Photochemical activation of chloroethenes leading to destruction of photosynthetic pigments[1]

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Summary. All major photosynthetic pigments in spruce needles *(Picea abies)* are rapidly destroyed when the latter are UV-irradiated in the presence of tri- or tetrachloroethene at concentrations about 10-fold higher than those present in the lower atmosphere of industrialized countries. Uptake of chlorocarbons by spruce needles is fast, and the partition coefficients between the lipophilic compartments, such as the cuticle and cellular lipid membranes, and air are high. Volatile halogenated hydrocarbons are therefore suspected of being initiators of a phytotoxic phenomenon usually referred to as new forest decline. In conjunction with the fact that they seem to be preferentially deposited along the western slopes of mountain ranges receiving high precipitation, the phenomena reported here may indicate the initiation of a severe ecotoxicological manifestation by these widely used compounds.

Key words. Forest decline; halogenated hydrocarbons; photochemical activation; photosynthetic pigments; phytotoxicity.

The tree injuries observed in recent years in clean-air regions in Central Europe have most properly been described as the 'air-and-light' syndrome. There is general agreement that the phenomenon represents a phytopathological process which is initiated by anthropogenic air pollutants[2,3], but the actual causes still remain to be elucidated. The primary ultrastructural and biochemical disturbance seems to be a disruption of thylacoid and other endoplasmic membranes[4] and loss of photosynthetic pigments[5]. Mainly conifers are affected, notably fir *(Abies alba)* and spruce *(Picea abies)*, but recently also some deciduous trees of the beech family (Fagaceae). The most evident damage to conifers is early shedding of needles; under undisturbed conditions a fir retains up to 12 needle generations, whereas an injured tree may have lost all needles older than four years. Beeches have small chlorotic leaves with necrotic spots along the light-exposed contours of a tree. Particularly strongly damaged are isolated trees and those protruding beyond the canopy of a forest. The most dramatic aggravation of the tree injuries took place during 1982 and 1983, both years with warm summers and long periods of sunshine. Assessment of the geographical distribution in Germany exhibits a peculiar pattern which generally follows the topography of the mountain ranges of medium and high elevation[6], with the heaviest damage along the western slopes. Conifers in remote mountain regions exhibited the symptoms earlier and to a greater extent than those growing close to urban centers which have been exposed to much higher concentrations of the 'classical' air pollutants for a long time.

To date, sulphur dioxide, nitrogen oxides, ozone or various metals have been discussed as potential causative agents. However, experimental testing by exposure of trees to controlled concentrations of these air pollutants has largely failed to reproduce the particular symptoms of forest decline. While in the field the oldest needles are most strongly affected, in most experiments involving exposure of trees or branches to relatively high concentrations of the air pollutants mentioned the youngest and most delicate needles are damaged; at relatively high ozone levels little or no damage occurs, and least with those species which are usually most heavily damaged in nature[7,8].

Table 1. Partition coefficients of tetrachloromethane, trichloroethene and tetrachloroethene between lipid and air; temperature 22 °C

	CCl_4	C_2HCl_3	C_2Cl_4
Beeswax	140	270	740
Total lipid and wax*	400	1000	2200
Spruce needles	17	44	95

*The total lipid and wax content of needles of *Picea abies*, extracted with cyclohexane and chloroform/methanol (2:1, v/v), was 4.5% of the fresh weight.

Table 2. Residual pigments in 5-year-old spruce needles *(Picea abies)* after UV-irradiation for 5 h in the absence and presence of trichloroethene and tetrachloroethene, in percent relative to untreated needles (n = 4)

	UV	$UV+C_2HCl_3$		$UV+C_2Cl_4$	
		180 ppb	2 ppb	180 pbb	2 ppb
Chlorophyll a	96 ± 9	33 ± 12	68 ± 8	33 ± 16	48 ± 9
Chlorophyll b	96 ± 8	64 ± 17	88 ± 15	56 ± 23	74 ± 7
β-Carotene	90 ± 16	43 ± 14	59 ± 7	41 ± 13	42 ± 9
Luteine/zeaxanthine	103 ± 7	68 ± 16	88 ± 17	64 ± 24	71 ± 9

Considering the symptoms and the geographical distribution of the new tree disease, we assumed that a photochemical process is involved[9]. Conspicuous is the fact that the damage is greatest in 'clean-air' regions. This led us to assume that atmospheric pollutants participate which have sufficiently high tropospheric stability to escape the primary emission zones of industrial centers and to be transported into remote areas. Several volatile halogenated hydrocarbons with widespread technical use as degreasers show considerable tropospheric persistence[10-12]. In a field experiment we have demonstrated that tetrachloroethene, at concentrations about 10-fold greater than those present nowadays in rural areas of industrialized countries, are capable of eliciting similar symptoms to those observed in the field[13]. It became apparent that the extent of damage depends upon the intensity and length of exposure to solar irradiation.

