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THE EFFECTS OF IRRADIATION AND CHEMICAL SPROUT SUPPRESSANTS ON STORED POTATOES

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Contents

Summary

This thesis contains a description and discussion of work carried out to investigate the use of irradiation as a means of inhibiting the sprouting of potatoes during extended storage. In particular some emphasis was put on a comparison between irradiation and the chemical treatments currently used for the control of potato sprouting.

In Chapter 2 the relationship between irradiation dose and the degree of sprout inhibition observed was investigated. The logistic regression technique and linear regression of the square root of the percentage sprouting by weight were found to be useful in modelling the dose-inhibition relationship for two cultivars, cv. Record and cv. Desiree.

Irradiation treatment has been shown to cause increases in the free sugar contents of potatoes. This is of concern as the processing of potatoes with high free sugar contents results in low quality products. In Chapter 3 a storage experiment was carried out to compare the free sugar contents of irradiated and chemically treated potatoes. The initially high levels of reducing sugars and sucrose observed in irradiated potatoes when compared to controls fell gradually during storage. After approximately 5 months storage at $8^{o}C$ the sugar levels in irradiated potatoes were comparable with control and chemically treated potatoes. Senescent sweetening did not appear to be accelerated by irradiation treatment although some difference in the behaviour of the two cultivars was noted.

In Chapter 4 the rates of weight loss from sprout suppressed potatoes stored at $8^{o}C$ were investigated. Irradiated potatoes, deliberately wounded before treatment, were found to lose water at a

greater rate than controls. Wounded potatoes treated with Chlorpropham or methanol, a commonly used solvent in the application of Chlorpropham, were also found to lose weight more quickly than controls. Irradiation and Chlorpropham treatment did not affect weight loss from non-wounded potatoes cured at 25^{o}C before treatment. This suggests that irradiation and Chlorpropham treatment affect the development of potato periderm after harvest and adds further weight to the recommendation that their use should be delayed until after curing has taken place.

Attempts in Chapter 5 to measure the effect of treatments on potato periderms by isolating periderms from treated tubers and measuring their permeabilities were inconclusive. It was found that there was a great deal of variability in the permeabilities measured in periderms isolated from tubers within a treatment and also in periderms isolated from individual tubers. The variability observed was too great for any effect of treatment on periderm permeability to be detected.

The combination of irradiation and chemical treatments is anticipated in the work carried out in Chapter 6. Solutions of two sprout suppressants, Chlorpropham and Tecnazene, were irradiated in methanol and hexane solutions and the rates of their degradation in the two solvents measured. Commercial formulations of Tecnazene were also irradiated and the rate of Tecnazene degradation in the formulations assessed. The differing sensitivities of the sprout suppressants to irradiation in the two solvents and in the formulations are discussed. Several radiolytic products of Chlorpropham and Tecnazene were observed when solutions were

irradiated within the 0 - 50 kGy dose range. The predominant radiolytic product of Chlorpropham in methanol and hexane solution was identified as Propham and a possible mechanism for its formation is suggested. The relevance of such studies to current potato storage practices is discussed.

Recommendations for further work are made in Chapter 7 and the viability of the use of irradiation as a means of sprout inhibition in the U.K. is discussed.

Declaration

Preliminary results forming part of the work contained in Chapters 3 and 4 have been reported in the following publications;

MUIR, J.W., BOYD, I.M.G. and DUNCAN, H.J. (1987). A comparison of the effect of irradiation and chemical sprout suppressant treatments on sugar levels in tubers. EAPR Abstr. Conf. Pap. 10, 164-165.

WILSON, L.A., BOYD, I.M.G., MUIR, J.W. and DUNCAN, H.J. (1987). The effect of sprout suppressant treatments on weight loss of potato tubers during storage. EAPR Abstr. Conf. Pap. 10, 337-338.

Chapter 1

Introduction

1.1 The need for food irradiation

On the 21st of June 1989 the Minister of Agriculture, Fisheries and Food, at that time Mr. John MacGregor, announced in the House of Commons the Government's intention of permitting the preparation and sale of irradiated food in the U.K.. Much of the contentious debate surrounding the introduction of food irradiation centres on whether food treated in this way is wholesome and nutritious. Ironically, in his statement to the House, Mr. MacGregor justified lifting the ban on irradiated food as a means of improving food safety. In his statement Mr. MacGregor said that, "irradiation has a useful contribution to make ... to the reduction of food-borne disease" (Anon., 1989a).

Concern has arisen over the increasing incidence of bacterial contamination of some foods, for example, contamination of eggs and poultry with *Salmonella* spp. and various foods with *Listeria* spp.. Such concern has provided a timely opportunity for proponents of irradiation to call for its introduction, as irradiation has been shown to be able to reduce the bacterial contamination of food. Control of *Salmonella* contamination of poultry has been demonstrated using doses of approximately 5 kGy but a comparable reduction in eggs results in unacceptable organoleptic deterioration (Urbain, 1986).

The process of food irradiation can be defined as the exposure of food to ionising radiation to affect some change in the food. The object of this study, however, is not to investigate the use of irradiation as a sterilisation or pasteurisation technique. Food

irradiation can be used to induce other changes in foods. Amongst the proposed uses of food irradiation are; control of microbial spoilage, inactivation of pathogenic organisms, insect disinfestation, delay of ripening and senescence of fruits and vegetables and inhibition of sprouting of some vegetables. Excellent reviews of the food irradiation process and its uses have been published by Josephson and Peterson (1983) and Urbain (1986).

The central theme of this thesis is the study of some of the consequences of irradiating potatoes. Irradiation of potatoes has been shown to be an effective method of sprout control (this effect is more fully discussed in Chapter 2). Control of sprouting is required when potatoes are to be stored over a period of months. At present potato sprouting is normally controlled by the use of sprout suppressant chemicals. Concern has been expressed that the residues of storage chemicals in food ought to be reduced where possible. Irradiation treatment has been suggested as an alternative to chemical sprout control treatments.

If irradiation treatment is to replace chemical treatment as a means of sprout control a comparison of the relative merits of the treatments must be made. Some elements of that comparison will hopefully be made in this study. In order to make such a judgement it is necessary to first discuss the methods of sprout control available at present.

1.2 Methods of sprout suppression

In most countries the limitations of the climate mean that a continuous growing season is not possible. Crops must therefore be stored from harvest until they are required, or imported from other localities with different growing seasons. In the U.K. potatoes are stored by the manufacturers of processed potato products during the period between harvests in order to avoid purchasing more expensive imported produce.

During these months of storage losses occur. One of the principal contributions to storage losses is the sprouting of potatoes. Potato sprouts represent an economic loss as any weight in the sprout at the time of processing will be lost when the potatoes are trimmed. Additionally, water loss, and hence weight loss, is greater through sprout tissue than through the potato skin. This is discussed in greater depth in Chapter 4. These losses are important when the final processed product is sold by weight.

Such losses have led to the development of several methods for controlling sprouting during storage. The methods of sprout control currently employed include the control of storage temperature or storage atmosphere, diffuse light control, chemical sprout inhibition and, in several countries, irradiation.

1.2.1 Inhibition of sprouting by the control of storage temperature

After harvest potato tubers often do not sprout immediately, they undergo a period of dormancy. Definitions of dormancy vary but it is essentially the period of time between harvest and the initiation of sprout growth (Harris, 1978). At storage temperatures below $5^{o}C$ the break of dormancy of potatoes and the subsequent growth of sprouts from them will be slow. Sprout growth can therefore be inhibited by the storage of potatoes at low temperatures to a level where it does not represent an economically important loss. (Storage temperatures below $0^{o}C$ can result in damage due to the freezing of water within potatoes).

Storage of potatoes at low temperatures has some important disadvantages. Refrigeration of large stores is expensive. A great deal of work has been carried out to investigate the effect of storage temperature on the reducing sugar content of potatoes and its influence on the quality of processed potato products. This subject is discussed more fully in Chapter 3, however it can be stated here that the adverse effect of low storage temperatures on sugar contents of stored potatoes is a major drawback to the use of temperature controlled sprout inhibition in long term storage.

1.2.2 Inhibition of sprouting by the use of diffuse lighting

Exposure to light is a method widely used to control the sprouting of potatoes, particularly of seed potatoes (Short and Shotton, 1968; Nash, 1978; Bishop and Maunders, 1980; McGee *et al.*, 1987). Diffuse light storage is a useful method of inhibiting the sprouting of seed potatoes as it is reversible. On returning tubers whose sprouting had been controlled by exposure to light to dark conditions it was found

that the ability of those tubers to sprout had not been permanently impaired by storage in the light (McGee et al., 1987). It is assumed that the effect of light on sprouting is mediated through the phytochrome system (Harris, 1978).

Reversibility is an essential requirement for temporarily inhibiting sprouting in the storage of seed potatoes as seed must be able to grow normally after storage. However, when storing ware grade potatoes the ability to overcome any sprout inhibition at a later date is not required and in fact is a distinct disadvantage as complete control for the duration of storage is the aim. Control of the sprouting of ware potatoes using diffuse light has further disadvantages. To control sprouting by this method diffuse light must fall on all of the potatoes to be inhibited (in seed stores potatoes are laid out on trays). This would require a costly re-design of stores if the method were to be applied to potatoes to be used in processing, resulting in the use of much larger stores. The quantity of potatoes to be stored in this way is also much greater than that stored for seed and additional handling costs would also be incurred.

A further problem caused by the storage of potatoes in this way is that exposure of potatoes to light has been shown to stimulate the production of chlorophyll and the poisonous alkaloid solanine (Burton, 1966). The presence of chlorophyll causes a marked green discolouration in potatoes making the potatoes unmarketable. The consumption of potatoes with high solanine contents can cause alkaloid poisoning. As seed potatoes are not eaten the presence of chlorophyll and solanine is of no concern in the storage of seed potatoes by exposure to diffuse lighting. They would, however, cause grave

problems in the manufacture of potato products from ware potatoes stored in that way.

1.2.3 Chemical sprout suppressants

A wide range of chemicals have been investigated for their ability to control sprouting (Dalziel, 1978; Beveridge et al., 1981a), however only a few are used commercially. The vast bulk of potatoes whose sprouting is inhibited chemically are treated with one of only four sprout suppressants; Maleic Hydrazide (MH), Tecnazene (TCNB), Chlorpropham (CIPC) or Propham (IPC).

Maleic Hydrazide

Maleic Hydrazide (6-hydroxy-3(2H)-pyridazinone) is a colourless crystalline non-volatile mono-basic acid which is normally formulated as its diethanolamine or potassium salt. Maleic Hydrazide is exceptional within the group of chemical sprout suppressants as it is applied to the growing potato plant in the field rather than directly to the harvested tubers. Maleic Hydrazide is normally applied at a rate of approximately 4 kg hectare^{-1}. Application of Maleic Hydrazide is carried out a few weeks before harvest and the timing of the application is of critical importance if it is to have the desired effect (Nash, 1978). Maleic Hydrazide is absorbed through the leaves of the potato plant and translocated via the xylem and phloem to the tubers. Within tubers Maleic Hydrazide is thought to control sprouting by inhibiting cell division in sprout tissue as it has been shown to inhibit cell division in other species (Nooden, 1972). The mode of action of this chemical is one of the concerns which has limited its introduction as a potato sprout suppressant in the U.K.. This aspect of chemical sprout inhibition will be discussed later in

the text.

As Maleic Hydrazide is applied to the foliage of the plant the amount of the chemical reaching the site of action in the tubers is dependent on the evenness of application of the chemical and an even distribution amongst the tubers of an individual plant (Dalziel, 1978). To maintain even application Maleic Hydrazide must be applied under stable climatic conditions: dry, windless conditions are preferred (Perlasca, 1956).

Stable climatic conditions may occur at the appropriate point in the growing season in some parts of the world, for example in the U.S.A., however the variable conditions which predominate in the U.K. severely limit the usefulness of Maleic Hydrazide. Other sprout suppressant chemicals have until now been chosen in preference to Maleic Hydrazide for this reason in the U.K.. A restricted acreage has, however, been approved for Maleic Hydrazide application as part of a recent feasibility study, the results of which should be of some interest (McKenzie, 1989).

Tecnazene

Tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene) is a colourless crystalline compound which is appreciably volatile at room temperature. Tecnazene was initially introduced as a fungicide for the control of Fusarium spp. the causative agent of the fungal dry rot of potatoes. Its sprout suppressant properties were subsequently observed and an application rate of 135 g tonne^{-1} potatoes is recommended for sprout control of both ware and seed potatoes, although it is applied to seed potatoes nominally as a fungicide.

Incorrect use of Tecnazene on seed potatoes can result in reduced yield and delayed emergence. These effects can be minimised by chitting before planting. Potatoes are chitted by laying them out under diffuse lighting on wooden trays in a well ventilated store at 4 - 12°C. This initiates sprout growth but also encourages the formation of the short vigorous sprouts required for planting. Dalziel (1978) recommends a chitting period of 6 - 8 weeks at 10°C to alleviate the effects of Tecnazene by allowing the chemical to volatilise into the atmosphere.

Tecnazene is normally applied to potatoes as a dust or granular formulation from which it is slowly released. The mode of action by which it inhibits sprouting is not known. Tecnazene's sprout suppressant effect lasts only as long as an effective concentration of the chemical is in contact with the potato sprouts. Thus the length of the sprout control period depends on the design of the store and the rate of ventilation from it. Generally speaking by the beginning of January in the U.K. significant sprout growth has occurred on Tecnazene treated tubers and further steps must be taken to continue sprout inhibition in ware stocks. The re-application of Tecnazene would involve costly handling losses and so alternatives are usually sought. Commercial ware storage regimes often combine the treatment of potatoes at harvest with Tecnazene followed by Chlorpropham/Propham treatments at a later date.

Propham and Chlorpropham

Propham (isopropyl phenylcarbamate) and Chlorpropham (isopropyl 3-chlorophenylcarbamate) are crystalline compounds of appreciable volatility at room temperature. They were first introduced as members

of the phenylcarbamate group of pre-emergence herbicides and have subsequently been used to inhibit sprouting in potatoes (Sawyer and Dallyn, 1956). Their use as sprout suppressants has been reviewed by Boyd (1988). Propham is seldom applied individually, but is used in some countries in a combined formulation with Chlorpropham. At the present time Chlorpropham is the most commonly used sprout suppressant in the storage of potatoes for processing in Europe. In the U.K. it is normally applied at a rate of between 10 and 30 g $tonne^{-1}$ potatoes.

Chlorpropham is normally applied to potatoes as a fog dissolved in a suitable organic solvent such as methanol or dichloromethane. In some stores Chlorpropham or Chlorpropham/Propham is applied in a single dose, in others it is applied in several smaller doses throughout the storage period. Sprouting can be controlled for the full storage period required by processors with the proviso that a 6 week delay is observed between the final dose and use.

Chlorpropham has been shown to inhibit the normal wound healing process in potatoes after harvest. (This subject is covered in greater detail in Chapter 4). For this reason the application of Chlorpropham is frequently delayed so that wound healing can proceed normally and Tecnazene is often applied as a combined fungicide and sprout suppressant for the initial period of storage.

Chlorpropham inhibits cell division, and hence sprouting, by interfering with the correct function of microtubule organisation centres during the division of cells (Oliver et al., 1978). Evidence for similar effects caused by Propham were found by Hepler and Jackson (1969). As Chlorpropham and Propham have also been shown to affect

the cell division of human cells *in vitro* (Timson, 1970) there has been some concern that the ingestion of these chemicals through the consumption of processed potato products may be harmful (Timson, 1970; Oliver *et al.*, 1978).

1.2.4 Other methods of sprout suppression

As stated previously several chemicals have been suggested as potential potato sprout suppressants. Beveridge *et al.* (1981a) assessed the sprout inhibiting properties of some 20 volatile substances the most promising of which were benzothiazole and 1,4-dimethylnaphthalene. Investigations of the sprout inhibiting properties of naphthalene derivatives has been carried out by several workers (Hartmans and Van Es, 1986; Duncan, 1986; O'Hagan *et al.*, 1987; Ashall, 1988). Naphthalene derivatives have the advantage of being naturally produced by the plant itself, their application may be more appealing than the use of novel organic chemicals on food.

Current advances in the field of plant breeding may provide cultivars in the future with low free sugar contents allowing storage of potatoes to take place at lower temperatures thus eliminating the need for sprout suppressant treatments. Studies are also being carried out to develop cultivars with long dormancy periods with which sprouting can be avoided during storage, although problems may arise in stimulating the growth of seed potatoes of long dormany cultivars.

Another potential technique which may be used for the inhibition of sprouting in stored potatoes is the control of the content of the store atmosphere. It has been found that a carbon dioxide content of greater than 15% of the storage atmosphere at 10^{o}C inhibits sprouting (Burton, 1960). Sprouting is also inhibited by an increase in the

oxygen content of the store atmosphere above the normal atmospheric content although increased rotting may occur at oxygen contents greater than 50% at $10^{o}C$ (Burton, 1960).

The above techniques are still undergoing development and therefore any judgement of their capabilities must lie in the future.

1.3 The effect of irradiation treatment on food

The following discussion is a brief description of how the exposure to ionising radiation can cause changes in foods at the molecular level. This is a complex phenomenon and the treatment that follows is not definitive but is given as an introduction to the subject which is covered in greater depth in Hughes (1973), Swallow (1973), Elias and Cohen (1977), Elias and Cohen (1983), Josephson and Peterson (1983) and Urbain (1986).

Three forms of ionising radiation have been used to irradiate food; x-rays, gamma rays and high speed electrons generated by, for example, linear electron accelerators. The most commonly used of these methods is the exposure of food to a source of gamma rays such as ^{60}Co or ^{137}Cs. The radiation source used in the experiments in this study was ^{60}Co. ^{60}Co is an emitter of gamma photons which can be thought of as electromagnetic waves, part of the electromagnetic spectrum. Gamma rays are of very high frequency, $>10^{18}$ Hz, and as a consequence of equation 1.1 are of high energy.

$$E = h \times f \qquad \text{(equation 1.1)}$$

where E is the energy of an electromagnetic wave, f is its frequency and h is a universal constant (Planck's constant).

When food is exposed to a source of gamma photons one of two events will occur. As matter is very largely open space, an individual photon may simply pass through the food causing no change. Alternatively a photon may be absorbed by its interaction with a constituent atom of the food. If a photon is absorbed some or all of its energy is transferred to the atom with which it interacts.

Three types of energy transfer effects are important in irradiation; the photoelectric effect, the Compton effect and pair production. The photoelectric effect occurs when a gamma photon interacts with an electron in an atom of the food. All of the photon's energy is transferred to the electron which is then emitted at high speed from the atom. The Compton effect occurs when a photon loses only part of its energy to the electron. It results in the emission at high speed of the electron and the scattering of the gamma photon. Pair production occurs when a gamma photon is absorbed near the nucleus losing all of its energy in the emission of an electron-positron pair. Photons emitted by a ^{60}Co gamma ray source are of energies of 1.17 and 1.33 MeV. For photons of these energies the principal mechanism for the transfer of their energy to food is by the Compton process.

In all three of the above processes the absorption of a gamma photon leads to the transfer of energy and the release of a high speed electron. It is these electrons which cause the chemical changes in irradiated food. High energy electrons are also produced in food by exposure to x-rays and the use of linear electron accelerators, thus all three irradiation techniques rely on the production of high speed electrons within food.

High speed electrons are very reactive as they are of high energy and carry a negative charge. Any target molecule that they react with may be changed in a number of ways. The target molecule may undergo a change in reactivity as it absorbs the energy of the electron. It may, alternatively, be broken down to form radiolytic products. Some possible reactions of high speed electrons with target molecules are shown below. (M is a target molecule, A and B are fragments of it).

(1) Capture by positive ions

$$M^+ + e^- \longrightarrow M^* \quad \text{(excited species)}$$

(2) Capture by a neutral molecule

$$M + e^- \longrightarrow M^-$$

(3) Capture by a neutral molecule and dissociation

$$M + e^- \longrightarrow A + B^- \quad \underline{\text{or}} \quad A^\cdot + B^\cdot \text{ radicals}$$

Many of the species produced by the reactions of electrons may themselves react further resulting in many possible end products. In physiologically active foods such as potatoes these reactions can cause alterations within important biochemical pathways. If a great enough radiation dose is received this may alter the progress of normal physiological processes. One example of this is the inhibition of normal sprouting of potatoes after exposure to radiation.

Irradiation dose

When using radiation to alter the behaviour of biological systems, for example when irradiating food to kill bacteria or inhibit sprouting, it is important to quantify the extent to which the biological system has been exposed to radiation in order to determine how large the effect of irradiation may be. The extent of exposure to radiation is defined as "radiation dose". The exposure to irradiation can be related to the effect of such exposure by expressing "radiation dose" as the number of ion pairs produced per unit mass of the sample irradiated. An ion pair is simply one pair of charged species produced by the absorption of radiation on a molecule of the food.

As the average energy required for the formation of an ion pair does not depend on the energy of the radiation used, the amount of energy transferred from radiation to resultant ionisation is also a measure of "radiation dose" termed "absorbed dose". This is more convenient as it avoids the measurement of the number of ion pairs produced. "Absorbed dose" is defined as the quantity of energy transferred from the incident radiation per unit mass of material exposed to the radiation. The S.I. unit of absorbed dose is the Gray (Gy) which is equivalent to the absorption of 1 Joule kg^{-1}. When the word "dose" has been used in this thesis it refers to "absorbed dose" unless specifically stated otherwise. (Other units for absorbed dose are still used and may appear in older literature. The most commonly used superseded unit is the rad. 1 rad is equivalent to 0.01 Gy).

The greater the activity of a radioactive source the greater the dose a sample will receive at a fixed distance from it in the same time. That is to say the rate of irradiation is greater. Therefore a

quantity called dose rate is defined to describe the rate of energy transfer per unit time. For example a typical dose rate could be 2.5 kGy h^{-1}.

1.4 Sprout inhibition by irradiation treatment

The ability of radiation to inhibit the sprouting of potatoes has been known since the pioneering work of Sparrow and Christensen (1954). This effect has been the subject of an excellent and detailed review by Matsuyama and Umeda (1983) and has also been reviewed by Burton (1960), Thomas (1984) and Urbain (1986). As the irradiation of potatoes has been so comprehensively covered in an accessible form in the literature it was thought unnecessary to repeat that review procedure here. Therefore it was decided to summarise only the most important general aspects of the process in this section and to discuss more fully the studies carried out in the relevant research fields in the introduction sections of each subsequent chapter.

Several possible mechanisms have been suggested to explain how sprouting of potatoes is inhibited by irradiation.

(1) The interruption of nucleic acid synthesis.

(2) Disruption of essential phosphorylation reactions in biochemical pathways.

(3) The induction of chromosomal aberations in the meristematic tissue of sprouts.

(4) Interference of the synthesis of Auxins.

Each of these effects could affect the development of normal sprouts and all of the above processes have been observed in

irradiated potatoes. It is unclear at the present time which process is responsible for inhibiting sprouting. It is possible that the result of any or a combination of all of the above effects may be to inhibit sprouting. Some of these effects may in fact be manifestations of the same primary effect: the disruption of nucleic acid structure could lead to all of the above effects. Further work is needed to determine the exact mechanism of sprout suppression.

A number of factors influence the effectiveness of radiation for sprout inhibition the most important of which is the magnitude of the dose received by the potatoes. Most workers publish the radiation dose required to completely inhibit sprouting, in most cases this is in the range between 0.05 - 0.15 kGy. Indeed the minimum and maximum dose limits set by the United States Food and Drug Administration (F.D.A.) are 0.05 and 0.15 kGy respectively (Matsuyama and Umeda, 1983). Maximum and minimum doses have to be set as due to the attenuation of radiation with distance from the source a spread of doses will be received by potatoes within one treated sample. The width of the range of doses received will be determined by the geometry of the radiation source used. One object of this thesis, discussed in Chapter 2, is to study the relationship between radiation dose and degree of sprout inhibition.

The dose required for complete sprout inhibition is influenced by cultivar and the dose rate at which irradiation is carried out. The dose required for complete sprout suppression has been shown to be cultivar dependent. This is perhaps to be expected as the vigour of sprout growth has long been known to be a function of cultivar. Optimum doses therefore need to be found for each variety to be

irradiated. In Chapter 2 two cultivars commonly grown in the U.K. were studied.

Lower doses can be used to inhibit sprouting if a higher dose rate is used (Mathur, 1963). The most effective rate will be determined by what is possible within the operating constraints of each particular irradiation plant. A single dose rate was used in the experiments carried out in this study.

Irradiation was found to be most effective when carried out during the period of dormancy of the potato.

The process of sprout inhibition by irradiation treatment is irreversible under normal storage regimes, therefore it offers good long term sprout control. (In studies to investigate the mechanism of radiation induced sprout inhibition Mathur (1961) has shown that inhibition can be reversed by treatment of irradiated tubers with Gibberellic acid after irradiation. This, however, is not of practical concern in the long term storage of potatoes for processing). One further implication of the irreversibility of irradiation treatment under normal storage conditions is that irradiated potatoes cannot later be used as seed.

The temperature of storage of potatoes after irradiation at a sprout inhibiting dose is not of critical importance to the degree of subsequent sprouting (Urbain, 1986). Irradiation may therefore allow the storage of potatoes at higher temperatures, although this must be balanced against an increase in storage diseases at higher storage temperatures. In addition only one sprout inhibition treatment is necessary.

Irradiation treatment is not without disadvantages. Radiation affects many biochemical systems within potatoes, some of them to the detriment of the quality of the stored potato. Increases in sugar contents have been reported. The severity of storage losses caused by rotting during storage are also greater in irradiated potatoes. These problems are the subjects of investigation in Chapters 3 and 4 respectively.

Irradiation has been implicated as a cause of discolouration in both raw and cooked potatoes. Although much work has been done in this field the results are often variable and sometimes contradictory. The reason why discolouration may be observed on one occasion and not another is probably because of the natural variability in the chemical content of potatoes between seasons, locations and cultivars. This complicates the investigation of what seems to be a real effect of irradiation treatment.

While other disadvantages of irradiation treatment do exist, such as a reduction in the content of some vitamins, effective control of sprouting can by achieved by this method.

1.5 The safety of sprout suppression methods

One motivation for replacing chemical sprout control by irradiation treatment is to remove the need for the use of potentially hazardous chemical sprout suppressants. In order to decide whether the replacement of chemical control by irradiation is justified it is necessary to judge whether irradiated potatoes are safer to eat than chemically treated potatoes.

Concern has been raised over residue levels of various agricultural

chemicals applied to food crops. Some potato sprout suppressants, unlike most other agricultural chemicals, are applied directly to the harvested crop at the beginning of, or during, storage. Thus there is no question that a residue of the sprout suppressant remains in potatoes sold to consumers. The important questions must therefore be; how large are the residues, is the sprout suppressant harmful if ingested and are any potentially harmful metabolites or by-products formed from the sprout suppressant in the food?

Pesticide residues

The residue levels of a number of agricultural chemicals in food in the U.K. have been monitored in studies carried out under the auspices of the Ministry of Agriculture, Fisheries and Food (MAFF). The results of these studies were published in the Report of the Working Party on Pesticide Residues: 1985-1988 (Anon., 1989b). Of the potato sprout suppressants only Chlorpropham and Tecnazene were monitored by MAFF as Maleic Hydrazide is not in common use in the U.K.. In potatoes provided by growers and packers in 1985-1986 Chlorpropham was found in 9 of the 69 samples tested, residue levels of between 0 and 5.7 mg kg^{-1} (average 0.3) whole potatoes were measured. Tecnazene was found in 51 of the 69 samples at levels of between 0 and 7.1 mg kg^{-1} (average 1.6) whole potatoes. In retail potatoes the residue ranges were 0 to 0.8 (average 0.02) for Chlorpropham and 0 to 2.1 (average 0.2) for Tecnazene.

Recent legislation in the U.K. on the use of pesticides on food crops, the Food and Environment Protection Act (F.E.P.A.), was introduced in 1985 in anticipation of an E.C. directive. One result of the F.E.P.A. is the imposition of statutory Minimum Residue Limits

(M.R.L.s) on a number of pesticides in specific foods.

The proposed M.R.L. for Tecnazene, published in the MAFF consultative document (Anon., 1988) of 1 mg Tecnazene kg^{-1} whole potatoes did not appear in the final legislation. It is not known if Tecnazene is to be included at a later date. It should be noted that in the MAFF residue study discussed previously (Anon., 1989b) 37 out of 67 retail potatoes samples measured had Tecnazene residues greater than the proposed M.R.L..

The use of Chlorpropham, although not covered by either the consultative document or the F.E.P.A., is due for review by F.A.O./World Health Organisation (W.H.O.) in 1990 and an M.R.L. is expected to be set for potatoes following the publication of their report. It would appear likely that the M.R.L. will be set at a value consistent with limits presently in force in other E.C. countries, probably at 5 mg Chlorpropham kg^{-1} whole potatoes.

It should be noted that the M.R.L.s specified above refer to the residue levels measured in the whole potato. Both Tecnazene and Chlorpropham are applied to the outside of the tuber and several studies have shown that most of these chemicals remain in the peel fraction of the tuber and the residue in the whole tuber can therefore be reduced by airing (Dalziel et al., 1980; Coxon and Filmer, 1985) and peeling (Ritchie et al., 1983). Residues in peeled tubers were found to be only 10 - 20% of those found in the whole tuber (Ritchie et al., 1983). The inclusion of unpeeled tubers in some potato products is a practice worthy of discouragement.

The distribution of residues within the tuber previously described does not, of course, apply to Maleic Hydrazide. Maleic Hydrazide has

been shown to be distributed throughout the flesh of the potato (McKenzie, 1989) and has been detected in processed products at levels similar to those found before processing. Maleic Hydrazide, as it is not applied to any great extent in the U.K. at present, is not included under the F.E.P.A.. The W.H.O., however, have set a limit of 50 mg Maleic Hydrazide kg^{-1} whole potatoes on Maleic Hydrazide residues. As with all residue limits it may be difficult to strike a balance between getting an effective concentration of the sprout suppressant to the site of action and remaining within the residue limits. This is particularly so when application is by as imprecise a method as is used for Maleic Hydrazide and when little remedial action can be taken to reduce residues after harvest.

The toxicology of sprout suppressants

Table 1.1 summarises some of the data available on the acute toxicity of Tecnazene, Chlorpropham and Maleic Hydrazide.

Table 1.1 Acute oral LD50 values* of some commonly used chemical sprout suppressants.

Sprout suppressant	Acute oral LD50 for rats (mg kg^{-1})
Chlorpropham	5000
Tecnazene	7500
Maleic Hydrazide (sodium salt)	6950
(diethanolamine salt)	2340

* source of information Hartley and Kidd (1983).

These values show that all of the above chemicals are at the lower end of the acute toxicity range.

As was discussed previously Chlorpropham has been shown by in vitro studies to affect mitosis and result in the unequal distribution of DNA after mitosis in isolated human cells (Timpson, 1970; Oliver et al., 1978). The ingestion of a compound capable of such an effect is not conclusive proof that these effects will be observed in live animals which may be able to metabolise Chlorpropham. Such evidence does, however, add impetus to further investigations of the toxicology of Chlorpropham.

Toxicology studies on Tecnazene have led to its classification as a neoplastigen of moderate to high mammalian toxicity (Sax, 1984). For this reason the Food and Agriculture Organisation of the World Health Organisation (FAO/WHO) have recommended an Acceptable Daily Intake (ADI) of 0 - 0.01 mg Tecnazene per kg body weight (Yess, 1988).

The health risks of Maleic Hydrazide have been reviewed by Ponnampalam et al. (1983). It has been suggested that Maleic Hydrazide is degraded to hydrazine (Biswas et al., 1967; Peddie et al., 1986) although no conclusive evidence has yet established this. Hydrazine is a highly toxic chemical (Sax, 1984) which is defined by the International Agency for Research on Cancer as a class 2B substance, carcinogenic to animals and possibly carcinogenic to humans (Anon., 1987a). Evidence that Maleic Hydrazide has been found to be associated with the nucleic acid fraction (Coupland and Peel, 1971) and other structural considerations have led to the the suggestion that Maleic Hydrazide acts as a pyrimidine antagonist or analogue (Nooden, 1972). Further weight is lent to this hypothesis by the observation of the synthesis of abnormal proteins in plants treated with Maleic Hydrazide (Peterson and Naylor, 1953; Coupland and Peel,

1971).

Similarities between plant and animal systems and the effects of these chemicals on humans should not be over-emphasised, the data available from studies on humans is limited. Results of studies on the toxicology of these chemicals have sometimes been equivocal and are often based on studies carried out under protocols which have since been superseded, hence further investigations are necessary. More studies are also required on the possible chronic effects of ingesting small amounts of sprout suppressants.

Sprout suppressant metabolites

It is not only the residue levels of the sprout suppressants themselves which may be of concern, consideration must also be given to their metabolites.

As Chlorpropham is used primarily as a herbicide much work has been done to investigate its metabolism in the soil. Soil is present on potatoes in stores and Chlorpropham is applied to that soil as well as to the potato skin therefore the soil metabolism of Chlorpropham is of interest in any safety study. Chlorpropham has been shown to be cleaved by microbial degradation to 3-chloroaniline (Freitag et al., 1984). The presence of 3-chloroaniline raises the possibility of a further condensation reaction to form the toxic compound 3,3'-dichloroazobenzene. In order for these reactions to take place anaerobic conditions must exist in the soil associated with the potatoes. The moisture content of the soil must therefore be high enough to allow anaerobic conditions to occur. Under properly controlled commercial storage conditions the occurrence of anaerobic

conditions should be minimised, however anaerobic micro-environments may still exist. The presence of these compounds in soil on potatoes is under investigation at this time at Glasgow University.

Chlorpropham is effective as a herbicide applied to monocot l dons but is ineffective in the control of dicot l dons. Studies carried out to determine the reason for the specificity of Chlorpropham action led to the discovery of the hydroxylated metabolites of Chlorpropham isopropyl 3-chloro-2-hydroxycarbanilate (2-OH Chlorpropham) and 3-chloro-4-hydroxycarbanilate (4-OH Chlorpropham) in soya beans by Still and Mansager (1972). This was confirmed to be the first step in the mechanism of detoxification of Chlorpropham by some plants. Hydroxylation is followed by the attachment of a sugar moitey to form a glucoside with no herbicidal activity.

The hydroxylated derivatives of Chlorpropham have not yet been identified in potatoes, although Heikes (1985) has isolated isopropyl 3-chloro-4-methoxycarbanilate (4-methoxy chlorpropham) which may be the methylated derivative of 4-OH Chlorpropham. More recently trace levels of 3-chloroaniline (Worobey et al., 1987) and 3,3' dichloroazobenzene (Worobey and Sun, 1987) have been identified in potatoes. The formation of aniline and azobenzene metabolites of Chlorpropham is likely to be of greater concern than the formation of hydroxy or methoxy metabolites. This is due firstly to the relative toxicity of the former two chemical classes and secondly as mammalian cells can more readily metabolise and detoxify hydroxylated compounds to glucuridones and sulphate esters which can then be excreted. The presence of methoxy, aniline and chloroazobenzene metabolites of Chlorpropham in potatoes have yet to be confirmed by other workers.

Metabolism of Tecnazene in soil has been found to result in the formation of 2,3,5,6-tetrachloroaniline (McGibbon, 1984). This metabolite was found in greater quantities under anaerobic soil conditions especially when a glucose amendment had been added. The addition of mercuric chloride amendments resulted in a decrease in the amount of the metabolite formed. These facts suggest that 2,3,5,6-tetrachloroaniline is a metabolite formed by anaerobic soil bacteria.

Heikes et al. (1979) found the following compounds to be present in Tecnazene treated potatoes; 2,3,5,6-tetrachloroanisole, -tetrachloroaniline, -tetrachlorothioanisole, -tetrachloro-p-nitroanisole, -tetrachloro-p-anisidine, -tetrachlorobenzene, trichloronitrobenzene and pentachloronitrobenzene. The final three compounds on the above list were also found as contaminants in Tecnazene formulations. It is not clear whether the other compounds detected are the products of bacterial degradation or potato metabolism. The formation of 2,3,5,6-tetrachloroaniline found in the above studies once again suggests the possibility of coupling to form chlorinated azobenzenes, the formation of such compounds in potatoes has not been observed.

Information on the routes which exist for the metabolism of Tecnazene in mammals is provided by a study on rabbits by Bray et al. (1953). The metabolic routes include; mercapturonic acid and glucuronide conjugation and the formation of 4-amino-2,3,5,6-tetrachlorophenol, 2,3,5,6-tetrachloroaniline and its sulphate ester. Only some 34% of the Tecnazene dose administered was absorbed.

Little evidence has come to light with regard to the metabolites of Maleic Hydrazide in potatoes possibly because of the difficulties

inherant in the analysis of that compound. As Maleic Hydrazide is applied to the foliage, soil metabolites need not be considered in a discussion on its use in potatoes. A β-D-glucoside of Maleic Hydrazide has been identified in wheat (Towers et al., 1958) and in tobacco plants (Frear and Swanson, 1978). Attempts to characterise this glucoside in potato juice by McKenzie (1989) proved inconclusive.

The nature of some of the compounds potentially present in or associated with potatoes as a result of treatment with chemical sprout suppressants is of concern and further investigation is required to confirm whether they pose a significant hazard to health if present and ingested at the levels found in potatoes. The above discussion does, however, provide a valid justification for the search for alternative sprout inhibition techniques.

Irradiation

The safety of the irradiation process has been a matter of great discussion. Reports have been published by the relevant departments of several governments and academic institutions (Anon., 1955; Anon., 1986a; Metlitsky et al., 1967; Pim, 1983; Urbain, 1986). Some consumer groups have made their contribution to the debate, including, in the U.K., the Consumer Association (Anon., 1989c) and the London Food Commission (Webb, 1985). Articles have also appeared in the media; television, magazines and newspapers have commented on its proposed introduction, particularly as the legislation allowing its introduction has made its way through parliament.

The most commonly raised questions concerning the safety of food irradiation are as follows.

(1) Is irradiated food radioactive after treatment?

(2) Are the compounds which may be formed in irradiated food by the irradiation process harmful?

(3) Is the freshness of irradiated food merely cosmetic, masking it s deterioration?

(One other important question, whether it is possible to determine that food has been irradiated, is discussed in Chapter 7).

(1) Induced radioactivity

Whether exposure to radiation can induce radioactivity in food can be answered unequivocally. A small increase in activity as a consequence of irradiation has been calculated (Urbain, 1986; Anon., 1986a). The magnitude of the increase is related to the dose received. Even at the maximum dose envisaged by U.K. authorities of 10 kGy the amount of induced radioactivity produced is regarded as of negligible risk to human health (Anon., 1986a) and is small in comparison with the level of radioactivity naturally present in food. The choice of radioisotopes which emit gamma photons of energy lower than 5 MeV such as ^{60}Co and ^{137}Cs limits the number of radioisotopes which can be induced by activation to three, ^{2}H, ^{17}O and ^{13}C. The half-lives of those species are short and they decay to stable products (Urbain, 1986), ^{1}H, ^{16}O and ^{12}C.

The level of induced radioactivity falls as radioactive elements decay during storage. The overall radioactivity may, in fact, fall below pre-irradiation levels during storage due to the decay of

naturally occurring radioisotopes such as ^{40}K. Irradiation of potatoes to inhibit sprouting requires far lower doses, of the order of 0.1 kGy, than the proposed maximum permissible dose of 10 kGy and any initial increase in radioactivity will therefore be smaller than will occur when irradiation is carried out for other purposes.

(2) Radiolytic products

The question of whether irradiation treatment results in the production of toxic radiolytic products is more difficult to answer conclusively. If a harmful (mutagenic, carcinogenic or toxic) compound were to be formed by irradiation it could be isolated and quantified. There are, however, many possible potentially harmful compounds which could be formed in many diverse foodstuffs. Thus it is difficult to guarantee that no such compound has been formed in every food or processed combination of foods likely to undergo irradiation.

The authorities in several countries have investigated this problem thoroughly (Urbain, 1986). In the U.K. the view of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (C.O.T.) is that there is "no evidence to suggest that any toxicological hazard to human health would arise from the consumption of food irradiated up to an overall dose of 10 kGy" (Anon, 1986a). Urbain (1986) summarises the available toxicological data as follows.

(1) No identified radiolytic product has been shown to be significantly toxic.

(2) Radiolyic products identified can be found in non-irradiated food and are similar to those produced by other preservation and cooking techniques.

(3) The quantities of radiolytic products are small at normal doses.

