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Studies on the phytotoxic effects of some organotin(IV) compounds on the germination of the mung bean seed, *Phaseolus aureus*

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A selected range of organotin(IV) compounds of formula R_xSnX_{4-x} ($x=2$ or 3; R = alkyl, cycloalkyl or aryl), as well as the adducts, $(p\text{-}ZC_6H_4)_3SnCl.Ph_3PO$ (Z = H, Me) were examined *in vivo* for their phytotoxic effects relative to the more powerful *s*-triazine herbicides against the mung bean seed, *Phaseolus aureus*. Phytotoxicity was assessed in terms of changes in (a) weights of primary leaves, (b) weights of cotyledons, (c) seedling heights and (d) root morphology. The triorganotin compounds were more phytotoxic than the diorganotins, and within the R_3Sn structural class, inhibitory potency was greater for alkyltins than for aryltins. Decreasing the alkyl chain lengths or placing electron-donating substituents on the aromatic rings increased the phytotoxicity. A significant phytotoxic effect, comparable with that of Ph_3SnCl, was manifested by tricyclohexyltin chloride, in marked contrast to the well-known low phytotoxicity of tricyclohexyltin hydroxide.

Using Ph_3SnCl as the model compound, it was further demonstrated that the total chlorophyll content decreased with increasing concentrations of Ph_3SnCl, but the ratio of chlorophyll *a* to chlorophyll *b* was constant. It was also found that tin uptake in the seedlings was directly proportional to Ph_3SnCl test concentrations; the magnesium and iron levels in the treated seedlings, however, were unchanged relative to the control.

Keywords: Organotins, *in vivo* phytotoxicity, mung bean, structure–activity relationships, chlorophyll, growth retardation

INTRODUCTION

Studies on the biological properties of organotin(IV) compounds have received considerable attention[1–5] since the pioneering efforts in the field by van der Kerk[6,7] in the early 1950s. Commercial interest in organotin biocides has focused on the active triorganotin structural class and, specifically, on tri-n-butyl-, triphenyl-, and tricyclohexyl-tins which possess low mammalian toxicities[8] and display favourable environmental properties.[9]

The tri-n-butyltins find use as industrial biocides,[1] particularly in wood preservation and antifouling coatings, while the triphenyltin and tricyclohexyltin compounds are used principally in agriculture[1,10] as fungicides and miticides, respectively.

The high phytotoxicity of tri-n-butyltins precludes their use in agriculture. The higher alkyl derivatives such as tri-n-octyltins are non-phytotoxic[11] but are also bereft of any useful biological activity. The tricyclohexyltin compounds exert negligible[11] phytotoxicity but their antifungal properties are less pronounced compared with the triphenyltins, Ph_3SnX. With the latter compounds, the phytotoxicity was found to be influenced[12,13] by the nature of the anionic X moiety on tin, and was greatest for X = chloride and sulphate, and less when X = hydroxide or acetate. Although Ph_3SnOH and Ph_3SnOAc appear to be well tolerated by many plants at the concentrations required for fungicidal action, nevertheless they pose a general phytotoxic problem which has hindered their widespread use. This has encouraged[11] efforts aimed at moderating their phytotoxicity through appropriate structural modifications, and among claims for reduced phytotoxicity based on this approach are those[14] reported in the patent literature for $(Ph_3Sn)_2S$ and for compounds of the general formulation[15] $[Ar(SO_n)_mC(X){:}NOSnPh_3]$ (X = CN or CO_2Et; Ar = aryl; $n=0$–2; $m=0$ or 1), which exert an antidotal action even when used with phytotoxic agrochemicals. However, there appear to be no *in vivo* biochemical studies to date on

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organotin phytotoxicity. This has prompted the work described herein on the phytotoxic effects of a selected range of organotin compounds on the germination of the mung bean seed (*Phaseolus aureus*).

MATERIALS AND METHODS

Reagents

With the exception of Me_3SnCl and Me_2SnCl_2, which were commercially procured samples, the remainder of the organotin(IV) compounds used in this study were synthesized according to established methods[16–18] and their purity established by elemental analyses.

The *s*-triazine herbicides (atrazine and simazine) were a gift from Ciba–Geigy Chemical Co., Basel, Switzerland and were used without further purification.

