

**CENTRE FOR ECOLOGY AND HYDROLOGY
NATURAL ENVIRONMENT RESEARCH COUNCIL**

Final Report

**Potential effects of volatile organic pollutants on vegetation
Critical review
DEFRA New Horizons Research Programme**

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EXECUTIVE SUMMARY

1. **Volatile organic compounds** (compounds with up to 8 C atoms) are ubiquitous in both rural and urban air, with both natural and anthropogenic sources. The main concern over VOC emissions and atmospheric concentrations has been their role as precursors of photochemical ozone, which is known to be toxic to plants at concentrations that are currently experienced over most of the United Kingdom.
2. **Effects of VOCs on plants**
 - **Laboratory experiments** to study the potential direct effects of VOCs on plants have used very high concentrations (relative to ambient air) and short exposure periods. The concentrations of VOCs required to elicit adverse effects have been substantially greater than those observed outdoors, even in polluted air. Laboratory experiments have rarely considered the role of UV light as a potential contributory factor to VOC toxicity.
 - However, there have been very few **field experiments**, or experiments that simulate field conditions over long time periods (weeks to months) at concentrations relevant to atmospheric exposure.
 - **Field measurements**, downwind of a major industrial source, have identified subtle, pollutant-specific effects on plants of ethylene, which is a plant hormone.
 - A very **wide range of tolerance** to VOCs among plant species has been demonstrated. Bean (*Phaseolus vulgaris*) plants have been shown to be among the most sensitive, with decreases in the harvested pod weight. Other studies have identified **effects on flowering and seed formation**, including mutagenic effects, in the absence of visible damage or effects on growth.
 - **Plants can metabolise** many VOCs or transport them from leaves to roots. The metabolites may be further degraded, or may be conjugated and accumulate within the leaf, in vacuoles or cell walls. It is possible that the **breakdown products are toxic** to herbivores or phytophagous insects.
3. **Risks to vegetation in the UK**
 - While **it appears unlikely that VOCs pose a direct threat to plant health in the UK**, there is no information on their effects in conjunction with other gaseous pollutants or environmental stresses such as drought, nutrition or temperature extremes.
 - **The greatest risk** from exposure to VOCs in the UK is likely to be to **horticultural crops** grown in or close to urban areas, and to garden plants in cities. The most sensitive stage of plant growth appears to be flowering and seed/fruit production.
 - **Native plant species** and **agricultural crop plants** at greatest risk are those close to industrial sources or major roads.
 - **Adverse effects at the ecosystem level** are more likely to be caused by the accumulation of VOC metabolites in plants, and consequent effects on the food chain, than by direct effects on plant growth.
4. **Gaps in knowledge and potential solutions**
 - There have been **no relevant experimental studies of the long-term effects** of VOCs on plants that include effects on flowering, seed production and viability, the accumulation of metabolites and interactions with phytophagous insects and herbivores.
 - **Open-top chambers** may be used to determine dose-response relationships by delivering known concentrations of individual VOCs or mixtures, but adequate replication is expensive.
 - **Field fumigation** experiments are easier to control for VOCs (normally liquids at ambient temperatures) than for inorganic pollutant gases such as ozone, and can be conducted on native vegetation and crops with minimal disturbance to the normal growing environment. Monitoring of the applied VOC concentrations across a gradient of exposure may permit dose-response relationships to be estimated.
 - Several promising **biomarkers of VOC exposure** have been suggested, but to date no VOC-specific biomarker/biomonitor has been identified.

3. Note on units of air concentration measurements

Air concentrations of gases are measured either as mixing ratios, expressed as parts per billion (ppb = parts in 10^9 by volume) or parts per million (ppm = parts in 10^6 by volume), or as mass per unit volume (mg or $\mu\text{g m}^{-3}$). These two sets of units can be interconverted, but the conversion factor depends on the molecular weight of the molecule, and the temperature and pressure at which the measurement was made. Where concentrations are reported in this document, they are given in the units used by the original authors. Approximate conversion factors, based on an air temperature of 15 °C and 1 atmosphere pressure, are given below for the more important VOCs. Conversion factors for **ppb to $\mu\text{g m}^{-3}$** are the same as for **ppm to mg m^{-3}** , and *vice versa*.

VOC	Molecular formula	Molecular weight	Conversion factor 15 °C ppb -> $\mu\text{g m}^{-3}$	Conversion factor 15 °C $\mu\text{g m}^{-3}$ -> ppb
Ethylene	C_2H_4	28	1.18	0.84
Isoprene	C_5H_8	68	2.88	0.35
Benzene	C_6H_6	78	3.30	0.30
Toluene	C_7H_8	92	3.89	0.26
Xylene	C_8H_{10}	106	4.49	0.22
Trichloroethane	$\text{C}_2\text{H}_3\text{Cl}_3$	133.5	5.65	0.18
Tetrachloroethylene	C_2Cl_4	166	7.02	0.14
Formaldehyde	CH_2O	30	1.27	0.79
PAN	$\text{CH}_3\text{CO}_2\text{ONO}_2$	121	5.12	0.20
Methanol	CH_3OH	32	1.35	0.74

1. INTRODUCTION

1.1 Air pollution effects on plants

Historically, the main interest in the effects of air pollutants on vegetation has been with inorganic gases produced by fossil fuel combustion, such as sulphur dioxide and nitrogen oxides, and the acids derived from them in 'acid rain'. Such studies go back to the early years of this century, and also considered direct effects of smoke, regarded as a non-reactive contaminant, which might occlude stomata or inhibit light interception by leaves. More recently, interest has been expressed in the deleterious effects of photochemically produced oxidants such as ozone and peroxyacetyl nitrate (PAN). The direct effects of these gases have been known for over 30 years, following the classic studies in the San Bernardino mountains of California (Miller and McBride, 1998). The relationship between exposure to ozone and reduction in growth or crop yield has now been established for several key plant species, and forms the basis for describing the risk to vegetation from ozone in Europe in terms of the AOT40 (Accumulation Over Threshold of 40 ppb) statistic, or the SUM60 (Accumulated sum of exposure over 60 ppb) in the USA (Mauzerall and Wang, 2001). Such statistics are used to develop strategies for emission reduction.

1.2 Chronic and acute exposure to pollutants

One of the findings of the early research in the late 1970s was that the effects of pollutant gases on plants caused by short-term exposure to large concentrations ('acute' exposure) could not be used to predict the effects of long-term exposure to much smaller concentrations ('chronic' exposure) (Garsed and Rutter, 1982). Experimental testing of plants, to establish 'safe' exposure thresholds to individual pollutants, has therefore moved out of the laboratory and into the field. Exposure systems using open-top chambers, and direct field fumigation, have been developed in order to avoid the artefacts introduced by growing plants indoors in controlled environments. It is now recognised that the growing conditions and environment during long-term exposure play an important role in plant responses to pollutants. Moreover, it is now clear, although there has been comparatively little experimental work, e.g. (Freer-Smith, 1984), that plants often respond in a non-additive way to mixtures of pollutants, i.e. that the effect of a given pollutant on a given plant species may be enhanced or reduced by the simultaneous presence of other pollutants.

1.3 Definition of Volatile Organic Compounds (VOCs)

For the purposes of this review, in which the focus is on VOCs, the compounds in question contain up to 8 carbon atoms (e.g. xylene), and may contain nitrogen, oxygen or halogen atoms. There is no absolute distinction between VOCs and SVOCs, given the gradation in vapour pressures at normal temperatures for organic compounds found as gases in the atmosphere. Specifically excluded from this study are compounds such as polychlorinated biphenyls (PCBs), dioxins and polycyclic aromatic hydrocarbons (PAHs), where a large fraction of the material in the atmosphere may exist in the particulate phase rather than as a gas.

