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Chlorinated Pesticides and Plant Foliage: Translocation Experiments

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Chlorinated pesticides such as hexachlorobenzene (HCB), α- and γ-hexachlorocyclohexane (HCH), and p,p'DDT with its principal derivative p,p'DDE have become constituents of the biosphere, even though they are xenobiotics. This is a consequence of their great use all over the world, their high stability in several natural conditions, and their mobility in the environment.

Physico-chemical properties of these compounds indicate that soils and sediments can be considered as the principal environmental reservoirs (Mackay and Paterson 1981; Gaggi et al. 1985). Despite their low (HCH) or very low (DDT) vapor pressure (Spencer and Cliath 1970; Dobbs and Cull 1982; Rothman 1980) vapor movements of these contaminants from polluted soils probably constitute the main route of their long-range transportation (Spencer and Cliath 1972; Atlas and Giam 1981).

The remarkable lipoaffinity of these chemicals (Veith et al. 1979) indicates the potential of bioconcentration and bioaccumulation in biological system. Plant foliage uptake of vapors of these contaminants has been previously reported (Whitacre and Ware 1967; Nash and Beal 1970).

In a recent paper (Bacci and Gaggi 1985) it was demonstrated that the level of PCBs found in the foliage of beans, broad beans, tomatoes and cucumbers cultured 28 d in PCB-fortified sand or in 'clean' sand in small green-houses was independent of the contamination level of the soil where they were grown. Levels found in foliage were mainly due to the PCB-vapor concentration in the air of the green-houses.

The present study was undertaken to investigate the significance of the root uptake (translocation) in the determination of foliar levels of HCB, HCH isomers, p,p'DDT and DDE, in a test species:

Phaseolus vulgaris (dwarf bean).

MATERIALS AND METHODS

Two 60-cm-wide cubic glass boxes, mantained at a constant temperature by means of a warm water system in the bottom and continuosly illuminated by 3x20 W True-Lite fluorescent tubes, were used (Bacci and Gaggi 1985). The temperature was 24°C at the bottom and 27°C at the top, due to influence of the lamps. The culture area was located in a glass tray to reduce the impact of air flowing in through a 3-cm-diameter hole placed near the bottom. Two other similar holes and slight gaps between the box and its cover which simply rested on the walls, guaranteed a sufficient air turnover. The air-turnover time of each box, calculated from the decay of a tracer (γ-HCH), was about 80 min. The air volume of the boxes being 200 l, this means that the air input (and output) was 2.5 l min^{-1}.

A Pliocene sand, pH 7.5 (Aubert 1978), containing 0.15% organic carbon (Gaudette et al. 1974), classifiable as a 'fine sandy medium sand' (Doeglas 1968), was used as culture soil. The sand was oven-dried (24 h at 80°C) and, after cooling in a dessicator, equilibrated with n-hexane (for pesticide residue analysis, 1 l kg^{-1}, 3 d). Fortification was done with HCB, α- and γ-HCH, p,p'DDT and DDE, by adding 75 mg of pure compound to different 300 g aliquots of sand (one for each contaminant). The nominal concentration of each pollutant in the fortified sand was 250 mg kg^{-1}. The solvent was then completely evaporated by vacuum rotary evaporator.

Dwarf bean was selected as test species. Bean seeds were activated in water (1 h at 40°C), seeded in 'clean' sand and kept at 35°C for a week; then the small sprouts were transferred to the green-houses and acclimatated at 24-25°C for a second week. After this (preliminary culture), the main culture began: each bean sprout was put into a glass vessel containing either 65 g of 'clean' sand, or 25 g of fortified sand sandwiched between two layers of 'clean' sand, 30 g at the bottom and 10 g on top. The sandwich was made to avoid foliage contamination by polluted sand (the top) and to reduce the amount of polluted sand to be used (the bottom). The tangle of the roots inside the vessels during the experiment guaranteed a direct contact between roots and polluted sand.

