Available online at www.sciencedirect.com
SCIENCE DIRECT®
Ecotoxicology and Environmental Safety 61 (2005) 128–133
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow by gavage administration

Ayla Çelik[a,*], Birgül Mazmanci[a], Yusuf Çamlica[a], Ülkü Çömelekoğlu[b], Ali Aşkin[a]

[a]*Department of Biology, Faculty of Science and Letters, Mersin University, 33342 Mersin, Turkey*
[b]*Department of Biophysics, Faculty of Medicine, Mersin University, Mersin, Turkey*

Received 17 November 2003; received in revised form 17 June 2004; accepted 2 July 2004
Available online 22 September 2004

Abstract

In this study, a synthetic pyrethroid insecticide, lambda-cyhalothrin (LCT), was administered to adult female albino rats (Wistar rats) by gavage dose of 6.12, 3.06, 0.8 mg/kg b.w. repeated for 13 days at 48 h intervals. The cytotoxic and genotoxic effects of LCT were investigated in bone marrow cells, using the structural chromosomal aberration (SCA) and micronucleus (MN) test systems. Mitomycin C (MMC) was also used as positive control (2 mg/kg b.w.). All the doses of LCT increased the number of SCAs and the frequency of micronucleated erythrocytes, with respect to the control group. Only the highest dose of LCT significantly increased the MN frequency compared with control ($P<0.01$). It was also observed that LCT caused a significant decrease in the number of polychromatic erythrocytes compared with controls ($p<0.001$). These observations indicate the in vivo suspectibility of mammals to the genetic toxicity and cytotoxicity potential of LCT.

Keywords: Lambda-cyhalothrin; Structural chromosome aberrations; Pyrethroid insecticide; Micronucleus; Polychromatic erythrocytes; Wistar rat

1. Introduction

According to 1997 market estimates, approximately 5684 million pounds of active pesticide ingredients are applied annually throughout the world (USEPA, 2001). The World Health Organization (WHO) (WHO, 1992) reported that roughly three million pesticide poisonings occur annually and result in 220,000 deaths worldwide. Many of these chemicals are mutagenic (Galloway et al., 1987; Garaj-Vrhovac and Zeljezic, 2000), linked to the development of cancers (Leiss and Savitz, 1995) or may lead to developmental deficits (Arbuckel and Server, 1998). Since several studies have shown that exposure to pesticides may induce genotoxic effects in occupationally exposed human populations (Börzsönyi et al., 1984; Dulot et al., 1985; Nehéz et al., 1988), the evaluation of the genotoxicity of pesticides in use is of immediate concern.

The synthetic pyrethroid insecticides are analogs of naturally occuring pyrethrins and have been developed with the aim to improve the specificity and activity of natural insecticide pyrethrum (Sogorb and Vilanova, 2002). Synthetic pyrethroids are a group of potent insecticides that are environmentally compatible by virtue of their moderate persistence, low volality, and poor aqueous mobility in soil. The favorable properties of this class of insecticides have promoted widespread application in virtually all sectors of food protection and pest control. With regard to effectiveness and toxicity, synthetic pyrethroids appear to be the first-choice insecticides for this type of use pattern because pyrethroids are much more effective against a wide spectrum of pests than the other insecticides particularly, organochlorine, organophosphate, and carbamate insecticides (Pauluhn, 1999). With the use of pyrethroids steadily rising, there may be an urgent need to identify

*Corresponding author. Fax: +90-324-3610047.
E-mail address: a.celik@mersin.edu.tr (A. Çelik).

0147-6513/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2004.07.009

the adverse effects that may be associated with their use. Genotoxic potentials of some pyrethroid insecticides were shown in previous studies (Carbonell et al., 1989; Puig et al., 1989; Surralles et al., 1990). The carcinogenic potential of pyrethroids has been discussed in a review by Litchfield (1985). Lambda-chyalothrin (LCT) is a newer pyrethroid insecticide used all over the world. The cytogenetic effects of LCT were investigated in human and different animal species using different endpoints such as micronucleus (MN), chromosomal aberrations, sister chromatid exchange (Agarwal et al., 1994; Campana et al., 1999; Fahmy and Abdalla, 2001).

The data reported on the genotoxicity of synthetic pyrethroids, including LCT, are rather controversial. The aim of the present work was, therefore, to authenticate the in vivo potential genotoxic effects of LCT in bone marrow cells of laboratory female rats using chromosomal aberrations and MN assays as genetic endpoints. The ratio of PCEs to normochromatic erythrocytes (NCEs) was also calculated to evaluate cytotoxic effects of LCT in bone marrow.

