vities is that of the calculation of the ratio extracellular/total activity, an internal parameter, that may be an improved indicator of stress, as proposed elsewhere[6].

Finally, the possibility of a practical application of these biochemical assays for detecting ozone stress to Norway spruce is encouraging. Saplings placed in the chamber fumigated with ambient air and trees maintained outside exhibited biochemical values which were close to those of saplings fumigated with ozone; i.e., the levels of pollutants in ambient air were high enough to induce a change in those biochemical parameters. More work is needed with large trees, in situ, to separate the possible effects of season, and several intrinsic tree variables, from changes induced by ozone and other stresses.

Abbreviations used: ACC, 1,aminocyclopropane-1-carboxylic acid; EDTA, ethylenediaminetetraacetic acid; NBT, nitrobluetetrazolium; SOD, superoxide dismutase; G6PDH, glucose-6-P-dehydrogenase; HPLC, high performance liquid chromatography.

Acknowledgments. This work was supported in part by Grant Number 4849-0.85 (NFP 14) from the Swiss National Fund of Scientific Research. We thank Dr F. Cupelin and Dr J.Cl. Landry from the Service d'Ecotoxicologie, Genève, who provided the facilities for the monitoring of ozone levels. We also thank A. Mesrobian, A. Rossier and D. Voluntaru for technical assistance. Du Pont de Nemours International is gratefully acknowledged for the gift of TEDLAR (polyvinyl fluoride film) to build up the fumigation chambers.

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Trialkyllead. Occurrence, biological interactions, and possible impact on forest decline

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Summary. Biochemical studies and experiments with cells have shown that trialkyllead (R_3Pb^+), a degradation product of tetraalkyllead (R_4Pb) antiknock agents, is highly cytotoxic. Trialkyllead may occur in rain and fog in concentrations much higher than those reported so far. From these data it has been proposed that trialkyllead may represent one of the factors responsible for the present forest decline. This article reviews the chemistry of trialkyllead, its molecular interactions, its toxic effects on cells and on plant cells in particular, and its occurrence in the environment. Evidence for and against the involvement of trialkyllead in forest decline will be discussed.
Key words. Organolead; phytotoxicity; rain water; acid rain; glutathione.

Experientia 43 (1987), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Introduction

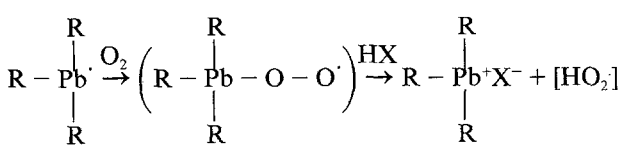
Several years ago, trialkylated lead derivatives were used as biocides[8, 12, 33, 62]. Large scale application did not occur because of the high toxicity of these compounds for many species, including mammals. Obviously, the compounds must interact with basic biological processes in lower and higher organisms.

Since 1962 it has been known that trialkyllead disturbs the function of mitochondria[2]. In the following years it was shown that organolead compounds inhibit enzymes such as glutathione-S-aryl transferase[31], or serum choline esterase[24]. Furthermore, membrane-bound ATPases providing the energy for transport processes were found to be susceptible to the toxin[26]. It was recently reported that trialkyllead interferes with cytoskeletal proteins, inhibiting, for example, the assembly of tubulin[20, 57, 73]. Considering the evidence coming from the various biochemical studies, the high cytotoxicity of trialkyllead compounds can no longer be regarded as a matter of controversy. Clearly, the ecological significance of these compounds depends on whether they occur in the environment, and if so, at what concentrations.

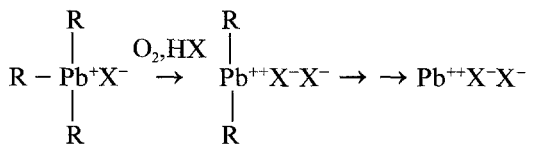
The problem of organolead (R_3Pb^+, R_4Pb, R = methyl, ethyl) in the environment was addressed several years ago by numerous laboratories, which analyzed air, surface water, or marine organisms[9, 11, 14]. Most of the analytical approaches aimed at tetraalkylated lead compounds, while determinations of trialkyllead were rare and ambiguous, until in 1983 De Jonghe et al.[15] described a procedure for aqueous solutions, which was applied to a few rain water samples collected in Belgium. In 1984 we started rain water sampling at two locations on the ridge of the Black Forest, using the inhibition of tubulin assembly for the quantitation of trialkyllead. The toxin was found in about 20% of all samples, in a few of them in considerable concentrations[21]. Both sampling stations in the Black Forest were located in areas with progressive forest decline. In 1985 additional sampling stations were established, for example in a city, and close to a highway junction. Furthermore, we have continued our studies on the effects of trialkyllead on various biochemical and biological systems. Results of both kinds of work will be described here.

Chemical nature

In order to understand the occurrence and the biological activities of organolead compounds it is useful to consider briefly some aspects of their chemical properties. Tetraalkylated lead compounds (R_4Pb), added to gasoline as antiknock agents, are instable. On exposure to light[49] or thermal energy, one of the 4 Pb-C bonds is easily broken into radical fragments. The resulting trialkyllead radical undergoes oxidation by oxygen, eventually leading to the formation of trialkyllead cation:



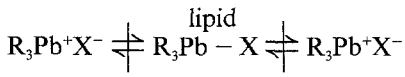
In the cationic trialkyllead (R_3Pb^+) the Pb-C bonds are more stable than in R_4Pb as concluded from a slower degradation of these compounds[35]; nevertheless, decomposition may proceed until only inorganic lead (Pb^{++}) remains:



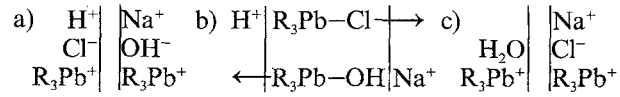
Evidence exists that the degradation of R_3Pb^+ likewise may include radical intermediates. Schnabl and Youngman found recently in experiments with Vicia faba protoplasts that triethyllead chloride ($Et_3Pb^+Cl^-$) caused strong peroxidation of membrane lipids, presumably triggered by radical fragments of the organolead compound (personal communication).

Trialkyllead exists in two forms[36]. The dissociated form ($R_3Pb^+X^-$) prevails in water due to solvation, particularly of the anion. The energy of solvation probably also represents the driving force for the transition of trialkyllead from the air into condensing water droplets of fog or clouds. In the absence of water, or in organic solvents, trialkyllead compounds exist in the undissociated form (R_3Pb-X). When, for example, an aqueous solution of triethyllead chloride is brought to dryness, the residue can be easily dissolved in benzene, but is only slowly redissolved by water. By thin layer chromatography on silica the undissociated compound can be distinguished from the salt form by a different R_F-value.

