposures as low as 0.05 Gray can be detected if pre-exposure internal controls are available.

3.5 Methodological Issues

3.5.1 Tissue Types Used

Several tissue types have been utilized for the micronucleus assay, including polychromatic erythrocytes from bone marrow, peripheral blood erythrocytes, peripheral blood lymphocytes, and epithelial cells from the bladder, buccal cavity and nasal epithelia. The bone marrow method is generally considered too invasive for population studies, although they have been used in the past. Micronucleated erythrocytes are quickly removed from the blood stream by the spleen, and therefore experiments are limited to splenectomized subjects. Epithelial cells are relatively easy to obtain from mucosal swabs or urine samples, but their turnover is fairly rapid, and so the exposure to be studied must be recent.

Peripheral blood lymphocytes can also be obtained by fairly non-invasive techniques. No disease has so far been directly associated with elevated micronucleus levels in lymphocytes, but as they migrate back and forth between blood and other tissues, they act as surrogate markers for effects throughout the body. They have long life-spans, and low repair rates, and tend to accumulate damage. They are widely used in population studies.

3.5.2 Peripheral Blood Lymphocytes and the Micronucleus Assay

There are two main types of peripheral blood lymphocytes, T-cells, and B-cells, which account for approximately 70% and 30% of lymphocytes in adults (IAEA, 1986). Approximately 80% of the peripheral lymphocytes belong to the "redistributional pool" which means that the lymphocytes are able to leave the blood and pass through various tissues in the body before returning to the peripheral blood circulation. It is estimated that the recirculation time is approximately 12 hours, and that the lymphocytes spend approximately 30 minutes of this time in the peripheral blood. Thus assays of the peripheral lymphocyte system are potentially capable of detecting damage which has occurred throughout the body.

Peripheral blood lymphocytes normally remain in a permanent "resting" state or "G_0" state until stimulated by an antigen response, or until they are stimulated to divide artificially by a chemical mitogen. Several

such mitogens are available. Phytohemagglutinin (PHA) preferentially stimulates T-lymphocytes, and does not induce MN.

3.5.3 Timing of Sampling

The life-span of the T-lymphocyte is not well understood. Different sub-populations are thought to have different life-spans, but there is controversy over their lengths. The mean life-span of T-lymphocytes has been suggested as 4.3 years (i.e. half-life of 3 years, Vine, 1990). However, other work (reviewed by Sprent and Tough, 1994) provides evidence that while some sub-populations are extremely short lived, others may have an almost indefinite life span. Because some T-lymphocytes are fairly long-lived, and have low repair rates (Vine, 1990), they can accumulate damage over time, and integrate the effect of exposure duration (Osanto *et al.*, 1991).

In studies of genotoxicity it may be useful to adjust exposure to account for normal lymphocyte turnover by weighting by time since exposure. Braselmann *et al.* (1994), used this technique in a study of historical radiation exposure, and they termed the weighted value the "equivalent acute exposure" or EAE. This value represents the level of exposure as if it were received at the time of the study.

The appearance of the micronuclei marker (or more accurately, the latent damage which will produce a micronucleus) is immediate following insult by a genotoxin (Vine, 1990), and T-lymphocytes are relatively long-lived; therefore samples may be taken any time after exposure, although reduction of apparent effect will be caused by lymphocyte turnover with time.

B-lymphocytes are not as long-lived, and may have a different sensitivity to chemical mutagens. This means that B-lymphocytes could be used both in determinations of more acute exposures, because the accumulate damage over a much shorter time interval, and to complement T-lymphocyte analysis, because they respond differently and may actually be more sensitive.

One author has noted that chronically exposed individuals may have heightened repair capability which could potentially reduce the expressed effect over time to a greater degree than simple lymphocyte turnover (Vine, 1990).

3.5.4 Kinetics of Micronucleus Formation

Early micronucleus assay techniques had serious problems with cell kinetics. To form a micronucleus, a cell must divide. Therefore, in an assay, only those cells which have divided should be enumerated, as only they were eligible to produce micronuclei. However, the lymphocyte assay requires division to be artificially stimulated using a mitogen such as PHA, which is not 100% efficient, and so many cells do not divide. In addition, some cells may complete more than one division before enumeration begins. Therefore simply counting all cells during the enumeration lowers the true micronucleus frequency, because of the inclusion of many ineligible cells in the denominator. Worse, the inter-individual response to PHA varies, and so the error is variable among different individuals.

Several mechanisms were proposed to deal with this issue, and one, the cytokinesis-block method (Fenech and Morely, 1985) has been widely adopted. This modification of the MN assay utilizes cytochalasin-B (CB), a chemical which prevents the dividing lymphocytes from undergoing cytokinesis. Although the cell undergoes normal nuclear division, it does not divide, and remains viable. Therefore cells which have divided once are easily recognizable by their binucleated (twin - nuclei) appearance. CB itself does not induce micronuclei (Fenech and Morely, 1985). Individuals' sensitivity to CB may vary, but because there is no preferential micronucleation of cytochalasin-blocked cells, this will not bias the assay. CB allows collection of baseline data, and comparison of individuals who might respond differently to PHA. A typical binucleated cell is shown in Figure 3-3. Figure 3-4 shows a binucleated cell with a micronucleus.

3.5.5 Differentiating Mechanism of Micronucleus Formation

Although the micronucleus assay is sensitive to both clastogenic and aneuploidogenic mechanisms, until recently, there was not a simple method of distinguishing the results of the two. This problem was solved by the introduction of the kinetochore-antibody adaption of Eastmond and Tucker (1989) which utilizes the presence of kinetochore proteins to distinguish MN formed by clastogens from MN formed by aneuploidogens.

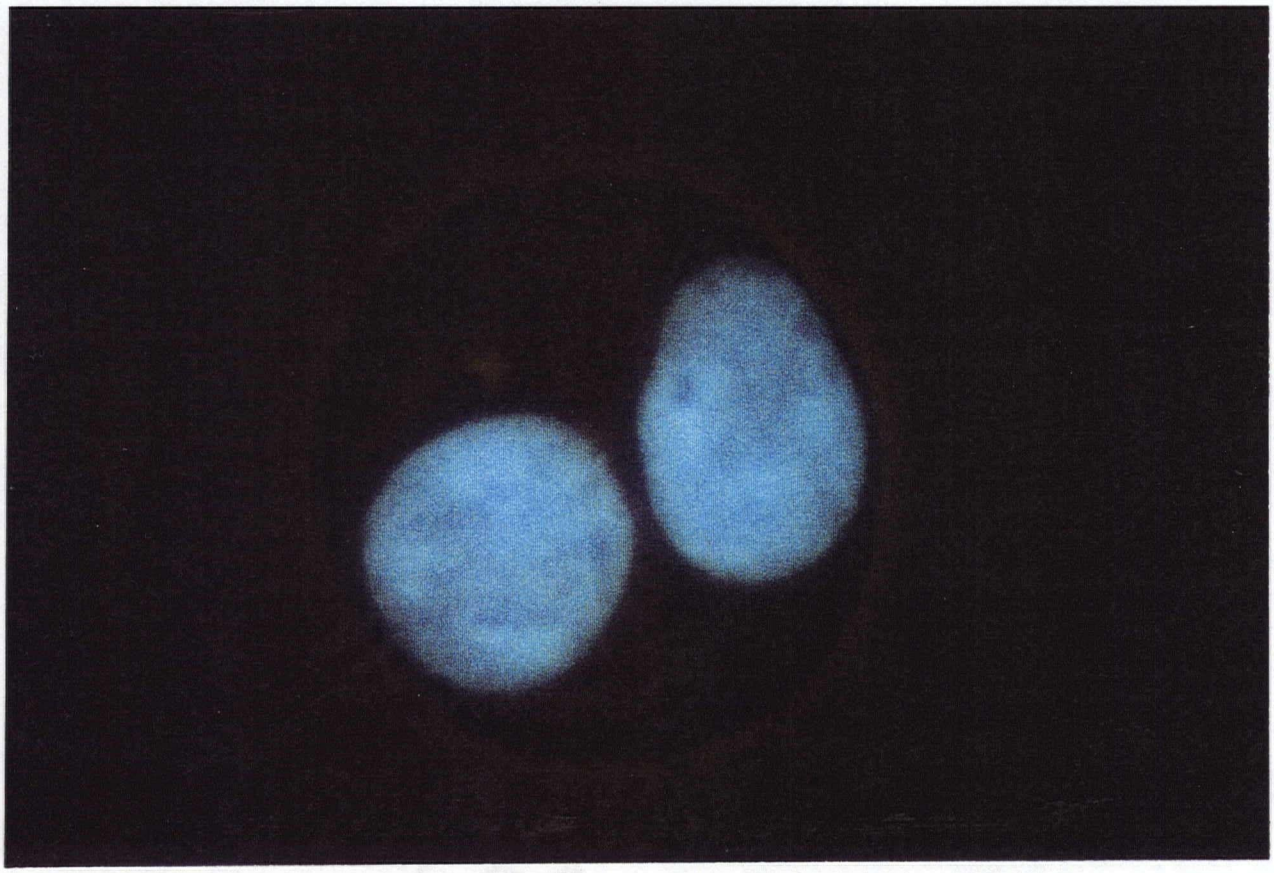

FIGURE 3 - 3. CYTOKINESIS-BLOCKED LYMPHOCYTE WITH BINUCLEATED APPEARANCE.

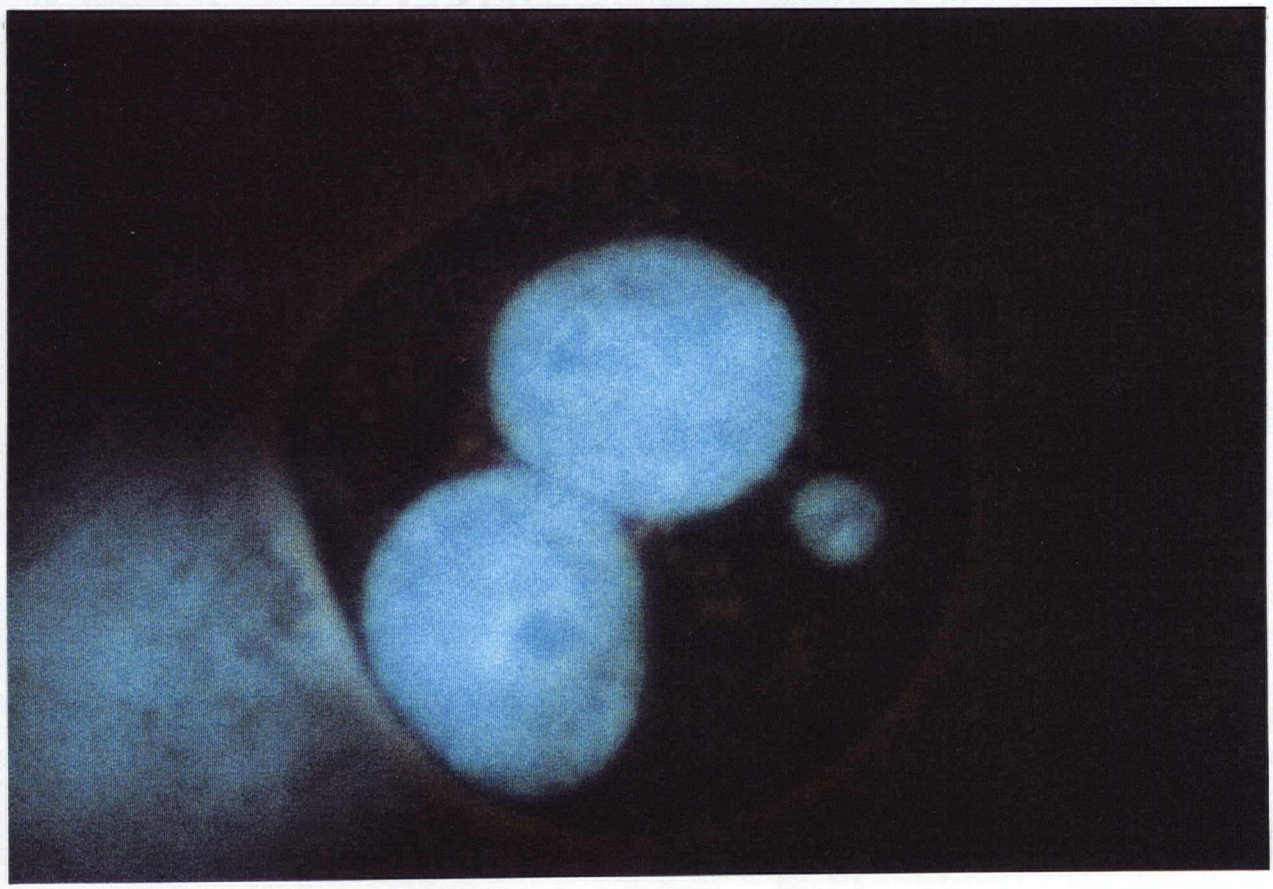

FIGURE 3 - 4 CYTOKINESIS BLOCKED LYMPHOCYTE WITH BINUCLEATED APPEARANCE AND SINGLE MICRONUCLEUS

The kinetochore is a protein structure which in conjunction with the centromere forms an attachment point for the mitotic spindle during mitosis (cell division). Each chromosome has only a single kinetochore. Antibodies to the human kinetochore protein are isolated from patients suffering from the CREST syndrome, an autoimmune disease. These antibodies attach to the kinetochore proteins on chromosomes inside the nucleus or micronucleus. A second treatment with fluorescently labeled goat-anti-human antibody will attach to the antikinetochore antibody, and cause the kinetochore, if present, to fluoresce. Assaying for whole chromosome or acentric fragments within micronuclei becomes a simple assessment of the presence or absence of a fluorescent spot. Figure 3-5 shows a typical binucleated cell, with a small micronucleus. Figure 3-6 shows the same cell, now with the labeled kinetochores fluorescing. Note that in this case, there is no fluorescent signal from the micronuclei, indicating that this micronucleus probably contains an acentric fragment. Figure 3-7 shows another binucleated cell, with 3 micronuclei. Figure 3-8 shows the same cell under illumination to fluoresce to kinetochore stain. This time it can be clearly seen that the two left-hand micronuclei have positive kinetochore signals, indicating that they probably contain whole chromosomes.

3.6 Variability, Sensitivity and Specificity of the Micronucleus Assay

3.6.1 Sensitivity and Specificity

Testing of the sensitivity and specificity of the micronucleus assay has not been conducted in humans, although there is evidence from studies of the assay in mice that demonstrates that it is efficient in identifying and distinguishing mutagens and non-mutagens. Waters *et al.*, (1994), showed that in a retrospective analysis of the results of *in vivo* rodent bone marrow micronucleus tests, a sensitivity of 96% (22/23 mutagens correctly identified) was obtained, and a specificity of 63% (12 out of 19 non-mutagens correctly identified). This matched the performance of the technically more demanding chromosome aberration assay. The chemical used in the assay had previously repeatedly demonstrated their mutagenicity (or non-mutagenicity) in germ cell mutation assays which met US Environmental Protection Agency's evaluation criteria (Waters *et al.*, 1994).

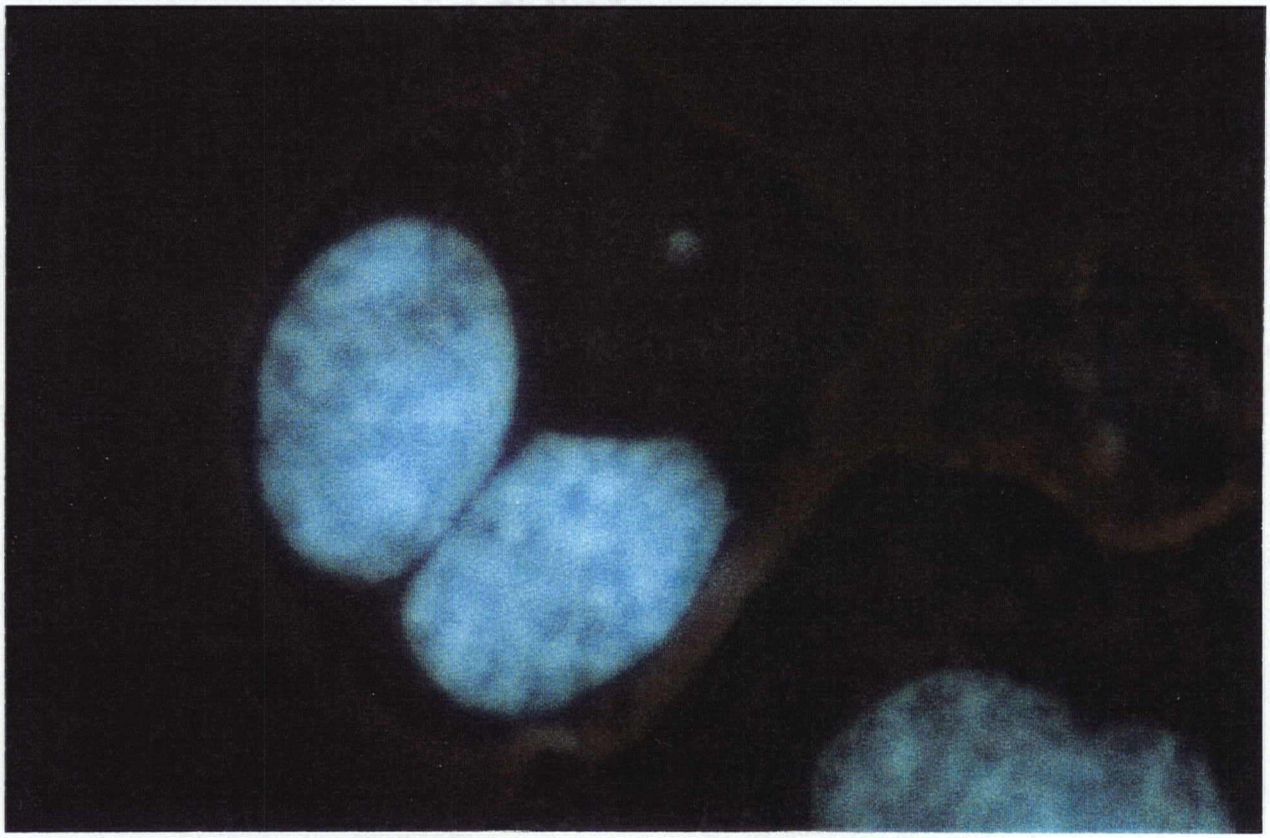

FIGURE 3 - 5. CYTOKINESIS BLOCKED LYMPHOCYTE WITH BINUCLEATED APPEARANCE AND SINGLE MICRONUCLEUS

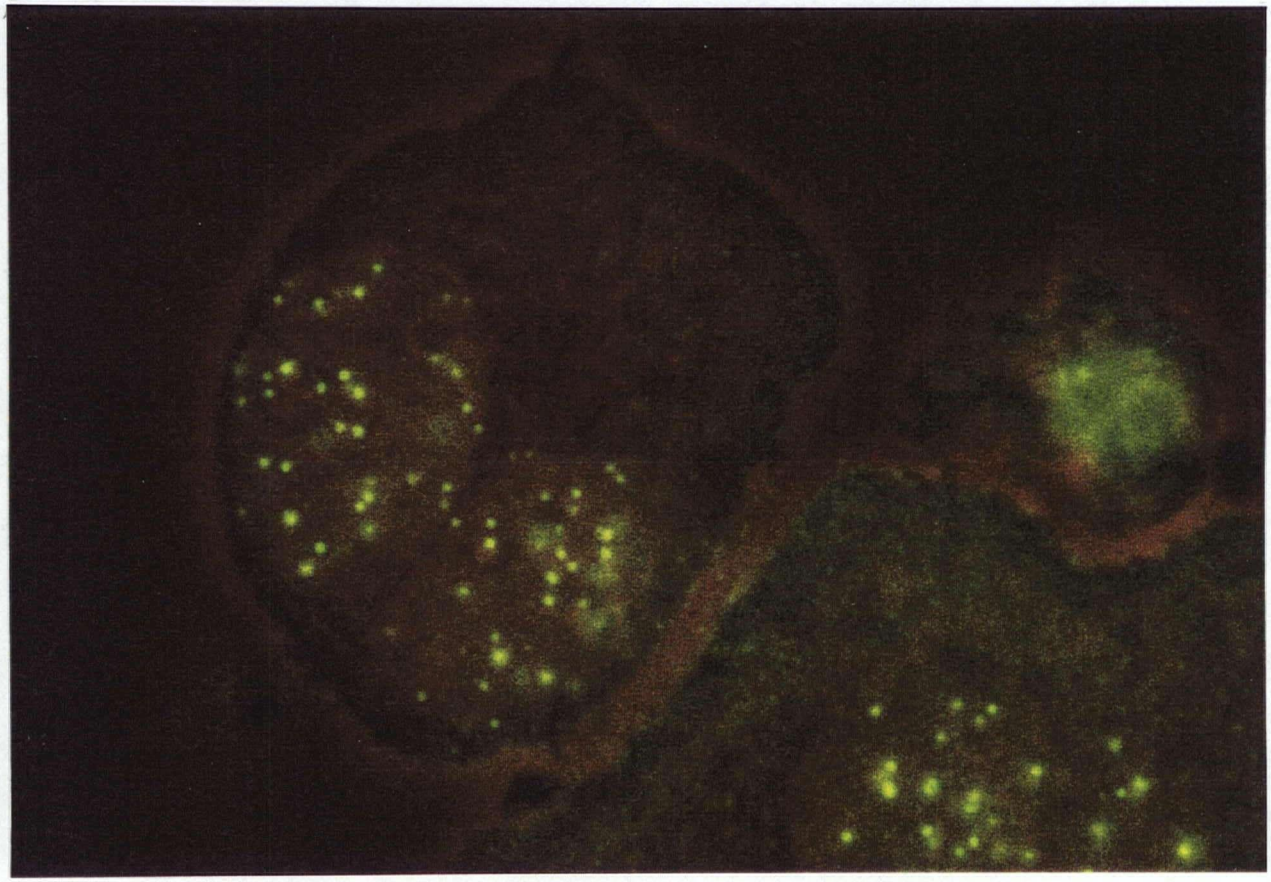

FIGURE 3 - 6. SAME BINUCLEATE CELL AS FIGURE 3 - 5, SHOWING KINETOCHORES. ABSENCE OF A KINETOCHORE SIGNAL IN THE MICRONUCLEUS INDICATES THAT IT PROBABLY CONTAINS AN ACENTRIC FRAGMENT

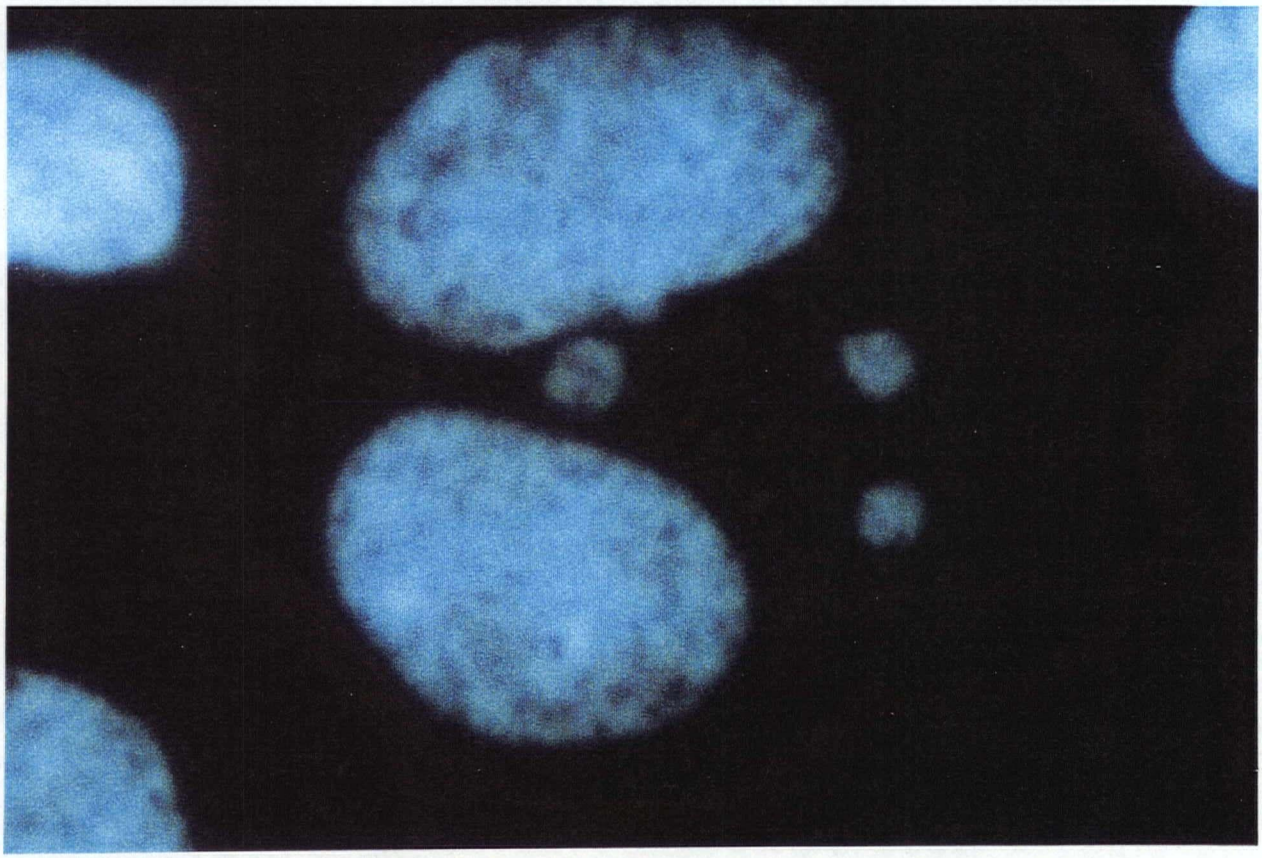

FIGURE 3 - 7. CYTOKINESIS BLOCKED LYMPHOCYTE WITH BINUCLEATED APPEARANCE, AND MULTIPLE MICRONUCLEI

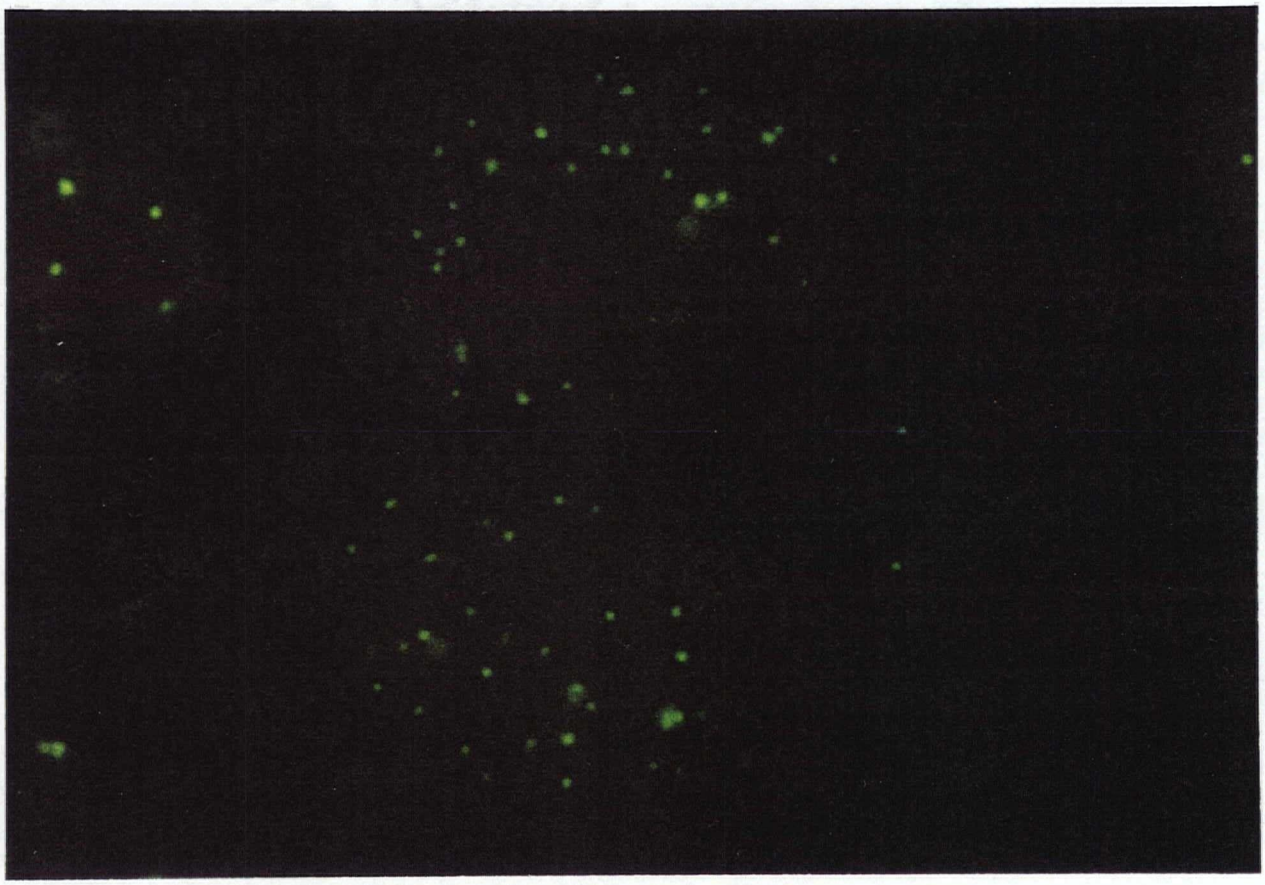

FIGURE 3 - 8. SAME BINUCLEATE CELL AS FIGURE 3 - 7, SHOWING KINETOCHORES. KINETOCHORE SIGNALS IN TWO OF THE MICRONUCLEI INDICATE THAT THEY PROBABLY CONTAIN WHOLE CHROMOSOMES.

