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Vacuolar-type H^+-Translocating ATPase is the Target of Tributyltin Chloride

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Abstract—Tributyltin is broadly used as a preserving agent mainly for wood textiles and in antifouling paints on ships, leading to severe contamination of aquatic ecosystems. We here examined the effect of tributyltin chloride (TBT–Cl) on the vacuolar-type proton-translocating ATPase (V-ATPase), a proton pump, distributed in a variety of acidic organelles and specialized plasma membranes, playing a fundamental role in cellular pH homeostasis. TBT–Cl inhibited the V-ATPase activity of the vacuolar membrane vesicles from *Saccharomyces cerevisiae* with the IC_{50} of about 200 nM. The growth of *S. cerevisiae* was inhibited by TBT–Cl with the IC_{50} of about 1 μM, and fluorescence microscopic observation of quinacrine showed that vacuolar acidification of *S. cerevisiae* cells was blocked by this reagent. TBT–Cl inhibited growth of human culture cell lines, extraskeletal myxoid chondrosarcoma H-EMC-SS. Fluorescence microscopic observation of acridine orange as the pH indicator showed that TBT–Cl blocked the internal pH acidification of acidic organelles, such as lysosomes, in cells. These results suggest that V-ATPase is the possible target of tributyltin chloride in disruption of pH homeostasis.

Keywords: tributyltin, vacuolar ATPase, lysosome, pH, homeostasis

INTRODUCTION

Organotin compounds are of widespread use in many different areas for more than 60 years. Tributyltin is broadly used as a preserving agent mainly for wood textiles and in antifouling paints for marine vessels, quays and buoys. It is also used as a biocide in cooling systems and as an organometallic chemical in diverse applications (Fent, 1996; IPCS, 1999; USEPA, 2003). Triphenyltin is applied in agriculture as a pesticide. Dioctyl-, mono-, and dibutyltin serve as stabilizers in plastics and are therefore found in many products of daily use. Tributyltin is extremely stable and resistant to natural degradation in water. Although the use

and production of tributyltin are now strictly regulated, tributyltin and its degradation products, dibutyltin and monobutyltin, were leading to severe contamination of aquatic ecosystems (Iwata *et al.*, 1995; Tanabe *et al.*, 1998; USEPA, 2003).

Tributyltin is lipophilic and easily accumulated in animal tissues, being toxic to mammalian species. There is, however, limited information available on human exposure to this compound. Based on immunological toxicity in rats, a proposed tolerable daily intake of 0.25 μg/kg body weight per day was calculated for humans (Penninks, 1993). Human exposure is possible via contaminated water or food, or professional exposure due to the use of tributyltin-containing wood and paints (USEPA, 2003). Since butyltin compounds have bee used to stabilize polyvinyl chloride, they have been found in drinking water, municipal wastewater and sludge. Tributyltin is mainly found in fish, meat and dairy products (Kannan *et al.*, 1995).

The general toxicity of organotin compounds is complex and not well understood. Studies with animals have suggested that the spectrum of potential adverse chronic systemic effects of organotins in human includes primary immunosuppressive, endocrinopathic, neurotoxic, metabolic, and enzymatic activity, as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary, gastrointestinal, blood dyscrasias, reproductive/teratogenic/developmental, liver, kidney, bioaccumulative, and possibly carcinogenic activity (Antizar-Ladislao, 2008).