The following alphabetical codes are used to represent the test compounds whose data are depicted in the histograms (Fig. 1a–c):

A, control with distilled water; B, control with $2\,cm^3$ ethanol dissolved in $1\,dm^3$ distilled water; C, $Ph_3SnCl.Ph_3PO$; D, Bu_3SnOAc; E, Me_2SnCl_2; F, Ph_2SnCl_2; G, Ph_3SnCl; H, Ph_3SnOAc; I, Ph_3SnOH; J, Cyh_3SnCl; K, $(p\text{-}ClC_6H_4)_3SnCl$; L, Ph_3Sn(indole-3-acetate); M, $(p\text{-}MeC_6H_4)_3SnCl.Ph_3PO$; N, $(p\text{-}MeC_6H_4)_3SnCl$; O, Me_3SnCl; P, Et_3SnOAc; Q, atrazine; R, simazine.

Abbreviations used: Me, methyl; Et, ethyl; Bu, n-butyl; Ph, phenyl; Cyh, cyclohexyl.

Germination of mung bean seeds

Mung bean seeds were first immersed for 16 h in running tap-water, after which their seed coats were removed prior to sowing. Ten healthy seeds of uniform size and radicle length were sown per petri dish (80 mm in diameter), which was layered with cotton gauze. Each experimental group was represented by five dishes which were arranged randomly in a uniformly-lit growth chamber ($150\,cm \times 50\,cm \times 50\,cm$), which was well-protected from insects.

The lighting arrangement consisted of four fluorescent tubes (each of 120 cm length and 60 W power) arranged parallel and equidistant to each other and positioned 45 cm from the surface of the petri dishes.

The germinating seedlings were watered daily with fixed volumes of $15\,cm^3$ distilled water or treatment solution applied directly to the cotton gauze. The seedlings were harvested as and when required for evaluation of phytotoxic effects.

Preparation of control and treatment solutions

One control consisted of distilled water while the other was $2\,cm^3$ ethanol made up to $1\,dm^3$ with distilled water.

Treatment solutions were prepared by dissolving weighed amounts of the organotin or triazine test compounds in $2\,cm^3$ ethanol and making up to the mark in a one-litre standard flask with glass-double-distilled water to yield final concentrations of $10\,\mu mol\,dm^{-3}$. Detailed studies with Ph_3SnCl were performed with 0.5, 1.0, 1.5 and $2.0\,\mu g\,g^{-1}$ concentrations.

Evaluation of phytotoxicity

Seedlings grown in the presence and absence of organotin compounds for seven days were washed, dried on paper towels and subjected to the following measurements. The entire length of the seedling was measured from the primary root tip to the shoot tip with the aid of a string. The weights of primary leaves and the cotyledons for each seedling were taken. Additionally, visual observations pertaining to the general appearance of the root, stem, cotyledons and leaves of the seedlings were made.

Extraction and estimation of chlorophyll

Seven-day-old seedlings grown in various concentrations of Ph_3SnCl were harvested and their chlorophyll content estimated in the following manner. The harvested primary leaves were washed and dried on paper towels, after which 1 g of leaf tissue per sample was homogenized with traces of calcium carbonate, acid-washed sand and $10\,cm^3$ of 80% (v/v) aqueous acetone in a Silverson Blender Emulsifier. The homogenate was filtered through a sodium sulphate filter-cake in a Buchner funnel and the brie washed free of chlorophyll with further amounts of the aqueous acetone. The extract and washings were combined and made up to $50\,cm^3$ in a standard flask. The entire procedure was conducted under reduced-light conditions. The above chlorophyll extract was subsequently diluted three-fold with the 80% aqueous acetone and spectrophotometrically assayed by the method of Harborne[19] for total chlorophyll content as well as for chlorophyll *a* and chlorophyll *b*.

Preparation of dry matter from mung bean seedlings for tin, magnesium and iron analyses

Seven-day-old seedlings grown in various concentrations of Ph_3SnCl were harvested and washed with distilled water followed by deionized water. The seedlings were dried using paper towels and weighed. They were then dried to constant weight in an oven at 70°C.

The dried material from each experiment was ground to a fine powder using liquid nitrogen in a mortar and pestle. Dried material (4 g) from each sample was added to a 250 cm^3 digestion flask and digested with a concentrated acid mixture, consisting of sulphuric and nitric acids in 15:10 volume proportions and a few drops of perchloric acid, for 45 min in a fume cupboard until evolution of NO_2 ceased and the solution in the flask became clear. Acid blanks were similarly digested.

The clear digest was diluted to 50 cm^3 with deionized water and then filtered through a Whatman 40 filter paper. The filtered digest was used for the determination of tin, magnesium and iron.

The analyses of these metals in the respective digests were carried out using the automated Plasmascan System (Model 710 ICP). For calibration purposes, standards of each element were prepared in glass-double-distilled, deionized water. The samples were analysed in a sequential manner using the respective element disc programmes.