The ambivalency of trialkyllead probably enables the toxin to pass lipid membranes and enter cells without hindrance[66]:



By a similar mechanism trialkyllead has been claimed to dissipate proton gradients, for example at the inner mitochondrial membrane. It is assumed that by a shuttle mechanism, chloride is transported in one and hydroxide ion in the other direction[3,6]:



A basis for understanding the various toxic effects of trialkyllead in biochemical and cellular systems may be a similar interaction with nucleophilic groups of proteins. It seems that particularly thiol residues, when located in lipophilic pockets of proteins, can form strong complexes with trialkyllead. This was concluded from the finding that in ($\alpha + \beta$) tubulin, which through the addition of triethyllead chloride had lost its polymerizability, two out of a total of 18 thiol groups had lost their ability to react with typical thiol reagents[20]. Likewise, the inhibition of some ATPases[26] may be explained by assuming that thiol groups which are known to be essential for this kind of

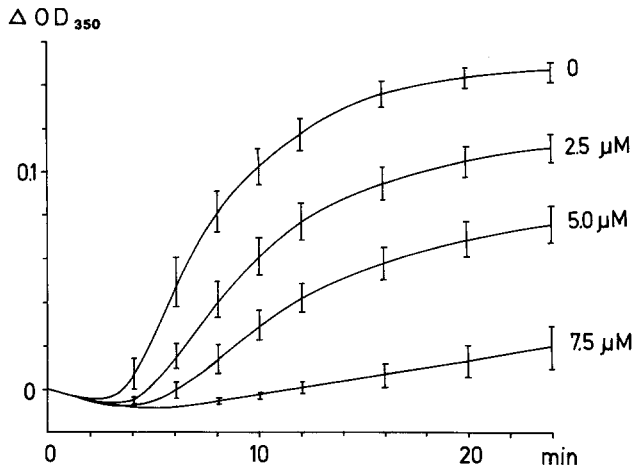


Figure 1. Inhibition of tubulin polymerisation by various concentrations of triethyllead chloride in the presence of microtubule-associated proteins (MAPS). Formation of microtubules was monitored by light-scattering measured as the increase in optical density at 350 nm. For experimental details see ref. 21.

enzymes, are blocked by the toxin. Other nucleophilic groups like imidazol nitrogen (= N-), phenolic (OH), or carboxylate ($-COO^-$)[60], located in hydrophobic domains of proteins may react in a similar way. Reactions with nucleophiles shielded from solvent possibly represent a common basis for understanding most of the seemingly diverse toxic effects of trialkyllead compounds.

Molecular toxicity

One of the targets of trialkyllead is tubulin[20, 57, 73]. In the presence of triethyllead chloride, for example, pig brain ($\alpha + \beta$) tubulin is no longer able to form microtubules. Corresponding to the inhibition of assembly, preformed microtubules are disrupted when triethyllead chloride is added. When monitored by the decrease of light scattering[18], 50% inhibition of pig brain tubulin assembly (IC_{50}) was found at a concentration of 5 μM triethyllead chloride (fig. 1). A calibrated assay system of this kind was one of the methods used in our laboratory for measuring the concentration of trialkyllead in rain water samples. Even higher sensitivity to trialkyllead seems to occur in another group of cytoskeletal proteins, the so-called intermediate filaments. Zimmermann and Plagens[74] showed that in non-epithelial cells the organization of the 10 nm filaments (desmin, vimentin, neurofilaments) was altered in the presence of 0.01 μM triethyllead chloride.

In 1984 it was discovered that distinct ATPases present in the plasma membranes of opossum kidney cells (PtK_1) can be inhibited by triethyllead chloride[26]. The inhibitory effect is strongest for the ouabain-sensitive Na^+/K^+ dependent ATPase. In HeLa cells, 50% inhibition (IC_{50}) of the corresponding enzyme required a concentration of about 12 μM triethyllead chloride (fig. 2). Recently, Weber et al. have shown that also in homogenates of plant cells, for example, of soybean (*Glycine max.* L.) a corresponding, triethyllead-sensitive fraction of ATPases exists, although not identifiable by ouabain susceptibility (personal communication). In mammalian mitochondria trialkyllead inhibits oxidative phosphorylation[2,3]. Recent experiments in our laboratory suggest that F_1-ATPase, which is part of the ATP-synthesizing system, may represent the target for the toxin. As shown for rat liver mitochondria, F_1-ATPase activity is inhibited by 50% at a concentration of 16 μM triethyllead chloride (fig. 2). In good agreement with this, Münter et al. measured an IC_{50} value of 17 μM for the inhibition of ATP synthesis (unpublished results).

Most recently, Schnabl and Youngman showed that triethyllead chloride interferes with fundamental metabolic processes of plant cells, such as photosynthesis and the CO_2-fixation by ribulose 1,5-biphosphate carboxylase. Inhibition of both systems starts at triethyllead concentrations distinctly lower than those for most other biological processes affected. Details of this work will be published in the near future (personal communication).

Phytotoxic effects

Phytotoxic effects of trialkyllead compounds have been studied in cultured cells, plant tissue and whole plants. In most of these studies the molecular mechanism of cytotoxicity remains to be elucidated.

Among the first organisms to be investigated for their sensitivity to alkylated lead compounds were fungi. Inhibition of growth by tributyl- or tripentyllead in *Aspergillus, Penicillium* and *Botrytis* spp. were observed at concentrations of circa 1 μM[37]. In such experiments triethyllead was more than 100 times less toxic for these organisms than the corresponding butyl- or pentyl-compounds. In mosses like *Funaria* or *Marchantia,* the toxic effects detected with 1–20 μM tetraethyllead are presumably those of the degradation product triethyllead, formed during incubation[43,55]. Algae have been investigated for their susceptibility to organolead compounds since 1970[67–69]. Their sensitivity was found to be similar to that of mosses. For example, the growth of the marine alga *Enteromorpha* was found to be inhibited with 2 μM tributyllead[64]. Concentrations of 0.4–4 μM alkyllead inhibited photosynthesis[46,47,63]. Detailed morphological studies were performed in the chrysophytic alga *Poterioochromonas malhamensis.* In this species triethyllead, and in

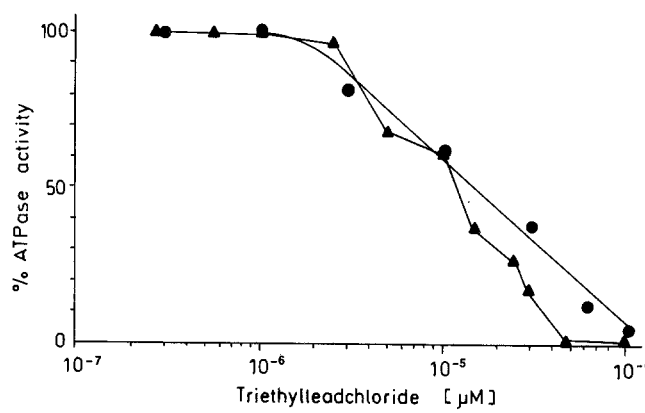


Figure 2. Inhibition of mammalian ATPases by triethyllead chloride; mitochondrial ATPase of rat liver (F_1-ATPase), (●); Na^+/K^+ – dependent ATPase of plasma membranes of HeLa cells, (▲) (unpublished results).

the presence of light also its precursor tetraethyllead, caused the formation of giant cells by inhibiting cytokinesis. A concentration of 25 µM triethyllead was found to be lethal for this organism[55,56]. From electron micrographs it was obvious that after several days' treatment with 10 µM triethyllead changes in number and volume of nuclei, contractile vacuoles, chloroplasts and dictyosomes may occur[58].

In our own studies we were able to show that suspension cultures of higher plant cells are very sensitive to trialkyllead. For example, growth of soybean cells, or their protoplasts, was inhibited in the presence of triethyllead chloride at concentrations of 1 µM, or 0.3 µM, respectively (fig. 3) (Stournaras et al.[66], and Weber et al., unpublished results). In tissues of higher plants like lettuce (*Lactuca sativa*)[32,61] or onion (*Allium cepa*)[1,53] mitotic disturbances in meristematic root cells were observed upon treatment with tetramethyllead (70-700 µM), or trimethyllead (1-100 µM). As to the molecular mechanism of this toxicity, it was suggested that thiol groups, as present in the mitotic spindle, may be attacked. Toxic effects were also reported from corn plants grown in soil treated with tetraalkyllead-containing water. At concentrations of 10 ppm (circa 30 µM), yield of dry matter (grain, glumes, straw) was found to be decreased by circa 75%[17].

