Mutagenesis vol. 20 no. 2 pp. 125–129, 2005 doi:10.1093/mutage/gei020

Induction of micronuclei by lambda-cyhalothrin in Wistar rat bone marrow and gut epithelial cells

Ayla Çelik[1,*], Birgül Mazmanci[1], Yusuf Çamlica[1], Ali Aşkin[1] and Ükü Çömelekoğlu[2]

[1]Department of Biology, Faculty of Science and Letters and [2]Department of Biophysics, Faculty of Medicine, Mersin University, Mersin, Turkey

We have investigated the genotoxicity of lambda-cyhalothrin (LCT) (CAS registry No. 91465-08-06), a pyrethroid insecticide, in bone marrow cells and in colonic crypt epithelial cells of groups of four rats per dose treated *in vivo* by gavage at doses of 0.8, 3.06 and 6.12 mg/kg body weight (body wt). We measured genotoxicity using the micronucleus (MN) assay, scoring 2000 polychromatic erythrocytes (PCEs) per animal for bone marrow and 1000 colonic crypt epithelial cells per animal for the colon. We assessed cytotoxicity in bone marrow by calculating the ratio of PCEs to normochromatic erythrocytes, and in the colonic crypt epithelium by observing the frequency of binucleate cells and the mitotic index in 1000 cells. Apoptosis in colonic crypt epithelial cells was measured by observing the frequency of karyorrhexis and karyolysis in 1000 cells. We found that LCT induced a statistically significant dose-related increase in MN formation in the bone marrow and the colonic crypt. The colonic epithelium was more sensitive to the clastogenic effects of LCT than the bone marrow as judged by the significantly higher frequencies of MN in the colon than in the bone marrow at doses of 3.06 and 6.12 mg/kg body wt.

Introduction

In recent years, the use of pesticides in agriculture has been increasing steadily. The harmful effects of many pesticides, such as organochlorines, organophosphates and carbamates, have led to the use of pyrethroids as alternatives. Pyrethroids are analogs of naturally occurring pyrethrins and widely used in agriculture in many countries (1). Generally, it is known that these pesticides possess high activity against a broad spectrum of insect pests (adult and larvae) (2–4). For these reasons, pyrethroid insecticides are extensively used in forestry and household products.

Synthetic pyrethroids are a diverse class of >1000 powerful broad spectrum insecticides by virtue of their moderate persistence, low volatility and poor aqueous mobility in soil (5). With the steadily increasing use of pyrethroid insecticides, there is an urgent need to identify their possible effects on living organisms. The genotoxic potential of some pyrethroid insecticides has been shown in previous studies (6–10). The carcinogenic potential of pyrethroids has been discussed in a review by Litchfield (11).

Lambda-cyhalothrin (LCT) is a newer pyrethroid insecticide used all over the world (12). Campana *et al*. (12) reported that LCT induced micronuclei (MN) in erythrocytes of the fish *Cheirodon interruptus* and in tadpoles of the frog *Rana catesbeiana* (13). The cytogenetic effects of LCT have been investigated in humans and laboratory animals using different endpoints, such as the formation of MN or induction of chromosomal aberrations and sister chromatid exchange (9,14–16). In our previous studies, (17) we demonstrated that an intraperitoneal (i.p.) administration of LCT has genotoxic and cytotoxic effects on the rat bone marrow and, in another study (18), shown to have similar effects when given through a gavage.

The rodent bone marrow MN test is the most widely used short-term *in vivo* assay for identification of genotoxic effects, such as structural or numerical chromosome mutations (19–21).

However, as indicated by Hayashi *et al*. (22) for some compounds or their active metabolites that are too short lived to reach bone marrow cells, tissues other than bone marrow may be more sensitive for the detection of clastogenic or aneugenic effects. Vanhauwaert *et al*. (23) demonstrated that the *in vivo* gut MN test is a sensitive method for the identification of the genotoxic potential of chemicals given through gavage. Increases in MN frequency in gut epithelial cells by various chemicals have been reported in various studies (15,24). Environmental pollutants, such as pesticides and insecticides, may enter the human body through the food chain. Therefore, it is appropriate to consider the epithelium of gut for *in vivo* screening of chemicals because it is the first tissue to come into substantial contact with potentially hazardous chemicals and because cancers of the digestive system are some of the most common forms of cancer in some human populations.

