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# Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures

J. Surrallés [a], N. Xamena [a], A. Creus [a], J. Catalán [b], H. Norppa [b], R. Marcos [a,*]

[a] *Unitat de Genètica, Departament de Genètica i de Microbiologia, Edifici Cn, Universitat Autònoma de Barcelona, 08193 Bellaterra (Cerdanyola del Vallès), Spain*

[b] *Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Helsinki, Finland*

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**Abstract**

Five pyrethroid insecticides: cypermethrin, deltamethrin, fenpropathrin, fenvalerate and permethrin, were tested for their ability to induce micronuclei in both whole-blood (WB; three donors) and isolated human lymphocyte (IL, 2 donors) cultures, by using the cytokinesis-block method with 6 μg/ml cytochalasin B (Cyt-B). Fenvalerate and permethrin were tested with two different concentrations of Cyt-B (3 and 6 μg/ml).

At the concentration ranges tested, all the five pyrethroids induced clear dose dependent cytotoxic effects, fenpropathrin being the most toxic. Nuclear division index (NDI) and the newly introduced index of cytotoxicity, the cytokinesis block proliferation index (CBPI), reflected the dose dependency more accurately than the percentage of binucleated cells did. CBPI is similar to NDI except that it estimates the average number of cell divisions that the cell population has gone through, and, therefore, classifies both trinucleate and tetranucleate cells into the same category.

Cypermethrin and fenpropathrin slightly increased the number of MN and micronucleated cells in WB lymphocyte cultures from two out of the three donors. Deltamethrin produced a positive response only in WB cultures of one donor and in IL cultures of another donor. Permethrin gave mostly negative results, although it increased the MN frequency in WB cultures of one donor when 6 μg/ml Cyt-B was used. Fenvalerate did not significantly induce MN. With certain reservations to the purity and isomer composition of each pesticide, the existing information appears to support the idea that pyrethroid insecticides have a weak (cypermethrin, deltamethrin and fenpropathrin) or nule (fenvalerate and permethrin) genotoxic activity in vitro.

*Keywords:* Micronucleus; Human lymphocyte; Pyrethroid insecticide; Cytochalasin-B; Cytotoxicity

## 1. Introduction

Synthetic pyrethroids are highly active insecticides characterized by photostability, relatively low mammalian toxicity and low production of residues in plants (Papadopoulou-Mourkidou, 1983; Vijverberg and van der Bercken, 1990). Deltamethrin, as an example of a synthetic pyrethroid, is 600 times more active than DDT against *Anopheles stephensi* (Zerba, 1988).

Because of their obvious advantages, pyre-

* Corresponding author.


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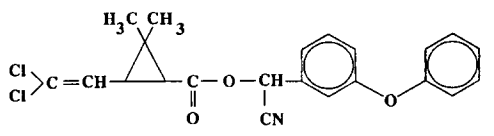
throid insecticides are becoming widespread and, therefore, studies on the biological effects of these pesticides are of immediate concern. Numerous studies on their toxicity both in insects and mammals have been reported in the literature (for detailed description on biological activity, mode of action, metabolism and toxicity, see Aldridge, 1990). The genotoxicity of these chemicals has been an important research line in our laboratory (Barcelona) during the last 10 years (Batiste-Alentorn et al., 1986, 1987; Puig et al., 1989; Carbonell et al., 1989; Surrallés et al., 1990; Pardo et al., 1993). Although pyrethroid insecticides have consistently shown negative results in microbial genotoxicity tests, the outcome of other assays has been variable and it has not been possible to draw definite conclusions about the genotoxicity of this group of pesticides.

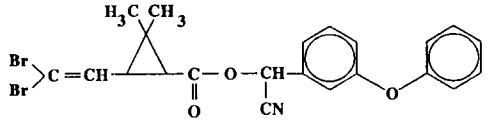
The present study deals with the genotoxicity of five synthetic pyrethroid insecticides currently used in modern agriculture in great extent: cypermethrin (Cyp), deltamethrin (Del), fenpropathrin (Fep), fenvalerate (Fev), and permethrin (Per). These chemicals are carboxylic acid esters with three chirial centres located at carbons 1 and 3 of the cyclopropane ring and at the $\alpha$-carbon of the alcohol moiety. All of them, except permethrin, have a cyano group which has been related to mammalian toxicity (see Fig. 1).

The genetic end-point chosen for this study was the micronucleus assay in cultured human lymphocytes. Micronuclei (MN) are acentric chromosome fragments or whole chromosomes that are left behind during mitotic cellular division and appear in the cytoplasm of interphase cells as small additional nuclei. The cytokinesis block micronucleus (CBMN) assay (Fenech and Morley, 1985), using cytochalasin-B (Cyt-B) to identify cells that have divided once in vitro, has made the identification of MN a useful tool for assessing genetic damage.

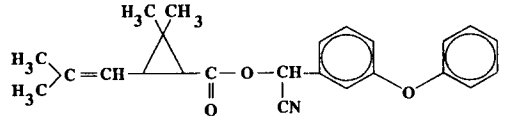
Among the five pyrethroids studied here, Fen and Per have earlier been shown to induce MN in human lymphocytes (Surrallés et al., 1990; Barrueco et al., 1992). Thus, the main aim of this study was to investigate the ability of the five pyrethroids to induce MN and to reevaluate the MN induction by Fev and Per by using a different concentration of Cyt-B and a different methodology.



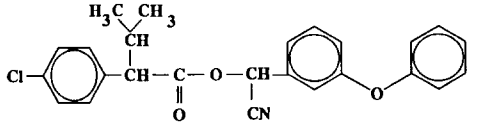
**Cypermethrin**



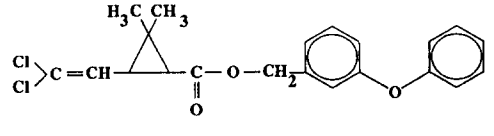
**Deltamethrin**



**Fenpropathrin**



**Fenvalerate**



**Permethrin**

Fig. 1. Chemical structure of the pyrethroid insecticides tested.

Previous studies have demonstrated that the concentration of Cyt-B is an important variable affecting the efficiency of the cytokinesis block (Littelfield et al., 1989; Surrallés et al., 1992, 1994) and the baseline and induced frequency of MN (Surrallés et al., 1992, 1994). We have sug-

gested that, especially when a suboptimal Cyt-B concentration (such as 3 μg/ml) is used to block cytokinesis, binucleated cells that have actually divided twice are included in the scoring (Lindholm et al., 1991; Norppa et al., 1993; Surrallés et al., 1994). This fact could lead to an overestimation of genetic damage (Surrallés et al., 1994).

