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TESTING THE POTENTIAL CLASTOGENIC/ CYTOTOXIC EFFECTS OF PESTICIDE CALYPSO 480 SC

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ABSTRACT

The detection of chromosomal damage serves as a tool for the verification of the genotoxic effects of chemical substances *in vitro*. We used conventional cytogenetic analysis in order to test for the potential genotoxic action of the insecticide thiacloprid (the active ingredient in commercial preparation CALYPSO 480 SC). The test cultures of bovine lymphocytes obtained from the peripheral blood were incubated with the insecticide in concentrations of: 30, 120, 240 and 480 μg.ml^{-1} for 24 and 48 hours. After 24 hours of incubation, we observed that the increasing concentrations resulted in a significant ($P < 0.05$; $P < 0.01$) increase in the frequency of DNA damage. Our experiments showed the presence of aberrations of a non-stable type (chromatid and chromosome breakage). The conventional chromosome analysis was supplemented with fluorescence *in situ* hybridization for the detection of numeric and stable structural aberrations. Whole chromosome probes for bovine chromosomes 1, 5 and 7 (BTA 1, BTA 5 and BTA 7) were used in the experiments.

Key words: conventional cytogenetics; chromosomal aberrations; fluorescence *in situ* hybridization

INTRODUCTION

Pesticides are a heterogeneous group of chemical substances designed specifically for the protection against pests. The intensive application of these substances in agriculture results in heavy contamination and degradation of the environment, which is reflected in the health of humans and other animals, and the presence of various residues in the food of animal origin [14].

Genotoxicity and its impact on the body are considered the most serious side effects of agricultural chemicals [2]. Their negative influence is amplified by other factors, such as environmental pollution, presence of heavy metals, nitrates and many other contaminants in our food and water.

There is available, a range of methods and models that can be used in a multistage system of evaluation of genetic risk related to the action of mutagens. One of the most important biological parameters reflecting exposure to geno-

toxic substances are chromosomal aberrations (CA) detected in lymphocytes of the peripheral blood [12]. They include changes in the structure or number of chromosomes in the cell genome that considerably contribute to the development of tumorous diseases, reproduction disorders (reduced fertility, increased embryonal mortality, abortions, perinatal mortality) and inherent malformations. In addition to the evaluation of chromosomal aberrations, the genotoxic effects of chemicals can be confirmed by other cytogenetic markers, such as sister chromatid exchanges (SCE) and micronucleus frequences (MN).

Neonicotinoids are one of the most important groups of insecticides used in agriculture. Due to their high selectivity, they are suitable also for controlling skin parasites in dogs and cats [15]. They are broad-spectrum systemic insecticides acting as stomach and contact poisons. They persist little in the outer environment and do not accumulate in the tissue of mammals, so their toxicity to mammals is lower than that of the older classes of insecticides [3].

Their mechanism of action consists in blockage of the post-synaptic nicotinic acetylcholine receptors, which results in the disturbance of the transfer of impulses and subsequent paralysis and death. In insects, these receptors are localised exclusively in the CNS and thus they are much more sensitive to these insecticides than mammals [11].

The aim of our study was to test the potential clastogenic/cytotoxic effects of commercial insecticide CALYPSO 480 SC, with the effective ingredient of thiacloprid, in bovine lymphocytes. Fluorescence *in situ* hybridization was used for the detection of stable and numeric aberrations.

MATERIALS AND METHODS

The experiments were carried out using the peripheral blood from two clinically healthy young bulls of the Slovak spotted breed. Mitomycin C (MMC, Sigma, St. Louis, MO, USA, 0.4 $\mu g.ml^{-1}$) and ethylmethane sulphonate (EMS, Sigma, St. Louis, MO, USA, 250 $\mu g.ml^{-1}$) served as a positive control. Thiacloprid (CALYPSO 480 SC; thiacloprid 480 $g.l^{-1}$) was dissolved in water (negative control) and added before the final 24 and 48 hours of cultivation at concentrations of: 30, 120, 240 and 480 $\mu g.ml^{-1}$. Colchicine (Merck, Darmstadt, Germany) with a final concentration of 5 $\mu g.ml^{-1}$ was added 90 min before the termination of the cultivation.

