Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 539 (2003) 91–97
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow

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

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

Received 5 March 2003; received in revised form 7 May 2003; accepted 22 May 2003

Abstract

In this study, the genotoxic and cytotoxic potential of lambda-cyhalothrin (LCT), a synthetic pyrethroid insecticide, was investigated in Wistar rat bone-marrow cells, using the structural chromosomal aberration (SCA) and micronucleus (MN) test systems. LCT was administrated to adult female albino rats as repeated i.p. doses of 6.12, 3.06, 0.8 mg/kg BW for 13 days at 48 h intervals. Mitomycin C (MMC) was used as a positive control (2 mg/kg BW). All the doses of LCT increased the number of structural chromosomal aberrations and the frequency of micronucleated erythrocytes, compared with the control group. It was also observed that LCT caused a significant decrease in the number of polychromatic erythrocytes. Our results demonstrate that LCT has a clastogenic/genotoxic potential as measured by the bone marrow SCA and MN tests in Wistar rats.

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

1. Introduction

The synthetic pyrethroid insecticides are analogs of naturally occurring pyrethrins. Synthetic pyrethroids are a group of potent insecticides that are environmentally compatible by virtue of their moderate persistence, low volatility and poor aqueous mobility in soil. A wide margin of safety to mammals also makes these compounds ideal insecticides for widespread use in agriculture, veterinary and public health programs [1].

With the use of pyrethroids steadily rising, it may be an urgent need to identify the possible adverse effects that may be associated with their use. The genotoxic potential of some pyrethroid insecticides has been shown in previous studies [2–4]. Lambda-cyhalothrin (LCT) is a newer pyrethroid insecticide used all over the world. The cytogenetic effects of LCT were investigated in humans and in various animal species using different endpoints such as micronucleus (MN) formation, induction of chromosomal aberrations, and sister chromatid exchange [5–7]. The rodent bone-marrow MN and CA tests are the most widely used short-term in vivo assays for identification of genotoxic effects such as chromosome damage and aneuploidy, associated with mutagenesis and carcinogenesis [8,9].

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 potential in vivo genotoxic effects of LCT in bone marrow cells of female laboratory rats using chromosomal aberrations and micronuclei as genetic

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

1383-5718/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1383-5718(03)00159-1

endpoints. The ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) was also calculated to evaluate the cytotoxic effects of LCT in bone marrow.

2. Materials and methods

2.1. Chemical

LCT, a synthetic pyrethroid insecticide with the trade name 'KARATE' (Fig. 1), CAS chemical name (*R* + *S*)-α-cyano-3-(phenoxyphenyl)methyl-(1*S* + 1*R*)-cis-3-(2-2chloro-3,3,3-trifluoroprop-1-enyl)-2-2-dimethyl-cyclopropane-carboxylate, CAS registry number 91465-08-06, was from ZENECA agrochemicals, UK (2.5%).

2.2. Animals and treatment

Healthy adult female swiss albino rats (Wistar rat) (6–8 weeks of age, with average body weight (BW) of 180–200 g) were used in this study. Rats were obtained from the Experimental Animal Center, University of Gaziantep, Turkey. The rats were randomly selected and housed in polycarbonate boxes (three or four rats per box) with stell wire tops and rice husk bedding. They were maintained in a controlled atmosphere with a 12 h:12 h dark/light cycle, a temperature of 22 ± 2 °C 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 diluted with isotonic saline as required. LCT was intraperitoneally administered to adult female albino rats at the doses of 6.12, 3.06 and 0.8 mg/kg BW, one dose per 48 h given for 13 days. Three animals were used in each group per assay. The animals received a total of seven injections of LCT during the present study.

