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Frequency of sister chromatid exchange and hematological effects in pesticide-exposed greenhouse sprayers

by Flemming Lander, PhD,[1] Mogens Rønne, PhD[2]

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Objectives A cross-sectional study was conducted to investigate whether exposure to pesticides in greenhouses causes hemato- or genotoxic damage in sprayers.

Methods The frequency of sister chromatid exchange (SCE) in cultured lymphocytes and the number of blood erythrocytes, leucocytes, and thrombocytes were studied among 134 greenhouse sprayers exposed to a complex mixture of almost 50 insecticides, fungicides, and growth regulators and among 157 referents.

Results The hematological profiles did not differ between the exposed and unexposed groups. The SCE frequency was elevated in nonsmoking, but not in currently smoking sprayers when compared with the referents. There was a slight tendency towards an increased SCE frequency with decreasing degree of protection during pesticide applications. The frequency of pesticide applications, lifetime pesticide exposure, and in-season plasma-cholinesterase inhibition (as an estimate of current exposure to organophosphates and carbamates) did not influence the SCE frequency or any of the hematological parameters.

Conclusions The present results suggest a genotoxic effect from combined subtoxic occupational pesticide exposure, whereas no hematogenic effects could be observed at the current exposure level.

Key terms hematology, pesticides.

While the acute toxic effects of overexposure to pesticides are well known, there is little information on the possible hematotoxic or cytogenetic hazards from subtoxic and long-term occupational exposure. Observations of cytotoxic effects from single pesticides or mixtures of pesticides are mainly derived from a variety of short-term test studies or case reports (1—5). In previous epidemiologic investigations significant increases in chromosome damage have been observed in cultured lymphocytes from pesticide-exposed workers (6—10). Other studies have failed to detect cytogenetic damage (11—14). This contradiction is probably the result of variation in pesticide exposure because of differences in the crops, work habits, and pesticides used among the agricultural workers studied.

In temperate countries, the most intense agricultural pesticide exposure is likely to occur in the enclosed and poorly ventilated environment of greenhouses. The applicators repeatedly spray with one or more compounds several times a month, especially in the plant growing season (15—17). Measurements of airborne or dermal residues after spray and fog applications in greenhouses indicate that the potential hazards to workers are much greater than in similar outdoor operations (15, 18). Although workers and applicators may be aware of the danger associated with exposure to pesticides, many workers do not wear adequately protective clothing because of the relatively high humidity and temperature in a greenhouse. Handling the foliage of recently sprayed plants and cut flowers can cause additional pesticide exposure from dislodgeable residues among greenhouse workers (19). Hence the aim of this study was to investigate whether exposure to pesticides in greenhouses causes hemato- or genotoxic damage in sprayers.

Subjects and methods

Subjects

The present investigation was carried out as a cross-sectional study. The exposed group consisted of 134

1 Danish Labor Inspection, Odense, Denmark.
2 Institute of Biology, Department of Zoology, Aarhus University, Aarhus, Denmark.

Reprint requests to: Dr Flemming Lander, Danish Labor Inspection, 5000 Odense, Denmark.

Table 1. Pesticides used by the 134 greenhouse sprayers during the five months preceding the sampling, listed in decreasing order of frequency. (N = number of users)

Pesticide	N
Organophosphates	
Dichlorvos	36
Mevinphos	34
Acephate	25
Azinphos-methyl	17
Parathion	17
Etrimphos	16
Diazinon	13
Phoxim	11
Malathion	7
Pyrazophos	4
Heptenophos	2
Oxydemeton-methyl	2
Carbamates	
Pirimicarb	64
Aldicarb	38
Propamocarb	28
Methomyl	22
Oxamyl	14
Carbofuran	3
Mercaptodimethur	2
Polychlorinated insecticides	
Dienochlor	53
Dicofol	29
Endosulfan	26
Lindan	10
Pyrethroid insecticides	
Permethrin	52
Deltamethrin	35
Cypermethrin	2
Fenvalerat	2
Pyrethrin	1
Fungicides	
Benomyl	75
Captan	51
Estridiazol	49
Mancozeb	26
Vinclozolin	22
Iprodion	18
Furalaxyl	18
Chlorothalonil	14
Prochloraz	8
Triforin	6
Thiram	5
Fenarim	4
Growth regulators	
Daminoazid	54
Chlormequat-chloride	41
Ancymidol	8
Dikegulac	2

healthy greenhouse sprayers (118 men and 16 women) employed in 52 floricultural producing nurseries situated in the Odense municipality. The mean nursery size in terms of the area covered by glass was 14 000 m^2 (range 1700—45 000 m^2).

The pesticides used by the greenhouse sprayers during the preceding five months before the blood sampling consisted of a mixture of almost 50 different compounds which included insecticides, fungicides, and growth regulators, whereas the application of herbicides was negligible (table 1).

