

# Organolead Toxicity in Plants: Triethyl Lead ( $\text{Et}_3\text{Pb}^+$ ) Acts as a Powerful Transmembrane $\text{Cl}^-/\text{OH}^-$ Exchanger Dissipating $\text{H}^+$ -Gradients at Nano-Molar Levels

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Triethyl Lead ( $\text{Et}_3\text{Pb}^+$ )-Toxicity,  $\text{H}^+$ -ATPase (Tonoplast), Anion Antiporter, Elongation Growth, *Zea mays*

Triethyl lead ( $\text{Et}_3\text{Pb}^+$ ), a highly toxic oxidation product of the anti-knock agent tetraethyl lead ( $\text{Et}_4\text{Pb}$ ) was shown to act as anion ( $\text{Cl}^-/\text{OH}^-$ ) antiporter in plant membranes, dissipating energy-dependent ion gradients, membrane potentials, and consequently turgor. This mechanism was demonstrated with tonoplast-type vesicles isolated from coleoptiles of *Zea mays* L. The ATP-driven  $\text{H}^+$  accumulation within those vesicles was abolished already at nano-molar levels of  $\text{Et}_3\text{Pb}^+$ , but only in the presence of  $\text{Cl}^-$ .

In intact cells the turgor dependent indole-3-acetic acid induced elongation growth of coleoptile segments of *Avena sativa* L. was inhibited by  $\text{Et}_3\text{Pb}^+$  at micro-molar levels and after a lag of 15–20 min. This lag might be due to a slow penetration of the agent through the waxy cuticle and the cell wall.

## Introduction

Tetraethyl lead ( $\text{Et}_4\text{Pb}$ ) is used as anti-knock agent in motor fuel. Its degradation product triethyl lead ( $\text{Et}_3\text{Pb}^+$ ) is toxic to cells of mammalian origin [1–5] as well as of algae and higher plants [3, 6–9]. Recently, triethyl lead was suggested to be one of the factors causing progressive damage of European forests [10–12] (but see [13]). The toxic effect of  $\text{Et}_3\text{Pb}^+$  to cells was attributed to an inhibition of microtubule assembly [2, 3, 5]. In *in vitro* experiments it has been found that  $\text{Et}_3\text{Pb}^+$  ( $> 1 \mu\text{M}$ ) interacts with thiol groups present in tubulin dimers. As a result tubulin loses its capability for microtubule assembly [4]. In the present study, evidence will be given that in plant cells, demonstrated with isolated vacuolar vesicles from *Zea mays* L.,  $\text{Et}_3\text{Pb}^+$  also acts as a potent trans-membrane  $\text{Cl}^-/\text{OH}^-$  exchanger. Thereby it dissipates ion gradients at nano-molar concentrations, *i.e.*, a range which is 1000-fold lower than that affecting microtubules [4].

## Materials and Methods

The preparation of microsomal and tonoplast vesicles from coleoptiles of *Zea mays* L., and the separa-

tion of membrane fractions by density gradient centrifugation was performed according to [18, 21]. The ATP-dependent intravesicular acidification of tonoplast-type vesicles was demonstrated with a dual wavelength method and neutral red ( $40 \mu\text{M}$ ) as pH indicator [15, 19, 21].  $\text{Et}_3\text{Pb}^+$  and  $\text{Et}_4\text{Pb}$  were purchased from Ventron, Karlsruhe, FRG.

## Results and Discussion

The energy-dependent transport of ions and solutes into the vacuole of a plant cell (necessary for the formation of turgor) can be studied by using isolated vacuoles or membrane vesicles derived from the tonoplast (reviewed in [14]). The primary driving force for the accumulation of osmotic compounds within the vacuole or vacuolar vesicles was shown to be an ATP-dependent  $\text{H}^+$ -pump [15]. A second, pyrophosphate-driven  $\text{H}^+$ -pump localized at the tonoplast was demonstrated only recently [16–18]. The transport of  $\text{H}^+$  strictly depends on a cotransport with  $\text{Cl}^-$  [18–20] or organic anions, such as malate [21], whereas the uptake of the osmotically important  $\text{K}^+$  ion occurs *via* a  $\text{K}^+/\text{H}^+$  exchange mechanism [18, 21, 22]. Furthermore, in some cases the active  $\text{H}^+$  transport is responsible for the uptake of sugars, metabolites, and natural products [23], thereby increasing the osmotic potential of the cell sap.

Fig. 1 depicts the ATP-driven uptake of  $\text{H}^+$  ions into tonoplast vesicles of coleoptiles of *Zea mays*.

