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The effect of low doses of chlorpyrifos on blood and bone marrow cells in Wistar rats

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The aim of this study was to investigate the genotoxic potential of low doses of chlorpyrifos (CPF) on blood and bone marrow cells in adult male Wistar rats. CPF was administered by oral gavage at daily doses of 0.010, 0.015, and 0.160 mg/kg of body weight (bw) for 28 consecutive days. Positive control (PC) was administered 300 mg/kg bw/day of ethyl methane sulphonate (EMS) for the final three days of the experiment. Toxic outcomes of exposure were determined with the *in vivo* micronucleus (MN) assay and alkaline comet assay. The 28-day exposure to the 0.015 mg/kg CPF dose, which was three times higher than the current value of acute reference dose (ARfD), reduced body weight gain in rats the most. The *in vivo* MN assay showed significant differences in number of reticulocytes per 1000 erythrocytes between PC and negative control (NC) and between all control groups and the groups exposed to 0.015 and 0.160 mg/kg bw/day of CPF. The number of micronucleated polychromatic erythrocytes per 2000 erythrocytes was significantly higher in the PC than the NC group or group exposed to 0.015 mg/kg bw/day of CPF. CPF treatment did not significantly increase primary DNA damage in bone marrow cells compared to the NC group. However, the damage in bone marrow cells of CPF-exposed rats was much higher than the one recorded in leukocytes, established in the previous research. Both assays proved to be successful for the assessment of CPF-induced genome instability in Wistar rats. However, the exact mechanisms of damage have to be further investigated and confirmed by other, more sensitive methods.

KEY WORDS: alkaline comet assay; body weight changes; genotoxicity; *in vivo* micronucleus assay; low doses

Owing to biomagnification and presence in all environmental compartments, pesticides pose a significant threat to plant, animal, and human health (1, 2). Their harmful effects are mostly mediated by reactive oxygen species (ROS), oxidative tissue damage, or their covalent bonds with cellular macromolecules (3, 4). Although the number of studies focused on biological effects of currently used pesticides has increased in the last two decades (5–17), the issue of pesticide genotoxicity remains controversial.

Chlorpyrifos (CPF) (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), which is the subject of our study, is an organophosphate (OP) insecticide with known genotoxic and teratogenic potential (18–28). Animal studies report that it causes hepatic dysfunction (18, 29, 30), immunological abnormalities (31–33), embryo- and foetotoxicity (26, 34–36), neurobehavioral (37) or neurochemical changes (38), and testicular damage (39–41).

In order to establish the best experimental model for this research in line with the EU Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides (42), CPF doses we selected were based on toxicological reference values, including the acceptable daily intake (ADI), acute reference dose (ARfD), and the acceptable operator exposure level (AOEL). Previous studies tested CPF at high doses delivered via different routes of exposure over different lengths of time.

We chose bone marrow model for more detailed evaluation since CPF toxicity at this level is almost unknown, especially at low, real-life doses. Bone marrow is a tissue where blood cells are produced and consists of a number of dividing cells, which makes it suitable for the assessment of DNA damage using the comet assay. This method is a well-established for sensitive detection of genome instability (43, 44). It is widely used in genetic toxicology and environmental biomonitoring. It can detect short-lived DNA damage, namely single- and double-strand breaks, DNA adducts, and DNA–DNA or DNA–protein cross-links (45–48). It can also be used to study DNA repair (49, 50) and alkylation damage (51).

Little is known about CPF genotoxicity in the bone marrow. One study that investigated DNA damaging effect of CPF formulation on rat bone marrow cells was that of Yahia and Ali (52). While their approach was to combine comet assay with chromosome aberration analysis, in our research we applied the rodent *in vivo* micronucleus (MN) assay instead. This method relies on the detection of micronucleated polychromatic erythrocytes (MN-PCE) originated from the bone marrow of rodents. Increased

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MN frequency in the blood of exposed animals specifically shows damage to the chromosomes or mitotic spindle (53, 54).

The aim of our study was therefore to expand the knowledge of CPF genotoxicity in the bone marrow with new data by investigating toxic outcomes of exposure to low oral daily doses of CPF over 28 consecutive days in adult male Wistar rats. We also wanted to gain new data about the occurrence of MN in rat erythrocytes after CPF exposure, which has not been investigated so far.

MATERIAL AND METHODS

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee and the Croatian Ministry of Agriculture (Reg. No.: 525-10/0255-14-2; file class UP/I-322-01/14-01/75 of 12 September 2014) as well as the Ethics Committee of the Institute for Medical Research and Occupational Health (IMROH) (Reg. No.: 100-21/14-6; file class: 01-18/14-02-2/6 of 11 June 2014). It was carried out in compliance with international standards and national legislation for animal welfare protection.

