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Plant growth regulating properties of sulphonamides and some aspects of phenoxyacetate herbicide photochemistry.

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I would like to dedicate this thesis to J.B. and Chris.

CONTENTS

SUMMARY

The work contained in this thesis is primarily an investigation of the biological and chemical properties of a variety of herbicides and related compounds. Two classes of compounds were studied. One included asulam and structurally related sulphonamides. The other was the phenoxyacetate salts and esters. The work can be summarised as follows:

Chapter 2: All of the test sulphonamides were found to reduce the fresh weight of test plant seedlings. Growth inhibition of cress seedlings was overcome by adding p-aminobenzoic acid. Results indicated the competitive inhibition of the enzyme dihyropteroate synthetase. Field experiments involving bracken were inconclusive.

Chapter 3: Test sulphonamides containing aliphatic and five-membered heterocyclic groups were more effectively able to penetrate bean leaves than those containing six membered heterocyclic groups. Low humidity reduced foliar uptake in all cases. Tween 20 was found to improve penetration in most cases. Tween 80 and ammonium thiocyanate (NH_4SCN) had no effect on penetration.

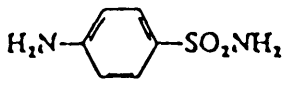
Chapter 4: Asulam and three test sulphonamides were rapidly degraded in both soil types used, particularly over the first few days of the experiments. Asulam was less readily degraded in an aged soil sample (3 months). There was some chromatographic evidence for the generation of sulphanilamide as a metabolite.

Chapter 5: Literature review.

Chapter 6: Irradiation of 4-chlorophenoxyacetate (CPA) and 2-methyl-4-chlorophenoxyacetate (MCPA) with light from a medium pressure mercury arc lamp in the presence of NH_4SCN generated the thiocyanate and isothiocyanate derivaties respectively. Two isomeric phenylacetic acid photoproducts were generated from the irradiation of sodium phenoxyacetate (PPA).

Chapter 7: Using the butylesters of PAA, 2,4-D and MCPA, vitamin C was the most effective photostabiliser in the presence of UV light. Vitamin E, phenothiazine, p-nitrophenol and 8-hydroxyquinoline showed useful activity but only at higher concentrations. Potassium iodide (KI) and NH_4SCN were least effective.

Structures of some of the compounds studied



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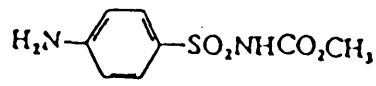

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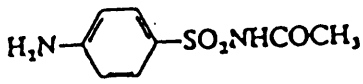

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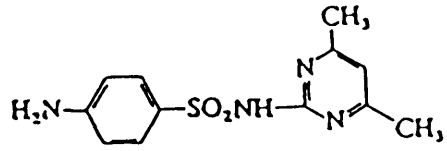

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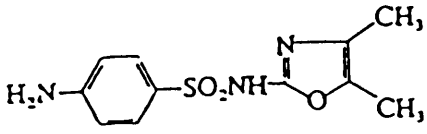

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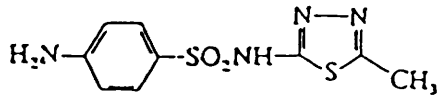

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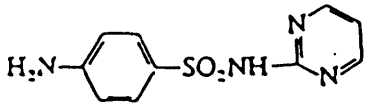

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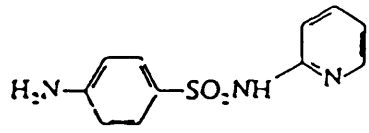

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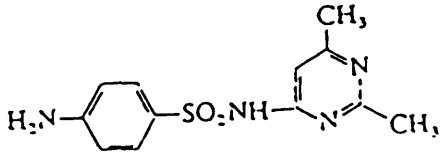

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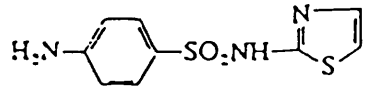

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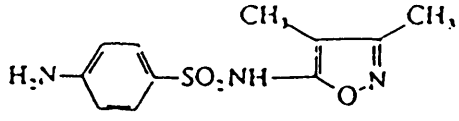

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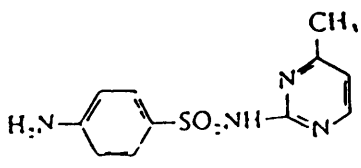

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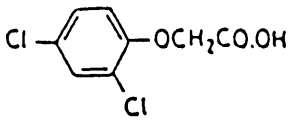

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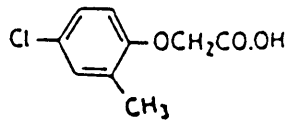

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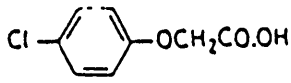

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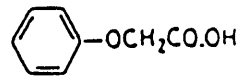


phenoxyacetic acid

CHAPTER 1

INTRODUCTION AND THESIS OBJECTIVES

Initially one of the objectives of this work was to consider in some detail the biological activity of a group of sulphonamide compounds in relation to that of the herbicide asulam. It has only relatively recently been discovered that asulam probably functions as a herbicide through the inhibition of folic acid biosynthesis in plants. On the other hand, it has been known for more than forty years that the sulphonamide drugs, which are structural analogues to asulam, exhibit their bacteriostatic properties by competitively inhibiting the enzyme dihydropteroate synthetase and hence the synthesis of dihydrofolate. Investigating the biological activity of the sulphonamides in plants was aimed at indirectly examining the most likely mode of action of asulam and also the possible application of these compounds as plant growth regulators. There have been a number of studies in the past, most notably Brian (1944), Audus and Quastel (1948), Hotson (1953) and Rudd-Jones (1956) which have observed impairment of plant growth by a variety of sulphonamide drugs. It came as some surprise that there has been no detailed investigation of their potential use as herbicides in light of these observations and in light of the success of asulam in this role.

Stephen (1983) carried out a detailed investigation of the behaviour of some bracken control chemicals in plant soil systems. It was clear from his work that there was scope for the introduction of new herbicides for the control of bracken. As asulam is the only herbicide officially recommended for selective bracken control (for non-selective, non-crop situations only asulam, chlorthiamid, dichlobenil and glyphosate are recommended, Heywood (1982)), one can see that there was good reason to examine the behaviour of chemicals which the literature suggested might function in a similar way.

Bracken has a world wide distribution, being found in humid and sub-humid regions. In Scotland it occupies at least 200,000 hectares of hill land (Taylor, 1980) and is one of the most persistent weeds present in upland pastures in Great Britian. Not only can it cause stock poisoning but it often occupies soils which are potentially useful pasture land (Stephen, 1983). It is an extremely robust plant with considerable reserves in its underground rhizome food storage system (Heywood, 1982). The main spread of bracken in established colonies does in fact occur through this extensive underground rhizome system (Conway, 1959).

Heywood (1982) describes the problems facing both existing and any new bracken control chemicals. Any herbicide which is applied to bracken faces a greater

dilution than is the case with most other weed situations. This arises because the dry weight of the fronds in a strong stand can be up to 14 tonnes per hectare. This apparently corresponds to a dilution factor of a typical herbicide of about 20,000 (Heywood, 1982). Not only this but the underground rhizome system can have a fresh weight of up to 125 tonnes per hectare which represents a further ten times dilution. Heywood (1982) concluded that as a consequence of this, an effective bracken control herbicide must either possess very high activity or must be concentrated selectively at the site of action.

The situation is even more complicated than this however. For more than half the year there is no exposed living part of the plant. In addition, the rhizomes are effectively protected up to 0.75 metres below the ground (Braid, 1957). During the growing season application of systemic herbicides is restricted to only a very short interval. In Scotland, for example, the most effective time for control is during a four week period from about mid-July to mid-August. If herbicides are applied too early then partially unfolded fronds represent a small and unreceptive target whereas if application is carried out too late the leaf cuticle will be hard and glossy, representing an effective barrier for the penetration of most chemicals (Heywood, 1982). Also, at this time it is likely that any chemical which did overcome the cuticle

barrier, would be immobilised in the dying bracken ferns before it could be translocated to the site of action.

It is hardly surprising, then, that in general, bracken control has been erratic. There has been a considerable amount of research, particularly in this Department, into ways of improving bracken control. It seemed to me that the knowledge and experience which was gained from the various studies should be utilised and developed to investigate the possible herbicidal behaviour of test sulphonamides. By including asulam in such an investigation it was possible to relate the results to the practical situation.

From the literature it became apparent that one of the methods employed to try and increase the effectiveness of asulam in the field was to formulate the herbicide with various additives. The purpose behind doing this was to improve the uptake of the chemical into the weed leaves. An enhanced rate of foliar uptake or cuticular penetration means that an applied herbicide is exposed to potentially adverse weather conditions for the minimal amount of time. In the case of asulam, for example, which is formulated as the sodium salt to increase its solubility in aqueous solution, rainfall after application greatly reduces its activity (Stephen, 1983). Through the use of chemical additives such as the surfactant Tween 20 (Babiker 1974a) or the emulsifiable oil Dessipron (Heywood, 1982) it has been possible to

improve the rate of uptake of asulam and hence weed control. Consequently, Chapter 3 was concerned with uptake behaviour of asulam and test sulphonamides using bean plants (Phaseolus vulgaris L.c.v. Canadian wonder) as a model system. By altering the humidity of test conditions and by utilising various additives it was possible to examine the type of environment and formulation which would be conducive to cuticular penetration. There is always a danger involved in extrapolating test conditions to field conditions, however.

The study was then extended to investigate the persistence of asulam and selected test sulphonamides in soil. Some consideration has been given in the past to the pre-emergence application of asulam to the soil. This route of application would overcome many of the problems associated with post-emergence foliar application. Asulam has generally been found to undergo rapid degradation in the soil although there have been some reports of effective weed control after soil application. Using a soil from an area which was infested with bracken it was decided to establish whether asulam was, in fact, as readily degraded as most other in vitro studies had suggested. In addition, it was decided to examine the rate of degradation of selected examples of the sulphonamides. The physical properties of the test sulphonamides were quite different from those of

asulam, therefore there was some reason to believe that their rate of soil degradation might be different. The fact that they all had bacteriostatic properties enhanced this view.

In the past, some of the phenoxyalkanoic herbicides have been employed to control bracken. Stephen (1983) mentioned the use of 4-chlorophenoxyacetic acid (p-CPA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) for this purpose. Common features associated with these chemicals have been inconsistencies in performance at different sites, in different years, and the ability of the weed to regenerate after treatment (Stephen, 1983). Both Stephen (1983) and Jaff (1982) pointed out that the application of p-CPA to bracken had caused scorching of the foliage. This scorching was not unlike that observed when aminotriazole was applied to bracken foliage without the addition of the free radical scavenger, ammonium thiocyanate, to formulations. Photochemical oxidation was thought to account for the observed scorching (Cook, 1979). The obvious possibility was that 4-CPA underwent a similar form of transform within bracken fronds causing the same scorching. Jaff (1982) went on to demonstrate that 2,4-D was readily photo-oxidised in vitro in the presence of the photosensitizer riboflavin. He found that thiocyanate could reduce the rate of this photochemical transformation. The work of Jaff further

suggested that photodegradation may be an important means of reducing the effectiveness of this group of herbicides. The work in this area was concluded at a relatively early stage, however, and there seemed to be scope for developing some aspects further. In particular, the nature of the photochemical transformations of these herbicides was of some interest theoretically and practically. In addition, the potential use of additives such as thiocyanate to impart some control over the rate of degradation was worthy of further research. Consequently the latter half of this thesis has been dedicated to considering the photochemistry of the phenoxyacetates.

Only a brief introduction to the areas of research has been given here as more detail has been given in the respective Chapters. As a broad look at the environmental disposition of different herbicides and potential herbicides, it was felt that the thesis as a whole would be better presented with a more detailed introduction given to individual Chapters.

CHAPTER 2

THE EFFECT OF SULPHONAMIDES ON PLANT GROWTH

Introduction

The aim of this Chapter was to investigate the growth regulating properties of a range of test sulphonamides in different plant species. It has been found that asulam probably functions as a plant growth inhibitor through behaving as a sulphonamide, although it has yet to be shown conclusively that it does not have carbamate activity also. As a consequence, there seemed to be a need to explore the possibility of plant growth regulation by sulphonamide analogues.

Mode of action of sulphonamides in relation to that of asulam

(i) bacteriostatic activity of therapeutic sulphonamides

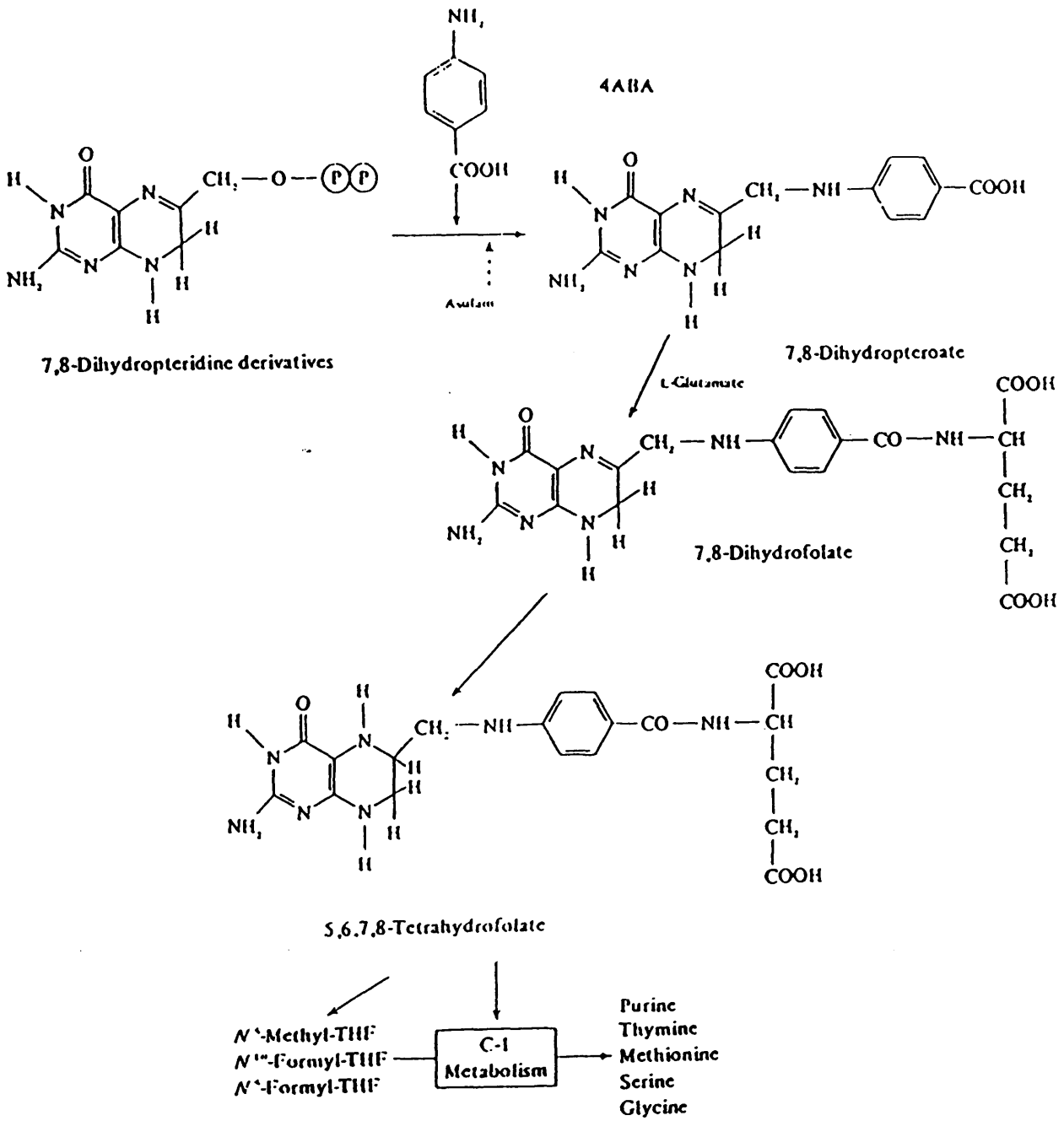
That sulphonamide drugs exhibit bacteriostatic properties through the inhibition of folic acid biosynthesis has been demonstrated with bacterial cultures and at the enzyme level. Lampen and Jones (1946; 1947) found that sulphonamides competitively inhibited the growth of some strains of Streptococcus fecalis, Lactobacillus arabinosus and L. plantarum in media containing p-aminobenzoic acid (PABA). The

non-competitive reversal of this inhibition by folic acid suggested that it might be a product of the inhibited reaction. Similarly, Nimmo-Smith et al. (1952) demonstrated inhibition of folic acid biosynthesis in non-growing suspensions of L. plantarum and competitive reversal of this inhibition by PABA. At the enzyme level, Brown (1962) using the enzyme system extracted from E. Coli demonstrated that sulphonamides could inhibit the biosynthesis of dihydropteroate from PABA. Brown (1962) and later Shiota et al. (1964) found that the inhibition was of a competitive nature as long as the sulphonamide and the PABA substrate were added simultaneously or the PABA added first. Diagram (2.1) illustrates the biosynthesis of folic acid and the involvement of sulphonamides as inhibitors of the pathway. Folic acid and folate derivatives play an important role in biological methylations and inhibition of their biosynthesis effectively inhibits nucleotide and hence protein biosynthesis.

(ii) asulam as a plant growth regulator

Although asulam is a substituted sulphonamide it can also be considered to be a carbamic acid derivative. Early reports suggested that its herbicidal activity bore a close similarity to that of other carbamate herbicides. Its morphological effects were similar, for example, causing stunted growth, chlorosis and eventually death by

Fig. 2.1 Competitive inhibition of the enzyme dihydropteroate synthetase by asulam



interfering with cell division and expansion in apical and axial meristems (Cottrell and Heywood, 1965). Sterrett and Fretz (1975) reported that in onion root tips asulam inhibited mitosis by arresting metaphase and producing chromosomal aberrations in a similar manner to other carbamate herbicides. Veerasekaran (1977) reported that one of the biochemical effects of asulam was to inhibit oxidative phosphorylation in bracken rhizome buds. The author considered this as evidence to substantiate similar findings with other carbamate herbicides. Similarly, Veerasekaran et al. (1976, 1977) demonstrated that asulam significantly inhibited both RNA and protein synthesis as carbamates do, particularly in meristematic regions.

Evidence that asulam might function as an inhibitor of folic acid biosynthesis has come from a number of observations, largely based on the ability of PABA and folic acid to overcome its effects. Killmer et al. (1980) demonstrated that the growth inhibition of carrot cell suspension cultures by asulam could be reversed by adding PABA or folic acid to the growth medium. The inhibition was found to be competitive. A variety of components of C1 metabolism were found to be ineffective in reversing asulam inhibition. Veerasekaran et al. (1981) also investigated the antagonistic interaction of asulam and PABA. They found that germination of wheat seeds (Triticum aestivum L.) in

asulam solution resulted in marked inhibition of root growth but that this inhibition could be partially reversed by the addition of PABA. Similarly, on foliar application asulam significantly reduced the fresh weight yield of wheat, wild oat, flax and s. media. This reduction was partially overcome by the addition of PABA to spray solutions. Similar results were found with sulphanilamide but this compound was a much less effective growth inhibitor. The authors saw these results as evidence of action on folic acid synthesis.

Stephen (1983) germinated bean, pea, wheat and oat seedlings in the presence and absence of asulam and sulphanilamide and recorded inhibition of growth with both compounds, although asulam was much more effective at doing so. This inhibition was effectively overcome by the addition of PABA or folic acid to growth solutions. Structural analogues of PABA were ineffective at overcoming inhibition. Like earlier experiments by Veerasekaran (1981), Stephen (1983) demonstrated that foliar application of asulam to wheat plants reduced growth and that PABA could overcome this reduction.

More recently Hewertson and Collin (1984) examined the mechanism of action of asulam in celery tissue cultures to determine whether the mechanism involved an inhibition of folic acid synthesis. It was found that asulam, sulphanilamide and the carbamate herbicide barban all inhibited growth of the cultures.

However, the addition of folic acid or PABA almost completely reversed the inhibitory effect of only asulam and sulphanilamide. This suggested that asulam was behaving as a sulphonamide rather than a carbamate.

Direct evidence for the inhibition of folic acid biosynthesis came with the observation that asulam and also sulphanilamide inhibited the enzyme 7,8-dihydropteroate synthetase in cell free extracts of wheat seedlings (Veerasekaran et al. 1981). Both compounds effectively inhibited the formation of 7,8-dihydropteroate from PABA and 2-amino-7,8-dihydro-4-hydroxy-6-(hydroxymethyl) pteridine. Asulam was found to be a slightly more effective inhibitor of the enzyme that sulphanilamide. It was also shown that plants with a high folate content were relatively less susceptible to asulam.

Kidd et al. (1982) also demonstrated that asulam impaired the folic acid biosynthetic pathway. Peas soaked in asulam solutions showed a build-up of purine precursors. The 5'-phosphoribosyl glycineamine was found to accumulate as a consequence of reduced levels of $N^{5,10}$methenyltetrahydrofolate and at lower asulam levels, 5'-phosphoribosylaminoimidazole carboximide was found to accumulate as a consequence of reduced levels of N^{10}-formyltetrahydrofolate. The later step was found to be more sensitive than the former. These findings illustrated that inhibition of folic acid biosynthesis

effectively inhibits the biosynthesis of purine bases.

(iii) Plant growth regulation by sulphonamide drugs

There are various reports that the growth regulating properties of the sulphonamide drugs are not restricted solely to bacteria and fungi.

Sulphanilamide, for example, which is the simplest member of the sulphonamide class was shown by Brian (1944) to inhibit the growth of wheat. The inhibition, however, was completely reversed by the addition of PABA. Audus and Quastel (1948) demonstrated that at concentrations as low as 10 μgcm^{-3} sulphanilamide, sulphapyridine, sulphathiazole and sulphaguanidine could effectively inhibit the growth of cress (Lepidum sativum). Like the earlier study by Brian (1944), the addition of PABA was found to overcome the growth inhibition.

It was later shown by Hotson (1953) that although sulphadiazine could be successfully used to control wheat stem rust when applied as a foliar spray, the sulphonamide had an adverse effect on the plants themselves. Growth was stunted and chlorosis of leaves was observed although plants were found to recover and resume normal growth after a period of 7 days. Similarly, Rudd-Jones (1956) concluded that injury to the host plant was a serious problem in the use of sulphonamides for controlling fungal infections.

Sulphanilamide was particularly phytotoxic although sulphacetamide, sulphathiazole and sulphaguanidine all reduced the fresh weight yield of tomato plants at a concentration of 100μgcm^{-3}. Typical symptoms of damage were reported to include stunted roots and shoots, chlorosis, necrotic lesions on the leaves and a marked drop in fresh weight. Strangely, in these experiments sulphadiazine stimulated plant growth.

Iwai et al. (1962) showed that sulphonamides could inhibit the growth of germinating wheat and pea seedlings. Reversal of the inhibition was demonstrated by the addition of PABA or various folates. It was later found by Iwai et al. (1968) that sulphonamides could competitively inhibit the synthesis of 7,8-dihydropteroate by the enzyme 7,8-dihydropteroate synthetase in cell free extracts from peas.

More recently, Kadoya and Kawamoto (1979) reported the use of a mixture of sulphonamides as a herbicide formulation. They report also that sulphapyridine at a level of 6 μgcm^{-3} controlled Echinochloa crus-galli and Setaria virides and showed phytotoxicity to radish and chinese cabbage at a concentration of greater than 100 μgcm^{-3}.

Thus, there is reasonable evidence to suggest that sulphonamide compounds do have plant growth regulating properties. On the whole, however, previous studies have been limited to experiments involving only a

few test chemicals. It was felt that a more comprehensive study of the plant growth regulating behaviour of this class of compounds was relevant, particularly in light of the known herbicidal activity of asulam.

Experimental

(i) Effect of test sulphonamides on the growth of cress seeds

Fifteen cress seeds (Lepidum sativum) were placed randomly on to filter paper on the bottom of sterile petri dishes containing 4 cm^3 of test solution. Petri dishes were kept in the dark in a temperature controlled incubator at 21°C. Seedling numbers were reduced to twelve after 1-2 days. This was considered to be necessary since it was not possible to determine whether the non-germination of a few seeds, in some instances, was due to a lack of vigour or an inhibition by the test chemicals. After a period of 4 days the root and shoot lengths of individual seedlings were measured and recorded. Four petri dishes per treatment were employed. As described in similar experiments by Audus and Quastel (1948), the pH of all test solutions was adjusted to a value of 6.8.

(ii) Effect of PABA on the growth inhibition of cress seeds by selected sulphonamides

The same methods as described previously were used in these experiments except that PABA was formulated with selected sulphonamides in some test solutions. Sulphonamides were formulated at a concentration of $5\mu gcm^{-3}$ only, since it was found that any further increase in concentration generally did not increase the degree of growth inhibition.

(iii) Effect of test sulphonamides on the growth of pea seeds

Pea seeds (Pisum sativum L.c.v. meteor) were placed in 500 ml beakers containing 36 g of 2 mm sieved vermiculite (it was shown previously by Stephen (1983) that the particle size of vermiculite affects its water holding capacity therefore fine particles were removed). Six pea seeds were added to each beaker and covered with a further 5g of vermiculite. Three beakers per treatment were used and test chemicals were added in a volume of 200 ml. Beakers were placed in a temperature controlled growth room at 26±0.75°C, 74±5% r.h. and a 16 h day length. The number of seedlings was reduced to four after 3-4 days leaving the most uniformly germinated. The weights of beakers were adjusted daily by the addition of de-ionised water. The fresh weight of roots and shoots were recorded after 8 days.

(iv) Effect of test sulphonamides on the growth of bean seeds

French bean seeds (Phaseolus vulgaris L.c.v. Canadian wonder) were sown at a rate of four seeds per beaker, this number being reduced to three after 3-4 days. The conditions and treatments were exactly as discribed previously for pea seeds.

(v) Effect of test sulphonamides on the growth of wheat seeds

Wheat seeds (Triticum aestivum L.c.v. sicco) were added at a rate of ten per 100 ml beaker containing 9g of 2 mm sieved vermiculite. A further 2 g of vermiculite was added to the beakers and test chemicals applied in a volume of 50 ml. Seeds were germinated and grown under the conditions described for pea and bean. The number of seedlings was reduced to six per beaker after 3-4 days and root and shoot fresh weights recorded after 7 days. Four beakers per treatment were employed and weights were adjusted daily with de-ionised water.

(vi) Effect of test sulphonamides upon foliar application to bean plants

Individual French bean seeds (Phaseolus vulgaris L.c.v. Canadian wonder) were sown in compost (Fisons Levington) in 6 cm plastic pots. Plants were grown in a temperature controlled growth room as described

previously. At the time of treatment the primary leaves of individual plants were fully expanded (12 days). Each test chemical was formulated as its sodium salt and applied to bean leaves as very fine droplets from a Shandon spray gun. From the results of foliar uptake experiments (see Chapter 3) spray conditions were selected which had been found to maximise uptake through bean leaves. Tween 20 (0.1% w/v) was formulated with spray solutions and after application, spray droplets were allowed to dry and plants sealed below polythene bags for 24 hours (to maximise relative humidity). The polythene bags were removed and plants allowed to grow under the previously described growth room conditions for a total period of 21 days, when fresh weight yields were recorded. Ten plants per treatment were employed. Control plants were sprayed with an aqueous solution containing Tween 20 (0.1% w/v) only.

(vii) Effect of test sulphonamides upon foliar application to wheat plants

Twelve wheat seeds (Triticum aestivum L.c.v. sicco) were sown in compost in 6 cm plastic pots as described previously. The same growth conditions as before were used and seedling numbers were reduced to six on the basis of uniformity of size and condition. Plants were treated after twelve days of growth in exactly the same manner as the bean plants. Fresh weight yields were

recorded after 21 days. Four pots per treatment were employed. Controls were as described previously.

(viii) Effect of test sulphonamides upon foliar application to bracken in the field

From the results of growth room experiments it was decided to restrict this study to a smaller number of test chemicals. Asulam, sulphacetamide and sulphamoxole were included as they had shown inhibition of bean plant growth upon foliar application. Sulphadiazine was also tested as it had shown visual symptoms of growth impairment without actually reducing fresh weight yields.

Bracken plots were marked out at Carbeth, Stirlingshire (Grid reference NS527798). Test chemicals were sprayed from an ICI Mark 3 Knapsack sprayer at an output pressure of 30 p.s.i.. Application was carried out around the period of full frond development when a systemic herbicide such as asulam should be most effective (Stephen, 1983).

Test chemicals were applied at a rate of 1.1 $kgha^{-1}$. Although this is considerably less than the recommended dosage rate of 4.4 $kgha^{-1}$ (Anon, 1971), 1.1 $kgha^{-1}$ was the application rate used in other experiments. It was considered that at a lower value differences between the effectiveness of test chemicals could be more readily established. In retrospect this

was not a good choice since control was very poor even with Asulox.

Bracken plots were laid down as 5 m^2 squares with 1 m^2 paths between plots. Test chemicals were applied to triplicate 5 m^2 plots and were formulated as their sodium salts in aqueous solution containing 0.1% Tween 20. Asulam was applied as asulox (May and Baker Ltd).

Application was carried out on 31/6/87 and control was assessed in the following season by measuring the height of 20 fronds within 5 x 1 m^2 quadrats from the central 9 m^2 of each plot.

Results

For the purpose of clarity of data presentation results have been expressed in the form of a percentage inhibition of growth of controls. It was felt, however, that an example of the raw data for one of the test chemicals should be presented in order to illustrate the range of values recorded. Asulam was selected randomly for this purpose and data from experiments involving this test chemical are tabulated at the end of this section.

Analysis of data was carried out by one-way analysis of variance followed by LSD testing and Tukey's Honestly Significance test for comparing means (Dowdy and Wearden, 1983). This applies to subsequent Chapters also.

(i) Effect of test sulphonamides on the growth of cress seeds

(i)a Asulam

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	(+)9	0
0.5	41	9
1.0	76	39
5.0	81	50
10.0	79	52
25.0	81	55
50.0	79	55
100.0	78	50

(+) denotes an increase in length
* denotes a non-significant difference at the 5% level

(i)b Asulox

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	(+)9	*2
0.5	39	18
1.0	75	47
5.0	77	51
10.0	80	42
25.0	78	51
50.0	80	49
100.0	78	53

(i)c Sulphacetamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	(+)15	*(+)2
0.5	28	7
1.0	71	36
5.0	77	43
10.0	80	48
25.0	78	48
50.0	78	52
100.0	82	50

(i)d Sulphamoxole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*(+)2	7
0.5	34	7
1.0	74	36
5.0	75	51
10.0	80	56
25.0	80	53
50.0	82	51
100.0	83	56

(i)e Sulphanilamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*2	*(+)2
0.5	5	7
1.0	*2	0
5.0	41	47
10.0	64	16
25.0	72	27
50.0	73	36
100.0	77	40

(i)f Sulphadiazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	0	*(+)2
0.5	30	7
1.0	77	33
5.0	75	51
10.0	77	56
25.0	78	51
50.0	77	49
100.0	78	53

(i)g Sulphisomidine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*3	0
0.5	13	5
1.0	46	18
5.0	71	39
10.0	75	45
25.0	79	48
50.0	76	50
100.0	79	48

(i)h Sulphisoxazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*2	(+)7
0.5	32	0
1.0	75	29
5.0	77	55
10.0	78	52
25.0	80	55
50.0	78	50
100.0	77	57

(i)i Sulphamerazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*(+)3	0
0.5	22	7
1.0	65	43
5.0	77	57
10.0	77	61
25.0	75	55
50.0	77	59
100.0	78	59

(i)j Sulphamethazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*2	0
0.5	*3	*(+)5
1.0	56	14
5.0	74	57
10.0	75	60
25.0	72	60
50.0	77	57
100.0	74	62

(i)k Sulphamethizole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*(+)3	*4
0.5	20	7
1.0	74	38
5.0	75	51
10.0	74	51
25.0	77	49
50.0	77	47
100.0	75	51

(i)l Sulphapyridine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	(+)8	(+)2
0.5	8	0
1.0	41	10
5.0	71	31
10.0	75	40
25.0	73	45
50.0	78	48
100.0	76	50

(i)m Sulphathiazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
0.1	*2	0
0.5	10	*(+)2
1.0	69	36
5.0	74	48
10.0	77	48
25.0	75	50
50.0	77	52
100.0	79	48

(ii) Effect of PABA on the growth of cress seeds by selected sulphonamides (5 μgcm^{-3} concentration)

The rate of sulphonamide:PABA is expressed on a molar basis.

(ii)a Asulam

Treatment	Reduction in length (%)	
	Root	Shoot
no PABA	79	48
1:5 PABA	71	45
1:2 PABA	59	36
1:1 PABA	39	39

(ii)b Sulphacetamide

Treatment	Reduction in length (%)	
	Root	Shoot
no PABA	80	48
1:5 PABA	74	50
1:2 PABA	48	38
1:1 PABA	35	31

(ii)c Sulphamoxole

Treatment	Reduction in length (%)	
	Root	Shoot
no PABA	78	50
1:5 PABA	64	41
1:2 PABA	58	39
1:1 PABA	39	30

(ii)d Sulphanilamide

Treatment	Reduction in length (%)	
	Root	Shoot
no PABA	39	2
1:5 PABA	16	2
1:2 PABA	*3	*(+)2
1:1 PABA	*2	0

(ii)e Sulphisomidine

Treatment	Reduction in length (%)	
	Root	Shoot
no PABA	73	45
1:5 PABA	55	39
1:2 PABA	31	30
1:1 PABA	27	20

(ii)f Sulphapyridine

Treatment	Reduction in length (%) Root	Shoot
no PABA	69	33
1:5 PABA	42	19
1:2 PABA	32	7
1:1 PABA	20	*(+)2

(iii) Effect of test sulphonamides on the growth of pea seeds

(iii)a Asulam

Concentration (μgcm^{-3})	Reduction in length (%) Root	Shoot
5	58	22
15	80	49
25	87	56
50	90	69

(iii)b Asulox

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	57	29
15	83	63
25	84	64
50	89	70

(iii)c Sulphacetamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	60	17
15	83	57
25	87	64
50	90	71

(iii)d Sulphamoxole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	66	35
15	89	79
25	91	88
50	93	89

(iii)e Sulphanilamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	*4	*2
15	38	8
25	56	29
50	62	42

(iii)f Sulphadiazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	64	24
15	85	64
25	89	70
50	92	72

(iii)g Sulphisomidine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	47	17
15	73	39
25	75	51
50	86	50

(iii)h Sulphisoxazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	46	12
15	73	55
25	77	58
50	81	61

(iii)i Sulphamerazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	34	12
15	61	25
25	64	35
50	70	47

(iii)j Sulphamethazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	35	11
15	72	40
25	77	50
50	81	56

(iii)k Sulphamethizole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	31	*3
15	49	25
25	67	39
50	71	48

(iii)l Sulphapyridine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	10	6
15	40	16
25	62	24
50	66	44

(iii)m Sulphathiazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	37	6
15	55	22
25	69	33
50	73	45

(iv) Effect of test sulphonamides on the growth of bean seeds

(iv)a Asulam

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	54	16
15	67	54
25	72	59
50	73	60

(iv)b Asulox

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	61	28
15	77	58
25	67	70
50	79	71

(iv)c Sulphacetamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	48	19
15	60	42
25	68	62
50	70	67

(iv)d Sulphamoxole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	54	23
15	61	54
25	69	60
50	76	68

(iv)e Sulphanilamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	*(+)8	*(+)1
15	29	8
25	15	8
50	31	12

(iv)f Sulphadiazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	51	21
15	65	49
25	69	51
50	74	61

(iv)g Sulphisomidine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	0	0
15	25	8
25	42	28
50	52	36

(iv)h Sulphisoxazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	14	*1
15	31	*6
25	38	13
50	42	21

(iv)i Sulphamerazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	29	19
15	58	33
25	68	48
50	69	44

(iv)j Sulphamethazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	31	13
15	52	29
25	63	38
50	68	40

(iv)k Sulphamethizole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	21	4
15	42	16
25	50	19
50	63	24

(iv)l Sulpapyridine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	13	*1
15	20	*7
25	24	12
50	23	19

(iv)m Sulphathiazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	14	9
15	53	32
25	61	44
50	67	61

(v) Effect of test sulphonamides on the growth of wheat seeds

(v)a Asulam

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	57	7
15	80	53
25	86	59
50	88	68

(v)b Asulox

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	49	7
15	77	51
25	88	61
50	89	71

(v)c Sulphacetamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	41	6
15	64	21
25	68	40
50	81	57

(v)d Sulphamoxole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	45	5
15	48	17
25	79	41
50	82	54

(v)e Sulphanilamide

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	8	*2
15	36	16
25	52	26
50	68	33

(v)f Sulphadiazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	50	12
15	71	49
25	88	58
50	88	73

(v)g Sulphisomidine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	42	6
15	60	31
25	75	36
50	75	47

(v)h Sulphisoxazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	45	*1
15	49	*1
25	67	13
50	77	24

(v)i Sulphamerazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	40	7
15	53	30
25	64	41
50	74	57

(v)j Sulphamethazine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	40	7
15	59	21
25	76	34
50	80	49

(v)k Sulphamethizole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	29	8
15	48	13
25	66	27
50	75	39

(v)l Sulphapyridine

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	16	*2
15	28	6
25	50	18
50	65	29

(v)m Sulphathiazole

Concentration (μgcm^{-3})	Reduction in length (%)	
	Root	Shoot
5	41	*3
15	48	8
25	63	21
50	74	43

(vi) Effect of test sulphonamides upon foliar application to bean plants

Test chemical	Inhibition of growth (%)
Asulam	56
Asulox	60
Sulphacetamide	15
Sulphamoxole	46
Sulphanilamide	*1
Sulphadiazine	*0
Sulphisomidine	*2
Sulphisoxazole	*2
Sulphamerazine	*0
Sulphamethazine	*3
Sulphamethizole	11
Sulphapyridine	*+3
Sulphathiazole	*1

(vii) Effect of test sulphonamides upon foliar application to wheat

Test chemical	Inhibition of growth (%)
Asulam	24
Asulox	27
Sulphacetamide	*4
Sulphamoxole	18
Sulphanilamide	*3
Sulphadiazine	*3
Sulphisomidine	*3
Sulphisoxazole	*2
Sulphamerazine	0
Sulphamethazine	*5
Sulphamethizole	*6
Sulphapyridine	*1
Sulphathiazole	*1

(viii) Effect of test sulphonamides upon foliar application to bracken in the field

Test chemical	Frond height (cm)
Control	115.2 (11.9)
Asulox	*114.4 (11.3)
Sulphacetamide	*111.9 (5.3)
Sulphamoxole	*117.6 (9.9)
Sulphadiazine	*117.7 (11.8)

Values are mean values.
Figures in parentheses are standard deviations (n-1 D.F.)
* Denotes a non-significant difference at the 5% level.

Raw data for the results of experiments involving Asulam

(i) Effect of Asulam on the growth of cress

Concentration (μgcm^{-3})	Root and shoot length (mm)	
	Root	Shoot
Control	68 (18)	44 (6)
0.1	74 (11)	44 (5)
0.5	40 (11)	40 (7)
1.0	16 (5)	27 (6)
5.0	13 (4)	22 (6)
10.0	14 (5)	21 (5)
25.0	13 (4)	20 (4)
50.0	14 (5)	20 (5)
100.0	15 (6)	22 (5)

All values are mean values.
Figures in parenthesis are standard deviations (n-1 D.F.)

(ii) Effect of PABA on the growth inhibition of cress by Asulam

Concentration (μgcm^{-3})	Root and shoot length (mm)	
	Root	Shoot
Control	66 (8)	44 (6)
No PABA	14 (4)	23 (6)
1:5 PABA	19 (4)	24 (5)
1:2 PABA	27 (7)	28 (6)
1:1 PABA	40 (11)	27 (5)

All values are mean values.
Figures in parathensis are standard deviations (n-1 D.F.)

(iii) Effect of Asulam on the growth of pea seeds

Concentration (μgcm^{-3})	Root and shoot fresh weight (g)	
	Root	Shoot
Control	0.4175(0.0362)	0.4106(0.0195)
5	0.1785(0.0283)	0.3212(0.0533)
15	0.0832(0.0191)	0.2095(0.0403)
25	0.0563(0.0148)	0.1826(0.0369)
50	0.0401(0.0066)	0.1288(0.0080)

(iv) Effect of Asulam on the growth of bean seeds

Concentration (μgcm^{-3})	Root and shoot fresh weight (g)	
	Root	Shoot
Control	0.5837(0.0942)	1.5034(0.1170)
5	0.2452(0.0341)	1.2555(0.2186)
15	0.1795(0.0230)	0.6846(0.1113)
25	0.1503(0.0220)	0.6229(0.2497)
50	0.1450(0.0176)	0.6020(0.1304)

(v) Effect of Asulam on the growth of wheat seeds

Concentration (μgcm^{-3})	Root and shoot fresh weight (g)	
	Root	Shoot
Control	0.0921(0.0232)	0.1052(0.0188)
5	0.0392(0.0053)	0.0976(0.0176)
15	0.0180(0.0075)	0.0496(0.0240)
25	0.0126(0.0041)	0.0431(0.0191)
50	0.0115(0.0052)	0.0337(0.0110)

(vi) Effect of Asulam upon foliar application to bean plants

Application rate ($kgha^{-1}$)	Fresh weight yield (g)
Control	11.55 (0.66)
1.1	5.10 (0.66)

(vii) Effect of Asulam upon foliar application to wheat

Application rate ($kgha^{-1}$)	Fresh weight yield (g)
Control	0.7722(0.0581)
1.1	0.5836(0.0330)

Discussion

The results of the experiments carried out in sections (i) to (v) illustrate that a range of structurally related sulphonamides have the capacity to inhibit the growth of different plant species. In the Introduction various publications were cited which provided evidence that this class of compounds could interfere with plant growth. The trends observed in this work supports these earlier observations. Some

sulphonamides were more effective than others, although it was difficult to generalise as there were variations between plant species. Possible reasons for these differences in phytotoxicity will be discussed more fully later.

Under the conditions employed in the experiments conducted in sections (vi) to (vii) it was found that most of the test chemicals were ineffective plant growth inhibitors when foliar applied. It was the case with several chemicals that the results of sections (i) to (v) had indicated effective growth inhibition and that the results of Chapter 3 had demonstrated a reasonable degree of foliar uptake under the experimental conditions employed. It may be reasonable to conclude that other plant related factors have an important influence on the activity of these particular test chemicals. This too will be discussed in greater detail later.

Growth inhibition of cress, pea, bean and wheat ((ii) - (v))

It was found that asulam and its commercial equivalent Asulox along with sulphamoxole, sulphacetamide and sulphadiazine were the most phytotoxic test chemicals. Sulphanilamide and sulphapyridine were the least phytotoxic with the other 5 and 6 membered heterocyclic N^1-substituted analogues showing activity between the upper and lower limits.

Growth inhibition of cress seeds reached a maximum at 5 μgcm^{-3} in most cases, although in the case of the less inhibitory chemicals such as sulphanilamide and sulphapyridine, a higher concentration was required to inhibit growth. It was apparent in these experiments and in those involving pea, bean and wheat that roots were more effectively inhibited than shoots. Similar experiments carried out by Stephen (1983) showed this trend also. Perhaps some parameter of tissue growth such as cell division was occurring more rapidly in roots than it was in shoots and that this was subsequently restricted through a deficiency in folates and hence nucleic acids and proteins.

The addition of PABA to sulphonamide solutions was found to overcome the inhibition of cress growth and this is in agreement with other work, such as that of Audus and Quastel (1948) and Stephen (1983). This is further evidence that sulphonamides function as folic acid biosynthesis inhibitors in plants. Six test chemicals were used for this experiment giving a range of growth inhibition at the 5 μgcm^{-3} level. It was observed that a larger proportion of PABA was necessary to overcome the inhibitory effect of the most phytotoxic chemicals. For example, at an equivalent concentration to the test chemical, PABA completely overcame the growth inhibition of cress by sulphanilamide. In the case of the other test chemicals, inhibition was only partially

overcome.

Similar observations were reported by Audus and Quastel (1948). They found that at a 1:1 ratio PABA overcame the inhibition of cress growth by sulphanilamide and three other sulphonamides at both 10 μgcm^{-3} and 100 μgcm^{-3} levels. They also found that growth was never completely inhibited even at a concentration of 1 $mgml^{-1}$. Similarly in this work there was never complete inhibition of cress, pea, bean or wheat by any of the test chemicals. It seems likely that endogenous folates allow germination and some growth until biosynthesis of more folic acid is inhibited.

One point of interest was the apparent stimulation of cress growth by asulam, Asulox and sulphacetamide at the 0.1 μgcm^{-3} level. It is difficult to explain this, but perhaps at sub-lethal concentrations growth stimulation rather than inhibition occurs, provided the chemical is present in low enough quantities.

On the whole, trends in the growth inhibition of pea, bean and wheat were the same. That is, asulam, Asulox, sulphacetamide and sulphamoxole and sulphadiazine were the most effective and sulphanilamide and sulphapyridine the least so. The other test chemicals gave an intermediate degree of inhibition and without any obvious pattern amongst them. As with cress, inhibition reached a certain level after which a further increase in

test chemical concentration only slightly further reduced growth. It was observed that beans were less susceptible than pea or wheat. Stephen (1983) reported this also. He suggested that perhaps bean seedlings have higher endogenous levels of PABA or folates and that this confers some resistance on them.

Foliar application of test chemicals to bean, wheat and bracken

From the results of the experiments conducted in sections (vi) to (viii) it can be seen that only a few of the test chemicals showed any activity when foliar applied to bean, wheat and in selected cases bracken.

Although conditions were optimised to give maximum uptake into bean leaves, only asulam, Asulox, sulphacetamide and sulphamoxole reduced plant growth. Although it was observed that sulphadiazine caused chlorosis of new tissue there was no reduction in fresh weight yield. Typically, asulam, Asulox and sulphamoxole caused effective inhibition of growth of new tissue. Necrosis and dessication of existing tissue was observed. Although sulphamoxole caused exactly the same symptoms as asulam and Asulox, there was a slightly greater development of new tissue before plants began to manifest these symptoms. Sulphacetamide was found to stunt growth although plants did continue to develop tissue. There was extensive chlorosis of leaves although no necrosis or

dessication.

Only asulam, Asulox and sulphamoxole inhibited the growth of wheat. Visual symptoms included a reduction in plant size and chlorosis. Other published data suggested that asulam and Asulox should be more effective inhibitors of wheat growth than was observed. Stephen (1983) reported an 80% inhibition of wheat growth by the foliar application of asulam, although different experimental conditions were employed and asulam was applied at a rate of 2.2 $kgha^{-1}$. In addition, Veerasekaran (1981) reported a 74% reduction in the growth of wheat sprayed with asulam at a lower level of 1.1 $kgha^{-1}$ under growth room conditions. However, the duration of the experiments in both these studies was considerably longer than those in this work. Perhaps over a longer period a greater degree of growth inhibition would have been observed.

All four test chemicals selected were ineffective in controlling bracken in the field. The apparent lack of control by Asulox was something of a surprise. Although a relatively low application rate of 1.1 $kgha^{-1}$ was used one still might expect some level of control. Veerasekaran (1976), for example, reported a 97% reduction in the frond density of bracken one year after the treatment with Asulox at an application rate of 4.4 $kgha^{-1}$. On the other hand, Preest (1975) reported very poor control of bracken with asulam. Perhaps at a higher

level, the test sulphonamides might have shown some degree of control. Alternatively, application may have been carried out at a stage in the season when conditions were, perhaps, less conducive to uptake into bracken. In any case, one would expect plants grown outdoors to develop a thicker, more robust and less permeable cuticle which would obviously be a greater barrier to the uptake of surface applied chemicals. In addition, chemicals applied under field conditions would be exposed to differences in light intensity, wind, humidity and temperature and these parameters can all influence uptake (Hammerton, 1967). A reduction in the translocation of assimilates from the leaf to the rhizomes would also account for inactivity of phloem translocated herbicides.

Structure in relation to plant growth regulation

It may be that other factors such as differences in the inherent phytotoxicity of individual test chemicals accounted for the range of growth inhibition observed.

Recently, Anand (1979) discussed the differences in the bacteriostatic properties of sulphonamides in their role as therapeutic agents. Some of the areas can, perhaps, be extrapolated to the plant situation.

Generally, N^1-monosubstitution of the sulphonamide structure has produced more active

compounds. Activity was enhanced further by heteroaromatic substitution. The results of this study perhaps suggest that a similar situation exists with plants. Sulphanilamide was the least active of all the test chemicals. Chemicals substituted in the N^1 position were much more active although there was no evidence to suggest that heteroaromatic substitution resulted in more activity than aliphatic substitution.

There has been some discussion as to the likely reasons why some sulphonamides show greater bacteriostatic activity than others. Initially it was considered that N^1-substitution could influence the primary animo group. This functional group has been shown to be vital in producing bacteriosis. Any form of N^4-substitution, for example, caused complete loss of activity. It was concluded that the amount of negative charge on the aromatic amino group was the significant factor in producing activity. Anything which alters the amount of negative charge, ie. the ionic strength, interfered with the activity. However, substitution at the other end of the molecule ie. N^1-substitution, was found to have no influence on the ionic strength of the primary animo group. All of the bacteriostatic sulphonamides have a basic dissociation constant of 2 which is close to that of PABA (Anand, 1979). Thus, variations in the biological activity of the test sulphonamides in this study do not arise through an

effect of N^1-substituents on the primary amine group of the molecule.

However, the influence of N^1-substitution on the acid dissociation constant of sulphonamides is considered to play a central role in the differences in activity. Acid dissociation constants of the sulphonamide group varies from 3 to 11 (Anand, 1979). However it has been found that the most active chemicals have pKa values in the physiological range. The type of N^1-substituent does of course influence the pKa value and hence the bacteriostatic activity. It is generally considered that the explanation for this observation lies in the fact that in the ionised state a sulphonamide will more closely resemble the p-aminobenzoate anion. Ionisation results in enhanced polarization of the sulphonyl group which then bears a greater resemblance to the geometrical and electronic characteristics of PABA.

It seems feasible that this may be the case in the inhibition of plant growth also. In this study, sulphapyridine, for example, was less active than the other test chemicals with N^1-substituents. This compound has a pKa of 8.4 (Anand, 1979) which is higher than the other test chemicals. However, asulam, sulphacetamide, sulphamoxole and sulphadiazine were all very active (section (i)-(v)) but have different pKa values. The former two have pKa values in the acid range and the latter around neutral. Obviously, however, other factors

such as differences in penetration, translocation and metabolism, to more or less active compounds, may have an important bearing on this study.

CHAPTER 3

THE FOLIAR UPTAKE OF SULPHONAMIDES INTO BEAN LEAVES

Introduction

It had been decided to investigate the growth regulating properties of asulam and the other test suphonamides after foliar application to bean and wheat. However, since uptake from the leaf surface into the leaf interior was a necessary pre-requisite for systematic activity, it was decided to consider the likelihood of uptake in the case of the test chemicals. In addition, since the group of chemicals being studied were structurally related, it was an aim to possibly relate the ease of uptake into bean leaves with chemical structure. The possibility of enhancing uptake through the use of additives and selection of optimal environmental conditions was also investigated.

Asulam - the need for rapid uptake

The success of asulam as a herbicide for controlling the growth of bracken relies on effective uptake of the chemical into bracken fronds and translocation in the phloem to underground rhizomes. Studies were carried out by Veerasekaran et al. (1977) on the absorption and translocation of foliage-applied

ring-labelled [^{14}C]-asulam into glasshouse and field-grown bracken plants. They demonstrated that uptake was rapid initially then slowed and that increased humidity and temperature enhanced penetration into the bracken fronds. Uptake was found to diminish with increasing frond age. Once inside the bracken frond, translocation of asulam from the treatment area was found to follow a "source to sink" pattern with accumulation in the metabolically active sinks such as rhizome apices, frond buds, root tips and young frond tissue.

The general erratic performance of asulam (Holroyd et al., 1970) has been attributed to climatic factors although it was suggested by Babiker and Duncan (1975a) that slow penetration of the herbicide could, in part, be responsible for this. The results of field trials carried out by Holroyd et al. (1970) would seem to substantiate this as they demonstrated increased activity when 0.1% Agral 70 (w/v) (a surfactant) was added to asulam in the presence of rain.

There have been several studies aimed at enhancing the uptake of asulam and reference is made later in this Chapter to the work of Babiker and Duncan (1974, 1975) and Jaff (1983) in particular.

These studies were aimed at enhancing foliar uptake of asulam through the formulation of the herbicide with different adjuvants. Their aim, essentially, was to facilitate effective and rapid herbicide uptake by

modifying the local environment encountered on the leaf surface or possibly by altering the properties of the leaf barrier itself. Ultimately, the result of doing this successfully would be to improve cost-effectiveness, since less herbicide will be required, improve selectivity and reduce environmental contamination (Turner, 1974).

An alternative, though less effective, method of improving efficiency of uptake of asulam and other foliar applied herbicides is to select a suitable environment (Brian, 1970), or to control the timing of application. This, however, limits the use of herbicides to particular regions, restricts spraying times and ultimately increases the cost of treatment.

The leaf cuticle as a barrier to foliar uptake

The process of foliar uptake is complicated and involves interaction between the environment, the spray droplet and applied pesticide and the plant cuticle.

The cuticle is the first barrier to foliar uptake. Its highly lipophilic nature means that it is an effective barrier to transport into and out of the leaf and it represents a discontinuity between the aqueous interior of the leaf and the aqueous pesticide solution applied to the outer surface (Price, 1982). It is not a complete barrier however as water and solutes are able to cross it as leachates (Tukey, 1970) and pesticides do

enter the plant after external application.

Permeability of the cuticle is known to vary amongst different plant species and even amongst different plants of the same species as a result of age, temperature, rainfall, wind, humidity and light conditions (Jaff, 1983). The cuticles of young leaves have been shown to be thinner and more permeable than those of mature leaves (Leon and Bukovac, 1978), for example, and decreased permeability has been reported during leaf development (Bukovac et al., 1979). It was demonstrated by Veerasekaran and Kirkwood (1972) using excised pinnules from field bracken, that the retention of asulam increased as the plant matured, but uptake decreased. It was suggested that this probably reflected development of cuticle wax as the bracken fronds matured. From the thickness of leaf cuticle and its much lower protein content in comparison with the cell membrane, it has been suggested that specific active transport mechanisms of the cell membrane type do not exist (Price, 1982). Diffusion is accepted as being a much more likely mechanism for the movement of pesticides across the cuticle.

Thus, in the case of asulam and the test sulphonamides used in this study, foliar uptake essentially involves diffusion from an aqueous droplet across the cuticle and into the aqueous interior of the leaf. In the field, where this process only occurs to a

limited extent or where it is slow, the chemical may be removed from the leaf surface physically by rain and wind, or can be exposed to photodecomposition. The aim obviously would be to achieve rapid and complete penetration. In the past this has not been the case with asulam, particularly where bracken is concerned (Babiker and Duncan, 1974), although uptake has been considerably improved through the addition of surface active compounds to formulations.