The weight of the evidence gathered so far suggests that no health hazard is presented by the consumption of irradiated food but it cannot, however, absolutely guarantee that such hazards do not exist. The continuation of research into the effects of radiation on food is prudent.

(3) Freshness

The concept of "false freshness" arises because of the manner in which people identify harmful food. A cook can often tell when food is "off" simply by its colour and smell. These signs are produced by the growth of spoilage organisms such as Pseudomonas spp. which indicate that significant microbial growth has occurred and therefore that more harmful organisms may be present.

Microorganisms show varying sensitivities to radiation. Some bacteria may be greatly reduced in numbers by irradiation at a given dose allowing the subsequent unimpaired growth of other species. An example of a radiation-resistant pathogen is the spore-forming Clostridium botulinum some strains of which can grow to hazardous levels without producing noticeable spoilage. A further problem caused by this organism is that it can produce a toxin which will remain in irradiated foods even when the microorganisms responsible for its production have been reduced to safe levels by irradiation. In each of these sets of circumstances the consumer will be given a

false impression that such food is perfectly safe as, due to the reduction in number of more radiation sensitive spoilage organisms, it will look and smell no different from uncontaminated food.

The solution to this problem lies in the application of good hygiene standards before and after irradiation and sensible storage conditions for irradiated food. Consumers should also be made aware that they must treat irradiated food differently to fresh food. Therefore further important safeguards for the safe use of irradiated food should include the provision of information for consumers on the subject of irradiated food and a clear food labelling policy which indicates that food has been irradiated and the length of time over which the food is safe to use. (It should be noted that the doses used to inhibit sprouting are not great enough to eliminate spoilage organisms).

Is irradiation a safer method of controlling the sprouting of potatoes than chemical sprout suppression? From the above discussion it would appear that irradiation has much to offer as an alternative sprout suppression treatment to chemical sprout control. It is, however, worthwhile to periodically reassess the relative merits of each method as new information on their safety becomes available.

In investigations of the effect of irradiation on foods most attention has been paid, quite correctly, to the effect on the major food constituents such as carbohydrates, proteins and lipids as well as important more minor constituents such as, for example, vitamins. However, even the most minor constituent of irradiated food is worthy of interest as some substances are of very high toxicity even at very low concentrations.

When agricultural produce is irradiated residues of agricultural chemicals may be present and they will, of course, be irradiated with the food itself. In the specific case of the irradiation of stored potatoes circumstances can be envisaged where chemicals may be applied prior to irradiation. Some investigations of the combination of these two treatments will be made in this study.

1.6 Thesis objectives

As has been previously stated this study concentrated on investigations into the use of irradiation as an alternative to conventional methods used to inhibit sprouting in stored potatoes. Investigations were made into some of the problems encountered in the use of irradiation in order to assess the viability of irradiation for that purpose. Emphasis was placed on a comparison of the quality of potatoes stored after irradiation treatment with those stored under current chemical storage regimes. Initial studies were also carried out to investigate the possible combination of chemical and irradiation treatments.

Chapter 2

The effect of gamma radiation on sprout growth

2.1 Introduction

As was discussed in Chapter 1 one of the major problems encountered during the storage of potatoes is that of sprout growth. In the discussion in section 1.2 several techniques used to control sprouting were described. In this chapter the use of irradiation for the control of potato sprouting is investigated.

In section 1.4 of Chapter 1 the general aspects of the inhibition of the sprouting of potatoes by irradiation were outlined. The most important factors influencing the success of irradiation were identified as irradiation dose, dose rate, cultivar and the timing of treatment. Recent comprehensive reviews on the inhibition of potato sprouting by irradiation treatment have been published by Matsuyama and Umeda (1983) and Thomas (1984) and that review process will not be duplicated here.

Thomas (1984) provides a wide-ranging summary of studies carried out on sprout inhibition by irradiation treatment from which he concludes that an irradiation dose of 0.1 kGy is sufficient to inhibit sprouting irreversibly in most circumstances regardless of cultivar irradiated or subsequent storage temperature. Matsuyama and Umeda (1983) and Thomas (1984) summarise the evidence of a number of studies on the effect of irradiation on the sprouting after irradiation treatment of different cultivars grown in many locations throughout the world. They confirm that the radiation dose required for complete sprout inhibition is cultivar dependent.

Although many studies have been carried out on the effect of irradiation on the sprouting of potatoes, and studies of the other effects of irradiation on potatoes often include some description of the effect that irradiation had on sprouting, these studies describe the degrees of sprouting at various irradiation doses without seeking to investigate the nature of the relationship between dose and sprout growth. It is that topic on which this study concentrates.

Most previous studies have only qualitatively described the degree of sprouting after irradiation treatment, normally stating the irradiation dose required for complete sprout inhibition, for example Burton and Hannan (1957) or Heiligman (1957). Other workers have expressed sprout levels as a percentage of tuber weight (e.g. Sparrow and Christensen (1954)) which is a method commonly used, along with the mean length of the longest sprout growing on a tuber, to describe the levels of sprouting in non-irradiated potatoes. Mathur (1963) has used the percentage of potatoes sprouting at each irradiation dose as the criterion for comparing the degree of sprout growth. It was decided to use both the mean of the lengths of the longest sprouts on tubers and the weight of sprouts (expressed as a percentage of the weight of the tubers on which they were growing - the percentage sprouting by weight) as methods of describing the levels of sprouting. It was thought that these measurements could be used as a basis for developing models to relate the degree of sprout growth to the irradiation dose applied.

Several factors which have not as yet been discussed can greatly influence the effectiveness of irradiation treatment. The effectiveness of the radiation dose received is dependent on the

nature of the material irradiated as governed by the relationship:

$$I = I_o e^{-ux}$$

where I_o is the intensity of the incident radiation, I its intensity after passing through the absorbing material, x the thickness of the material and u the linear coefficient of absorption of the material (u varies according to the energy of the radiation and the nature of the absorbing material). A dense material, such as concrete, absorbs more radiation than a less dense material, for example, air. Potatoes are of relatively low density but only a small number of radiation-induced changes are required to alter the sensitive physiological processes responsible for sprout development.

The size of the radiation dose received is dependent on the amount of radiation incident on a unit area of the sample. If the distance between the source and the sample is increased fewer gamma photons will be incident on the sample and a lower dose will be administered to the sample. The geometry of the source and the positioning of samples within it are therefore of critical importance in determining the dose or range of doses received by samples.

All materials irradiated in the course of this work were treated at the ^{60}Co gamma radiation source in the Scottish Universities Research and Reactor Centre (S.U.R.R.C.) at East Kilbride. The relationship between distance from the source and the dose received as described above is relatively simple for a single point source of radioactivity, however the ^{60}Co irradiation facility at the S.U.R.R.C. consists of several sources mounted in a cylindrical arrangement. This makes the dose-distance relationship more complex.

Diagram 2.1. Structure of the ^{60}Co gamma radiation source at the S.U.R.R.C..

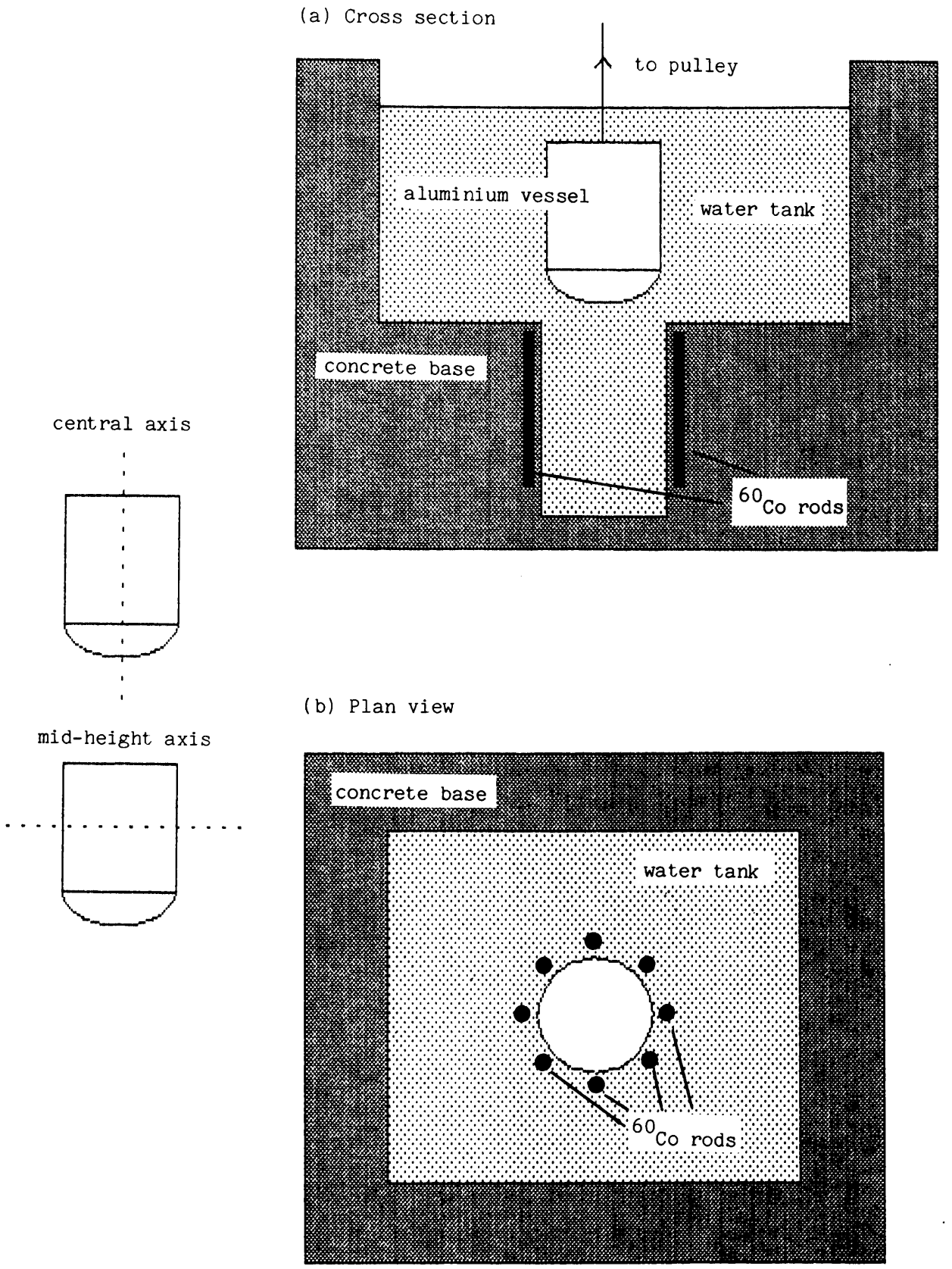
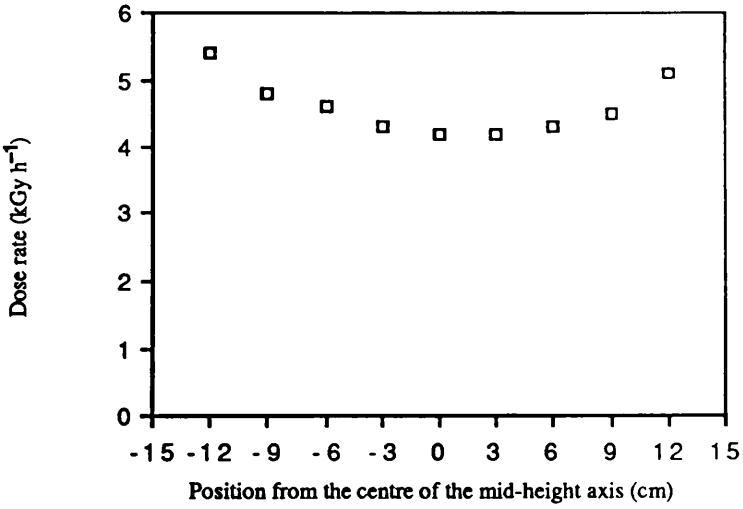


Diagram 2.1 represents the structure of the source at the S.U.R.R.C.. It consists of a cylindrical arrangement of metal rods containing pellets of the isotope ^{60}Co into the centre of which an aluminium vessel can be introduced by the use of a pulley system. The source is sunk into the concrete structure of the reactor itself, at the base of a tank of water through which the vessel travels to deliver the sample to the source. Samples to be irradiated are placed inside the vessel which is made airtight before being lowered into the source.

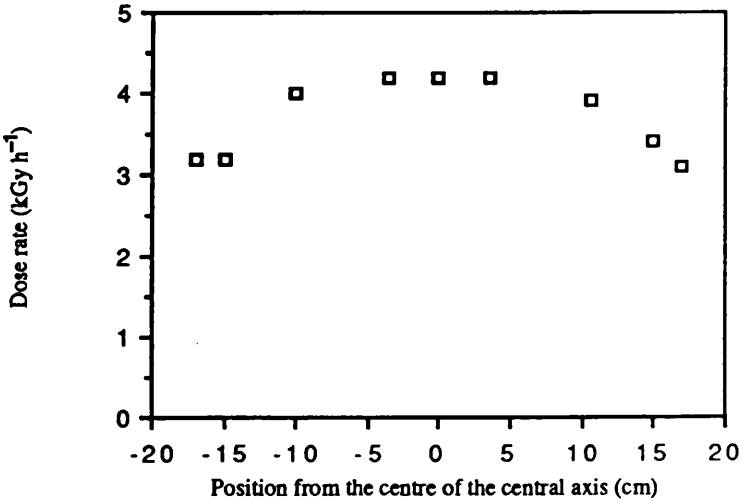
Constructing a radiation source with cylindrical geometry is an attempt to minimize the range of doses received by the different parts of samples irradiated. However, some variability is inescapable as the finite dimensions of the vessel ensure that some parts of the sample are closer to the rods than others.

The amount of variability experienced throughout the sample has been estimated by the technical staff of the S.U.R.R.C.. Some results of their investigations are presented in graphs 2.1 and 2.2. These graphs show that the maximum dose is received at the circumference of the vessel at the mid-point of the central axis. The minimum dose is received at the top or bottom of the central axis.

Graph 2.1. Variation in dose rate received at various positions across the mid-height axis of the S.U.R.R.C. ^{60}Co source vessel.



Graph 2.2. Variation in dose rate received at various positions on the central axis of the S.U.R.R.C. ^{60}Co source vessel.



2.2 Preliminary investigation

2.2.1 Introduction

The following small scale unreplicated study was carried out in order to measure the degree of inhibition of sprout growth caused by various irradiation doses. This information was to be used to determine the dose range suitable for further investigation.

2.2.2 Experimental

A sample of 40 kg of cv. Record tops from a seed crop were donated by United Biscuits (Agriculture) Ltd. through an Aberdeenshire seed supplier as part of a larger potato storage study (for details of that study refer to Chapter 5). The potatoes were harvested on September 27 (1986) and arrived at Glasgow University on October 2 where they were stored at $8^{o}C \pm 1^{o}C$ in a constant temperature room until November 13 when they were irradiated.

On November 13 the potato sample was split into 5 kg sub-samples and transported to the S.U.R.R.C., a journey of approximately 15 miles, where irradiation was to be carried out. Potato samples irradiated in this experiment were placed into the vessel above an aluminium spacer. This positioned the samples at the middle of the central axis of the vessel ensuring that the doses received by samples were as even as possible. However, because potato samples are by nature bulky the samples filled the width of the vessel completely. The 10 kg samples used in later experiments were larger and therefore the spacer had to be removed in order to irradiate all of the potatoes in one sample at one time. The dose rate of the source was obtained from staff of the S.U.R.R.C. (who monitor it routinely), the duration

of exposure to the source for each sample was calculated and the samples irradiated.

The doses used in this study were; 0, 0.01, 0.025, 0.05, 0.1, 0.15, 0.3, 0.5 kGy at a dose rate of 2.5 kGy h^{-1}. One 5 kg sample was irradiated at each dose.

After exposure to the source for the appropriate period of time the vessel was removed from the source and the samples transferred into closed cardboard boxes of dimensions 385 mm X 310 mm X 155 mm. These samples were returned to the constant temperature room and stored at $8^{o}C \pm 1^{o}C$.

On March 24, 131 days after the samples were returned to the constant temperature room, the degree of sprout growth in each sample was assessed. Thirty tubers were randomly selected from each treatment and the length of the longest sprout growing on each tuber in mm $\pm$ 1 mm was determined. Thirty tubers amounted to almost the entire sample in most cases. A visual examination was carried out to determine the effect of irradiation on the apical dominance of sprouting and the appearance of the sprouts. Finally all of the tubers in each treatment were desprouted and both the sprouts and the desprouted tubers were weighed on an Oertling JB52 electronic balance. Since there was little soil adhering to the tubers it was not thought necessary to wash them prior to weighing.

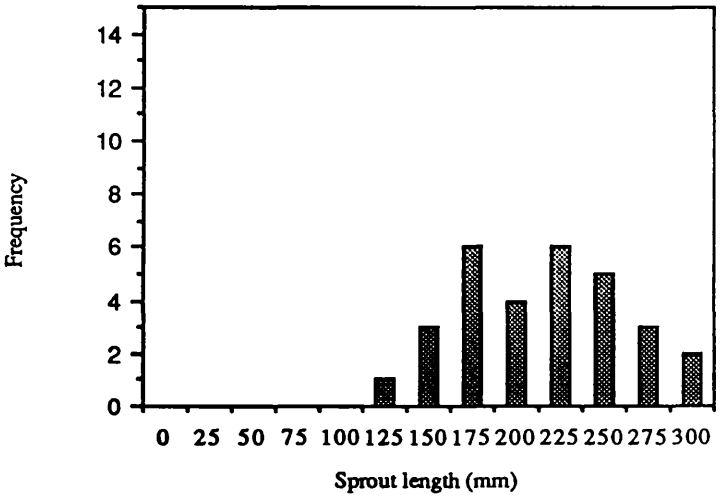
2.2.3 Results

It was found that at irradiation doses of 0.1 - 0.5 kGy no significant sprout growth occurred. It was noted that at these doses sprouts of less than 1 mm were present and that most sprout eyes were

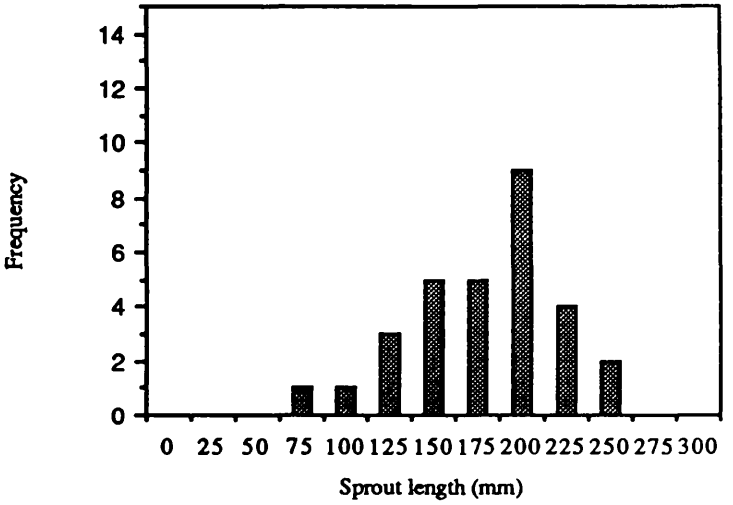
open. It was also found that these sprouts consisted of many small leaves but no sprout elongation had taken place. It was also found that the growth of any small sprouts (2 - 3 mm) that had been present at the time of irradiation had been halted by the irradiation treatment and the tissue of such sprouts had become necrotic. Apical dominance in treated tubers was broken, the magnitude of the effect appeared to increase with increasing irradiation dose.

Inspection of the sprout measurement data at the doses at which sprouting occurred revealed that at some doses sprouting was completely inhibited or greatly reduced in a number of tubers, while in other tubers in the same treatment appreciable sprouting took place. This probably occurred as a consequence of the range of doses received within one sample as previously discussed. Histograms showing the distribution of sprout lengths in treatments in which sprouting occurred are presented as graphs 2.3 to 2.6.

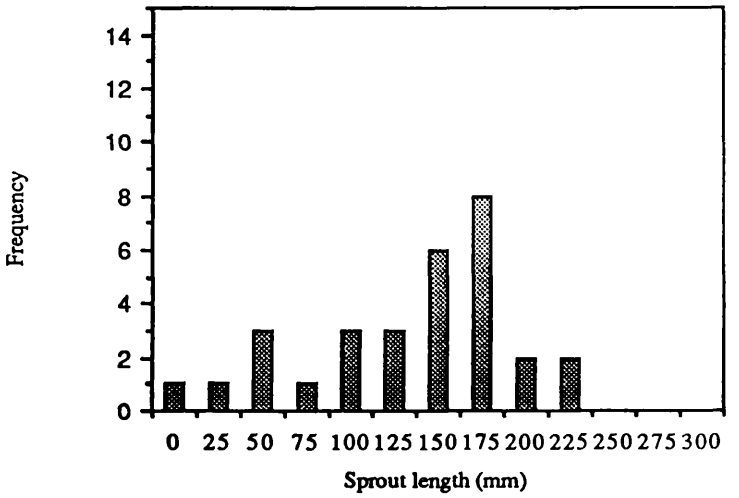
Graph 2.3. The sprout length distribution within a 30 tuber sample of non-irradiated cv. Record potatoes stored for 131 days at $8^{o}C$.



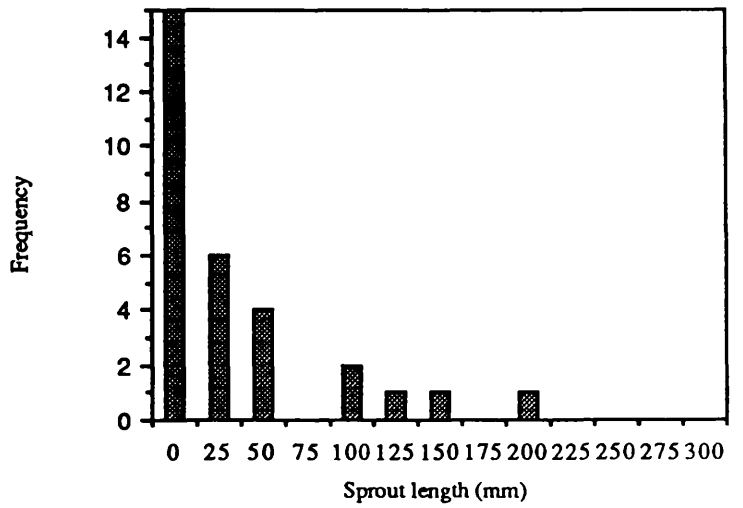
Graph 2.4. The sprout length distribution within a 30 tuber sample of cv. Record potatoes irradiated at a dose of 0.01 kGy and stored for 131 days at $8^{o}C$.



Graph 2.5. The sprout length distribution within a 30 tuber sample of cv. Record potatoes irradiated at a dose of 0.025 kGy and stored for 131 days at 8°C.



Graph 2.6. The sprout length distribution within a 30 tuber sample of cv. Record potatoes irradiated at a dose of 0.05 kGy and stored for 131 days at 8°C.



Measurements of the length of longest sprout were categorised into two groups, those greater than 2 mm and those less than or equal to 2 mm. Those potatoes with sprouts greater than 2 mm were categorised as sprouting while those with sprouts less than or equal to 2 mm were categorised as not sprouting. The value of 2 mm was chosen as the method of measurement employed had an accuracy of $\pm$ 1 mm and therefore a maximum non-sprouting length of 1 mm would include measurements of up to 2 mm.

The means of the lengths of the longest sprout of sprouting tubers were calculated for each irradiation dose and are tabulated with the number of measurements upon which they are based in table 2.1.

Table 2.1. The effect of irradiation dose on mean sprout length of longest sprout of sprouting tubers.

Irradiation dose (kGy)	No. potatoes sprouting (> 2 mm)	No. potatoes not sprouting (<= 2 mm)	Mean length of longest sprout (mm)	Standard deviation
0.00	30	0	213	45.0
0.01	30	0	178	42.8
0.025	30	0	138	46.5
0.05	22	8	48[a]	51.3
0.10	0	30	-	-
0.15	0	30	-	-
0.30	0	30	-	-
0.50	0	30	-	-

[a] Mean = 35 if sprouts of less than or equal to 2 mm are included.

The percentage sprouting by weight was calculated thus:

$$\% \text{ Sprouting by weight} = \frac{\text{Weight of sprouts removed (g)}}{\text{Weight of desprouted potatoes (g)}} \times 100$$

The values of percentage sprouting by weight are presented in table 2.2.

Table 2.2. The effect of irradiation dose on percentage sprouting by weight.

Irradiation dose (kGy)	% Sprouting by weight (g sprouts 100 g^{-1} desprouted potatoes)
0.00	9.36
0.01	7.25
0.025	5.64
0.05	0.89
0.10	0.00
0.15	0.00
0.30	0.00
0.50	0.00

2.2.4 Discussion

The initial aim of this experiment was to examine the relationship between dose of radiation received and sprout growth. It was found that at doses of 0.10 kGy and greater no sprout growth took place during the storage period studied. This preliminary investigation also showed that at doses of radiation below the level required for complete sprout inhibition a range of sprout lengths could be observed. A subjective assessment concluded that apical dominance was overcome in irradiated potatoes.

The size of the samples irradiated in this initial work preclude any formal statistical analysis of the results. However, the results obtained were thought promising enough to justify a more rigorous study from which a clearer picture might emerge.

2.3 Full scale box storage study

2.3.1 Introduction

The results obtained in section 2.3 were thought to be of sufficient interest to justify repeating the experiment in a more rigorous manner. Since doses greater than 0.1 kGy were effective in inhibiting sprouting completely, the spread of doses used in this study was altered from the range used in the preliminary study. The changes consisted of inserting an extra dose at the lower dose range and the omission of higher dose treatments. A second cultivar was included in order to observe if sprout growth after irradiation displayed cultivar dependence. Cv. Desiree was chosen as the second cultivar to be used as it is generally a more vigorously sprouting cultivar than cv. Record and also because it was to be used in a subsequent study. Greater replication was introduced in order to take into account variability present in the results due to box and position effects. Finally measurements of the degree of sprouting were made at two points in the storage period rather than one and the period of storage studied was lengthened.

2.3.2 Experimental

140 kg of cv. Desiree potatoes were purchased from J. M. Fairlie, Kirkton Farm, Kirkton of Monikie, Tayside on December 14. A further 140 kg of cv. Record top potatoes from a seed crop were purchased from T. McGregor, Murdoch Cairnie Farm, Couper Rethillet, Fife on December 18. These potatoes were stored at $8^{o}C \pm 1^{o}C$ and a relative humidity of 90% $\pm$ 1% in a constant temperature room until December 22 when they were transported to the S.U.R.R.C. for irradiation.

Two replicate 10 kg samples of each cultivar were irradiated at each dose. The doses used were; 0, 0.01, 0.025, 0.035, 0.05, 0.075 and 0.15 kGy. Due to the large number of samples to be irradiated it was not possible to irradiate all of the samples on the same day. Samples irradiated at 0.15, 0.075 and 0.05 were treated on December 22 while the remaining doses, 0.035, 0.025 and 0.01 kGy, were treated on December 23. Irradiations were carried out as described in section 2.2.2, exposure times were calculated as outlined in that section. Control potatoes were transported to the S.U.R.R.C. with the potatoes to be irradiated and remained there for the same length of time and under the same conditions.

All potatoes were returned to the temperature control room on December 23. Potatoes were then stored at $8^{o}C \pm 1^{o}C$ for 193 days and sprouting was assessed twice during that storage period.

At the first assessment on March 27, 96 days after the first irradiations, the length of the longest sprout on each of 45 randomly selected tubers from each box was measured to a precision of $\pm$ 1 mm. The percentage sprouting by weight in each box was then measured. An approximately 3 kg sub-sample was randomly selected from each box, the potatoes desprouted and the sprouts and desprouted potatoes weighed on an Oertling JB52 electronic balance. The remaining potatoes were then returned to the sample boxes and the sample boxes were returned to the potato store.

The second sprout assessment was carried out on July 2, 193 days after the first irradiations. It was initially intended that this sprout assessment should have followed the procedure carried out at the first assessment, however, on inspecting the samples it was

apparent that in some treatments where sprout growth had occurred sprouting was so extensive that the sprouts had become strongly entangled. This hampered measurement greatly as removal of potatoes from the boxes often resulted in other tubers being inadvertently desprouted. It was therefore decided to assess sprouting in this case by measuring the percentage sprouting by weight only. As this was the last measurement to be made all of the remaining tubers were desprouted and the sprouts and desprouted tubers weighed as in section 2.2.2.

2.3.3 Results and discussion

2.3.3.1 Proportion of sprouting inhibited by irradiation

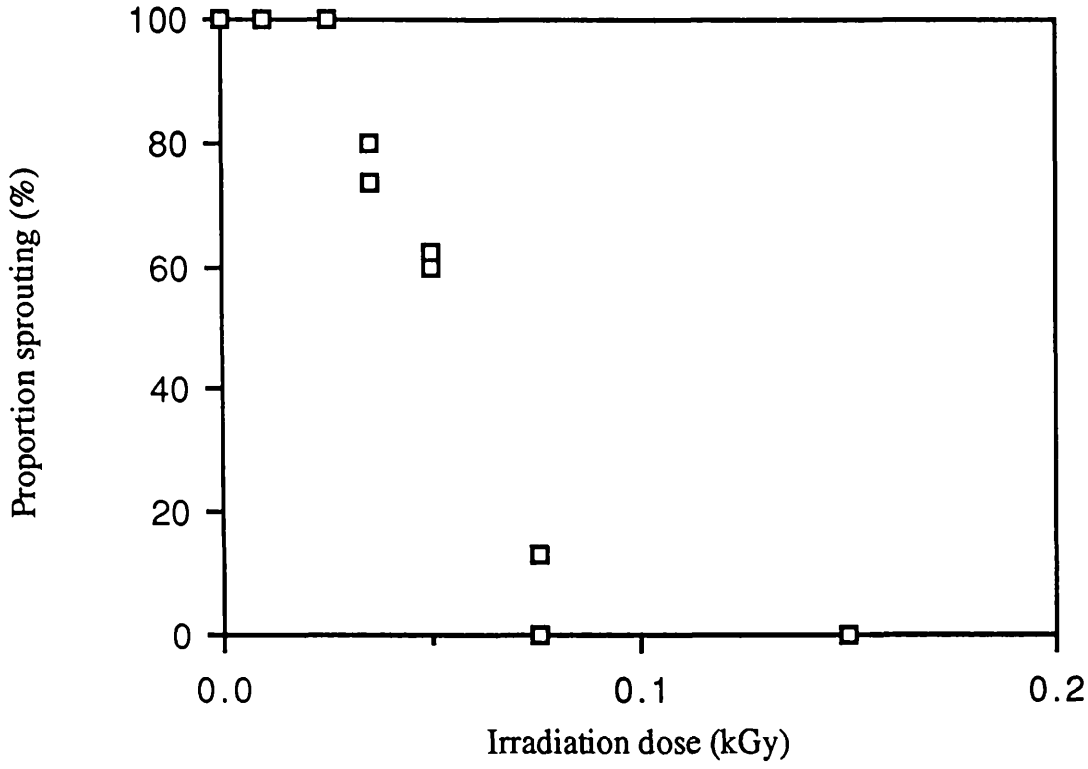
An initial examination of the sprout lengths in treatments where sprouting had taken place indicated that the range of sprout lengths was similar to that found in the preliminary study, i.e. groups of low measurements and high measurements within one treatment box. Linear regression is therefore not an appropriate technique to describe the relationships between sprouting and irradiation dose because, as can be seen in the histograms 2.3 to 2.6 prepared for potatoes in the preliminary study, the lengths of sprouts within a treatment box are not distributed normally. In fact the mean of the length of the longest sprout on tubers in such samples is meaningless in the context of such a distribution of sprout lengths as it does not adequately describe the degree of sprouting in sprouting tubers or non-sprouting tubers. However, if the sprout measurements are categorised into two groups, tubers sprouting and tubers not sprouting as defined in section 2.2.3, a more valid statistical model can be fitted to the data from which the prediction of the proportion of tubers that will

sprout at a given irradiation dose can be made.

The logistic regression model

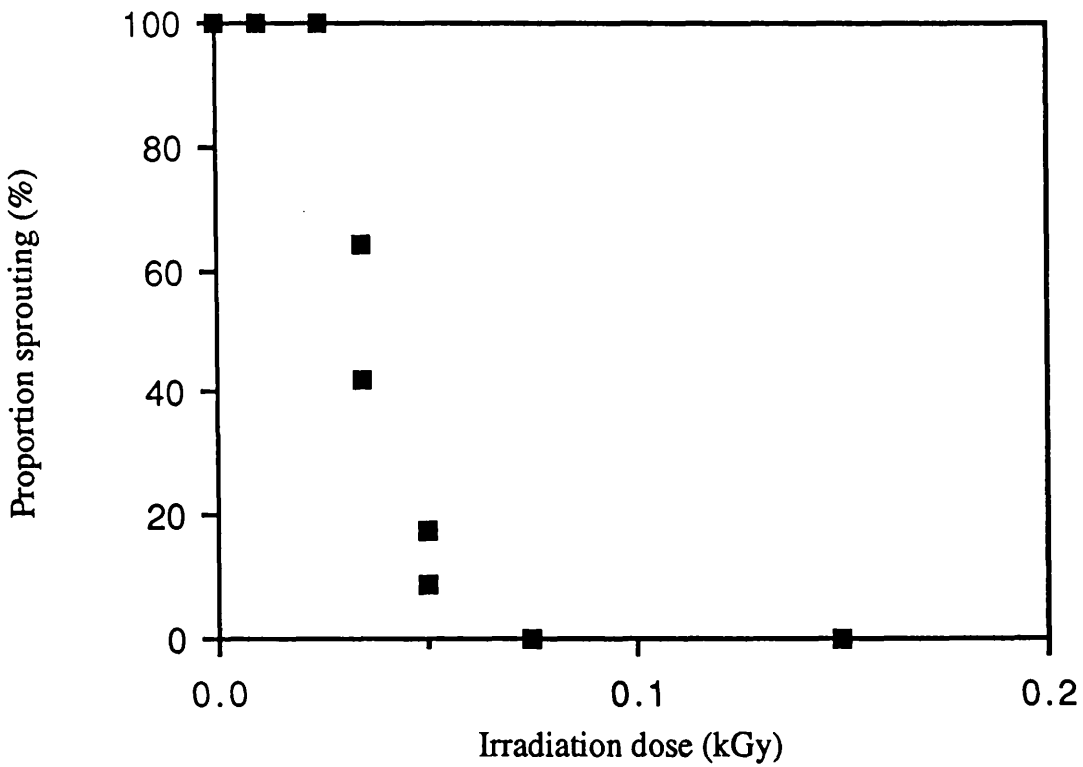
On the advice of Dr. T. Aitchison of the Statistics department of Glasgow University it was decided to use a logistic regression model to describe the relationship between sprouting and irradiation dose for each cultivar. Logistic regression was chosen because logistic regression models grow to a stable maximum or decay to a stable minimum level. The latter description fits the data obtained here, the proportion of tubers sprouting decreases with increasing irradiation dose to a stable level of zero as can be seen from graphs 2.7 and 2.8.

Graph 2.7. The effect of irradiation dose on the proportion of potatoes sprouting*. Cv. Record potatoes stored at 8°C for 96 days.



* each point represents a 45 tuber treatment.

Graph 2.8. The effect of irradiation dose on the proportion of potatoes sprouting*. Cv. Desiree potatoes stored at 8°C for 96 days.



* each point represents a 45 tuber treatment.

The logistic model has the general form:

$$y = \frac{e^{a+bx}}{1+e^{a+bx}}$$

(equation 2.1)

Logistic regression is applied to derive a model relationship from a set of data when one of the parameters measured can have only two possible outcomes, in this case where potatoes either sprout or do not sprout. In order to carry out a logistic regression the data was first transformed by calculating the logit of the sprouting data for each box. The logit is defined as the logarithm of the odds of an event occurring, that is:

$$\text{logit} = \log_e \frac{\text{Probability of success}}{\text{Probability of failure}}$$

In this specific case the logit could have been calculated thus:

$$\text{logit} = \log_e \frac{\text{Number of tubers successfully sprouting}}{\text{Number of tubers not sprouting}}$$

However, in order to make the final model more convenient to use the following transformation was used:

$$\text{logit} = \log_e \frac{\text{Number of tubers successfully sprouting}}{\text{Total number of tubers in a treatment}}$$

This operation has the advantage of describing the relationship between dose and proportion sprouting, rather than between dose and proportion not sprouting as the first operation does.

By transforming equation 2.1 it can be rewritten as:

$$y = a + bx$$

(equation 2.2)

where y is the logit of the proportion of tubers sprouting and x is the irradiation dose. The data has therefore been transformed into the form of the equation of a straight line on which regression can be performed.

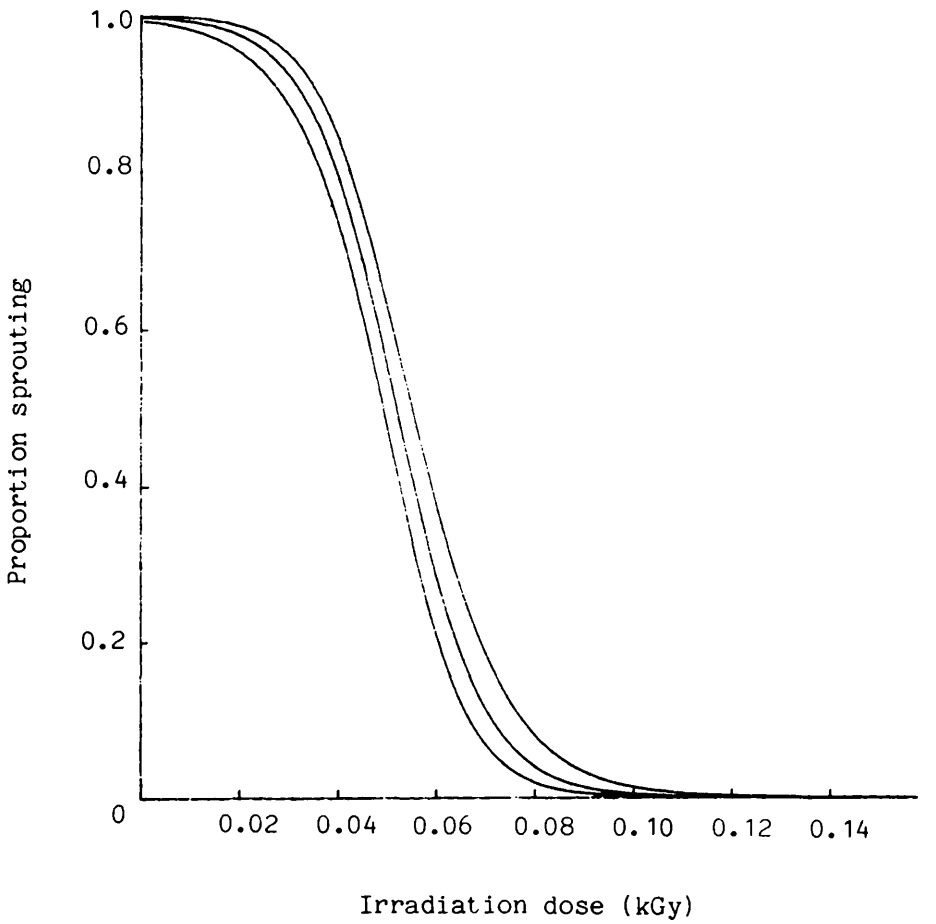
Inferences from logistic regression

The data was transformed as described above and a logistic regression of dose with the logit of sprouting was carried out for each cultivar. The equations of the model relationships are presented in table 2.3. The curves are plotted with 95% confidence intervals in graphs 2.9 and 2.10.