The aim of the present study was to determine whether the *in vivo* rat gut MN test could be a more sensitive method to detect the effect of LCT given through gavage than the *in vivo* bone marrow MN test.

Materials and methods

Chemicals

LCT (Figure 1), a synthetic pyrethroid insecticide with the trade name 'KARATE', CAS chemical name (*R* + *S*)-α-cyano-3-(phenoxyphenyl) methyl-(1*S* + 1*R*)-*cis*-3-(2-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate, CAS registry No. 91465-08-06, was obtained from ZENECA agrochemicals, UK. The commercial product consisted of 2.5% w/w LCT formulated in aqueous propylene glycol.

Animals and treatment

Healthy adult female Swiss albino rats (Wistar rats) [6–8 weeks of age, with average body weight (body wt) 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 (four rats per box) with steel wire tops and rice husk bedding. They were maintained in a controlled atmosphere with a 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 commercially available dry food pellets. For each dose group, four animals were used and the animals were allowed to acclimatize for 7 days before treatment.

*To whom correspondence should be addressed. Tel: +90 324 3610001; Fax: +90 324 3610047; Email: a.celik@mersin.edu.tr

Doses

The animals were exposed to the highest possible dose with minimal toxic symptoms. Toxic symptoms were commonly of a neurological nature observed as unusual behaviour, such as aggressiveness. In studies performed by some researchers, LCT was reported to cause thinness in the myelin sheath and axonal degeneration (25–27). Some pyrethroid insecticides were reported to cause behavioral changes (28). It has been determined that the LD_{50} of KARATE is 612 mg/kg body wt for rats. Therefore, the highest dose was set at 6.12 mg/kg body wt i.e. 1% of the LD_{50} dose. The lowest dose was determined to be 0.8 mg/kg body wt because this dose did not affect the animals neurologically. KARATE was diluted with isotonic saline as required and was administered by gavage to groups of four adult female rats at doses of 0.8, 3.06 and 6.12 mg/kg body wt (representing 0.02, 0.077 and 0.153 mg/kg of LCT, respectively), one dose per 48 h given for 13 days (total 7 doses/animal).

Mitomycin C (MMC) (2 mg/kg), as a single i.p. dose, was used as a positive control in this study. The positive control and untreated control rats were treated identically with equal volumes of normal saline. It is acceptable that a positive control is administered by a route different from or the same as the test substance and that it is given only a single time (20).

In vivo gut MN test

Animals were killed by cervical dislocation and colons excised and flushed free of faeces with phosphate-buffered saline (PBS). The colon was everted on a glass rod and placed in trypsin–EDTA solution at 30°C for 15 min. Following this treatment, crypts were isolated from the colon by vibrating the glass rod for 5 min. The isolated crypts at this stage were intact and retained their oblong shape (29). They were then dispersed into single cells by pipetting with Pasteur pipettes. The cells were collected by centrifugation at 84 *g* for 10 min, re-suspended in a small amount of PBS, and then fixed in methanol:acetic acid (3:1, v/v) (Carnoy's fixative). This method was performed according to Matsuoka *et al*. (30) with slight modifications. Thus, cells were re-suspended in a small amount of fresh fixative, dropped onto clean slides, allowed to dry and stained with May-Grünwald and Giemsa protocol. Slides were scored at a magnification of 1000× using a light microscope.

Scoring

At least 1000 intact cells that retained their oblong shape or microvilli, and in which the nucleus was well identified but not fragmented and surrounded by cytoplasm with a clear boundary, were analyzed per animal for the presence or absence of MN. A micronucleated epithelial cell was defined as a cell with a normal nucleus and one or two MN, which neither exceeded one-third of the diameter of the main nucleus nor overlapped with it. Any ambiguous MN-like inclusions were not scored as MN. In addition to this analysis, in order to determine the cytotoxicity of LCT on the gut epithelial cells, binucleated cells were counted in 1000 cells within the crypt of colon. Cells with two nuclei were considered as binucleates. Cells with mitotic figures were identified as cells in which the chromatin was condensed and visible as chromatids. Normal cells were those not showing any of these features. Karyorrhexis (KR) and karyolysis (KL) frequency were calculated to determine apoptosis in gut epithelial cells. Nuclei fragmented into irregular pieces were scored as displaying KR; nuclear dissolution, the ghost-like image of the nuclear remains, was evaluated as KL.