Migliore et al. (1989) recommended that whole-blood (WB) cultures should be used in the CBMN assay. However, it was recenty suggested that the sensitivity of the CBMN assay may be enhanced by using isolated lymphocytes (IL) instead of WB (Van Hummelen and Kirsch-Volders, 1992; Elhajouji et al., 1993). Therefore, the five pyrethroids were tested in both WB and IL cultures, to see if one type of culture is superior to the other.

## 2. Materials and methods

*Cytogenetic methods*

Blood was obtained by venipuncture from healthy non-smoking donors aged from 25 to 30 years and with no previous known contact with high concentrations of pesticides.

WB cultures were set up as described by Surrallés et al. (1992). Briefly, 0.5 ml of blood was added to 4.5 ml of culture medium containing RPMI 1640, 15% heat-inactivated foetal calf serum (FCS), antibiotics, L-glutamine and phytohaemagglutinin (PHA). IL cultures were based on the method described by Lindholm et al. (1991). Mononucleated cells were isolated in Lymphoprep and a total of one million cells were added to 5 ml PRMI 1640 medium supplemented with 15% FCS, antibiotics, L-glutamine, and PHA. All the cultures were incubated at 37°C for 72 h.

Treatments were performed at 24 h after culture initation and were prolonged until the end of the culture. Cyt-B (Sigma, St. Louis, MO, USA) was added at 44 h, at a concentration of 6 μg/ml (all treatments) or 3 μg/ml (parallel cultures of Fev and Per, donor C).

The WB cultures were harvested at 72 h by gently rinsing the cells in serum-free RPMI 1640 medium at room temperature. Following a short hypotonic treatment with KCL (0.075 M at room temperature), the cells were fixed three times in a solution consisting of 5 parts of methanol and 1 part of acetic acid. Finally, the centrifuged cells were dropped onto clean slides and stained with Giemsa (Surrallés et al., 1992). The IL cultures were harvested by centrifugation and, after two rinses in PBS at room temperature, the cells were centrifuged directly onto slides using a cytocentrifuge (Shandon Cytospin 3, Astmoor, UK; 5 min at 500 rpm), fixed with methanol, and stained in May-Grünwald-Giemsa (Lindholm et al., 1991).

*Treatments*

Cypermethrin ((R,S)-α-cyano-3-phenoxy benzyl-2,2-dimethyl (1R,1S)-cis,trans-3-(2,2-dichlorovinyl)cyclopropanecarboxylate, CAS No. 52315-07-8, purity 90%), deltamethrin (S-α-cyano-3-phenoxybenzyl-(1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, CAS No. 52918-63-5, purity 99.9%), fenpropathrin (α-cyano-3-phenoxybenzyl-1-2,2,3,3-tetramethylcyclopropanecarboxylate, CAS No. 39515-41-8, purity 99.6%), fenvalerate ((R,S)-α-cyano-3-phenoxybenzyl(1R,1S)-2-(4-chlorophenyl)-3-methyl-1-butyrate, CAS No. 51630-58-1, purity 97.9%) and permethrin (3-phenoxybenzyl-(1R,1S)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, CAS No. 52645-33-1, purity 97%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Serial dilutions were made in dimethyl sulphoxide (DMSO, purity > 99%, Panreac, Barcelona, Spain) in sterile conditions and 50 μl (1% v/v) were added to each culture. Negative and positive control cultures receiving 50 μl DMSO and mytomicin C (MMC, Sigma, St. Louis, MO, USA), respectively, were also established in parallel. The final concentrations of MMC were 0.4 μM for the WB and 0.6 μM for the IL cultures

The highest concentration of each pesticide was chosen taking into account pH changes, maximum solubility in DMSO and medium, reduction of mitotic index by > 50%, and general toxicity found in this and previous studies conducted in our laboratory (Carbonell et al., 1989; Puig et al., 1989; Surrallés et al., 1990) and elsewhere (Hoellinger et al., 1987; Dolara et al., 1992; Barrueco

et al., 1992). Although pH changes were not marked at the concentrations chosen, doses higher than 200 μg/ml resulted in chemical precipitation (Cyp and Del) or in complete lack of cell growth (Fep, Fev and Per).

A range of 5–6 concentrations (including negative controls) of each pesticide was studied in the WB cultures from the three donors. The two highest effective doses of each pesticide were also tested in the IL cultures, with two donors.

*Cell scoring*

Toxicity was evaluated by classifying 500 cells according to the number of nuclei. Two well known cytotoxicity indexes were used: percentage of binucleated (BN) cells (Fenech and Morley, 1985) and nuclear division index (NDI), which was calculated following the formula: NDI = (MI + 2MII + 3MIII + 4MIV)/total, where MI-MIV represent the number of cells with one to four nuclei (Eastmond and Tucker, 1989). A new index for measuring cell proliferation kinetics was also applied, which was called *cytokinesis block proliferation index* (CBPI). This new index is based on the formula: CBPI = [MI + 2MII + 3 (MIII + MIV)]/total, where MIII and MIV are equally considered to be in their third cell cycle. While NDI is the average number of nuclei per cell, CBPI indicates the average number of cell cycles undergone by a given cell, which may be biologically more relevant in the case of cytokinesis-blocked cells.

The induction of MN was evaluated by scoring a total of 1000 binucleated (BN) cells with well preserved cytoplasm at 1000 × magnification. BN cells were analyzed according to the number of MN so that total MN counts (MN) and total number of micronucleated BN cells (BNMN) could be recorded. Only binucleated cells were included in the microscopic analysis.

The WB and IL experiments were done in different laboratories (Barcelona and Helsinki, respectively) but using the same pesticide stocks. All scoring was performed by the same person on coded slides.

*Statistical analysis*

The frequency of BNMN and MN in the treated cultures were compared with their respective controls by using the 'one tailed' Fisher's exact test and the Kastenbaum and Bowman (1970) test, respectively. Correlation coefficients were calculated by plotting toxicity indexes against pesticide concentrations, and the slopes were compared to zero using the *t*-test with the null hyphotesis considering that pesticide concentrations and toxicity indexes are not related. Correlation coefficients derived from the three different toxicity indexes were compared by performing an analysis of variance (ANOVA).