1.4 Current DEFRA research

Volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) are produced by a range of human activities, such as motor vehicles, solvent use and many industrial processes. The emissions in the UK are well characterised in the UK Emissions Inventory, which is part of the DEFRA Programme (ref. EPG 1/3/103). The ambient urban concentrations of VOCs are monitored through DEFRA's Enhanced Urban Network ((Derwent, et al., 2000), ref. EPG 1/3/105 +106), and SVOCs are measured as part of DEFRA's research into TOMPs (Toxic Organic Micro Pollutants) ((Tremolada, et al., 1996), ref. EPG 1/3/149, EPG 1/3/154).

1.5 Sources of VOCs in the UK

The major sources of anthropogenic VOCs in the UK atmosphere are industrial processes (solvents and manufacturing), oil refining and distribution, and transport (exhaust emissions and unburnt fuel) (NAEI 1998). In addition there are biogenic sources of VOCs, of which the most important are isoprene (C₅) and monoterpenes (C₁₀), although very many different VOCs are emitted by plants (Kesselmeier and Staudt, 1999). Some of the VOCs normally considered as anthropogenic are also produced and emitted by plants. For example, ethylene is a plant hormone, ethane may be emitted by plants which are under stress (Kimmerer and Kozlowski, 1982; Wolfenden, et al., 1988), methanol and longer-chain alcohols are emitted by many species (Macdonald and Fall, 1993), formaldehyde production has been observed in acorns (Albert, et al., 1998), and even toluene has been reported to be produced by some species (Heiden, et al., 1999). This may mean that plants may be either tolerant of external VOCs, because there are in-built mechanisms for metabolising them, or they may be particularly sensitive to anthropogenic VOCs that are used as signalling molecules within the plant.

1.6 Typical concentrations of VOCs in polluted UK air

In the UK a fully automatic network of 11 urban background and one rural site has operated successfully since 1996, with hourly measurements of 26 hydrocarbons with between 2 and 8 C atoms. The data for 1996 have been analysed in depth, and compared with similar results from the Netherlands and the United States (Derwent, et al., 2000). More recent data, available on the web at <http://ariadne.aeat.co.uk/netcen/airqual/statbase/cones/hcarb.html>, are tabulated in Table 1, showing the annual mean concentration and maximum hourly concentration for a sub-set of the measured hydrocarbons for 1998-2000. It is striking that the 'background' urban concentrations are very similar at all the sites. The site at London (Marylebone Road), which is situated at the kerbside of a busy thoroughfare, shows much larger average concentrations than the other sites. The sites at Southampton and Middlesbrough also have slightly larger values, and the maximum hourly ethylene concentrations at Middlesbrough probably reflect the site's proximity to major petrochemical industry. The 'rural' site at Harwell has annual average concentrations which are approximately half those of the urban background sites. Although the annual average concentrations are small, the maximum hourly concentrations over the 3-year period can be up to 100 times greater than the annual average, giving maximum toluene concentrations over 100 µg m⁻³ even at the rural site.

Close to industrial sources, or landfill sites, concentrations may be very much greater than recorded in the 'background' network data. Measurements of VOCs in UK landfill gas gave concentrations of aromatic hydrocarbons up to 2 mg m^{-3} , and of halogenated compounds up to 1.2 mg m^{-3} (Allen, et al., 1997). Although such large concentrations will be dispersed fairly rapidly by the wind, their source at ground level means that there may be local fumigation of the surrounding area with concentrations well above the maxima recorded in the urban network.

Table 1: Average annual concentrations ($\mu\text{g m}^{-3}$) and hourly maximum concentration at UK hydrocarbon network sites in 1998-2000

site	benzene		Max hour	toluene		max hour	ethylene		max hour		
	1998	1999		2000	1998		1999	2000		1998	1999
URBAN BACKGROUND											
Belfast	2	2	1	55	5	4	117	2	2	1	45
Birmingham	3	2	1	62	6	5	461	3	2	2	74
Bristol	3	3	1	64	6	5	142	3	3	2	51
Cardiff	3	3	2	63	7	5	384	3	3	3	48
Edinburgh	2	2	1	33	5	5	90	2	1	1	123
Leeds	3	3	2	50	6	6	339	3	3	3	50
Liverpool	2	2	2	58	6	6	354	1	2	2	59
London Eltham	3	3	2	45	6	5	134	2	2	2	32
London UCL	4	4	2	61	9	8	125	3	3	4	69
London Marylebone Rd	13	11	6	69	33	29	203	19	15	15	93
Middlesbrough	3	3	2	217	4	4	91	5	4	4	918
Southampton	5	4	2	62	10	9	208	4	3	4	67
RURAL											
Harwell	1	1	1	12	2	1	169	1	1	1	14

2. UPTAKE OF VOCs BY PLANTS

2.1 Conceptual models

The uptake of VOCs by plants has been measured, irrespective of any potential deleterious effects, as part of studies to examine the fate of VOCs released to the atmosphere. One of the requirements for simple modelling of VOCs and their interaction with vegetation is knowledge of the *partition ratio*, which is defined as the relative enrichment of VOCs physically dissolved in plant tissue in equilibrium with a given atmospheric concentration. Because the ultimate objective is to be able to model the behaviour of a very wide range of VOCs, based on their known or calculable physical properties, such studies have tended to use large concentrations under laboratory conditions, and may have looked at uptake by individual parts of a plant, such as the leaf cuticle (Baur, et al., 1996; Welke, et al., 1998). The data are then used to develop conceptual models of VOC uptake that can be applied using the measured relationship to properties such as the octanol-air partition coefficient (K_{oa}) (Bell and Failey, 1991; Hiatt, 1998; Paterson, et al., 1990; Riederer, 1995). Many studies relate to SVOCs, which are absorbed and desorbed by leaves more slowly than VOCs, and which may accumulate in the field over days or months. The partition ratios for SVOCs may also be much greater than for VOCs, leading to fears of accumulation in the food chain of compounds such as PCBs and PAHs, many of which are known carcinogens (Jones and de Voogt, 1999).

2.2 Direct measurements

Laboratory measurements of VOC absorption can show very large differences among species and different plant parts, and for different chemical compounds (Collins, et al., 2000; Omasa, et al., 2000). Measurements suggest that the degree of enrichment at low concentrations may be greater than at high concentrations (Frank and Frank, 1989), implying that the processes involved may vary with VOC concentration. Experiments performed at large concentrations cannot therefore be used in a simplistic manner to predict effects at lower concentrations. Field observations (for example by exposing plants alongside highways) have shown that VOCs do accumulate in leaves (Brown, et al., 1999; Hiatt, 1999; Keymeulen, et al., 1993), but the extent of this (reversible) accumulation depends both on the individual VOC and the plant species. These partitioning measurements have assumed that there is no active role of the plant leaf in altering the internal concentrations of VOCs, for example by metabolising or transporting the gas absorbed within the leaf. This assumption may prove to be erroneous for some VOCs (see below).