A model system for studying transport processes in plants introduced by Kriedemann and Beevers[42] and investigated in great detail by Komor[39] are seedlings of

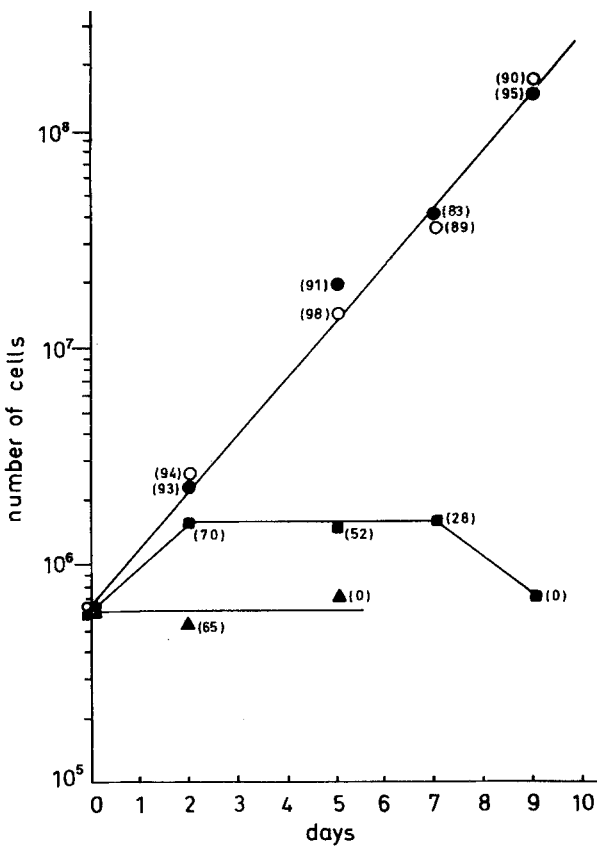


Figure 3. Inhibition of soybean cell growth by various concentrations of triethyllead chloride. (Numbers in parentheses mean percentage of live cells) Control: (o); 0.3 µM: (●); 1 µM: (■); 10 µM: (▲). For experimental details see ref. 66.

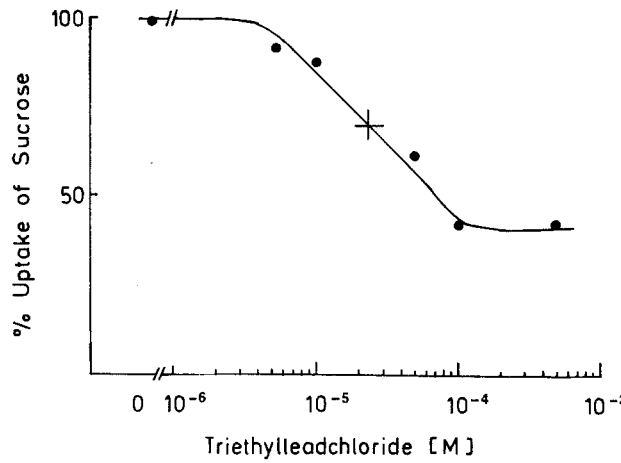


Figure 4. Inhibition of the uptake of [^{14}C]sucrose by excised cotyledons of castor beans by triethyllead chloride. The residual transport activity is energy-independent. (Unpublished results. Methods according to Komor[39], with modifications).

castor beans (*Ricinus communis* L.). During several days after germination the cotyledons have the ability to incorporate [^{14}C]-sucrose. Experiments in our laboratory have shown that the energy-dependent part of the transport process (circa 60%) can be completely blocked by triethyllead chloride (fig. 4). The IC_{50} value of this system (22 µM) is similar to that of the ATPases, and indeed it has been suggested that a plasmalemma-bound ATPase provides the energy for this kind of sucrose transport[40,41]. Further experiments will show whether triethyllead chloride also impairs the following transport of [^{14}C] sucrose to stem and root cells. Of course it would be of considerable importance for the subject discussed here if trialkyllead could be shown to inhibit the phloem transport of plants.

Occurrence of organolead in the environment

Tetraalkylated lead compounds, e.g. tetraethyllead or tetramethyllead are very effective antiknock agents and are used world-wide for improving the quality of gasoline. While in Canada, and some Western European countries like France, Italy and Spain, organolead in gasoline is still very high (0.4 to 0.77 g Pb per liter), it has been reduced to 0.15 g Pb per liter by most others. Some actual data for Europe and North America are compiled in the table.

Maximum lead contents of gasoline (May 1985) and estimated gasoline market (1984) of some countries of Europe and North America[50]

Country		g Pb/liter	Tons/year (× 10^6)
Germany (West)		0.15	23.7
Austria, Switzerland		0.15	5.6
Sweden, Norway, Denmark		0.15	7.3
France		0.40	18.3
Italy		0.40	11.7
Germany (East)		0.42	—
Poland		0.33–0.55	2.8
Hungary		0.40–0.70	—
Spain		0.48–0.65	5.5
USA	leaded	0.15–0.22	115.4*
	unleaded	0.01	169.0
Canada	leaded	0.77	13.0
	unleaded	0.01	11.4

*includes premium only, not regular.

By far the largest part of the organolead additives is fully combusted in engines to inorganic lead salts, e.g. $PbBr_2$. Small amounts of tetraalkyllead may, however, escape combustion or evaporate during handling of gasoline. For example, engines under normal use exhaust circa 1% of their total lead emission as organolead, and the amount may rise significantly when the engine is cold-choked or idling[44]. According to information provided by the petrol industry, circa 0.6% of the total gasoline production evaporates during transport[22]. Huntzicker et al.[34] have suggested that about 1.3% of the total tetraalkyllead is lost at filling stations. In total, it is estimated that up to 4% of all gasoline, together with the organolead additives, is wasted[27,52]. Once liberated, degradation of tetraalkyllead to toxic trialkyllead occurs, e.g. by microsomal oxidation in the liver of animals inhaling the vapor[65]. In the atmosphere trialkyllead is formed under the influence of light and oxygen[28] (see under heading 'Chemical nature'), or possibly, by energy-rich oxygen particles like hydroxyradical ($HO^{\cdot}$), or ozone (O_3)[4,48,49].

Analytical procedures for the determination of organolead pollution in air have been developed by several laboratories[14,27]. In most cases researchers distinguished between the lead fraction retained by filters – taken as inorganic lead (PbX_2) – from the non-retainable part of lead considered to be organolead. Typical concentrations of the non-retainable part of lead in the air in rural areas were in the range from 2 ng/m^3 to 20 ng/m^3, in one case up to 230 ng/m^3 [29]. In general, organolead in the air accounted for 0.1 to 1% of the total lead. Accordingly, most authors regarded organolead as having no immediate ecological significance for the environment.

When the organolead fraction was further analyzed for tetraalkyllead, part of it remained unidentifiable. De Jonghe et al.[13,14] pointed out that the difference may be accounted for by degradation products of tetraalkyllead, such as trialkyllead (R_3PbX) and dialkyllead (R_2PbX_2). In addition to the possible existence of a gaseous fraction, trialkyllead may be adsorbed to the surface of airborne particles. In this case, tri- and dialkyllead would represent part of the 'inorganic lead'.

At the moment when moisture in air condenses to fog or clouds (dew point), trialkyllead both in the gaseous and in the solid form would be solvated by water droplets ('rain out')[25]. Particularly the solid form, neglected so far, may contribute considerably to trialkyllead contamination of rain and fog. A further enrichment with trialkyllead may occur when rain drops on their way from clouds to the surface pass air layers which contain trialkyllead ('wash-out')[25]. Since both these processes of entering rain have not been described exactly as yet, the trialkyllead content of rain can at present not be calculated or even estimated from the available analytical data for trialkyllead in air.

