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GENOTOXIC EVALUATION OF NEWLY SYNTHESIZED ORGANOMETALLIC COMPOUNDS OF TIN

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ABSTRACT

The genotoxic effects of organometallic tin(II) and tin(IV) complexes namely $L_{OEt}SnCl$ (**5**), $L^*_{OEt}SnCl$ (**6**), $L_{OEt}SnPh_3$ (**7**), $L^*_{OEt}SnPh_3$ (**8**), incorporating the oxygen tripodal ligands $[(\eta^5\text{-}C_5R_5)Co\{P(OEt)_2O\}_3]^-$, {R = H, ($L_{OEt}^-$) (**3**); R = Me ($L^*_{OEt}^-$) (**4**)} (Klaui type ligands), were investigated using the Cytokinesis Block Micronucleus assay in human lymphocytes cultures. For comparison the precursors NaL_{OEt} (**3**), NaL^*_{OEt} (**4**), $SnCl_2 \bullet 2H_2O$ (**1**) and Ph_3SnCl (**2**), were also studied.

Statistically significant differences in comparison with the control in the micronuclei frequencies were seen at the concentrations: 75 μM for complex (**5**), 50 μM for complex (**6**), 20, 50, 75 μM for complex (**8**). No statistically significant differences were observed between controls and all the rest tested concentrations for all chemicals examined.

The cytotoxic effect was evaluated by the Cytokinesis Block Proliferation Index. Regarding this index, the precursor (**1**) is not cytotoxic at all tested concentrations. Complexes (**3**), (**4**) and (**5**) induced cytotoxicity at the concentrations of 20, 50 and 75 μM, while complexes (**6**) and (**8**) were cytotoxic at all tested concentrations. Complex (**7**) was cytotoxic at 5, 10 and 20 μM but extremely toxic at 50 and 75 μM. Finally complex (**2**) was extremely toxic at all tested concentrations except at 1 μM.

Keywords: Cytotoxicity, Cytokinesis Block Micronucleus assay, Genotoxicity, Klaui ligands, Organotin compounds

Abbreviations: Binucleated (BN) cells, Cytochalasin-B (Cyt-B), Cytokinesis Block Micronucleus (CBMN), Cytokinesis Block Proliferation Index (CBPI), Dimethyl sulfoxide (DMSO), Micronuclei (MN), Mitomycin-C (MMC)

1. Introduction

Organotin complexes displaying typical metal to carbon single bonds (Sn-C) represent an important class of compounds. They are generally represented by the formula: $R_xSn(L)_{(4-x)}$, where R is a typical organic group such as methyl, butyl, octyl, etc. For the organotin stabilizers and catalysts, x is either 1 or 2, while for organotin biocides and pesticides, x equals to 3 and the R group is usually a butyl, cyclohexyl or phenyl group. Once these compounds are made and isolated, the R groups maintain their connection to tin; they do not transfer from one tin to another under normal conditions of use. Organotin compounds present a broad range of industrial applications such as PVC stabilizers, catalysts, biocides and pesticides,

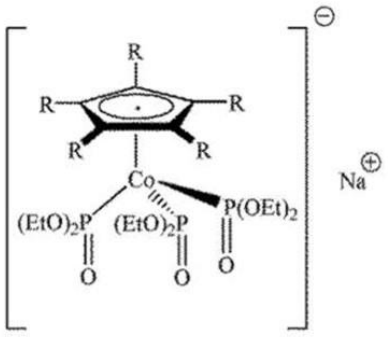
intermediates in chemical synthesis, antifouling paints, glass coverings and veterinary as well as human medicines (Greenwood and Earnshaw, 1997; Appel, 2004; Government of Canada, 2010).

The toxicity of inorganic forms of tin toward microorganisms is relatively low, but the more lipid soluble organotins are thought to be more toxic (Cima *et al.,* 2003). Tri-substituted (R_3SnX) organotins were reported to be more toxic than di-(R_2SnX_2) and mono-substituted ($RSnX_3$) compounds, while the anion (X^-) appeared to have little influence on the toxicity of the tin compound (Gadd, 2000).