2.3 Rates of uptake – dry deposition

Many of the models of plant uptake of organic compounds from the atmosphere are based on equilibrium properties such as the octanol-air equilibrium constant. The dynamics of uptake, and the approach to equilibrium, are more difficult to address experimentally. For SVOCs, equilibrium may never be truly achieved, in that the time-scales involved are long compared to temperature and concentration changes in the air, and the phenological state of the plant. For VOCs, however, the approach to equilibrium is relatively rapid, occurring over a few hours (Binnie, et al., 2001; Brown, et al., 1998; Keymeulen, et al., 1995). As a consequence, the description of

air-plant transfer in terms of a deposition velocity, as applied to inorganic gases such as ozone, is not appropriate. The simple description of air-plant transfer as a unidirectional flux does not take into account the development of a dynamic equilibrium between plants and the atmosphere. At equilibrium the net deposition would be zero. In practice, measured 'deposition velocities' for VOCs to vegetation are usually calculated by measuring the net uptake of a VOC by a plant over a fixed time, which may or may not be appropriate to the development of a dynamic equilibrium. The resultant flux (amount absorbed per unit time and leaf area) divided by the air concentration (mass per unit volume) has the dimensions of a deposition velocity (length/time) but is not a representation of the (equilibrium) rate of VOC exchange at the plant surface. The net deposition velocity derived from long-term exposures after equilibration has occurred may represent the loss of the absorbed VOC from the plant by processes other than atmospheric exchange, e.g. metabolism, or active transport to the roots (Deinum, et al., 1995; Jen, et al., 1995). The reported deposition velocities are consequently very much smaller ($\mu\text{m s}^{-1}$) (Duyzer and van Oss, 1997; Wesely, et al., 2000) than those observed for inorganic gases like ozone which are irreversibly removed from air by plants, with maximum deposition velocities measured in cm s^{-1} .

3. EFFECTS OF VOCS ON PLANTS

3.1 Existing databases of information

The United States EPA maintains a web-searchable database (ECOTOX), and a related database of ecotoxicological studies on plants (PHYTOTOX). URLs for both these sites are given in the reference list. The PHYTOTOX database contains, *inter alia*, data on experiments where plants have been exposed to substances in the vapour phase. To quote the site introduction:

“The PHYTOTOX database is a computerized information resource that permits the rapid retrieval and comparison of data pertaining to lethal and sublethal responses, excluding residue effects, of terrestrial plants to the application of chemicals. Both natural and synthetic organic compounds administered to native, crop, or weed species have been considered.....
.....Independently compiled laboratory data files that include PHYTOTOX parameters and meet the quality assurance criteria are also included.”

A manual search of this database for VOCs (as defined in section 1.3) yielded 793 entries. Different exposure levels in the same experiment are treated as separate entries. However, of these, only 23 entries involve fumigation with concentrations of 100 ppb (parts per billion, parts in 10^9) or less, as reported in 5 published papers, all investigating the effects of ethylene (C_2H_4). Most of these ‘low concentration’ studies were also of relatively short duration, with only 10 that lasted for 10 days or longer. The results from this database search illustrate the problem – although experiments have been done on a wide variety of plants using a wide variety of VOCs, almost all have used unrealistically large concentrations (factors of 100 or more greater than those observed) and have been conducted for exposure times typically of hours.

If any effects are observed under these conditions, then they may point to the more sensitive species and plant parts exposed to very high concentrations (e.g. in case of a chemical spillage or accident). However, there is no guarantee that those species or plant parts would be susceptible to much longer exposures at much smaller concentrations. The converse is also true – the lack of response to a short-term exposure to high concentrations does not mean that that species or plant part would respond similarly to long-term exposure at low concentrations.

3.2 Published literature reviews

3.2.1 VOCs and ‘forest decline’

The concern over ‘forest decline’ (neuartige Waldsterben) in Germany in the late 1980s and early 1990s prompted interest in the potential for VOCs to be responsible for the apparent loss of tree vitality across a wide area of central Europe, and several reviews of the effects of VOCs on plants were published (Schulze and Stix, 1990; Smidt, 1992; Smidt, 1994). This interest in Germany mirrored earlier short-term experiments on the potential effects of VOCs on plants in relation to other pollutants (van Haut and Prinz, 1979), which is discussed in more detail below. These reviews are summarised here, with the emphasis on long-term experiments, before considering individual VOCs in greater detail.

Schulze and Stix (Schulze and Stix, 1990) summarised results for ethylene and formaldehyde, as examples of potentially important organic air pollutants. In the

longest experiment, with ethylene, a concentration of $8 \mu\text{g m}^{-3}$ over 100 days led to a 22% reduction in flowering of oats (Reid and Watson, 1985). Results for formaldehyde suggested a long-term effect on plant growth at concentrations around $20 \mu\text{g m}^{-3}$ (van Haut and Prinz, 1979).

The earlier paper by Smidt (Smidt, 1992) gives a table showing global estimates of emissions of several VOCs, and typical concentrations in background, forest and urban air. He gives a detailed review of the effects of PAN (peroxyacetyl nitrate), noting that most work has been done on plant species relevant to southern California, rather than central Europe. No long-term experiments with ethylene are cited, but the author points out that ethylene concentrations in vehicle exhaust may be as high as 1000 ppm. Chlorinated hydrocarbons are considered, and 'other' organic air pollutants including formaldehyde and several SVOCs such as herbicides and polycyclic aromatic hydrocarbons (PAHs). Assumed threshold concentrations for damage are quoted as $12 \mu\text{g m}^{-3}$ for formaldehyde, $3 \mu\text{g m}^{-3}$ for PAN and $2 \mu\text{g m}^{-3}$ for acetic acid. The later paper (Smidt, 1994) lists the demonstrated direct effects of VOCs on forest plants as follows:

- Changes in leaf composition
- Increased activity of glutathione-S-transferase
- Oxidation of sulphhydryl groups (and consequent inhibition of SH enzymes and decrease in reduction potential)
- Partial or total destruction of organelles and other ultrastructural changes
- Destruction of needle pigments, development of chlorosis
- Disturbance to photosynthesis
- Reduction in transpiration
- Premature leaf fall

Most of these effects were derived from short-term experiments with very high (relative to ambient air) concentrations of VOCs.

In addition to a summary of the effects of man-made hydrocarbons, the potential physiological role of biogenic hydrocarbons such as isoprene or terpenes was also briefly considered, both directly, and after reaction with ozone to form more toxic free radicals and peroxides (Möller, 1988). The major (indirect) effect on plants of these biogenic VOCs is, however, recognised in their contribution to the formation of photochemical ozone. The role of peroxyacetyl nitrate (PAN) was reckoned to be relatively unimportant in forested areas of central Europe, if measured concentrations were less than 4 ppb (based on limited data), but the possibility of phytotoxic compounds being formed by reaction of nitrate (NO_3) radicals with biogenic VOCs was also noted. This possibility has not yet been explored. The following conclusions were reached:

- VOCs are ubiquitous in the atmosphere
- The effects of uptake of several compounds can be observed in conifer needles
- Several compounds are enriched in leaf organs and the soil
- The effects of most individual VOCs are not likely to be the cause of the widely observed forest damage, but there may be synergistic effects with other damaging factors, or indirect effects
- Indirect effects are more likely to be a cause of damage than direct effects
- The uptake pathways and toxicity to forest trees is known for some compounds

- In badly polluted areas, concentrations of some compounds may cause damage to sensitive trees

These reviews addressed the perceived problem of 'forest decline' (neuartige Waldschäden), and therefore concentrated on the potential effects of VOCs on forests in regions distant from major point sources and urban areas.

3.2.2 *United States review of Priority Pollutants*

A major review of the ecological exposure and effects of organic chemicals in the atmosphere on terrestrial plants was published a decade ago (Foster, 1991). The author noted the emphasis on experiments to determine the uptake and accumulation of potentially toxic materials in crop plants, and the dearth of experimental data on the fate and effects of toxic organics on non-crop terrestrial plants. To quote:

"little is known concerning deposition rates of organics to terrestrial ecosystems, rates of plant uptake, and subsequent transport and metabolism".