Chemicals and reagents

Chlorpyrifos 99.9 % (CAS No. 2921-88-2) was purchased as analytical standard PESTANAL® (Sigma-Aldrich Laborchemikalien GmbH; Seelze, Germany). The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA): ehylenediaminetetraacetic acid (EDTA) disodium salt dehydrate, ethyl methane sulphonate (EMS), Tris-HCl, Triton X-100, ethidium bromide, low-melting point (LMP) and normal melting point (NMP) agarose, and acridine orange. Kemika (Zagreb, Croatia) supplied ethanol, NaOH, KCl, and NaCl. Foetal bovine serum (FBS) was purchased from Gibco® Life Technologies (Grand Island, NY, USA).

Animals

Thirty male Wistar rats used in this study were bred until adult age (three months) at the IMROH Animal Breeding Unit (Zagreb, Croatia). We selected only male rats to avoid potential sex-related differences in results. Before the experiment, they were randomised into six groups of five rats. The decision to minimise the sample was based on the EU Directive 2010/63 (55). Body weights of the selected rats were in the range 286–338 g. Rats were kept at 22 °C in stable microenvironmental conditions, with 12 h light/dark cycle. They had free access to tap water and food (4RF21 Complete feed for mice and rats, Mucedola srl, Settimo Milanese, Italy).

Experimental design

The stock pesticide solution was prepared by reconstitution of CPF powder in ethanol and diluted with saline for treatment of rats. The percentage of ethanol in CPF solutions administered to animals did not exceed 0.03 % [this concentration was used as solvent control (SC)].

We tested the effects of CPF at daily doses of 0.010 mg/kg bw (10× the current ADI and AOEL values), 0.015 mg/kg bw (3× the current value of ARfD), and 0.160 mg/kg bw [taken from the World Health Organization (WHO) (56) and the European Food Safety Authority (EFSA) report (57)].

Chlorpyrifos was administered orally with the gastric tube (1 mL of CPF solution per rat a day) for 28 days. Body weights were monitored once a week, and CPF doses adjusted accordingly.

Appropriate negative (NC), SC, and positive control (PC) groups were studied in parallel. Negative control (NC) received 1 mL of PBS instead of CPF. The PC group was receiving a well-known genotoxic agent ethyl methane sulphonate (EMS) (58) at a daily dose of 300 mg/kg bw over the final three days of the experiment.

All animals were inspected daily by a licensed veterinarian at IMROH to assess survival, clinical signs of toxicity, and body weight changes. Body weight of each animal (expressed in grams) was measured at the beginning and the end of the experiment. Body weight gain was calculated according to the following formula: [(Final weight - Initial weight) × 100] / Initial weight.

The experiment was terminated 24 h after the final gavage. Animals were humanely euthanised by exsanguination under intraperitoneal anaesthesia with a combination of xylazine (Xylapan, 12 mg/kg bw) and ketamine (Narketan, 80 mg/kg bw), both produced by Vetoquinol UK Ltd. (Towcester, UK). During dissection, the rats were examined by a licensed veterinarian for possible internal organ abnormalities. Blood samples for the *in vivo* MN assay were collected from the carotid artery, without anticoagulant, and immediately pipetted onto slides pre-coated with acridine orange (AO). Bone marrow samples were collected simultaneously and processed as described below.

MN *in vivo* – supravital acridine orange (AO) staining

AO-coated microscope slides were prepared using a water solution of the stain (1 mg/mL, V =10 μL per slide), which was carefully applied on the surface of the slide, as recommended by Hayashi et al. (59). A drop of blood (V=5 μL) was then pipetted on the pre-coated slide, covered with a coverslip, and stored in a box protecting it from light until the analysis. Slides were analysed for the presence of micronuclei in reticulocytes and erythrocytes with a 1000× magnification fluorescence microscope (Leitz Orthoplan, Oberkochen, Germany) equipped with a blue excitation filter of 502–525 nm.

Reticulocytes, i.e. young RNA-containing erythrocytes (or polychromatic erythrocytes, PCE), emitted orange-red fluorescence. Cells with a few red fluorescent dots were not regarded as reticulocytes. Mature erythrocytes (or normochromatic erythrocytes, NCE) had green colour. Round structures which emitted a bright yellow-green fluorescence were recorded as micronuclei (MN).