An important aspect may be the high lipophilicity of chlorocarbons and the high permeability of the cuticle to them, as expected from a previous publication[14] and model experiments with beeswax. We determined the partition coefficients between waxes and lipids extracted from spruce-needles *(Picea abies)* and air (table 1). A comparison of these values with those obtained with fresh spruce needles and calculated on the basis of their wet weight suggests that chlorocarbons with high boiling points are taken up from the surrounding air into the cuticles and functional membranes of mesophyll-cells. This could explain the species-selectivity of the new tree disease: the specific structure and composition of the cuticle probably has a strong influence upon the uptake of lipophilic compounds; all heavily damaged species have well-developed, coherent epicuticular wax layers which increase with the age of the needles or leaves and with exposure to light[15].

The uptake of the chlorocarbons by spruce needles is fast; figure 1 shows the time curve of equilibrium establishment for a beeswax coating on the inner surface of a round-bottomed flask (filled symbols) and for fresh spruce needles (open symbols) of similar surface area; for all three chlorocarbons studied in this experiment the initial slope of the invasion curve for spruce needles is about five-fold steeper than for beeswax.

Chlorocarbons in general may be activated by UV-light to radicals and triplet-states which in turn may yield phosgene, dichloroethyne, chloroacetylchloride, atomic chlorine and others[16]. Photosynthetic pigments in an electronically excited state are expected to react with chloroethenes with concomitant destruction, a photochemical reaction analogous to the well known addition of chloroethenes to electronically excited dienes and polyenes[17,18].

We exposed single needles of spruce *(Picea abies)* at 20 °C to UV-light in the presence of trichloroethene or tetrachloro-

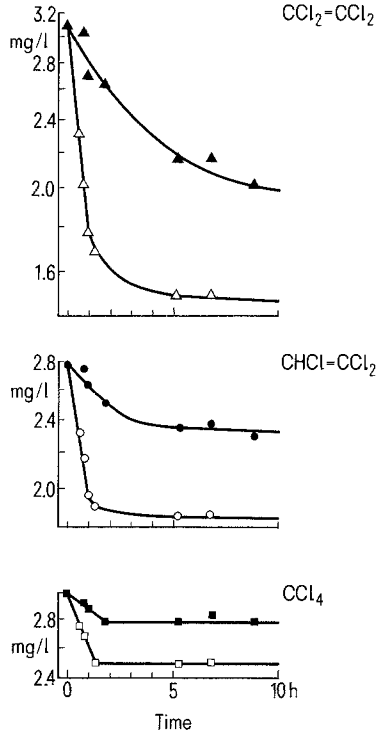

Figure 1. Time dependence of the partitioning of tetrachloroethene, trichloroethene and tetrachloromethane between air and lipid-compartment (beeswax: filled symbols; spruce needles: open symbols); in both cases a 2-L round-bottom flask was used; the inner surface of the flask was coated with beeswax; for the experiments with spruce needles amounts (25 g of fresh needles) with similar surface areas (800 cm^2) were used. Ordinate: air-concentration determined at the respective time after introduction of the corresponding amount of solvent-vapor.

ethene; a high-pressure mercury lamp (Hanau TQ 150) was used as light source; layers of 3 mm quartz and 3 cm of water were between lamp and needles. The experiments were performed at two concentrations in the gas phase, i.e. 180 and 2 ppbv. The faces of the needles exposed to direct irradiation in the presence of 180 ppbv of each chloroethene for 5 h showed a strong alteration of the apparent color, changing from clean dark green to a dirty brown-green; tetrachloroethene is in this respect the more efficient compound and the bleaching was already visible after one hour of irradiation. This was only slightly weaker when the needles were exposed to the chloroethenes at concentrations of 2 ppbv, suggesting that at high concentration saturation is reached or that the residual pigment molecules may be located at sites relatively well protected against UV-irradiation or the invasion of the chlorocarbons.