RESULTS AND DISCUSSION

A range of organotin(IV) compounds including several known for their antifungal properties were screened for their phytotoxicity based on the inhibition of the rate of germination of the mung bean seed. In addition, their effects were compared with those of the *s*-triazine herbicides, atrazine and simazine.

The results, displayed in the histograms of Fig. 1, revealed that, in general, a greater degree of inhibition of germination of the mung bean seedlings was exerted by the alkyltin compounds than by the aryltins.

When the trimethyl-, triethyl-, and tributyl-tin compounds were compared, the degree of inhibition of the germination rate was found to decrease with increasing alkyl chain length. Replacement of one alkyl group by an anionic moiety appeared to decrease the inhibitory effect, as could be seen by comparing the histograms for Me_2SnCl_2 and Me_3SnCl. In the triphenyltin series, no significant differences in the overall rate of inhibition were noted when the inorganic residue was changed from the chloro to the hydroxy or acetate group.

However, a variation in the ester function from acetate to indole-3-acetate resulted in a greater reduction in primary leaf weight. Indole-3-acetic acid is a well-known plant growth hormone[20–22] and it was anticipated that its attachment to a triorganostannyl moiety would promote greater uptake of the organotin substrate by the plant. The higher phytotoxicity of the indole-3-acetate derivative relative to Ph_3SnOAc suggests this to be the case, and is supported by the observed correlation between reduction in leaf weight and tin content in the Ph_3SnCl-treated seedlings (*vide infra*). As in the alkyltin case, mono-dearylation of Ph_3SnX led to an appreciable decrease in phytotoxicity.

The introduction of an electron-withdrawing group such as halogen in the phenyl rings decreased the inhibitory potency as noted from the results obtained with $(p\text{-ClC}_6H_4)_3SnCl$. However, introduction of an electron-donating *p*-methyl group enhanced the inhibitory potency, as borne out by the data on $(p\text{-MeC}_6H_4)_3SnCl$ and its triphenylphosphine oxide adduct.

Tricyclohexyltin chloride manifested the same degree of inhibitory potency as Ph_3SnCl. Inasmuch as Cyh_3SnOH is essentially non-phytotoxic, the above result underscores the importance of the anionic moiety in determining the overall phytotoxicity of a given triorganotin compound.

The lower trialkyltin derivatives are known to be highly toxic to mammals, unlike their triaryltin counterparts.[8] Since triphenyltin derivatives have already found some application as agricultural fungicides, it was felt useful to investigate the phytotoxic effects of this class of organotin