There were the experiments using a variety of cell types, including thymocytes (Gennari *et al.*, 2000), neuronal cells (Viviani *et al.*, 1995), Jurkat lymphoma cells (Stridh *et al.*, 1999), natural killer cells (Whalen *et al.*, 1999) and hepatocytes (Jurkiewicz *et al.*, 2004; Grondin *et al.*, 2007), to investigate the direct effect of organotin. Tributyltin and triphenyltin inhibited osteoclast differentiation (Yonezawa *et al.*, 2007) and the aromatase activity in human granulose-like tumor cell line (Saitoh *et al.*, 2001). Furthermore, it was reported that tributyltin induced apoptosis in a variety of cell lines (Viviani *et al.*, 1995; Stridh *et al.*, 1999; Whalen *et al.*, 1999; Gennari *et al.*, 2000; Jurkiewicz *et al.*, 2004; Grondin *et al.*, 2007). Apoptosis is an active cellular death process characterized by distinctive morphological changes that include chromatin condensation, cell shrinkage, nuclear disintegration, plasma membrane blebbing, to become known as apoptotic bodies. Although many pathways were proposed for apoptotic signaling along with various cell types, activation of initiator caspase is the first step of apoptosis occurring with the cellular events, such as activation of cytokine mediated death receptor pathway, cytochrome c release from mitochondria, up-regulation of cytoplasmic calcium and cellular acidosis (Karwatowska-Prokopczuk *et al.*, 1998; Kim *et al.*, 2006; Bernardi and Rasola, 2007). Tributyltin induced cytochrome c release from isolated rat mitochondria (Nishikimi *et al.*, 2001; Gogvadze *et al.*, 2002). In addition, it was suggested that tributyltin could be related to changes in intracellular calcium ion and inducing apoptosis (Aw *et al.*, 1990; Chow *et al.*, 1992; Viviani *et al.*, 1995; Reader *et al.*, 1999; Stridh *et al.*, 1999; Gennari *et al.*, 2000; Nakatsu *et al.*, 2006; Grondin *et al.*, 2007). However,

there is no observation for the effect of tributyltin, as a disrupter of cellular ion homeostasis, on cellular acidosis. We here examined the effect of tributyltin chloride (TBT–Cl) on the vacuolar-type proton-translocating ATPase (V-ATPase), a proton pump, distributed in a variety of acidic organelles and specialized plasma membranes, playing a fundamental role in cellular pH homeostasis (Nishi and Forgac, 2002).

MATERIALS AND METHODS

Cell culture and chemicals

H-EMC-SS (Extraskeletal Myxoid Chondrosarcoma) and KU812 (basophilic leukemia) cells were obtained from RIKEN Cell Bank (Tukuba, Japan). Cells were cultured in the medium of RPMI1640 containing 10% fetal bovine serum (FBS; GIBCO). TBT–Cl was purchased from Sigma-Aldrich. Concanamycin A and acridine orange were obtained from Wako (Tokyo, Japan). Phosphate buffer saline (PBS) was prepared by using ready-made salt as a manufacture's instructions (NISSUI PHARMACEUTICAL Co., Ltd., Tokyo, Japan). Trypan blue and trypsin were purchased from GIBCO. *Saccharomyces cerevisiae* (BY4741) cells were aerobically cultured at 30°C in the standard complex medium YPD (2% polypeptone, 1% yeast extract, 2% glucose).

Effect of TBT–Cl on the cell growth

Human cell lines (5×10^5 cells) were cultured in 35 mm culture dishes in the medium of RPMI1640 containing 10% FBS and various concentration of TBT–Cl or dimethyl sulfoxide (DMSO) as a control. After 40 hours, KU812 cells were stained by trypan blue (final concentration was 0.02%) and then viable cells were counted by counting chamber. In case of H-EMC-SS cells, after culture medium was removed, cells were once washed by 2 ml of PBS and treated by 0.1 ml of 0.25% trypsin/PBS for strip the cells. After trypsin activity was inhibited by adding 0.1 ml of FBS, cells were suspended in PBS containing 0.02% trypan blue to a final volume of 1 ml. Viable cells were counted by cell counting chamber. Growth inhibition effect was calculated by % of viable cells against control of DMSO. For the growth of *S. cerevisiae*, TBT–Cl at a concentration of 0, 1, 5 or 15 μM was added to the YPD culture at $OD_{660} = 0.1$. Effect of TBT–Cl on the cell growth was followed by the measurement of turbidity.