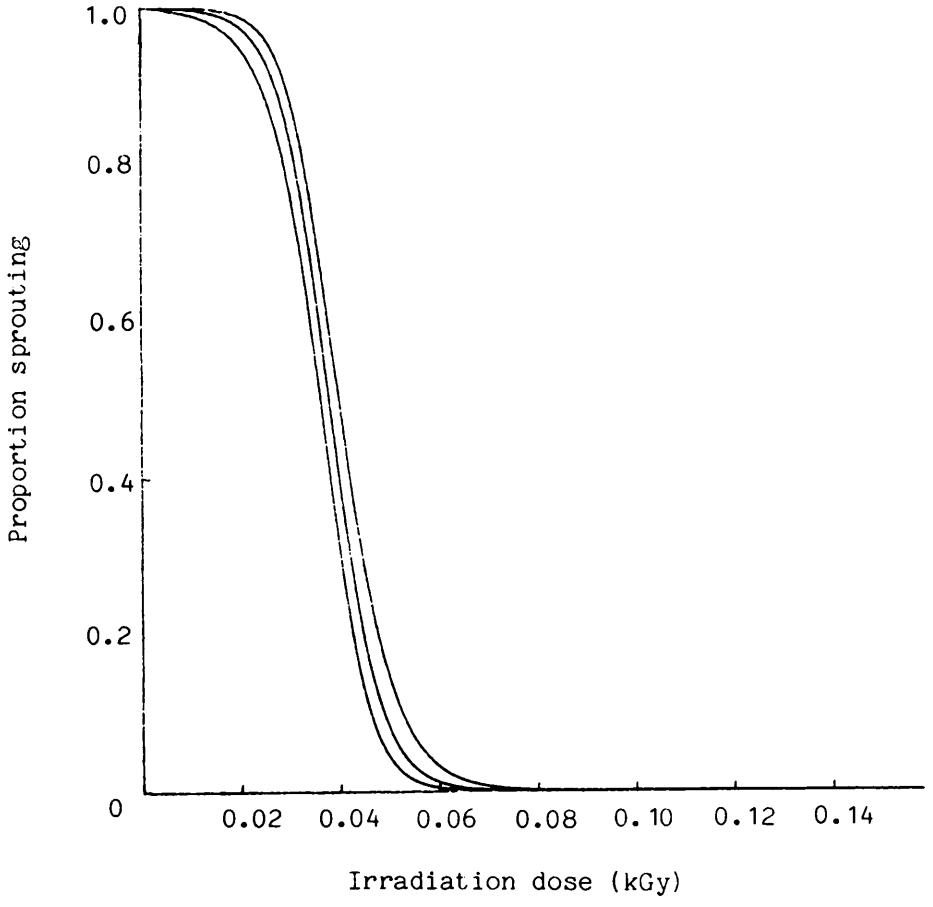
Table 2.3. Logistic regression coefficients and standard errors (s.e.) for the relationship between irradiation dose and the logit of the proportion of tubers sprouting.

Cultivar	a (y-intercept)	s.e.	b (slope)	s.e.
Record	5.92	0.502	-1.14	0.103
Desiree	7.74	0.764	-2.03	0.205

Graph 2.9. Logistic regression curve and 95% confidence intervals describing the relationship between the proportion of potatoes sprouting and irradiation dose. Cv. Record potatoes stored at $8^{o}C$ for 96 days.



Graph 2.10. Logistic regression curve and 95% confidence intervals describing the relationship between the proportion of potatoes sprouting and irradiation dose. Cv. Desiree potatoes stored at $8^{o}C$ for 96 days.



For an experiment with the appropriate number of degrees of freedom a ratio of a to its standard error of greater than 1.96 or less than -1.96 indicates that the intercept of the regression line with the y-axis (proportion sprouting) is significantly different from zero. Similarly if the ratio of b to its standard error is greater than 1.96 or less than -1.96 the slope of the regression line is significantly different from zero, indicating that the proportion sprouting is influenced by increasing the irradiation dose. From table 2.3 it can be seen that both parameters in each cultivar were significantly different from zero. This shows that there is a relationship between proportion of tubers sprouting and irradiation dose.

Chi-square goodness-of-fit tests were carried out to test if the relationships between proportion sprouting and irradiation dose are adequately described by the equations derived from logistic regression. Grouping low values of observed and expected proportion sprouting together, Chi-square values of 17.02 and 16.38 were calculated for cv. Record and cv. Desiree respectively. The tabulated critical Chi-square value for the appropriate number of degrees of freedom at the 5% significance level is in this case 21.03. The smaller Chi-square values calculated from the sprout data indicate that the models derived by logistic regression adequately describe the relationships between the proportion of tubers sprouting and irradiation dose.

The influence of cultivar on the proportion of tubers sprouting was investigated by calculating the differences between the regression parameters of the two cultivars as shown in table 2.4. The parameters are subscripted $_R$ to indicate cv. Record and $_D$ to indicate cv. Desiree.

Table 2.4. Differences in logistic regression parameters between cultivars Desiree$_{(D)}$ and Record $_{(R)}$.

$a_D - a_R$	$s.e._D - s.e._R$	$b_D - b_R$	$s.e._D - s.e._R$
1.82	0.261	-0.89	0.103

a is the y-intercept, b the slope and s.e. the standard error.

When the differences between the a and b parameters of the two cultivars were divided by their respective differences in standard error the results were both greater than 1.96 or less than -1.96. This indicates that there is a significant effect of cultivar on the proportion of tubers sprouting.

By comparing graphs 2.9 and 2.10 it can be seen that the slope of the regression relationship for cv. Desiree is steeper than that for cv. Record. This can be illustrated by reference to table 2.3 in which it is indicated that the slope coefficient b of the cv. Desiree regression is larger, and hence the slope of the line steeper, than that of cv. Record. In addition the curve describing the relationship in cv. Record potatoes is displaced further along the x-axis, complete sprout inhibition is reached at a higher irradiation dose. This is confirmed by the significant differences found between the regression constants in the regression equations of the two cultivars in table 2.4. These differences show that, if the proportion of tubers sprouting is used as the criterion, the sprouting of cv. Desiree potatoes is controlled by a lower irradiation dose than the sprouting of cv. Record potatoes. This implies that cv. Desiree is less resistant to the effect of irradiation on sprouting than is cv. Record.

The irradiation dose required to completely inhibit sprouting has been shown to be dependent on the cultivar to be irradiated and will probably also depend on other factors such as the previous growing and storage conditions of the potatoes. From the results obtained from this particular study for cv. Desiree a dose of 0.07 - 0.08 kGy should be sufficient for complete sprout inhibition, in the case of cv. Record the dose required would be 0.10 - 0.12 kGy. This dose range, it must be remembered, is based on sprouting data from the first sprout assessment in this study and thus represents sprout growth until the end of March. The interpretation of these results should be tempered by reference to the results of the second sprout assessment.

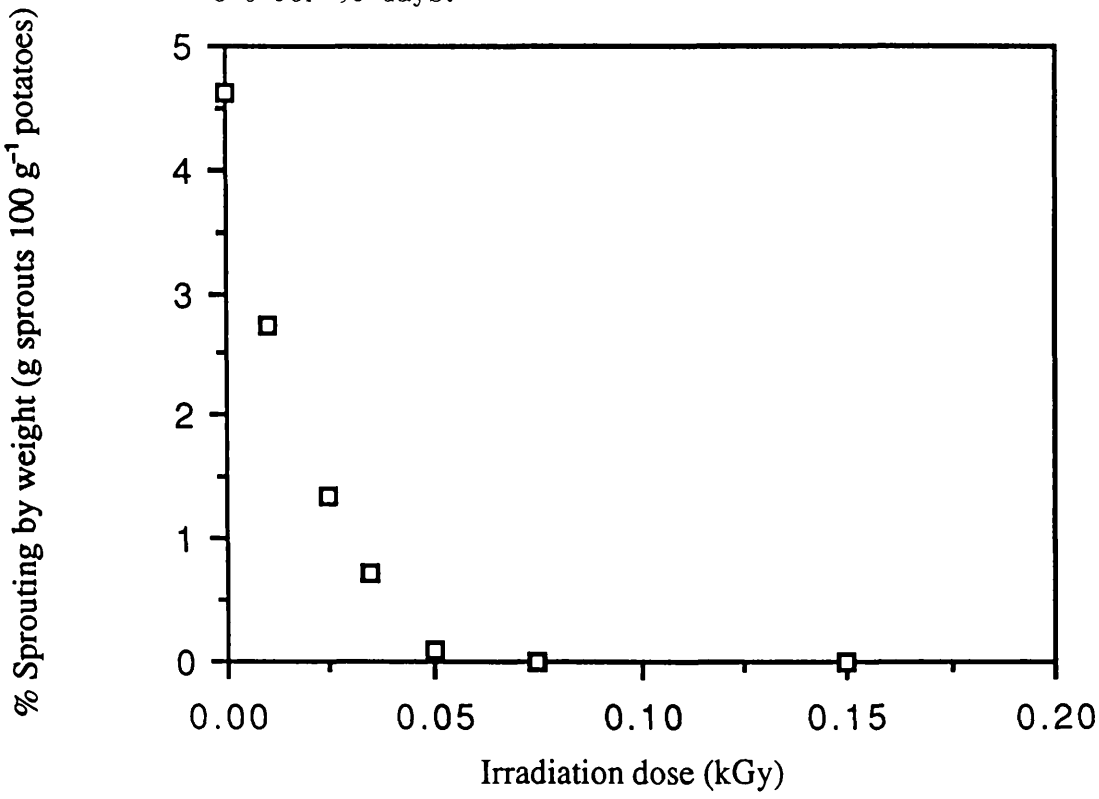
2.3.3.2 Percentage sprouting by weight

Linearisation of percentage sprouting by weight data

Plots of the percentage sprouting by weight against irradiation dose received showed that the relationship between them is non-linear as can be seen, for example, in graph 2.11. No sprouting was observed on March 27 at doses of 0.075 kGy or greater in either cultivar. By July 2 a small degree of sprouting had taken place at 0.075 kGy in both cultivars but in the 0.10 and 0.15 kGy treatments sprouting was still completely inhibited.

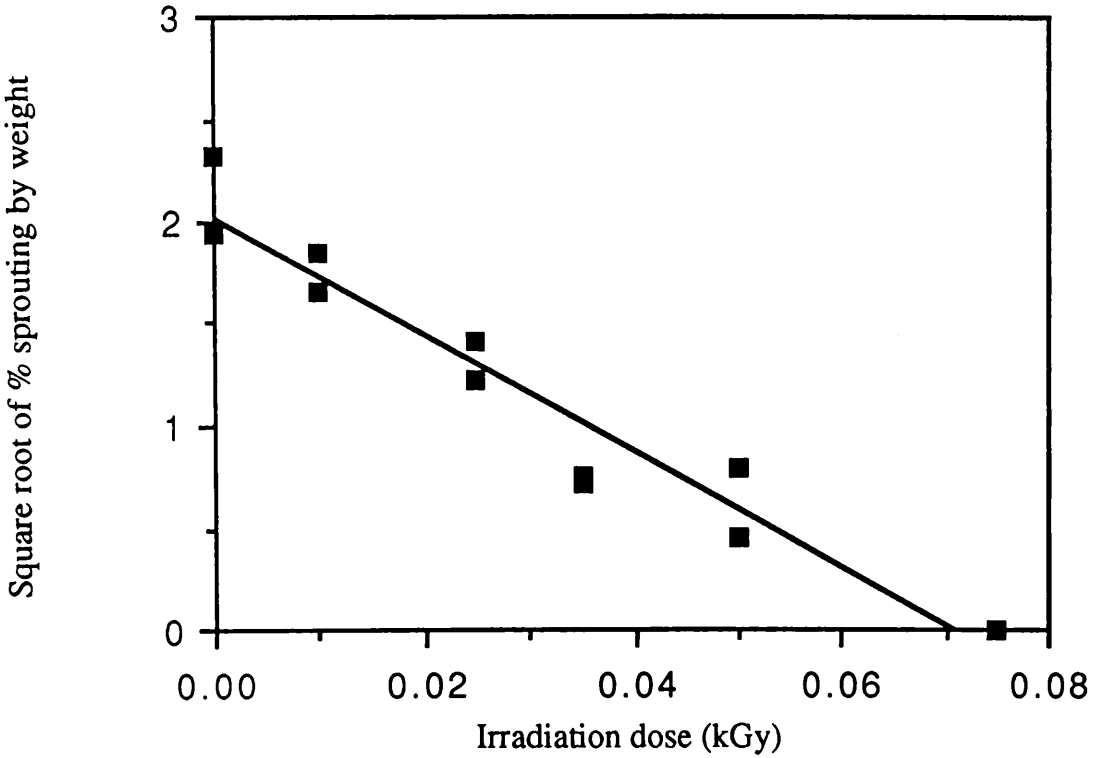
To simplify the interpretation of the relationships between the degree of sprouting and irradiation dose the data was transformed to give straight line relationships. Several transformations were investigated, the best results being achieved by plotting the square root of the percentage sprouting by weight against irradiation dose. Those plots are presented as graphs 2.12 to 2.15.

Graph 2.11. The effect of irradiation dose on the percentage sprouting by weight* of cv. Desiree potatoes stored at 8°C for 96 days.



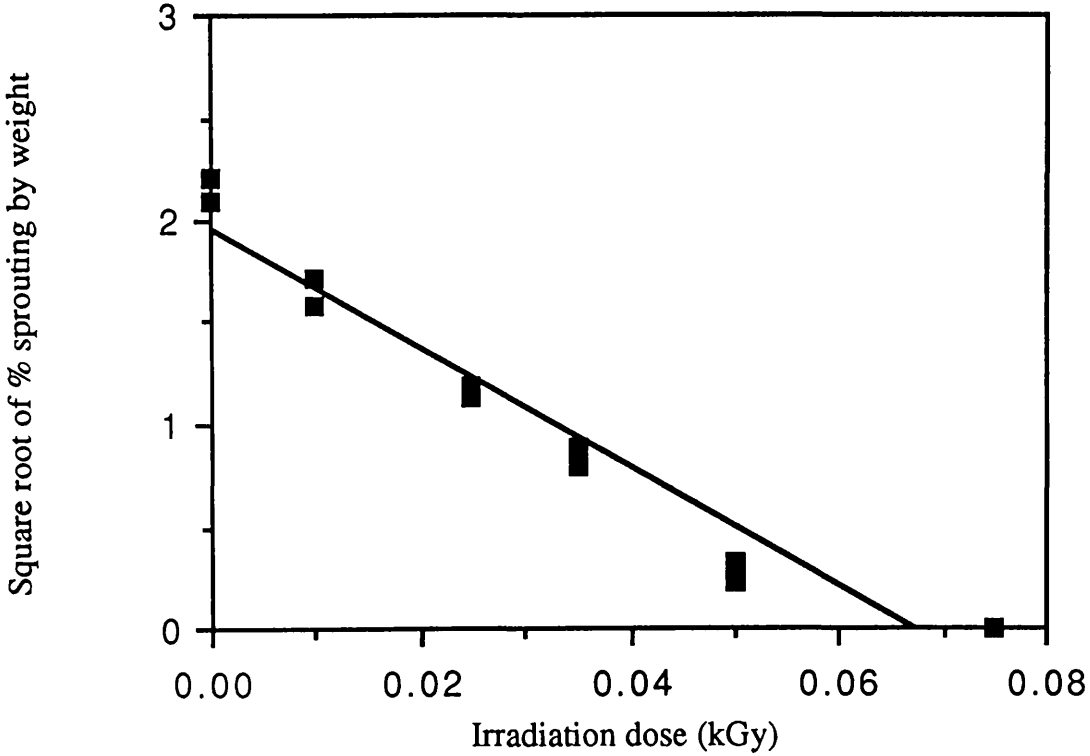
* each point represents a 45 tuber treatment.

Graph 2.12. The relationship between irradiation dose and the square root of the percentage sprouting by weight of cv. Record potatoes after 96 days of storage at 8°C.



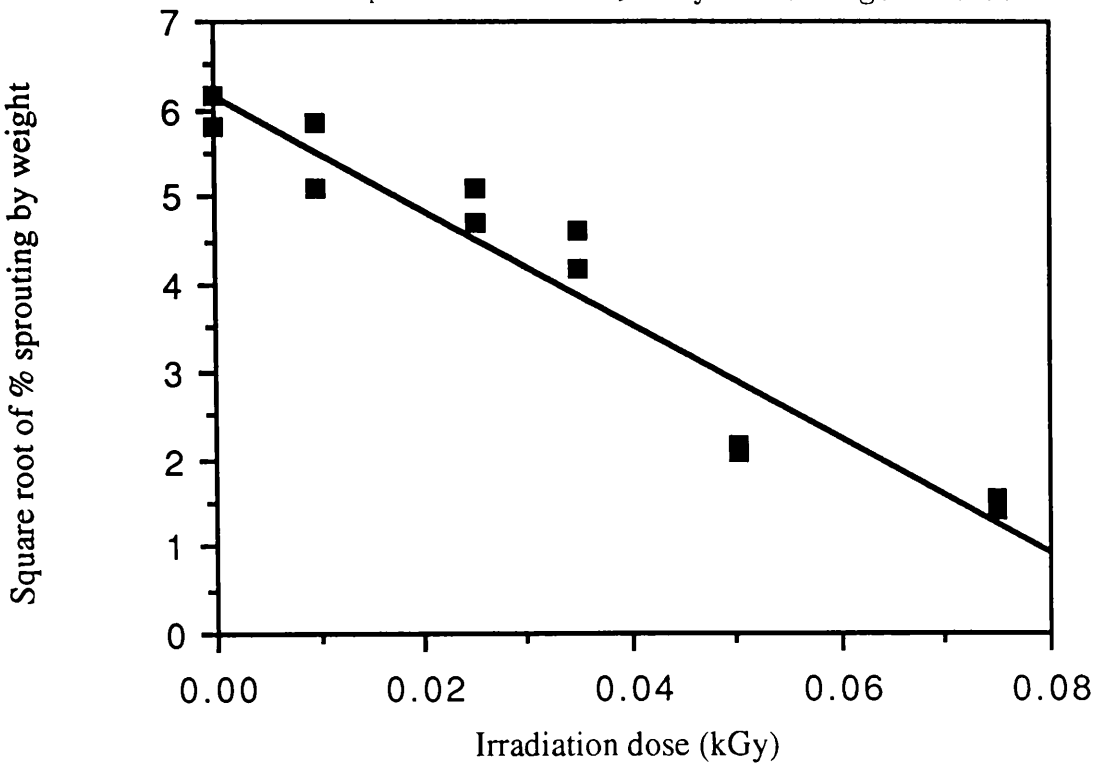
* each point represents a 45 tuber treatment.

Graph 2.13. The relationship between irradiation dose and the square root of the percentage sprouting by weight of cv. Desiree potatoes after 96 days of storage at $8^{o}C$.



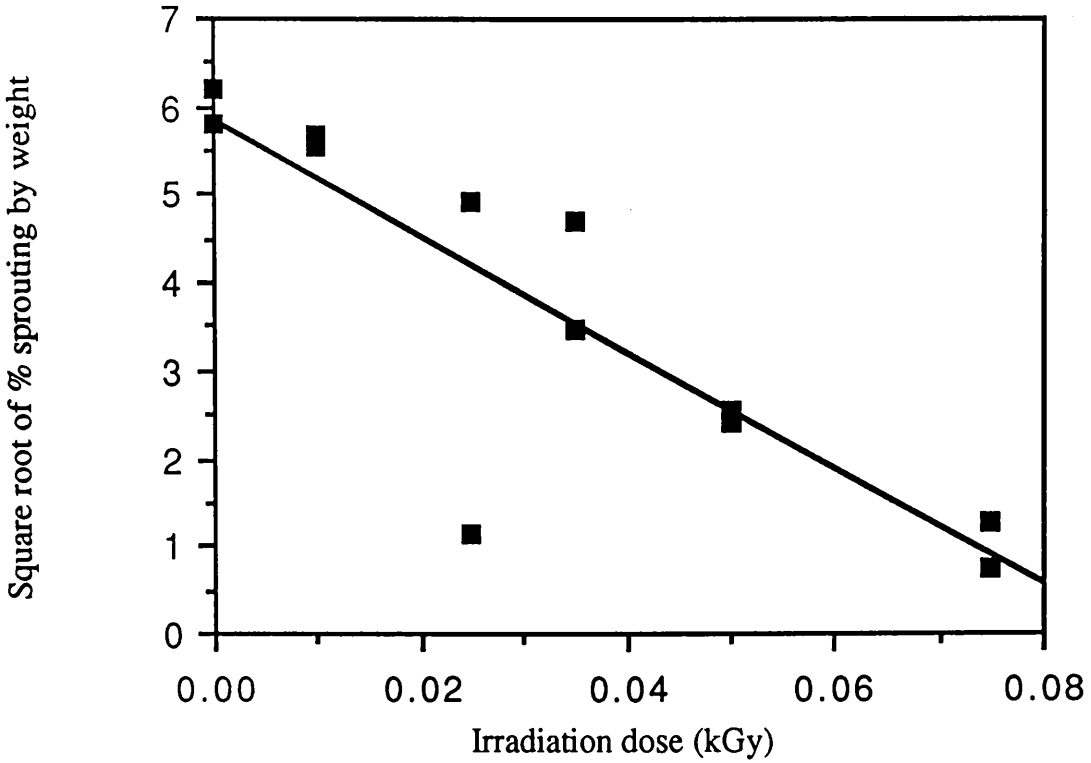
* each point represents a 45 tuber treatment.

Graph 2.14. The relationship between irradiation dose and the square root of the percentage sprouting by weight of cv. Record potatoes after 193 days of storage at 8°C.



* each point represents a 45 tuber treatment.

Graph 2.15. The relationship between irradiation dose and the square root of the percentage sprouting by weight of cv. Desiree potatoes after 193 days of storage at 8°C.



* each point represents a 45 tuber treatment.

Inferences from the regression of percentage sprouting by weight data

Linear regressions of the square root of percentage sprouting by weight and irradiation dose were carried out and the regression information obtained is presented in table 2.5. As irradiation doses of 0.1 kGy or greater completely inhibited sprouting only the data from irradiation treatments at doses lower than 0.1 kGy were regressed.

Table 2.5. Regression parameters for the relationships between irradiation dose and the square root of percentage sprouting by weight.

(Square root of % sprouting by weight = a + b * Irradiation dose).

Cultivar	Date	a (y-intercept)	b (slope)	t	t_{tab}
Desiree	March 29	2.09	-0.36	-27.16	2.31
	July 2	5.86	-0.66	-5.25	2.23
	[a]July 2	6.25	-0.70	-14.14	2.26
Record	March 29	2.08	-0.32	-8.83	2.31
	July 2	6.18	-0.65	-10.76	2.23

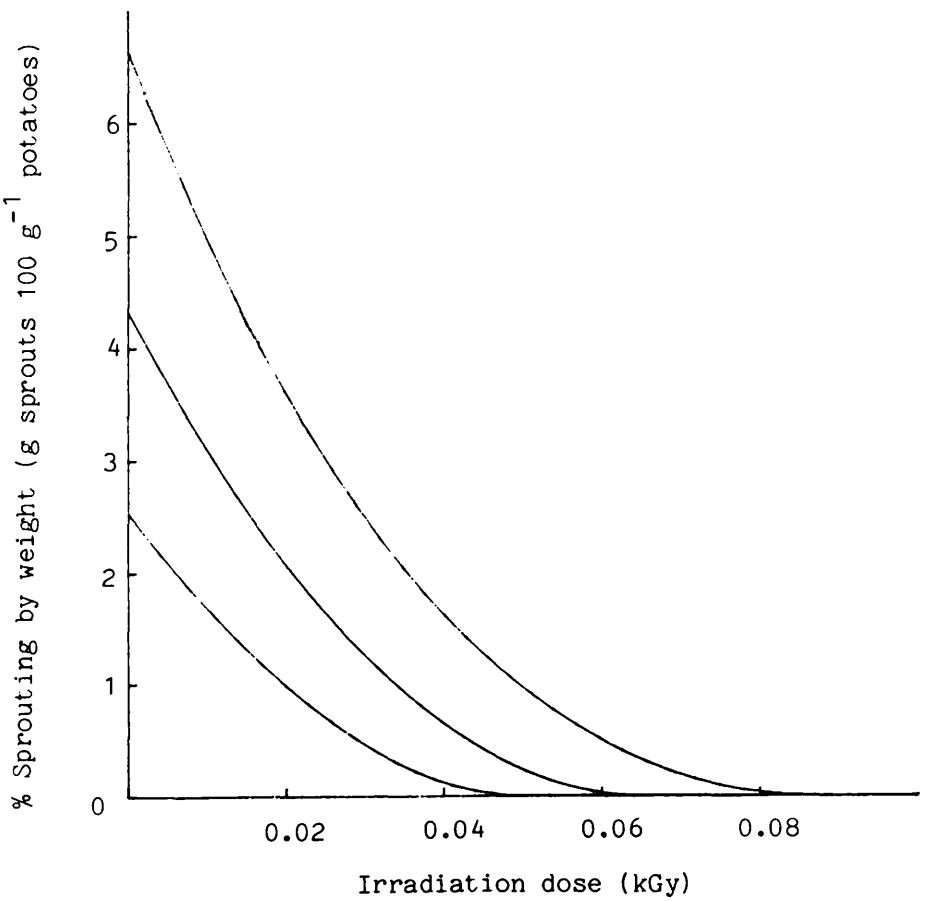
[a]regression parameters after removal of outlying point (see below).

t is the calculated t-ratio of the slope b and t_{tab} is the tabulated t-ratio for the appropriate number of degrees of freedom at the 5% significance level.

For convenience the percentage sprouting by weight values predicted by the regression equations were plotted against irradiation dose with 95% confidence intervals in graphs 2.16 to 2.19.

Graph 2.16.

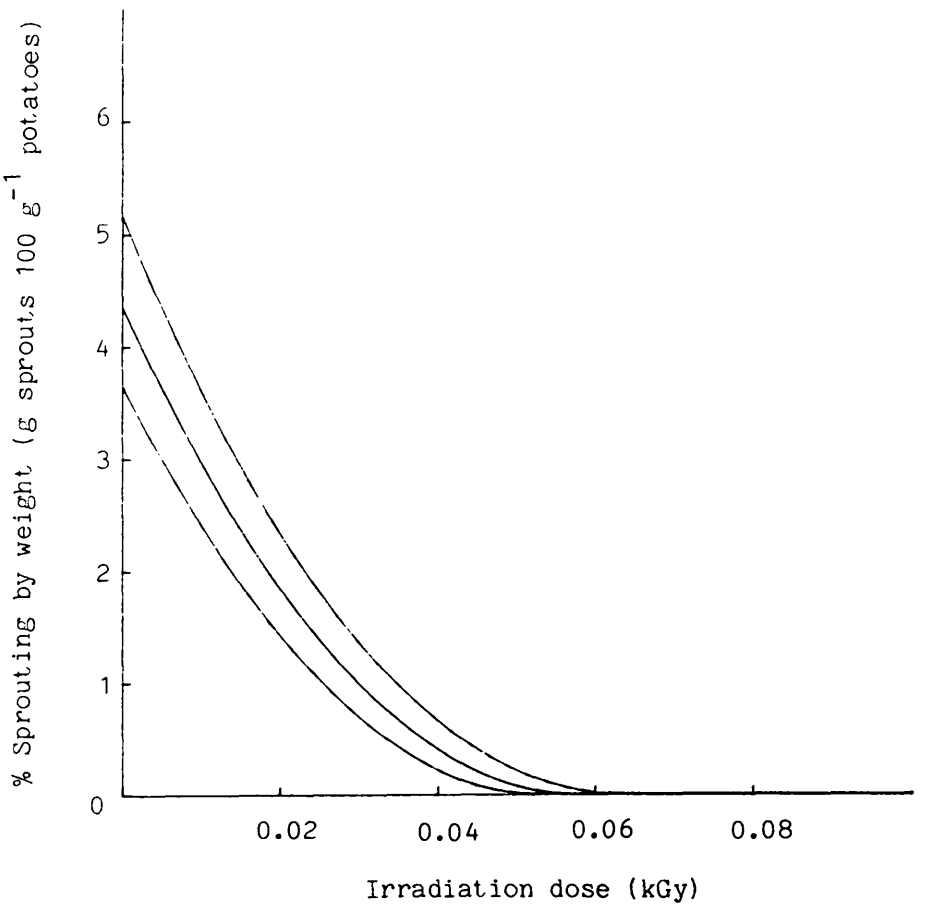
The relationship between irradiation dose and percentage sprouting by weight of cv. Record potatoes after 96 days storage at 8°C. Relationship derived by regressing irradiation dose with the square root of percentage sprouting by weight.



Outer lines represent 95% confidence intervals.

Graph 2.17.

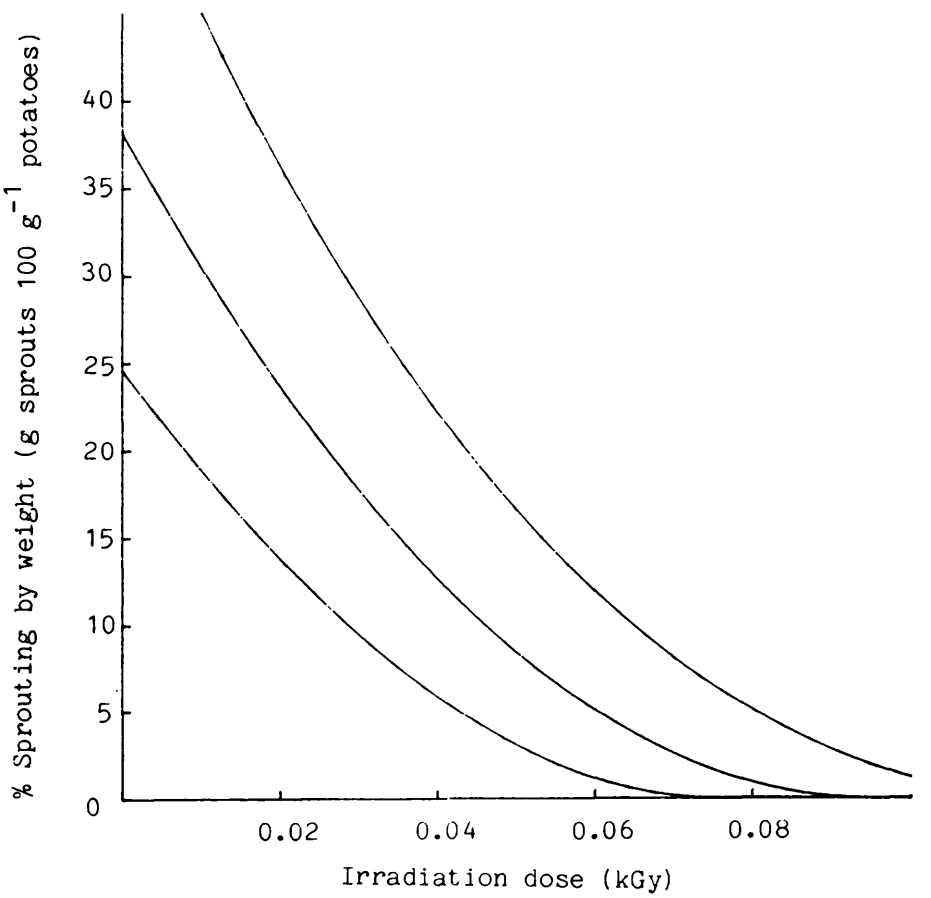
The relationship between irradiation dose and percentage sprouting by weight of cv. Desiree potatoes after 96 days storage at 8°C. Relationship derived by regressing irradiation dose with the square root of percentage sprouting by weight.



Outer lines represent 95% confidence intervals.

Graph 2.18.

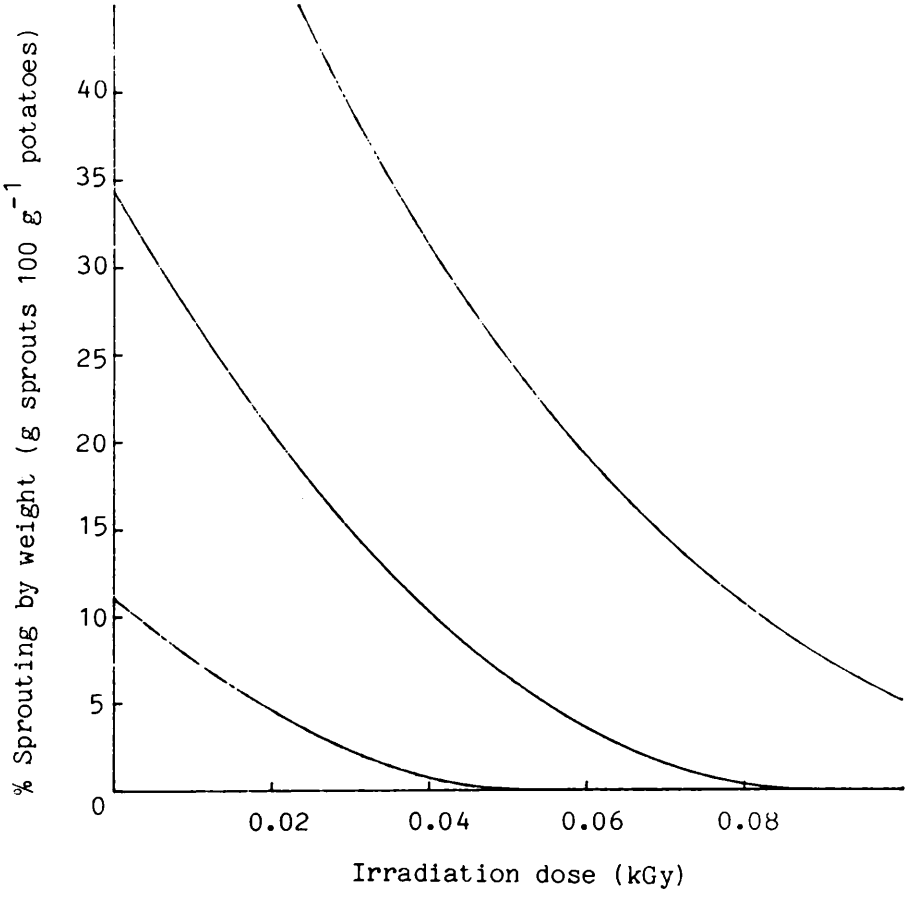
The relationship between irradiation dose and percentage sprouting by weight of cv. Record potatoes after 193 days storage at 8°C. Relationship derived by regressing irradiation dose with the square root of percentage sprouting by weight.



Outer lines represent 95% confidence intervals.

Graph 2.19.

The relationship between irradiation dose and percentage sprouting by weight of cv. Desiree potatoes after 193 days storage at $8^{o}C$. Relationship derived by regressing irradiation dose with the square root of percentage sprouting by weight.



Outer lines represent 95% confidence intervals.

From table 2.5 it can be seen that the calculated values of the t-ratios of the slope parameters of all five lines greatly exceed the tabulated values. This indicates that the slope b is significantly different from zero, i.e. that irradiation dose is linked to the square root of percentage sprouting by weight in all cases.

Correlations were carried out to test the goodness-of-fit of the regression lines and the results are contained in table 2.6.

Table 2.6. Correlation parameters for the relationships between irradiation dose and the square root of percentage sprouting by weight.

Cultivar	Date	R^2	r	r_{tab}	Degrees of freedom
Desiree	March 29	98.9	0.99	0.63	8
	July 2	73.4	0.86	0.58	10
	[a]July 2	95.7	0.98	0.60	9
Record	March 29	90.7	0.95	0.63	8
	July 2	92.0	0.96	0.58	10

[a] correlation parameters after removal of outlying point.

R^2 is the coefficient of determination expressed as a percentage.

r is the calculated linear correlation coefficient.

r_{tab} is the tabulated linear correlation coefficient at the 5% significance level.

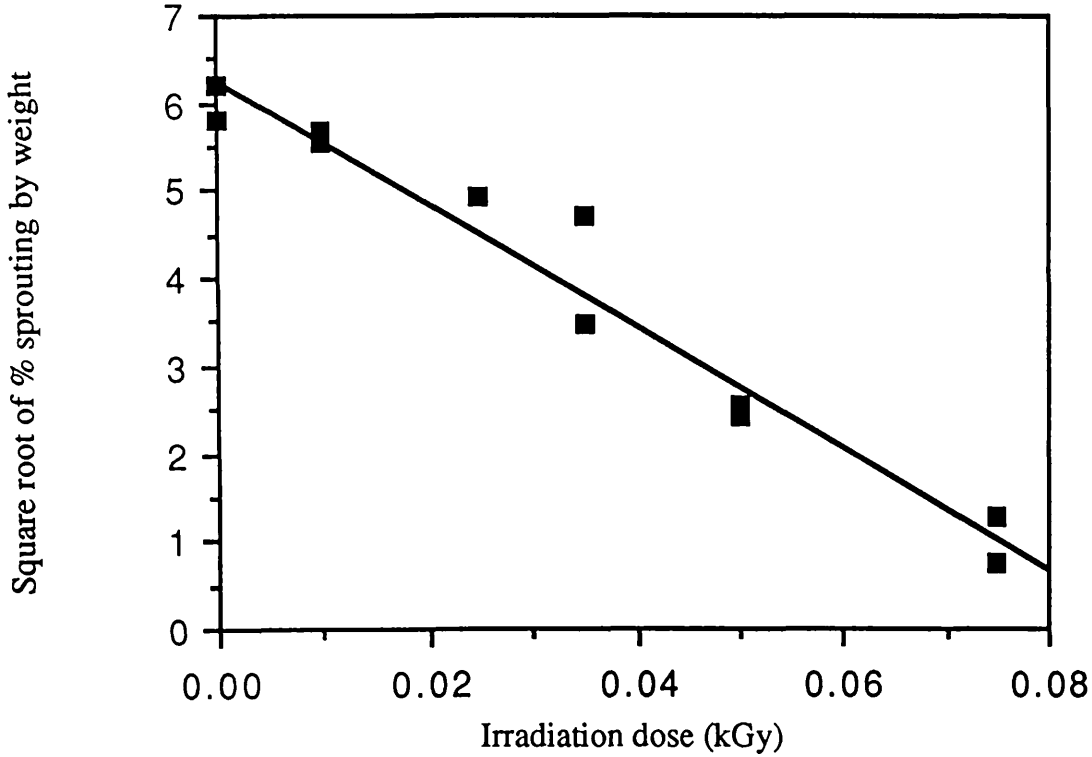
From table 2.6 it can be seen from the r values that the linear correlation coefficient is significant in all cases indicating a strong relationship between the square root of percentage sprouting by

weight and irradiation dose. From the R^2 values it can be seen that a large proportion of the variability (> 90% with the exception of one case (see below)) in percentage sprouting can be accounted for by its relationship with irradiation dose.

In graph 2.15 of the percentage sprouting by weight at the second cv. Desiree measurement on July 2 the percentage sprouting values calculated for the pair of replicate boxes irradiated at 0.025 kGy were much more variable than for the other pairs of replicates measured on that date. This resulted in very wide confidence intervals for the regression relationship for that set of data. Examination of the individual data points showed that this was due to the influence of only one of the replicate boxes at that irradiation dose. This data point was excluded and the data replotted as graph 2.20.

The regression line obtained by excluding the outlier has the equation given at [a] in table 2.5 and correlation parameters given at [a] in table 2.6. Comparison of this equation with the table 2.5 regression information shows that the outlying point has a very marked influence on the position of the regression line and its exclusion also narrows the 95% confidence intervals considerably as is shown in graph 2.21.

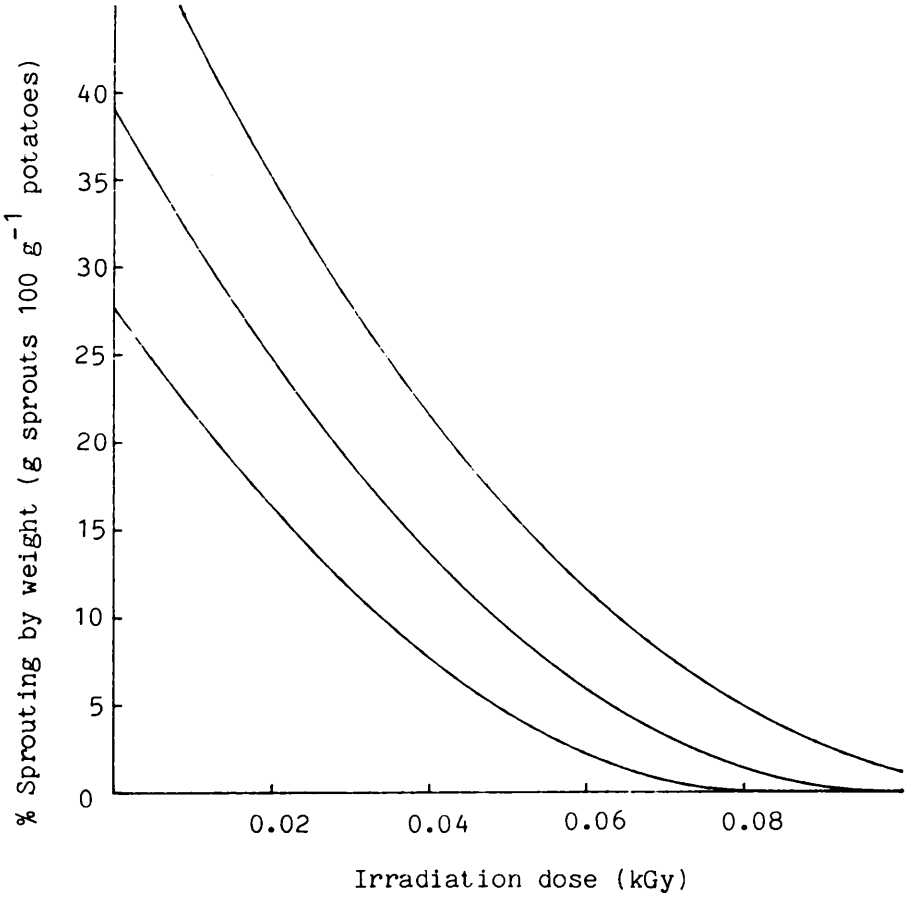
Graph 2.20. The relationship between irradiation dose and the square root of the percentage sprouting by weight of cv. Desiree potatoes after 193 days of storage at 8°C after exclusion of outlying replicate.