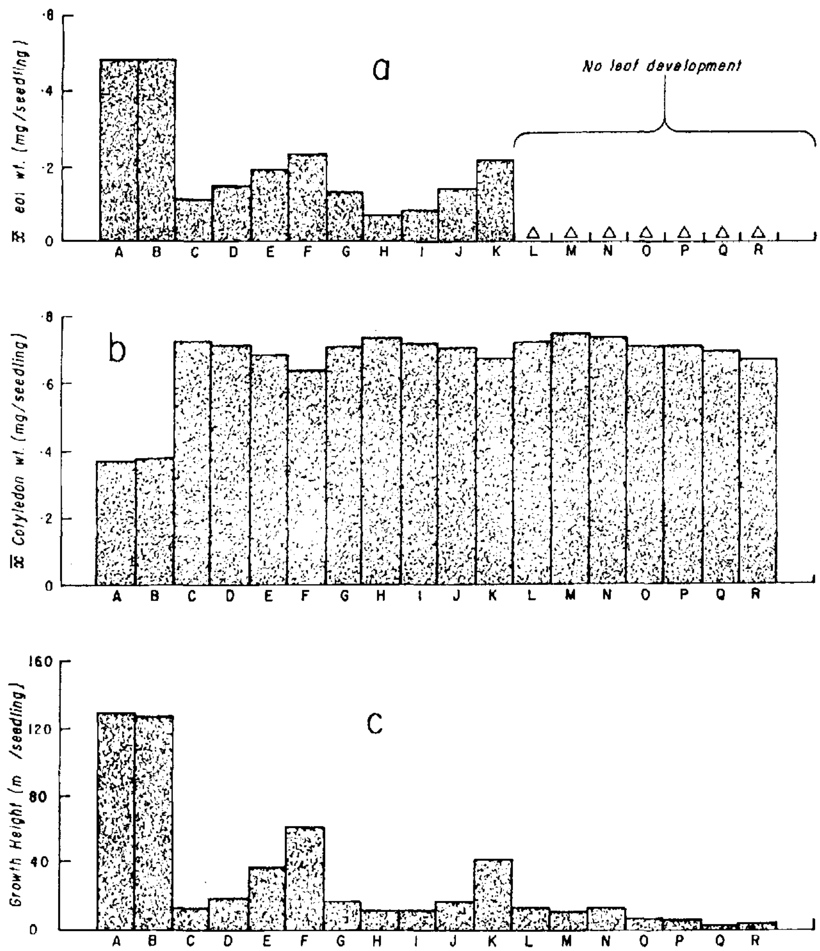

Figure 1 Comparison of the effects of some organotin compounds and *s*-triazines on (a) mean primary leaf weight, (b) mean cotyledon weight, and (c) growth height of treated mung bean seedlings.

compounds using Ph_3SnCl (Brestanol; Hoechst) as a model compound. The latter was selected over Ph_3SnOH (Duter; (Philip–Duphar) and Ph_3SnOAc (Brestan; Hoechst) on account of its increased water solubility.[9]

Effect of Ph_3SnCl on overall growth of germinating mung bean seedlings

The concentration-dependent effects of Ph_3SnCl on both visual and physical parameters of the mung bean seedlings were investigated in a range of concentrations of up to $2.0\,\mu g\,g^{-1}$. Phototoxicity was assessed (see Fig. 2) in terms of retarded growth height, poor root development, delayed emergence of primary leaves, reduction in leaf colour and the delayed shrinking of the cotyledons.

Jones and Everett[23] reported foliar damage when Ph_3SnOH was used in controlling fungal diseases of cucumber. Kranz[24] reported a similar observation when Ph_3SnOAc was used to treat banana leaf spot. Apparently, the greatest damage by Ph_3Sn compounds is to the roots. This is especially evidenced in the studies of Solel,[25] Mukhopadhyay and Thakur,[26] Singh and Sharma[27] and Mukhopadhyay and Upadhay.[28] Ph_3SnCl and Ph_3SnOAc were tested by these workers on sugar beet seedlings at concentrations of 100 and $500\,mg\,cm^{-3}$. Both these compounds were found to affect root development in the seedlings. A similar finding is noted in the present study on the mung bean seedlings using these compounds.

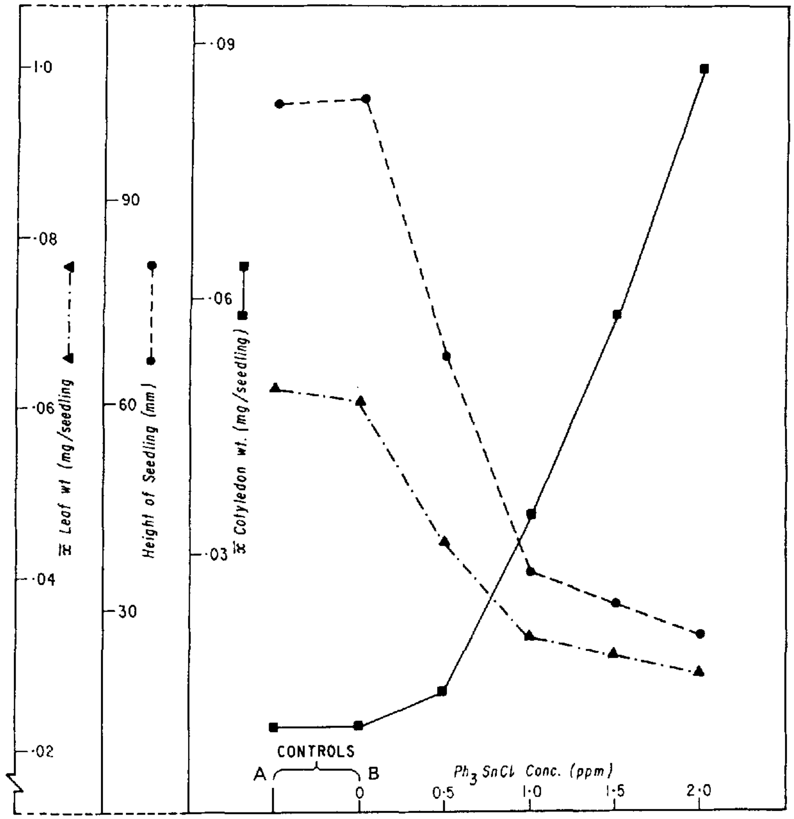

Figure 2 Effect of various concentrations of Ph_3SnCl on growth height, mean primary leaf weight and mean cotyledon weight of mung bean seedlings.