Detection of pH change in acidic organelles

Change of acidic organelles in human cell lines were done by staining with acridine orange after treated with TBT–Cl or concanamycin A, a specific H^+-V-ATPase inhibitor (Dröse and Altendorf, 1997). H-EMC-SS cells were cultured on 35 mm culture dishes 70% confluently, the cells were incubate in Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4–$2H_2O$, 1 mM $MgCl_2$–$6H_2O$, 12 mM $NaCO_3$, 1.8 mM $CaCl_2$–$2H_2O$, pH 7.4) containing DMSO, TBT–Cl or Concanamycin A for 10 minutes. Remove the reagents from the dish, cells were

incubated at room temperature in acridine orange solution (5 μM) for 10 minutes. After wash the cells three times by 2 ml of Tyrode's buffer, and then cells were observed using fluorescence microscope (Nicon). The acidification of yeast vacuoles was monitored by following the accumulation of quinacrine (Rothman *et al.*, 1989). Approximately 4–5 $\times$ 10^6 yeast cells were harvested, resuspended in 500 μl of YPD buffered with 100 mM Hepes (pH 7.6) containing 200 μM freshly prepared quinacrine and incubated with shaking at room temperature for 5 min. Cells were sedimented at 10,000 $\times$ *g* for 5 s and resuspended in 50 μl of 2% glucose buffered with 100 mM Hepes (pH 7.6). 3 μl samples were applied to a microscope slide and visualized immediately with fluorescence microscope. Cells were viewed under phase contrast optics to observe cell morphology and in fluorescence mode using a fluorescein filter with a 40× objective to observe quinacrine staining.

The ATPase assay

The vacuolar membrane vesicles were isolated from *S. cerevisiae* BY4741 cultured in the standard YPD medium as described previously (Ohsumi and Anraku, 1981; Kakinuma *et al.*, 1981). The ATP hydrolytic activities of the vacuolar membrane vesicles were measured as described previously (Kakinuma *et al.*, 1981).

RESULTS AND DISCUSSION

Inhibition by TBT–Cl of S. cerevisiae vacuolar ATPase

Organotins have been known for decades to act as potent inhibitors of the F_0F_1 ATP synthase distributed in mitochondria, chloroplast and oxidative bacteria (Barrett and Selwyn, 1976; Cain and Griffiths, 1977; Matsuno-Yagi and Hatefi, 1993). It is now accepted that inhibition by TBT–Cl of F_0F_1 ATP synthase was caused by binding of TBT–Cl on the ion channel moiety, the a subunit, of F_0F_1 ATP synthase (von Ballmoos *et al.*, 2004). Vacuolar-type H^+-translocating ATPase (V-ATPase) is the proton pump widely distributed in from bacteria to higher organisms (Nishi and Forgac, 2002). V-ATPase resembles to F_0F_1 ATP synthase in structure and molecular mechanism. It is expected that organotin compound such as tributyltin chloride acts on the vacuolar ATPase. However, the toxic effect of organotin to the V-ATPase was not well investigated; it has been reported that tributyltin inhibited the ATPase activity of the vacuolar ATPase in a plant (Smith *et al.*, 1984). We here examined the ATP hydrolytic activity of the V-ATPase of the vacuolar membrane vesicles of *S. cerevisiae*, and found that the V-ATPase is inhibited by TBT–Cl with the Ki value of approximately 200 nM. In the previous paper (Uchida *et al.*, 1985), interestingly, it has been reported that the ATPase activity of the V1 catalytic headpiece of V-ATPase was inhibited by 100 μM. These results suggest that the target site of tributyltin in V-ATPase is the membrane-embedded portion V0, likely Vph1 subunit (a subunit) as well as the a subunit of F_0F_1 ATP synthase.