On the average each exposed person had applied pesticides about 6 (range 1—21) times a month with an average cumulated spraying duration of about 13 (range 1—57) h a month. Especially when mixing the pesticides the operators were potentially exposed to highly concentrated compounds. None of the greenhouse sprayers had exhibited acute pesticide intoxication during the past spraying season. The mean employment time as gardeners was 17 (range 1—50) years.

For each exposed person work habits were obtained in relation to the application of anticholinesterase (anti-ChE) agents and other pesticides, including the use of face masks, rubber or synthetic gloves, and protective clothes such as rubber aprons or whole-body clothes. Almost each piece of whole-body clothing was made of lightweight and chemically resistant material. Correct wearing or the intactness of the equipment was not registered.

Four hundred and ninety school teachers and clerical staff members from 11 banks formed the potential reference group. Randomly selected, 157 healthy referents (137 men and 20 women) were chosen from this study base.

The characteristics of all of the exposed subjects and referents are shown in table 2. The age distributions were uniform in both study groups, and the drinking and smoking habits did not differ significantly. The non-smokers included a few ex-smokers, who had not smoked for at least one year. The smoking quantities did not differ significantly between the exposed and reference groups.

Venous blood samples for the in-season analysis of sister chromatid exchange (SCE) frequency, hematologi-

Table 2. Population characteristics.

Group	Number of subjects	Age (years)			Drinks per week			Smoking status			
		Mean	SD	Range	Mean	SD	Range	Number of smokers	Cigarettes per day		
									Mean	SD	Range
Greenhouse sprayers	134	35	12	21—76	5	7	0—48	48	15.4	8.1	1—35
Referents	157	37	12	21—66	8	9	0—63	62	14.6	8.6	1—50

cal parameters, and plasma cholinesterase (plasma ChE) activity were collected in the total study group at each workplace in August or September 1988, the end of the period in which pesticides are traditionally the most extensively used in greenhouses in Denmark. The base-line plasma-ChE samples were drawn in January 1989.

Blood samples were anticoagulated with heparin, and transferred within 2—4 h to the Cytogenetic Laboratory, Institute of Anatomy, Odense University, for the SCE analysis and to the Department of Clinical Chemistry, Odense University Hospital, for the blood cell count. Plasma was separated within 3 h, stored at –20°C, and later transferred to the Department of Clinical Chemistry, Randers Hospital, for the determination of the plasma ChE activity.

Sister chromatid exchange analysis

Lymphocyte cultures for the analysis of SCE were set up within 24 h after venipuncture. Each sample was cultured and processed as follows: Aliquots of 0.5 ml of peripheral blood in 9.5 ml of growth medium (GIBCO, RPMI 1640) supplemented with 10% fetal calf serum, heparin (5 IU · ml^{-1}), gentamycin (50 µg · ml^{-1}), 0.2 ml of stock solution of phytohemagglutinin, and 5-bromodeoxyuridine (5 µg · ml^{-1}) were incubated in airtight dark brown 100-ml glass bottles for a total of 72 h in 5% carbon dioxide, with colcemid (0.2 µg · ml^{-1}) added 2 h before the harvest. Further processing, including hypotonic treatment, fixation, slide production, microscopic techniques, and differential fluorescence photolysis giemsa staining (FPG staining) of sister chromatids for the detection of SCE, has been described elsewhere (20). To ensure a uniform quality of handling, all culture procedures and processing including slide production were performed by the same very experienced technician. To prevent sampling bias, all screening was performed by the same operator. For each sample 30 randomly selected FPG-stained metaphases in the second division were examined for SCE. Despite the rather long culture time, it was no problem to find and examine 30 second-division metaphases with well-defined exchange per sample. Hence replication indices were not calculated. All of the examinations were performed on coded slides as double-blind experiments. The numbers of exchanges per metaphase cell (SCE/C) were scored and listed for later calculation.

Hematological analysis

Blood smear preparation and clinical chemical analysis were performed within 4—6 h of venipuncture. Erythrocyte, thrombocyte, and total leucocyte counts were taken by standard methods (Coulter Counter S+2).

Plasma cholinesterase analysis

The analytical determination was performed by a kinetic assay using butyrylthiocholine (6 mmol · l^{-1}) as the substrate. All of the samples were run in duplicate and the mean value was used.

Exposure estimates

The following five measures of exposure were used: (i) ever exposed, (ii) individual protection during the application of pesticides, (iii) the frequency of monthly spraying during the last five months, (iv) in-season individual plasma ChE inhibition, and (v) lifetime pesticide exposure. Each of these exposure indicators could, in theory, measure a different dimension of exposure. Because little is known about the types of exposure that would be the most likely to produce cytogenetic damage, we employed several measures.