In a standard battery of tests, pesticidal organotin compounds are generally negative although some have been shown to be genotoxic (Jensen *et al.,* 1991; Sasaki *et al.,* 1993). *In vitro* exposure of human lymphocytes to organotin compounds resulted in statistically significant increases in the frequency of hyperdiploid cells. Indications pointed that some organotin compounds were able to induce aneuploidy in human peripheral lymphocytes *in vitro*, probably by affecting spindle function (Jensen *et al.,* 1991). In the meantime there were reports that tributyltin and triphenyltin compounds increase the frequency of chromosome aberrations in Chinese hamster ovary cells (CHO-K1) (Sasaki *et al.,* 1993). Mutagenicity studies of several organotin compounds, including butyltin, phenyltin and methyltin derivatives, as well as the inorganic tin tetrachloride, were performed using the Salmonella mutagenicity assay (Ames test) (Hamasaki *et al.,* 1993).

Trimethyltin chloride induced micronuclei (MN) and chromosome aberrations in healthy male and female human lymphocytes (Ghosh *et al.,* 1990; Ghosh *et al.,* 1991). Isolated lymphocytes of different age groups were exposed to two concentrations of trimethyltin chloride (0.1 and 0.2 μg ml^{-1}). In this study, the lower concentration of trimethyltin chloride induced more micronucleated cells than did the higher dose, and male individuals showed higher frequencies of MN than female ones. No correlation between MN frequencies and donor age was observed. However, evidence was provided that butyltin compounds significantly inhibiting apoptosis in human cells (Whalen *et al.,* 1999).

The potential of organometallic compounds of tin for adversely affecting animal and human health is well documented. Nevertheless, data on the genotoxicity as well as on the mechanisms of cellular action of organotin compounds are scarce (Penninks and Seinen, 1984; Gielen, 2003; Chasapis *et al.,* 2004; Dopp *et al.,* 2004; Tabassum and Pettinari, 2006).



R = H, L_{OEt} (3); R = Me, L^*_{OEt} (4)

Figure 1. The molecular structure of NaL_{OEt} (**3**) and NaL^*_{OEt} (**4**).

Design and biological evaluation of new organometallic tin compounds is an issue of current research (Gielen et al., 2005; Basu Baul, 2008; Hadjikakou and Hadjiliadis, 2009; Pizarro *et al.,* 2010). In this respect a series of organometallic tin(II) and tin(IV) complexes incorporating the oxygen tripodal ligands $[(\eta^5\text{-}C_5R_5)Co\{P(OEt)_2O\}_3]^-$, {R = H, ($L_{OEt}^-$) (**3**); R = Me ($L^*_{OEt}^-$) (**4**)} (Fig. 1) were synthesized, according to a previously described procedure followed for the synthesis of the germanium(II) analogues (Filippou *et al.,* 2000). The four-coordinate $L_{OEt}SnCl$ (**5**) and $L^*_{OEt}SnCl$ (**6**) chlorides were obtained by a metathetical exchange reaction of $SnCl_2$ with the starting materials (**3**) and (**4**). The corresponding six-coordinated tin(IV) derivatives $L_{OEt}SnPh_3$ (**7**) and $L^*_{OEt}SnPh_3$ (**8**) were formed from the reaction of the Ph_3SnCl (**2**)

precursor with (**3**) and (**4**) respectively. The choice of the ligand is based on the chemical inertness of these ligands along with their thermal and hydrolytic stability, which is of crucial importance especially in the field of organometallic chemistry (Klaui *et al.,* 1987).

In the present genotoxicity study, the Cytokinesis Block Micronucleus (CBMN) assay was employed for the detection of MN in the cytoplasm of interphase cells, under the influence of various concentrations of the four newly synthesized organotin compounds **(5), (6), (7)** and **(8)** and their precursors $SnCl_2 \bullet 2H_2O$ **(1**), Ph_3SnCl **(2), (3)** and **(4).**

MN may originate from acentric chromosome fragments or whole chromosomes that are unable to migrate towards the poles during the anaphase stage of cell division. The simplicity, rapidity, sensitivity as well as the validation of the CBMN assay renders it a valuable tool for genotoxicity screening. The assay detects the potential clastogenic and aneugenic activity of chemicals in cells that have undergone cell division after exposure to chemicals under investigation. The use of cytochalasin-B, an inhibitor of actin polymerization, which prevents cytokinesis, while permitting nuclear division leads to formation of binucleated (BN) cells which are scored for the presence of MN. OECD acceptance of the *in vitro* MN test led to its widespread international application (OECD, 2014).