The International Programme on Chemical Safety (IPCS, 1990) recommended the mouse bone marrow (BM) micronucleus as a primary *in vivo* test system for established *in vitro* genotoxins. They compared the results of over 97 investigators and 50 different *in vivo* techniques, and concluded that the mouse BM micronucleus assay was robust, sensitive and reproducible. Each investigator involved studied two structurally related pairs of compounds, one genotoxic, the other non-genotoxic. The mouse BM micronucleus assay correctly identified the two genotoxins 97% of the time, and correctly identified the non-genotoxic analogue 97% of the time, in a total of over 60 tests. In another study of 143 chemicals evaluated by mouse micronucleus assay, the researchers found a specificity of 80% but a sensitivity of only 58%. The authors chose chemicals based on their ability to induce malignant tumours in animal studies, and the results of epidemiological studies in humans. They noted that the sensitivity of the micronucleus assay could have been improved by counting more cells (reviewed in Vine, 1990).

Norppa *et al.* (1993) discussed the sensitivity of the micronucleus assay with respect to its ability to detect aneuploidogens and clastogens. They argue that aneuploidogens must be present during mitosis to act; and that because lymphocytes are artificially stimulated to divide, it is unlikely that the action will be captured. They demonstrated *in vitro* how the timing of aneuploid treatment with respect to mitosis changed the frequency of micronuclei which contain whole chromosomes; those treated 24 hours before mitogen stimulation showed the weakest response. This work has not been duplicated *in vivo* to my knowledge. In the same review, the authors also discuss the ability of the assay to accurately detect chemical clastogens *in vivo*, pointing to the lack of positive studies reported. However, since the publication of their review several positive studies have been published (Al-Sabti *et al.*, 1992, Di Giorgio *et al.*, 1994, Franchi *et al.*, 1994, Ribeiro *et al.*, 1994, and Anwar *et al.*, 1994).

Fenech (1993) concluded that the cytochalasin-B micronucleus assay detects 60 - 90% of acentric fragments.

3.6.2 Variability

There are three main sources of variability: systematic, or technical variability, and inter- and intra-individual variability.

Systematic variability can be introduced first of all by the technique itself. As mentioned earlier, before the use of cytochalasin-B, there tended to be an over counting of non-dividing cells, resulting in an underestimate of true micronucleus levels. This makes the CB assay more sensitive than non-CB assay (Maki-Paakkanen, 1991). It also means that care must be taken in comparing the results of MN assays, and the method used must be taken into account.

Other large systematic variability can be introduced during the preparation of the sample for scoring. Cell proliferation kinetics must be consistent, so that cytochalasin-B is added at the same part of the cell cycle. This is ensured if consistent protocols are followed.

Another important source of systematic variability is in scoring. Högstedt (1984) had two scorers independently score the same 24 preparations. Although the correlation between the two scores was high ($r=0.95$) one scorer systematically scored 30% below the other. This demonstrates the need for a single scorer for an experiment, and clear and objective scoring guidelines.

Inter-individual variability

Many factors are suspected of having influence on micronucleus frequencies, and most would be expected to vary between individuals. Exogenous influences include health factors such as viral infections, X-ray exposure, chemotherapy, and vaccination; dietary factors include the intake of carcinogens or anti-carcinogens in meats and vegetables, and physiologic folic acid levels. Smoking is believed to increase micronucleus levels. Endogenous factors include age, gender, and individual susceptibility through physiological factors; these include: enhanced metabolic activation or detoxification of proto-genotoxins, and genetic susceptibility via reduced DNA repair capability (Olden, 1994).

An example of an inherited factor is that ataxia telangiectasia (AT) heterozygotes (approximately 4% of the general population) suffer from impaired DNA repair capabilities, although their cancer frequency is not as elevated as that of homozygotes. Several metabolic enzymes involved in the biotransformation of toxic chemicals are also known to be genetically polymorphic (i.e. many forms of the protein), and to have phenotypes associated with an elevated risk of cancer (Idle, 1991). An example is the CYP1A1 gene,

which codes for the P450 enzyme P4501A1, associated with aryl hydrocarbon hydroxylase activity. A polymorphism in this gene appears to produce both high and low-inducibility hydroxylases. The polymorphism has been demonstrated to be associated with lung cancer, such that the high-inducibility phenotype was found in 30.0% of the cases, but only 9.4% of the controls, while the low-inducibility enzyme was found in 4.0% of the cases and 44.7% of the controls.

A review of 7 recent studies which all used the same assay methodology (cytochalasin block in lymphocytes) showed a range of coefficient of variations in unexposed controls from 21% to 80% with an average of 47% (Di Giorgio *et al.*, 1994, Franchi *et al.*, 1994, Ribeiro *et al.*, 1994, and Anwar *et al.*, 1994, Migliore *et al.*, 1991a, Yager *et al.*, 1994, Sarto *et al.*, 1991).

Intra-individual Differences

Within an individual, variability may be attributed to age, changes in health, hormonal cycles, recent vaccination, and the effects of exogenous agents, both physical and chemical (Carrano and Natarajan, 1988, Yager, 1990).

Repeated sampling of the same individuals over time obtained agreement within 15% (Köteles, *et al.*, 1993). Fenech (1993) found good correlation ($r=0.85$, $p<0.001$) between repeated measurements taken on 12 individuals taken 12 months apart.

3.7 *In vivo* Studies Using the MN Assay

The micronucleus assay has been widely used in many *in vivo* human studies. Authors have investigated background levels in normal populations (Köteles *et al.*, 1993) accidental radiation exposure, and radiation therapy (da Cruz *et al.*, 1994, Bauchinger *et al.*, 1989), effects of chemotherapy (Osanto *et al.*, 1991, Sarto *et al.*, 1990, Migliore *et al.*, 1991b), the effects of age and gender (Fenech and Morely, 1985, Di Giorgio *et al.*, 1994, Köteles *et al.*, 1993), various exogenous factors including smoking (Tomanin *et al.*, 1991, Dave *et al.*, 1991), and many occupational exposures including styrene (Al-Sabti *et al.*, 1992, Maki-Paakkanen *et al.*, 1987, 1991, Yager *et al.*, 1994, Högstedt *et al.*, 1984, and Meretoja *et al.*, 1977), paint (Di Giorgio *et al.*, 1994), benzene (Högstedt *et al.*, 1991), chromium (Migliore *et al.*, 1991b), PAH's (Carstenen *et al.*,

1993), piperizine (Högstedt *et al.*, 1988), ethylene oxide (Sarto *et al.*, 1988, Ribeiro *et al.*, 1994), mercury (Barregård *et al.*, 1991, Franchi *et al.*, 1994), vinyl chloride (Sinués *et al.*, 1991), toluene (Nise *et al.*, 1991), cadmium (Forni *et al.*, 1994), cytotoxic drugs (Anwar *et al.*, 1994), and pesticides (Bolognesi *et al.*, 1993).

3.8 Experimental Demonstrations of Pesticide-Induced Micronuclei

The micronucleus assay has been widely used in *in vitro* assays and *in vivo* animal studies examining the genotoxicity of pesticides.

The organophosphate insecticide malathion has been shown to significantly elevate micronucleus frequency in the bone marrow cells of mice treated cutaneously (Dulout *et al.*, 1982). Rats exposed to the pyrethroid insecticide deltamethrin also had significantly elevated micronucleus frequencies in bone marrow cells (Agarawal *et al.*, 1994). Captan has been shown to elevate micronucleus levels in a dose-dependent manner in mouse bone marrow cells (Feng and Lin, 1987).

4. STUDY DESIGN

4.1 Study Goal

The goal of this study was to investigate whether seasonally employed farmworkers occupationally exposed to pesticide residues had elevated levels of chromosome damage. This damage would be measured by determining micronucleus (MN) frequency in peripheral blood lymphocytes.

The hypothesis that was to be tested was that seasonal farm workers hired to harvest berry crops in British Columbia had a higher frequency of MN in their peripheral lymphocytes when compared to a matched control group consisting of similar individuals who had not been employed as seasonal farmworkers.

4.2 Study Objectives

The following specific objectives were established for the study:

a) to identify and recruit a study group representative of the British Columbia seasonally employed farm worker population, and to identify and recruit a suitable control population;

b) to obtain blood samples from the farmworker group (post-season) and concurrently from the controls; to develop a questionnaire and collect personal data on exposure variables, potential confounders, and general demographic information;

c) to determine the frequency of micronucleated lymphocytes in blood samples; if evidence of damage is present, compute the ratio of MN caused by aneuploidy to MN caused by chromosome breakage, using the technique of Eastmond and Tucker (1989).

d) to determine: (i) whether there was a statistically significant difference in MN frequency between the study and control groups, (ii) whether a statistically significant difference existed in aneuploid vs. breakage type of MN observed in the exposed group, and finally (iii) to determine if there were associations between any of the exposure variables and MN frequency; and

e) to inform workers of the study results.

4.3 Issues With Respect To Study Design

It was decided that the study population would be women who worked in the berry fruit fields in the Fraser Valley during the summer of 1993. Because gender influences MN frequency, males and females must be analyzed separately. Our resources only permitted the study of one sex, and females were selected because: (a) they are more representative of the farm worker population, comprising approximately 73% of the seasonal farm workers (Oja *et al.*, 1990); (b) previous studies have traditionally examined only male populations, resulting in a lack of data on females; (c) reduced folate levels in females (compared to males) may elevate micronuclei frequencies; (d) study of females may also permit comparison of MN levels in pre- and post-menopausal women. To be eligible for the study, workers would have to have worked a minimum of 12 weeks during 1993.

It was desirable to further restrict the analysis to one ethnic group to reduce background genetic variability. It was decided to limit the study to individuals of East Asian descent, because they form the largest ethnic group in the farmworker community.

4.3.1 Control Group

Internal controls, or "pre-exposure" and "post-exposure" measurements are considered highly desirable for cytogenetic studies because they reduce the amount of variability introduced by individual susceptibility to genotoxins (Carrano and Natarajan, 1988, Gebhart, 1982). However, resources and timing restrictions restricted our opportunity to collect samples, and so only an external control group was possible. The control group was to be recruited from a population with the same ethnic background as the study group, to be of the same gender, and in the same age-range. The same exclusion criteria were to apply for controls as for the farmworker group. The controls were not to have been employed as farmworkers during the summer of 1993.

4.3.2 Study Group Size

The target size for the study and control groups was 25 individuals. This number of participants was based on a power calculation using an alpha equal to 0.05, beta equal to 0.25, and a coefficient of variation equal to 0.4.

4.3.3 Exposure Assessment

No direct exposure assessment was possible because the study did not have access to any work sites. Additionally, the exposure profile of the workers would have been extremely complicated with a variety of different chemical exposures over the course of a work season, and with continually varying levels of exposure.

Exposure was thus determined indirectly, using employment duration as a surrogate index for pesticide exposure. Data was collected on both short-term employment history (weeks worked in 1993) and long-term (years worked, lifetime).

5. METHODS - POPULATION RECRUITMENT, DATA GATHERING AND WORKER NOTIFICATION

5.1 Contacting the Population

5.1.1 Study Recruitment - Initial Attempts

The ideal study population would have been one which permitted quantitative measurements of pesticide residue exposure, or detailed observations of the pesticide formulations to which the subjects were exposed. Access to farms would have been required for this, but several early attempts to gain employer support for the study were unsuccessful.

In the earliest phases of this study, potential employers were contacted through three different routes. First, a mailing list of Fraser Valley berry farmers was obtained from the British Columbia Federation of Agriculture (BCFA), with their permission to contact members and solicit volunteer farms. Second, farmers were approached directly by a presentation given to the Fraser Valley Strawberry Growers Association (FVSGA) annual general meeting in Surrey, British Columbia. Third, intermediaries who were familiar with the aims of the study approached farmers known to them, and asked for their assistance.

Sixty letters were mailed to berry farms identified from the BCFA mailing list. There were no responses to this mailing. The presentation given to approximately 25 farmers at the FVSGA annual general meeting similarly resulted in no positive responses. Of the individual farmers approached, one expressed a willingness to participate, but did not respond to later communication. From our contact with the growers, it appeared that their general feeling was that the study would only serve to raise public concern about chemical contamination of fruit products, which they considered unfounded. They also thought that applicators were at much higher risk, and that they should be studied rather than farmworkers.

One organization, the Canadian Farmworkers Union (CFU), was identified as being the largest organization representing seasonal farmworkers in the Fraser Valley Region. Their enrollment in 1992 when this phase of the study was begun was approximately 1000. A presentation was made to the attendees of the CFU annual general meeting at the Sunset Community Hall in Vancouver on April 28, 1993. Approximately 250

individuals were in attendance. The presentation involved a description of the nature of the study, requirements of individual participants, the benefits of the study, plus information on participants' rights, and worker notification. As the audience was predominantly Sikh, the presentation was translated into Punjabi. Executives from the CFU spoke encouragingly on behalf the project and its aims.

Following the presentation, attendees were contacted by telephone by Punjabi speaking assistants. Individuals were asked if they had attended the meeting, if they had heard of the study, and if they would be working in the upcoming season as farmworkers.

Approximately 25 individuals were contacted. The results were not encouraging. Many individuals contacted had not attended the annual general meeting. Apparently the contact list we were provided was not accurate in that respect. Other individuals contacted were not working, or were union supporters, but not active workers. Others had not decided whether to work that summer. There were no definite volunteers for the study.

Following these attempts, it was decided to undertake the study by recruiting individual workers through community organizations.

5.1.2 Study Recruitment - Final Recruitment Methods

Farmworkers were successfully recruited through the MOSAIC organization, which provides services to Vancouver ethnic communities. The agency organizes weekly social groups for both male and female seniors within the East Asian community for the purpose of social interaction (religion, arts, politics) and information dissemination (public health, etc.). Through contacts within the community, it was known that many attendees were seasonally employed as farmworkers.

The non-farmworker controls were recruited in part from the same community group, and the remainder contacted through employees of a second social services agency, the Surrey Delta Immigrant Services Society (SDISS), and through friends and relatives of a research assistant.

To contact the study population, permission was first obtained from the community group organizer and from its leaders to approach. At a subsequent gathering, where about 30 individuals were in attendance, a short presentation was made which described the project, explained the requirements of individual participants and the aims of the study, informed them regarding their rights as participants (e.g. right to withdraw at anytime, right to confidentiality, etc.) and explained worker notification. The presentation was given in Punjabi by a research assistant. Questions were encouraged and answered. At the end of the presentation, the attendees were told that the researchers would return to the community center the following week to recruit individuals, and take blood samples. In accordance with UBC policy, this gave individuals more than 24 hours to consider before actual recruitment. Information sheets were provided in both English and Punjabi with all the details of the study (Appendix A).

Some of the non-farmworker participants (those known to the research assistant, and those recruited through SDISS) were contacted first by letter or by personal meeting. The first contact in either case was to explain the purpose of the study and the requirements of the participants, and to explain the rights of the participants. Only on secondary contact (after a minimum of 24 hours) did recruitment occur.

5.2 Recruiting

At a subsequent meeting of the MOSAIC group, individuals interested in participating presented themselves in a temporary clinic established at the community center where provision had been made for interviewing and blood sampling. Each prospective participant was again explained the purpose of the study, risks to the participant, the requirements for the participant, and the participant's rights. These details were also contained in the study consent form (Appendix B) which was made available both in English and Punjabi. The research assistant conversed in the language of choice for the participant. If the individual chose to participate, they were asked to sign the consent form. A copy of the consent form was provided to the participant.

Non-MOSAIC participants were recontacted by research assistants to ask if they would participate. If they agreed, a meeting was arranged at the participant's home to obtain the blood sample. The same process as above was followed with respect to obtaining consent.

Because many of the participants were volunteers and there was no random selection process, it was not possible to determine a refusal rate. However, an estimated 60% of those approached at the MOSAIC meeting volunteered, and of those contacted directly by research assistants the refusal rate was very low, about 10%. The refusal rate of those contacted through SDISS is not known.

5.3 Inclusion and Exclusion criteria

Only individuals who were female and of East Asian descent were enrolled. Participants had to be over 18 years old, and had to have worked as a seasonal farmworker for at least 12 weeks in the 1993 season (May 1993 to November 1993). Individuals who were recruited as control subjects had not worked as farmworkers during the 1993 season. Eligibility was ascertained by questioning the candidate prior to obtaining a blood sample.

Only individuals who did not meet the inclusion criteria or who were considered unfit for blood sampling were excluded. Reasons to be considered unfit were if the individual was at increased risk of side-effects from the phlebotomy, i.e. excessive bruising in individuals taking blood thinning drugs, and if an individual could not provide a sufficient blood sample, i.e. if the phlebotomy procedure failed for some reason.

Attempts were made to match control participants based on age, especially for individuals recruited outside of the seniors group meetings. Due to low numbers of participants, however this matching criteria was not rigorously implemented.

5.4 Questionnaire

The primary purpose of the questionnaire (Appendix C) was to gather employment history data, and data relating to potential confounders. Additional questions gathered demographic data, and investigated various determinants of exposure to pesticide residues.

The questionnaire was based on a draft questionnaire prepared specifically for population monitoring of cytogenetic and gene mutation endpoints (Cole, 1991). The questionnaire was then tailored to farm work to

elicit detailed employment information. A removable coversheet contained all the participant's identifying information, and an identity number which linked it to the rest of the questionnaire.

The questionnaire was translated into Punjabi so that it could be administered in the language of choice for the participant. The Punjabi version was proofread for accuracy by an independent party, who translated the Punjabi version back to English and then compared the resulting version with the original English version. A guide was also prepared for the interviewer, to assist them with the interpretation of questions into Punjabi.

The questionnaire was not tested prior to use. Instead, responses were reviewed with the interviewer after the first 6 interviews (which included both controls and farm workers) had been completed. No significant problems were identified at this time, but questions regarding the intent of certain questions and the required form of the answers were reviewed with the interviewers. Questionnaires were primarily done over the phone, and took approximately 20 - 30 minutes to complete for a farmworker, less for controls.

Following questionnaire completion, cover sheets which contained participant identification information were removed, and stored in a secure cabinet in a locked room. Only the author and his supervisor had access to confidential data.

Following review of the completed questionnaires, data was entered onto Microsoft Excel™ on a Macintosh™ SE/30. Errors and omissions in questionnaire completion were identified, and participants recontacted by phone if necessary to repeat question or pose supplementary questions.

6. METHODS - MICRONUCLEUS ASSAY

The Micronucleus assay with cytokenesis block and kinetochore staining as described in section 3.6 was used (Eastmond and Tucker, 1989).

6.1 Laboratory Supplies and Equipment

Equipment required for the micronucleus assay is listed in Table 6 - 1. Supplies and reagents are listed within the methods.

TABLE 6 - 1. LABORATORY EQUIPMENT REQUIRED FOR THE MICRONUCLEUS ASSAY

Item	Manufacturer	Type	Comments
Biosafety Cabinet	Nu-Aire	Nu 425-600	
CO_2 Incubator	Nu-Aire	IR Autoflow	
Centrifuge	Hitachi	CT5DL	
Microscope	Nikon	LABOPHOT2-POL	
Fluorescence Attachment	Nikon	EF-D	
Light Source	Nikon	Hi-Intensity HG100W	
Objective Lens	Nikon	100X Phase Contrast	E Flat Field
Filter Blocks	Nikon	V2B	EX380-425;DM430;BA460
		B2A	EX450-490;DM510;BA520
Refrigerator	Foster	+4°C	
Freezer	General Freezer	-20°C	
Autoclave	Market Forge	Sterilmatic	
Mechanical Shaker	Fisher	Model 127	
Cytofuge	Miles Scientific	Cytotec	
Camera	Nikon	N2000	With MC12-A remote cord

6.2 Procedures

6.2.1 Blood Samples

Blood samples were collected from participants during the period of October to December 1993.

Blood samples were collected from each participant by a registered nurse or a trained phlebotomist by forearm venipuncture with a 21 gauge needle and Vacutainer® tubes (Becton Dickinson, Rutherford, NJ)

containing anti-coagulant. One 15 mL tube (with sodium heparin) and one 5 mL (with ethylenediaminetetraacetic acid or "EDTA") were drawn. Tubes were thoroughly mixed to avoid coagulation.

One sample (containing sodium heparin) was used in the micronucleus assay. The second sample (containing EDTA) was used to determine red blood cell (RBC) folate concentration for analysis as a potential confounder.

6.2.2 Blood storage

Blood samples were kept at room temperature (approximately 18°C) and processed within 24 hours of the blood draw. The tube containing EDTA was taken as soon as possible to the Department of Hematology at Vancouver General Hospital for RBC folate analysis.

6.2.3 Lymphocyte Separation

All procedures involving blood or cell cultures, up to harvesting, were done using sterile techniques.

Blood was diluted 1:1 with phosphate buffered saline ("PBS", Sigma, St. Louis, MO) and 30 mL of the resulting blood/PBS mixture was carefully layered using a 25 mL pipette over 13 mL of Ficoll-Paque (Pharmacia, Baie D'Urfe, QB) in a 50 mL centrifuge tube (Corning, New York, NY). The tubes containing the blood/PBS and Ficoll-Paque were centrifuged at 400 x *g* for 30 minutes (no braking). The lymphocytes were then removed to another tube, by careful extraction of the white lymphocyte layer using a plastic transfer pipette. Care was taken not to transfer any red blood cells.

The white blood lymphocytes were then washed twice with RPMI 1640 culture media ("Rosewood Park Memorial Institute 1640", Terry Fox Laboratory, Vancouver, BC). First the lymphocyte suspension was brought up to 50 mL with RPMI, well mixed and then spun at 400 x *g* for 10 minutes. The supernatant was then aspirated down to 5 mL; the pellet was resuspended in the remaining supernatant, and transferred to a 15 mL tube (Corning, New York, NY). This tube was brought up to 15 mL with RPMI, mixed thoroughly,

and spun at 400 x *g* for 5 minutes. The supernatant was then aspirated down to 1 mL, and the lymphocytes resuspended thoroughly in remaining supernatant.

Packed red blood lymphocytes and excess whole blood were retained for possible future analysis.

6.2.4 Lymphocytes Viability

The lymphocytes were counted using a hemocytometer (Fisher Scientific, Vancouver) according to the protocol of Sigma (1992). Sample was diluted 1:10, i.e. 2 parts lymphocyte solution to 3 parts trypan blue solution (0.4%) to 15 parts PBS.

6.2.5 Lymphocyte Cultures

Culture and treatment conditions were performed as previously described (Eastmond and Tucker, 1989). Lymphocytes were cultured in inclined 15 mL centrifuge tubes at 37°C for 72 hours in a 5% CO_2 atmosphere at an initial density of 1.0 x 10^6 lymphocytes/mL. Two cultures were initiated for each blood sample. Culture medium consists of 2 mL RPMI 1640 supplemented with 2 mM L-glutamine, 100 units/mL penicillin, 100 mg/mL streptomycin, 20% fetal bovine serum ("FBS"; all from Terry Fox Laboratory, Vancouver, BC) and 10 µg/mL phytohemagglutinin-P ("PHA", Sigma, St. Louis, MO).

Cytochalasin B ("Cyt-B"; Sigma, St. Louis, MO) at a 6 µg/mL final concentration was added at 44 hours. Lymphocytes were harvested onto slides at 72 h.

6.2.6 Harvesting Slides

Each culture was vigorously agitated, then mixed thoroughly by several dozen passes through a pasteur pipette to ensure lymphocyte clumps were disaggregated. Lymphocytes were spun onto slides (Fisherbrand Superfrost®/Plus, Fisher, Vancouver, BC) using a Cytotek cytofuge (Miles Scientific, Eckhart, IN), for 8 minutes at 600 RPM, using 1 ml specimen chambers, and filter type 4333. For each culture a test slide was made at an initial concentration determined by best judgment (usually 75-150 µL). After reviewing the lymphocyte density on the test slides using phase contrast microscopy, a minimum of 3 further slides were made for each culture at an optimized concentration if necessary. Ideal lymphocyte density was considered

to be closely neighbouring lymphocytes each separated from its neighbours so that distortion due to clumping did not occur.