A number of workers have demonstrated that in healthy seedlings, cotyledons functioning as storage organs empty out as other parts of the embryo develop.[29–33] Further, the decrease in weight of the cotyledons during germination is correspondingly reflected in the growth of the seedlings. These effects have been substantiated in seedlings belonging to the two control groups but not in the Ph_3SnCl-treated seedlings (Fig. 2).

Evaluation of tin, iron and magnesium levels in Ph_3SnCl-treated seedlings

Since phytotoxic effects were pronounced in the seedlings grown in the presence of increasing concentrations of Ph_3SnCl, it became important to establish tin uptake in the seedlings and also the concentrations at which tin was present in the seedlings. For this reason, seedlings grown for seven days in the presence of varying concentrations of Ph_3SnCl were analysed for metal content as described under Experimental. It was found that the concentration of tin in the plants was proportional to the test concentration of Ph_3SnCl used (Table 1). Furthermore, the con-

Table 1 Measurements of Ph_3SnCl uptake by germinating mung bean seedlings

Treatment	Tin content[a] ($\times 10^{-3}\,\mu g\,g^{-1}$ fresh tissue)
Control A[b]	Nil
Control B[c]	Nil
$0.5\,\mu g\,g^{-1}$ Ph_3SnCl	1.4
$1.0\,\mu g\,g^{-1}$ Ph_3SnCl	2.0
$1.5\,\mu g\,g^{-1}$ Ph_3SnCl	2.3
$2.0\,\mu g\,g^{-1}$ Ph_3SnCl	2.8

[a]Results averaged from three separate experiments.
[b]With distilled water.
[c]With $2\,cm^3$ ethanol dissolved in $1\,dm^3$ distilled water.

Table 2 Effects of Ph_3SnCl on chlorophyll content

Treatment	Total chlorophyll ($mg\,g^{-1}$ fresh wt)	Chlorophyll *a* ($mg\,g^{-1}$ fresh wt)	Chlorophyll *b* ($mg\,g^{-1}$ fresh wt)	Ratio of chlorophyll *a*: chlorophyll *b*
Control A[a]	3.5	2.7	0.8	3.2
Control B[b]	3.5	2.6	0.9	3.1
$0.5\,\mu g\,g^{-1}$ Ph_3SnCl	3.3	2.5	0.8	3.1
$1.0\,\mu g\,g^{-1}$ Ph_3SnCl	2.8	2.1	0.7	3.2
$1.5\,\mu g\,g^{-1}$ Ph_3SnCl	1.8	1.3	0.4	3.0
$2.0\,\mu g\,g^{-1}$ Ph_3SnCl	1.5	1.1	0.4	3.0

[a]With distilled water.
[b]With $2\,cm^3$ ethanol dissolved in $1\,dm^3$ distilled water.

centration of tin present in the plants was directly proportional to the degree of phytotoxicity manifested by the seedlings. No detectable levels of tin were found in the control plants. In all cases, however, the levels of magnesium and iron remained essentially constant at 0.2 and $5.4 \times 10^{-3}\,mg\,g^{-1}$ of fresh tissue, respectively.

It was noticed that the colour of primary leaves of Ph_3SnCl-treated seedlings appeared less intense with increasing concentrations of Ph_3SnCl when compared with the controls. When the total amount of chlorophyll in these seedlings was estimated, it was found that the total chlorophyll content decreased with increasing concentrations of Ph_3SnCl (Table 2). However, the ratio of chlorophyll *a*: chlorophyll *b* remained constant regardless of the tin concentrations used in this study. Inasmuch as magnesium levels in both the treated and untreated seedlings did not vary significantly, the decrease in total chlorophyll content in the treated seedlings could not be attributed to a decrease in magnesium concentration in the treated seedlings. Similarly, the constancy of the level of iron (an essential component of the respiratory chain) in the plants rules out deficiency of the metal in the treated seedlings as being the cause of retarded growth. Although several reasons are possible, it is tempting to explain the above results in terms of the inhibition of ATP synthesis. Pertinent to this consideration is the *in vitro* study on potato and mung bean mitochondria by Moore *et al.*,[34] who found the chlorotin derivative, n-$Bu_2MeSnCl$, to be a potent electron-transport inhibitor localized on the substrate side of the ubiquinone–cytochrome *b* region of the respiratory chain. Further work is in progress to elucidate the biochemical features of the phytotoxic effects revealed in this study.

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