Effect of Tin and Lead Chlorotriphenyl Analogues on Fruit Fly *Drosophila hydei* and Liposomes Membrane

Slawomir Mitrus,[1] *and Dariusz Man*[2]

[1]Department of Biosystematics, Opole University, Oleska 22, 45-052 Opole, Poland; E-mail: *smitrus@uni.opole.pl*

[2]Institute of Physics, Opole University, Oleska 48, 45-052 Opole, Poland

Received 3 October 2011; revised 29 November 2011; accepted 17 December 2011

ABSTRACT: This article presents the results of a study investigating the biological activity of triphenyltin chloride (TPhT) and two metalloorganic compounds, triphenyllead chloride (TPhL) and triphenylmethane chloride (TPhC), in their interaction with model membranes and the living organisms of fruit flies *Drosophila hydei*. The study of model membranes (sonicated liposomes) was conducted using the electron spin resonance (ESR) spin probe technique, whereas the experiment on fruit flies involved investigating their viability on media containing the studied compounds. The test results clearly demonstrate that TPhT affects fruit flies more actively than TPhL (complete lethality after 7 days of culture with a TPhT-containing medium). No toxic effect of TPhC on fruit flies was shown. The results of the biological experiment were reflected in the physical experiment involving an ESR study of liposomes: TPhT activity manifested itself as a considerable increase in fluidity of the central region of the liposome lipid bilayer. © 2012 Wiley Periodicals, Inc. J Biochem Mol Toxicol 26:162–167, 2012; View this article online at wileyonlinelibrary.com. DOI 10:1002/jbt.21403

KEYWORDS: ***Drosophila hydei*; EYL Liposomes; ESR Method; Chlorotriphenyl Analogues**

INTRODUCTION

Owing to their high biological activity, metalloorganic compounds have been the subject of intensive research by ecologists, biologists, geneticists, biochemists, and biophysicists. The scientific community has long been acquainted with the toxic properties of lead, mercury, and cadmium derivatives [1–3], all of which contain metals commonly used in industry in the past century. Currently, the progress of civilization has brought about global computerization, which has entailed expansion of the electronic industry, thereby generating new technologies, which have become a source of environmental pollution with different metals to those mentioned above. Tin is one such an example, as a substance widely applied in electronics and usually considered a low-toxicity metal. However, because of its widespread presence in numerous products and the ability to accumulate in the organism, it can cause severe poisoning. Many studies [4–7] suggest that organic tin compounds can hamper the oxidative phosphorylation process and accelerate erythrocyte hemolysis, as well as alter the physical properties of model lipid membranes. The ionized forms of tin compounds (e.g., chlorides) show particular activity toward lipid membranes [8–10]. Man [11] demonstrated the strong influence of triphenyltin chloride (TPhT), consisting of three aromatic rings, on the central region of the lipid bilayer. After penetrating the liposomes, the compound significantly fluidized their structure, which may imply a detrimental effect with reference to the function of the biological membranes, namely, destruction of the cell. In biology we know that TPhT inhibits pig testicular 17 β-hydroxyestriol dehydrogenase activity and suppresses testicular testosterone biosynthesis [12], whereas in rat Leyidig cells (LC-540) the substance induces apoptosis [13]. Triphenyllead chloride (TphL) hemolyzed pig erythrocytes when used in micromolar concentrations [14]. Man [15,16] carried out comparative studies on the impact of TPhL, TPhT, and triphenylmethane chloride (TPhC) on the development of simple unicellular organisms [*Saccharomyces cerevisiae*, *Escherichia coli*, and HEK (human embryonic kidney cells)]. The results obtained clearly indicate that TPhT destroys the tested organisms even when applied at very low concentrations (1.0 $\mu g \times mL^{-1}$and 2.5 $\mu g \times mL^{-1}$ liquid medium, for *S. cerevisiae* and *E. coli*, respectively), and that it is particularly harmful to HEK cells (the lowest used concentration—0.05 $\mu g \times mL^{-1}$—produced 100% cell mortality).

Correspondence to: S. Mitrus.

© 2012 Wiley Periodicals, Inc.

