Genotoxicity of the Organophosphorus Insecticide Chlorpyrifos Based on Human Lymphocyte Culture

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Chlorpyrifos, O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate is a broad spectrum insecticide, marketed under the names Dursban, Lorsban and DOWCO 19. Organophosphorus compounds such as chlorpyrifos are popular insecticides because of their effectiveness as cholinesterase inhibitors, high degree of water solubility and rapid biodegradation. However, many of these compounds and their breakdown products are known alkylating agents of DNA (Bedford and Robinson 1972). It is therefore imperative to continue to assess potential genetic damage from limited exposure to organophosphorus insecticides (Degraeve *et al.* 1984).

Materials and methods

Peripheral blood while fasting was used from three non-smoking healthy adults. Their GTG banded karyotypes were normal. Total volume of 8.0 ml. was attained by using 7.1 ml. of Difco chromosome culture medium, 0.8 ml. of fresh whole blood and 0.1 ml. of DMSO (dimethyl sulfoxide) with or without treatments. Four treatments (0.02, 0.2, 2.0, 20 $\mu\text{g/ml}$) of chlorpyrifos and two controls (no treatment and 0.1 ml. DMSO) were used in close accord with Preston *et al.* (1981) protocol. Chlorpyrifos (>99% pure) was provided by the U.S. Environmental Protection Agency. All solutions were prepared within 48 hours of their use. Four-hour exposure was used for assessment of the chromosome damage from direct action of the compound, and 24-hour exposure was used to detect indirect interactions of chlorpyrifos and its metabolites (Bloom 1981). Single replication of sister chromatid exchanges (SCE) from 24-hour exposure in 10 $\mu\text{g/ml}$ of BrdU (5-Bromodeoxyuridine) was used for mutagenicity testing, using the Latt *et al.* (1979) method.

Standard culture technique for the whole blood was used at 37°C for 72 hours in a Labline CO₂ incubator. Slide labels were disguised while taking the data by a Zeiss Photomicroscope III. Data were taken from 50 cells per treatment and controls giving a total of 150 cells per replication of 4- and 24-hour treatments. Data for the clastogenic and mutagenic studies were analyzed using ANOVA and Dunnett's multiple comparison test. Dunnett's test is most applicable when comparing a control group to one or more experimental groups (Roscoe 1975). Since treatments spanned more than one order of magnitude, log transformed data was also analyzed (Margolin 1985).

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Results and discussion

The most common chromosome aberrations observed from chlorpyrifos exposure were interstitial gaps and breaks. Most aberrations were single events in individual cells. Thus, the means are not skewed results from a few cells with large numbers of aberrations.

The summary of three 4-hour replications (150 calls per replication) is presented in Table 1. The two controls exhibited the smallest numbers of gaps, breaks and gaps plus breaks. Chromosome damage tended to increase slightly (statistically nonsignificant) as the concentrations

Table 1. Chromosome aberrations per cell in Chlorpyrifos treated human lymphocytes, 4-hour exposure summary

Concentration ($\mu\text{g/ml}$ Chlorpyrifos)	Gaps	Breaks	Gaps plus breaks	Abnrmal cells (%)
No treatment control	0.060	0.047	0.107	10.7
DMSO control	0.053	0.033	0.086	8.6
0.02	0.080	0.040	0.120	12.0
0.2	0.080	0.047	0.127	12.7
2.0	0.080	0.053	0.133	13.3
20.0	0.060	0.060	0.120	12.0
F value ¹			2.278	
Df ²			1,16	
F value ³			4.49	

Means are based on 150 cells per treatment.

¹ Calculated F value.

² Degrees of freedom for the replicate.

³ Table value which F must equal or exceed to be statistically significant ($P < 0.05$).

Table 2. Chromosome aberrations per cell in Chlorpyrifos treated human lymphocytes, 24-hour exposure summary

Concentration ($\mu\text{g/ml}$ Chlorpyrifos)	Gaps	Breaks	Gaps plus Breaks	Abnormal cells (%)
No treatment control	0.067	0.047	0.114	11.4
DMSO control	0.053	0.047	0.100	10.0
0.02	0.040	0.033	0.073	7.3
2.0	0.087	0.047	0.134	13.4
20.0	0.053	0.080	0.133	13.3
F value ¹			1.010	
Df ²			1,16	
F value ³			4.49	

Means are based on 150 cells per treatment.

¹ Calculated F value.

² Degrees of freedom for the replicate.

³ Table value which F must equal or exceed to be statistically significant ($P < 0.05$).

of chlorpyrifos increased, reaching a peak of 0.133 gaps plus breaks per cell at 2.0 $\mu\text{g/ml}$ level. The aberration levels between the two controls and between the three donors were also non-significant.

The summary of three 24-hour replications is presented in Table 2. The smallest number of gaps, breaks and gaps plus breaks occurred at 0.02 $\mu\text{g/ml}$ followed by the DMSO control. The aberration levels increased slightly, reaching a peak at 2.0 and 20.0 $\mu\text{g/ml}$ where the values for gaps and breaks per cell were 0.134 and 0.133, respectively. The observed pattern of chromosome damage was nonsignificant and generally quite similar to the 4-hour exposure results.