HPLC-analysis and quantitative determination of the pigments[19–21] of needles irradiated in the presence of the chloroethenes corroborated the qualitative observation. Compared to non-irradiated needles (fig. 2a) and those irradiated in the absence of the chloroethenes (fig. 2b), the concentrations of all pigments in needles exposed to tri- or tetrachloroethene were strongly depressed (fig. 2c, table 2); in addition, a new peak could be seen, supposedly a degradation product. Most strongly affected were chlorophyll-a and β-carotene. UV-irradiation alone entailed loss of violaxanthine, but other pigments and the apparent color of the needles did not change. Exposure of needles to the chloroethenes without UV-irradiation had no effect.

In naturally affected needles from a spruce suffering from the 'air-and-light' syndrome (fig. 2d) all major pigments are depressed to about half, relative to apparently undamaged needles from the same tree. A peak with a similar elution volume to that of the assumed degradation product is also present; however, it must be kept in mind that an identical elution volume does not prove structural identity. In any case, typical for both the artificially damaged needles and those from a diseased tree are low contents of β-carotene and of both chlorophylls; the main biochemical injury seems to be loss of photosynthetic pigments and a corresponding depression of photosynthetic capacity[22].

These findings suggest that a significant cause for the new forest decline observed in clean-air regions and mountain ranges at elevated altitudes has been identified. In conjunction with evidence provided by others, a hypothetical sequence of events may be envisaged: on the western slopes of the Black Forest, an area with heavy forest damage, a strong increase in the concentrations of several chlorocarbon solvents has been found in the soil with increasing elevation[23]; this seems to indicate that chlorocarbons are collected by clouds above the low-lying industrialized areas of Northwestern Europe, swept by the prevailing northwesterly winds toward the mountain ranges in Central Europe and are there deposited along the slopes receiving large amounts of precipitation as ascending rain. The high lipophilicity of the chlorocarbons, the rapid uptake of these compounds by the foliage and the high photochemical reactivity with light-activated photosynthetic pigments may constitute the amplifying stages of an ecotoxicological sequence.

This explains why the strongest damage is found in 'clean-air' regions with high solar flux and a relatively high intensity of short-wave irradiation; in low-lying and heavily polluted areas the solar flux, especially in the UV-A and UV-B bands, is attenuated by Raleigh- and aerosol scattering. The continuous photochemical stress of the needles may lead to fast aging and shedding of needles, earlier than under undisturbed conditions. The surprising similarity of the observed effects upon lipid mem-

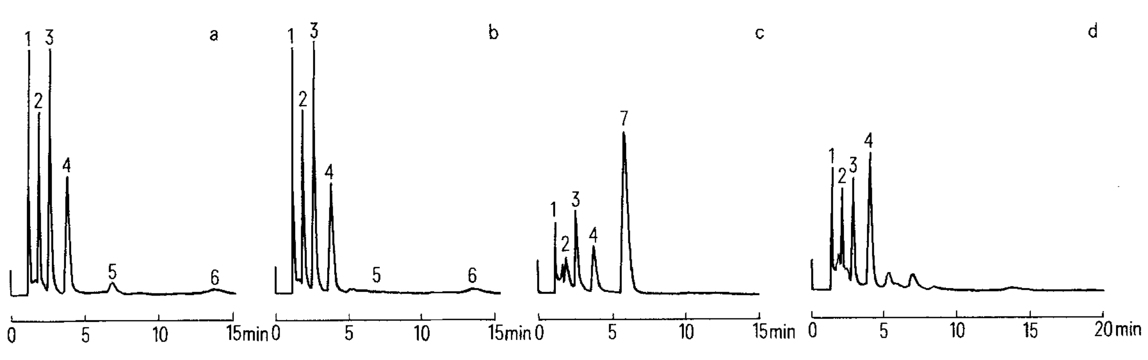

Figure 2. *a* HPLC-separation of pigments of needles of *Picea abies*; *b* irradiated for 5 h at 22°C with a UV-mercury lamp (Hanau TQ 150); *c* irradiated with UV-light in the presence of 180 ppb tetrachloroethene; *d* typical HPLC-chromatogram of a naturally affected needle. 1: β-carotene, 2 = chlorophyll a, 3 = chlorophyll b, 4 = luteine/zeaxanthine, 5 = violaxanthine, 6 = neoxanthine, 7 = non-identified.