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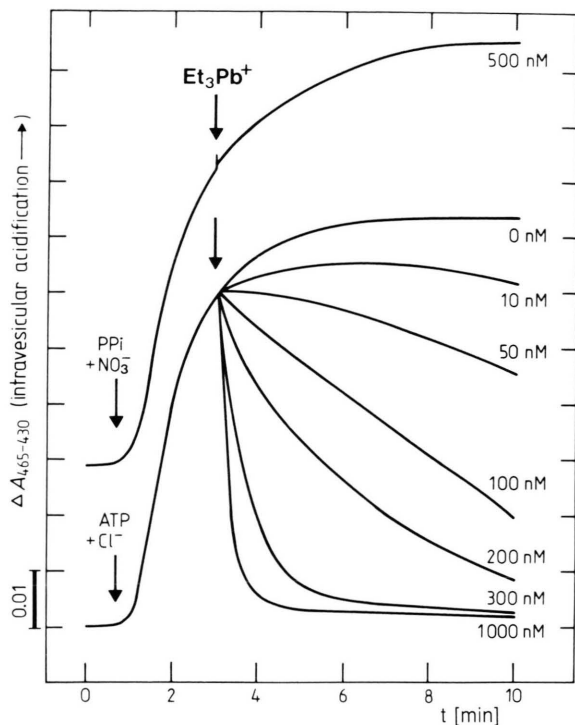


Fig. 1. Inhibition by  $\text{Et}_3\text{Pb}^+$  of the ATP-driven intravesicular acidification of tonoplast vesicles from coleoptiles of *Zea mays* in the presence of 50 mM KCl. In the presence of  $\text{NO}_3^-$  (instead of  $\text{Cl}^-$ ) the pyrophosphate (PPi)-driven acidification (which is  $\text{NO}_3^-$  insensitive in contrast to the  $\text{H}^+$ -ATPase [18]) is not abolished by  $\text{Et}_3\text{Pb}^+$ .

Under the given experimental conditions  $\text{Cl}^-$  is co-transported with the  $\text{H}^+$  [18, 19]. Addition of  $\text{Et}_3\text{Pb}^+$  at various concentrations caused an immediate destruction of the  $\text{H}^+$  gradient. Even in the low concentration range of 10 nM the toxin stopped the ATP-dependent accumulation of protons immediately and a decrease of the  $\text{H}^+$  concentration was initiated. A prerequisite for this drastic effect of  $\text{Et}_3\text{Pb}^+$  is the presence of  $\text{Cl}^-$ . As shown in Fig. 2 the intravesicular acidification occurring in the presence of the anion fumarate was not inhibited by  $\text{Et}_3\text{Pb}^+$ . Addition of  $\text{Cl}^-$  at the 3<sup>rd</sup> minute increases the  $\text{H}^+$  transport rate in the absence of  $\text{Et}_3\text{Pb}^+$ . In its presence, however,  $\text{Cl}^-$  induced a decrease of the  $\text{H}^+$  concentration within the vesicles. This effect can best be explained by the assumption that the  $\text{Et}_3\text{Pb}^+$  cation solubilized within the membrane is acting as a powerful  $\text{Cl}^-/\text{OH}^-$  exchanger (Fig. 5). The disappearance of accumulated protons can only occur if  $\text{Cl}^-$  ions transported into the vesicles are exchanged by  $\text{OH}^-$  from the medium, neutralizing the protons within the vesicles. Organic acids, such as fumarate, can not be exchanged for  $\text{OH}^-$  via  $\text{Et}_3\text{Pb}^+$  (Fig. 2). A further indication for  $\text{Cl}^-/\text{OH}^-$  antiporter properties of  $\text{Et}_3\text{Pb}^+$  is the fact that if  $\text{Cl}^-$  is substituted by  $\text{NO}_3^-$  the intravesicular acidification of tonoplast vesicles which is driven by the pyrophosphate (PPi)-dependent  $\text{H}^+$ -pump (insensitive to  $\text{NO}_3^-$  in contrast to the