The use of additives to enhance the uptake of asulam and related sulphonamides

(i) Surfactants

In practice a variety of different surfactants have been added to asulam spray formulations to improve uptake. Holroyd et al. (1970) found that Agral 70 at a concentration of 0.1% (w/v) increased the activity of asulam against pot grown bracken. Soper (1972) on the other hand reported no increased acitivity of asulam on field bracken when Shellestol was included in formulations at 0.025% (w/v). The use of Tergitol-7 at 0.1% (w/v) by Veerasekaran et al. (1977) was mentioned earlier and they reported increased uptake and activity of asulam against bracken. Veerasekaran et al. (1978) later reported a 10% improvement in the reduction of bracken frond density when asulam was formulated with 1% ethylan CP. In addition, Tween 20 has been used very

successfully on a number of occasions to improve the uptake of asulam and enhance its performance (Babiker and Duncan, 1975a; Jaff, 1983). Babiker and Duncan (1975a) actually demonstrated that although Tween 20 gave the greatest improvement in the uptake of asulam into bean leaves, Triton GR-5, Tergitol and Triton X-67 all gave a significant increase in penetration compared with an aqueous control.

More recently Harper and Appleby (1984) reported increased uptake of asulam into the leaves of Medicago sativa L. plants when a surfactant was included at a concentration of 0.2%, although the identity of the surfactant was not revealed.

The use of Tween 20 by Babiker and Duncan (1974) to enhance asulam uptake into bracken was prompted by the work of Crowdy et al. (1958) who used the surfactant to enhance sulphonamide uptake into plant foliage. In addition it had earlier been demonstrated by Hotson (1953) that by dissolving sulphamerazine in a 1% solution of Tween 20, it was possible to control stem rust in wheat at the equivalent of 14 lb/acre whereas 80 lb/acre was required in the absence of the surfactant. In the same year, Livingston (1953) formulated sulphanilamide with Triton X45 to control cereal rusts. (As a point of interest, the work of Hotson (1953), Livingston (1953) and Crowdy et al. (1958) illustrates that many of the sulphonamides used in this study do seem to be

translocated from the leaves to the stems, in cereals at least).

(ii) The effect of surfactants on the uptake process

The selection of a particular surfactant or combination of surfactants for a certain purpose is considered to still be a rather empirical procedure as optimal amounts have to be found by trial and error (Hassall, 1982). In addition, there is often uncertainty as to the precise way in which a particular surfactant enhances pesticide uptake.

The ability to reduce surface and interfacial tension is one way in which they can increase uptake. This has the effect of increasing spray retention when the cuticle is of a low natural wettability, although it has been shown to decrease retention when the cuticle is of a high natural wettability (Holy, 1976). It also causes increased droplet spreading and elimination of air films between the spray droplets and the leaf surface, resulting in an increased contact with the leaf surface. Cook (1979) pointed out, however, that often the major influence of surfactants on herbicide activity are found at concentrations beyond which the greatest changes in surface and interfacial tensions occurs. This might suggest another role for the surfactant such as co-solvency, solubilisation of epicuticular wax or hydration of the cuticle.

In the case of the test chemicals used in this study, Tween 20 seemed to be a particularly appropriate surfactant to employ. Its success with asulam and other sulphonamides and also its known ability to function as a cosolvent for sulphonamides and carbamates (discussed later) were good reasons to investigate its potential for increasing the foliar uptake of the test chemicals.

Tween 20 has a relatively high HLB value (hydrophobic-lipophilic balance) and it was considered that this might not be entirely compatible with some of the less polar sulphonamides. Consequently it was decided to use Tween 80 in some cases as it has a lower HLB value and hence is more lipophilic. The selection of surfactants and their concentrations are discussed more fully later.

(iii) Inorganic salts

Other types of additives which have received some attention in recent years are inorganic salts. It was demonstrated by Jaff (1983) that salts of monovalent cations and anions were particularly effective at enhancing uptake of asulam. The effects were not as spectacular as those reported for other herbicides such as aminotriazole, MCPA, picloram and glyphosate (Jaff, 1983).

It is generally considered that the leaf surface has a low density negative charge and therefore behaves

like an ion-exchange membrane (Crafts and Foy, 1962; Cook and Duncan, 1983). Negatively charged pesticides such as asulam are repelled by the negative charge and in this way uptake is inhibited. Jaff (1983) found that uptake could be enhanced by monovalent cations through suppression of this low density negative charge and possibly through swelling of the cuticle as a result of the large shell of hydration of the monovalent species.

Since the test chemicals used in this study were formulated in the anionic form, it seemed appropriate to employ such an inorganic salt in these circumstances. More details of the choice of salt and experimental conditions are given in the next section.

EXPERIMENTAL

Materials

Asulam (99.5% purity) was obtained from the National Physical Laboratory, sulphanilamide from May and Baker Ltd. and the ten other sulphonamides used, from Sigma London Chemical Co. Ltd.. Asulox, the commercial formulation containing 40% w/v asulam as the sodium salt, was purchased from May and Baker Ltd.. Tween 20 (polyoxyethylene 20 sorbitan monolaurate) and Tween 80 (polyoxyethylene 80 sorbitan monolaurate) were purchased from Koch-Light Laboratories Ltd. and ammonium thiocyanate from Hopkin and Williams Ltd. at the purest

grade available.

Husbandry

All foliar uptake experiments were carried out in a growth room adjusted to a temperature of 25±2°C and a 16 h day length. Bean seeds (Phaseolus vulgaris var. Canadian wonder) were germinated in trays of vermiculite under growth room conditions and seedlings selected for experiments on the basis of uniformity of size and condition. Seedlings were transplanted to pots at an early stage (two leaf) and grown under growth room conditions until an age of 10 - 15 days when the primary leaves were fully expanded.

Application of test material

All solutions were applied to bean leaves as discrete droplets (2.5 μl) from a 50 μl Hamilton syringe. Droplets were placed randomly on the upper leaf surface in a total volume of 30 μl/leaf. It has been observed that the point of application of droplets can greatly influence the extent of pesticide uptake from the leaf surfaces (Leonard, 1958). Therefore, care was taken to avoid main veins and to apply solutions to the same upper area of leaves in each case.

In the past, droplets have been applied from an Eppendorf pipette (Babiker and Duncan, 1975; Cook and Duncan, 1978; Jaff, 1983) but it was found that use of a

graduated syringe gave better control of droplet size. It was also found that when volumes greater than 30 μl were applied to bean leaves, solutions tended to spread over the leaf surface, rather than persist as discrete droplets. Consequently, 30 μl was the largest volume applied in these experiments.

For each treatment 12 leaves were treated and after 24 hours residues were washed from the leaf surface into a 25 cm^3 volumetric flask using distilled water. As in previous studies (Cook et al., 1977; Jaff, 1983), preliminary work confirmed that total recovery of all test chemicals was possible, provided leaves were washed immediately after treatment.

Humidity conditions

After application of test chemicals, plants were exposed to two different humidity conditions.

In the first case, treated plants were left exposed in the growth room. Droplets then dried out and test chemicals were subjected to low humidity conditions. This treatment has been referred to subsequently as the low humidity level (LHL).

Alternatively, treated plants were placed within polythene bags for the duration of experiments. Since the relative humidity below sealed polythene bags is considered to approach 100% (Clor et al., 1962), chemicals under these conditions were exposed to near

maximal or high humidity conditions. This treatment has been referred to as the high humidity level (HHL).

A third experimental condition was employed. This involved allowing droplets to dry out under low humidity conditions, then placing treated plants under polythene bags, exposing test chemicals to high humidity conditions for the duration of the experiment. Droplets dried in approximately 20 - 30 minutes, and timing of experiments began after this time. This has been referred to as the second high humidity level (HHL2).

Selection of humidity conditions

The choices of humidity for the foliar uptake experiments was based on the results of earlier research into factors affecting aminotriazole penetration into bean leaves (Cook and Duncan, 1977). These authors found that penetration from initially dry droplets was approximately twice that from wet droplets, where plants were held at the high humidity level. Further, penetration could be increased by transforming plants from the low humidity level to the high humidity level.

Although the effects of humidity on the leaf surface barrier were considered to play a major role in the increased penetration under these conditions, it was found that there was increased aminotriazole uptake following an increase in aminotriazole concentration. This was seen to support the idea that under low humidity

conditions, applied chemicals will concentrate as droplets dry out, then form a relatively highly concentrated film of solution on the leaf surface upon transfer to high humidity conditions. This idea was also proposed by Middleton and Sanderson (1965) to account for increased penetration under similar conditions. The uptake of the test chemicals in this study may be influenced by humidity conditions in a similar fashion to the chemicals used in these earlier studies. Any such humidity effects could have an important bearing on the types of environmental conditions under which the test chemicals would be expected to perform most effectively, provided foliar uptake was a limiting factor.

Effect of additives on the foliar uptake of test chemicals

Since a large number of chemicals were used in this study, only relatively few additives were used to investigate the possibility of improving their foliar uptake. It was decided to use additives which had either been successfully used in the past to improve the uptake of asulam or other herbicides under similar conditions, or which had been shown to improve the uptake of sulphonamide drugs.

(a) Tween 20

Tween 20 has been used in a number of studies to enhance the uptake of asulam and other herbicides.

Cook et al. (1977) demonstrated that at both high and low humidity levels this surfactant could greatly increase the uptake of aminotriazole from aqueous droplets into bean leaves. More significantly, Babiker and Duncan (1974) and Jaff (1983) found that the incorporation of Tween 20 into aqueous formulations of asulam resulted in marked improvements in the uptake of the herbicide into bean leaves. Improvements were found under both low and high humidity conditions.

Babiker and Duncan (1974) had earlier shown that the inclusion of Tween 20 increased spray retention and doubled the uptake of asulam into the bracken frond. From this evidence, it seemed feasible that this surfactant might be successfully employed to increase the uptake of the structurally related sulphonamides.

Direct evidence for a role for Tween 20 in increasing the uptake of the sulphonamides came from the results of Hotson (1953) and Jones and Wignal (1955). Their aim was to improve the uptake of selected sulphonamides into cereal leaves thereby giving greater control of cereal rusts present on the stems of infected plants.

Hotson (1953) demonstrated that the addition of Tween 20 to formulations at a concentration of 1% greatly improved the uptake of sulphamerazine into wheat leaves and hence gave improved control of wheat stem rust. At this concentration, the surfactant was also found to

increase the uptake of other sulphonamides, including sulphanilamide, sulphadiazine, sulphapyridine and sulphamethazine.

The concentration of Tween 20 in formulations has been found to influence the uptake of test chemicals. Cook et al. (1977) reported details of the effect of Tween 20 on aminotriazole uptake into bean leaves. He concluded that the ratio of herbicide to surfactant in formulations was critical to the process of penetration.

Similar results were reported by Babiker and Duncan (1975) on the effect of Tween 20 on the uptake of aminotriazole into bracken fronds. The surfactant gave improved uptake at the three concentrations tested, compared with an aqueous control, although it was most effective at a concentration of 0.2% (w/v) rather than 0.1% or 0.3% (w/v).

Babiker and Duncan (1975a) also added Tween 20 to asulam formulations at a concentration of 0.2% (w/v) to increase the uptake of asulam into bean leaves. Jaff (1983), on the other hand, included Tween 20 at a concentration of 0.1% (w/v) in his investigations and found that at both concentrations the surfactant increased the uptake of asulam into bean leaves.

As a consequence of these results, it was decided to use Tween 20 at a concentration of 0.1% (w/v). It seemed reasonable to expect that any effect of the surfactant on the uptake of the test chemicals into bean

leaves would be manifested at this level. The surfactant was formulated with all test chemicals under the three previously described humidity conditions.

(b) Tween 80

The role of surfactants as co-solvents has been discussed already and it was felt that perhaps a less polar surfactant of the Tween series might be more compatible with some of the less polar test chemicals. Hence, Tween 80 was included in selected cases at a concentration of 0.1% (w/v).

(c) Ammonium thiocyanate

Early studies by Cook (1979) showed that both ammonium thiocyanate (NH_4SCN) and potassium iodide (KI) could effectively improve the uptake of aminotriazole into bean leaves at different humidity levels. Increases were observed at both 1/1 and 2/1 molar ratios (salt:herbicide), with higher ratios giving no further increase in uptake.

More recently, Jaff (1983) examined the effect of a number of inorganic salts at different concentrations, on the uptake of asulam by bean seedlings. It was concluded that uptake could be greatly improved by the addition of inorganic salts. Salts of monovalent cations and monovalent anions were more successful than divalent cations and anions. In this study, NH_4SCN seemed to be particularly effective. For this reason and because Cook

(1979) had demonstrated the role of NH_4SCN in improving aminotriazole uptake, it was decided to formulate NH_4SCN at a molar ratio of 1:1 (salt:sulphonamide) with selected sulphonamides.

Summary of experimental conditions

All sulphonamides were formulated at a concentration of 5 $mgcm^{-3}$ based on the recommended field application rate for Asulox, the commercial formulation of asulam.

Many of the chemicals were found to be only sparingly soluble in aqueous solution at this concentration, so all test chemicals were formulated as their sodium salt by titration with dilute sodium hydroxide to a value of pH 9.0. This value was kept constant in all cases.

Experiment I

All thirteen test sulphonamides were formulated as aqueous solutions (pH 9.0) and uptake was studied under three humidity conditions.

Experiment II

All thirteen test sulphonamides were formulated as aqueous solutions (pH 9.0) containing 0.1% (w/v) Tween 20 and uptake was studied under three humidity conditions.

Experiment III

Asulam, sulphathiazole, sulphamoxole, sulphadiazine and sulphapyridine were formulated as aqueous solution (pH 9.0) containing 0.1% (w/v) Tween 80 and uptake was studied under three humidity conditions.

Experiment IV

Same as Experiment III except aqueous solutions formulated with NH_4SCN at 1:1 molar ratio.

Residue quantification

The concentration of test sulphonamide present in the washings from bean leaves was determined using either a colorimetric method or by HPLC (reverse phase). In practice the method used was dependant upon the availability of equipment.

(i) Colorimetry

Sulphonamide concentrations were determined by the Bratton-Marshall reaction as described for asulam by Brockelsby and Muggleton (1973). The method can be summarised as follows:

(1) Add 1.0 cm^3 of 6N HCl to 5 cm^3 of test solution.
(2) Add 1.0 cm^3 of 0.5% sodium nitrite, shake and leave for 15 minutes.
(3) Add 1.0 cm^3 of 5% ammonium sulphamate, shake and leave for 10 minutes.

(4) Add 1.0 cm^3 of 0.2% N-1-napthylethylenediamine dihydrochloride, shake and read the absorbance at 545 nm within 10 minutes.

(ii) High Pressure Liquid Chromatography (HPLC)

This method offered a rapid means of analysis and was particularly time-saving since a large number of samples could be analysed unattended using the auto-injection function.

The presence of residues in aqueous solution was suitable for reverse phase conditions and since solutions were simply washings, there were no interfering compounds and hence no need for a clean-up method.

Variations in the polarity of the test chemicals resulted in differences in their respective retention times on the C18 stationary phase. As a consequence, it was necessary to vary the proportions of each of the two components of the mobile phase ie. methanol:0.1% aqueous acetic acid in order to optimise retention times and hence peak shape.

For example, in the case of a relatively non-polar sulphonamide such as sulphamethazine a greater proportion of methanol was required otherwise the compound was too strongly retained, resulting in peak spreading and tailing. Three examples are shown below along with relevant HPLC conditions.

Chemical	Mobile phase (methanol:acetic acid (0.1%))	Retention time (minutes)
Asulam	20:80	2.98
Sulphathiazole	20:80	2.81
Sulphamethiazine	70:30	4.53

(see Figures 3.1 - 3.3)

For HPLC analysis a phenyl phase column (150 x 4.6 mm, particle size 5 μm) was attached to a Perkin Elmer Series 400 solvent delivery system, a Perkin Elmer 155-100 sampling system, a Perkin Elmer u.v. detector (u.v. absorption measured at 254 nm) with a Perkin-Elmer LC1-100 integrator.

RESULTS

Experiment I

Test sulphonamides formulated as sodium salts in aqueous solution. No additives.

(a) LHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	142.4	5.1 (0.7)
Asulox	142.4	5.1 (0.6)
Sulphacetamide	143.1	4.6 (0.6)
Sulphamoxole	144.3	3.8 (0.4)
Sulphanilamide	141.7	5.5 (1.4)
Sulphadiazine	141.9	5.4 (0.7)
Sulphisomidine	140.7	6.2 (1.0)
Sulphisoxazole	141.9	5.4 (0.9)
Sulphamerazine	145.2	3.2 (0.3)
Sulphamethazine	141.9	5.4 (0.7)
Sulphamethizole	142.2	5.2 (0.6)
Sulphapyridine	143.1	4.6 (0.4)
Sulphathiazole	142.8	4.8 (0.6)

Figures in parentheses are standard deviations (n-1 D.F.)

(b) HHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	82.3	45.1 (9.4)
Asulox	69.5	53.7 (17.4)
Sulphacetamide	64.9	56.7 (15.4)
Sulphamoxole	93.1	37.9 (6.7)
Sulphanilamide	142.6	4.9 (0.4)
Sulphadiazine	130.8	12.8 (1.8)
Sulphisomidine	122.1	18.6 (1.7)
Sulphisoxazole	59.8	60.1 (11.0)
Sulphamerazine	133.3	11.1 (1.4)
Sulphamethazine	126.1	15.9 (2.4)
Sulphamethizole	98.4	34.4 (5.4)
Sulphapyridine	136.2	9.2 (1.4)
Sulphathiazole	101.7	32.2 (4.5)

(c) HHL II

Test sulphonamide	Mean amount remaining on leaf surface (µg)	Mean uptake (%)
Asulam	35.1	76.6 (9.4)
Asulox	44.7	70.2(20.5)
Sulphacetamide	104.2	30.5(11.1)
Sulphamoxole	91.4	39.1(10.8)
Sulphanilamide	142.4	5.1 (0.6)
Sulphadiazine	130.1	13.3 (3.1)
Sulphisomidine	124.7	16.9 (4.3)
Sulphisoxazole	56.4	62.4(15.4)
Sulphamerazine	134.6	10.3 (1.7)
Sulphamethazine	124.1	17.3 (3.6)
Sulphamethizole	82.2	45.2 (7.4)
Sulphapyridine	137.3	8.5 (1.2)
Sulphathiazole	105.9	29.4 (6.4)

Experiment II

Test sulphonamides formulated as sodium salts in aqueous solution containing 0.1% (w/v) Tween 20.

(a) LHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	142.5	5.0 (0.5)
Asulox	142.8	4.8 (0.5)
Sulphacetamide	142.5	5.0 (0.6)
Sulphamoxole	143.1	4.6 (0.6)
Sulphanilamide	142.4	5.1 (0.7)
Sulphadiazine	142.4	5.1 (0.5)
Sulphisomidine	141.7	5.5 (0.9)
Sulphisoxazole	142.0	5.3 (0.5)
Sulphamerazine	143.4	4.4 (0.5)
Sulphamethazine	142.2	5.2 (0.7)
Sulphamethizole	141.9	5.4 (0.7)
Sulphapyridine	142.5	5.0 (0.6)
Sulphathiazole	141.9	5.4 (0.6)

(b) HHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	61.2	59.2(13.4)
Asulox	46.2	69.2(14.6)
Sulphacetamide	64.1	57.3(10.2)
Sulphamoxole	86.9	42.1 (8.3)
Sulphanilamide	142.8	4.8 (0.7)
Sulphadiazine	128.9	14.1 (2.2)
Sulphisomidine	122.0	18.7 (3.6)
Sulphisoxazole	62.0	58.7(14.3)
Sulphamerazine	129.7	13.5 (1.9)
Sulphamethazine	129.2	13.9 (2.4)
Sulphamethizole	63.9	57.4(10.0)
Sulphapyridine	137.1	8.6 (1.2)
Sulphathiazole	106.5	29.0 (7.6)

(c) HHL II

Test sulphonamide	Mean amount remaining on leaf surface (µg)	Mean uptake (%)
Asulam	10.1	93.3(10.2)
Asulox	21.6	85.6(13.2)
Sulphacetamide	24.2	83.9(14.2)
Sulphamoxole	62.1	58.6(11.4)
Sulphanilamide	142.2	5.2 (0.5)
Sulphadiazine	129.9	13.4 (2.8)
Sulphisomidine	119.8	20.1 (4.1)
Sulphisoxazole	35.6	76.3(14.8)
Sulphamerazine	132.6	11.6 (2.1)
Sulphamethazine	101.6	32.3 (9.6)
Sulphamethizole	43.5	71.0(12.6)
Sulphapyridine	134.7	10.2 (1.2)
Sulphathiazole	77.9	48.1 (9.6)

Experiment III

Asulam, sulphathiazole, sulphamoxole, sulphadiazine and sulphapyridine formulated as sodium salts in aqueous solution containing 0.1% (w/v) Tween 80.

(a) LHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	142.8	4.8 (0.3)
Sulphathiazole	143.1	4.6 (0.5)
Sulphamoxole	141.9	5.4 (0.7)
Sulphadiazine	142.2	5.2 (0.4)
Sulphapyridine	144.0	4.0 (0.4)

(b) HHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	87.4	41.7 (8.9)
Sulphathiazole	96.9	35.4(10.2)
Sulphamoxole	100.2	33.2 (9.0)
Sulphadiazine	128.5	14.3 (3.2)
Sulphapyridine	139.7	6.9 (1.2)

(c) HHL II

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	45.3	69.8(14.6)
Sulphathiazole	105.6	29.6 (5.8)
Sulphamoxole	96.3	35.8 (8.4)
Sulphadiazine	132.6	11.6 (2.3)
Sulphapyridine	133.3	11.1 (2.0)

Experiment IV

Asulam, sulphathiazole, sulphamoxole, sulphadiazine and sulphapyridine formulated as sodium salts in aqueous solution containing NH_4SCN (1:1 molar ratio).

(a) LHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	142.0	5.3 (0.7)
Sulphathiazole	142.6	4.9 (0.5)
Sulphamoxole	142.0	5.3 (0.5)
Sulphadiazine	142.5	5.0 (0.6)
Sulphapyridine	141.9	5.4 (0.7)

(b) HHL

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	74.7	50.2(12.2)
Sulphathiazole	111.6	25.6 (6.4)
Sulphamoxole	91.2	39.2 (9.6)
Sulphadiazine	129.4	13.7 (3.8)
Sulphapyridine	139.8	6.8 (1.0)

(c) HHL II

Test sulphonamide	Mean amount remaining on leaf surface (μg)	Mean uptake (%)
Asulam	29.7	80.2(13.2)
Sulphathiazole	100.4	33.1 (8.6)
Sulphamoxole	99.3	33.8 (7.4)
Sulphadiazine	135.3	9.8 (2.1)
Sulphapyridine	135.5	9.7 (1.3)

Fig. 3.1 HPLC trace of Asulam reference standard ($1\mu gcm^{-3}$)

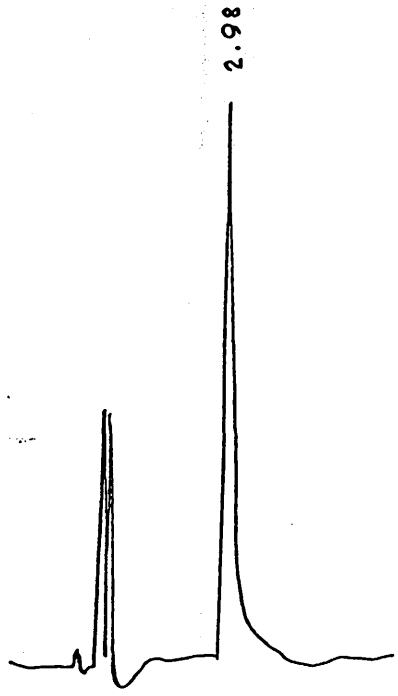


Fig. 3.2 HPLC trace of sulphathiozole reference standard ($1\mu gcm^{-3}$)

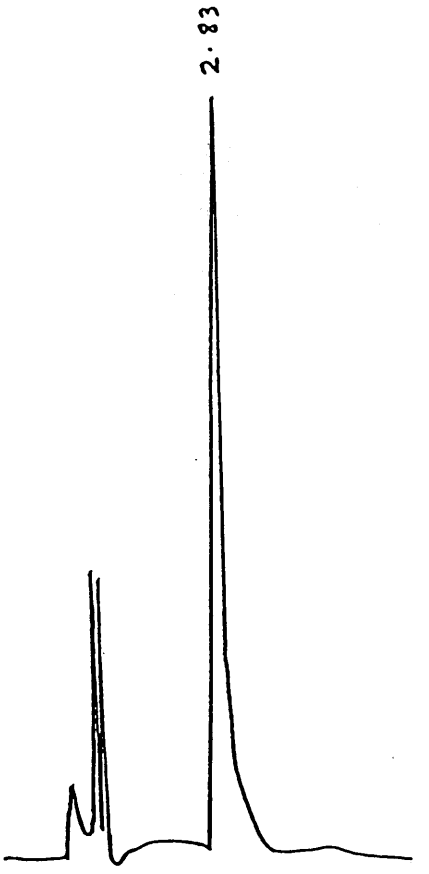
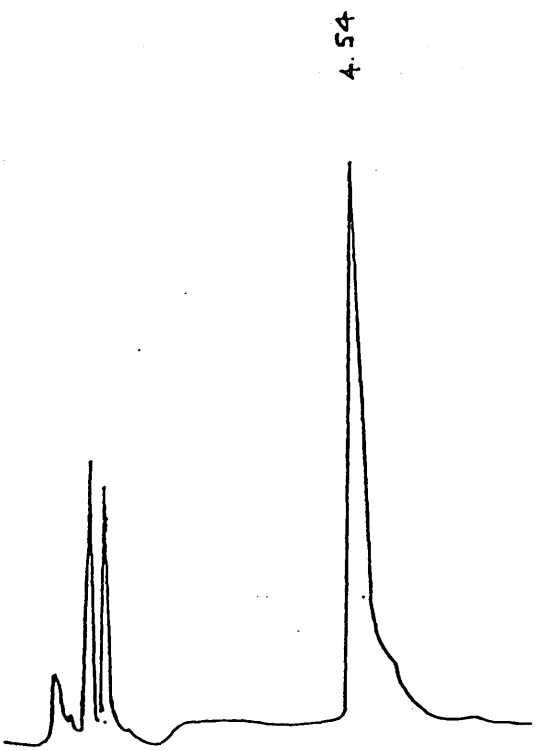


Fig 3.3 HPLC trace of sulphamethazine reference standard ($1\mu gcm^{-3}$)



DISCUSSION

The influemce of humidity and additives on the foliar uptake of sulphonamides by bean leaves is worth considering in some detail. The interaction of humidity with additives in this context is also worth consideration. Similarly, the possible significance of chemical structure on the ease with which the sulphonamides are taken up is a point of interest.

Without exception uptake under the low humidity conditions was minimal and rarely greater than 5% of the total applied chemical. It was observed on each occasion that droplets dried out very rapidly leaving a crystalline residue on the leaf surface. It seems likely that this re-crystallisation reduced the amount of chemical left in solution and in this way uptake was minimised.

Although it has been observed that such droplet drying can lead to the formation of concentrated films of solution on the leaf surface, leading to increased uptake under favourable humidity conditions (Middleton and Sanderson, 1965; Cook, 1979), it seems likely that under the conditions employed in these experiments, droplets dried out too quickly for this concentration effect to be of significance.

When experiments were carried out under high relative humidity conditions a significant increase in

the foliar uptake of all test chemicals was observed with the exception of sulphanilamide which was poorly taken up under all conditions. It is difficult to explain why it was taken up to the extent of about only 5%, particularly since an increase in the uptake of all other test chemicals was observed. Perhaps solubility was a problem in this case. Being a base, sulphanilamide was not formulated as the sodium salt and perhaps re-crystallisation from solution made the chemical less available for uptake. Alternatively it may be that the chemical and physical properties of sulphanilamide were incompatible with the route and mechanism by which this class of compound traverse the bean leaf cuticle. It is, perhaps, worth noting that sulphapyridine was only marginally more effectively taken up than sulphanilamide.

The enhanced uptake of the other sulphonamides under high humidity conditions is consistent with reports for other herbicides (Van Overbeek, 1956; Prasad et al., 1967; Cook, 1977). Where test chemicals were formulated in aqueous solution without any additives and droplets exposed to high humidity conditions immediately ie. Experiment Ib, it was found that Asulox, asulam, sulphisoxazole and sulphacetamide were taken up to the greatest extent. The other three sulphonamides containing five-membered heterocyclic groups, namely sulphamoxole, sulphamethizole and sulphathiazole were

taken up to a lesser extent and the five chemicals containing six membered heterocyclic groups, namely sulphadiazine, sulphamerazine, sulphamethazine, sulphisomidine and sulphapyridine were least effectively taken up.

The overall increase in uptake at high relative humidity may result as a consequence of cuticular hydration under these conditions. This might make the cuticle more permeable to the sulphonamides. It has been suggested that modification of internal factors related to the water continuum of the plant may also occur under high relative humidity conditions and contribute to increased cuticular penetration (Babiker and Duncan, 1975a). Another possible effect of higher humidity is to reduce the rate at which aqueous droplets dry. This would in effect keep the chemicals in solution and therefore in a physical state more compatible with a foliar uptake pathway.

In Experiment Ic (where droplets were allowed to dry out before exposure to high relative humidity conditions) the same pattern of uptake was observed. That is, test chemicals containing five membered heterocyclic groups were taken up to a greater extent than those containing six membered heterocyclic groups. A significant decrease in the uptake of sulphacetamide and a significant increase in the uptake of asulam and sulphamethizole was noted, in comparison with the results

of Experiment Ib.

It would seem that, on the whole, drying droplets under low humidity conditions then exposing the chemical residues to high humidity conditions results in the same patterns of uptake as those observed when droplets were exposed to high relative humidity conditions from the outset (ie. Experiment Ic compared with Experiment Ib). This seems to suggest that dried residues re-wet when humidity is increased. One might expect to observe an increased uptake since the chemicals will be in a more concentrated form upon re-wetting (Cook, 1979), but this was only the case with two of the test chemicals. Perhaps differences in solubilities accounts for the observed behaviour under these particular conditions.

The additions of Tween 20 at a concentration of 0.1% (w/v) caused marked changes in the uptake of several sulphonamides and with the exception of sulphamethizole and asulam this only occurred when droplets were allowed to dry out under low humidity (ie. Experiment IIc).

Under low humidity conditions uptake of all chemicals was poor therefore it would seem that in such conditions Tween 20 at this level has no effect of foliar uptake. From the results of Experiment IIb (where droplets were exposed to high relative humidity conditions immediately after application), it can be seen that, like before, the sulphonamides are much more effectively taken up under high relative humidity

conditions. It was observed that the surfactant significantly increased the uptake of only sulphamethizole compared with the equivalent experiment with only aqueous formulation. Babiker and Duncan (1975a) observed previously that Tween 20 at a concentration of 0.2% (w/v) resulted in a twofold increase in the uptake of asulam by bean leaves under the same conditions, compared with aqueous controls. In this experiment only a 14% increase was observed. Perhaps the concentration of Tween 20 was critical for the improvement of uptake. When droplets were first allowed to dry before exposure to high humidity conditions, 0.1% Tween 20 significantly increased the uptake of Asulox, asulam, sulphacetamide, sulphamethazine and the five sulphonamides containing five membered heterocyclic groups. The uptake of the other test chemicals was of the same order of magnitude as before.

The enhanced uptake of nine of the test chemicals compared with aqueous formulations under the same conditions (Experiment Ic) is in agreement with the results reported by Cook (1979) for aminotriazole. Although it is not easy to differentiate between the possible effects of Tween 20 on cuticle permeability and its effects on the solute, Cook (1979) suggested that solubilisation of aminotriazole in the surfactant played a major role in its penetration. Similarly, there is evidence to suggest that Tween 20 acts as a cosolvent for

asulam as well as other carbamates and sulphonamides, since it is known to prevent molecular association and aggregation (it is thought to reduce hydrogen bonding between the imino group on one molecule and the carbonyl of the next, (Barker et al., 1948; Chaplin and Hunter, 1937)). In the case of asulam, it was suggested that this effect of Tween 20 would result in a finer dispersion of the herbicide and because Tween 20 is non-volatile, recrystallisation of the asulam on the leaf surface would be prevented (Babiker and Duncan, 1975a). It might be the case that Tween 20 is acting as a cosolvent for most, but not all, of the test chemicals under these conditions. If one compares the results of Experiment IIc with those of IIb it can be seen that Tween 20 only had this effect when droplets had first been allowed to dry out before exposure to high relative humidity conditions (Experiment IIc). Perhaps this indicates the formation of highly concentrated films of chemical in Tween 20 which were available for uptake since they were in solution (unlike the equivalent aqueous situation where droplets would completely dry out before exposure to high humidity conditions). Babiker and Duncan (1975a) suggested that the hygroscopic nature of Tween 20 will encourage condensation to take place on the leaf surface at the site of deposition of the spray droplets. In this way a continuous supply of chemical may be maintained and contact with the aqueous continuum

of the cuticle established.

Possibly different solubilities of the test chemicals in Tween 20 might account for the variation in the degree of enhancement in uptake. The sulphonamides containing six membered heterocyclic groups are less polar than the other test chemicals and therefore, perhaps, less compatible with the Tween 20 cosolvent. The increased uptake of sulphamethazine, however, is in disagreement with this. The results of Hotson (1953) is also in disagreement with this.

One cannot ignore a possible effect of the surfactant or the cuticle itself, although it would seem likely that an increased permeability through a direct effect on the cuticle would be observed in the case of all test chemicals.

Cook (1979) reported that Tween 20 is indeed taken up by bean leaves and this is consistent with other observations (Price, 1976). It has been suggested that surfactants taken up in this way could change the viscosity of the cuticle locally by mixing or comicellizing with amphipathic acids and alcohols of the wax (Price, 1982). Generally, perhaps by modifying the hydrophilic-lipophilic interfaces within the cuticle, Tween 20 increases the size and accessibility of aqueous routes.

Babiker and Duncan (1975a) suggested that if Tween 20 is a cosolvent then its distribution will

influence the uptake of asulam (and other chemicals for which the surfactant behaves as a cosolvent) in that uptake is a function of the equilibrium distribution ratio of the herbicide between the carrier and the leaf surface. This might explain the variation in uptake seen in Experiment IIc but only if there were differences in the solubility of the test chemicals in the surfactant.

It may even be possible that differences in size and in particular molecular radii of the test sulphonamides could account for the differences in foliar uptake. The precise significance of the molecular radius of a chemical in diffusion across the cuticle is not fully understood. It has been suggested, however, that considering the heterogeneity and molecular sieve characteristics of the cuticle, the minimum molecular radius of a chemical may be important for uptake (Price, 1982). It might be that the sulphonamides containing six membered heterocyclic groups have a larger molecular radius than the others and penetrate the cuticle less readily as a consequence. In view of the increased uptake observed when Tween 20 was added to formulations, it might be that the surfactant does have some direct effect on the cuticle and that this effect results in an increased permeability to sulphonamides of a certain molecular radius ie. sulphonamides containing aliphatic and five membered heterocyclic groups.

From the results of Experiments III and IV it can

be seen that there was no significant increase in the uptake of the test chemicals when Tween 80 and ammonium thiocyanate were included in formulations.

It had been considered feasible that Tween 80 may have been a better choice of surfactant for the less polar sulphonamides. However this proved not to be the case at the particular concentration used. Perhaps a different concentration would have been more successful. Results do show that it had no detrimental influence on foliar uptake.

The ineffectiveness of ammonium thiocyanate in improving the uptake of the test chemicals was surprising particularly in view of its success in the past (Jaff, 1983; Cook, 1979). It can be concluded that under the experimental conditions employed in this experiment the salt did not function in the same way as described by the above authors, or did so, but not in a way which improved cuticular penetration.

CHAPTER 4

THE DEGRADATION OF SELECTED SULPHONAMIDES IN SOIL

Introduction

It was shown in Chapter 2 that most of the test chemicals were effective, to varying degrees, at reducing the growth of test plant seeds when in intimate contact with the seeds. This type of activity could be useful as a means of controlling weeds in the field through soil application. Soil application for the control of bracken was an area in particular where there was scope for new herbicides. The general erratic performance of foliar applied herbicides for this purpose and their inability to provide long-term control of bracken was highlighted by Stephen (1983). He further suggested that bracken control by soil application would have the advantage that application of the herbicide could be carried out when farm labour was less urgently needed for other operations. Another practical advantage would be that it would be easier to cover difficult terrain when the ground was visible.

Use of asulam as a soil applied herbicide

There is conflicting evidence as to the effectiveness of asulam when applied to the soil. Ball

et al. (1965) reported that asulam showed a high level of activity when applied to a range of soils including a sample with a high organic matter content. Although it was found that the herbicidal activity of asulam was not influenced by soil type, persistence studies did reveal that there was significant loss of activity over a three week incubation period. They concluded that both asulam and its 4-nitroanalogue were rapidly degraded in soil.

Menges et al. (1972) showed that asulam gave good control of weeds in loamy sand soils planted with water-melon. The organic matter content of the soils was reported to be low (0.7 - 0.8%). Similarly, Liefstingh et al. (1971) reported that asulam gave good weed control and selectivity in poppy fields. At a level of 1.8 $\mu g g^{-1}$ in the soil, asulam was found to give 50% inhibition of oat seedling roots.

In contrast to this, Brock et al. (1972) observed that asulam gave only short-term pre-emergence control of docks on a sandy loam soil.

Williams (1980) also reported that asulam had very limited activity as a soil applied herbicide for the control of bracken. Although the reasons for this lack of activity were not discussed, it was suggested by Stephen (1983) that difficulties encountered in reaching developing and dormant buds of the rhizome system could account for this lack of activity. In fact, it had been reported that soil conditions likely to result in high

rates of microbial activity would result in low asulam persistence in the soil and hence poor weed control (Anon, 1971). These conditions included high levels of organic matter, high temperatures and high moisture contents. Since soils found under bracken often have top soils high in organic matter (Mitchell, 1973) it seemed likely that the observations of Williams (1980) were stimulated by rapid asulam degradation in treated soils.

Low residence time of asulam in soils - microbial breakdown

Rapid microbial breakdown has limited the use of asulam as a pre-emergence herbicide. Babiker and Duncan (1977) reported rapid rates of breakdown in top-soil. In one soil type only 21% of the rapid asulam remained in a sample of the topsoil after 5 days. In samples of subsoil from the same profile, persistence was much greater and increased as the profile descended. In addition, amendment of subsoil with yeast was found to enhance breakdown. From these observations it was concluded that conditions conducive to microbial activity ie. higher organic matter and readily available substrates, would reduce asulam persistence in soils.

Similar results were reported by Smith and Walker (1977). Asulam is commonly used in Canada for the control of wild oat (Avena fatua L.) in flax (Linum usitissimum L.). Using a soil sample from an area under

flax, they found that asulam degradation was rapid. At temperatures in the range of 20 - 35°C and at soil moisture contents greater than 50% of field capacity. Asulam was found to have a half-life of approximately 7 days. When one considers that the organic matter content of the soil used in this study was much lower than is commonly found in soils under bracken in Scotland, then this rate of breakdown was indeed rapid.

Using samples from two soils found under bracken, Stephen (1983) also observed that asulam degradation was very rapid. Degradation was greatest in soil with the highest organic matter and moisture content. Application of a variety of surfactants and carbamate additives were not found to significantly reduce the rate of asulam degradation.

Smith and Milward (1984), while investigating the products of asulam metabolism in three Canadian soils, found that asulam was rapidly degraded over a three week period. During the first 4 days of incubation, 40% of the herbicide was broken down in all three soils.

Thus, asulam has generally been found to have a low residence time in several different soils. The herbicide seems to be particularly susceptible to microbial breakdown and insufficient contact with roots and with weed seeds would account for the widely observed poor field performance.

Use of sulphonamides as soil-applied herbicides

Investigation of their suitability for soil application seemed appropriate for several reasons.

Their biological activity during bioassays was similar to that of asulam and several chemicals were as phytotoxic. However, variation in the chemical and physical properties of these chemicals, as a consequence of differences in structure, might reasonably be expected to lead to different soil behaviour in comparison with that of asulam. That is, variation in their solubilities and in the degree of ionisation of the imino proton would possibly be expected to give different patterns of adsorption, leaching and biodegradation. The table below illustrates the different values for the chemical studied in this Chapter.

Sulphonamide	Solubility in water mg/100cm^3 at 25°C	pKa
asulam	500	4.8
sulphadiazine	8	6
sulphamoxole	soluble	7.14
sulphacetamide	670	5.4

(data from Anand, (1979))

Since sulphonamides are used chemically as bacteriostatic agents, the possibility seemed to exist that the test chemicals might be less readily degraded by the soil microflora. Should this be the case then an increased residence time in the soil would facilitate contact with roots and promote weed control, should the chemicals be sufficiently phytotoxic.

Investigation of sulphonamide breakdown in soil

Microbial breakdown was considered to be the parameter most likely to limit the use of these chemicals in the soil. The main aim of this work, therefore, was to investigate their persistence in soil. Two Scottish soils, one infested with bracken, were selected for this purpose.

Asulam was included in the study as a means of comparing its persistence with that of the other test chemicals. In addition it was apparent from previous studies that although the herbicide was rapidly degraded in a variety of soil types, there was very little information about the metabolites formed. In some cases sulphanilamide was identified as a metabolite but it was found in quantities that accounted for only a fraction of the asulam metabolised. Other studies were unable to identify any such primary amine products. This will be discussed more fully later.

Thus, another aim of the work was to investigate the likely pattern of metabolism of asulam and test chemicals to predicted breakdown products such as sulphanilamide.

EXPERIMENTAL

Materials

Asulam (90.5% purity) was purchased from the National Physical Laboratory. Sulphanilamide and sulphanilic acid were purchased from May and Baker Ltd. All reagents were obtained at the purest grades available. All HPLC solvents were purchased from Rathburn Chemicals Ltd. at HPLC grade and analar grade solvents were used for all TLC analyses.

The soils used in the study were collected from A horizons of two different soil types found in West-Central Scotland. The soils are described as follows:

Darleith: site located at Carbeth in Strathblane, Dumbartonshire. The Grid reference is NS 521785. The soil belongs to the Darleith Association which is developed on till derived from Carboniferous age igneous rock. The soil series is Darleith and the soil sample was taken from the organic rich A horizon of a freely drained brown forest soil,

characteristic of steeper slopes. The area was covered with bracken.

Dreghorn: site located at Arkleston Farm, Paisley. The Grid reference number is NS 508655. The soil belongs to the Dreghorn Association which is formed from raised beach deposits. The soil series is Dreghorn and the soil has been classed as a freely drained brown forest soil. It is used for intensive cultivation of potatoes, barley and oilseed rape.

Relevant analytical data of the soils is presented in Table 4.1.

Table 4.1 Analytical data for the soils used in the study

Soil	Darleith	Dreghorn
pH	5.5	5.6
% sand	46.7	51.2
% silt	30.2	25.7
% clay	22.9	23.1
% OM	15.3	6.5
Soil type	Clay loam	Sandy clay loam

Data is by courtesy of Dr Quasim

Soil preparation

(i) Treatment and storage

Soil samples were collected as close as possible to the start of degradation studies. This was designed to reduce possible deterioration of the microbial biomass during storage. Fresh samples were sieved through a 2 mm sieve to remove stones and other unwanted debris and were stored in sealed polythene bags at a temperature of 8 ± 1°C. This temperature would reduce microbial activity thereby preventing possible exhaustion of natural substrates and deterioration of the microflora. Samples were allowed to equilibrate for 2 - 3 days at room temperature before the start of experiments.

The exception to this was a sample of Dreghorn soil which was intentionally stored at room temperature for a period of eight weeks before use in certain degradation experiments. The purpose of storing the soil under these conditions was to try and reduce microbial populations through exhaustion of natural substrates. Comparison of the rate of degradation of test chemicals in this soil with that in fresh soil might illustrate the involvement of the microflora in the breakdown process. Regular aeration of samples was carried out to prevent the development of anaerobic conditions.

(ii) Moisture content

In the past soils have been incubated at a variety of different moisture contents during degradation studies. Since soil moisture content can have a profound effect on the rates of breakdown of pesticides (Anderson, 1981), it is important that soils are incubated at the same moisture content when a comparison of degradation rates is a requirement.

The Environmental Protection Agency (EPA), for example, has stipulated that for the purpose of pesticide registration, soil moisture contents should be maintained at 75% of 0.33 bar throughout degradation studies. (0.33 bar is a measure of the soil moisture content at field capacity.) One third bar is considered to represent the upper limit of soil moisture for microbial activity, and 75% of this value a safe and convenient level within the optimum range.

Bartha and Pramer (1972) suggested that soils should be incubated at a moisture content in the range 50 - 70% of the water holding capacity.

In the experiments carried out in this Chapter, degradation studies were carried out with soil moisture contents adjusted to 0.1 bar. From the physical state of the soil samples, this value was considered to be the most suitable one for maintaining an optimum environment for the soil microflora. It also allowed a sufficient volume of solution to be added when adjusting fresh soil

moisture contents to give an even distribution of test chemicals without soils becoming sticky. This would risk a loss of structure and possible development of anaerobic conditions.

Since both soil types used in this study were incubated at the same moisture content during experiments, it was possible to draw conclusions about the effect of other soil parameters, such as organic matter content, on test chemical degradation. It was a feature of the work of Stephen (1983) that the two soils used in that study were not incubated at the same moisture content. This made it impossible to differentiate between the effects of different moisture contents and the effects of different organic matter contents, on degradation by microflora.

Degradation studies

(i) Application of test chemical

Each of the four test chemicals, asulam, sulphacetamide, sulphamoxole and sulphadiazine, formulated as the sodium salts (pH 9) were added at an appropriate concentration to duplicate 150 g samples of each soil type (O.D., oven-dry, basis). The chemicals were applied in a sufficient volume of solution to raise the moisture content of the soils to 0.1 bar. Control incubations were set up by adding the appropriate volume

of water (pH 9) to duplicate soil samples.

In each case, the test chemicals were applied at a rate of 50 μg/g soil on an equal mole to mole basis. In the studies mentioned earlier, asulam was applied to soils at rates ranging from 2 μg/g to 8 μg/g of soil. Although it is often advisable to apply herbicides at the rate at which they are likely to be applied in the field, three of the test chemicals used in the study had no field use. The rate selected, therefore, was chosen to enable quantification of residues over a reasonable period of incubation, without levels falling too low for accurate quantification. Identification of metabolites was also considered when selecting the rate of application.

Studies were initiated by applying the appropriate volume of test solution to soil samples in 500 cm^3 capacity amber glass screw-cap bottles. The solution was added dropwise over the surface of each soil and samples mixed thoroughly with a spatula. This technique was found in preliminary studies to give even incorporation of test chemical into soils without disrupting the soil structure.

An alternative method of incorporating test solutions into soil samples is to place the appropriate volume of solution on the bottom of the incubation vessel, add the soil and allow the solution to move into the soil by capillary action (Bartha and Pramer, 1972).

Although this may more effectively maintain the integrity of the soil structure, it is likely to take some time before samples become homogenous. For early sampling points this would be a problem.

(ii) Sampling intervals

Duplicate 5 g samples of soil (O.D. basis) were removed from each incubation jar at the following times over a three week period:

0 hour (immediately after application of test material), 3,6,9,12,15,18 and 21 day. Residue extraction and quantification was carried out immediately after the removal of duplicate samples (see residue extraction procedures).

The choice of incubation period and sampling frequency was based on the results of other studies.

Babiker (1977) sampled soils after 5, 10 and 18 days during an investigation into asulam persistence whereas Smith and Walker (1977) measured asulam residue levels at 7 and 14 days over a two week incubation period. Smith and Milward (1984), on the other hand, sampled soil incubates at 4, 8, 14 and 21 days whereas Stephen (1983) quantified asulam levels at the beginning and end of an 8 day study.

All four studies reported rapid rates of asulam degradation. Consequently, in this study, soils were sampled more frequently to give a more realistic

breakdown pattern. This was particularly true in the early stages of the study.

(iii) Soil aeration and moisture

Bartha and Pramer (1972) reported that 1 g of an average soil will exhaust the oxygen content of 1 cm^3 of air over a 24 hr period. Consequently, they recommended that if the ratio of vessel volume:sample weight is 10 or less, vessels should be aerated daily. Since this ratio was small in these studies, samples were aerated daily by simply removing a cotton wool plug from the neck of the incubation jars and allowing gaseous exhange with the atmosphere.

Extraction and quantification of residues

A preliminary study was carried out to determine which solvents were most suitable for the extraction of residues from soil samples. The main aim was to select a solvent which could efficiently extract both parent chemical residues as well as metabolite residues from the soil. A volatile organic solvent which could be reduced in volume and which met this criterion would be ideal. Such properties would facilitate accurate quantification of parent residues. Concentration of low levels of metabolites would be an aid to their subsequent identification.

The main factors which limit the use of an extraction solvent are the solubility of a test chemical in that solvent and the ability of the solvent to remove the test chemical from a biological matrix. However, a preferred method of clean-up and/or analysis can impose restrictions on the choice of extraction solvent as well. For example, to use the Bratton-Marshall (Bratton and Marshall, 1939) method for the quantification of aromatic amines, the test chemical would have to be present in a solvent which was miscible with the aqueous reagents of this method. Similarly, use of a solvent of low polarity to extract sulphonamide residues would make it difficult to use HPLC (reverse phase) directly for the quantification of residues. It might be possible to use a solvent partition or to remove the immiscible extraction solvent under reduced pressure. This can be time consuming and can lead to poor recoveries.

(i) Sulphonamide clean-up

A method reported by Haagsma and Van de Water (1985) described the extraction of five test sulphonamides, including sulphanilamide and sulphadiazine, from swine tissue using chloroform/acetone (1:1). Extracts were passed through solid phase cation-exchange extraction columns and bound residues eluted from the columns using methanol. HPLC (reverse phase) was used for residue separation and

quantification.

Although the initial extraction of the test chemicals was based on their respective solubilities in the solvent used, and therefore might not be suitable for all sulphonamides, the clean-up was based on their amphoteric properties. Use of the clean-up method for other sulphonamides was recommended.

There was reason to believe, therefore, that sulphonamide residues could be extracted and purified from soil samples using this method. Residues would also be in a solvent which was compatible with either of the analysis methods used (see later).

However, in practice, the cation-exchange extraction columns were not available so the method was not developed further. It should be pointed out, though, that theoretically, there seems to be no obvious reason why this method could not be used for the extraction and purification of asulam as well as sulphonamide residues from biological samples.

(ii) Extraction solvents (preliminary study)

It was apparent from the work of Haagsma and Van de Water (1985) that chloroform:acetone (1:1) gave efficient extraction of five sulphonamide drugs of considerably different solubilities. This particular solvent seemed like a useful one to use for the extraction of the test chemicals from soil samples. An

earlier publication by the same authors (Haagsma et al., 1984) recommended the use of dichloromethane for sulphonamide extraction although they stated that, generally, chloroform:acetone (1:1) is a common solvent to use for extracting sulphonamides from biological tissues. For these reasons, it was decided to test both solvents for the extraction of the test chemicals from soils.

In the past a wide range of different solvents have been used for the extraction of asulam and its metabolites. Babiker and Duncan (1977) and Stephen (1983) extracted asulam residues and possible metabolites from soil by shaking soil samples with acetate buffer (pH 5.6) for 3 hours. In both cases, asulam residues were coupled with the Bratton-Marshall reagent N-(1-naphthyl)ethylene diamine dihydrochloride and concentrated by extracting the coloured complex into a smaller volume of butan-1-ol. Although this procedure is useful for asulam quantification, it seemed unlikely that extracts could be concentrated sufficiently to allow metabolite identification. In addition it was reported in an earlier publication (Smith and Walker, 1977) that the coloured complex formed between sulphanilic acid and the Bratton-Marshall reagent was insoluble in butan-1-ol. This would make it difficult to detect this compound and there was reason to believe that it could be generated as an asulam degradation product.

For these reasons it seemed unlikely that acetate buffer would be of much use in this study. In addition, direct concentration of an aqueous extract would be difficult. It was decided, however, to employ this extractant in the preliminary study in order to compare it with other solvents.

Smith and Walker (1977) used calcium hydroxide for the extraction of asulam, sulphanilic acid and sulphanilamide from soils. Franci et al. (1981) used calcium hydroxide as well as water and ethanol for the extraction of residues.

It was decided, however, to test methanol and acetone as extractants along with chloroform:acetone (1:1), dichloromethane and acetate buffer (pH 5.6) as described previously. Smith and Milward (1984) used methanol to extract asulam, sulphanilamide and sulphanilic acid residues from soil, whereas Guardigli et al. (1984) successfully used it to extract asulam from plant samples. Although acetone had never been used to extract asulam residues from the soil it had been effectively used to do so from plant samples. Brockelsby and Muggleton (1973) and Veerasekaran (1976) all used acetone for this purpose.

Hence in the preliminary study, five solvents were tested as extractants for each of the test chemicals:

1. acetone:chloroform (1:1)
2. dichloromethane
3. acetate buffer
4. acetone
5. methanol

(iii) Experimental procedure for testing extraction solvents

1. Duplicate 5 g (O.D. basis) soil samples were weighed into 100 cm^3 capacity screw cap top bottles (teflon tape was used to seal the tops and prevent loss of solvent).
2. Each of the test chemicals were applied to the soil samples in aqueous solution, at the same concentration used in the main study.
3. A 50 cm^3 volume of extractant was added to each of the screw-top bottles. 15 g of anhydrous sodium sulphate was added and the bottles placed on an end over end shaker for either one hour or three hours (anhydrous sodium sulphate was omitted in the case of the acetate buffer).
4. Extracts were filtered through Whatman No. 42 filter paper into amber glass screw-top bottles.
5. Aliquots were removed from the storage bottles and residues quantified by the Bratton-Marshall method (see Chapter 3).

Notes

(i) Sodium sulphate facilitated a better contact between the less polar solvents and the soil particles. By removing water associated with the soil it also prevented partition of residues into the soil water rather than the extraction solvent. In the main study, it gave dry samples which were then readily concentrated.

(ii) In the case of chloroform:acetone (1:1) extracts and dichloromethane extracts, solvent was removed under reduced pressure and residues re-dissolved in methanol prior to quantification by the Bratton-Marshall method.

(iv) Choice of extraction solvent

It was found that acetate buffer and methanol were the most efficient extractants in all cases. Acetone gave high recoveries of test chemicals but a precipitate was found to form during quantification by the Bratton-Marshall method. Filtration of extracts through a 0.45 micron cellulose-nitrate membrane overcame this problem but was time consuming.

In the main study, therefore, methanol was used as the extractant since it gave efficient recovery of all test chemicals as well as the potential metabolites sulphanilic acid and sulphanilamide (>90%). In addition, methanol extracts could be readily reduced in volume.

Samples were extracted for one hour in the main study. A three hour extraction as reported by Stephen (1983) gave no advantage over a one hour extraction. In fact, in the case of sulphadiazine, recoveries were lower when soil samples were extracted for three hours.

(v) Definitive extraction procedure

1. Duplicate 5 g (O.D. basis) soil samples were taken from each incubation flask and weighed directly into 100 ml plastic screw-top bottles.

2. Exactly 50 cm^3 of methanol was added to each bottle. 15 g of anhydrous sodium sulphate was added and the bottles placed on an end over end shaker for one hour.

3. Extracts were filtered through Whatman No. 42 filter paper into amber glass screw-top storage bottles.

4. Aliquots were removed from the storage bottles and residues either quantified directly or after concentration by the Bratton-Marshall method.

Metabolism

Smith and Walker (1977) suggested that the most likely products of asulam soil metabolism would be sulphanilamide and sulphanilic acid. To determine whether this was the case, they treated soil samples with asulam and incubated them under a variety of conditions.

Residues were extracted with calcium hydroxide, coupled with N-(1-napthyl) ethylene diamine dihydrochloride and separated on silica gel 60 Fe_{254} TLC plates in an acetone:ammonium hydroxide (19:1) solvent system. Although asulam degradation was rapid they failed to identify either sulphanilamide or sulphanilic acid. It was concluded that hydrolysis to these products was not a major pathway for the herbicide.