Microscopic analysis included MN counts per 2000 polychromatic erythrocytes (PCE), reticulocyte (or PCE) count per 1000 NCE, and MN frequency in 4000 randomly selected reticulocytes per rat (two independent evaluations on two replicate preparations were made).

Bone marrow dissection and isolation of bone marrow cells

Both femurs were taken out by dissection, cleaned to remove muscles and other tissues. The epiphyses were cut off and the bone marrow gently flushed with 2 mL of foetal bovine serum (FBS) and aspirated with a syringe, and then centrifuged at 390 *g* for 10 min). The obtained pellet was washed with phosphate buffered saline (Ca^{2+} and Mg^{2+} free PBS) to remove FBS. Cells were resuspended in PBS and used to prepare slides for the alkaline comet assay.

Alkaline comet assay

Microscope slides (Vitrognost Plus Ultra, Biognost, Zagreb, Croatia) pre-coated with 1 % NMP agarose were prepared earlier and stored in a tightly closed plastic box until use. Shortly after collection, bone marrow samples were further processed according to the standard comet assay protocol (60) with some adjustments as reported elsewhere (61). For each experimental group, two replicate preparations were made. The first layer of microgel consisted of 0.6 % NMP agarose. To prepare the second layer, bone marrow cell suspension (V=20 μL per slide) was mixed with 0.5 % LMP agarose (V=100 μL per slide). The top microgel layer consisted of 0.5 % LMP agarose. Preparations were transferred into a cold freshly prepared lysis buffer (2.5 mol/L NaCl, 100 mmol/L EDTA, 10 mmol/L Tris, 10 % DMSO, 1 % Triton X-100, pH 10, 4 °C). Lysis lasted for 1 h at +4 °C, and was followed by denaturation in cold alkaline buffer (pH>13), composed of 300 mmol/L NaOH and 1 mmol/L EDTA. After 20 min of denaturation, the preparations were placed in an electrophoresis unit (Horizon 11.14, Whatman, Florham Park, NJ, USA) filled with the same buffer. The electrophoresis was run for 20 min at +4 °C, and 0.86 V/cm (61). Thereafter the microgels were neutralised in 0.4 mol/L Tris-HCl (pH 7.5), rinsed with re-distilled water, dehydrated in 70 % and 96 % ethanol for 10 min each, and allowed to dry at room temperature.

The slides were stained with 20 μg/mL ethidium bromide immediately before analysis under a fluorescence microscope (Olympus BX-51, Olympus, Tokyo, Japan) with a charge coupled device (CCD) camera. Comets were captured at 200× magnification.

Slides were analysed with the Comet Assay IV™ image analysis system (Instem-Perceptive Instruments Ltd., Suffolk, Halstead, UK). One hundred and fifty randomly selected comets per slide were scored (representing a total of 300 comets per animal or 1500 comets per experimental group). Microgel areas near to slide edges or around entrapped air bubbles were excluded from the scoring (62). Since the nucleoids with no or small head and long diffuse tails may indicate cytotoxicity-related DNA damage (63, 64), we recorded only those nucleoids which had <80 % DNA in the tail region. We used tail intensity (% DNA in comet tail) as a descriptor of the DNA damage.

Statistical analysis

For statistical analysis we used the STATISTICA software (Data Science Workbench, version 14; License No. 14.0.0.15; TIBCO Software Inc., 2020; Palo Alto, CA, USA).

The homogeneity of variances of *in vivo* MN assay data was tested with Levene's test at a significance level of 5 %. If variances of the data were homogenous, we used one-way ANOVA. The means were further compared using *post hoc* Tukey's HSD test. If variances were not homogenous even after log-normalisation, we used the non-parametric Mann-Whitney *U* test.

Comet assay results were evaluated with one-way ANOVA, after we tested the normality of distribution with the Shapiro-Wilk test. For *post hoc* analysis, we used Tukey's HSD test.

Differences between groups were considered significant when $p<0.05$.

RESULTS

All animals survived the 28-day treatment with orally applied low-doses of CPF without signs of internal organ abnormalities. Exposure-related changes in body weights are shown in Figure 1. Exposure to 0.015 mg/kg bw/day of CPF lowered body weight gain the most. Statistically significant differences in the body weight gain were: PC vs NC (p=0.0098), PC vs SC (p=0.0004), SC vs NC (p=0.0105), SC vs 0.010 mg/kg bw/day of CPF (p=0.0183), SC vs 0.015 mg/kg bw/day of CPF (p=0.0004), and SC vs 0.160 mg/kg bw/day of CPF (p=0.0373).