The aim of the present study was to investigate the biological activity of TPhT in comparison to the similar compounds: TPhL and TPhC, in the interaction with model membranes, as well as with living organisms, which are more complex than unicellular ones. For the purpose of testing convenience, fruit flies were chosen for the study. The experiment is intended to elucidate how the molecular structure and the ion transported by the molecule to the membrane determine the activity of a given compound. Moreover, we expect to find the answer to the question of what type of interactions induced by the additive within the membrane is responsible for the toxic effect. To this end, the ESR method has been applied to examine the impact of TPhT, TPhL, and TPhC on the dynamic properties of liposome membranes formed from egg yolk lecithin (EYL).

MATERIALS AND METHODS

ESR Method—Chemicals and Spin Labels

Liposomes were obtained from EYL via a sonication process in distilled water using an ultrasonic disintegrator UD-20 (Techpan, Warsaw, Poland). The total sonication time of a single sample was 3 min in six alternate cycles: 30 s of sonication and 60 s of cooling. The concentration of lecithin in each sample was 0.04 M. After finishing the sonication, a spin label with a concentration of 0.1% relative to the lecithin (molar ratio to lipid particles is equal to 0.001) was added to the liposome water dispersion. Two different spin labels (purchased from Sigma–Aldrich, Poznan, Poland) were used in the ESR tests: (1) 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and (2) 2-ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxy (16-DOXYL). Admixtures of the investigated compounds were introduced in the labeled liposome samples. The concentration of the dopants was 1% relative to lecithin. For doping of the biological material, pure-grade chemicals TPhT (CTP-Sn), TPhL (CTP-Pb), and TPhC (CTP-C) (general chemical structure of the compounds is presented in [16]), were purchased from Sigma–Aldrich. Analytical grade dimethylformamide (DMF) was used to prepare solutions of the above-mentioned compounds.

The dissolved chlorides added to the liposomes were mixed for 15 min, including an undoped control sample. Subsequently, all samples were left for 60 min to stabilize. In the next step, liposomes containing the admixtures incorporated into the membrane were placed in the ESR spectrometer chamber in thin glass capillary tubes (ca. 1-mm diameter). ESR spectroscopy was applied in the investigations, and the spectra were recorded within a period of 180 h, starting from the moment the spin probes were introduced in the liposomes at a constant temperature of 25°C. Between the measurements, the samples were dark-stored at the same temperature. Two spin probes, TEMPO and 16-DOXYL-stearic acid methyl ester located in different sites inside the liposome membrane, were used. The TEMPO probe was placed both in the hydrophobic part of the membrane, as well as in the water ambient, whereas the 16-DOXYL-stearic acid methyl ester probe was placed deep within the hydrophobic layer of the membrane. From the ESR spectrum of the TEMPO probe the spectroscopic partition parameter (F), reflecting the probe distribution between the membrane and its ambient, was determined. A measure of the parameter F is the ratio of the high-field line amplitude in the ESR spectrum of the probe dissolved in the water ambient (P), to the amplitude of the low-field line (H) in the lipid environment (Figure 1a). The value of F, among other parameters, is connected with the membrane fluidity [17]. From the spectrum of the 16-DOXYL-stearic acid probe, the spectroscopic parameter τ was determined. Its value depends, among other