* each point represents a 45 tuber treatment.

Graph 2.21.

The relationship between irradiation dose and percentage sprouting by weight of cv. Desiree potatoes after 193 days storage at $8^{o}C$. Relationship derived by regressing irradiation dose with the square root of percentage sprouting by weight after exclusion of outlying replicate.



Outer lines represent 95% confidence intervals.

It is also worth noting the improvement of the R^2 value and thus the goodness-of-fit found when the outlying point is excluded from the July 2 regression of cv. Desiree. The exclusion of that data point would therefore seem well justified although the reason for its deviation from the regression line is unclear (one possible reason for this effect may be that the storage conditions at the position occupied by that replicate box within the store were different from those in the rest of the store). The corrected regression equation for that relationship was therefore used in preference to the initial equation. The final regression equations can therefore be said to be adequate models from which to predict irradiation doses required for subsequent experiments.

The effect of cultivar on percentage sprouting by weight in irradiated tubers was investigated by testing the homogeneity of the regression slopes. This t-test information is summarised in table 2.7.

Table 2.7. T-values from tests of homogeneity of the slopes of regression lines relating percentage sprouting by weight and irradiation dose.

Comparison	t calculated	$t_{0.05}$ tabulated
cv. Record March 29 and cv. Desiree March 29	1.14	2.12
cv. Record July 2 and cv. Desiree July 2	0.20	2.09
cv. Record March 29 and cv. Record July 2	43.38	2.10
cv. Desiree March 29 and cv. Desiree July 2	5.02	2.11

The slopes of the regression equations of the two cultivars were t-tested at the 5% significance level for both dates of measurement. No significant difference was found between the slopes of the two cultivars at either date indicating that in this experiment cultivar had no significant effect on percentage sprouting by weight. These results are in contrast to those of section 2.3.3.1 in which it was found that the proportion of tubers sprouting was smaller at a given irradiation dose in the case of cv. Desiree samples than it was in the case of cv. Record samples.

There are several possible explanations of why the two models differ on whether cultivar effects are important. Firstly the precision of the models limits such comparisons, the limitations of the models are more fully discussed in section 2.3.4 but include the

size of samples and the timing of measurements of sprouting. As a result of differences in the way in which the data was collected one model may be precise enough to detect a significant cultivar effect that another model may not.

Secondly the measurements made for the two types of models used, sprouting >= 2 mm and percentage sprouting by weight, may measure different aspects of sprouting and thus are not truely comparable. Proportion sprouting only measures whether a tuber is sprouting or not by the length of its longest sprout, percentage sprouting by weight takes account of the mass of the sprout i.e. both thickness and length of all sprouts in relation to the mass of the tuber. Therefore one model suggests that the proportion of tubers sprouting is cultivar dependent while the second model indicates that the percentage sprouting by weight is not. These are not necessarily contradictory views.

A comparison of the slopes of the two regression equations of cv. Record showed that the slope of the regression line derived from the July 2 data was significantly greater at the 5% level than that derived from the March 29 data. The same significant difference was found in cv. Desiree. These differences were to be expected since in the time between measurements sprout growth in tubers where sprouting was unaffected by irradiation would have taken place. Hence to limit percentage sprouting by weight to a particular level by July 2 requires a higher dose than until March 29.

2.3.4 Conclusions

The use of sprout-dose regression models for prediction

The development of regression models in this chapter has shown that data obtained by the measurement of the proportion of tubers sprouting and percentage sprouting by weight can be used to predict effective irradiation doses for the control of sprouting.

The prediction of effective irradiation doses using the logistic regression models can be illustrated by fitting data into the model equation for each cultivar. A value of proportion of tubers sprouting equal to 0.01 may be chosen, i.e. one tuber in a hundred will sprout, as it is a proportion which for practical purposes can be said to represent complete sprout inhibition. Irradiation doses of 0.061 kGy for cv. Desiree and 0.092 kGy for cv. Record would, the model predicts, lead to that proportion of tubers sprouting under the conditions used in this study.

Using the proportion of tubers sprouting to develop model relationships has the advantage that it is possible to simply categorise the stored potatoes into two groups, those sprouting and those not sprouting. If the cut-off level of 2 mm was to be used to determine which category a tuber belonged to, sprout assessments would be speeded up considerably as fewer tubers would require measurement. However, the cut-off level for categorisation could be set at a different level according to requirements.

Potato processors may alternatively wish to use the percentage sprouting by weight as the method of sprout assessment as it is a measure of sprouting with which they are more familiar and for which

data exists from previous studies with which comparisons can be made. If a level of sprouting acceptable to processors is chosen, e.g. 1% sprouting by weight, the irradiation doses calculated from the regression equations which would result in sprouting developing to that level by March 29 are 0.038 kGy and 0.033 kGy for cultivars Record and Desiree respectively. To keep sprouting to that level by July 2 doses of 0.080 kGy and 0.075 kGy would be required for the same respective cultivars. Similar predictions were used to choose effective doses for subsequent experiments.

Comparison of the regression models derived from the percentage sprouting by weight data with those derived from data describing the proportion of tubers sprouting should only be attempted with some care. The reason for such caution is that although both measurements describe the way in which increasing the irradiation dose affects sprouting they describe that effect in different ways.

For example if a particular dose of radiation inhibits sprouting in 99% of tubers this provides no information on how great the sprouting is within the remaining 1%. Similarly, if a given irradiation dose results in a percentage sprouting by weight of 1%, this does not describe how that weight of sprouts is distributed within the sample. Thus the two models can provide complementary information which when combined can give a more complete understanding of the effect of radiation on sprouting.

Restrictions on the use of regression models

There are, however, limitations to the application of the models developed in this study. Firstly only one storage season and two cultivars were studied. This limits the validity of the models derived from such data. The inclusion in the models of data from several growing seasons, as growing conditions introduce seasonal variation, and several locations would widen the applicability of the models. Experiments carried out on a potato store scale rather than the box storage scale carried out here would provide data from which models of sprouting in irradiated potatoes in full scale potato stores could be derived. Other factors such as the timing of irradiation treatment and the influence of the individual irradiation plant would also be of practical interest. One further limitation on the logistic regression models derived in this study are that they are based on the level of sprouting in potatoes stored only until March. The duration of storage studied could be expanded in any subsequent experiments.

These problems can, however, be addressed by further experimentation. The principal of deriving regression models of the relationships between irradiation dose and the level of sprouting has been established by this study.

Further applications of logistic regression

The technique of logistic regression modelling may also be of use in describing the sprouting of potatoes treated with some chemical sprout suppressants. In box experiments where tubers were treated with Tecnazene differences in the concentration of Tecnazene in different parts of a sample due to the volatilisation of the chemical at the edges of the boxes have been shown to result in variability in

the levels of sprouting within a box. Complete control of the sprouting of tubers was found at the centre of boxes while tubers at the edges exhibit significant sprout growth (this effect is discussed in Chapter 3). Another example of variability in the level of sprouting in potato samples can be found in potatoes treated with Chlorpropham in large stores where uneven application of Chlorpropham may lead to differences in the level of control within different areas of the store. In both of these examples it may be appropriate to assess sprouting using the proportion of tubers sprouting as the basis of measurement. Logistic regression could then be employed to describe the relationships between the degree of sprouting and the rate of application of the sprout suppressant.

Chapter 3

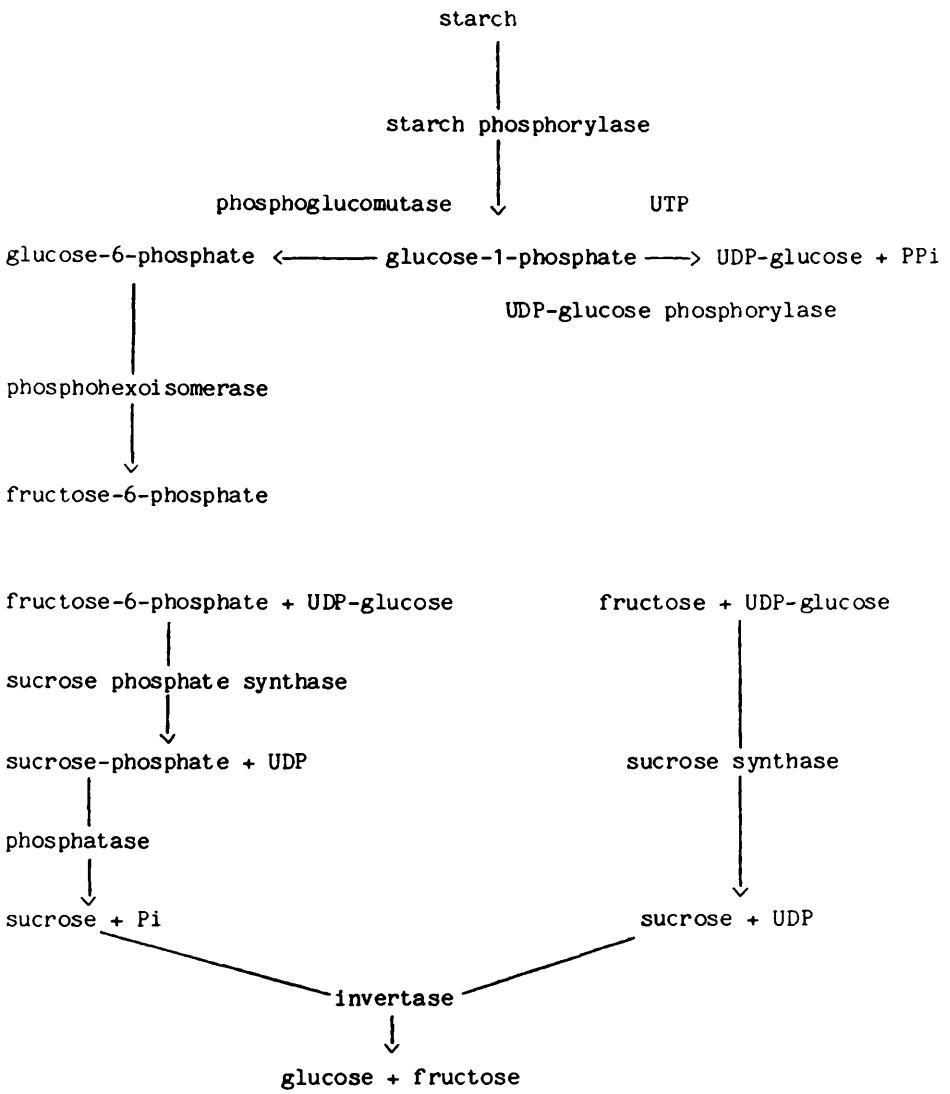
Sugar levels of irradiated and chemically sprout suppressed potatoes during storage

3.1 Introduction

Chapter 2 discussed the use of irradiation treatment as a means of controlling the sprouting of potatoes during storage. In this chapter another effect of irradiation treatment on potatoes is considered, the accumulation of free sugars. Free sugars are those sugars found in free solution within the tuber, that is to say not bound in an insoluble polysaccharide such as in starch or structural polysaccharides. In potatoes the free sugars which predominate are sucrose, glucose and fructose and their associated phosphorylated derivatives (Dalziel, 1978). The levels of phosphorylated sugars are normally very low in comparison to the levels of glucose, fructose and sucrose. They account for only a small error when included in measurements of sugar contents (Schwimmer et al., 1955; Dalziel, 1978).

Sugars are synthesized in the foliage of the potato during the growing season and translocated to the tubers in the form of sucrose. Sucrose is then converted into the storage polysaccharide starch. The interconversions of glucose, fructose and sucrose are made through phosphorylated derivatives as shown in diagram 3.1. A dynamic equilibrium exists between these three free sugars and starch although the vast majority of carbohydrate will be in the form of starch (Burton, 1966). Burton notes that in mature tubers stored at 10°C - 20°C approximately 98% of carbohydrate is in the form of starch.

Diagram 3.1. Sugar interconversions in potatoes.*



UTP = Uridine triphosphate

PPi = Inorganic pyrophosphate

UDP = Uridine diphosphate

Pi = Inorganic phosphate

* compiled from Burton (1966) and Iritani and Weller (1980).

The importance of free sugars in processing

Both fructose and glucose possess a reducing group while sucrose, bonded at the reducing end of both of its ring systems, does not. The combined glucose and fructose content is therefore often referred to as the reducing sugar content while sucrose is the major non-reducing sugar found in potatoes (Dalziel, 1978). This distinction is important as the reducing group is free to react during frying with other potato constituents. At normal crisp frying temperatures, approximately 200^{o}C, glucose and fructose can react quite readily. At higher temperatures (normally avoided by processors) of approximately 220^{o}C, sucrose can also react (Burton, 1966).

The interaction of reducing sugars and α-amino groups, present largely in the form of free amino acids, in the frying process is of particular interest and has been reviewed by Burton (1966). This interaction is an important initial step in a series of reactions known as the Maillard reaction, the final polymeric condensation products of which are dark coloured (Ellis, 1959). These dark coloured products discolour crisps or chips and make them unacceptable to the consumer (Hughes and Fuller, 1984; Sowokinos, 1978).

Burton (1966) suggests that the limiting factor in such discolouration reactions in potatoes will generally be the reducing sugar content as α-amino groups will normally be present in excess. Wunsch and Schaller (1972) whilst accepting the major role played in determining product colour by reducing sugars show that the content of free amino acids also contributes significantly to the final colour of fried potato products.

Since reducing sugars play a vital role in this unwanted process

they are measured at harvest in potatoes to be used for processed products. A range of between 0.1% and 0.3% reducing sugars (fresh weight) is regarded as acceptable (Wunsch and Schaller, 1972; Rumpf, 1973; Burton and Wilson, 1978), although levels of reducing sugars measured at harvest do not guarantee acceptable levels at the time of processing.

Factors affecting free sugar levels in potatoes

Several variables can affect the level of reducing sugars in potatoes including maturity, temperature of storage and cultivar (Burton, 1966). In immature tubers before harvest sucrose is still being translocated from the foliage to the tubers. Directly after harvest interconversion of that sucrose can lead to a subsequent increase in reducing sugars. Therefore sucrose is often also measured at harvest as an indicator of the degree of maturity in order to predict whether potatoes will be suitable for storage for processing (Sowokinos, 1978).

Potatoes of different cultivars may have very different reducing sugar levels. Burton (1965) observed a range of 0.06% to 0.45% of reducing sugars (fresh weight) in a study of 7 cultivars. Processors therefore favour specific cultivars known to have low reducing sugar contents. Cv. Record was chosen to be used in this study as it is a cultivar widely used for processing into crisps. It should, however, be noted that significant variation in reducing sugar levels has been observed even within samples of the same cultivar and crop (Burton, 1965).

The degree of variability in reducing sugar contents at harvest is

added to by the observation that the temperature of subsequent storage can have a marked effect on reducing sugar levels (Burton, 1965; Schippers, 1975). At storage temperatures between 2°C and 7°C reducing sugars can rise to unacceptable levels (Burton, 1965; Pollock and Ap Rees, 1975; Isherwood, 1976). Temperatures lower than 0°C are avoided as they result in chilling injury due to water freezing within potatoes. Thus although sprouting is inhibited at lower temperatures, in order to maintain acceptably low reducing sugar levels a higher storage temperature is normally set (Schippers, 1975; Burton and Wilson, 1978).

The accumulation of reducing sugars due to low temperature sweetening can under some circumstances be reversed by "reconditioning" sweetened potatoes. This is carried out by raising the storage temperature to approximately 20°C for a short time allowing the reducing sugar contents to fall, presumably due to an increased rate of respiration at the higher storage temperature (Burton and Wilson, 1978).

In contrast, storage of potatoes at temperatures of 10°C - 20°C result in little accumulation of sugars taking place during most of the storage period (Burton, 1965). The higher storage temperature, however, results in increased sprout growth. Therefore a compromise temperature of approximately 10°C is normally used to limit low temperature sweetening and reduce sprouting (Burton and Wilson, 1978). Even at 10°C sprout growth is considerable and a chemical sprout suppressant is considered necessary to inhibit sprouting (Burton, 1966).

Towards the end of storage a large increase in reducing sugars

takes place (Burton, 1965; Burton, 1975). This increase is irreversible and reconditioning merely accelerates the rise (Burton 1965). This effect, known as senescent sweetening, is thought to be due to a deterioration of cell membranes due to aging (Isherwood, 1976). A rise in sucrose levels has been observed prior to the reducing sugar rise found in association with senescent sweetening. The size of the sucrose increase has been shown to be greater at higher storage temperatures (Burton, 1965). Elevated sucrose levels have therefore been used as an indicator of oncoming senescence (Sowokinos, 1978).

The effect of irradiation on sugar levels

Several studies have shown that irradiation can increase the level of free sugars present in potatoes (Sparrow and Christensen, 1954; Schwimmer et al., 1957; Burton, et al. 1959; Kodenchery and Nair, 1972; Hayashi and Kawashima, 1982; Metlitsky et al., 1967). This effect has also been reviewed by Matsuyama and Umeda (1983) and Thomas (1984).

Immediately following irradiation treatment at sprout inhibiting doses there is a rapid rise in both the reducing and non-reducing sugar contents of potatoes (Burton, 1975). The increase in reducing sugars was found to be greater the later in the season that irradiation took place (Burton et al., 1959). The sweetening of irradiated tubers was also affected by factors known to affect sweetening in non-irradiated potatoes such as temperature and cultivar (Schwimmer et al., 1957; Burton and Hannan, 1957).

Sucrose levels also exhibit a considerable post-irradiation increase (Burton et al., 1959; Rumpf, 1973; Hayashi and Kawashima, 1982). A typical report of this effect showed that in cv. King Edward

irradiated at 0.1 kGy and stored at 10°C, sucrose rose to approximately 1.0% (fresh weight) by five days after treatment and then decreased to control level within a further three weeks (Burton, 1959).

The rise in sugar levels in irradiated tubers is thought to be linked to an increased activity of the enzyme phosphorylase induced by irradiation. Phosphorylase in potatoes is responsible for the degradation of starch, via phosphorylated intermediates, into glucose and ultimately to sucrose (Schwimmer et al., 1958; Kodenchery and Nair, 1972). Schwimmer et al., 1958) suggest that the increased enzyme activity may be a result of the rate of the synthesis of phosphorylase exceeding the rate of its degradation, resulting in a net increase in phosphorylase present and thus an increase in its activity.

Hayashi and Aoki (1985) postulate an important role in the sweetening process for sucrose phosphate synthase, which catalyses the combination of UDP-glucose and fructose-6-phosphate in the synthesis of sucrose-6-phosphate. In the above radiolabelling study increased uptake of labelled glucose into sucrose by the sucrose phosphate synthase route was observed. This implies that starch is being broken down to glucose-1-phosphate by phosphorylase and is then converted into sucrose by the sucrose phosphate synthase pathway.

In the same study Hayashi and Aoki support Schwimmer's mechanism to explain how enzyme activities increase in response to irradiation. Cycloheximide treated irradiated potato tissue was found not to convert labelled glucose into sucrose as readily as irradiated tissue not treated with cycloheximide. Since cycloheximide is known to

inhibit ribosome functions this effect is evidence for the involvement of protein synthesis in irradiation induced sweetening and supports the theory that the synthesis of key enzymes may be inhibited by irradiation, the consequence of which is a net accumulation of reducing sugars and sucrose.

After the initial post-irradiation rise, reducing sugar levels were found to gradually lower during storage (Burton, 1959; Burton, 1975). The extent of the rise and the subsequent rate of fall was found to be dependent on the date of irradiation, although this effect was influenced by the previous storage regime (Burton, 1959).

The dependence of reducing sugar levels on the date of irradiation restricts the use of irradiation treatment to the early part of the storage season when there is still enough time prior to processing for a reduction in sugar levels to an acceptable level (Burton, 1959).

Burton observed the occurrence of senescent sweetening earlier in the storage period in irradiated potatoes than in controls. Once senescent sweetening had begun, its effects were found to be more pronounced in irradiated potatoes than in untreated potatoes. Therefore after the onset of senescent sweetening irradiated potatoes deteriorated faster and were unacceptable for processsing earlier in the storage season (Burton, 1959). Burton also regarded the early senescent sweetening of irradiated potatoes to be linked to sprout inhibition as early senescence was also observed in chemically sprout suppressed potatoes (Burton, 1959).

Irradiation, therefore, initially increases the contents of both reducing sugars and sucrose in potatoes and is thought to accelerate

the effects of senescent sweetening later in storage. Such effects can be minimised to some extent but some initial elevation in sugar contents is unavoidable in order to maintain a low level of sprouting in irradiated potatoes.

The effect of chemical sprout suppressants on sugar levels

The most widely used method for the sprout suppression of potatoes stored for processing in the U.K. at present is the use of chemical sprout suppressants and in particular Chlorpropham. The effect of chemical sprout suppressants on the sugar contents of potato tubers has not yet been fully investigated and much of the information obtained in this field has been contradictory. It does however appear that the sugar contents of potatoes treated with Chlorpropham are generally unaffected by that treatment for most of the storage period in comparison with controls (Isherwood and Burton, 1975). It is only towards the end of storage, when senescent sweetening intervenes that sucrose contents of Chlorpropham treated potatoes rise above those of controls.

The influence of Tecnazene on sugar levels in potatoes has not been widely investigated. It has been observed by some workers that in general sweetening is greater if sprouting is chemically suppressed (Burton and Wilson, 1978; Rumph, 1973) hence in Tecnazene treated tubers some sugar accumulation would be expected. Dalziel (1978) refutes this suggestion and states that sugar contents may be preserved at levels below those of controls by the use of Tecnazene treatment. Dalziel further showed that in early storage sugar contents remained unaffected by Tecnazene treatment when stored at $8^{o}C$ but that sugar contents increased in the latter half of storage with

the onset of sprouting.

The exact nature of the effect of sprouting on sugar levels has been the subject of some debate, both reductions and increases in sugar levels have been observed in sprout suppressed potatoes. A definitive picture of the influence of sprouting has not yet been drawn. On the basis of the studies presently available it is this author's view that while sprouting is inhibited sugar levels remain low until senescence occurs.

Analytical method

Due to the importance of glucose, fructose and sucrose in biological systems and their importance in food processing many analytical methods have been developed for their measurement. This offers a wide choice of potential analytical methods for the analysis of those sugars in this study. The following discussion will only present examples of each type of analysis and does not set out to definitively review the methods available.

The criteria that such an analytical method must fulfill to be of use in this instance are; reproducible quantification at the sensitivity required, specificity of measurement to the three sugars to be analysed and the ability to cope with the large number of analyses required in a reasonable period of time.

The available methods fall into the following categories.

(1) Paper chromatography

(2) Gas chromatography

(3) High performance liquid chromatography (H.P.L.C.)

(4) Spectrophotometry

Sugar measurements by paper chromatography (e.g. Chan and Cain, 1966; Jarvis and Duncan, 1974) are hard to readily quantify and could not cope with the large number of analyses required. Therefore this technique was not suitable for this study.

Gas chromatography has been widely used in the past for sugar analysis in potatoes (Rumpf, 1973; Dalziel, 1978) and is a reproducible and specific method. By this technique glucose, fructose and sucrose can be separated and measured simultaneously in one sample injection. The main drawback of gas chromatography is the requirement for a time consuming derivatisation of the sugars, as the sugars themselves are not volatile enough for gas chromatographic analysis at normal operating temperatures. Several types of sugar derivatives are reviewed in Blau and King (1978) and Knapp (1979). The derivatisation step makes gas chromatographic analysis of sugars a lengthy process and therefore a less time consuming method was sought.

Some H.P.L.C. methods that have been developed for sugar analysis require derivatisation to attach a U.V. absorbing group to the sugar (Lawson et al., 1979) and are therefore as lengthy as gas chromatographic methods. Other H.P.L.C. methods (e.g. Wilson et al., 1981) use more specific refractive index detectors and so retain the advantages of gas chromatography whilst shortening analysis time. Those methods would therefore have met all of the criteria set for this study. However the necessary equipment was unavailable at that time.

Spectrophotometric methods of sugar analysis fall into two categories, chemical and enzymic. Chemical methods (e.g. Jarvis et

al., 1974) suffer from a low degree of specificity, to overcome which multi-step analysis of a single sample is necessary. In addition they lack adequate reproducibility for the purposes of this study. Enzymic methods are based on enzymically catalysed reactions which are highly specific to the substrates glucose, fructose and sucrose. The problem of reproducibility can be solved by the use of an automated continuous flow system allied to a direct comparison with standard sugar solutions. This system also allows the analysis of a large number of samples in a relatively short period of time and so satisfies all of the criteria stipulated.

The method employed in this study was the automated spectrophotometric determination of Coenradie and Nederpel (1976) based on the enzymic analysis technique of Schmidt (1961). The details of this method are discussed in section 3.2.3 of this chapter.

Experimental objectives

In the previous discussion the variability of potato sugar levels has been emphasised. Variability arises from cultivar dependence, level of maturity, seasonal variation and storage regime. It can, therefore, be difficult to readily compare one study with another. It was therefore thought worthwhile to make a direct comparison of sugar levels found in control, irradiated and chemically sprout suppressed potatoes from the same crop of potatoes grown and stored under the same conditions.

Rumpf (1973) has carried out a study of this nature on cv. Saturna and cv. Tasso potatoes. The latter study involved a direct comparison of Chlorpropham/Propham treated tubers with irradiated tubers but did not however include an untreated control. No sprout data was

published, the storage temperature ranged from 8°C - 16°C and sugar levels were only determined at a few discrete sample dates. In the following study the sugar levels and degrees of sprouting in control, Tecnazene, Chlorpropham and irradiated tubers will be compared throughout a normal storage season at a constant storage temperature of 8°C. The inclusion of the Tecnazene treatment, which is likely to partially inhibit sprouting, may hopefully clarify the effect of sprout inhibition on sugar accumulation by providing information on the effect of intermediate sprout inhibition on sugar contents.

Cultivar dependence was investigated by comparing two cultivars, cv. Record, for reasons already outlined, and cv. Desiree a commonly grown, vigorously sprouting cultivar. If differences in sugar levels are connected to the degree of sprouting this cultivar comparison may be of some use in discovering the relationship between them. Experiments carried out by previous workers have naturally concerned the commonly grown cultivars in the locality of the study and at the time the study took place, in some cases more recently introduced cultivars have now replaced them. As has already been discussed sugar levels have been shown to be cultivar dependent and it would be of interest to have information on the response to irradiation of the major cultivar used by the potato crisp processing industry in the U.K. at present. At the time this study was undertaken no such information was available.

It is further hoped that the results obtained will help to clarify the effect that irradiation has on the process of senescent sweetening.

3.2 Experimental

3.2.1 Sprout suppressant treatments

Cv. Desiree potatoes used in this investigation were harvested on October 15 at Arkleston Farm, Renfrew as part of a field trial carried out by M. Leonard to investigate the effect of Tecnazene on subsequent seed growth (Leonard, 1988). Potatoes from the control treatment in that field experiment were graded and 200 kg of ware grade (>45 mm) tubers were transported to Glasgow University for use in this study. A further 200 kg of cv. Record potatoes, tops from a seed crop, were purchased from D. Russel, Mawhill Farm, Mawhill, Kinross in Scotland on November 12. Potatoes of both cultivars were split into 10 kg subsamples each of which was placed into a cardboard box of dimensions 38.5 cm X 31 cm X 15.5 cm. All boxes were then stored in a constant temperature room at $8^{o}C \pm 1^{o}C$ and relative humidity of 84% $\pm$ 1% until November 19.

The potatoes of each cultivar were divided into the following five treatments; control (untreated), two irradiation doses, Chlorpropham treated and Tecnazene treated. Four replicate boxes were filled for each treatment of each cultivar, giving 40 boxes in total. The two irradiation doses were chosen, first of all, to inhibit sprouting to a negligible level. The second consideration was to chose two doses which were far enough apart to allow any dose dependence of sugar contents to be apparent while still remaining within the dose range that would normally be used to prevent sprouting. For these reasons, after consideration of the models derived in Chapter 2 and allowing for a margin of error, doses of 0.10 and 0.15 kGy were chosen.

All boxes were transported to the S.U.R.R.C. on November 19 where

those to be irradiated were treated. The exposure times required for each treatment were calculated as outlined in section 2.2.1 of Chapter 2. Each sample was transferred from the box to the irradiation vessel, exposed to the source for the required time and returned to the box. No spacer was placed below samples during these irradiations as the 10 kg samples almost completely filled the irradiation vessel. All boxes were then returned to Glasgow University where they were placed in a constant temperature room under the conditions described above.

Chemical sprout suppressant treatments were applied at the normal full commercial dose for each chemical, that is 20 mg Chlorpropham kg^{-1} potatoes or 135 mg Tecnazene kg^{-1} potatoes. An alumina dust formulation of each chemical was used for application in order to achieve even distribution of the chemical throughout the box.

The weight of Chlorpropham (Sigma Chemicals, U.S.A.) required for the treatment of 8 boxes (1.6 g) was dissolved in approximately 2-5 cm^3 of analytical grade hexane (Rathburn Chemicals, U.K.) and poured into a 500 g screw capped glass jar containing 200 g of grade 0 alumina (Spence and Sons, Scotland). The jar was left open to the atmosphere for several minutes to allow the solvent to evaporate after which the jar was sealed. The jar was shaken vigorously by hand for 2 minutes and then placed in an oven at $35^{o}C$ for 1 hour to ensure that the chemical was volatilised. The jar was then transferred to an end-over-end shaker and shaken overnight at room temperature to evenly adsorb the Chlorpropham onto the alumina. The weight of Tecnazene (Aldrich Chemicals, U.K.) required to treat a further 8 boxes (10.8 g) was adsorbed onto a second 200 g of alumina in the same way.

Boxes of potatoes to be treated with Chlorpropham or Tecnazene were removed from the constant temperature room on November 20. The weight of dust formulation required to treat one box of each of the two chemical treatments was calculated and the two formulations subdivided. Each box of potatoes was treated by scattering the dust throughout the box as evenly as possible taking care to dust the corners and edges of the boxes.

After treatment the boxes were closed and returned to the constant temperature room where they were positioned randomly and stored at $8^{o}C \pm 1^{o}C$.

3.2.2 Sampling and sugar extraction procedure

Sampling

At various intervals in the storage period a representative sample was removed from each box and the levels of reducing sugars and sucrose determined in each. Samples were taken more frequently at the beginning of the storage period as it has been observed that larger fluctuations in sugar contents occur in irradiated potatoes soon after treatment (Burton, 1959; Burton, 1965; Schwimmer et al., 1957; Hayashi and Kawashima, 1982). If the date of irradiation treatment is defined as day 0, samples were taken on days 6, 13, 28, 49, 84, 120, 161, 203 and 273, i.e. with the interval between samples gradually increasing. By the end of the storage period in mid-August nine samples had been removed from each box and there was insufficient material remaining to continue the experiment.

Six tubers were sampled from each box, four of average size, one larger than average and one smaller than average. As far as possible the sampled tubers were taken randomly from throughout the whole box. If sprouts were present on any of the tubers sampled the length of the longest sprout on the tuber was measured in mm $\pm$ 1 mm. The tubers from each sample were desprouted, washed, bulked and weighed and the combined weight of their sprouts also noted.

Each tuber was cut to the desired size and then "chipped" using a household potato-chipping device with blades 7.5 mm apart (Brabantia, Holland). The potato chips from all six tubers were bulked, mixed and a 100 g subsample weighed into a pre-weighed 600 cm^3 beaker.

Sugar extraction procedure

When the cells of the potato are ruptured by cutting, subsequent enzymic activity can alter the concentration of sugars present, free sugars can be interconverted and starch broken down to release more free sugars. In attempting to measure the levels of free sugars in stored potatoes by extraction it is necessary to completely disrupt the cells of the potatoes used. It is essential to avoid as much interconversion and degradation as possible in order to make as accurate a measurement as possible of sugar contents. Burton and Hannan (1957) recommend the freezing of samples to -20^{o}C prior to extraction.

To minimise these changes the extraction procedure in this study was carried out as quickly as was reasonably possible. The extractant chosen was methanol as glucose, fructose and sucrose are soluble in methanol at the concentrations required and also because only negligible enzymic activity can take place in methanol solution. Additionally extracting the sugars with methanol has the further advantage of allowing a delay between the extraction and analysis of sugar solutions. It has been observed that a delay of over a year in properly stored extracts caused no change to measurable sugar contents (Boyd, 1986). As a further safeguard methanol used for extractions was pre-cooled by the addition to it of solid CO_2 (Distillers, U.K.). (The sublimation temperature of CO_2 is -78.5^{o}C).

The weighed 100 g subsample of tuber material was then transferred into the glass base of a top-drive macerater (Townson and Mercer, U.K.) and 250 cm^3 of CO_2-cooled methanol was added. The potato chips were blended with the methanol for three minutes at the end of which

the mixture and washings were filtered under reduced pressure using 9.0 cm diameter Whatman No.1 filter paper (Whatman, U.K.). The residual solids were washed with two 100 cm^3 volumes of methanol, the filtrate decanted into a 500 cm^3 volumetric flask with washings and the extract was made up to 500 cm^3 with methanol. The flask contents were mixed well and two replicate sub-samples of approximately 10 cm^3 of the extract were transferred into two 15 cm^3 sample bottles and retained for analysis.

3.2.3 Analysis of extracts

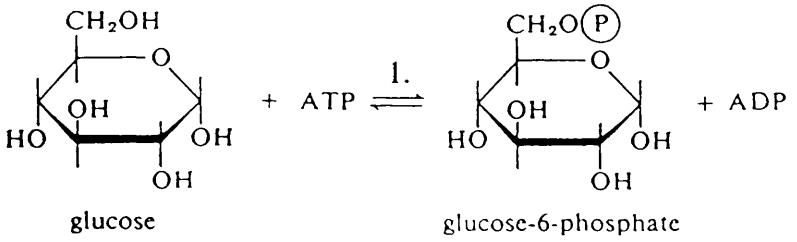
Procedure for glucose measurement

Glucose was measured by linking its enzymic conversion to gluconate-6-phosphate with the reduction of $NADP^+$ shown in diagram 3.2. In diagram 3.2 enzymes 1. and 2. are hexokinase (E.C.2.7.1.1) and glucose-6-phosphate dehydrogenase (E.C.1.1.1.49). Glucono-δ-lactone-6-phospate is highly unstable and hydrolyses readily to gluconic acid-6-phosphate (Yudkin and Offord, 1975).

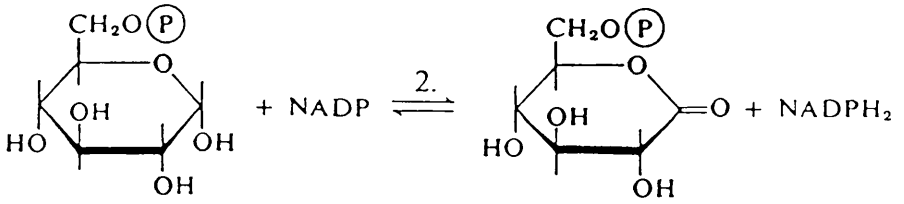
As can be seen from graph 3.1 NADPH absorbs light at 340 nm, in the U.V. wavelength range, while $NADP^+$ does not. The conversion of glucose-6-phosphate to gluconate-6-phosphate is stoichiometric and therefore the amount of glucose present in the sample can be measured by the comparison of the U.V. absorbances of reacted glucose standard solutions to samples within the linear range of the method.

A single mixed Glucose reagent for this method is supplied by Technicon Diagnostics (Holland) as a freeze-dried preparation which is reconstituted by the addition of distilled water. When reconstituted the reagent contains >1000 units hexokinase and >800 units glucose-6-phosphate dehydrogenase per litre. In addition the reconstituted reagent contains ATP (1.4 mmolar), $NADP^+$ (0.8 mmolar) and the enzymic co-factor Mg^{2+} (10 mmolar).

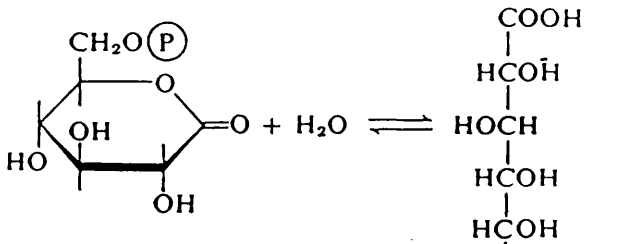
Diagram 3.2 The enzymic conversion of glucose to gluconate*.



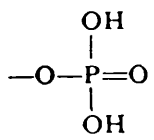
glucose glucose-6-phosphate



glucose-6-phosphate gluconolactone-6-phosphate

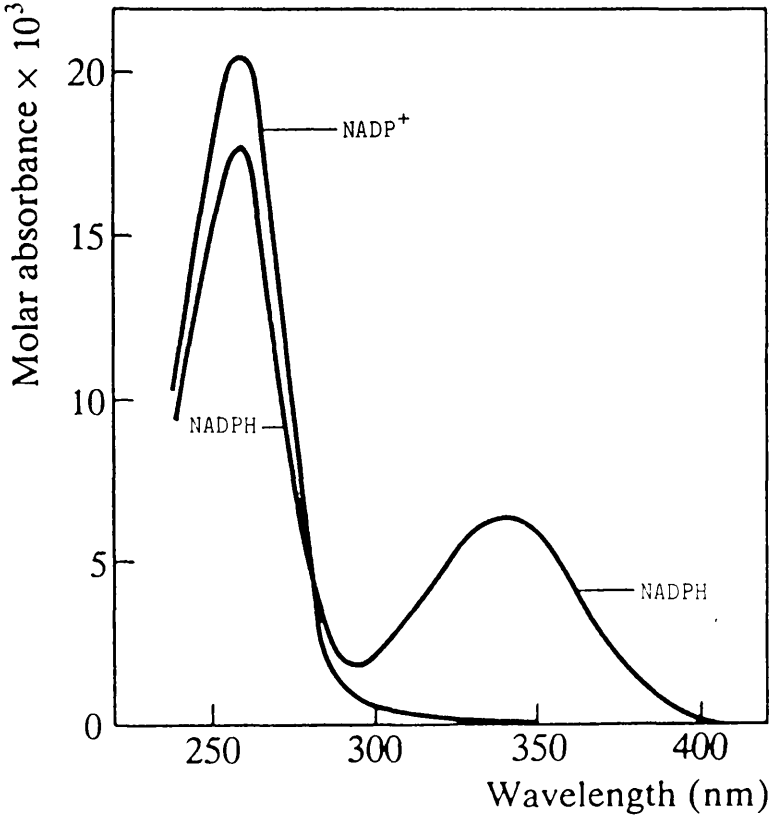


gluconolactone-6-phosphate gluconic acid-6-phosphate

(P) is equivalent to the phosphate group 

* for a description of the enzymes involved see text.

Graph 3.1 The absorbance spectra* of NADPH and $NADP^+$.



* After Dixon, M. and E.C.Webb (1979).

Procedure for reducing sugar measurement

Reducing sugars were measured by converting fructose enzymically to glucose-6-phosphate as shown in diagram 3.3. The enzymes 1. and 3. in diagram 3.3 are hexokinase and phosphoglucose isomerase (E.C.5.3.1.9) respectively. The total glucose-6-phosphate concentration was then measured as described above and thus represented the combined contributions of glucose and fructose, i.e. reducing sugars.