Taken into consideration the previously described reasons, in this study, we aimed to comparatively investigate the cytotoxic and genotoxic effects of the four new organometallic compounds of tin using the CBMN assay in human lymphocytes cultures.

2. Methodology

2.1. Chemicals

The new organometallic tin(II) and (IV) compounds comprise the amphiphilic spectator ligands (**3**) and (**4**) described previously (see introduction). The ligands are abbreviated as NaL_{OEt} (**3**) for the case that the R group of the cyclopentadienyl ligand is hydrogen (Klaui, 1979) and NaL^*_{OEt} (**4**) where R = Me (Roman *et al.*, 1986; Klaui *et al.,* 1997).

All chemicals were synthesized by Assist. Prof. A. I. Philippopoulos (Faculty of Chemistry, National and Kapodistrian University of Athens) in collaboration with Prof. Dr. A. C. Filippou (University of Bonn, Department of Chemistry) (paper under preparation) were of high purity (≥ 98% in solid state checked by elemental analysis and by multinuclear NMR spectroscopy in solution). All tested chemicals were dissolved in DMSO following the OECD (2014) guideline for testing of chemicals with the *in vitro* mammalian cell micronucleus assay as well as the Human micronucleus project (Bonassi *et al.,* 2001).

Stannus chloride dehydrate (CAS no.: 10025-69-1) and triphenyl tin chloride (CAS no: 639-58-7) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.2. CBMN assay in human lymphocytes in vitro

Blood samples were obtained from two non-smoking healthy young individuals not undergoing any drug treatment, viral infection or X-ray exposure in the recent past. Blood donors were aware of the present study that was conducted according to the University of Patras Ethics Committee and gave their consent.

Blood samples were kept under sterile conditions in heparinized tubes. Whole blood (0.5 ml) was added to 6.5 ml Ham's F-10 medium (Gibco, Grand Island, NY), 1.5 ml foetal bovine serum (Gibco) and 0.3 ml phytohaemagglutinin (Gibco) to stimulate cell division.

The various chemical solutions were added to final concentrations of 1, 5, 10, 20, 50 and 75 μM. All tested chemicals were dissolved in DMSO. DMSO concentration in cultures did not exceed 0.75 %. The appropriate solutions were added 41 h post culture initiation. For each tested chemical, two identical sets in two independent experiments were conducted for all aforementioned concentrations as well as for positive and negative controls. The reported results represent the pooled data from the two donors' replicated cultures.

Mitomycin-C (MMC) (Sigma-Aldrich Chemie GmbH) at final concentration of 1.5 μM served as positive control in all respective experiments (Stivaktakis *et al.,* 2010). Subsequently, 3 h after the addition of the appropriate chemical solutions, 6 μg ml^{-1} Cytochalasin-B (Cyt-B) (Sigma) were added to the culture medium, at 44 h post culture initiation. This concentration of Cyt-B was selected so as to obtain a higher percentage of BN cells and a lower baseline MN frequency (OECD, 2014).

Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere. 72 h after the initiation of culture, cells were harvested and collected by centrifugation. Collected cells were processed according to standard protocol (OECD, 2014) with minor modifications. A mild 3 min, at room temperature, hypotonic treatment with 75 mM KCl was followed by 10 min fixation (for at least 3 times) with a fresh 3:1 solution of methanol/acetic acid before the cells were stained with 7% Giemsa.

In total, 4000 BN cells with preserved cytoplasm scored per experimental point. Standard criteria were used for scoring MN (Fenech *et al.,* 2003).

2.3. Cytotoxicity evaluation

In order to determine possible cytotoxic effects, the Cytokinesis Block Proliferation Index (CBPI) was calculated by counting at least 2000 cells for each experimental point (500 cells per culture of each donor). CBPI is given by the equation: $CBPI = [M_1 + 2M_2 + 3(M_3 + M_4)]/N$ where M_1, M_2, M_3 and M_4 correspond to the numbers of cells with one, two, three and four nuclei and N is the total number of cells (Surrales *et al.*, 1995).

2.4. Statistical analysis

All results are expressed as the mean frequency (‰) ± standard error [MF (‰) ± se]. The statistical analysis of the obtained data was conducted with the use of the Minitab statistical software (Minitab Inc., Pennsylvania, USA) and the SPSS Inc.17. MN data analysis was conducted using the *G*-test for independence on 2x2 tables, while CBPI data set was analyzed using the chi-square (χ^2) test. Significant levels were established as $p<0.05$ in all cases.