For a long time suitable analytical methods for the measurement of trialkyllead in rain were lacking. In 1983 De Jonghe et al.[15] established a valuable separation procedure based upon the distribution of the Pb^{++}/EDTA complex and the R_3Pb^+/diphenylthiocarbazone complex between water and organic solvents, with quantitation of lead by atomic absorption spectrometry. Most recently, Chakraborti et al.[10] and Harrison and Radojevic[30] reported another analytical procedure using n-butylmagnesium halogenide for converting di- and trialkylated lead into tetraalkylated species with subsequent detection of the products by gas chromatography/atomic absorption spectrometry.

Only a few applications of these analytical procedures have been made known so far. De Jonghe et al. analyzed several rain samples collected in, or near, Antwerp, and reported average values of 28 ng trialkyllead/l from a rural site and 60–330 ng/l in the city[16]. Single measurements performed in suburbs of Antwerp[10,72] confirmed these results (circa 30 ng trialkyllead/l). At various sites in the U.K., trialkyllead in rain was shown to be present in the range of 40–100 ng/l[30].

Trialkyllead in southwestern Germany

In 1984 and 1985 rain was collected at 6 locations in the southwestern part of Germany, and analyzed for lead and trialkyllead. Sampling stations were situated at altitudes of 650–1400 m in the Black Forest (3) where severe forest damage has been reported; further on in the city of Heidelberg (1), and at a highway junction (1); and finally, in a remote area (Oberschwaben) with insignificant forest damage (1). Rain samples were collected without interruption for 4–5 months each year. After determination of pH value, the total content of lead was measured by atomic absorption spectrometry. All samples containing lead levels above 7×10^{-8}M were further analyzed for trialkyllead. In 1985, two analytical procedures were used in parallel, the tubulin assay as described above (fig. 1) and the chemical approach established by De Jonghe et al.[15]. The results obtained with the two methods were in good agreement with each other and are summarized in figures 5 and 6.

The graphics show that the occurrence of trialkyllead may vary widely with the location. Areas occur in which only circa 2% of all rain samples contained trialkyllead, while in others this proportion rose to 13%. Concentrations of trialkyllead between 0.1 and 0.9 μM were found in some samples. This is the range where toxic effects were observed in several biological in vitro systems (see chapter 4). Analytical data published by others[71] include values of trialkyllead 1000 times lower than those reported here. However, the data of 1985 presented in this study are based upon two independent methods yielding consonant results. Therefore we believe that the disagreement is not due to inadequate analytical work but to a different procedure for sampling. While our samples were collected in an area located circa 1000 km inland, the other samples were from regions with a typical maritime climate, such as the North Sea coast or England. Moreover, the number of samples taken by other workers was very small compared to ours. The number of samples is important, because it may happen that even in regions where trialkyllead imission is usually high the toxin is undetectable during a whole month. Finally, organolead contamination of rain is sometimes confined to small areas. For example, only circa 100 km east of the Feldberg, the location with the highest trialkyllead immission, we found trialkyllead only once in 5 months (location Oberschwaben, fig. 6). This shows that extrapolation of analytical data from one location to another, even a small distance away, can lead to wrong conclusions.

A simple correlation between the occurrence of trialkyllead and forest injury was not expected to exist, because local factors like intensive sun radiation, or other pollutants, may contribute to forest damage as well. Although the existence of such a correlation was suggested by our analytical data, which showed high concentrations of trialkyllead at places with severe forest decline (Feldberg, Loffenau) and low values in areas with a low level of forest damage (Oberschwaben, Heidelberg), this relation is regarded as not significant, because of the short sampling period. On the other hand, the presence of high concentrations of trialkyllead at places with severe forest decline suggests that this particular pollutant deserves our most careful attention.

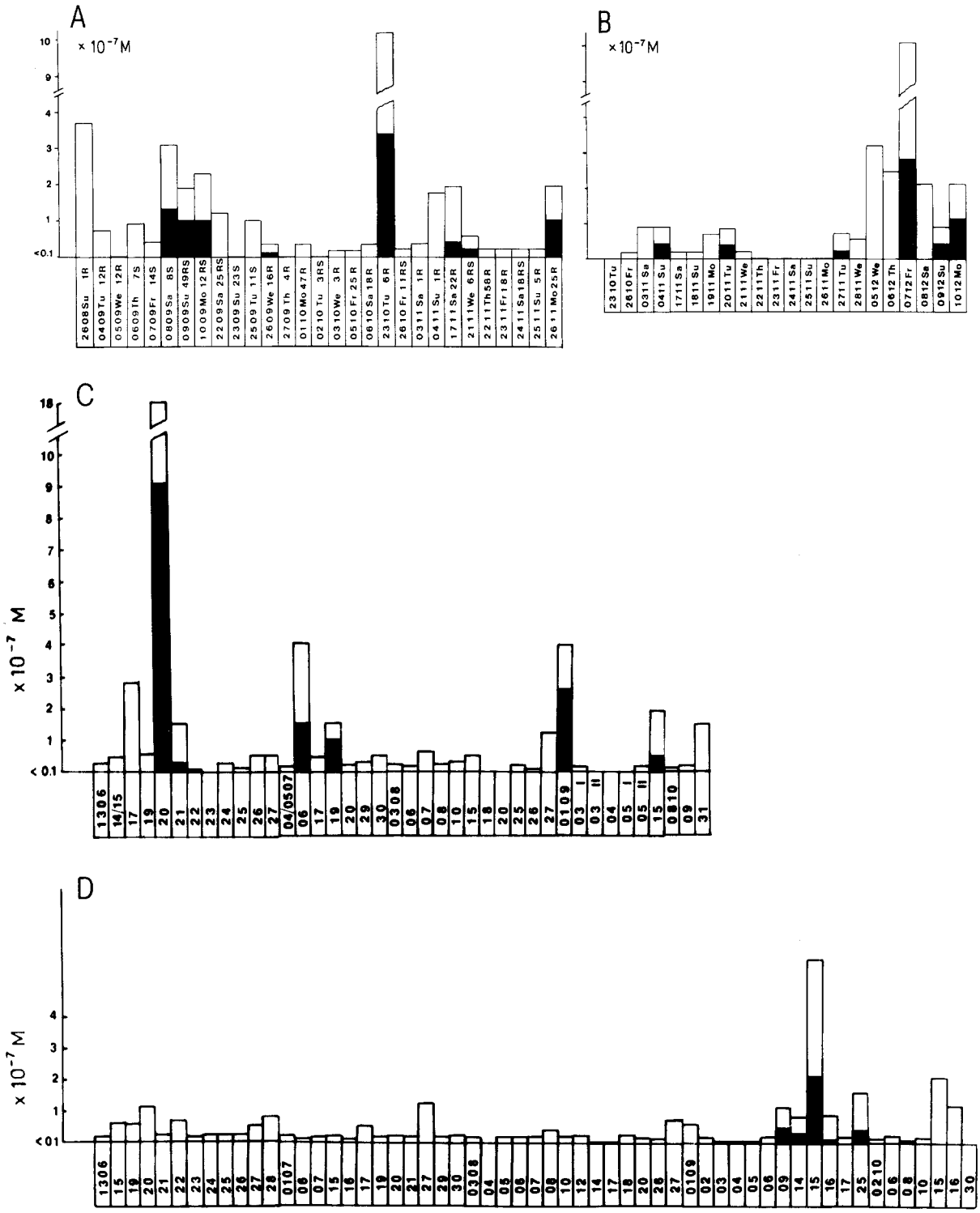


Figure 5. Occurrence of lead (white bars) and trialkyllead (black bars) in precipitation at two locations in the Black Forest during 1984 and 1985. Feldberg 1984 (A), 1985 (C); Loffenau 1984 (B), 1985 (D). (Measured according to refs 21 and 15).