For reducing sugar measurements 1 cm^3 of phosphoglucose isomerase suspension (Boehringer, F.D.R.), 0.25 cm^3 of hexokinase/glucose-6-phosphate dehydrogenase suspension and 50 mg ATP were added to and gently mixed with the Technicon glucose reagent. Additional ATP and hexokinase/glucose-6-phosphate dehydrogenase were required as a larger amount of glucose-6-phosphate was to be converted.

Procedure for sucrose measurement

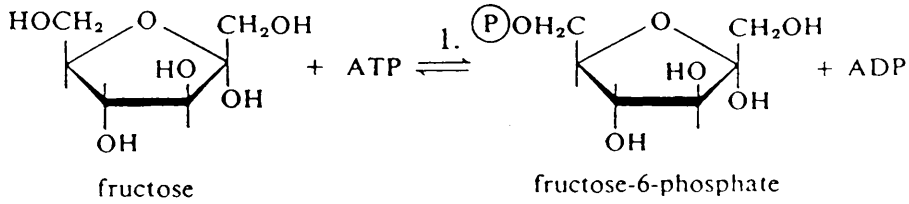
Sucrose was measured by enzymically catalysed hydrolysis of sucrose to fructose and glucose, conversion of those monosaccharides to glucose-6-phosphate and the determination of total glucose-6-phosphate concentration as carried out for the fructose measurements described above. The hydrolysis reaction is described in diagram 3.4. The enzyme 4. in diagram 3.4 is β-fructosidase (E.C.3.2.1.26).

Sucrose hydrolysis was carried out using a β-fructosidase solution prepared by mixing 250 mg of β-fructosidase (Boehringer, F.D.R.) with 100 cm^3 of distilled water, filtering through Whatman No.1 filter paper (Whatman, U.K.) and making the filtrate up to 100 cm^3 in a volumetric flask. Sucrose hydrolysis is more pH sensitive than the other reactions in the analytical procedure and had to be buffered to

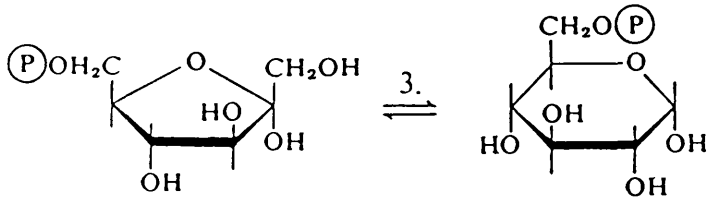
obtain consistent results. A sodium hydroxide/acetic acid buffer at pH 4.6 was therefore used for sucrose measurements.

Sucrose concentration was calculated by subtracting the contribution made by glucose and fructose from the total glucose-6-phosphate concentration. Therefore to measure reducing sugars and sucrose small aliquots of each sample were analysed on the sucrose and reducing sugars systems and sugar concentrations calculated by difference.

Diagram 3.3 The enzymic conversion of fructose to glucose-6-phosphate*.

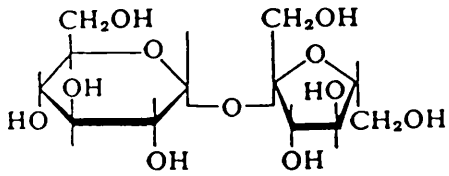


fructose fructose-6-phosphate

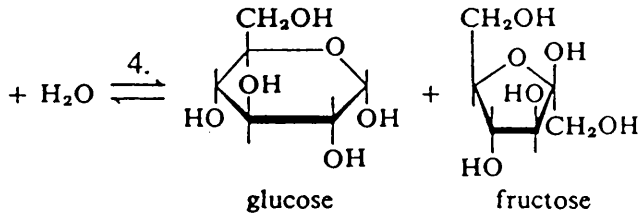


fructose-6-phosphate glucose-6-phosphate

Diagram 3.4 The enzymic conversion of sucrose to glucose and fructose*.



sucrose



glucose fructose

* for a description of the enzymes involved see text.

Analytical instrumentation

Sugar analysis was carried out using the Technicon AutoAnalyzer II system comprising - Sampler IV autosampler, Proportioning pump III, water bath at constant temperature (35^{o}C) and single-channel colorimeter. Absorbance levels were monitored by the connection of the colorimeter to a Dynamaster chart recorder and to a BBC B microcomputer. Computer software designed specifically for the task of data collection and processing on the Autoanalyser II system was written by Dr.T.H.Flowers of the Agricultural Chemistry section of Glasgow University.

It was estimated that by the end of the sugar extraction process the final extraction solution was 85% v/v methanol as some water was co-extracted from the potatoes with the sugars. For this reason standard sugars solutions were prepared in 85% v/v methanol. The standard solutions used were 1 and 2mg cm^{-3} glucose, fructose and sucrose (Sigma, U.S.A.) depending on the concentration of the sample solutions to be measured.

Glucose solutions were used as the primary standards throughout for the analysis of all three sugars. Conversion within the system of fructose and sucrose to glucose-6-phosphate was periodically checked during measurement by the analysis of fructose and sucrose standard solutions, whose absorbance was then compared to that of a glucose standard of the same concentration.

3.3 Results

Sprouting

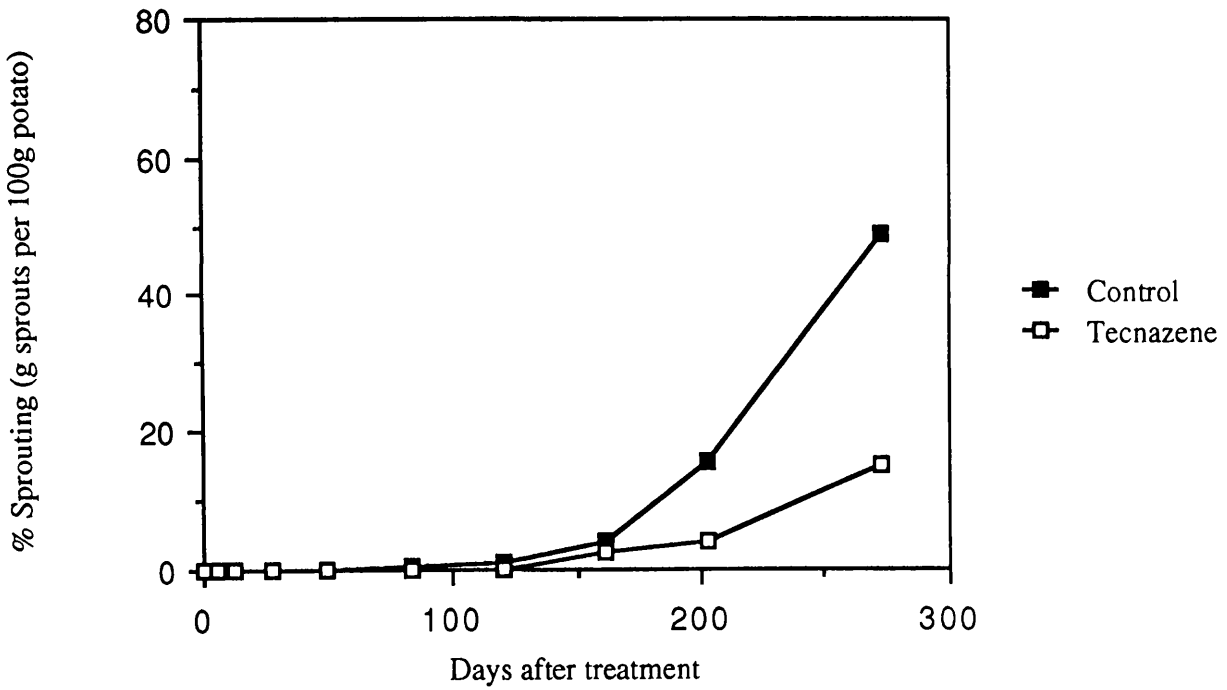
No appreciable sprouting was observed in either of the irradiation treatments or in the Chlorpropham treated potatoes throughout the period of storage of 10 months, from November to August. Sprouting was observed, however, on control and Tecnazene treated tubers. Within boxes of potatoes treated with Tecnazene sprouting was notably uneven, much greater sprouting was observed at the four edges of the boxes than in the middle. The degrees of sprouting in the Tecnazene and control treatments of cv. Record and cv. Desiree are displayed in graphs 3.2(a) and 3.2(b) respectively.

Sugar contents

A standard curve of absorbance, measured as chart recorder peak height in mm, against glucose concentration is presented as graph 3.3. From graph 3.3 it can be seen that there is a linear response range for the method between approximately 0 mg and 5 mg glucose cm^{-3}. All samples analysed in this study were found to be within that linear concentration range.

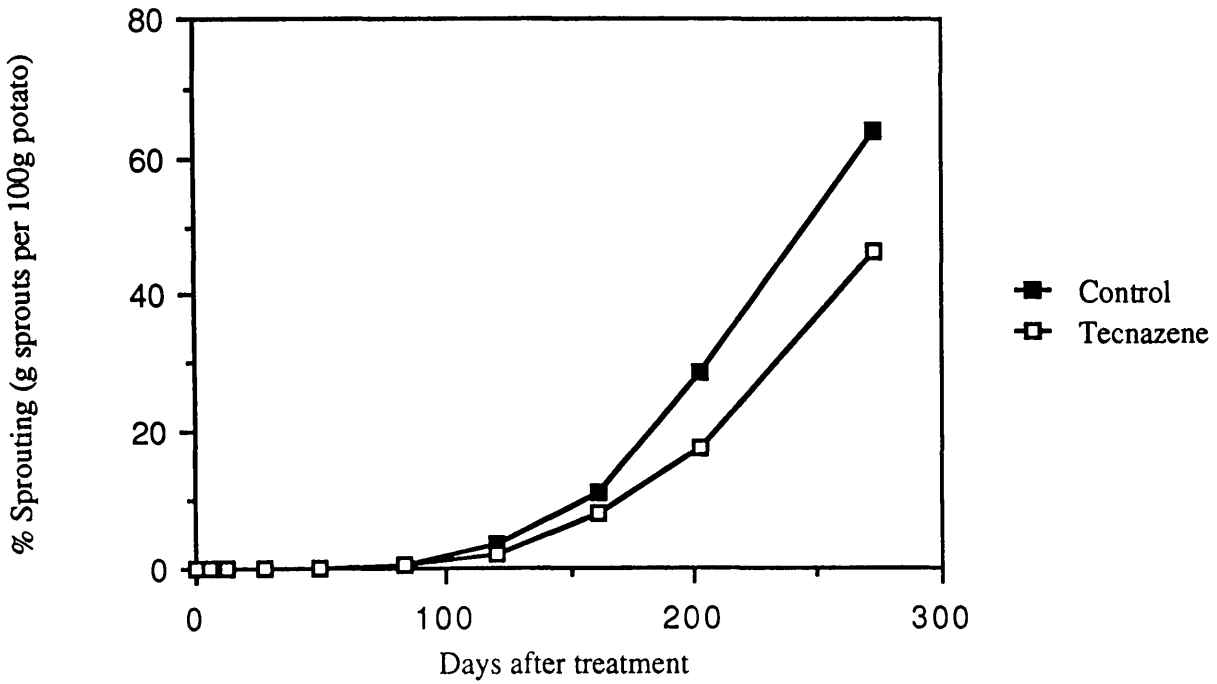
Reducing sugars and sucrose contents were calculated as previously outlined and expressed as g sugar(s) per 100 g potato on a fresh weight basis (f.wgt.). The levels of reducing sugars and sucrose for each cultivar throughout the storage period are plotted in graphs 3.4 to 3.7.

Graph 3.2(a) Percentage sprouting* by weight (g sprouts/100g potato) in control and Tecnazene treated cv. Record potatoes during 273 days of storage at 8°C.



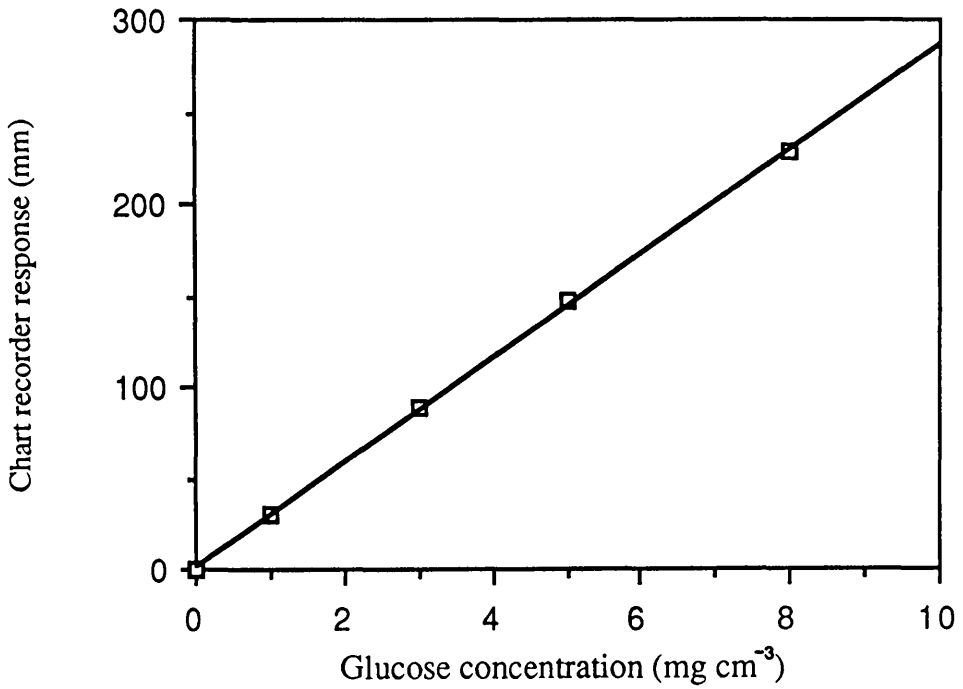
* each point is the mean of four replicates.

Graph 3.2(b) Percentage sprouting* by weight (g sprouts/100g potato) in control and Tecnazene treated cv. Desiree potatoes during 273 days of storage at 8°C.



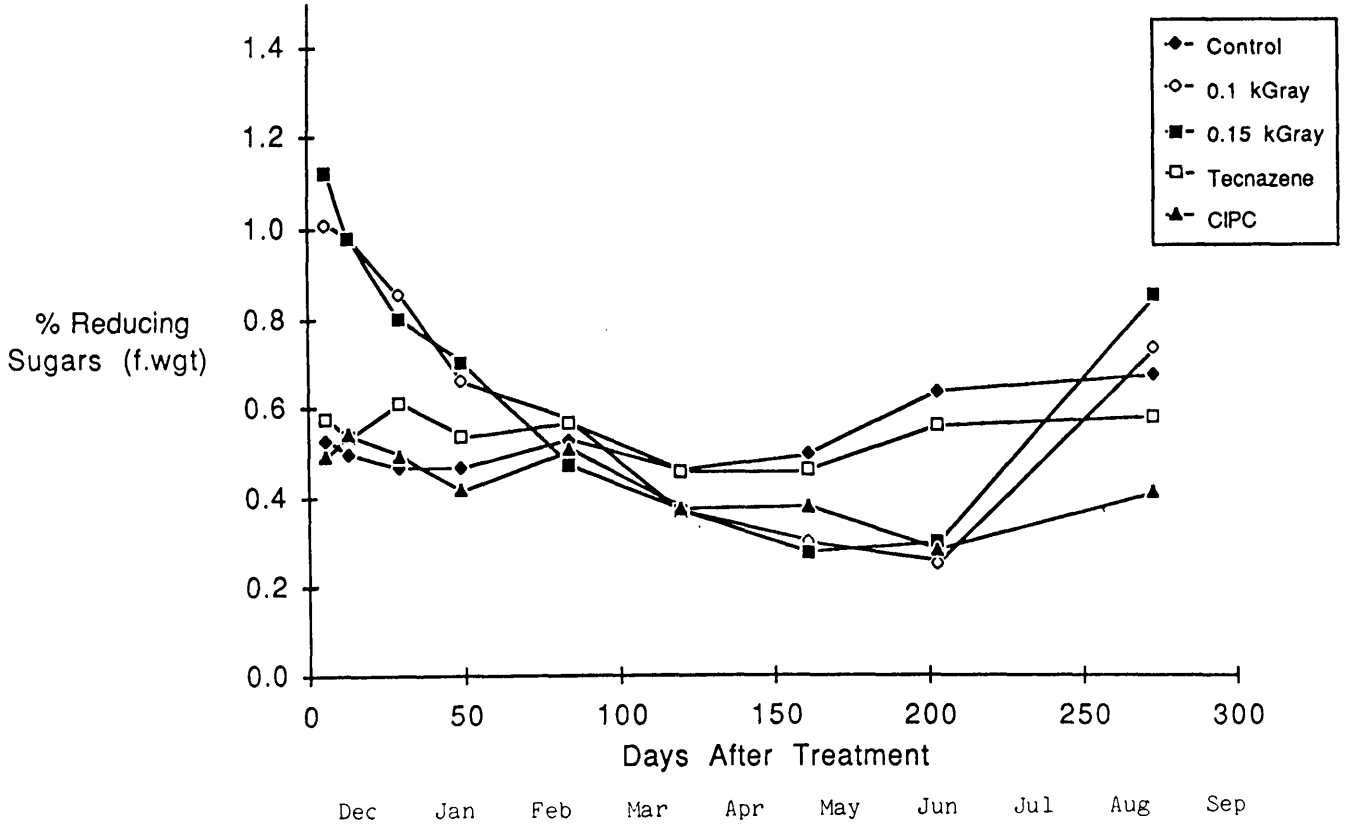
* each point is the mean of four replicates.

Graph 3.3 Standard curve of Technicon autoanalyser system response to glucose concentration.



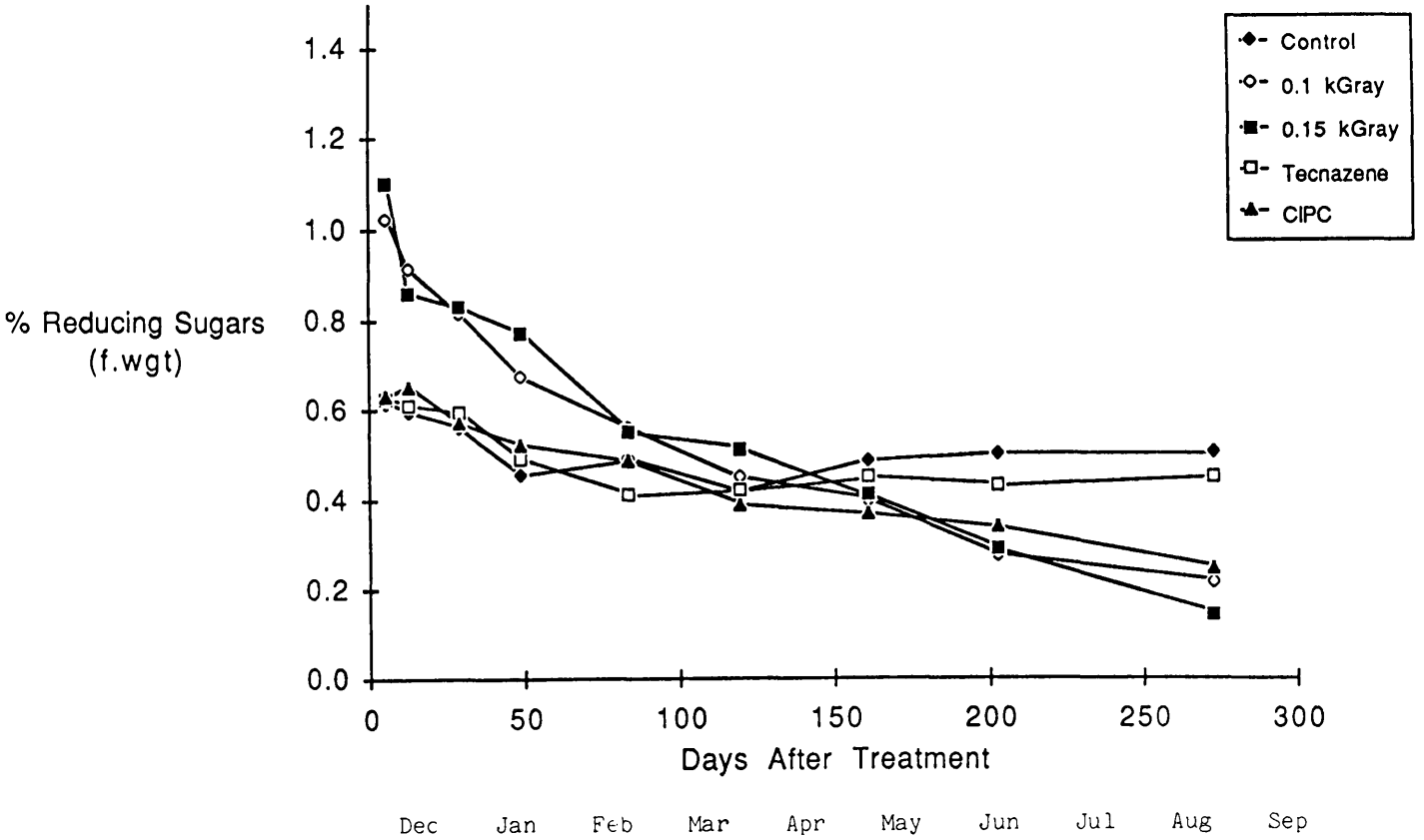
Each point is the mean of four replicates.

Graph 3.4 Percentage reducing sugar contents* of cv. Desiree potatoes stored for 273 days at 8°C.



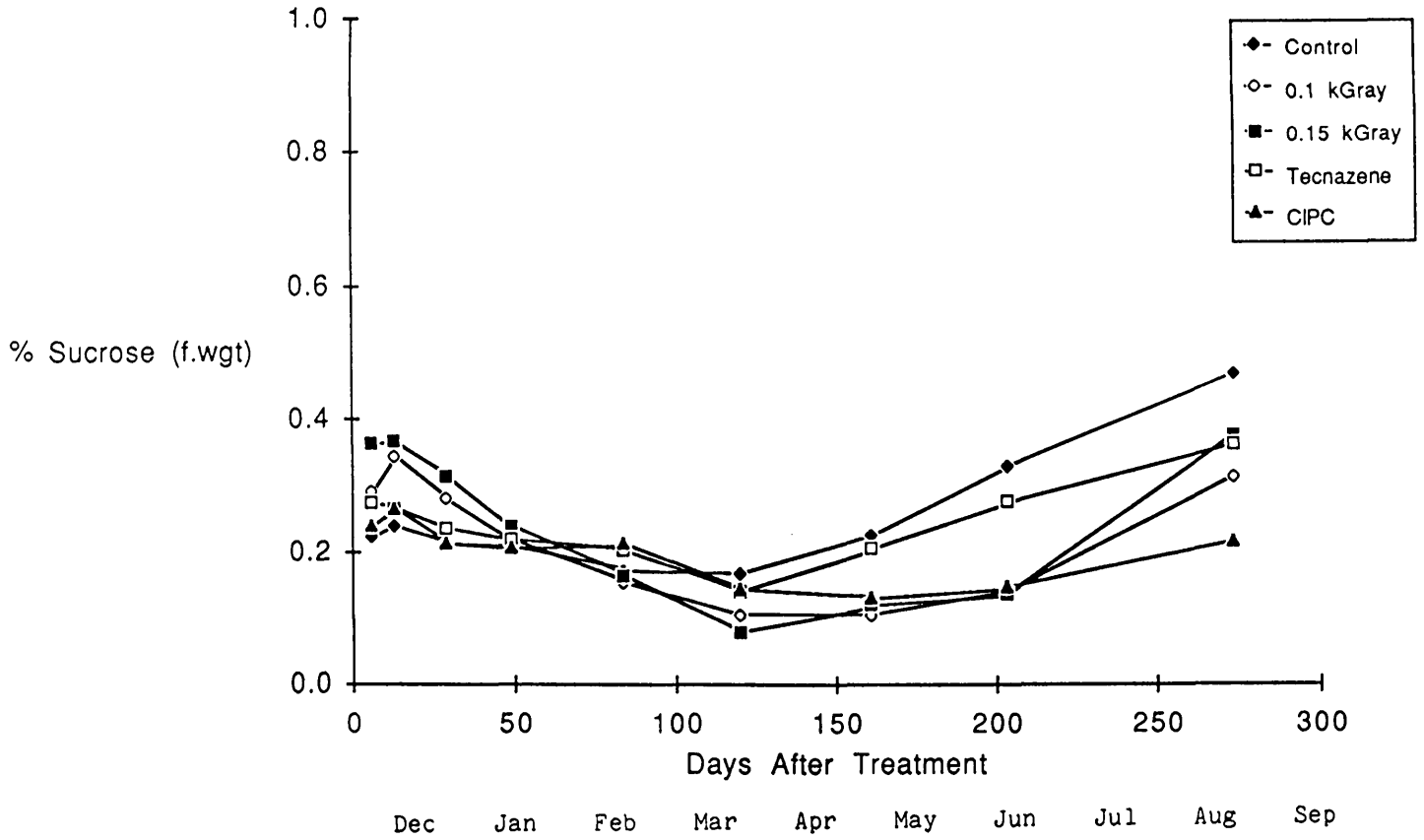
* each point is the mean of four replicates.

Graph 3.5 Percentage reducing sugar contents* of cv. Record potatoes stored for 273 days at 8°C.



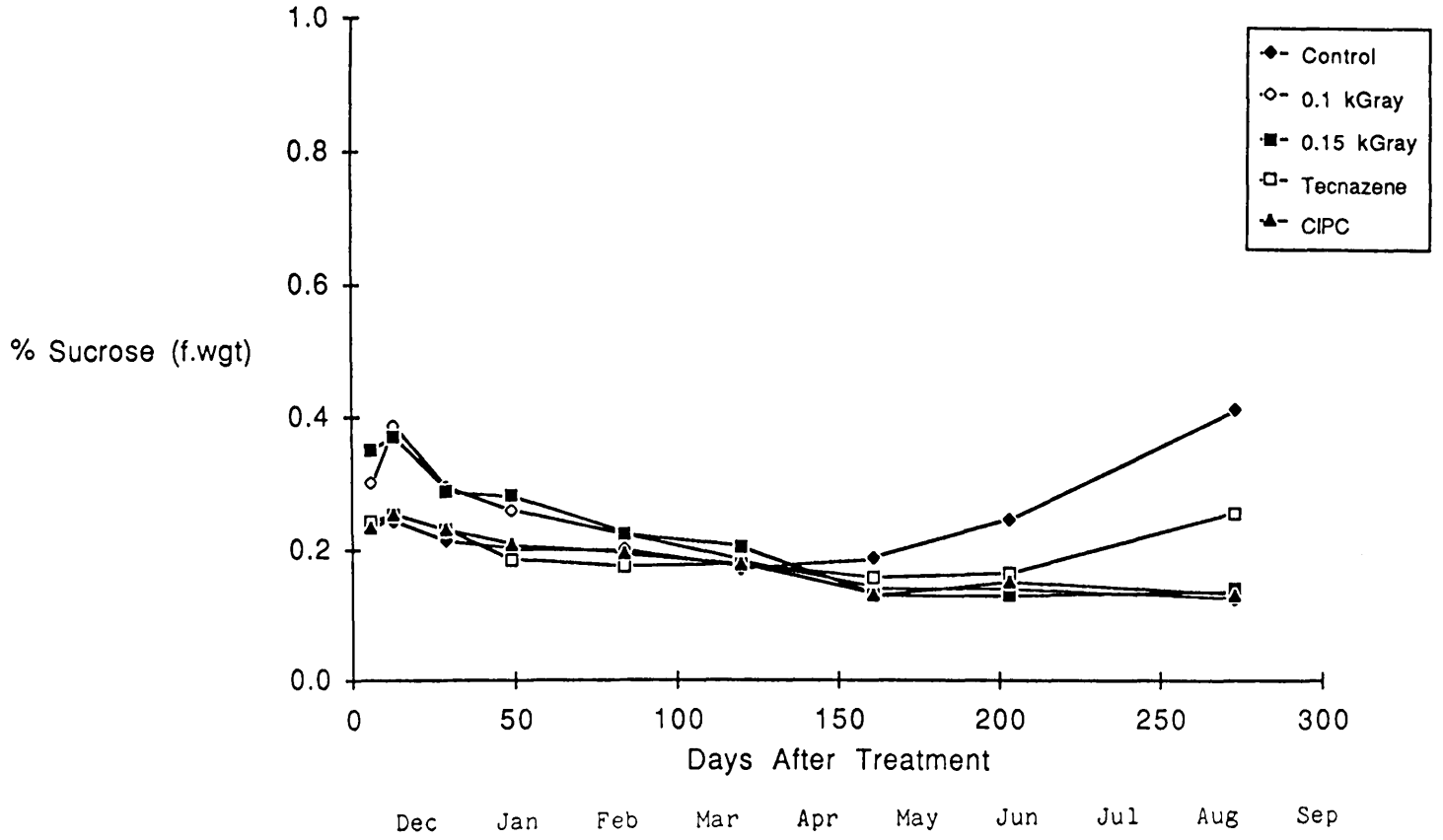
* each point is the mean of four replicates.

Graph 3.6 Percentage sucrose contents* of cv. Desiree potatoes stored for 273 days at 8°C.



* each point is the mean of four replicates.

Graph 3.7 Percentage sucrose contents* of cv. Record potatoes stored for 273 days at 8°C.



* each point is the mean of four replicates.

Effect of cultivar on sugar contents

An analysis of variance procedure was carried out to determine whether there was any overall significant difference between the sugar contents of the two cultivars. The results of that procedure are contained in table 3.1.

Table 3.1. The effect of cultivar on the mean percentage sugar contents of cv. Desiree and cv. Record potatoes over a complete storage period.

	Mean sugar content (% fresh weight)		
Cultivar	Reducing Sugars	Sucrose	Total Sugars
Desiree	0.550	0.233	0.781
Record	0.531	0.233	0.751
F statistic	0.73	1.62	1.04

The F statistic gives a measure of the probability of a difference in means arising because of a real difference in the effect of treatments rather than by chance. Tabulated F values represent the highest value a difference in means could have if the difference arose by chance at a given probability. For the test of significance of the data in table 3.1 in 95% of cases the F statistic calculated would exceed the tabulated value if there was a real difference between the means. Thus the F value calculated in tables for the appropriate number of degrees of freedom is compared with F values calculated from the experimental data. If the tabulated value for the 5% significance

level is exceeded then there is a 95% probability that there is a significant difference between the means. The F-statistic for the appropriate number of degrees of freedom in this case is 3.9, therefore in this study there was no significant overall difference between cultivars at the 5% significance level.

A study of graphs 3.4 to 3.7, however, reveals that differences in sugar contents can be seen within cultivars at various points in the storage period. The comparisons in table 3.1 were made over the whole storage period and more subtle shorter term differences in sugar contents occurring may not be being detected by an overall cultivar comparison. The data from each cultivar was therefore analysed independently because of the differences in sugar contents at particular points in the storage period. For this reason it was also thought advisable to compare treatments at individual sample dates in any further statistical analysis.

Effect of treatment on sugar contents

To determine if there were any differences between the sugar contents in control potatoes and those whose sprouting had been inhibited a multiple comparison procedure was carried out. The procedure used was Duncan's New Multiple Range Test (DMRT) which tests the significance of a difference in treatment means by initially ranking them according to size and testing each rank against a different test value. Duncan's New Multiple Range Test was chosen as it is slightly more conservative than Fisher's Least Significant Difference which, because of the variable nature of the data obtained in this experiment, could result in differences being found where no real differences exist. Duncan's New Multiple Range Test is not,

however, so conservative as to obscure many real differences. Readers requiring a more detailed discussion of Duncan's New Multiple Range Test are referred to Dowdy and Wearden (1983).

The results of the Duncan's New Multiple Range Tests are contained in tables 3.2 and 3.3.

Table 3.2. Reducing sugar and sucrose contents of control, irradiated and chemically sprout suppressed cv. Desiree potatoes stored at 8°C.

		Sugar content (% fresh weight)								
	Days after treatment	6	13	28	49	84	120	161	203	273
	Treatment									
Reducing Sugars	Control	0.52	0.50	0.46	0.46	0.52	0.46	0.49	0.63	0.66
	0.1 kGy	0.98*	0.97*	0.84*	0.64*	0.56	0.36	0.29*	0.25*	0.72
	0.15 kGy	1.10*	0.97*	0.79*	0.69*	0.46	0.36	0.27*	0.29*	0.84
	Tecnazene	0.56	0.53	0.60	0.52	0.56	0.45	0.46	0.55	0.57
	Chlorpropham	0.49	0.54	0.49	0.41	0.50	0.37	0.38	0.28*	0.40*
Sucrose	Control	0.22	0.24	0.21	0.21	0.17	0.17	0.23	0.33	0.47
	0.1 kGy	0.29*	0.34*	0.28*	0.22	0.15	0.10*	0.10*	0.14*	0.32*
	0.15 kGy	0.36*	0.37*	0.32*	0.24	0.16	0.08*	0.12*	0.14*	0.38*
	Tecnazene	0.27	0.27	0.24*	0.22	0.20*	0.14*	0.21	0.28*	0.36*
	Chlorpropham	0.24	0.27	0.21	0.21	0.21*	0.14	0.13*	0.15*	0.22*

* denotes a significant difference from the control value at the 5% significance level in Duncan's New Multiple Range Test.

Table 3.3. Reducing sugar and sucrose contents of control, irradiated and chemically sprout suppressed cv. Record potatoes stored at 8°C.

		Sugar content (% fresh weight)								
	Days after treatment	6	13	28	49	84	120	161	203	273
	Treatment									
Reducing Sugars	Control	0.61	0.58	0.56	0.45	0.48	0.41	0.48	0.50	0.50
	0.1 kGy	1.02*	0.90*	0.81*	0.68*	0.56	0.45	0.40	0.27*	0.22*
	0.15 kGy	1.09*	0.85*	0.83*	0.77*	0.55	0.52*	0.41	0.28*	0.14*
	Tecnazene	0.62	0.60	0.59	0.48	0.41	0.42	0.44	0.42	0.44
	Chlorpropham	0.62	0.64	0.56	0.52	0.48	0.39	0.36*	0.33*	0.24*
Sucrose	Control	0.25	0.24	0.21	0.20	0.20	0.17	0.19	0.25	0.41
	0.1 kGy	0.30*	0.39*	0.30*	0.26*	0.22	0.18	0.14*	0.14*	0.12*
	0.15 kGy	0.35*	0.37*	0.29*	0.28*	0.22*	0.20*	0.13*	0.13*	0.14*
	Tecnazene	0.24	0.26	0.23	0.19	0.17	0.18	0.16*	0.16*	0.26*
	Chlorpropham	0.23	0.25	0.23	0.21	0.19	0.18	0.13*	0.15*	0.13*

* denotes a significant difference from the control value at the 5% significance level in Duncan's New Multiple Range Test.

3.4 Conclusions

Initial sugar contents

As has been outlined previously reducing sugar levels are critical in determining the suitability of potatoes for processing, the initial level prior to storage being tested routinely at harvest. From tables 3.2 and 3.3 it can be seen that the initial level of reducing sugars in cv. Desiree was 0.52% (fresh weight) while in cv. Record the level was 0.61% (fresh weight). These values would be considered rather high and potatoes with such levels would not be used for the manufacture of crisps where levels of 0.1 - 0.3% are considered more suitable (Burton and Wilson, 1978). The fact that these values are greater than normally accepted shows once more the variability in sugar levels that can be observed between different studies.

The high initial reducing sugar contents found in this study were probably due to a combination of factors. Firstly most potatoes used in processing come from an area several hundred miles to the South of those used in this experiment. Scotland's potato crop is largely used for domestic consumption or seed production. At the end of the growing season in Scotland the ambient temperature is generally likely to be several degrees Celsius lower than in the southern potato producing areas. The effect of temperature on sugar contents can be critical and a lower ground temperature may have resulted in a degree of low temperature sweetening of the potatoes used in this study.

Another important factor in post-harvest sweetening is the level of maturity of potatoes. Slightly above average sucrose levels in control samples of both cultivars appear to indicate some degree of immaturity at harvest which could have led to the accumulation of

reducing sugars immediately after harvest, although the levels of 0.25% in cv. Record and 0.22% in cv. Desiree on the first date of measurement do not greatly exceed a typical value of approximately 0.1 - 0.2% for mature tubers (Burton and Wilson, 1978). At the earliest measurement date in November most of the sucrose may already have been converted to reducing sugars as such conversion can occur very soon after harvest (Burton, 1966). With hindsight it would have been informative to have measured sucrose and reducing sugars directly at harvest, however, unfortunately that would not have been possible in this case as the cv. Record potatoes were purchased from a third party.

Some further evidence that immaturity could have been responsible for the observed high reducing sugar levels is found in the way reducing sugar contents themselves change during the early stages of storage. From graphs 3.4 and 3.5 it can be seen that reducing sugars fall slightly in control treatments in the early part of storage which may indicate that immature tubers are converting reducing sugars into starch. This pattern of reducing sugar fall can also occur in mature tubers as they adjust to the temperature of the store and should not be taken as conclusive evidence of immaturity. It is, however, not inconsistent with immaturity. The reducing sugar contents of the two chemically treated batches tend to fluctuate more than those in the controls during the early period of storage, although this may have been caused by removing them from the constant temperature room to be treated.

Whether the sugar contents of the potatoes studied here were higher due to low temperature sweetening or immaturity the initial levels

were higher than would be normally accepted. Consequently the effect of irradiation and chemical sprout suppressants on sugar contents must be interpreted bearing in mind such initially high sugar levels.

Effect of irradiation on reducing sugars

The most dramatic effect of treatment visible in graphs 3.4 and 3.5 is the rapid rise in the reducing sugar contents of both cultivars after irradiation treatment.

From tables 3.2 and 3.3 it can be seen that the reducing sugar contents of both irradiated treatments in both cultivars are significantly greater than the controls from the beginning of storage until mid-February. The reducing sugar levels measured on the first sample date in November may not represent the maximum content of reducing sugars reached after irradiation as the levels only fall from that point. The maximum contents may have been reached earlier as, for example, Burton indicated that a sharp rise and fall in the early days of storage takes place (Burton, 1959). In fact the maximum value of reducing sugars reached is not the most critical parameter of interest to processors. In practice processors may be more interested in how quickly the reducing sugar levels fall after treatment and most importantly what level they can expect at time of processing.

Although the initially measured sugar levels may not represent the maximum value achieved they are still high enough to make the potatoes of no use to processors at that time. However, the levels present fall from that point in both cultivars until June.

The pattern of reducing sugar levels from the first sample date onwards is less consistent but follows a general downward trend. By

mid-February there was no significant difference from controls found in either cultivar and there were significantly lower levels of reducing sugars in irradiated tubers after the mid-February sampling date in cv. Record, and after the April sampling date in cv. Desiree.

From early June onwards the reducing sugar levels in cv. Record continued to fall but in cv. Desiree there was a rapid rise to a level above that of the controls. This sharp rise in reducing sugars in cv. Desiree may have been indicative of senescence occurring in cv. Desiree. The discussion of sucrose levels in cv. Desiree in the following text adds weight to that supposition.

In cv. Desiree by the end of the study in mid-August the reducing sugar levels were no longer significantly lower than in controls as the levels of reducing sugars in irradiated samples rose steeply. The reducing sugar levels measured in June in both cultivars would be acceptable to processors for crisp manufacture, while the levels in the controls of both cultivars are too high for crisp manufacture. However, a delay in the use of cv. Desiree potatoes for processing until the end of the storage period studied would allow the reducing sugar contents to rise again to unacceptable levels.

These fluctuations in reducing sugars indicate that in potatoes sweetened by irradiation treatment, subsequent desweetening can result in the lowering of reducing sugar levels during storage to a level acceptable for processing, even in tubers that exhibited unacceptably high reducing sugar levels before irradiation. The observed trend in reducing sugar levels underlines the need for appropriate timing of irradiation treatment so that irradiation takes place early enough in the storage season to allow a period of desweetening. Delaying the

processing of irradiated potatoes until desweetening to the required degree has occurred is also essential, although an excessive delay may be counterproductive as senescent sweetening may intervene.

Comparisons were carried out using Duncan's New Multiple range test to determine whether the sugar levels in irradiated tubers were dose dependent within the dose range studied. No significant difference between the reducing sugar levels of 0.10 and 0.15 kGy were found throughout the storage period in either cultivar. This result is consistent with the view of Schwimmer et al. (1957) who found little difference in reducing sugar contents within this dose range.