Although some progress has been made during the past decade, the problem has still hardly been addressed. The review covers the processes of deposition and uptake of atmospheric organic compounds (VOCs and SVOCs), and transportation of organics from roots, largely based on information from studies of herbicide behaviour. There is hardly any reference to the actual or potential effects of VOCs as defined in section 1.3, other than citation of the studies of van Haut and Prinz (van Haut and Prinz, 1979), described below.

3.3 **Direct comparison with other air pollutants**

Van Haut and Prinz (van Haut and Prinz, 1979) examined the phytotoxicity of 11 VOCs relative to sulphur dioxide, in controlled laboratory exposures of several plant species over 14 days to a range of concentrations. Their philosophy, of relating growth reductions in response to a VOC by comparison with exposure to known concentrations of a more studied pollutant (SO₂) over a short time (14 days), is an interesting approach, but begs the question as to whether the modes of uptake and effects within the plant are similar. However, it is a useful starting point for identifying potential variations in sensitivity in this type of screening experiment. They took as their effects threshold a reduction in plant dry weight of 20% - a relatively high threshold for a short-term experiment. The range of SO₂ concentrations used was from 500 to 3000 µg m⁻³, very much higher than would be observed in ambient air. Their results are summarised in Table 2. What is of greatest interest, in the light of longer term effects at lower concentrations, more relevant to polluted air, was the consistent increase in the effects of the VOCs relative to SO₂ as concentrations decreased. The authors suggested that a simple extrapolation to annual exposures would mean that exposure of plants to ethylene at an average concentration of 10 µg m⁻³ or less would have the same effect as an annual mean concentration of 80 µg m⁻³ SO₂, which is 4 times the UNECE Critical Level for SO₂ exposure for natural and semi-natural vegetation (Ashmore and Wilson, 1994). They also noted that visible effects such as epinasty (leaf droop) may precede effects on growth, and/or occur at even lower concentrations. In the same way, they predicted a similar degree of growth reduction from exposure to an annual mean concentration of 20 µg m⁻³ formaldehyde, but a smaller effect than caused by SO₂ for the other compounds studied.

This attempt to relate effects of VOCs to the effects of a more widely studied pollutant is an interesting approach to the problem, but does not address the more important issue of long-term exposures to low concentrations.

Table 2. Effects on plant dry weight of selected VOCs on 6 plant species relative to effects of sulphur dioxide in a 14-day experiment under laboratory conditions (van Haut and Prinz, 1979).

VOC	Max conc. With no effect (mg m^{-3})	Conc. of SO_2 ($\mu\text{g m}^{-3}$) to produce same effect as $1000 \mu\text{g m}^{-3}$ VOC	Conc. of SO_2 ($\mu\text{g m}^{-3}$) to produce same effect as $500 \mu\text{g m}^{-3}$ VOC	Conc. of SO_2 ($\mu\text{g m}^{-3}$) to produce same effect as $100 \mu\text{g m}^{-3}$ VOC	Predicted annual mean VOC conc. with same effect as $80 \mu\text{g m}^{-3}$ SO_2
dichloromethane	100				
toluene	60				
trichloroethylene	100				
acetone	60				
xylene	160				
ethylene		1100	1050	810	< 10
formaldehyde		1600	1000	330	20
acetic acid					100
dimethylformamide					500-2000
methanol					400-2500
propylene					3600-15000

3.4 Effects of individual VOCs

Experiments with VOCs from as early as the 19th century indicated that direct short-term effects of ambient VOC concentrations ('acute' exposure) were unlikely, given the rather large concentrations needed to induce measurable changes in plant leaves over time scales of a few hours. However, there have been few experiments that extend for longer than a day, or that use VOC concentrations typical of polluted air. Given the difference in plant responses to 'acute' and 'chronic' exposure to inorganic pollutants such as SO_2 (Garsed and Rutter, 1982), it is not safe to conclude that long-term exposure to low concentrations of VOCs will have no effect, even though few 'acute' effects have been observed. The following summarises the known effects of individual VOCs, or groups of VOCs, with particular emphasis on long-term (weeks rather than days or hours) exposure to concentrations that are within a factor of 10 times measured ambient concentrations.

3.4.1 Hydrocarbons

Some of the earliest experiments on the effects of VOCs on plants were done almost a century ago in Germany (Heller, 1904). Saturated air concentrations of solvent vapours (petroleum ether, 'petroleum', benzene, xylene) caused death of mustard (*Sinapis*) and cabbage (*Brassica*) seedlings in times from a few hours (benzene) to several days (petroleum). However, these results probably reflect the relative volatility of the different hydrocarbons used, rather than any inherent difference in toxicity. The consensus nowadays is that alkanes and monoaromatic compounds (benzene, toluene, xylene etc.) have no direct effects on plants at ambient concentrations, even in grossly polluted air. The one exception relates to experiments testing directly the effects of motor vehicle exhaust emissions on plants. Although

several papers have reported the damaging effects of vehicle exhausts on plants, either through controlled experiments (Kammerbauer, et al., 1987; Viskari, 2000; Viskari, et al., 2000a; Viskari, et al., 2000b; Ziegler-Jöns, et al., 1990) or by exposure of plants beside motorways (Sauter, et al., 1987), the effects cannot be attributed simply to VOC emissions. The major effective pollutant appears to be nitrogen oxides rather than VOCs (Kammerbauer, et al., 1987), despite some simple experiments which apparently showed similar degradation of leaf surface waxes after exposure to aromatic hydrocarbon 'fumes' to that observed after roadside exposure of young conifer trees for up to 13 weeks (Sauter and Pambor, 1989).

The assumption in many studies is that these VOCs are biologically inactive, so that uptake from the atmosphere can be regarded as a simple physico-chemical process, with an equilibration between concentrations in leaves and concentrations in air (Hiatt, 1999; Keymeulen, et al., 1993; Keymeulen, et al., 1995). In a 3-month laboratory exposure of horticultural crops to benzene at a concentration of 1 mg m^{-3} no visible effects were noted, although the experiment was designed to study uptake of benzene rather than effects (Collins, et al., 2000). The uptake of benzene was shown to be variable with time, and between species, and did not correspond to a simple air-plant equilibrium. In an open-top chamber experiment in which grass (*Lolium perenne*) was exposed to several VOCs at concentrations ranging upwards from 10 ppb, concentrations of benzene and toluene measured in the leaves were lower than expected on the basis of simple partitioning between air and leaf, implying the involvement of active removal processes (transport to roots, or metabolism) within the plant (Binnie, et al., 2001). Similarly, experiments investigating the fate of benzene in contaminated soil have failed to identify significant concentrations of benzene in plant tissues, even at high doses (Ferro, et al., 1997). These results all suggest that the active metabolism of benzene and toluene, which has been shown to occur at high concentrations (see below, section 4), also occurs at ambient concentrations, and reduces concentrations in leaves below the equilibrium concentrations expected for exposure to a given air concentration. The implications of this will be discussed later.