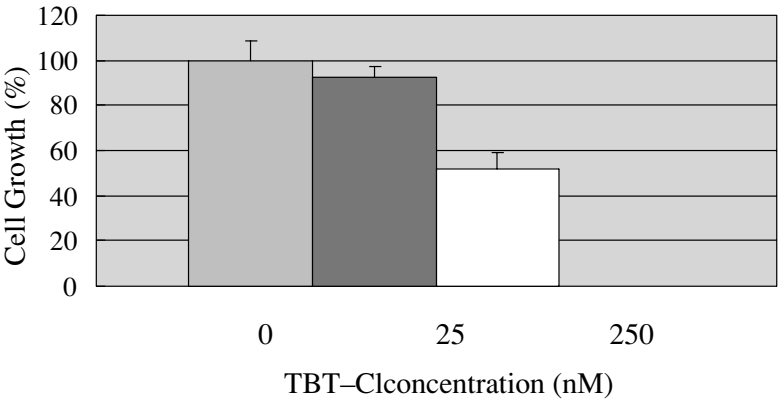

Fig. 1. Growth inhibition by TBT–Cl of H-EMC-SS. H-EMC-SS cells were cultured in various concentrations of TBT–Cl and DMSO as a control. Percentage of viable cell number against control was calculated (n = 3). EC_{50} was 260 nM.

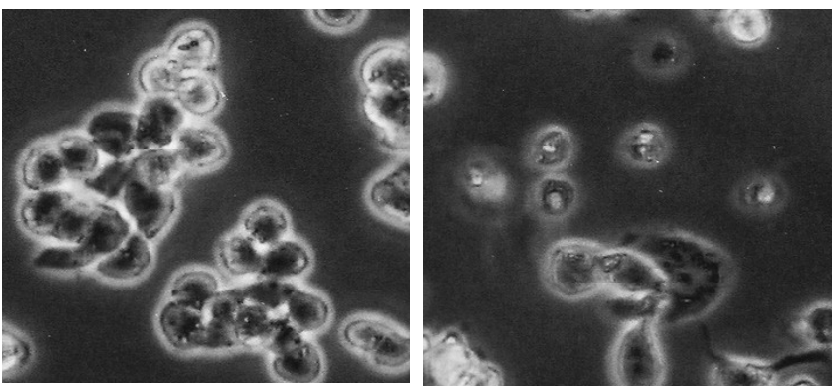

Fig. 2. Morphological change of H-EMC-SS cells in the presence of TBT–Cl. H-EMC-SS cells were cultured in the medium 10% FBS/RPMI1640 containing DMSO or 250 nM TBT. After 56 hrs, apoptotic cells were observed under a microscopy.

Inhibition by TBT–Cl of vacuolar acidification in S. cerevisiae

Vacuolar acidification of *S. cerevisiae* is important for cell physiology, such as cytoplasmic pH homeostasis, protein degradation, metabolite compartmentation in yeast (Wada and Anraku, 1994). The V-ATPase is indispensable for acidification of vacuoles in yeast. The mutant defective in the V-ATPase activity did not grow at standard pH condition (Anraku *et al.*, 1992). The effect of TBT–Cl on the