It was also reported by Babiker and Duncan (1977) that asulam degradation was rapid but no aromatic amines such as sulphanilamide were detected using TLC separation. Similarly, Stephen (1983) was unable to identify any aromatic amine metabolites of asulam when soil extracts were separated by TLC.

Fusi et al. (1981) described the non-biological degradation of asulam when the herbicide was adsorbed by clays, and the complex heated. Breakdown products including sulphanilamide and sulphanilic acid were separated on silica gel 60 Fe_{254} TLC plates in a chloroform:methanol (5:2) solvent system. The condition employed in these experiments cannot reasonably be extrapolated to environmental conditions however.

More recently, Smith and Milward (1984) reported that although no degradation products had previously been reported from soils treated with asulam, sulphanilamide and sulphanilic acid were the most likely products of metabolism. They applied asulam to three different soil

types and at intervals, residues were extracted with methanol and separated on silica gel 60 Fe_{254} TLC plates using chloroform:methanol (1:1) and acetic acid:methanol:benzene (1:1:3) solvent systems. A fluorescamine spray was used to detect primary amines down to a level of 10 ng.

They found no trace of sulphanilic acid in any of the soil extracts. Sulphanilamide, on the other hand, was recovered from all of the soils at all sampling dates. The amounts detected were dependent upon soils type although less than 10% of the applied herbicide was converted to sulphanilamide despite its rapid degradation.

Using fluorescamine to detect low levels of primary amines, Kurucova and Josef (1983) reported the detection of asulam residues indirectly through the identification of its main metabolite, sulphanilamide, in waters, soils and plants. Residues were separated by two-dimensional TLC.

Thus, the evidence from the literature suggested that although asulam metabolism was rapid, degradation products were identified only occasionally, and at levels which did not account for the amount of parent herbicide degraded. The aim of this study was to chromatograph soil extracts against sulphanilamide and sulphanilic acid reference standards and determine whether these chemicals were the likely products of sulphonamide metabolism in

the soil.

Thin-layer Chromatography

(i) Method development

Since Smith and Milward (1984) discussed the highly specific and sensitive nature of fluorescamine as a means of detecting low levels of primary amine metabolites, this compound was used as a chromogenic reagent for developed TLC plates. Preliminary investigations revealed that it was possible to detect 10 ng levels of all 4 test sulphanilamides as well as sulphonamide and sulphanilic acid.

A variety of solvent systems were tested as a means of separating test chemicals from sulphanilamide and sulphanilic acid on silica gel 60 Fe_{254} plates:

1. Acetonitrile:ammonia (10:1)

This solvent system was used by Veereskaran et al. (1976) to separate the products of asulam metabolism in biological samples. Although TLC plates developed in this solvent separated the four test chemicals from sulphanilamide and sulphanilic acid both sulphacetamide and asulam were found to chromotograph poorly as tailing peaks.

2. Acetonitrile:acetic acid (10:1)

This solvent was tested as a comparison with the

one detailed above. It was found to prevent tailing of the test chemicals but did not resolve them.

3. Chloroform:methanol (1:1)

Smith and Milward (1984) used this solvent to separate asulam from sulphanilamide and sulphanilic acid in soil extracts. Although it was found to resolve the test chemicals from sulphanilic acid it did not separate them from sulphanilamide.

4. Butanol:chloroform (1:4)

Haagsma and Gortemaker (1984) reported a rapid thin-layer chromatographic screening method for five different sulphonamides in animal tissues. From their success it was decided to test this solvent system as a means of separating the test sulphonamides in this study. It was found to effectively resolve sulphamoxole and sulphadiazine from sulphanilamide and sulphanilic acid but not asulam and sulphacetamide.

5. Butanol:chloroform (4:1)

The test chemicals were chromatographed but with the two solvents in the opposite proportions from that used before. This system was found to separate all 4 test chemicals from sulphanilamide and sulphanilic. It was decided, therefore, to chromatograph the soil extracts with this solvent system.

Further investigation revealed that this solvent still gave good resolution when test chemicals were

applied as a mixture or when mixtures were dissolved in blank soil extracts the chromatographed.

Chemical	Rf
asulam	0.49
sulphacetamide	0.61
sulphamoxole	0.55
sulphadiazine	0.60
sulphanilamide	0.77
sulphanilic acid	origin

(iii) Sample treatment

It was earlier described how each test chemical was applied to duplicate 100 g (O.D. basis) samples of each soil type. Duplicate 5 g (O.D basis) samples were removed from each replicate at specific time intervals. This effectively generated 4 x 50 cm^3 methanol extracts from each soil type at each sampling interval.

Rather than chromatograph each extract, a 10 cm^3 aliquot was taken from each of the four extracts, the solution pooled and evaporated to dryness. The residue was then re-dissolved in 250 μl of methanol and either 10 μl or 25 μl of this solution applied to a silica gel 60 Fe_{254} TLC plate. Each plate was developed in butanol:chloroform (4:1) with extract spots being co-chromatographed against a reference mixture of test

chemical, sulphanilamide and sulphanile acid.

After development, solvent fronts were marked and plates left to dry at room temperature. Each plate was then sprayed with fluorescamine solution (12 μg in 100 cm^3 acetone) in the dark. After a period of 20 minutes plates were viewed under long-wave ultraviolet light where aromatic amines appeared as a fluorescent spot on a purple background.

HPLC

This technique was used in an earlier chapter to quantify sulphonamide residues in aqueous solution. A method was developed in this study which was found to separate each of the test chemicals from sulphanilamide (sulphanilic acid was not included since this chemical was absent from TLC chromatograms).

Initially the aim was to chromatograph extracts for eact test chemical from each soil at each sampling interval. In practice it was found that extracts from the Darleith soil, which had a high organic matter content, were too discoloured to put through the HPLC system. HPLC analysis was, therefore, confined to extracts from Dreghorn soil samples. Only pooled extracts from two sampling points (6 days and 21 days) were chromatographed in the case of asulam, sulphacetamide and sulphadiazine. Before sulphamoxole extracts could be chromatographed, the HPLC column was

found to have blocked irreversibly. Therefore, only TLC separations were used. One of the major problems was that residues were not purified from co-extracts from the soil. Impurities such as this lead to poor chromatography and column deterioration. Details of the method are given as follows:

Sample treatment

(i) A 10 cm^3 aliquot was taken from each of the 4 x 50 cm^3 methanol extracts for each of the test chemicals (except sulphamoxole) at the Day 6 and Day 21 sampling points.

(ii) The 4 x 10 cm^3 aliquots were pooled and the solvent evaporated to dryness under reduced pressure.

(iii) Residues were re-dissolved in 10 cm^3 of mobile phase (see below) and samples filtered through a 0.45 micron cellulose-nitrate filter prior to injection onto the HPLC column.

Chromatography

The HPLC system detailed in Chapter 3 was used for the analysis of soil extracts. In this study 10 μl injections of sample solutions were applied to the phenyl column and eluted with a mobile phase consisting of methanol:acetic acid (0.1%). Isocratic conditions were sufficient to separate each of the test chemicals from

sulphanilamide. The mobile phase consisted of the two solvents in the proportions 20:80 respectively. Under these conditions the following retention times were recorded:-

Chemical	Retention time (mins)
sulphanilamide	1.58
asulam	3.18
sulphacetamide	2.29
sulphadiazine	3.29

(see Figures 4.1 - 4.7)

RESULTS

(a) Asulam

Sampling point (days)	Amount of asulam remaining (μg/g O.D. soil)	
	Dreghorn A	Darlieth A
3	36.3 (1.2)	28.9 (0.4)
6	28.4 (0.9)	15.1 (1.1)
9	22.3 (1.1)	12.4 (0.7)
12	17.9 (0.8)	7.7 (0.4)
15	14.4 (0.6)	5.0 (0.4)
18	11.7 (0.7)	1.3 (0.2)
21	8.3 (0.2)	nd

Figures in parathensis are standard deviations (n-1 D.F.)

(b) Sulphacetamide

Sampling point (days)	Amount of sulphacetamide remaining (μg/g O.D. soil)	
	Dreghorn A	Darleith A
3	34.5 (0.9)	20.9 (1.0)
6	24.7 (0.7)	13.5 (0.8)
9	18.8 (0.7)	9.2 (0.7)
12	16.2 (0.8)	3.3 (0.4)
15	13.3 (0.4)	1.0 (0.1)
18	11.5 (0.7)	nd
21	6.6 (0.5)	nd

(c) Sulphamoxole

Sampling point (days)	Amount of sulphamoxole remaining (μg/g O.D. soil)	
	Dreghorn A	Darleith A
3	29.0 (0.9)	26.3 (0.7)
6	22.3 (1.2)	16.4 (0.9)
9	19.1 (1.1)	8.8 (0.7)
12	15.1 (0.7)	5.7 (0.3)
15	12.2 (0.4)	1.9 (0.1)
18	9.9 (0.4)	nd
21	8.3 (0.3)	nd

(d) Sulphadiazine

Sampling point (days)	Amount of sulphadiazine remaining (μg/g O.D. soil)	
	Dreghorn A	Darleith A
3	30.4 (1.2)	29.7 (0.8)
6	22.3 (0.4)	18.1 (0.6)
9	17.6 (0.9)	10.5 (0.4)
12	15.1 (0.5)	6.3 (0.8)
15	10.4 (0.5)	3.1 (0.5)
18	8.0 (0.3)	1.0 (0.2)
21	5.1 (0.4)	nd

(e) Asulam (2 month old Dreghorn soil)

Sampling point (days)	Amount of asulam remaining (μg/g O.D. soil)
	Aged Dreghorn A
3	41.8 (1.6)
6	36.6 (1.3)
9	30.3 (0.9)
12	24.5 (1.2)
15	19.7 (0.7)
18	16.8 (0.9)
21	14.9 (0.5)

Metabolism

(1) Thin-layer chromatography

(a) Asulam

Sampling point (days) (Dreghorn)	Rf values of fluorescent spots		
3	0.49	0.77	0.94
6	0.49	0.77	0.94
9	0.49	0.77	0.94
12	0.49	0.77	0.94
15	0.49	0.77	0.94
18	0.49	0.77	0.94
21	0.49	-	0.94
Control			0.94

Sampling point (days) (Darleith)	Rf values of fluorescent spots		
3	0.49	0.77	0.94
6	0.49	0.77	0.94
9	0.49	0.77	0.94
12	0.49	0.77	0.94
15	0.49	0.77	0.94
18	0.49	-	0.94
21	0.49	-	0.94
Control			0.94

(b) Sulphacetamide

Sampling point (days) (Dreghorn)	Rf values of fluorescent spots		
3	0.61	-	0.94
6	0.61	0.77	0.94
9	0.61	0.77	0.94
12	0.61	0.77	0.94
15	0.61	0.77	0.94
18	0.61	-	0.94
21	0.61	-	0.94
Control			0.94

Sampling point (days) (Darleith)	Rf values of fluorescent spots		
3	0.61	0.77	0.94
6	0.61	0.77	0.94
9	0.61	0.77	0.94
12	0.61	0.77	0.94
15	0.61	-	0.94
18	0.61	-	0.94
21	0.61	-	0.94
Control			0.94

(c) Sulphamoxole

Sampling point (days) (Dreghorn)	Rf values of fluorescent spots		
3	0.55	-	0.94
6	0.55	-	0.94
9	0.55	0.77	0.94
12	0.55	0.77	0.94
15	0.55	0.77	0.94
18	0.55	0.77	0.94
21	0.55	-	0.94
Control			0.94

Sampling point (days) (Darleith)	Rf values of fluorescent spots		
3	0.55	-	0.94
6	0.55	0.77	0.94
9	0.55	0.77	0.94
12	0.55	0.77	0.94
15	0.55	0.77	0.94
18	0.55	-	0.94
21	0.55	-	0.94
Control			0.94

(d) Sulphadiazine

Sampling point (deys) (Dreghorn)	Rf values of fluorescent spots		
3	0.60	-	0.94
6	0.60	-	-
9	0.60	0.77	0.94
12	0.60	0.77	0.94
15	0.60	0.77	0.94
18	0.60	0.77	0.94
21	0.60	0.77	0.94
Control			0.94

Sampling point (days) (Darleith)	Rf values of fluorescent spots		
3	0.60	-	0.94
6	0.60	-	0.94
9	0.60	0.77	0.94
12	0.60	0.77	0.94
15	0.60	0.77	0.94
18	0.60	0.77	0.94
21	0.60	-	0.94
Control			0.94

Fig 4.1 HPLC trace of sulphadiazine extracted from soil (Dreghorn - Day 6)

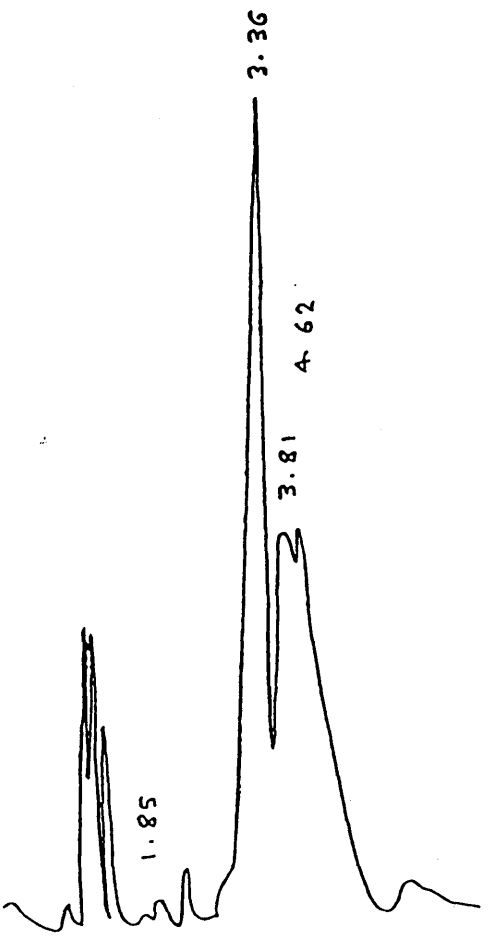


Fig 4.2 HPLC trace of sulphadiazine extracted from soil (Dreghorn - Day 21)

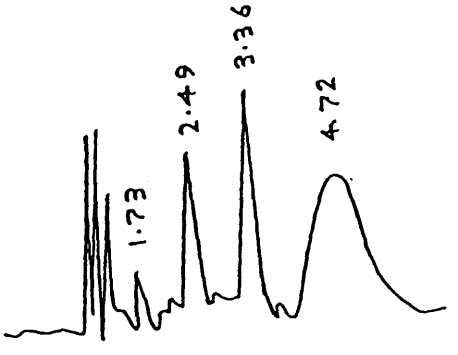


Fig 4.3 HPLC trace of sulphacetamide extracted from soil (Dreghorn - Day 6)

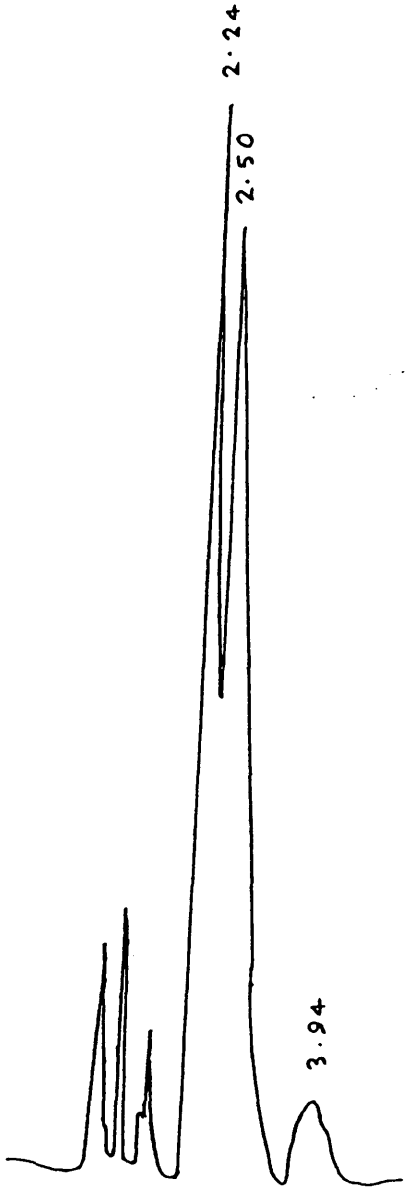


Fig 4.4 HPLC trace of sulphacetamide extracted from soil (Dreghorn - Day 21)

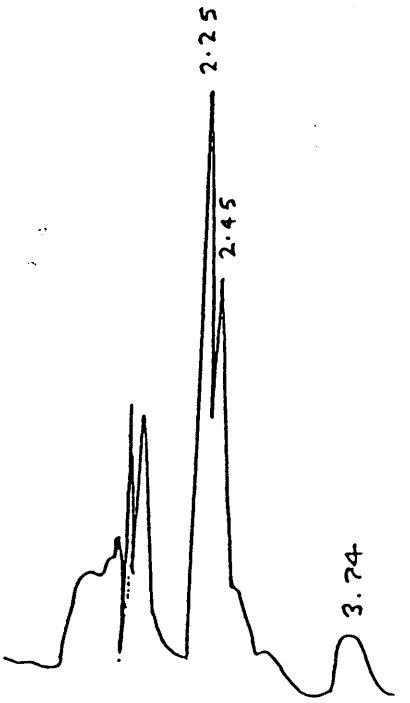


Fig 4.5 HPLC trace of Asulam extracted from soil

(Dreghorn - Day 6)

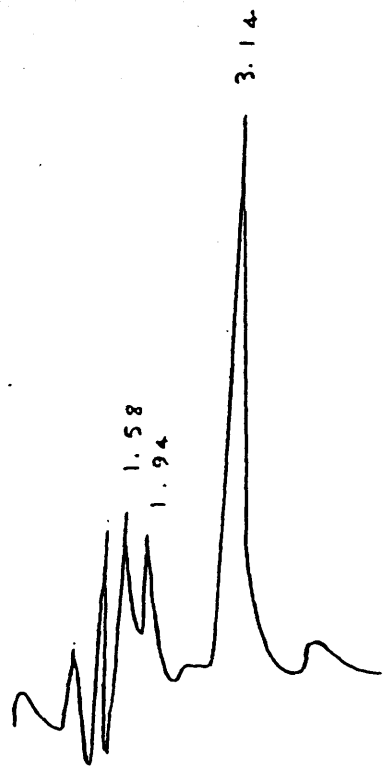


Fig 4.6 HPLC trace of Asulam extracted from soil (Dreghorn - Day 21)

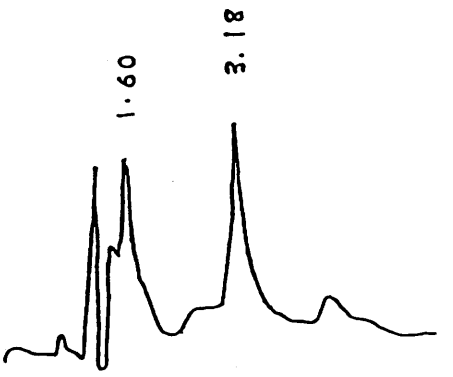
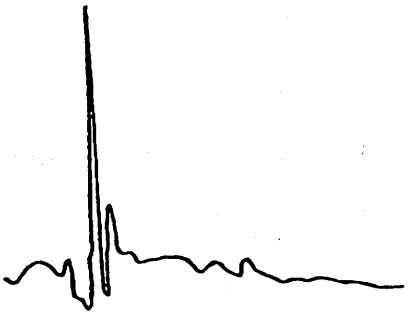


Fig 4.7 HPLC trace of blank methanol extract from soil (Dreghorn)



DISCUSSION

It was found that all four chemicals were rapidly degraded in both soil types. Typically, degradation was most rapid over the first three days of incubation, proceeding more slowly after this. Each of the sulphonamides was more readily degraded in the Darleith soil, with residues falling below the level of detection at Day 18 in the case of sulphacetamide and sulphamoxole and Day 21 in the case of asulam and sulphadiazine.

The Darleith soil had considerably higher organic matter content than the Dreghorn soil and it might be that the greater degree of breakdown arose as a consequence of a larger microbial population associated with this increased organic matter.

The ease with which asulam disappeared from soil samples was in general keeping with earlier publications. To some extent there was reason to believe that the other test chemicals might be more persistent. Kaufman (1967) conducted an investigation into the influence of physicochemical properties on the biodegradation of several phenylcarbamates. It was his conclusion that the structural and electronic properties of a herbicide must be understood before any reasonable basis for predicting its susceptibility to microbial breakdown can be established. Although amide hydrolysis was the mechanism of breakdown in his study, his observations can, to some degree, be extrapolated to sulphonamide metabolism.

Three molecular parameters were found to influence the rate of enzymatic hydrolysis of the test carbamates:-

(i) the size of the alcohol group bonded to the amide moeity was important. Large and bulky alcohols reduced the rate of hydrolysis possibly by reducing the ease with which the carbonyl group could be attacked by some reactive group on an enzyme surface.

(ii) any feature of the carbamates which caused a strong electron withdrawing effect away from the reactive site of the molecule, thereby increasing the relative acidity of the imino nitrogen proton, was found to make hydrolysis more facile.

(iii) the overall size and bulk of the molecule significantly altered the rate of hydrolysis.

Kaufman (1967) suggested that these observations about carbamate hydrolysis may have broader implications in terms of biodegradability of other herbicides. The sulphonamide linkage of the test chemicals used in this study was subject to similar molecular variations. All four chemicals were found to be equally susceptible to breakdown however. It may be that the metabolic processes involved in sulphonamide breakdown were less specific than those involved in carbamate hydrolysis. It would seem that the test chemicals would be no more suitable for soil application than asulam, certainly in

the case of high organic matter soil types.

It was mentioned earlier that it has been a feature of early studies that asulam metabolism in the soil has proceeded via intermediates which have largely remained unidentified. In some cases there was no identification of amine products whereas in others only very small amounts of sulphanilamide were detected.

Bacterial species capable of utilising asulam as a growth substrate have been identified. Walker (1978) isolated a Flavobacterium which was able to grow and degrade both asulam and sulphanilamide. Balba et al. (1979) were able to show that the same Flavobacterium could be induced to metabolize sulphanilate and p-phenolsulphonate, which it generated as products of asulam metabolism, as well as benzene-1,2,4-triol. After a few sub-cultures it lost the ability to degrade asulam and sulphanilamide as did another unclassified Gram-negative bacterium isolated from sulphanilate treated soil (this observation may, in part, explain why the rate of asulam degradation declines with time).

Balba et al. (1979) also isolated three bacterial species from asulam treated soils which were capable of growing in a medium containing the herbicide. One species belonged to the Curtobacterium group and the other two were found to be Pseudomonae. Together the bacterial populations completely metabolized asulam at a concentration of 0.1 g litre^{-1} in 2 - 4 days. Extraction

of the culture fluid and TLC analysis of the extracts showed that sulphanilamide, sulphanilate and p-phenolsulphonate were present as metabolites.

Evidence that the amide linkage in certain sulphonamides is labile came from observations on the metabolism of chlorsulphuron and metsulphuron in the soil. It was reported by Smith and Hsiao (1985) that 15% of chlorsulphuron applied to a sandy loam soil was recovered as 2-chlorobenzenesulphonamide. Similarly, Smith (1986) found that 2-carboxymethylbenzene-sulphonamide was formed as a metabolite of metsulphuron in three soil types while 2-chlorobenzenesulphonamide was generated from chlorsulphuron metabolism.

In this study co-chromatography against reference standards revealed that sulphanilamide was a likely product of metabolism of all four sulphonamides tested. Breakdown of asulam to this product may proceed by a mechanism similar to that observed for chlorsulphuron and metsulphuron. The quantity of sulphanilamide generated, however, could not account for the amount of sulphonamide degraded. It was only present in minor quantities. This suggests that the test chemicals were converted to some metabolite other than an aromatic amine eg. a 4-nitro product, or that aromatic amines were produced but were very rapidly metabolized themselves to some other non-amine products.

CHAPTER 5

AN INTRODUCTION TO PESTICIDE PHOTOCHEMISTRY

The aim of this Chapter is to introduce the reader to the area of photochemistry and to illustrate through the use of selected examples the diverse number and type of photochemical transformations which are known to exist.

A large part of the discussion has been dedicated to the role of natural photosensitising compounds in environmental pesticide photochemistry. It was felt that the increasing number of publications in this area justified their discussion in some detail.

A general introduction is now given and will be followed by a discussion of the electronic changes caused by the absorption of electromagnetic radiation. An understanding of these changes is a prerequisite for the discussion of direct and indirect photochemical reactions.

Introduction

Direct photochemical transformation involves the absorption of light and results in the generation of electronically excited singlet and triplet states. From these excited states various reactions can occur, for example expulsion of halogen atoms from aromatic rings or homolytic re-arrangement involving free radicals.

Pesticides which do not absorb light in the wavelength spectrum of natural sunlight, or which do but not efficiently, will not undergo direct transformation. However, they can be transformed indirectly through interaction with photosensitisers and photo-oxidants such as superoxide, hydroperoxide and hydroxyl free radicals.

The photochemical transformation of pesticides has several very important implications and they should be outlined at the outset.

In most cases there is a loss of activity through transformation to biologically inactive or less active products and this may necessitate the need for large or repeated applications of the parent compound to bring about the necessary level of pest control. This, in effect, may raise the cost of control and in some cases may result in the release of larger amounts of potentially toxic materials into the environment.

Photochemical transformation has also been shown to generate photoproducts about whose environmental behaviour and toxicology very little was known. It may even be possible for the formation of photoproducts which could be more toxic than the parent pesticide.

An extreme example of this was the formation of 1,2,3,4,5,6,7,8-octachlorodibenzo-p-dioxin from the irradiation of an alkaline solution of pentachlorophenol albeit at concentrations unlikely to occur under natural conditions (Wong and Crosby, 1981). In addition, there

is the potential formation of dibenzo-p-dioxins from the widely used phenoxyacetate herbicides. Although no such compound has been identified from any photochemical study of these herbicides, except those present as low level contaminants in some formulations, it is mechanistically feasible that under appropriate conditions dibenzo-p-dioxins could be generated. This is discussed briefly in the next Chapter.

It can be seen from these considerations that by using chemical additives which can reduce or inhibit the photodecomposition of pesticides, it may be possible to manipulate the effective life of residues. Extending the life of residues too much could lead to persistence problems but controlled extension could potentially improve cost-effectiveness by reducing the amount of pesticide required for control.

The environmental implications of this are obvious also, because successful manipulation of pesticide residence time could mean a reduction in both the amount of active chemical reaching the environment, and also the amount of photoproduct generated.

(i) Light absorption and the electronic excited state

When a molecule is irradiated with visible or ultraviolet light it can udergo an electronic transition during which it absorbs a quantum of energy with an electron being excited from its ground state orbital to

another of higher energy. For this to occur the frequency of the irradiating light must correspond to the energy separation between the two orbitals. These two paramenters are linked by the equation, $E = hv$, and this relationship between frequency and energy can be re-arranged to reveal that energy is inversely proportional to the wavelength of the incident light:

$$E = \frac{hc}{\lambda}$$

where h = Planck's constant

v = frequency of light

λ = wavelength of light

c = velocity of light

This means, in effect, that shorter wavelength light can supply more energy, thereby increasing the likelihood of a photochemical transformation.

Electronic excitation, therefore, causes a re-organisation of the valence within a molecule and not surprisingly the chemical behaviour of molecules in the electronically excited state is often considerably different from that of ground state molecules.

Reference to a Jablonski diagram reveals in more detail the electronic changes which can occur when electromagnetic radiation is absorbed by a molecule. Almost without exception, molecules in the ground state exist as singlets and because transitions between states

of different multiplicity are forbidden, electronic excitation leads to the generation of excited singlet states. The amount of available energy determines which state is generated.

Since any electron can be promoted to an empty orbital and the empty orbitals can be antibonding orbitals such as π^* or σ^* or higher atomic orbitals such as 3d or 4p, for example, there are many possible excited states.

This Law of Conservation of Spin means, in effect, that direct conversion from the ground state singlet, S_0, to the first excited triplet state, T_1, is of exceedingly low probability (singlet states have spin paired electrons whereas in the triplet state the molecule is a diradical having electrons with anti-parallel spins). Triplet states are much longer lived than singlet states and it is from the triplet state that the majority of photochemical reactions occur. This usually means that for an excited molecule to react photochemically, the triplet state has to be generated. This can occur through a process known as intersystem crossing, or it can proceed via the interaction of a donor photosensitiser molecule with a receptor molecule in the ground state.

If the excited molecule is in a higher singlet state such as S_2, for example, it returns to the singlet state of lowest energy by a series of radiationless

transitions known as internal conversion. It is from this lowest excited singlet state that intersystem crossing can occur, generating the first excited triplet state by this radiationless crossover involving spin flipping.

Alternatively, the excited molecule can react chemically from the excited singlet state, or it can give up its excess energy as heat, or it can return to the ground state singlet by emitting radiation as fluorescence.

Generation of the triplet state means that photochemical reaction is more likely but it can be possible for an excited electron to return directly from the triplet state to the ground state singlet. This is a process known as phosphorescence but such conversions are of low probability.

The importance of generation of the triplet state for photochemical reaction has been mentioned but for many molecules intersystem crossing from the first excited singlet state is not an efficient process. This may not totally exclude the possibility of photochemical reaction, however, because it may be possible to generate the triplet state through a sensitiser molecule.

Sensitisers are molecules which can be converted, on absorption of light of sufficiently high energy, to the triplet state in relatively high yields. If the energy of the sensitiser triplet is higher than that of

the receptor molecule triplet, it may be possible for the direct transfer of the sensitiser excitation energy to the receptor molecule converting it to the first excited triplet state.

This transfer of energy requires overall spin conversion and the end result is that the receptor molecule is converted to the first excited state triplet while the sensitiser returns to the ground state singlet.

In effect, it may be possible for sensitisers to facilitate photochemical transformations under conditions which could not normally be conclusive to this. These compounds were mentioned earlier and it is becoming increasingly clear that they may have an important role to play in the environmental photochemical transformation of pesticides. Photosensitisers have been identified in plants (Dodge and Knox, 1986) and have also been identified as important intermediates in the photodecomposition of pesticides in soil (Khan and Gamble, 1983) and in natural and agricultural waters (Khan and Schnitzer, 1978; Miller et al., 1980 and Ross and Crosby, 1973).

This concludes the discussion on the electronic re-arrangement caused when a molecule absorbs light. In practice, the likelihood of a photochemical transformation depends on whether the wavelength of the irradiating light is low enough to supply sufficient

energy, and whether the pesticide can efficiently absorb this light.

(ii) Selected examples of pesticide phototransformations under laboratory and natural conditions

a) Direct transformation on absorption of ultraviolet light

The aim of this section is to provide the reader with selected examples of pesticide photochemical transformations, as a means of illustrating the types of reactions which have been shown to occur under both laboratory and natural conditions.

On reviewing the literature on this area, it soon became apparent that virtually any pesticide could theoretically be transformed by the absorption of sufficiently energetic light. Further, it was found that those which could not be transformed directly either because their absorption maximum was outside the spectrum of irradiating light, for example the phenoxyacetates, or because they were inefficient absorbers in the particular region of the spectrum available to them, could be transformed indirectly through the action of photochemically generated oxidants, as well as through the action of natural photosensitisers.

Since there was such a diverse number and type of published studies of pesticide photochemistry, examples

were selected which were either related to the compounds investigated in the next Chapter, or which could demonstrate the particular susceptibility of pesticides to light. In this way, it was possible to emphasize the need and relevance of further research into the area of pesticide photochemistry and at the same time highlight the potential application of chemical additives to modify this photochemistry.

From the literature, it was noticed that even apparently chemically stable compounds could be transformed. This was illustrated by investigations of the behaviour of both DDT [1,1 bis(p-chlorophenyl)ethane] and pentachlorophenol. Both would normally be considered to be inherently stable compounds but they have been found to undergo photochemical degradation readily in aqueous solution, organic solvents and air (Moilanen, Crosby and Soderquist, 1974; Moilanen and Crosby, 1975; Crosby and Moilanen, 1977; Crosby, 1979; Wong and Crosby, 1981).

DDT, for example, has been shown to undergo photochemical transformation to a variety of products and it can be seen that the vapour phase transformation closely parallels that of the aqueous phase transformation.

Initial reaction of the light energised DDT molecule is dehydrochlorination to produce DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene], and it

can be seen, therefore, that DDE photochemistry parallels that of DDT.

From the identification of the intermediate [1,1-dichloro-2,2-bis-(p-chlorophenyl)ethylene oxide], it was postulated that DDE subsequently undergoes photo-epoxidation and that re-arrangement of the epoxide to a carbonyl group generates 4,4-dichlorobenzophenone. This intermediate was shown to undergo decarbonylation to form the dichlorobiphenyl product, which was stable to further photodecomposition and hence represented the end-product of DDT phototransformation.

Pentachlorophenol photochemistry has also been studied extensively and the photodecomposition of this compound was the first example of the complete degradation of a pesticide to mineral products (Wong and Crosby, 1981).

Crosby and Wong (1976) examined the photo-degradation pathway of pentachlorophenol in aqueous solution at alkaline pH when exposed to either natural sunlight or light from a specially designed photoreactor. The first step involved photonucleophilic substitution of hydroxide ions for chloride to produce three isomeric tetrachlorodiols, which then underwent air oxidation to the corresponding quinones.

Further displacement of chloride ions from one of the quinones was then observed, with eventual fragmentation to mineral products proceeding via such a

substituted quinone.

These photonucleophilic substitutions are ionic in nature and under natural conditions would rely upon the presence of hydroxide ions and hence would be pH dependant. It can also be seen that photoreduction can occur and under natural conditions only reduced products seem to have been found. From this it seems reasonable to conclude that the formation of specific photoproducts under laboratory conditions does not necessarily mean that the same photoproducts will be formed under natural conditions.

It has been shown that if pentachlorophenol was present in high enough concentrations and in alkaline solution, then exposure to ultraviolet light could result in the generation of 1,2,3,4,5,6,7,8,-octachlorobenzene-p-dioxin (Crosby and Wong, 1976).

This product is related in structure to the highly toxic TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a trace contaminant found in certain phenoxyacetate herbicide formulations (Woolson et al., 1972), and the potential formation of compounds such as these in the environment is obviously of concern.

It was found, however, that even primary photo products themselves can undergo photochemical transformations and octachlorodibenzo-p-dioxin was found to undergo photoreduction to hexachlorodibenzo-p-dioxin (Crosby, 1978). In addition, it was observed in an

earlier study that in the presence of either natural or simulated sunlight 2,7-dichloro-, 2,3,7-trichloro- and 2,3,7,8-tetrachlorodibenzo-p-dioxin were all entirely photodecomposed within a few hours (Crosby, Moilanen and Wong, 1973).

It may be that despite the detrimental effects of sunlight discussed so far, photodecomposition may have important advantages and may be a means by which toxic waste products and contaminants can be removed from the environment.

A good example of this was actually described in an article by Crosby (1978), where TCDD contamination was partially overcome through photodecomposition.

The dioxin was thermally generated when a still overheated at a trichlorophenol plant in Serveso, Italy. It was released in a cloud of sodium trichlorophenate and contaminated hundreds of acres of agricultural land and buildings.

Despite the known susceptibility of TCDD to sunlight, residues were found to dissipate only very slowly until it was finally realised that there was no adequate source of photochemical proton donors. As a consequence, contaminated areas were sprayed with olive oil as a ready source of protons and this was found to facilitate the photo-reduction reactions characteristic of TCDD photochemistry. A rapid reduction in residue levels was then observed.

The examples of DDT and pentachlorophenol photochemical transformations illustrate how even pesticides which appear to be chemically stable can be effectively photodegraded in the presence of natural or simulated sunlight. There are, however, numerous examples of even more facile photochemical transformations involving agriculturally important pesticides.

These are reported studies where exposure to sunlight has resulted in rapid and almost complete loss of some pesticides. Immediate soil incorporation of trifluralin, for example, is recommended by the manufacturer, since three to four times as much chemical is needed for effective weed control when the herbicide is applied as a surface spray.

Although trifluralin has a relatively high vapour pressure and there may be significant losses through volatilisation from the soil surface, it has been shown that its susceptibility to photodegradation may also be responsible for the need for immediate soil incorporation.

It was shown that in both aqueous and organic solvents, exposure to either natural or simulated sunlight caused rapid photodecomposition of trifluralin.

In 10% aqueous methanol, the identification of the monoalkylated photoproduct (2,6-dinitro-N-n-propyl-$\alpha\alpha\alpha$-trifluoro-p-toluidine) after 30 seconds exposure to

natural sunlight and the further identification of the principal photoproducts after only a few minutes, revealed that the photodecomposition of trifluralin in solution is rapid indeed.

It can be seen that oxidative dealkylation, nitroreduction and cyclisation reactions control the solution photochemistry of trifluralin. The photochemical N-dealkylation apparently proceeds by a free radical oxidation mechanism involving atmospheric oxygen and the nitro-reduction in methanol by proton abstration from the solvent. The mechanism of photoreduction in aqueous solution was not known in this study and it was apparently difficult to explain the source of reducing power in other studies also, where photoreduction was observed (Leitis and Crosby, 1974).

A vapour phase study involving the irradiation of trifluralin in a reactor specially designed to simulate natural sunlight conditions, revealed that similar reactions to those mentioned before controlled the vapour phase photochemistry of trifluralin (Soderquist et al., 1975).

It was also shown in this study that the same vapour phase reactions could control the photochemistry of trifluralin under environmental conditions. In a field experiment, soil was treated with trifluralin formulation and air samples were taken from above the area of treatment. It was found that where the

formulation was merely sprayed onto the surface of the soil, four photoproducts, identical to four identified in the photoreactor experiment, were generated. Soil incorporation of the formulation resulted in the production of only one photoproduct, identified earlier as being the first product of vapour phase photodecomposition.

These results showed, through identification of common photoproducts, that laboratory photochemistry experiments could, to a reasonable degree, simulate the natural situation. In the particular case of trifluralin, the ease with which the herbicide can be photodecomposed in the environment was demonstrated and the need for immediate soil incorporation further illustrated.

The final example given in this section is one involving a study of the photochemistry of the phenoxyacetate herbicide, MCPA [(4-chloro-2-methyl-phenoxy)acetic acid].

This example is particularly relevant because MCPA was one of the four phenoxyacetates selected for study in subsequent Chapters. Another reason for its selection as an example was because its in vivo and in vitro photochemistry was studied. Consequently, it was possible to illustrate further the probable likelihood of photodecomposition under environmental conditions and the potential which exists for the application of additives

to control it.

Although a brief review of the photochemistry of the phenoxyacetate herbicides used in the present study is detailed in Chapter 6, the relevant examples of MCPA photochemical transformation have been included here because they particularly emphasize the facile environmental photodecomposition of this herbicide.

The rapid breakdown of MCPA under environmental conditions was observed when a rice field was sprayed with an MCPA formulation (Soderquist and Crosby, 1975). Samples of water, rice plants and mud were collected and it was concluded that rapid transformation, both chemical and in this case biological also, reduced residues to very low levels after only 14 days.

It was shown in this same study that exposure of aqueous solutions of MCPA to natural sunlight, or to simulated sunlight in a specially designed photoreactor, resulted in rapid photodecomposition to produce 4-chloro-2-methylphenol, as the major photoproduct and smaller amounts of o-cresol and 4-chloro-2-formyl phenol.

More recent work has considered both the bulk solution photochemistry of MCPA and the effect of light on formulated MCPA spray droplets.

A comprehensive study revealed that the sunlight irradiation of an aqueous spray solution of MCPA, formulated as its dimethylamine salt, resulted in over

80% loss of the herbicide within 6 days (Crosby and Bowers, 1985). The chemical 4-chloro-2-methylphenol was again the major photoproduct although 13 other photoproducts were also identified.

An interesting finding in this study was the identification of (4-chloro-2-methylphenyoxy)-N,N-dimethylacetamide as a photoproduct. It was claimed that the amine salts of the other phenoxyacetate herbicides could also be converted photochemically to the corresponding amides and this illustrates that the nature of pesticide formulations can have an influence over the types of photoproducts formed.

A recent study on the photochemical loss of MCPA from simulated spray droplets was reputedly one of the first reported cases which investigated the effect of light on a pesticide within the environment of an aqueous spray droplet (Freiberg and Crosby, 1986). It was pointed out by the authors that the micro-environment of an airborne droplet is likely to be unique, having a maximum surface to volume ratio, maximum exposure to oxygen and other atmospheric oxidants, as well as full exposure to sunlight. As a consequence, it would seem likely that the fate of pesticides in airborne droplets may be considerably different from that of pesticides present in solution or on surfaces.

It was shown by Freiberg and Crosby (1986) that only 4.6 days exposure to natural sunlight was required

for 50% loss of MCPA from aqueous droplets. From the reaction pathway, it can be seen that a variety of photoproducts were identified of which 4-chloro-2-methylphenol was again the major component. The photochemical transformation of MCPA to the phenol is supported by identification of the photoproduct in the field (Freiberg and Crosby, 1986).

Whether additives could be used to confer protection against the action of sunlight under these particular circumstances is difficult to assess. Presumably if they could inhibit photodecomposition of a given pesticide in bulk solution, then they could do also if present in an aqueous airborne droplet, provided the mechanism was the same in both cases.

MCPA does, then, provide an excellent example of the impact which light can have on the field residence time of some pesticides. The economic significance of the photolability of MCPA becomes apparent when one considers that at least 19 European countries use the herbicide as salts or esters (Anon, 1986) and even more use it in combination with other herbicides. In the USA in 1979, for example, it was estimated that almost 80% of the rice produced was sprayed with MCPA (Crosby and Bowers, 1985). Considering the results of the aforementioned studies, one wonders what proportion of these amounts were rendered ineffective by the action of light and what quantity of herbicide would actually have

been required for effective control, had there been some way to counteract the observed effect of light.

(ii) Selected examples of pesticide phototransformations under laboratory and natural conditions

b) Indirect photochemical transformations (absence of uv light absorption by pesticide)

The importance of photosensitising compounds and natural oxidants for the indirect photochemical transformation of pesticides was mentioned earlier.

It is becoming increasingly clear that reactive intermediates such as these may have a more significant role to play in pesticide photodecomposition than was previously thought. Their presence on soil surfaces, in soil solution and in natural waters, has been demonstrated on many occasions in the last 15 years.

Not only have photosensitisers and photo-oxidants been found to increase the rate of photodecomposition of photochemically labile pesticides, but it has been shown that they can have a particularly important role to play in the photodecomposition of pesticides which are normally stable to light.

A number of examples have been selected to illustrate these points and to convey the generally accepted view that indirect photochemical transformation of pesticides may be just as important as direct transformation.

Details were given earlier of the mechanism by which photosensitisers can facilitate the generation of a pesticide triplet state. It was said that provided the excitation energy of the sensitiser triplet state was greater than that of the pesticide triplet state, then direct interaction between the two could lead to generation of the pesticide triplet. From the triplet state, which is relatively long lived, direct photochemical reaction was then more likely.

This is one way in which photosensitisers can bring about pesticide photodecomposition. There is another way, which does not involve the transfer of excitation energy and generation of the pesticide triplet.

This involves the direct reaction of the excited sensitiser with the pesticide, or the direct reaction of some intermediate, generated by the sensitiser, with the pesticide. Proton abstraction from the pesticide could be considered to be a direct interaction between the sensitiser and the pesticide:-

$$^3_{sens.} + P \rightarrow sens\text{-}H_2 + \text{oxidised products}$$

where $^3_{sens.}$ is the sensitiser in the triplet state and P is a pesticide in the ground state. Reduction of the sensitiser back to the ground state generates sens-H_2 and oxidised pesticide products.

On the other hand, generation of singlet oxygen, $^1O_2$, through the interaction of ground state triplet oxygen (oxygen exists as a triplet diradical in the ground state), with the excited sensitiser, would be considered an example of the generation of a reactive intermediate, which is then available to oxidise the pesticide (Silber et al., 1976):-

eg. $^3O_2 + {}^3_{sens.} \rightarrow sens. + {}^1O_2$

$^1O_2 + P \rightarrow$ oxidised products

It has been shown in subsequent examples that singlet oxygen generated directly by the absorption of light, not by the transfer of triplet energy, can actually react with sensitisers through an electron transfer quenching mechanism. This has been shown to produce superoxide anion radicals, which can oxidise pesticides themselves, or which can indirectly oxidise them, through intermediates such as hydroxyl or hydroperoxide free radicals. In addition, it has been found that superoxide can also be generated directly through an electron transfer reaction between an excited sensitiser and ground state triplet oxygen, rather than excited state singlet oxygen. The mechanisms by which these reactive intermediates can be generated, and their interaction with pesticides, will now be considered in some detail.

Simple photosensitising compounds

The importance of photosensitised reactions in natural waters was first suggested in a publication in 1973, where it was found that the toxic trace contaminant, ethylenethiourea (imidazolidine-2-thione), although stable to natural sunlight was rapidly photodecomposed in the presence of either acetone or riboflavin in light. It was further shown that the observed photosensitised reaction of ethylenethiourea, in vitro, could also be caused by irradiation in natural drainage waters (Ross and Crosby, 1973).

Ross and Crosby (1973) suggested that sensitisers such as riboflavin would be expected to occur in natural waters, and that these compounds would be important for the indirect photochemical transformation of otherwise stable pesticides.

There was further evidence for indirect photochemical transformations. It was suggested that dissolved organic substances might have been responsible for the increased rate of photodecomposition of MCPA in rice paddy water (Soderquist and Crosby, 1975). It was found, for example, that in distilled water, 20 days of irradiation in sunlight resulted in only 50% photodecomposition of the MCPA, whereas after sunlight irradiation of the herbicide in rice paddy water, only 25% of the original amount remained after 6 days. This not only illustrated the susceptibility of MCPA to the

action of light, but it also suggested the presence of some component which could potentiate the photochemical breakdown of the herbicide in the field water.

A few years later, the amino-acid tryptophan was directly implicated in the observed increased rate of molinate (S-ethyl N,n-hexamethylene thiocarbamate) photodecomposition in water samples taken from a treated field.

It was found that dilute aqueous solutions of molinate were actually stable to sunlight irradiation, but that the addition of tryptophan caused photo-decomposition to 1-[(ethylsulphinyl)carbonyl]hexahydro-1H-azepine, S-ethylhexahydro-21H-azepine-1-carbothioate and hexamethyleneimine, photoproducts which had not been previously identified. From the identification of these photoproducts in water samples taken from a treated field, it was suggested that tryptophan, present in soil solution, might have been responsible for the photosensitised breakdown of molinate (Soderquist et al., 1977).

A few years later, it was found that the sunlight irradiation of the herbicide Sustar resulted in a much greater rate of degradation in natural water compared to buffered distilled water (Miller and Crosby, 1978). The authors suggested that photosensitisation by natural solutes, or by photochemically generated oxidants, might be responsible for the enhanced rate.

More recently, there have been several publications which have suggested a role for tryptophan as a natural photosensitiser, and in each case a mechanism for its action has been postulated.

The generation of superoxide by simple organic molecules, such as tryptophan, was the subject of a study by Draper and Crosby (1983).

They found that the irradiation of aqueous solutions containing aniline, anthranilic acid, N-acetylanthranilic acid, tryptophan, kynureline, tyrosine, p-cresol and acetophenone, with simulated sunlight, resulted in the photochemical generation of superoxide radical anions. Three different mechanisms were suggested to account for the generation of the superoxide radical from tryptophan and the other test compounds:-

i) Photo-ionisation of the dissolved sensitiser could lead to the generation of solvated electrons, which might then be scavenged by dissolved oxygen to produce the superoxide

$$\text{eg. sens.} \rightarrow (\text{sens.}^{+})^{\cdot} + \text{electron(aq)}$$

$$\text{electron(aq)} + O_2 \rightarrow (O_2^{-})^{\cdot}$$

ii) Reduction of a photochemically excited sensitiser by the test compounds leading to the formation of a radical anion which, upon auto-oxidation, would generate the

superoxide radical.

eg. $^3sens. + R\text{-}H_2 \rightarrow {}^3sens.^-\text{-----}\ R\text{-}H_2^+$

$\rightarrow (sens.^-)^\cdot + (RH_2^+)$

$(sens.^-)^\cdot + O_2 \rightarrow sens. + (O_2)^\cdot$

iii) Quenching of photochemically generated singlet oxygen, involving complete electron transfer between the test compounds and the excited singlet oxygen.

eg. $sens. + {}^1O_2 \rightarrow (sens.^+)^\cdot + (O_2^-)^\cdot$

The first mechanism was considered to be the most likely one because it could best account for the experimental observation that each of the test compounds could also photosensitise the displacement of chloride atoms from chloracetic acid in solution. Although superoxide was considered a strong nucleophile under certain conditions, this observation was taken as strong evidence for the generation of solvated electrons from the test compounds.

It was concluded from this study, that simple organic molecules in soil solution and in natural field waters could function as sensitisers through the generation of superoxide, and that superoxide could have been responsible for the photo-oxidations observed in other studies.

As mentioned previously, under some circumstances, superoxide can itself behave as a strong nucleophile and this behaviour could be responsible for the photonucleophilic displacement reactions characteristics of some pesticides in aqueous solution.

However, it has been suggested that the actual oxidising agent was more likely to be the hydroxyl radical. In aqueous solution, superoxide can be reduced to hydrogen peroxide which has been shown to undergo homolytic fission to generate hydroxyl free radicals, in the presence of light

$$\text{eg.} \quad 2(O_2^-) + 2H^+ \rightarrow H_2O_2 + O_2$$

$$2H_2O_2 \rightarrow 2HO^{\cdot}$$

A recent review by Crosby suggested that hydroxyl free radicals and superoxide ($HO_2^{\cdot}$) might be the photo-oxidants responsible for the oxidation of some pesticides in aqueous solution (Crosby, 1982).

Crosby (1982) described a study where the irradiation of phenol in sea water, in the presence of nitrite ions, resulted in the formation of hydroquinone and bromo- and chlorophenols. This was as a consequence of the photochemical generation of hydroxyl radicals by nitrite anions and reaction of the hydroxyl radicals with chloride and bromide ions, present in the sea water, to generate the halogen atoms. Photonucleophilic attack of the phenol by the radicals then produced the observed

photoproducts.

$$\text{eg. } NO_2^- + H_2O \xrightarrow{\text{light}} NO + HO^\cdot + HO^-$$

$$HO^\cdot + Cl^- \longrightarrow Cl^\cdot + HO^-$$

$$HO^\cdot + Br^- \longrightarrow Br^\cdot + HO^-$$

A similar study showed that the sunlight and near ultraviolet irradiation of some important pesticides, in dilute hydrogen peroxide, lead to photo-oxidation reactions (Draper and Crosby, 1984). It had earlier been shown that hydrogen peroxide levels as high as 30 μM had been found in some natural water samples (Draper and Crosby, 1981), and levels in this range were used for the irradiation of the pesticides.

In each case there was an observed increase in the rate of photo-oxidation and this increase was most pronounced with molinate, carbofuran and MCPA, the pesticides with the lowest near ultraviolet extinction coefficients and hence lowest rates of direct photodecomposition. It would seem that photosensitised oxidation might be most significant in the case of pesticides which have the least susceptibility to the direct effects of light.

The results of another study by Ross and Crosby (Ross and Crosby, 1985), further implicated hydrogen peroxide as a photochemical oxidant in natural waters. An interesting feature of this study was that it provided

a possible mechanism for the photodecomposition of ethylenethiourea, the contaminant studied in the first publication to consider the role of photosensitisers in natural waters.

It was shown that the irradiation of natural field waters with sunlight or near ultraviolet light could cause the photo-oxidation of not only ethylenethiourea, but also thiobencarb, DDE and aldrin. None of these pesticides were found to absorb appreciably in the sunlight wavelength spectrum, yet they were effectively photo-oxidised in the field water samples, suggesting the presence of photosensitisers.

Further irradiation experiments were carried out where either tryptophan or tyrosine was added to solutions of the pesticides, and it was shown that both amino-acids could effect the same photo-oxidations as the photosensitisers present in the natural field waters.

Like the previous experiments involving molinate (Soderquist et al., 1977), these results suggested a possible role for simple amino-acids as photosensitisers in natural waters. Ross and Crosby (1985), however, went on to propose that the amino-acids could function as photosensitisers by generating solvated electrons on absorption of light. The solvated electrons might then cause reduction of dissolved oxygen to produce hydrogen peroxide, and although the hydrogen peroxide was considered to be the actual oxidant, there was

experimental evidence for the presence of another photo-oxidant.

This came from the finding that ethylenethiourea did not react with hydrogen peroxide in the dark, but was still found to undergo oxidation. It was suggested that in the case of tryptophan, a reactive indolenyl hydroperoxide might be generated in the dark and could bring about the observed oxidation of ethylenethiourea. It had been demonstrated in other studies that this oxidant can actually be formed from reaction between tryptophan and hydrogen peroxide.

Complex photosensitising molecules

Up until this point, the discussion has been restricted to the role of simple organic molecules in the photosensitised oxidation of pesticides. There is some opinion, however, that more complex compounds, like humic and fulvic acids, might also be responsible for some of the observed photosensitised reactions of pesticides.

The studies reported by Zepp and co-authors in consecutive years, (1976 and 1977), directly implicated organic polymers in the photosensitised breakdown of selected chemicals.

The first of these publications, (Zepp et al., 1976), reported that the photodecomposition of methoxychlor in pure water was much slower than that in sterilised natural water samples. They went on to report

that the absorption spectrum of a commercial humic acid preparation was very similar to that of dissolved organic compounds identified in the natural waters. In addition, irradiation of the methoxychlor in the presence of the commercial humic acid resulted in an increased rate of photodecompostion.

The evidence pointed to the involvement of humic acid or a similar organic polymer in the photodecomposition of the pesticide in the natural waters. In this study, however, it was difficult to provide a likely explanation for the experimental observations.

Energy transfer sensitisation was not considered possible because of the magnitude of the triplet energy of the insecticide molecule. Those sensitisers present in natural waters whose triplet energy was high enough to generate the reactive methoxychlor triplet, were found to absorb only low wavelength ultraviolet light. It was also found that the insecticide was unreactive towards singlet oxygen, so this means of oxidation was unlikely. It was suggested that attack by free radicals might be more likely and it could be that superoxide free radicals, generated through the humic acid, might explain their results.

Later Zepp et al. (1977) reported the results of an investigation into the occurrence of singlet oxygen in natural waters. They suggested the involvement of fulvic

acid and other organic polymers in the photochemical transformation of pollutants and other biologically important substances, in lakes and rivers.

Using 2,5-dimethylfuran to chemically trap singlet oxygen, it was shown that the highest rates of formation occured in highly coloured water samples, containing high levels of organic substances. Fulvic acid was found to be a major component of this organic fraction, and it was concluded that the light energy absorbed by sensitisers, such as fulvic acid, was probably transferred to ground state triplet oxygen to generate the reactive singlet oxygen. Reaction of organic molecules, including pesticides, with the singlet oxygen would result in oxidation to produce peroxides.

Because of the widespread occurrence of fulvic acid in natural waters and soils, it was concluded that the photochemical generation of singlet oxygen was probably a widespread phenomenon.

Miller et al. (1980) were interested in the effects of dissolved organic substances on the rate of photodecomposition of 3,4-DCA(3,4-dichloroaniline), a compound which is a common hydrolysis product of several herbicides, for example, diuron, linuron and propanil. They found that the sunlight irradiation of 3,4-DCA in natural water samples resulted in both direct and sensitised photo-reactions and that the photoproduct, TCAB ($3,4,3^1,4^1$-tetrachlorobenzene), was identified only

during irradiation in natural water samples and not in distilled water samples.