## 3. Results

Tables 1–5 show the results obtained from the WB cultures with the five pyrethroids tested by using 6 μg/ml Cyt-B. Table 6 shows MN induction by Fen and Per in WB cultures of donor C at two concentrations of Cyt-B. The results obtained with the IL cultures of two donors with two concentrations of the five insecticides are summarized in Table 7. None of the five pyrethroid tested was able to induce a clear dose-dependent increase of MN frequencies, although Cyp (200 μg/ml) and Fep (10 and 50 μg/ml) produced a slight but statistically significant increase in the frequency of MN and BNMN in WB cultures of two out of the three donors. A significant increase was also found for Del (100 and 200 μg/ml), both in the WB and in IL, but only in cultures of one donor in each case. A slightly positive result was likewise obtained for Per only at one concentration with one donor in the WB study and, therefore, this increase can be considered as marginal. A possible suggestion of an effect was also seen for Fev (Tables 4, 6, and 7) but no statistically significance differences were obtained. MMC showed a highly significant induction of MN with all donors ($P < 0.001$).

From cytotoxicity data presented in Tables 1–5, correlation coefficients were calculated by plotting pesticide concentrations against the three toxicity indexes. The results of this analysis are shown in Table 8. From these correlations, it is obvious that the percentage of BN cells did not reflect the reduction of cell proliferation since the correlation coefficients were quite low and in

Table 1
Induction of micronuclei and cytotoxicity by 48-h treatment with cypermethrin in human whole-blood lymphocyte cultures

| Donor | Treatment | Distribution of BN cells according to No. MN | | | | | MN | BNMN | Distribution of cells according to No. nuclei | | | | % BN | NDI | CBPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | > 3 | | | 1 | 2 | 3 | 4 | | | |
| A | DMS0 1% | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 44 | 329 | 65 | 62 | 65.8 | 2.29 | 2.17 |
| | 10 μg/ml | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 69 | 333 | 51 | 47 | 66.6 | 2.15 | 2.06 |
| | 25 μg/ml | 991 | 8 | 1 | 0 | 0 | 10 | 9 | 52 | 350 | 57 | 41 | 70.0 | 2.17 | 2.09 |
| | 50 μg/ml | 991 | 9 | 0 | 0 | 0 | 9 | 9 | 106 | 355 | 30 | 9 | 71.0 | 1.88 | 1.87 |
| | 100 μg/ml | 993 | 6 | 1 | 0 | 0 | 8 | 7 | 194 | 302 | 4 | 0 | 60.4 | 1.62 | 1.62 |
| | 200 μg/ml | 980 | 18 | 1 | 1 | 0 | 23** | 20** | 303 | 196 | 1 | 0 | 39.2 | 1.40 | 1.40 |
| | MMC 0.4 μM | 951 | 47 | 1 | 1 | 0 | 52*** | 49*** | 71 | 324 | 67 | 38 | 64.8 | 2.14 | 2.07 |
| B | DMSO 1% | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 20 | 332 | 86 | 62 | 66.4 | 2.38 | 2.26 |
| | 10 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 10 | 318 | 105 | 67 | 63.6 | 2.46 | 2.32 |
| | 25 μg/ml | 992 | 7 | 1 | 0 | 0 | 9 | 8 | 19 | 375 | 58 | 48 | 75.0 | 2.27 | 2.17 |
| | 50 μg/ml | 998 | 1 | 1 | 0 | 0 | 3 | 2 | 94 | 380 | 11 | 15 | 76.0 | 1.89 | 1.86 |
| | 100 μg/ml | 995 | 4 | 1 | 0 | 0 | 6 | 5 | 216 | 282 | 2 | 0 | 56.4 | 1.57 | 1.57 |
| | 200 μg/ml | 991 | 7 | 1 | 1 | 0 | 12* | 9 | 365 | 135 | 0 | 0 | 27.0 | 1.27 | 1.27 |
| | MMC 0.4 μM | 954 | 44 | 2 | 0 | 0 | 48*** | 46*** | 85 | 328 | 48 | 39 | 65.6 | 2.08 | 2.00 |
| C | DMS0 1% | 994 | 5 | 1 | 0 | 0 | 7 | 6 | 42 | 312 | 77 | 69 | 62.4 | 2.35 | 2.21 |
| | 10 μg/ml | 993 | 7 | 0 | 0 | 0 | 7 | 7 | 47 | 343 | 50 | 60 | 68.6 | 2.25 | 2.13 |
| | 25 μg/ml | 993 | 7 | 0 | 0 | 0 | 7 | 7 | 62 | 341 | 68 | 29 | 68.2 | 2.13 | 2.07 |
| | 50 μg/ml | 994 | 5 | 1 | 0 | 0 | 7 | 6 | 147 | 306 | 33 | 18 | 61.2 | 1.86 | 1.82 |
| | 100 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 226 | 271 | 2 | 1 | 54.2 | 1.56 | 1.55 |
| | 200 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 953 | 44 | 3 | 0 | 0 | 50*** | 47*** | 49 | 349 | 68 | 34 | 69.8 | 2.17 | 2.11 |

*T*, 100% toxicity; MN, total MN; BNMN, binucleated cells with MN; %BN, percentage of BN cells; NDI, nuclear division index; CBPI, cytokinesis blocked proliferation index; Probabilities: *, **, *** = $P < 0.05$, 0.01, 0.001 respectively (Fisher's exact test and Kastenbaum and Bowman test for BNMN and MN respectively).