After drying, slides were fixed for 15 minutes in absolute methanol at room temperature, dried, and stored, desiccated, in sealed Zip-Loc® bags (Dow, Paris, ON) under N_2 at -20°C, according to the method of Eastmond and Tucker (1989). For ease of blinding, and to minimize thaw/refreeze damage to lymphocytes, slides were aliquoted into scoring "sets" each of eight slides, containing four control and four study slides.

6.2.7 Staining Slides

All staining was done under reduced light conditions, or under a gold fluorescent bulb. Slides were removed from the freezer and allowed to come to room temperature. They were then washed in 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20, Sigma, St. Louis, MO) in PBS for 5 min. Excess wash was drained and 50 ml of anti-kinetochore antibody ("Antinuclear antibody test positive control serum centromere", PDI Bioscience, Aurora, ON) diluted 1:1 in 0.2% Tween-20 in PBS was added to each slide. The slides were then coverslipped (24 X 60 mm, Fisher Scientific, Vancouver) excluding all air bubbles, and arranged in a glass tray lined with damp filter paper. This was partially covered with Handiwrap® (Dow, Paris, ON) and incubated at 37°C for 1 hour in the CO_2 incubator.

Following incubation, the coverslips were removed, and the slides irrigated with filtered phosphate buffered saline (PBS, Sigma, St. Louis, MO.) with a minimum of 10 mL for each slide. The slides were then washed 3 times in fresh filtered PBS, each for 10 minutes, with agitation.

Excess PBS was drained, and 50 mL of fluoroscein isothiocyanate (FITC)-conjugated goat anti-human antibody (PDI Biosciences, Aurora, ON) diluted 1:50 in 0.05% Tween-20 in PBS was added. This reagent is highly light sensitive. Each slide was cover slipped (24 X 60 mm) and all bubbles carefully excluded. The slides were placed on a damp towel in a tray, partially covered with Handiwrap®, and incubated for 37°C for 1 hour.

Following incubation, the coverslips were removed, and the slides irrigated with fresh, filtered PBS (minimum 10 mL each slide). The slides were then washed 3 times with fresh filtered PBS, each for 10 minutes, with agitation.

Finally, the slides were counterstained with 35 µL 4,6-diamidino-phenylindole (DAPI , Sigma, St. Louis, MO). DAPI is light sensitive so the stain was dissolved in 0.1 mg/ml of an antifade solution (Johnson and Nogueira Araujo, 1981). The slides were coverslipped and pressed under filter paper (Whatman, Maidstone, UK) to remove bubbles and excess moisture.

Slides were stored in 4°C awaiting scoring. The majority of the slides were scored within 4 days, although some were scored up to 8 days after staining.

6.3 Randomization and Coding

Slides were randomized and coded to blind the scorer as to lymphocyte origin. Following staining the identification number on the slide was obscured, and a new, randomly selected identification number was added by someone other than the scorer. The key to the coding was not accessible to the scorer. Each time a batch of slides (approximately 8 slides) was stained prior to scoring, an equal number of exposed and control slides were processed. Following scoring, the key was broken for the batch, to permit additional slides to be prepared for a subject, if required. When additional slides were prepared, they were mixed in with the next randomized batch, so that at all times the scorer was blind to the source of the slides.

All slides were scored by the author, to avoid inter-scorer variability. A minimum of 2000 binucleated lymphocytes per participant scored for micronuclei when possible (1000/duplicate culture).

6.4 Scoring Criteria

Only binucleate lymphocytes which met the following criteria (Fenech, 1993) were scored:

1) lymphocytes should have two nuclei of approximately equal size [included within preserved cytoplasm (Tomanin *et al.*, 1991)].
2) lymphocytes should not contain more than six MN
3) main nuclei may be attached by a fine nucleoplasmic bridge
4) main nuclei may overlap slightly or touch each other at the edges

5) main nuclei are round, oval or slightly kidney shaped with smooth nucleoplasmic boundaries

The following criteria for identifying micronuclei were used (Fenech, 1993, Eastmond and Tucker, 1989, Tomanin *et al.*, 1991, Titenko-Holland *et al.*, 1994):

1) MN are morphologically identical to, but smaller than, normal nuclei, with similar chromatin structure, and similar refraction
2) MN should have diameter between 1/16 and 1/3 of main nuclei
3) MN should be distinctly separate from the main nucleus and located within binucleate lymphocytes with intact cytoplasm and nuclear membranes
4) MN should be coplanar with main nuclei
5) MN would have smooth oval or round shape

The following criteria for identifying kinetochores was used:

1) MN kinetochores only scored if nuclear kinetochores also visible
2) MN kinetochores only scored if clearly distinguishable above background FITC staining.

6.5 Photography

Photographs of micronuclei preparations (see chapter 3) were taken on Kodak Ektachrome P1600 professional slide film, shot at 1600 ASA. Phase contrast, and DAPI (470 nm emitted) images were shot using an automatic setting. FITC images (560 nm emitted) were shot with automatic aperture and 4 - 8 seconds shutter speed. Processing required two stop pushing.

6.6 Scoring Protocol

Scoring started at the top left corner of the microscope slide, and progressed was from left to right, until the right hand edge was reached. The view was then moved down one field. Progression was then right to left until the left edge was reached (Figure 6-1). This pattern was continued until the entire slide was covered, or 1000 binucleated cells, had been scored.

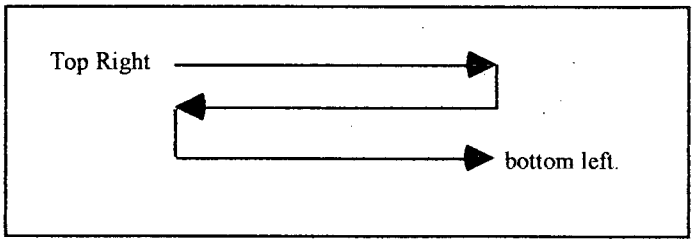

FIGURE 6 - 1. SLIDE SCORING PROGRESSION

Identification of binucleate cells in cell groups required careful visual examination for the individual cell boundaries of the cytoplasm (Fenech and Morley, 1985). Simultaneous phase contrast and DAPI using Nikon filter cube # V-2B gave optimal visualization of cytoplasmic boundaries, and nuclei.

Where a binucleated cell contained micronuclei, filters were changed to a fluorescein filter (Nikon filter cube # B-2A) and kinetochore signal status was determined.

Every binucleate cell which was considered to contain a micronucleus was recorded whether it was finally decided to score the micronucleus or not. This information was required to enable accurate rescoring of slides (see section 6.7). The following information was recorded for every possible micronucleated cell:

1) positive or negative for micronuclei
2) location of binucleate cell on slide (stage coordinates)
3) number of micronuclei in cell
4) kinetochore status of each micronucleus (if scorable)
5) photo roll/frame number, if photograph shot
6) comments on reason for rejecting micronucleus or reason for unscorability of kinetochore.

To monitor cell proliferation in culture, four hundred cells (200 per duplicate culture) were scored for mononucleate cells (1N), binucleate cells (2N) and cells with three or more nuclei (3N). Then a replication index (RI) was calculated as: $RI = (1N + 2(2N) + 3(3N))/1N + 2N + 3N$.

6.7 Rescoring

To reduce scoring variability over the scoring period, slides from a recent prior batch were periodically rescored. Selected slides were rescored one to two weeks after they had originally been scored. Slides were initially rescored "blind" for replicative index, frequency of micronuclei, and percent micronuclei that were

kinetochore positive. These scores were compared to the original scores to ensure reproducibility. The original score sheet contained data not only on cells which had scored positive for micronuclei, but also those which had been considered but rejected. Therefore it was possible to relocate all these cells and blindly rescore them for MN. The scoring decision could be compared to the original as an indication of consistency of scoring criteria for MN. Inconsistencies were evaluated, and criteria for scoring reviewed. No changes were made to existing scores, but the exercise assisted the scorer in minimizing temporal drift by ensuring consistent application of MN and kinetochore identification criteria.

At an early point in the scoring, 5 stained slides were sent to Dr. M.T. Smith's laboratory at University of California, Berkeley. These slides were reviewed by Dr. Nina Titenko-Holland for quality, and assessed for their scorability. Dr. Titenko-Holland also made a semi-quantitative assessment of micronucleus frequency to validate early study results.

6.8 Statistical Analysis

All statistical analysis was performed on an Apple Macintosh SE/30 with floating point unit (FPU), using STATVIEW 4.0™ (Abacus Concepts, Berkeley, CA). Multiple regression analysis was performed using STATVIEW™ and SAS/PC ™ (SAS Institute).

The primary outcome of this study was the frequency of micronuclei within binucleated lymphocytes. Other data was collected regarding potential exposure to pesticides as measured by farmworker employment status, and duration, and data on potential confounders.

All data were first subject to univariate analysis to determine distribution characteristics and to investigate missing data and potential outliers. Where appropriate, data was transformed to produce a better fit to the normal distribution.

Group comparisons were done using ANOVA, using Scheffé's post-hoc test to identify sources of differences. The Student's t-test was used where appropriate for comparing two groups. Chi-squared and Fisher's Exact tests were used for comparing nominal variables.

Multiple regression analysis was performed to investigate the effect of potential confounders on the relationship between farmwork and micronuclei frequency.

For all tests , a result was considered significant if $p < 0.05$. Details of testing are provided in the results section.

6.9 Worker notification

6.9.1 Intermediate Results

Part of the red blood cell folate analysis done by the hematology laboratory included a hematocrit test, which was unanticipated by the study design. Consent had not been obtained to send the results directly to a physician, and therefore the results were sent to the participant. They were accompanied by a letter of explanation, requesting that they take them to their physician on their next visit. A letter to their physician was also included which explained the general circumstances of the tests, but maintained the individual's confidentiality (see Appendix D). For some of the participants a physician assisting the study recommended prompter medical attention, and a different letter was mailed to this effect (Appendix D).

6.9.2 Final Results

Final results were reported back to the participants by mail. A letter to all participants explained the general findings of the study, and conclusions regarding the risks involved in working as a seasonal farmworker (Appendix E). Group results, but not individual results, were shown (see section 3.4.1). A contact person who spoke Punjabi was identified to participants for clarification or further information. Sources for additional health and safety resources were included.

7. RESULTS

7.1 Recruitment and Testing of Individuals

The study group comprised 39 individuals, all women from the cities of Vancouver, Burnaby and Surrey, in the province of British Columbia. All were volunteers, recruited as described in section 5.2. Eighteen had been employed harvesting berry crops (strawberries, raspberries and blueberries) in the Fraser Valley during the summer of 1993. Twenty one individuals who had not worked on farms during the same period acted as a control group. Eight of this second group had some prior farm work experience, however. Blood samples were obtained from farmworkers and controls as they were recruited, i.e. no attempt was made to randomize or match workers with controls for the purpose of blood sampling. Table 7 - 1 summarizes the results of recruitment and sampling.

TABLE 7 - 1. DETAILS OF SAMPLING CLINICS, BIOLOGICAL SAMPLE COLLECTION

			Samples Obtained							
			Farmworker		Control					
Trip	Date/Time	Location	MN	Folate	MN	Folate	Translator	Nurse	Date cells Cultured	Date cells Harvested
1	19/10/93 18:00 - 20:00	Homes in East Vancouver	3	3	5	5	HS	NM	20/10/93	23/10/93
2	21/10/93 13:00 - 15:00	Community Center	5	5	2	2	HS	NM	22/10/93	25/10/93
3	28/10/93 12:30 - 15:00	Community Center	6	6[a]	5	5[a]	MR	NM	29/10/93	1/11/93
4	2/11/93 18:15 - 20:00	Homes in East Vancouver	4	4	0	0	VY	NM	3/11/93	6/11/93
5	14/12/93 14:30 - 18:00	Homes in Surrey and East Vancouver	0	0	9	8[b]	SB, HS	CT	15/12/93	18/12/93

[a] Folate samples collected, but lost due to technical error at testing laboratory.

[b] One participant could not provide enough blood for folate sample

One participant was unable to provide a sample large enough for both tests, and so the specimen was reserved for the micronucleus analysis. Eleven samples delivered to a contract analytical laboratory responsible for the RBC folate analysis were spoiled by premature freezing prior to analysis. Therefore only 27 RBC folate levels were finally recorded.

Questionnaires were administered immediately following blood sampling, during the period of November 14, 1993 to December 18, 1993. One questionnaire was not completed until June, 1994, as the participant was out of the country for several months. All individuals who provided a blood sample completed the subsequent questionnaire.

7.2 Description of Study Group

7.2.1 General

Table 7-2 shows the general characteristics of the farmworker and control groups. The data for the control group is further divided by those who had previous experience as a farmworker, and those who had never been employed as farmworkers.

All participants were female, between the ages of 37 and 72 years The mean age was 55.9 years. All participants reported their ethnic group as East Asian, and from the individuals' names, it was estimated that 87% were of the Sikh religion (traditionally, Sikh women adopt Kaur as a middle name). As followers of the Sikh religion observe certain rules and traditions governing diet and lifestyle, this was a useful factor in assessing non-occupational risk factors for micronucleus formation. All participants were born outside of Canada, and immigrated to Canada between 1963 and 1992. Those working as farmworkers in 1993 tended to be older, and had immigrated more recently than the members of the control group .

None of the participants had ever smoked, and only two were exposed to environmental tobacco smoke (one farmworker, and one control; both individuals reported exposure > 17 hours/day). Tea was consumed by all but 3 of the study group (all controls), but coffee was consumed by only seven (2 farmworkers, 5 controls; one participant did not record whether she consumed coffee). A majority of participants were

TABLE 7 - 2. GENERAL CHARACTERISTICS OF FARMWORKER AND CONTROL GROUPS [Mean (± SD)]

		Controls		
	1993 Farmworkers	All	Previously Farmworkers	Never Farmworker
n	18	21	8	13
Age (years)	57.9(±6.1)	54.1(±12.0)	63.0(±5.1)	48.8(±11.9)
Age Distribution (years):				
30-39	0	5	0	5
40-49	2	3	0	3
50-59	8	3	2	1
60-69	8	9	5	4
70-79	0	1	1	0
DIET				
% Vegetarian	88.9	38.1	50.0	30.8
Tea intake (cups/day)	3.3(±0.9)	2.6(±1.7)	2.5(±0.5)	2.7(±2.1)
% Consume coffee[a]	11.1	25.0	12.5	33.3
% Consume soft drink/juice	83.3	71.4	75.0	69.2
RBC Folate (nMol/L)[b]	452.6(±98.6)	497.1(±170.6)	462.5(±143.4)	509.7(±184.2)
ENVIRONMENTAL				
Hours outside (per day)	9.7(±1.0)	1.0(±1.5)	1.8(±2.1)	0.6(±0.8)
Years since immigration	8.1(±8.3)	15.2(±8.6)	17.1(±6.4)	14.0(±9.9)
HEALTH				
% Recent vaccination	38.9	14.3	37.5	0.0
% X-Ray in past two years	27.7	47.6	50.0	46.2
X-rays in last 10 years	2.6(±3.4)	4.4(±4.7)	4.0(±4.9)	4.6(±4.8)
Years Since Menopause	6.7(±6.1)	9.0(±1.7)	14.9(±7.4)	5.5(±5.9)

[a] 38 observations
[b] 27 observations

vegetarian (61.5%), and farmworkers were more likely to be vegetarian (88.9%) than controls (38.1%). Red blood cell (RBC) folate levels were recorded for 27 of the group, and the mean value was 477.3 ± 142.5 nMol/L. All tests were within the laboratory reference range (160 nMol/L - 900 nMol/L). Farmworkers had a slightly lower mean RBC folate level than the controls.

7.2.2 Health and Medication

All but 4 participants (1 farmworker and 3 controls) had received at least one X-ray in the preceding 10 years. The mean number of X-rays in the farmworker group was 2.6 (±3.4), versus 4.4 (±4.7) in the control group. Fifteen (5 farmworkers, and 10 controls) had received a recent X-ray (within the past two years). Thirty two of the participants were post-menopausal (17 farmworkers and 15 controls) and the mean

number of years since menopause was 6.7(±1.4) and 9.0(±1.7) respectively. As expected, the number of years since menopause was closely correlated to age (correlation coefficient = 0.66). Ten participants had received recent vaccination (7 farmworkers and 3 controls), within 3 months prior to blood sampling. All vaccinations were for influenza with the exception of one farmworker who received a vaccination for tetanus. None of the participants had ever had cancer, and none had received chemotherapy, or therapeutic radiation treatment.

The study group was generally healthy. Two farmworkers and 6 controls reported existing illness at the time of blood sampling. Acute infections were reported by 1 farmworker (a cold) and one control (an ear infection). Chronic health problems were reported by one farmworker (asthma) and 5 controls (high cholesterol, high blood sugar, high blood pressure, a heart condition, and an individual who reported a psychiatric disorder). Two controls also reported having the flu in the month prior to the blood sampling. One farmworker reported currently having hepatitis, but the type was not given.

Three farmworkers and 5 controls were taking prescription medicines at the time of their blood sample. Medications included an asthma inhaler, cholesterol medication, cytotec with lectopam (farmworkers), diclofemal, haldol, surgam, capaten, and glyburide with glucose (controls).

Participants spent an average of 5.1 hours outdoors each day. As might be expected, individuals who worked as farmworkers spent more time on average outdoors (9.7 ± 1.0 hours) than did those in the referent group (1.0 ± 1.5 hours).

7.2.3 Socio-Economic Status and Lifestyle Factors

No direct measure of socio-economic status was attempted. However, from data collected related to employment status, country of origin, from the individuals name, and from observations relating to primary language and place of residence, it was observed that certain controls might be considered to belong to a higher socio-economic category than the rest of the group. Specifically, 5 controls were identified who shared certain distinguishing characteristics: all used non-Sikh names and were born in Kenya, and of whom at least 4 spoke fluent English, and 3 held office jobs.

7.2.4 Employment Data

Table 7-3 summarizes general employment data gathered for the individuals who worked on farms in 1993. All work was done between May 1, 1993, and November 17, 1993.

TABLE 7 - 3. EMPLOYMENT DATA FOR FARMWORKERS EMPLOYED IN 1993

	Farmworkers
Weeks worked in 1993[a]:	
mean	19
range	16-27
Hours worked in 1993[a]:	
mean	1412
range	896-2100
Hours worked per day:	
mean	10.9
range	8-13
Days per week:	
mean	6.8
range	6-7

[a]n=17. One individual reported only that they had worked more than 90 days.

None of the farmworkers reported mixing or applying pesticides, and none reported any direct exposure to pesticides by any means, including overspray (e.g. by an aerial application), or by drift, leak or spillage. None reported knowingly entering a field which had recently been treated with pesticides, and none reported becoming sick while actually in the fields.

Those in the farmworker group worked on between 1 and 6 farms during the season, the average being 3.6 farms. One had worked only on strawberry farms, but the rest (n=17) worked on three different crops: strawberry, raspberry and blueberry. Three of the 17 also worked on vegetable farms, where the crops included cauliflower, carrots, onions, beans, peas, cabbage, peppers, corn, and potatoes. None of the farmworkers worked on cranberry farms, which is another large berry fruit crop in British Columbia, but highly mechanized and not a large employer.

Of the 21 individuals in the control group, 8 reported having prior experience as a farmworker. Table 7-4 shows the cumulative employment duration for the 26 individuals who had some experience as a

farmworker (18 farmworkers and 8 controls with previous farmworker experience), and their first and last year worked. No detailed information was collected regarding employment prior to the 1993 season.

7.2.5 Other Employment

None of the participants reported any experience or activity outside of employment as a farmworker which would indicate exposure to pesticides. Participants were specifically asked about prior employment in chemical plants, in laboratories, X-ray facilities, garages, or other places where they may have been exposed to genotoxic substances. None reported such experience. Nine of those not employed as farmworkers were otherwise currently employed, 3 as secretaries, 2 as chambermaids, and 4 who reported their jobs as cook, cashier, seamstress, and family counselor. None of the farmworkers reported any other concurrent employment. One farmworker reported having had experience as a permanent farm employee,

TABLE 7 - 4. CUMULATIVE FARMWORKING EXPERIENCE

	First years worked	Last year worked	Cumulative Exposure (years)
Farmworkers			
	1968	1993	24
	1970	1993	23
	1980	1993	14
	1973	1993	14
	1979	1993	14
	1980	1993	10
	1987	1993	7
	1990	1993	4
	1990	1993	4
	1991	1993	3
	1990	1993	3
	1991	1993	3
	1992	1993	2
	1992	1993	2
	1992	1993	2
	1993	1993	1
	1993	1993	1
	1993	1993	1
Controls			
	1980	1992	13
	1980	1990	11
	1975	1982	8
	1977	1988	5
	1983	1985	3
	1985	1985	1
	1978	1978	1
	1970	1970	1

and one other did not respond to the question of other employment. None of the participants reported any hobbies or pastimes which might have resulted in exposures to genotoxic agents.

7.2.6 Personal Protective Equipment

For those who worked in 1993, all reported covering arms, legs, head and feet with garments, but none reported wearing any special protective materials. 12 workers (66.7 %) wore gloves while picking. One indicated using leather gloves, but the glove material for the others was not recorded. Respiratory protection was not worn by any worker.

7.2.7 Personal Hygiene

Workers reported access to handwashing facilities at all farm sites, and all reported washing their hands before eating. Meals were eaten in the fields. All workers reported changing out of their work clothes when returning to their homes. 16 of the 18 reported washing their work clothes on a daily basis, and wearing clean clothes everyday. 2 individuals indicated wearing the same clothes for up to two days, and only laundering work clothes every two days.

7.3 Micronucleus Assay Results

7.3.1 Cell Proliferation and Scorability

All cultures had successful lymphocyte growth, with no contamination.

Cell proliferation was assessed by the replicative index (RI), and by the percentage of cells which were binucleated, indicating that they had undergone one mitotic division. The mean RI for all cultures scored was 1.267 (± 0.109) and the mean percent binucleated cells (BN%) was 23.7 (±9.1) %. This provided ample binucleates to be scored for most cultures. 20.5% of the cultures were scored from just one slide (i.e. one slide contained over 1000 binucleates). The remainder required multiple slides to be scored. The approximate time to score a slide for 1000 binucleates was 3 to 4 hours. A total of 170 slides, and 80,207 binucleated cells, were scored for micronuclei. An average of 2057 binucleated cells were scored for each individual, with a minimum of 1776, and maximum of 3009.

7.3.2 Internal Validation of Scoring

The results of the rescoring are shown in Table 7-5. One slide could not be rescored because the FITC fluorescence had faded too much over the 27 days between first and second scoring. Blind rescoring for replicative index was consistent, with between a 9.5% deficit and a 1.8% elevation. The frequency of micronucleated cells varied more widely, between a deficit of 22% to an elevation of 33%. Two of the four rescores were very close, however, between 1 and 5% difference over the two scorings. Kinetochore rescoring showed a consistent deficit of between 34% and 41%, with the exception of the last slide, where a 42% increase in kinetochore positive micronuclei was noted. As a measure of scoring criteria reproducibility, MN rescore was between 72% and 96%, with a mean of 88.75%.

TABLE 7 - 5. INTERNAL SCORING VALIDATION: RESCORING RESULTS

	Scored	Rescored	Δ RI%	Δ MN%	Δ K%
1	14/5/94	5/6/94	-9.52%	5.22%	-41.37%
2	2/6/94	11/6/94	1.17%	31.72%	-34.63%
3	14/6/94	26/6/94	-2.52%	-22.68%	-35.83%
4	4/7/94	15/7/94	1.80%	1.45%	41.79%

Δ RI%: change in replicative index; Δ MN%: change in micronucleus count;
Δ K%: change in kinetochore positive frequency

Another measure of the internal consistency of scoring was the variation of micronucleus frequency between the two cultures prepared from the same individual. The paired t-test was used to evaluate the difference between the first and second cultures of all individuals. The mean difference was -0.66 micronuclei per 1000 binucleates, which was not significantly different from zero ($p = 0.440$).

Replicates of 5 slides sent to the laboratory of Dr. M. T. Smith at University of California, Berkeley were reviewed by Dr. Nina Titenko-Holland, for scoring quality. She assessed the slides as adequate, and supporting "accurate and unambiguous scoring". The slides reviewed by Dr. Titenko-Holland had already been scored in Vancouver, and so a comparison of results was possible. Although Dr. Titenko-Holland considered the frequency reported by the Occupational Hygiene laboratory as high relative to the reported literature, she concurred with the results.

7.4 Measures of Micronucleus Frequency

Two measures of micronucleus frequency were made: Frequency of micronucleated cells (FMC), which was the number of binucleated cells containing at least one micronucleus, per 1000 binucleates; and total micronucleus frequency (TMF), which was the total number of micronuclei counted per 1000 binucleates. The distribution of micronuclei within binucleates is shown in Figure 7-1. The two measures are highly correlated (correlation coefficient = 0.993), because the majority of micronucleated cells contained only one micronucleus. FMC will be used throughout for statistical analysis.

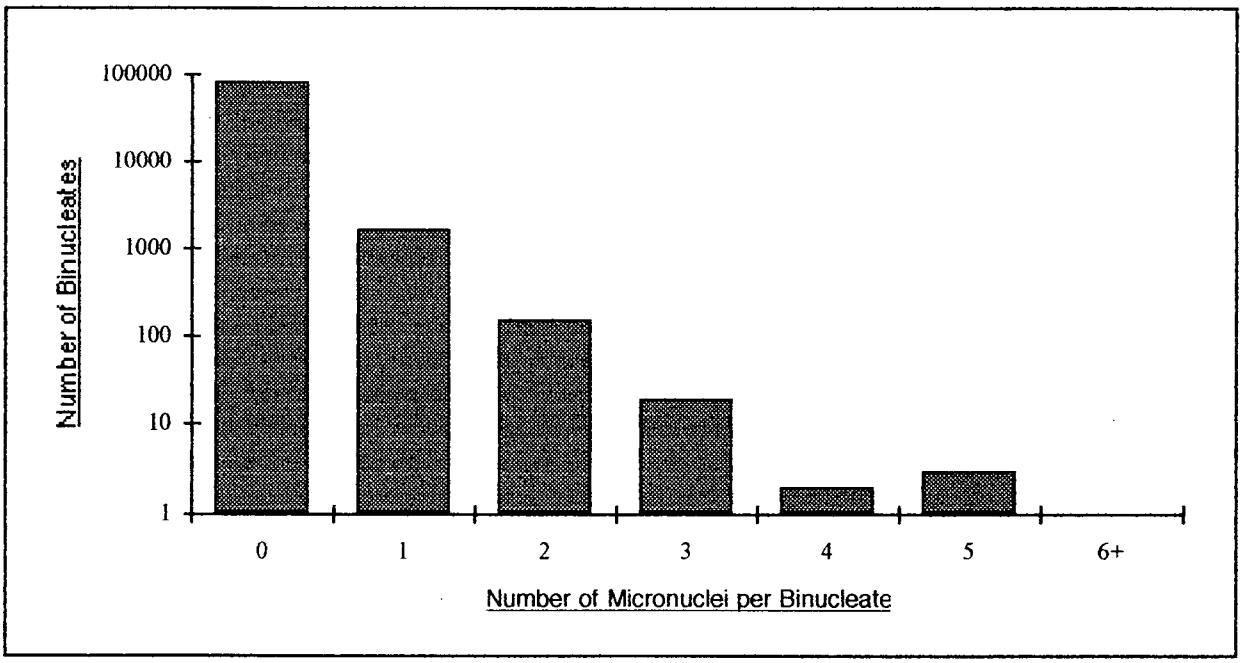

FIGURE 7 - 1. DISTRIBUTION OF MICRONUCLEI WITHIN BINUCLEATES

Details of each individual's results including frequency of micronucleated cells (FMC), total micronucleus frequency (TMF), kinetochore positive micronuclei (KPM), Percentage of binucleated cells (BN%) and the replicative index (RI), are presented in Table 7-6. Descriptive statistics are shown in Table 7-7. The coefficient of variation for micronucleus frequency in this study was 44.7 %.