The preparations with chromosomes in metaphase were prepared by a standard cytogenetic method. Structural aberrations were evaluated in 100 metaphases for each donor and concentration. The value of the mitotic index was determined by the calculation from the number of metapahases per total number of 1000 cells. The statistical significance of the effects of the insecticide tested on the induction of chromosomal aberrations and reduction of mitotic index in lymphocytes of bovine peripheral blood was determined by parametric $\chi 2$ test. The standard deviations (SD) were calculated using variation analysis.

The preparations intended for fluorescence *in situ* hybridization were subjected to the process following a 3-day storage. Whole chromosome fluorescence-labelled probes BTA 1, BTA 5 and BTA7 were used in the experiments. The probes were applied to lymphocyte cultures after a 48-hour exposure to the pesticide in a concentration of 30 $\mu g.ml^{-1}$. The results of hybridization were evaluated and recorded under a fluorescence microscope Nikon (Labophot 2A/2) by means of dual filters.

RESULTS

The hybridization experiments failed to detect chromosomal aberrations of a stable type. Of the numeric aberrations of the type of aneuploidia and polyploidia, we observed one polyploidy (Fig. 1).

The frequency of the induced chromosomal aberrations following the exposure to thiacloprid for the last 24 and 48 h is presented in Figures 2 a) b). A significant increase in aberrations was detected after the exposure to the two highest concentrations of 240 and 480 $\mu g.ml^{-1}$ ($P < 0.05$ and $P < 0.01$). In both donors, we detected a moderate reduction in the mitotic index (MI) which was insignificant with the exception of the concentration of 480 $\mu g.ml^{-1}$ in Donor 1 ($P < 0.05$) (Fig. 3a). Chromatid and chromosomal breakages were the most frequently detected types of chromosomal aberrations in both donors. We also evaluated gaps (achromatic lesions) which were not subjected to statistical analysis. After the prolonged action of the insecticide (48 h), we observed a moderate insignificant increase in the frequency of breakages in comparison with the control cultures. The decrease in the mitotic activity was dose-dependent and the changes were significant after exposure to the highest concentration of 480 $\mu g.ml^{-1}$ ($P < 0.001$) in