The results of the *in vivo* MN assay are presented in Figures 2–4. Significant differences for the number of RETs per 1000 erythrocytes were observed between PC and NC samples and between the groups treated with 0.015 and 0.16 mg/kg bw/day of CPF (Figure 2). Figure 3 shows the number of MN-PCE scored in 2000 PCE per each experimental group. This descriptor is regarded as an indicator of genotoxicity. The highest number of MN-PCE was found in the PC sample (Figure 3) and was significantly higher than in the NC and the group treated with 0.015 mg/kg bw/day of CPF. As for the number of MN in 4000 reticulocytes, again, the PC group had the highest damage level, significantly higher than all other control and CPF-treated groups.

Table 1 shows the results of the alkaline comet assay in bone marrow cells. CPF treatment did not cause statistically significant increase in primary DNA damage compared to NC. The PC group had the highest level of primary DNA damage, significantly higher compared to all other groups. No other inter-group differences in DNA damage were significant.

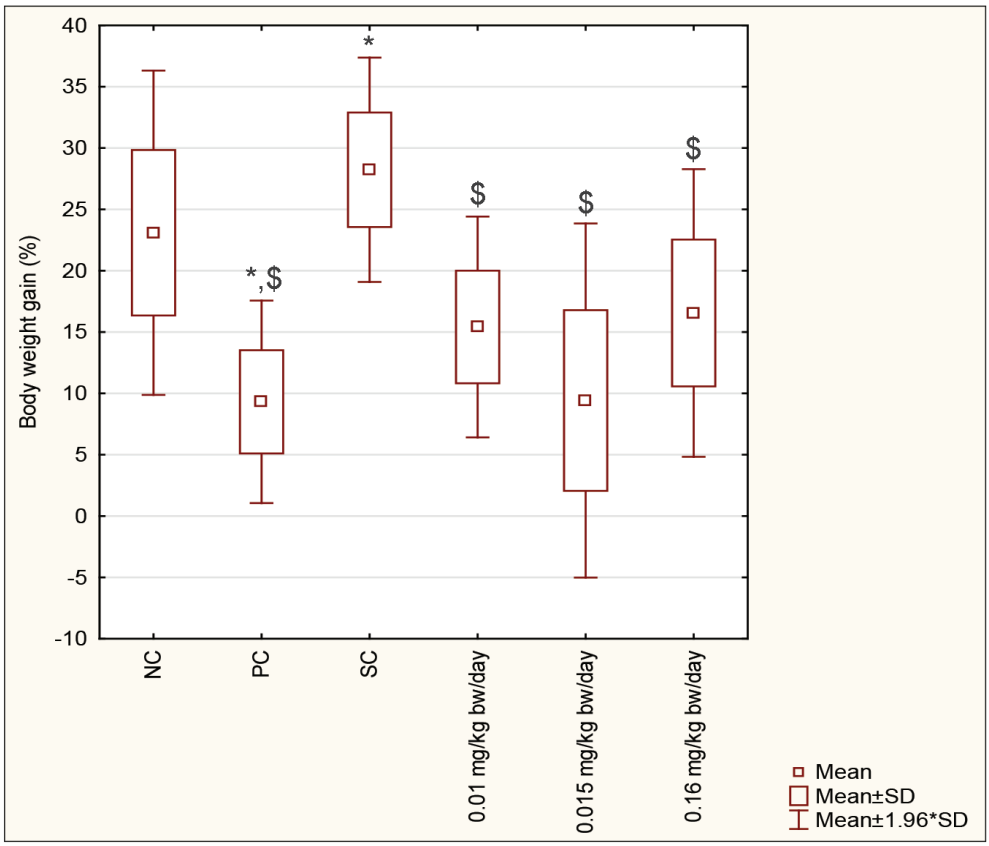

Figure 1 Effect of sub-chronic oral 28-day exposure to chlorpyrifos (CPF) on body weight gain (%, mean ± standard deviation) in adult male Wistar rats (N=5 per group). NC – negative control (NC); PC – positive control (ethyl methane sulphonate); SC – solvent control (0.03 % ethanol). * – significantly different from NC; $ – significantly different from SC ($p<0.05$)