Table 2
Induction of micronuclei and cytotoxicity by 48-h treatment with deltamethrin in human whole-blood lymphocyte cultures

| Donor | Treatment | Distribution of BN cells according to No. MN | | | | | MN | BNMN | Distribution of cells according to No. nuclei | | | | % BN | NDI | CBPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | >3 | | | 1 | 2 | 3 | 4 | | | |
| A | DMSO 1% | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 44 | 329 | 65 | 62 | 65.8 | 2.29 | 2.17 |
| | 10 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 60 | 337 | 46 | 57 | 67.4 | 2.20 | 2.09 |
| | 25 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 40 | 356 | 59 | 45 | 71.2 | 2.22 | 2.13 |
| | 50 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 134 | 339 | 18 | 9 | 67.8 | 1.80 | 1.79 |
| | 100 μg/ml | 985 | 13 | 2 | 0 | 0 | 17* | 15* | 251 | 246 | 2 | 1 | 49.2 | 1.51 | 1.50 |
| | 200 μg/ml | 989 | 10 | 0 | 0 | 1 | 15* | 11 | 267 | 233 | 0 | 0 | 46.6 | 1.47 | 1.47 |
| | MMC 0.4 μM | 951 | 47 | 1 | 1 | 0 | 52*** | 49*** | 71 | 324 | 67 | 38 | 64.8 | 2.14 | 2.07 |
| B | DMSO 1% | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 20 | 332 | 86 | 62 | 66.4 | 2.38 | 2.26 |
| | 10 μg/ml | 992 | 8 | 0 | 0 | 0 | 8 | 8 | 32 | 353 | 71 | 44 | 70.6 | 2.25 | 2.17 |
| | 25 μg/ml | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 34 | 396 | 40 | 30 | 79.2 | 2.13 | 2.07 |
| | 50 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 80 | 398 | 16 | 6 | 79.6 | 1.90 | 1.88 |
| | 100 μg/ml | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 205 | 291 | 2 | 2 | 58.2 | 1.60 | 1.60 |
| | 200 μg/ml | 993 | 7 | 0 | 0 | 0 | 7 | 7 | 301 | 198 | 1 | 0 | 39.6 | 1.40 | 1.40 |
| | MMC 0.4 μM | 954 | 44 | 2 | 0 | 0 | 48*** | 46*** | 85 | 328 | 48 | 39 | 65.6 | 2.08 | 2.00 |
| C | DMSO 1% | 994 | 5 | 1 | 0 | 0 | 7 | 6 | 42 | 312 | 77 | 69 | 62.4 | 2.35 | 2.21 |
| | 10 μg/ml | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 53 | 306 | 80 | 61 | 61.2 | 2.30 | 2.18 |
| | 25 μg/ml | 992 | 8 | 0 | 0 | 0 | 8 | 8 | 64 | 333 | 64 | 39 | 66.6 | 2.16 | 2.16 |
| | 50 μg/ml | 995 | 4 | 0 | 1 | 0 | 7 | 5 | 88 | 308 | 68 | 36 | 61.6 | 2.10 | 2.03 |
| | 100 μg/ml | 994 | 5 | 1 | 0 | 0 | 7 | 6 | 219 | 278 | 1 | 2 | 55.6 | 1.57 | 1.57 |
| | 200 μg/ml | 993 | 6 | 1 | 0 | 0 | 8 | 7 | 304 | 195 | 1 | 0 | 39.0 | 1.39 | 1.39 |
| | MMC 0.4 μM | 953 | 44 | 3 | 0 | 0 | 50*** | 47*** | 49 | 349 | 68 | 34 | 69.8 | 2.17 | 2.11 |

Legend: see Table 1.

Table 3
Induction of micronuclei and cytotoxicity by 48-h treatment with fenpropathrin in human whole-blood lymphocyte cultures

| Donor | Treatment | Distribution of BN cells according to No. MN | | | | | MN | BNMN | Distribution of cells according to No. nuclei | | | | % BN | NDI | CBPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | > 3 | | | 1 | 2 | 3 | 4 | | | |
| A | DMSO 1% | 992 | 8 | 0 | 0 | 0 | 8 | 8 | 82 | 298 | 70 | 50 | 59.6 | 2.18 | 2.08 |
| | 5 μg/ml | 992 | 7 | 1 | 0 | 0 | 9 | 8 | 88 | 314 | 49 | 49 | 62.8 | 2.12 | 2.02 |
| | 10 μg/ml | 995 | 4 | 1 | 0 | 0 | 6 | 5 | 61 | 306 | 70 | 63 | 61.2 | 2.27 | 2.14 |
| | 25 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 65 | 347 | 39 | 49 | 69.4 | 2.14 | 2.05 |
| | 50 μg/ml | 992 | 7 | 1 | 0 | 0 | 9 | 8 | 168 | 317 | 10 | 5 | 63.4 | 1.70 | 1.69 |
| | 100 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 951 | 45 | 4 | 0 | 0 | 53*** | 49*** | 86 | 286 | 74 | 54 | 57.2 | 2.19 | 2.08 |
| B | DMSO 1% | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 98 | 329 | 37 | 36 | 65.8 | 2.02 | 1.95 |
| | 5 μg/ml | 997 | 2 | 1 | 0 | 0 | 4 | 3 | 86 | 343 | 33 | 38 | 68.6 | 2.05 | 1.98 |
| | 10 μg/ml | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 80 | 354 | 36 | 30 | 70.8 | 2.03 | 1.97 |
| | 25 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 79 | 390 | 14 | 17 | 78.0 | 1.94 | 1.90 |
| | 50 μg/ml | 979 | 19 | 2 | 0 | 0 | 23*** | 21** | 165 | 326 | 6 | 3 | 65.2 | 1.69 | 1.69 |
| | 100 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 924 | 73 | 3 | 0 | 0 | 79*** | 76*** | 69 | 335 | 55 | 41 | 67.0 | 2.14 | 2.05 |
| C | DMSO 1% | 994 | 5 | 1 | 0 | 0 | 7 | 6 | 42 | 312 | 77 | 69 | 62.4 | 2.35 | 2.21 |
| | 5 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 77 | 307 | 71 | 48 | 61.4 | 2.19 | 2.10 |
| | 10 μg/ml | 984 | 15 | 1 | 0 | 0 | 17* | 16* | 95 | 297 | 58 | 50 | 59.4 | 2.13 | 2.03 |
| | 25 μg/ml | 993 | 7 | 0 | 0 | 0 | 7 | 7 | 101 | 312 | 58 | 29 | 62.6 | 2.03 | 1.97 |
| | 50 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 198 | 292 | 8 | 2 | 58.4 | 1.63 | 1.62 |
| | 100 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 953 | 44 | 3 | 0 | 0 | 50*** | 47*** | 49 | 349 | 68 | 34 | 69.8 | 2.17 | 2.11 |

Legend: see Table 1.