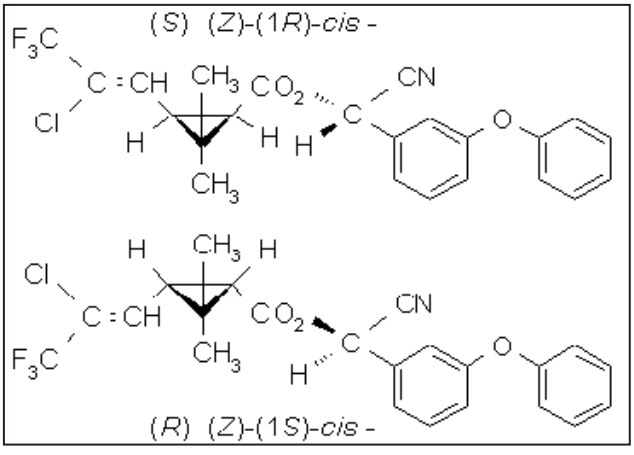

Fig. 1. Chemical structure of LCT.

In vivo bone marrow MN test

Rats were killed by cervical dislocation 30 h after the last treatment. The frequency of micronucleated erythrocytes in femoral bone marrow was evaluated according to the procedure of Schmid (31), with the slight modifications of Agarval and Chauhan (32). The bone marrow was flushed out from both femora using 1 ml of fetal calf serum and centrifuged at 336 *g* for 10 min and 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.

Scoring

For the analysis of MN, 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 PCE to normochromatic erythrocytes (NCEs). A micronucleated PCE was defined as a cell with one or two MN, which neither exceeded one-third of the diameter of the main nucleus nor overlapped with it. Any ambiguous MN-like inclusions were not scored as MN. The frequency of micronucleated PCEs is the principal endpoint in bone marrow. MN were calculated only in PCEs. In bone marrow, PCEs appear as blue and NCEs appear as orange/pink.

Statistical analysis

The arc-sin square root transformation was applied to all the data. These transformations are generally applied where the variable p is the proportion of successes and consequently bounded by 0 and 1 (33). Statistical analyses were performed by the repeated measure test. Multiple comparisons were analyzed using the least significant difference (LSD) test. $P < 0.05$ was considered as the level of significance. In this study, the Pearson correlation test was used.

Results

LCT treatment caused significant dose-related increases in the MN formation in bone marrow and colonic crypt epithelial cells ($P < 0.001$). In both bone marrow and gut epithelial cells, an interaction was found between all the doses of LCT and MN frequency (Table I).

A 0.8 mg/kg (body wt) dose of LCT similarly affected both the bone marrow and gut epithelial cells. However, the 3.06 and 6.12 mg/kg doses of LCT caused a larger increase in MN frequency in gut epithelial cells than in bone marrow. While there was a significant difference among all doses of LCT in gut epithelial cells, there was no difference between the 0.8 and 3.06 mg/kg doses of LCT in bone marrow cells. The cytotoxic effect of LCT in both bone marrow and gut epithelial cells was tested by using different parameters. LCT caused a decrease in the proportion of PCEs (in PCEs + NCEs) in bone marrow.