Concerning individual protection, the greenhouse sprayers were divided into three groups of exposure according to information on optimal or suboptimal protection during applications of either anti-ChE agents or other pesticides. Optimal protection was defined as the combined wearing of a face mask, protective gloves, and protective clothes. Persons with low exposure included those fully protected during the application of all types of pesticides. Medium-exposed persons included those fully protected during the application of anti-ChE agents but suboptimally protected during the application of other pesticides. The highly exposed persons included those who applied all types of pesticides while suboptimally protected.

Statistics

The results were evaluated from a stepwise multiple regression analysis, an analysis of variance (ANOVA), and a Student's t-test for independent samples using the SPSS/PC+ software package. The level of significance was taken as $P < 0.05$.

Results

In the present study a significant increase in SCE frequency by age [correlation coefficient (r) = 0.19, P = 0.002] and smoking (r = 0.20, P = 0.0005) was observed. The number of leucocytes was significantly increased by smoking (r = 0.45, P = 0.00005), but independent of age. Neither the number of erythrocytes nor thrombocytes was associated with age or smoking habits.

Table 3 shows the arithmetic mean SCE frequency and the red blood cell counts, total white blood cells, and

Table 3. Frequency of sister chromatid exchange per cell (SCE/C) and the hematological profiles for the greenhouse sprayers and referents, and for the same groups stratified into current smokers and nonsmokers.

Group	Number of subjects	SCE/C frequency		Erythrocytes ($10^{-12} \cdot l^{-1}$)		Leucocytes ($10^{-9} \cdot l^{-1}$)		Thrombocytes ($10^{-9} \cdot l^{-1}$)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Greenhouse sprayers	134	7.2	3.5	4.8	0.4	6.7	2.1	273	58
Smokers	48	7.3	3.7	4.7	0.3	8.2	2.6	288	60
Nonsmokers	86	7.1	3.4*	4.8	0.4	5.8	1.2	264	54
Referents	157	6.8	2.3	4.8	0.4	6.4	2.1	271	57
Smokers	62	7.4	2.4	4.7	0.5	7.5	2.4	271	45
Nonsmokers	95	6.3	2.1	4.8	0.4	5.8	1.6	272	63

* ANOVA, adjusted for age between nonsmokers, P = 0.04.

platelets in the exposed subjects and the referents. No statistically significant differences were found between the groups (Student's t-test). To analyze the effects of exposure to pesticides on SCE frequency and the number of leucocytes independent of smoking habits, we stratified the exposed subjects and referents into groups of nonsmokers or current smokers. The nonsmoking greenhouse sprayers displayed a significantly higher SCE frequency than the nonsmoking referents adjusted statistically for age, whereas no difference was observed between the current smokers (ANOVA) (table 3). Furthermore, the number of leucocytes, as well as the number of erythrocytes and thrombocytes, did not differ significantly between the exposed and unexposed smokers and nonsmokers (Student's t-test).

Figure 1 shows the mean SCE frequency for the greenhouse sprayers with low, medium, and high exposure according to individual optimal and suboptimal protection during the application of either anti-ChE agents or other pesticides. Information on protective measures was missed for 10 persons (N = 124). The figure shows a borderline dose-related increase of the mean SCE frequency (ANOVA, F = 2.40, P = 0.1). The hematological parameters were not associated with individual protection.

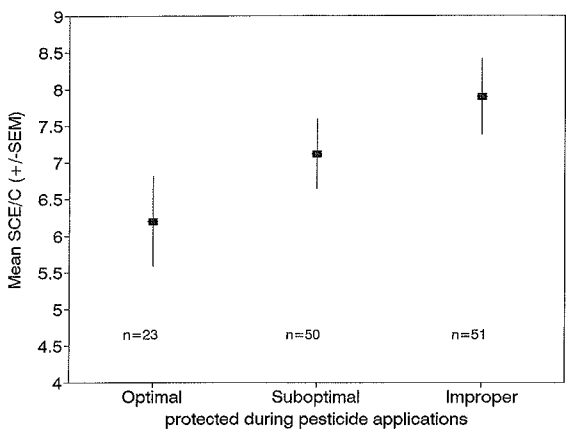

Figure 1. Mean sister chromatid exchange per cell (SCE/C) in blood lymphocytes in greenhouse sprayers classified according to individual protection during pesticide applications (ANOVA, F = 2.40, P = 0.1).

No correlation was found between individual in-season plasma ChE inhibition and SCE frequency or blood cell counts. Neither was monthly spraying frequency, including all types of pesticides, associated with SCE frequency or the hematological parameters. The lifetime pesticide exposure was not associated with SCE frequency or the hematological parameters.