Another factor with only local relevance may be polluted fog, or cloud water. It was reported that the areas with the most severe forest damage are in most cases located at altitudes around 1000 m. In these regions fog or clouds are frequent and may last for days. Provided a pollutant is taken up at a high rate the continuing moistening of the surface of needles and leaves can cause an intensive transfer of the pollutant[70]. So far, only a few fog samples have been collected in the southern Black Forest, in which trialkyllead was found in concentration of 0.2 µM. One of these places was Münstertal, an area from which severe forest decline was reported, but where the trialkyllead content of rain was found to be low. We therefore suppose that in some areas advection of trialkyllead by clouds may contribute considerably, if not exclusively, to the wet deposition of organolead.

It is well documented that at locations with heavy traffic relatively high concentrations of organolead can occur in the air[14]. This is not necessarily paralleled by high concentrations of trialkyllead in rain, as illustrated by figure 6A, B. They show that in the city and near the highway trialkyllead in rain was distinctly lower than, for example, in the Feldberg area. It seems that trialkyllead is not necessarily deposited by rain at the places where it is emitted. Rather, it can be transported over large distances and so may appear in rain or in clouds at rural sites.

Trialkyllead and acid rain

All rain water samples collected in 1985 were routinely assayed for their pH. We found values in the range of 2.7 to 6.0. There was no significant difference between the pH values measured at the various locations. Strongly acidic pH-values were found near the highway as well as in the city of Heidelberg or on top of the Black Forest hills.

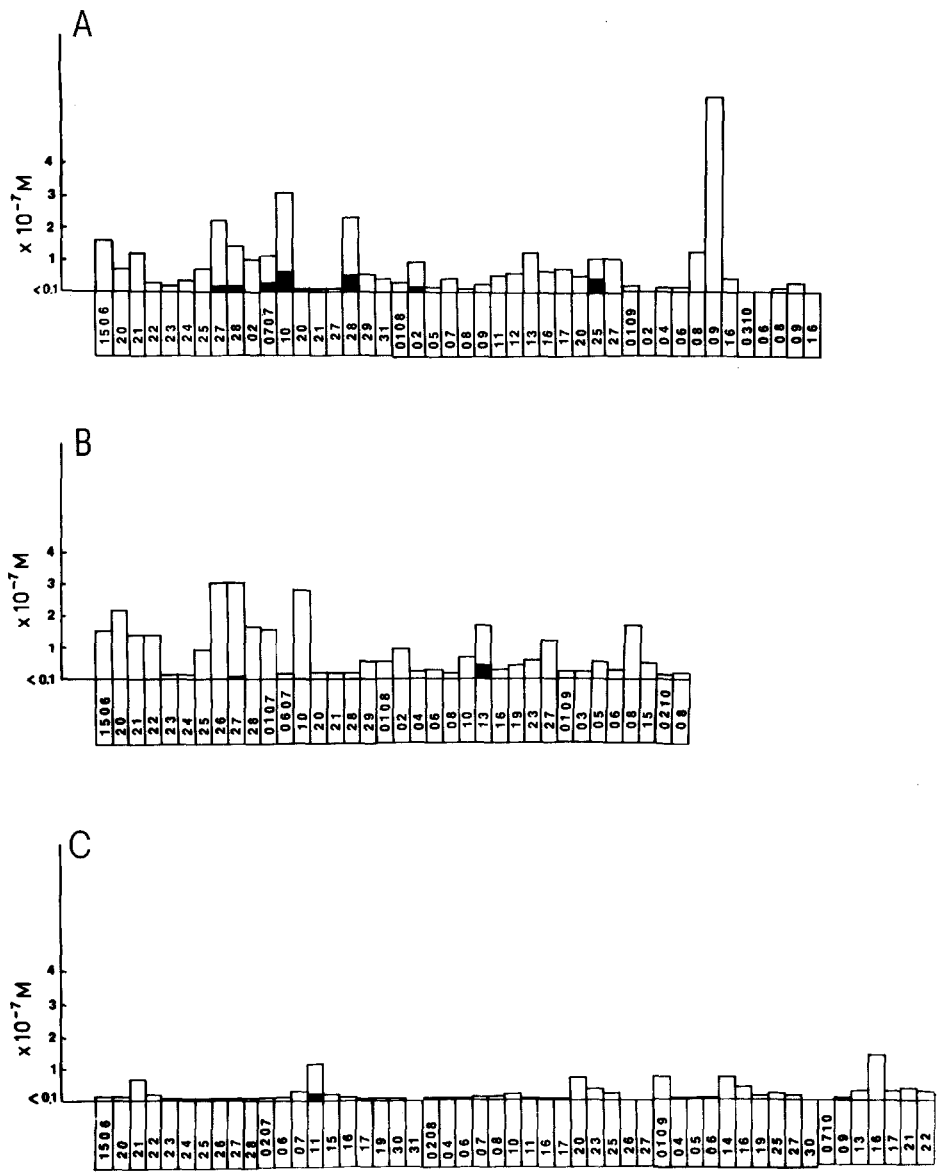


Figure 6. Occurrence of lead (white bars) and trialkyllead (black bars) in precipitation collected in 1985 in the city of Heidelberg (A); in the immediate environment of Heidelberg close to the highway (B); and at a remote area in Oberschwaben (north of Bodensee) (C).

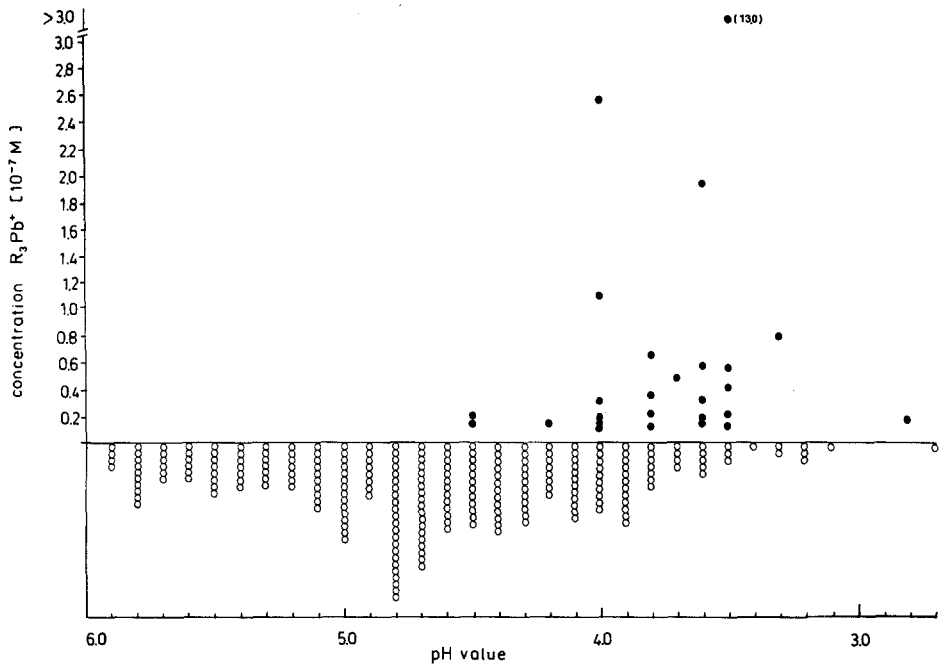


Figure 7. Correlation of acidity and trialkyllead content of rain. About 90% of all trialkyllead-contaminated samples (full circles) have pH 4.0. Rain samples containing no trialkyllead (limit of detection 0.01 μM) are plotted according to their pH-values (open circles).