3. Results

3.1. Cytokinesis-block micronucleus (CBMN) assay

Complexes (**5-8**) have been tested for the possible inductions of MN frequencies, while precursors (**1-4**) were used as controls in order to evaluate whether the observed effects resulted or not from the chemicals under investigation.

As positive control Mitomycin-C (MMC) at final concentration of 1.5 μM was used. The positive control frequencies of MN (63.75±16.25‰) in our experiments are in accordance with published values in the used cytogenetic end point (Clare *et al.,* 2006).

The results of MN analysis (mean values for MN induction as well as for CBPI) obtained from human peripheral blood lymphocyte cultures treated with different concentrations (1, 5, 10, 20, 50 and 75 μM) of the studied chemicals (**1**, **3**, **4**, **5**, **6**, **7, 8)** are shown in Figure 2 (a-g).

Ph_3SnCl (**2**) was extremely cytotoxic towards the lymphocytes from the concentration of 5 μM, thus no histogram could be produced.

The mutagenic potential of the various compounds examined is depicted in Table 1. A comparative analysis of the genotoxicity of the various compounds, in the tested concentrations, points that the precursors $SnCl_2 \bullet 2H_2O$ (**1**), NaL_{OEt} (**3**) and NaL^*_{OEt} (**4**) do not induce MN frequencies (no genotoxic effect) towards human peripheral blood lymphocytes. Statistically significant differences ($p< 0.05$) in comparison with the control in the MN frequencies were seen only at the higher tested concentration of (**5**) (75 μM) as well as at the concentration of 50 μM of (**6**), while (**6**) at the highest concentration (75 μM) was lethal for the lymphocytes. In this respect, complex (**7**) was not genotoxic at the concentrations of 1, 5, 10 and 20 μM. However complex (**7**) appeared to be lethal for the lymphocytes at 50 and 75 μM. Finally, complex

(**8**) revealed statistically significant differences ($p< 0.05$) in comparison with the control in the MN frequencies at 20, 50 and 75 μM.

3.2. Cytotoxic index evaluation

The cytotoxic effect was evaluated by the CBPI index. Regarding this index, as can be seen in Figure 2, compounds (**3**), (**4**), and (**5**) at 20, 50 and 75 μM, while compound (**6**) induced statistically significant decrease ($p<0.001$) on CBPI values at all tested concentrations except of the 75 μM where no cells could be observed, possibly due to extremely toxic effect. Compound (**7**) induced statistically significant decrease ($p<0.001$) on CBPI values at the concentrations of 5, 10 and 20 μM, while it was lethal at the 50 and 75 μM concentrations. In the meantime compound (**8**) induced statistically significant decrease ($p<0.001$) on CBPI values at all tested concentrations. $SnCl_2 \bullet 2H_2O$ (**1**) did not exert cytotoxic effect at all tested concentrations as it can be seen in Figure 2. Ph_3SnCl (**2**) was apparently extremely toxic to lymphocytes except at the concentration of 1μM, thus no histogram could be drawn and presented.

The cytotoxicity results in comparison to the genotoxicity ones are summarized in Table 1.