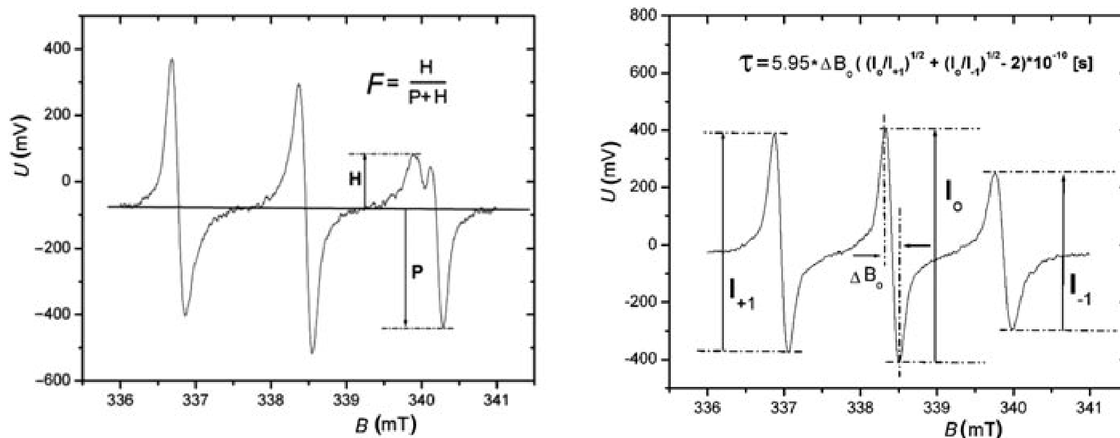

FIGURE 1. ESR spectra provided by the TEMPO (a) and 16-DOXYL spin labels (b).

things, on the degree of the membrane fluidity and is greater, the more rigid (better ordered) the ambient of the probe [18]. In the case of an isotropic environment, τ is the rotation correlation time of the probe (Figure 1b).

Fruit Fly Cultures

Fruit flies *Drosophila hydei* were cultured in 32 containers, each of 330 mL in volume. The medium in each of them was prepared from 25 g of cooked apples mixed with 10 mL of hot water containing 1.80 g of a mixture comprising wheat starch, agar, and sugar in a weight ratio of 3:2:2. While such medium was still warm, 0.005 g of the studied compound dissolved in 100 μL of dimethylformamide (DMF) was administered to it: 0.005 g of $(C_6H_5)_3CCl$ (TPhM) was added to medium in eight containers, 0.005 g of $(C_6H_5)_3PbCl$ (TPhL) in another eight, and 0.005 g of $(C_6H_5)_3SnCl$ (TPhT) in yet another eight vessels. One hundred microliter μf DMF was added to the medium in the remaining eight containers (treated as control containers). After the medium had cooled, several drops of baker's yeast solution were applied to its surface and an approximately 7 cm × 8 cm piece of a plastic mesh net (ca. 3 mm) was placed inside the container, to increase the space where the fruit flies could stay.

Three days after preparation of the medium containers, 25 fruit flies were released into each container. Subsequently, the number of surviving individuals in each container was recorded at ca. 24-h interval. The cultures were kept for 14 days in a thermostatic cabinet Pol-Eko ST 1. A daily cycle: 10 h of dark ("night") in temperature 15°C and 14 h of light ("day") in 25°C was maintained throughout the experiment.

Statistical Analysis

The survival rates of individuals depending on the medium used were plotted for the data considered separately for each individual. In total, survivability in 800 individuals was analyzed: Three groups of 200 individuals (25 flies in a container × 8 containers) kept on a medium with an admixture of $(C_6H_5)_3CCl$ (TPhC), $(C_6H_5)_3PbCl$ (TPhL), and $(C_6H_5)_3SnCl$ (TPhT), and also 200 individuals in the control containers.

The survival rates for flies kept on a medium, with tin and lead compounds added, were compared using the Mann–Whitney U test for determining the mean survival time in each of the eight containers with tin compounds added and in each of eight containers with lead compound added. In those cases where flies survived after 14 days, the time of 14 days was used in the analyses.

The statistical analyses were performed using the Statistica 6.1. software package [19].

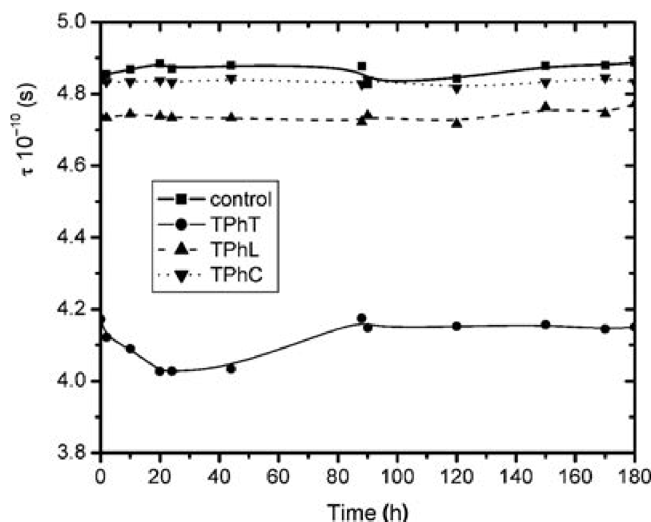

FIGURE 2. Dependence on time, of the value of the spectroscopic parameter τ (rotation correlation time) of the 16-DOXYL-stearic acid methyl ester probe, dissolved in the EYL liposome membranes containing admixtures of 1% TPhT, TPhL, and TPhC.