Remarkably, all rain samples containing trialkyllead were, on average, more acidic than those without. In figure 7 (upper part, full circles) the pH-values of all trialkyllead-containing samples of 1985 are plotted against pH. The lower part of figure 7 (open circles) shows the statistical distribution of pH-values of all samples in which trialkyllead could not be detected. While the mean pH-value in this group was circa 4.8, it was 3.6 in the group contaminated with trialkyllead. In fact, all except 3 of the samples containing trialkyllead had pH-values $\leqslant 4.0$.

From this correlation one may deduce that trialkyllead is stabilized in an acidic medium. Stabilization may occur already during its generation, in that acidic components (HX) of the atmosphere rapidly convert intermediates of the process, such as trialkyllead peroxides ($R_3Pb\text{-}O\text{-}O^{\cdot}$, $R_3Pb\text{-}O\text{-}O\text{-}H$), into more stable salts ($R_3Pb^+X^-$). Another possibility is that mineral salts of trialkyllead (sulfate, chloride) are more stable than, for example, trialkyllead carbonate or hydroxide.

Probably because of this stabilization, trialkyllead can account for a high portion of total lead, in most cases 10–20%, in the Black Forest up to 50%. This suggests that the major part of inorganic lead found in the rain samples may have originated from organolead compounds, transported over long distances.

Even in the presence of mineral acids trialkyllead suffers degradation, ultimately to inorganic lead. Stability data for the tetraalkylated compounds Et_4Pb and Me_4Pb, show half-lives of 20 h to 7 days in winter, and 2–8 h in summer[49]. Trialkylated lead is considered to be more stable, so Et_3PbX and Me_3PbX will at least persist as long as their precursors and probably longer. Shielding from bright sunshine, as for example in the lower part of a cloud, will prolong their half-lives, and no degradation at all is to be expected during the night. In conclusion, the instability of trialkyllead compounds seems not to be a valid argument against their possible importance in the environment.

Availability for plants

Very low concentrations of trialkyllead were found in surface water[16]. This is in good agreement with laboratory experiments showing that organolead compounds are rapidly degraded in the presence of iron and copper ions[35]. These ubiquitous transition metal ions are known to act as catalysts in oxygen-dependent oxidations. Accordingly, the half-life of trialkyllead must be short in contact with soil. We therefore only investigated the uptake of trialkyllead from contaminated water by foliage. We found that leaves or needles of trees took up triethyllead chloride from an aqueous solution within a few minutes. Saturation was reached within 5–10 min for needles of spruce, fir, and pine, (fig. 8A) and also for leaves of oak, beech and maple (fig. 8B). As figure 8a shows, uptake of triethyllead chloride by needles continued even when the concentration had fallen to 10% of that at the beginning. This indicates that triethyllead chloride is resorbed from water even in concentrations as low as 3×10^{-8} M. By comparison, more than 50% of all our rain samples in which trialkyllead was detected, contained the toxin in concentrations of 3×10^{-8}M, or higher.

The high rate of uptake may be particularly crucial when foliage comes into contact with trialkyllead-contaminated fog or cloud water. When intercepted, for example, by needles of conifers the droplets will form a continuously flowing water film, which, when it is in contact with the surface for at least a few minutes, would make possible a nearly complete transfer of the trialkyllead loading. This process of trialkyllead scavenging from cloud water may be especially important for isolated trees, or those overtopping the canopy.

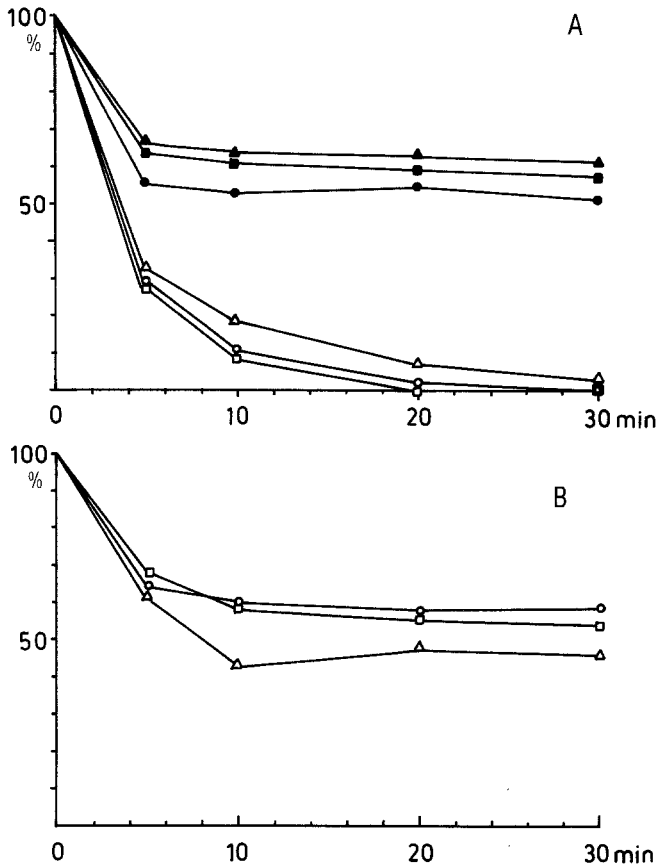


Figure 8. *A* Kinetics of uptake of triethyllead chloride into isolated needles of fir (●,○) spruce (■,□) and pine (▲,△) from a 3×10^{-7} M aqueous solution (empty symbols) and from a 3×10^{-6}M aqueous solution (full symbols). Uptake is expressed as % decrease of lead caused by 10 g of fresh plant tissue in 40 ml of incubation medium at 20 °C. Lead concentration was measured by atomic absorption spectrometry; the values were corrected for the amount of triethyllead chloride adsorbed to the glass surface of the incubation vessels.
B Kinetics of triethyllead chloride uptake into isolated leaves of oak (○), beech (△) and maple (□) from a 3×10^{-7} M aqueous solution. Uptake is expressed as % decrease of lead caused by 10 g of fresh plant tissue in 40 ml of incubation medium at 20 °C. The values were corrected for the amount of triethyllead chloride adsorbed to the glass surface of the incubation vessels.

It is not yet known where the triethyllead chloride taken up by needles and leaves actually resides. In control experiments, needles loaded with triethyllead were shaken in distilled water for 30 min, resulting in a release of only 5–10% of the amount of lead absorbed. We conclude that triethyllead chloride must be tightly bound either onto or in the plant tissue. Since trialkyllead salts can exist in an undissociated form also, we expect that the toxin can penetrate into the fatty matter of the cuticula. If not trapped there it would be able to penetrate also into aqueous compartments of the plant tissue, such as the extracellular space or cytoplasm. This problem certainly needs to be addressed in more detail.
As is obvious from figure 8A and B, needles as well as leaves reach saturation points, reflecting different capacities for the uptake of triethyllead chloride. Coniferous trees, which in Germany were affected earlier and to a higher extent, can accumulate amounts of triethyllead chloride up to 15 times higher than broad-leaved trees (fig. 9). One must also consider that coniferous trees bear their needles for several years, while broad-leaved trees shed their leaves and thus excrete absorbed pollutants once a year.