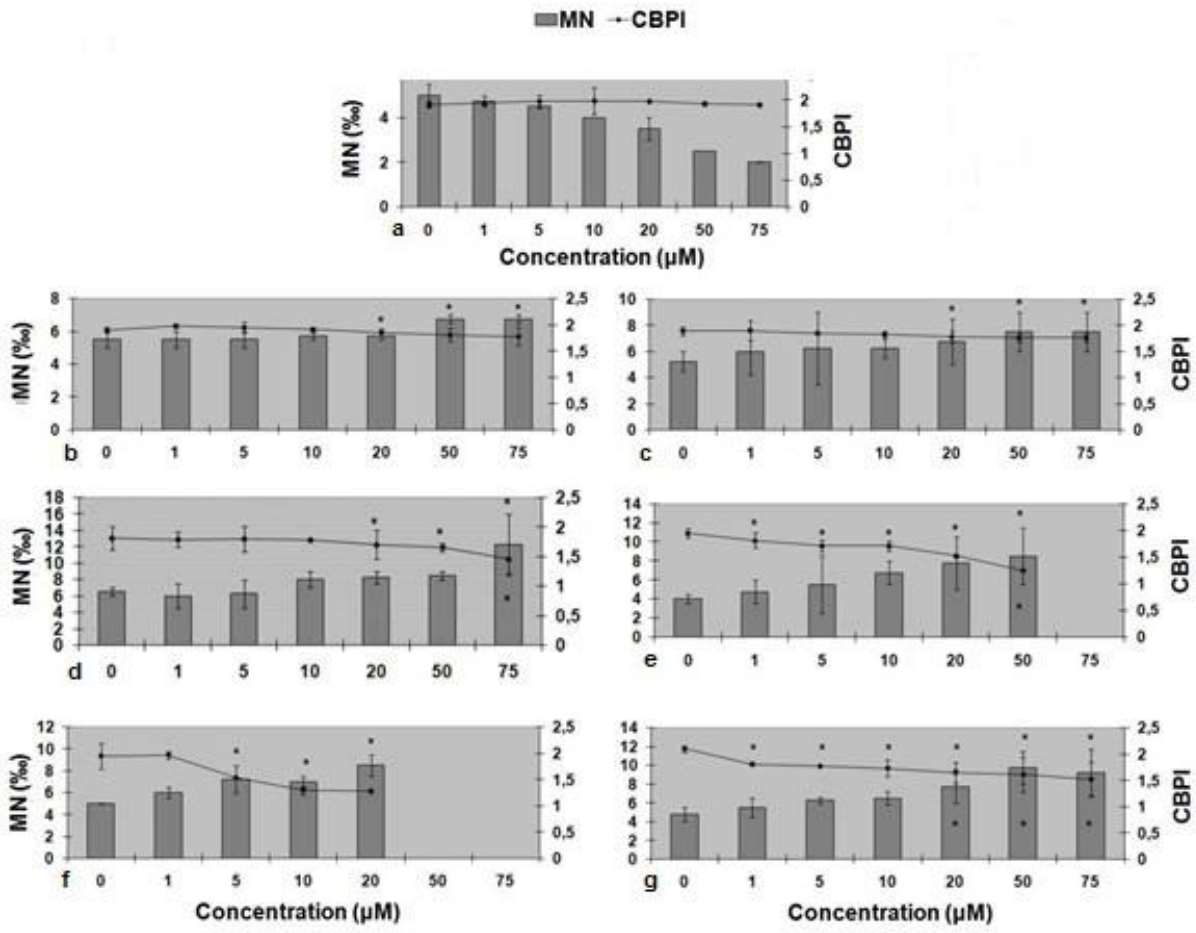


Figure 2. Induction of MN and CBPI values in human lymphocytes treated with **a.** SnCl2•2H2O **(1), b.** NaLOEt **(3), c.** NaL*OEt **(4)**, **d.** LOEtSnCl **(5)**, **e.** L*OEtSnCl **(6), f.** LOEtSnPh3 **(7)** and **g.** L*OEtSnPh3 **(8).** 4000 binucleated cells scored per experimental point, * $p< 0.05$ [G-test for MN]; *$p<0.001$ [$\chi 2$ for CBPI

4. Discussion

Based on the broad range of industrial applications of organotin complexes (Greenwood and Earnshaw, 1997; Appel, 2004; Government of Canada, 2010) new organotin compounds are continuously