Humic acid was implicated as one of the organic components which might have been responsible for the observed photosensitised generation of TCAB, since irradiation of 3,4-DCA in a solution of commercial humic acid gave similar results to the irradiation in natural water, with TCAB being identified in this case also.

Hydrogen abstraction by the excited organic sensitiser was proposed as the initial stage in the mechanism by which the azobenzene photoproduct could be formed. This was based on earlier model studies, where it had been found that in the presence of riboflavin or benzophenone, irradiation of 3,4-DCA resulted in the formation of TCAB.

It had been reported that the triplet state energies of the two sensitisers were 69 and 43.5 K cal mol^{-1} respectively whereas the triplet energy of 3,4-DCA was likely to be no less than 73 K cal mol^{-1} and it was not possible, therefore, to generate the 3,4-DCA triplet state by direct transfer of triplet energy. Proton abstraction by the sensitisers was considered a more likely process in the model study and in the study by Miller et al. (1980).

Complex photosensitisers in soils

More recently, research on the herbicide prometryne [2-(methylthio)-4,6-bis(isopropylamino)-s-triazine], also revealed that humic and fulvic acid polymers, present in soils and natural waters, may have a more widespread role as photosensitisers (Khan and Gamble, 1983).

In this study, humic acid and fulvic acids were actually isolated from soil profiles and solutions of prometryne containing the polymers were irradiated with ultraviolet light. Both were found to alter the rate of transformation of the herbicide and, in addition, a third photoproduct, not generated in their absence, was produced during irradiation.

The photochemical de-alkylation of the prometryne observed in this study was suggested to have been caused by hydroxyl free radicals. A reaction between photochemically generated singlet oxygen and the two polymers, could produce organic hydroperoxides and the subsequent decay of the hydroperoxides would release hydroxyl free radicals into solution.

An alternative mechanism, based on earlier studies on tryptophan sensitised reactions, could be the following:-

$$\text{Humic} \rightarrow (\text{Humic}^{+})^{\cdot} + \text{electron(aq)}$$

$$\text{electron(aq)} + O_2 \rightarrow (O_2^{-})^{\cdot}$$

$$2H^{+} + 2(O_2^{-})^{\cdot} \rightarrow H_2O_2 + O_2$$

$$H_2O_2 \rightarrow 2HO^{\cdot}$$

where a solvated electron, released from the excited humic and fulvic acids, could reduce dissolved oxygen to generate superoxide. Reduction of the superoxide to hydrogen peroxide and photodecomposition of the hydrogen peroxide could then generate reactive hydrogen peroxide free radicals.

The observations of Khan and Gamble (1983) were supported by the finding that humic substances present in natural water could enhance the rate of 2,4,5-T photodecomposition, producing 2,4,5-trichlorophenol as the major photoproduct (Skurlatov et al., 1983).

The following mechanisms were proposed to account for the experimental observations:-

i) A direct reaction between the herbicide and the electronically excited humic sensitiser or some oxidising agent derived from it:-

$$\text{Humic} \rightarrow \text{humic}^{*}$$

$$\text{Humic}^{*} + R\text{-}CO_2^{-} \rightarrow \text{products}$$

ii) The reversible formation of a photo-reactive complex between the humic sensitiser and the herbicide:-

$$\text{Humic} + \text{R-CO}_2^- \rightarrow \text{Humic-----R-CO}_2^- \rightarrow \text{products}$$

iii) Electron transfer from the herbicide anion to the humic sensitiser to form a herbicide free radical which could decompose:-

$$\text{Ar-OCH}_2\text{CO}_2^- + \text{humic} \rightarrow \text{Ar-OCH}_2\text{CO}_2^{\cdot} + (\text{humic}^-)^{\cdot}$$

$$\text{Ar-OCH}_2\text{CO}_2^{\cdot} \rightarrow \text{Ar-OCH}_2^{\cdot} + \text{Ar-OH}$$

The third mechanism probably most adequately accounts for the formation of the 2,4,5-trichlorophenol photoproduct. It was also shown in an earlier study that the acetoxy radicals of various phenoxyacetates in water were unstable and readily decomposed to the corresponding phenol (Brown et al., 1964).

The same mechanisms could account for the oxidative side chain cleavage observed in this study, and it can be seen that it bears a close resemblance to the one proposed to account for the photochemical side chain cleavage of phenoxyacetates in an earlier study (Crosby and Wong, 1973).

So far, the role of naturally occuring organic monomers and polymers as photosensitisers and the mechanisms by which they can facilitate pesticide photo-oxidation, has been discussed in some detail. All the experimental evidence would seem to suggest that

compounds such as tryptophan, fulvic acid and humic acid, and probably many other naturally occuring organic compounds, have an important role to play in the photochemical transformation of pesticides.

There have been reports, however, which have suggested the involvement of organic surfaces in the indirect photochemical transformation of pesticides.

Gohre and Miller (1983), were interested in the role of inorganic components in the formation of singlet oxygen on soil surfaces. Using TME (tetramethylene ethylene) and DMF (2,5-dimethylfuran), to chemically trap singlet oxygen, they showed that the generation of characteristic oxidation products, during the sunlight irradiation of soil samples, was due to singlet oxygen.

Although the authors suggested that other strong oxidants, such as peroxyl, hydroxyl, superoxide and other free radicals could be the products of soil irradiation, singlet oxygen was considered to be the most likely product. This oxidant would, of course, then be available for the oxidation of any pesticide which might come into contact with the soil.

In this report, it was suggested that the organic fraction of the soil could have been responsible for the observed photosensitised generation of singlet oxygen. It was pointed out, however, that in the past inorganic compounds, such as oxides of titanium and zinc, had been implicated in the formation of the oxidant and it was

concluded that inorganic surfaces may have been involved in this study.

A later report by the same authors proved conclusively that inorganic components could generate singlet oxygen upon light irradiation (Gohre and Miller, 1985).

They had observed, while carrying out the research for their earlier publication, that the irradiation of 2,3-dimethylbut-2-ene, adsorbed onto chromatographic silica gel and alumina, had resulted in the production of the singlet oxygen oxidation product, 2,3-dimethylbut-1-en-3-ol.

This finding prompted a more detailed investigation into the formation of singlet oxygen on oxide surfaces. Two different chemical traps were employed this time. The chemical 1,2-dimethylcyclohexene was chosen because it was found to react with the oxidant to form characteristic products, which were readily distinguished from the products of radical oxidation. Similarly, deuterated 2,3-dimethylbut-2-ene was selected because of its specificity for singlet oxygen.

From the nature and distribution of oxidation products formed on soil surfaces during irradiation with fluorescent light, it was concluded that singlet oxygen was readily produced, photocatalytically, on the surfaces of aluminium, iron and magnesium oxides.

There are other examples which illustrate the catalysis of pesticide photodecomposition by soil surfaces. It was shown by Slade (1966) that whereas paraquat was readily decomposed by the light from a mercury vapour lamp, it was stable to natural sunlight irradiation. This was found to be as a consequence of its sharp uv absorption band at 257 nm, a value outwith the natural sunlight spectrum.

However, it was demonstrated that when paraquat was absorbed onto a thin layer of silica gel, photodecomposition in natural sunlight was then possible, generating the same photoproducts as the mercury lamp irradiation. It was suggested that this could have been due to an observed shift in the wavelength maximum of the paraquat, to a value of 275 nm, resulting in a broader absorption band. This would allow sufficient absorption of light to bring about a photochemical reaction.

In the light of the recent results of Gohre and Miller (1985), it could be that there is actually an involvement of singlet oxygen, in Slade's (1966) work, photochemically generated by the silica gel. The identification of oxidative photoproducts by Slade might seem to support this possibility.

Adsorption onto soil surface has been shown to facilitate the breakdown of other inherently photochemically stable pesticides. Moilanen et al. (1974) reported that in the vapour phase, parathion

[0,0-diethyl,0-(p-nitrophenol)] phosphorothioate was stable to uv irradiation and they found that even after extended exposure to uv light, the insecticide did not photodecompose.

However, when applied to a fine road dust and then irradiated, photodecomposition was found to be rapid. Identification of the same oxidation photoproducts in field samples suggested that soil surfaces could have an important role in the indirect photochemical transformation of pesticides in the environment.

It was also found, under both laboratory and field conditions, that parathion was readily photo-oxidised to paraoxon and it was again suggested that soil surfaces were catalysing the oxidation. This time, though, it was further proposed that atmospheric ozone might have been an intermediate in the oxidation (Woodrow et al., 1978).

The final example which has been selected to illustrate the catalytic effect of soil surfaces on pesticide photo-oxidation, is a study by Smith et al. (1978). The aim of their work was to develop a method for studying the behaviour of methidathion (S-[5-methoxy-2-oxo-1,3,4-thiadiazol-3(2H)-yl)-methyl]) 0,0-dimethylphosphorodithioate, in air dry soils under different environmental conditions, with a view to determining the effects of soil type and ozone on its

dissipation and oxidation.

It was found that in the absence of sunlight or atmospheric oxidants, the rate of photodecomposition of methidathion was slow, whereas in their presence it was significantly faster and considerable amounts of the oxidation products of methidathion were produced. Like the previous example, photo-oxidation was the major reaction and it was suggested that atmospheric ozone, around soil particles, might have been involved.

It was suggested by Gohre and Miller (1985) that these results were more likely to suggest the involvement of singlet oxygen, generated photochemically by the oxide surface.

In conclusion, it is apparent that both organic and inorganic components in soils, plants and natural waters, are available to facilitate and enhance the rates of pesticide photodecomposition.

Where re-arrangement of valence on direct absorption of light is absent, or insufficient to bring about direct chemical reactions, natural sensitisers and oxidants will be particularly important for photochemical reaction.

In the vapour phase, it has also been demonstrated that oxidants may be important. Radical reactions of hydroxyl and triplet oxygen and polar reactions of ozone, are thought to be particularly important (Crosby, 1982).

The relevance of this Chapter to the content of the following Chapters should become apparent.

CHAPTER 6

PHOTOCHEMICAL REACTIONS OF PHENOXYACETATES IN AQUEOUS SOLUTION

Introduction

Before introducing the specific areas of research in this Chapter a brief account of the research which has been reported on the photochemistry of the selected test chemicals is now given. It is hoped that this will be interesting from a historical point of view and informative from a chemical one.

The history of phenoxyacetate photochemistry

Although the effects of light on the activity of these compounds were first reported in 1947, there have been recent investigations into the interaction of the herbicides with light, as discussed in Chapter 5. Therefore there is still interest in the photochemical behaviour of the phenoxyacetate herbicides.

On examination of the literature for information on this photochemical behaviour, it was found that over a period of 40 years there has emerged both biological and direct chemical evidence for a variety of diverse reactions, leading to many different photoproducts, the nature of which was found to depend upon factors such as the spectral range of the irradiating light and the

physical form of the herbicide.

It was the research of Penfound and Minyard as long ago as 1947, which first indicated the possible detrimental effect of light on the biological activity of the phenoxyacetate herbicides. Although the authors themselves did not relate their results to a possible interaction between the herbicides and light, the findings of their study may be the first to have illustrated such an interaction.

The herbicide, 2,4-D, formulated as its butyl ester in kerosene was applied to both water hyacinths and red kidney beans under different light intensity conditions. Less epinasty and necrosis was observed in the case of water hyacinths grown in full sunlight, compared to those grown under shaded conditions after application of the herbicide. In the case of red kidney beans, although the effects of the herbicide were the same regardless of whether the plants were placed in direct sunlight, diffuse light or darkness, it was observed that ultimate survival of the plants was better where plants had been placed in direct sunlight. This may be the first example of the photodecomposition of a phenoxyacetate herbicide to less toxic photoproducts with an observed reduction in biological activity.

It has been suggested that the reduction in biological activity under the higher intensity conditions could have been due to loss of the 2,4-D ester through

volatilisation from the relatively warmer leaf surface. It is difficult to determine with any certainty, though, if the ester would actually be volatile enough to be lost in this way, under those conditions.

Most of the research in the early stages was of a biological nature and there were four further studies which investigated the effect of light on the biological activity of 2,4-D. Many studies were interested particularly in 2,4-D because of its known properties and widespread use.

Payne and Faults (1947) observed that the irradiation of various salts and esters of 2,4-D with both filtered and unfiltered ultraviolet light resulted in the production of solutions whose contents, when bio-assayed, caused an increased stimulation of cell elongation in the pea slit-stem test.

Bio-assays were again used in the early 1950's to demonstrate the interaction between light and the phenoxyacetates. A study was carried out on the biological activity of the sodium salt of 2,4-D and 2-(2,4-dichlorophenoxy)ethyl sulphate and it was found that both herbicides could inhibit the growth of cucumber seedling roots over a range of concentrations. It was further observed, however, that in the presence of fluorescent lighting this inhibition could be overcome if riboflavin was present in the herbicide solution, (Carroll, 1952). It had been observed earlier by the

same author, in a different study, that the inhibition of corn root elongation caused by the sodium salt of 2,4-D, could be overcome if riboflavin had first been added to solutions and the solutions exposed to light (Carroll, 1949).

One of the first studies to illustrate both the in vivo and in vitro inactivation of 2,4-D by riboflavin in the presence of light, was that reported by Hansen and Buchholtz (1952). In vivo inactivation was demonstrated by the incubation of corn seedlings on nutrient agar containing 2,4-D, with and without the addition of riboflavin. On exposure to light, it was found that the presence of riboflavin could effectively overcome the root inhibition caused by the herbicide. This confirmed the findings of Carroll but the study went on to further show that riboflavin could inactivate 2,4-D in vitro.

This time solutions containing 2,4-D and riboflavin were first exposed to light then assayed for biological activity with corn seedlings. Like the in vivo experiments, the inhibitory action of the herbicide alone was overcome upon addition of riboflavin and exposure to light.

It was noticed in this study that there was evolution of a strong phenolic odour during illumination of 2,4-D/riboflavin solutions and for the first time an attempt was made to characterise the product(s) of 2,4-D photodecomposition.

A positive substitution reaction was observed on the addition of bromine to the illuminated herbicide/ riboflavin solutions and this was taken as evidence for breakdown of 2,4-D to a phenol, possibly 2,4-dichlorophenol - it was to be proved conclusively later that this was the major product of 2,4-D photodecomposition in aqueous solution.

The in vitro results of Hansen and Buchholtz (1952) were confirmed in the following year by a study by Hamilton and Aldrich (1953), who exposed solutions of the triethylamine salts of 2,4-D and 2,3,5-triiodobenzoic acid and the ethyl ester of trichlorobenzoic acid to fluorescent light in the presence and absence of riboflavin. After a defined period of illumination, the biological activity of the three chemicals was measured by determining the effect of the irradiated solutions on the growth of cucumber seedling roots.

Like before, it was found that the presence of riboflavin effectively overcame the root inhibition caused by each of the chemicals alone.

By this stage there was a growing awareness that light could actually have a profound effect on the biological performance of the phenoxyacetates and that this was the result of some form of photochemical transformation.

Bell (1956) published the results of research which had been aimed at studying the chemistry of the

photochemical transformation of 2,4-D and not the biological consequences of the transformation. His work involved an investigation of the riboflavin photosensitised breakdown of 2,4-D and related phenoxyacetates and aryl ethers, as well as an investigation of the effect of unfiltered low wavelength ultraviolet light on 2,4-D alone.

The test compounds including 2,4-D were dissolved in a saturated solution of riboflavin and exposed to fluorescent light. In addition, 2,4-D alone was irradiated with light from a mercury arc lamp.

Using a colorimetric test to detect phenols, it was found that 2,4-D and the related phenoxyacids, 2,6-D [(2,6-dichlorophenoxy)acetic acid)],2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid)],4-CPA [(4-chlorophenoxy)-acetic acid)] and phenoxyacetic acid were rapidly photodecomposed in the presence of the riboflavin photosensitiser in light. It was also demonstrated that 2,4-D was readily photodecomposed by the low wavelength ultraviolet light from the mercury lamp and that there was a fall in the pH of the solution, although no phenol was detected. It was suggested that the absence of phenol from the ultraviolet irradiated solution was as a consequence of the experimental observed instability of phenols themselves to ultraviolet light. Despite this, it was shown, using paper chromatography, that five new photoproducts were generated from the 2,4-D irradiation.

The formation of compounds with more than one aromatic nucleus was observed and this was thought to occur by an intermolecular process. In view of the results of more recent studies, this could be the first evidence for the formation of the polyquinoid humic components which were the characteristic end products of many subsequent aqueous phenoxyacetate photodecomposition experiments.

An interesting finding in this study was the variation in the susceptibility of the different test compounds to photosensitised breakdown. Bell (1956) proposed that the structure of the side chain determined whether the phenoxyacetates photodecomposed to phenol. Compounds containing a carboxyl group α to the oxygen bridge underwent more facile breakdown and this was thought possibly to be as a consequence of the electronegative carboxyl group weakening the ether link.

Hence, compounds such as the previously mentioned phenoxyacetates, 2,4-D,2,6-D,2,4,5-T,p-CPA and phenoxyacetic acid, were all photodecomposed much more rapidly than others such as, 3-(2,4-dichlorophenoxyl)-propionic acid, 3-(phenoxy)propionic acid and 2-(2,4-dichlorophenoxy)-ethanol.

Similarly, Carroll (1952) had shown in his earlier study that whereas riboflavin and light could effectively counteract the growth regulating activity of 2,4-D, they were much less effective at doing so with the

herbicide Sesone [2-(2,4-dichlorophenoxy)ethyl sodium sulphate]. This would seem to be in agreement with Bell's findings.

Without digressing too much, Bell's proposed mechanism of side chain cleavage could be taken to suggest a homolytic ether cleavage and the electron withdrawing effect of the a carboxyl could facilitate this, with generation of a phenoxy radical. Formation of the phenol, however, would require hydrogen abstraction from the aqueous solvent and this is, perhaps, less likely. The more recent mechanism proposed by Crosby and Wong (1973) could account for Bell's observation that phenol formation was much less facile with the longer chain phenoxyacids.

This involves expulsion of a solvated electron by the excited phenoxyacetates and loss of carbon dioxide to form a free radical, which then forms a peroxy free radical on reaction with dissolved oxygen and finally re-arranges to form a phenol via a phenol formate. This would be less likely to occur with the longer propionate side chain, since formation of the phenyl formate does not seem possible.

Finally, Bell (1956) made the observation that the potency of the phenoxyacetates in a field situation may be controlled by the amount of ultraviolet light reaching them and from the earlier example of MCPA photo-decomposition (Chapter 5) this would seem to bear some

truth.

The results of a study by Aly and Faust (1964) a few years later showed some similarity to those described previously.

Aqueous solutions of the sodium salt and the isopropyl and butyl esters of 2,4-D were irradiated with ultraviolet light from a mercury lamp, mainly at a wavelength of 253.7 nm. Like before, the pH of the solutions was found to decrease through loss of the side chain and in each case 2,4-dichlorophenol was produced as the major photoproduct.

It was also shown that photodecomposition was actually pH dependent, with an increase in the rate being observed at higher pH. This was also found in subsequent studies, for example that of Crosby and Wong (1973).

The subsequent 2,4-dichlorophenol was much more readily photodecomposed than the parent acid. Its absence in the solutions adjusted to a pH value of pH 9 before irradiation reflected its photochemical consumption as rapidly as it was produced from the acid.

It was concluded, quite reasonably, that the solar ultraviolet spectrum ranges from 292 nm to 400 nm and therefore, that the increased energy, supplied by the low wavelength light emitted by mercury vapour lamps, could facilitate reactions which might not actually be likely in natural sunlight.

It was further concluded that the presence of suspended matter and organic material in surface waters, and presumably soil solution also, could greatly reduce the effects of solar radiation on the phenoxyacetate herbicides present in these solutions. From the discussion in Chapter 5 of the indirect photodecomposition of pesticides by sensitisers and photo-oxidants, it seems equally likely that the organic components present in natural waters may actually increase the rate of breakdown of some pesticides.

In the same year as this publication, Kelly and Pinhey (1964), for the first time, produced evidence for the photo-rearrangement of some mono-substituted phenoxyacetates when they were exposed to the ultraviolet light from a high pressure mercury lamp.

Irradiation of an ethanolic solution of phenoxy-acetic acid, the simplest member of the class, resulted in oxidative cleavage of the ether linked side chain to produce phenol while photo-rearrangement generated 2- and 4-hydroxyphenylacetic acid.

The compound 2-coumaranone was identified also but it was likely that this component was not a direct photoproduct. Its formation from the intramolecular re-arrangement of 2-hydroxyphenylacetic acid was the most likely mechanism of formation.

An identical study with p-chlorophenoxyacetic acid resulted in identification of similar photoproducts

to those formed from phenoxyacetic acid photo-transformation (Pinhey and Rigby, 1969). Although no p-chlorophenol or 2-hydroxy-5-chlorophenylacetic acid were identified (they would be the equivalent of the phenol and 2-hydroxyphenylacetic acid identified during phenoxyacetic acid irradiation), it was suggested that the experimentally rapid reduction of these two compounds under the same experimental conditions, may indicate that aryl ether bond homolysis and re-arrangement is followed by reduction and this could be an alternative pathway by which the parent phenoxyacetate photodecomposed to the conserved products.

A more comprehensive study confirmed the observations of these two reports and discussed in more detail the mechanism of the photochemical re-arrangement (Kelly, Pinhey and Rigby, 1969). The mechanism will be discussed in more detail later.

Around the same time as the publications of Kelly et al. (1969), Crosby and various associates investigated the effect of natural and simulated sunlight on the stability of different phenoxyacetate herbicides in aqueous solution.

In 1966 a study on the photochemical transformation of 2,4-D in aqueous solution was published, and for the first time provided evidence for photonucleophilic as well as homolytic oxidative re-arrangement reactions (Crosby and Tutass, 1966).

It was found that irradiation with sunlight or with light from a mercury vapour lamp generated essentially the same photoproducts. Oxidative cleavage of the side chain to produce 2,4-dichlorophenol was the major reaction and was followed by photonucleophilic substitution of hydroxyl for chloride to form 4-chlorocatechol.

Direct substitution of hydroxide for chloride in the parent acid was also observed and accounted for the formation of 2-hydroxy-4-chlorophenoxyacetic acid. Further dehalogenation and ether bond cleavage resulted in the formation of 1,2,4-benzenetriol which was then rapidly air oxidised and polymerised to a mixture of polyquinoid humic acids by a light independent process. This was considered to be the final product from phenoxyacetate photodegradation.

A similar study in 1973 was the first to identify the photoproducts from the sunlight irradiation of p-CPA in aqueous solution (Crosby and Wong, 1973).

Most of the earlier studies concerning the photochemistry of the phenoxyacetates had involved irradiation of solutions with ultraviolet light from mercury vapour lamps. Although it was mentioned in the previous study that irradiation of 2,4-D with natural sunlight, or with light from a medium pressure mercury vapour lamp, resulted in the formation of the same products, it should again be stressed that lamps will

produce ultraviolet light of much shorter wavelength than will ever reach the earth's surface and consequently may induce unnatural reactions.

The study by Crosby and Wong (1973), however, used natural sunlight as well as light from a reactor designed to produce light in the same spectral region as natural sunlight, in order to illustrate the likely photoproducts from environmental photodecomposition of 4-CPA.

4-chlorophenol was identified as the major product, although phenol, hydroquinone, p-chlorophenol formate, phenoxyacetic acid, p-hydroxyphenoxyacetic acid and humic acid were all produced in varying amounts.

Oxidative cleavage of the side chain to generate p-chlorophenol was found to be the rate limiting step and like the photochemical reactions of 2,4-D under the same conditions, photoreduction and photonucleophilic substitution of hydroxyl for chloride were found to be the major reactions. Polymerisation of unstable intermediates to humic substances was found to be the final stage in the photodecomposition of CPA, as it was in the photodecomposition of 2,4-D.

Thus, from the similarity between the photochemical reactions of p-CPA and 2,4-D under the same conditions, it was suggested that the photochemical pathway described for CPA was likely to be the one by which all members of the class photodecomposed under

natural conditions.

A later study with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) supported this suggestion (Crosby and Wong, 1973), although it should be pointed out that in the case of 2,4,5-T, as in the earlier case of 2,4-D, formation of the 2,4,5-trichlorophenol was not found to proceed via the phenylformate.

In contrast, formation of p-chlorophenol from CPA was found to proceed via the phenylformate, and similarly the formation of 4-chloro-o-cresol from the irradiation of MCPA was found to proceed through the intermediate 4-chloro-2-methyl-phenyl formate.

Although this represents only a small difference in the respective photochemical pathways, it shows that the pathways for each of the phenoxyacetates were not, in fact, exactly the same.

Apart from this, however, the nature of the other reactions would seem to be common to all the phenoxyacetates under natural sunlight conditions.

One significant feature which emerges from the results of the various studies is that it is apparently possible for these herbicides to undergo photochemical transformation in sunlight, even though the spectrum of irradiating light would be outwith their maximum absorption range. Perhaps this implies a role for photosensitisers or for photochemically generated oxidants.

At this stage, then, evidence had emerged for a variety of different photochemical reactions involving the phenoxyacetate herbicides. Re-arrangements and substitutions were shown to occur, although the use of different reaction conditions made it difficult to compare the results of the various studies.

Using the methyl esters of 2,4-D, CPA and phenoxyacetic acid, Binkley and Oakes (1974) attempted to provide evidence for a general photochemical reaction pathway. They irradiated methanol solutions containing each of the methyl phenoxyacetates with ultraviolet light from a high pressure mercury lamp. Filters were used to expose the herbicides to specific wavelength bands.

A variety of photoproducts were identified and from the selective irradiation of the herbicides with different light wavelengths the authors demonstrated that methyl 2,4-D was transformed into methyl 2-chlorophenoxy-acetate methyl 4-chloroacetate and methyl phenoxyacetate, presumably by a photoreduction reaction. Two hydroxyphenyl acetate isomers and phenol were also identified.

That the methyl 2,4-D was transformed only into the named methyl chloroacetates and not into methyl phenoxyacetate despite its presence, was shown by irradiating the methyl 2,4-D with light of a wavelength greater than 285 nm. This was achieved using a pyrex filter to filter low wavelength ultraviolet light.

Since the ultraviolet adsorption of 2,4-D begins at a longer wavelength than that of the monochlorinated products, it was possible to facilitate selective absorption by the 2,4-D ester and demonstrate that irradiation yielded only the two monochlorinated esters. The generation of methyl phenoxyacetate using lower wavelength light was, therefore, brought about by the transformation of the monochlorinated esters and not by transformation of the 2,4-D ester.

Since it was not experimentally feasible to facilitate selective light absorption by either of the monochlorinated esters or by the methyl phenoxyacetate, it was not possible to provide direct evidence for the exclusive formation of methyl phenoxyacetate from the two monochlorinated esters or for the formation of the hydroxyphenylacetates and phenol from only the methyl phenoxyacetate.

Indirect evidence did, however, come from the conservation that the ratio of methyl 2- and 4-hydroxy-phenylacetates and phenol to each other was exactly the same, independent of whether the methyl 2-chloro or 4-chlorophenoxyacetate was used as the starting material in the irradiation. This was seen to suggest the presence of a common intermediate, in this case methyl phenoxyacetate.

Further, irradiation of methyl phenoxyacetate generated exactly the same three photoproducts as did

irradiation of the monochlorinated esters and in exactly the same ratio. This was also seen to suggest that methyl phenoxyacetate was derived from the monochlorophenoxy- acetates and that the two hydroxyphenylacetates and phenol were generated exclusively from the photodecomposition of methylphenoxyacetate.

This study provided evidence for the sequential photodecomposition of phenoxyacetate esters to simpler members of the class and finally ether bond cleavage and re-arrangement of the simplest member to phenol and hydroxyphenylacetates.

Many of the early studies which investigated the photochemical pathways of the phenoxyacetates had used salts of the respective acids. The work of Binkley and Oakes (1974) was one of the first studies where the reaction pathway of the esters were examined.

Crosby and Tutass (1966) had first succeeded in the characterisation of the photoproducts from the irradiation of the sodium salt of 2,4-D, but the attention was drawn by Binkley and Oakes (1974) to the fact that 2,4-D, and other members of the class were often formulated as alkyl esters.

They subsequently went on to show in another study, that the photochemical reactions of the ethyl, butyl and 2-methylheptyl esters of 2,4-D differed significantly from those of the sodium salt (Binkley and

Oakes, 1974).

Each of the esters was dispersed in water and irradiated with light from a high pressure mercury lamp, fitted with a pyrex sleeve to filter out ultraviolet light with a wavelength less that 285 nm.

They found that reductive dechlorination was the only light induced reaction and that this accounted for the identification of 2- and 4-chlorophenoxyacetate esters. Hydrochloric acid was also produced and although the authors suggested that this was responsible for the observed formation or the free 2,4-D acid, through hydrolysis, there has been more recent evidence which shows that de-esterification may be the direct result of a light induced intramolecular re-arrangement, analogous to the McClafferty re-arrangement (Que Hee and Sutherland, 1979).

There was no evidence of the hydroxylation or oxidative side chain cleavage reactions which characterised the aqueous photochemical transformation of 2,4-D salts. It was also pointed out in this study that whereas photoproducts from the irradiation of 2,4-D salts continue to absorb light and react via a series of secondary photochemical transformations, the ester photoproducts were stable to further photodecomposition.

Thus, it was concluded that at light wavelengths of greater that 285 nm the esters of the phenoxyacetate herbicides would be expected to undergo different

photochemical reactions from the corresponding salts.

A study by Que Hee et al. (1979) concerning the photochemical transformation of formulated mixed butyl esters of 2,4-D, in both hexane and aqueous solution, supported these earlier observations.

They suggested that the presence of surfactants, sequestering agents and inert compounds in commercial formulations could influence the nature of photochemical pathways or rates of photodecomposition. From the discussion in the next Chapter, it can be seen that there is some evidence to support this.

Irradiation of the esters with ultraviolet light of wavelengths of 300 nm and 350 nm resulted in the formation of butyl(p-chlorophenoxy)acetate by a reductive dechlorination reaction, free 2,4-D acid by a Norrish Type 2 reaction (similar to the McClafferty re-arrangement) and butyl 2-hydroxyphenylacetate by a Photo-Fries type reaction.

Like previous studies involving irradiation of the esters of 2,4-D, there was no evidence for the photonucleophilic hydroxylations such as those observed by Crosby and Tutass (1966) for 2,4-D salts. This fact, in conjunction with the generation of only reduced photoproducts under these conditions, was taken as evidence for the presence of ester micelles in aqueous solution.

It was suggested that the hydrophobic phenyl moeity could be emmersed in a hydrocarbon-like environment and the hydrophilic areas of the surfactants could be in contact with water molecules in these mixed micelles.

This type of environment was considered to favour the reductive photochemical pathways observed in this study, rather than the oxidative ones characteristic of the salts in aqueous environments.

Further evidence for the existence of these micelles was seen in the formation of a Photo-Fries product during irradiation of the ester formulation in aqueous solution, and its absence during the irradiation of the pure ester in hexane. It was considered that formation of tight radical cages, necessary for the Photo-Fries reaction to occur, could be facilitated by the mixed micelles and that this was more evidence for their existence.

The final study, which will be discussed as part of this summarised review on the known photochemistry of some of the phenoxyacetate herbicides, was another study by Que Hee and Sutherland (1979).

Earlier studies had indicated that the ultraviolet irradiation of aqueous and hexane solutions of the butyl ester of 2,4-D resulted in reductive dechlorination in the ortho position. From the known volatility of the butyl ester the authors considered that

it would be worthwhile to study the photodecomposition in the gas as well as the vapour phase (Que Hee and Sutherland, 1979).

Irradiating the pure butyl ester in both the liquid and the vapour phase, with light of 300 nm, generated a variety of photoproducts.

The main products in both the vapour and the liquid phases were 2,4-dichlorophenol, formed by a homolytic ether cleavage, the methyl ester of 2,4-D, formed as a result of degradation of the butyl side chain and the butyl ester of (5-chloro-2-hydroxyphenyl)acetic acid, generated by the Photo-Fries re-arrangement of the butyl ester of 4-CPA, itself a photoproduct formed from reductive dechlorination of the parent ester. The Photo-Fries product was not observed in any of the earlier studies which used hexane as the solvent under the same conditions.

From the nature of the photoproducts it was concluded that the reactions were largely of a free radical nature. The vapour phase photodecomposition was equated with the field situation, where butyl ester molecules would probably exist as single molecules and the liquid phase photodecomposition was seen to simulate the situation where pure 2,4-D ester would be present alone, once the carrier and any additives had evaporated.

Specific photochemical reactions of the phenoxyacetates

This has, perhaps, given some insight into the known photochemical reactions of this class of herbicide. Certain aspects of this photochemistry were found to be worthy of further research.

(i) The generation of hydroxyphenylacetic acid isomers as photoproducts

Light induced side chain migration to generate o- and p-hydroxyphenylacetic acid from phenoxyacetic acid has already been discussed. This seemed to be a particularly interesting reaction and it was decided to determine whether it could be reproduced in aqueous solution. From the literature on this subject it was found that the reaction was apparently only typical of phyenoxyacetic acid. Only on one occasion, for example, was 4-chlorophenoxyacetic acid found to undergo this reaction and this was in ethanol not aqueous solution. Hence it was decided to irradiate both compounds with uv light in aqueous solution and determine whether the hydroxyphenylacetates were generated. Conclusions about the likelihood of their formation in natural water systems ie. irrigation waters or within the plant leaf, can, perhaps, be drawn.

(ii) Photonucleophilic reactions

The generation of highly reactive free radicals in the presence of uv light has been demostrated and was discussed earlier. Most of the hydroxylated photoproducts of the phenoxyacetates, for example, are thought to involve the photonucleophilic displacement of ring substituents by hydroxyl free radicals. One reaction in particular which seemed to have several practical implications was the possible photonucleophilic displacement of ring chlorines by thiocyanate in aqueous solution. Evidence that this type of reaction was feasible came from the identification of 4-cyanophenoxy-acetic acid during the irradiation of 4-chlorophenoxy-acetic acid in the presence of cyanide ions in aqueous solution. Such a reaction involving thiocyanate might prove useful from a growth regulation point of view.

1. Organic thiocyanates as growth regulators

Inorganic thiocyanates have been demonstrated to have plant growth regulating properties. It was shown by Ranjan and Kaur (1954) that treatment of potatoes with thiocyanates resulted in rapid sprouting. Swets and Addicot (1955) found that ammonium thiocyanate caused leaf abscission in Phaseolus vulagaris and that there was also a rapid reduction in the level of IAA in treated plants. Brian (1976) on the other hand observed a stimulation of root formation on the stems of beans and

geranium plants. Stephen (1983) reported that at relatively low levels thiocyanate caused chlorosis and defoliation in Phaseolus vulgaris L. whereas at higher levels, rapid freezing and dessication was apparent. From the observation that thiocyanate free radicals could be generated in solution and that they had a high degree of selectivity for the indole nucleus (Adams et al. 1972) it was concluded by Stephen (1983) that the results of his work could be explained by an interaction between thiocyanate and IAA.

Some attention has been given to the synthesis of organic thiocyanates with biological activity. Allias (1970) gave details of the synthesis of 1-(carbamoyloxy)-4-thiocyanate benzenes as pesticides. Mussell (1968) discussed the synthesis of 3-chloro-4-hydroxyphenylthiocyanate, 3,5-dibromo-4-hydroxyphenylthiocyanate and 2-methyl-4-hydroxyphenylthiocyanate, which were found to have herbicidal properties, killing 4 inch tall plants of a variety of species. Similarly Arder (1975) discussed the synthesis and use of 3,5-dichloro-4-hydroxyphenyl-isothiocyanate as a fruit abscission agent.

An interesting publication by Tippe and Eckstein (1974) gave details of a laborious five step synthesis of several [(mercaptoaryl)oxy] alkane carboxylic acids including 4-thiocyanatophenoxyacetic acid. All of these compounds were found to have systemic fungicidal activity against Phytophthora infestans in potato tubers. More

recently, Colin and Loubinoux (1983) discussed the synthesis of new aryloxyacetic acid derivatives with biological activity. Through the use of different solvent systems and reaction conditions thiocyanato- and isothiocyanato- derivatives of 2,4-D were produced where substitution occurred on the acetic side chain.

2. Photochemical synthesis of organic thiocyanates

The most common methods for synthesising thiocyanate derivatives involve the reaction of organic starting materials with:-

§ a) isothiocyanic acid and its salts

b) thiocyanogen and related reagents

c) cyanide derivatives Guy (1977)

Tippe and Eckstein (1974) used a combination of these reactions in their complex synthesis of the thiocyanate equivalent of 4-CPA and MCPA. It seemed feasible that the photonucleophilic displacement of chlorine atoms by thiocyanate in the presence of uv light could be exploited to generate similar products from 4-CPA and MCPA starting products.

EXPERIMENTAL

Materials

Phenoxyacetic acid, 4-chorophenoxyacetic acid, 2-methylphenoxyacetic acid, 2-hydroxyphenylacetic acid,

4-hydroxyphenylacetic acid, phenol, o-cresol, 4-chlorophenol, 2,4-dichlorophenol, and 2-methyl-4-chlorophenol were purchased from Sigma Chemical Co. at the purest grades available. 2,4-dichlorophenoxyacetic acid was purchased as an analytical standard from National Physical Laboratories. 2-methyl-4-chlorophenoxyacetic acid was extracted and purified from formulation granules. Both phenyl formate and 4-chlorophenylformate were synthesized and purified as described below. Ammonium thiocyanate was purchased from Hopkin and Williams Ltd., boron trifluoride in methanol from B.D.H. Ltd and "reacti-vials" (5 ml) from Pierce Chemical Company. All organic solvents were used at the purest grades available.

Extraction and purification of 2-methyl-4-chlorophenoxy-acetic acid (MCPA)

MCPA formulation granules were dissolved in hot ethanol/water (80:20) and the solution cooled with the addition of more distilled water. A white precipitate formed and was separated from a brown deposit. The white precipitate was further re-crystallised from ethanol/water in this way, producing fine crystals on cooling. Crystals were recovered by filtration, washed with distilled water, then re-dissolved in distilled water with the addition of dilute sodium hydroxide. The pH was maintained at a neutral value and any

contaminating phenols were extracted into diethyl ether. The remaining aqueous solution was acidified and MCPA extracted into diethyl ether. This solution was dried over anhydrous sodium sulphate, evaporated to dryness under reduced pressure and the crystalline residue re-crystallised twice from diethyl ether/petroleum ether. Purity was checked by HPLC (see later for details of method).

Both phenoxyacetic acid and 4-chlorophenoxyacetic acid were purified using the same techniques.

Synthesis of phenyl formates

Both phenyl formate and 4-chlorophenyl formate had already been identified as intermediates during the photochemical transformation of phenoxyacetates in other studies. The formation of 4-chlorophenol from 4-CPA was found to proceed via 4-chlorophenyl formate, for example, and similarly the generation of 2-methyl-4-chlorophenol from MCPA was found to proceed via 2-methyl-4-chlorophenyl formate (Crosby and Bowers, 1985). However, since 4-chlorophenol is also a photoproduct of MCPA it seemed feasible that 4-chlorophenylformate and possibly phenyl formate may appear as intermediates during the photochemical transformation of more substituted phenoxyacetates such as MCPA. Consequently there was a need to obtain these compounds as reference standards. Since they were not available from commercial sources,

they were synthesized according to the method detailed by Van Es and Stevens (1965). The formates were purified using preparative silica columns by the organic chemistry technical staff. Their purity was checked by TLC (chloroform solvent system) and by gas chromatography (G.C.). They were identified using mass spectrometry and infra red spectroscopy. These compounds were found to be unstable at room temperature. Immediately after synthesis and purification only one peak was visible on G.C. chromatograms. However, after 24 hr storage a second peak corresponding to the starting phenol was observed.

Ultraviolet irradiation of phenoxyacetates

Each of the test chemicals was irradiated at a concentration of 1 g litre^{-1} in distilled water at pH 8. Solutions were irradiated with light from a medium pressure mercury arc lamp (Englhard Hanovia Lamps Ltd.). The lamp emitted principally at a wavelength of 254 nm. Solutions were maintained at room temperature with a cooling system and were stirred continuously. Samples were exposed to the uv light for up to 12 hours or until about 25% of starting material was transformed. In the case of solutions containing NH_4SCN this took longer. These solutions were found to turn a deep purple colour, possibly due to the formation of thiocyanogen and its reaction with water (Gardner and Weinberger, 1939).

Ammonium thiocyanate was added to test solutions as a 3:1 molar excess. Phenoxyacetic acid was irradiated under these conditions and 4-CPA were irradiated in both the absence and presence of NH_4SCN.

Fractionation of photoproducts

To aid in the purification and subsequent identification of some of the photoproducts, irradiated solutions were fractionated by solvent partitioning at different pH values.

Typically, the pH of irradiated solutions would fall below neutral except in the case of solutions containing NH_4SCN where it changed little. All solutions were adjusted to a neutral value and extracted three times with diethyl ether. Phenols and neutral products were removed into the organic phase. The phenolic components were separated from any neutral components by extracting the diethyl ether with 2N sodium hydroxide. The sodium hydroxide was neutralised and phenols re-extracted into ether.

The original aqueous phase was then acidified and the phenoxyacetic acids partitioned into diethyl ether. Diethyl ether extracts were combined at each stage of the fractionation, washed with distilled water, dried over anhydrous sodium sulphate and reduced in volume. It was observed that a brown polymeric material formed during the first solvent partitioning in some cases and might be

the humic type polymer described by Crosby and Tutass (1966). At the end of the fractionation, three diethyl ether solutions containing neutral, phenolic and acidic photoproducts respectively were chromatographed where required.

Chromatographic and spectroscopic analysis of photoproducts

Photoproducts were separated from each other using HPLC, G.C. and TLC. The behaviour of different chemicals under the chromatographic conditions employed was compared with that of reference standards where possible and this used as an aid to identification. Chemicals which were isolated and purified using preparative TLC were sent for routine infra-red and mass spectral (probe) analysis as a means of a more definite identification. If reference standards were not available, spectra were compared with those of similar compounds or were interpreted using literature references.

HPLC

Crosby and Bowers (1985) described the use of HPLC to separate photoproducts. The chromatographic system involved application of samples to a reverse-phase C18 column, elution with a 50% aqueous methanol mobile phase and detection at 254 nm. These conditions were

found to be unsuitable in this study, giving a poor separation of disubstituted phenols and incomplete resolution of acidic compounds. Systems were developed which gave good separation of phenolic compounds from one another and which resolved all potential acidic photoproducts. The HPLC equipment described in Chapter 3 was used except that a reverse phase C18 column was employed.

i) Separation of phenols

Either methanol or acetonitrile in 0.02 M phosphate buffer (pH 4) was found to be the most suitable mobile phase. A gradient system where the proportion of acetonitrile was increased from 20% to 80% over 15 minutes gave the following retention times:

Chemical	Retention time (mins)
phenol	1.41
o-cresol	2.68
4-chlorophenol	4.06
2-methyl-4-chlorophenol	7.04

see trace 6.1

ii) Separation of acidic components

The same mobile phase as described for the separation of phenols was found to be the most suitable in this case also. A gradient system where the proportion of acetonitrile was increased from 5% to 95% over 30 minutes gave the following retention times.

Chemical	Retention time (mins)
phenoxyacetic acid	2.27
p-hydroxyphenylacetic acid	2.86
o-hydroxyphenylacetic acid	3.39
4-chlorophenoxyacetic acid	6.36
2-methylphenoxyacetic acid	7.07
2-methyl-4-chlorophenoxyacetic acid	2.86

see trace 6.2

Gas chromatography

Photoproducts were separated and co-chromatographed against reference standards using G.C. methods developed for this purpose. Packed columns were used most frequently although a capillary G.C. method was also employed.

For packed column G.C. a Pye 4500 gas chromatograph fitted with a flame ionisation detector (FID) was used. Glass columns of 1 m length and 4 mm internal diameter were packed with Gas chrom Q diatomaceous earth support (Applied Science, USA) coated with 1.8% OV 17/1.95% OV 202 and 2% Carbowax stationary phases (Phase separations, UK). Gas flow rates for analysis were as follows; nitrogen carrier gas 30 cm^3min^{-1}, air for FID 180 cm^3min^{-1} and hydrogen for FID 30 min^3min^{-1}. The detector temperature was maintained at 250°C. For the separation of photoproducts a temperature gradient was used where samples were applied to the column at 85°C, this temperature held for 5 minutes and then raised to 200°C at $4°Cmin^{-1}$ or $8°Cmin^{-1}$. A 10 μl Hamilton Series 701 microlitre syringe was used to make 5 μl sample injections. Data was monitored using a Shimadzu C-RIB chromatopac or Spectra Physics SP 4290 integrator.

Capillary G.C. was carried out using a Shimadzu Gas Chromatograph GC-8A fitted with a DB5 - 30 metre column of one microlitre thickness. A temperature gradient was again used to separate components. From an initial temperature of 75° at injection, the column temperature was raised to 200°C at $4°Cmin^{-1}$.

Due to the non-volatile nature of phenoxyacetic acids, the acidic components were methylated using BF_3 in methanol to facilitate G.C. analysis. This was carried out by transferring an aliquot of the ether solution

containing these components into a 5 ml reacti-vial. The ether was removed under nitrogen, then 1 ml of 14% BF_3 in methanol solution was added, the contents mixed and heated for 30 minutes at 60°C with continuous stirring. The solution was allowed to cool and 1 ml of 2% aqueous sodium sulphate solution added to stop any further reaction. The contents were mixed and extracted with 3 x 1 ml hexane. The hexane extracts were passed through a column of anhydrous sodium sulphate and the eluant collected, pooled and analysed by G.C. Standard solutions of each of the standard phenoxyacetic acids were methylated in the same way, individually and as a mixture. This process was not suitable for the methylation of the phenylacetic acid isomers, therefore G.C. was not used for their identification.

Reaction times for phenols were as follows:-

Chemical	Retention time (mins) OV17/202
phenol	3.84
o-cresol	5.83
4-chlorophenol	11.71
2-methyl-4-chlorophenol	14.14

Chemical	Retention time (mins) carbowax
phenol	7.53
o-cresol	8.12
4-chlorophenol	9.06
2-methyl-4-chlorophenol	11.19

Chemical	Retention time (mins) DBS
phenol	9.61
o-cresol	12.10
4-chlorophenol	16.54
2-methyl-4-chlorophenol	18.97

Retention times for acidic components were as follows:-

Chemical	Retention time (mins) carbowax
phenoxyacetic acid	3.78
4-chlorophenoxyacetic acid	5.77
2-methyl-4-chlorophenoxyacetic acid	7.25

Chemical	Retention time (mins) DBS
phenoxyacetic acid	7.17
4-chlorophenoxyacetic acid	13.47
2-methyl-4-chlorophenoxyacetic acid	17.26

Retention times for formates were as follows:-

Chemical	Retention time (mins) OV17/202
phenyl formate	4.36
4-chlorophenyl formate	5.89

Chemical	Retention time (mins) carbowax
phenyl formate	5.73
4-chlorophenyl formate	9.71

Chemical	Retention time (mins) DBS
phenyl formate	8.03
4-chlorophenyl formate	11.87

TLC

Thin layer chromatography was only used for the separation of acidic photoproducts as problems were encountered in trying to develop a system for the separation of phenols. Initially, two solvent systems were examined as a means of separating phenoxyacetic acid, 2-hydroxyphenoxyacetic acid and 4-hydroxyphenoxy-acetic acid on silica gel F_{254} plates (0.25 mm thickness). The first system was hexane:acetone:acetic acid (80:19.5:0.5) but this was unsuccessful in separating the two phenylacetic acids from each other. The second system tested was diethyl ether: petroleum ether:acetic acid (20:5:1) but all 3 test chemicals were found to migrate with the solvent front. However this same solvent system in the proportions (5:20:1) gave a good separation of the three standards.

For preparative scale TLC separations ie. 1mm thickness plates, it was often necessary to develop plates more than once, in particular to enable an adequate separation of mixtures of acidic components. For example, both MCPA and CPA were irradiated with uv light in the presence of a 3:1 M excess of NH_4SCN. When photoproducts from the acidic fraction of irradiated solutions were concentrated and developed in this TLC system, up to six different components could be seen including two main bands. One of the bands in each case was absent in solutions which had been irradiated without

the addition of NH_4SCN. For effective separation of this component, TLC plates were developed three times for CPA solutions and five times for MCPA solutions. The same solvent was used in each case and plates were allowed to dry between development. Altering the proportions of the different solvents in this particular system did not give an adequate separation.

After the final development, plates were removed from their tanks and left to dry at room temperature. The band containing the unknown component was marked out under uv light (254 nm) and removed from the rest of the plate. The compound was extracted from the silica with diethyl ether. Solvent was removed under reduced pressure and the crystalline deposit recovered and purified by re-crystallisation from diethylether: petroleum ether. Samples were then sent for mass spectral and infra-red analysis.

The same procedure was used to separate the acidic components present in a solution of 2,4-D which had been irradiated in the presence of NH_4SCN. However, in this case only one major band was isolated and purified.

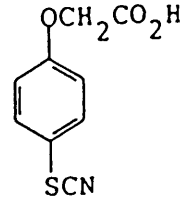
Results

Identification of major photoproducts

1. Thiocyanate substituted CPA photoproduct

Elemental micro-analysis revealed the presence of both nitrogen and sulphur. This was taken as some evidence for the substitution of chlorine by thiocyanate to generate a nitrogen and sulphur containing acidic compound. Further evidence that this had occured came from the mass spectrum results (see Figure 6.3). It had been found that both aryl isothiocyanates and thiocyanates exhibit a molecular peak (Ben-Efrain, 1977). This being the case, it was likely that the peak at 209 amu corresponded to the molecular ion and hence the molecular weight of the photoproduct. In addition, the fragmentation pattern revealed the presence of fragments corresponding to M^+, $M^+(-CN)$, $M^+(-CS)$, $M^+(-SCN)$ and $M^+(-SCN-C_2H_4)$. This form of fragmentation is common for aryl thiocyanates (Ben-Efrain, 1977). A molecular weight value of 209 amu corresponds exactly with an empirical formula of $C_9H_7NO_3S$. Final confirmation that the suspected photonucleophilic substitution reaction had occured came from infra-red analysis. From Figure 6.4 it can be seen that there is a single sharp peak at 2165 cm^{-1}. Commonly, the asymmetric stretching vibration of aromatic thiocyanates appears around 2170 cm^{-1} as a sharp peak of medium intensity (Ben-Efrain, 1977). Tippe and

Eckstein (1974) reported that all of the phenoxyacetic acid-thiocyanate derivatives they synthesized showed an absorption in the 2170 cm^{-1} - 2140 cm^{-1} region which was assigned to the CN stretch. Their results included the compound 4-thiocyanato phenoxyacetic acid which was the identity of the photoproduct. The nmt spectrum of the photoproduct revealed two proton doublets at 7.0 and 7.5 ppm which corresponded to aromatic protons in a compound substituted in the para position (Figure 6.4a). Thus, all of the spectroscopic evidence suggests the following structure:-



It could be argued that isothiocyanate was the substituting species. Such a compound would have the same molecular weight as the thiocyanate equivalent. However, although this would generate the same molecular ion during mass spectral analysis, the fragmentation pattern would probably be different. For example, phenyl thiocyanate shows M^+, $M^+(-CN)$, $M^+(-CS)$, $M^+(-SCN)$, and $M^+(-SCN-C_2H_4)$. On the other hand, phenyl isothiocyanate shows only M^+, $M^+(-CN)$, $M^+(-SCN)$ and $M^+(-SCN-C_2H_4)$ (Ben-Efrain, 1977).

In addition, it was possible to distinguish between an aryl thiocyanate and an aryl isothiocyanate by infra-red spectroscopy. The thiocyanate usually shows a sharp peak of medium intensity around 2170 cm^{-1} whereas the isothiocyanate usually shows a strong band which is broad or a doublet at a lower frequency ie. 2100 cm^{-1} to 2040 cm^{-1} (Ben-Efrain, 1977). Figure 4.5 shows the infra-red spectrum of phenylisothiocyanate as a pure reference standard. It can seen that the peak at 2080 cm^{-1} is strong and broad with signs of a doublet. It is completely different from the peak in the spectra obtained from the photoproduct.

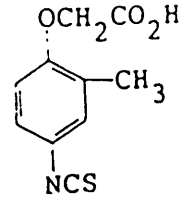
Figures 6.6 and 6.7 show the mass spectrum and infra-red spectrum for the compound which was located furthermost from the origin on the preparative TLC plates. Comparison of these spectra with those for a sample of CPA standard (Figures 6.8 and 6.9) show that the compound was unreacted CPA.

2. Isothiocyanate substituted MCPA photoproduct

Elemental micro-analysis revealed the presence of both nitrogen and sulphur in this compound also. The mass spectrum showed the presence of a molecular ion at 223 amu which corresponds with the molecular weight of a compound with empirical formula $C_{10}H_9NO_3S$. The fragmentation pattern is the same as that discussed previously and is indicative of a thiocyanate substituted

MCPA molecule (Figure 6.10).

The infra-red spectrum, however, does not show the same sharp peak of medium intensity at 2170 cm^{-1} as that of the CPA derivative (Figure 6.11). Instead absorption is relatively broad and appears as a doublet with peaks at 2080 cm^{-1} and 2161 cm^{-1}. It is not quite as pronounced as the peak seen in the spectrum of the phenylisothiocyanate standard. The likely structure of this compound is:-



Figures 6.12 and 6.13 show the mass spectrum and infra-red spectrum for the compound which was located furthermost from the origin on preparative TLC plates. This compound was found to be unreacted MCPA (Figure 6.14 shows the mass spectrum for a sample of standard MCPA).

3. No major photoproduct from 2,4-D/NH_4SCN irradiation

TLC gave a poor resolution of the acidic photoproducts of 2,4-D irradiation. The furthermost band was isolated and purified. From the mass spectrum and infra-red spectrum of this compound (Figures 6.15 and 6.16) it can be seen that it was, in fact, unreacted

2,4-D. Three other bands were observed under low wavelength uv but the compounds were not present in sufficient quantities to warrant extraction from the TLC plate and re-purification.

4. Rearrangement reaction of phenoxyacetic acid

The acidic fraction of a solution of phenoxyacetic acid which had been irradiated with uv light was chromatographed under the TLC conditions described earlier. Plates were developed three times to yield three discrete bands which were then isolated and re-purified. Mass spectrum and infra-red spectrum results are shown in Figures 6.17 and 6.18 and comparison with the spectra for a sample of phenoxyacetic acid standard (Figures 6.19 and 6.20) show that the furthermost compound on the plate was probably parent chemical. The spectra for the component below phenoxyacetic acid are shown in Figures 6.21-22. Comparison of these spectra with those for 2-hydroxyphenylacetic acid (Figures 6.23 - 24) indicate that this is the likely identity of this component. Spectra for the third component which was located nearest the origin are shown in Figures 6.25 and 6.26. They are similar to the spectra for 4-hydroxy- phenylacetic acid (Figures 6.27 - 28) and suggest that this is the identity of the third component.

Further, reference standards of each of the three compounds were separated once on analytical TLC plates (silica gel F_{254}) giving the following Rf values:-

Compound	Rf value
phenoxyacetic acid	0.41
2-hydroxyphenylacetic acid	0.28
4-hydroxyphenylacetic acid	0.19

(diethyl ether:petroleum ether:acetic acid (5:20:1)

The compounds isolated from the irradiated solution chromatographed in exactly the same order and with the same Rf values as the reference standards in this TLC system.