RESULTS AND DISCUSSION

ESR

Figure 2 presents changes in the value of the spectroscopic parameter τ of the 16 DOXYL probe introduced in EYL liposomes, dependent on time and the dopant added. The following conclusions can be drawn from the Figure 2:

1. The greatest changes compared to the control sample were observed in liposomes containing the TPhT admixture. Compared with the control τ, the spectroscopic parameter τ was altered by 18%. The decrease in the parameter value indicates a faster rotation of the probe in the bilayer center–the structure was fluidized. In the time range from 0 to 80 h, the parameter τ fluctuated, reaching its minimum $\tau_{\min} = 4.02$ after about 20 h. The parameter changes may reflect complex processes that occur inside the bilayer under the influence of TPhT and time.
2. The other admixtures applied, TPhL and TPhC, changed the τ parameter to a small extent, which may suggest the weak impact of these agents on the central part of the bilayer. The changes induced by TPhC fell within the tolerance of the measurement error, whereas those caused by TPhL differed from the values in the control samples by a mere 3%.

Figure 3 presents changes in the value of the spectroscopic parameter F of the TEMPO probe introduced in EYL liposomes, dependent on time and the dopant

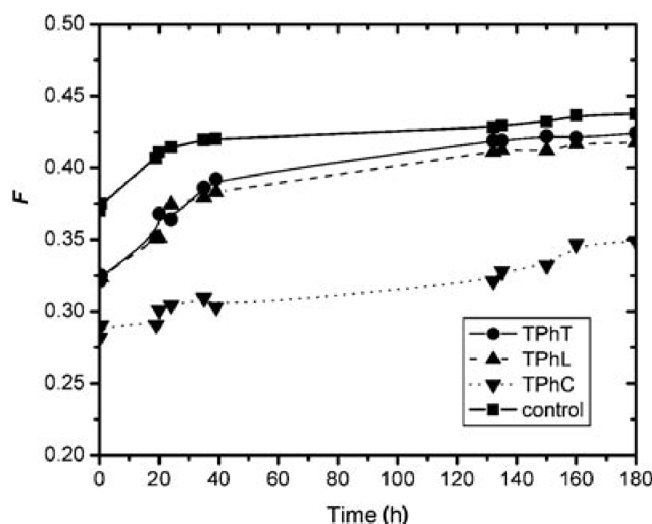

FIGURE 3. Time dependence of the value of the spectroscopic parameter *F* of the TEMPO probe, dissolved in the water suspension of EYL liposomes containing admixtures of 1% TPhT, TPhL, and TPhC.

added. The following conclusions can be drawn from the figure:

1. The greatest changes compared to the control sample were observed in liposomes containing the TPhC admixture. The spectroscopic parameter F was 27% different compared with the control F. The decrease in the parameter value indicates probe displacement from the lipid bilayer to water–the structure was stiffened. TPhT and TPhL induced slightly smaller changes amounting to about 13%.
2. The parameter F gradually increased within the range from 0 to 30 h for the control sample and for the liposomes containing admixtures of TPhL and TPhT, which suggests an increase in liposome membrane fluidity over time. No such change was observed in the liposomes doped with TPhC, which could indicate a stiffening of the surface layer by the admixture and blocking of the probe trapped in the lipids from the water phase. The admixture of TPhC acted as a membrane stabilizer in this case.

Figure 4 shows changes in the ESR spectrum of the TEMPO probe placed in EYL liposomes, occurring under the influence of admixtures of TPhC, TPhT, and TPhL. The last line in the spectrum (high-field line) splits into two components (circled area). The peak closer to the center represents the lipid phase. The control sample illustrates a starting point, where the lipid peak is considerably higher than the neighboring one. After adding a 2% admixture, the lipid peak for TPhC fells markedly, with smaller drops of peaks for TPhT and TPhL. Increasing the doping level to 10% demonstrates this process more clearly, although the changes are not as intense as the concentration gradient would suggest. This effect implies a strong interaction of TPhC with the membrane surface layer, which results in a stiffening of the structure. The considerable changes in the admixture concentration (an increase from 2% to 10%) affected the height of the peaks to a smaller extent than the 2% admixture in comparison with the control. This occurred presumably as a consequence of the limited solubility of dopants in liposomes.