synthesized and their antitumor and cytotoxic potential is under investigation (Shpakovsky *et al.,* 2014; Zhao *et al.,* 2014; Khan *et al.,* 2014). The synthesis of a series of organotin complexes with Sn-S bonds of formulae $Me_2Sn(SR)_2$ (1); $Et_2Sn(SR)_2$ (2); $(n\text{-}Bu)_2Sn(SR)_2$ (3); $Ph_2Sn(SR)_2$ (4); $R_2Sn(SR)_2$ (5); Me_3SnSR (6); Ph_3SnSR (7) (R = 3,5-di-tert-butyl-4-hydroxyphenyl) that derived from the precursor R_2SnCl_2 (8) was reported and the *in vitro* cytotoxicity was investigated against normal human fetal lung fibroblast cells (MRC-5), as well as against human breast (MCF-7) and human cervix (HeLa) adenocarcinoma cells (Shpakovsky *et al.,* 2014). Complexes 2 - 4 and 8 exhibited significantly lower cytostatic activity against the normal MRC-5 cell line compared to the tumor cell lines MCF-7 and HeLa. A high activity against both cell lines 250 nM (MCF-7) and 160 nM (HeLa) was determined for the triphenyltin complex 7 while the introduction of hindered phenol groups decreases the cytotoxicity of the complexes against normal cells. In the meantime twenty one novel mixed ligand di-n-butyltin(IV) complexes [n-Bu_2SnAL] (A = substituted 4-acyl-5-pyrazolone, and L = fluorinated benzoic acid) were synthesized and their cytotoxicity against Hela and KB cancer cell lines compared to cisplatin was studied by means of the 3-(4,5-dimethyl-2-thiazolyl)-2,5diphenyl-2*H*-tetrazolium bromide (MTT) assay (Zhao *et al.,* 2014). All twenty one synthesized complexes were found more effective than cisplatin. Additionally, triorganotin(IV) complexes with six different *R*N-2-X-benzohydroxamic acid ligands having general formula *R*'C(O)N(*R*N)OH (*R*' = alkyl/aryl; *R*N = alkyl/aryl or H), (X = –I, $–NO_2$, $–OCH_3$, –Br and R = $–CH_3$, $–C_6H_5$ and $–C_6H_4–CH_3$) were synthesized (Khan *et al.,* 2014). They showed significantly higher cytotoxic activities than doxorubicin toward K-562, Jurkat, HepG2 and L929 cells using the MTT assay.

Table 1. Comparison of the genotoxic and cytotoxic action of the tested chemicals

Compounds	Genotoxicity						Cytotoxicity					
	Concentration (µM)						Concentration (µM)					
	1	5	10	20	50	75	1	5	10	20	50	75
$SnCl_2•2H_2O$ (**1**)	-	-	-	-	-	-	-	-	-	-	-	-
Ph_3SnCl (**2**)	-	NC	NC	NC	NC	NC	-	NC	NC	NC	NC	NC
NaL_{OEt} (**3**)	-	-	-	-	-	-	-	-	-	+	+	+
NaL^*_{OEt} (**4**)	-	-	-	-	-	-	-	-	-	+	+	+
$L_{OEt}SnCl$ (**5**)	-	-	-	-	-	+	-	-	-	+	+	+
$L^*_{OEt}SnCl$ (**6**)	-	-	-	-	+	NC	+	+	+	+	+	NC
$L_{OEt}SnPh_3$ (**7**)	-	-	-	-	NC	NC	-	+	+	+	NC	NC
$L^*_{OEt}SnPh_3$ (**8**)	-	-	-	+	+	+	+	+	+	+	+	+

-: negative; +:positive; NC: No cells

In the present study a series of new four-and six-coordinated organotin complexes containing the oxygen tripodal ligand $[(\eta^5\text{-}C_5R_5)Co\{P(OEt)_2O\}_3]^-$, {R = H, ($L_{OEt}^-$) (**3**); R = Me ($L^*_{OEt}^-$)} (**4**) (Klaui, 1979; Roman *et al.,* 1986; Klaui *et al.,* 1987; Klaui *et al.,* 1997) were studied with regard to their genotoxic and cytotoxic potential against human peripheral lymphocytes. In order to investigate their potential, the corresponding precursors, inorganic and organic ones, were studied as well. It should be noted, that since the introduction of the Klaui's oxygen tripodal ligand to a tin(II) and (IV) metal centre no genotoxic or cytotoxic study has been ever reported about this class of compounds while the work of Lloyd *et al.* (2006) emphasized on the synthesis and characterization of six-coordinate organotin(IV) complexes with the $[(\eta^5 C_5R_5)Co\{P(OMe)_2O\}_3]^-$, {R = H, ($L_{OMe}^-$) and ($L_{OEt}^-$)} ligands.

In a previous study, where various inorganic and organic tin(II) and tin(IV) salts were tested for possible genotoxic and cytotoxic activity, a lack of genotoxicity was revealed, while they were cytotoxic at several concentrations in the absence of metabolic activation (Damati *et al.,* 2014). A low toxicity of inorganic tin forms towards microorganisms was reported, while organotin compound's toxicity was related to their lipophilicity (Cima *et al.,* 2003). Trisubstituted (R_3SnX) organotins appeared to be more toxic than disubstituted (R_2SnX_2) and monosubstituted ($RSnX_3$) compounds, while the anion (X^-) seemed to have little influence on the toxicity of the tin compound (Gadd, 2000). Furthermore it was reported that tributyltin and triphenyltin compounds showed higher toxicities against the red killifish *Oryzias latipes* among 29

organotin compounds, while their toxicities did not depend upon their hydrophobic character (Nagase *et al.*, 1991). In the meantime organotins are regarded as membrane permeable because of their lipophilicity and thus the possible site of their action may be the cytoplasmic membrane (Florea and Busselberg, 2006).