Although CPA was irradiated under the same conditions. The only visible band was that of unreacted parent CPA. There was no evidence of any side chain migration to generate phenylacetic acid, despite the fact that methylation and G.C. analysis indicated the presence of a compound which behaved the same as phenoxyacetic acid under the same chromatographic conditions (ie. retention time of 3.61 min on carbowax column and 6.93 min on DBS column. Conditions detailed earlier). Perhaps a longer irradiation period would have been more conclusive to re-arrangement of the phenoxyacetic acid photoproduct.

Discussion

This limited look at some photochemical aspects of selected phenoxyacetates generated some interesting results. It seems clear that photochemical reactions such as these may have a role to play in some areas of synthetic organic chemistry. The relatively facile generation of thiocyanate/isothiocyanate derivatives by comparison with existing published methods for their synthesis suggests that ultra-violet light might be an important synthetic tool. Although the exact mechanism of chlorine replacement by thiocyanate/isothiocyanate was not known, it seems likely that the aromatic substrate would be susceptible, in an energetically excited state, to a nucleophilic substitution reaction of ionic nature. The carbon-chlorine bond is known to be susceptible to homolytic fission in the presence of ultraviolet light, generating free radicals, but this is not considered to be the likely mechanism in circumstances such as these (Plimmer, 1970). In the case of nucleophilic substitution of chlorine by thiocyanate, careful selection of reaction conditions might ensure that there is no involvement of isothiocyanate (Colin and Loubinoux, 1983). Both CPA and MCPA were susceptible to photonucleophilic substitution but 2,4-D apparently was not. It may be that the TLC conditions employed were inadequate in resolving photoproducts, as a large unresolved area of quenching was observed under low

wavelength uv light. However, it may be that the pressure of a chlorine atom in the meta position to the other chlorine atom interferes with the reaction. The position and nature of other substituents on aromatic compounds is known to influence their photochemical behaviour (Plimmer, 1970). From the observed substitution of the chlorine on the MCPA molecule, it would seem that a methyl group in the meta position does not affect the reaction.

The influence of thiocyanate on the biological activity of phenoxyacetic acid herbicides has been given some consideration in the past. Hitchcock and Zimmerman (1948) reported synergism between 2,4-D and thiocyanate in tomatoes. Jaff (1982) found that 2,4-D underwent oxidation by in-vitro free-radical generating systems and that thiocyanate could inhibit this. Stephen (1983) suggested that formulation of this group of herbicides with thiocyanate salts might improve their field performance in certain circumstances through inhibition of photochemical transformation within plants. However, the results of this study might suggest that rather than inhibiting such a photochemical reaction, thiocyanate could be a part of it. Jaff (1982) suggested the in vivo generation of a 2,4-D derivative substituted in the 6 position of the aromatic nucleus. A more likely reaction might be substitution of chlorine in the same manner as that observed in vitro. The substitution of chlorine by

cyanate was observed by Crosby and Wong (1973) and was considered to proceed by a photonucleophilic mechanism. Like this study, they used CPA as the substrate for the reaction but also found that there was a substitution of the acetic acid side chain. It may be that such a reaction could occur under natural conditions generating another thiocyanate photoproduct.

In general terms, one can quite reasonably question the relevance of using ultraviolet light from a lamp to simulate the natural situation. Is it possible to extrapolate the results of experiments such as these to the natural environment? Well, obviously low wavelength uv light of this nature and intensity will never reach the earth's surface as long as the ozone layer exists to act as a filter. However, on reviewing the literature it was apparent that essentially the same types of reactions were found to occur in both natural sunlight and light from mercury and fluorescent lamps. The rate of reaction will differ as this is dependent on both the light spectrum and intensity at specific wavelengths. In the natural environment the widespread occurence of photosensitisers creates the possibility of enhanced photochemical transformation through sensitisation reactions. To some extent this counterbalances the enhanced reaction rates observed under laboratory conditions. In relation to this, Boval (1972) discussed the influence of photoproducts

themselves on the rate of 2,4-D photodecomposition. To make a mechanistic study possible he maintained both wavelength distribution and intensity of irradiation at constant values. He concluded that 2,4-dichlorophenol and other reaction products increased the disappearance of 2,4-D. It was propsed that these photoproducts accelerated the transformation of 2,4-D in the initial stage of its reaction. When one considers that Draper and Crosby (1983) found simple aromatic compounds such as p-cresol to function as photosensitisers, the possibility exists that the nature and abundance of photoproducts themselves could have a bearing on the subsequent rate and nature of photochemical transformation of parent chemicals.

In the experiments carried out in this Chapter, loss of side chain from CPA and MCPA were observed. Crosby and Wong (1973) suggested that this probably occurs by autoxidation of the ether followed by re-arrangement of the resulting peroxy radical and hydrolysis. A similar reaction was described by Brown et al. (1964). Loss of ring substituents was observed both before and after side chain loss generating phenols and phenoxy acid photoproducts. The same types of reactions have already been observed in other studies. No hydroxylated photoproducts were identified in this study. Perhaps more sophisticated analytical techniques could have made their separation and identification possible.

The formation of brown polymer material during some experiments may be indicative of hydroxylation followed by polymerisation.

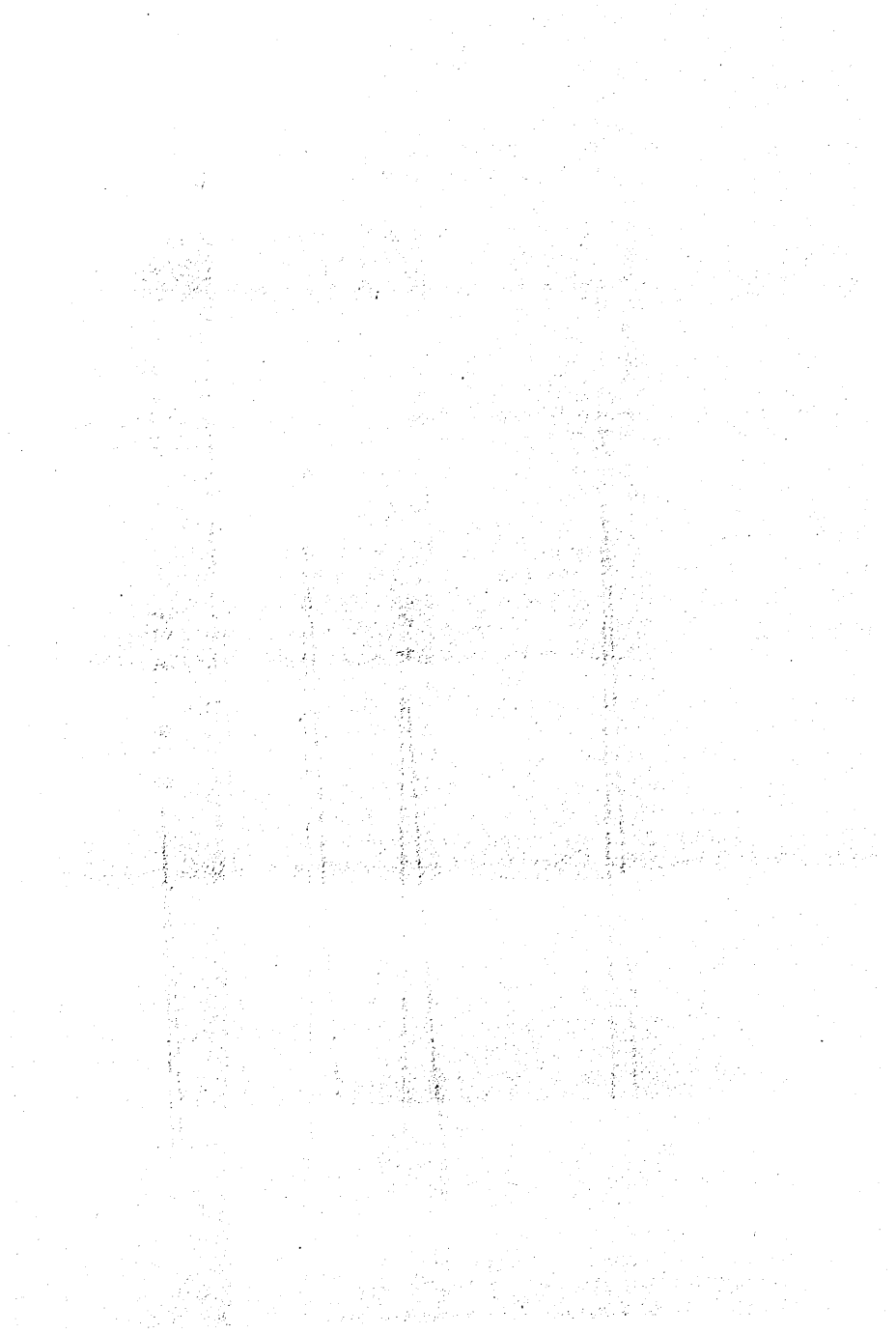


Fig. 6.1 HPLC trace of phenol reference standards

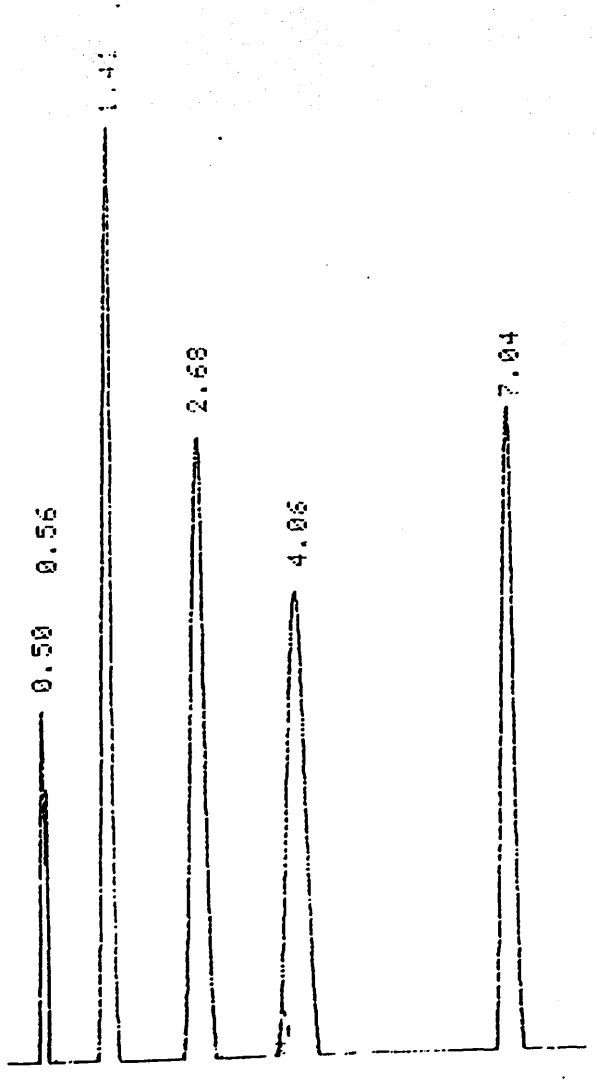


Fig. 6.2 HPLC trace of phenylacetic acid and phenoxyacetic acid reference standards