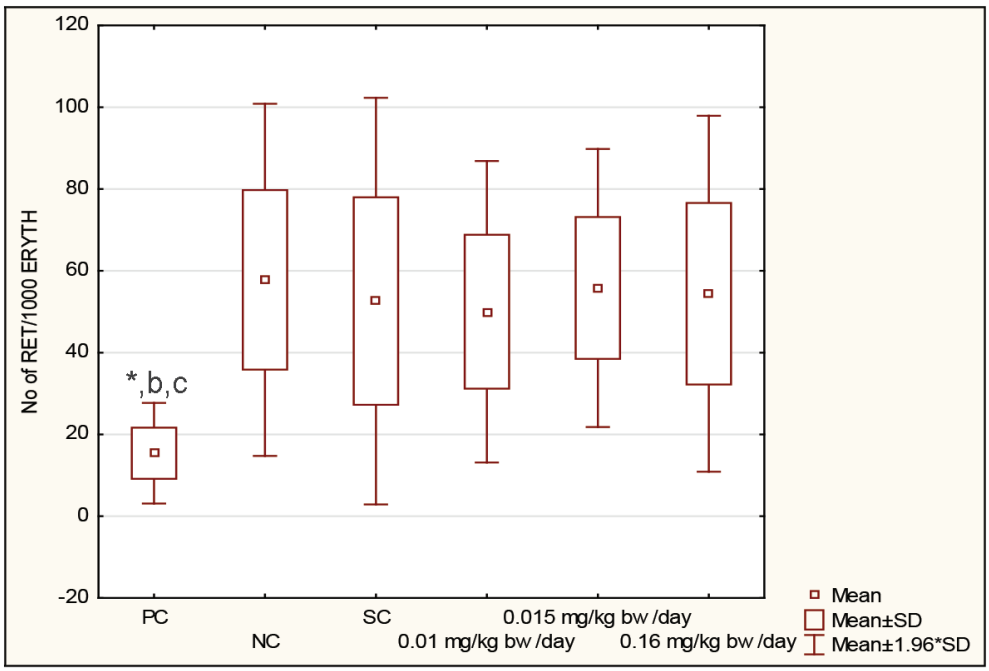

Figure 2 Number of reticulocytes (mean ± standard deviation) per 1000 erythrocytes in adult male Wistar rats (N=5 per group) after sub-chronic oral 28-day exposure to chlorpyrifos. NC – negative control (NC); PC – positive control (ethyl methane sulphonate); SC – solvent control (0.03 % ethanol). * – significantly different from NC; b – significantly different from 0.015 mg/kg bw/day of CPF; c – significantly different from 0.015 mg/kg bw/day of CPF ($p<0.05$)

Table 1 Effects of sub-chronic, oral, 28-day exposure to chlorpyrifos on primary DNA damage (tail DNA %) in bone marrow cells of adult male Wistar rats (N=5 per group) assessed with the alkaline comet assay

Experimental group	Tail intensity (DNA %)
Negative control	3.00±0.12 0.47 0–27.83
Positive control (ethyl methane sulphonate)	16.33±0.32* 15.33 0–47.55
Solvent control (0.03 % ethanol)	3.58±0.15 0.46 0–31.96
CPF 0.010 mg/kg bw/day	3.09±0.12 0.48 0–30.68
CPF 0.015 mg/kg bw/day	3.21±0.14 0.42 0–38.85
CPF 0.160 mg/kg bw/day	2.70±0.13 0.31 0–54.96

All measurements were done in duplicate. The results are shown as mean value ± standard error of the mean (first row), median (second row), and range (third row). * – significantly different from all other experimental groups [one-way ANOVA with Tukey's HSD *post hoc* test (F=123.6822; p=0.000)]

DISCUSSION

To the best of our knowledge, there are not many studies with CPF in a rat model that could be used for reliable comparison. What makes our study unique is the very low level of the tested doses. Most pesticide toxicity studies used significantly higher dose levels, which produced much higher genome damage. They also differ greatly in exposure routes.

When we planned this research, we chose the oral route because it is relevant to humans and could maximise the delivery of pesticide to the target tissue, while gavage was used to allow more precise dosing (65). In this preliminary phase of the study, we selected only male rats to avoid potential hormone-related effects in results. Furthermore, we conducted the research on the smallest possible number of animals. Both assays we used were previously confirmed as sensitive to detect the genome damage produced after exposure to various xenobiotics. In their work, Gianotti et al. (66) showed that comet assay can detect the short-lived DNA damage. Krishna and Hayashi (67) showed that the *in vivo* micronucleus assay detects the structural and numerical chromosomal damage.