Possible effects on trees

All mechanisms for the toxic effects of trialkyllead elucidated so far interfere with fundamental processes of plant metabolism. Some of these processes, like photosynthesis, CO_2-fixation, or microtubular activity, are located inside cells, so that their inhibition would require penetration of trialkyllead into cells. Other toxic events may occur in the extracellular space, for example destruction of plasma membranes or inhibition of their component enzymes. Finally, corrosive effects on the cuticle can be expected when trialkyllead is trapped by the wax layer and degraded by sun light.
Even if trialkyllead is in fact able to enter the cytoplasm of plant cells, toxic effects such as inhibition of photosynthesis, CO_2-fixation, or microtubular assembly may fail to appear, owing to the protective effect of cytoplasmic thiol compounds such as glutathione. Protection by glutathione from trialkyllead poisoning was demonstrated, for example, for tubulin. Here the inhibition of microtubular assembly, as caused by 10 µM triethyllead

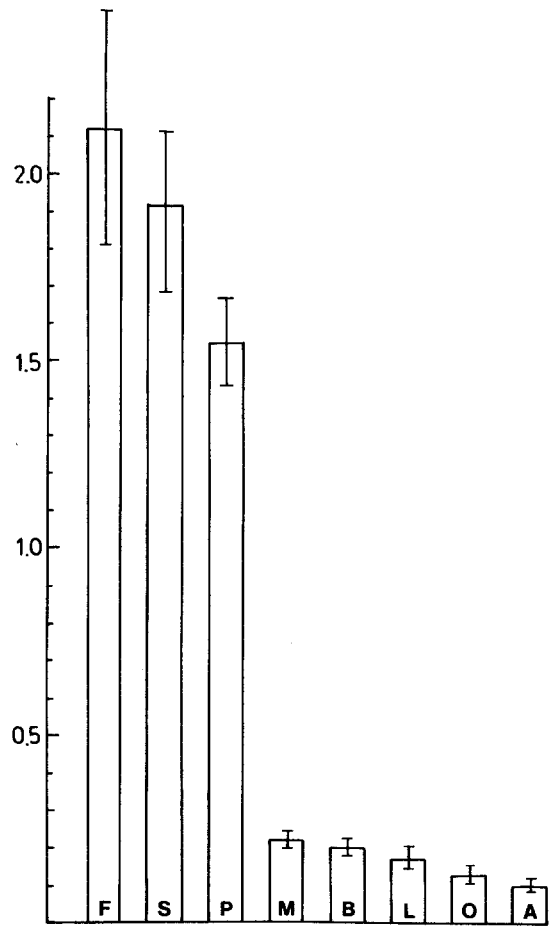


Figure 9. Saturation values of isolated needles and leaves of several common forest trees after repeated incubation in a 3×10^{-7} M solution of triethyllead chloride at 20 °C, expressed in µg triethyllead chloride taken up per g fresh weight. F(fir), S(spruce), P(pine), M(maple), B(Beech), L(larch), O(oak), A(ash).

chloride, was found to be fully reversed by the addition of 1 mM glutathione (fig. 10). Glutathione, a reducing and detoxifying agent, is present in mammalian cells, as well as in plant tissue, for example in chloroplasts, in concentrations up to 4 mM[7,23,54]. The protective effect of glutathione may, however, be decreased or even disappear under the influence of other toxic compounds requiring glutathione for their detoxification, e.g. peroxides. Accordingly, in the presence of peroxides or ozone, the toxicity of trialkyllead may be enhanced, and vice versa. More likely than the inhibition of the cytoplasmic processes by trialkyllead is the attack of plant cells from the extracellular (or interstitial) space. At least in mammals this compartment does not contain reduced glutathione and is therefore unprotected against trialkyllead. Possible targets would be the plasmamembrane-bound ATPases, which establish ion gradients providing the energy for the transport of metabolites (see chapter 4). Since phloem transport is also assumed to depend on such ATPases[40,41], their inhibition could affect the transport of assimilated substances into lower parts of the plant. Typical features of the present forest decline, such as decrease of stem growth, roots and mycorrhiza could be understood as a consequence of an insufficient supply with products of assimilation. This in turn would impair the balance of water and minerals within the trees.

Other toxic effects in trees could result from the fact that decomposition of trialkyllead by sunlight may lead to the formation of radicals. This obviously occurs in *Vicia faba* protoplasts, as shown by Schnabl and Youngman (personal communication). These workers have demonstrated that lipids of biomembranes are destroyed when exposed to light in the presence of triethyllead chloride. Similar destruction may occur with carotenes, or photoreceptors like chlorophylls. Moreover, in a reaction similar to that of the phospholipids of membranes with trialkyllead, the waxes of the cuticle could be destroyed. 'Corrosion' of the cuticle has been documented by several authors from electron microscopic studies of e.g. spruce needles. This degenerative process has so far been considered to represent one of the toxic actions of ozone[19,51], but considering the effects of trialkyllead on membranes, destruction of this protective layer could be induced by organolead compounds as well.

Discussion

Reports of forest damage are also known from previous centuries[38]. At no time, however, has forest decline affected all species of forest trees. Furthermore, it has never before shown such rapid progress, with only a few signs of slowing or reversal. Most scientists concerned with the problem agree that the present situation is not comparable to any previous one.

Anticipating that air pollution is involved in this process, it appears most promising to look for its origin among waste products associated with the technical developments of the last decades. Of particular interest are gaseous waste products, which are distributed by wind to areas far away from their sources. Most of the air pollutants under discussion, such as O_3, unsaturated hydrocarbons, NO_x, or SO_2, are not exclusively man-made, but may stem from natural sources as well, although at lower concentrations. Living organisms have been in contact with most of these components for a long period of time, and, therefore, may have developed responses to these environmental challenges. In contrast to these, alkylated lead is a typical man-made pollutant, and has been used in Europe on a large scale for only about 30 years.

The criticism has been made that the onset of accelerated forest decline was seen just at the time (1976) when the level of lead additives in West Germany was lowered by law. However, we note that a reduced annual growth of coniferous trees has been reported since the 1960[5,45]. From the same time onwards, a decrease in branching in

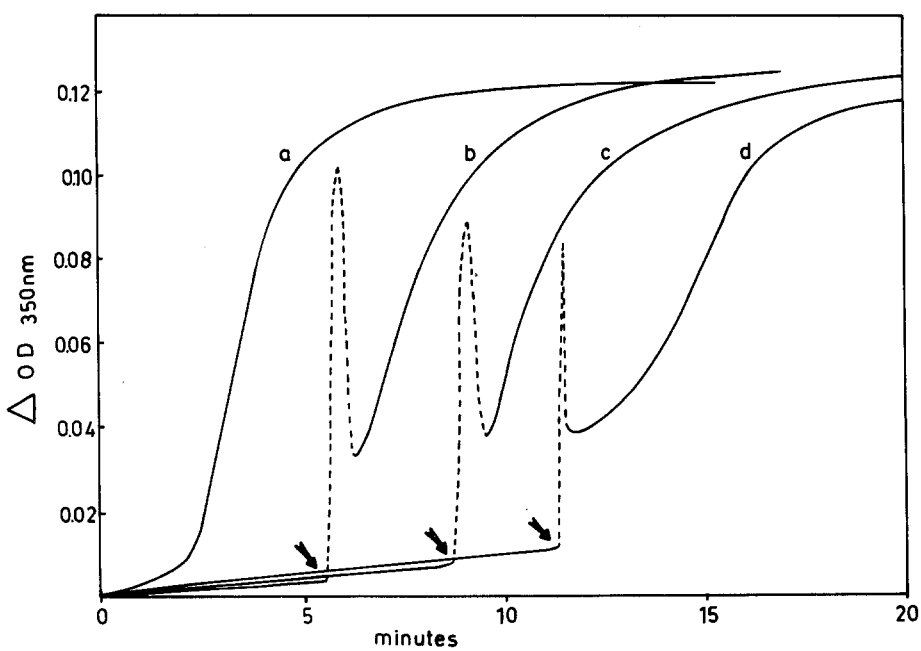


Figure 10. Reversal of inhibition of microtubule assembly (10 µM triethyllead chloride) by the addition of glutathione (arrows, final concentration 1 mM) after various periods of time. Non-inhibited assembly of microtubules for control. Experimental details as in figure 1.

part of the beech population has been reported[59]. This shows that the onset of the actual toxic events may be dated back to many years ago. On the other hand the interval between 1950 and 1976 represents the period of time when car traffic in West Germany was greatly expanding and levels of lead additives were high (0.64 g Pb/l until 1972 and 0.40 Pb/l until 1976). Unfortunately, no analysis of trialkyllead in rain or fog was made at that time.