The ESR method demonstrated that the studied compounds exerted a different impact on the membrane's interior (fluidizing it) and the surface layer (stiffening it). This process is only slightly affected by the incubation time. TPhT considerably fluidizes the center of the bilayer, which may lead to the membrane's disintegration. On the other hand, TPhC stabilizes liposomes by stiffening their surface layer, without affecting the fluidity of the bilayer center. TPhL produced a moderate effect. Such a substantial difference between TPhT and TPhC regarding their influence on liposome membranes was surprising. It indicates the important role played by the ion inside the molecule in determining the compound's activity in its interaction with membranes.

Biological Experiment

All flies kept on the medium containing tin compounds died within 7 days from the start of the experiment, whereas seven flies cultured on the medium with lead compounds (all from the same container) survived till the end of the experiment (Figure 5). During 14 days of observation, only a few individuals died on the medium with an admixture of 0.005 g of $(C_6H_5)_3CCl$ (TPhC) (seven flies from five different containers), and on the control medium (15 flies from six different containers). The survival rate on the medium containing lead compounds was higher than on the medium containing tin compounds (Mann-Whitney U test, $U = 4$, $N_1 = 8$, $N_2 = 8$, and $P = 0.0033$).

CONCLUSIONS

The studies conducted demonstrate that TPhT is a highly toxic substance even at a low concentration–all *D. hydei* flies kept on the medium with an admixture of this compound died throughout 7 days TPhL also proved to be toxic for fruit flies, although significantly less than TPhT: the probability of survival till the seventh day on a TPhL-containing medium was 20%. No