Our study demonstrated reduced body weight gain by the CPF dose of 0.015 mg/kg bw/day (three times higher than the current value of ARfD). Previous studies reported body weight changes with much higher doses of CPF. Several studies (18, 31, 32, 68, 69) found that CPF exposure caused an increase in rat bw compared to controls. The observed gain was attributed to an increase in adipose tissues. Other researchers (70, 71) showed that rats exposed

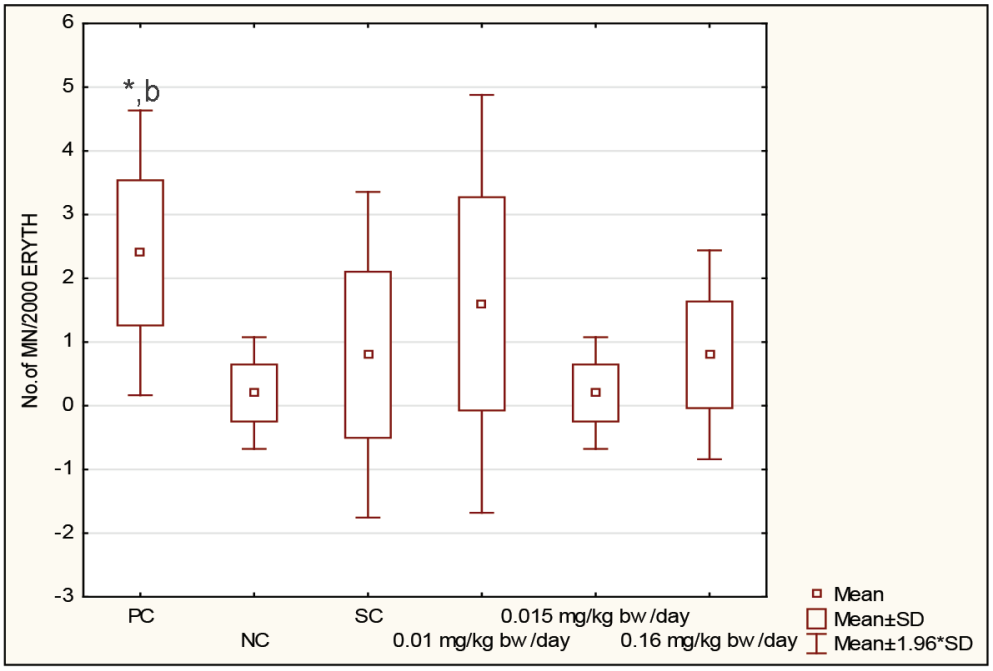

Figure 3 Number of micronuclei (mean ± standard deviation) in 2000 polychromatic erythrocytes in adult male Wistar rats (N=5 per group) after sub-chronic oral 28-day exposure to chlorpyrifos. NC – negative control (NC); PC – positive control (ethyl methane sulphonate); SC – solvent control (0.03 % ethanol). * – significantly different from NC; b – significantly different from 0.015 mg/kg bw/day of CPF ($p<0.05$)