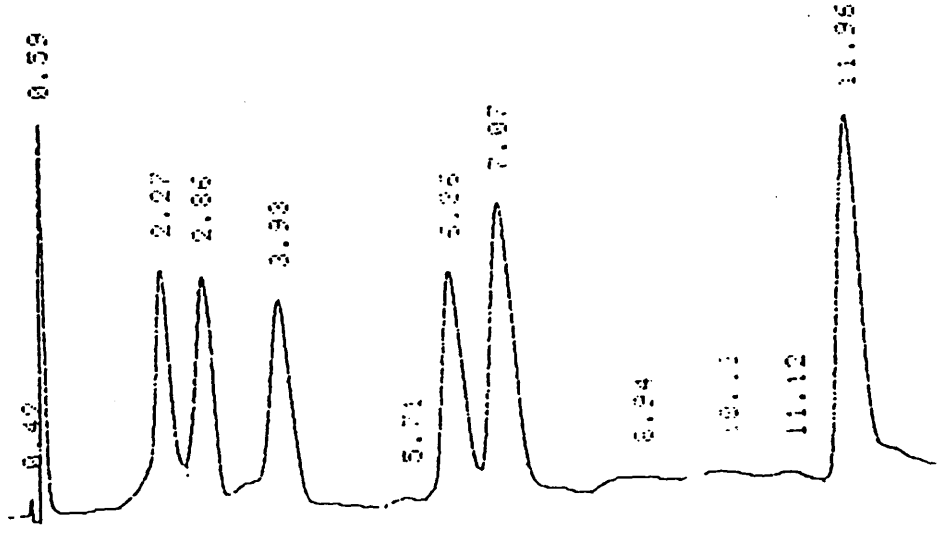


Fig. 6.3 Mass spectrum of thiocyanate substituted CPA photoproduct

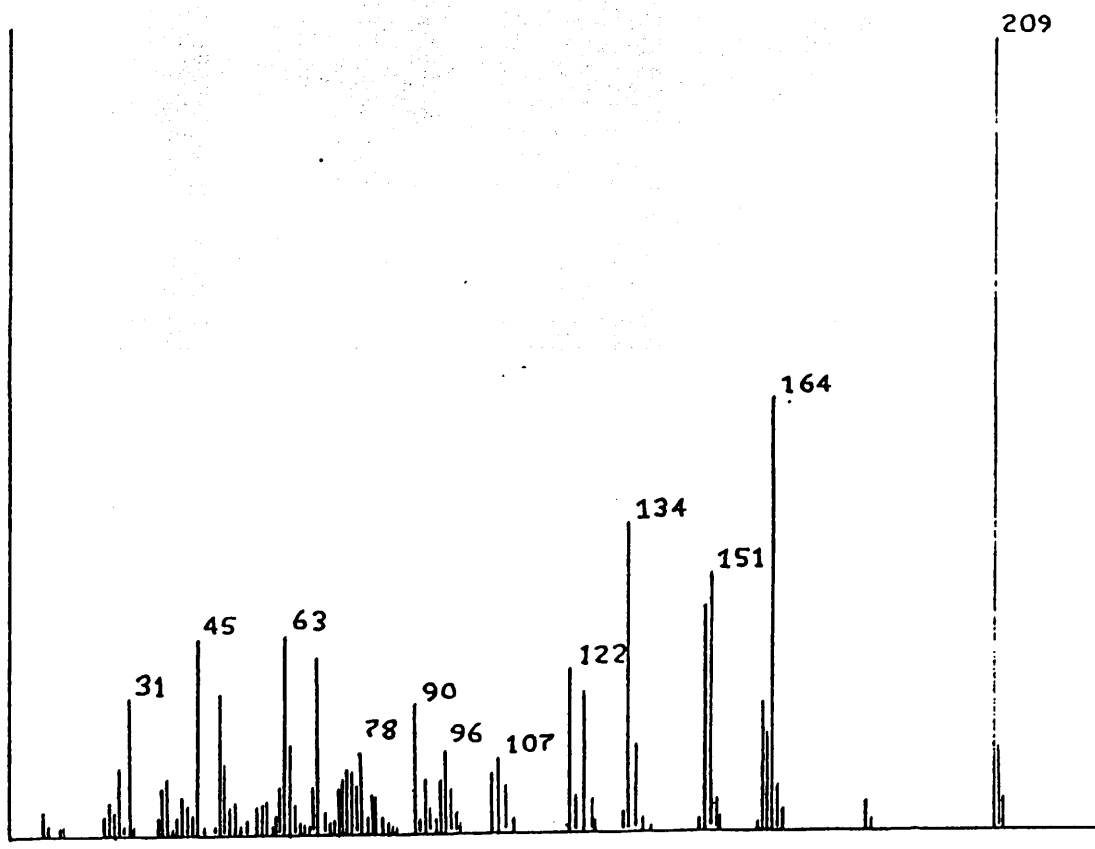


Fig. 6.4 Infra-red spectrum of thiocyanate substituted CPA photoproduct

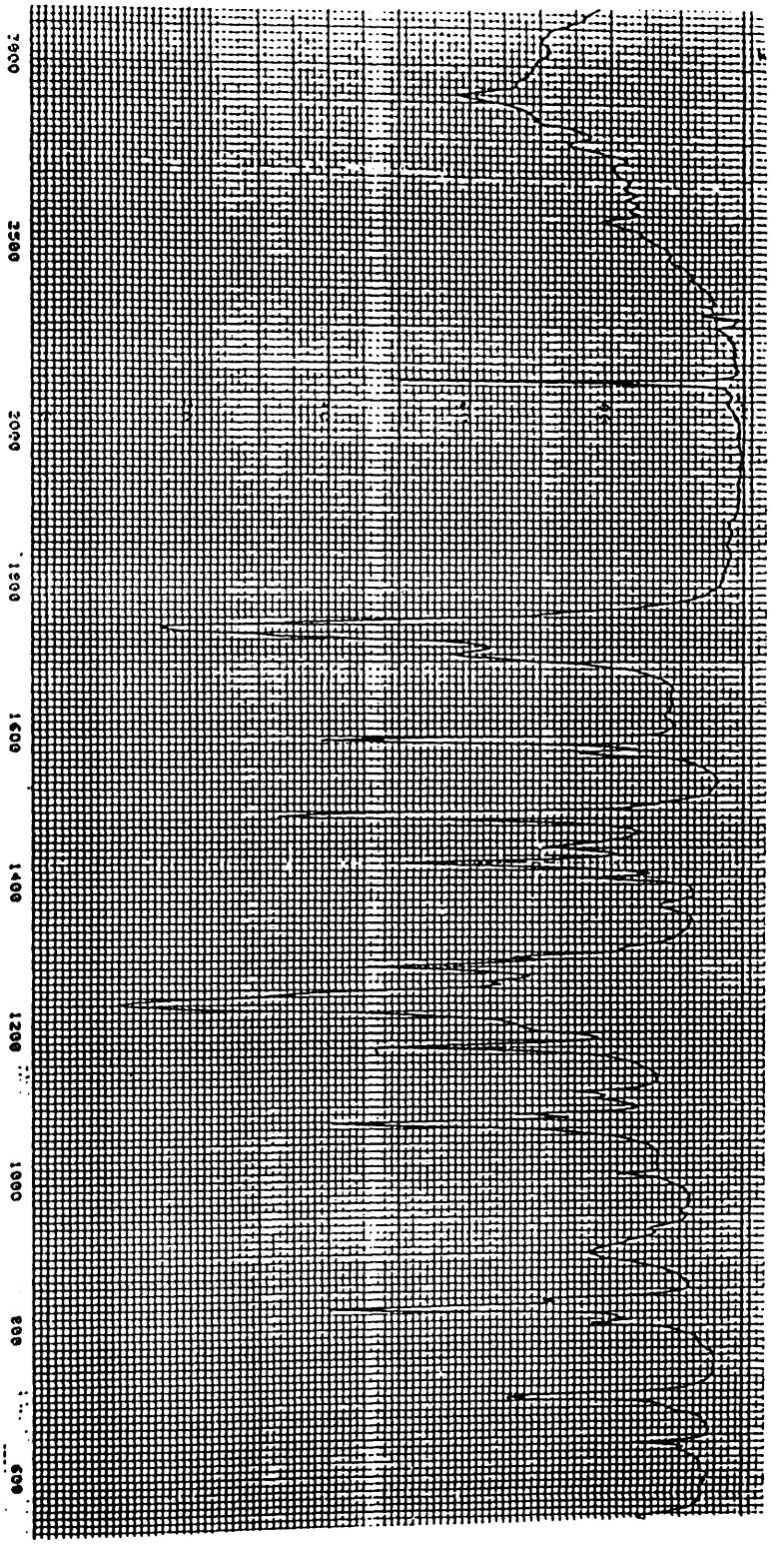


Fig. 6.4a nmv spectrum of thiocyanate substituted photoproduct

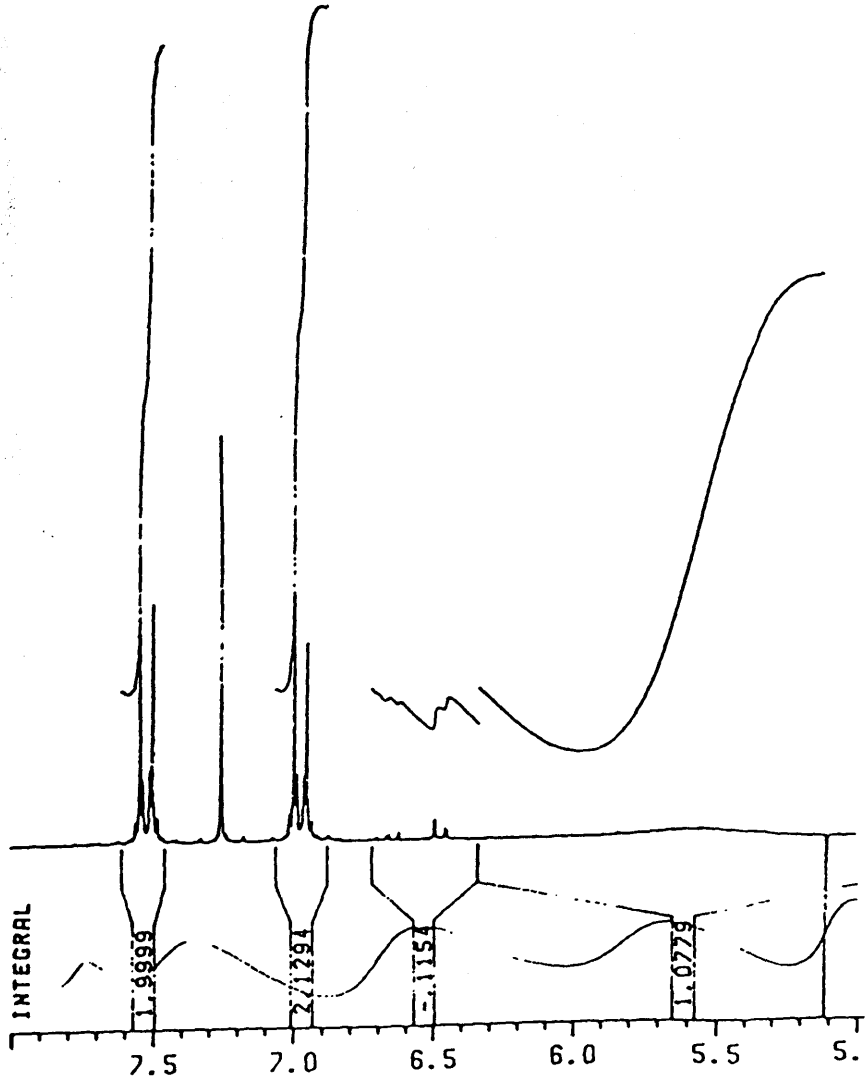


Fig. 6.5 Infra-red spectrum of phenylisothiocyanate

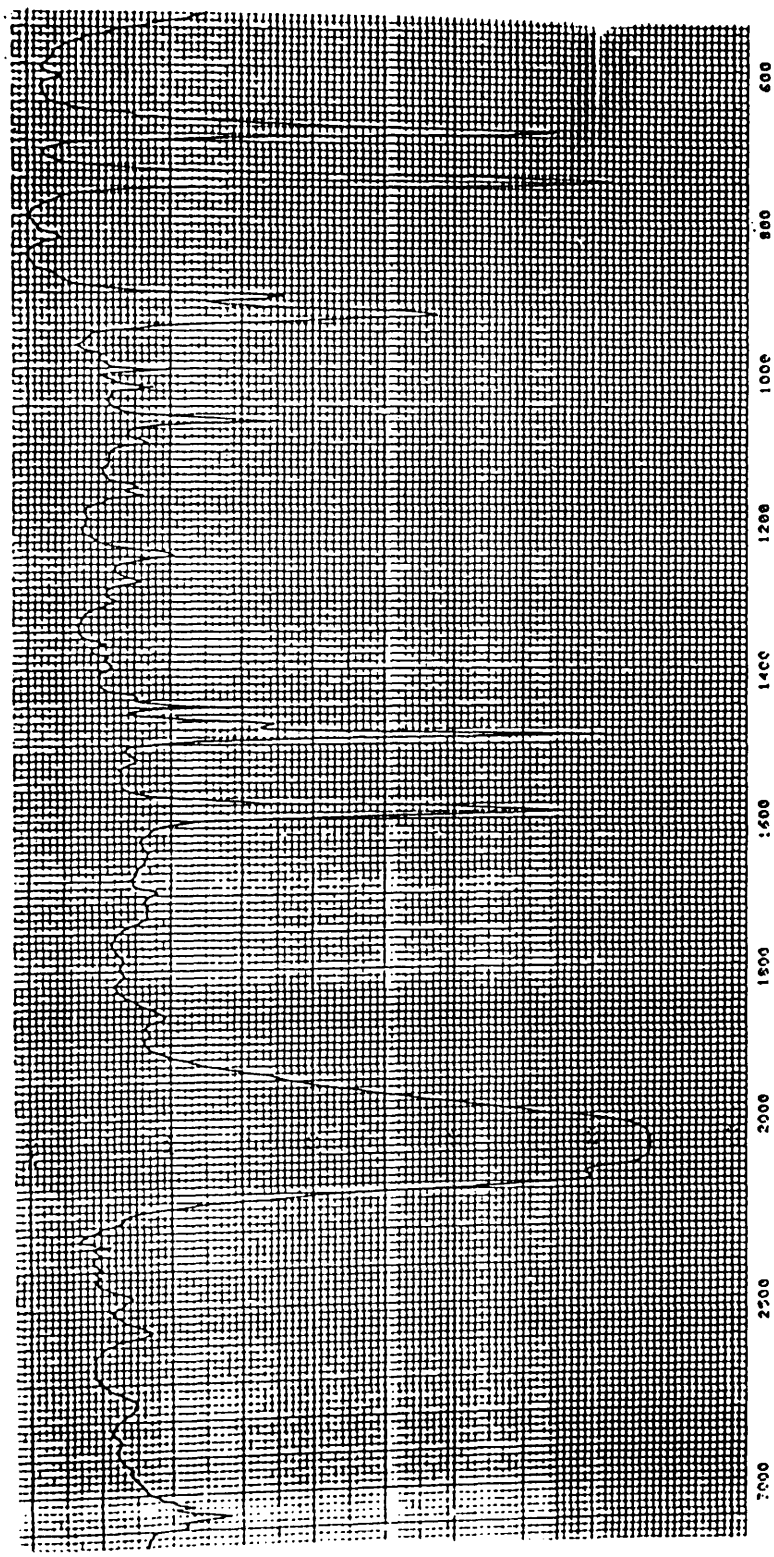


Fig. 6.6 Mass spectrum of unreacted CPA

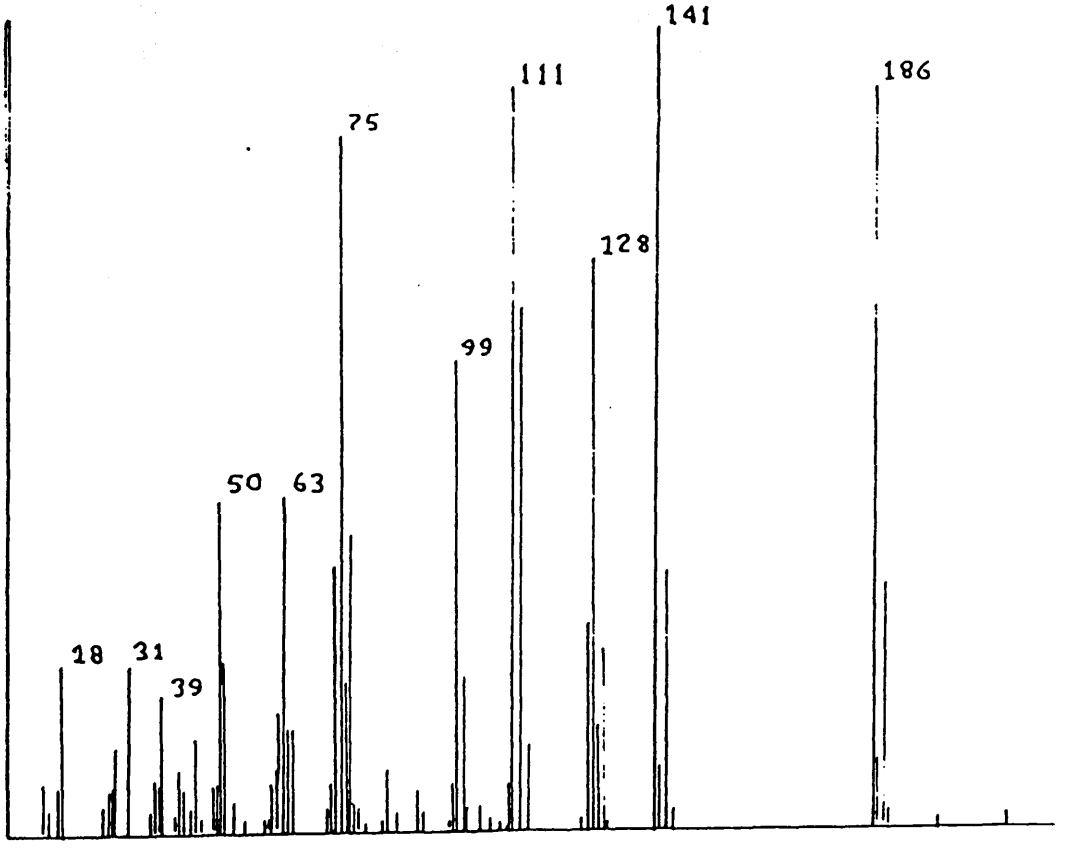


Fig. 6.7 Infra-red spectrum of unreacted CPA

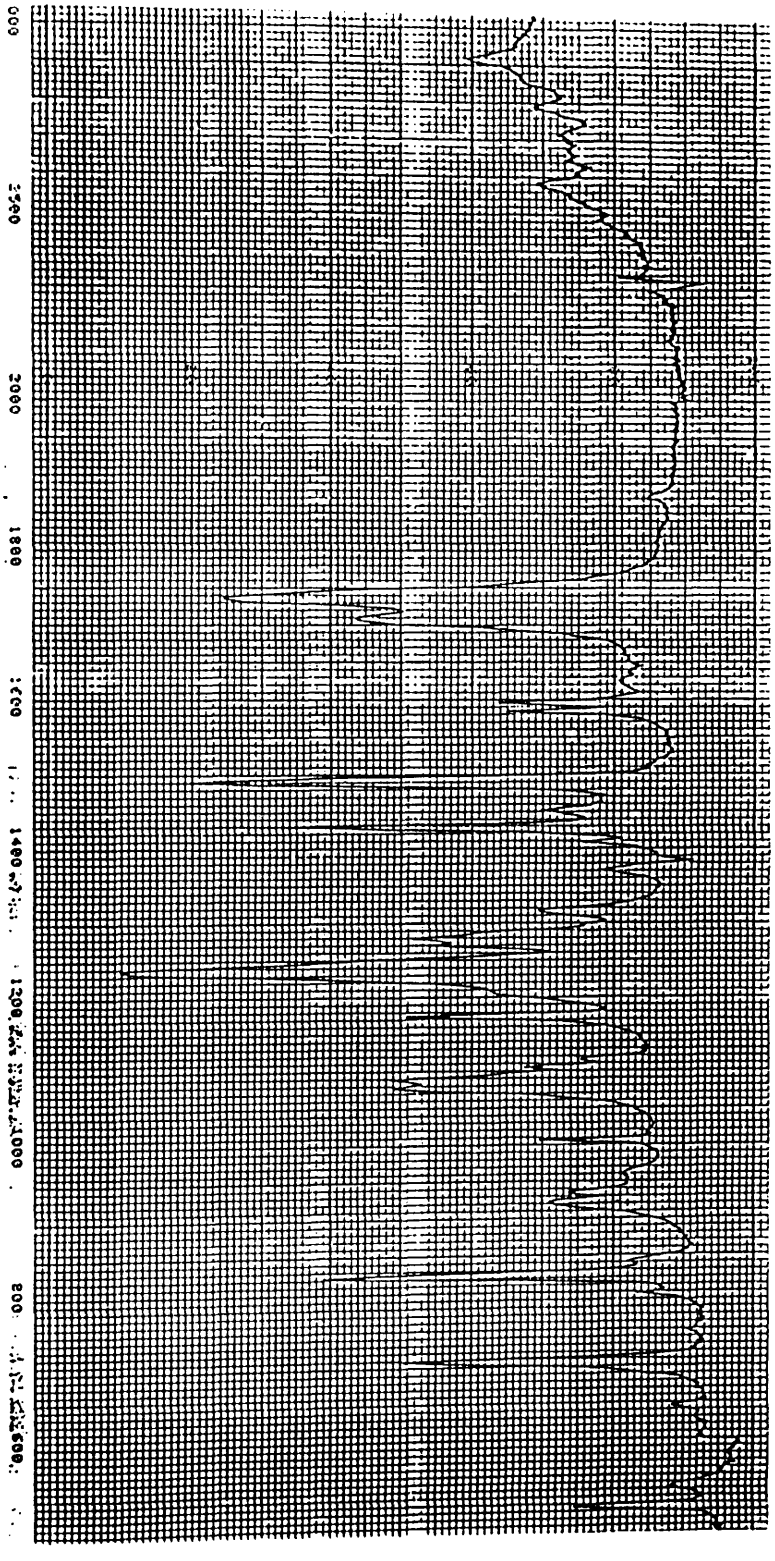


Fig. 6.8 Mass spectrum of CPA reference standard

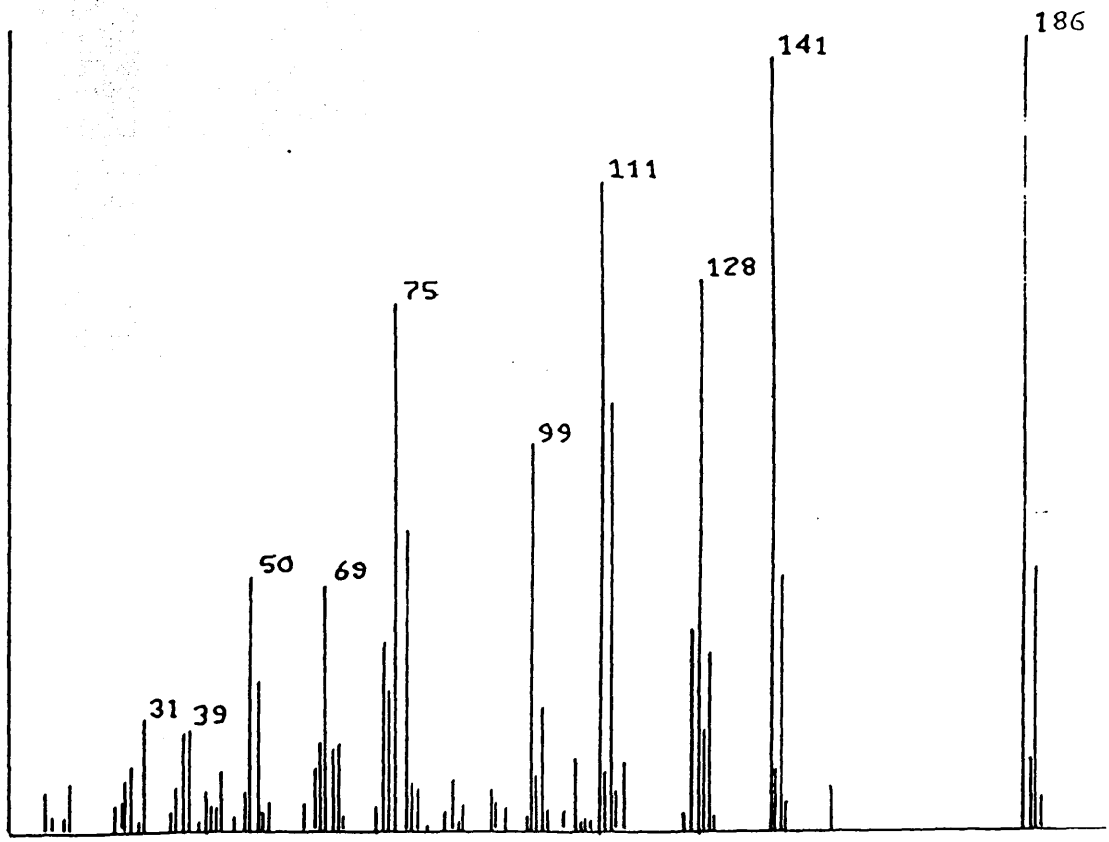


Fig. 6.9 Infra-red spectrum of CPA reference standard

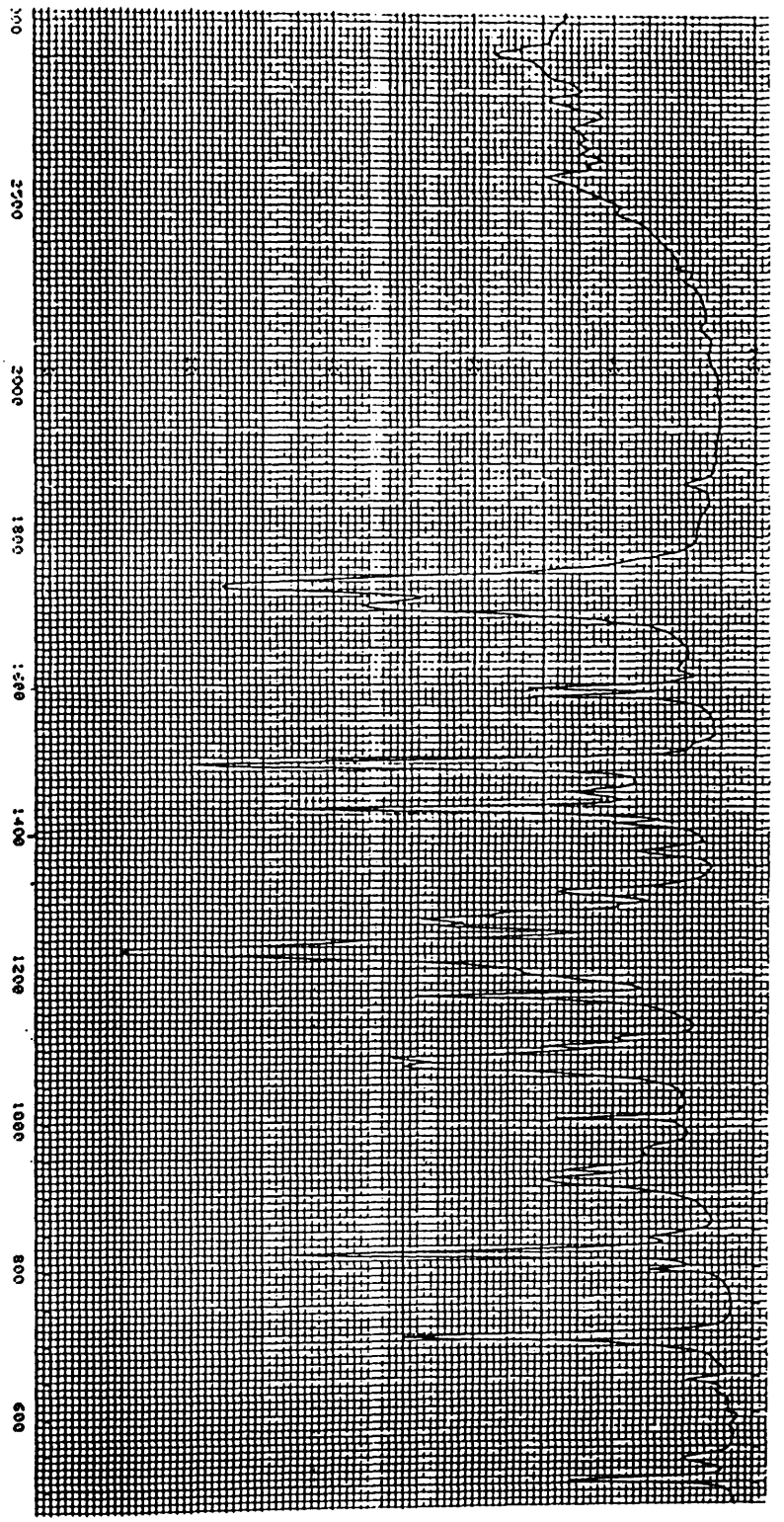


Fig. 6.10 Mass spectrum of isothiocyanate substituted MCPA photoproduct

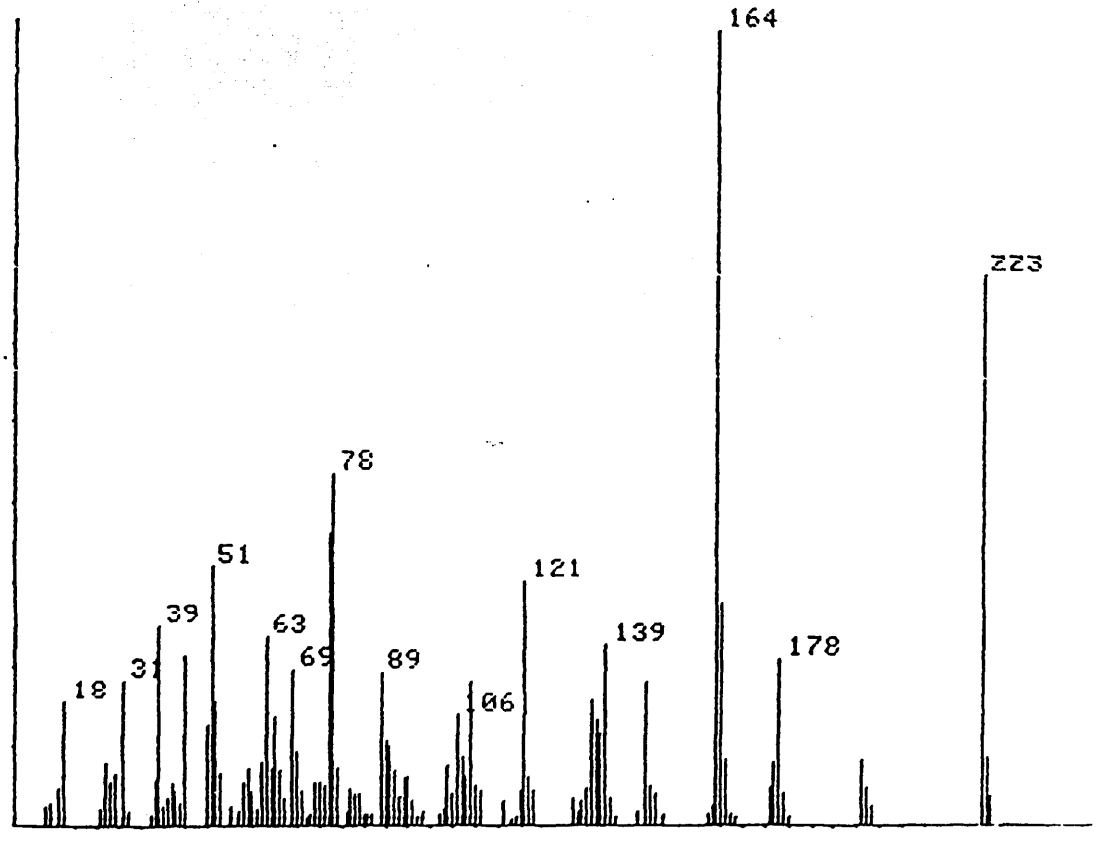


Fig 6.11 Infra-red spectrum of isothiocyanate substituted MCPA photoproduct

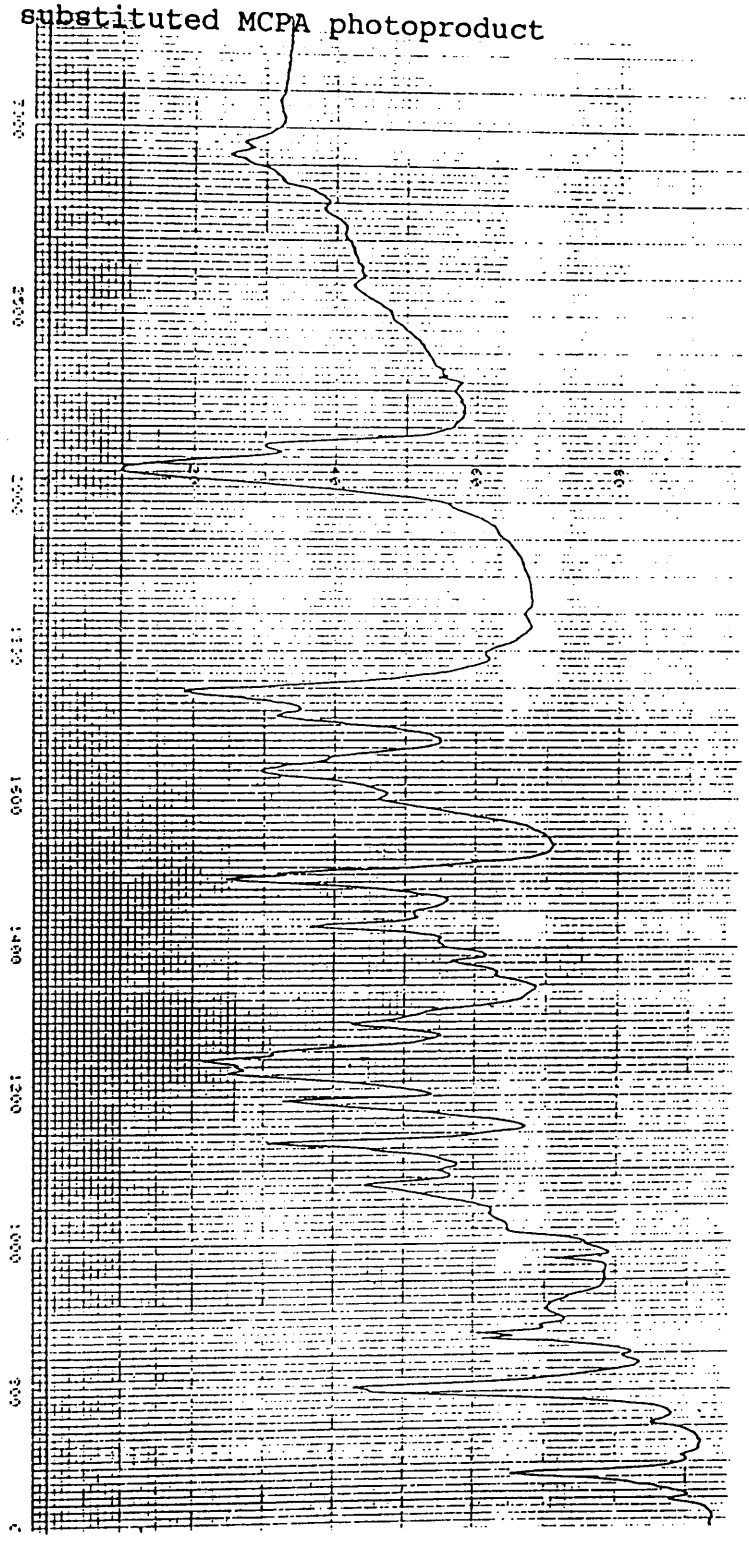


Fig. 6.12 Mass spectrum of unreacted MCPA

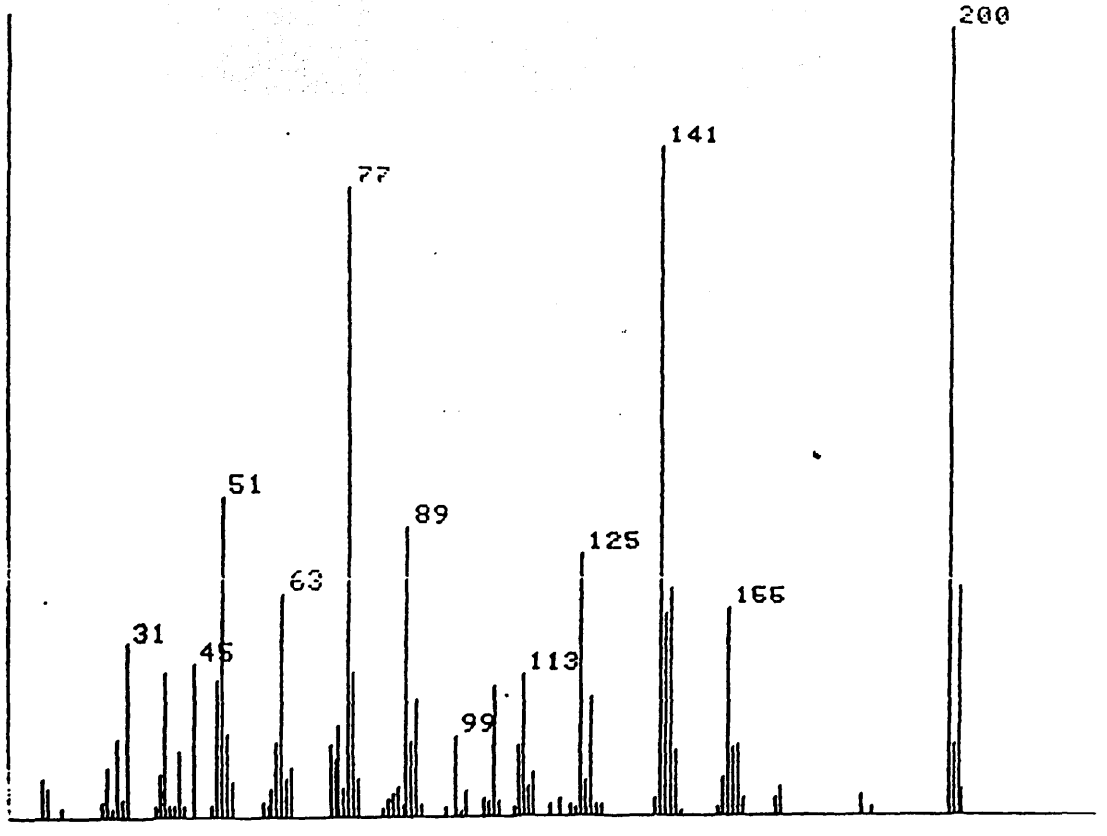


Fig. 6.13 Infra-red spectrum of unreacted MCPA

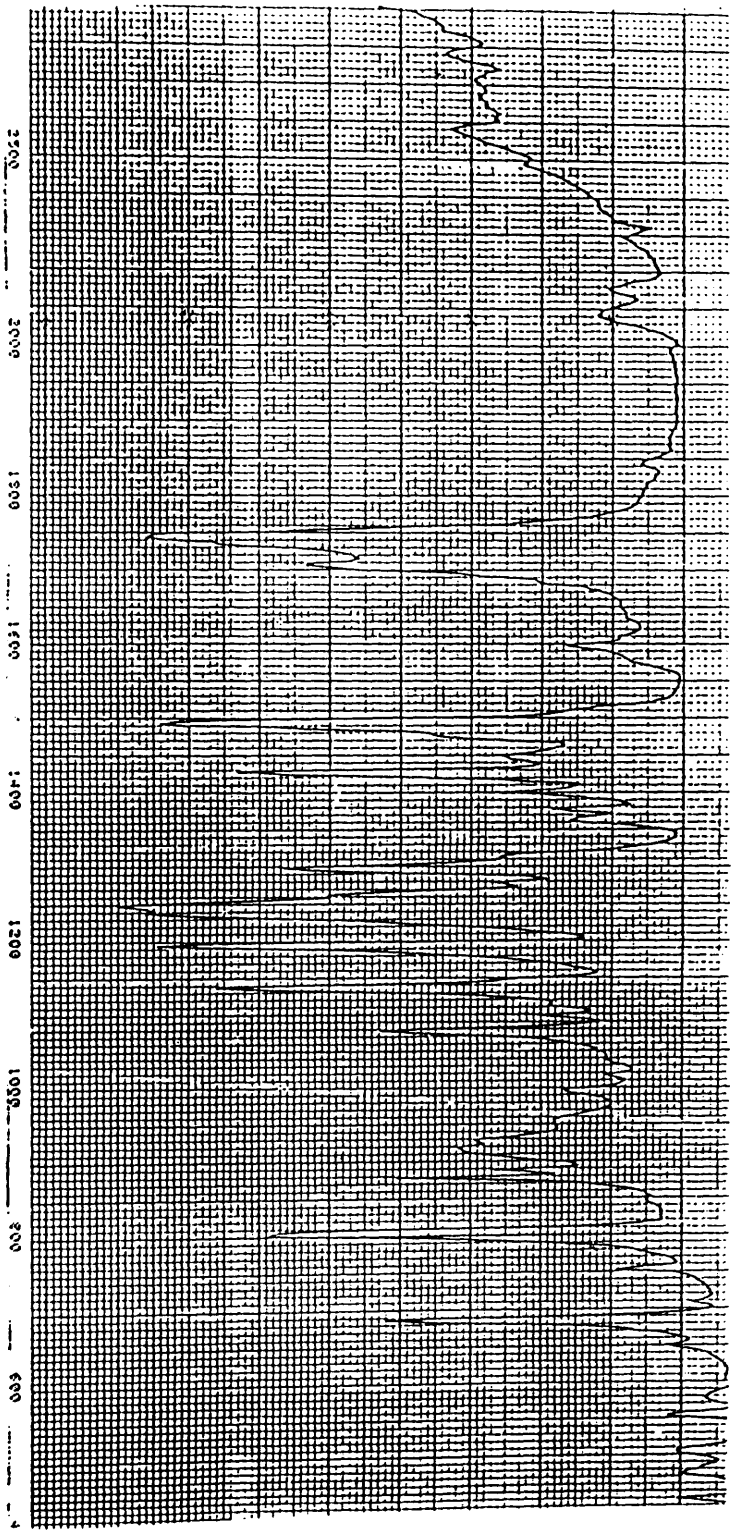


Fig. 6.14 Mass spectrum of MCPA reference standard

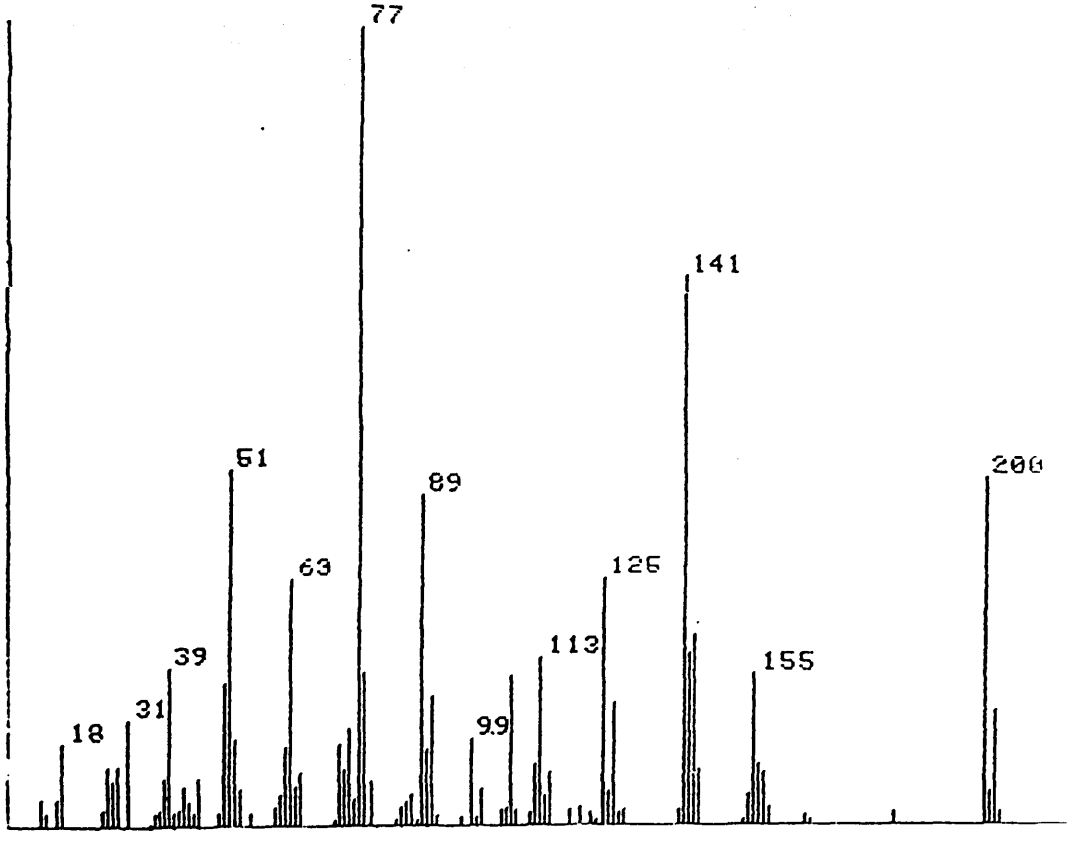


Fig. 6.15 Mass spectrum of unreacted 2,4-D

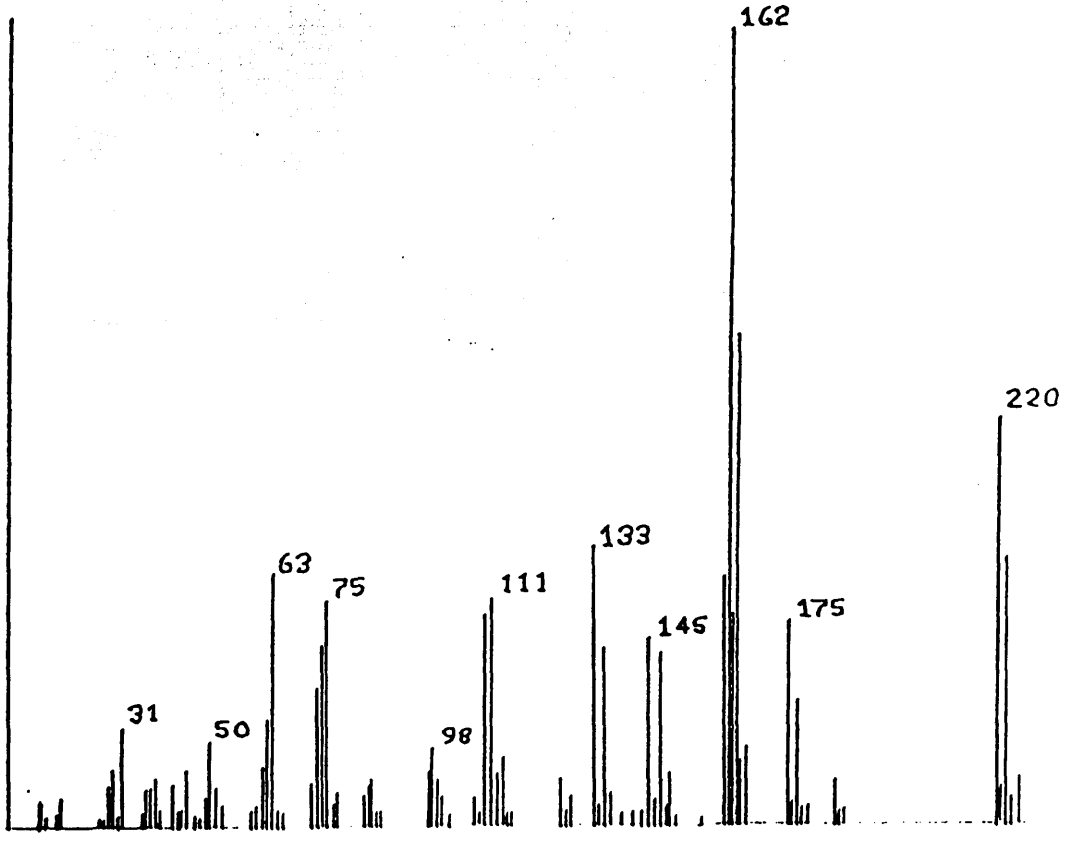


Fig. 6.16 Infra-red spectrum of unreacted 2,4-D

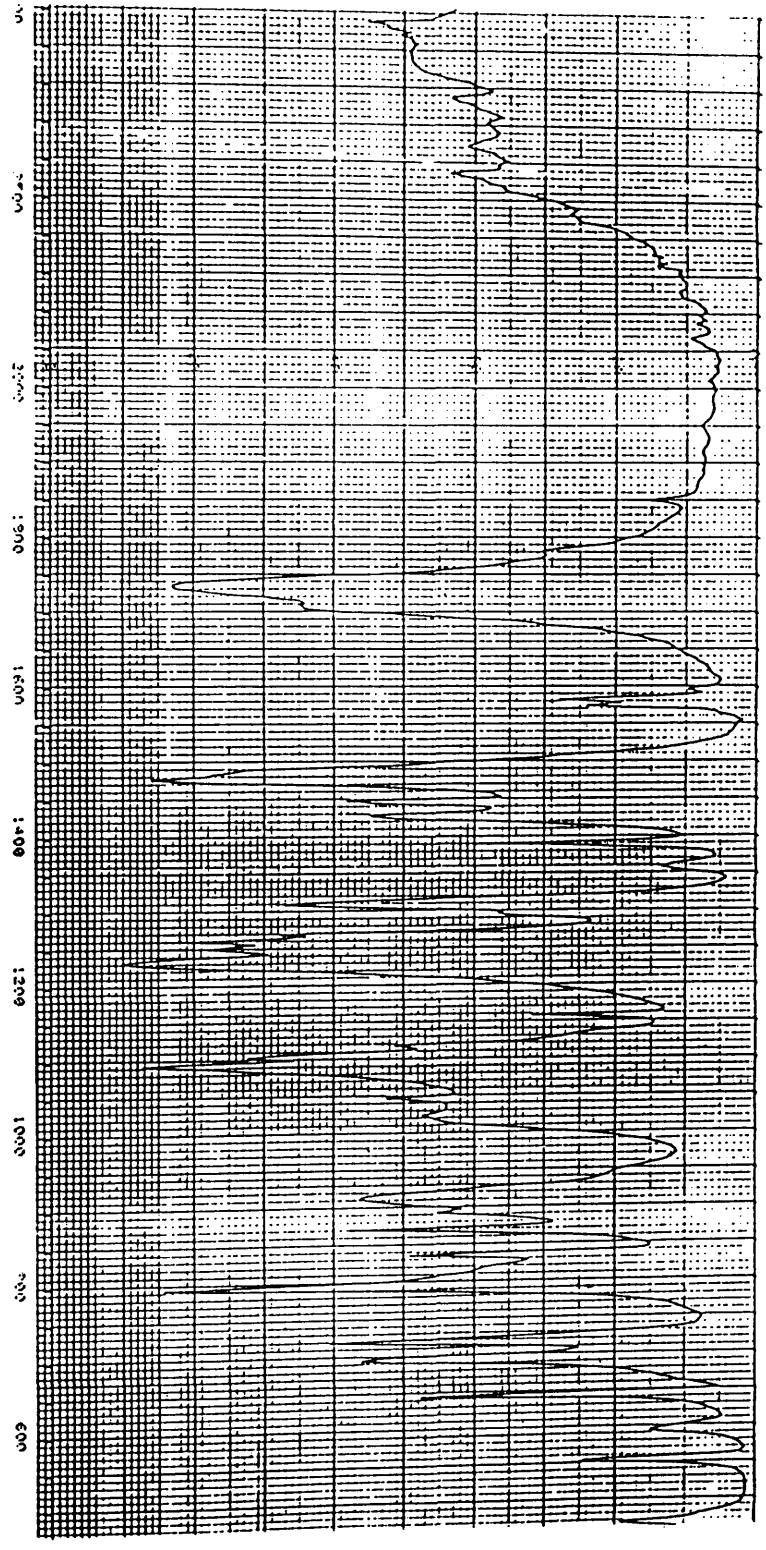


Fig. 6.17 Mass spectrum of unreacted phenoxyacetic acid

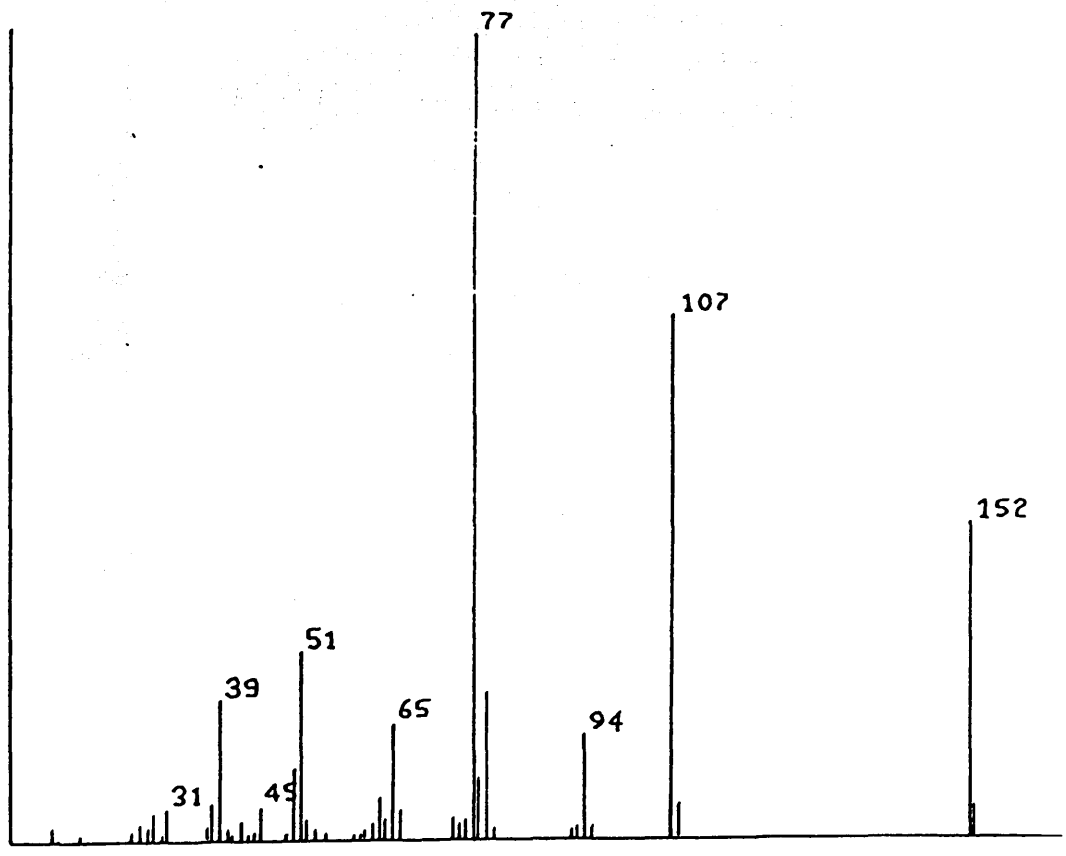


Fig. 6.18 Infra-red spectrum of unreacted phenoxyacetic acid

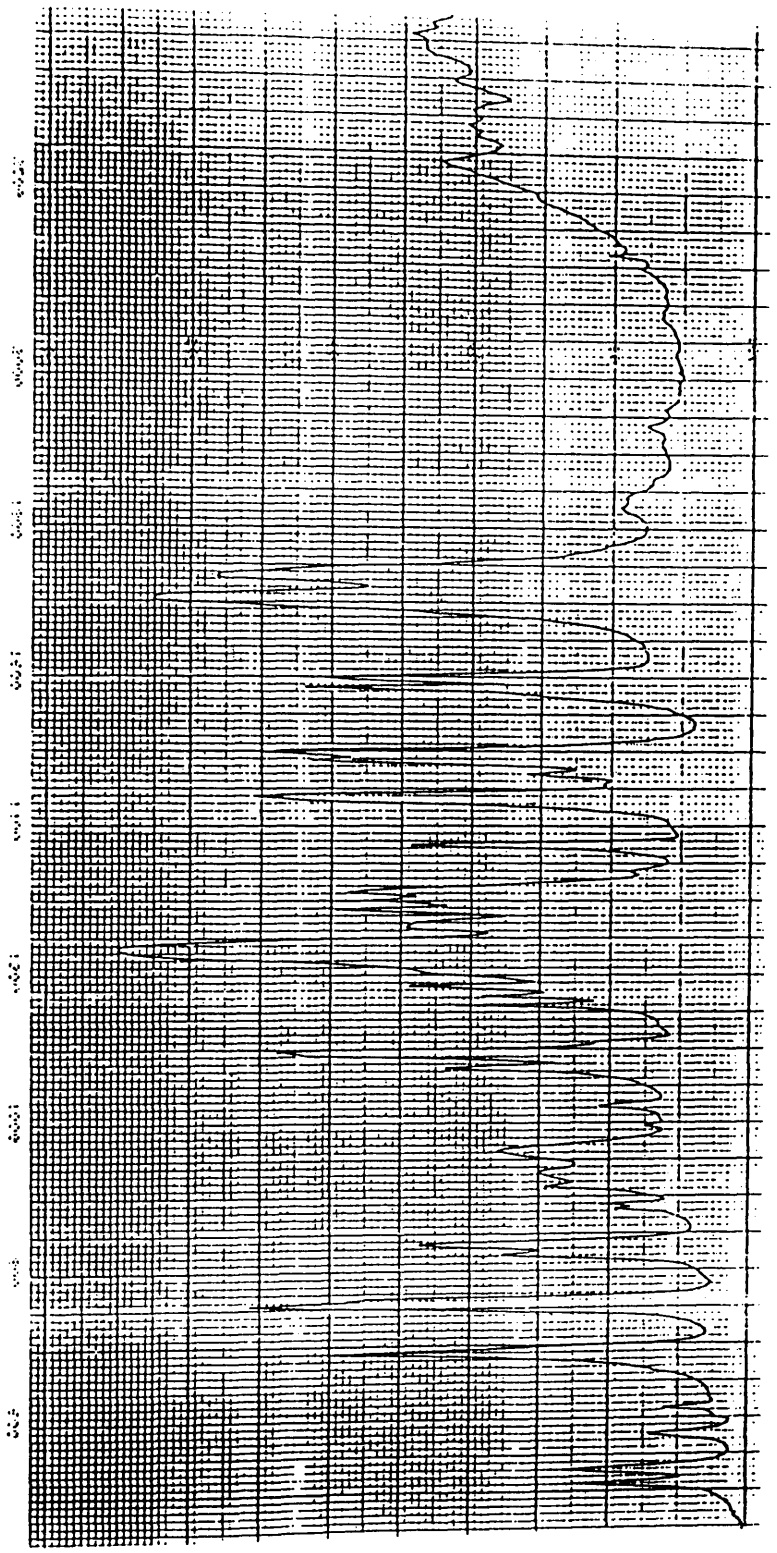


Fig. 6.19 Mass spectrum of phenoxyacetic acid standard

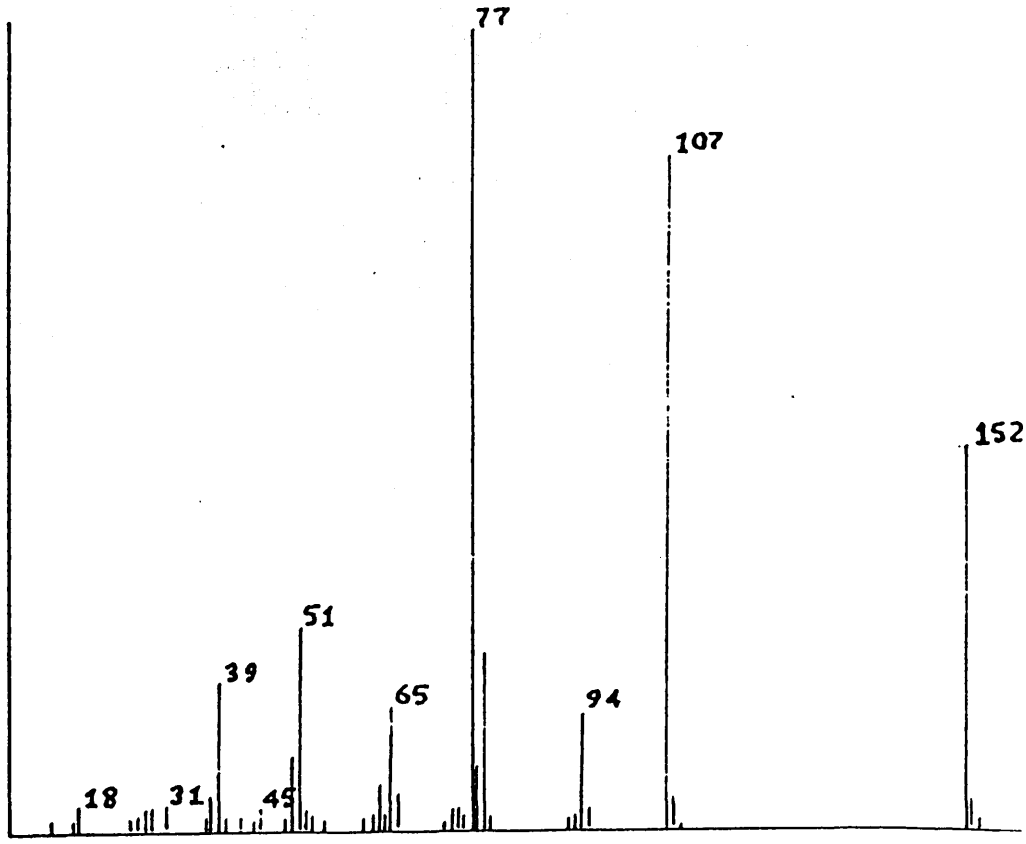


Fig. 6.20 Infra-red spectrum of phenoxyacetic acid reference standard

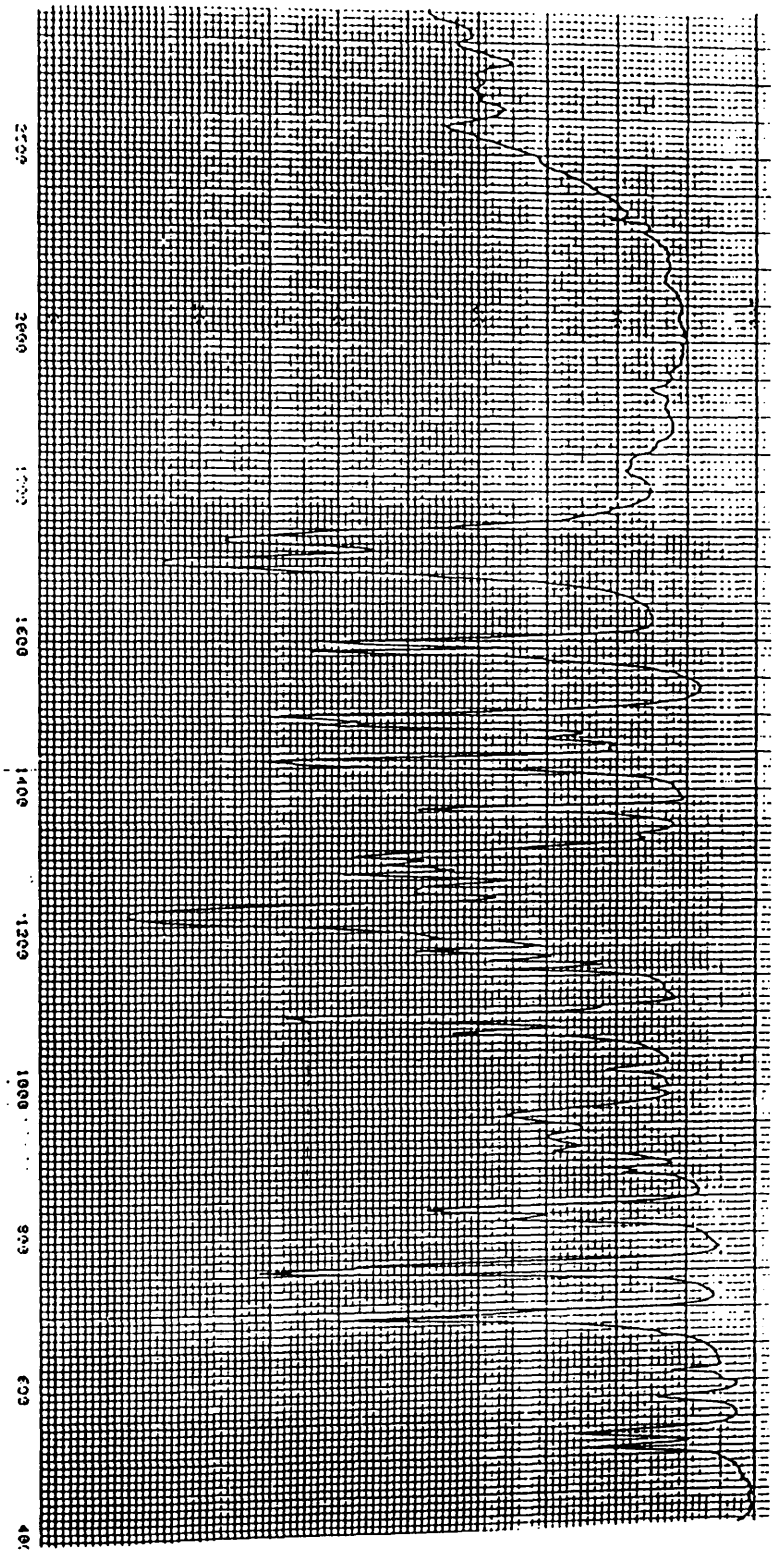


Fig. 6.21 Mass spectrum of 2-hydroxyphenylacetic acid photoproduct

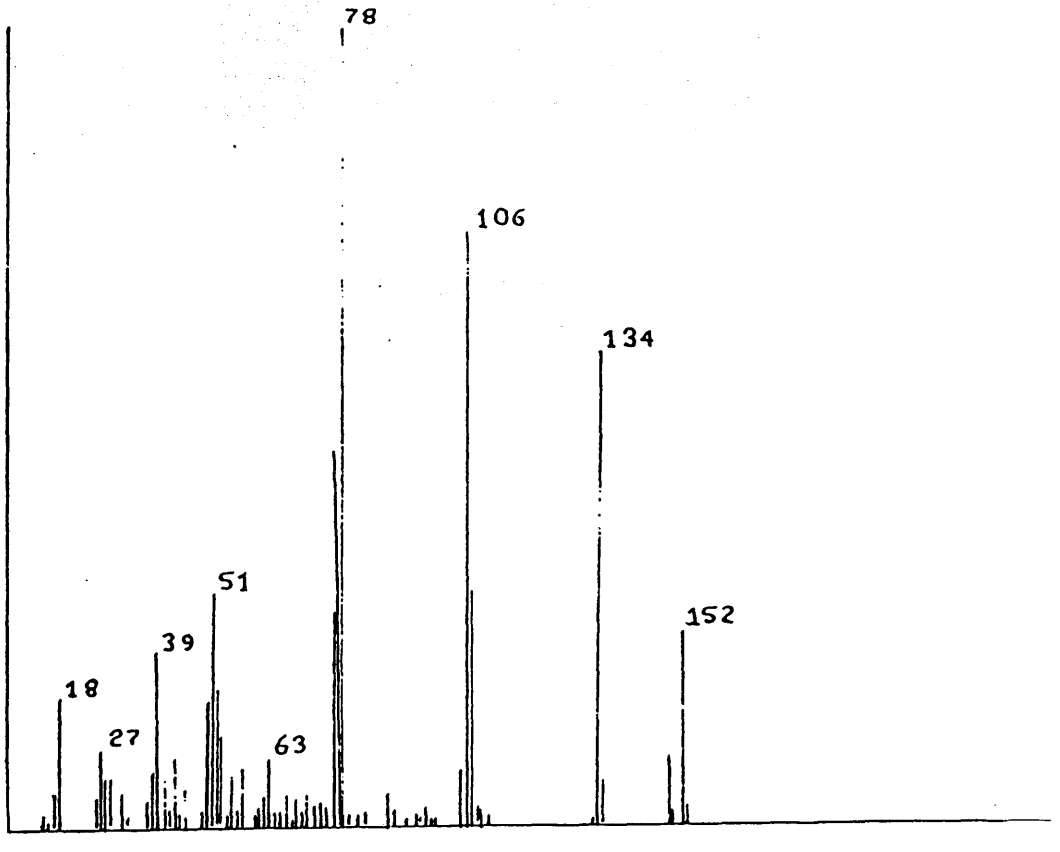


Fig. 6.22 Infra-red spectrum of 2-hydroxyphenylacetic acid photoproduct

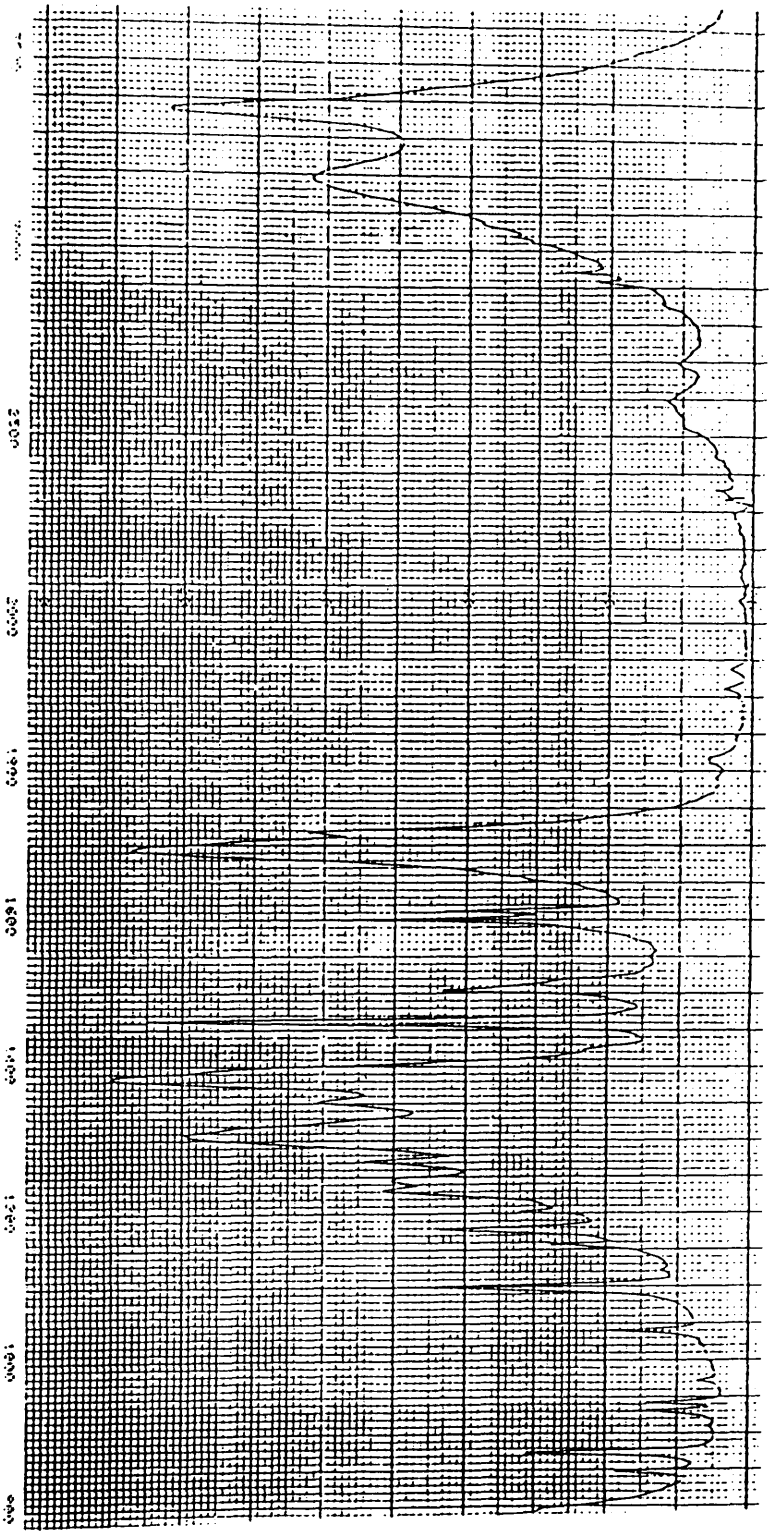


Fig. 6.23 Mass spectrum of 2-hydroxyphenylacetic acid reference standard

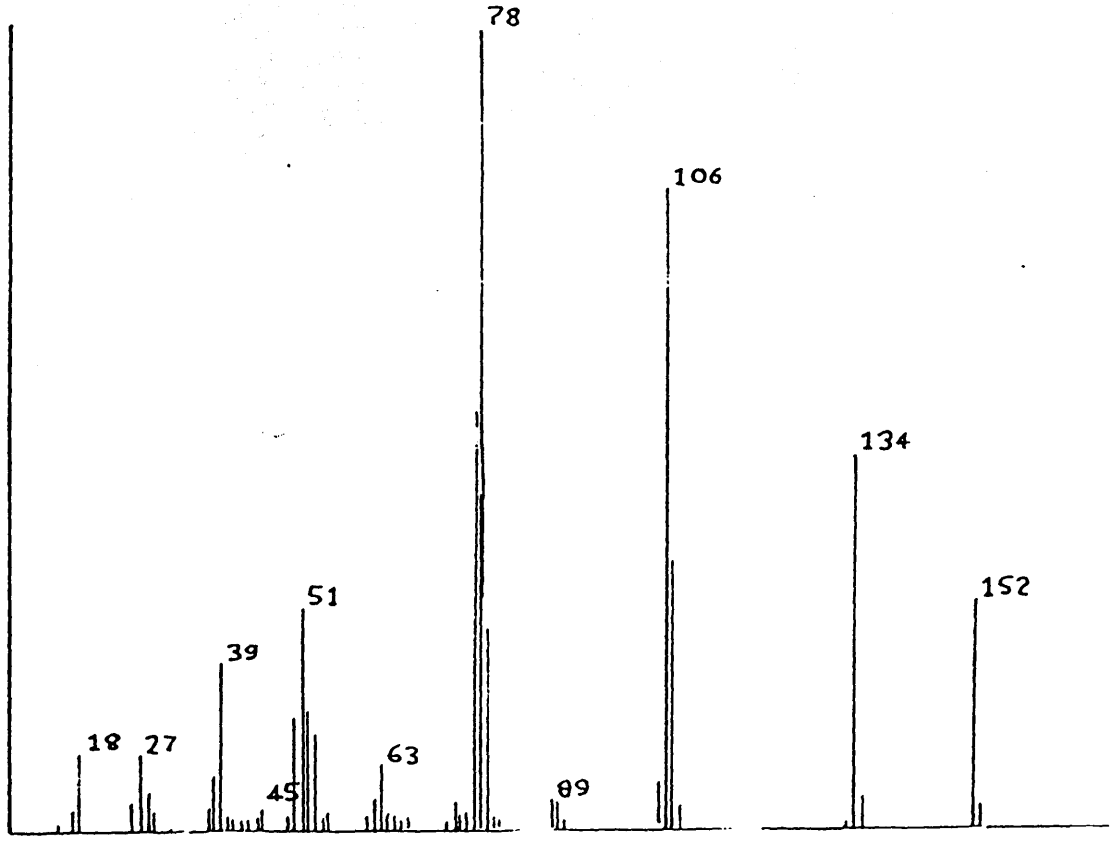


Fig. 6.24 Infra-red spectrum of 2-hydroxyphenylacetic acid reference standard

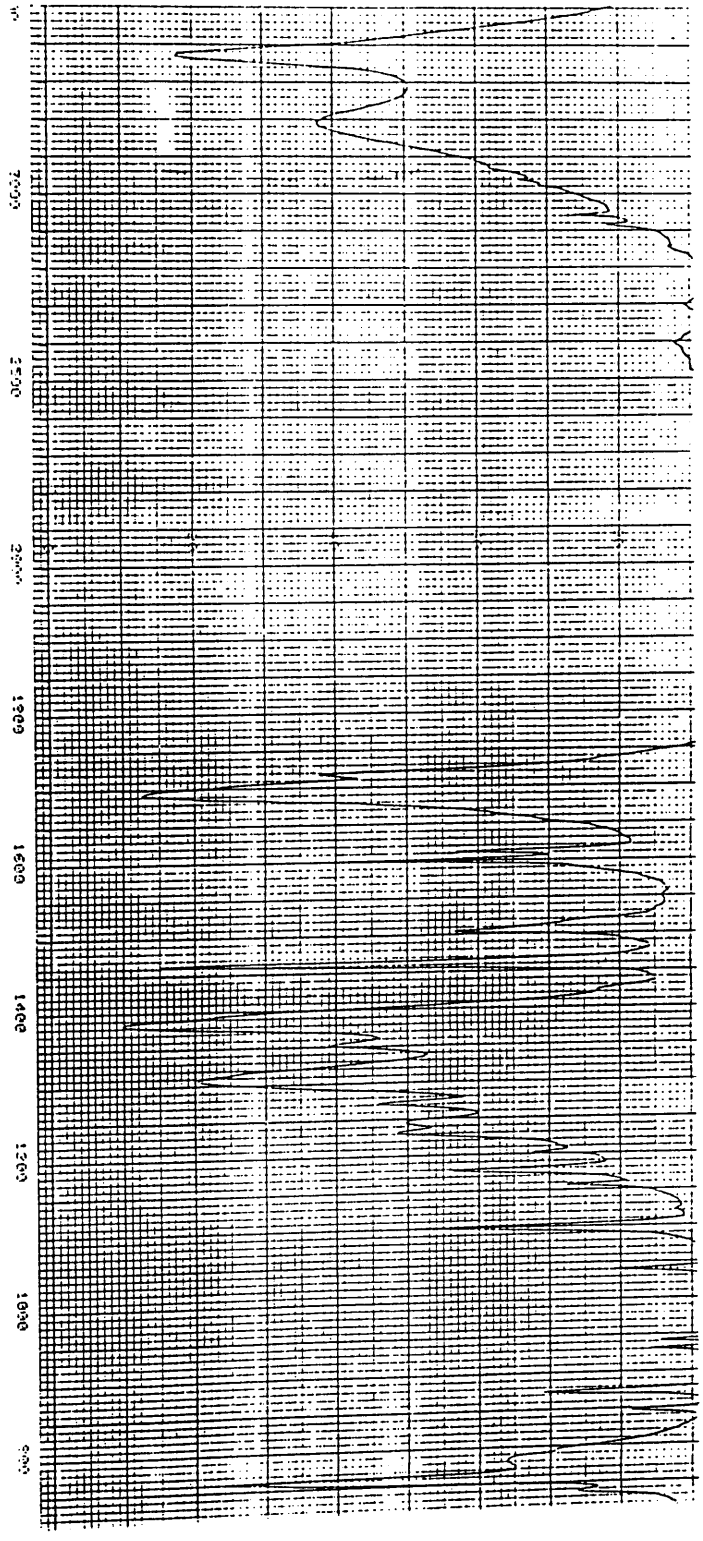


Fig. 6.25 Mass spectrum of 4-hydroxyphenylacetic acid photoproduct

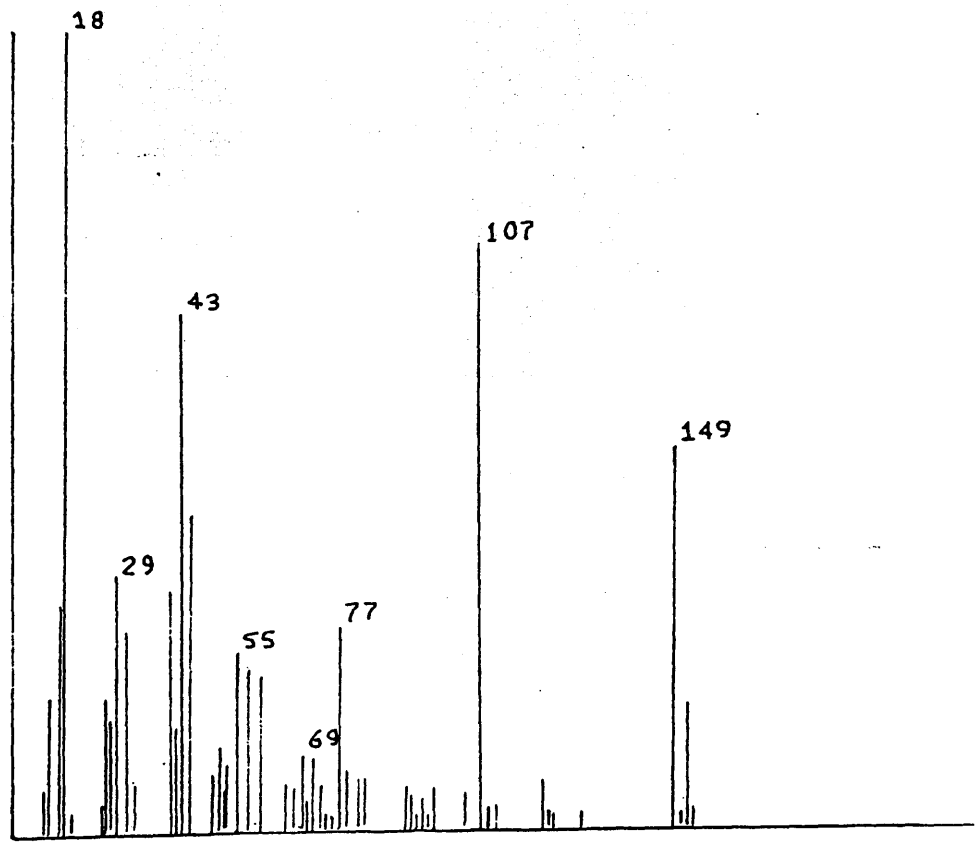


Fig. 6.26 Infra-red spectrum of 4-hydroxyphenylacetic acid photoproduct

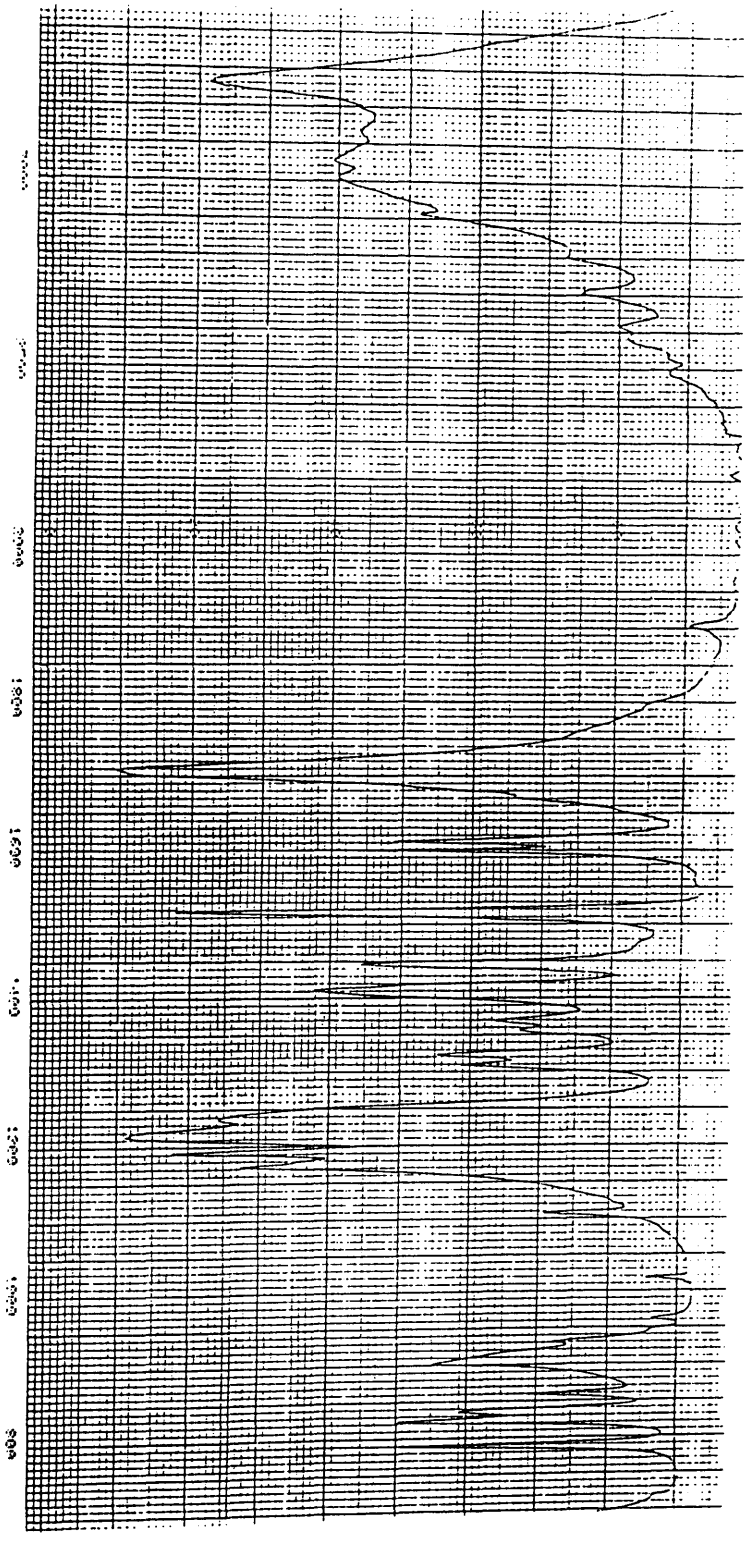


Fig. 6.27 Mass spectrum of 4-hydroxyphenylacetic acid reference standard

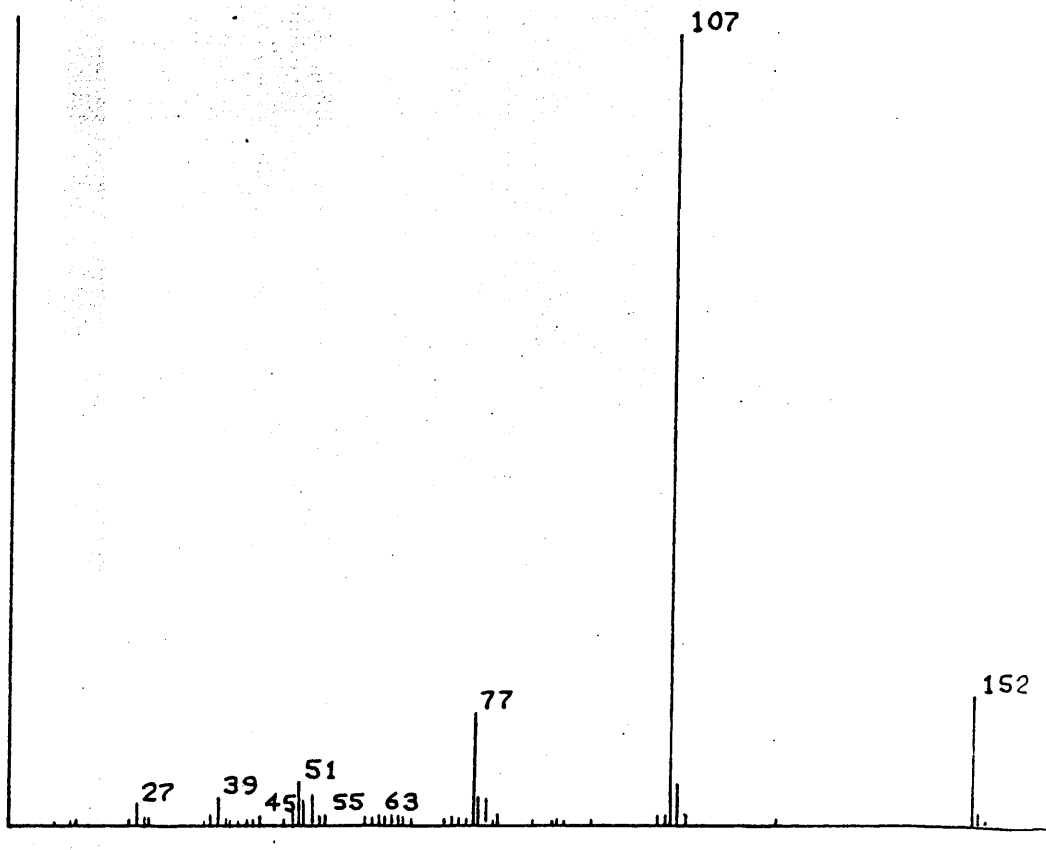
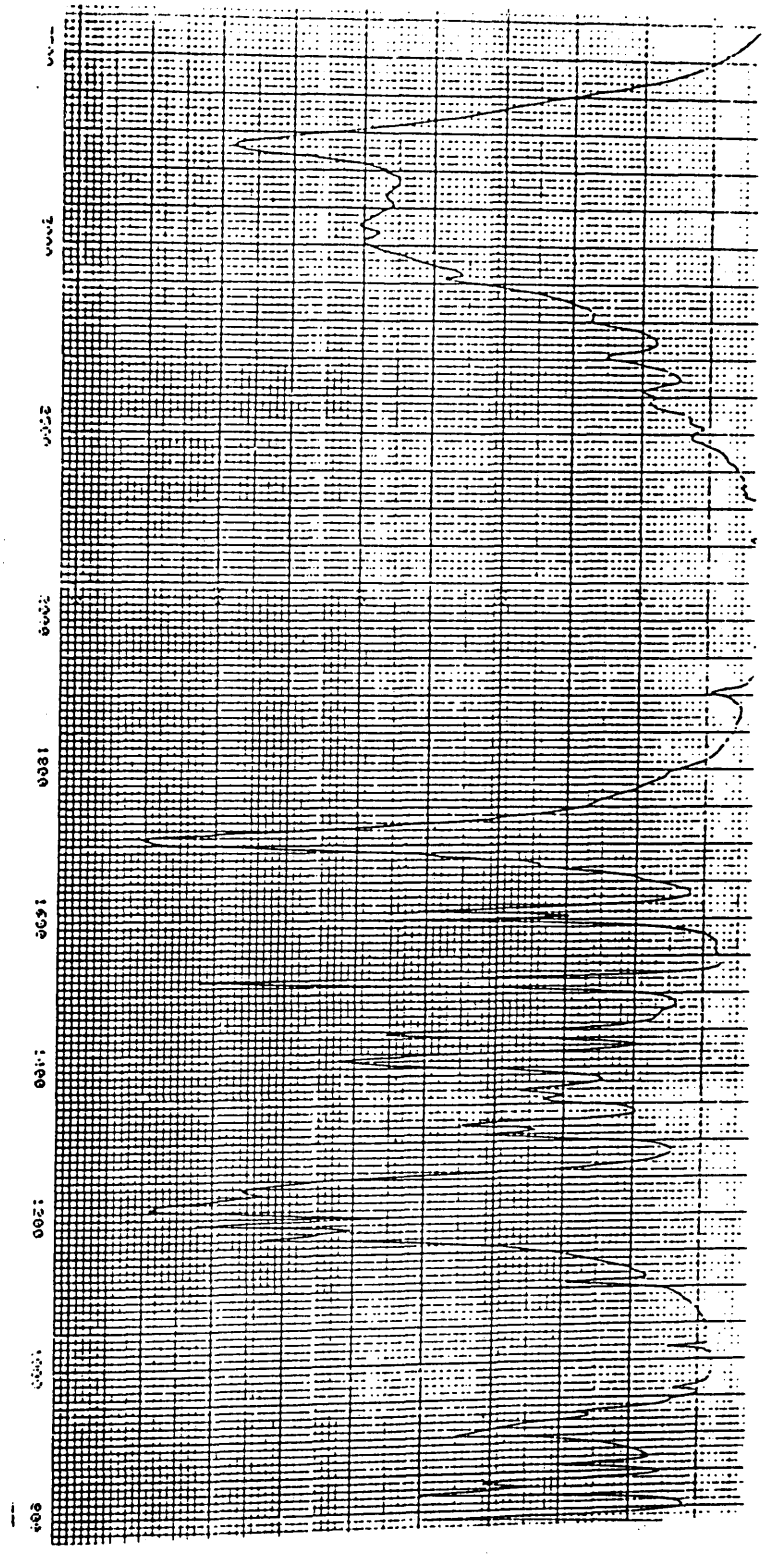


Fig. 6.28 Infra-red spectrum of 4-hydroxyphenylacetic acid reference standard



CHAPTER 7

THE USE OF ADDITIVES TO REDUCE THE PHOTODEGRADATION OF PHENOXYACETATE BUTYL ESTERS

Introduction

The object of this Chapter was to investigate the potential use of both organic and inorganic additives for controlling the photochemical transformation of selected herbicides. Although the present study examines the photochemistry of only three selected herbicides, it has already been emphasized that virtually any pesticide can be photochemically transformed. The results, therefore, may have more far-reaching implications.

Additives used to modify pesticide photochemistry

i) Inorganic additives

Some early work in this area was carried out in this Department and a summarised account seems appropriate. Cook (1979) assessed the potential use of ammonium thiocyanate and other free radical scavenger additives for reducing the photodecomposition of aminotriazole (3-amino-1,2,4-triazole). Two free radical generating systems were used to oxidise the chemical, namely the photochemical riboflavin system (Castelfranco and Brown, 1963) and the ferrous sulphate - hydrogen peroxide chemical system (Plimmer et al. 1967).

Under the conditions used in this study it was demonstrated that aminotriazole could be readily oxidised by the hydroxide free radicals and photodecomposed in the presence of the riboflavin photosensitiser. Losses in both instances were significantly reduced when additives were employed. Ammonium thiocyanate and sodium iodide were particularly effective and thioacetamide also reduced losses but to a lesser extent.

Improved bracken control through the addition of ammonium thiocyanate to aminotriazole formulations was observed by Cook (1979), with elimination of the scorching normally associated with the application of formulations containing aminotriazole alone. This scorching was considered to effectively inhibit the translocation of aminotriazole down to the bracken rhizomes and in this way give extremely poor control of the weed, since destruction of the rhizomes was essential for effective, long-term control.

Field results from Cook's research revealed that although sodium iodide was an effective inhibitor of aminotriazole photodecomposition in vitro, it gave poor results when applied to bracken in formulation with aminotriazole. The sodium iodide was found to cause severe scorching of the bracken fronds, impairing aminotriazole translocation and hence effectiveness (the scorching is actually caused by the oxidation of iodide to iodine within the bracken fronds).

Stephen (1983) also considered the potential use of ammonium thiocyanate and other additives, as a means of controlling aminotriazole photodecomposition and detoxification by weed species.

The earlier observations by Cook (1979) prompted a search by Stephen (1983) for a more rapid bioassay technique for assessing additives in vivo and several useful methods were developed. Using these bioassays it was shown that ammonium thiocyanate, in particular, as well as other additives could be successfully employed to effectively reduce aminotriazole scorching in several different plant systems.

The conclusion from both these studies was that the general scorching and poor weed control exhibited by aminotriazole when formulated alone was as a consequence of photo-oxidation within the leaves of the different plant species. Endogenous free radical generating systems such as porphyrin containing chlorophylls, quinones, furanocoumarins, polyacetylenes and thiophenes have all been shown to exist in plants (Dodge and Knox, 1986), and it is possible that systems such as these could be responsible for the generation of aminotriazole free radicals. Such free radicals could then conjugate with other plant constituents and this is thought to be the mechanism by which aminotriazole induces scorching when applied alone.

Conjugation with endogenous amino acids and sugars is actually considered to be a major de-toxification mechanism in higher plants (Ashton and Crafts, 1973) and it has been postulated that the generation of aminotriazole free radicals is the first step in the de-toxification pathway (Cook, 1979).

The presence of free radical scavengers would certainly be likely to inhibit, or at least reduce, this conjugation with plant constituents and it was the ability of ammonium thiocyanate, sodium iodide and the other tested additives to function as free radical scavengers that was thought to account for the effects observed by both Cook (1979) and Stephen (1983).

The thiocyanate anion, in fact, has been shown to be a free radical scavenger, functioning as such through a charge transfer mechanism (Adams et al., 1972), and it has been demonstrated that ammonium thiocyanate can effectively inhibit the formation of (3-[3-amino-1,2,4-triazole-1-yl]-2-aminopropionic acid), a major aminotriazole metabolite (Carter, 1975) formed through conjugation with serine. This conjugate is both less toxic and less mobile than aminotriazole alone and is thought to be responsible for the observed weed scorch.

Similar findings were reported earlier when it was demonstrated that aminotriazole could be incorporated into protein upon incubation with riboflavin in the presence of light, and that this photosensitised reaction

could be inhibited by ammonium thiocyanate and to a lesser extent by cystine and methionine (Carter, 1969).

Aminotriazole does, then, provide one of the most obvious and best known examples of impairment of pesticide function through photochemical transformation, and almost complete elimination of this impairment through the use of additives. In the particular case of aminotriazole, the observed transformation was a photosensitised one and it was stressed by both Cook (1979) and Stephen (1983) that the extent of aminotriazole scorching is dependant upon free radical generating capacity within a particular plant species.

This may seem obvious, but it is a critical factor in determining the effectiveness of a particular additive in a practical situation and will determine the optimum amount of additive to use.

This concludes the introduction to the application of inorganic additives for the inhibition of pesticide photodecomposition. There has been recent interest in the use of organic compounds for the same purpose.

ii) Organic additives

Dureja et al. (1984) reported the results of a study which was aimed at testing compounds for the control of pyrethroid insecticide photodecomposition. This class of insecticide has been found to be

particularly photolabile and natural pyrethroids, particularly, have had limited use for crop protection because they are so rapidly destroyed by light.

One technique used to impart photostability has been to protect photolabile sites on the insecticides by synthesizing molecules containing halogenated and other stable functional groups. Other methods have involved formulation of the pesticides with anti-oxidants and uv screens. An example of this was the protection of the non-halogenated pyrethroids, bioethanomethrin and resmethrin, and the photolabile natural pyrethrins, from photodecomposition by artificial and solar radiation, using a formulation containing 1% of mixed diaryl-p-phenylenediamines (Pieper and Rapperport, 1982).

Dureja et al. (1984), however, concentrated on the use of dinitroanilines and other related compounds as potential photostabilisers. Over one hundred different compounds were tested for this type of activity, and using cyphenothrin as a test chemical, it was found that thirty-nine different compounds could stabilise the insecticide against photodecomposition.

The dinitroanilines were particularly effective photostabilisers, with trifluralin giving the best results. Along with trifluralin, p-nitrophenol, phenothiazine and 8-hydroxyquinoline were most effective, although it was concluded that, in general, most of the compounds having high photostabilising activity were

those with an aromatic ring, with one or more electron-withdrawing groups and one or more electron-donating groups, ortho or para to an electron-withdrawing.

At high ratios of stabilizer to insecticide, it was suggested that the photostabilising action of the test compounds was probably due to competitive light absorption. However, the effective stabilisation observed at trace levels of additive was not attributable to this, and it was considered likely that it was due to quenching of the excited pyrethroid state, possibly through a charge transfer mechanism.

This seemed particularly relevant in relation to the phenoxyacetates, because it had been shown that the photonucleophilic and oxidative side chain reactions, characteristic of their photochemistry in aqueous solution, occurs from the excited state. It seemed feasible that by quenching this excited state, using compounds such as those suggested by Dureja et al. (1984), it might be possible to confer stability upon the phenoxyacetates. Consequently, three of the chemicals which were shown to be the most effective by these authors were tested.

A related area which has received some attention in recent years, has been the effect of surface active agents on pesticide photochemistry. Although this field was not the focus of the research presented in this

Chapter, it would seem to be an area worthy of consideration because of its relevance to the area of pesticide photostability.

Effect of surface active additives on pesticide photostability

Tanaka et al. (1979) stated that whereas any studies concerning pesticide photochemistry concentrate on the use of pure samples of selected pesticides, a large number of pesticides are applied in formulation with different types of adjuvants. In many cases, it was claimed, almost as much adjuvant as active ingredient can be present in formulations, and as a consequence the authors saw the need for an investigation of the effect of surfactant additives on the rate and nature of pesticide photodecomposition.