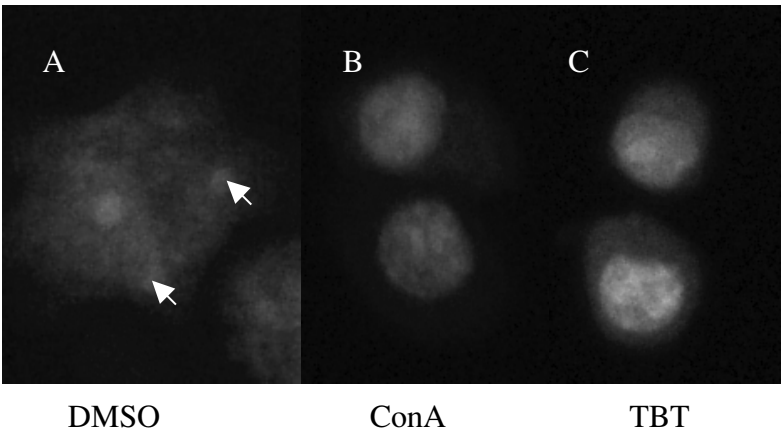

Fig. 3. Fluorescence of acridine orange in cells treated with TBT–Cl or concanamycin A, a specific V-ATPase inhibitor. DMSO, treated with dimethyl sulfoxide; TBT, 100 nM TBT–Cl; ConA, 50 nM concanamycin A. White arrows indicate acidic organelles stained with acridine orange in red color. Many red pigments like these were observed in the cell treated with DMSO, but in the cells treated with TBT–Cl or concanamycin A were not, only green fluorescence (see color image in the web data).

growth and vacuolar acidification in *S. cerevisiae* was examined. The cell growth was inhibited with IC_{50} of about 1 μM, and microscopic observation of quinacrine fluorescence revealed that vacuolar acidification was impaired by the addition of 500 nM TBT–Cl (data not shown). It has been reported in *S. cerevisiae* that Pdr5 multidrug resistance transporter five-fold increased the resistance of yeast to tributyltin (Golin *et al.*, 2000). Although it is likely that TBT–Cl attacks not only the V-ATPase but also other physiologically important reactions, these results suggest that the V-ATPase indispensible for the growth of yeast is one of the crucial targets of TBT–Cl.

Inhibition by TBT–Cl of cell proliferation of H-EMC-SS and KU812 cell line

Effect of tributyltin on a variety of cell types, including thymocytes, neuronal cells, Jurkat lymphoma cells, natural killer cells, keratinocyte and hepatocytes, were studied. However, there is no report on the effect of TBT–Cl on extraskeletal myxoid chondrosarcoma cells (H-EMC-SS) (Fig. 1) and basophilic leukemia cells (KU812) (data not shown). The growth of these cells was inhibited by TBT–Cl with EC50 for H-EMC-SS of 260 nM (Fig. 1) and for KU812 of 320 nM (data not shown), respectively. Considering the efficacy of TBT–Cl to the growth of *S. cerevisiae* as described above, the sensitivities to TBT–Cl of these mammal cell lines is nearly equal to the sensitivity of yeast. When H-EMC-SS and KU812 cells were incubated longer with TBT more than 2 days, apoptosis was induced (Fig. 2). It has been reported that the cytoplasmic acidification triggers apoptosis (Gottlieb *et al.*, 1996; Barriere *et al.*, 2001). The specific

inhibitor of the vacuolar ATPase, such as concanamycin A (Dröse and Altendorf, 1997), induced apoptotic cell death of a variety of cells (Nishihara *et al*., 1995). Therefore, it is likely that the blockage by TBT–Cl of the V-ATPase induced apoptosis of H-EMC-SS and KU812 cells through the cytoplasmic acidification.

Inhibition by TBT–Cl of organelle acidification in cultured cells

Organelle acidification such as lysosome is monitored by fluorescence of acridine orange (Hong *et al*., 2006). Fluorescence microscopic observation of acridine orange showed that organelle acidification was inhibited by addition of concanamycin A at 50 nM (Fig. 3B). When cells were treated with 100 nM TBT, acidification of organelles was also inhibited (Fig. 3C). Based on the results of the effects of TBT–Cl on the V-ATPase and the cell growth of *S. cerevisiae*, these results suggest that TBT inhibited organelle acidification via inhibition of the lysosomal V-ATPase. Although the cytoplasmic acidification was not clearly by observation of acridine orange fluorescence, it is likely that the vacuolar ATPase is the target of TBT–Cl, inducing apoptosis likely via the cytoplasmic acidification. Recently it was reported that TBT–Cl induced cytoplasmic acidification in hepatocytes (Grondin *et al*., 2007). As TBT–Cl-induced cytoplasmic acidification was stopped by DIDS, the inhibitor of Cl^-/HCO_3^- exchanger (Rothstein and Ramjeesingh, 1980) or Bapta-AM, calcium chelator, it was speculated that a change in calcium homeostasis triggers apoptosis (Grondin *et al*., 2007). However, there is so far no evidence for the direct interaction of TBT–Cl with the machinery, such as calcium channel, for calcium homeostasis. Instead, we consider that the effect of TBT–Cl on the V-ATPase of acidic organelles is crucial for pH homeostasis as well as calcium homeostasis (Aw *et al*., 1990; Chow *et al*., 1992; Viviani *et al*., 1995; Reader *et al*., 1999; Stridh *et al*., 1999; Gennari *et al*., 2000; Grondin *et al*., 2007; Nakatsu *et al*., 2006), inducing apoptosis. This is the first report suggesting the possibility of that the V-ATPase is one of the critical targets of TBT–Cl although the details of cytoplasmic acidification requires further investigation. In any case, the intracellular ion homeostasis (Schreiber, 2005) is important for understanding the toxicity of organotin compounds.