Table 4
Induction of micronuclei and cytotoxicity by 48-h treatment with fenvalerate in human whole-blood lymphocyte cultures

| Donor | Treatment | Distribution of BN cells according to No. MN | | | | | MN | BNMN | Distribution of cells according to No. nuclei | | | | % BN | NDI | CBPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | >3 | | | 1 | 2 | 3 | 4 | | | |
| A | DMSO 1% | 992 | 8 | 0 | 0 | 0 | 8 | 8 | 82 | 298 | 70 | 50 | 59.6 | 2.18 | 2.08 |
| | 10 μg/ml | 991 | 7 | 1 | 1 | 0 | 12 | 9 | 58 | 302 | 72 | 68 | 60.4 | 2.30 | 2.16 |
| | 25 μg/ml | 990 | 10 | 0 | 0 | 0 | 10 | 10 | 106 | 335 | 31 | 28 | 67.0 | 1.96 | 1.91 |
| | 50 μg/ml | 993 | 7 | 0 | 0 | 0 | 7 | 7 | 164 | 308 | 14 | 14 | 61.6 | 1.76 | 1.73 |
| | 100 μg/ml | 989 | 8 | 1 | 1 | 1 | 17 | 11 | 272 | 226 | 2 | 0 | 45.2 | 1.46 | 1.46 |
| | MMC 0.4 μM | 951 | 45 | 4 | 0 | 0 | 53*** | 49*** | 86 | 286 | 74 | 54 | 57.2 | 2.19 | 2.08 |
| B | DMSO 1% | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 98 | 329 | 37 | 36 | 65.8 | 2.02 | 1.95 |
| | 10 μg/ml | 990 | 10 | 0 | 0 | 0 | 10 | 10 | 66 | 367 | 30 | 37 | 73.4 | 2.08 | 2.00 |
| | 25 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 76 | 370 | 16 | 38 | 74.0 | 2.03 | 1.96 |
| | 50 μg/ml | 990 | 8 | 2 | 0 | 0 | 12 | 10 | 232 | 263 | 3 | 2 | 52.6 | 1.55 | 1.55 |
| | 100 μg/ml | T | | | | | | | | | | | | | |
| | 200 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 924 | 73 | 3 | 0 | 0 | 79*** | 76*** | 69 | 335 | 55 | 41 | 67.0 | 2.14 | 2.05 |
| C | DMSO 1% | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 65 | 332 | 54 | 49 | 66.4 | 2.17 | 2.06 |
| | 10 μg/ml | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 53 | 318 | 49 | 72 | 63.6 | 2.25 | 2.10 |
| | 25 μg/ml | 995 | 4 | 1 | 0 | 0 | 6 | 5 | 133 | 316 | 25 | 26 | 63.2 | 1.89 | 1.84 |
| | 50 μg/ml | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 109 | 355 | 17 | 19 | 71.0 | 1.89 | 1.85 |
| | 100 μg/ml | 994 | 4 | 2 | 0 | 0 | 8 | 6 | 168 | 319 | 6 | 7 | 63.8 | 1.70 | 1.69 |
| | MMC 0.4 μM | 952 | 45 | 3 | 0 | 0 | 51*** | 48*** | 157 | 304 | 20 | 19 | 60.8 | 1.80 | 1.76 |

Legend: see Table 1.

Table 5
Induction of micronuclei and cytotoxicity by 48-h treatment with permethrin in human whole-blood lymphocyte cultures

| Donor | Treatment | Distribution of BN cells according to No. MN | | | | | MN | BNMN | Distribution of cells according to No. nuclei | | | | % BN | NDI | CBPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | > 3 | | | 1 | 2 | 3 | 4 | | | |
| A | DMSO 1% | 992 | 8 | 0 | 0 | 0 | 8 | 8 | 82 | 298 | 70 | 50 | 59.6 | 2.18 | 2.08 |
| | 25 μg/ml | 988 | 10 | 2 | 0 | 0 | 14 | 12 | 69 | 339 | 44 | 48 | 67.8 | 2.14 | 2.05 |
| | 50 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 167 | 301 | 25 | 10 | 60.2 | 1.77 | 1.75 |
| | 100 μg/ml | 982 | 17 | 1 | 0 | 0 | 19* | 18* | 328 | 169 | 3 | 0 | 33.8 | 1.35 | 1.35 |
| | 200 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 951 | 45 | 4 | 0 | 0 | 53** | 49*** | 86 | 286 | 74 | 54 | 57.2 | 2.19 | 2.08 |
| B | DMSO 1% | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 98 | 329 | 37 | 36 | 65.8 | 2.02 | 1.95 |
| | 10 μg/ml | 995 | 5 | 0 | 0 | 0 | 5 | 5 | 55 | 358 | 40 | 47 | 71.6 | 2.16 | 2.06 |
| | 25 μg/ml | 988 | 12 | 0 | 0 | 0 | 12 | 12 | 73 | 382 | 30 | 15 | 76.4 | 1.97 | 1.94 |
| | 50 μg/ml | 989 | 11 | 0 | 0 | 0 | 11 | 11 | 219 | 279 | 1 | 1 | 55.8 | 1.57 | 1.57 |
| | 100 μg/ml | T | | | | | | | | | | | | | |
| | 200 μg/ml | T | | | | | | | | | | | | | |
| | MMC 0.4 μM | 924 | 73 | 3 | 0 | 0 | 79** | 76*** | 69 | 335 | 55 | 41 | 67.0 | 2.14 | 2.05 |
| C | DMSO 1% | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 65 | 332 | 54 | 49 | 66.4 | 2.17 | 2.08 |
| | 10 μg/ml | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 106 | 331 | 30 | 33 | 66.2 | 1.98 | 1.98 |
| | 25 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 56 | 336 | 45 | 63 | 67.2 | 2.23 | 2.10 |
| | 50 μg/ml | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 136 | 328 | 15 | 21 | 65.6 | 1.84 | 1.80 |
| | 100 μg/ml | 994 | 6 | 0 | 0 | 0 | 6 | 6 | 224 | 266 | 8 | 2 | 53.2 | 1.58 | 1.57 |
| | MMC 0.4 μM | 952 | 45 | 3 | 0 | 0 | 51** | 48*** | 157 | 304 | 20 | 19 | 60.8 | 1.80 | 1.76 |

Legend: see Table 1.