TABLE 7 - 6. DETAILED CYTOGENETIC RESULTS

ID	# BN Scored	FMC (per 1000 binucleates)	TMF(per 1000 binucleates)	% BN 'd Lymphocytes	RI
2	2000	21.5	22.0	32.96	1.39
7	1973	22.3	23.3	11.56	1.12
32	1976	17.7	21.8	17.06	1.19
33	1866	5.9	5.9	15.26	1.17
34	2022	15.8	17.3	32.96	1.41
38	1964	9.7	10.2	19.93	1.21
41	1998	14.5	15.0	27.62	1.32
43	2000	12.0	12.5	26.11	1.32
44	2065	20.4	21.9	25.31	1.27
45	2002	15.5	16.5	25.42	1.28
47	2000	25.5	30.0	19.21	1.20
51	1929	24.2	27.7	38.45	1.48
52	3001	34.7	35.7	31.04	1.38
53	3009	27.7	29.4	14.94	1.16
54	1967	9.2	9.7	34.30	1.41
55	2000	27.5	33.0	19.81	1.24
59	2062	26.8	30.6	25.10	1.28
61	1959	49.1	56.2	9.36	1.11
63	2314	16.0	16.8	28.04	1.36
78	2019	21.3	21.8	26.31	1.30
85	1972	31.9	36.5	26.41	1.28
86	2015	21.3	22.8	35.22	1.42
107	2000	19.0	21.0	13.61	1.17
108	2097	13.7	14.7	26.04	1.31
114	1776	32.7	40.2	8.67	1.09
117	2023	14.2	15.7	11.54	1.13
121	2002	27.5	32.0	21.15	1.23
125	2021	18.8	19.8	11.69	1.13
134	2001	45.5	56.0	13.40	1.15
147	2000	16.0	18.0	37.28	1.42
163	2000	20.0	24.0	32.52	1.41
171	2018	35.7	40.6	14.99	1.16
172	2000	15.0	16.0	36.09	1.42
183	2006	41.4	44.9	17.60	1.21
185	2137	10.7	12.2	25.07	1.28
190	2094	33.5	39.2	16.49	1.19
192	2000	33.5	37.0	19.35	1.21
195	1919	13.5	15.6	16.63	1.18
200	2000	24.5	28.0	45.14	1.51

Abbreviations: FMC, Frequency of micronucleated cells; TMF, Total micronucleus frequency; BN, Binucleate cells; RI, Replicative index.

Frequency distributions of the untransformed and (natural) log-transformed FMC are shown in Figures 7-2 and 7-3, respectively, with overlaid normal distributions. Visual examination of the frequency distributions

suggested that the log normal distribution was a good fit, and this is supported by Figure 7-4, which shows probability plots for log-transformed and untransformed data.

Evidence of the log-normality of the distribution was also provided by the ratio-metric test (Waters *et al.*, 1991). This test is based on the ratio of the direct estimate of the mean and the maximum likelihood estimate of the mean of the lognormal distribution, which will be close to 1 when the distribution is lognormal. The ratio for the micronucleus frequency data was computed as 0.9918. This falls within the 95% CI for a sample of size of 50, and a CV of 0.5 (both conservative estimates, actually, n=39 and CV= 0.44), and therefore it was concluded that the distribution was adequately described by the log-normal distribution. Although some researchers have suggested that a Poisson distribution is the most appropriate for micronucleus data, we noted that in this sample the mean was not equal to the standard deviation, which is a characteristic of the Poisson distribution. The Poisson distribution was not considered further.

TABLE 7 - 7. DESCRIPTIVE STATISTICS FOR FREQUENCY OF MICRONUCLEATED CELLS DATA

Statistic	Value
n	39
Mean	22.710
Median	21.30
Mode	~16
Standard deviation	10.152
Coefficient of Variation	44.7 %
Mean of Ln(FMC)	3.022
Standard Deviation of Ln(FMC)	0.467
Geometric Mean	20.54
Geometric Standard Deviation	1.60
Minimum	5.90
Maximum	49.10

On the strength of the data presented above, it was decided to log transform the micronucleus frequency data and then use parametric statistical tests throughout.

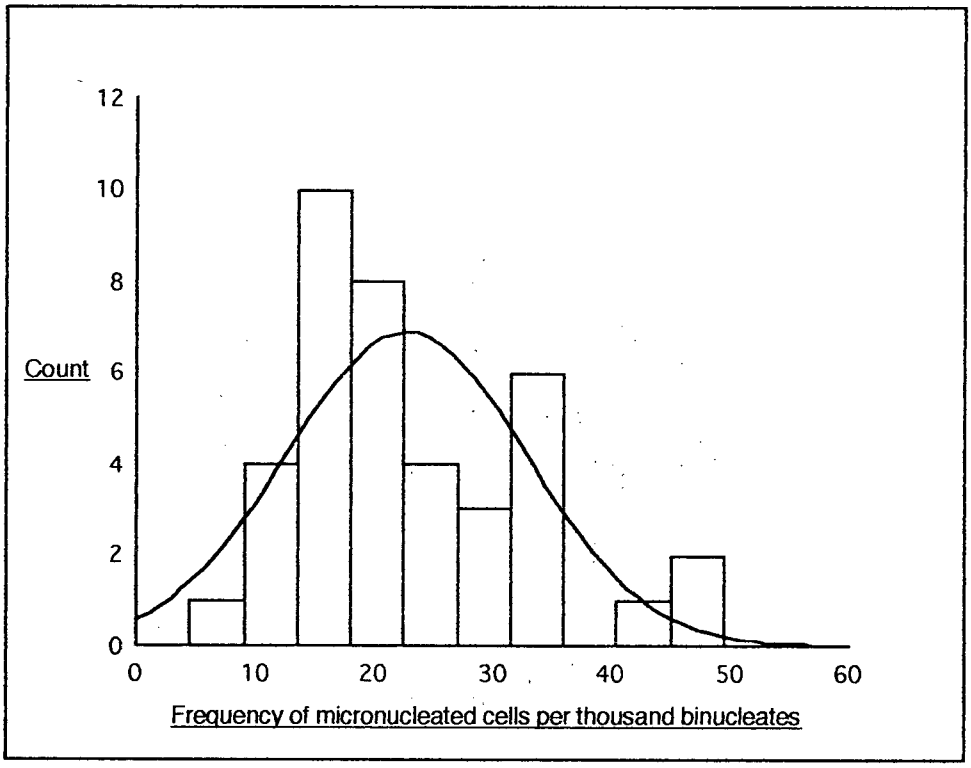

FIGURE 7 - 2. FREQUENCY DISTRIBUTION: UNTRANSFORMED DATA

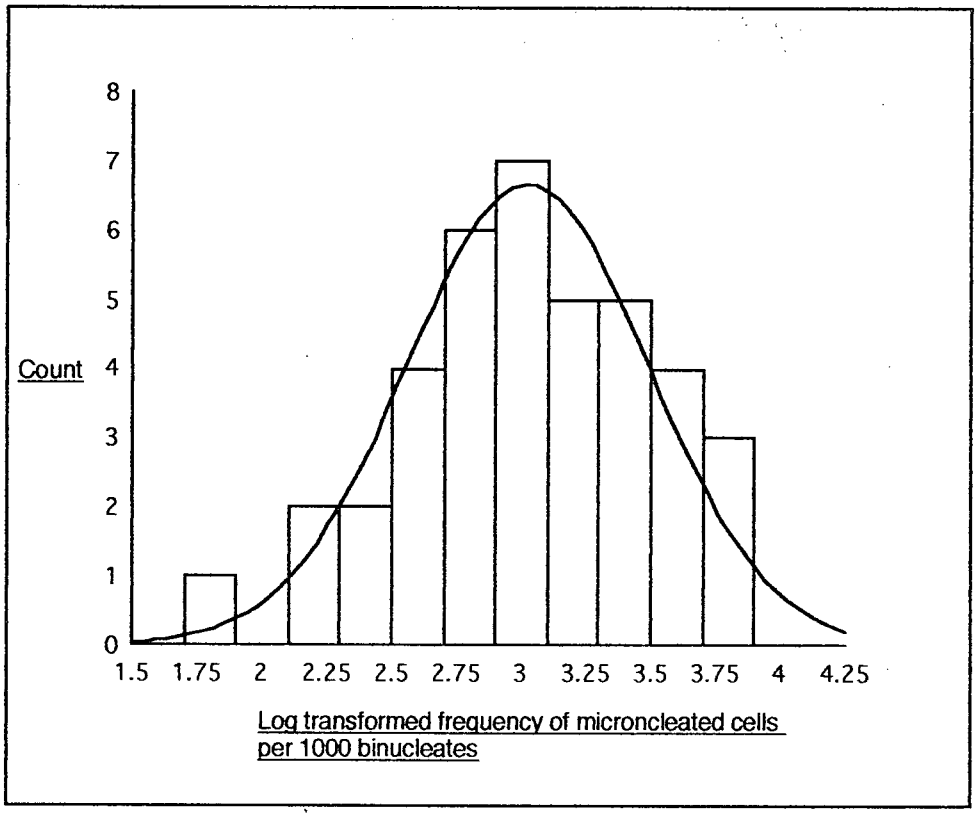

FIGURE 7 - 3. FREQUENCY DISTRIBUTION: LOG TRANSFORMED DATA

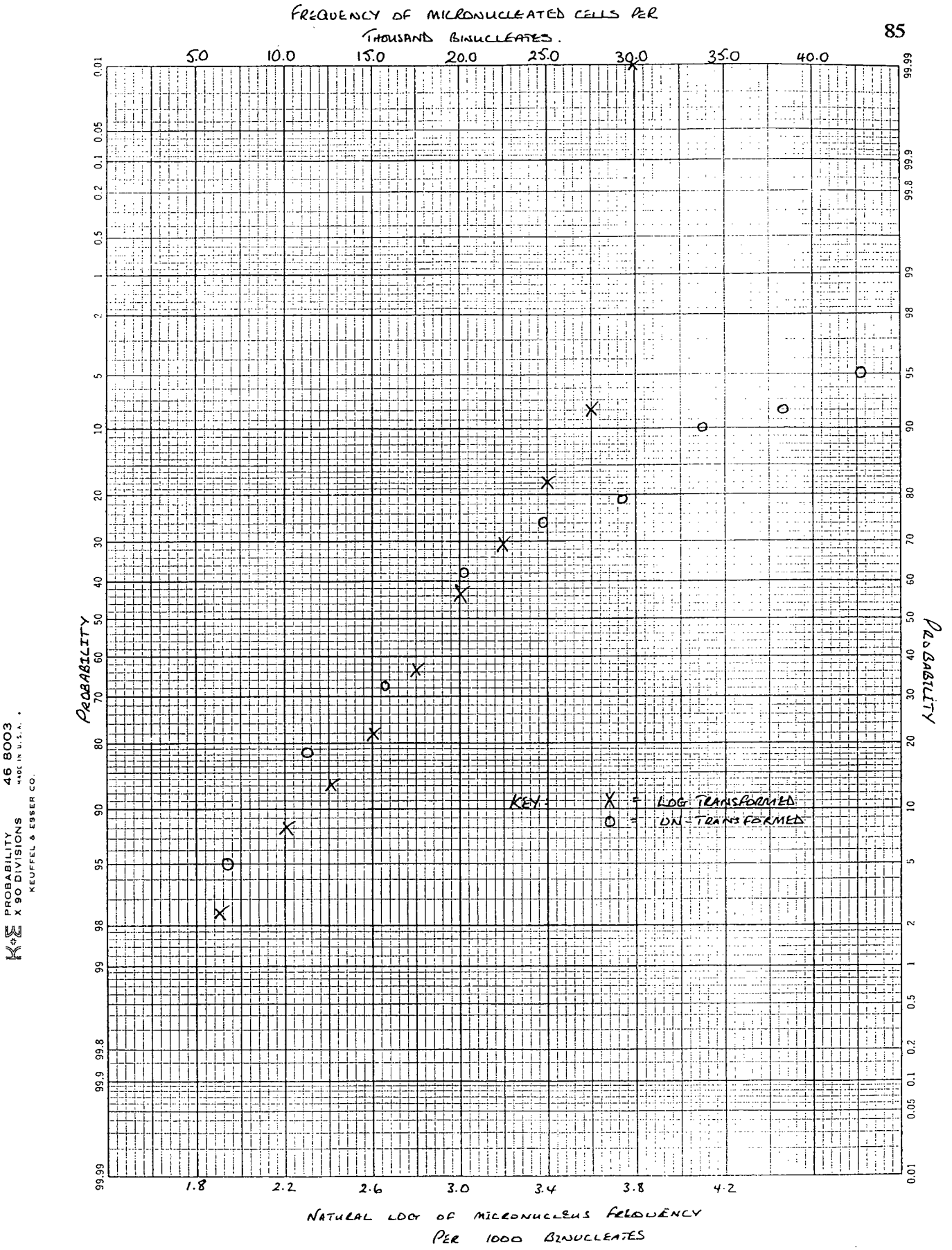

FIGURE 7 - 4. PROBABILITY PLOT OF UNTRANSFORMED DATA AND LOGNORMAL DATA

7.5 The Effect of Employment as a Farmworker on Micronucleus Frequency

From the data collected, it was possible to divide the study group into various exposure categories, based on current and historical employment. This permitted several analyses to be performed: (i) levels of micronuclei in farmworker group versus control groups (section 7.5.1); (ii) levels of micronuclei in groups with different cumulative life-time farm employment (section 7.5.2); and (iii) levels of micronuclei in farmworkers with different short-term cumulative farm employment (i.e. weeks worked in 1993, section 7.5.3).

7.5.1 Micronucleus Frequency in Farmworker vs. Control Groups

Table 7-8 shows the results of the first analysis, which compared the frequency of micronucleated binucleate cells (FMC) in farmworkers with the frequency in the control group, and with two control subgroups: previous-employed and never-employed. FMC is given as the geometric mean and standard deviation as the data was log-transformed for the analysis.

Comparison between specific pairs of FMC results were made using Student's t-test. The farmworker group did not differ significantly from the control group. When compared to the two control sub-groups, the geometric mean of the farmworker group was depressed over 23% with respect to the mean of the never-employed group, and slightly elevated with respect to the previously exposed group. Neither difference was significant. The never-employed sub-group was significantly elevated with respect to the previously exposed sub-group. There were no significant differences between the replicative indexes (RI) for any of the categories.

To investigate the reason for this apparent heterogeneity in the control group further, an analysis was performed of the contribution of the higher SES subset of controls (see 7.2.3). The mean FMC of this subset (27.7 per 1000 binucleates) was elevated in comparison to the rest of the never-employed group (23.5 per 1000 binucleates). This variable was retained and included in future analyses.

TABLE 7 - 8. FREQUENCIES OF MICRONUCLEATED CELLS IN FARMWORKERS AND CONTROLS

	n	FMC (per 1000 binucleates)[a]	RI
Farmworkers	18	19.20(±1.70)	1.24(±0.11)
Controls	21	21.76(±1.50)	1.30(±0.11)
- Previously-Exposed	8	17.32(±1.37)*	1.32(±0.12)
- Never-Exposed	13	25.05(±1.50)	1.29(±0.11)

[a] Geometric mean ± GSD

* $P < 0.05$, Student's t-test, compared with Never-Exposed group

7.5.2 Cumulative Employment

The basic analysis of farmworkers vs. controls is a suitable test if the lymphocyte life span is short - e.g. less than one year. However, to account for accumulation of DNA-damaged lymphocytes if their life span is greater than one year, we considered individuals with a history of farmwork as part of the potentially exposed group. As the life of the T-cell population as a whole is not infinite, however, the cumulative exposure were adjusted to reflect the natural loss of some fraction of the cells over time.

An adjusted life-time cumulative employment duration ("equivalent acute exposure" or EAE) was calculated for farmworkers and previously-employed farmworkers to determine remaining effect after *n* half-lives of the T-cells. The following formula was used: $EAE = \sum (D_j)e^{-j/m}$; where D_j is the annual "dose" received j years before blood sampling (and always equals 1 in this study, for 1 years employment) and m is the mean life-time of lymphocytes. Because there is still much discussion about the true mean life span of T-lymphocytes, the estimate of Braselmann *et al.* (1994), m=4.3 was used. Cumulative exposure was assumed to be continuous up until the last year worked. Table 7 - 9 shows the adjusted cumulative exposures in descending order of magnitude.

Four categories of EAE were created of equal duration: never employed (0 years), low EAE (< 1.67 year); medium EAE(1.67 - 3.33) and high EAE (> 3.33 years). Table 7 - 10 shows micronucleated cell frequency (FMC) with respect to adjusted cumulative years of employment (EAE). The group with the longest cumulative farmwork employment (> 3.33 years) showed a 63% increase in FMC (25.28 per 1000 binucleates) over the medium group (1.67 - 3.33 years, 15.54 per 1000 binucleates) and a 53% increase

TABLE 7 - 9. CUMULATIVE EXPOSURE DATA ADJUSTED FOR T-CELL HALF LIFE OF 3 YEARS

ID	First years worked	Last year worked	Cumulative Exposure (years)	EAE (m=4.3 years)
134	1968	1993	24	4.81
171	1970	1993	23	4.80
7	1980	1993	14	4.63
86	1973	1993	14	4.63
125	1979	1993	14	4.63
51	1980	1993	10	4.35
114	1987	1993	7	3.87
41	1980	1992	13	3.63
190	1990	1993	4	2.92
59	1990	1993	4	2.92
38	1991	1993	3	2.42
47	1990	1993	3	2.42
33	1991	1993	3	2.42
63	1980	1990	11	2.21
45	1992	1993	2	1.79
43	1992	1993	2	1.79
195	1992	1993	2	1.79
200	1977	1988	5	1.04
54	1993	1993	1	1.00
44	1993	1993	1	1.00
32	1993	1993	1	1.00
117	1983	1985	3	0.38
185	1975	1982	8	0.32
147	1985	1985	1	0.16
121	1978	1978	1	0.03
78	1970	1970	1	0.00

over the low group (<1.67 years, 16.48 per 1000 binucleates). Interestingly, the control group (24.75 per 1000 binucleates) was also elevated compared to the medium or low groups. Analysis of variance indicated statistically significant departure from homogeneity among the group ($p < 0.05$), but a Scheffé

TABLE 7 - 10. FREQUENCY OF MICRONUCLEATED CELLS BY ADJUSTED CUMULATIVE YEARS OF EMPLOYMENT

	n	FMC (per 1000 binucleates)[a*]	RI
Controls	14	24.75(±1.48)	1.29(±0.11)
EAE (years):			
< 1.67	8	16.48(±1.47)	1.27(±0.12)
1.67 - 3.33	9	15.54(±1.72)	1.28(±0.10)
> 3.33	8	25.28(±1.45)	1.23(±0.15)

[a]GM (± GS)

* $p < 0.05$, ANOVA

post-hoc test found no significant pair-wise differences. No significant differences were apparent between the RI for any groups.

To determine if there was a linear relationship between the EAE and FMC, linear correlation analysis was performed. Figure 7-5 includes all 39 data points, and no relationship is evident. When the analysis is restricted to only those individuals who have ever been employed as farmworkers (n=26), however, a positive relationship between the two variables emerges (Figure 7-6). The relationship is statistically significant (p=0.040), but the adjusted-R^2 value is 0.13, indication that only 13% of the variability in the FMC in the farmworkers is explained by an cumulative employment experience.

7.5.3 Weeks Worked in 1993

The individuals who were employed as farmworkers during the summer of 1993 worked between 16 and 27 weeks. Farmworkers were grouped into three categories of equal duration (16 to 20 weeks, 20 to 23 weeks, and 23 to 27 weeks), and these categories were compared to test for an association between increasing short-term employment duration and elevated micronucleus frequency. The results are shown in Table 7-11. The group that worked for the longest duration in 1993 shows an 17% increase in FMC over the control group, and an increase of 55% and 7% over the group employed for < 20 weeks and 20 - 23 weeks, respectively. None of these elevations were statistically significant (ANOVA, $p < 0.05$). There was no significant difference between the replicative index (RI) values of any of the groups (ANOVA).

TABLE 7 - 11. MICRONUCLEUS FREQUENCIES BY NUMBER OF WEEKS WORKED DURING 1993

	n	FMC (per 1000 binucleates)[a]	RI
Controls	21	21.76(±1.50)	1.30(±0.11)
Weeks worked:			
< 20	11	16.44(±1.71)	1.26(±0.13)
20 - 23	4	23.78(±1.65)	1.20(±0.09)
> 23	3	25.43(±1.66)	1.20(±0.06)

[a]GM ± GS

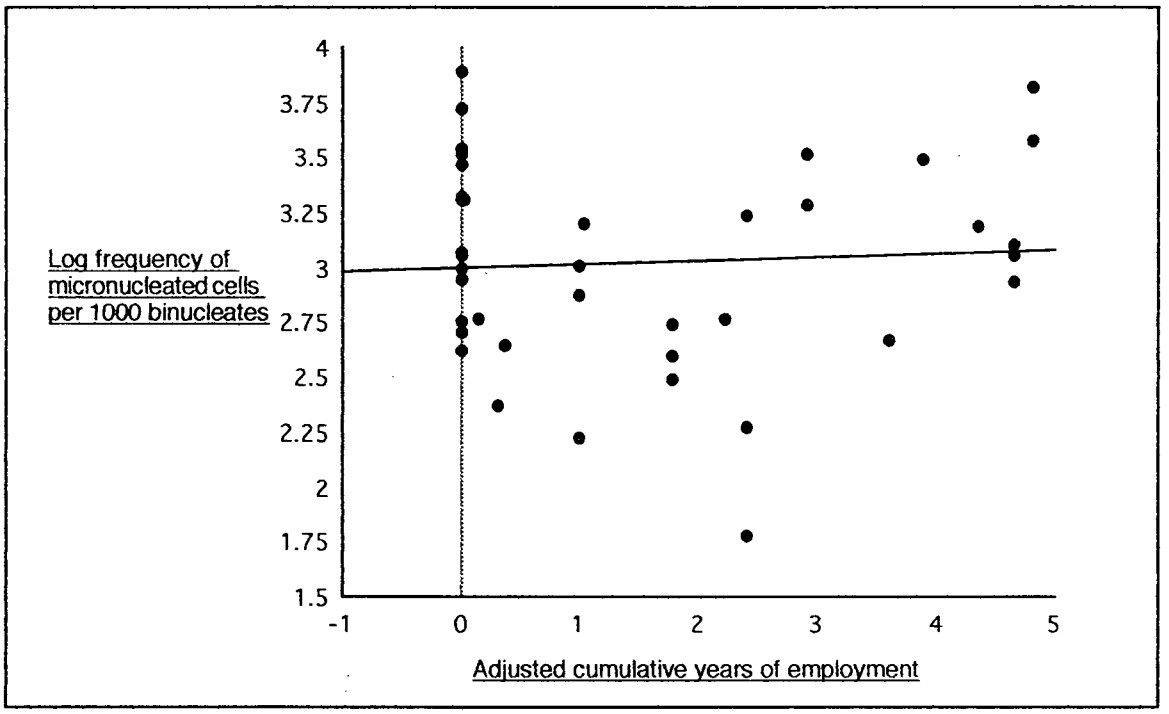

FIGURE 7 - 5. REGRESSION PLOT, MICRONUCLEUS FREQUENCY VS. ADJUSTED CUMULATIVE YEARS OF EMPLOYMENT, ALL PARTICIPANTS (N=39)

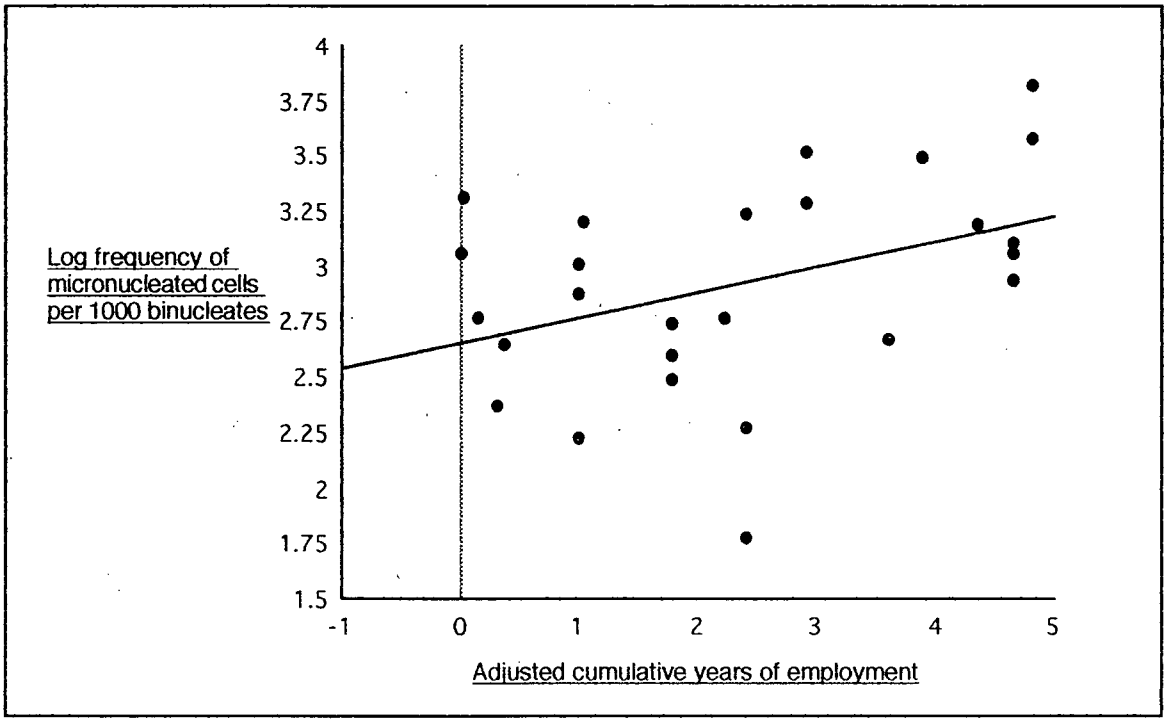

FIGURE 7 - 6. REGRESSION PLOT, MICRONUCLEUS FREQUENCY VS. ADJUSTED CUMULATIVE YEARS OF EMPLOYMENT, INDIVIDUALS WITH FARMWORK EXPERIENCE ONLY (N=26)

To determine if there was a linear relationship between the cumulative weeks of employment during 1993 and FMC, a linear correlation analysis was performed. Figure 7-7 shows the results when all data points are included. No relationship is demonstrated. When the analysis is restricted to only those individuals who worked during 1993, however, a positive relationship between the two variables emerges (Figure 7-8), with 6.9 % of the variance in micronucleus frequency explained.