Two green-houses were used. The first (box 1) was testing for

α-HCH (12 bean sprouts in 12 vessels with α-HCH-fortified sand) and p,p'DDE (12 sprouts in 12 vessels with p,p'DDE-fortified sand); 12 sprouts cultured on 'clean' sand were also placed in the same box. Similarly, in the second box (box 2) 12 sprouts were cultured in HCB-, 12 in γ-HCH- and 12 in p,p'DDT-fortified sand in addition to 12 sprouts grown on 'clean' sand. Vessels were placed in the culture area of the boxes at random.

A 'clean' area, away from the laboratory, was used for a culture of 10 plants in 'clean' sand at room temperature, as controls.

Water was supplied once a day directly on the sand to keep the soil moist.

Sampling times were 7, 14, 21 and 60 days (main culture), taking foliage from 3 separate sprouts for each kind of treatment, by using a little noose introduced into the boxes through one of the lateral aeration holes. Even when sprouts cultured on polluted sand were completely used up for sampling (or heavily damaged during sampling), the sand was watered daily to avoid reduction in evaporation rate of pesticides (Spencer and Farmer 1980).

Analyses were carried out on air and foliage samples. Air samples were collected on Florisil using disposable Pasteur pipets according to Giam et al. (1975), with minor modifications. Apparent sample volumes were measured by soap-bubble flow-meter connected in series between the air trap and a tap-water vacuum pump. These volumes were corrected only by pressure (about half an atmosphere), measured by a manometer on the aspiration line. Pesticide residues adsorbed onto the Florisil were eluted with 45 ml n-hexane in glass chromatographic-columns containing 0.5 g anhydrous Na_2SO_4 at the bottom. After a suitable concentration the samples were injected into a Perkin-Elmer F-22 gas-chromatograph equipped with on-column injectors, Nickel^{63} ECDs and a data station; 2 m x 2 mm i.d., silane treated, borosilicate glass-column, packed with GP 4% SE-30 6% SP-2401 on 100/120 mesh Supelcoport (routine packing) and GP 1.5% SP-2250 1.9% SP-2401 on 100/120 mesh Supelcoport, were used. The carrier gas was argon-methane 95/5%; flows 60 and 40 (as scavenger) ml min^{-1}. Injector, oven and detector temperatures were 210, 200 and 280°C respectively.

Foliage samples were partially dried overnight at 50°C. Subsamples were used to detect the residual water content (24 h, 105°C). Extractions were carried out in a Soxhlet apparatus, using n-hexane. The clean-up procedure was a sulfuric acid treatment

Table 1. Results from translocation experiments. Concentration in the bean leaves are in $\mu g\ g^{-1}$, dry weight (pool of 3 plants). CS = 'clean sand'.

t (d)	BOX 1			BOX 2			
	Treatment	α-HCH	p,p'DDE	Treatment	HCB	γ-HCH	p,p'DDT
7	CS	5.7	12.7	CS	0.4	26.4	1.7
	α-HCH	**9.5**	17.7	HCB	**1.0**	23.6	1.3
	p,p'DDE	5.2	**17.4**	γ-HCH	0.8	**27.3**	1.5
				p,p'DDT	0.9	38.2	**1.3**
14	CS	6.1	22.7	CS	1.5	48.6	3.0
	α-HCH	**5.4**	16.8	HCB	**0.7**	19.9	1.4
	p,p'DDE	7.0	**34.1**	γ-HCH	0.9	**29.3**	1.6
				p,p'DDT	1.2	37.6	**1.8**
21	CS	8.0	17.4	CS	1.1	48.5	3.1
	α-HCH	**7.3**	13.5	HCB	**1.8**	57.0	3.5
	p,p'DDE	6.4	**19.3**	γ-HCH	1.2	**46.0**	3.2
				p,p'DDT	0.8	45.5	**2.4**
60	CS	4.8	28.3	CS	0.7	42.7	4.0
	α-HCH	**3.8**	64.0	HCB	**1.3**	48.0	9.7
	p,p'DDE	4.5	**25.0**	γ-HCH	0.7	**40.6**	3.0
				p,p'DDT	1.4	27.3	**5.4**

followed by the Florisil chromatography.

