SUMMARY
The biological activity of organotin compounds is well known owing to their practical applications as fungicides, bactericides, biocides and pesticides (Pellirito et al 2002, Gielen and Tieken 2005). However, one of the fields that have been more studied and reviewed is the activity of such compounds against cancer (Pellirito et al. 2002, Barbieri et al 2002).

The organotin(IV) compounds that were first tested were those that were available or easily synthesized, like tri- or diorganotin(IV) halides. It was reported that diorganotin(IV) oxides and hydroxides, which either contain tin-oxygen bond or are capable of forming such a bond upon hydrolysis, have antitumor potential (Crowe 1987, Gielen et al 1995).

Diorganotin(IV) compounds, $R_2SnCl_2$, are often tetrahedral, and when appropriate nitrogen-chelating ligands are co-ordinated to the central metal, octahedral complexes $R_2SnCl_2L$ (L=bidentate ligand) are obtained (Crowe et al. 1984a). Diorganotin(IV) compounds structurally resemble the active platinum compounds, i.e cisplatin and carboplatin, and consequently a large number of such complexes have been tested for antitumor activity.

Three primary factors are involved in the structure-activity relationship for organotin(IV) derivatives $R_nSnX_{4-n}(L)_x$: the nature of the organic group $R$, of halide or pseudohalide $X$, and of donor ligand $L$. Examination of the structures of organotin(IV) compounds containing a N-donor atom when tested for antitumor activity revealed that in the active Sn complexes the average Sn-N bond lengths were >2.39Å, whereas the inactive complexes had Sn-N bonds <2.39Å. This implies that predissociation of the ligand may be an important step in the mode of action of these complexes, while the coordinated ligand may favour transport of the active species to the site of action in the cells, where they are released by hydrolysis (Pellerito et al 2002). A structural correlation with biological activity for diorganotin(IV) complexes has shown that active species are associated with complexes having Sn-N bonds longer than 2.39Å which in turn determines the formation of a tin-DNA complex (Crowe et al 1984). In view of these, a series of diorganotin(IV) dichloride complexes of N-(2-pyridyl-methylene)arylamine (nitrogenchelating ligands) have been synthesized and characterized on the basis of IR, NMR and $^{119}$Sn-Mössbauer studies (Basu Baul et al 1998, Koch et al 2008). The observed Sn-N bond lengths in
Me$_2$SnCl$_2$L$^1$, Et$_2$SnCl$_2$L$^2$ and Bu$_2$SnCl$_2$L$^2$ is Sn-N(1) = 2.452 (6) Å and Sn-N (2) = 2.559 (6) Å which is >2.39Å and therefore, better formation of tin-DNA complex can be expected. In line with these developments, the anti-proliferative and cytotoxic effect of Me$_2$SnCl$_2$L$^1$, Et$_2$SnCl$_2$L$^2$ and Bu$_2$SnCl$_2$L$^2$ have been investigated both in vivo and in vitro (Basu Baul et al 1998, Syng-ai et al 2002, Koch et al 2008). The cytotoxic effect of several diorganotin(IV) and triorganotin(IV)-meso-tetra(4-sulfonatophenyl)porphine derivatives was tested and only the (Bu(2)Sn)(2)TPPS and the (Bu(3)Sn)(4)TPPS showed cytotoxicity on A375 human melanoma cells (Costa et al 2006). It was shown earlier that the antitumor activity of Et$_2$SnCl$_2$L$^2$ was improved after depleting endogenous glutathione (GSH) by buthionine sulfoximine (BSO) (Syng-ai et al. 2001). The majority of anti-tumor drugs are DNA targeted and antimitotic (Schneider et al 2003), however, the mode of biological action of Et$_2$SnCl$_2$L$^2$ has not yet been clearly understood. More experimental data must be collected in order to understand the biological (including antitumor) activity of organotin(IV) complexes. Therefore, the present study, was carried out to investigate the comparative anti-tumor and anti-proliferative effect of diorganotin(IV) complexes with different alkyl groups (methyl, ethyl and butyl). It was also shown that present diorganotin(IV) complexes are toxic to mice was more toxic to mice and therefore, the present study was carried out to investigate the comparative anti-tumor and anti-proliferative effect at low doses of diorganotin(IV) complexes with different alkyl groups (methyl, ethyl and butyl). In order to improve the antitumor potentiality of these diorganotin(IV) compounds, X-rays was combined with these diorganotin(IV) compounds instead of increasing the dose of diorganotin(IV) compounds. It has been observed that the radiation and chemical combined therapy has led to improved local control and disease-free survival (Einhorn et al 2003).