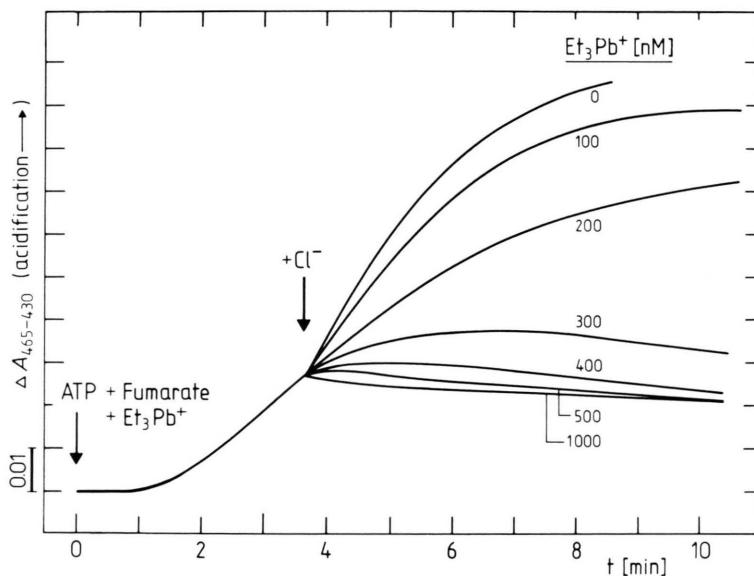


Fig. 2. ATP-driven intravesicular acidification of tonoplast vesicles in the presence of  $\text{Et}_3\text{Pb}^+$ . Initially fumarate was the anion, cotransported with the proton. Addition of  $\text{Cl}^-$  after the third minute enhances the  $\text{H}^+$  uptake, but in the presence of  $\text{Et}_3\text{Pb}^+$   $\text{Cl}^-$  decreases the  $\text{H}^+$  accumulation.

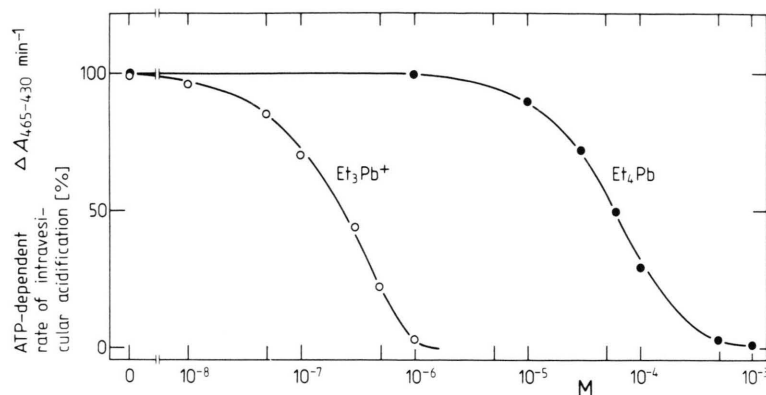


Fig. 3. Initial rate of the ATP-dependent intravesicular acidification of tonoplast-type vesicles in the presence of various concentrations of  $\text{Et}_3\text{Pb}^+$  and  $\text{Et}_4\text{Pb}$ .

ATP-driven  $\text{H}^+$ -pump; see Fig. 5) could not be inhibited by the toxin (Fig. 1).

The functioning of  $\text{Et}_3\text{Pb}^+$  as  $\text{Cl}^-/\text{OH}^-$  antiporter corresponds with similar mechanisms reported for triethyl-, tripropyl- or triphenyltin [24, 25].

It should be mentioned that in concentrations higher than  $1 \mu\text{M}$  an additional inhibitory effect of  $\text{Et}_3\text{Pb}^+$  was observed. The tonoplast-type  $\text{H}^+$ -pump activity depends on regulatory thiol groups on the enzyme [21]. SH-blocking agents, such as *p*-hydroxymercuribenzoate, or an oxidation of these sulfhydryl groups to disulfides, *e.g.*, by blue light or by  $\text{H}_2\text{O}_2$ , inactivated the enzyme reversibly; a rereduction by GSH restores the activity [21, 26].  $\text{Et}_3\text{Pb}^+$  interacts with these thiols of the  $\text{H}^+$ -ATPase at concentrations comparable with those employed for the inhibition of microtubule assembly [4]. But this SH-blocking effect might not be of importance under *in vivo* conditions because  $\text{Et}_3\text{Pb}^+$ , acting as  $\text{Cl}^-/\text{OH}^-$  exchanger, already disturbs cell metabolism in a much lower, nano-molar concentration range.

A comparison of the effects of  $\text{Et}_3\text{Pb}^+$  and  $\text{Et}_4\text{Pb}$  on the ATP-dependent rates of the acidification of tonoplast vesicles (Fig. 3) shows that the oxidized charged molecule is 1000-fold more effective in abolishing the proton accumulation within tonoplast vesicles than  $\text{Et}_4\text{Pb}$ . The relatively small inhibitory effect of  $\text{Et}_4\text{Pb}$  may probably be caused by contamination with  $\text{Et}_3\text{Pb}^+$  molecules, which are permanently formed in small amounts by oxidation from  $\text{Et}_4\text{Pb}$ .

The strong inhibitory effect of  $\text{Et}_3\text{Pb}^+$  on the accumulation of ions within vacuolar vesicles should result in an immediate collapse of turgor of the intact cell. However, in experiments with coleoptiles auxin-induced elongation growth, which depends on a suf-

ficient osmolarity of the cell sap, was inhibited only slowly and at higher concentrations of  $\text{Et}_3\text{Pb}^+$  only (Fig. 4). This retarded effect of the toxin could be due to absorption (cuticle; cell wall) and, consequently, a poor penetration into the cytoplasm.