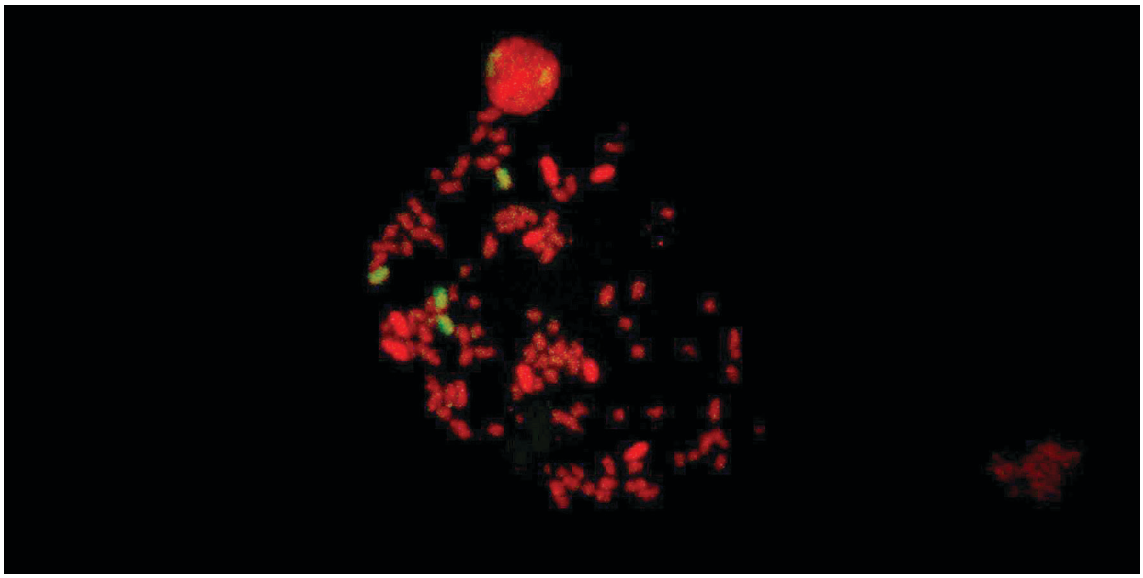

Fig. 1. Metaphase HD, 60 (XY), BTA 1 and BTA7-red, BTA5-green

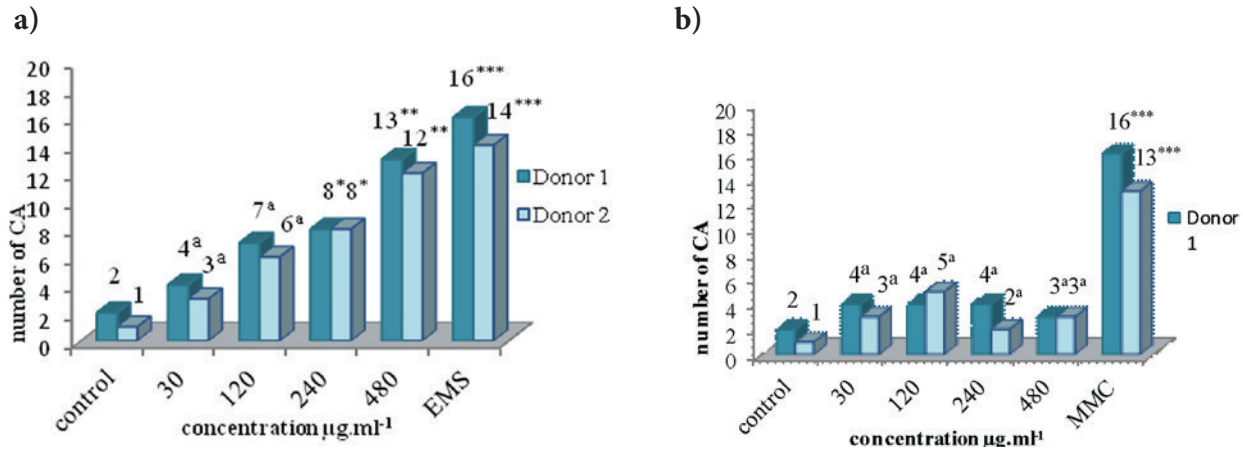

Fig. 2. Frequency of chromosomal aberrations after 24h (a) and 48 h(b) exposure to thiacloprid

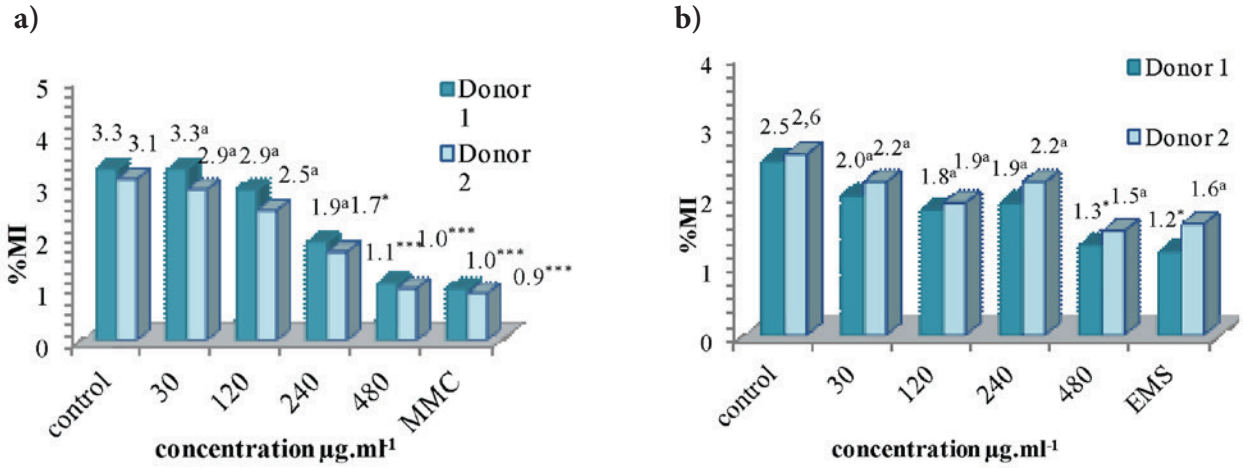

Fig. 3: Reduction in mitotic index after 24 h (a) and 48 h (b) exposure to thiacloprid