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REFERENCES

Anraku, Y., R. Hirata, Y. Wada and Y. Ohya (1992): Molecular genetics of the yeast vacuolar H^+-ATPase. *J. Exp. Biol*., **172**, 67–81.

Antizar-Ladislao, B. (2008): Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. *Environ. Int*., **34**, 292–308.

Aw, T. Y., P. Nicotera, L. Manzo and S. Orrenius, (1990): Tributyltin stimulates apoptosis in rat thymocytes. *Arch. Biochem. Biophys*., **283**, 46–50.

Barrett, R. H. and M. J. Selwyn (1976): Effects of triphenylsulphonium ions on mitochondria. Inhibition of adenosine triphosphatase activity. *Biochem. J*., **156**, 315–322.

Barriere, H., C. Poujeol, M. Tauc and J. M. Blasi (2001): CFTR modulates programmed cell death by decreasing intracellular pH in Chinese hamster lung fibroblasts. *Am. J. Physiol. Cell Physiol.*, **281**, 810–824.

Bernardi, P. and A. Rasola (2007): Calcium and cell death: the mitochondrial connection. *Subcell. Biochem.*, **45**, 481–506.

Cain, K. and D. E. Griffiths (1977): Studies of energy-linked reactions. Localization of the site of action of trialkyltin in yeast mitochondria. *Biochem. J.*, **162**, 575–580.

Chow, S. C., G. E. Kass, M. J. McCabe and S. Orrenius (1992): Tributyltin increases cytosolic free Ca^{2+} concentration in thymocytes by mobilizing intracellular Ca^{2+}, activating a Ca^{2+} entry pathway, and inhibiting Ca^{2+} efflux. *Arch. Biochem. Biophys.*, **298**, 143–149.

Dröse, S. and K. Altendorf (1997): Bafilomycins and concanamycins as inhibitors of V-ATPases and P-ATPases. *J. Exp. Biol.*, **200**, 1–8.

Fent, K. (1996): Ecotoxicology of organotin compounds. *Crit. Rev. Toxicol.*, **26**, 1–117.

Gennari, A., B. Viviani, C. L. Galli, M. Marinovich, R. Pieters and E. Corsini (2000): Organotins induce apoptosis by disturbance of $[Ca^{2+}]_i$ and mitochondrial activity, causing oxidative stress and activation of caspases in rat thymocytes. *Toxicol. Appl. Pharmacol.*, **169**, 185–190.

Gogvadze, V., H. Stridh, S. Orrenius and I. Cotgreave (2002): Tributyltin causes cytochrome C release from isolated mitochondria by two discrete mechanisms. *Biochem. Biophys. Res. Commun.*, **292**, 904–908.

Golin, J., A. Barkatt, S. Cronin, G. Eng and L. May (2000): Chemical specificity of the PDR5 multidrug resistance gene product of *Saccharomyces cerevisiae* based on studies with tri-*n*-alkyltin chlorides. *Antimicrob. Agents. Chemother.*, **44**, 134–138.