Table I. The MN frequency in bone marrow and gut epithelial cells

Treatment group	Rat number	MN (bone marrow) mean (‰) ± SE	MN (gut epithelial cells) mean (‰) ± SE
Vehicle control			
Isotonic saline	4	2.25 ± 0.25	2.00 ± 0.40
LCT (mg/kg body wt)			
0.8	4	3.50 ± 0.28[d]	4.25 ± 0.25[a,c]
3.06	4	4.00 ± 0.40[d]	6.75 ± 0.25[b,c]
6.12	4	6.00 ± 0.40[d]	8.25 ± 0.47[a,c]
PC MMC (2 mg/kg body wt)	4	22.2 ± 1.25[d]	20.25 ± 0.85[a,c]

Data were given as mean ± standard error. LCT, lambda-cyhalothrin; PC, positive control; MMC, mitomycin C.
[a]No difference between bone marrow and gut epithelial cell.
[b]There is a significant difference between bone marrow and gut epithelial cell.
[c]There is a significant difference between control and LCT dose group in gut epithelial cell.
[d]There is a significant difference between control and LCT dose group in bone marrow.

There was a significant difference between the three doses of LCT and negative controls ($P < 0.001$) (Table II). The decrease in the number of PCEs was dose-dependent (correlation coefficient: $r = -0.87$).

Table III shows the results for binucleated cells. LCT caused an increase in the frequency of binucleated cells in gut epithelial cells. There is a significant difference between LCT doses and negative controls for binucleated cell frequency. Statistical analyses show that there is a correlation between LCT doses and the frequency of binucleated cells (correlation coefficient: $r = 0.81$).

In this study, nuclear changes (KR, KL and binucleated cells) were scored in 1000 epithelial cells in the gut. There was a dose-dependent increase in nuclear changes and this increase is statistically significant compared with controls.

There was also a dose-dependent increase of nuclear changes that is statistically significant compared with control animals. The frequency of KR increased with increasing doses of LCT and statistically significant differences were observed compared with negative controls ($P < 0.05$), except at the doses of 0.8 and 3.06 mg/kg (Table III).

The frequency of KL increased with the LCT dose administered and there was a statistically significant difference between LCT doses and negative control groups ($P < 0.05$), except at the dose of 0.8 mg/kg (Table III).

To determine the cytotoxic effects of LCT on gut epithelial cells, mitotic index values were also calculated in 1000 gut epithelial cells. LCT induced a statistically significant decrease in the values of mitotic index compared with negative control animals ($P < 0.001$).

The present study implies that oral administration of LCT not only has an inhibiting potential on the erythropoiesis in bone marrow but also a clastogenic effect in both bone marrow and gut epithelial cells. Doses of LCT of 3.06 and 6.12 mg/kg (body wt) were more effective in gut epithelial cells than that in bone marrow for MN formation. Thus, for all LCT doses tested, the *in vivo* gut MN test was as sensitive or even more sensitive than the *in vivo* bone marrow MN assay.

Table II. Proportion of PCEs (in PCEs + NCEs) in LCT-exposed rat bone marrow

Treatment group	Rat number	Examined erythrocyte number (four rats)	PCE/200 erythrocytes mean (‰) ± SE
Vehicle control			
Isotonic saline	4	800	100.75 ± 2.49
LCT (mg/kg body wt)			
0.8	4	800	74.0 ± 3.34[a]
3.06	4	800	60.5 ± 3.22[a]
6.12	4	800	50.5 ± 2.02[a]
PC MMC (2 mg/kg body wt)	4	800	47.5 ± 0.70[a]

PC, positive control; MMC, mitomycin C; PCE, polychromatic erythrocytes. Data were given as mean ± standard error.
[a]$P < 0.001$ compared with control.

Discussion

Fundamental similarities in cell structure and biochemistry between animals and humans provide a valid basis for prediction of the likely effects of chemicals on human populations (34). The data on the genotoxic effects of synthetic pyrethroids are rather controversial as different studies have reported different results depending on the test system, administration route or organism used in the experiments. Most toxicological studies on pyrethrins and pyrethroids have been performed in vertebrates or laboratory rodents and limited data are available for other species. In our previous study, we demonstrated that an i.p. administration of LCT caused clastogenic and cytotoxic damage in rat bone marrow. The decrease in the number of PCEs show that LCT caused an inhibition in cell proliferation in bone marrow.