Table 6
Induction of micronuclei and cytotoxicity by fenvalerate and permethrin at two concentrations of cytochalasin-B in whole-blood cultures

| Treatment | 3 μg/ml Cytochalasin-B | | | | | 6 μg/ml Cytochalasin-B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MN | BNMN | % BN | NDI | CBPI | MN | BNMN | % BN | NDI | CBPI |
| DMSO 1% | 11 | 11 | 55.4 | 1.70 | 1.66 | 2 | 2 | 66.4 | 2.17 | 2.07 |
| Fev 10 μg/ml | 5 | 5 | 45.6 | 1.58 | 1.56 | 2 | 2 | 63.6 | 2.25 | 2.11 |
| Fev 25 μg/ml | 7 | 7 | 45.0 | 1.57 | 1.54 | 6 | 5 | 63.2 | 1.89 | 1.84 |
| Fev 50 μg/ml | 4 | 4 | 49.0 | 1.62 | 1.59 | 2 | 2 | 71.0 | 1.89 | 1.85 |
| Fev 100 μg/ml | 4 | 4 | 37.4 | 1.41 | 1.40 | 8 | 6 | 63.8 | 1.70 | 1.69 |
| Per 10 μg/ml | 10 | 9 | 52.2 | 1.63 | 1.61 | 2 | 2 | 66.2 | 1.98 | 1.91 |
| Per 25 μg/ml | 4 | 4 | 43.2 | 1.55 | 1.53 | 6 | 6 | 67.2 | 2.23 | 2.10 |
| Per 50 μg/ml | 5 | 5 | 46.4 | 1.59 | 1.56 | 2 | 2 | 65.6 | 1.84 | 1.80 |
| Per 100 μg/ml | 5 | 5 | 39.2 | 1.42 | 1.41 | 6 | 6 | 53.2 | 1.58 | 1.57 |
| MMC 0.4 μM | 64 *** | 59 *** | 58.6 | 1.86 | 1.81 | 51 *** | 48 *** | 60.8 | 1.80 | 1.76 |

Legend: see Table 1.

some cases positive, with only 6 out of 15 being statistically significant. On the other hand, both NDI and CBPI were reduced in a linear manner with increasing concentrations of the five pesticides. The ANOVA showed no significant difference between correlation coefficients calculated for NDI and CBPI but both indexes were significantly different from the correlation coefficients calculated for the percentage of BN cells ($P < 0.01$).

## 4. Discussion

Initially considered to be non hazardous to mammals and human health, pyrethroid insecticides were regarded as non-carcinogenic to humans. Further studies of synthetic pyrethroids have, however, revealed a possible genotoxic activity in a wide range of organisms. Most of the pyrethroids were more recently classified in group 3 by IARC (1991), since the studies performed could not be interpreted as showing either the presence or absence of a carcinogenic effect, because of major qualitative or quantitative limitations. Therefore, and bearing in mind the widespread environmental occurrence of pyrethroid insecticides, the study of their possible genotoxicity has been of immediate concern during the last two decades.

The data reported on the genotoxicity of synthetic pyrethroids are rather controversial, depending on the genetic system or the assay used. Mutagenicity studies in bacteria have been performed for Cyp, Del, Fev and Per, and they consistently gave negative results (Brooks et al., 1976; Suzuki et al., 1977; Kavlock et al., 1979; Pluijmen et al., 1984; Herrera and Laborda, 1988). In *Drosophila melanogaster*, the sex-linked recessive lethal test was negative for Fep, Fev, and Per, but positive for Cyp, while none of these four pyrethroids induced nondisjunction (Woodruff et al., 1983; Batiste-Alentorn et al., 1986, 1987; Gupta et al., 1990). Only Fep has been tested in the somatic mutation and recombination test (SMART) of *D. melanogaster*, with a negative outcome (Pardo et al., 1993). In plant meristems, Cyp induced mitotic abnormalities in *Vicia faba* (Amer and Aboul-ela, 1985) and Del, chromosome aberrations (CA) in *Allium cepa* (Chauhan et al., 1986).

In Chinese hamster cells treated in vitro, Fev inhibited intercellular communication in fibroblasts, whereas Del did not (Floodstrom et al., 1988); Del, Fev and Per were negative in the hprt mutation assay in V79 cells, but induced CA in

Table 7
Induction of micronuclei and cytotoxicity by 48-h treatment with the five pyrethroids in isolated human lymphocyte cultures

| Donor | Treatment | Distribution of BN cells according to No. MN | | | | | MN | BNMN | Distribution of cells according to No. nuclei | | | | % BN | NDI | CBPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | > 3 | | | 1 | 2 | 3 | 4 | | | |
| A | DMSO 1% | 995 | 4 | 1 | 0 | 0 | 6 | 5 | 202 | 293 | 1 | 4 | 58.6 | 1.61 | 1.60 |
| | Cyp 100 μg/ml | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 371 | 129 | 0 | 0 | 25.8 | 1.26 | 1.26 |
| | Cyp 200 μg/ml | 998 | 2 | 0 | 0 | 0 | 2 | 2 | 402 | 98 | 0 | 0 | 19.6 | 1.20 | 1.20 |
| | Del 100 μg/ml | 997 | 3 | 0 | 0 | 0 | 3 | 3 | 291 | 209 | 0 | 0 | 41.8 | 1.42 | 1.42 |
| | Del 200 μg/ml | 498 | 2 | 0 | 0 | 0 | 2 | 2 | 341 | 159 | 0 | 0 | 31.8 | 1.34 | 1.34 |
| | Fep 25 μg/ml | 995 | 4 | 0 | 0 | 1 | 8 | 5 | 287 | 213 | 0 | 0 | 42.6 | 1.43 | 1.43 |
| | Fep 50 μg/ml | NB | | | | | | | 442 | 58 | 0 | 0 | 11.6 | 1.14 | 1.14 |
| | Fev 50 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 379 | 121 | 0 | 0 | 24.2 | 1.24 | 1.24 |
| | Fev 100 μg/ml | NB | | | | | | | 369 | 128 | 3 | 0 | 25.6 | 1.27 | 1.27 |
| | Per 50 μg/ml | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 298 | 202 | 0 | 0 | 40.4 | 1.40 | 1.40 |
| | Per 100 μg/ml | 496 | 3 | 1 | 0 | 0 | 5 | 4 | 450 | 50 | 0 | 0 | 10.0 | 1.10 | 1.10 |
| | MMC 0.6 μM | 968 | 29 | 2 | 1 | 0 | 36*** | 32*** | 161 | 326 | 9 | 4 | 65.2 | 1.71 | 1.70 |
| D | DMSO 1% | 996 | 4 | 0 | 0 | 0 | 4 | 4 | 132 | 336 | 8 | 24 | 67.2 | 1.85 | 1.80 |
| | Cyp 100 μg/ml | 993 | 7 | 0 | 0 | 0 | 7 | 7 | 470 | 30 | 0 | 0 | 6.0 | 1.06 | 1.06 |
| | Cyp 200 μg/ml | NB | | | | | | | 467 | 32 | 0 | 1 | 6.4 | 1.07 | 1.07 |
| | Del 100 μg/ml | 991 | 6 | 2 | 1 | 0 | 13* | 9 | 317 | 183 | 0 | 0 | 36.6 | 1.37 | 1.37 |
| | Del 200 μg/ml | 988 | 11 | 1 | 0 | 0 | 13* | 12* | 359 | 141 | 0 | 0 | 28.2 | 1.28 | 1.28 |
| | Fep 25 μg/ml | 991 | 9 | 0 | 0 | 0 | 9 | 9 | 371 | 129 | 0 | 0 | 25.8 | 1.26 | 1.26 |
| | Fep 50 μg/ml | NB | | | | | | | 476 | 24 | 0 | 0 | 4.8 | 1.05 | 1.05 |
| | Fev 50 μg/ml | 990 | 10 | 0 | 0 | 0 | 10 | 10 | 407 | 92 | 1 | 0 | 18.4 | 1.19 | 1.19 |
| | Fev 100 μg/ml | NB | | | | | | | 456 | 44 | 0 | 0 | 8.8 | 1.09 | 1.09 |
| | Per 50 μg/ml | 994 | 5 | 1 | 0 | 0 | 7 | 6 | 361 | 139 | 0 | 0 | 27.8 | 1.28 | 1.28 |
| | Per 100 μg/ml | 992 | 8 | 0 | 0 | 0 | 8 | 8 | 457 | 42 | 1 | 0 | 8.4 | 1.09 | 1.09 |
| | MMC 0.6 μM | 955 | 43 | 2 | 0 | 0 | 47*** | 45*** | 141 | 326 | 12 | 20 | 65.2 | 1.82 | 1.78 |