The lack of significant increases from the treatments ranging from 0.02 to 20 $\mu\text{g/ml}$ both in 4-hour and 24-hour exposures indicates that chlorpyrifos and its metabolites have a limited, if any, influence in increasing chromosome aberrations. This is in agreement with the findings of Muscarella *et al.* (1984) and Thompson *et al.* (1971), who reported chlorpyrifos to be non-clastogenic in small mammals, and those of Sobti *et al.* (1982), who observed the compound to be nonclastogenic in humans.

Sister chromatid exchanges, based on the average of 25 cells each for the two controls were compared with the four treatment levels (Table 3). No treatment control had statistically nonsignificant differences compared to all four treatment levels. The DMSO control had a nonsignificant difference at 0.2, 2.0 and 20.0 $\mu\text{g/ml}$ concentration levels, and a significant increase at 0.02 $\mu\text{g/ml}$ level. Compared to the DMSO control all treatment levels had enhanced SCE values, though only at 0.02 $\mu\text{g/ml}$ it reached significance. Thus, results of this investigation do not depict chlorpyrifos as an agent that results in consistent increase of SCE levels, at these dosages. However, Sobti *et al.* (1982) have reported increased SCE levels at 2.0 and 20.0 $\mu\text{g/ml}$ of chlorpyrifos in human lymphocytes in culture. Chen *et al.* (1981) did not observe

Table 3. Sister Chromatid Exchange in Chlorpyrifos treated human lymphocytes

Concentration ($\mu\text{g/ml}$ Chlorpyrifos)	Number of exchanges observed	Mean number per cell (%)
No treatment control	151	6.04
DMS control	119	4.76
0.02	165*	6.60*
0.2	124	4.96
2.0	124	4.96
20.0	148	5.92
F value ¹	0.124	0.124
Df ²	1, ∞	1, ∞
F value ³	3.84	3.84

Means are based on 25 cells per treatment.

¹ Calculated F value.

² Degrees of freedom for the replicate.

³ Table value which F must equal or exceed to be statistically significant ($P < 0.05$).

* Significant at ($P < 0.05$) compared to the DMSO control.

significant increases in rates of SCE or delay in cell cycle induced by this insecticide in cultured mammalian cells. Though our data tend to indicate a lack of significant increase in rates of SCE from chlorpyrifos exposure, we have reported a significant increase in SCE rates from exposure to another organophosphate, Malathion (Herath *et al.* 1989).

The common structural element of all organophosphates are the phosphorus and carbon as the electrophilic key reactive sites (Wild 1975). Chlorpyrifos and its two primary metabolites, namely 3,5,6-trichloro-2-pyridinol and diethyl-3-5-6-trichloro-2-pyridyl phosphate, have the nucleophilic sites that can cleave the carbon-oxygen bond resulting in alkylation (Wild 1975). Most of the chlorpyrifos is eliminated in animals as 3,5,6-trichloro-2-pyridinol through urine (Marshall and Roberts 1978). Genotoxic investigations of all compounds used widely as insecticide is indeed imperative but particularly those known to be nucleic acid alkylating agents must undergo close scrutiny.

Single acute exposures for a duration of 4- or 24-hours of chlorpyrifos at concentrations ranging from 0.02 to 20 $\mu\text{g/ml}$ did not have statistically significant chromosome damage, though the rates of increase of sister chromatid exchanges did reach significance level at 0.02 $\mu\text{g/ml}$ compared to one of the two controls. The LD₅₀ (lethal dosage at 50% kill) for mammals for

chlorpyrifos ranges from 62 to 6730 mg/kg body weight (Marshall and Roberts 1978). It therefore seems that chlorpyrifos is an insecticide that causes relatively limited chromosome damage at low dosage exposures. These results must be treated with caution since they do not reflect genotoxic influence from repeated or chronic exposures. Efforts must continue to procure safer compounds that are not reactive to nucleic acids.

Summary

Chlorpyrifos is one of the widely used organophosphorus insecticides. The compound and two of its main metabolites are known nucleic acid alkylating agents. Potential of the compound as a genotoxic agent was determined on the basis of chromosome damage and rates of sister chromatid exchanges in cultured human lymphocytes. Four treatments (0.02, 0.20, 2.00 and 20.00 $\mu\text{g/ml}$), a solvent control and an untreated control were used in three replications of 4-hour and 24-hour exposures to assess damage to chromosomes. One replication of the four treatments and two controls based on 24-hour exposure was used to determine sister chromatid exchanges. Chromosome damage assessed primarily as gaps and breaks did not increase at a statistically significant level. The trend was almost similar for sister chromatid exchanges though for one of the controls, significance was reached for one treatment level. It seems that short term exposure to chlorpyrifos at these dosages is relatively safe. Efforts however, must continue for procurement of safer compounds that are not reactive to nucleic acids.

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