The purposes of genotoxicity testing are (i) to assess the mutagenity of chemicals, in order to protect the human gene pool and (ii) to identify potential carcinogens. Genotoxicity of chemicals in mammalian cells can be determined in different ways: by identification of DNA damage, DNA breaks, DNA repair, gene mutations, chromosome aberrations, chromosome breakage and chromosome loss (35). The rodent bone marrow and peripheral blood MN tests have been used widely as tools to evaluate the genotoxicity of environmental mutagens (19,20). However, since the target of these tests is restricted to the hematopoietic cells, the detection of a genotoxin that may target sites other than bone marrow cannot be assured. Therefore MN tests using organs other than bone marrow, such as liver (36), skin (37), testis and colon (38) have been developed (39).

The purpose of the MN assay is to identify a substance that causes cytogenetic damage, which results in the formation of

Table III. Frequency of nuclear changes in gut epithelial cells

Treatment group	Rat number	Examined epithelial cell number (for four rats)	BN mean (‰) ± SE	KR mean (‰) ± SE	KL mean (‰) ± SE	MI mean (‰) ± SE
Vehicle control						
Isotonic saline	4	4000	2.25 ± 0.25	1.25 ± 0.25	1.25 ± 0.25	7.75 ± 0.75
LCT (mg/kg body wt)						
0.8	4	4000	3.50 ± 0.28[b]	1.75 ± 0.75	1.75 ± 0.75	4.75 ± 0.47[c]
3.06	4	4000	3.75 ± 0.25[b]	2.25 ± 0.25	2.50 ± 0.28[a]	2.50 ± 0.28[c]
6.12	4	4000	6.00 ± 0.57[c]	3.25 ± 0.25[a]	3.50 ± 0.28[a]	1.25 ± 0.25[c]
PC MMC (2 mg/kg body wt)	4	4000	11.75 ± 0.35[c]	11.75 ± 0.62[b]	12.00 ± 0.70[c]	3.75 ± 0.25[c]

KR, karyorrhexis; KL, karyolysis; BN, binucleated cells; MI, mitotic index; PC, positive control; MMC, mitomycin C. Data are given as mean ± standard error.
[a]$P < 0.05$ compared with control.
[b]$P < 0.01$ compared with control.
[c]$P < 0.001$ compared with control.

MN containing lagging chromosome fragments or whole chromosomes. An increase in MN frequency in buccal epithelial cells of workers occupationally exposed to pesticides was reported (40). It has been reported that the gut epithelial MN test is an effective method for identifying the potential genotoxicity of chemical given by gavage (23,39).

It seems logical to consider the epithelium of the gut for *in vivo* screening of chemicals given by gavage since it is the first tissue to come into substantial contact with potentially hazardous chemicals (food contaminants or pesticides) and since colon cancer is one of the most important types of cancer in some populations. Gut epithelial cells are suitable cells for the MN assay because they have a high turnover and the MN test depends on a population of dividing cells (41). The present study is the first to report the genotoxic, cytotoxic, apoptotic and antimitotic effects of LCT in both bone marrow and gut epithelial cells.

Genotoxic activity in bone marrrow is normally indicated by a statistically significant dose-related increase in the incidence of micronucleated PCEs compared with a concurrent control group. In the present study, we observed the genotoxic and cytotoxic effects of LCT in both bone marrow and gut epithelial cells. LCT elevated the MN frequency in both tissues. The lowest dose of LCT (0.8 mg/kg body wt) showed a similar genotoxic effect in both bone marrow and gut epithelial cells. Pyrethroid insecticides have been reported to have genotoxic effects in the bone marrow of rodents in several studies (9,15,42).

The results of this study show that the 3.06 and 6.12 mg/kg doses of LCT cause an increase in MN frequency in both bone marrow and gut epithelial cells. Statistical analysis indicates that these doses are more effective in the formation of MN in gut epithelial cells than in bone marrow. This is also in agreement with previous studies performed in gut epithelial cells to identify the genotoxic potential of the substances given by gavage (38,39,43–45).

In this study, we analyzed the cytotoxicity of LCT by calculating the ratio of PCEs to NCEs in the bone marrow of rats to provide the cytotoxicity index (46). In normal bone marrow, the PCE:NCE ratio is generally ~1:1 (47). LCT induced a decrease in the number of PCEs in bone marrow in a dose dependent manner (Table II). This finding indicates that LCT is a potent inhibitor of mitosis as shown in studies reported earlier (48). Similar results were reported in studies performed with different pyrethroid insecticides (7,49,50).