RESULTS AND DISCUSSION

The concentration of HCB, α- and γ-HCH, p,p'DDT and DDE in the control bean plants after 60 d of treatment (main culture) were always lower than 20 ng g^{-1}, dry weight.

General conditions of the plants were good: by the end of the experiments flowers and small fruits had developed on the plants that had been disturbed less during sampling. No evidence of toxicological effects due to treatment was observed.

The results shown in Table 1 demonstrate that the levels of the tested chlorinated pesticides in the foliage of the bean plants are not significantly dependent on the contamination level of the soil where they have been grown. Probably the mobility of these contaminants through the root is very slight, as was previously stated in similar experiments on PCBs (Bacci and Gaggi 1985). Therefore levels found in the bean foliage are mainly due to the vapor uptake from the contaminated air of the boxes.

Pesticide vapor concentrations in the air of the green-houses were measured during the translocation experiment. As is reported in Table 2, there is a good direct proportionality between these levels and the vapour pressure values of the studied compounds

Table 2. Mean concentrations (μmol l^{-1}) of the vapors of the chemicals in the air of the boxes during the translocation experiments and vapor pressure values (P°, as mmHg at 25°C).

	γ-HCH	α-HCH	HCB	p,p'DDE	p,p'DDT
$\bar{x}$	$6.6\ 10^{-5}$	$2.5\ 10^{-5}$	$1.1\ 10^{-5}$	$2.9\ 10^{-6}$	$2.5\ 10^{-7}$
n	14	19	14	18	13
%CV	31	29	51	34	22
P°	$6.3\ 10^{-5}$	$3.0\ 10^{-5}$	$1.7\ 10^{-5}$	$3.2\ 10^{-6}$	$4.3\ 10^{-7}$

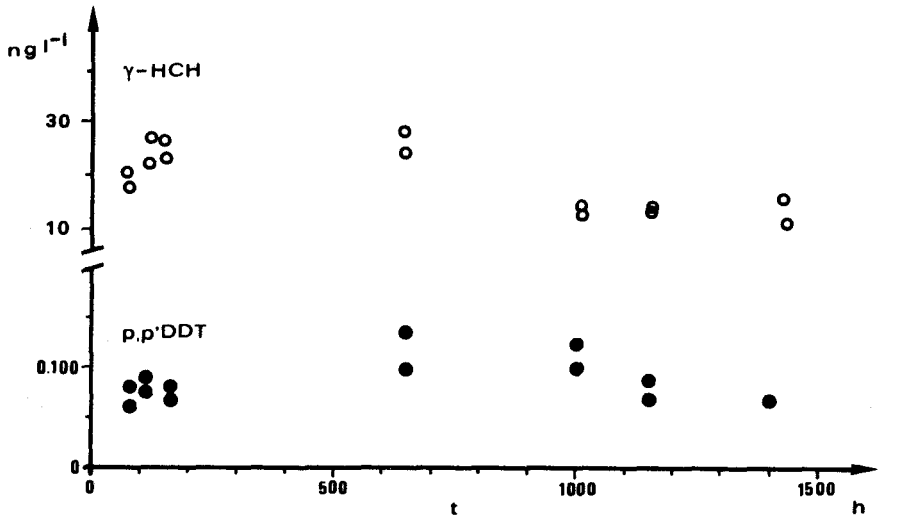

Figure 1. Trends of the levels of γ-HCH and p,p'DDT vapors in the air of the green-house (box 2) during translocation experiments.

(Spencer and Cliath 1970; Dobbs and Cull 1982; Farmer et al. 1980; Spencer and Cliath 1972; Rothman 1980). Considering that the molecular weight of these chemicals ranges from 284.8 (HCB) to 354.5 (DDT) and that the air turnover-time as well as other experimental conditions were kept almost constant, these findings are in agreement with the literature (Hartley 1969; Dobbs and Cull 1982).