To determine if there might be an additive effect between these two cumulative values, a multiple regression analysis was conducted with both weeks worked in 1993 and adjusted cumulative years (EAE) as independent variables. The resulting model is described by the equation:

$$\text{Ln (MN per 1000 BN)} = 1.09 + 0.06\ (\text{1993 weeks worked}) + 0.22\ (\text{EAE})$$

P-values were 0.053 and 0.006 for weeks-worked and EAE, respectively. This analysis demonstrated that taken together, the two variables are good predictors of micronucleus frequency. The multiple regression analysis had an adjusted-R^2 value of 0.42, meaning 42% of the variance in micronucleus frequency was explained by these two variables. This compares to 6.9% or 29.2% for weeks-worked alone or EAE alone, respectively (only n=18, 1993 farmworkers compared).

7.6 Non-Occupational Factors

Data was gathered regarding non-occupational factors which were thought to influence micronucleus frequency: age, X-ray exposure (ten-year cumulative exposure and recent X-ray), vegetarian status, tea and coffee consumption, vaccinations received in prior 6 months, number of years since menopause, and red blood cells (RBC) folate levels (see Table 7-2). Data regarding the number of hours spent outside was excluded from the analysis because it was closely correlated to exposure status. Because of the low numbers in the study, data from all groups were pooled and then used to assess the relationship between the non-occupational factors and MN frequency.

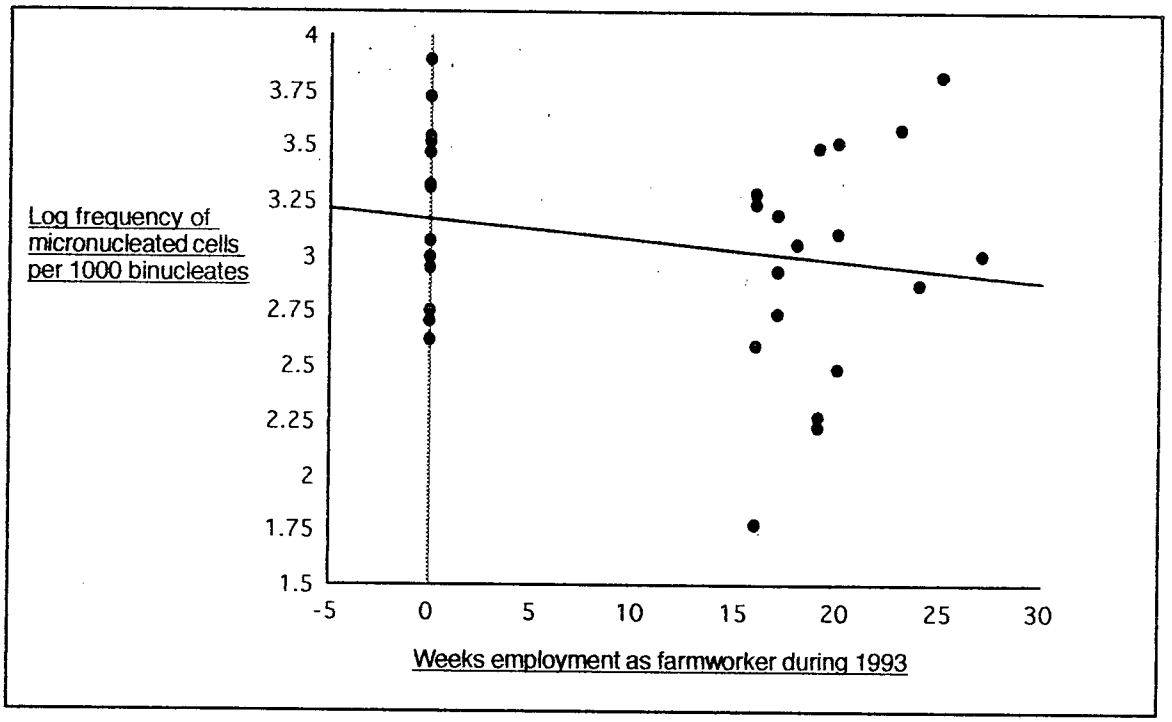

FIGURE 7 - 7. REGRESSION PLOT, MICRONUCLEUS FREQUENCY VS. WEEKS WORKED IN 1993, ALL PARTICIPANTS (N=39)

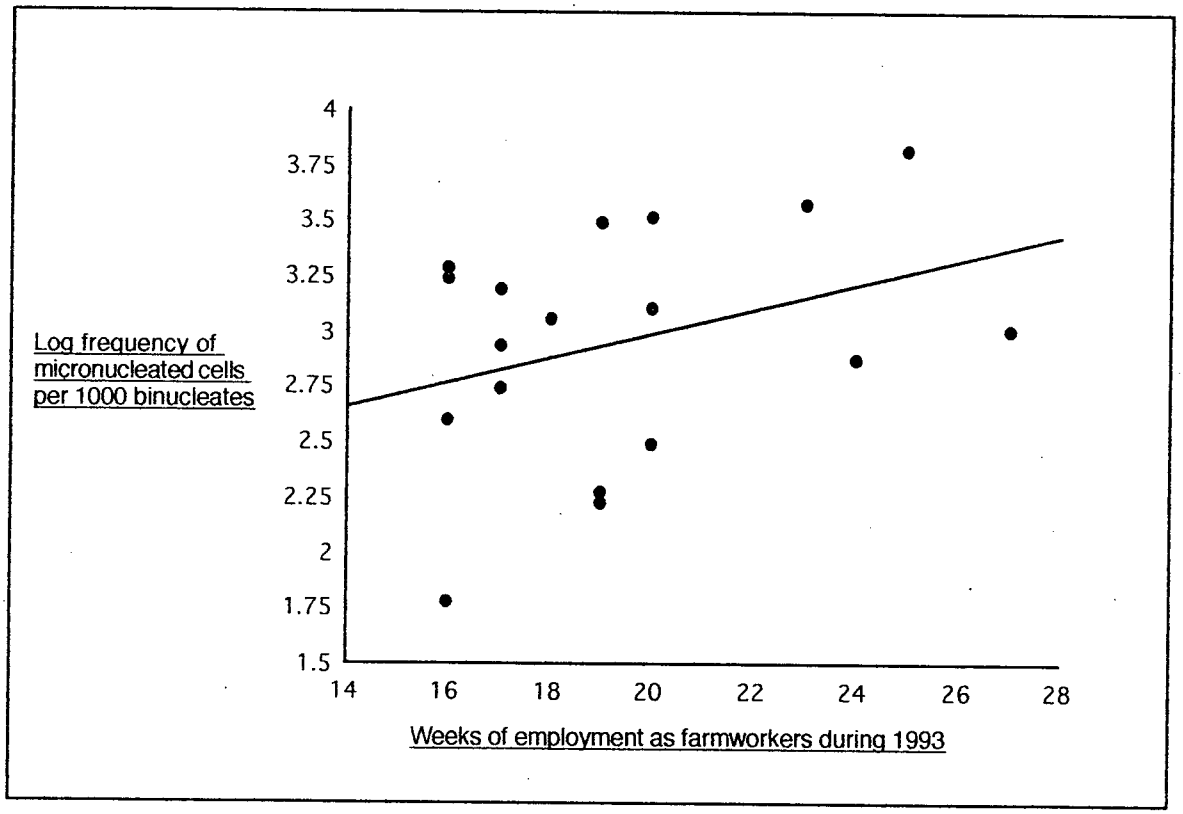

FIGURE 7 - 8. REGRESSION PLOT, MICRONUCLEUS FREQUENCY VS. WEEKS WORKED IN 1993, ONLY THOSE WHO WORKED IN 1993 (N=18)

7.6.1 Non-Occupational Factors - Descriptive

Table 7-12 shows the relationship between non-occupational factors and micronucleus frequency. Age, the number of X-rays in past ten years, X-ray in past 2 years, coffee consumption, menopause status, and years since menopause had little apparent influence on micronucleus frequency in this study. None showed significant differences in micronucleus frequency among the different groups. While still not significant, RBC folate level demonstrates a positive trend with micronucleus frequency increasing with increasing folate levels ($p = 0.256$). Individuals who have had a recent vaccination also show elevated micronucleus frequency, although again not significant ($p=0.220$). Those that consumed more tea also showed elevated micronucleus frequencies, although not statistically significant. Finally, those who characterized themselves as vegetarian showed an almost significant ($p=0.054$) decrease in micronucleus frequency when compared to those who consume meat. On this basis, RBC folate level, tea consumption, vegetarian status and vaccination variables were retained for future multivariate analysis.

TABLE 7 - 12. MICRONUCLEUS FREQUENCY AND NON-OCCUPATIONAL FACTORS

	N	MN (per 1000 binucleates)[a]	p Value*
AGE (years)			0.615
≤50	11	21.14(±1.50)	
51 - 60	10	18.08(±1.66)	
≥61	18	21.65(±1.12)	
RBC Folate level (nmol/L)[b]			0.256
≤400	9	16.76(±1.76)	
401 - 600	13	19.91(±1.44)	
≥601	5	25.97(±1.66)	
Tea Consumption (cups/day)			0.761
0	3	18.71(±1.59)	
1 - 3	26	20.05(±1.57)	
≥4	10	22.51(±1.72)	
Recent X-ray (in previous 2 yr.'s)			0.577
No	25	21.20(±1.59)	
Yes	14	19.40(±1.63)	
10 year X-ray			0.917
0	4	19.89(±1.32)	
1 - 3	22	21.12(±1.74)	
≥4	13	19.79(±1.44)	

TABLE 7 - 12. Continued

	N	MN (per 1000 binucleates)[a]	p Value*
Meat consumption			0.054
No	24	18.34(±1.58)	
Yes	15	24.63(±1.54)	
Coffee Consumption[c]			0.314
No	31	21.29(±1.61)	
Yes	7	17.39(±1.60)	
Vaccinations			0.220
No	29	19.45(±1.67)	
Yes	10	24.05(±1.27)	
Menopause			0.430
No	7	18.07(±1.60)	
Yes	32	21.12(±1.60)	
1 - 7 yrs	11	20.11(±1.79)	0.807
8 - 14 yrs	13	20.91(±1.58)	
≥ 15 yrs	8	22.97(±1.48)	
Socio-economic Status			
High	5	27.74(±1.47)	0.128
Low	34	19.65(±1.59)	

*ANOVA
[a] GM (±GS)
[b] 27 observations
[c] 38 observations

7.6.2 Multivariate Analysis

To take into account the potential confounding effects of non-occupational factors, a multivariate analysis was performed. Variables were selected for inclusion in the analysis if they demonstrated a relationship (even weak) to the outcome variable, or if they demonstrated differential distribution among the exposure categories. Table 7-13 shows the non-occupational factors from Table 7-12, and their distribution between the four cumulative exposure groups. The differences between the groupings were analyzed using ANOVA or chi-squared tests.

Tea consumption, RBC folate, number of X-rays in past ten years, recent last X-ray and number of years since menopause were all similar among the four exposure groups.

Referring to Table 7 - 13, the never-employed group had a mean age (49.6 ± 11.9 years) significantly lower than the medium (59.9 ± 5.6 years) or short-employed (59.8 ± 6.8 years) groups, but not significantly different from the long-employed group. The never-employed group also varied significantly from the high and medium-employed groups with respect to vegetarianism and coffee consumption. Only 28.6 % of the never-employed group reported being vegetarian, while 75.0 % and 88.9 % of the high and medium groups, respectively, were vegetarian. 30.1 % of the never-employed group reported drinking coffee, while only 12.5 % and 0.0 % of the respective high and medium employed groups drank coffee. On this basis, age and coffee consumption were added to the variables to be retained for further analysis. Finally, socio-economic status (SES) was included, which appeared to influence micronucleus frequency (see section 7.2.3) and was differentially distributed across employment groups.

In summary , age and RBC folate level (as continuous variables), vegetarian status, tea consumption, vaccination status, coffee consumption , and socio-economic status (as nominal variables) and cumulative

TABLE 7 - 13. NON-OCCUPATIONAL CHARACTERISTICS OF PARTICIPANTS BY EXPOSURE CATEGORY

	Adjusted Cumulative Employment (EAE, years)			
	> 3.33 (high)	1.67 - 3.33 (medium)	< 1.67 (short)	Never Employed
n	8	9	8	14
Age (years)*	58.4(±7.3)	59.9(±5.6)	59.8(±6.8)	49.6(±11.9)
Range	49-68	48-66	51-72	37-67
DIET				
% Vegetarian**	75.0	88.9	75.0	28.6
Tea intake (cups/day)	3.0(±0.5)	3.2(±1.3)	3.1(±0.6)	2.6(±2.1)
% Consume coffee[d]	12.5	0.0	25.0	30.7
RBC Folate[e]:				
n	5	5	5	12
nmol/L	525.00(±82.89)	400.00(±75.95)	402.20(±84.86)	521.00(±180.00)
HEALTH				
% Recent vaccination**	62.5	22.2	25.0	7.1
X-rays in last 10 years	3.63(±4.87)	3.22(±4.55)	2.50(±1.93)	4.29(±4.81)
% X-ray in last 2 years	25.0	33.3	50.0	35.7
Years since menopause	12.00(±8.50)	6.67(±5.00)	9.00(±8.82)	5.93(±5.90)
% High Socio-economic Status	0.0	0.0	0.0	35.7

Mean (±SD.)

* $P < 0.05$, ANOVA

** $P < 0.05$ Chi-squared

[d] 38 observations

[e] 27 observations

exposure level (as three dummy variables) were input to a multiple regression analysis, and then all possible competing models were assessed (SAS PROC REG, with SELECTION = ADJRSQ). There was one missing value for coffee consumption, and so the analysis was performed on 38 observations. Because there were missing RBC folate samples (n=27), the analysis was repeated with and without RBC folate observations. RBC Folate level was not found to contribute to explaining variation in the model, so the final analysis was performed on n=38 observations, without RBC folate.

The best fitting model which included all employment (EAE) groups, was selected, based on highest adjusted-R^2. This removed tea consumption, age and socio-economic status from the analysis as they did not contribute to the model. The final model, which included vegetarianism, coffee consumption, recent vaccination, and the three EAE levels, explained 28.1 % of the total variation in the micronucleus frequency. Table 7-14 shows the results from the regression equation. Table 7-15 shows the frequency of micronucleated cells (FMC) for each of the employment groups, after adjustment, and the relative change in frequency. Because the data was log-transformed, coefficients were exponentiated for presentation.

TABLE 7 -14. SUMMARY OF RESULTS FROM REGRESSION ANALYSIS REGARDING THE INFLUENCE OF NON-OCCUPATIONAL FACTORS ON THE RELATIONSHIP BETWEEN ADJUSTED CUMULATIVE YEARS OF EMPLOYMENT AND MICRONUCLEUS FREQUENCY (OUTCOME VARIABLE = LN[MN])

Variable	Coefficient	Standard error	P-value
Intercept	3.122	0.159	
Employment category:			
< 1.67 yrs	- 0.352	0.194	0.080
1.67 - 3.33 yrs	- 0.463	0.199	0.027
> 3.33 yrs	- 0.061	0.211	0.774
Non-vegetarian	0.293	0.161	0.080
Recent vaccination	0.233	0.167	0.174
Consume Coffee	- 0.399	0.183	0.037

The dose-dependent increase in FMC is still apparent after adjustment for potential confounders. All the employed groups FMC have dropped below that of the never employed as farmworker group. Only the decrease in the medium employment category (1.67 - 3.33 years) was significant ($p < 0.05$). The

consumption of meat, and recent vaccination elevate the frequency of micronucleated cells, although neither significantly. Coffee consumption significantly depresses the level of micronucleated cells ($p < 0.05$).

TABLE 7 - 15. MULTIPLE REGRESSION ANALYSIS FOR FREQUENCY OF MICRONUCLEATED CELLS, BY EXPOSURE CATEGORY

	FMC (per 1000 binucleates)	95% CI of FMC	FMC relative to baseline	95% CI of Relative FMC
Baseline[a]	22.69		1.00	
Employment Category				
< 1.67 years	15.96	10.66 - 25.19	0.70	0.47 - 1.11
1.67 - 3.33 years	14.28	9.53 - 21.32	0.63	0.42 - 0.94
> 3.33 years	21.35	13.84 - 32.67	0.94	0.61 - 1.45
Consume meat	30.41	21.78 - 42.20	1.34	0.96 - 1.86
Recent vaccination	28.59	20.42 - 40.16	1.26	0.90 - 1.77
Consume Coffee	15.20	10.44 - 22.24	0.67	0.46 - 0.98

[a]Baseline represents predicted micronucleus frequency when individual is vegetarian, non-coffee drinker, no recent vaccination, and has no history of farmwork

Following a similar adjustment for meat consumption, coffee consumption, and for recent vaccination, the differences between the farmworker group and the two control subgroups also remained (see Table 7 - 8). The adjusted frequency of micronucleated cells (FMC) for the never-employed group was 22.42 micronuclei per 1000 binucleates; 36% higher than the FMC for farmworkers and 75% higher than for the previously exposed, which had 16.44 and 12.81 micronuclei per 1000 binucleates respectively.

7.7 Kinetochore-Positive Micronuclei

The quality of the anti-kinetochore antibody staining system was highly variable. Even on a single slide, the intensity of FITC staining of nuclear kinetochores varied from very intense areas (e.g. see Figure 3-6) to areas which did not stain at all. The variability was probably caused by a degradation of the kinetochore antigen over time, perhaps as a result of hydrolysis of disulfide bonds in kinetochore proteins (Eastmond, 1994).

Because high frequencies for unscorable micronuclei led to unstable proportions of kinetochore-positive micronuclei, only slides which had high levels of scorable micronuclei (greater than 70%) were kept for further analysis.

Frequencies of kinetochore positive micronuclei (KPM) are recorded in Table 7-16. Five individuals were not included because of lack of data due to poor FITC staining. An ANOVA with post-hoc Scheffé analysis was used to examining the differences in KPM frequency. The never-employed group was found to be significantly elevated over the farmworker group.

TABLE 7 - 16. KINETOCHORE ASSAY RESULTS

	n	Kinetochore positive Binucleates (%)
Farmworker	16	44.98(±17.69)
Previous	7	51.48(±20.67)
Never	11	64.34(±14.49)[a]

[a]p = 0.027, ANOVA, Scheffé

8. DISCUSSION

8.1 Influence of Farmwork on Micronucleus Frequency

8.1.1 Micronuclei Frequency in 1993 Farmworkers

In this study, we compared the frequency of micronucleated cells in peripheral blood lymphocytes of female farmworkers employed during the summer of 1993 with a concurrent, non-farmworker control group. The frequency in farmworkers (19.2 micronuclei/1000 binucleates) was slightly lower than the frequency in the control group (21.76 micronuclei/1000 binucleates), but the difference was not statistically significant. Several of the subjects in the control group had prior experience as farmworkers, however. As the micronucleus frequency in the control group might have been elevated due to their prior exposure to pesticides, the frequency of micronuclei in two control sub-groups was compared. Those with previous farm work experience actually had a lower micronucleus frequency than both the never-employed group ($p < 0.05$) and the current farmworkers. The never-employed group had a higher level than the current farmworkers.

Adjustment for potential confounders, including meat consumption, coffee consumption, and vaccinations did not affect this ranking. Following adjustment, the never-employed group had 1.75 times the frequency of micronuclei of the previously exposed group, and 1.36 times the frequency of the current farmworkers.

The elevated levels found in control group may have been due to the selection of inappropriate individuals for comparison. Seasonal farmwork requires long hours of strenuous outdoor work. Particularly in the older age range that this study looked at (our mean age was 55.9 years), there would be a tendency for only stronger, healthier individuals to remain in this kind of work. Health information collected by the questionnaire shows that the farmworker group, although slightly older than the control group, had fewer chronic health problems, and that the control group tended to be more sedentary. The number of X-rays received by the control group was elevated over the farmworker group, and although X-ray frequency was not associated with elevated micronucleus frequency, it perhaps indicated generally poorer health in the control group. Upon meeting the farmworkers, we noted their healthy and strong physical appearance,

given the advanced age of some. Exercise and a healthy lifestyle are factors associated with reduced risk of cancer, and thus we might expect the farmworker group to show reduced micronucleus frequency (in the absence of exogenous genotoxins such as pesticides) when compared to the general population.

Although there are several studies which investigated biomarkers of genotoxicity in pesticide applicators (Rupa *et al.*, 1988, 1991, Yoder *et al.*, 1973), studies of farmworker groups who do not engage in pesticide application are uncommon. A literature search found no studies which explicitly included only individuals whose primary exposure was through contact with treated crops. Several cytogenetic studies were reviewed (Table 8-1) which investigated agriculture or floriculture workers whose tasks were similar to our study participants. However they varied in the crops worked, pesticides used and climate, etc., and so direct comparison is not possible. Only one of the studies utilized the micronucleus assay, the others using either sister chromatid exchange or chromosomal aberrations assays.

TABLE 8 - 1. CYTOGENETIC STUDIES IN AGRICULTURAL WORKERS

Author (year)	Tasks performed	n (exposed)	Pesticides[a]	Assay[b]	Results
Dulout *et al.* (1985)	Floriculture; 49% non- sprayers	37	OP, C, OC, and Captan	SCE CA	+VE
Carbonell *et al.* (1990)	Agriculture workers	27	OP, P, Captan, and Benomyl	SCE	- VE
De Ferrari *et al.* (1991)	Floriculture; Some spraying	64	H, F, OP, P, and OC	SCE CA	+VE +VE
Gómez-Arroya *et al.* (1992)	Agriculture workers	94	OP, C, OC, and Triazines	SCE	- VE
Bolognesi *et al.* (1993)	Floriculture Agriculture workers	71	OP, OC	MN	+VE
Carbonell *et al.* (1993)	Fruit and floriculture; Some spraying	70	OP, C, P, and Captan	SCE CA	- VE +VE

[a]OP - organophosphate; OC - organochlorine; C - carbamate; P - pyrethrin

[b]SCE - sister chromatid exchange; CA - chromosome aberration, MN - micronucleus

The negative results in this study concur with those of Gómez-Arroyo *et al.* (1992), and Carbonell *et al.* (1990, 1993), who also reported no association between employment as agricultural workers and elevated biomarker levels. Gómez-Arroyo *et al.* looked at agricultural workers in rural Mexico, using the sister

chromatid exchange (SCE) assay. The crops there were primarily vegetables, but the range of chemical used on the crops was similar to those used on the berry crops in the Fraser Valley. No details were provided about the nature of the exposure - i.e. whether it occurred during application of pesticides or on re-entry to treated fields. They concluded that the exposures were probably too low to cause detectable changes. Carbonell *et al.* (1990), investigated floriculture workers who were commonly exposed to a wide variety of pesticides, and used several pesticides also used here in British Columbia, including deltamethrin, captan and benomyl. The nature of their exposure was not explicitly defined. The authors found no association between the frequency of sister chromatid exchange and employment as a floriculturist, and they concluded that the exposures were too low to cause a measurable effect.

In a second study, Carbonell *et al*. (1993) studied a group of 70 floriculturists, some of whom also cultivated strawberries. Again, a wide variety of pesticides were identified as being used, and captan was used by 26% of the participants. The authors found no SCE activity, but did find elevations in chromosomal aberrations (CA) in the pesticide-exposed group. Some of these workers, however, applied pesticides as part of their duties (on average, 170 hours per year, range 0 - 1266 hours/ year), and the majority of this work was also done indoors in greenhouses. Both of these facts would contribute to higher exposure than would be experienced by non-applicators.

De Ferrari *et al.* (1991), studied two groups of pesticide-exposed floriculturists, one healthy, and the other hospitalized for bladder cancer. There were 32 individuals in each group. Again these groups had mixed exposures, involving mixing and applying pesticides, but also included the handling of treated plants. Both the exposed groups demonstrated increased SCE and CA frequencies when compared to a healthy, non-exposed control group. Dulout *et al*. (1985), studied a group of floriculturists, utilizing primarily organophosphate and carbamate insecticides, who worked in plastic greenhouses. Approximate 50% of the participants were also classified as applicators. They found significant elevations in CA frequency in the exposed workers. In another part of the same study they found SCE, but not CA, levels elevated in a subgroup of the exposed population who presented with symptoms of pesticide intoxication, when compared to a group who were asymptomatic.

Bolognesi *et al*. (1993), utilized the micronucleus assay (with cytochalasin-B modification) to study the genotoxic effects of pesticide exposure to floriculturists in Italy. They found that individuals exposed to pesticides had a statistically significant 25% increase in micronucleus frequency over unexposed controls (local blood donors). Only forty percent of the 71 participants worked solely outdoors however, the rest working at least part of their time indoors. No details of pesticide exposure were provided, except that dithiocarbamates, organophosphates and chloro-organics were used. The group was selected from individuals applying for government permits to use hazardous pesticides, and so we may assume that they probably engaged in some application activity. The author also states that "all exposed subjects worked with pesticides on a regular basis 8 h per day".

The variation among the findings of these studies might be attributed to a number of factors. Three different genotoxicity assays were used, different genotoxic tests measure different mutation events, and have different sensitivities. For example, SCE's which are highly sensitive to radiation, and to radiomimetic agents, are insensitive to smoking, which is considered a moderately powerful clastogen. Because of these differences we might expect some tests to be more sensitive to pesticides than other tests. Also previously noted, the conditions of application varied. Several of the studies involved indoor application, which would be expected to elevate exposure. Use of personal protective equipment (PPE) was not indicated in any of the studies, although some (Dulout *et al.*, 1985) noted that sometimes conditions caused the applicators to remove clothes. Exposure misclassification and exposure variability between studies is probably the major influence, however. None of these studies obtained detailed exposure information. Generally, the investigators were only able to identify the most commonly used pesticides, or the pesticides used most recently by their subjects. This problem stems from the huge variety of pesticides available, and because the pesticides are generally applied in combinations, at different rates, in different formulations, and in different climatic and natural environments. A group which is identified as "exposed to pesticides" may be very heterogeneous with respect to individual agents. Given that the degree of genetic toxicity of different pesticidal agents is very different, then an exposed group probably contains individuals who are exposed to a wide range of levels of each pesticide. This results in a serious misclassification bias, resulting in a dilution of the observed effect.

8.1.2 Micronuclei and Cumulative Work Experience

Associations between micronucleus frequency and duration of employment as a farmworker both over one season, and over a lifetime, were investigated. All individuals with farmworker experience were analyzed for association between years worked as a farmworker and micronucleus frequency; current farmworkers were also analyzed for an association between the number of weeks worked in 1993 and micronucleus frequency.