They showed that in the presence of selected non-ionic surfactants, ring hydroxylation reactions, which had been characteristic of the herbicide monuron in an earlier study (Tanaka et al., 1977), were no longer in evidence. Instead only dealkylation, coupling and reductive dehalogenation reactions were observed.

Not only did the surfactants alter the nature of the photochemical reactions, but they were found to increase the rate of monuron photodecomposition. It was found that as the surfactant concentration was increased, the rate of photodecomposition increased. This was

considered to be as a consequence of the surfactant partitioning the herbicide from the aqueous phase into the organic phase of the surfactant micelles. The increased rate was thought to be caused by differences in bond dissociation energies. The bond dissociation energies of carbon-hydrogen bonds within the hydrocarbon interior would be lower than those of the covalent oxygen-hydrogen bonds in the aqueous environment. Photo-reduction would, therefore, be expected to be more facile within the micelles. Exclusion of water molecules from the hydrophobic interior accounted for the lack of photo-oxidation.

However, it was observed that aryl substituted surfactants have the ability to photosensitise pesticide photodecomposition, and it was suggested that this may be an alternative way by which non-ionic surfactants could increase pesticide photodecomposition.

This photosensitising action of some surfactants was the focus of a later study (Tanaka et al., 1981), where a wider range of herbicides was selected to look for more general trends.

Four different classes of herbicides were chosen to give a range of different structures, patterns of substitution, and solubilities. In order to investigate the effects of both surfactant micellar solubilisation and surfactant photosensitisation on the rate of photo-decomposition, two different non-ionic surfactants were

selected. One did not absorb uv light, so it was possible to observe the effect of solubilisation, whereas the other did contain a uv absorbing chromophore, therefore it was possible to examine possible photo-sensitisation.

It was found that the rates of photodecomposition of herbicides having a low water solubility and containing chloro substituents on the aromatic ring, were significantly increased by the addition of a solubilising surfactant. It was also observed that where the triplet energies of the herbicides were lower than that of the uv absorbing surfactant, photosensitised degradation was possible.

Tanaka et al. (1981), discussed a report in which it was found that the rate of photodecomposition of the methyl ester of 2-4-D was increased by the addition of surfactants. This, apparently, was attributed to increased water solubility of the herbicide, as well as to a shift in its uv maxima to longer wavelengths.

More recently, Harrison and Wax (1985), reported an increase in the rates of photodecomposition of three herbicides, including 2,4-D, in the presence of formulation additives.

Emulsifying agents and oils were tested along with a non-uv absorbing surfactant, and it was found that the oil concentrates were able to increase the rates of photodecomposition of all three herbicides, although the

surfactant was less efficient at doing so. It was suggested that the rapid degradation of the pesticide may have been due to photosensitisation by the aryl groups present on the adjuvants. Like before, though, it was considered feasible that micellar solubilization may have been responsible, at least in part, through the lower bond dissociation energies within the hydrocarbon interior.

Thus, these studies seem to suggest that through the careful selection of formulation adjuvants, it might be possible to actively inhibit or potentiate the rate of photodecomposition of selected pesticides. Tanaka et al. (1981), mention a reduction in the rate of breakdown of some herbicides in the presence of surfactants, but they also suggest that selection of a surfactant having a triplet energy greater than that of a particular pesticide may be a means by which one could reduce the residence time of persistent chemicals. Increasing pesticide solubility through surfactants may be another way of achieving this.

On selecting an adjuvant specifically for this effect, however, one would need to consider the effects on the adjuvant itself. For example, it was shown that six out of nine selected herbicides could actually sensitise the breakdown of a non-ionic surfactant, (Tanaka et al., 1986). It would seem, therefore, that selection of a surfactant to confer stability upon a

given pesticide, would necessitate consideration of the effects on the properties of the formulation as a whole.

This concludes the introduction to the area of modification of pesticide photochemistry by chemical additives. The practical implications of successfully reducing pesticide photodegradation are obvious. The aim of this study was to explore such a possibility using a variety of chemicals as photostabilisers. Selected phenoxyacetate butyl esters were used as model compounds. The known susceptibility of this class of herbicide to photochemical transformation has already been discussed.

Experimental

Synthesis of phenoxyacetate butyl esters

The esters were synthesized by refluxing the parent acid in butanol in the presence of an acid catalyst. The reaction was followed by chromatographing samples from the reaction mixture on TLC plates (silica gel F_{254}) in a chloroform solvent system. Esters travelled with the solvent front whereas the parent acid travelled some distance between the origin and the solvent front. It was not considered necessary to record Rf values for this purpose.

Although the actual proportions of alcohol and acid varied, approximately 5 - 10 g of acid were refluxed in an excess of alcohol (30 - 40 cm^3) in the presence of

1 cm^3 of sulphuric acid. Reaction mixtures were refluxed for up to 24 hours. Esterification took considerably longer with 2,4-D and MCPA substrates. Esterification of phenoxyacetic acid and 4-CPA was a relatively more facile reaction.

To terminate the reaction, the reaction mixture was allowed to cool then poured into an approximately equal volume of distilled water in a separating funnel. The upper layer of crude ester was washed with 2 x 100 cm^3 of distilled water followed by 25 cm^3 of saturated sodium bicarbonate solution and finally 50 cm^3 of distilled water. The crude ester was dried over 5 - 6 g of anhydrous sodium sulphate and finally purified by distillation. Samples were then sent for mass spectral and infra-red analysis (see Figs. 7.1 - 7.8).

Difficulties were encountered with the purification of 4-CPA. Whereas the other three esters were recovered as liquids, the 4-CPA butyl ester was recovered as a solid. Time did not permit resolution of this problem therefore the study was confined to the butyl esters of phenoxyacetic acid, MCPA and 2,4-D.

Formulation and irradiation of test chemicals

The following seven additives were tested in the study: ammonium thiocyanate (NH_4SCN), potassium iodide (KI), vitamin C (vit. C), vitamin E (vit. E), phenothiazine (PT), 8-hydroxyquinoline (8-HQ) and

p-nitrophenol (pNP).

Each additive was formulated with each of the three test esters in molar ratios 1:10, 1:2, 1:1, 3:1 and 5:1. The esters were dissolved in methanol at a concentration of 100 $\mu g\ cm^{-3}$ and solutions were irradiated with the light from a medium pressure mercury arc lamp. Solutions were stirred constantly in a specially designed glass vessel. The vessel was constructed with a side arm which allowed sub-samples to be removed from the test solution at specific intervals. The lamp was fitted with a cooling system which dissipated any heat.

Initial experiments indicated that the butyl esters of MCPA and 2,4-D were much more stable than that of phenoxyacetic acid (PAA). Consequently, it was decided to irradiate solutions of these test chemicals for a longer period of time. This was found to be necessary in order to more clearly observe the influence of the additives on the extent of degradation of parent ester. Solutions containing the butyl esters of MCPA and 2,4-D were irradiated for 60 minutes whereas those containing PAA were irradiated for 30 minutes. Sub-samples of test solutions were removed every two minutes and injected directly into a Gas Chromatograph to enable quantification of parent residues.

Quantification of parent esters

Residues of parent esters were quantified using Gas Chromatography (G.C.). The Pye-Unican 4500 system described in Chapter 6 was used. In this case, however, only the OV17/202 packed column was employed (see Chapter 6 for details). The use of the methanol solvent was found to produce a broad solvent front on chromatograms. For effective resolution of esters from the solvent front, it was necessary to use a temperature gradient. In the case of 2,4-D and PAA butyl esters, samples were injected onto the column at a temperature of 150°C. The temperature was then raised at 6°C min^{-1} to a value of 220°C. Under these conditions, the retention times were recorded as 8.90 mins. and 4.33 mins. respectivley. In the case of solutions containing MCPA butyl ester, samples were injected at a column temparature of 190°C. The temperature was then raised to 250°C at 6°C min^{-1}. The retention time of the ester was found to be 4.85 mins. MCPA butyl ester quantification was faster using this particular gradient.

Table 7.1 Phenoxyacetic acid butyl ester : additive (10:1)

Time	% PAA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
2	94.81	94.63	95.12	98.99	94.15	94.33	93.89	94.13
4	64.58	62.14	70.14	92.63	54.34	74.38	62.15	59.84
6	13.20	17.34	28.27	86.81	16.15	25.24	30.25	26.21
8	9.41	10.11	16.75	81.42	11.11	14.45	18.93	14.59
10	4.53	4.43	9.9	73.43	4.23	9.21	8.52	9.89
12	3.14	3.71	5.43	69.49	3.18	6.82	4.73	3.91
14	ND*	ND	3.91	68.17	ND	3.99	4.02	3.54
16	-	-	ND	59.06	-	ND	ND	ND
18	-	-	-	54.55	-	-	-	-
20	-	-	-	50.18	-	-	-	-
22	-	-	-	46.79	-	-	-	-
24	-	-	-	43.89	-	-	-	-
26	-	-	-	40.77	-	-	-	-
28	-	-	-	38.65	-	-	-	-
30	-	-	-	35.91	-	-	-	-

ND* - not detectible

Table 7.2 Phenoxyacetic acid butyl ester : additive (2:1)

Time	% PAA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
2	94.81	95.13	94.73	98.23	97.18	95.14	95.31	94.91
4	64.58	52.86	71.24	95.42	69.15	73.24	66.14	65.99
6	13.20	23.34	30.21	90.29	35.34	33.89	26.85	28.83
8	9.41	12.63	18.79	85.78	18.13	16.54	18.43	17.91
10	4.53	8.54	11.45	80.13	10.15	8.91	12.15	8.96
12	3.14	4.32	8.76	77.14	6.79	4.59	8.53	6.24
14	ND	3.56	4.18	74.23	4.91	3.79	4.14	4.23
16	-	ND	3.14	71.89	3.81	ND	3.43	3.49
18	-	-	ND	69.18	ND	-	ND	ND
20	-	-	-	66.73	-	-	-	-
22	-	-	-	64.89	-	-	-	-
24	-	-	-	61.73	-	-	-	-
26	-	-	-	58.92	-	-	-	-
28	-	-	-	54.43	-	-	-	-
30	-	-	-	51.89	-	-	-	-

Table 7.3 Phenoxyacetic acid butyl ester : additive (1:1)

Time	% PAA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
2	94.81	95.43	96.14	99.91	96.36	97.2	100.2	97.18
4	64.58	76.93	84.18	98.12	78.31	89.25	99.17	92.01
6	13.20	31.18	62.83	98.01	64.71	69.15	99.99	82.18
8	9.41	21.85	51.79	97.79	58.13	47.83	98.14	74.76
10	4.53	14.89	38.91	96.61	38.77	38.14	96.54	63.15
12	3.14	5.17	29.80	96.13	29.83	26.28	93.23	60.21
14	ND	3.89	20.34	96.65	23.15	16.58	83.90	58.32
16	-	ND	16.58	91.14	21.99	16.52	74.31	55.13
18	-	-	9.79	88.01	14.33	12.15	68.52	54.38
20	-	-	6.81	86.23	10.02	10.99	60.13	50.91
22	-	-	3.24	84.81	6.25	9.39	52.07	45.76
24	-	-	ND	83.79	4.13	7.01	45.91	38.91
26	-	-	-	81.24	ND	5.43	41.88	31.41
28	-	-	-	80.09	-	4.17	36.71	27.31
30	-	-	-	79.07	-	3.23	34.76	20.27

Table 7.4 Phenoxyacetic acid butyl ester : additive (1:3)

Time	% PAA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
2	94.81	93.79	94.89	100.1	93.35	95.17	96.65	97.16
4	64.58	76.46	91.24	99.98	84.37	90.76	94.15	94.34
6	13.20	43.39	83.67	100.7	76.19	81.79	87.65	85.18
8	9.41	31.17	77.23	99.11	67.35	75.15	83.18	76.95
10	4.53	20.19	64.54	99.02	54.31	67.19	75.43	69.99
12	3.14	16.54	59.89	98.76	38.79	55.81	72.18	64.38
14	ND	10.98	51.28	98.23	26.71	47.14	69.87	59.89
16	-	5.43	47.89	96.1	21.29	44.76	66.18	56.16
18	-	3.79	44.59	95.98	18.34	40.89	61.89	54.38
20	-	ND	39.85	94.59	16.14	37.65	58.61	50.93
22	-	-	35.76	94.1	14.93	34.89	54.82	47.65
24	-	-	34.38	93.78	13.51	33.15	48.61	38.23
26	-	-	31.29	93.11	12.23	27.83	44.32	34.32
28	-	-	28.58	92.17	11.18	25.12	42.89	31.89
30	-	-	26.24	91.87	10.89	28.17	39.93	29.97

Table 7.5 Phenoxyacetic acid butyl ester : additive (1:5)

Time	% PAA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
2	94.81	96.29	96.89	100.2	99.13	96.23	99.13	95.87
4	64.58	83.15	93.12	99.98	98.54	93.84	98.76	90.18
6	13.20	43.24	88.12	100.1	97.62	84.81	97.54	82.98
8	9.41	29.81	82.53	100.4	96.14	78.61	96.18	76.13
10	4.53	18.56	77.57	99.99	94.43	73.71	94.64	72.23
12	3.14	12.35	74.33	100.1	92.89	68.91	90.73	65.76
14	ND	8.76	69.89	100.0	91.13	62.73	88.13	60.79
16	-	4.39	66.42	101.0	90.64	59.21	86.78	57.17
18	-	3.85	63.94	99.76	90.53	54.89	85.67	55.73
20	-	ND	60.14	99.91	89.18	50.76	84.09	53.24
22	-	-	58.71	99.75	88.43	46.17	80.14	51.78
24	-	-	52.31	98.99	87.99	41.82	79.77	49.98
26	-	-	50.89	99.13	87.39	39.94	76.34	45.66
28	-	-	48.76	98.43	86.89	37.76	72.18	43.25
30	-	-	45.32	97.76	85.09	32.18	69.38	40.89

Table 7.6 MCPA butyl ester : additive (10:1)

Time	% MCPA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	98.89	99.13	98.76	99.99	97.84	98.89	98.24	99.24
8	96.22	97.15	94.81	97.79	94.31	96.55	95.43	97.23
12	92.13	94.79	91.76	96.85	92.89	93.89	92.18	96.75
16	87.13	89.19	84.39	91.25	86.19	88.43	90.77	94.29
20	84.33	81.89	81.72	89.76	82.57	85.99	86.23	88.76
24	81.09	80.54	78.58	87.85	78.43	83.77	81.88	83.89
28	77.52	75.46	76.12	86.14	74.85	78.90	77.98	81.24
32	73.89	71.82	72.91	83.21	73.76	74.92	76.21	74.71
36	71.01	69.63	69.42	81.76	69.18	71.63	73.18	71.23
40	69.52	67.52	65.81	80.89	68.79	70.85	68.57	67.88
44	67.10	64.87	63.79	78.59	67.24	68.54	64.98	66.76
48	66.23	61.88	61.24	76.25	63.49	65.91	61.03	64.31
52	63.49	59.71	60.89	74.14	62.58	65.86	60.57	61.88
56	62.13	57.42	59.36	73.24	61.79	63.75	59.99	60.91
60	59.45	54.83	57.39	71.29	58.99	62.48	57.76	60.24

Table 7.7 MCPA butyl ester : additive (2:1)

Time	% MCPA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	98.89	98.93	99.19	99.93	99.01	98.43	99.00	98.79
8	96.22	98.82	97.63	100.1	96.59	98.32	97.89	97.32
12	92.13	95.43	95.31	99.76	95.84	97.59	95.54	94.88
16	87.13	89.14	91.28	97.73	91.93	95.81	95.31	90.53
20	84.33	85.63	85.43	97.18	86.43	92.76	93.37	87.65
24	81.09	83.24	81.79	96.54	82.08	84.32	86.76	82.12
28	77.52	77.13	78.24	92.18	77.91	81.76	81.24	74.43
32	73.89	76.89	74.41	91.78	70.23	76.53	76.58	72.12
36	71.01	74.42	70.96	88.15	69.78	70.54	72.92	68.44
40	69.52	70.85	68.13	87.73	67.32	70.12	69.58	61.76
44	67.10	67.85	67.99	86.93	63.28	65.53	66.91	57.37
48	66.23	64.29	65.44	84.21	60.98	60.12	64.32	54.32
52	63.49	62.18	64.32	80.74	59.14	59.83	60.91	51.77
56	62.13	60.73	61.86	77.34	57.63	56.72	58.76	50.98
60	59.45	58.39	58.31	74.32	53.21	51.72	57.33	48.88

Table 7.8 MCPA butyl ester : additive (1:1)

Time	% MCPA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	98.89	99.13	100.2	100.4	99.99	98.79	100.1	99.4
8	96.22	96.58	98.76	99.79	100.1	99.14	98.43	99.76
12	92.13	93.17	97.13	100.1	100.0	98.41	99.12	98.99
16	87.13	90.12	93.21	100.12	98.54	97.79	98.76	99.01
20	84.33	89.76	92.99	98.72	97.65	94.43	96.54	98.13
24	81.09	83.12	86.54	99.73	96.98	85.00	96.18	96.42
28	77.52	74.32	81.76	100.9	91.43	94.11	94.43	97.01
32	73.89	72.18	77.43	99.13	88.78	90.76	90.12	94.38
36	71.01	69.76	72.18	89.79	85.43	88.84	89.76	94.17
40	69.52	66.81	70.17	98.11	82.17	85.41	87.18	92.19
44	67.10	62.17	70.08	98.13	79.19	82.13	85.82	87.65
48	66.23	59.18	68.76	97.65	76.43	80.78	81.76	85.18
52	63.49	56.89	65.43	96.14	74.54	80.13	78.34	82.76
56	62.13	55.09	64.79	94.38	73.18	79.01	77.12	80.79
60	59.45	54.17	64.18	93.21	72.14	78.54	76.97	80.21

Table 7.9 MCPA butyl ester : additive (1:3)

Time	% MCPA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	98.89	99.79	99.43	100.9	99.97	99.76	100.5	99.91
8	96.22	98.75	98.73	100.2	98.91	99.68	100.1	99.43
12	92.13	94.32	98.14	99.43	98.72	100.5	99.98	98.99
16	87.13	93.21	94.43	99.76	97.41	98.74	99.41	99.12
20	84.33	87.65	93.12	99.19	96.31	98.59	99.91	97.63
24	81.09	85.43	90.17	100.1	93.28	99.11	98.76	96.93
28	77.52	88.49	91.76	100.9	90.52	99.00	97.12	97.21
32	73.89	79.91	89.73	99.76	87.63	97.65	97.43	95.89
36	71.01	74.83	86.58	98.54	87.14	96.99	96.65	96.12
40	69.52	71.77	86.12	100.2	86.32	96.53	94.38	95.81
44	67.11	71.41	85.43	99.77	86.08	65.19	95.41	93.23
48	66.23	68.76	83.21	100.5	85.79	96.73	94.81	93.89
52	63.49	66.63	81.89	99.17	84.13	95.12	93.27	91.76
56	62.13	64.32	80.99	98.99	83.58	94.91	92.79	91.43
60	59.45	63.78	80.41	99.01	83.74	94.81	92.19	90.67

Table 7.10 MCPA butyl ester : additive (1:5)

Time	% MCPA ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	98.89	99.14	100.2	100.5	100	99.10	99.77	100.3
8	96.22	98.99	100.9	100.7	99.97	99.76	99.93	100.8
12	92.13	98.76	99.71	99.19	100.3	100.7	99.91	99.98
16	87.13	99.10	100.1	100.5	100.8	99.34	99.12	98.78
20	84.33	97.56	99.33	100.32	100.0	99.09	99.42	99.91
24	81.09	95.14	98.76	99.89	98.99	98.54	99.57	100.2
28	77.52	95.79	98.71	97.63	99.76	99.65	99.01	100.7
32	73.89	94.32	99.83	98.77	100.1	98.73	100.4	99.12
36	71.01	92.08	95.43	99.52	100.0	99.96	98.82	98.71
40	69.52	88.79	94.18	100.3	99.23	100.1	99.13	99.71
44	67.10	88.10	92.76	99.87	100.5	98.73	99.04	98.54
48	66.23	85.43	90.53	99.48	100.2	99.29	98.76	99.07
52	63.49	83.21	88.19	99.46	98.54	99.52	99.51	98.76
56	62.13	80.89	87.84	98.79	98.53	97.21	98.93	98.10
60	59.45	76.54	86.59	99.13	97.79	97.63	98.50	98.01

Table 7.11 2,4-D butyl ester : additive (10:1)

Time	% 2,4-D ester remaining							
(min)	Control	SN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	99.76	100.2	99.81	99.91	100.4	100.3	99.75	100.1
8	99.53	99.81	98.32	99.56	97.69	98.91	99.49	99.56
12	99.32	97.32	99.01	99.51	98.99	98.10	99.10	100.3
16	97.81	96.99	97.54	98.71	99.01	98.02	89.45	100.2
20	96.57	96.18	96.31	98.56	98.14	97.54	89.13	96.45
24	95.81	95.14	95.66	97.89	96.53	97.32	88.54	93.12
28	93.78	94.76	92.16	95.13	95.42	94.81	89.12	95.89
32	91.29	91.82	91.77	94.22	92.78	91.23	89.01	91.77
36	90.85	89.32	90.54	91.88	91.71	90.54	88.31	90.33
40	88.99	87.54	88.19	90.01	87.63	87.52	87.38	86.21
44	86.81	85.18	87.93	87.65	85.43	87.12	85.32	83.29
48	84.92	84.17	87.34	86.43	81.88	83.17	83.02	77.81
52	83.77	82.98	96.93	83.21	80.90	81.10	82.96	75.43
56	81.99	76.4	86.18	82.88	78.23	80.91	82.53	74.60
60	80.89	74.51	86.81	81.28	77.89	80.73	81.29	71.81

Table 7.12 2,4-D butyl ester : additive (2:1)

Time	% 2,4-D ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	99.76	100.1	98.91	100.4	100.4	99.99	99.76	100.1
8	99.53	99.80	99.95	100.2	98.91	99.39	100.10	99.89
12	99.32	99.83	99.76	99.54	98.76	99.10	99.44	99.42
16	97.81	98.42	98.32	99.01	98.04	98.52	99.14	97.65
20	96.57	97.18	97.38	98.51	96.12	97.09	96.31	97.08
24	95.81	96.57	95.32	96.29	94.39	94.18	93.18	96.79
28	93.78	94.81	94.98	94.18	92.18	92.32	91.99	94.12
32	91.29	92.79	92.71	92.39	91.67	91.73	90.17	90.86
36	90.85	91.28	90.08	91.01	90.28	90.54	89.98	89.98
40	88.99	88.67	89.12	88.58	87.15	88.18	88.76	89.97
44	86.81	85.38	87.36	85.11	87.04	85.09	87.11	88.14
48	84.92	83.92	86.28	84.67	83.28	84.91	83.28	85.32
52	83.77	82.77	83.14	81.54	82.19	82.61	81.49	84.17
56	81.99	80.18	81.54	81.28	81.73	80.77	80.32	82.99
60	80.89	79.39	80.76	80.99	79.54	78.21	79.11	81.01

Table 7.13 2,4-D butyl ester : additive (1:1)

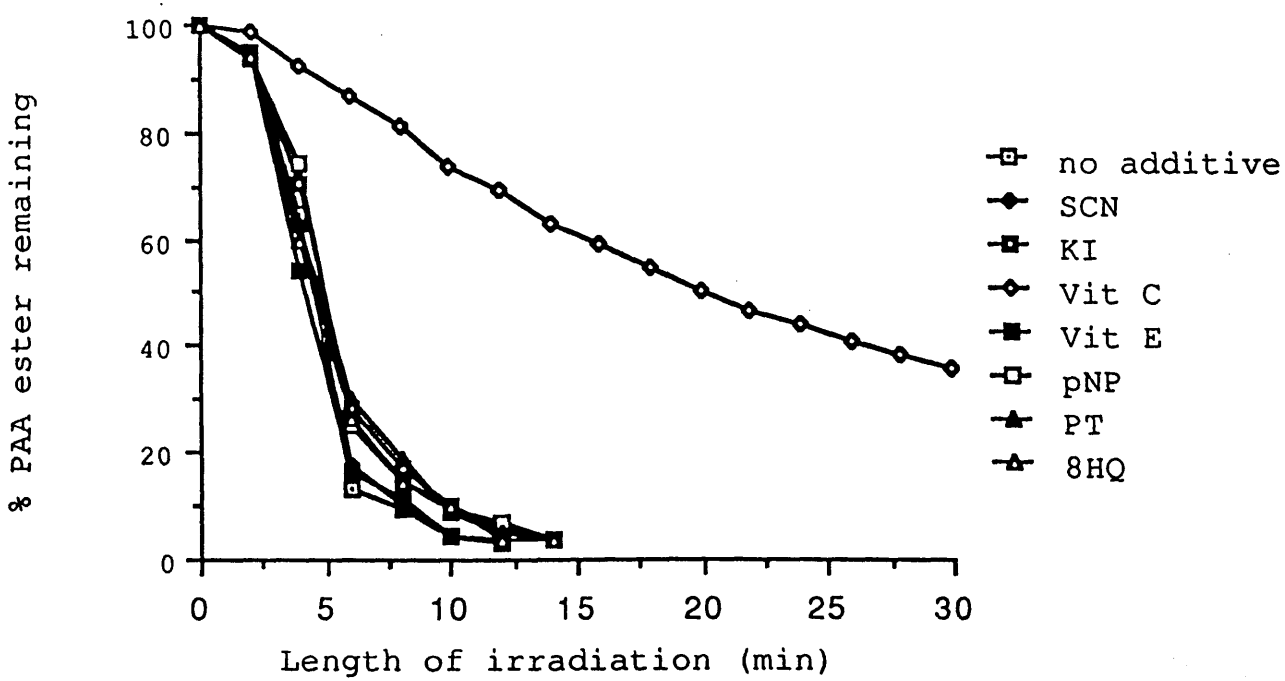
Time	% 2,4-D ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	99.76	99.59	100	99.91	100.6	99.98	100.3	99.81
8	99.53	99.28	99.73	99.76	99.98	100.5	99.86	99.76
12	99.32	99.76	99.58	98.98	98.26	98.91	98.33	96.44
16	97.81	98.54	98.91	98.52	97.94	97.43	98.01	93.21
20	96.57	97.12	98.93	98.31	95.84	96.91	96.55	93.11
24	95.81	94.32	98.14	96.98	95.13	94.33	94.13	92.76
28	93.78	91.88	95.57	95.43	93.28	86.18	92.89	91.34
32	91.29	90.55	93.21	92.18	92.16	85.43	88.54	89.14
36	90.85	90.23	89.98	90.11	88.46	84.13	86.32	88.76
40	88.99	87.68	89.16	87.99	86.19	82.18	83.18	86.21
44	86.81	87.31	86.54	83.28	85.43	79.71	83.09	83.56
48	84.92	84.17	79.17	83.79	83.29	77.63	81.79	84.76
52	83.77	81.78	74.32	82.88	81.76	75.13	81.43	83.81
56	81.99	78.12	73.17	81.54	81.84	74.79	79.61	82.11
60	80.89	76.88	70.89	81.11	78.23	72.66	77.54	81.76

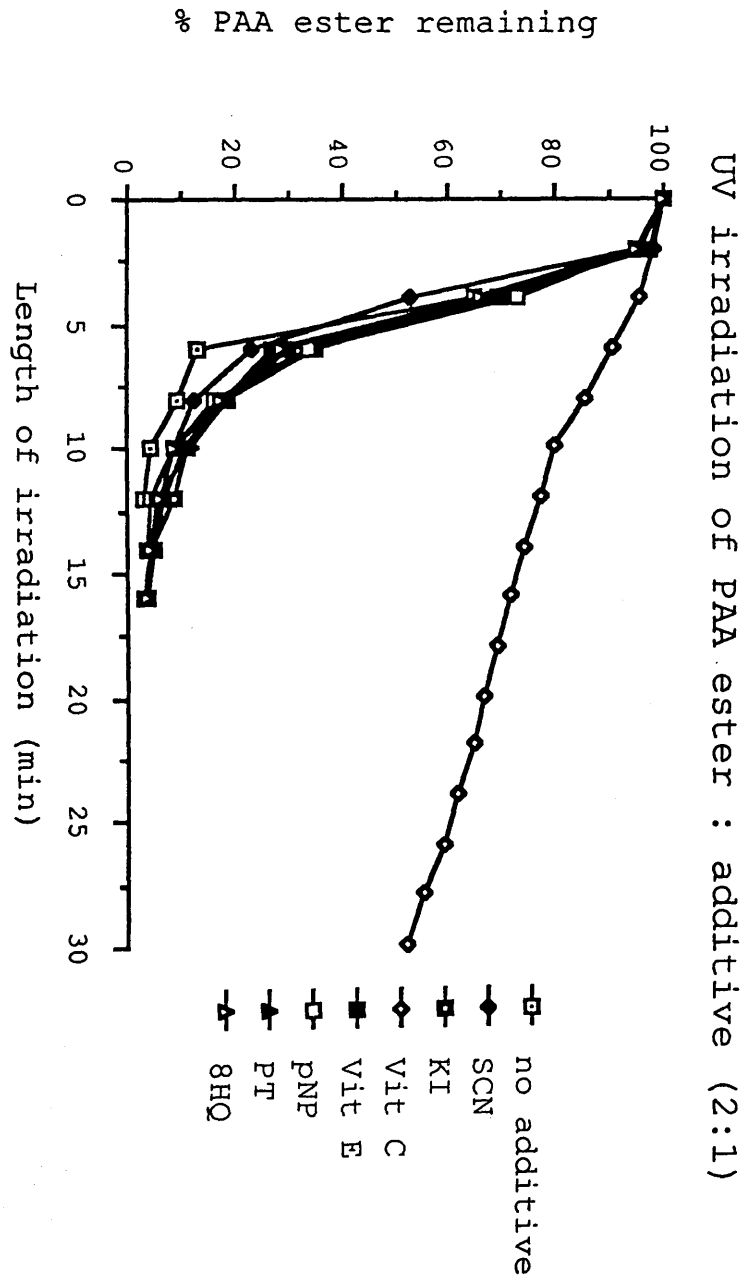
Table 7.14 2,4-D butyl ester : additive (1:3)

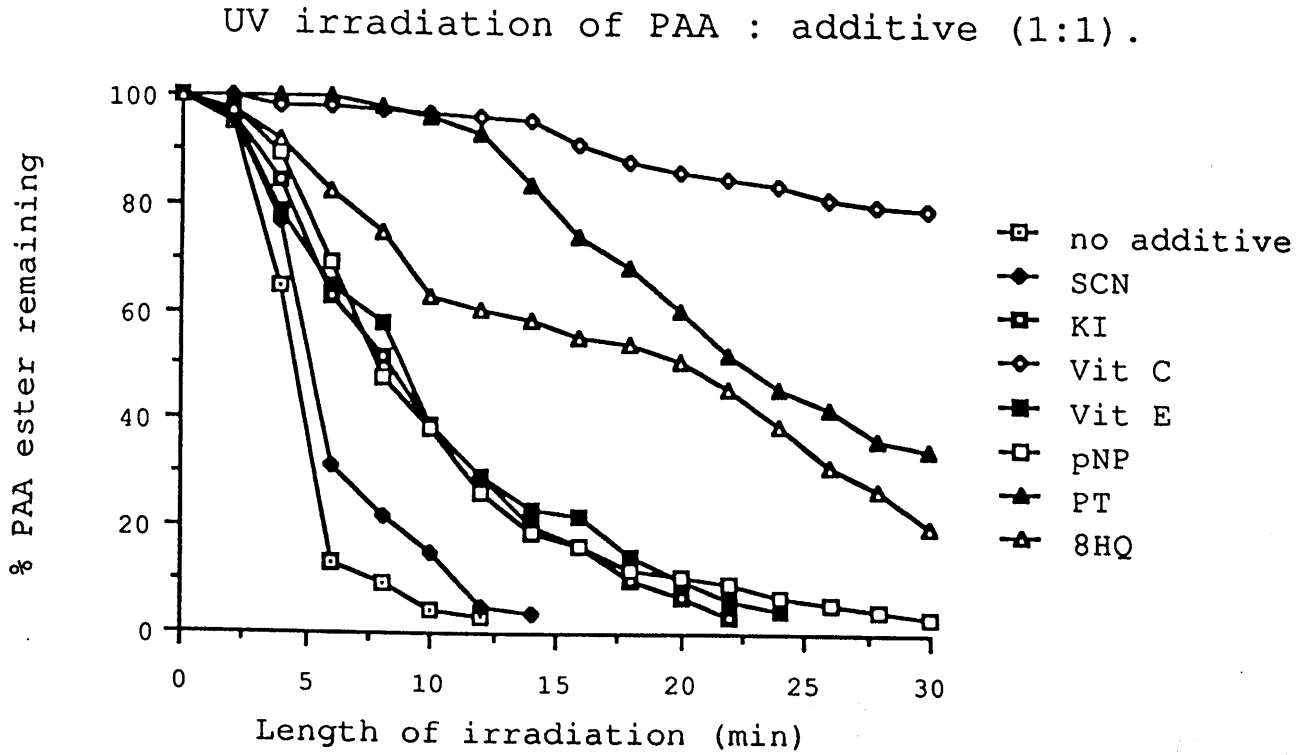
Time	% 2,4-D ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	99.76	100.3	99.89	99.79	99.83	100.7	100.2	99.59
8	99.53	100.7	100.1	100.2	99.18	100.2	99.71	99.68
12	99.32	100.2	100.4	99.13	99.09	99.73	99.65	100.2
16	97.81	99.77	99.37	99.01	97.65	99.14	98.43	99.13
20	96.57	100.1	99.71	98.65	96.32	98.76	98.14	98.76
24	95.81	99.36	98.65	98.43	96.18	94.32	97.65	98.15
28	93.78	98.76	98.91	98.11	95.43	91.76	98.13	97.43
32	91.29	98.43	97.13	98.01	92.17	90.70	97.43	97.02
36	90.85	99.21	96.54	95.91	86.15	88.54	96.54	96.54
40	88.99	95.43	94.32	95.81	86.07	88.18	95.42	95.38
44	86.81	93.21	94.18	94.32	84.38	86.32	93.17	95.01
48	84.92	91.79	93.76	94.09	81.28	82.78	92.17	91.49
52	83.77	89.13	88.76	93.59	80.76	83.01	91.86	88.11
56	81.99	87.65	82.88	91.76	78.54	81.79	89.73	87.29
60	80.89	86.17	81.76	85.71	77.57	79.67	88.14	85.43

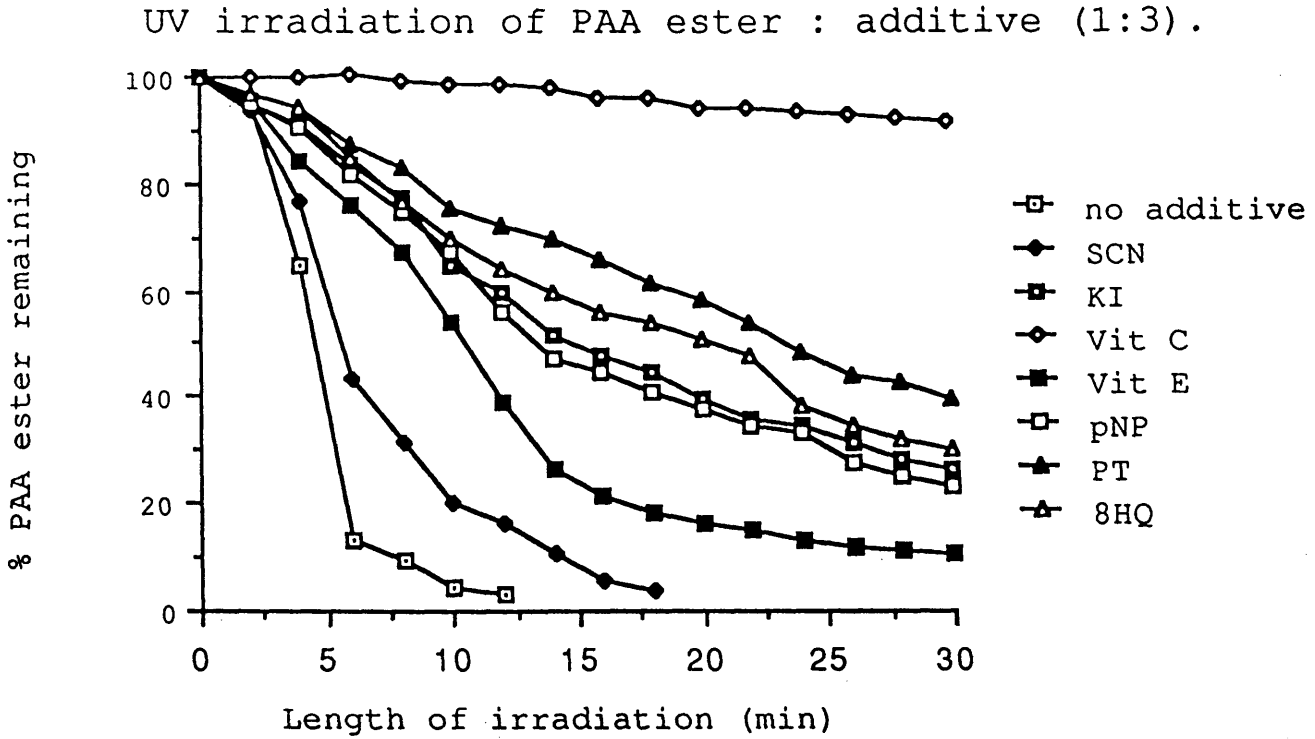
Table 7.15 2,4-D butyl ester : additive (1:5)

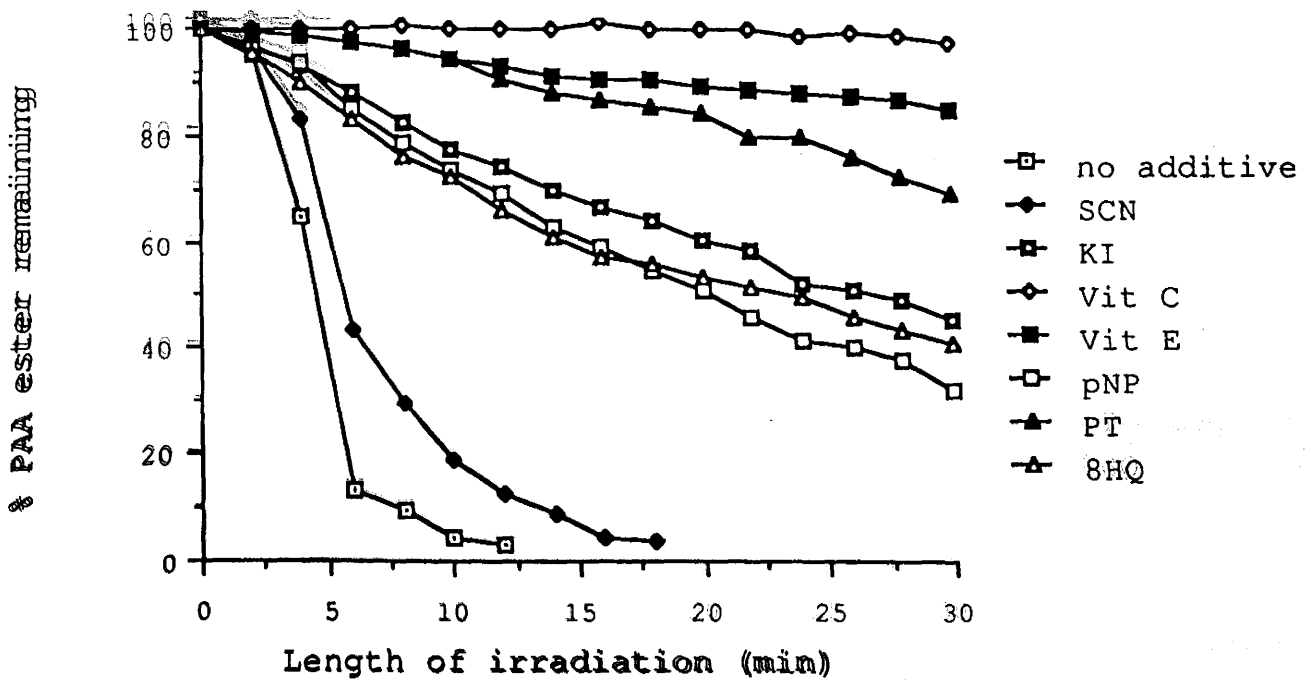
Time	% 2,4-D ester remaining							
(min)	Control	SCN	KI	Vit C	Vit E	pNP	PT	8HQ
0	100	100	100	100	100	100	100	100
4	99.76	100.2	99.91	101.3	99.76	99.89	101.1	99.83
8	99.53	101.1	100.3	100.2	100.3	99.71	99.86	100.9
12	99.32	100.4	99.86	100.0	99.76	99.84	99.73	100.1
16	97.81	99.71	99.43	99.91	99.80	98.99	99.43	99.39
20	96.57	99.60	99.09	100.4	98.54	98.33	99.14	98.99
24	95.81	98.79	98.65	98.73	99.02	99.31	99.30	99.18
28	93.78	97.14	99.18	99.01	98.31	99.30	98.99	98.77
32	91.29	96.54	98.01	99.65	97.64	99.65	99.04	98.34
36	90.85	95.84	96.53	99.43	97.99	98.81	100.2	97.71
40	88.99	95.71	84.10	98.10	96.84	98.65	89.42	96.63
44	86.81	93.93	92.13	97.99	93.28	97.42	99.13	95.39
48	84.92	93.93	92.13	97.99	93.28	97.42	99.13	95.38
52	83.77	92.92	90.76	97.64	93.01	96.88	98.98	95.01
56	81.99	91.11	90.81	97.41	92.19	96.09	98.56	94.39
60	80.89	90.76	90.43	97.32	93.29	95.56	98.73	94.71


UV irradiation of PAA ester : additive (10:1).
% PAA ester remaining
100
80
60
40
20
0
0
5
10
15
20
25
30
Length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ

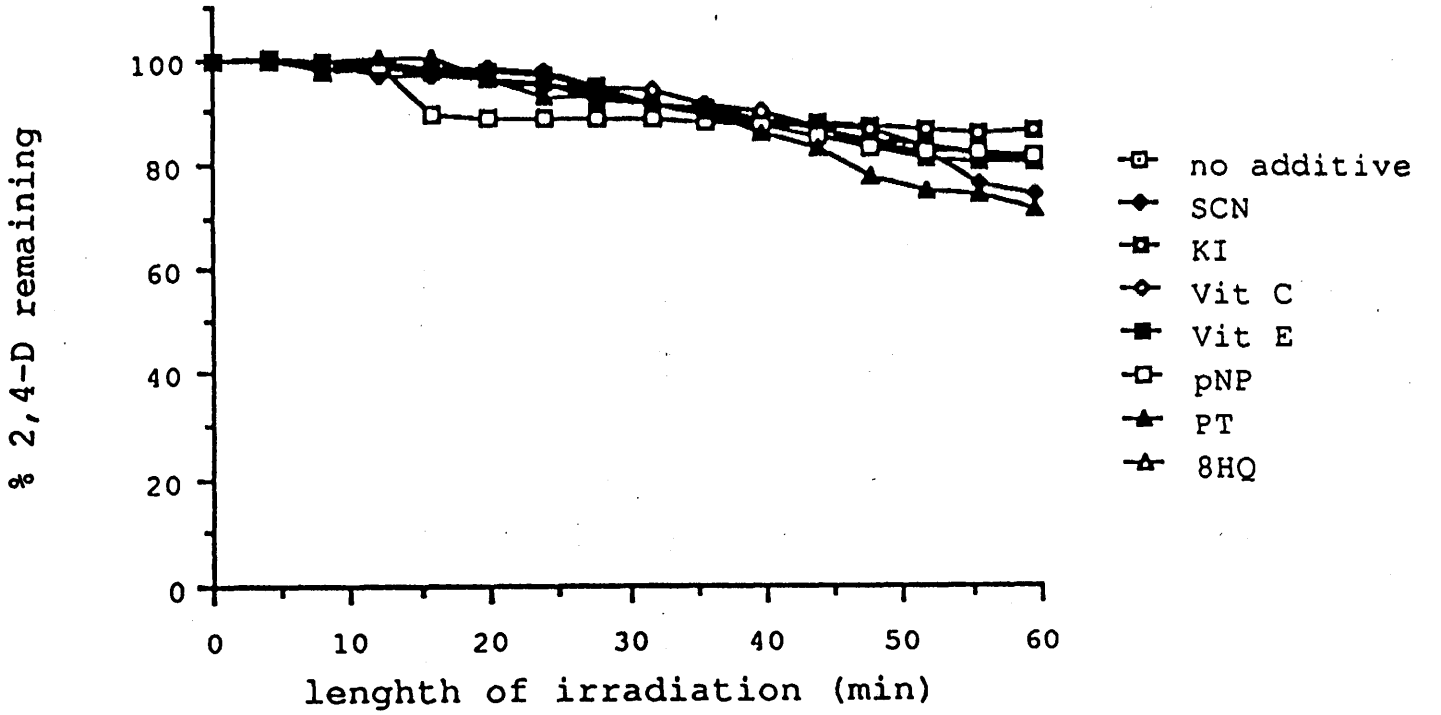

UV irradiation of PAA ester : additive (2:1)
% PAA ester remaining
0
20
40
60
80
100
0
5
10
15
20
25
30
Length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ


UV irradiation of PAA : additive (1:1).
% PAA ester remaining
100
80
60
40
20
0
0
5
10
15
20
25
30
Length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ

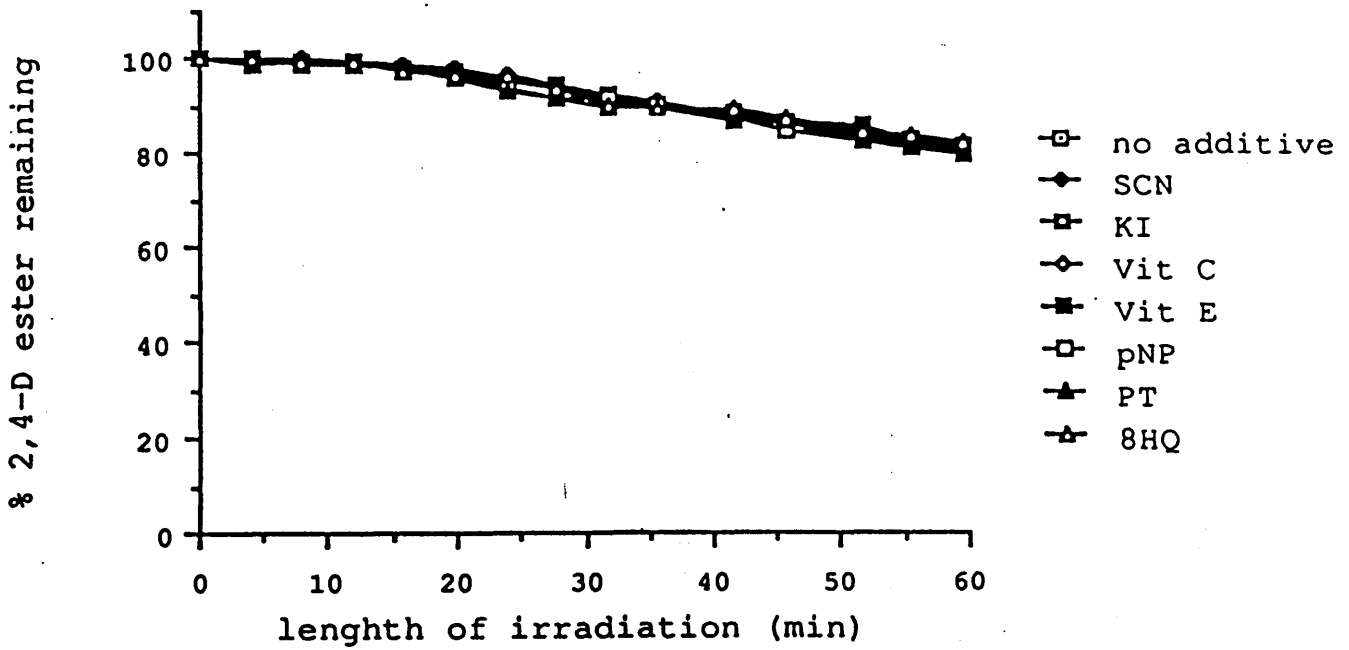

UV irradiation of PAA ester : additive (1:3).
% PAA ester remaining
100
80
60
40
20
0
0
5
10
15
20
25
30
Length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ


UV irradiation of PAA ester : additive (1:5).
% PAA ester remaining
100
80
60
40
20
0
0
5
10
15
20
25
30
Length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ

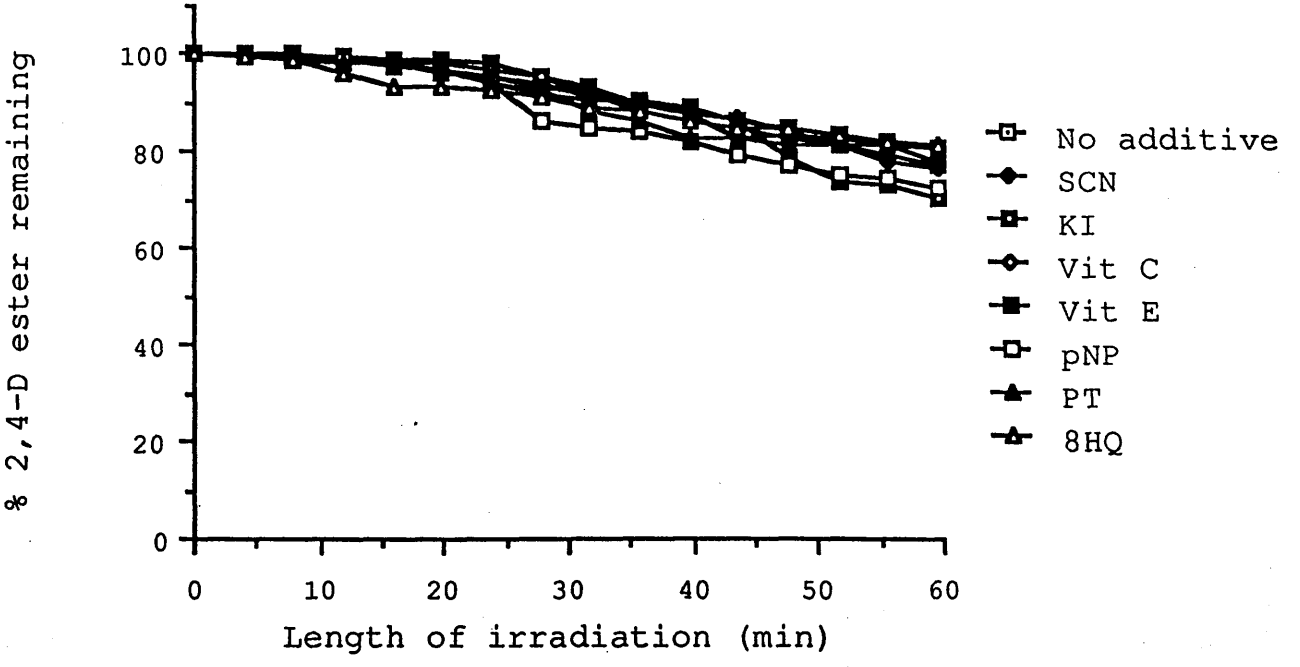
UV irradiation of 2,4-D ester : additive (10:1).