2. Materials and methods

2.1. Chemical (substance)

LCT is a synthetic pyrethroid insecticide with the trade name "Karate". Cas, chemical name (R + S) α-cyano-3-(phenoxyphenyl)methyl-(1S + 1R)-*cis*-3(2-2chloro-3,3,3-trifluoroprop-1-enyl)-2-2-dimethylcyclopropane carboxylate, CASRN 91465-08-06, was from Zeneca Agrochemicals, England (2.5%).

2.2. Animal and treatment

Healthy adult female Swiss albino rats (Wistar rat) (6–8 weeks of age and average body weight (b.w.) 180–200 g) were used in this study. Rats were obtained from the Experimental Animal Center, University of Gaziantep, Turkey. The study was approved by the research and ethical committee at the University of Mersin. The rats were randomly selected and housed in polycarbonate boxes (three or four rats per box) with steel wire tops and rice husk bedding. They were maintained in a controlled atmosphere of 12 h dark/light cycle, 22 ± 2 °C temperature, and 50–70% humidity, with free access to pelleted feed and fresh tap water. The animals were supplied with dry food pellets commercially available. For each dose group, the animals were allowed to acclimate for 5 days before treatment. LCT was suitably diluted with isotonic saline. The animals received by gavage an aqueous solution of LCT at three different doses (0.8 mg/kg b.w., 3.06 mg/kg b.w., 6.12 mg/kg b.w.) per 48 h for 13 days. Three animals were used in each group/assay. The animals received a total of seven injection of LCT during the present study.

2.3. Doses

The animals tolerated the highest dose with minimal toxic symptoms. The toxic symptoms were mostly neurological. However, the animals recovered within 2 h of the treatment. It has been determined that the LCT LD_{50} dose was 612 mg/kg for mammals. Therefore, the highest dose was determined as 6.12 mg/kg b.w., 1% of the LD_{50}. The lowest dose was determined as 0.8 mg/kg b.w. because this dose did not neurologically affect the rats. Groups of three rats each were treated with three doses of LCT, 6.12 mg/kg b.w., 3.06 mg/kg b.w., and 0.8 mg/kg b.w. per 48 h for 13 days, to investigate genotoxic and cytotoxic effects on rat bone marrow cells. Therefore, the cumulative doses of LCT given to rats were 42.84 mg/kg b.w., 21.42 mg/kg b.w., and 5.6 mg/kg b.w.

Mitomycin C (MMC) (2 mg/kg) was used as a positive control. Positive control and untreated control rats were identically treated with equal volumes of normal saline only via gavage (per os). It is acceptable that a positive control may be administered by a different route or the same route as the test agent sampled at only a single time (Hayashi et al., 1994). MMC was given as a single dose. Same-dose regimes were used in both chromosomal aberration and MN assays.

2.4. Chromosome aberrations assay

The rats were sacrificed 24 h after last dose administration for a chromosome aberration assay. Cytogenetic analysis was performed on bone marrow cells according to the recommendations of Adler (Adler, 1984), with slight modifications. Experimental animals were injected (i.p.) with colchicine (4 mg/kg) 1.5 h prior to sacrifice. Both femurs were dissected out and cleaned of any adhering muscle. Bone marrow cells were collected from both the femurs by flushing in KCl (0.075 M, at 37 °C) incubated at 37 °C for 25 min. Material was centrifuged at 2000 rpm for 10 min, fixed in acetomethanol (acetic acid:methanol, 1:3, v/v). Centrifugation and fixation (in cold) were repeated five times at an interval of 20 min. The material resuspended in a small volume of the fixative, was dropped onto chilled slides, flame-dried, and stained on the following day in 5% buffered Giemsa (pH 6.8). At least 75 good metaphases containing 42 ± 2 chromosomes were examined per animal to score different types of aberrations.

2.5. Micronucleus test

Rats were killed by cervical dislocation 30 h after treatment. The frequency of micronucleated erythrocytes

in femoral bone marrow preparation was evaluated according to the procedure of Schmid (Schmid, 1976) with slight modifications by Agarwal and Chauhan (Agarwal and Chauhan, 1993). The bone marrow was flushed out from both femurs using 1 mL of fetal calf serum (FCS) and centrifuged at 2000 rpm for 10 min. Supernatant was discarded. Evenly spread bone marrow smear was stained using the May–Grünwald and Giemsa protocol. Slides were scored at a magnification of 1000 × using a light microscope.