Legend: see Table 1 (NB, not enough binucleated cells).

Table 8
Correlation coefficients and probabilities of dose-cytotoxic effect relationships with different indexes obtained in whole blood cultures treated with five pyrethroid insecticides

| Pyrethroid | Donor | Higher dose tested | | % BN | | NDI | | CBPI | |
|---|---|---|---|---|---|---|---|---|---|
| | | μg/ml | viability | r | P | r | P | r | P |
| Cyp | A | 200 | V | −0.91 | 0.0110 | −0.96 | 0.0026 | −0.97 | 0.0013 |
| | B | 200 | V | −0.88 | 0.0195 | −0.95 | 0.0033 | −0.96 | 0.0019 |
| | C | 200 | T | −0.82 | 0.0924 | −0.99 | 0.0008 | −0.99 | 0.0007 |
| Del | A | 200 | V | −0.88 | 0.0197 | −0.89 | 0.0177 | −0.90 | 0.0159 |
| | B | 200 | V | −0.85 | 0.0314 | −0.95 | 0.0038 | −0.96 | 0.0022 |
| | C | 200 | T | −0.95 | 0.0043 | −0.96 | 0.0028 | −0.96 | 0.0023 |
| Fep | A | 100 | T | +0.45 | 0.4456 | −0.87 | 0.0537 | −0.87 | 0.0562 |
| | B | 100 | T | −0.02 | 0.9689 | −0.96 | 0.0086 | −0.95 | 0.0136 |
| | C | 100 | T | −0.63 | 0.2562 | −0.97 | 0.0025 | −0.98 | 0.0030 |
| Fev | A | 100 | V | −0.77 | 0.1244 | −0.92 | 0.0097 | −0.97 | 0.0057 |
| | B | 100 | T | −0.67 | 0.3316 | −0.88 | 0.1236 | −0.87 | 0.1269 |
| | C | 100 | V | +0.02 | 0.9756 | −0.89 | 0.0435 | −0.90 | 0.0363 |
| Per | A | 200 | T | −0.84 | 0.1578 | −0.98 | 0.0220 | −0.98 | 0.0211 |
| | B | 200 | T | −0.54 | 0.4588 | −0.89 | 0.1098 | −0.88 | 0.1249 |
| | C | 100 | V | −0.90 | 0.0394 | −0.88 | 0.0474 | −0.94 | 0.0182 |

V, viable culture; T, not viable culture (toxic); % BN, percentage of BN cells; NDI, nuclear division index; CBPI, cytokinesis blocked proliferation index; r, correlation coeficient; P, probability (t-test).

Chinese hamster ovary (CHO) cells; Del and Fev also produced sister-chromatid exchanges (SCE) in CHO cells (Pluijmen et al., 1984; Caballo, 1991; Caballo et al., 1992; Barrueco et al., 1994).

In rodents in vivo, Cyp, Del, and Fev were reported to induce CA in mouse bone marrow cells (Bhunya and Pati, 1988, 1990; Pati and Bhunya, 1989), while one study on Del yielded negative results in the same cell system (Polaková and Vargová, 1983). Fev caused CA also in rat bone marrow (Chatterjee et al., 1982) and MN in polychromatic erythrocytes of mice (Pati and Bhunya, 4989). Two studies on Cyp found induction of MN in mice (Amer and Aboul-ela, 1985; Bhunya and Pati, 1988), although a negative report has also been published (Hoellinger et al., 1987). Both positive and negative findings were likewise obtained in the mouse MN test for Del (Hoellinger et al., 1987; Bhunya and Pati, 1990). Per weakly induced MN in mice (Hoellinger et al., 1987). From the available published data, it appears that the genotoxicity of these pyrethroids seems not to be modulated by animal metabolic activation.

With reference to carcinogenesis studies, Del and Fev were not carcinogenic in mice and rats (Parker et al., 1983, 1984; Cabral et al., 1990), whereas Fev showed a weak positivity in mice hepatocytes (Cabral and Galendo, 1990).