Like other air-borne pollutants deposited in Germany, trialkyllead is partly imported from neighboring countries, and the law that came into force in Germany in 1976 did not necessarily reduce this source of trialkyllead. For example, gasoline in France or Italy still contains tetraalkyllead in amounts corresponding to 0.40 g Pb/l. Since most of the rain or cloud water in Germany comes with the south west wind, and part of it then precipitates on the slopes of the Black Forest, the high concentrations of trialkyllead found in the rain on top of the Feldberg may at least partly be imported from neighboring countries.

An important question is how trialkyllead concentrations of almost 1 μM in rain can arise from the low concentrations of organolead in air. Here one must note that in the presence of water trialkyllead is a salt, and dissociates. At the moment of its formation from tetraalkyllead one can assume that it is as finely distributed in air as its gaseous precursor. However, at dew point the finely dispersed salt is forced into the droplets of clouds or fog. For physical reasons, one would not expect an equilibrium distribution of trialkyllead between water and air, but a complete transition into the liquid phase (rain-out). A simple calculation shows that by the entrance of trialkyllead into the small volume of water corresponding to air humidity (circa 10 g per m^3 air in Continental Europe) the concentration must be increased by a factor of 10^5. This number does not include a possible further enrichment of rain with trialkyllead when drops are on their way from clouds to surface (wash-out).

Another objection occasionally made is that trialkyllead may be too unstable to exert toxic activities. As outlined above, this argument is invalid because the half-lives of trialkyllead compounds may range from one day to several days. This is in line with preliminary experiments in our own laboratory showing that triethyllead chloride in a 10^{-4} M aqueous solution persisted for more than 8 hours when exposed to the day light on a cloudy day. The half-life of trialkyllead in plant tissue is not known. As suggested above, however, the light-induced decomposition to radicals in plant tissue may itself represent a toxic process.

An argument for the involvement of trialkyllead in forest decline is the observation that the poison was actually found in relatively high concentrations at places with considerable forest decline, but located far away from traffic and industry centers. Through the use of two independent analytical procedures, the occurrence of trialkyllead in rain and fog can no longer be seriously questioned. However, one may object that the rarity of such toxic events is inconsistent with the severe forest damage observed. Here we note that trialkyllead is a poison which inhibits fundamental biological processes in a very specific way. This must be concluded from the strong toxic activities on several biological model systems, some of which occur in the nM range. Correspondingly, one would expect that trialkyllead can exert toxic effects on plants at lower concentrations and at less frequent exposures than those required for other pollutants. Furthermore, the analytical values of 1985 do not necessarily reflect those before 1976, when the pollution with trialkyllead in the Black Forest may have been 2–3 times higher and also more frequent than at present.

Conclusions

The present study shows in much detail that trialkyllead exists in the environment. Although its precursor, tetraalkyllead, is distributed by air, the toxin probably exerts its activities not as an airborne pollutant, but dissolved in rain, fog, or cloud water.

In the past years analytical work on pollutants was focussed on compounds found in the environment in impressive concentrations. Specific poisons requiring only minute concentrations to become active may have been overlooked. Such a poison is trialkyllead, which at some locations has been found in rain in potentially dangerous concentrations. Therefore, analytical efforts in this field should be extended to other areas with severe forest decline. As well as rain, contamination of fog and cloud water is certainly a matter of interest.

Many essential biological processes in mammalian and plant cells are specifically inhibited by trialkyllead, while structures susceptible to radical attack may be destroyed. It seems unlikely that trees would be insensitive to these kinds of toxic interactions. Therefore, biological experiments with triethyllead chloride, which have been performed so far in model systems only, should be extended to forest trees, particularly in their own habitat. Exposure of leaves to aqueous solutions of trialkyllead will show whether typical symptoms of forest decline, such as bleaching or fall of foilage, can be produced.

The present study reveals a strong correlation between the occurrence of trialkyllead and acid rain. Here, it is completely unknown whether acid rain in some way influences the uptake or the biological activities of trialkyllead. Experiments on this are in progress.

Considering our present knowledge about trialkyllead we strongly recommend that research work in this field should be intensified in order to find out to what extent these treacherous compounds are involved in the process of European forest decline.

Note added in proof: In circa 500 rain ramples collected during 1986 trialkyllead concentration was in no case $\geqslant 10^{-8}$ M. The analytical work will be continued.

Acknowledgment. The authors wish to thank Prof. W. Hasselbach for his support of this work, Dr K. Münter for his experiments with mitochondria, and Miss S. Zobeley for technical assistance. We further gratefully acknowledge the cooperation of the Forstliche Versuchs- und Forschungsanstalt, Freiburg, the Community of Loffenau, the Meteorological Station of Feldberg and the Stadtwerke Heidelberg. Financial support for this study was kindly provided by the Bundesministerium für Forschung und Technologie, the Ministerium für Ernährung, Landwirtschaft, Umwelt und Forsten Baden-Württemberg, the Max-Planck-Gesellschaft, und Boehringer Mannheim GmbH.

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0014-4754/87/020115-13$1.50 + 0.20/0
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Criteria for permissible browse impact on sycamore maple *(Acer pseudoplatanus)* in mountain forests

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Summary. A naturally regenerated young-growth in the 'Buchser Hochwald' area in the Canton of St. Gallen, Switzerland, was used to determine the silviculturally permissible browsing limit for sycamore maple *(Acer pseudoplatanus)*. The investigated area is situated at an altitude ranging from 1280 to 1310 m a.s.l. on an *Abieti-Fagetum typicum* site. Browsing was done by red deer *(Cervus elaphus)* and roe deer *(Capreolus capreolus)*. A total of 57 sycamore maples, 1.30 m high, were examined. The actual browse impact on these plants was assessed by determining the frequency of browsing marks on the stem axis. To this end, the plants were cut into sections of 5 cm each and then split radially. On this basis it was possible to calculate permissible shares of browsed plants for four different size categories between 0.10 and 1.30 m. The permissible share of plants with two or more visible browsing marks on the stem axis amounted to 37.8% as an average value for the total risk period. This corresponds to a quota of 26.9% browsed terminal shoots per annum.
Key words. Sycamore maple *(Acer pseudoplatanus)*; game browsing; red deer and roe deer *(Cervus elaphus* and *Capreolus capreolus)*; browse impact limits.

1. Introduction

A considerable amount of literature is available on deer browsing, but previous research has dealt almost exclusively with game distribution, the varied causes and effects, suitable preventive measures, or the need for game population control. None of these investigations can yet supply satisfactory information for the purposes of ecology and game policy on what criteria would be suitable to show excessive browse impact on the young forest generation.

Some ten years ago Mayer[8] showed that browsing had become a serious problem for forest regeneration in large parts of the Alps. Mountain forests are particularly liable to game browsing. The reasons for this are the frequent lack of sufficient young growth and the slow growth of