2.6. Scoring

For MN analysis, 2000 polychromatic erythrocytes (PCEs) per animal were scored to calculate the MN frequencies, and 200 erythrocytes (immature and mature erythrocytes) were examined to determine the ratio of PCEs to normochromatic erythrocytes (NCEs).

2.7. Statistical analysis

Data were compared by one-way variance analysis. Statistical analysis was performed using SPSS for Windows 9.05. Multiple comparisons were performed by the least significant difference (LSD) test. $P<0.05$ was considered as the level of significance.

3. Results

The metaphase analysis of the bone marrow cells revealed various types of chromosomal aberrations, which consisted of chromatid and isochromatid types of break, double minute (including isochromatid breaks), exchange, dicentric chromosomes, and fragments (Table 1). Numerical aberrations were not scored in this study. The results of SCAs, the ratio of PCE to NCE, and the MN frequency in bone marrow cells after gavage treatment with LCT are represented in Table 1 and Table 2, respectively. The frequency of SCAs also increased with increasing concentrations of LCT, but statistically significant differences from the controls were not observed only at the dose of 0.8 mg/kg ($P>0.05$). The mean of the induced SCAs reached 5.7 ± 1.17, 7.5 ± 1.59, and 10.1 ± 0.43 at the doses of LCT 0.8, 3.06, and 6.12 mg/kg, respectively. There is no significant difference between LCT doses. It was observed that LCT induced a dose-related increase in MN frequency. There was a linear dose–response relationship between the frequency of micronucleated cells and the concentration of the pyrethroid insecticide, LCT. Only the highest dose (6.12 mg/kg) of LCT significantly increased the frequency of the MN when compared to the control ($P<0.01$). No significant difference was observed between the two doses (0.8 and 3.06 mg/kg) and the control ($P>0.05$). Comparing doses of LCT each other, it is determined that through a significant difference between the highest dose (6.12 mg/kg) and the other two doses, 3.06 and 0.8 mg/kg ($P<0.05$, $P<0.01$, respectively). The ratio of PCE:NCE in whole bone marrow samples are depicted in Table 2. All three doses of LCT used in this study decreased the ratio of PCE:NCE when compared to control ($P<0.001$). The present study implies that gavage administration of LCT has not only the potential in inhibiting the erythropoiesis but also mutagenic effect.

4. Discussion

Experience and fundamental similarities in cell structure and biochemistry between animals and humans provide a general valid basis for prediction of likely effects of chemicals on the human population (Meyer, 1993). Most toxicologic research on pyrethrins and pyrethroids has utilized invertebrates or laboratory rodents, and limited data are available for other species.

The genetic toxicity/mutagenicity studies concerning the synthetic pyrethroids have produced controversial results depending on the compound or the assay used.

Table 1
Frequency of chromosome aberrations in the bone marrow cells of rat exposed to LCT by gavage

Dose groups	Fixation time	Total number of examined metaphases/*n*	Chromatid break	Chromosome break	Fragment	Disc	TSCA	Mean (% SCA) ± SE (excluding gaps)
Control (isotonic saline)	24	225/3	3	3	1	—	7	3.06 ± 0.46
LCT (mg kg^{-1}b.w.t)								
0.8	24	225/3	4	3	4	2	13	$5.76\pm1.17^{**}$
3.06	24	225/3	4	5	6	2	17	$7.50\pm1.59^{***}$
6.12	24	225/3	6	10	4	3	23	$10.1\pm0.43^{****}$
MMC (2 mg kg^{-1})	24	225/3	15	16	13	—	44	$19.5\pm2.35^{*}$

Note: *n*:number of animals; TSCA: total structural aberrations; dic: Dicentric chromosome, LCT: lambda-cyhalothrin, MMC: Mitomycin C.
$^{*}P<0.001$; $^{**}P>0.05$; $^{***}P\leqslant0.05$; $^{****}P<0.01$ compared to control group.