Donor 1 and to the two highest concentrations ($P < 0.05$ and $P < 0.001$) in Donor 2 (Fig. 3b).

DISCUSSION

The progressive development of industry and agriculture results in the increasing introduction of complex chemical substances of varying character and origin into the environment, such as agrochemicals, heavy metals, medicines and many various industrial wastes. We refer to them by the common name of xenobiotics. One of the most serious classes of xenobiotics are pesticides that are intensively applied in the agroindustry sector to control pests and weeds in order to ensure adequate protection of crop and increase agricultural yields. Pesticides belong among the most used chemicals around the world [4] and their negative impact on the environment is increasingly noticeable.

Farm animals exposed to the influence of pesticides may present risk to human health as they are an important part of the human food chain. Genotoxicity and its impact on the health of individuals is considered the most serious side-effect of pesticides [2]. Studies were conducted reporting the effects of the commercial fungicide Raxil with the active ingredient of tebuconasole [6], tolylfluanid [10], herbicide bifenox [14], insecticide bendiocarb [8] and others.

By the Decision of the European Comission from 2013, the use of three types of neonicotinic insecticides (imidacloprid, thiamethoxam and clothianidin) is currently limited due to their negative effect on honey bees. The fact that bees favour crops containing these substances raises a suspicion that they act as drugs and can gradually induce addiction in bees. At the same time, neonicotinic pesticides disturb their immune system, making them more susceptible to viral infections to which they are normally resistant [5].

The basis of confirmation of the genotoxic effects of a chemical *in vitro* is the detection of chromosomal damage linearly dependent on concentrations of the active ingredient, while the lowest dose should not cause any significant changes. The highest frequency of the induced chromosomal aberrations is observed during the first cellular cycle and is decreased to about half in subsequent divisions [1].

In our experiments, after a 24 h exposure, we observed a significant increase in aberrations after the application of the two highest concentrations of 240 and 480 $\mu g.ml^{-1}$ ($P < 0.05$ and $P < 0.01$). After a longer exposure to the insecticides (48 h), no clastogenic effect of thiacloprid was observed.

Chromosomal aberrations are recognised as a valuable biological marker of genotoxic effects and probably the only internationally standardized and validated cytogenetic biomarker [1]. Despite extensive use of thiacloprid there have not been published many studies involved in testing of its genotoxic effect. In V79 cells of Chinese hamsters no statistically or biologically important increase in chromosomal aberrations (96.8—97.2 %) was observed after their exposure to pure thiacloprid [13]. Kocaman et al. [9] described, in their study, genotoxic and cytotoxic/cytostatic effects of thiacloprid *in vitro* in human lymphocytes, where they had observed an increased frequency of chromosome aberrations, sister chromatid exchanges and micronucleus after application of all tested concentrations. Galdíková et al. [7] carried out complex evaluations of potential genotoxic effects of commercial preparation based on thiacloprid (CALYPSO 480 SC) *in vitro* in lymphocytes of the peripheral blood of cattle. In agreement with our results, they observed a significant increase in breakages starting from concentration of 120 $\mu g.ml^{-1}$, after 24 h of exposure to thiacloprid.

In the hybridization experiment we failed to record stable aberrations and observed one polyploidy included among numeric aberrations. The inability to detect any translocations in our experiments can be ascribed to the fact that we had at our disposal only three whole-chromosome probes and thus could investigate only a small percentage of the genome.

CONCLUSION

Our results obtained by conventional cytogenetic analysis allowed us to report the genotoxic effects of the insecticide thiacloprid on bovine peripheral blood lymphocyte chromosomes.

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