Discussion

Smoking and age are well known determinants of SCE frequency, and they also significantly influenced the SCE frequency in our study. After adjustment for these confounding variables, a significantly higher mean SCE frequency was observed for the nonsmoking greenhouse sprayers when they were compared with the nonsmoking unexposed referents. This result indicates that handling pesticides may result in genetic damage. No negative effects of pesticide exposure were observed in relation to the blood cell counts.

The almost equal SCE frequency in the pesticide-exposed smokers and the exposed nonsmokers indicates no cumulative or synergistic effects on the SCE frequency of pesticides and smoking. Our results differ from those of Rupa et al (9, 10), who observed a higher SCE frequency among smoking cotton field workers than nonsmoking ones. The workers were equally exposed to pesticides. To our knowledge no other studies have been published which have investigated the interactive effects between smoking and pesticide exposure.

In general, neither SCE nor variability in hematological parameters was found to be associated with any of

the dose exposure estimates such as individual protection during pesticide application, in-season plasma ChE inhibition (short-term exposure effect of organophosphates and carbamates), current spraying frequency or accumulated lifetime pesticide exposure (chronic exposure effect). However, a borderline dose-related increase in SCE frequency was observed regarding individual protection. In fact, the degree of protection during spraying displayed a strong association with the amount of individual pesticide uptake. Hence it seems probable that poor individual protection during the handling of potentially mutagenic pesticides might result in an increased frequency of both SCE and other cytogenetic effects. Previous studies have reported a significant increase in SCE frequency in pesticide sprayers and correlated this observation with a lack of appropriate protective measures during spraying (8—10, 21). Shane et al (22) showed that greenhouse sprayers who did not wear respirators during spraying had a significantly higher urinary mutagenic activity than unexposed persons. These findings agree with our results and indicate the importance of proper work practices in preventing genetic damage.

Given the complex pesticide exposure with almost 50 compounds, it is not possible to know which agent(s) might have been responsible for the observed induction of SCE in the greenhouse sprayers. Some of the organochlorinated insecticides, organophosphates, and carbamates, in addition to some fungicidal groups of pesticides, used by the sprayers in the present study have been reported to be inducers of cytogenetic effects in experimental test systems (1—3, 23— 25). A few studies have also provided evidence for a pesticide-induced increase in SCE frequency. Floriculturists exposed to pesticides with chronic intoxication symptoms had increased SCE frequencies (21). Crossen et al (8) observed a significant increase in mean SCE frequency in 5 of 57 pesticide and herbicide sprayers. Rupa et al (9, 10) observed a significant increase in SCE frequencies as the duration of exposure increased among cotton field sprayers handling organochlorinated, organophosphorus, and synthetic pyrethroid insecticides.

Although the exact mechanism leading to an increased exchange of segments between sister chromatids is not known in detail, the SCE analysis has been adopted as a sensitive indicator of genotoxicity. The health significance of increased levels of SCE is unknown, and it remains uncertain whether a high rate of SCE indicates increased cancer susceptibility (26, 27). However, an increase in the SCE level has been observed in persons at higher cancer risk due to occupational or environmental exposure to a wide variety of carcinogens (28). Epidemiologic data provide evidence for a pesticide-induced increased risk for certain malignant neoplasms (eg, lung cancer, neoplasms of the lymphatic and hematopoietic tissue, and soft tissue sarcomas) (2). In a recent cohort study an increased risk of lymphatic and hematopoietic neoplasms and soft-tissue sarcomas was observed for 4000 Danish gardeners. The study cohort primarily comprised nonspraying greenhouse workers (29).

Thus any agent capable of damaging deoxyribonucleic acid (DNA) should be treated with care, and workers using pesticides commercially (eg, greenhouse sprayers) should take adequate protective measures to prevent long-term effects such as neoplasms.

From a literature review it appears that exposure to certain organochlorines and possibly the organophosphates may be associated with aplastic anemia, as well as with other hematologic diseases (1, 4, 5). A review of several case reports suggests that dichlorodiphenyltrichloroethane (DDT) and lindane may be important etiologic agents of aplastic anemia. The present negative hematological results could have several explanations. First of all, the levels of exposure may have been too low to induce even subclinical hematological changes in the greenhouse sprayers. Second, the pesticides used by the sprayers may have mostly been nonhazardous, although many individuals had been exposed to potentially hematotoxic polycholorinated compounds (eg, lindane, endosulfan and dicofol). However, our results are in accordance with those of some other epidemiologic studies showing that hematological effects due to subtoxic pesticide exposure are either nil or minimal (14, 30, 31, 32).

In conclusion the results suggest that SCE in human lymphocytes, but not the number of blood cells, is predictive of putative cytotoxic effects of in vivo subtoxic pesticide exposure.

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