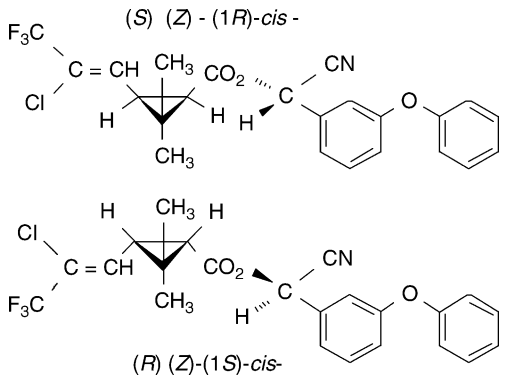

Fig. 1. Structure of the pyrethroid insecticide lambda-cyhalothrin (LCT, $C_{23}H_{19}ClF_3NO_3$).

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 LD_{50} dose for LCT was 612 mg/kg BW for mammals. Therefore, the highest dose was set at 6.12 mg/kg BW, i.e. 1% of the LD_{50} dose. The lowest dose was determined to be 0.8 mg/kg BW because this dose did not neurologically affect the rats. Groups of three rats were treated at three different LCT dose levels, 6.12, 3.06 and 0.8 mg/kg BW. Each rat received a total of seven doses at 48 h intervals, and genotoxic and cytotoxic effects were investigated in the bone marrow. The cumulative doses of LCT given to rats were thus 42.84, 21.42, and 5.6 mg/kg BW.

MMC (2 mg/kg) was used as a positive control. The positive control and the untreated control rats were identically treated with equal volumes of normal saline only via intraperitoneal (i.p.) injection. It is acceptable that a positive control is administered by a route different from or the same as the test agent and that it is given only a single time [10]. MMC was given as a single dose. The same dose regimes were used in both the chromosome aberration and MN assay.

2.4. Chromosome aberration assay

The rats were sacrificed 24 h after administration of the last dose for chromosome aberration assay. Cytogenetic analysis was performed on bone marrow cells according to the recommendations of Adler [11], with slight modifications. Experimental animals were injected (i.p.) with colchicine (4 mg/kg) 1.5 h prior to sacrifice. Both femora were dissected out and cleaned of any adhering muscle. Bone-marrow cells were collected from both the femora by flushing in KCl (0.075 M, at 37 °C) and incubated at 37 °C

for 25 min. Material was centrifuged at 2000 × *g* for 10 min, fixed in aceto-methanol (acetic acid:methanol, 1:3, v/v). Centrifugation and fixation (in the cold) were repeated five times at an interval of 20 min. The material was resuspended in a small volume of the fixative, dropped onto chilled slides, flame-dried and stained 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 servical dislocation 30 h after the last treatment. The frequency of micronucleated erythrocytes in femoral bone marrow was evaluated according to the procedure of Schmid [12], with slight modifications of Agarwal and Chauhan [13]. The bone marrow was flushed out from both femora using 1 ml of fetal calf serum (FCS) and centrifuged at 2000 × *g* for 10 min. The supernatant was discarded. Evenly spread bone-marrow smears were stained using the May-Grünwald and Giemsa protocol. Slides were scored at a magnification of 1000× using a light microscope.

2.6. Scoring

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

2.7. Statistical analysis

Data were compared by one-way variance analysis. Statistical analysis was performed using the SPSS for Windows 9.05 package program. Multiple comparisons were performed by least significant difference (LSD) test. $P < 0.05$ was considered as 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 gaps and breaks, double minute (included isochromatid breaks), exchanges, dicentric chromosomes and fragments. Chromatid gaps and breaks were noted to be more frequent than others (Table 1). Relatively higher frequencies of gaps were observed for all the doses tested. A quantitative assessment of the distribution of breaks and gaps revealed that the distal regions of the long chromosomes were more vulnerable to the effects of LCT. Numerical aberrations were not scored in this study.

The results of the chromosome aberration assay, the ratio of PCE to NCE and the MN frequency in bone marrow cells after intraperitoneal treatment with LCT are summarized in Tables 1 and 2, respectively. The frequency of structural chromosomal aberrations (SCAs) also increased with increasing concentrations of LCT (Fig. 2), and statistically significant differences from the control were observed, except at the dose of 0.8 mg/kg ($P > 0.05$). The mean of the induced SCA reached 3.5 ± 0.46, 7.1 ± 0.90, 10.6 ± 0.77, at LCT doses of 0.8, 3.06, and 6.12 mg/kg, respectively. Such values are much lower than those induced by the positive control Mitomycin C (2 mg/kg BW) (19.5 ± 2.35).