branes[4] with the radical-initiated lesion of hepatocellular membranes in mammals by tetrachloromethane indicate that irrespective of the mode of generation of the primary radicals the secondary reactions may be very similar, i.e. cross-linking and oligomerization of unsaturated fatty acids and direct and indirect effects on vital membrane functions[24].

These observations also suggest that uptake and photodegradation in the phytosphere contribute strongly to the elimination of anthropogenic chlorocarbons from the environment.

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Photodynamic effect of the porphyrin derivative meso-tetra (4-N-methylpyridyl) porphine on sister chromatid exchanges in meristematic cells

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Summary. The porphyrin derivative meso-tetra (4-N-methyl-pyridyl) porphine tetraiodide (TMpyP) was tested for its ability to induce sister chromatid exchanges (SCE) in *Allium cepa* roots. A significant increase in the SCE frequency was observed when BrdU-substituted chromosomes were treated with TMpyP during the G_1 period of the second-division cycle and also when treatment with TMpyP was followed by blue light irradiation.

Key words. Allium cepa chromosomes; meso-tetra (4-N-methylpyridyl) porphine; sister chromatid exchanges; photodynamic effect.

Sister chromatid exchanges (SCE) have been shown to be sensitive indicators of the effects of chemical mutagens and carcinogens on eucaryotic chromosomes[1–3]. It is known that certain drugs and dyes (e.g. psoralens[4–7], mitomycin C[8], pyronin Y[9]) enhance their ability as inducers of SCE when treatments are followed by irradiation with a suitable light. Porphyrin derivatives are photosensitizing agents[10], and several authors have proved that treatment with hematoporphyrin or a hematoporphyrin derivative, followed by various irradiation treatments, increases the SCE frequency in animal cells[11,12]. The aim of this paper was to determine for the first time the combined effect of a basic porphyrin derivative and light in an in vivo plant system. The synthetic porphyrin derivative meso-tetra (4-N-methylpyridyl) porphine tetraiodide (TMpyP) (fig.1) was chosen because it has been identified as a photosensitizing agent[13] and DNA intercalator[14–17].

Material and methods. Root meristems of *Allium cepa* bulbs (15–30 g in weight) were grown in the dark at a constant temperature of 25 ± 0.5°C in tap water (renewed every 24 h) and aerated by continuous bubbling at a rate of 10–20 cm^3/min. BrdU-substitution of DNA was achieved throughout the first 20 h in 10^{-4} M 5-bromo-2′-deoxyuridine (BrdU) and 10^{-7} M 5-fluor-2′-deoxyuridine, after which the bulbs were placed in 10^{-4} M unlabeled thymidine (dT) for an equal period. Treatments with 5×10^{-8} M TMpyP (Ventron) were performed in the presence of dT throughout the G_1 period of the second-division cycle (4 h). The duration of interphase periods corresponding to the cycle time was estimated according to González-Fernández et al.[18]. The treatment with TMpyP throughout the G_1 period alone was performed in order to study the effect of a postirradiation at the beginning of the S period, when cells are highly sensitive[9].

The irradiation was applied for 30 min at the end of the G_1 period (4 h after the start of dT treatments). The growing roots were placed in glass receptacles containing tap water at a mean distance of 5 cm from the light source. A 60 W incandescent blue

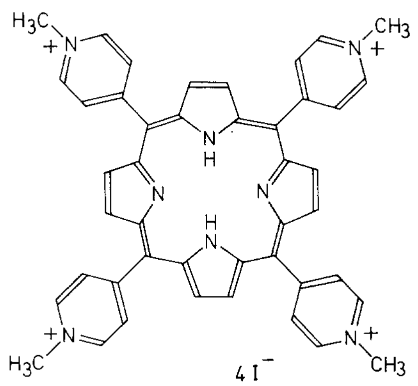

Figure 1. Chemical structure of meso-tetra (4-N-methylpyridyl) porphine tetraiodide (TMpyP).