Based on these above background the following objectives will be addressed:

- To evaluate and compare the antitumor activity of the dialkyl-series (Methyl, ethyl and n-butyl) of Organotin compounds with respect to endogenous GSH and the structure of tin compounds,
- To investigate the cytotoxic potentialities of these compounds and
• To ascertain the influence of these organotin compounds on the expression of cell cycle regulatory proteins.

Determination of Lethal dose

Most anticancer drugs have a relatively poor therapeutic index. For this reason the majority of antiproliferative agents in current use are given at very close to the maximum tolerated dose (MTD). This precludes the use of healthy volunteers that makes such studies potentially hazardous. It has been the standard practice to establish the MTD in rodents and extrapolates to man using some form of stepwise escalation to reach a maximum tolerant dose (Phase I), which can be applied in tumor-specific studies of anti-tumor activity (Phase II).

A number of different schemes have been used for determination of a safe starting dose based on animal toxicology. The NCI, US reviewed the data from mice and dogs and concluded that one-tenth the LD$_{10}$ (Lethal dose 10% is the dose of drug which will kill 10% of the exposed animals within 30 days) in mice is a safe starting dose provided this is tolerated in dogs in Phase I clinical trial (Judson 1995).

In this study, a few preliminary experiments were carried out so as to determine an appropriate dose for use in all further studies. The Lethal Dose (LD$_{10}$) was determine by taking at least 20 mice each and the LD concentration for Me$_2$SnCl$_2$, Et$_2$SnCl$_2$ and Bu$_2$SnCl$_2$, were found to be 28, 18 and 14 mg kg$^{-1}$, respectively. For antitumor activity, 5 mg kg$^{-1}$ and 20 mg kg$^{-1}$ diorganotin (IV) was used.

Materials and Methods: The cytotoxicity of these diorganotin(IV) compounds was studied in human peripheral lymphocytes (HPBL) and in mouse bone marrow cells (BMC). The antitumor activity was assessed in Dalton’s lymphoma cells. The involvement of proteins that regulate the cell cycle and apoptosis was investigated to elucidate the mechanism of their action. The diorganotin(IV) dichloride complexes of N-(2-pyridylmethylene)arylamine, e.g., Me$_2$SnCl$_2$.L$^1$ (OTC-1), Et$_2$SnCl$_2$.L$^2$ (OTC-2) and Bu$_2$SnCl$_2$.L$^2$ (OTC-3) were synthesized as previously described (Basu Baul et al 1998). The stock solutions of OTC-1, OTC-2 and OTC-3 were prepared in 5% (v/v) ethanol/water.
keeping a concentration of 3mg ml\(^{-1}\). The stock solutions of OTCs were freshly diluted with distilled water to reach a planned concentration before each experiment. Heparinized peripheral blood from five healthy male donors was used immediately after venipuncture. Rules of 'Ethical Guidelines for Biomedical Research on Human Subjects' of the Indian Council of Medical Research, India were followed in all the experiments. One of the three diorganotin(IV) complex (2\(\mu\)g ml\(^{-1}\)) was added to the 1ml blood for 2hr. At the end of 2hr treatment, the treated samples were washed twice with pre-warmed medium and were cultured in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum following phytohaemagglutinin stimulation and incubated at 37°C. If radiation was used in combination to diorganotin(IV) compounds then at the end of 2hr treatment, the treated samples were washed twice with pre-warmed medium and were irradiated with Faxitron Cabinet X-ray Systems (Model No. 43855D, 110kVp, 3mA, Beryllium window thickness 0.76mm) at the dose rate of 1.5 Gy min\(^{-1}\). All the samples were kept at 37°C for an hour after irradiation to allow normal cellular repair before setting up cultures in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum following phytohaemagglutinin stimulation and incubated at 37°C. To obtain differential sister chromatid staining, 5\(\mu\)g ml\(^{-1}\) 5-bromodeoxyuridine was added to the cultures at the time of initiation. Cells were harvested at 52 and 72hr and colcemid (0.1 \(\mu\)g ml\(^{-1}\)) was added 3hr prior to that.