Unsaturated hydrocarbons such as ethylene and isoprene are actively produced by many plants, and ethane can be released in response to severe stress leading to cell death (Kimmerer and Kozlowski, 1982). Ethylene is a plant hormone, and is also emitted as a non-specific response to stress. Its relationship to pollutant exposure has been reviewed recently (Lucas and Wolfenden, 1996), in particular its role in affecting the response of some plants to ozone. As a plant hormone, it might be expected that even low concentrations of man-made ethylene in air would have measurable effects on plants, and this appears to be the case. Laboratory experiments using a wide range of ethylene concentrations showed a large stimulation of oat and rice mesocotyl growth at 100 ppb (the lowest concentration tested) over a few days (Suge, 1971). Later experiments showed significant effects on pea epicotyl elongation at concentrations of 20 ppb or less (Goeschl and Kays, 1975), and enhanced rolling of flower rib segments from Morning Glory (*Ipomoea tricolor*) after a few hours exposure to 10 ppb (Hanson and Kende, 1975). More recent studies relating to fruit and vegetable ripening have been reviewed by Saltveit (Saltveit, 1999), who noted that internal ethylene concentrations in plants can exceed 100 ppm in ripening fruit. Concentrations as low as 30 ppb cause softening of kiwifruit, although concentrations in the range 1-1000 ppm are more usually used to control fruit ripening. Interferences of other hydrocarbons with the role of ethylene have also been reported; exposure of

bananas to cyclopropene or 1-methylcyclopropene (0.5 ppb) for 6 h made them insensitive to the ripening effects of ethylene for 12 days (Sisler, et al., 1996).

There is also direct evidence of the effects of ethylene on plants in the field, close to a polyethylene factory. This is admittedly a rather extreme example, with average ethylene concentrations over 8 years measured at $78 \mu\text{g m}^{-3}$ (65 ppb) at midnight and $38 \mu\text{g m}^{-3}$ (32 ppb) at midday. Epinasty (leaf droop) in potato plants was observed for 5% of the growing season (on average), at a mean ethylene concentration around $50 \mu\text{g m}^{-3}$ (42 ppb), with a greater response in younger plants. The response also depended on temperature and vapour pressure deficit (Tonneijck, et al., 1999). This short-term response was reversible, and appeared to have no effect on tuber yield (Tonneijck, et al., 2000).

Unsaturated hydrocarbons react rapidly with ozone to produce more reactive, and possibly more toxic, intermediates. The reaction of ethylene with ozone inside the stomatal cavity has been postulated as a mechanism for increased damage by ozone in ethylene-producing leaves (Mehlhorn, et al., 1991). Products of the reaction of ozone with isoprene and other biogenic hydrocarbons (organic hydroperoxides) have also been postulated as potentially phytotoxic compounds (Möller, 1988; Salter and Hewitt, 1992). Other unsaturated hydrocarbons have been shown to have subtle effects on plant biochemistry; propylene, although shown to be 1000 times less toxic than ethylene in short-term exposures at very large concentrations, caused measurable effects on the leaf biochemistry of cherry trees in a long-term controlled exposure, particularly when applied early in the growing season (Terry, et al., 1995b). Exposure of several plant species to isoprene concentrations of 50 – 150 ppb accelerated the onset of flowering (Terry, et al., 1995a).

3.4.2 Chlorinated hydrocarbons

3.4.2.1 'Forest decline' and Frank's hypothesis

The potential role of volatile chlorinated hydrocarbons in 'forest decline' was postulated by Frank in the mid 1980s (Frank and Frank, 1985; Frank and Frank, 1986). The hypothesis was that chlorinated hydrocarbons were rapidly absorbed by leaves, and were then decomposed by the UV component of sunlight to produce free radicals that attacked plant pigments. Initial experiments demonstrated the possibility of such reactions occurring even at relatively low concentrations of solvent vapour (2 ppbV), and the relative effectiveness of different chlorinated hydrocarbons was demonstrated in laboratory experiments with β -carotene as a 'model' pigment molecule (Grimmer and Schmidt, 1986). Subsequent research showed that substances such as tetrachloroethylene were absorbed by leaves from the atmosphere; of greatest interest was the observation that the partitioning of this substance between the atmosphere and Norway spruce needles appeared to increase at lower concentrations, i.e. the bioconcentration factor (ratio of concentration in leaf to concentration in air) was not constant, but varied with air concentrations (Frank and Frank, 1989). This result meant that laboratory studies of the uptake of chlorinated VOCs (and possibly other VOCs) by leaves in conditions where the air concentrations used were very large relative to those in ambient air could not be simply used to predict the partitioning of such substances into leaves under field conditions. These conclusions were confirmed by later work with Scots pine (Brown, et al., 1998; Brown, et al., 1999).

3.4.2.2 Effects of exposure to high concentrations over short times

The potential for uptake of chlorinated VOCs to cause deleterious effects on plants was confirmed by several short-term laboratory experiments, using large air concentrations. Metabolic processes of spruce needles were rapidly affected by 25 ppb trichloroethylene (Schröder, et al., 1991) and 25 ppb tetrachloroethylene (Schröder and Weiss, 1992). Longer term experiments (4 weeks) exposing spruce shoots to much larger concentrations of a range of VOCs including trichloroethane (11-15 mg m⁻³ = 2-3 ppm) showed large effects on shoot growth (Kristen, et al., 1992). These, and other experiments from the late 1980s and early 1990s, were summarised in Smidt's review (Smidt, 1994), in which he concluded that the air concentrations of chlorinated hydrocarbons in forest areas were unlikely to reach phytotoxic levels.

3.4.2.3 The role of trichloroacetic acid

Although these experimental approaches to determining the toxicity of chlorinated hydrocarbons were all relatively short-lived, and used larger concentrations than observed in the field, it was noted that exposure to chlorinated hydrocarbons led to an accumulation of trichloroacetic acid (TCAA) inside leaves. TCAA was widely used as a herbicide in the 1950s and 1960s, and its accumulation in leaves was consequently identified as a likely causal factor for plant damage (Frank, et al., 1993). It was not clear, however, whether the TCAA in the plant came indirectly from the uptake of chlorinated hydrocarbons and their subsequent oxidation in the leaf, or whether TCAA was taken up directly by leaves from rain, or by roots from the soil. The role of chlorinated solvents in the production and accumulation of TCAA in the environment is a topic of some debate in the literature (Franklin, 1994; Hoekstra and Juuti, 1999; Jordan and Frank, 1999; Juuti and Hoekstra, 1998), with the discovery that it and related compounds can be formed directly in soil by microbial activity (Haiber, et al., 1996; Haselmann, et al., 2000; Hoekstra and de Leer, 1993).

3.4.2.4 Long-term open-top chamber experiments

The potential of volatile chlorinated hydrocarbons to damage plants at relatively low concentrations over a long exposure period, especially in the presence of sunlight, was not studied systematically until relatively recently. In response to concern over the suggested role of chlorinated hydrocarbons in 'forest decline', and tetrachloroethylene in particular, the European Chlorinated Solvents Association commissioned a long-term study of the direct effects of fumigation of a range of different plant species, with a view to establishing a dose-response relationship and a 'no effect' concentration for realistic environmental exposures. The experiments were conducted by Plant Research International at Wageningen in the Netherlands, and used 3 crop species, 3 tree species, 3 'wild' herbaceous species and 3 mosses, exposing them in open-top chambers for up to 6 months to 6 different concentrations of C₂Cl₄ between 10 (control) and 2000 µg m⁻³ (1-300 ppb). The most sensitive indicator tested in terms of the measured responses was the pod weight of bean (*Phaseolus vulgaris*), for which the predicted no-effect concentration (NOEC) was 46 µg m⁻³ (7 ppb) over 7 weeks in June and July. A repeat of the experiment in July and August showed no effect even at the largest concentration tested (> 2 mg m⁻³). The authors concluded that time of year and age of plant determined plant sensitivity (young conifer needles were affected but not older ones). No apparent difference was observed between shade and sun needles, in contradiction of earlier reports (Frank and Frank, 1985). The different results at different times of year were attributed to higher temperatures

and lower UV light levels later in the summer, with the possibility that damage was increased under conditions where photoinhibition occurred (low temperature, high light levels) earlier in the year. Formation and accumulation of trichloroacetic acid was also observed in response to the fumigation with tetrachloroethylene (Franzaring, et al., 2000). However, even the lowest concentrations used in the experiment exceeded the concentrations likely to be found in polluted urban air, so it appears that direct effects of tetrachloroethylene on plants are unlikely to occur except close to major industrial sources, or as a result of a chemical accident. The longer-term accumulation of TCAA may still be a problem, but the uncertainties relative to the role of the natural production of TCAA in some soils are still very large.