Binucleus formation and a decrease in mitotic index are considered as indicators of cytotoxicity in epithelial tissues (51). In order to determine the cytotoxicity in gut epithelial cells, we calculated the frequency of binucleated cells in 1000 gut epithelial cells in this study. LCT has similar cytotoxic effects in both bone marrow and gut epithelial tissue. In addition to binucleus formation, LCT induced not only a decrease in mitotic index but also apoptosis, measured by an increase in the frequency of KR and KL in gut.

In conclusion, data from the present study indicate that LCT has not only cytotoxic and genotoxic effects but also antimitotic and apoptotic effects on gut epithelial cells. Thus, the gut MN test seems to be a useful method for identifying the biomarkers of oral exposure of chemicals given through gavage. We conclude from the present study that rats are sensitive to LCT, the pyrethroid insecticide tested in this study, and that LCT genotoxicity is dose-dependent.

References

1. Leahey,J.P. (1985) *Pyrethroid Insecticides*. Taylor & Francis, London.
2. Papodopoulou-Markidou,E. (1983) Analysis of established pyrethroid insecticides. *Residue Rev*., **89**, 179–208.
3. Zerba,E. (1988) Insecticidal activity of pyrethroids on insects of medical importance. *Parasitol. Today*, **4**, 53–57.
4. Vijveberg,H.P.M. and Van Der Bercken,J. (1990) Neurotoxicological effect and the mode of action of pyrethroid insecticides. *Crit. Rev. Toxicol*., **21**, 105–126.
5. Erstfeld,K.M. (1999) Environmental fate of synthetic pyrethroids during spray drift and field run off treatments in aquatic microcosms. *Chemosphere*, **39**, 1737–1769.
6. Carbonell,E., Puig,M., Xamena,N., Creus,A. and Marcos,R. (1989) Mitotic arrest induced by fenvalerate in human lymphocyte cultures. *Toxicol. Lett*., **48**, 45–48.
7. Puig,M., Carbonell,E., Xamena,N., Creus,A. and Marcos,R. (1989) Analysis of cytogenetic damage induced in cultured human lymphosytes by the pyrethroid insecticides cypermethrin and fenvalerate. *Mutagenesis*, **4**, 72–74**.**
8. Surrales,J., Carbonella,E., Puig,M., Xamena,N., Creus,A. and Marcos,R. (1990) Induction of mitotic micronuclei by the pyrethroid insecticide fenvalerate in cultured human lymphocytes. *Toxicol. Lett*., **54**, 151–155.
9. Agarwal,D.K., Chauhan,L.K.S., Gupta,S.K. and Sundararaman,V. (1994) Cytogenetic effects of deltametrin on rat bone marrow. *Mutat. Res*., **311**, 133–138.
10. Institoris,L., Undeger,U., Siroki,O., Nehez,M. and Desi,I. (1999) Comparison of detection sensitivity of immuno and genotoxicological effects of subacute cypermethrin and permethrin exposure in rats. *Toxicology*, **137**, 47–55.