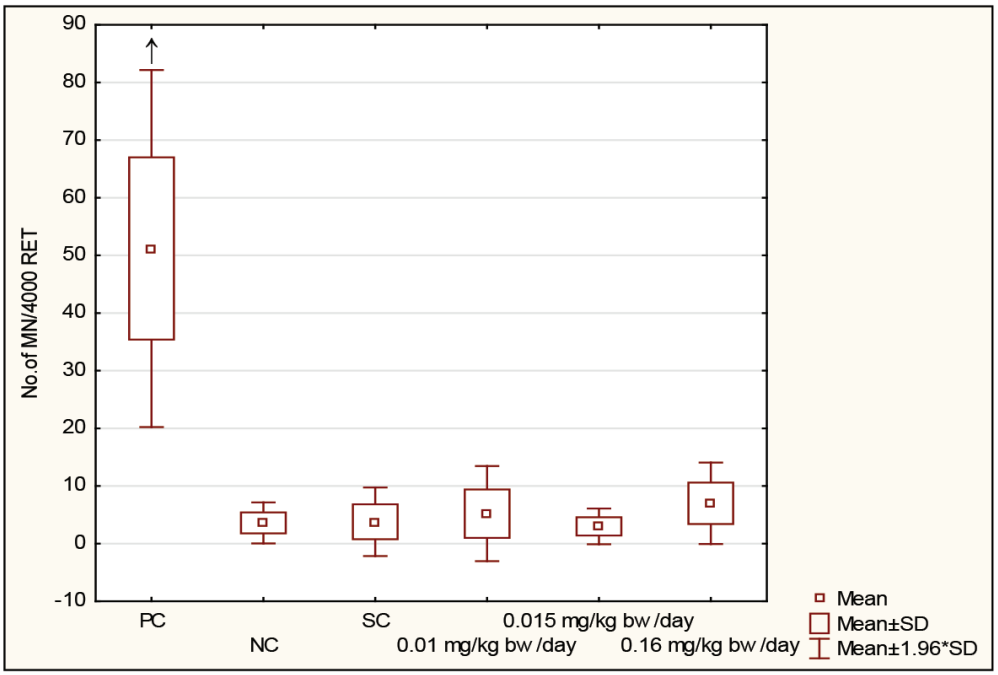

Figure 4 Number of micronuclei (mean ± standard deviation) in 4000 reticulocytes in adult male Wistar rats (N=5 per group) after sub-chronic oral 28-day exposure to chlorpyrifos (CPF). NC – negative control (NC); PC – positive control (ethyl methane sulphonate); SC – solvent control (0.03 % ethanol). ↑ – significantly different from all experimental groups ($p<0.05$)

to CPF doses of 7–10 mg/kg per day had fluctuations in body weight. The mechanisms behind the effects on body weight gain observed in our study remain to be elucidated by further research. Even though we cannot explain them presently, such response has already been reported with other organophosphate insecticides (fenitrothion, diazinon) (72, 73).

As for the frequency of MN-PCE as an indication of chromosome damage (53, 74), the two higher CPF doses tested in our study showed significantly higher damage and genotoxic potential towards haematopoietic progenitor cells. Ezzi et al. (18) reported that CPF induced the formation of bone marrow micronuclei in a dose-dependent manner by clastogenic and aneugenic mode of action. Similar clastogenic mechanism of action of other OP insecticides was observed in *in vivo* studies on mice (75–77). Furthermore, these studies showed that the PCE to NCE ratio is an indicator of toxicity affecting cell formation in the bone marrow. Our observations match well literature findings and provide additional evidence of CPF potential to induce MN as one of the mechanisms behind genotoxicity of this insecticide *in vivo*.

Since there are no related comet assay studies on CPF-exposed rats, it is not possible to draw a parallel between our findings and the existing literature sources or to propose mechanisms behind the observed primary DNA damage. Our earlier study (19) showed that 28-day oral exposure to low doses of CPF resulted in a significant increase in primary DNA damage in rat leukocytes compared to control. Mean tail intensities varied between 1.08 % and 2.16 % of DNA in the comet's tail. In bone marrow cells in this study mean tail intensities were even higher and varied between 2.70 to 3.21 % of DNA in the comet's tail, yet they did not differ significantly from negative control or any other group. The reason for this may be in inherent differences between leukocytes and bone marrow cells. They differ in their capacity for DNA repair and also have different potential for replacement of highly injured cells. In addition, higher DNA damage in bone marrow cells may be related to higher metabolic activity in this cellular matrix, but this assumption has to be verified by future studies.

As can be seen in Table 1, the comet assay did not show a clear dose response with CPF. Tail intensities in the 0.010 and 0.015 mg/kg bw/day CPF groups were both higher than those in the 0.160 mg/kg bw/day group. It is possible that the highest CPF dose produced such high cell damage that they were destroyed during microgel processing for the comet assay and lost from scoring, as has been reported elsewhere (64), so the damage level in that sample mostly refers to cells with lower damage. In such conditions, less damaged nucleoids will be measured, and the obtained values would be lower than the real damage. Another explanation could be more efficient DNA repair (78).

Yahia and Ali (52) tested much higher doses than those applied in our study (the CPF dose applied orally twice a week for 30 days was 8 mg/kg bw, which corresponded to 1/20 of its LD_{50}). They found a significant decrease in red blood cells count, haemoglobin, haematocrit, mean cell haemoglobin concentration, and red cell distribution width. There was a significant decrease in lymphocyte and monocyte counts. They observed a significant time-related DNA damage. The comet assay predictors they measured (tail length, tail moment, and the percentage of tail DNA) also increased significantly compared to control. They also observed polyploidy and various types of chromosomal aberrations in CPF exposed groups (breaks, deletion, attenuation, chromosome ring, gap and fragments). Over four weeks of CPF exposure in rats, Ezzi et al. (18) also documented