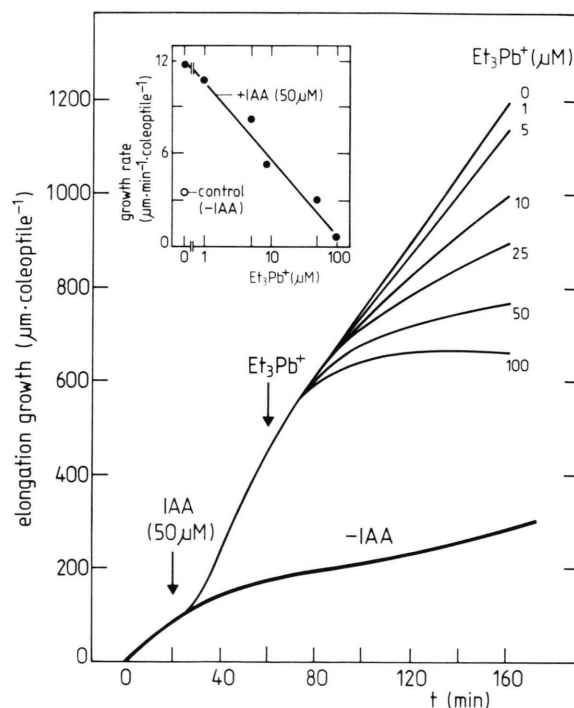


Fig. 4. Inhibition of auxin(IAA)-induced elongation growth of *Avena* coleoptile segments (1 cm in length) by various concentrations of  $\text{Et}_3\text{Pb}^+$ . Insert: Rate of elongation growth of coleoptile segments 3 h after addition of IAA ( $50 \mu\text{M}$ ) and  $\text{Et}_3\text{Pb}^+$  (various concentrations). IAA = Indole-3-acetic acid. Method see [28].

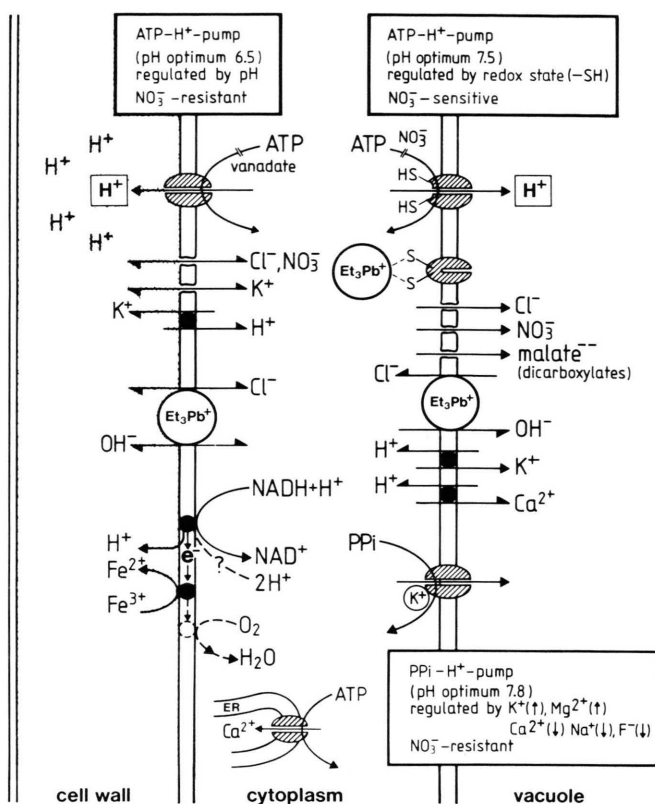


Fig. 5. Schematic presentation of the primary and secondary energized ion transport mechanisms in a plant cell as demonstrated in [18] and other recent publications [21, 26–29; 14, 30], and the sites of Et<sub>3</sub>Pb<sup>+</sup> action as Cl<sup>-</sup>/OH<sup>-</sup> antiporter and SH-blocker, effective in nmolar and μmolar concentrations, respectively.

Therefore, the disappearance of the toxin from a solution containing fresh needles of conifers [10] can not give evidence to what degree cellular processes will be inhibited.

The effects of Et<sub>3</sub>Pb<sup>+</sup> on plant cells by acting as an anion antiporter and a thiol blocker of the tonoplast-type H<sup>+</sup>-pump are summarized in Fig. 5. The experi-

mental basis of this scheme is provided in some recent publications [18 and 15, 19, 21, 26–29; 14, 30].

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