The "equivalent acute exposure" or "EAE" is a measure of lifetime cumulative exposure adjusted for the half-life of the T-Lymphocyte. The group with the highest EAE (>3.33 years) had a micronucleus frequency of 25.28 per thousand binucleates (BN), approximately 58% higher than either the medium group (1.67 - 3.33 years, 15.54 MN/1000 BN) or lowest EAE group (< 1.67 years, 16.48 MN/1000 BN). The frequency of micronucleated cells in the never-employed control group was 24.75 MN/1000 BN.

A trend test with all subjects (n=39) did not show a correlation between cumulative employment duration and micronucleus frequency. However, when only those individuals with prior experience as farmworkers were included, the correlation analysis showed a small but statistically significant increase in micronucleus frequency related to increasing years of employment as a farmworker. The increase was approximately 13% for every year worked. The adjusted-R^2 value was only 0.13 however, meaning that cumulative years of exposure only accounted for 13% of the total variation. This supports the contention that the control group in our study represented a different base population. Bolognesi *et al.* (1993) also demonstrated a dose-response relationship in their MN study of pesticide-exposed floriculturists. They found a 23% increase in MN frequency in individuals with 19-30 years of exposure, and a 71% increase in those with > 30 years of exposure. Carbonell *et al*. (1993), in his study of floriculturists found a cumulative effect, with the frequency of chromosome aberrations increasing significantly with increasing duration of exposure. Increased micronucleus frequency associated with increasing years at work was also seen in other occupational groups including reactor workers, X-ray workers and mercury miners (Al-Sabti *et al.*, 1992).

Evidence for long-lasting cumulative damage has been demonstrated in non-occupational studies. Elevated frequencies of micronucleated cells were found in the peripheral blood lymphocytes of patients treated with chemotherapy for testicular cancer up to nine years after their treatments ended (Osanto *et al.*, 1991). In another study, individuals who had completed combined chemotherapy and radiation therapy 3 to 12 months previously still showed higher levels of micronuclei when compared to a non-treated group (Migliore *et al.*, 1991b). In individuals exposed to nuclear fallout, increased levels of chromosomal aberrations could be detected several decades after the exposure occurred (referenced in Bauchinger *et al.*, 1989).

The relationship between the frequency of micronuclei and the increasing amount of time spent in the fields during one season was explored. Individuals who worked during the summer of 1993 were divided into three groups. Those in the longest-employed groups (>23 weeks) had a 57% increase in the frequency of micronuclei (25.43 MN/1000 BN) over the individuals who worked less than 20 weeks (16.44 MN/1000 BN). This difference was not found to be statistically significant, perhaps because of the small sample size. Individuals who worked between 20 and 23 weeks, had an intermediate micronucleus frequency (23.78 MN/1000 BN). Linear regression analysis demonstrated a weak positive correlation between weeks worked and micronucleus frequency.

An analysis of the combined effects of lifetime cumulative exposure and recent exposure in the farmworker group demonstrated that when taken together these variables were fair predictors of micronucleus frequency. A mechanism that might support this kind of additive effect is one in which the assay enumerates two sub-groups of T-lymphocytes, both stimulated by PHA, but with different mean life-spans. Then the longer-lived would be responding to the historical exposure, while the shorter lived cells would present recently accrued damage. This is supported by the view that mature T-lymphocytes have an almost indefinite life span, while naïve T-lymphocytes, unless rescued by an encounter with an antigen, die within a few weeks (Sprent and Tough, 1994).

8.2 Non-Occupational Factors and Micronucleus Frequency

The mean micronucleus frequency in this study for both farmworker and controls appears to be somewhat higher than reported values in the literature for similar occupational studies, which used similar techniques (Table 8-2). This is probably primarily due to the influence of female sex and age, both of which have been positively associated with micronucleus frequency.

Drawing on Table 8-2 and other studies, we find that although some authors did not find a gender-related effect on micronucleus frequency (Brenner *et al.*, 1991, Di Giorgio *et al.*, 1994), several authors have noted that females tend to have higher background micronucleus frequency than males. Fenech (1994) studied 24 age-matched male and female pairs. He chose married couples to maximize the similarity of lifestyle factors which might influence micronucleus frequency. The female's micronucleus frequency was on average 1.4 times greater than the male's. In a concurrent study, he found that although the micronucleus frequency of both males and females increased with age, the females increased at a higher rate. Yager *et al.* (1994) studied 14 age-matched male and female pairs, a subset of a styrene exposed study group. She found that the females had 2.3 times the number of micronuclei found in the male group. Bolognesi *et al.* (1993) in his study on floriculturists noted a 1.45 times higher micronucleus frequency in females over males. An association of micronucleus frequency with gender would cause the study group in the current study to appear to have a higher background rate than other occupational studies, most of which are confined to males. Both the studies in Table 8-2 that investigated significant numbers of females (Yager, 1994, Anwar, *et al.*, 1994) still had lower MN frequencies than the current study, but they also had a lower mean age and increased age has been linked to elevated MN frequency.

The elevated frequency in females is thought to be due aneuploidy involving the loss of an X-chromosome in females. Richard *et al.* (1994), tested this theory by probing micronuclei for involvement of the X-chromosome in women of different ages. Richard *et al.* showed that in females, the X-chromosome was involved between 2 to 5 times more frequently than would be predicted if there was random involvement of all chromosomes. Additionally, the increase was positively associated with age, therefore also demonstrating that over involvement of the X-chromosome increases with age.

While some studies (Di Giorgio *et al.*, 1994) did not show an effect of age on micronucleus frequency, many others have demonstrated a positive association (Högstedt, 1984, Högstedt *et al.*, 1991, Migliore *et al.*, 1991a, Bolognesi *et al.*, 1993, Köteles *et al.*, 1993, Yager *et al.*, 1994, Sorsa *et al.*, 1988, and Fenech, 1993). Fenech studied 224 healthy individuals, and found a highly significant correlation between age and MN frequency. His data suggests that the MN frequencies in our study are normal or even a little low for the mean age. An analysis of a sub-group of 24 women with a mean age of 49.8 years showed that they had

TABLE 8 - 2. OCCUPATIONAL EXPOSURE STUDIES UTILIZING THE CYTOCHALASIN BLOCK (CB) TECHNIQUE

Author	Exposure	Average Age(yrs)	n Ex/con	Micronucleated Cells per 1000 Binucleates		Sex M/F
				Exposed	Control	
Maki-Paakkanen (1991)	Styrene	34.0	17/17	14(±6)	12(±8)	-
Migliore, *et al.*. (1991a)	Chromium	40.2	38/38	7.44(±4.21)[a] 12.01(±6.23)[b]	7.28(±3.64) 12.95(±10.41)	76/0
Sarto, *et al.* (1991)	EtOH	39.7	10/10	11.55(±4.7)	9.52(±5.4)	-
Ribeiro, *et al.* (1994)	EtOH	33.0	75/22	38.4(±3.7)*	10.6(±2.2)	97/0
Franchi, *et al.* (1994)	Hg	44.7	51/0	8.7(±2.47)[c,*]		51/0
Anwar, *et al.* (1994)	Cytotoxic drugs	29.6	20/20	10.05(±4.71)*	5.4(±2.22)	0/40
Di Giorgio, *et al.* (1994)	Paint	43	33/200	18.30 (±7.39)*	9.87(± 3.1)	155/78
Yager, *et al.* (1994)	Styrene	F41.1, M33.1	48/0	8.9 (±6.2)[c]		26/28
Bolognesi, *et al.* (1993)	Pesticides	44.7	71/75	8.57(±4.8)*	6.67(±3.1)	122/24
This Study (1995)	Pesticides	55.9	18/21	19.20(±1.70)	21.76(±1.50)	0/39

*Statistically significant

[a]Group A

[b]Group B

[c]Regression analysis

a mean micronucleus frequency of 25.5 per thousand binucleates, while his regression model would predict a frequency of nearly 40 MN per thousand binucleates for the mean age of our study group (55.9 years). In

a study of 188 individuals, Köteles *et al.* (1993), also found a significant correlation between age and MN frequency. Their regression model would predict a frequency of approximately 19.5 MN per thousand binucleates for age 55.9 years. The current study did not show an age relationship for micronucleus frequency, probably because there was a relatively narrow range of ages (80% of participants between 47 and 72 years), and relatively few subjects. Age effects may be due to an accumulation of DNA lesions over a lifetime, or may be related to true factors of aging, such as reduced DNA repair capability (Högstedt, 1984).

From our multivariate analysis, effects associated with meat consumption, coffee consumption and with vaccinations were also detected.

Approximately 35% of all cancer deaths are believed to be related to diet (Obe, 1982). Meat consumption may contribute to an elevated risk of cancer, particularly colorectal cancer, although the mechanism is not understood. Cooking meat produces heterocyclic amines and aminoimidazoazaarenes (AIA's), however, and these are known to be genotoxic and carcinogenic (Fenech and Neville, 1992, Fenech and Neville, 1993). Meat in a diet also presumably displaces vegetable components which may contain anti-mutagenic or anti-oxidant factors. The genotoxic effect of meat consumption has been investigated using the micronucleus assay in both animals and humans. Fenech and Neville (1992) found elevated levels of micronuclei in mice fed pan-fried and barbecued meats (73% and 136% increases respectively) over controls. In a similar study in humans, Tucker *et al*. (1993), noted increases in micronucleated polychromatic erythrocytes in individuals who were fed a diet high in fried beef for four consecutive days. The effect was only noted in individuals with clinically low folate levels, however. The current study found an increase in micronucleus frequency of 34% in individuals who consumed meat, which remained consistent after adjustment for farmwork, coffee consumption and vaccinations. This finding may be of some significance, because the subjects' eating practices were traditional (Assanand, *et al.*, 1990) and the non-meat eaters are likely to have been vegetarian from birth; therefore a stronger MN response to vegetarianism may be expected in this population. Because vegetarianism is related through traditionalism to other cultural practices however, it is possible that the response is to something associated with vegetarianism and not the effects of meat itself.

The results of this study showed a marked decrease in micronucleus frequency of 18% in those consuming coffee. This result contradicts the general finding that coffee and its constituents, including caffeine, damage DNA and that their effects can be measured by increases in markers of genotoxicity such as chromosome aberrations and micronuclei. Although some researchers have found no effect of coffee on genetic markers (Yager, 1990), caffeine is believed to interact with a variety of cell mechanisms, some of which relate to DNA synthesis and repair. It is thought that caffeine reduces the incorporation of thymidine into DNA, and inhibits the elongation of DNA strands in *E. coli*. It has been found to inhibit the *de novo* synthesis of purines (Waldren, *et al.*, 1992). Other mutagenic compounds are commonly found in coffee, including hydrogen peroxide, methylglyoxal and benzo-(a)-pyrene (Jensen, 1985, Nagao, 1985). McGregor (1988) noted that both caffeinated coffee and tea were associated with elevated frequency of micronuclei in erythrocytes, and that conditions of low folate combined with caffeine consumption also caused enhanced micronucleus formation, as well as the formation of fragile DNA sites. Chen *et al.* (1989) reported that individuals who consumed more than 4 cups of coffee a day had significantly higher percentage of cells with chromosome aberrations, and that this effect was linear with dose. Reidy *et al.* (1988) also found a positive association between coffee consumption and SCE.

The depressed levels of micronuclei associated with coffee consumption found in this study may be due solely to chance. It is also possible that coffee consumption is highly correlated to another, unidentified factor which has a strong negative influence on micronucleus frequency.

An association was also found between recent vaccination and MN frequency. Individuals who had received a recent vaccination had a 24% increase in micronucleus frequency. Other studies have reported a similar effect. Knuutila *et al.* (1978), demonstrated a statistically significant increase in SCE's in 10 women who had recently received smallpox vaccinations.

Data was collected regarding several other factors thought to potentially influence MN frequency. X-ray exposure, socio-economic status and menopause status were found not to influence MN frequency in this study. Other variables, such as viral illness, and medication status did not provide enough data points for analysis. Because we had no smokers in the study group, we were unable to ascertain its effect, but it has

been associated with increased MN rates in many studies. Two individuals in this study were exposed to environmental tobacco smoke, and it is possible that their micronucleus frequencies were influenced by this exposure.

There was a concern that exposure to sunlight might be a potential confounder, given that exposure to UV radiation is associated with cancer of the skin and lip (both significantly in excess in agricultural populations), and that hours spent outdoors was highly correlated with farmworker status in this study. However, there is no literature to suggest that the lymphocytes are detectors of UV damage to the skin. Darker skinned people are also at a reduced risk of skin cancers, presumably because of the protection afforded by their heavier skin pigmentation (Mathias, 1986). This would also be expected to modulate chromosomal damage in cells.

Individual susceptibility to DNA damage (genetic polymorphism) was not accounted for but there is no reason to believe that individuals with elevated susceptibility would be preferentially distributed between the groups, except with regard to the effect this had on their general health, and their ability to work.

8.3 Mechanism of Micronucleus Formation

Technical difficulties meant that only 70% of slides could be scored for kinetochore positive micronuclei. The variation in staining quality may have been due to loss of epitope over time. The samples which were stored the least time had best staining. Eastmond (1994) noted similar poor results in his early attempts with this assay, and eventually concluded it was a humidity problem. Our slides were originally stored under N_2 which we assumed to be bone dry; desiccant was also added, but not until several months after the slides had first been stored.

In farmworkers, 44% of the micronuclei were Kinetochore positive (K +VE) compared to 66% of the controls. This inferred that a greater proportion of micronuclei in the farmworkers were acentric, and suggested the result of a clastogenic mechanism. The difference also supported the idea that micronucleus frequencies were elevated for different reasons in the farmworkers and controls.

The author is not aware of published studies which have utilized the kinetochore method in *in vivo* human studies. *In vitro* studies have been used to validate the assay, but it has come under some recent questioning regarding its use *in vivo*, because aneuploidogens are generally thought to be direct acting, e.g. they have to be present at mitosis to interfere with the spindle mechanism. However, if pesticides are clastogens, they will depress K +VE proportion and so it is still a valid test.

8.4 Factors Influencing Study Outcome

8.4.1 Exposure Assessment

A weakness of this study, and one which plagues many studies of its type, was a lack of detailed exposure data. Because there was no access to farms, it was not possible to determine the types of pesticide used. Farmworkers themselves were not asked because it was considered unlikely that farmworkers would have knowledge of the farmers' pesticide application practices (Mentzer and Villalba, 1988). Farmworkers in this study worked on between 1 and 6 farms each during the summer of 1993, and potentially, all may have worked at different farms. This diversity of worksite was also noted by Oja *et al.*, (1990) who found 70% of farmworkers working on more than 3 farms in a summer. This would suggest a highly heterogeneous exposure to pesticide types, although probably within the group of high-risk pesticides identified in chapter 1.

Quantitatively, the exposure data is weaker still. Access to a farm site would have permitted exposure monitoring, but because of the changing conditions related to application schedule, climate, agent degradation, etc., any sampling strategy would have had to have been highly intensive and complex. A feasible alternative would be estimation of exposure by sampling the crop foliage and calculating exposure from dermal transfer coefficients for the crops and some of the pesticides (Zweig *et al*., 1985), but the dermal transfer coefficients have not been validated in British Columbia, and its particular climatic and environmental conditions.

In the future, because of new Workers Compensation Board requirements for pesticide application records, it may be practical to construct a retrospective exposure profile. Changes to the Employment Standards Act

which require more comprehensive record keeping on behalf of the labour contractors may also help in this regard.

8.4.2 External validity of results

As mentioned earlier, precise demographic data regarding the farmworker population in British Columbia is not available and so it is difficult to judge the representativeness of this study group. However recent local studies of farmworkers' social issues, including employment standards, health, and pesticide issues, determined that between 50 and 75% of farmworkers were female, and that their average age was 45 years (Oja *et al.*, 1990). A more recent study showed that over 46% of the 340 farmworkers surveyed were over 50 years (Basran, 1994). Both studies noted that the majority of farmworkers in the region were East Asian. Based on this information, the current study which only studied East Asian females, and which had a mean age of 55.9 years, could be said to be at least moderately representative of the Fraser Valley farmworker community. It may not representative of other ethnic minorities however, who might have different genetic susceptibilities, and it may not be representative of workers on crops other than strawberries, raspberries, and blueberries, because crop type and specifically canopy size and shape plays an important role in determining the exposure potential.

Our choice of contacting non-residential farmworkers through the community itself, rather than contacting through the farms, meant that our farmworker group probably had a more heterogeneous exposure. Non-residential farmworkers would be expected to be exposed to more of a variety of pesticide agents, and therefore more combinations because they work on many farms, and choice of pesticide agent is determined to some extent by the farmer, as are various application parameters which might affect exposure availability. This increase in heterogeneity might be expected to lower the apparent risk by diluting the effect of genotoxic pesticides or methods which elevated the exposure potential. Therefore any effect seen in this group could be expected to be amplified in a sub-population whose exposure is of a more homogeneous nature to genotoxic agents (i.e. residential farmworkers).

The study is representative of the labour-pool farmworker, though, and restriction of the study to a single farm site would limit the external validity further, although perhaps increasing the power of the study to detect relationships between specific agents and genotoxic outcomes.

8.4.3 Selection Bias

It is possible that the farmworker group was differentially biased because all participants were volunteers, and individuals who perceive themselves to be at risk may be more likely to volunteer. This would have the effect of artificially increasing the apparent risk in the farmworker group. However, it is unlikely that individuals would know their own micronucleus frequency or their personal susceptibility to genotoxic damage, and so they wouldn't volunteer based on knowledge of this health outcome. Those who perceived themselves at greater risk of exposure, however, may have self-selected, and this is more difficult to account for. No farmworkers reported any acute exposure episode during the season though, and this might have been expected from a group if its members were sensitized to pesticide exposure.

8.4.4 Sample Size and Control Group Selection

Although the study was successful in demonstrating the feasibility of assembling a study group and obtaining biological samples and associated questionnaire data, it did suffer from small sample size. This was partly due to low numbers of women attending the community center in which recruitment was based. Although other community recruitment sites were identified, recruitment was curtailed because of time constraints. The low numbers of non-farmworkers at the community center also caused us to recruit controls from within family and social groups with potentially different socio-economic backgrounds to the community center group. This may have introduced individuals with different lifestyle factors, which in part could have been responsible for the high micronucleus frequency seen in the control group when compared to the farmworker group. Indeed the control group as a whole tended to be slightly younger in age than the farmworkers, and to be less likely to be vegetarian, more likely to drink coffee, and be less recent immigrants. A subgroup of controls, whose members differed with respect to their employment, language and place of birth, had an elevated micronucleus frequency when compared to the rest of the control group.

It is possible that the control group is affected in some way, perhaps by generally poorer health than the farmworker group, and that they are an inappropriate comparison group. Comparisons between groups of workers and the non-employed often show this health bias, which is termed the healthy worker effect (Checkoway *et al.*, 1989). A comparison to working individuals would be more satisfactory; ideally farmworkers from organic farms where pesticides are not used. Because of the labour contract system, however, it may be difficult to find individuals who are employed solely on non-chemical treated farms. For a male population, other outdoor workers, such as construction workers who also have a similarly strenuous job, without obvious exposure to genotoxins, would be suitable.

Other potential confounders were perhaps inadequately controlled. No information was obtained with respect to alcohol consumption, although it was considered to be very rare among Sikh females because of cultural restrictions. Neither is alcohol consumption thought to be associated with elevated levels of micronuclei. Data on pesticide use in the home was not collected. This is potentially more serious, because repetitive use of household and garden pesticides could represent a significant exposure.

Stronger application of exclusion criteria would help reduce some of the inter-individual variability. In a study with a larger population base to draw on, individuals with existing illness could be excluded, along with those having had recent vaccinations, recent X-rays, or on any medication, etc.

8.4.5 Technical Issues

Generally the micronucleus assay worked well, with a few noticeable shortcomings, however. Cell proliferation and binucleate production was adequate, although higher percentages of binucleated cells was possible and would have reduced scoring time.

Internal validation of scoring was generally good, however, there were significant differences with the key metric of micronucleus frequency. Differences of 20 - 30 % were due to the rescoring covering different fields of view on the microscope slide. The degree of difference was exaggerated by the small number of binucleates rescored. When large number of cells were scored (i.e. 2000 binucleates) this effect would be reduced.

The low efficiency of the kinetochore labeling using antikinetochore antibodies was disappointing. It was attributed to elevated humidity during storage, but not proven. Additional testing is obviously required.

The coefficient of variation of micronucleus frequency in this study , 44.7 % for all participants, is comparable to other studies of its type (see Section 3.7.2).

9. CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

First and foremost, this study has established the feasibility of conducting a cytogenetic investigation in a seasonal farmworker population, which was regarded by many as a difficult population to study because of their transient work patterns, and language and possibly social differences. The study appears to be novel in the fact that it is the first cytogenetic study to focus solely on an occupational group whose primary exposure is through dermal contact with pesticide residues on crop foliage and fruit.

While it suffered a small study group size, had a questionable comparison group, and lacked a quantitative assessment of exposure, it still functions well as a pilot study. Using exploratory analysis it was able to demonstrate a perhaps important association between cumulative duration of employment as a farmworker and increasing micronucleus frequency. As micronuclei are an index of chromosome damage, which in turn is linked to various adverse health outcomes including cancer and birth defects in the offspring of individuals of reproductive age, this would indicate that individuals in this population, particularly those who have many years of experience, may in fact be at some elevated risk.

The association was only identified within the farmworker group, and comparison to a control group of largely unemployed individuals did not show any significant difference in micronucleus frequencies. The control group appeared to be generally less healthy than the farmworker group, and also differed in other aspects of diet and socio-economic indicators. One conclusion would be that this is an example of the healthy worker effect, and that elevations in the farmworker group due to pesticide exposure are masked by elevations in the control group due to generally poorer health. The results of the kinetochore assay suggested that the micronuclei in farmworkers were predominantly the result of clastogenic action, while the micronuclei in the control group more predominantly aneuploidogenic; this also indicated that the micronucleus frequency in the two groups is elevated for different reasons.

The positive findings of this study support the general weight of evidence from experimental and epidemiological work which find many pesticides to be genotoxic, and links pesticide exposure to certain cancers. The findings are consistent with many other cytogenetic studies which have investigated the effect of pesticide exposure on the health of other occupational groups such as applicators. Many, if not all of these studies, suffered from the same problem of poor exposure assessment. Because this would likely to lead to a misclassification of exposure, and result in weakening of any apparent effect, the true frequencies of cytogenetic markers in exposed populations would be expected to be higher than those reported.

The study provides valuable population data for a group which has not been widely studied - East Asian peri-menopausal females. It has shown that micronuclei frequency in this group is higher than that seen in typical occupational groups. However the elevated levels would be predicted because of the effects of gender and age on micronucleus frequency.

The study also demonstrated associations of micronucleus frequency with recent vaccination, coffee consumption and vegetarianism. The negative correlation of micronucleus frequency with coffee intake was opposite to the generally reported findings. Recent vaccination apparently elevates micronucleus frequency, which has been previously recognized. The association with meat consumption, however, is particularly interesting. Although an association between meat diets and risk of certain cancers has been proposed, other cytogenetic investigations have focused on short-term diet modifications to elicit effects in subjects. This is the first study to the author's knowledge which has investigated the effect of a meat diet on chromosomal damage, and included in its study group individuals who are likely to have been life-long vegetarians. Unfortunately, insufficient data was collected on the specific dietary habits of the individuals involved to permit more in depth analysis of this phenomenon.

Finally, the conclusions of this study may be generalized to a large portion of the seasonal farmworker population, because our study group is similar to the majority of farmworkers with respect to age, gender and ethnic origin. However, additional studies with males, and individuals of other ethnic backgrounds are still required.

9.2 Recommendations

Regarding the execution of this study, several recommendations can be made. Technically, the kinetochore assay perhaps needs further work to improve the staining efficiency, and increased cell proliferation would also produce more binucleated cells, and improve scoring times.

The variability in the study could be greatly reduced by the use of internal controls, in addition to external controls. Internal controls, or pre-exposure and post-exposure measures, would remove much inter-individual variability. They could also provide a time-series of measurements which can be correlated with seasonal exposures and show dose-response relationships. External controls could be greatly improved by selecting closer comparison groups, with respect to working status, and physical activity on the job. Two examples are farmworkers on organic farms, and construction workers. If larger pools of individuals were available to select from, then stricter application of exclusion criteria could remove many individuals with strong confounding potential, i.e. viral disease, medicated, recently vaccinated, smoker, etc..

Additional studies are recommended to broaden the external validity of the investigations. Similar studies in males, and other ethnic groups should be undertaken. Additional tests, such as the sister chomatid exchange and the chromosomal aberration assays could be added to the study to increase its chances of detecting genotoxic damage, by broadening the effects that could be detected. New studies should attempt to recruit larger populations to increase the statistical power of the study, as well as providing more control over the selection of participants. In sufficiently large populations, random selection should be a goal. To obtain larger pools of participants, additional community sources, such as religious centers and educational centers could be targeted. To this extent, males might be easier to recruit in some ethnic groups because they participate in community group activities more than women.

Finally, improvements must be made in the area of exposure assessment. A comprehensive analysis of farmworker's job activities and frequencies, and their coincidence with pesticide application is needed. This

would assist in determining the extent of the exposure problem, and identify pesticides posing the greatest hazard; this would permit prioritization and targeting of study efforts.

Qualitative assessment may be aided by new Workers Compensation Board of British Columbia requirements for pesticide application and reentry records. These may make retrospective exposure assessment easier, and new record keeping requirements for the farm labour contractors may permit tracing a of farmworker's movements among farms. Biomonitoring for pesticide residues in urine or blood, or cholinesterase testing, could be used to qualitatively demonstrate the occurrence of pesticide contamination, and possibly to identify pesticide contaminants.

Quantitative exposure assessment would be extremely challenging because of the constantly changing exposure conditions as applications are made, and residues degrade. Biological monitoring would have the advantage of integrating these fluctuations over time, if a suitable metabolite with the required kinetics could be identified. Modeling of exposures using the dislodgable foliar residues and dermal transfer coefficients could be an adequate intermediate step once the published transfer coefficients were validated for use here in British Columbia.

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APPENDIX A - STUDY HANDOUT

APPENDIX B - STUDY CONSENT FORM

THE UNIVERSITY OF BRITISH COLUMBIA

Occupational Hygiene Programme
Faculty of Graduate Studies
3rd Floor, Library Processing Centre
2206 East Mall
Vancouver, B.C. Canada V6T 1Z3
Tel: (604) 822-9595
Fax: (604) 822-9588

Pesticide Exposure Study Consent Form

This summer, Dr Jenny Quintana, an assistant professor with the Occupational Hygiene Programme, and a graduate student, Hugh Davies, will be conducting a study of pesticide exposure among farmworkers in the British Columbia. The purpose of this study is to test a new method of measuring pesticide exposure, for potential use in future studies.

Farmworkers doing manual work amongst crops may be exposed to low levels of pesticide residues, which they may absorb through their skin, as well as inhale and ingest. Current techniques cannot accurately measure the level of this exposure. This study will investigate a new exposure assessment method which measures the frequency of a particular component of blood cells, called micronuclei. Elevated levels of micronuclei may be associated with pesticide exposure. If they are, then they may be useful in the future as a way of determining safe levels of exposure, or determining if an individual has been overexposed.