In Figure 1 the trend of the levels of the vapors of γ-HCH (the most volatile) and p,p'DDT (the least) are plotted to show that over two months the levels of contaminant vapor in the air of the boxes remained almost constant. Similar trends were found for the other chemicals studied, indicating that these small green-houses could also be used for kinetics experiments (e.g. the uptake of these or similar vapors by plants or animals). In this case the use of non-growing (or rather with negligible growth) biological systems is recommended to evaluate the exposure time of the examined materials; in fact the slight mobility of the studied compounds and their high bioaffinity indicate that accumulation phenomena are very likely, as was observed, in natural conditions, for pine needles (Thomas et al. 1984; Gaggi and Bacci 1985).

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REFERENCES

Atlas E, Giam CS (1981) Global transport of organic pollutants: ambient concentration in the remote marine atmosphere. Science 211:163-165

Aubert G (1978) Methodes d'analyses des sols. CNDP, CRDP Marseille

Bacci E, Gaggi C (1985) Polychlorinated biphenyls in plant foliage: translocation or volatilization from contaminated soils? Bull Environ Contam Toxicol 35:673-681

Dobbs AJ, Cull MR (1982) Volatilisation of chemicals-Relative loss rates and the estimation of vapour pressure. Environ Pollut Ser B 3:289-298

Doeglas DJ (1968) Grain-size indices, classification and environment. Sedimentology 10:83-100

Farmer WJ, Yang MS, Letey J, Spencer WF (1980) Hexachlorobenzene: its vapor pressure and vapor phase diffusion in soil. Soil Sci Soc Am J 44:676-680

Gaggi C, Bacci E (1985) Accumulation of chlorinated hydrocarbon vapours in pine needles. Chemosphere 14:451-456

Gaggi C, Bacci E, Calamari D, Fanelli R (1985) Chlorinated hydrocarbons in plant foliage: an indication of the tropospheric contamination level. Chemosphere 14:1673-1686

Gaudette HE, Flight WR, Toner L, Folger DW (1974) An inexpensive titration method for the determination of organic carbon in recent sediments. J Sed Petrol 44:249-253

Giam CS, Chan HS, Neff GS (1975) Rapid and inexpensive method for detection of polychlorinated biphenyls and phthalates. Anal Chem 47:2319-2320

Hartley GS (1969) Evaporation of pesticides. In: Gould RF (ed) Pesticides formulation research. American Chemical Society, Washington

Mackay D, Paterson S (1981) Calculating fugacity. Environ Sci Technol 5:1006-1014

Nash RG, Beal ML Jr (1970) Chlorinated hydrocarbon insecticides: root uptake versus vapor contamination of soybean foliage. Science 168:1109-1111

Rothman AM (1980) Low vapor pressure determination by the radiotracer transpiration method. J Agric Food Chem 28:1225-1228

Spencer WF, Cliath MM (1970) Vapor density and apparent vapor pressure of Lindane (γ-HCH). J Agr Food Chem 18:529-530

Spencer WF, Cliath MM (1972) Volatility of DDT and related compounds. J Agr Food Chem 20:645-649

Spencer WF, Farmer WJ (1980) Assessment of the vapor behavior of toxic organic chemicals. In: Haque R (ed) Dynamics, exposure and hazard assessment of toxic chemicals. Ann Arbor Science, Ann Arbor, p 143

Thomas W, Rühling Å, Simon H (1984) Accumulation of airborne pollutants (PAH, chlorinated hydrocarbons, heavy metals) in various plant species and humus. Environ Pollut Ser A 36:295-310

Veith GD, DeFoe DL, Bergstedt BV (1979) Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Res Board Can 36:1040-1048

Whitacre DM, Ware GW (1967) Retention of vaporized lindane by plants and animals. J Agr Food Chem 15:492-496

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