3.4.2.5 Chlorinated hydrocarbons in contaminated soils

In experiments to investigate the role of plants in decontaminating soil, the transport of chlorinated hydrocarbons through plants from roots to leaves, and then into the atmosphere, has been observed. In one experiment with alfalfa plants grown on soil fed with water contaminated with either 1,1,1-trichloroethane or trichloroethylene, the headspace above the plants accumulated solvent concentrations at rates between 0.5 and 2 ppm h⁻¹, but the authors reported no effects on the plants (Narayanan, et al., 1995). In a similar experiment with both poplar trees and axenic cell culture, it was shown that poplar cells were capable of transforming trichloroethylene *in vitro*, and that there was a 15% growth reduction over 8 months in trees growing in soil treated with trichloroethylene. Trichloroethylene transpired by the leaves gave air concentrations around the leaves of up to 300 µg m⁻³ (55 ppb), so internal leaf concentrations were probably higher (Newman, et al., 1997). These results suggest that (for these species at least) chlorinated hydrocarbons are not directly phytotoxic even at very large concentrations relative to polluted air, and that metabolism of the chlorinated solvent to chlorinated alcohols and acids leads eventually to its total breakdown to carbon dioxide.

3.5 Oxygenated hydrocarbons – alcohols, aldehydes, ketones

3.5.1 Biogenic emissions and plant metabolism

Short-chain alcohols, aldehydes and ketones have been identified as major biogenic VOCs, with methanol contributing significantly to total biogenic VOC emissions (Baker, et al., 2001; Guenther, et al., 1996; Macdonald and Fall, 1993; Schade and Goldstein, 2001). The available evidence points to methanol being beneficial to plants, at least under some conditions, when it can be metabolised as a source of carbon (McGiffen and Manthey, 1996; Nonomura and Benson, 1992).

Concern was raised over the potential damaging effects of formaldehyde on plants arising from the increasing use of methanol and methyl t-butyl ether as vehicle fuel additives (Barker, et al., 1996). The conclusion was that some scenarios of future fuel use in the United States could increase air concentrations of formaldehyde to 90 ppb, a concentration that would be phytotoxic to some species, on the basis of rather limited experimental data. Formaldehyde is formed naturally in plant tissue (Albert, et al., 1998; Gullner and Tyihak, 1991; Sardi and Tyihak, 1998; Tyihak, et al., 1989), and therefore biochemical pathways exist to regulate its concentration inside plants, as demonstrated by experiments using cell cultures of several plant species (Giese, et al., 1994).

The tolerance of many tree species to short-term exposures (8 h) to up to 2 ppm formaldehyde, and rapid metabolism within the leaves, has been the basis of a

suggestion that trees could be planted to reduce formaldehyde concentrations in urban areas (Kondo, et al., 1996). A similar suggestion was made for higher aldehydes, although deciduous trees were shown to be more sensitive than evergreens in short-term experiments (Kondo, et al., 1998). No significant effects of formaldehyde on Douglas fir needles were seen in response to treatment with mist containing concentrations up to 1 mM (Muir and Shirazi, 1996). However, lower concentrations of formaldehyde in solution (0.3 mM) were shown to affect Douglas fir pollen *in vitro* (Shirazi and Muir, 1998), and exposure to gaseous formaldehyde has been observed to affect the growth of bean plants (Mutters, et al., 1993).

3.5.2 Plants as 'air purifiers' indoors

Additional evidence for active metabolism of formaldehyde comes from investigations into the use of plants to control indoor air pollution. Although uptake and metabolism of many compounds (benzene, toluene, chlorinated hydrocarbons, formaldehyde) has been demonstrated (Cornejo, et al., 1999; Dingle, et al., 2000; Godish and Guindon, 1989; Wood, et al., 2000), the rates of absorption and metabolism are claimed to be too small to be practicable for use as indoor 'air purifiers' (Schmitz, et al., 2000). These experiments have not shown obvious detrimental effects of the VOCs studied on the prospective 'air purifying' plants, even at concentrations of the order of 1 ppm.

3.5.3 Long-term exposure to VOC mixtures at realistic concentrations

In an experiment similar to that described for tetrachloroethylene above (section 3.4.2.4) 6 different wild plant species were exposed to a mixture of VOCs in a replicated open-top chamber experiment during summer 2001 (Cape, et al., 2001). The VOC mixture was dominated by acetone, with smaller quantities of ethanol, methyl t-butyl ether, acetonitrile, dichloromethane and toluene. The continuous exposure represented a 'worst-case' scenario for solvent emissions from a factory. Very few statistically significant effects of the exposure were observed during or at the end of the experiment. The clearest effect was seen in an acceleration of the formation of seed pods on birdsfoot trefoil (*Lotus corniculatus*), where seed pods were present in all 3 treated open-top chambers, but only one of 30 plants in the 3 control chambers had formed seed pods at the end of the experiment after 7 weeks exposure.

3.6 Peroxyacyl nitrates (PAN and higher analogues)

The direct effects of PAN on plants were first noted in the 1960s and 1970s in the Los Angeles basin during smog episodes. Several literature reviews of the effects have been published (Cape, 1997; Kleindienst, 1994; Smidt, 1994; Temple and Taylor, 1983). After a period of intense experimental activity in the 1970s (Thompson and Kats, 1975) there were few experiments on the effects of PAN on plants until the 1990s, when interest was stimulated in the Far East in response to visible injury to plants in major urban areas (Sun and Huang, 1995). Subsequent experimental assessments of the damage caused by PAN have been short-term and used large concentrations relative to those measured even in polluted air. There have been no recent long-term experiments to investigate the possibility of chronic effects of PAN on vegetation in the absence of visible injury. There is a wide range of sensitivity among different plant species to acute exposure to PAN, and susceptibility does not appear to be related to uptake rates (primarily through stomata), suggesting that it is

the metabolic detoxification mechanism rather than leaf uptake that determines sensitivity (Okano, et al., 1990). PAN usually occurs during photochemical 'episodes' when ozone concentrations are also high, and the simultaneous presence of both pollutants appears to be less damaging than PAN on its own (Cape, 1997).

It appears unlikely, on the basis of the published evidence, that PAN concentrations in the UK pose a risk to vegetation. The World Health Organization suggested an air quality guideline to protect vegetation of 15 ppb over 8 h (WHO, 1987). This would rarely, if ever, be exceeded in the UK on the basis of existing PAN measurements (Cape and McFadyen, 2001). However, there is still too little information on the spatial and temporal variation in PAN concentrations in the UK to be certain that no damage would occur during a long dry summer when elevated PAN concentrations (several ppb) could arise over a long period.

Although the phytotoxicity of the higher peroxyacyl nitrates (e.g. peroxypropionyl nitrate, PPN) is greater than that of PAN (Smidt, 1994), concentrations are very much smaller than those of PAN, rarely exceeding 1 ppb even in areas susceptible to photochemical smog (Grosjean, et al., 2001).