LCT induced a dose-related increase in MN frequency (Fig. 3). The two high doses (3.06 and 6.12 mg/kg BW) of LCT significantly increased the

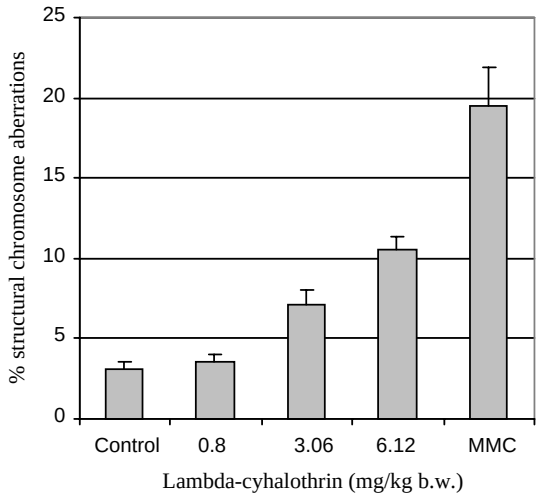

Fig. 2. Frequency of structural chromosomal aberrations (%) in relation to lambda-cyhalothrin doses in rat bone marrow. MMC: mitomycin C (positive control) (2 mg/kg BW).

Table 1
Frequency of chromosome aberrations in bone marrow cells of female Wistar rat induced by lambda-cyhalothrin

Dose group	Fixation time	Total number of examined metaphases (*n*)	*Chromatid* gap	break	*Chromosome* gap	break	Fragment	Dic	TSCA	Mean ± % SCA (excluding gaps)
Isotonic saline (control)	24	225/3	5	3	3	3	1	–	15	3.06 ± 0.46
LCT (mg/kg BW)										
0.8	24	225/3	5	4	4	3	–	1	17	3.50 ± 0.46[a]
3.06	24	225/3	9	7	7	6	2	1	32	7.10 ± 0.90[b]
6.12	24	225/3	14	12	10	8	4	–	50	10.6 ± 0.77[c]
MMC (mg/kg BW)										
2	24	225/3	23	15	18	16	13	–	85	19.5 ± 2.35[d]

n: number of animals; TSCA: total structural aberrations; Dic: dicentric chromosome; LCT: lambda-cyhalothrin; MMC: mitomycin C.
[a] $P > 0.05$ compared to control group.
[b] $P < 0.05$ compared to control group.
[c] $P < 0.01$ compared to control group.
[d] $P < 0.001$ compared to control group.

Table 2
Micronucleus induction and number of PCEs in LCT-exposed rat bone marrow

Treatment group	Rat number	MN per 2000 PCEs	Fixation time (h)	PCEs per 200 erythrocytes
Isotonic saline (vehicle control)	1	2		110
	2	2		100
	3	3		98
Mean ± S.E.		2.33 ± 0.33	30	102.6 ± 3.71
LCT (mg/kg BW, i.p.)				
0.8	1	4		70
	2	4		69
	3	3		68
Mean ± S.E.		3.66 ± 0.33	30	69 ± 0.57[a]
3.06	1	5		64
	2	5		66
	3	6		58
Mean ± S.E.		5.3 ± 0.33[b]	30	62.6 ± 2.40[a]
6.12	1	7		60
	2	7		56
	3	8		55
Mean ± S.E.		7.33 ± 0.33[a]	30	57 ± 1.52[a]
MMC (mg/kg BW)				
2 (positive control)	1	22		47
	2	21		45
	3	24		48
Mean ± S.E.		22.3 ± 0.88[a]	30	46.6 ± 0.88[a]

Abbreviations: LCT, lambda-cyhalothrin; PCEs, polychromatic erythrocytes; MN, micronucleus; MMC, mitomycin C.
[a] $P < 0.001$ compared with the control group.
[b] $P < 0.01$.