Each study participant will donate two samples, one at the beginning of the picking season, and another later in the summer. The blood samples will be quite small (20 mL, or less than two tablespoons) and taken from the arm by a registered nurse. This procedure may cause some discomfort, and slight bruising. On rare occasion infection may occur. The participant will also be required to answer some questions about their diet, health, and work history.

All information gathered about an individual will be kept confidential. Anonymity of individuals is guaranteed in any publication. Names and other identifying information will be kept in a secure place, separate from the data.

Pesticide Exposure Study Consent Form, Page 2/2

Each blood sample will take only a few minutes, and the questionnaire less than five minutes. To compensate the subject for lost earnings while participating in the study, the investigators will pay the subjects $20 per sample/interview.

Dr. Jenny Quintana and Hugh Davies may be contacted at any time at 822-9595 if you have questions or concerns about your participation in the study.

A subject may decline to participate in the study, and after signing this form a subject remains completely free to withdraw from the study at any time without any adverse effect on employment or health care.

Statement by subject: I acknowledge receipt of a copy of this consent form. The investigators have explained its contents to my satisfaction, and I understand that I may withdraw from the study whenever I wish. I hereby consent to participate:

Signature _____

Date _____

Name (Please Print) _____

Witness _____

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ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਦੀ ਵਰਤੋਂ ਬਾਰੇ ਅਧਿਐਨ ਸਹਿਮਤੀ ਦਾ ਫਾਰਮ

ਇਸ ਸਾਲ ਗਰਮੀਆਂ ਵਿਚ ਯੂਨੀਵਰਿਸਟੀ ਆਫ ਬ੍ਰਿਟਿਸ਼ ਕੋਲੰਬੀਆਂ (ਯੂ.ਬੀ.ਸੀ.) ਦੇ ਆਕੁਪੇਸ਼ਨਲ ਹਾਈਜਨ (ਕਿੱਤੇ ਤੇ ਅਰੋਗਤਾ) ਪ੍ਰੋਗਰਾਮ ਨਾਲ ਕੰਮ ਕਰਦੇ ਅਸਿਸਟੈਂਟ ਪ੍ਰੋਫੈਸਰ ਡਾ: ਜੈਨੀ ਕੁਇਨਤਾਨਾ ਅਤੇ ਗਰੈਜੁਏਟ ਵਿਦਿਆਰਥੀ ਹਿਉ ਡੇਵਿਸ ਫਾਰਮਵਰਕਰਾਂ ਤੇ ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਦੇ ਹੋਣ ਵਾਲੇ ਅਸਰਾਂ ਬਾਰੇ ਇਕ ਅਧਿਐਨ ਕਰਨਗੇ। ਇਸ ਅਧਿਐਨ ਦਾ ਮਕਸਦ ਭਵਿੱਖ ਵਿਚ ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਦੇ ਹੋਣ ਵਾਲੇ ਅਸਰਾਂ ਨੂੰ ਮਾਪਣ ਲਈ ਇਕ ਨਵੇਂ ਢੰਗ ਨੂੰ ਟੈਸਟ ਕਰਨਾ ਹੈ।

ਉਹਨਾਂ ਖੇਤ ਮਜ਼ਦੂਰਾਂ, ਜਿਹੜੇ ਖੇਤਾਂ ਵਿਚ ਕੰਮ ਕਰਦੇ ਹਨ, ਦਾ ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਨਾਲ ਵਾਹ ਪੈਂ ਸਕਦਾ ਹੈ ਬੇਸ਼ੱਕ ਦਵਾਈਆਂ ਦੀ ਮਾਤਰਾ ਬਹੁਤ ਥੋੜੀ ਹੀ ਹੋਵੇ। ਇਹ ਦਵਾਈਆਂ ਚਮੜੀ ਰਾਹੀਂ, ਸਾਹ ਰਾਹੀਂ ਜਾਂ ਮੂੰਹ ਰਾਹੀਂ ਉਹਨਾਂ ਅੰਦਰ ਜਾ ਸਕਦੀਆਂ ਹਨ। ਹੁਣ ਦੇ ਤਕਨੀਕੀ ਸਾਧਨਾਂ ਨਾਲ ਸਰੀਰ ਵਿਚ ਰਚਣ ਵਾਲੀਆਂ ਕੀੜੇਮਾਰ ਦਵਾਈਆਂ ਦੀ ਮਿਕਦਾਰ ਨੂੰ ਸਹੀ ਤਰ੍ਹਾਂ ਮਿਣਨਾ ਮੁਸ਼ਕਿਲ ਹੈ। ਇਹ ਅਧਿਐਨ ਇਕ ਨਵੇਂ ਢੰਗ ਬਾਰੇ ਖੋਜ ਕਰੇਗਾ ਜਿਸ ਵਿਚ ਖੂਨ ਦੇ ਸੈੱਲਾਂ ਦੇ ਇਕ ਹਿੱਸੇ, ਮਾਈਕਰੋਨਿਊਕਲਾਈ, ਦੇ ਅਨੁਪਾਤ ਨੂੰ ਮਿਣਿਆ ਜਾਵੇਗਾ। ਹੋ ਸਕਦਾ ਹੈ ਮਾਈਕਰੋਨਿਊਕਲਾਈ ਦੀ ਉੱਚੀ ਪੱਧਰ ਦਾ ਕਾਰਨ ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਦਾ ਸਰੀਰ ਅੰਦਰ ਰਚਨਾ ਹੋਵੇ। ਜੇ ਇਸ ਤਰ੍ਹਾਂ ਹੋਵੇ ਤਾਂ ਹੋ ਸਕਦਾ ਹੈ ਕਿ ਇਸ ਤਰ੍ਹਾਂ ਹੋਣਾ, ਭੱਵਿਖ ਵਿਚ, ਸਰੀਰ ਵਿਚ ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਦੇ ਰਚਨ ਦੀ ਸੁਰੱਖਿਅਤ ਹੱਦ ਨਿਸ਼ਚਤ ਕਰਨ ਵਿਚ ਫਾਇਦੇਮੰਦ ਹੋਵੇ ਜਾਂ ਇਹ ਨਿਸ਼ਚਤ ਕਰਨ ਵਿਚ ਮਦਦ ਕਰੇ ਕਿ ਕੀ ਇਕ ਵਿਅਕਤੀ ਦੇ ਸਰੀਰ ਅੰਦਰ ਕੀੜੇ ਮਾਰ ਦਵਾਈ ਹੱਦ ਤੋਂ ਜ਼ਿਆਦਾ ਤਾਂ ਨਹੀਂ ਰਚ ਗਈ।

ਇਸ ਅਧਿਐਨ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਵਾਲੇ ਵਿਅਕਤੀ ਨੂੰ ਟੈਸਟ ਲਈ ਆਪਣੇ ਖੂਨ ਦੇ ਨਮੂਨੇ ਦੋ ਵਾਰ ਦੇਣੇ ਪੈਣਗੇ। ਇਕ ਵਾਰ ਸੀਜ਼ਨ ਸ਼ੁਰੂ ਹੋਣ ਤੋਂ ਪਹਿਲਾਂ ਅਤੇ ਦੂਜੀ ਵਾਰ ਇਹਨਾਂ ਗਰਮੀਆਂ ਦੇ ਅੰਤ ਵਿਚ ਕਿਸੇ ਸਮੇਂ। ਟੈਸਟ ਕਰਨ ਲਈ ਤੁਹਾਡੇ ਖੂਨ ਦਾ ਨਮੂਨਾ ਬਹੁਤ ਥੋੜੀ ਮਿਕਦਾਰ ਵਿਚ ਲਿਆ ਜਾਵੇਗਾ– 20 ਮਿਲੀ ਲੀਟਰ ਭਾਵ ਦੋ ਚਮਚਿਆਂ ਤੋਂ ਵੀ ਥੋੜਾ। ਇਹ ਖੂਨ ਇਕ ਰਜਿਸਟਰਡ ਨਰਸ ਤੁਹਾਡੀ ਬਾਂਹ ਵਿਚੋਂ ਲਵੇਗੀ। ਹੋ ਸਕਦਾ ਹੈ ਇਸ ਨਾਲ ਤੁਹਾਨੂੰ ਮਾਮੂਲੀ ਅੱਖ ਹੋਵੇ ਅਤੇ ਬਾਂਹ ਤੇ ਮਾੜਾ ਜਿਹਾ ਨੀਲ ਪੈ ਜਾਏ। ਬਹੁਤ ਹੀ ਘੱਟ ਕੇਸਾਂ ਵਿਚ ਲਾਗ ਵੀ ਲੱਗ ਸਕਦੀ ਹੈ। ਇਸਦੇ ਨਾਲ ਹੀ ਹਿੱਸਾ ਲੈਣ ਵਾਲੇ ਵਿਅਕਤੀ ਕੋਲੋਂ ਉਸਦੇ ਕੰਮ, ਖੁਰਾਕ ਅਤੇ ਸਿਹਤ ਬਾਰੇ ਸਵਾਲ ਵੀ ਪੁੱਛੇ ਜਾਣਗੇ।

APPENDIX C - QUESTIONNAIRE

CONFIDENTIAL

Occupational Hygiene Farmworker Pesticide Study

I am going to ask you several questions about your work history, your present and recent health, and about your diet, drinking and smoking habits. It is very important that you answer the questions accurately, so that the true extent of your exposure to pesticides can be determined. Your answers to these questions are being kept very confidential, and only the researchers will have access to the information, which will be used only for this study.

Your name is: _____

Your Current Mailing Address is: _____
_____
_____
_____

Your permanent Address (if different from you mailing address) is:
_____
_____
_____
_____

Your current phone number is: (____)_____-_____

Subject ID: _____

Interviewer ID: _____

Date:___/____/___ Location:_____

Subject ID: _____

1) Background Information:

a) Your age is: _____yrs

b) Your Date of Birth is: __ /___ /__ (DD/MM/YY)

c) Sex: M or F

d) Ethnic Group : Caucasian East Asian Oriental

Native North American Hispanic

e) Where were you born? _____

f) If you were born outside Canada, when did you emigrate to Canada? 19___

Subject ID: _____

2) Occupational Data

A) Have you ever worked as a seasonal farmworker? Y or N

If NO, go to (B), If Yes:

a) What was the first year you worked as a seasonal farmworker? 19___

b) How many years have you worked as a seasonal farmworker?
(Include this year, but don't include any years when you didn't work) ____ yrs

c) What was the last year you worked as a seasonal farmworker? 19___

B) Did you work as a seasonal farmworker this summer (1993)? Y or N

If No:

During the summer, pproximately how many hours each day did you spend outdoors in the sun? ____ hrs

Go to (C)

If Yes:

a) When did you start work this summer ? __ /___ /__ (DD/MM/YY)

b) When did you (will you) finish work this summer ? __ /___ /__ (DD/MM/YY)

c) Did you work continually over this period? Y or N
If No, how many weeks missed? _____ Weeks missed

d) On average, how many days per week did you work? ___ days

Subject ID: _____

e) Approximately, how many days in total did you work this summer:

Less than 30 days __
30 - 59 days __
60 - 90 days __
more than 90 day __

f) On average, how many hours did you work each day? ____ hours

g) What crops did you work on this summer, and for how long?

Strawberry	Y or N	_____days
Raspberry	Y or N	_____days
Cranberry	Y or N	_____days
Blueberry	Y or N	_____days
Vegetables or others (specify)	_____	_____days
	_____	_____days
	_____	_____days
	_____	_____days

h) Did you mix or apply any pesticides this year Y or N

If Yes:

What presticides? _____
How many times? _____ times
On average, how many hours, each time? _____ hours

i) How many different farms did you work on this summer? ____ Farms

Subject ID: _____

j) At any time this year were you directly exposed to pesticides, by:

1) accidental overspray by applicators?	Y or N
2) drift from application on neighbouring fields?	Y or N
3) leak or spill from container?	Y or N
4) spill while loading pesticides?	Y or N
5) Other (specify)	_____

- If the answer to any of 1 - 5 is YES, give details of frequency, duration, and name of pesticide, if known:

_____
_____
_____
_____

k) This summer, did you work in fields that were recently sprayed with pesticides? Y , Don't know

- If Yes:

how recently had the field been sprayed?	_____ hours/days
how often did this occur	_____days
What pesticide was used?	_____

l) When working in a field, do your cover:

a) your hands	Y or N	with what?_____ (material) _____
b) your arms	Y or N	with what?_____ (material) _____
c) your feet	Y or N	with what?_____ (material) _____
		(open/closed shoes?)_____
d) your legs	Y or N	with what?_____ (material) _____
e) your head	Y or N	with what?_____ (material) _____
f) your mouth/nose	Y or N	with what?_____ (material) _____

Subject ID: _____

m) This summer, were you ever sick during your work in the fields? Y or N

-Symptoms _____
-Action taken _____

n) On the farms that you worked at, was there:

	None	Some farms	All farms
Facility for hand washing	____	_____	_____
Drinking water available	____	_____	_____
Emergency medical care			
Posted information	____	_____	_____
Transportation	____	_____	_____
Safety instruction for workers	____	_____	_____
Information about pesticides used in field?	____	_____	_____
Posted warning signs	____	_____	_____

Did you ever enter a field with a posted warning sign? Y or N

o) Washing

ii) Where did you eat your meals while working? _____

iii) Did you usually wash your hands before eating? Y or N

iv) Did you usually change your work clothes at the end of a work shift? Y or N

v) Did you usually wear the same work clothes for more than one day? Y or N

vi) How many days would you wear the same work clothes for ? _____days

viii) How often were these work clothes laundered ? every _____ days

ix) If you were resident at a farm, were laundry and showering facilities provided? Y or N

Subject ID: _____

C) All respondants:

a) Have you ever worked on a permanent basis as a farmer or farmworker? Y or N

If yes, How many years _____ yrs,
Period 19__ to 19__

b) Do you have any other regular work, at any time? Y or N

If yes, what other work do you do? _____

c) Have you ever worked in:
(i) A chemical plant? Y or N
(ii) X-ray clinic or laboratory? Y or N
(iii) Auto body shop or garage, where you may have been exposed to gasoline or solvents Y or N

d) Do you have a hobby or any other work that might expose you to gasoline or solvents? Y or N

- If the answer to any of (p) or (q) is YES, give details of frequency and duration:

_____
_____
_____
_____

Subject ID: _____

3) Recent Medical History

a) Are you currently suffering from:

i) A cold or the flu, or other disease (please specify) _____

ii) Hepatitis Y or N
- If YES, type ? _____

iii) Any cancer or other major disease (please specify) _____

b) Have you been sick in the past month? Y or N

If yes: What did you have? _____

c) Have you ever had chemotherapy Y or N

If yes, When? ___/___ to ___/ ____ (MM/YY)

d) Have you ever had radiation treatment? Y or N

If yes, When? ___/___ to _____/ ____ (MM/YY)

e) Have you had any vaccinations recently (e.g.. for Flu, tetanus, Hepatitis, etc.)? Y or N

If yes: For what? _____
When? ___/___/___ (DD/MM/YY)

f) Have you taken any medicines in the past year prescribed by your doctor?

Medication	Commenced (DD/MM/YY)	Ended (DD/MM/YY)?

g) When was your last X-ray? ___/___/___ (DD/MM/YY)

h) How many X-rays do you think you have had in the past 10 years (include dental X-rays) _____ x-rays

i) If female, have you been through menopause? Y or N
Number of children _____ children

j) Have any of your immediate family (alive or deceased) suffered from cancer? Y or N

Relative (e.g., mother, brother) _____ type: _____
_____type:_____
_____type:_____
_____type:_____

k) Have you travelled abroad in the past 5 years? Y or N

- Which countries _____
- When, how long? _____
_____

4) Smoking

a) Do you currently smoke, or have you ever smoked? Y or N

If YES, Specify age started and stopped smoking: _____.
average number of cigarettes per day. _____cigs/day

b) Do you come into regular contact with someone,
at home or at work, who smokes? Y or N

-If Yes: duration of contact (hrs/day) _____hrs per day
for how many years? _____yrs

Subject ID: _____

5) Diet

1) Do you eat meat?	Y or N
2) Do you drink Coffee?	Y or N
If yes: How many cups a day?	____ cups reg
Regular or decaffeinated?	____ cups decaf
3) Do you drink tea?	Y or N
How many cups a day?	____ cups
4) Do you drink soft drinks like Coca cola, 7 - UP, etc? (Specify)	_____, __cups/day
	_____, __cups/day
	_____, __.cups/day

Thank you. That is the end of the questionnaire.

ਗੁਪਤ
CONFIDENTIAL
ਕਾਰੋਬਾਰੀ ਸਿਹਤ ਸੰਬੰਧੀ (ਹਾਈਜੀਨ) ਖੇਤ ਮਜ਼ਦੂਰਾਂ ਦਾ ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ (ਪੈਸਟੀਸਾਈਡ) ਬਾਰੇ ਅਧਿਅਨ
Occupational Hygiene Farmworker Pesticide Study

ਮੈਂ ਤੁਹਾਨੂੰ ਤੁਹਾਡੇ ਕੰਮ ਦੇ ਇਤਿਹਾਸ ਬਾਰੇ, ਤੁਹਾਡੀ ਹੁਣ ਦੀ ਜਾਂ ਪਿਛਲੇ ਕੁਝ ਸਮੇਂ ਦੀ ਸਿਹਤ ਬਾਰੇ, ਅਤੇ ਤੁਹਾਡੇ ਖਾਣੇ, ਪੀਣੇ ਅਤੇ ਤੁਮਾਕੂ ਆਦਿ ਦੀ ਵਰਤੋਂ ਸੰਬੰਧੀ ਕਈ ਸਵਾਲ ਪੁੱਛਣੇ ਹਨ। ਇਹ ਬਹੁਤ ਜ਼ਰੂਰੀ ਹੈ ਕਿ ਤੁਸੀਂ ਸਵਾਲਾਂ ਦਾ ਜਵਾਬ ਠੀਕ ਠੀਕ ਦੇਵੋ ਤਾਂ ਕਿ ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ ਨਾਲ ਤੁਹਾਡੇ ਪਏ ਵਾਹ ਦਾ ਪੂਰਾ ਪੂਰਾ ਪਤਾ ਲਾਇਆ ਜਾ ਸਕੇ। ਤੁਹਾਡੇ ਦਿੱਤੇ ਹੋਏ ਜਵਾਬ ਪੂਰੀ ਤਰ੍ਹਾਂ ਗੁਪਤ ਰੱਖੇ ਜਾਣਗੇ ਅਤੇ ਇਹਨਾਂ ਨੂੰ ਸਿਰਫ ਖੋਜੀ ਹੀ ਦੇਖ ਸਕਣਗੇ ਅਤੇ ਇਹ ਜਾਣਕਾਰੀ ਸਿਰਫ ਏਸੇ ਅਧਿਅਨ ਵਾਸਤੇ ਹੀ ਵਰਤੀ ਜਾਵੇਗੀ।

I am going to ask you several questions about your work history, your present and recent health, and about your diet, drinking and smoking habits. It is very important that you answer the questions accurately, so that the true extent of your exposure to pesticides can be determined. Your answers to these questions are being kept very confidential, and only the researchers will have access to the information, which will be used only for this study.

ਤੁਹਾਡਾ ਨਾਂ ਹੈ: _____

ਤੁਹਾਡਾ ਮੌਜੂਦਾ ਪਤਾ ਹੈ: _____
_____
_____
_____

ਤੁਹਾਡਾ ਪੱਕਾ ਪਤਾ (ਜੇ ਉਪਰਲੇ ਨਾਲੋਂ ਵੱਖਰਾ ਹੋਵੇ) ਹੈ:
Your permanent Address (if different from you mailing address) is:
_____
_____
_____
_____

ਤੁਹਾਡਾ ਮੌਜੂਦਾ ਟੈਲੀਫੋਨ ਨੰਬਰ ਹੈ:
Your current phone number is: (____) _____- _____

Subject ID: _____

Interviewer ID: _____

Date: ___ /___/___ Location: _____

Subject ID: _____

1) ਪੁਛਲੀ ਜਾਂ ਪਿਛੋਕੜ ਦੀ ਜਾਣਕਾਰੀ:

a) ਤੁਹਾਡੀ ਉਮਰ ਹੈ _____ yrs

b) ਤੁਹਾਡੀ ਜਨਮ ਤਰੀਕ ਹੈ __ /__ /__ (DD/MM/YY)

c) ਲਿੰਗ: M or F

d) ਐਥਨਿਕ ਗਰੁੱਪ : Caucasian East Asian Oriental

Native North American Hispanic

e) ਤੁਸੀਂ ਕਿਥੇ ਪੈਦਾ ਹੋਏ ਸੀ? _____

f) ਜੇ ਤੁਸੀਂ ਕਨੇਡਾ ਤੋਂ ਬਾਹਰ ਜੰਮੇ ਸੀ ਤਾਂ ਤੁਸੀਂ ਕਨੇਡਾ ਵਿਚ ਕਿਹੜੇ ਸਾਲ ਆਏ ਸੀ? 19 ___

Subject ID: _____

2) ਕਾਰੋਬਾਰ ਸੰਬੰਧੀ ਜਾਣਕਾਰੀ

A) ਕੀ ਤੁਸੀਂ ਕਦੇ ਕੰਮ ਦੇ ਮੌਸਮ ਦੌਰਾਨ (ਸੀਜ਼ਨਲ) ਖੇਤ ਮਜ਼ਦੂਰ ਦੇ ਤੌਰ ਤੇ ਕੰਮ ਕੀਤਾ ਹੈ? Y or N

ਜੇ ਨਹੀਂ: ਤਾਂ (C) ਤੇ (ਸਫਾ 7) ਜਾਵੋ

ਜੇ ਹਾਂ,

a) ਪਹਿਲਾ ਸਾਲ ਕਿਹੜਾ ਸੀ ਜਦੋਂ ਤੁਸੀਂ ਕੰਮ ਦੇ ਮੌਸਮ ਦੌਰਾਨ ਖੇਤ ਮਜ਼ਦੂਰ ਦੇ ਤੌਰ ਤੇ ਕੰਮ ਕੀਤਾ? 19___

b) ਕੰਮ ਦੇ ਮੌਸਮ ਦੌਰਾਨ (ਸੀਜ਼ਨਲ) ਖੇਤ ਮਜ਼ਦੂਰ ਦੇ ਤੌਰ ਤੇ ਤੁਸੀਂ ਕਿੰਨੇ ਸਾਲ ਕੰਮ ਕੀਤਾ ਹੈ?
(ਹੁਣ ਦਾ ਸਾਲ ਵੀ ਇਸ ਵਿਚ ਗਿਣ ਲਵੋ ਪਰ ਜਿਸ ਕਿਸੇ ਸਾਲ ਤੁਸੀਂ ਕੰਮ ਨਹੀਂ ਕੀਤਾ ਉਹ ਨਾ ਗਿਣੋ।) ____ yrs

c) ਤੁਹਾਡਾ ਕੰਮ ਦੇ ਮੌਸਮ ਦੌਰਾਨ (ਸੀਜ਼ਨਲ) ਖੇਤ ਮਜ਼ਦੂਰ ਦੇ ਤੌਰ ਤੇ ਕੰਮ ਕਰਨ ਦਾ ਅਖੀਰਲਾ ਸਾਲ ਕਿਹੜਾ ਸੀ? 19___

B) ਕੀ ਤੁਸੀਂ ਇਹਨਾਂ ਗਰਮੀਆਂ ਵਿਚ (1993) ਕੰਮ ਦੇ ਮੌਸਮ ਦੌਰਾਨ (ਸੀਜ਼ਨਲ) ਖੇਤ ਮਜ਼ਦੂਰ ਦੇ ਤੌਰ ਤੇ ਕੰਮ ਕੀਤਾ? Y or N

ਜੇ ਨਹੀਂ: ਤਾਂ (C) ਤੇ (ਸਫਾ 7) ਜਾਵੋ

ਜੇ ਹਾਂ:

a) ਕੰਮ ਕਰਨਾ ਕਦੋਂ ਸ਼ੁਰੂ ਕੀਤਾ __ /___ /__ (DD/MM/YY)

b) ਇਹ ਕੰਮ ਤੁਸੀਂ ਕਦੋਂ ਖਤਮ ਕੀਤਾ ਜਾਂ ਕਰੋਗੇ? __ /___ /__ (DD/MM/YY)

c) ਕੀ ਤੁਸੀਂ ਇਸ ਸਾਰੇ ਸਮੇਂ ਦੌਰਾਨ ਕੰਮ ਕਰਦੇ ਰਹੇ? Y or N
ਜੇ ਨਹੀਂ, ਤਾਂ ਕਿੰਨੇ ਹਫਤੇ ਨਹੀਂ ਕੀਤਾ? _____ Weeks missed

d) ਔਸਤਨ ਇਕ ਹਫਤੇ ਵਿਚ ਤੁਸੀਂ ਕਿੰਨੇ ਦਿਨ ਕੰਮ ਕਰਦੇ ਸੀ? ___ days

Subject ID: _____

e) ਇਹਨਾਂ ਗਰਮੀਆਂ ਦੌਰਾਨ ਤੁਸੀਂ ਅਦਾਜ਼ਨ ਕੁਲ ਕਿੰਨੇ ਦਿਨ ਕੰਮ ਕੀਤਾ?

Less than 30 days
30 - 59 days
60 - 90 days
More than 90 day

f) ਔਸਤਨ ਇਕ ਦਿਨ ਵਿਚ ਤੁਸੀਂ ਕਿੰਨੇ ਘੰਟੇ ਕੰਮ ਕਰਦੇ ਸੀ? ____ hours

g) ਤੁਸੀਂ ਇਹਨਾਂ ਗਰਮੀਆਂ ਵਿਚ ਕਿਹੜੀਆਂ ਫਸਲਾਂ ਤੇ ਕੰਮ ਕੀਤਾ, ਅਤੇ ਕਿੰਨੇ ਸਮੇਂ ਵਾਸਤੇ ਕੰਮ ਕੀਤਾ?

ਸਟ੍ਰਾਬੇਰੀ	Y or N	_____ days
ਰਸਬੇਰੀ	Y or N	_____ days
ਕਰੇਨਬੇਰੀ	Y or N	_____ days
ਬਲੂਬੇਰੀ	Y or N	_____ days
ਸਬਜ਼ੀਆਂ ਜਾਂ ਦੂਜੀਆਂ ਫਸਲਾਂ (ਵਿਸ਼ੇਸ਼ ਦੱਸੋ)	_____	_____ days
	_____	_____ days
	_____	_____ days
	_____	_____ days

h) ਇਸ ਸਾਲ ਤੁਸੀਂ ਕੋਈ ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ ਰਲਾਈਆਂ (ਮਿਕਸ ਕੀਤੀਆਂ) ਜਾਂ ਛਿੜਕੀਆਂ? Y or N

ਜੇ ਹਾਂ:

ਕਿਹੜੀਆਂ ਦਵਾਈਆਂ? _____

ਤਾਂ ਕਿੰਨੀ ਕੁ ਵਾਰੀ ਕੀਤੀਆਂ _____ times

ਔਸਤਨ ਹਰ ਵਾਰੀ ਕਿੰਨੇ ਘੰਟੇ? _____ hours

i) ਇਹਨਾਂ ਗਰਮੀਆਂ ਵਿਚ ਤੁਸੀਂ ਕਿੰਨੇ ਵੱਖਰੇ ਵੱਖਰੇ ਫਾਰਮਾਂ ਵਿਚ ਕੰਮ ਕੀਤਾ? ____ Farms

Subject ID: _____

j) ਇਸ ਸਾਲ ਕਿਸੇ ਸਮੇਂ ਵੀ ਤੁਸੀਂ ਸਿੱਧੇ ਤੌਰ ਤੇ ਹੇਠ ਲਿਖੇ ਤਰੀਕਿਆਂ ਨਾਲ ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ ਦੀ ਮਾਰ ਵਿਚ ਆਏ?