4. METABOLISM AND FATE OF VOCs IN PLANT LEAVES

4.1 Evidence for metabolism within plants

The metabolism of VOCs inside plants, whether from leaf or root uptake, has already been mentioned above (sections 3.4.2.5, 3.5.2), and has been observed or inferred in a large number of studies. Metabolism of VOCs such as benzene and toluene by plants has been known for many years (Jansen and Olson, 1969). The use of radiolabelled VOCs allows the labelled atoms incorporated into the plant tissue, or evolved as $^{14}\text{CO}_2$, to be followed (Ugrekheldze, et al., 1997). Such studies have shown that stomatal uptake is an important pathway, and that absorbed VOCs (or their metabolic products) can be translocated from leaf to root (Jen, et al., 1995). These experiments, while conducted at concentrations more than 1000 times greater than observed in urban air, demonstrate the possibility of active metabolism, but this has been assumed to be negligible in many partitioning or uptake studies. There can be a wide variation in behaviour with different plant species and different VOCs (Omasa, et al., 2000). Our own recent studies (Binnie, et al., 2001) have shown that benzene and toluene are actively removed within grass, at rates approaching the uptake rate from air, even at relatively low concentrations (tens of ppb), although removal of chlorinated hydrocarbons occurs more slowly, if at all. This observation may explain why benzene is rarely observed to accumulate in plant leaves (Collins *et al.*, 1998; 2000), unlike some of the chlorinated hydrocarbons.

4.2 Fate of metabolised VOCs

The use of radiolabelled VOCs has established that the first products of metabolism of monoaromatic VOCs (benzene, toluene) are phenols, with further oxidation leading to breaking of the aromatic ring to give unsaturated dicarboxylic acids (Durmishidze, et al., 1974a; Durmishidze, et al., 1974b). The eventual breakdown product is CO_2 . The mechanisms of breakdown of xenobiotics (substances foreign to normal plant metabolism) have been studied in great detail, and parallels have been drawn with detoxification mechanisms in animals. Sandermann introduced the idea of the 'green liver' to emphasise the parallel processes in plants and animals (Sandermann, 1994). As early as 1940, and without the help of radiolabels, the metabolism of relatively simple compounds such as ethylene chlorohydrin (1-chloro, 2-hydroxy ethane) into β -D-gentiobiosides was demonstrated (Miller, 1940). The fate of many xenobiotics can be described in terms of transformation (e.g. oxidation, reduction, hydrolysis), conjugation, and storage in vacuoles or cell walls of the conjugated or transformed products.

Most of the detailed work has been done on herbicides and SVOCs that accumulate in plant tissue, such as PAHs, and several of the plant enzymes involved have been identified. These include cytochromes P450, glutathione S-transferases, carboxylesterases, O-glucosyltransferases, O-malonyltransferases, N-glucosyl transferases and N-malonyltransferases (Sandermann, 1994). There has been some debate over the relative importance of cytochrome P450 and peroxidases in the primary step of metabolising absorbed xenobiotics (Zaalishvili, et al., 2000). The induction of plant cytochrome P450 has been shown to occur in response to different stresses (wounding, pathogens), as well as to chemicals (Durst, et al., 1997).

The fate of xenobiotics in plants has recently been extensively reviewed (Korte, et al., 2000; Zaalishvili, et al., 2000), and the processes of conjugate

formation, oxidative degradation and enzymatic reactions summarised. Xenobiotics may also be transported without reaction from leaves to roots and excreted. The first stage in the metabolism of alcohols and phenols is often glycosylation. This may also be the fate of the products of initial oxidation of an alkane or alkyl group, where a hydroxyl group is formed. However, phenol itself appears to be conjugated with low molecular weight peptides in intact plants.

These detoxification processes appear to be generalised, and capable of degradation of small doses of xenobiotics without any obvious detrimental effect on the plant (Korte, et al., 2000). The enzyme activities may well be induced by exposure to xenobiotics, and increase the detoxification potential of the plant.

4.3 Implications of plant metabolism of VOCs

The metabolites of benzene and toluene (phenols) are more toxic than the parent compounds, so active metabolism has implications not only for the plant itself, but potentially also for any animal that feeds on the plant. Whereas the concentrations of VOCs in leaves may be in dynamic equilibrium with air concentrations, and never reach toxic concentrations, metabolic products are likely to be much less volatile, and may accumulate in leaves. There appear to be no studies of the consequences of exposure to VOCs for the subsequent ingestion of plant material by herbivores, although studies of exposure pathways and accumulation of SVOCs have been conducted (Jones and de Voegt, 1999). In the case of SVOCs the extent of accumulation in vegetation of the original compound is much greater than for VOCs, given their much smaller volatility, and the rate of metabolism of these larger molecules is likely to be much slower than for VOCs. There have also been no studies on the effects of VOC exposure, and any consequent metabolic products, on feeding or performance of phytophagous (plant-eating) insects.

4.4 Genotoxic effects of VOCs and potential for biological monitoring

Recent studies of DNA from plants exposed along roadside verges showed that DNA adducts were formed, indicative of genotoxicity (Weber-Lotfi, et al., 2000). Recent correspondence with Professor Dietrich, however, suggests that these adducts are not specific to VOC exposure, and therefore would have only limited application for biomonitoring.

Bioassays based on genetic mutations in *Tradescantia* plants have been used to indicate exposure to pollutants. Two bioassays have been developed, based on *Tradescantia*-Micronucleus (MCN) and *Tradescantia*-Stamen-Hair-Mutation (SHM) (Grant, 1999; Ma, et al., 1996; Rodrigues, et al., 1997). These tests have been used with exposure to single VOCs, with the MCN bioassay being the more sensitive. While such tests have been developed as bioindicators of air pollution, and have been deployed widely (Guimaraes, et al., 2000; Ma, 1999), it is clear that these bioassays are not specific to VOCs, but respond to a wide range of other pollutants including ozone (Rodrigues, et al., 1996) and heavy metals (Evseeva and Zainullin, 2000; Steinkellner, et al., 1998).

5. SUMMARY OF THE LITERATURE REVIEW

- Volatile organic compounds are ubiquitous in both rural and urban air, with both biogenic and anthropogenic sources. The main concern over VOC emissions and atmospheric concentrations has been their role as precursors of photochemical ozone, which is known to be toxic to plants at concentrations that are currently experienced over most of the United Kingdom (NEGTAP, 2001).
- Laboratory experiments to study the potential *direct* effects of VOCs on plants have used very high concentrations (relative to ambient air) and short exposure periods. The involvement of UV light, as a potential contributory factor to VOC toxicity, has not always been appreciated in laboratory experiments. In short-term experiments the concentrations of VOCs required to elicit any effect have been substantially greater than those observed outdoors, even in polluted air.
- There have been few field, or near-field, experiments over long time periods (weeks to months) at concentrations relevant to atmospheric exposure. Subtle, but pollutant-specific, effects of ethylene (a plant hormone) have been recorded in the field downwind of a major industrial source.
- A very wide range of tolerance to VOCs among plant species has been demonstrated. Bean (*Phaseolus vulgaris*) plants have been shown to be among the more sensitive, with decreases in the harvested pod weight. Other studies have also identified effects on flowering and seed formation, including mutagenic effects, in the absence of visible damage or effects on growth.
- Plants can metabolise many VOCs or transport them from leaves to roots. The metabolites may be further degraded, or may be conjugated and accumulate within the leaf, in vacuoles or cell walls. It is possible that these breakdown products are toxic to herbivores or phytophagous insects.
- While it appears unlikely that VOCs pose a direct threat to plant health in the UK, there is no information on their effects in conjunction with other gaseous pollutants or environmental stresses such as drought, nutrition or temperature extremes. There have been no long-term studies of the potential effects on genetic diversity, competition in natural ecosystems, or interactions with phytophagous insects and herbivores.