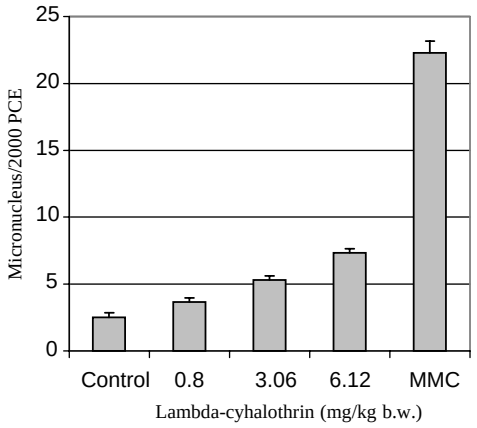

Fig. 3. Frequency of micronuclei in relation to lambda-cyhalothrin doses on rat bone marrow. MMC: mitomycin C (positive control) (2 mg/kg BW) and PCE: polychromatic erythrocyte.

frequency of micronuclei compared with the control ($P < 0.01$ and $P < 0.001$). No significant difference was observed between the lowest dose (0.8 mg/kg BW) and the control ($P > 0.05$).

The ratios PCE:NCE measured in whole bone marrow samples are summarized in Table 2. All three doses of LCT decreased the PCE:NCE ratio when compared with the control (Fig. 4).

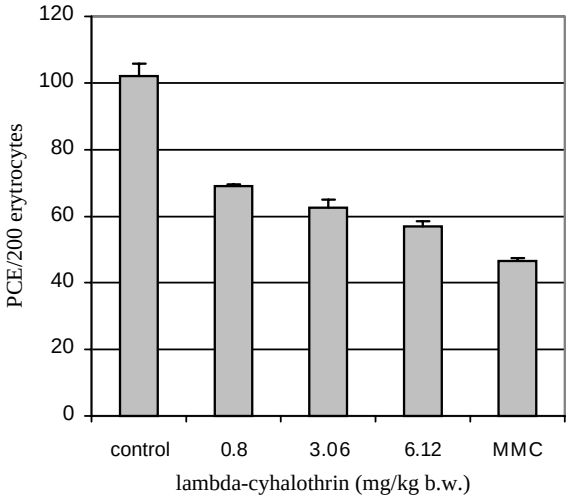

Fig. 4. The number of PCEs as a function of the lambda-cyhalothrin dose in rat bone marrow. MMC: mitomycin C (positive control) (2 mg/ml) and PCE: polychromatic erythrocyte.

4. Discussion

The genetic toxicity and mutagenicity studies concerning the synthetic pyrethroids have produced controversial results depending on the compound or the assay used. In some studies, pyrethroids were reported to be mutagenic in rodent bone marrow [6,14–17], human peripheral lymphocyte cultures [2,4], and in aquatic organisms [7].

The induction of SCAs and micronucleated erythrocytes following exposure to LCT indicates a potential for clastogenicity. The frequency of SCAs (excluding gaps) following intraperitoneal exposure to three different concentrations of LCT (0.8, 3.06, and 6.12 mg/kg BW) remained significantly higher than in controls. This is in agreement with previous reports on the clastogenic potential of synthetic pyrethroids as manifested in rodent bone-marrow [4,5,18–24], in human peripheral lymphocyte cultures [4,18–20], and in aquatic organism [25].

In the present study, the observed inhibition of cell proliferation in the rat bone marrow illustrates the cytotoxicity of LCT. These data indicate the cytotoxic potential of LCT. This is also in agreement with previous reports on the cytotoxic potential of pyrethroid insecticides. Similar results were observed in studies performed with different pyrethroid insecticides [2,24,26,27]. Cavas and Ergene-Gozukara [25] tested the cytotoxic potential of LCT using nucleolar biomarkers on fin cells of the fish *Gara rufa*. They reported that LCT has the ability to repress the nucleolar activity.

Conflicting results have been obtained in whole animal studies of the induction of DNA damage after exposure to pyrethroid insecticide [24,26]. It was reported that the pyrethroid insecticide, cypermethrin, induced numerical chromosome aberrations in rats, but did not change the number of SCAs [28].