Effect of chemical treatments on reducing sugars

From tables 3.2 and 3.3 it can be seen that there was no significant difference, in either cultivar, between the reducing sugar contents of control tubers and those treated with either of the two chemical treatments until the beginning of April. Reducing sugar levels were significantly lower in Chlorpropham treated tubers than in controls of both cultivars from April until early June. From graphs 3.4 and 3.5 it can be seen that the differences are a consequence of a fall in the reducing sugar levels of Chlorpropham treated potatoes while the reducing sugar contents of controls remained stable. This effect may be linked to the different degrees of sprouting found in Chlorpropham and control treatments.

The reducing sugar contents of Tecnazene treated tubers follow those of the controls most closely of all the sprout inhibition treatments. Towards the end of storage however Tecnazene treated tubers appear to be slightly lower in reducing sugars than controls. From tables 3.2 and 3.3 however it can be seen that the reducing sugar

levels in Tecnazene treated tubers are not significantly different from those of the control at any sampling date, although graphs 3.4 and 3.5 show that as storage progresses there is a trend for Tecnazene treated tubers to have consistently slightly lower reducing sugar contents than controls.

Effect of sprouting on reducing sugars

Differences in sugar contents between treatments towards the end of the storage period appear to show that the degree of sprouting does exert some influence on reducing sugar levels. In treatments where no sprouting has taken place, that is in irradiated and Chlorpropham treated potatoes, there is a decline in reducing sugar contents until senescent sweetening intervenes. In Tecnazene treated potatoes and controls where sprouting does occur a more stable level of reducing sugars, similar to initial levels, is maintained, slightly increasing as storage progresses. The differences apparent between Tecnazene treated potatoes and controls may simply be one of degree. The sprouting of potatoes within the Tecnazene treatment was partially held back by Tecnazene resulting in more uneven, less extensive sprouting than in controls. Thus the process responsible for the increase in reducing sugars observed in vigorously sprouting controls may be at work to a lesser extent in the unevenly sprouting Tecnazene treated potatoes.

Effect of treatment on sucrose

It has been argued that the initial sucrose levels found in the potatoes used in this study may indicate some degree of immaturity. It can be seen from graphs 3.6 and 3.7 that the pattern of behaviour

of sucrose levels bears some resemblance to the pattern of behaviour of reducing sugars. Graphs 3.6 and 3.7 show that the initial rise in the level of sucrose in irradiation treatments does not appear to be as extreme as that observed in the level of reducing sugars. However this may be misleading, as Burton observed a rapid rise in sucrose in irradiated tubers followed by a reduction in sucrose content (Burton, 1959). Therefore, as was also noted in the discussion of reducing sugar levels, the maximum level may have been reached prior to the first date of measurement in this study.

After the initial temporary rise in sucrose in those treatments graphs 3.6 and 3.7 chart a slow fall in sucrose until mid-March in both cultivars. Sucrose levels in irradiated potatoes were significantly greater than control levels until mid-December in both cultivars and even until the beginning of April in cv. Record. No significant difference was found between irradiated and control treatments during the period from early January until mid-March in cv. Desiree.

From mid-March onwards the changes in sucrose levels within the two cultivars are different. In cv. Record those potatoes treated with Chlorpropham or irradiated continue to slowly decline in sucrose content. From table 3.3 it can be seen that sucrose levels from the beginning of April until the end of storage in irradiated and Chlorpropham treated cv. Record tubers were significantly lower than those in controls. In cv.Desiree the sucrose levels in Chlorpropham treated and irradiated potatoes level off during the period from mid March until early June. Between that time and the end of storage there is a sharp rise in the sucrose content of irradiated tubers and a less steep rise in that of Chlorpropham treated tubers, to a level

still below that of the controls. This can be clarified by reference to table 3.3 from which it can be seen that sucrose levels from mid-March until the end of storage in irradiated cv. Desiree tubers are significantly lower than in controls. Sucrose levels in Chlorpropham treated tubers are also significantly lower during that period with the exception of the mid-March measurement when there is no significant difference. Although there is a swift rise in sucrose content by the end of storage in irradiated treatments the sucrose level is still significantly lower than in controls at that date in cv. Desiree.

The sucrose contents of irradiated potatoes of cv. Desiree taken together with the information regarding their reducing sugar contents at the end of the storage season provides some strong evidence that senescent sweetening is occurring in cv. Desiree. The occurrence of senescent sweetening appears to be linked to treatment and the degree of sprouting present in each treatment. Senescent sweetening does not appear to be taking place within the storage period studied in irradiated or Chlorpropham treated cv. Record potatoes. This difference may be due to the effect of cultivar or to differing levels of maturity in the two cultivars.

The sucrose contents of Tecnazene treated tubers in the early part of the storage period closely follow those of controls. In both cultivars after the mid-March sampling date sucrose levels in Tecnazene treated and control tubers rose steadily, sucrose levels in Tecnazene potatoes lagging slightly behind those of the controls. There was no significant difference between the sucrose contents of Tecnazene treated and control potatoes from early December until mid-

March in cv. Record, however after the beginning of April sucrose levels were significantly lower. In cv. Desiree the pattern of sucrose fluctuations is more variable than was found in cv. Record, but generally speaking sucrose levels in Tecnazene treated tubers followed a similar trend to those in cv. Record. By the end of the storage period the sucrose contents of Tecnazene treated tubers of cv. Desiree were lower than those of the controls.

A comparison of the sucrose levels throughout storage in irradiated cv. Record potatoes shows that there was a significant difference between the two different doses on only one sampling date. This isolated difference seems unlikely to be reflecting a real effect. In cv. Desiree there are three significant differences in sucrose content between the two irradiation doses, however there is no clear trend. Increasing the radiation dose from 0.10 kGy to 0.15 kGy does not appear to have a marked effect on sucrose levels.

Effect of sugar changes on the viability of radiation treatment

The results of this study show that irradiation treatment in the dose range of 0.10 kGy to 0.15 kGy of cv. Record and cv. Desiree potatoes has a marked initial effect on both reducing sugar and sucrose levels. This effect is temporary and with time the contents of these sugars can fall to levels acceptable to processors.

The growth or inhibition of sprouting appears to have an effect on free sugar contents in the later part of the storage period, control of sprouting does not appear to lead to the accumulation of free sugars as has been previously suggested. In this study sugar contents were lower by the end of storage in those treatments where sprouting was inhibited completely.

Cv. Record did not suffer from early senescent sweetening induced by irradiation in this study and no special problems of that nature are anticipated in irradiating cv. Record.

If irradiation is to be considered as a method for the control of sprouting in potatoes stored for processing the initial rise in sugar contents need not be seen as an insurmountable obstacle. If a sufficient period of storage is allowed after irradiation treatment, free sugar levels will fall to adequately low levels.

A delay between the treatment and processing of irradiated potatoes becomes even more essential if, due to high free sugar contents at harvest, the sugar contents of potatoes prior to irradiation are already high. Routine monitoring of sugar contents at harvest should go some way towards anticipating such problems.

One further consideration should be made in deciding when irradiated potatoes should be processed. This study has not clearly shown whether or not senescent sweetening occurs earlier in the storage season in irradiated potatoes. Further work is required in this area. Irradiated potatoes should not, however, be stored indefinitely in the hope of lowering sugar levels to a minimum as the risk of senescent sweetening occurring becomes greater as the storage period continues.

Chapter 4

The rate of water loss from irradiated and chemically treated potatoes

4.1 Introduction

Weight losses from stored crops can represent a financial loss of some importance to processors, particularly when the final product is sold by weight as is the case with potato products. Potatoes lose weight during prolonged storage by two main mechanisms; respiration and evaporation. Of the two, evaporative water loss is by far the more economically important, as loss due to respiration is only responsible for approximately 0.1% loss in weight per month of storage (Nash, 1978). This value can be compared to a typical total weight loss of about 6% after 6 months of storage at $7^{o}C$ - $10^{o}C$ (Nash, 1978). Bishop and Maunders (1980) reported a 5.1% loss in weight from potatoes stored for 15 weeks at $10^{o}C$ and 83% relative humidity. A loss of 5 - 6% would still be regarded as tolerable by manufacturers although losses greater than 10% would be regarded as unacceptably high (Nash, 1978).

Mechanisms of weight loss

Nash (1978) lists the potential sites of evaporative water loss as; lenticels, skin, sprouts, disease lesions and cuts and abrasions. Nash (1978) states that by far the greatest part of water loss takes place through the relatively impermeable skin. The role of the potato skin as a barrier to weight loss will be discussed in more detail in the following text.

Lenticels, the equivalent of leaf stomata, through which gas exchange between the tuber and the store atmosphere takes place, cover

less than 1% of the potato tuber surface. Lenticels are covered in hydrophobic wax outgrowths which may limit transpiration while accommodating free gas exchange with the environment (Hayward, 1974).

The water lost through evaporation from sprouts can be responsible for a significant proportion of the total weight loss from potatoes as water loss occurs at a rate 30 to 40 times faster through sprouts than through the skin (Burton, 1966). The importance of sprouting to weight loss depends on the degree of sprout growth, which in turn is dependent on many environmental factors such as the storage temperature and humidity of the store, or other factors such as cultivar, whether sprouting has been deliberately inhibited and the length of time that potatoes have been in storage. It has been estimated by Burton (1966) that for every 1% by weight of sprout growth, weight loss increases by 0.08% $week^{-1}$ mb^{-1} VPD^{-1} (the vapour pressure deficit (VPD) is a measure of the capacity of the store air to hold more water vapour). Weight lost due to water loss through sprouts is compounded by the loss represented by the dry matter of the sprout itself. Burton (1972) reports an example of weight losses under normal farm storage conditions for 6 months from potatoes whose sprouting was not controlled chemically. Weight loss was found to be distributed as follows; evaporation 3.6%, sprouting 1.5% and respiration 0.6% (these results exclude losses due to disease the degree of which can fluctuate markedly).

Damage and disease work in tandem to influence weight loss. Damage to potatoes caused by harvesting or subsequent handling, in the form of surface "scuffing" or compression bruises or internal damage such as cuts or scrapes, leads to increased weight loss as the resultant

breaks in the skin provide a route for greater evaporative water loss. Damage to the potato skin also provides areas open to bacterial or fungal infection. Infected areas lose water at a greater rate than intact skin as the normal mechanisms by which water loss is minimised no longer operate properly in those areas. Those mechanisms are discussed in more depth later in the text.

Storage diseases can be categorised as surface diseases or diseases which cause internal rotting. Surface diseases are important to potato retailers, as unsightly infection may discourage consumers from buying their goods. However, in this study of potatoes to be used for processing surface infection is of little consequence as the potatoes are peeled before processing and the flesh below is largely unaffected by surface infections (McGee, 1984). Internal rotting can, however, be a major source of loss to processors. The storage diseases which cause internal rotting commonly encountered in the U.K. are Phytophthora infestans (blight fungus), Erwinia spp. (bacterial soft rot), Phoma spp. (gangrene fungus) and Fusarium spp. (fungal dry rot). The extent of losses due to infection by bacteria and fungi depends on the storage conditions, the condition of potatoes as they are brought into store and how quickly and effectively damage can be repaired by the potato's natural wound healing system. Therefore a wide variation in the degree of infection can be observed from store to store and season to season.

In this chapter an investigation is carried out to consider how various sprout inhibition treatments can affect the rate of weight loss from potatoes. As the rate of weight loss from potatoes is largely dependent on the effectiveness of the skin as a barrier to evaporation and pathogenic infection, the discussion will inevitably

centre on the effect such treatments have on the development and effectiveness of the skin. Therefore to more fully understand the effects of sprout suppressant treatments on weight loss some essential background information on the structure of potato periderm and how it forms is required.

The wound healing response of potato tubers

The development of potato periderm after harvest is reviewed by Peterson et al. (1985). Investigations have not yet progressed sufficiently for a full description of all of the stages of periderm development to be made. The periderm does, however, appear to develop in a broadly similar way to wound periderm, although initiation of periderm development and processes complementary to periderm formation may differ in each case.

The response of the potato to wounding has been the subject of much research and is reviewed by Burton (1966), McGee (1984), Kollatukudy (1984) and Peterson et al. (1985). Wounding potato tissue initiates an increase in DNA and protein synthesis in cells adjacent to the wound (Borchert and McChesnesy, 1973). This is followed by a breakdown in the intracellular membranes of such cells resulting in a complete disintegration of the endoplasmic reticulum (Camm and Towers, 1973). The endoplasmic reticulum then reconstitutes and produces droplets of lipid material which move outwards to the plasmalemma, pass through it and form a lipid layer known as suberin or suberin complex within the cell wall and between adjacent cells (Fox et al, 1972; Kollatukudy, 1984). Suberin has been shown to be a polymer of predominately ω-hydroxy and dicarboxylic fatty acids (Kollatukudy and Dean, 1974). The simultaneous deposition of shorter chain length

waxes within the suberin layer provides a completed hydrophobic layer.

In the 2 - 3 day period after wounding when the suberin layer is forming, lignin, a polymer of phenyl propanoids, is also deposited in the cell wall of the suberising cells. Kollatukudy (1978) postulates a structure for the suberin complex consisting of the linkage of suberin to the cell wall via lignin. Kollatukudy (1978) provides evidence that the link from lignin to suberin is made through ferulic acid, a lignin monomer.

This structure presents a two-fold barrier to infection. Lignin prevents the depolymerisation of the cell wall polysaccharides as it is resistant to the enzymic attack of pathogens, and suberin and its associated waxes form a hydrophobic layer blocking the access of pathogenic enzymes to the cell wall. The suberin layer also provides an impermeable layer through which water cannot easily pass.

After the formation of the suberised layer at the wounded surface, the cells directly below the wound begin to divide. The cell walls of sub-surface cells then become impregnated with suberin and waxes. Finally the surface cells collapse and die resulting in the formation of a dead skin with suberised coating above a layer of suberised live cells several cells deep. This process takes place on a rather longer time scale of 2 - 3 weeks when compared to the 2 - 3 days over which suberisation occurs.

Therefore wound healing can be seen as the combination of two distinctive processes; suberisation, marked by a laying down of lipid material at the wound surface, and periderm formation, marked by cell division and programmed cell death.

The effect of storage conditions on wound healing

Storage conditions during curing have a major effect on the rate of formation of wound periderm. The temperature of storage is one environmental factor of great significance, faster development of periderm was observed by Artschwager(1927) as storage temperature increased over the range of 2.5°C to 21°C. Wigginton (1974) found a three-fold increase in the rate of wound-healing between 5°C and 10°C and a further three-fold increase between 10°C and 20°C. Thomas (1982) observed that wound healing occurred most rapidly at 25°C, higher temperatures of 35°C retarded suberisation and prevented periderm formation. Dean (1989) measured the formation of aliphatic suberin monomers and alkanes and found the optimal temperatures for their syntheses to be 26.4°C and 18.6°C respectively.

The relative humidity of the store also influences the rate of wound healing. At low relative humidity (R.H.) desiccation of the surface occurs inhibiting wound healing, Wigginton (1974) found that at a R.H. of 30% and a storage temperature of 20°C suberisation was completely prevented. Higher humidities have been found to encourage more rapid wound healing (Artschwager, 1927; Wigginton, 1974) although at humidities approaching 100% proliferation can take place. Humidities of the order of 80 - 90%, are recommended by most researchers for optimal wound healing in a post-harvest curing period (Metlitsky et al., 1967; Wigginton, 1974; Nash, 1978). Burton (1966) advocates storage at as high as possible R.H. while warning that water must on no account be allowed to condense on the surface of potatoes in store.

Optimal conditions for wound healing may therefore be recommended

as greater than 80% R.H. and storage at 20°C - 25°C. The transport of potatoes to the store from different farms means that potatoes arrive at the store at different times and the process of putting a large amount of potatoes into a store also takes several weeks in a large store of 2000 - 5000 tonne capacity. These considerations mean that optimal conditions for wound healing are seldom, if ever, achieved.

The effect of sprout inhibition treatments on wound healing

Chlorpropham

The effect of Chlorpropham on wound healing and the rate of pathogenic infection of treated potatoes has been assessed by Craft and Audia (1959), Audia et al., (1962), Reeve et al., (1963), McGee (1984), Leonard et al., (1986) and Hide and Cayley (1987). In all of the above studies the inhibition of periderm formation and decay of Chlorpropham treated tubers was noted, although most workers reported no inhibition of the suberisation process. Audia et al., (1962) reported retardation of periderm formation on slices of potato tissue dipped in a 25 ppm solution of Chlorpropham and total inhibition of periderm formation when tissue was exposed to a 100 ppm solution. McGee (1984) found marked inhibition of periderm formation when potato tissue discs were exposed to both 10 and 100 ppm Chlorpropham solutions.

Several researchers have speculated that Chlorpropham may affect wound healing by disrupting the mitosis of periderm cells (Craft and Audia, 1959; McGee, 1984) by the same mechanism as the related chemical Propham. This theory is borne out by the evidence so far, as periderm formation is dependent on cell division while suberisation is not, and Chlorpropham affects periderm formation but does not appear

to affect suberisation. If this is indeed the mechanism by which Chlorpropham affects wound healing it demonstrates the importance of cell division in the formation of periderm to act as a barrier to water loss, as McGee (1984) and Leonard et al. (1986) found significantly lower resistance to water loss from discs of normally suberised, Chlorpropham treated potato tissue. A definitive experiment proving conclusively that Chlorpropham affects wound healing by inhibiting cell division has yet to be performed.

Tecnazene

The effect of Tecnazene on wound healing has been studied by Cunningham (1953), McGee (1984) and Leonard et al. (1986). Cunningham (1953) reported no inhibition of suberisation but did note some delay in periderm formation. Surprisingly, however, he did not state the application rate of Tecnazene which delayed suberisation and additionally Tecnazene treatment did not appear to control Fusarium infection in his study. McGee (1984) using resistance to water loss with time as a measure of the rate of wound healing, by the method of Jarvis and Duncan (1979), found that Tecnazene stimulated wound healing when potato tissue discs were exposed to a 10 ppm solution. In store experiments using a histological assessment of wound healing the same worker found inhibition of wound healing by Tecnazene but suggested that the inhibition may have been due to possible contamination from the store by Chlorpropham. Leonard et al. (1986) found that Tecnazene did not inhibit wound healing when assessed by either histological or water loss methods and that fungal disease development was reduced by Tecnazene development. The consensus of such investigations is unclear, but on balance it seems that Tecnazene

shows no great inhibitory effect on wound healing in the more rigorous studies. As yet no mechanism to explain how Tecnazene may stimulate wound healing has been advanced.

Dimethylnaphthalene

Little investigation of the effect of the Dimethylnaphthalenes (DMN's) on wound healing has taken place. The work carried out so far has been summarised by Duncan and Van Es (1988). Several DMN and trimethylnaphthalene isomers have been found to be active sprout suppressants (Meigh et al., 1973; Beveridge, 1979; Beveridge et al., 1981a; Filmer and Rhodes, 1984), their relative effectiveness has been linked to differences in volatility (O'Hagan et al., 1987). McGee (1984) found that the wound healing of potato tissue discs was inhibited by exposure to DMN solutions of 100 and 200 ppm but not when discs were exposed to a 10 ppm solution. McGee (1984) considered the effect of DMN to be similar to that of Chlorpropham, the rotting found in Chlorpropham treatments also occurred with DMN application. Water loss from treated discs of potato tissue was used to assess wound healing in McGee's study and no whole tuber study was carried out.

Application of a single dose of 100 mg DMN dust kg^{-1} of whole wounded potatoes was found to result in a small increase in water loss during the first month of storage (Duncan and Van Es, 1988) which may indicate some effect of the chemical on wound healing. Duncan and Van Es (1988) were of the opinion that a larger dose of DMN would be required to inhibit wound healing than would be necessary to control sprouting. Little is known regarding the mechanism by which DMN may affect wound healing.

Methanol

The effect of methanol treatment on wound healing is of interest as some chemicals, for example Chlorpropham, are applied as methanol aerosols in the U.K.. Some organic solvents, including ethanol and methanol, have been shown to inhibit wound healing (Jarvis and McGee, unpublished). McGee (1984) states that methanol slows down wound healing particularly in the early stages of development, wound healing was inhibited when potato tissue was exposed to a 1% aqueous methanol solution.

Irradiation

The effect of irradiation on wound healing of potatoes has been reviewed by Matsuyama and Umeda (1983) and Thomas (1984). In histological studies Thomas (1982) showed that suberisation was not inhibited by irradiation doses of up to 0.1 kGy, but with doses as low as 0.02 and 0.03 kGy wound periderm formation was prevented. This effect was presumably the result of radiation-induced inhibition of cell division in periderm cells below the surface which would normally divide successfully to form wound periderm.

Isleib (1957) noted that irradiation doses from 0.015 kGy to approximately 2 kGy prevented periderm formation and delayed suberisation, although the suberin layer was ultimately as well developed as that of controls. Henriksen (1960) found slower suberisation in potatoes irradiated at 0.1 kGy than in controls and total inhibition of periderm formation in the irradiated treatment. Henriksen (1960) also suggested that the inhibition of wound healing by irradiation treatment would make irradiated potatoes more susceptible to attack by bacteria and fungi.

Metlitsky et al. (1967) reported that irradiation treatment did indeed decrease the natural resistance of potatoes to infection by microorganisms. Potato slices irradiated at a dose of 0.1 kGy and inoculated with blight fungus became infected to an extent 4 times greater than unirradiated slices. In a storage experiment reported by the authors in the same study 29.7% of potatoes stored after a 0.1 kGy irradiation treatment were infected by pathogens while only 20.8% of control tubers were infected. A total of 3.8% of irradiated tubers were completely rotted in comparison with only 0.8% of controls.

Experimental objectives

The work carried out in this study sets out to investigate the weight losses over a typical storage period from cv. Record potatoes that had been irradiated or treated with various chemical sprout suppressants. The relative weight losses from each treatment were of interest as an indicator of the effect of the treatments on the effectiveness of the potato periderm as a barrier to water loss.

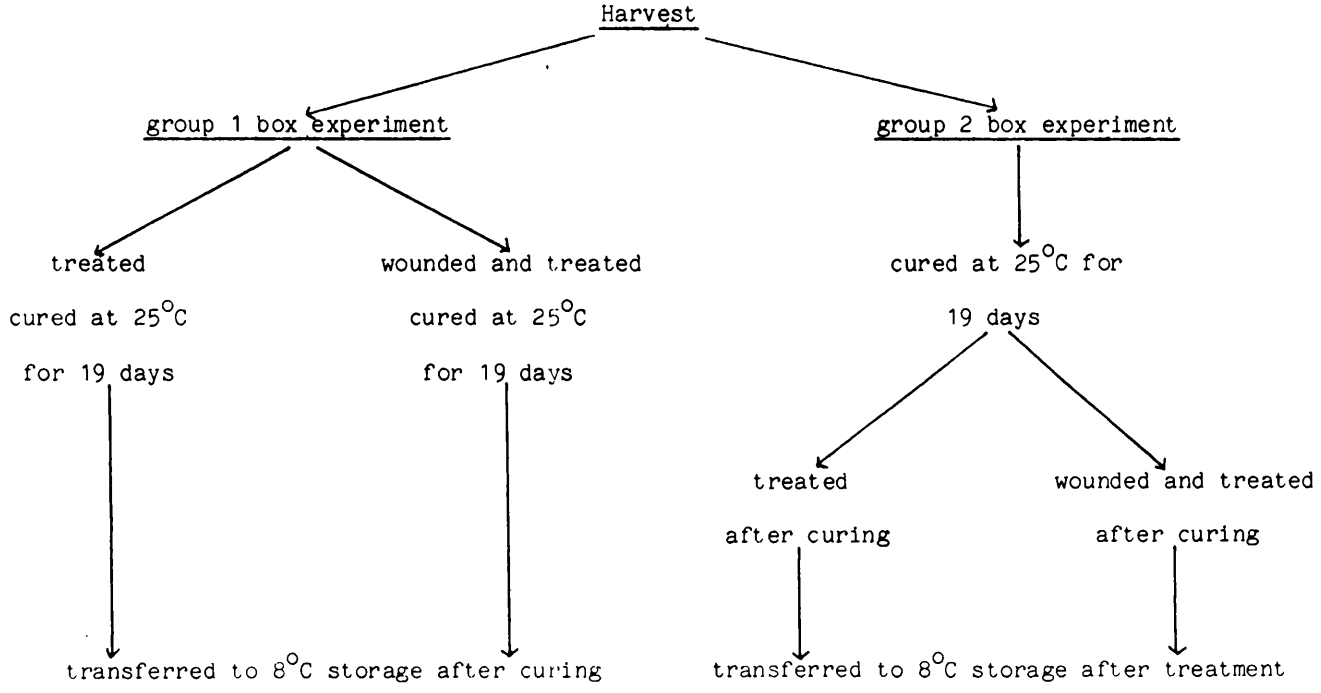
The investigation undertaken was divided into several comparisons. Firstly the potatoes were divided into two groups; group 1 to be treated directly after harvest, cured and stored, and group 2 to be cured before treatment and storage. This comparison was intended to establish how large the effect of treatment was during the early stages of storage, as curing takes place after harvest (that is, when cell division and suberisation occur to form the potato periderm).

The second comparison carried out in the study was to deliberately wound half of the potatoes within each treatment of each group. This comparison was intended to determine the effect of treatment on the

normal wound healing response within groups 1 and 2. A more detailed description of the experimental design of this study is to be found in section 4.2 of this chapter. Diagram 4.1 gives a summarised description of the experimental design.

In was hoped by these comparisons to more clearly understand the gross effects of sprout inhibition treatments on weight losses from whole tubers on a large experimental scale and to discuss the effects found in the light of previous potato tissue studies.

Diagram 4.1. Summary of the experimental design of a study of weight losses from control, chemically treated and irradiated cv. Record potatoes.



4.2 Experimental

Potatoes used in this experiment were generously donated by United Biscuits (U.K.). 1600 kg of cv. Record potatoes were harvested as part of a crop grown under contract by H. and L. Aykroyds in Cushnie, Auchterless in Aberdeenshire for United Biscuits on September 27, 1986. These tubers were then transported as soon as possible to Glasgow University where they were divided into 10 kg lots and placed in pre-weighed cardboard boxes of dimensions 38.5 cm X 31.0 cm X 15.5 cm. As some moisture was still visible on the potatoes and the soil adhering to them at this time, the potatoes were aired overnight at room temperature. These replicate boxes were then divided into two groups.

Group 1 box experiment

The first group of boxes was treated immediately, six replicate boxes of each treatment. The first two boxes in each treatment were simply treated, closed and weighed. Tubers in a second pair of boxes were deliberately wounded prior to treatment. Wounding was carried out by cutting a slice with a knife from each tuber of approximately 3 cm in diameter and 5 mm in depth. The wounded tubers were then returned to the boxes, treated and weighed. These two pairs of boxes were to remain closed throughout storage and represented a comparison of the rates of water loss in wounded and non-wounded treated tubers. The third pair of boxes in each treatment were treated in the same way as the first pair. The use to which the third pair of boxes was put is described in the following text.

The treatments used in the group 1 study were control, Chlorpropham, Tecnazene, a mixed isomer preparation of Dimethylnaphthalene (Aldrich, U.K), and methanol. (The mixed isomer preparation of Dimethylnaphthalene, consisting of a mixture of 1,6-Dimethylnaphthalene and other isomers in smaller quantities, was used as it was thought that it was more likely to be used as a future commercial formulation, on economic grounds, than any of the pure isomers (Beveridge et al., 1981b). Chlorpropham, Tecnazene and DMN were absorbed onto 25 g of alumina using the method described in section 3.2.1 of Chapter 3 and applied as a dust. Chlorpropham and Tecnazene were applied at the normal commercial doses of 20 mg kg^{-1} potatoes and 135 mg kg^{-1} potatoes respectively. DMN was formulated and applied in the same way at a dose of 100 mg kg^{-1} potatoes, this represents the effective sprout suppressing dose for that chemical (Beveridge, 1979).

Control tubers were treated with 25 g of alumina to account for any effect on the rate of water loss that the alumina in sprout suppressant formulations may have had. Methanol (Rathburn, U.K.) was applied as a spray using a Laboratory Spray Gun (Shandon Southern Products, U.K.) at the application rate of 2.5 cm^3 kg^{-1} potatoes. The boxes treated with methanol were then aired for 2.5 hours after treatment before being closed and weighed.

It was intended to include two further treatments of irradiated potatoes in this group. This was not possible as at the time of harvest the facility at the S.U.R.R.C. within which the ^{60}Co source is situated was closed for essential maintenance. Maintenance was, however, completed by the end of the curing period and irradiation treatments were included in group 2.

After treatment on October 2 each box was weighed using a Salter model 1325 electronic balance to an accuracy of $\pm$ 0.05 kg. The boxes were placed in a constant temperature room and stored at 25°C $\pm$ 1°C for 19 days for curing after which they were transferred on October 21 to a constant temperature room at 8°C $\pm$ 1°C for the remainder of the experiment. Each box was subsequently weighed periodically throughout storage and the relative humidity of the constant temperature room monitored.

Group 2 box experiment

The potatoes in the second group of boxes were cured before treatment by storing them at a constant temperature of 25°C for 19 days. The treatments used in this group were the same as those used on group 1 boxes; control, Chlorpropham, Tecnazene, DMN and methanol, with the addition of two irradiation treatments of 0.10 kGy and 0.15 kGy and a further control treatment. The potatoes in the second control treatment were transported to the irradiation facility at S.U.R.R.C. with the samples to be irradiated and since those controls were for comparison with irradiated potatoes they were not dusted with alumina.

Chemical treatments were applied by the same method and at the same doses as for group 1 boxes. Treatments were carried out on October 21. Six replicate boxes were used - two wounded and four non-wounded. Irradiations were carried out as described in Chapter 2 and irradiated and control tubers returned to Glasgow University. All group 2 boxes were weighed after treatment and transferred to a constant temperature room at 8°C $\pm$ 1°C for the remainder of the experiment. They were

subsequently re-weighed periodically and the relative humidity of the constant temperature room was monitored.

In both group 1 and group 2 boxes, at each weighing date, samples were removed from one box of the third pair of replicates in each treatment. These samples were used for periderm isolation as outlined in Chapter 5. When samples were taken a visual assessment of the general level of sprout development and degree of infection was undertaken in that pair of boxes, as it was thought unwise to disturb the rate of water loss in the other four replicate boxes of each treatment.

Boxes were stacked in the constant temperature room in pairs, each pair consisting of a wounded and non-wounded replicate of a treatment. After each weighing top and bottom boxes were swapped to even out any resistance to water loss caused by box position.

The study was concluded on March 31 as it had become apparent that levels of sprouting and rotting in some treatments were having a great influence on weight losses by that date.

4.3 Results

Relative Humidity

The relative humidity in the constant temperature room in which curing took place at 25°C was found to be 41% ± 2%. This is considerably lower than would have been desirable for optimal wound healing, but other, more suitable facilities were not available. Under these conditions drying of the wounds on wounded potatoes was observed and the wounds of those tubers had powdery surfaces presumably due to the drying of starch.

The relative humidity of the constant temperature room used for storage at 8°C was measured to be 95% ± 2%.

Sprouting

Sprouting was completely inhibited throughout storage in potatoes treated with Chlorpropham or irradiated at 0.10 or 0.15 kGy. Control tubers had sprouts of approximately 20 mm by December 10 in both groups of potatoes, and by the end of the storage period sprouts were on average approximately 200 mm in length. Methanol did not appear to inhibit the sprouting of potatoes treated with it, the level of spouting in methanol treated tubers was visually indistinguishable from that in controls.

Tecnazene inhibited sprouting temporarily and in an uneven way as was previously observed in Chapter 3. Little sprouting was observed until the beginning of February in Tecnazene treated potatoes when it reached approximately 10 mm in both groups of potatoes. By the end of the storage period sprouts had grown to approximately 20 mm at the centre of each box but to nearly 100 mm at the edges of the boxes.

DMN did not appear to inhibit sprouting at all effectively in this study, although the sprouting of DMN treated tubers appeared to lag marginally behind control tubers. At the onset of sprouting the difference was small, sprouting was approximately 15 mm by December 10. By the end of the storage period the degree of sprouting in DMN treated potatoes was no different to that in controls.

Rotting

As was expected rotting occurred to a very much greater extent in wounded potatoes than in non-wounded potatoes. This was the case in potatoes of all treatments. Towards the end of the study all wounded potatoes of all treatments in both groups had severely rotted.

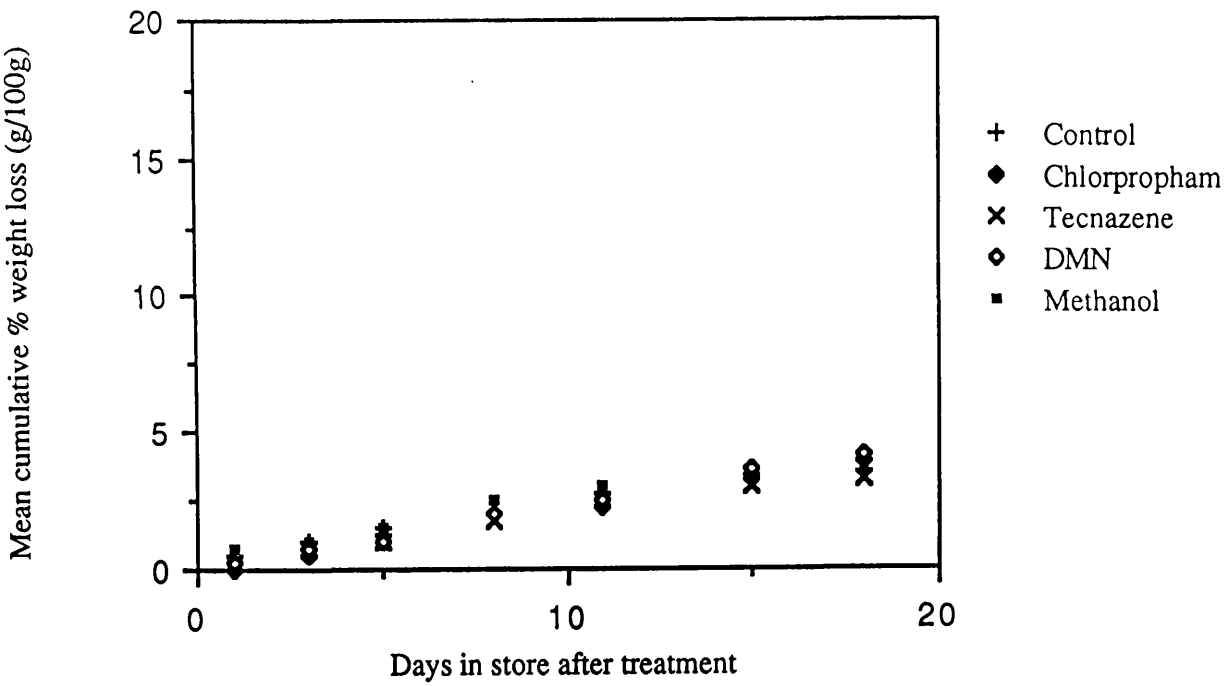
From the visual assessments carried out it was not possible to determine whether Tecnazene treatment restricted rotting in wounded potatoes to any appreciable extent. Similarly it was not possible to detect any obvious acceleration of rotting in other treatments such as Chlorpropham treated or irradiated potatoes when compared to controls.

Little rotting occurred in non-wounded treatments in either group 1 or group 2 studies by the end of the storage period studied. The amount of rotting observed, typically 2 - 3 tubers rotted in a box containing approximately 60 - 70 tubers, was too small for a valid comparison of the effects of the treatments used on the rotting of non-wounded tubers.

Weight loss

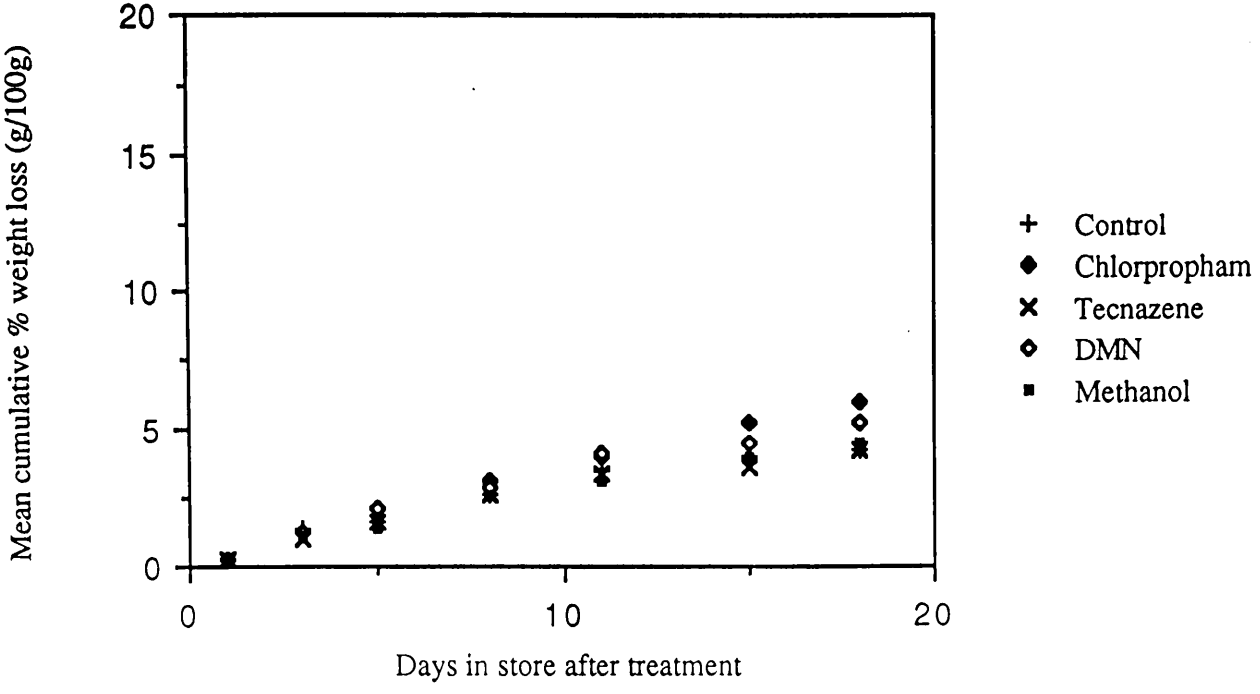
The cumulative weight loss from each box by each measurement date was calculated and expressed as a percentage of the weight of tubers after treatment. The mean of the two replicate boxes, wounded or non-wounded, in each treatment was calculated. These mean percentage weight losses were plotted against time and are presented as graphs 4.1 to 4.8.

Graph 4.1. Mean cumulative percentage weight losses* from non-wounded control and chemically treated cv. Record potatoes. Weight losses from harvest until the end of a 19 day curing period at 25°C from potatoes treated immediately after harvest (group 1).



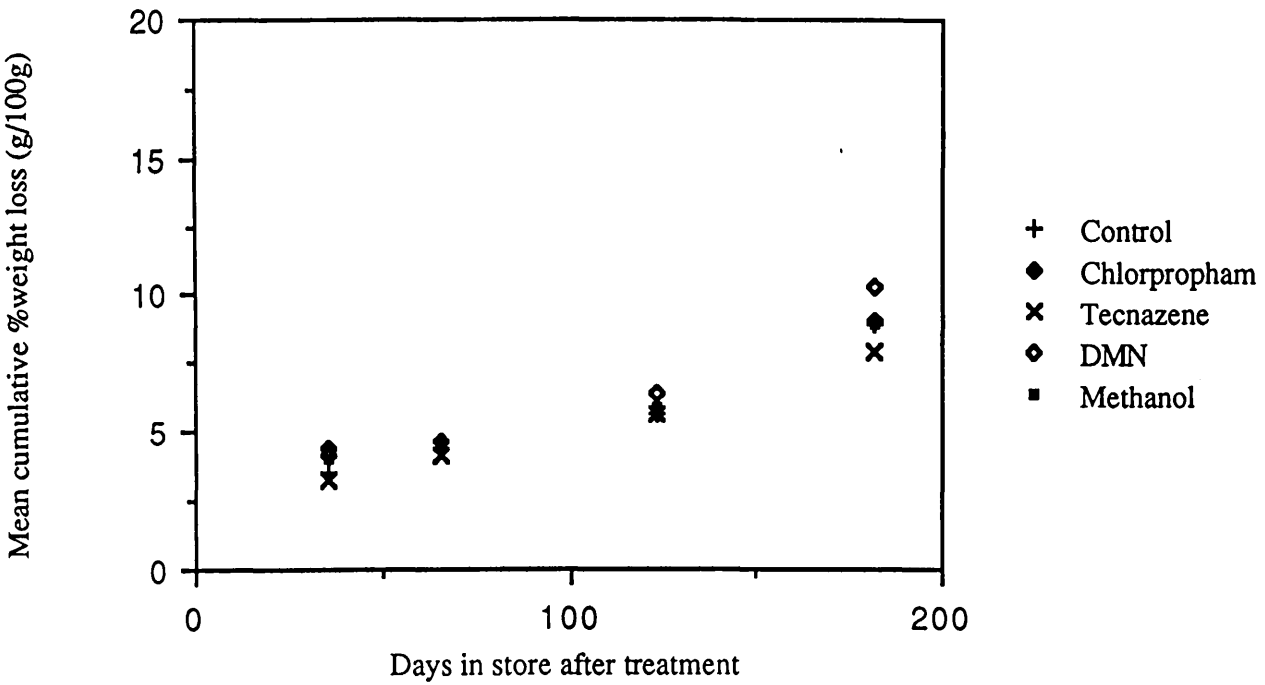
* each point is the mean of two replicates.