6. POTENTIAL STRATEGIES FOR FURTHER RESEARCH ON VOCs RELEVANT TO PLANT HEALTH IN THE UK

6.1 Defining plant species at risk

This review has indicated that short-term effects of VOCs on plants are unlikely, and long-term effects are also unlikely except where vegetation is exposed for long periods to elevated concentrations. Consequently, it is likely that most agricultural crop plants are not at risk. However, there may be implications for horticulture, given the proximity of much horticultural activity to urban and industrial areas, and the possibility of the accumulation of VOCs in enclosed growing areas such as greenhouses. Moreover, flowering has been shown to be one of the more sensitive stages of plant growth, so production of flowering plants may be the commercial area at greatest risk. However, the horticulture industry already manipulates flower and fruit development through the use of chemicals, both applied directly, and including VOCs such as ethylene. Although the risk to commercial growers may be greatest, potential effects on garden plants should also be considered, given the financial value of this sector of the economy. The apparent effects of several VOCs on plant reproduction may also have long-term implications for native species and biodiversity along roadsides or close to major industrial installations that emit VOCs.

Although this review has concentrated on the direct effects of VOCs on plants, the indirect effects of VOC metabolites, which may accumulate in plants, on herbivores and phytophagous insects are not known. Although more difficult to quantify, such effects operate at an ecosystem level involving bioconcentration up the food chain, or effects on pollinating insects, and may have a more profound influence over long time scales than occasional visible injury or modification of growth patterns of a few plant species.

6.2 Methods to assess the effects of VOCs on plants

There is a long history of experimental approaches to studying the effects of air pollution on plants, ranging from small-scale laboratory experiments over short times to large-scale open-air field experiments over several years. Although short-scale laboratory experiments have some value in identifying sensitive species and/or growth stages of plants, the very large VOC concentrations typically used, and the artificial growing regimes detract from accurate extrapolation to field conditions.

6.2.1 *Open-top chambers*

Longer studies in near-field conditions can be supplied by the use of outdoor chambers (e.g. open-top chambers), where light and temperature regimes are similar to outdoor conditions, although air turbulence is constant, and water status may be maintained artificially. Several chambers must be used to replicate the experimental treatments, making such experiments costly both in terms of equipment and manpower. The simplest experimental design, used to evaluate the effects of a polluted environment, is to situate the experiment in a polluted region, and provide half of the chambers with charcoal-filtered air. This approach has been used in the UK, predominantly to test for the effects of sulphur and nitrogen oxides (Fowler, et al., 1988) or ozone (Taylor and Dobson, 1989). However, this approach cannot be used for testing exposure to VOCs, which are minor components of the mixture of

pollutant trace gases that may influence plant growth and development. Any filtration process that removed VOCs would also remove gases, such as ozone, that are known to affect plants.

Open-top chambers have mostly been used to supply known concentrations of a pollutant gas, in order to determine dose-response relationships. This was the basis for the major NCLAN (National Crop Loss Assessment Network) study of ozone effects on crop plants in the United States in the 1980s (Lefohn and Foley, 1992), and has also been the technique used to estimate exposure thresholds for ozone in Europe (Fuhrer, 1996). The technique has also been used to study the effects of tetrachloroethylene (Franzaring, et al., 2000), and a mixture of VOCs (Cape, et al., 2001), on plants over a period of weeks to months.

While such experiments exercise a degree of control over the growing conditions, they do not perfectly replicate field conditions. They may be used as a framework for establishing a reproducible method for testing different VOCs against different plant types, in order to estimate 'no effect' concentrations, but do not integrate the exposure with environmental stresses such as drought.

6.2.2 Field fumigation

Field-scale fumigation has also been used as an experimental technique for evaluating the effects of gaseous pollutants on plants. The scale of the experiments is somewhat greater even than for open-top chambers, where the volume of treated air is determined by the number of chambers and the flow rate through them. In field fumigation systems, the amount of pollutant required depends on the wind speed across the site and the desired fumigation level. In the UK the largest field fumigation experiment was established at Liphook, Hampshire, to investigate the combined effects of sulphur dioxide and ozone on young trees over several years (McLeod, 1995; McLeod, et al., 1992). In principle, field fumigation with VOCs would be easier than using ozone, in that the active agent is a liquid, rather than having to be produced on site, as in the case of ozone.

The Liphook experiment used several exposure rings, each of diameter 50 m, with computer control of the gas release depending on wind speed and direction. Alternative, simpler, release systems are possible, which generate horizontal gradients in air concentration downwind of a release point or line, and which only release gas when the wind is coming from a given sector (usually the prevailing wind direction). Such systems have been used for experiments with ammonia gas (Theobald, et al., 2001).

One of the advantages of such an approach is the exposure of naturally growing plants to a spatial gradient of air concentrations that may be similar to natural exposure from point or line source emissions, yet is under the control of the experimenter. In principle, dose-effect relationships can be derived if air concentrations are measured across the fumigated area. The large scale of the experiment may also permit additional studies, such as insect interactions, to be conducted as well as direct experiments on the plants. The requirement for electrical power and complex infrastructure is also greatly reduced in comparison with open-top chamber systems, so that experiments can be sited away from urban areas, where there is an increased risk of vandalism. One of the features of field fumigation systems is that they should be deployed in 'background' regions where ambient concentrations of the pollutant of interest are as low as possible.

6.3 Biomarkers of VOC exposure

This review has highlighted the characteristics of plants that may be most sensitive to VOC exposure, namely the timing or extent of flowering and fruiting. The tests applied to experimental plants in long-term experiments with VOCs may therefore have to be rather different from those applied in studies of inorganic pollutants, which have usually relied on measuring changes in growth, yield, or nutritional status. Although VOCs themselves do not appear to accumulate in plant tissues, metabolic breakdown products may be retained in the plant, and may be useful as indicators of potential effects. Analysis for such products may also be useful in assessing secondary effects on herbivores. Measurement of VOC metabolites may also be a more specific diagnostic test than measurements of generalised stress reactions, whether expressed as DNA adducts or genetic bioassays. A recent review of the potential use of biomonitors in assessing the effects of VOCs on plants (Brown and Rumford, 1999) concluded that:

“at present there are no standard techniques for measuring the effects of point source emissions on the terrestrial environment. However, there are a number of techniques that have the potential to be utilised. These include: plant community surveys; active biomonitors and mimics; remote sensing; modelling.....their specific application to the biomonitoring of VOCs and/or inorganic air pollutants at individual manufacturing sites needs to be explored by field trials.”

6.4 Conclusions

- The greatest risk from exposure to VOCs in the UK is likely to be to horticultural crops grown in or close to urban areas, and to garden plants in cities. The most sensitive stage of plant growth appears to be flowering and seed/fruit production.
- The greatest risk to native plant species and agricultural crop plants is from exposure close to industrial sources or major roads.
- Adverse effects at the ecosystem level are more likely to be caused by the accumulation of VOC metabolites in plants, and consequent effects on the food chain, than by direct effects on plant growth.
- There have been no relevant experimental studies of the long-term effects of VOCs on plants that include effects on flowering, seed production and viability, and the accumulation of metabolites.
- Open-top chambers may be used to determine dose-effect relationships by delivering known concentrations of individual VOCs or mixtures, but adequate replication is expensive.
- Field fumigation experiments are easier to control for VOCs (normally liquids at ambient temperatures) than for inorganic pollutant gases such as ozone, and can be conducted on native vegetation and crops with minimal disturbance to the normal growing environment. Monitoring of the applied VOC concentrations across a gradient of exposure may permit dose-response relationships to be estimated.
- Several promising biomarkers of VOC exposure have been suggested, but to date no VOC-specific biomarker/biomonitor has been identified.

7. REFERENCES

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