Gottlieb, R. A., J. Nordberg, E. Skowronski and B. M. Babior (1996): Apoptosis induced in Jurkat cells by several agents is preceded by intracellular, acidification. *Proc. Natl. Acad. Sci. USA*, **93**, 654–658.

Grondin, M., M. Marion, F. Denizeau and D. A. Averill-Bates (2007): Tributyltin induces apoptotic signaling in hepatocytes through pathways involving the endoplasmic reticulum and mitochondria. *Toxicol. Appl. Pharmacol.*, **222**, 57–68.

Hong, J., A. Yokomakura, Y. Nakano, K. Ishihara, M. Kaneda, M. Onodera, K. Nakahama, I. Morita, K. Niikura, J. W. Ahn, O. Zee and K. Ohuchi (2006): Inhibition of vacuolar-type H^+-ATPase by the cytostatic macrolide apicularen A and its role in apicularen A-induced apoptosis in RAW 264.7 cells. *FEBS Lett.*, **580**, 2723–2730.

IPCS (1999): Tributyltin oxide. *Concise International Chemical Assessment Documents*, vol. 14. World Health Organization, Geneva.

Iwata, H., S. Tanabe, T. Mizuno and R. Tatsukawa (1995): High accumulation of toxic butyltins in marine mammals from Japanese Coastal Waters. *Environ. Sci. Technol.*, **29**, 2959–2962.

Jurkiewicz, M., D. A. Averill-Bates, M. Marion and F. Denizeau (2004): Involvement of mitochondrial and death receptor pathways in tributyltin-induced apoptosis in rat hepatocytes. *Biochim. Biophys. Acta*, **1693**, 15–27.

Kakinuma, Y., Y. Ohsumi and Y. Anraku (1981): Properties of H^+-translocating adenosine triphosphatase in vacuolar membranes of *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **256**, 10859–10863.

Kannan, K., S. Tanabe, H. Iwata and R. Tatsukawa (1995): Butyltins in muscle and liver of fish collected from certain Asian and Oceanian countries. *Environ. Pollut.*, **90**, 279–290.

Karwatowska-Prokopczuk, E., J. A. Nordberg, H. L. Li, R. L. Engler and R. A. Gottlieb (1998): Effect of vacuolar proton ATPase on pHi, Ca^{2+}, and apoptosis in neonatal cardiomyocytes during metabolic inhibition/recovery. *Circ. Res.* **82**, 1231–1233.

Kim, R., M. Emi and K. Tanabe (2006): Role of mitochondria as the gardens of cell death. *Cancer Chemother. Pharmacol.*, **57**, 545–553.

Matsuno-Yagi, A. and Y. Hatefi (1993): Studies on the mechanism of oxidative phosphorylation. ATP synthesis by submitochondrial particles inhibited at F_0 by venturicidin and organotin compounds. *J. Biol. Chem.*, **268**, 6168–6173.

Nakatsu, Y., Y. Kotake and S. Ohta (2006): Tributyltin-induced cell death is mediated by calpain in PC12 cells. *Neurotoxicology*, **27**, 587–593.

Nishi, T. and M. Forgac (2002): The vacuolar H^+-ATPases—nature's most versatile proton pumps. *Nat. Rev. Mol. Cell Biol.*, **3**, 94–103.

Nishihara, T., S. Akifusa, T. Koseki, S. Kato, M. Muro and N. Hanada (1995): Specific inhibitors of vacuolar type H^+-ATPases induce apoptotic cell death. *Biochem. Biophys. Res. Commun.*, **212**, 255–262.

Nishikimi, A., Y. Kira, E. Kasahara, E. F. Sato, T. Kanno, K. Utsumi and M. Inoue (2001): Tributyltin interacts with mitochondria and induces cytochrome c release. *Biochem. J.*, **356**, 621–626.