Graph 4.2. Mean cumulative percentage weight losses* from wounded control and chemically treated cv. Record potatoes. Weight losses from harvest until the end of a 19 day curing period at 25°C from potatoes treated immediately after harvest (group 1).



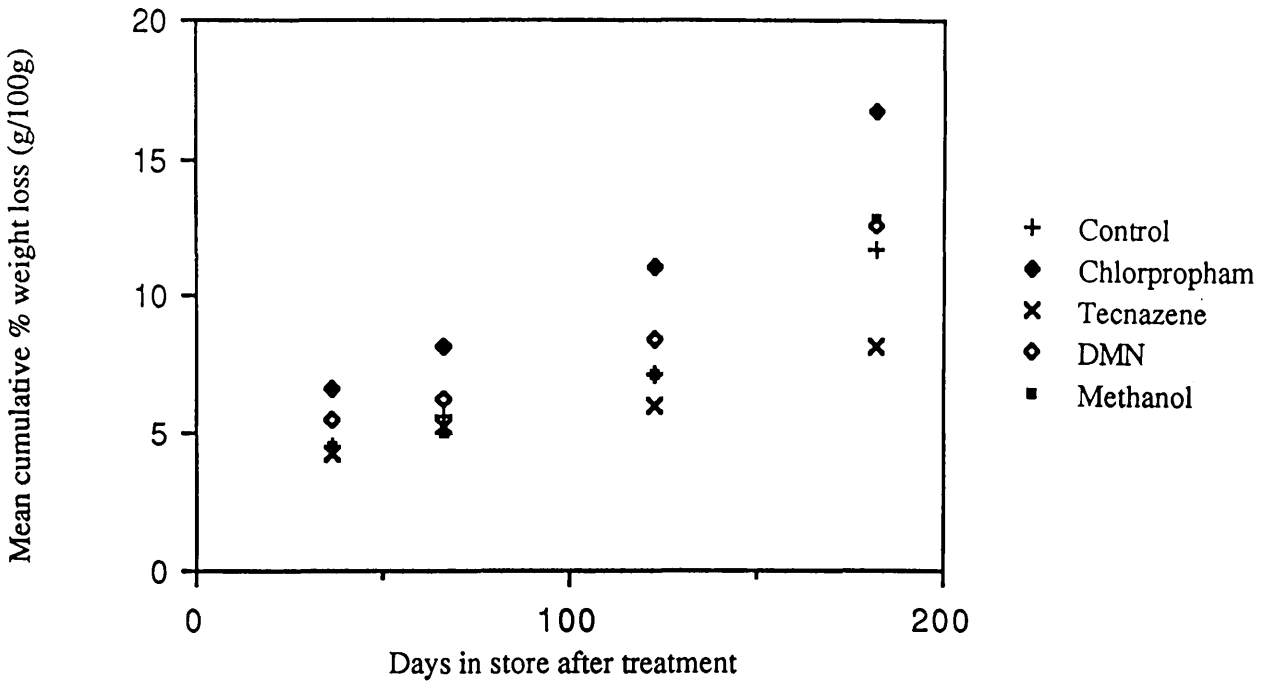
* each point is the mean of two replicates.

Graph 4.3. Mean cumulative percentage weight losses* from non-wounded control and chemically treated cv. Record potatoes. Weight losses from the end of a 19 day curing period at 25°C after harvest until 182 days after harvest. Potatoes treated immediately after harvest and stored at 8°C after curing (group 1).



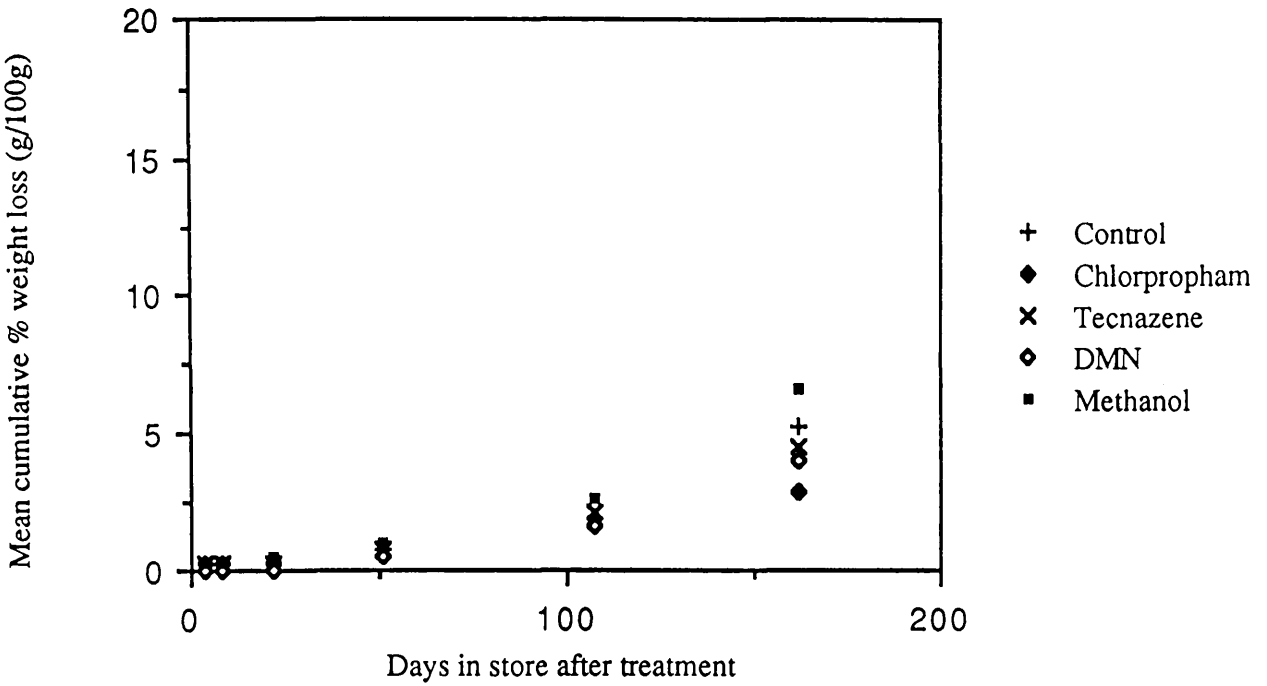
* each point is the mean of two replicates.

Graph 4.4. Mean cumulative percentage weight losses* from wounded control and chemically treated cv. Record potatoes. Weight losses from the end of a 19 day curing period at 25°C after harvest until 182 days after harvest. Potatoes treated immediately after harvest and stored at 8°C after curing (group 1).



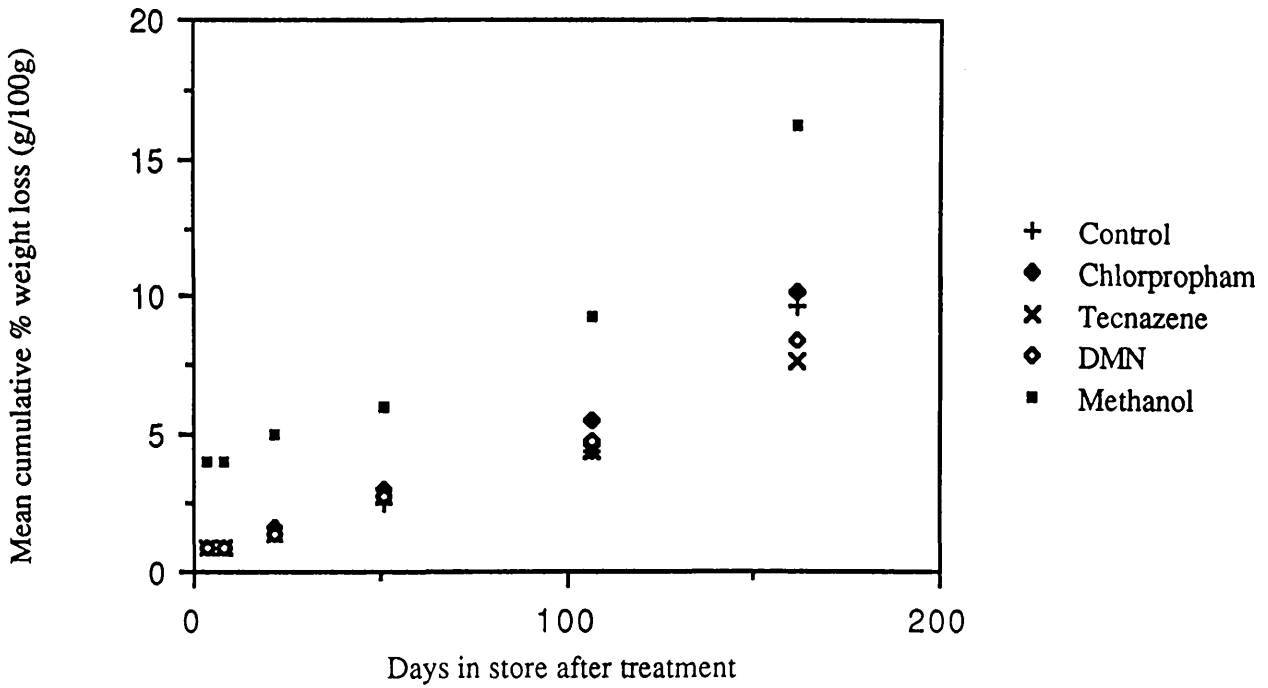
* each point is the mean of two replicates.

Graph 4.5. Mean cumulative percentage weight losses* from non-wounded control and chemically treated cv. Record potatoes. Weight losses from harvest until 162 days after harvest. Potatoes cured at 25°C for 19 days, treated and stored at 8°C (group 2).



* each point is the mean of two replicates.

Graph 4.6. Mean cumulative percentage weight losses* from wounded control and chemically treated cv. Record potatoes. Weight losses from harvest until 162 days after harvest. Potatoes cured at 25°C for 19 days, treated and stored at 8°C (group 2).

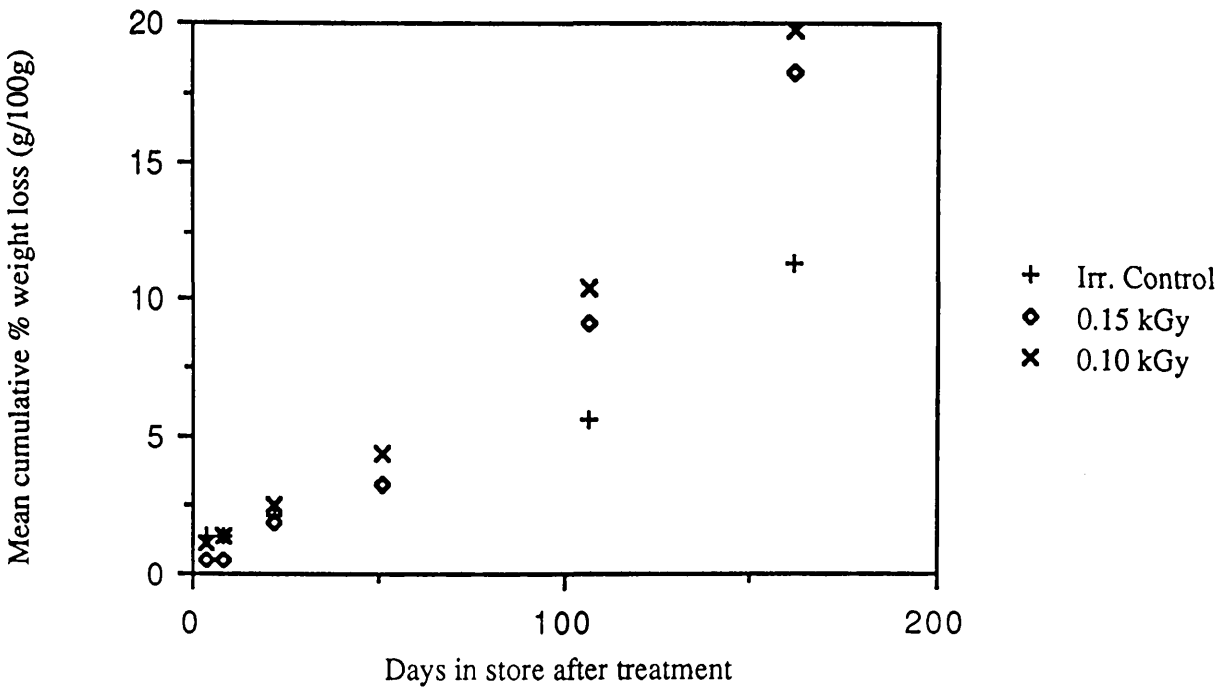


* each point is the mean of two replicates.

Graph 4.7. Mean cumulative percentage weight losses* from non-wounded control and irradiated cv. Record potatoes. Weight losses from harvest until 162 days after harvest. Potatoes cured at 25°C for 19 days, treated and stored at 8°C (group 2).

* each point is the mean of two replicates.

Graph 4.8. Mean cumulative percentage weight losses* from wounded control and irradiated cv. Record potatoes. Weight losses from harvest until 162 days after harvest. Potatoes cured at 25°C for 19 days, treated and stored at 8°C (group 2).



* each point is the mean of two replicates.

From graphs 4.1 to 4.8 it can be seen that although cumulative percentage weight losses increase with time of storage the relationships are non-linear. The data was therefore transformed into a linear form prior to attempts to find relationships between weight loss and time stored by regression. From graphs 4.1 to 4.4 it is also apparent that weight losses in the group 1 experiment occur at two different rates; during the early part of storage and subsequently. For that reason the two parts of those graphs were plotted and regressed separately.

Several different transformations of the data were investigated with varying degrees of success. It was found that of those transformations used a $\log_e$ transformation of the cumulative percentage weight loss gave the best improvement of the latter part of the group 1 data. Group 2 data was found to be best linearised by plotting the square root of the cumulative percentage weight loss.

None of the transformations studied improved the earlier section of group 1 data significantly compared with the simple linear regression model. As exhaustive testing of many different transformations would have resulted in the increasing probability of finding a nonexistent empirical relationship between the two variables it was decided to use the linear regression model for that data.

Regression constants a and b of the regression equations describing the weight loss relationships for each treatment are contained in tables 4.1 to 4.3. The equations are of the following form,

Group 1 (19 day curing period)	$y = a + bx$
Group 1 (post-curing storage period)	$\log_e y = a + bx$
Group 2 (curing and storage period)	$\sqrt{y} = a + bx$

where y is the cumulative percentage water loss and x is the time in store after treatment. The coefficient of determination values, R^2, for those equations are also contained in tables 4.1 to 4.3 as are the coefficients of determination of linear regression lines, R_L^2, for comparison with those of transformed data where appropriate.

The slope, b, of the least-squares regression line for each treatment was compared with the slope of the relevant control line in a test of homogeneity. The slopes of treatment lines that were significantly different at the 5% level from the control line slope are indicated in tables 4.1 to 4.3.

Table 4.1. Linear regression constants describing the relationships between cumulative percentage weight losses from control and chemically treated cv. Record potatoes and time stored. Weight losses during a 19 day curing period at 25°C from wounded and non-wounded potatoes treated immediately after harvest (group 1).

	Non-wounded			
Treatment	a	b	s.e. of b	R^2
Control	0.39	0.19	0.014	97.4
Chlorpropham	-0.10	0.23	0.012	98.7
Tecnazene	0.19	0.19	0.013	97.8
DMN	0.04	0.23	0.008	99.4
Methanol	0.68	0.19	0.018	95.5

	Wounded			
Treatment	a	b	s.e. of b	R^2
Control	0.49	0.23	0.024	95.0
Chlorpropham	0.27	0.33*	0.014	99.1
Tecnazene	0.42	0.23	0.024	94.9
DMN	0.47	0.28	0.027	95.7
Methanol	0.25	0.25	0.017	97.7

a is the y-intercept, b the slope, s.e. the standard error and R^2 the coefficient of determination. * indicates a slope significantly different from the control value at the 5% significance level.

Table 4.2. Linear regression constants describing the relationships between $\log_e$ of the cumulative percentage weight losses from control and chemically treated cv. Record potatoes and time stored. Weight losses from wounded and non-wounded potatoes stored at 8°C for 163 days following treatment immediately after harvest and a 19 day curing period at 25°C (group 1).

Non-wounded

Treatment	a	b	s.e. of b	R^2	R_L^2
Control	1.05	0.0061	0.00051	98.6	94.8
Chlorpropham	1.24	0.0051	0.00046	98.4	96.5
Tecnazene	1.00	0.0059	0.00014	99.9	99.0
DMN	1.14	0.0063	0.00055	98.5	94.4
Methanol	1.18	0.0053	0.00054	98.0	94.2

Wounded

Treatment	a	b	s.e. of b	R^2	R_L^2
Control	1.28	0.0063	0.00058	98.3	94.2
Chlorpropham	1.67	0.0062	0.00024	99.7	97.5
Tecnazene	1.33	0.0042	0.00052	97.1	96.8
DMN	1.48	0.0056	0.00037	99.1	95.9
Methanol	1.19	0.0071	0.00085	100.0	91.8

a is the y-intercept, b the slope, s.e. the standard error and R_L^2 and R^2 the coefficients of determination for linear and transformed data respectively. * indicates a slope significantly different from the control value at the 5% significance level.

Table 4.3. Linear regression constants describing the relationships between the square root of cumulative percentage weight loss from control, irradiated and chemically treated cv. Record potatoes and time stored. Weight losses from wounded and non-wounded cv. Record potatoes during a 19 day pre-treatment curing period at 25°C and subsequent storage at 8°C for 143 days (group 2).

	Non-wounded					Wounded				
Treatment	a	b	s.e. of b	R^2	R_L^2	a	b	s.e. of b	R^2	R_L^2
Control	0.028	0.014	0.00115	97.3	91.4	0.869	0.013	0.00090	98.2	94.2
Chlorpropham	0.412	0.008*	0.00049	98.4	96.4	0.895	0.014	0.00068	99.1	98.0
Tecnazene	0.390	0.010	0.00056	98.9	95.3	0.898	0.012	0.00069	98.6	98.4
DMN	-0.105	0.013	0.00090	98.1	93.5	0.891	0.012	0.00061	99.1	98.5
Methanol	0.416	0.013	0.00067	98.9	92.2	1.902	0.012	0.00084	98.2	95.2
Irrad^n Control	1.020	0.009*	0.00074	97.1	94.4	1.101	0.013	0.00071	98.9	95.4
0.10 kGy Irrad^n	0.853	0.007*	0.00036	99.0	98.0	1.023	0.021*+	0.00041	99.8	97.3
0.15 kGy Irrad^n	0.510	0.011	0.00064	98.7	98.0	0.695	0.022*+	0.00090	99.3	96.6

a is the y-intercept, b the slope, s.e. the standard error and R_L^2 and R^2 the coefficients of determination for linear and transformed data respectively. * indicates a significant difference from the control slope at the 5% level. + indicates a significant difference from the irradiation control slope at the 5% level.

Overall cumulative percentage weight losses were assessed using an analysis of variance procedure by selecting the total mean percentage weight loss at the end of the storage period from each treatment, wounded and non-wounded, in each group and comparing them to the appropriate control value. Additionally, for group 1 treatments the cumulative percentage weight losses by the end of the curing period were compared with the losses from controls at that point. Significant differences were identified using Duncan's New Multiple Range Test (DMRT). The results of the multiple comparison tests are contained in tables 4.4 and 4.5.

Table 4.4. Mean cumulative percentage weight losses from control and chemically treated wounded and non-wounded cv. Record potatoes. Potatoes treated immediately after harvest, cured for 19 days at 25°C and subsequently stored at 8°C for a further 163 days (group 1).

Weight losses at end of the 19 day curing period.

	Non-wounded		Wounded	
Treatment	Mean Percentage Weight Loss	Standard Deviation	Mean Percentage Weight Loss	Standard Deviation
Control	3.58	0.039	4.27	0.048
Chlorpropham	3.86	0.350	6.08*	0.329
Tecnazene	3.32	0.350	3.39	1.188
DMN	4.11	0.015	5.26	0.000
Methanol	3.83	0.361	4.54	0.344

Weight losses at the end of 182 days storage.

	Non-wounded		Wounded	
Treatment	Mean Percentage Weight Loss	Standard Deviation	Mean Percentage Weight Loss	Standard Deviation
Control	8.95	0.988	11.74	0.887
Chlorpropham	9.00	1.058	16.67*	0.249
Tecnazene	7.93	0.333	8.22	0.406
DMN	10.28	0.690	12.63	0.000
Methanol	8.93	0.361	12.83	0.659

* denotes a significant difference from the control value at the 5% level as determined using DMRT. Non-wounded and wounded losses were significantly different at the 5% level in a t-test.

Table 4.5. Mean cumulative percentage weight losses from control, irradiated and chemically treated wounded and non-wounded cv. Record potatoes. Potatoes were cured after harvest for 19 days at 25°C, treated and subsequently stored at 8°C for a further 143 days (group 2).

	Weight losses at the end of 162 days storage.			
	Non-wounded		Wounded	
Treatment	Mean Percentage Weight Loss	Standard Deviation	Mean Percentage Weight Loss	Standard Deviation
Control	5.32	0.040	9.67	0.315
Chlorpropham	2.92*	0.342	10.22	0.471
Tecnazene	4.51	0.426	7.72	1.588
DMN	3.99*	0.376	8.38	0.066
Methanol	6.63*	0.350	16.19*	2.058
Irradiation Control	5.32	0.670	11.35	1.529
0.10 kGy Irradiation	4.21*+	0.500	19.73*+	0.425
0.15 kGy Irradiation	5.14	0.000	18.28*+	1.026

* denotes a significant difference from the control value.

+ denotes a significant difference from the irradiation control.

Significant differences between treatments were determined using DMRT and are differences at the 5% significance level.

Weight losses from non-wounded and wounded potatoes were t-tested and were significantly different at the 5% level.

4.4 Discussion

4.4.1 Cumulative percentage weight losses

The effect of wounding on weight loss

The values obtained in tables 4.4 and 4.5 indicate that as expected wounded potatoes lost more weight during storage than non-wounded potatoes (significantly different at the 5% level in a pooled t-test) although the magnitude of that difference is dependent on treatment. Such differences emphasise the importance of maintaining an intact skin on potatoes to limit evaporative water loss.

The effect of curing conditions on weight loss

A comparison of the weight losses from cured potatoes and those measured from harvest onwards in table 4.4 reveals that a substantial proportion of the weight lost from potatoes of all treatments is lost in the initial curing period. This is not simply an effect of treatments on wound healing, as can be seen from the relative control values in tables 4.4. For example from table 4.4 it can be seen that in non-wounded potatoes during the 19 day curing period 3.6% of the initial weight is lost from group 1 controls, while only a further 5.4% is lost in the following 163 days of storage. More rapid weight loss is found from both wounded and non-wounded treatments.

It was observed that in tubers wounded and cured at 25^{o}C and 40% relative humidity the wounds became dry and had a powdery appearance. This effect was probably due to the deposition and drying of starch from disrupted cells at the surface of the wounds, such drying had been observed by Wigginton (1974). At a temperature of 8^{o}C and 95% relative humidity such drying and deposition did not take place. Thus

the conditions in the group 1 study may have limited weight loss in a different way to normal wound healing. This fact should be borne in mind in comparisons of group 1 and group 2 studies as they may not be strictly comparable in all cases.

The effect of treatment on weight loss

Methanol

The single exception to the pattern that cured potatoes lose less weight than those treated before curing is to be found with wounded methanol treated tubers. In tubers treated before curing methanol had no significant effect on weight loss as can be seen from table 4.4. However, table 4.5 shows that both non-wounded and wounded cured tubers treated with methanol immediately prior to removal to storage at $8^{o}C$ lost significantly more weight than controls. Reference to graph 4.5 reveals that there is a large difference between the last two measurements of weight loss from control and methanol treatments of non-wounded group 2 tubers. This rise is probably due to increased water loss through sprout tissue in those treatments as it coincides with increasing sprout growth. The difference between the control and methanol treatments although statistically significant is not numerically very large and may in fact be due simply to the natural variation in sprout levels often found in potatoes.

The effect of methanol on wounded group 2 tubers however is very large, the weight loss from methanol treated tubers is almost twice that measured from controls. The effect of methanol on the wound healing process at $8^{o}C$ may be due to the cytotoxic effect of methanol or to an interruption of suberisation of the wound or the structure of

the suberin layer in the non-wounded areas of the tubers. Organic solvents have been shown to dissolve wax material from the surface of periderms (Hayward, 1974) and surface waxes have been shown to be important in restricting water movement through the periderm (Soliday et al., 1979; Espelie et al., 1980).

Why then does methanol have no effect on the wound healing of tubers cured at 25°C after treatment? The answer may be that the important limit to weight loss under those conditions is the effect of starch deposition and drying rather than normal wound healing. If methanol treatment had little or no effect on that process it would not influence weight loss in those tubers.

Chlorpropham

Of all the chemical treatments applied before curing only wounded Chlorpropham treated tubers had lost significantly more weight than the controls by either the end of the curing period or the end of the study as can be seen from table 4.4. These results are as would be expected if Chlorpropham affects wound healing by the same mechanism as it inhibits sprouting, by interrupting cell division. When Chlorpropham is dusted onto potatoes, as in this study, it may not be able to penetrate the suberin layer sufficiently to reach the actively dividing periderm cells below in non-wounded tubers. In wounded tubers, however, at the surface of the wound those cells will be in direct contact with the dust formulation and thus with Chlorpropham. The inhibition of the division of those cells by Chlorpropham may result in a less well developed periderm which would be less effective as a barrier to water loss.

If this is the correct interpretation of the observed differences in weight losses, it would be expected that a Chlorpropham dust application would not increase the rate of weight loss from tubers if it was applied after curing when the suberin layer and periderm would be better developed. A difference would also be expected between wounded and non-wounded cured tubers, with a much greater weight loss expected from the exposed wounded tubers.

Table 4.5 however does not paint such a clear picture. The cumulative percentage weight losses from Chlorpropham treated non-wounded tubers treated after curing were not greater than from controls, they were, conversely, significantly lower. The weight losses from wounded Chlorpropham treated tubers in group 2 are also not significantly greater than those from controls.

These observations can be interpreted more clearly when the relative degrees of sprouting found in control and Chlorpropham treated tubers are taken into account. In the case of non-wounded tubers, sprouting controls lose more water through the relatively more permeable sprouts than through the skin. Chlorpropham treated tubers did not sprout at all and therefore would not have a contribution to weight loss from sprouting, resulting in lower weight losses from Chlorpropham treated tubers than from controls. Graphs 4.5 and 4.6 show that in controls there is a large increase in cumulative percentage weight loss in the final storage interval when sprouting was increasing substantially. Further evidence for the importance of sprouting to weight loss in the latter part of the storage season can be found by reference to Wilson *et al.* (1987) in which weight losses at an earlier stage of storage in this study point to less of a difference between Chlorpropham and control treatments at that time.

The cumulative percentage weight losses from wounded Chlorpropham treated tubers were much greater than from non-wounded Chlorpropham treated tubers. This may be the result of greater bacterial and fungal infection of less effectively wound healed Chlorpropham treated wounded tubers. Although no visible increase could be observed in the degree of infection when Chlorpropham treated tubers were compared to controls, other studies have concluded that greater infection does occur (Audia et al., 1962; Reeve et al., 1963; McGee, 1984; Leonard et al., 1986). The observation that there is no significant difference in weight losses between control and Chlorpropham treatments may once more be due to the difference in sprout growth in those two treatments. The effect of Chlorpropham on wound healing may be masked in this case by the effect of water loss from sprouts in controls.

Chlorpropham and Methanol

Chlorpropham was applied in this study as a dust formulation. However, in most commercial stores in the U.K. Chlorpropham is applied as a methanol aerosol using a thermal fogging apparatus such as a Swingfog machine (Bishop and Maunders, 1980). The combination of methanol and Chlorpropham in one treatment is likely to combine the adverse effects of both treatments on weight loss. Methanol may also afford greater penetration of the Chlorpropham into the periderm cells of uncured potatoes. In cured potatoes, periderm cells previously protected by the suberin layer may be exposed to attack by Chlorpropham. The importance of the effect of Chlorpropham on those cells is likely to depend on how well developed the periderm barrier is below the suberised cell layer.

Tecnazene

Tecnazene treated tubers lose consistently less weight than controls throughout the study although at no time significantly so. A possible explanation for this effect may be that sprouting was partially inhibited by Tecnazene treatment resulting in less evaporative water loss through sprouts but unlike Chlorpropham, Tecnazene did not affect periderm formation. This effect can be illustrated by comparing the weight losses from Tecnazene and Chlorpropham treated wounded tubers. (The weight losses from Tecnazene treated tubers were found to be significantly lower than from Chlorpropham treated tubers at the 5% significance level using DMRT in the case of cured and uncured wounded treatments). Tecnazene is used as a fungicide for the control of Fusarium in potatoes and may reduce the level of fungal infection which if unchecked could lead to increased weight loss. The fungicidal action of Tecnazene may contribute to the observed weight loss differences although no retardation of infection by Tecnazene was visible in this study. Other workers have noted similar effects in Tecnazene treated tubers. McGee (1984) observed some stimulation of wound healing when potato tissue discs were treated with a 10 ppm Tecnazene solution. Leonard et al. (1986) found that Tecnazene did not adversely affect wound healing but did in fact in some cases stimulate it, although the level of stimulation was by no means consistent. It should be stressed, however, that the lower weight loss found in this study from the Tecnazene treatment was not statistically significantly different from the weight loss from controls. This effect is, however, a phenomenon worthy of further study.

Dimethylnaphthalene

The cumulative percentage weight losses from DMN treated tubers treated before curing were not significantly different from controls. However non-wounded tubers treated after curing lose less weight than controls as shown in table 4.5. This difference may be due to the slight inhibition of sprouting by DMN. If applied after curing, this effect, although it appeared quite limited, may have significantly affected weight loss. (The assessment of sprouting in this study was not sophisticated enough to be able to quantify small differences in sprouting between treatments). In wounded tubers treated after curing, weight loss, although lower, was not significantly so. The difference may be smaller in this case simply because it is not only the degree of sprouting which determines weight loss in these wounded tubers but the loss from the wounds themselves. It appears from this study that if DMN has a deleterious effect on weight loss and by implication on wound healing, that effect is comparatively small. That conclusion is in agreement with the results of previous studies discussed in section 4.1 of this chapter. Differences between weight losses from DMN treated tubers and controls caused by the effect of DMN on rotting could not be determined from the results of this study as the degrees of rotting in treatments, including controls, could not be differentiated by the visual assessment used.

The failure of DMN to control sprouting at the recommended application rate may be due to a combination of its volatility and the humidity in the store. Beveridge et al. (1981b) emphasised that DMN will be least effective at high storage temperature and in humid conditions and where free ventilation of the compound from the

potatoes can take place. The importance of the volatility of DMN to weight loss may have been the cause of the lower weight losses found from non-wounded tubers treated immediately prior to storage at 8°C, where the loss of DMN due to volatilisation would be expected to be smaller than from potatoes treated and cured at 25°C. The effect that DMN has on weight loss appears likely to be due to its effect on sprout growth. Wounded tubers were not affected greatly by DMN treatment which implies that DMN may not greatly affect wound healing.

Irradiation

In the group 2 study the largest significant differences, shown in table 4.5, are those between controls and irradiation treatments. Both the 0.10 kGy and 0.15 kGy wounded irradiated treatments lost approximately twice as much weight as controls. There was no significant difference between the two irradiation doses. This implies that the healing of the wounded area in wounded tubers is greatly inhibited by irradiation.

That irradiation affects weight losses through the wound in wounded tubers and not through the intact periderm can be shown by comparing the large differences found between wounded treatments with those found between non-wounded treatments. In non-wounded treatments the weight losses from only the 0.10 kGy irradiation dose were significantly different from the control and the weight losses from those tubers were significantly lower than those from controls.

How then does irradiation reduce the weight loss from non-wounded potatoes when compared to controls? Irradiation has been observed to temporarily increase the rate of respiration after treatment (Gustafson et al., 1957; Kodenchery and Nair, 1972), but the effect of

respiration on weight loss is small in comparison to other mechanisms of weight loss (Burton, 1966). Sprouting was inhibited completely in irradiation treatments but, of course, not in controls. Therefore irradiated tubers could not lose water through sprouts while controls did.

It seems probable that the inhibition of sprouting by irradiation may explain the weight losses observed in non-wounded tubers but it does not explain why the 0.10 kGy treatment has a lower cumulative percentage weight loss than the 0.15 kGy treatment. This difference was probably due to random variability between boxes of potatoes as a real difference in weight loss over this dose range in the observed direction is not likely. The differences found in wounded potatoes, however, were most probably caused by the inhibition of periderm formation by irradiation in large wounded areas leading to increased water loss together with the effect of irradiation on sprouting.

The main point raised by the results obtained from irradiated treatments in this study is the importance of curing before irradiation this is in agreement with the findings of Metlitsky et al. (1967). As potatoes are moved into storage some damage occurs. Together with damage to the skin incurred during harvest and transportation this handling results in breaks and bruises to the potato skin which is simulated in this study by deliberate wounding. If irradiation treatment is carried out before such wounds have had time to heal little if any healing will take place and weight loss and rotting losses will occur on the scale found in this study. These losses would be even more serious if there was a high level of

moisture in the potato store due to, for example, a wet harvest as such conditions would further accelerate rotting. The evaporative weight loss from irradiated non-wounded tubers was not, however, greater than that from controls.

Effect of sprouting and rotting on weight losses

The effects of sprouting and rotting on weight losses have been discussed in the previous sections in relation to the effect of the treatments on them. In general, if sprouting is controlled by treatment its contribution to weight loss is eliminated. The effect of sprouting on weight loss is also much greater in the latter part of the storage period when the surface area of sprouts is larger. It should also be remembered that the loss in weight from potatoes represented by the dry matter of the sprout itself has not been considered here.

It has already been stated that it was not possible to directly determine the magnitude of the contribution made to the weight lost from potatoes in this study by infection. However the levels of infection were such in wounded tubers at the end of the study that losses due to rotting must have contributed significantly. Good management practices to reduce wounding during harvesting and store handling are to be recommended. Treatment with fungicides may also afford some reduction in losses caused in this way.

4.4.2 Weight loss regressions

Discussion of the cumulative percentage weight losses in the previous section has emphasised the complex interaction of several factors which lead to the weight losses measured. These factors; suberisation, periderm formation, starch deposition and drying, sprouting, fungal and bacterial infection and the effect of chemical treatments or irradiation on all of those processes contribute to various degrees to the relationships between weight loss and time. It is then perhaps not surprising that the plotted relationships in graphs 4.1 to 4.8 are non-linear, as each factor may have a greater or lesser influence at any particular part of the storage period.

Tables 4.2 and 4.3 however indicate that linearisation is possible for some of the weight loss - time relationships. The goodness-of-fit of the regression lines from the storage period after the curing of group 1 potatoes were all improved by regressing the natural logarithm of the cumulative percentage weight losses with time, as can be seen by comparing the R^2 and $R_L{}^2$ values in table 4.2. Indeed all but one R^2 value exceeds 98% after transformation. This improvement is also made to group 2 data, transformed by taking its square root, when regressed with time as can be seen from table 4.3. In that case, with two exceptions, all R^2 values exceed 98%. These improvements are found in both wounded and non-wounded tubers of groups 1 and 2 and R^2 values of that magnitude indicate strong relationships between cumulative percentage weight losses and time stored.

The nature of some of the suitable transformations found for group 1 data, suggesting the rate of weight loss is greater later in storage, implies that some factor or factors influenced weight losses

to a greater extent in the latter part of storage in those treatments. From other observations, of the degrees of sprouting and infection in wounded tubers, it would seem that those two factors may have influenced weight losses substantially after prolonged storage. They may, in some treatments, have interacted to produce the relationships found.

The relationships found between the square root of weight loss and time for the treatments in the group 2 data set indicate that the rate of weight loss is greatest particularly in the early stages of storage in those treatments. This effect is presumably due to the more rapid weight loss that occurs before curing is completed and the periderm is fully developed.

The failure of the linearisation technique to significantly improve the goodness-of-fit of the data from the early part of group 1 treatments, as can be seen from the R^2 values in table 4.1, does not mean that a better relationship could not be found between weight loss and time for that data. It merely emphasises that the formation of periderm in tubers treated immediately after harvest may be influenced by several factors simultaneously. As some treatments also affected the process of sprouting and possibly the rate of infection the relationships are likely to be difficult to describe simply. The R^2 values obtained using simple linear regression, however, are still indicative of a strong relationship between weight loss and time and are all greater than 94%. However, from graphs 4.1 and 4.2 it can be seen that the relationships are not simply linear, some degree of curvature is visible for most of the treatments. Linear regression is only used here as a convenient approximation to the real relationships.

Bearing in mind the goodness-of-fit of those lines to the data it is still possible to make a tentative comparison of the rates of weight losses calculated. With reference to tables 4.1 and 4.2 it can be seen that in potatoes treated before curing had taken place such a comparison produces broadly similar results to a comparison of the cumulative percentage weight losses. Only the Chlorpropham treatment was found to have a significantly different rate of weight loss from that of controls. One point of interest, however, is that it is only the rate of weight loss in the early part of storage that is significantly greater than the control's suggesting that the effect on weight loss of Chlorpropham is most important as periderm formation is taking place. This observation is consistent with the mode of action of Chlorpropham discussed in section 4.4.1. The plateau shaped curves formed by the Chlorpropham data in graphs 4.1 and 4.2 indicate once more that Chlorpropham affects weight loss to a greater extent in the early stage of storage when the periderm is forming.

From table 4.3 it can be seen that a significant difference was found between the slopes of the weight loss relationships of potatoes in the control and the irradiation control treatments in group 2. The slopes of the regression lines were significantly less steep at the 5% level in the case of the irradiated control but this difference was only found with non-wounded potatoes. The potatoes in the irradiation control were transported to and from the S.U.R.R.C. along with potatoes to be irradiated. Additionally, irradiation control potatoes were not dusted with alumina, as control potatoes for comparison with chemically treated potatoes were. It is not possible to decide from the information available whether it was the alumina dusting of

control potatoes or the transportation of the irradiation control potatoes that resulted in the difference between the two treatments. The fact that the irradiation control potatoes lost weight more slowly than control potatoes and that the difference was only found with non-wounded potatoes is also difficult to explain. This difference, however, justifies the inclusion of the irradiation control and it is with that control that the slopes of the relationships between weight loss and time of irradiated potatoes should be compared.

Significant differences in rates of weight loss found in table 4.3 from potatoes treated with Chlorpropham or irradiated after curing are in agreement with the differences described in table 4.5 of cumulative percentage weight losses and no reiteration of the discussion of those rates is required. However, the rates of weight loss obtained for DMN and methanol treated tubers were found to be not significantly different from controls, in contrast to those differences found in table 4.5.

In the case of group 2 DMN treated non-wounded tubers the weight losses from control and DMN treatments, as shown in graph 4.5, only really become different at the final measurement date. This difference is probably due to the variable influence of sprouting on weight loss. That single point could not alter the regression slope by a large enough amount for it to be significantly different in this case, but still gave rise to a difference in percentage weight loss in table 4.5. That difference probably does not represent a real effect of DMN on weight loss.

Weight losses from non-wounded methanol treated tubers seem to follow the pattern described above. The influence of one point on the

percentage weight loss is not reflected in a change in the slope of the regression line. That difference also probably does not represent a real effect of methanol on weight loss.