The present results indicate that the precursors $SnCl_2 \bullet 2H_2O$, NaL_{OEt} and NaL^*_{OEt} used to synthesize the new organotin compounds were not genotoxic, while NaL_{OEt} and NaL^*_{OEt} expressed a cytotoxic potential above the concentration of 20 μM. However, Ph_3SnCl was highly cytotoxic even from the concentration of 5 μM. In the meantime at the concentration of 1 μM did not increase the frequency of MN compared to the control. It must be noted that Ph_3SnCl was genotoxic against *Salmonella typhimurium*, *Escherichia coli* and *Bacillus subtilis* (Hamasaki *et al.,* 1992; Hamasaki *et al.,* 1993), while trimethyl tin chloride induced MN and chromosome aberrations in human lymphocytes (Ghosh *et al.,* 1990; Ghosh *et al.,* 1991).

The $L_{OEt}SnCl$ compound appeared slightly genotoxic inducing statistically significant increased MN frequencies at the highest concentration, while its cytotoxicity represented the one expressed by its precursor NaL_{OEt}. The $L^*_{OEt}SnCl$ complex was moderately genotoxic as induced statistically significant increased micronuclei frequencies at the concentration of 50 μM. Moreover our results show that $L^*_{OEt}SnCl$ complex was cytotoxic at all tested concentrations. The data indicate that the presence of the methyl group instead of hydrogens in the cyclopentadienyl ring renders $L^*_{OE}tSnCl$ more cytotoxic. In addition it was reported that tin (IV) chloride was not cytotoxic towards human lymphocytes (Damati *et al.,* 2014). The above indicate that the presence of methyl group may induce genotoxic as well as cytotoxic effects to human cells.

The tested compounds (**7**) and (**8**) containing the characteristic -Ph_3Sn moiety were highly cytotoxic; a result which is in accord with our observations about the extremely high toxicity of the Ph_3SnCl precursor. Triphenyl tin chloride was reported to induce chromosome aberrations in Chinese hamster ovary cells (CHO-K1) (Sasaki *et al.,* 1993). In the meantime, it was suggested that some organotin compounds are able to inducing aneuploidy in human peripheral lymphocytes *in vitro*, probably by affecting spindle function (Jensen *et al.,* 1991).

Our data could be summarized as follow:

Regarding the mutagenic potential, $SnCl_2 \bullet 2H_2O$ (**1**), NaL_{OEt} (**3**) and NaL^*_{OEt} (**4**) revealed not to be genotoxic, while $L_{OEt}SnCl$ (**5**) was genotoxic above 50 μM and $L^*_{OEt}SnCl$ (**6**) above 20 μM. Complex $L_{OEt}SnPh_3$ (**7**) was not genotoxic up to 20 μM compared to $L^*_{OEt}SnPh_3$ (**8**) that was genotoxic from the concentration of 20 μM.

Regarding the cytotoxic potential, $SnCl_2 \bullet 2H_2O$ (**1**) did not exert any cytotoxic effect, while NaL_{OEt} (**3**), NaL^*_{OEt} (**4**) and $L_{OEt}SnCl$ (**5**) were cytotoxic above the concentration of 20 μM. $L^*_{OEt}SnCl$ (**6**), $L_{OEt}SnPh_3$ (**7**) and $L^*_{OEt}SnPh_3$ (**8**) exerted various levels of cytotoxicity including lethal effects at the tested concentrations. In the meantime, Ph_3SnCl (**2**) was extremely toxic to lymphocytes except at the concentration of 1μM.

5. Conclusions

In conclusion the data of the present paper regarding the total genotoxic and cytotoxic potential of the tested chemicals, in the specific concentrations and experimental conditions, suggest that the compounds NaL_{OEt} (**3**), NaL^*_{OEt} (**4**) and $L_{OEt}SnCl$ (**5**) are very promising and could be potentially used for further biological applications.

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