Ohsumi, Y. and Y. Anraku (1981): Active transport of basic amino acids driven by a proton motive force in vacuolar membrane vesicles of *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **256**, 2079–2082.

Penninks, A. H. (1993): The evaluation of data-derived safety factors for bis(tri-*n*-butyltin)oxide. *Food Addit. Contam.*, **10**, 351–361.

Reader, S., V. Moutardier and F. Denizeau (1999): Tributyltin triggers apoptosis in trout hepatocytes: the role of Ca^{2+}, protein kinase C and proteases. *Biochim. Biophys. Acta*, **1448**, 473–485.

Rothman, J. H., C. T. Yamashiro, C. K. Raymond, P. M. Kane and T. H. Stevens (1989): Acidification of the lysosome-like vacuole and the vacuolar H^+-ATPase are deficient in two yeast mutants that fail to sort vacuolar proteins. *J. Cell Biol.*, **109**, 93–100.

Rothstein, A. and M. Ramjeesingh (1980): The functional arrangement of the anion channel of red blood cells. *Ann. N.Y. Acad. Sci.*, **358**, 1–12.

Saitoh, M., T. Yanase, H. Morinaga, M. Tanabe, Y. M. Mu, Y. Nishi, M. Nomura, T. Okabe, K. Goto, R. Takayanagi and H. Nawata (2001): Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN. *Biochem. Biophys. Res. Commun.*, **289**, 198–204.

Schreiber, R. (2005): Ca^{2+} signaling, intracellular pH and cell volume in cell proliferation. *J. Membr. Biol.*, **205**, 129–137.

Smith, J. A., E. G. Uribe, E. Ball, S. Heuer and U. Lüttge (1984): Characterization of the vacuolar ATPase activity of the crassulacean-acid-metabolism plant Kalanchoë daigremontiana. Receptor modulating. *Eur. J. Biochem.*, **141**, 415–420.

Stridh, H., D. Gigliotti, S. Orrenius and I. Cotgreave (1999): The role of calcium in pre- and postmitochondrial events in tributyltin-induced T-cell apoptosis. *Biochem. Biophys. Res. Commun.*, **266**, 460–465.

Tanabe, S., M. Prudente, T. Mizuno, J. Hasegawa, H. Iwata and N. Miyazaki (1998): Butyltin contamination in marine mammals from North Pacific and Asian Coastal Waters. *Environ. Sci. Technol.*, **32**, 193–198.

Uchida, E., Y. Ohsumi and Y. Anraku (1985): Purification and properties of H^+-translocating, Mg^{2+}-adenosine triphosphatasefrom vacuolar membranes of *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **260**, 1090–1095.

USEPA (United States Environmental Protection Agency) (2003): Ambient Aquatic Life Water Quality Criteria for Tributyltin.

Viviani, B., A. D. Rossi, S. C. Chow and P. Nicotera (1995): Organotin compounds induce calcium overload and apoptosis in PC12 cells. *Neurotoxicology*, **16**, 19–25.

von Ballmoos, C., J. Brunner and P. Dimroth (2004): The ion channel of F-ATP synthase is the target of toxic organotin compounds. *Proc. Nat. Acad. Sci. USA*, **101**, 11239–11244.

Wada, Y. and Y. Anraku (1994): Chemiosmotic coupling of ion transport in the yeast vacuole: its role in acidification inside organelles. *J. Bioenerg. Biomembr.*, **26**, 631–637.

Whalen, M. M., B. G. Loganathan and K. Kannan (1999): Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells in vitro. *Environ. Res.*, **81**, 108–116.

Yonezawa, T., S. Hasegawa, J. Y. Ahn, B. Y. Cha, T. Teruya, H. Hagiwara, K. Nagai and J. T. Woo (2007): Tributyltin and triphenyltin inhibit osteoclast differentiation through a retinoic acid receptor-dependent signaling pathway. *Biochem. Biophys. Res. Commun.* **355**, 10–15.

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