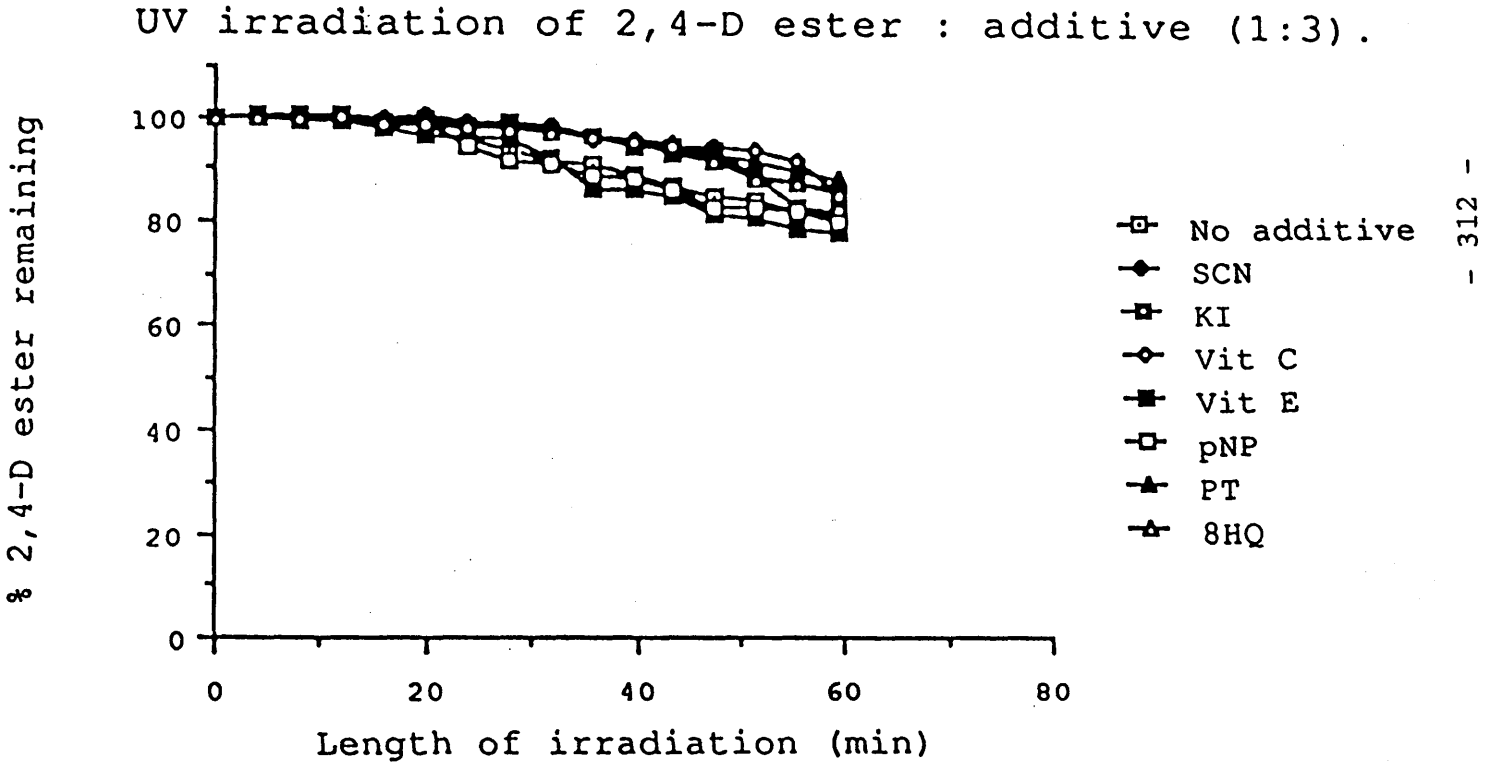


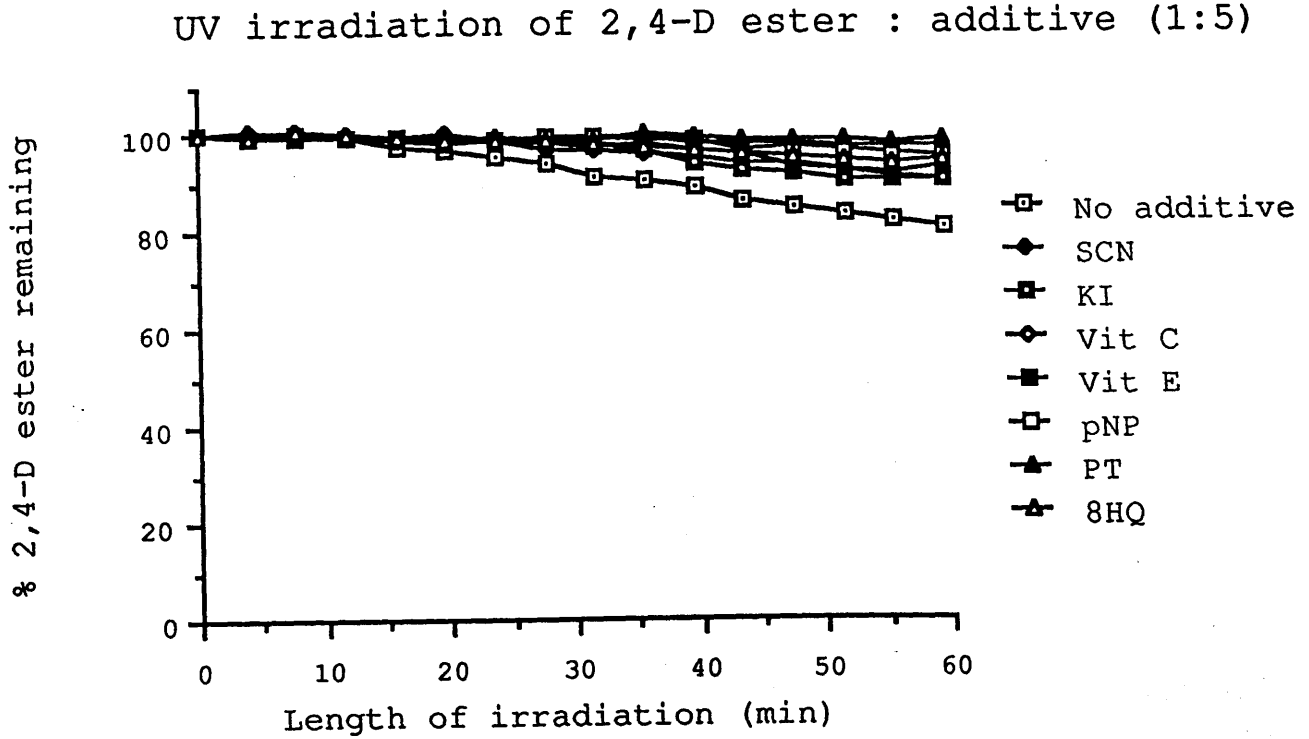
UV irradiation of 2,4-D ester : additive (2:1)

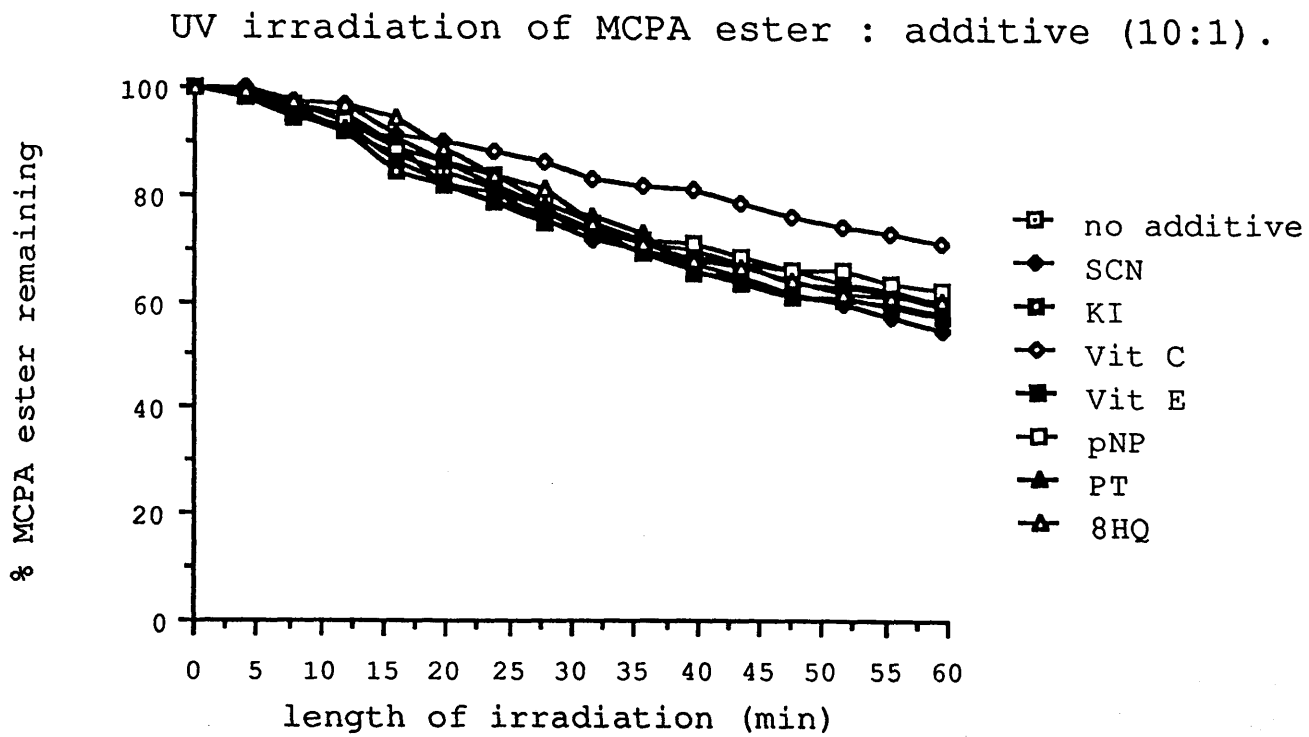


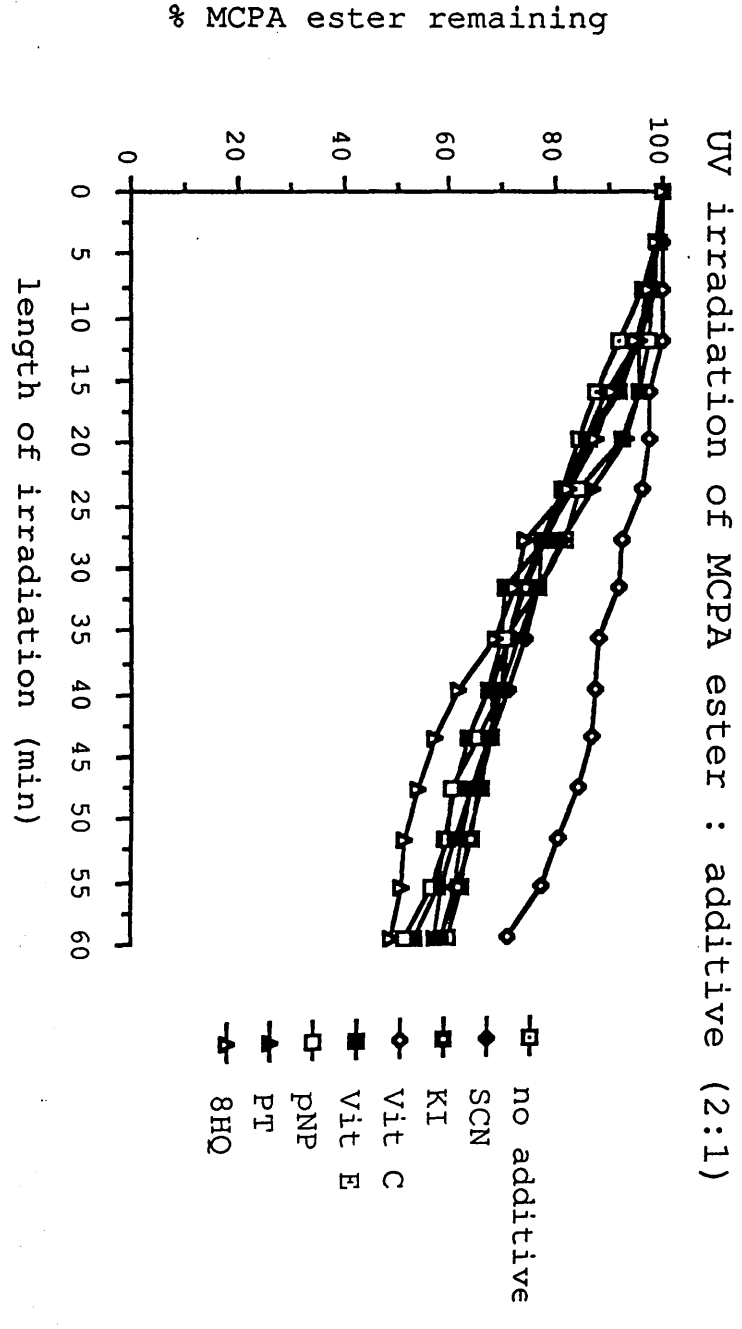
UV irradiation of 2,4-D ester : additive (1:1).

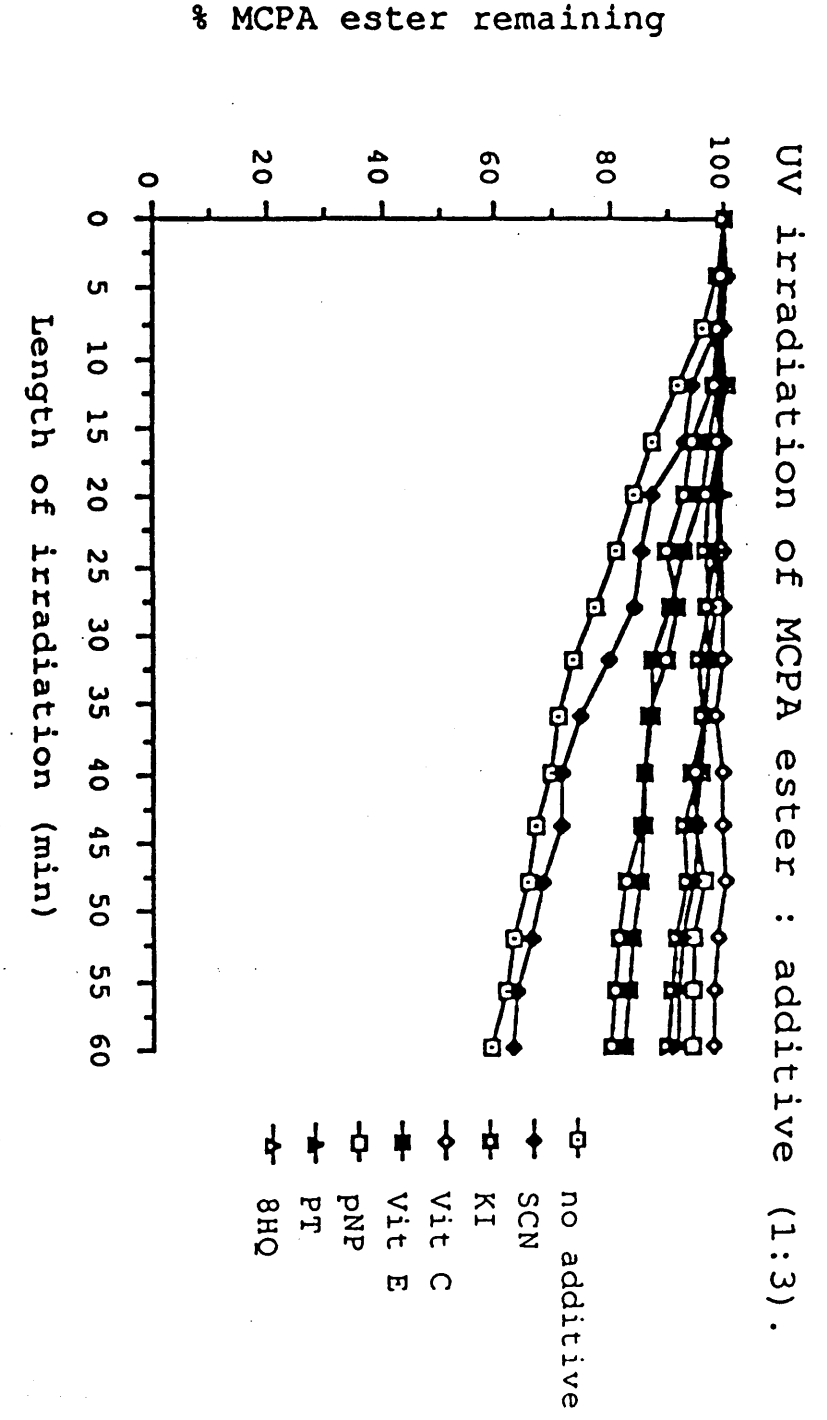



UV irradiation of 2,4-D ester : additive (1:3).
% 2,4-D ester remaining
100
80
60
40
20
0
0
20
40
60
80
Length of irradiation (min)
No additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ


UV irradiation of 2,4-D ester : additive (1:5)
% 2,4-D ester remaining
100
80
60
40
20
0
0
10
20
30
40
50
60
Length of irradiation (min)
No additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ


UV irradiation of MCPA ester : additive (10:1).
% MCPA ester remaining
100
80
60
40
20
0
0 5 10 15 20 25 30 35 40 45 50 55 60
length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ


UV irradiation of MCPA ester : additive (2:1)
% MCPA ester remaining
0
20
40
60
80
100
0 5 10 15 20 25 30 35 40 45 50 55 60
length of irradiation (min)
no additive
SCN
KI
Vit C
Vit E
pNP
PT
8HQ



UV irradiation of MCPA ester : additive (1:3).

Fig 7.1 Mass spectrum of phenoxyacetic acid butyl ester

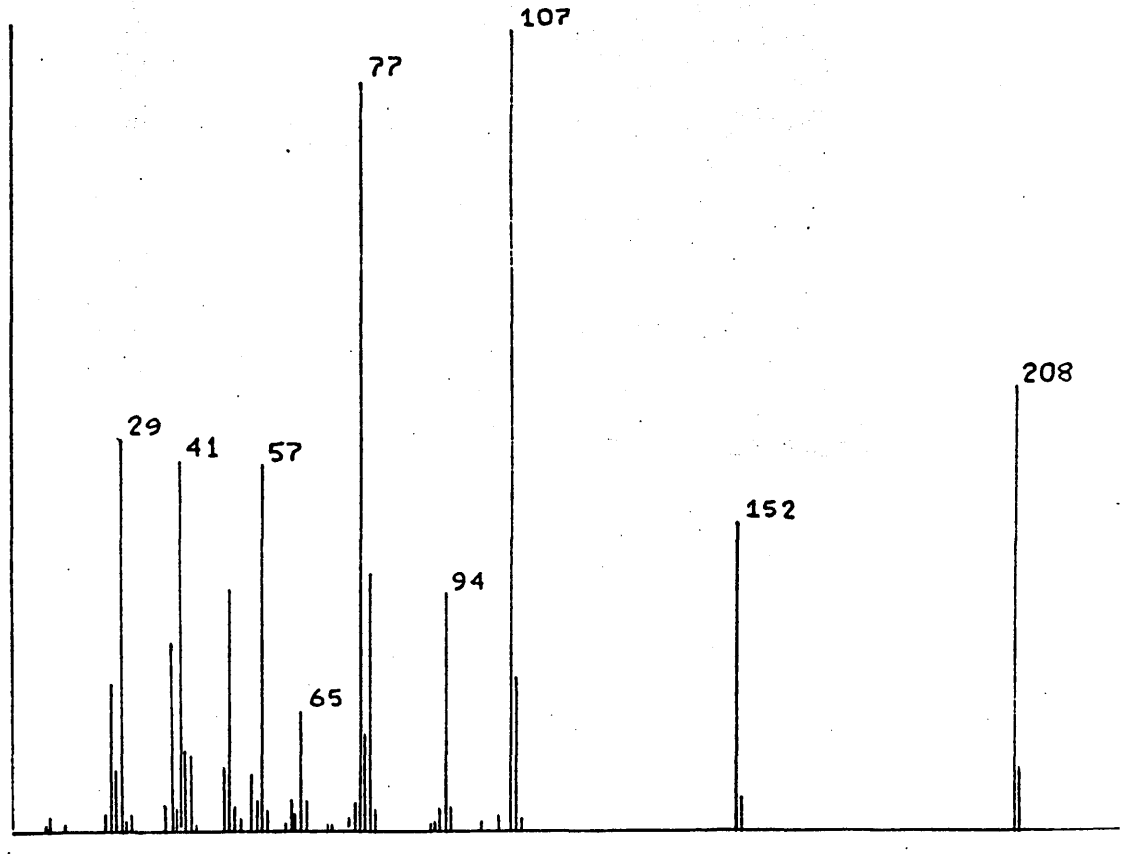


Fig 7.2 Infra-red spectrum of phenoxyacetic acid butyl

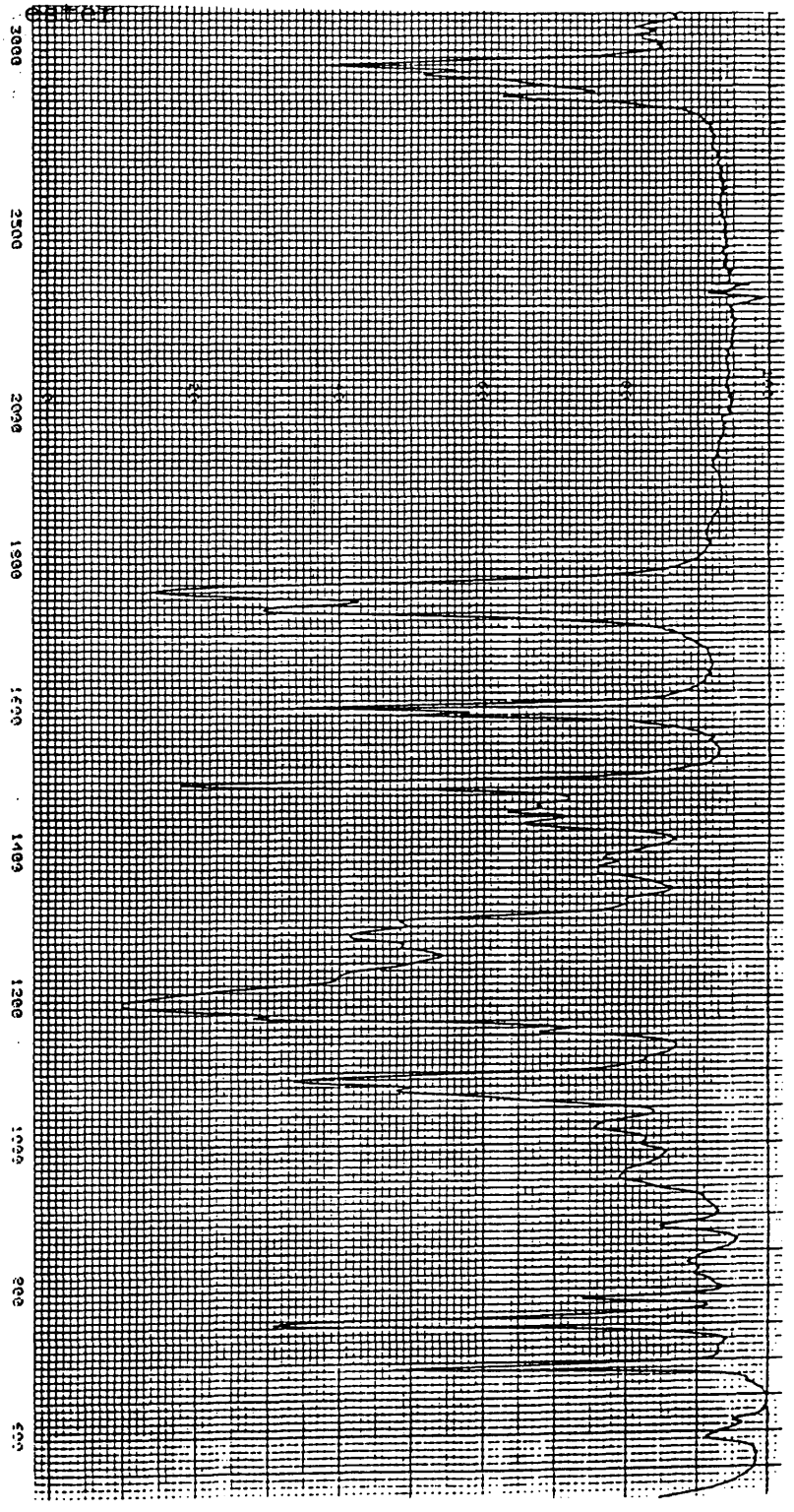


Fig 7.3 Mass spectrum of CPA butyl ester

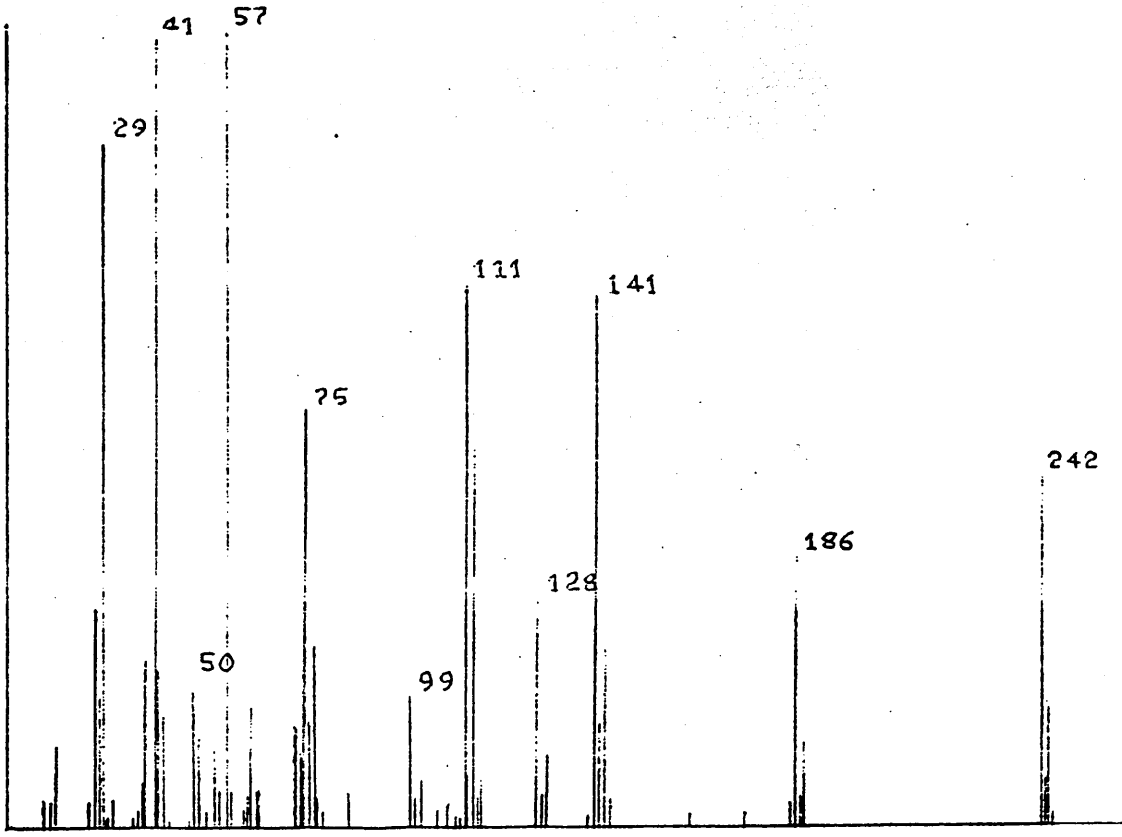


Fig 7.4 Infra-red spectrum of CPA butyl ester

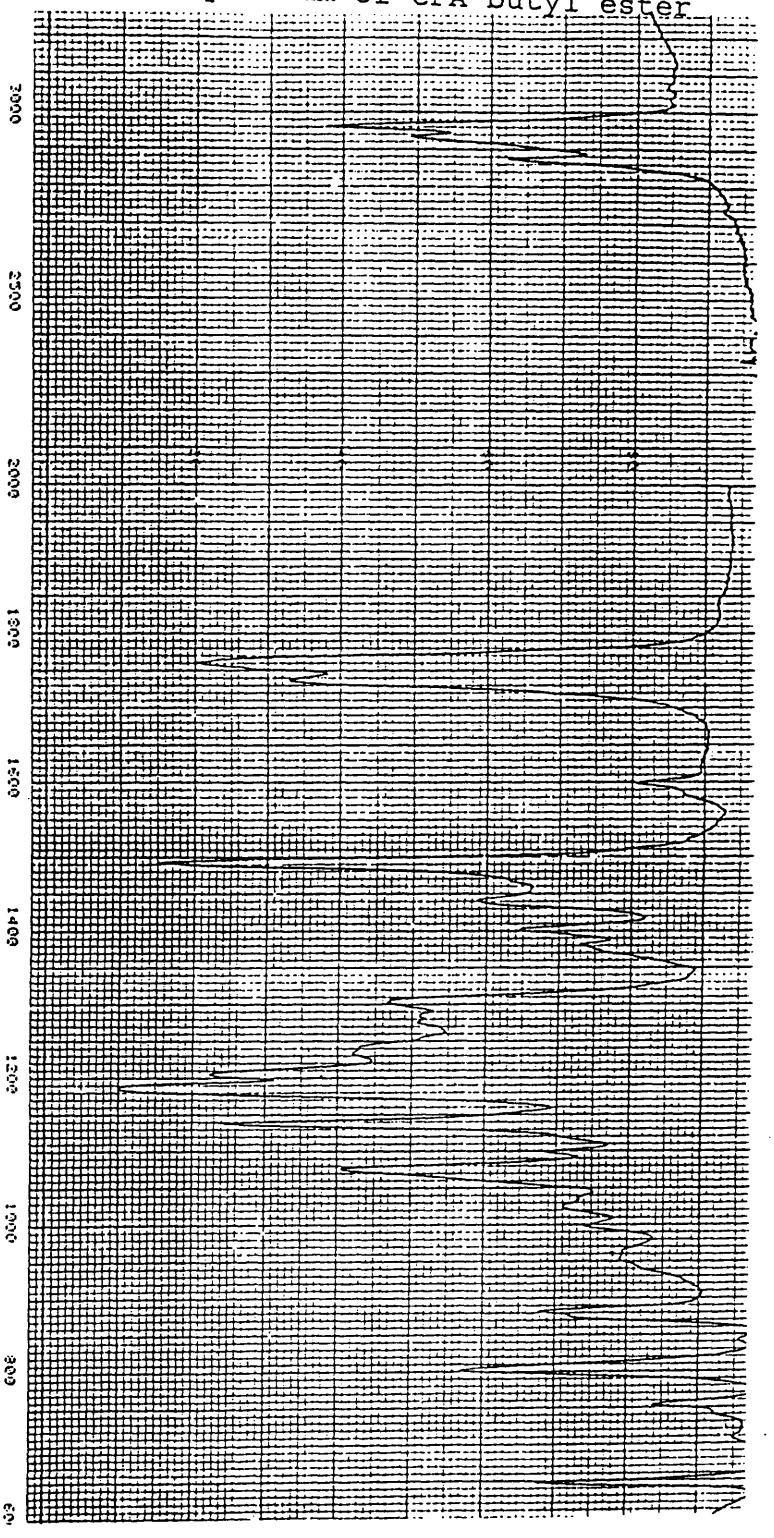


Fig 7.5 Mass spectrum of MCPA butyl ester

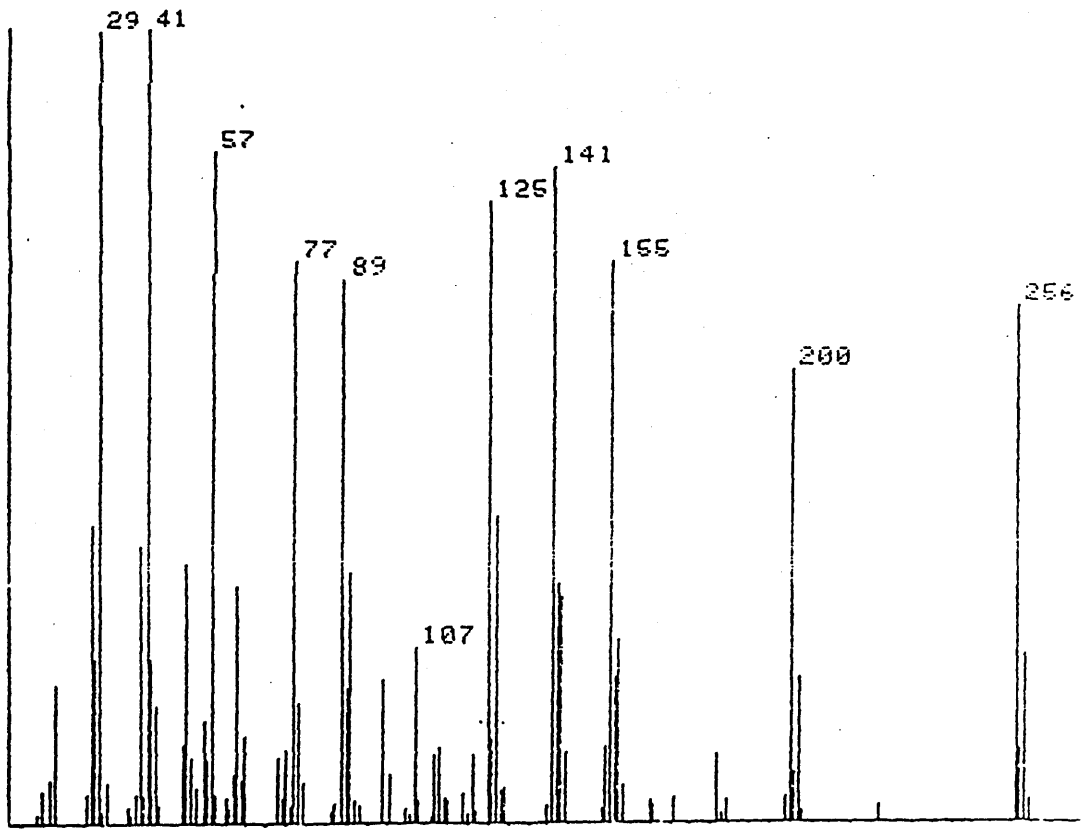


Fig. 7.6 Infra-red spectrum of MCPA butyl ester

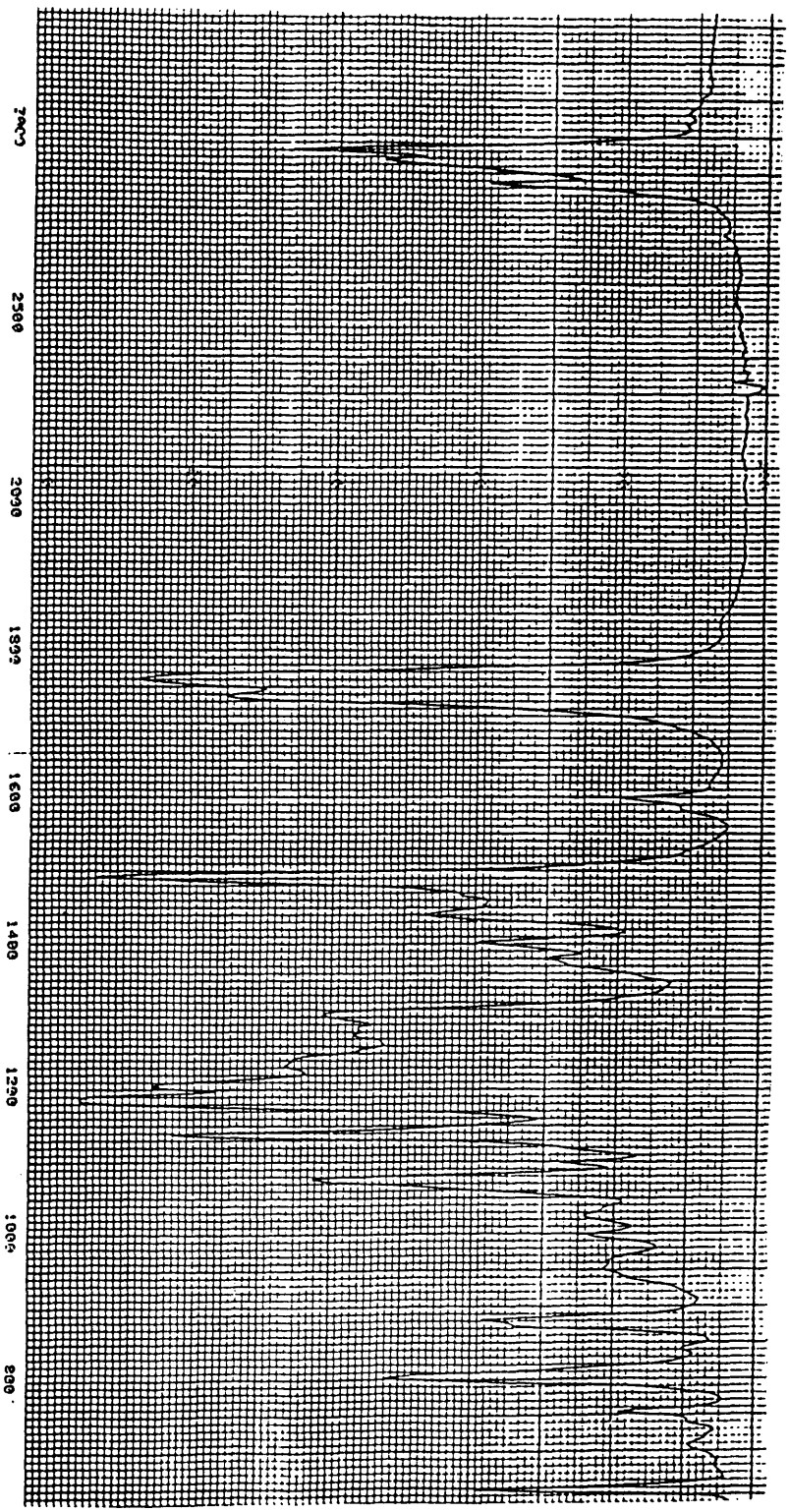


Fig 7.7 Mass spectrum of 2,4-D butyl ester

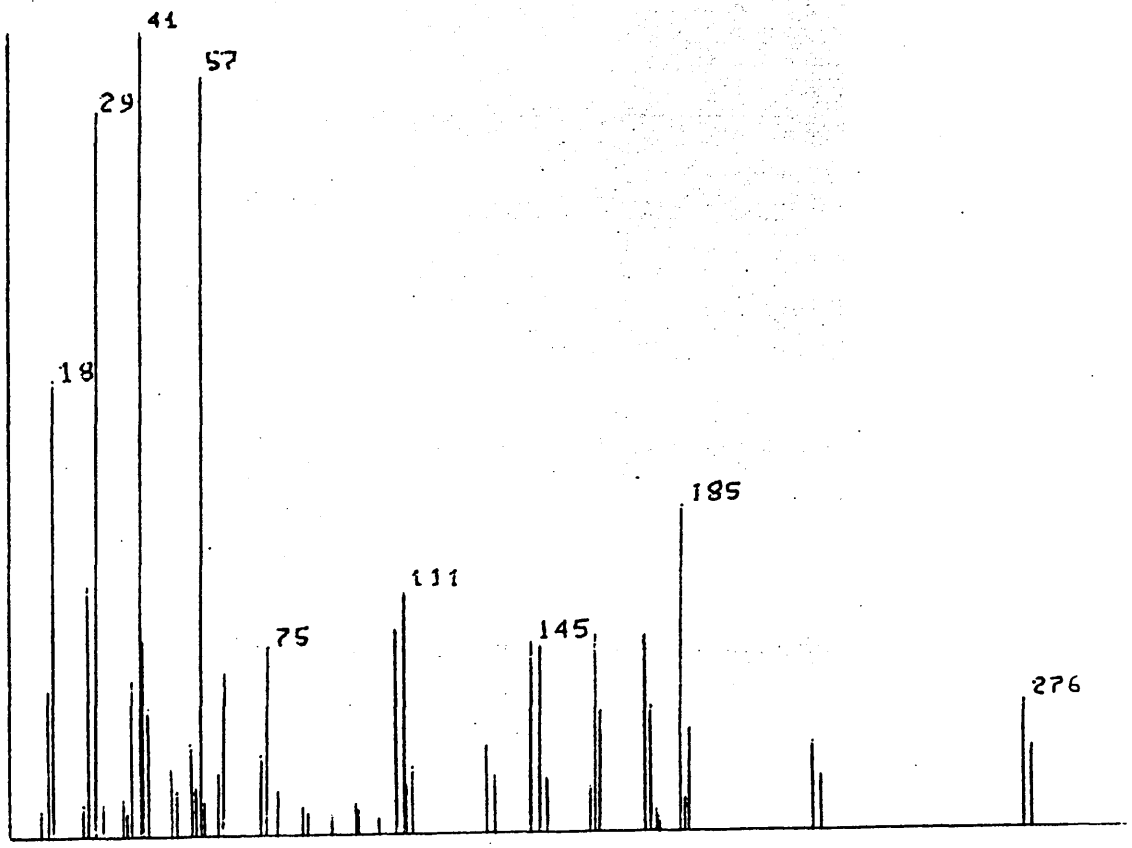
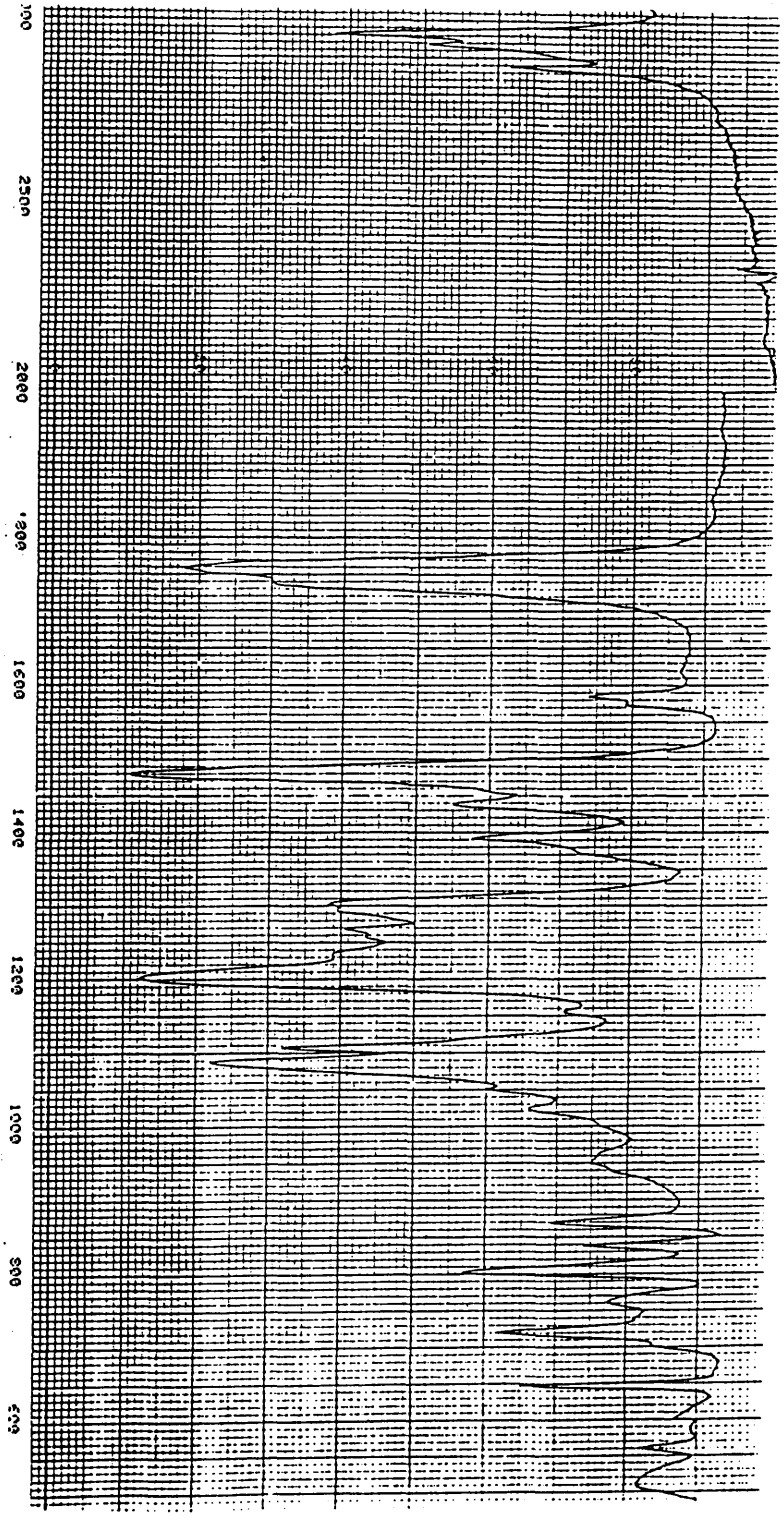


Fig 7.8 Infra-red spectrum of 2,4-D butyl ester



Discussion

There were a number of reasons for using phenoxyacetate butyl esters in this study, rather than the sodium salts. The esters themselves are widely used in commercial formulations. Therefore results had possible practical implications. Despite this fact many studies in the past have concentrated on the photochemistry of salts of the herbicides. This in itself was one reason for using the esters. In addition, using the herbicides in the form of relatively volatile esters made analysis easier. This enabled a larger number of experiments to be carried out than would have otherwise been possible.

Irradiation of the parent esters alone indicated that the butyl ester of phenoxyacetic acid was much more susceptible to photodecomposition than the butyl esters of MCPA and 2,4-D. It would seem that ring substitution confers a certain amount of stability to these compounds. Preliminary experiments involving irradiation of the sodium salts of the parent acids in methanol and aqueous solution were in agreement with this.

Generally, the addition of the test additives in excess caused a reduction in the extent of parent ester photodegradation. At lower levels it was possible to observe trends in the degree of effectiveness of additives. Vitamin C was found to be a particularly effective inhibitor of photodegradation and was the most useful in all instances. Vitamin E, p-nitrophenol,

phenothiazine and 8-hydroxyquinoline were also effective at higher concentrations than vitamin C. Potassium iodide was more effective than ammonium thiocyanate but less effective than the other additives.

Additives were really only effective at inhibiting the breakdown of 2,4-D butyl ester at the highest concentration tested (1:5). It may be that because of the relative stability of the ester over the duration of experiments, the effect of additives were not so readily observed.

The actual mechanism by which the additives were able to inhibit photochemical breakdown of the test chemicals remains unknown. In the case of p-nitrophenol, phenothiazine and 8-hydroxyquinoline, Dureja et al. (1984) suggested that at high levels these compounds may exhibit their photostabilising activity through competitive light absorption. At lower concentrations it was suggested that a more subtle interaction may be in evidence. Quenching of the photochemically generated excited state through a charge transfer mechanism was one possibility. Perhaps such a mechanism was in evidence in this study also.

Both vitamin C and vitamin E function as anti-oxidants and free radical scavengers in biological systems (Bindra, 1979). It seems feasible that they could inhibit the photochemical transformation of test chemicals by either inhibiting the action of photo-

oxidants or by reducing the levels of reactive free radicals. Perhaps under the experimental conditions employed, vitamin C was more efficient at doing this than vitamin E. In addition, it seems likely that both ammonium thiocyanate and potassium iodide function as free radical scavengers. Both Cook (1979) and Jaff (1982) proposed that this was the way in which thiocyanate and iodide salts were able to reduce the photo-oxidation of test chemicals in their experiments. Differences in the efficiency with which each of the anions behaved as free radical scavengers might account for the differences in the degree of breakdown of the test compounds.

In conclusion, the results of these experiments indicate that additives can be successfully used to stabilise herbicides and therefore other pesticides against the effects of ultraviolet light. In this study extreme conditions were used. Medium pressure mercury arc lamps emit light principally at a wavelength of 254 nm. This is particularly energetic light by comparison with that likely to reach an applied herbicide formulation under natural sunlight conditions. It seems reasonable to assume that any additive which shows a stabilising effect under such conditions would do so also under natural sunlight conditions.

CHAPTER 8

CONCLUSIONS

The aim of this Chapter was to link and put into perspective the different areas of work reported in this thesis. In doing this it was hoped that some clarity would be given to the rationale behind the different studies.

The experiments designed to investigate the potential use of sulphonamide drugs as plant growth regulators may seem unrelated to the studies conducted to study the photochemical behaviour of phenoxyacetate herbicides. Although they appear to be different areas of research they were in fact linked, not only in the general area of pesticide disposition in the evvironment, but also in other more direct ways. The reasoning behind the use of surfactants and salts as a means of increasing the foliar uptake of sulphonamides, for example, was not entirely different from the reasoning behind testing additives to reduce the rate of photo-degradation of phenoxyacetate butyl esters. Increasing the effective life of the parent compound, minimising the amount of active ingredient needed to achieve control and reducing cost are common factors to both.

Much of the work followed on from some of the recent work reported in these areas. Although the sulphonamide drugs have been in existence for a number of

years and there have been a number of studies concerning their biological activity, there did seem to be a genuine need to develop the understanding of their behaviour in plant systems and soil also. One of the main objectives of the work reported in Chapters 2-4 was to expand the knowledge and understanding of their behaviour.

The studies reported in Chapters 5-7 also developed various aspects of existing work. However, the experiments were designed to expand some relatively novel aspects of pesticide photochemistry. As a prelude to the practical research, the contents of Chapter 4 were organised and developed to present and inter-relate a variety of diverse but significant aspects of pesticide photochemistry. One of the objectives of this Chapter was to lay out and explain the important concepts which are involved in photochemical reactions. This was aimed at giving both the unfamiliar and familiar reader alike a better understanding of this area.

The work carried out in Chapter 6 was particularly satisfying. It was fascinating to learn about the theoretical aspects of photochemical reactions then to observe aspects of this in practice. To piece together chromatographic and spectroscopic data and be able to see electronic excitation and valence re-arrangement manifested as the products of re-arrangement and photonucleophilic reactions was quite fulfilling. Not only this but there are important

practical implications. For example, both inorganic thiocyanates and phenoxyacetate herbicide have growth regulating properties. The possibility exists that when both are combined as an organic compound, new biological properties might be produced. There now exists a relatively straightforward means of generating such compounds in situ.

The experiments carried out in Chapter 2 were similar in nature to some of the work carried out by Stephen (1983), concerning the phytotoxicity and mode of action of asulam and sulphanilamide in plants. It was felt that the relatively rapid bioassay tests involving cress, bean, pea and wheat enabled the screening of a larger number of test sulphonamides than might otherwise have been possible. In addition to this, the nature of the experiments were such that test chemicals were in intimate contact with the test species therefore genuine differences in their phytotoxicities were more likely to have been manifested. The foliar application of test chemicals to bean and wheat, on the other hand, involved other parameters such as cuticular penetration, translocation to active sites and metabolism. It was not possible to differentiate between the effects of these parameters on phytotoxicity and differences in the ability of the test chemicals to function as enzyme inhibitors. The same rationale applies to the bracken field experiments, although in these experiments there

was the further complication of variable effects of environmental factors on the test chemicals. In any case, the test application rate was a quarter of the value which is recommended for asulam. It has been emphasized that anything less than the recommended rate will usually lead to erratic or poor bracken control (Heywood, 1982). This possibly accounts for the results of the field experiment. Generally, the results of Chapter 2 revealed that all of the test compounds were capable of inhibiting plant growth. The magnitude of the inhibition, however, was dependant upon the nature of the test chemical, the concentration of the test chemical, the plant species employed and the root of application. Asulam or Asulox, the commercial form of the herbicide, showed a high level of phytotoxicity and this was consistent with most other reports. Several sulphonamides, however, also showed a high level of phytotoxicity indicating that these types of compound could have a use as plant growth regulators. The visual symptoms of plant growth inhibition by the foliar application of some selected sulphonamides was generally consistent with other reports. Not only this, but reduction in folate levels, through inhibition of the enzyme dihydropteroate synthetase, would account for the physical differences between control and treated plants. Existing endogenous folates probably maintained some growth of new tissue for a period of time immediately

after treatment. As test compounds inhibited or reduced the synthesis of new folates and the existing endogenous pool became depleted there was limited or no growth of new tissue, probably due to lack of essential nucleic acid and proteins. General necrosis, chlorosis and dessication, to varying degrees, probably reflected impairment of normal metabolism and membrane integrity for similar reasons. The similarity between the effects of asulam and sulphamoxole on the growth of bean plants was further evidence that asulam impairs growth in the same way as the sulphonamides ie. dihydropteroate synthetase inhibition.

The experiments carried out in Chapter 3 were designed to give some indication as to the extent of uptake of test compounds into bean leaves. Bean plants have been used on several occasions in the past as a useful and convenient model system (Jaff, 1982; Stephen, 1983). Although it was not feasible to extrapolate results further than laboratory conditions ie. field behaviour, it was possible to compare the pattern of uptake of different test chemicals through the bean cuticle. It was also possible, from the nature of the experiments, to draw conclusions regarding the possible influence of humidity on these patterns of uptake.

Without exception, low relative humidity (30-50% R.H.) severely reduced the level of uptake of test chemicals. High relative humidity, on the other hand,

significantly increased the uptake of all chemicals with the exception of sulphanilamide. Sulphonamides containing aliphatic and five membered heterocyclic groups were more efficient at penetrating the bean leaf cuticle than those containing six membered heterocyclic groups. The addition of Tween 20 at a concentration of 0.1% (w/v) significantly enhanced the uptake of nine test chemicals by comparison with aqueous formulations, but only when droplets were first allowed to evaporate to dryness before exposure to high relative humidity conditions.

Since both polarity and solubility are a function of molecular structure and functionality, it is difficult to determine the precise reason for the differences in the extent of penetration of individual test chemicals. That is, those compounds which most effectively penetrated the bean leaf cuticle were generally relatively polar. It might have been, therefore, that they were more soluble in aqueous droplets and hence remained in a form which was more available for uptake, for a longer period of time. It could also be argued that these compounds, being more polar, were more compatible with the suggested polar routes of uptake into and through the cuticle. The possible influences of molecular radius on uptake further complicated the issue.

A practical implication of the results of this Chapter was that the foliar uptake of asulam and the sulphonamides would be expected to be greatest during the night and early morning when one would expect the most frequent incidence of high relative humidity (Babiker, 1974). Theoretically, should the applied spray droplets dry out on the foliage during the day, then in the presence of Tween 20, one would expect uptake to increase further.

Both Tween 80 and ammonium thiocyanate at the levels employed gave no further increase in the uptake of test chemicals by comparison with aqueous controls. Perhaps those particular additives might have been effective at a different concentration. This, in fact, is one area which would have been worthy of further research. A detailed study of the influence of other additives, such as humectants and penetrants on uptake was not possible because of the number of test chemicals. However, it may have been possible to maximise the uptake of the chemicals through the use of such compounds and in this way determine whether cuticular penetration was a factor limiting the phytotoxicity of test chemicals when they were applied via the foliage. Both Babiker (1974) and Jaff (1982) explored the possibility of increasing the uptake of asulam through formulation with different surfactants and humectants, individually and in combination, at different concentrations. It might be

that such an approach could successfully be employed to improve the uptake of the sulphonamides and possibly increase their phytotoxicity. This is certainly an area which deserves further investigation.

The aim of Chapter 4 was to investigate the possible use of sulphonamides as plant growth inhibitors via soil application. Previous studies had reported that extremely rapid degradation limited the use of asulam as a soil applied herbicide. The evidence suggested that such facile degradation might also limit the use of sulphonamides. Rather than conduct experiments to assess their phytotoxicity to plants upon soil application, it seemed more productive to carry out a study of their persistence in soil. Not only this, but by including asulam in the study it was possible to further establish whether the herbicide was particularly susceptible to soil degradation. There also seemed to be a need to account for the products of asulam metabolism in soil, as existing reports were either lacking in data or conflicting.

Soils were selected which had high organic matter contents and which were likely, therefore, to maintain a large biomass. Such soils probably represented the extreme case in terms of pesticide metabolism by the biomass. Although the test chemicals were very rapidly degraded in these soils, this was not conclusive evidence that the same situation would exist in all soil types.

Asulam, for example, has had some success as a soil applied herbicide in soils with a low organic matter content. It was observed that the rate and extent of degradation was less in the test soil with a lower organic matter. Since most other soil parameters were the same for both soils, it would seem likely that organic matter content has a major influence on sulphonamide persistence. This is in keeping with the results of many other studies.

An attempt was made to determine the likely products of test chemical metabolism. Residues of parent compound were efficiently extracted from soils using methanol. Some of the extracts which were co-chromatographed against reference standards were found to contain two major compounds in addition to parent residues. One was unidentified but chromatographed near the solvent front under the conditions employed. The other compound was identified as sulphanilamide from its chromatographic behaviour.

The use of HPLC was largely unsuccessful as a means of metabolite identification. In some extracts there was a minor peak which chromatographed at a similar retention time to sulphanilamide. However, the compound was so weakly retained that one can only say with any real certainty that it was polar in nature. With further method development it might have been possible to purify extracts and resolve the components more efficiently.

Bonded phase extraction columns might have been a useful means of purifying the test compounds. In conclusion, it was observed, using a semi-quantitative TLC method, that only minor quantities of a compound which was possibly sulphanilamide were present in some extracts. It may be that either parent chemical or some product of metabolism was absorbed by soil constituents and that this accounted for the apparent inability to determine the total fate of parent chemicals. As was in the case in other reports, the amount of sulphonamide extracted did not account for the amount of parent lost. Adsorption is an inherent problem with aerobic degradation studies. Alternatively, perhaps some other less readily extracted or identified metabolite was produced. This area still requires further investigation.

The work carried out in Chapter 6 was originally stimulated by the observations of Cook (1979) and later by Jaff (1982) that thiocyanate could inhibit the in vitro photosensitised oxidation of test chemicals such as aminotriazole, asulam and 2,4-D. There appeared to be an obvious need to explore the use of additives to reduce photodecomposition. On reviewing the literature it became apparent that pesticide photochemistry was an area of intensive research. Not only this but there was considerable evidence to suggest that virtually any chemical could be photochemically transformed either through the direct absorption of light or through the

action of photosensitisers and photo-oxidants. The aim of Chapter 5 was to present some of this evidence. However, it was decided to study the nature and products of particular types of photochemical reactions, before the potential use of additives in this area was considered. The possible interaction of thiocyanate and 2,4-D was discussed by Jaff (1982) and this idea was developed and extended to include other phenoxyacetates. The outcome was the generation of thiocyanate and isothiocyanate derivatives of CPA and MCPA respectively. It was likely that these photoproducts were generated by a photonucleophilic reaction induced by the absorption of uv light. Other products of side chain migration, side chain cleavage and ring substitution reactions were identified.

Finally, various additives were tested as a means of reducing the photodegradation of phenoxyacetate butyl esters. It had been shown that the esters of these herbicides underwent the same types of photochemical reactions as the salts. Hence, it was decided to use the esters for the purpose of testing the various additives. This made analysis much easier and made it possible to test additives which were not always water soluble but which were more soluble in methanol. The outcome of this study was that it was indeed found to be possible to reduce photodegradation. Anions such as thniocyanate and iodide were less effective than the organic compounds

tested. Vitamin C and vitamin E were found to be very useful. The mechanism by which the various compounds reduced photodegradation was not known. Free radical scavenging, anti-oxidation or selective absorption of uv light were considered to be possible mechanisms.

There is a good deal of scope for further research in this area. An obvious extension of the work carried out in this study would be to assess the more successful additives under field conditions. Perhaps it would also be worth investigating the influence of additives on the absolute rate of photodegradation of test compounds. This would necessitate the use of carefully controlled experimental conditions and a means of quantifying the intensity of the irradiance. Although one would want to examine the possibility of enhanced weed control through the use of additives, a consideration of the reaction kinetics would be interesting and worthwhile.

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