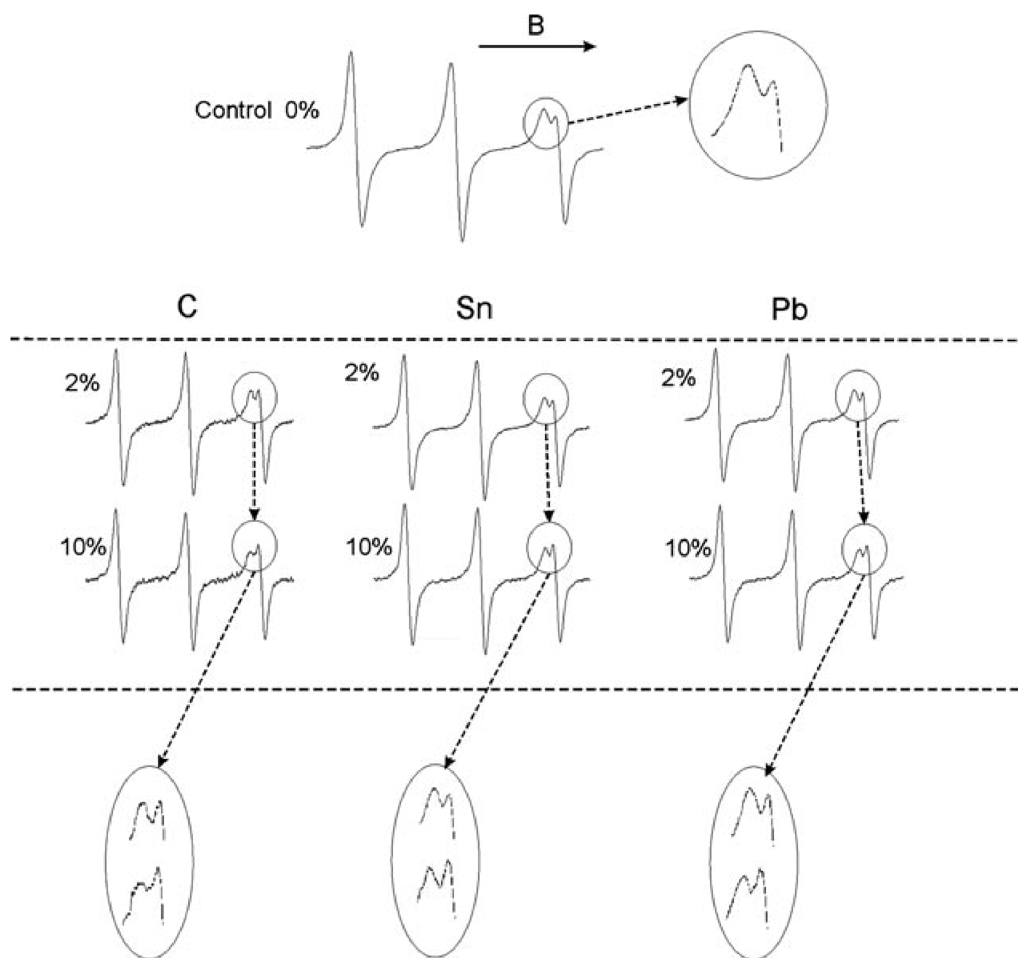

FIGURE 4. High-field line alterations in the ESR spectrum of the TEMPO probe introduced in liposome dispersion doped with TPhC (C), TPhT (Sn), TPhL (Pb) at 2% and 10% concentration with respect to EYL.

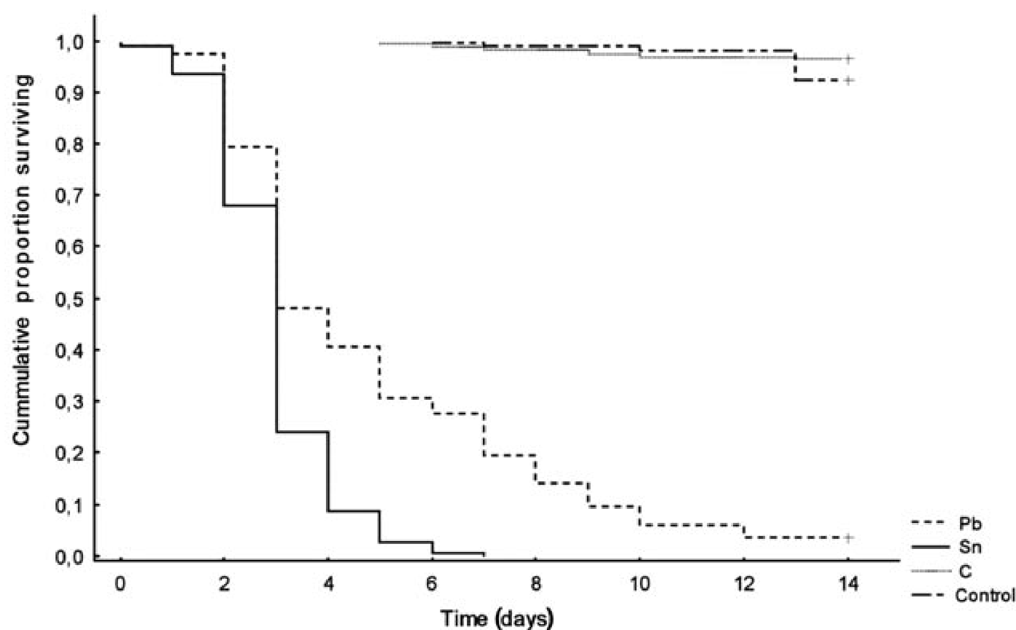

FIGURE 5. Survival probability for 800 fruit flies *D. hydei* kept in containers, each holding 25 individuals, in 8 duplicates, for the four media.

increase in survival rate compared to the control was noted on the medium with TPhC.

The investigated metallorganic compounds (TPhL and TPhC) are large molecules differing only in their central metal atom, hence finding such wide disparities in their toxicity is surprising. The ESR experiment showed significant differences in the effect of the studied compounds on the liposome membranes. The substance TPhT, which turned out to be the most toxic in the biological experiment, also exerted the strongest influence on the fluidity of the liposome bilayer central region, whereas TPhC, which did not have a significant toxic effect on fruit flies, stabilized liposome membranes (ESR study). Therefore, it can be supposed that the strong toxicity of a TPhT molecule is connected with the tin ion. This ion must play a crucial role in processes leading to the destruction of biological membranes and the consequent increased mortality of living organisms (both unicellular and complex). TPhT toxicity can involve considerable fluidization of the bilayer's hydrophobic inside, as membrane stability depends on the stability of this particular region. Excess fluidity may cause membrane disintegration or significantly alter transport processes—the cellular ion exchange system, resulting in cell death.