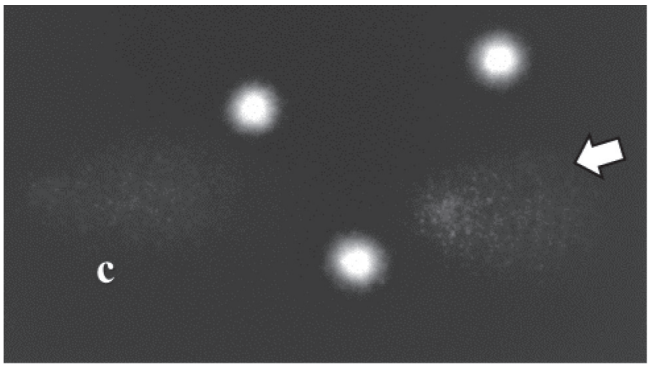

Figure 5 Photomicrograph of DNA damage in the bone marrow of adult male Wistar rats exposed to low doses chlorpyrifos by oral gavage for 28 consecutive days. The three bright nucleoids show low primary DNA damage; white arrow indicates a long-tailed nucleoid with extensive DNA damage. Letter **c** indicates a diffuse nucleoid with DNA damage possibly related to cytotoxicity

a significant increase in the comet tail length of blood cells. Similar findings were reported Muller et al. (20). Using alkaline comet assay, Ojha et al. (79) demonstrated that CPF induced DNA damage in the liver, brain, kidney, and spleen of rats even after administration of a single dose. Results reported by Sandhu et al. (22) indicate that shorter (seven- and 14-day) treatments of rats with 3 and 12 mg/kg bw of CPF produced a dose-related increase in DNA damage in lymphocytes, which was more pronounced in male rats. They also found a significant increase in the frequency of binucleated cells after treatment with 12 mg/kg bw of CPF.

CONCLUSIONS

Despite all limitations, we believe that our *in vivo* findings provide new useful information for the safety assessments of CPF. The MN and the alkaline comet assay have shown that 28-day exposure to low doses of CPF can produce genotoxic effects in bone marrow and blood cells of Wistar rats. Although both assays were sufficient to evidence CPF-induced genome instability, they cannot reveal the exact mechanisms behind the damage, which needs to be further investigated and confirmed by other, more sensitive methods such as flow cytometry and different enzyme-modified comet assay modifications that detect specific DNA lesions.

Conflicts of interest

None to declare.

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Utjecaj niskih doza klorpirifosa na krvne i stanice koštane srži štakora

Istražen je genotoksični potencijal niskih doza klorpirifosa na uzorcima krvi i stanica koštane srži u odraslih mužjaka štakora soja Wistar. Pokusnim je životinjama klorpirifos bio 28 dana oralno apliciran pomoću sonde u dnevnim dozama od 0,010 mg/kg t. m., 0,015 mg/kg t. m. i 0,160 mg/kg t. m. Kao pozitivna kontrola korišten je etil metan sulfonat (EMS) u dozi od 300 mg/kg t. m. tijekom posljednja tri dana pokusa. Toksični ishodi izloženosti klorpirifosu istraženi su primjenom *in vivo* mikronukleus (MN) testa i alkalnoga komet-testa. Utvrdili smo da je 28-dnevna izloženost klorpirifosu u dozi od 0,015 mg/kg t. m./dan, koja je trostruko viša od važeće vrijednosti akutne referentne doze, u najvećoj mjeri smanjila prirast tjelesne mase štakora. Rezultati MN-testa upućuju na značajne razlike u broju retikulocita na 1000 eritrocita između pozitivne i negativne kontrole te između obiju kontrola i skupina izloženih klorpirifosu u dnevnim dozama 0,015 i 0,160 mg/kg t. m. Broj polikromatskih eritrocita s mikronukleusima na 2000 eritrocita u pozitivnoj kontroli bio je značajno povećan u usporedbi s negativnom kontrolom te s uzorcima krvi štakora izloženih klorpirifosu u dnevnoj dozi od 0,015 mg/kg t. m. Izloženost CPF-u nije uzrokovala statistički značajan porast razine primarnih oštećenja DNA u stanicama koštane srži u usporedbi s razinama spontanih oštećenja DNA, izmjerenima alkalnim komet-testom u negativnoj kontroli. Međutim, razine oštećenja u stanicama koštane srži štakora izloženih klorpirifosu bile su značajno više od onih zabilježenih u leukocitima, koje su poznate iz prethodnih istraživanja. Oba su se testa pokazala uspješnima za procjenu nestabilnosti genoma izazvanih klorpirifosom u Wistar štakora. Međutim, točni mehanizmi oštećenja moraju se dodatno istražiti i potvrditi drugim osjetljivijim metodama.

KLJUČNE RIJEČI: alkalni komet-test; genotoksičnost; *in vivo* mikronukleus test; niske doze; promjene tjelesne mase