The antitumor activity of diorganotin(IV) compounds was assessed in Dalton’s lymphoma (DL) cells, in a different set of experiments using 2-3 months old male Swiss-albino mice. The rules of the Institutional Animal Care and Use Committee were strictly followed during the whole experiment and steps were taken to protect the welfare of the experimental animals. An inoculum size of \(10^6\) cells per mouse was used, and the animals were maintained by serial intraperitoneal transplantation. The day DL cells were transplanted was considered day ‘0’; diorganotin(IV) compound (10 or 20mg kg\(^{-1}\)) was administered on the first, fifth and ninth day. When BSO was used, it was given on the fourth and eighth day, that is, 24h before diorganotin(IV) compound treatment.

**Results:** The present study in human lymphocytes demonstrated that these diorganotin(IV) complexes could block the cell cycle progression and induce sister chromatid exchanges
(SCEs) significantly, however, with respect to the induction of chromosome aberrations (CAs) it was very mild. Moreover, due to structurally advantages of the present diorganotin(IV) complexes, particularly increased bond-length of Sn-N bond, it could be inferred that these compounds could bind on DNA more easily, and thus possibly induce delay in cell proliferation and SCEs.

OTC-3 showed better antiproliferative and antitumor activity than OTC-1 and OTC-2, both as alone or in combination with X-rays. The maximum enhancement of exchange aberrations and the level of p53 and p16 proteins were observed in the OTC-3 treated samples. The increase of exchange aberrations in the combined treatment is important since such aberrations carrying cells may die apoptotically (Bassi et al 2003). To validate the apoptotic induction from treatment of diorganotin(IV) complexes, DNA fragmentation analysis was performed. A typical laddering pattern, which is believed to occur at the later stage in apoptosis, was observed. The laddering pattern is only seen after 24h of treatment with OTC-3 alone or in combination with 1.5Gy X-rays. The antitumor activity was determined in accordance with the US National Cancer Institute (NCI) standard protocol for primary screening in Dalton's lymphoma that was maintained by serial intraperitoneal transplantation. The T/C (treated/control) value was increased (186% with OTC-3) when diorganotin(IV) was treated after transplantation. Both the level of p53 and p16 proteins was raised after diorganotin(IV) treatment and such induction was maximum in the OTC-3 treated samples. The value of T/C was further improved when BSO was given 24hr before the OTC-1 and OTC-3 treatment but not with OTC-2. These data suggest that at a lower endogenous GSH level, the OTC-1 and OTC-3 complexes increased its antitumor potential. These results indicate a different mechanism of action by OTC-2 than OTC-1 and OTC-3.

The present data suggest that the OTC-3 has better anti-proliferative and anti-tumor activity and endogenous glutathione level has no influence on the effect of OTC-3. It could be possible that after treatment with either OTC-3 alone or in combination with X-rays the Dalton's lymphoma cells may die apoptotically after inducing initial delay in cell cycle and thereby survivality of mouse bearing Dalton's lymphoma cells was increased significantly.