Table 2
Micronucleus induction and number of PCEs in bone marrow of rats exposed to LCT, by gavage

Treatment group	Number of rats	MN/2000 PCEs	Fixation time	PCEs/200 erythrocyte
Isotonic	1	2		108
Saline	2	2		100
(vehicle control)	3	3		98
	Mean ± SE	2.33 ± 0.33	30 h	102.0 ± 3.05
LCT	1	3		77
(mg/kg, i.p.)	2	4		82
0.8 mg kg^{-1}b.w.t	3	3		69
	Mean ± SE	3.33 ± 0.33*	30 h	76.0 ± 3.78***
3.06 mg kg^{-1} b.w.t	1	5		54
	2	3		66
	3	4		65
	Mean ± SE	4.0 ± 0.57*	30 h	61.6 ± 3.84***
6.12 mg kg^{-1}b.w.t	1	6		51
	2	5		56
	3	7		48
	Mean ± SE	6.00 ± 0.57**	30 h	51.6 ± 2.33***
MMC (2 mg/kg)	1	22		47
(positive control)	2	21		45
	3	24		48
	Mean ± SE	22.3 ± 0.88***	30 h	46.6 ± 0.88***

Note: Abbreviations: LCT, Lambda cyhalothrin; PCEs, polychromatic erythrocytes; MN, micronucleus; MMC, mitomycin C.
$^{*}P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to the control group.

In some studies, pyrethroids were reported to be mutagenic in rodent bone marrow (Agarwal et al., 1994; Amer and Aboul-Ela, 1985; Amer et al., 1993; Charterjee et al., 1982; Pati and Bhunya, 1989), human peripheral lymphocyte cultures (Puig et al., 1989; Surralles et al., 1990), and in aquatic organisms (Campana et al., 1999).

The induction of SCAs and micronucleated erythrocytes following exposure to LCT indicates a potential for clastogenicity. The frequency of SCAs (excluding gaps) following by gavage exposure to LCT (3.06 mg/kg b.w., 6.12 mg/kg b.w.) remained significantly higher than in controls, except at the lowest dose (0.8 mg/kg b.w.).

However, under in vivo conditions, the genotoxicity and in particular the clastogenic potential of an agent is evaluated using the chromosome aberrations assay (Preston et al., 1987). This is also in agreement with the previous reports on the clastogenic potential of synthetic pyrethroids as manifested in rodent bone marrow (Barrueco et al., 1992, 1994; Bhunya and Pati, 1988, 1990; Fahmy and Abdalla, 2001; Oraby 1997; Surralles et al., 1990, 1995; Tyrkiel et al., 2001) and human peripheral lymphocyte cultures (Barrueco et al., 1992, 1994; Surralles et al., 1990, 1995), and aquatic organisms (Campana et al., 1999). It was declined that pyrethroid insecticides apart from LCT, for example, permethrin, induced the frequency of sister chromatid exchange and micronuclei in the study performed by several researchers (Herrera et al., 1992).

When a bone marrow erythroblast develops into a PCE, the main nucleus is extruded; any MN that has been formed may remain behind in the otherwise anucleated cytoplasm. Visualisation of MN is fasiliated in these cells because they lack a main nucleus. An increase in the frequency of micronucleated PCEs in treated animals is an indication of induced chromosome damage. Genotoxic activity is normally indicated by a statistically significant dose-related increase in the incidence of micronucleated immature erythrocytes and/or chromosome aberrations for the treatment groups compared with the concurrent control group.

The observed inhibition of cell proliferation in the bone marrow illustrates the cytotoxicity of LCT. Similar results were reported in the previous studies performed on different pyrethroid insecticide (Agarwal et al., 1994; Dianovsky and Sivikova, 1995). Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant dose related decrease in the proportion of immature erythrocytes (PCEs); a very large decrease in the proportion would be indicative of a cytostatic or cytotoxic effect.

However, in the present study only a marginal decrease in the number of PCE (or depression in bone marrow) was found in the groups of animals receiving the three doses of LCT that were statistically significant (Table 2). This indicates that LCT is a very potent inhibitor of mitosis, as in studies reported earlier (Pati and Bhunya, 1989).

In the present study, the observed inhibition of cell proliferation in rat bone marrow illustrates the cytotoxicity of LCT. These data indicate the cytotoxic potential of LCT, pyrethroid insecticide. This is also in agreement with the previous reports on the cytotoxic potential of pyrethroid insecticides. Similar results were reported in studies performed with different pyrethroid insecticides (Dianovsky and Sivikova, 1995; Nakano et al., 1996; Puig et al., 1989; Tyrkiel et al., 2001). Çelik et al. (2003) reported that LCT has cytotoxic effects on wistar rat bone marrow by intraperitoneal treatment. In our previous study, it was determined that LCT showed the effect of cytotoxic and genotoxic on rat bone marrow at i.p. administration at same doses. These results imply that LCT shows the same effects on rat bone marrow in both gavage administration and intraperitoneal administration in same doses at under same experiment conditions.