Limited information is available on the cytogenotoxicity of LCT. Our results confirm that the increase in CA and MN, and the decrease in the PCE:NCE ratio was dose-dependent. We found that there is a linear relationship between the LCT dose used and the frequencies of chromosome aberrations and micronuclei. It is concluded that LCT has a cytotoxic effect on bone marrow in rats, because the decrease in the ratio PCE:NCE was observed at all doses. Such a decreased ratio is often used as an indicator

of bone marrow cytotoxicity or alterations in erythropoiesis. In normal bone marrow, the PCE:NCE ratio is generally around 1:1 [29].

Genotoxic activity is normally indicated by a statistically significant dose-related increase in the incidence of micronucleated PCEs compared with a concurrent control group. Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant dose-related decrease in the proportion of PCEs: a very large decrease in the ratio would be indicative of a cytostatic or cytotoxic effect. A decrease in the proportion of PCE is used as an indication of mutagen-induced bone marrow cytotoxicity or changes in erythropoiesis [30].

In conclusion, our data indicate that LCT induces structural chromosome aberrations and the occurrence of micronuclei. In addition to this clastogenic effect, our findings show that LCT is cytotoxic in the bone marrow. Our observations suggest the in vivo susceptibility of mammals to the genetic toxicity and cytotoxic potential of LCT.

Acknowledgements

The authors wish to express their thanks to Tolga Çavaş for his help in the statistic analyses.

References

[1] J.P. Leahey, Pyrethroid Insecticides, Taylor and Francis, London, 1985.
[2] M. Puig, E. Carbonell, N. Xamena, A. Creus, R. Marcos, Analysis of cytogenetic damage induced in cultured human lymphocytes by the pyrethroid insecticides cypermethrin and fenvalerate, Mutagenesis 4 (1989) 72–74.
[3] E. Carbonell, M. Puig, N. Xamena, A. Creus, R. Marcos, Mitotic arrest induced by fenvalerate in human lymphocyte cultures, Toxicol. Lett. 48 (1989) 45–48.
[4] J. Surralles, E. Carbonell, M. Puig, N. Xamena, A. Creus, R. Marcos, Induction of mitotic micronuclei by fenvalerate in cultured human lymphocytes, Toxicol. Lett. 54 (1990) 151–155.
[5] A.M. Fahmy, E.F. Abdalla, Cytogenetic effects by the natural pyrethrins and the synthetic lambda-cyhalothrin in mice in vivo, Cytologia 66 (2001) 139–149.
[6] D.K. Agarwal, L.K.S. Chauhan, S.K. Gupta, V. Sundararaman, Cytogenetic effects of deltamethrin on rat bone marrow, Mutat. Res. 311 (1994) 133–138.
[7] M.A. Campana, A.M. Panzeri, V.J. Moreno, F.N. Dulout, Genotoxic evaluation of the pyrethroid lambda-cyhalothrin using the micronucleus test in erythrocytes of fish *Cheirodon interruptus*, Mutat. Res. 438 (1999) 155–161.
[8] W. Schmid, Chemical mutagen testing on in vivo somatic mammalian cells, Agents Actions 3 (1973) 77–85.
[9] J.A. Heddle, A rapid in vivo test for chromosomal damage, Mutat. Res. 18 (1973) 187–190.
[10] M. Hayashi, R.R. Tice, J.T. Macgregor, D. Anderson, D.H. Blakey, M. Kirsch-Volders Jr., F.B. Oleson, F. Pacchierotti, F. Romagna, H. Shimada, S. Sutou, B. Vannier, In vivo rodent erythrocyte micronucleus assay, Mutat. Res. 312 (1994) 293–304.
[11] I.D. Adler, 1984. Cytogenetic tests in mammals, in: S. Venitt, J. M. Parry (Eds.), Mutagenicity Testing, A Practical Approach, IRL Press, Oxford, pp. 275–306.
[12] W. Schmid, 1976. The micronucleus test for cytogenetic analysis, in: A. Hollaender (Ed.), Chemical Mutagens, Principles and Methods for Their Detection, vol. 4, Plenum Press, New York, pp. 31–53.
[13] D.K. Agarwal, L.K.S. Chauhan, An improved chemical substitute for fetal calf serum for the micronucleus test, Biotechnol. Histochem. 68 (1993) 187–188.
[14] X.K. Charterjee, G. Tarukdan, A. Sharran, Effects of synthetic pyrethroids in mammalian chromosomes. I. Sumicidin, . Mutat. Res. 105 (1982) 102–106.
[15] S.M. Amer, E.I. Aboul-Ela, Cytogenetic effects of pesticide: induction of micronuclei in mouse bone marrow by the insecticides cypermethrin and rotenone, Mutat. Res. 55 (1985) 135–142.
[16] P.C. Pati, S.P. Bhunya, Cytogenetic effects of fenvalerate in mammalian in vivo test system, Mutat. Res. 222 (1989) 149–154.
[17] S.M. Amer, A.S. Ibrahim, K.M. El-Sherbeny, Induction of chromosomal aberrations and sister chromatid exchange in vivo and in vitro by the insecticide cypermethrin, J. Appl. Toxicol. 13 (1993) 341–345.
[18] C. Barrueco, A. Herrera, C. Cabolla, E. De La Penda, Induction of structural chromosomal aberrations in human lymphocyte cultures and CHO cells by permethrin, Teratog. Carcinog. Mutagen. 14 (1) (1994) 31–38.
[19] C. Barrueco, A. Herrera, C. Cabolla, E. De La Penda, Cytogenetic effects of permethrin in cultured human lymphocytes, Mutagenesis 7 (6) (1992) 433–437.
[20] J. Surralles, N. Xamena, A. Creus, J. Catalan, H. Norppa, R. Marcos, Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures, Mutat. Res. 341 (3) (1995) 169–184.
[21] S.P. Bhunya, P.C. Pati, Effect of deltamethrin, a synthetic pyrethroid, on the induction of chromosome aberrations, micronuclei ab sperm abnormalities in mice, Mutagenesis 5 (3) (1990) 229–232.
[22] S.P. Bhunya, P.C. Pati, Genotoxic effects of a synthetic pyrethroid insecticide, cypermethrin, in mice in vivo, Toxicol. Lett. 41 (3) (1988) 223–230.
[23] H.A. Oraby, Micronuclei formation in bone marrow cells of rats treated with meothrin (synthetic pyrethroid), J. Appl. Toxicol. 17 (6) (1997) 353–356.