REFERENCES

1. Kalajdzic P, Stamenkovic-Radak M, Andjelkovic M. The effect of different concentrations of lead on inversion polymorphism in *Drosophila subobscura*. Hereditas 2006;143:41–46.
2. Massadeh A, Al-Momani F, Elbetieha E. Assessment of heavy metals concentrations in soil samples from the vicinity of busy roads: influence on *Drosophila melanogaster* life cycle. Biol Trace Elem Res 2008;122:292–299.
3. Wiśniowska-Kielian B. Trace element contents in soil and winter wheat—part II: heavy metal contents. Chem i Inż Ekol 2003;10:1021–1030.
4. Falcioni G, Gabbianelli RR, Santini A, Zolese G, Griffits D, Bertoli E. Plasma membrane perturbation induced by organotin on erythrocytes from *Salmo iridens* tront. Appl Organomet Chem 1996;10:451–457.
5. Gray BH, Porvaznik M, Flemming C, Lee LH. Organotin-induced hemolysis, shape transformation and intramembranous aggregates in human erythrocytes. Cell Biol Toxicol 1987;3:23–38.
6. Kleszczynska H, Hładyszowski J, Pruchnik H, Przestalski S. Erythrocyte hemolysis by organic tin and lead compounds. Z Naturforsch 1997;52C:65–69.
7. Hamasaki T, Masumoto H, Soto T, Nagase H, Kito H, Toshioka T. Estimation of the hemolytic effects of various organotin compounds by structure-active relationships. Appl Organomet Chem 1995;9:95–104.
8. Gabrielska J, Sarapuk J, Przestalski S. Role of hydrophobic and hydrophilic interactions of organotin and organolead compounds with model lipid membranes. Z Naturforsch 1997;52:209–216.
9. Radecka H, Zielińska D, Radecki J. Interaction of organic derivatives of tin (IV) and lead (IV) with model lipid membranes. Sci Total Environ 1999;234:147–153.
10. Kleszczyńska H, Sarapuk J, Przestalski S. Destabilization of model membranes by organotin compounds. Folia Histochem Cyto 1999;37:31.
11. Man D. Fluidity of liposome membranes doped with organic tin compounds: ESR study. J Lipos Res 2008;18:225–234.
12. Ohno S, Nakajima Y, Nakajin S. Triphenyltin and Tributyltin inhibit pig testicular 17β-hydroxysteroid dehydrogenase activity and suppress testicular testosterone biosynthesis. Steroids 2005;70:645–651.
13. Watermann B, Grote K, Gnass K, Kolodzey H, Thomsen A, Appel KE, Candia-Carnevali D, Schulte-Oehlmann U. Histological alterations in ovaries of pubertal female rats induced by triphenyltin. Exp Toxicol Pathol 2008;60:313–321.
14. Kleszczyńska H, Bonarska D, Sarapuk J, Przestalski S. Protection of erythrocytes against organometals-induced hemolysis. J Fluoresc 2004;14:5–10.
15. Man D, Podolak M, Engel G, Boniewska E. Effect of chlorotriphenyl derivatives of Sn and Pb upon biophysical properties of membranes. J Biomed Biotech 2009;969480.
16. Boniewska-Bernacka E, Man D, Słota R, Broda MA. Effect of tin and lead chlorotriphenyl analogues on selected living cells. J Biochem Molec Toxicol 2011;25:231–237.
17. Shimshick EJ, McConnell HM. Lateral phase separation in phospholipid membranes. Biochemistry 1973;12:2351–2360.
18. Hemminga MA. Interpretation of ESR and saturation transfer ESR spectra of spin labeled lipids and membranes. Chem Phys Lipids 1983;32:323–383.
19. StatSoft Inc. STATISTICA:data analysis software system, version 6 2004. Available from: www.statsoft.com.