Our results complement previous data on the genotoxicity or cytotoxicity of LCT and other pyrethroid insecticide in rat bone marrow.

References

Adler, I.D., 1984. Cytogenetic tests in mammals. In: Venitt, S., Parry, J.M. (Eds.), Mutagenicity Testing, A Practical Approach. IRL Press, Oxford, pp. 275–306.

Agarwal, D.K., Chauhan, L.K.S., 1993. An improved chemical substitute for fetal calf serum for the micronucleus test. Biotechnol. Histochem. 68, 187–188.

Agarwal, D.K., Chauhan, L.K.S., Gupta, S.K., Sundararaman, V., 1994. Cytogenetic effects of deltamethrin on rat bone marrow. Mutat. Res. 311, 133–138.

Amer, S.M., Aboul-Ela, E.I., 1985. Cytogenetic effects of pesticide: induction of micronuclei in mouse bone marrow by the insecticides cypermethrin and rotenone. Mutat. Res. 55, 135–142.

Amer, S.M., Ibrahim, A.S., El-Sherbeny, K.M., 1993. Induction of chromosomal aberrations and sister chromatid exchange in vivo and in vitro by the insecticide cypermethrin. J. Appl. Toxicol. 13, 341–345.

Arbuckel, T.E., Server, L.E., 1998. Pesticides exposures and fetal death: a review of the epidemiologic literature. Crit. Rev. Toxicol. 28, 229–270.

Barrueco, C., Herrera, A., Cabolla, C., De La Penda, E., 1992. Cytogenetic effects of permethrin in cultured human lymphocytes. Mutagenesis 7 (6), 433–437.

Barrueco, C., Herrera, A., Cabolla, C., De La Penda, E., 1994. Induction of structural chromosomal aberrations in human lymphocyte cultures and CHO cells by permethrin. Teratogene. Carcinogen. Mutagen. 14 (1), 31–38.

Bhunya, S.P., Pati, P.C., 1988. Genotoxic effects of a synthetic pyrethroid insecticide, cypermethrin, in mice in vivo. Toxicol. Lett. 41 (3), 223–230.

Bhunya, S.P., Pati, P.C., 1990. Effect of deltamethrin, a synthetic pyrethroid, on the induction of chromosome aberrations, micronuclei ab sperm abnormalities in mice. Mutagenesis 5 (3), 229–232.

Börzsönyi, M., Török, G., Pintér, A., Surján, A., 1984. Agriculturally-related carcinogenic risk. In: Models, Mechanism and Etiology of Tumor Promotion. IARC Sci. Publ. 56, pp. 465–486.

Campana, M.A., Panzeri, A.M., Moreno, V.J., Dulout, F.N., 1999. Genotoxic evaluation of the prethroid lambda-cyhalothrin using the micronucleus test in erytrocytes of fish *Cheirodon interruptus*. Mutat. Res. 438, 155–161.

Carbonell, E., Puig, M., Xamena, N., Creus, A., Marcos, R., 1989. Mitotic arrest induced by fenvalerate in human lymphocyte cultures. Toxicol. Lett. 48, 45–48.

Çelik, A., Mazmancı, B., Çamlıca, Y., Aşkın, A., Çömelekoğlu, Ü., 2003. Cytogentic effects of lambda-cyhalothrin on wistar rat bone marrow. Mutat. Res. 539, 91–97.

Charterjee, X.K., Tarukdan, G., Sharran, A., 1982. Effects of synthetic prethroids in mammalian chromosomes. I. Sumicidin. Mutat. Res. 105, 102–106.

Dianovsky, J., Sivikova, K., 1995. In vivo and in vitro cytogenetic effect of supermethrin. Biomed. Environ. Sci. 8, 359–366.

Dulot, F.N., Pastori, M.C., Olivero, O.A., González Cid, M., Loria, D., Matos, E., Sobel, N., DE Bujan, E.C., Albiano, N., 1985. Sister chromatid exchanges and chromosomal aberrations in a population exposed to pesticides. Mutat. Res. 143, 237–244.