11. Litchfield,M.H. (1985) Toxicity to mammals. In Leahay,J.P. (ed.), *The Pyrethroid Insecticides*. Taylor & Francis, London, pp. 99–150.
12. Campana,M.A., Panzeri,A.M., Moreno,V.J. and Dulout,F.N. (1999) Genotoxic evaluation of pyrethroid lambda-cyhalothrin using the micronucleus test in erythrocytes of fish *Cheirodon interruptus*. *Mutat. Res*., **438**, 155–161.
13. Campana,M.A., Panzeri,A.M., Moreno,V.J. and Dulout,F.N. (2003) Micronuclei induction in *Rana catesbeiana* tadpoles by the pyrethroid insecticide lambda-chalothrin. *Genet. Mol. Biol*., **26**, 99–103.
14. Çavaş,T. and Ergene-Gözükara,S. (2003) Evaluation of the genotoxic potential of lambda-cyhalothrin using nuclear and nucleolar biomarkers on fish cells. *Mutat. Res*., **534**, 93–99.
15. Çelik,A., Çavaş,T. and Ergene-Gözükara,S. (2003) Cytogenetic monitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. *Mutagenesis*, **18**, 417–421.
16. Fahmy,A.M. and Abdalla,E.F. (2001) Cyogenetic effects by the natural pyrethrin and the synthetic lambda-cyhalothrin in mice *in vivo*. *Cytologia*, **66**, 139–149.
17. Çelik,A., Mazmanci,B., Çamlica,Y., Aşkin A. and Çömelekoğlu,Ü. (2003) Cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow. *Mutat. Res*., **539**, 91–97.
18. Çelik,A., Mazmanci,B., Çamlica,Y., Aşkin A. and Çömelekoğlu,Ü. (2005) Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow by gavage administration. *Ecotoxicol. Environ. Saf*., in press.
19. Schmid,W. (1973) Chemical mutagen testing on *in vivo* somatic mammalian cells. *Agents Actions*, **3**, 77–85.
20. Hayashi,M., Tice,R., Macgregor,J.T. *et al*. (1994) *In vivo* rodent erythrocyte micronucleus assay. *Mutat. Res*., **312**, 293–304.
21. Heddle,J.A. (1973) A rapid *in vivo* test for chromosomal damage. *Mutat. Res*., **18**, 187–190.
22. Hayashi,M., Macgregor,J.T., Gatehouse,D.G., Adler,I.D., Blakey,D.H., Dertinger,S.D., Krishna,G., Morita,T., Russo,A.N. and Sutou,S. (2000) *In vivo* rodent erythrocyte micronucleus assay. II. Some aspects of protocol design including repeated treatments, integration with toxicity testing and automated scoring. *Environ. Mol. Mutagen*., **35**, 234–252.
23. Vanhauwaert,A., Vanparys,P. and Kirsch-Volders,M. (2001) The *in vivo* gut micronucleus test detects clastogens and aneugens given by gavage. *Mutagenesis*, **16**, 39–50.
24. Surrales,J., Autio,K., Nylund,L., Jarventaus,H., Norppa,H., Veidebaum,T., Sorsa,M. and Peltonen,K. (1997) Molecular cytogenetic analysis of buccal cells and lymphocytes from benzene-exposed workers. *Carcinogenesis*, **18**, 817–823.
25. Douglas,A.C. and Doyle,G.G. (1991) Toxic responses of nervous system In Amdur,M.O., Daull,J. (eds), *Caserett and Daull's Toxicology: The*