The findings of the present study using the CBMN assay in human lymphocytes appear to suggest that pyrethroid insecticides have a weak (cypermethrin, deltamethrin and fenpropathrin) or nule (fenvalerate and permethrin) genotoxic potential in vitro. The slight MN induction observed for Del in WB and IL cultures of one donor appears to support the weak in vitro genotoxicity of Del, earlier suggested by modest SCE induction in human lymphocytes (Dolara et al., 1992). Fep and Cyp seemed to resemble Del in their ability to weakly induce MN. Fep has not previously tested in cytogenetic assays in vitro, while Cyp was earlier found to be negative in the SCE and CA test in human lymphocytes (Puig et

al., 1989). The present findings disagree with previous results also for Fev which was not able to significantly increase MN, despite the former positive results obtained using 24 h shorter treatment time and 3 μg/ml Cyt-B (Surrallés et al., 1990) as well as the induction of SCE, CA and C-mitotic figures found in cultured human lymphocytes (Puig et al., 1989; Carbonell et al., 1989). The relatively clear MN induction described by Barrueco et al., 1992 for Per using 3 μg/ml Cyt-B could not fully be confirmed in the present study either, as a slight increase in MN was obtained in a single concentration of Per in WB cultures of one donor only, using 6 μg/ml Cyt-B. Per was earlier also shown to produce CA and, marginally, SCE in human lymphocytes (Barrueco et al., 1992, 1994). However, taking into account that weak genotoxicity is difficult to demonstrate, especially if it occurs within a narrow and toxic concentration range, the results obtained with Cyp, Del and Fep are not necessarily in desagreement with those already published in the literature.

Several variables can affect the reproducibility and the sensitivity of the in vitro CBMN assay, including methodology, interindividual variability and the source, chemical composition and purity of the compound to be tested. The methodological aspects mainly include Cyt-B concentration, the presence or absence of erythrocytes in the lymphocyte culture and treatment schedule. In recent collaborative studies (Surrallés et al., 1992, 1994) we have demonstrated that the CBMN assay is reproducible and reliable when model genotoxins are tested using 6 μg/ml Cyt-B and the methodology applied in the present study. We showed that the use of an inefficient Cyt-B concentration (3 μg/ml Cyt-B) could lead to overestimation of genetic damage possibly due to the inclusion in the scoring of incompletely blocked binucleated cells that have divided twice in vitro. However, bearing in mind that both Cyt-B concentrations (3 and 6 μg/ml) yielded similar results in the present study, the possible overestimation of MN by Surrallés et al. (1990) and Barrueco et al. (1992, 1994) does not seem probable.

On the other hand, the possible effect of erythrocytes can be also excluded since both whole blood and isolated lymphocyte cultures have been performed. Our results suggest that the presumption of Van Hummelen and Kirsch-Volders (1992) and Elhajouji et al. (1993) on the superior sensitivity of IL cultures cannot be generalized. With reference to treatment schedule, Fev was added 24 h earlier in the present study than in our previous positive experiments (Surrallés et al., 1990). Barrueco et al. (1992, 1994) added Per at 48 h in their CA studies and 24 h (like in the present study) for MN experiments. Taking into account that Fev and Per mainly induced chromosome-type aberrations and were considered to act in a time-dependent but S-phase independent way (Puig et al., 1989; Caballo, 1992; Barrueco et al., 1992, 1994), the treatment schedule used in the present study should be as sensitive in detecting a possible genotoxicity of such chemicals as those described by Surrallés et al. (1990) and Barrueco et al. (1992, 1994).

Besides interindividual variability, which could not be controlled with the number of donors used in the present study, other important variables that could explain the differences found between the present and earlier studies could be the source, isomer composition, or purity of the insecticides used. It is important to point out that the purity of Fev but not Per was higher in the present study than in the previous experiments. The pyrethroids used in the present study were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) whereas in the previous studies, Per was obtained from Chem. Service, (West Chester, PA) and Fev from S.P.E. Shell (Madrid, Spain).

Cell cycle progression can be delayed as a consequence of cellular toxicity due to in vitro treatments. In order to control lymphocyte proliferation, the percentage of BN cells (Fenech and Morley, 1985) and, more recently, nuclear division index (NDI, Eastmond and Tucker, 1989) have been used. The need of including not only binucleated cells but also multinucleated cells was also pointed out by Van Hummelen and Kirsch-Volders (1992) who used the percentage of multinucleated cells as an indication of toxicity or cell cycle delay. The NDI reflects the average

number of nuclei per cell but is an indirect indication of cell cycle delay since it considers trinucleated cells separately from tetranucleated cells. We propose the cytokinesis block proliferation index (CBPI) in which both trinucleated and tetranucleated cells are supposed to be in their third cell cycle. This is acceptable if it is assumed that trinucleated and tetranucleated cells derive from binucleated cells undergoing one further mitosis after a multipolar (tri- and tetrapolar respectively) anaphase, as Schultz and Önfelt (1994) have recently shown by video time-lapse recording. Thus, CBPI indicates the average number of cell cycles the sampled cells have gone through, and can be considered as cell kinetic index or average lymphocytic division index with some similarities to the proliferation rate index (PRI) used for metaphase in cultures treated with BrdU.

Although we found no quantitative differences in measuring cell cycle delay between NDI and CBPI, CBPI can be considered biologically more relevant in measuring cell cycle delay. Our results show that CBPI and NDI are more effective in measuring cell cycle progression than the percentage of BN cells which is not always reduced by toxic treatments, because a decrease in the number of multinucleated cells may not affect or may even increase the percentage of BN cells.

According to our data, Fep appeared to be the most toxic pyrethroid tested, as 100 $\mu$g/ml resulted in a 100% toxicity in all donors studied. On the other hand, Cyp and Del caused a similar reduction of cell proliferation, and both pesticides were less toxic than Fev, which is opposite to what was found in our previous study (Puig et al., 1989). Per appeared to be as toxic as Fev. A relationship between the presence of the cyano group and toxicity to mammals has been proposed, but the only pyrethroid tested without a cyano group (Per) did not produce higher toxicity in cultured human lymphocytes when compared with the others. Therefore, the toxicity of these pyrethroids in vitro seems to be more related to the presence of methyl groups in the cyclopropane ring (see Fig. 1).

*In conclusion*, the five pyrethroids tested, except Fev, only weakly increased the frequency of MN. Nevertheless, as weak genotoxicity is difficult to demonstrate, more studies using an extended number of donors and different culture schedules as well as different source, isomer composition and purity of the pesticides, are required. A clear linear dose-dependent cytotoxic effect was found with all of the pesticides tested; this linearity could be accurately measured by means of the newly introduced cytokinesis block proliferation index.

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