Fahmy, A.M., Abdalla, E.F., 2001. Cytogenetic effects by the natural prethrins and the synthetic lambda-cyhalothrin in mice in vivo. Cytologia 66, 139–149.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, Brown, S.B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., Zeiger, E., 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells, evaluation of 108 chemicals. Environ. Mol. Mutagen. 10, 1–175.

Garaj-Vrhovac, V., Zeljezic, D., 2000. Evaluation of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis (SCGE) assay: pesticide genotoxicity revealed by comet assay. Mutat. Res. 469, 279–285.

Hayashi, M., Tice, R.R., Macgregor, J.T., Anderson, D., Blakey, D.H., Kirsch-Volders, Jr., M., Oleson, F.B., Pacchierotti, F., Romagna, F., Shimada, H., Sutou, S., Vannier, B., 1994. In vivo rodent erythrocyte micronucleus assay. Mutat. Res. 312, 293–304.

Herrera, A., Barrueco, C., Caballo, C., Pena de la, E., 1992. Effects of permethrin on the induction of sister chromatid exchange and micronuclei in cultured human lymphocytes 20, 218–222.

Leiss, J.K., Savitz, D.A., 1995. Home pesticide use and childhood cancer: a case control study. Am. J. Public Health 85, 249–253.

Litchfield, M.H., 1985. Toxicity to mammals. In: Leahay, J.P. (Ed.), The Pyrethroid Insecticides. Taylor & Francis, London, pp. 99–150.

Meyer, O., 1993. Implications of animal welfare on toxicity testing. Hum. Exp. Toxicol. 12, 516–521.

Nakano, E., Rabella-Gay, M.N., Pereira, C.A., 1996. Evaluation of the genotoxic potential of flumethrin in mouse bone marrow chromosomal analysis and micronucleus test. Teratogen. Carcinogen. Mutagen. 16 (1), 37–48.

Nehéz, M., Boros, P., Ferke, A., Mohos, J., Patolás, P., Vetró, G., Zimányi, M., Dési, I., 1988. Cytogenetic examination of people working with agrochemicals in the southern region of Hungary. Regul. Toxicol. Pharmacol. 8, 37–44.

Oraby, H.A., 1997. Micronuclei formation in bone marrow cells of rats treated with meothrin (synthetic pyrethroid). J. Appl. Toxicol. 17 (6), 353–356.

Pati, P.C., Bhunya, S.P., 1989. Cytogenetic effects of fenvalerate in mammalian in vivo test system. Mutat. Res. 222, 149–154.

Pauluhn, J., 1999. Hazard identification and risk assessment of pyrethroids in the indoor environment. Toxicol. Lett. 107, 193–199.

Preston, R.J., Dean, B.J., Galloway, S., Holden, H., McFee, A.F., Shelby, M., 1987. Mammalian in vivo cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. Mutat. Res. 189, 157–165.

Puig, M., Carbonell, E., Xamena, N., Creus, A., Marcos, R., 1989. Analysis of cytogenetic damage induced in cultured human lymphocytes by the prethroid insecticides cypermethrin and fenvalerate. Mutagenesis 4, 72–74.

Schmid, W., 1976. The micronucleus test for cytogenetic analysis. In: Hollaender, A. (Ed.), Chemical Mutagens, Principles and Methods for Their Detection, vol. 4. Plenum Press, New York, pp. 31–53.

Sogorb, A.M., Vilanova, E., 2002. Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol. Lett. 128, 215–228.

Surralles, J., Carbonell, E., Puig, M., Xamena, N., Creus, A., Marcos, R., 1990. Induction of mitotic micronuclei by fenvalerate in cultured human lymphocytes. Toxicol. Lett. 54, 151–155.

Surralles, J., Xamena, N., Creus, A.J., Catalan, H., Norppa, H., Marcos, R., 1995. Induction of micronuclei by five pyrethrpid insecticdes in whole-blood and isolated human lymphocyte cultures. Mutat. Res. 341 (3), 169–184.

Tyrkiel, E.B., Wiadrowska, J.K., Ludwicki, J.K., 2001. Comparative study of the effect of synthetic pyrethroids on the induction on genetic changes in mice somatic and sex cells depending on exposure route. Rocz. Panstw. Zakl. Hig. 52 (2), 97–109.

USEPA, 2001. Pesticide industry sales and usage: 1996 and 1997 market estimates, available at: www.epa.gov/oppbead1/pestsales/97pestsales/table1.htm.2001

WHO, 1992. Our Planet, Our Health: Report of the WHO Commission on Health and Environment. World Health Organization, Geneva.