1) ਛਿੜਕਾਵ ਕਰਨ ਵਾਲਿਆਂ ਵਲੋਂ ਘਟਨਾਵੱਸ (ਐਕਸੀਡੈਂਟਲੀ) ਵਾਧੂ ਛਿੜਕਾਵ ਨਾਲ	Y or N
2) ਨਾਲ ਲਗਦੇ ਖੇਤਾਂ ਵਿਚ ਹੁੰਦੇ ਛਿੜਕਾਵ ਦੀ ਧੂੜ ਨਾਲ	Y or N
3) ਦਵਾਈਆਂ ਵਾਲੇ ਬਰਤਨ ਵਿਚੋਂ ਚੋਣ ਜਾਂ ਭੁੱਲਣ ਕਾਰਨ	Y or N
4) ਕੀੜੇ ਮਾਰ ਦਵਾਈਆਂ ਨੂੰ ਲੱਦਣ ਸਮੇਂ	Y or N
5)ਕਿਸੇ ਹੋਰ ਤਰ੍ਹਾਂ (ਵਿਸ਼ੇਸ਼ ਦੱਸੋ)	_____

- ਜੇ ਉਪਰਲੇ 1 ਤੋਂ ਲੈ ਕੇ 5 ਤੱਕ ਸਵਾਲਾਂ ਵਿਚੋਂ ਕਿਸੇ ਦਾ ਵੀ ਜਵਾਬ ਹਾਂ ਹੋਵੇ ਤਾਂ ਉਸ ਬਾਰੇ ਵਿਸਥਾਰ ਦਿਓ ਕਿ ਕਿੰਨੀ ਵਾਰੀ ਇਹ ਗੱਲ ਵਾਪਰੀ ਅਤੇ ਕਿੰਨੇ ਸਮੇਂ ਲਈ ਵਾਪਰੀ?

_____
_____
_____
_____

k) ਇਹਨਾਂ ਗਰਮੀਆਂ ਵਿਚ ਕੀ ਤੁਸੀਂ ਉਹਨਾਂ ਖੇਤਾਂ ਵਿਚ ਕੰਮ ਕੀਤਾ ਜਿਹਨਾਂ ਉਪਰ ਕੁਝ ਦੇਰ ਪਹਿਲਾਂ ਕੀੜੇਮਾਰ ਦਵਾਈਆਂ ਛਿੜਕੀਆਂ ਗਈਆਂ ਸਨ? Y , N or Don't Know

- ਜੇ ਹਾਂ:

ਖੇਤ ਵਿਚ ਛਿੜਕਾਵ ਕਿੰਨੀ ਦੇਰ ਪਹਿਲਾਂ ਕੀਤਾ ਗਿਆ ਸੀ?	_____ Hours/ days
ਅਜਿਹਾ ਅਕਸਰ ਕਿੰਨੀ ਕੁ ਵਾਰ ਵਾਪਰਦਾ ਸੀ?	_____ days
ਕਿਹੜੀਆਂ ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ ਵਰਤੀਆਂ ਗਈਆਂ ਸਨ?	_____

l) ਜਦੋਂ ਤੁਸੀਂ ਖੇਤਾਂ ਵਿਚ ਕੰਮ ਕਰਦੇ ਹੋ ਤੁਸੀਂ ਹੇਠ ਲਿਖਿਆਂ ਵਿਚੋਂ ਕਿਹੜੇ ਅੰਗ ਢੱਕਦੇ ਹੋ?

a) ਆਪਣੇ ਹੱਥ	Y or N	ਕਾਹਦੇ ਨਾਲ?_____	(ਕਿਹੜਾ ਮਟੀਰੀਅਲ) _____
b) ਆਪਣੀਆਂ ਬਾਹਾਂ	Y or N	ਕਾਹਦੇ ਨਾਲ?_____	(ਕਿਹੜਾ ਮਟੀਰੀਅਲ) _____
c) ਆਪਣੇ ਪੈਰ	Y or N	ਕਾਹਦੇ ਨਾਲ?_____	(ਕਿਹੜਾ ਮਟੀਰੀਅਲ) _____
		(ਖੁੱਲੀ ਜਾਂ ਬੰਦ ਜੁੱਤੀ ਨਾਲ?)_____	
d) ਆਪਣੀਆਂ ਲੱਤਾਂ	Y or N	ਕਾਹਦੇ ਨਾਲ?_____	(ਕਿਹੜਾ ਮਟੀਰੀਅਲ) _____
e) ਆਪਣਾ ਸਿਰ	Y or N	ਕਾਹਦੇ ਨਾਲ?_____	(ਕਿਹੜਾ ਮਟੀਰੀਅਲ) _____
f) ਆਪਣਾ ਮੂੰਹ ਅਤੇ ਨੱਕ	Y or N	ਕਾਹਦੇ ਨਾਲ?_____	(ਕਿਹੜਾ ਮਟੀਰੀਅਲ) _____

m) ਇਹਨਾਂ ਗਰਮੀਆਂ ਦੌਰਾਨ ਤੁਸੀਂ ਖੇਤਾਂ ਵਿਚ ਕੰਮ ਕਰਦੇ ਸਮੇਂ ਕਦੀ ਬੀਮਾਰ ਵੀ ਹੋਏ ਸੀ? Y or N

- ਲੱਛਣ ਕੀ ਸਨ? _____
- ਕੀ ਉਪਾਅ ਕੀਤਾ? _____

n) ਜਿਹੜੇ ਫਾਰਮ ਵਿਚ ਤੁਸੀਂ ਕੰਮ ਕੀਤਾ ਸੀ ਕੀ ਉਥੇ ਇਹ ਸਵਿਧਾਵਾਂ ਸਨ:

	ਕਿਸੇ ਤੇ ਵੀ ਨਹੀਂ, None	ਕੁਝ ਥਾਂਹੀ, Some farms	ਸਾਰੇ ਫਾਰਮਾਂ 'ਤੇ All farms
ਹੱਥ ਧੋਣ ਲਈ ਸੁਵਿਧਾ	_____	_____	_____
ਪੀਣ ਵਾਲੇ ਪਾਣੀ ਦਾ ਮਿਲਣਾ	_____	_____	_____
ਐਮਰਜੈਂਸੀ ਤੇ ਡਾਕਟਰੀ ਦੇਖਭਾਲ ਲਿਖ ਕੇ ਲਾਈ ਜਾਣਾਕਰੀ	_____	_____	_____
ਲਿਆਉਣ/ਲਿਜਾਣ ਦਾ ਪ੍ਰਬੰਧ (ਟਰਾਂਸਪੋਰਟੇਸ਼ਨ)	_____	_____	_____
ਕਾਮਿਆਂ ਵਾਸਤੇ ਸੁਰੱਖਿਆ ਲਈ ਹਦਾਇਤਾਂ	_____	_____	_____
ਖੇਤਾਂ ਵਿਚ ਵਰਤੀਆਂ ਜਾਂਦੀਆਂ ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ ਸੰਬੰਧੀ ਜਾਣਕਾਰੀ	_____	_____	_____
ਲਿਖ ਕੇ ਲਾਏ ਚੇਤਾਵਨੀ ਦੇ ਸਾਈਨ	_____	_____	_____

ਕੀ ਤੁਸੀਂ ਕਦੀ ਕਿਸੇ ਉਸ ਖੇਤ ਵਿਚ ਵੜੇ ਹੋ ਜਿੱਥੇ ਚੇਤਾਵਨੀ ਸਾਈਨ ਲੱਗੇ ਹੋਏ ਸਨ? Y or N

o) ਹੱਥ ਪੈਰ ਧੋਣ ਬਾਰੇ:

i) ਕੰਮ ਦੌਰਾਨ ਤੁਸੀਂ ਆਪਣਾ ਖਾਣਾ ਕਿਥੇ ਖਾਂਦੇ ਹੁੰਦੇ ਸੀ? _____

ii) ਕੀ ਤੁਸੀਂ ਅਕਸਰ ਖਾਣਾ ਖਾਣ ਤੋਂ ਪਹਿਲਾਂ ਆਪਣੇ ਹੱਥ ਧੋਂਦੇ ਹੁੰਦੇ ਸੀ? Y or N

iii) ਕੀ ਤੁਸੀਂ ਅਕਸਰ ਕੰਮ ਦੀ ਦਿਹਾੜੀ ਤੋਂ ਬਾਅਦ ਕੰਮ ਵਾਲੇ ਕਪੜੇ ਬਦਲ ਦਿੰਦੇ ਹੁੰਦੇ ਸੀ? Y or N

iv) ਕੀ ਤੁਸੀਂ ਅਕਸਰ ਇਕ ਦਿਨ ਤੋਂ ਵਾਧੂ ਉਹੋ ਕਪੜੇ ਪਾਈ ਰੱਖਦੇ ਹੁੰਦੇ ਸੀ? Y or N

v) ਤੁਸੀਂ ਉਹੋ ਕੰਮ ਵਾਲੇ ਕਪੜੇ ਲਗਾਤਾਰ ਕਿੰਨੇ ਕੁ ਦਿਨ ਪਾਈ ਰੱਖਦੇ ਹੁੰਦੇ ਸੀ? _____ days

vi) ਕੰਮ ਵਾਲੇ ਤੁਹਾਡੇ ਕਪੜੇ ਕਿੰਨੀ ਕੁ ਵਾਰੀ ਧੋਤੇ ਜਾਂਦੇ ਸੀ? every _____ days

vii) ਜੇ ਤੁਸੀਂ ਫਾਰਮ ਤੇ ਰਹਿ ਰਹੇ ਸੀ ਤਾਂ ਕੀ ਉਥੇ ਕਪੜੇ ਧੋਣ ਅਤੇ ਨਹਾਉਣ ਧੋਣ ਦਾ ਪ੍ਰਬੰਧ ਮੌਜੂਦ ਸੀ? Y or N

C) ਪ੍ਰਸ਼ਨਾਵਲੀ ਭਰਨ ਜਾਂ ਉੱਤਰ ਦੇਣ ਵਾਲੇ ਸਾਰੇ ਵਿਅਕਤੀਆਂ ਵਾਸਤੇ:

a) ਇਸ ਗਰਮੀਆਂ ਦੇ ਮੌਸਮ ਵਿਚ, ਤੁਸੀਂ ਅੰਦਾਜ਼ਨ ਰੋਜ਼ ਕਿੰਨੇ ਘੰਟੇ ਬਾਹਰ ਧੁੱਪ ਵਿਚ ਬਿਤਾਉਂਦੇ ਸੋ? _____hours

b) ਕੀ ਤੁਸੀਂ ਕਦੀ ਪੱਕੇ ਤੌਰ ਤੇ ਫਾਰਮਰ ਜਾਂ ਖੇਤ ਮਜ਼ਦੂਰ ਬਣ ਕੇ ਕੰਮ ਕੀਤਾ ਹੈ? Y or N

ਜੇ ਹਾਂ, ਕਿੰਨੇ ਸਾਲ? _____ yrs,

ਕੰਮ ਕਰਨ ਦਾ ਸਮਾਂ? 19 __ to 19__

c) ਕੀ ਤੁਸੀਂ ਕੋਈ ਹੋਰ ਕੰਮ ਕਰਦੇ ਹੋ? Y or N

ਜੇ ਹਾਂ, ਤਾਂ ਕੀ ਕੰਮ ਕਰਦੇ ਹੋ? _____

d) ਕੀ ਤੁਸੀਂ ਕਦੀ ਇਹਨਾਂ ਥਾਵਾਂ ਤੇ ਕੰਮ ਕੀਤਾ ਹੈ?

(i) ਰਸਾਇਣਕ (ਕੈਮੀਕਲ) ਪਲਾਂਟ Y or N

(ii) ਐਕਸਰੇ ਕਲਿਨਿਕ ਜਾਂ ਪ੍ਰਯੋਗਸ਼ਾਲਾ Y or N

(iii) ਆਟੋ-ਬਾਡੀ-ਸ਼ਾਪ ਜਾਂ ਗੈਰਾਜ ਜਿਥੇ ਤੁਸੀਂ ਗੈਸ ਜਾਂ ਘੋਲਕ (ਰੋਗਨ ਆਦਿ ਘੋਲਣ ਵਾਲਾ ਰਸਾਇਣ) ਦੇ ਸੰਪਰਕ ਵਿਚ ਆਏ ਹੋਵੋ? Y or N

e) ਕੀ ਤੁਹਾਡਾ ਕੋਈ ਇਹੋ ਜਿਹਾ ਸ਼ੌਕ ਜਾਂ ਕੰਮ ਹੈ ਜਿਹਦੇ ਕਾਰਨ ਤੁਸੀਂ ਗੈਸੋਲੀਨ ਜਾਂ ਘੋਲਕ (ਰੋਗਨ ਆਦਿ ਘੋਲਣ ਵਾਲਾ ਰਸਾਇਣ) ਦੇ ਸੰਪਰਕ ਵਿਚ ਆ ਸਕਦੇ ਹੋਵੋ? Y or N

- ਜੇ ਉਪਰਲੇ ਸਵਾਲਾਂ ਵਿਚੋਂ ਕਿਸੇ ਦਾ ਵੀ ਜਵਾਬ ਹਾਂ ਵਿਚ ਹੋਵੇ ਤਾਂ ਕ੍ਰਿਪਾ ਕਰਕੇ ਵਿਸਥਾਰ ਦੇਵੋ ਕਿ ਇਹ ਕਿੰਨੀ ਕਿੰਨੀ ਦੇਰ ਬਾਅਦ ਵਾਪਰਦਾ ਸੀ ਤੇ ਕਿੰਨੀ ਕੁ ਦੇਰ ਲਈ ਵਾਪਰਦਾ ਸੀ?

_____

_____

_____

_____

Subject ID: _____

3) ਪਿਛਲੇ ਕੁਝ ਸਮੇਂ ਦੀ ਸਿਹਤ ਸਬੰਧੀ ਜਾਣਕਾਰੀ (ਰੀਸੈਂਟ ਮੈਡੀਕਲ ਹਿਸਟਰੀ)

a) ਕੀ ਤੁਸੀਂ ਇਸ ਵਕਤ ਹੇਠ ਲਿਖੀ ਕਿਸੇ ਬੀਮਾਰੀ ਦੇ ਮਰੀਜ਼ ਹੋ:

i) ਠੰਡ ਜਾਂ ਜ਼ੁਕਾਮ, ਜਾਂ ਕੋਈ ਹੋਰ ਬੀਮਾਰੀ (ਕ੍ਰਿਪਾ ਕਰਕੇ ਦੱਸੋ ਕਿਹੜੀ) _____

ii) ਜਿਗਰ ਦੀ ਸੋਜ਼ ਜਾਂ ਜਿਗਰ ਦੀ ਜਲਣ (Hepatitis) Y or N

- ਜੇ ਹਾਂ ਤਾਂ ਕਿਹੜੀ ਕਿਸਮ? _____

iii) ਕੋਈ ਕਿਸੇ ਕਿਸਮ ਦਾ ਕੈਂਸਰ ਜਾਂ ਕੋਈ ਹੋਰ ਵੱਡੀ ਬੀਮਾਰੀ (ਕ੍ਰਿਪਾ ਕਰਕੇ ਦੱਸੋ ਕਿਹੜੀ) _____

b) ਕੀ ਤੁਸੀਂ ਪਿਛਲੇ ਮਹੀਨੇ ਦੌਰਾਨ ਬੀਮਾਰ ਰਹੇ ਹੋ? Y or N

ਜੇ ਹਾਂ: ਤੁਹਾਨੂੰ ਕੀ ਹੋਇਆ ਸੀ? _____

c) ਕੀ ਤੁਸੀਂ ਕਦੀ ਰਸਾਇਣ ਚਿਕਿਤਸਾ (chemotherapy) ਦੁਆਰਾ ਇਲਾਜ਼ ਕਰਵਾਇਆ ਹੈ? Y or N

ਜੇ ਹਾਂ, ਤਾਂ ਕਦੋਂ ਕਰਵਾਇਆ ਸੀ? ___/___ to ___/ ____ (MM/YY)

d) ਕੀ ਤੁਸੀਂ ਕਦੀ ਰੇਡੀਏਸ਼ਨ ਟਰੀਟਮਿੰਟ ਵੀ ਕਰਵਾਇਆ ਹੈ? Y or N

ਜੇ ਹਾਂ, ਤਾਂ ਕਦੋਂ ਕਰਵਾਇਆ ਸੀ? ___/___ to _____/ ____ (MM/YY)

e) ਕੀ ਤੁਸੀਂ ਪਿਛਲੇ ਕੁਝ ਸਮੇਂ ਵਿਚ ਕੋਈ ਟੀਕਾ ਵੀ ਲਗਵਾਇਆ ਹੈ (ਜਿਵੇਂ ਕਿ ਫਲੂ, ਖਿੱਚ ਦੀ ਬੀਮਾਰੀ -ਟੈਟਨਸ - ਜਾਂ ਜਿਗਰ ਦੀ ਸੋਜ - ਹੈਪਾਟਾਈਟਸ ਆਦਿ)? Y or N

ਜੇ ਹਾਂ, ਤਾਂ ਟੀਕਾ ਕਿਹੜੀ ਬੀਮਾਰੀ ਵਾਸਤੇ ਸੀ? _____

ਕਦੋਂ ਲਗਵਾਇਆ ਸੀ? ___/___/___ (DD/MM/YY)

f) ਕੀ ਤੁਸੀਂ ਪਿਛਲੇ ਸਾਲ ਦੌਰਾਨ ਆਪਣੇ ਡਾਕਟਰ ਵਲੋਂ ਦੱਸੀ ਕੋਈ ਦਵਾਈ ਖਾਧੀ ਹੈ?

ਦਵਾਈ	ਸ਼ੁਰੂ ਕੀਤੀ (ਦਿਨ/ਮਹੀਨਾ/ਸਾਲ)	ਖਤਮ ਕੀਤੀ (ਦਿਨ/ਮਹੀਨਾ/ਸਾਲ)

g) ਤੁਹਾਡਾ ਆਖਰੀ ਵਾਰ ਐਕਸਰੇ ਕਦੋਂ ਹੋਇਆ ਸੀ? ___/___/___ (DD/MM/YY)

h) ਤੁਹਾਡੇ ਖਿਆਲ ਅਨੁਸਾਰ ਪਿਛਲੇ ਦਸਾਂ ਸਾਲਾਂ ਦੌਰਾਨ ਤੁਹਾਡੇ ਕਿੰਨੀ ਵਾਰੀ ਐਕਸਰੇ (ਜਿਹਨਾਂ ਵਿਚ ਦੰਦਾਂ ਦੇ ਐਕਸਰੇ ਵੀ ਸ਼ਾਮਲ ਹੋਣ) ਲਏ ਗਏ ਹੋਣਗੇ? _____ x-rays

i) ਜੇ ਤੁਸੀ ਇਸਤਰੀ ਹੋ ਤਾਂ ਕੀ ਤੁਹਾਡੀ ਮਾਹਵਾਰੀ ਸਦਾ ਲਈ ਬੰਦ (ਮੈਨੋਪਾਜ਼) ਹੋ ਚੁੱਕੀ ਹੈ? Y or N
ਜੇ ਹਾਂ ਤਾਂ ਕਿੰਨੀ ਦੇਰ ਪਹਿਲਾਂ?
ਤੁਹਾਡੇ ਕਿੰਨੇ ਬੱਚੇ ਹਨ? _____ children

j) ਤੁਹਾਡੇ ਆਪਣੇ ਟੱਬਰ ਦੇ ਕਿਸੇ ਮੈਂਬਰ (ਜੀਉਂਦਾ ਜਾਂ ਮਰ ਚੁੱਕਾ) ਨੂੰ ਕੈਂਸਰ ਦਾ ਰੋਗ ਹੋਇਆ ਹੈ? Y or N

ਰਿਸ਼ਤੇਦਾਰ (ਮਿਸਾਲ ਵਜੋਂ ਮਾਂ, ਭਰਾ)	
	_____type:_____
	_____type:_____
	_____type:_____

k) ਕੀ ਤੁਸੀਂ ਪਿਛਲੇ 5 ਸਾਲਾਂ ਵਿਚ ਮੁਲਕ ਤੋਂ ਬਾਹਰ ਸਫਰ ਕੀਤਾ ਹੈ? Y or N
- ਕਿਹੜੇ ਦੇਸਾਂ ਵਿਚ? _____
- ਕਦੋਂ ਅਤੇ ਕਿੰਨੇ ਸਮੇਂ ਵਾਸਤੇ? _____

Subject ID: _____

4) ਤੁਮਾਕੂ ਪੀਣਾ

a) ਕੀ ਤੁਸੀਂ ਅਜ ਕੱਲ ਤੁਮਾਕੂ ਪੀਦੇ ਹੋ, ਜਾਂ ਕੀ ਤੁਸੀਂ ਕਦੀ ਵੀ ਤੁਮਾਕੂ ਇਸਤੇਮਾਲ ਕੀਤਾ ਹੈ? Y or N

ਜੇ ਹਾਂ, ਤਾਂ ਦੱਸੋ ਕਿ ਕਿਹੜੀ ਉਮਰੇ ਸ਼ੁਰੂ ਕੀਤਾ ਸੀ ਤੇ ਕਿਹੜੀ ਉਮਰੇ ਬੰਦ ਕੀਤਾ? _____
ਦਿਨ ਵਿਚ ਅੰਦਾਜ਼ਨ ਕਿੰਨੀਆਂ ਸਿਗਰਟਾਂ ਪੀਦੇ ਸੀ? _____ cigs/day

b) ਕੀ ਤੁਸੀਂ ਘਰ ਜਾਂ ਕੰਮ ਉੱਪਰ ਕਿਸੇ ਤੁਮਾਕੂ ਪੀਣ ਵਾਲੇ ਦੇ ਸੰਪਰਕ ਵਿਚ ਆਉਂਦੇ ਹੋ। Y or N

ਜੇ ਹਾਂ, ਸੰਪਰਕ ਦੇ ਸਮੇਂ ਦੀ ਲੰਬਾਈ (ਘੰਟੇ/ਦਿਨ) _____hrs per day

ਕਿੰਨੇ ਸਾਲਾਂ ਤੋਂ? _____ yrs

Subject ID: _____

5) ਖਾਣਾ (ਡਾਇਟ)

1) ਕੀ ਤੁਸੀਂ ਮੀਟ ਖਾਂਦੇ ਹੋ?	Y or N
2) ਕੀ ਤੁਸੀਂ ਕੌਫੀ ਪੀਦੇ ਹੋ?	Y or N
ਜੇ ਹਾਂ, ਤਾਂ ਦਿਹਾੜੀ ਦੇ ਕਿੰਨੇ ਕੱਪ?	____ cups reg
ਥਾ ਕੌਫੀ ਰੈਗੁਲਰ ਜਾਂ ਡੀਕੈਫਿਨੇਟਡ ਪੀਦੇ ਹੋ	____ cups decaf
3) ਕੀ ਤੁਸੀਂ ਚਾਹ ਪੀਦੇ ਹੋ?	Y or N
ਰੋਜ਼ ਦੇ ਕਿੰਨੇ ਕੱਪ	____ cups
4) ਕੀ ਤੁਸੀਂ ਸਾਫਟ ਡਰਿੰਕ ਜਿਵੇਂ ਕਿ ਕੋਕਾ ਕੋਲਾ, ਸੈਵਨ-ਅੱਪ ਆਦਿ ਪੀਦੇ ਹੋ?	_____, __ cups/day
	_____, __ cups/day
	_____, __.cups/day

ਤੁਹਾਡਾ ਬਹੁਤ ਬਹੁਤ ਧੰਨਵਾਦ। ਇਹ ਇਸ ਪ੍ਰਸ਼ਨਾਵਲੀ ਦਾ ਅੰਤ ਹੈ।

Thank you. That is the end of the questionnaire.

APPENDIX D - INTERMEDIATE RESULTS NOTIFICATION

APPENDIX E - FINAL RESULTS PARTICIPANT NOTIFICATION

THE UNIVERSITY OF BRITISH COLUMBIA

1993 UBC Farmworkers and Pesticides Study

The study was conducted by the Occupational Hygiene Programme at the University of British Columbia. The programme teaches students and conducts research in the area of occupational and environmental exposures and related health risks.

This study looked at the effects of low-level pesticide exposure on human health. We took blood samples taken from people who worked as farmworkers in 1993 and compared them with blood samples from people who did not work as farmworkers, to see if an increase in damage to blood cells could be detected. Scientists had previously suggested that the frequency of a particular component of blood cells, called "micronuclei", increases in people who are exposed to pesticides. Such an increase is an indicator of contact with agents which might cause chronic illness such as cancer. If we found such an increase in the farmworkers participating in this study, this might indicate exposure to unacceptable levels of pesticides or their residues.

Thirty nine individuals participated in the project, from Vancouver and Surrey. Eighteen individuals had worked as farmworkers in 1993, and they were compared to 21 who did not work during 1993. We also collected information on each participant's work history, and so we were able to see if working as a farmworker for many years increased the cell damage, or if working many weeks during one summer increased cell damage.

In our results, we found no differences in blood cell damage (micronucleus frequency) between any of the groups compared. We found no difference between the group of 1993 farmworkers versus the non-workers. Neither were there differences between groups of individuals who had worked for several years versus those who had never worked and those who had worked for only one of two years. Finally, no differences were found between those farmworkers who worked for different lengths of time during the summer of 1993.

In conclusion, our study found that there were no detectable increase in micronucleus frequency associated with working as a farmworker, either due to recent exposure, or cumulative lifetime exposure. Therefore our study did not demonstrate any link between work as a farmworker and an increased risk of cancer. However, our study was limited by a small numbers of participants, and differences between the worker and non-worker groups which made it hard to determine the true effect of farmwork on cell damage. While our study had negative results, other studies which investigated the same effects have concluded that work which brings individuals into constant contact with low-levels of pesticides do cause cell-damaging effects, and it is generally accepted that agriculture workers continually exposed to certain pesticides are at increased risk from some cancers, and from other chronic health problems.

Detailed results will be published soon as an M.Sc. thesis entitled: "Micronucleus Frequency in B.C. Farmworkers", by Hugh. W. Davies., and may be viewed at the Woodward library, University of British Columbia, 2198 Health Sciences Mall, Vancouver, B.C., V6T 1Z3, phone 822-4440.