Basic Science of Poisons. McGraw Hill Company Pergmon Press, Toronto, p. 421.
26. Kandel,E.R., Schwartz,J.H. and Jessel,T.M. (2000) *Principles of Neural Science*, 4th edn. McGraw Hill Company, Toronto, pp. 146–147.
27. Griffin,J.W. (2000) Pathophysiology of periferal neurapathies. In Goldman,L. and Bennett,J.C. (eds), *Cecil Textbook of Medicine*, 21st edn. WB Saunders Co., p. 2198.
28. Mandhane,S.N. and Chopde,C.T. (1997) Neurobehavioral effects of low lewel fenvalerate exposure in mice. *Indian J. Exp. Biol.*, **35**, 623–627.
29. Tokumitsu,T. (1976) Chromosomal study on mouse intestinal mucosal cells, with a note on aberrations induced by a carcinogen, MNNG. *Proc. Jpn Acad.*, **52**, 513–516.
30. Matsuoka,A., Yamazaki,N., Suzuki,T., Hayashi,M. and Sofuni,T. (1993) Evaluation of micronucleus test using a Chinese hamster cell line as a alternative to the conventional *in vitro* chromosomal aberration test. *Mutat. Res.*, **272**, 223–236.
31. 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.
32. Agarval,D.K. and Chauhan,L.K.S. (1993) An improved chemical substitute for fetal calf serum for the micronucleus test. *Bioctech. Histochem.*, **68**, 187–188.
33. Rawlings,J.O. and Pantula,S.G. (2001) *Applied Regression Analysis: A Research Tool*, *Springer Text in Statistics*. Springer-Verlag, New York, p. 675.
34. Meyers,O. (1993) Implications of animal welfare on toxicity testing. *Hum. Exp. Toxicol.*, **12**, 516–521.
35. ICH (1997) Genotoxicity, A standard Battery for Genotoxicity Testing of Pharmaceuticals Guideline S2B. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.
36. Igarashi,M. and Shimada,H. (1997) An improved method for the mouse liver micronucleus test. *Mutat. Res.*, **391**, 49–55.
37. Nishikawa,T., Haresaku,M., Adachi,K., Masuda,M. and Hayashi,M. (1999) Study of a rat skin *in vivo* micronucleus test: data generated by mitomycin C and methyl methanesulfonate. *Mutat. Res.*, **444**, 159–166.
38. Ohyama,W. and Tokumitsu,T. (1996) An *in vivo* micronucleus test using colonic epithelial cells of mice. *Environ. Mut. Res. Commun.*, **17**, 265–269.
39. Ohyama,W., Gonda,M., Miyajima,H. *et al*. (2002) Collaborative validation study of the *in vivo* micronucleus test using mouse colonic epithelial cell. *Mutat. Res.*, **518**, 39–45.
40. Gomez-Arroyo,S., Diaz-Sanchez,Y., Meneses-Perez,M.A., Villalobos-Pietrini,R., De Leon-Rodriguez,J. (2000) Cytogenetic biomitoring in a Mexican floriculture worker group exposed to pesticides. *Mutat. Res.*, **466**, 117–124.
41. Heddle,J.A., Cosentino,L., Darwod,G., Swiger,R.R. and Paashuis- Lew,Y. (1996) Why do stem cell exist? *Environ. Mol. Mutagen.*, **28**, 334–341.
42. Dianovsky,J. and Sivikova,K. (1995) *In vivo* and *in vitro* cytogenetic effects of supermethrin. *Biomed. Environ. Sci.*, **8**, 359–366.
43. Wargovich,M.J., Goldberg,M.T., Newmark,H.L. and Bruce,W.R. (1983) Nuclear aberrations as a shortterm test for genotoxicity to the colon: evaluation of nineteen agents in mice. *J. Natl Cancer Inst.*, **71**, 133–137.
44. Migliore,L., Cocchi,L. and Scarpato,R. (1996) Detection of the centromere in micronuclei by fluorescence *in situ* hybridization: its application to the human lymphocyte micronucleus assay after treatment with four suspected aneugens. *Mutagenesis*, **11**, 285–290.
45. Blakey,D.H., Duncan,A.M.V., Wargovich,M.J., Goldberg,M.T., Bruce,W.R. and Heddle,J.A. (1985) Detection of nuclear anomalies in colonic epithelium of the mouse. *Cancer Res.*, **45**, 242–249.
46. Krishna,G. and Hayashi,M. (2000) *In vivo* rodent micronuceleus assay: protocol, conduct and data interpretation. *Mutat. Res.*, **455**, 155–166.
47. Schmid,W. (1975) The micronucleus test. *Mutat. Res.*, **31**, 9–15.
48. Pati,P.C. and Bhunya,S.P. (1989) Cytogenetic effects of fenvalerate *in vivo* test system. *Mutat. Res.*, **222**, 149–154.
49. Nakano,E., Rabella-Gay,M.N. and Pereira,C.A. (1996) Evaluation of the genotoxic potential of flumethrin in mouse bone marrow chromosomal analysis and micronucleus test. *Teratog. Carcinog. Mutagen.*, **16**, 37–48.
50. Tyrkiel,E., Wiadrowska,B. and Ludwicki,J.K. (2001) Comparative study of effect of synthetic pyrethroids on the induction on genetic changes n mice somatic and sex cells depeding on exposure route. *Rocz. Pantsw. Zakl. Hig.*, **52**, 97–109.
51. Surrales,J., Xamena,N., Creus,A., Catalan,J., Norppa,H. and Marcos,R. (1995) Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. *Mutat. Res.*, **341**, 169–184.

Received on October 20, 2004; revised on January 6, 2005; accepted on February 8, 2005