[24] E. Tyrkiel, B. Wiadrowska, J.K. Ludwicki, 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. Pantsw. Zakl. Hig. 52 (2) (2001) 97–109.
[25] T. Cavas, S. Ergene-Gozukara, Evaluation of the genotoxic potential of lambda-cyhalothrin using nuclear and nucleolar biomarkers on fish cells, Mutat. Res. 534 (1/2) (2003) 93–99.
[26] J. Dianovsky, K. Sivikova, In vivo and in vitro cytogenetic effect of supermethrin, Biomed. Environ. Sci. 8 (1995) 359–366.
[27] E. Nakano, M.N. Rabella-Gay, C.A. Pereira, Evaluation of the genotoxic potential of flumethrin in mouse bone marrow chromosomal analysis and micronucleus test, Teratog. Carcinog. Mutagen. 16 (1) (1996) 37–48.
[28] L. Institoris, U. Undeger, O. Siroki, M. Nehez, I. Dési, Comparison of detection sensitivity of immuno- and genotoxicological effects of subacute cypermethrin and permethrin exposure in rats, Toxicology 137 (1999) 47–55.
[29] W. Schmid, The micronucleus test, Mutat. Res. 31 (1975) 9–15.
[30] Y. Suzuki, Y. Nagae, J. Li, H. Sabaka, K. Mozawa, A. Takahashi, H. Shimizu, The micronucleus test and erythropoiesis: effects of erythropoietin and a mutagen on the ratio of polychromatic to normochromatic erythrocytes (P:N ratio), Mutagenesis 4 (1989) 420–424.