The weight losses from wounded methanol group 2 tubers are consistently higher than controls at all points in graph 4.6. The slope of the transformed regression line, however, is not significantly different from that of the control, but the intercept of that line with the y-axis is much higher. It may therefore be inferred that the large effect methanol is having on those tubers is happening soon after treatment and that the potato is able to recover from it to settle to a steady weight loss afterwards at a rate similar to that of controls. This may indicate that methanol treatment affects suberisation temporarily rather than affecting cell division permanently. Soliday et al. (1979) in studies of diffusion from potato tubers showed that the waxes associated with suberin rather than suberin itself were the main barrier to water loss. The disruption of the structure of the waxes associated with the periderm surface caused by contact with methanol may explain the pattern of water losses found from methanol treated tubers.

4.4.3 Implications of weight loss results

The greatest effects of treatments on weight loss in this study were found when treatments, such as Chlorpropham application or irradiation, which affect wound healing, were carried out on wounded or uncured tubers. This emphasises the importance of the timing of application of these sprout inhibition techniques. It would seem prudent to use Tecnazene to inhibit sprouting, if necessary, immediately after harvest, as it did not adversely affect subsequent weight loss. Once curing has been completed, other, more severe techniques can be considered for the long term sprout growth control that Tecnazene cannot offer. If irradiation is to be the chosen method of sprout suppression Tecnazene application may be unnecessary as long as a sufficient curing period can be used and as long as sprouting does not occur to any significant degree soon after harvest.

In this chapter much has been said regarding the presumed effects of sprout suppression treatments on periderm formation. In the following chapter it is hoped, by studying isolated periderms from treated tubers, to determine what effects the treatments have had on periderm formation by the direct measurement of periderm permeability.

Chapter 5

Development and permeability of isolated potato periderms

5.1 Introduction

The potato periderm, its structure and its role as the interface between the atmosphere and the internal structure of the potato have been discussed in Chapter 4. The exposure of the potato surface to some agricultural chemicals or radiation prior to development has been shown to affect the course of the development of the periderm (Isleib, 1957; Audia et al., 1962; Metlitsky et al., 1967; McGee, 1984; Thomas, 1982; Leonard et al., 1986) weakening its effectiveness as a barrier to fungal, and bacterial infection.

In Chapter 4 some effects of irradiation and some potato sprout suppressant chemicals on potatoes were measured indirectly by comparing the rates of water loss from treated and untreated tubers. However, factors such as the degree of sprouting and pathogenic infection were found to contribute to the level of water loss determined. Therefore to more directly measure the effect of the chemical and irradiation treatments periderms from treated potatoes were enzymically isolated and their water permeabilities measured. The permeabilities of periderms are of interest as reductions in the efficiency of the periderm of treated tubers caused by chemical or irradiation treatment could be due to either the inhibition of periderm development or the disruption of the structure of completely developed periderms. In either case the effect of treatment on the periderm can be investigated by measuring the permeability of the periderm as it develops, as fully developed periderm will be less permeable than periderm whose development has been inhibited by

treatment.

Periderms used in this study were isolated from potato tubers and their permeabilities measured using techniques originally developed for the study of plant cuticles. Cuticles have been prepared from a number of plant species using enzymic isolation techniques (Haas and Schonherr, 1979; Schonherr and Schmidt, 1979; Schonherr and Ziegler, 1980; Lendzian, 1982; Reiderer and Schonherr, 1986; Schonherr and Reiderer, 1988). Using similar techniques Schmidt and Schonherr (1982) isolated the periderms of potato tubers and investigated their structure.

The permeabilities of cuticles isolated in the above studies were measured using the diffusion of tritiated water through isolated cuticles as the means of determining permeability coefficients. Vogt et al. (1983) isolated potato periderms and measured their water permeability by the same technique. Schonherr and Lendzian (1981) describe a method for determining the permeability of cuticles isolated from several plant species based on measuring the rate of water loss through isolated cuticles gravimetrically.

It was thought that if the method of Schonherr and Lendzian (1981) could be applied to the measurement of the permeabilities of isolated potato periderms, the development of the periderm, its resistance to water loss and the effect of chemical and irradiation treatments on development could be investigated. The gravimetric method of Schonherr and Lendzian (1981) was preferred to the radioactive tracer technique used in other studies as it was more suitable for carrying out the large number of permeability measurements required in this study. Additionally, the use of tritiated water would have required

the use of special facilities.

The average weights of the periderms isolated from untreated potatoes were also measured in order to investigate the development of potato periderm over the storage period studied.

5.2 Isolation of periderms

As was described in section 5.1 the method used for the isolation of periderms in this study was an adaptation of the methods used by Schonherr (1976), Haas and Schonherr (1979), Schonherr and Lendzian (1981) and Vogt et al. (1983). Periderms were isolated from cv. Record potatoes used in the study of water loss from stored potatoes described in Chapter 4. For a description of the experimental design of that water loss study reference should be made to section 4.2 of that chapter, and also to diagram 4.1. The water loss study carried out in Chapter 4 involved the storage of two groups of potatoes at $8^{o}C$ for 182 days. During that storage period samples for periderm isolation were removed from replicate boxes of each treatment included in the study set aside for that purpose.

Three tubers were randomly sampled from a box within each treatment; one of average size, one larger and one smaller than average size. Samples were taken for isolation 3, 6, 9, 13, 20, 34, 65, 122 and 178 days after harvest from group 1 potatoes and after 19, 38, 67, 123 and 176 days after harvest from group 2 potatoes. Sampled potatoes were washed carefully by hand before several cores of 17 mm and 22 mm in diameter were taken from each potato. Although in previous isolation studies concerning cuticles care was taken to avoid areas of the leaf containing stomata, when taking cores for this

study it was not possible to avoid the inclusion of lenticels - which can be regarded as equivalent structures in potato periderms - in the core area as they were present over all of the surface of the potatoes. Most of the flesh below the skin of the cores was removed with a sharp knife taking care to leave a layer of 2 - 3 mm of flesh attached to the skin. The cores from each tuber were placed in a suspension of 0.1% cellulase (Sigma, U.S.A.) and 2% pectinase (ICN Biomedicals, U.S.A) buffered at pH 3.8 in a 150 cm^3 conical flask. The buffer used was 0.2 M sodium acetate/0.2 M acetic acid (May and Baker, U.K.). The flasks were stored at 25^{o}C for 14 days for the isolation of the periderms from the remaining attached flesh to take place.

At the end of the isolation period the cores were removed from the flasks and placed on a 100 mesh stainless steel sieve. Distilled water was sprayed onto the inside surface of the periderms to separate any cellular material still loosely adhering to the periderms. The isolated periderms were then rinsed and placed between single Whatman No.1 filter papers (Whatman, U.K.) and air dried at room temperature. Once dry the periderms were stored in this form between layers of tissue paper until required for permeability measurements at which time they were reconstituted by the method described in section 5.4.2.

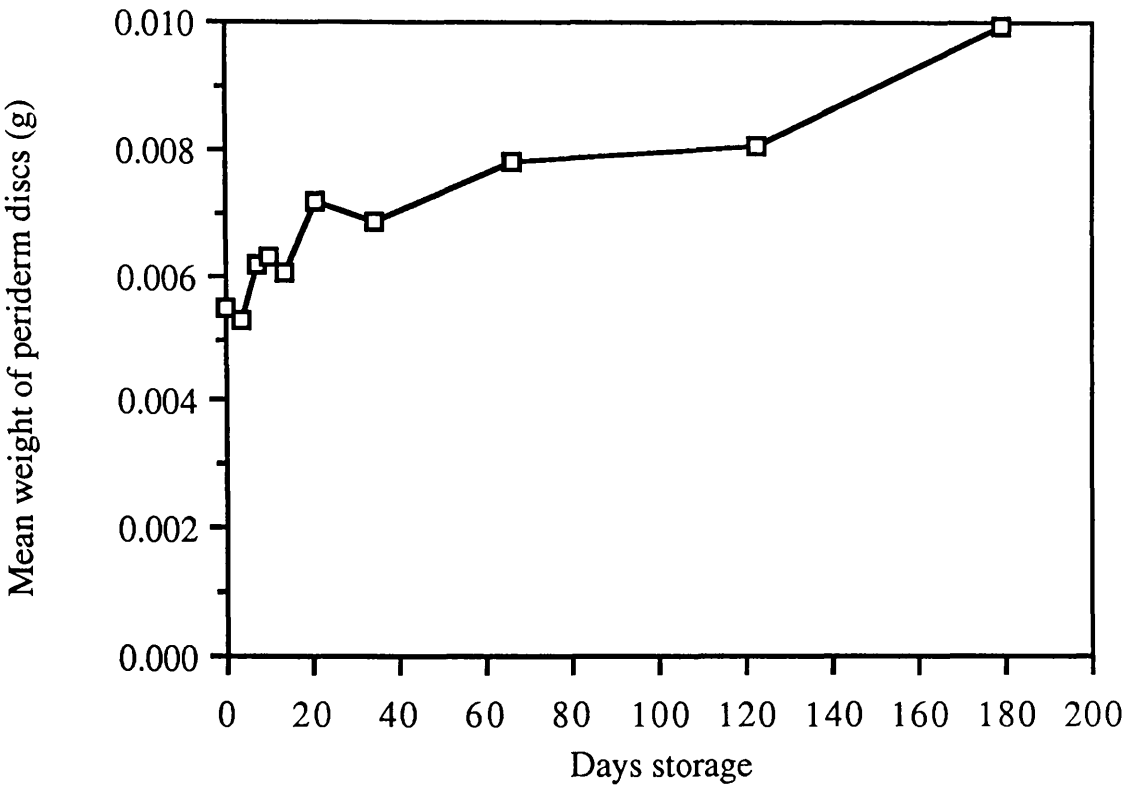
5.3 Weights of periderms

In order to follow the development of periderms in control potatoes it was decided to measure the average weights of isolated periderms, in a given surface area, over the storage period studied.

Experimental method and results

Air dried periderms isolated as described in section 5.2 from group 1 control tubers 3, 6, 9, 13, 20, 34, 65, 122 and 178 days after harvest were weighed to an accuracy of $\pm$ 0.0005 g using an Oertling LA 164 electronic balance. Group 1 potatoes were chosen for this study as periderms were isolated from a larger number of sampling dates in group 1 than in group 2. Five periderms isolated from each of the three tubers on each sampling date were weighed and the overall mean periderm weight at each isolation date calculated. The mean periderm weight on each sample date was plotted against storage time in graph 5.1 (potatoes in group 1 were stored for 19 days at 25^{o}C and subsequently for the remainder of the sampling period at 8^{o}C).

Graph 5.1. Mean weights of periderm discs isolated from untreated cv. Record potatoes stored at 25°C for 19 days and 8°C for a further 104 days (group 1).



* each point is the mean weight of 15 replicate periderm discs.

Discussion

From graph 5.1 it can be seen that in the initial 20 days of storage the mean weight of periderm isolated from a given potato surface area increases rapidly. As storage continues the rate of increase in weight diminishes.

This pattern is in agreement with the generally accepted understanding of how potato periderm develops during storage after harvest. It would be expected that in the immediate period after harvest, as curing takes place, a rapid development of the periderm occurs, marked initially by suberisation and followed by cell division and programmed periderm cell death. As storage continues some further development would take place, although more slowly.

It should, however, be noted that in the initial 19 day period of storage the storage temperature was 25^{o}C while during the remainder of the storage period the temperature was 8^{o}C. This may account for some part of the more rapid periderm weight accumulation observed in the initial storage period but since a higher storage temperature would be used for curing in common storage practice the weight accumulations found in this study may mimic the real development found during potato storage.

5.4 Periderm permeability

5.4.1 Calculation of periderm permeabilities

The permeabilities of isolated periderms were measured using the method described by Schonherr and Lendzian (1981) for the measurement of cuticle permeabilities. Isolated periderms were mounted on perspex cells containing water which was allowed to transpire through the periderm into a sealed chamber maintained at 0% relative humidity. The rate of transpiration through the periderm was determined by monitoring the rate of weight loss from cells over a period of time. Permeabilities were then calculated by the method of Schonherr and Lendzian (1981) of which the following discussion is a summary.

The transpirational flux through a periderm J_p, measured as the rate of water loss from the cell, can be calculated from equation 5.1.

$$J_p = \frac{J_t - J_c}{A_p \text{ X } d} \qquad \text{(equation 5.1)}$$

where J_t is the total transpirational flux (kg s^{-1}), A_p is the cross-sectional area of cell sealed by the periderm (m^2) and d is the density of water (kg m^{-3}).

As the perspex chambers have a small permeability the transpirational flux through the cell perspex (J_c (kg s^{-1})) must be subtracted from J_t. J_c was determined by mounting impermeable aluminium foil on cells and measuring the weight losses from these sealed cells. That procedure is described in section 5.4.4.

The total permeability coefficient P_t can be calculated from equation 5.2.

$$P_t = \frac{J_p}{\underline{a}}$$

(equation 5.2)

where $\underline{a}$ is the gradient of water activity from the surface of the water inside the cell to the silica gel desiccant used to maintain the atmosphere of the sealed chamber at 0% relative humidity. At the surface of the water within the cell in contact with the inner surface of the periderm the water activity = 1, while at the surface of the silica gel the water activity = 0. Therefore in this case $\underline{a}$ = 1.

The path length for the movement of water from the periderm surface to the silica gel is a finite distance. As the air inside the chamber is largely static it takes a finite time for water to diffuse from the periderm surface into the body of air within the chamber at 0% relative humidity. This effect, termed the unstirred layer effect by Schonherr and Lendzian (1981), slows diffusion from the cell and therefore makes a contribution to P_t as measured by the rate of water loss.

The transpirational flux from cells without periderms mounted on them (J^o) can be determined from the following equations.

$$J^o = \frac{J^o_t - J_c}{A_p \times d}$$

(equation 5.3)

$$P^o = \frac{J^o}{\underline{a}}$$

(equation 5.4)

Since the periderm and the path from the periderm surface to the surface of the silica gel act as resistances in series the periderm permeability (P_p) can be calculated from equation 5.5.

$$\frac{1}{P_p} = \frac{1}{P_t} - \frac{1}{P^o}$$

(equation 5.5)

5.4.2 Reconstitution of periderms

Periderms isolated, dried and stored by the methods described in section 5.2 were reconstituted before permeability measurements were made in the following way. Periderms isolated from each tuber from each treatment on each date were placed in separate 150 cm^3 conical flasks in buffer at pH 6 for 24 hours. The buffer used was that described by Vogt et al. (1983) and consisted of 0.01 M $CaCl_2$ (B.D.H., U.K.) and 0.01 2-[N-morpholino] ethanesulphonic acid (MES) (Sigma, U.S.A.) with the addition of NaN_3 (B.D.H., U.K.) to the concentration

of 1 mM to inhibit the growth of microorganisms in the medium.

5.4.3 Determination of J of unsealed cells

As was discussed in section 5.4.1 water within cells must diffuse a finite distance from the surface of the liquid inside the cell into desiccated air. Therefore when measuring the permeability of periderms such diffusion must be corrected for by calculating P^{o}. In order to calculate P^{o} the transpirational flux from unsealed cells J^{o} was measured in the following experiment.

Experimental

In the determination of J^{o} and in the subsequent measurement of periderm permeabilities a similar experimental procedure was used. Each cell was constructed by drilling a well 10 mm deep and diameter 10 mm into a cylinder of perspex 25 mm in diameter and 15 mm in height. Periderms were mounted on a rubber O-ring situated in a groove on the upper surface of the cell and below a perspex ring which was secured tightly by three screws sunk into the circumference of the cell. When sealed in this way water could only escape through the periderm or through the walls of the cell.

A 2 cm deep layer of regenerated silica gel was placed below a metal grill at the bottom of a polythene box of dimensions 205 mm X 235 mm X 70 mm. Cells were placed on the grill above Whatman No.1 filter papers (Whatman, U.K.), a hair hygrometer (Fischer, G.D.R.) was placed beside the cells to monitor the relative humidity within the box and the box was sealed airtight.

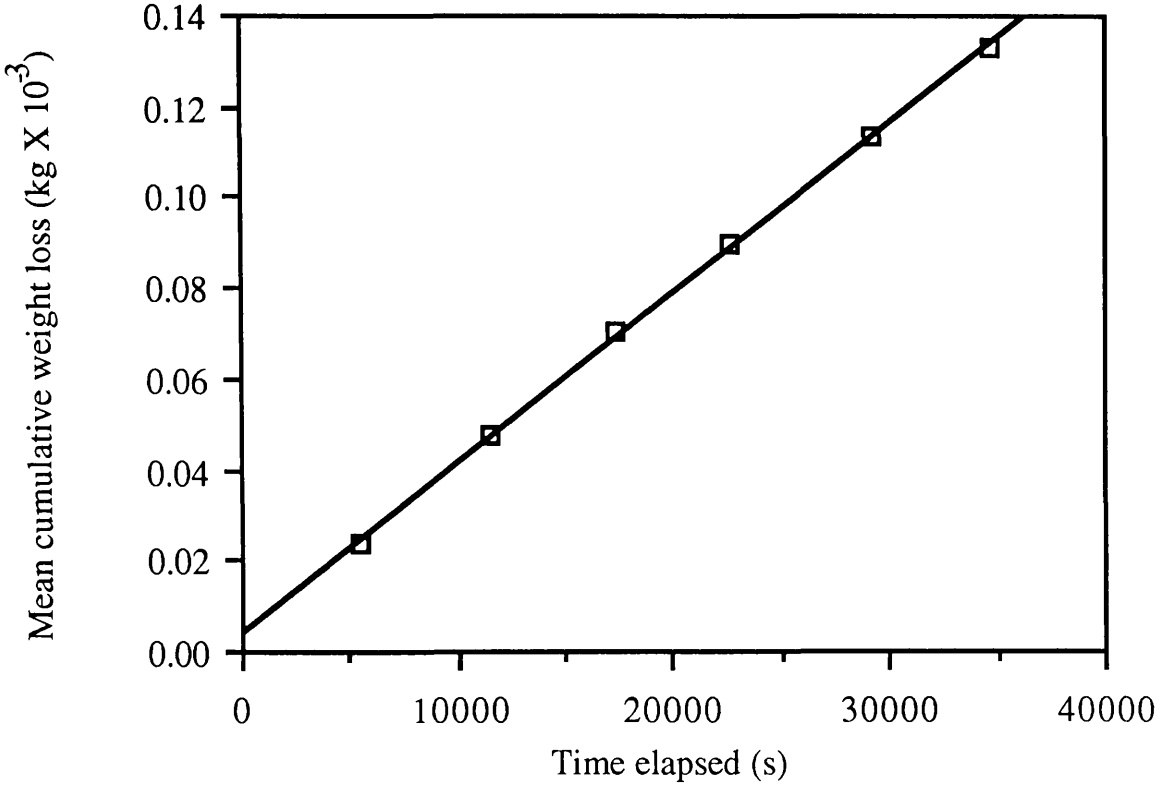
For the measurement of J^{o}, 0.5 cm^{3} of distilled water was pipetted

into the well of each of 20 cells using a 1 cm^3 adjustable Gilson Pipetman pipette (Gilson, France). Circles of aluminium foil were mounted on 10 of the cells and these cells were used to determine J_c. The cells were placed in the box together with a hair hygrometer (Fischer, G.D.R.) and the box was sealed and transferred to a constant temperature room maintained at $25^{o}C \pm 1^{o}C$. The weight of each cell was measured periodically to an accuracy of $\pm$ 0.0005 g using an Oertling LA 164 electronic balance. Measurements were made at 90 minute intervals seven times including the initial measurement. The relative humidity inside the box at each measurement was also noted.

Results and calculation

The relative humidity within the box remained at 0% throughout the experiment. The cumulative weight lost from each cell by the end of each storage interval was calculated. The mean cumulative weight lost from the 10 open cells by the end of each storage interval was calculated. The weight losses measured from the cells with foil seals over the duration of the experiment were within the experimental error of the weight measurements and therefore in this case J_c was assumed to be zero. The mean cumulative weight losses from the open cells were plotted against the time elapsed in graph 5.2.

Graph 5.2. Mean cumulative weight losses from open permeability cells stored at 0% relative humidity and at 25°C over an 10.5 hour period.



* each point is the mean of 10 replicate cell measurements.

The weight lost from open cells was regressed with time elapsed, the gradient of the regression line represents J^{o}_{t} in equation 5.3. For the cells described, of diameter 10 mm, the cross sectional area was calculated to be 7.85 X 10^{-5} m^{2} and at 25°C the density of water is 997.048 kg m^{-3} (Weast and Astle, 1979). Using equation 5.3 the value of J^{o}, and hence P^{o}, was calculated to be 4.79 X 10^{-8} kg s^{-1}.

5.4.4 Measurement of permeabilities

In order to determine whether treatment had any effect on periderm permeability the following experiment was performed. Periderms isolated at the beginning of the storage period (19 days after harvest) and towards the end of storage (123 days after harvest) were selected to represent the early and later stages of periderm development. The weight losses from periderms isolated from potatoes treated with Tecnazene, Chlorpropham and irradiated at doses of 0.10 kGy and 0.15 kGy together with control and foil sealed cells were measured using the procedure described in section 5.4.3 with the following modifications.

5 periderms isolated from 3 tubers sampled from each treatment on each sample date were reconstituted as described in section 5.4.2. Reconstituted periderms were mounted on the cells containing 0.5 cm^{3} of water and together with 5 cells sealed with aluminium foil were measured by the method described in section 5.4.3. Due to the large number of periderms to be measured it was necessary to divide them between two identical boxes. Each box contained a set of cells mounted with periderms from each of the 5 treatments isolated on one of the two dates to be measured plus a set of 5 foil sealed cells for the calculation of J_{c}. As water was lost much more slowly from these

cells than from open cells, measurements of weight loss and relative humidity were made at 24 hour intervals. The cells were randomly placed in the boxes above Whatman No.1 filter papers (Whatman, U.K.) with the periderm surfaces facing the silica gel. The boxes were stored under the conditions described in section 5.4.3 and cells were weighed at 24 hour intervals 7 times.

Results and calculation

The relative humidity within the boxes remained at 0% $\pm$ 2% throughout the experiment. As some water was present on the outside surfaces of the newly mounted periderms the weight losses measured after the first 24 hour period of the experiment were discarded as they were thought to be unrepresentative of the rate of water loss through the periderms. Daily cumulative weight losses from the beginning of the second 24 hour period until the end of the experiment were calculated for each cell.

Examination of the weight losses from individual cells revealed that some cells lost weight at a much greater rate than others. The greater rate of weight loss was in some cases as much as 10 times the rate of weight loss from other cells mounted with periderms from the same treatment. Extreme cuticle permeability measurements were observed by Schonherr and Riederer (1989) who concluded that such values were due to cells leaking rather than due to the loss of water by diffusion through the periderms. Leakage occurs when periderms are improperly mounted on cells or damaged prior to or in the process of mounting. Such damaged periderms were also identified by Geyer and Schonherr (1990) who were able to diagnose such damage by plotting weight losses from cells against time. Large deviations from a linear

relationship between weight loss and time indicated damage to periderm discs. Both Schonherr and Riederer (1989) and Geyer and Schonherr (1990) argued that such outlying measurements could be excluded from permeability calculations.

After the removal of the data from leaking cells the cumulative weight losses from each cell were regressed with time elapsed. The slope of the regression line for each cell represents J_t for the periderm mounted on it. All regression lines had R^2 values of greater than 90% indicating that a very large part of the variability in the weight losses measured in the experiment can be explained by the relationship between weight loss and time. P_p values were calculated from these J_t values by the method described in section 5.4.1 using the mean weight losses from the foil sealed chambers to calculate J_c. (A specific J_c was calculated for each isolation date as the permeabilities of periderms isolated on the two dates were measured separately). Mean P_p values for periderms isolated from each treatment on each date are presented in table 5.1.

Table 5.1. Periderm permeabilities (P_p) of periderms isolated from cv. Record tubers irradiated or treated with chemical sprout suppressants after 19 of storage at 25°C and after a further 104 days of storage at 8°C.

	19 days storage at 25°C	
Treatment	P_p (m s^{-1})	Standard deviation
Control	2.03×10^{-9}	0.39×10^{-9}
Chlorpropham	1.67×10^{-9}	0.36×10^{-9}
Tecnazene	2.42×10^{-9}	0.46×10^{-9}
0.10 kGy Irradiation	3.09×10^{-9}	0.34×10^{-9}
0.15 kGy Irradiation	1.90×10^{-9}	0.38×10^{-9}

	123 days storage (19 at 25°C, 104 at 8°C)	
Treatment	P_p (m s^{-1})	Standard deviation
Control	2.49×10^{-9}	0.57×10^{-9}
Chlorpropham	2.64×10^{-9}	0.66×10^{-9}
Tecnazene	2.75×10^{-9}	0.59×10^{-9}
0.10 kGy Irradiation	3.23×10^{-9}	0.57×10^{-9}
0.15 kGy Irradiation	2.93×10^{-9}	0.53×10^{-9}

A conventional analysis of variance procedure to determine if there was any significant effect of treatment on the permeability of periderms could not be carried out as the removal of the data from leaking cells resulted in an unequal number of replicate P_p values

calculated from each treatment. An alternative statistical procedure, the General Linear Model (GLM), was therefore used as it can analyse data from experiments with unequal numbers of replicates.

GLM analysis was carried out on the P_p data obtained for periderms isolated on each date and also on the combined data from both isolation dates. GLM analysis of variance showed that there was no significant effect of treatment on the permeability of periderms from the combined data at the 5% significance level. It also indicated that there was no significant effect of treatment on the permeability of periderms isolated on either individual date at the 5% significance level. GLM analysis of the combined data also indicated that there was no significant effect of the duration of storage on the permeability of isolated periderms at the 5% significance level.

P_p values for periderms isolated from control tubers after 19 days storage were calculated in order to compare P_p values from individual sample tubers. Those values are presented in table 5.2.

Table 5.2. Periderm permeabilities (P_p) of periderms isolated from untreated cv. Record potatoes stored for 19 days at $8^{o}C$.

Tuber	P_p ($m\ s^{-1}$)	Standard deviation
(1)	1.59×10^{-9}	0.66×10^{-9}
(2)	1.72×10^{-9}	0.81×10^{-9}
(3)	2.78×10^{-9}	0.51×10^{-9}
Mean	2.03×10^{-9}	0.39×10^{-9}

Discussion

The range of mean P_p values of between 1 and 3 X 10^{-9} m s^{-1} measured for cv. Record potatoes in this study is similar to the permeability coefficients of potato periderms measured by Vogt et al. (1983). In a study of periderms isolated from cv. Primura, cv. Syracuse and cv. Maritta potatoes Vogt et al. (1983) measured water permeabilities of the order of 1 - 3 X 10^{-10} m s^{-1}. Permeabilities in that study were found to reduce during storage and the presence of lenticels increased permeability by a factor of ten. As the periderms measured in this study contained lenticels, permeabilities of the order observed were to be expected.

The values obtained for P_p from periderms isolated after 123 days of storage appear to be generally larger than those obtained from periderms isolated after 19 days of storage. The difference is, however, not statistically significant. Larger values of P_p for periderms isolated after 123 days storage imply that water is lost at a greater rate through those periderms, i.e. they are more permeable. This is contrary to what would be expected as the periderm has been shown to develop further during storage and it would be expected that its permeability would decrease as curing occurred. The periderm may in fact already be almost fully developed by the end of the 19 day storage period. This suggests that any apparent differences in P_p values between the two isolation dates may be due to a change in the experimental conditions under which the two sets of periderms were measured rather than to a genuine difference in their permeabilities.

The fact that no significant differences in periderm permeability were found between control and treated tubers may be due to the

variability in the measurement of P_p values of individual periderms within sample tubers or to the variability of the permeability of periderms from different sample tubers within the same treatment. This can be illustrated by examining the standard deviations presented in table 5.1. Large standard deviations in the measurement of the mean P_p values determined for an individual treatment, the control, indicate that the differences between treatment means are not great enough to be detected above the large degree of variability in P_p values calculated for each treatment.

Table 5.2 contains P_p values calculated for individual control tubers from periderms isolated after 19 days storage. These results were chosen in order to show the degree of variability in P_p measurements experienced within one treatment on one isolation date. Permeability measurements of control tuber periderms were generally no more or less variable than the measurements from other treatments.

The results in table 5.2 show that there is a high degree of variability in mean P_p values calculated for each of the three tubers sampled, tuber (3) has mean P_p value almost twice that of the other two tubers. This degree of variability could be due to the natural variability between tubers within the same treatment or may be due to the fact that three differently sized tubers were sampled on each sampling date. Sampling was carried out in that way in order to ensure that samples were representative of the whole treatment box. However, it is conceivable that the three different sizes of potatoes were at different stages of growth and that their periderms were at different stages of development, especially at the first isolation date, and that this led to different P_p values for each tuber.

The standard deviations for individual tubers are also large indicating that there is a high degree of variability in P_p values measured from periderms isolated from the same tuber. This may be due to the condition of different areas of the periderm of a tuber. Surface blemishes and other damage or the presence of lenticels to varying degrees on different areas of the skin may result in substantially different permeabilities at different areas on the surface of a single tuber.

Several explanations of why no effect of treatment was observed can be suggested. Firstly the effect of treatment on periderm permeability may not be great enough to be observed by the method used to measure permeabilities. This view is supported by the degree of variability in P_p measurements discussed above. Treatment effects would have to be large in order to be visible above the variability in permeability coefficients observed within treatments.

Alternatively, treatments may affect the weight loss from potatoes, observed in Chapter 4, by affecting the incidence of disease or the degree of sprouting during storage rather than through a large effect on the permeability of potato periderm. That is to say the effect of treatment on permeability was not observed because it is not the mechanism by which treatments affect weight loss from whole potatoes.

Further experience with, and developments of, the method used in this study may lead to more precise measurements of P_p. This would enable the effects of treatment on the permeability of potato periderms, if such effects are great enough, to be studied. However, potato periderms may be so variable due to the presence of lenticels and to damage to the potato surface that the method employed here may

not be able to distinguish the effects of sprout suppressant treatment on periderm permeability from the random effects of periderm variability.

Chapter 6

Irradiation of Chlorpropham and Tecnazene

6.1 Introduction

Urbain (1986) has stressed that the irradiation of food has been one of the most closely investigated of food processes. The effect of irradiation on many food components has been studied (Elias and Cohen, 1977; Elias and Cohen, 1983; Josephson and Peterson, 1983; Urbain, 1986). However, in the preparation of some foods, substances are added to them and will be irradiated with the food. Two groups of such substances are food additives and agricultural chemicals.

Food additives are substances which are used by food processors to alter the appearance, shelf-life or processing characteristics of foods. Examples of food additives are colourings, preservatives, anti-oxidants and processing aids such as emulsifiers. The term "agricultural chemicals" is used here to include pesticides used on plants before or after harvest and hormones and antibiotics fed to livestock. The effect of irradiation on food additives and agricultural chemicals has not been investigated as fully as the effect of irradiation on the major food components.

In this study of the effects of irradiation on potatoes it was thought appropriate to investigate the effect of irradiation on some chemicals commonly applied to potatoes to be stored for subsequent processing. The agricultural chemicals commonly used to aid potato storage in the U.K. are Chlorpropham and its analogue Propham (sprout suppressants), Tecnazene (sprout suppressant/fungicide), Thiabendazole (fungicide) and 2-aminobutane (fungicide). These chemicals are

applied at the beginning of, or during, the storage period. If irradiation of chemically treated potatoes takes place subsequently, these chemicals will also be irradiated. Irradiation could take place during the storage period to inhibit sprouting or after storage if the potato or its products were used as ingredients in another irradiated product. It was decided to restrict these initial investigations to the two most commonly applied sprout suppressant chemicals, Chlorpropham and Tecnazene.

A review of the literature revealed that the research work conducted to investigate the effect of irradiation on pesticides is both limited and fragmented in its coverage of the field. Most studies carried out to date have been concerned with the irradiation of insecticides of the organophosphorus and organochlorine classes, although other classes of compounds have undergone more limited investigation. In the following discussion for the sake of brevity the common name of each pesticide is used rather than the more correct I.U.P.A.C. version. I.U.P.A.C. equivalents of the common names used are to be found in Hartley and Kidd (1983).

The effect of gamma irradiation on the degradation of some organophosphorus compounds was investigated by Getzin and Rosefield (1968). They found that the biological degradation of Malathion, Dichlorvos, Ciodin and Mevinphos in soil was enhanced by an irradiation pre-treatment at a dose of 40 kGy in comparison with autoclaved pre-treatments and controls. The rate of degradation of irradiated Parathion, Dimethoate and Diazinon treatments was comparable with pre-autoclaved treatments. It was also found that a

10 kGy dose of irradiation alone degraded Malathion, Diazinon and the nematicide Zinophos (Thionazin) by 15, 25 and 42% respectively when irradiated as silt or sand emulsions.

The degradation of Pirimphos-methyl by gamma radiation has been studied by Wiendl (1982) who found it was degraded by irradiation treatment when absorbed onto filter papers, especially at doses of 20 kGy and when present at low concentrations.

Grant et al. (1969) followed the radiolysis of Disulfoton and Phorate at doses of 0 - 40 kGy in hexane, acetone and aqueous solution finding that the degree of breakdown of those compounds increased with increasing radiation dose. Degradation was greater when irradiation was carried out in the organic solvents.

Lippold et al. (1969) studied the effect of a 50 kGy gamma radiation dose on the degradation of 5 ppm hexane solutions of a number of pesticides. They found that the concentration of the organochlorine insecticides DDT and Endosulfan were reduced by 75 and 45% respectively by this treatment. The concentrations of Parathion, Malathion and the acaricides Binapacryl and Tetradifon were reduced by 27, 71, 58 and 28% respectively.

Further studies of the radiation chemistry of DDT have been undertaken by Sherman et al. (1971) who studied its degradation in propan-2-ol. Woods and Akhtar (1974) irradiated DDT at low concentration in aqueous solution and also studied the radiolysis of Chloral Hydrate. In both studies it was found that irradiation treatment resulted in the dechlorination of DDT. The degradation of organochlorine pesticides has been of great interest as some chemicals of that class are notoriously persistent and can bioaccumulate in food

chains (Hassal, 1982). Gamma irradiation has been suggested as a possible method of destroying chloro-aromatics in industrial waste waters (Sherman et al., 1971; Woods and Akhtar, 1974; Vollner et al., 1975; Gilbert, 1976).

Research by Vollner and Korte (1974a) on the irradiation of Dieldrin in aqueous solution found that it was completely degraded by an irradiation dose of 70 kGy. Studies by Vollner and Korte (1974b) and Vollner et al. (1975) on the degradation of Dieldrin, Aldrin, Lindane, Chlordane and polychlorinated biphenyls (PCB's) have shown that progressive dechlorination of these compounds occurs when irradiated in aqueous and organic solutions.

No information could be obtained detailing studies of the gamma irradiation of either Tecnazene or Chlorpropham. Some experimental work has been carried out to investigate the photolysis of Tecnazene and Chlorpropham by U.V. light. U.V. photolysis is in some respects analogous to gamma radiolysis, however as with other analogies care must be taken not to draw the analogy too far as the two processes are not identical in their effects.

Hamadmad (1967) studied the U.V. photolysis of Tecnazene in hexane solution. The irradiation of Tecnazene with light of wavelength 253.7 nm resulted in the formation of 1,2,4,5-tetrachlorobenzene as the major product by the loss of the nitro group. Smaller amounts of 2,3,5-trichloronitrobenzene and 2,3,6-trichloronitrobenzene were also detected. 1,2,4,5-tetrachlorobenzene and trichloronitrobenzenes have been shown to be present as contaminants in commercial formulations of Tecnazene (Heikes et al., 1979), although presumably Hamadmad's study took that into account.

Guzik (1978) has investigated the U.V. photolysis of Chlorpropham in aqueous solution. The sole product identified was isopropyl-3-hydroxycarbanilate, that is the replacement of the ring chlorine by a hydroxyl group. Guzik (1978) also suspected the presence of other polymeric reaction products in photolysed solutions. In the U.V. photolysis of the structurally related herbicide Monuron (3(4-chlorophenyl)-1,1-dimethylurea) Tanaka et al. (1984) observed the formation of the dechlorinated derivative Fenuron (1,1-dimethyl-3-phenylurea) when irradiation took place in a solution with components, such as surfactants, possessing labile hydrogen atoms. The biphenyl compounds 2,4'-, 3,4'- and 4,4'-bis(N',N'-dimethylureido)biphenyl were also formed. In aqueous solution the following chlorinated biphenyls were formed; 2-chloro-4',5-bis(N',N'-dimethylureido)biphenyl and 5-chloro-2,4'-bis(N',N'-dimethylureido)biphenyl.

Experimental objectives

The investigations embarked on in this study set out to discover how Chlorpropham and Tecnazene are affected by gamma irradiation. These experiments fall into several areas. Firstly initial studies were carried out to develop an experimental method capable of measuring the degree of degradation of the chemicals by irradiation treatment. Irradiations of Chlorpropham and Tecnazene were then carried out in two different solvents to contrast the effects of irradiating the sprout suppressants in different chemical environments.

Methanol and hexane were chosen as solvents for this study so that comparisons of how each sprout suppressant behaved when irradiated in

polar and non-polar solutions could be made. Those solvents were also chosen because a clean-up step would not be required before analysis and because some information on the irradiation of hexane and methanol, and pesticide solutions in those solvents, already existed. Additionally, Chlorpropham is often applied as a methanol fog.

Commercial formulations of Tecnazene were irradiated to compare the sensitivity of Tecnazene to radiolysis in solution and as formulated for use in potato stores. Finally attempts were made to identify any radiolytic products formed as a result of irradiating Chlorpropham and Tecnazene.

6.2 Method development

These initial experiments were carried out to develop an experimental method for measuring the breakdown of Chlorpropham and Tecnazene in hexane or methanol solution as a result of irradiation treatment. The objectives of the initial experiments were; to find a suitable irradiation dose range within which observable breakdown of these compounds occurred, to determine whether other compounds were produced by the irradiation process which could interfere with the measurement of the concentration of the unaltered pesticide remaining and to identify factors responsible for any variability in measurements which could be eliminated prior to the quantification of the degree of radiolytic breakdown of Chlorpropham and Tecnazene.

In order to determine the sensitivity of Chlorpropham and Tecnazene to radiolytic breakdown it was decided to measure the percentage of the sprout suppressants remaining intact after irradiation at various radiation doses, i.e. the percentage retention. Methods were also required to qualitatively evaluate the breakdown products formed from

Chlorpropham and Tecnazene. Throughout these initial experiments the analytical technique used to quantify Chlorpropham and Tecnazene was Gas Liquid Chromatography (G.L.C.). An H.P.L.C. (High Performance Liquid Chromatograpy) method was also employed to gain further information on the nature of the reactions taking place at a later stage in the study.

G.L.C. was selected as it is a sensitive, specific and readily quantifiable technique (Dalziel and Duncan, 1974). G.L.C. is suitable for the analysis of both Chlorpropham and Tecnazene.

G,L.C. conditions

G.L.C. was carried out using a PYE PU4500 gas chromatograph fitted with a flame ionisation detector (F.I.D.). Gas flow rates for analysis were as follows; Nitrogen carrier gas 30 cm^3 min^{-1}, Air for F.I.D. 180 cm^3 min^{-1} and Hydrogen for F.I.D. 30 cm^3 min^{-1}.

Glass columns of length 1 m and internal diameter 4 mm were packed with Gas Chrom Q diatomaceous earth support (Applied Science Laboratories, U.S.A.) coated with either 3% OV17 or 1.8% OV17 / 1.95% OV202 stationary phases (Phase Separations, U.K.). Column temperatures of 170 - 190^{o}C were used unless stated otherwise. The injection port was maintained at 220^{o}C and the detector at 250^{o}C. Sample injections of 5 ul were injected using a 10 ul Hamilton series 701 microliter syringe (Hamilton Bonaduz, Switzerland).

Data was monitored by the connection of the PYE PU4500 chromatograph to a Shimadzu C-R1B Chromatopac or Spectra-Physics SP 4290 integrator.

A standard curve was prepared for Chlorpropham (Sigma, U.S.A.) and Tecnazene (Aldrich, U.K.) to determine the linear range of detection. From these it was decided to use 100 ppm solutions of each compound for irradiation as that concentration lay within the linear range in both cases and lower levels of Chlorpropham and Tecnazene would be detectable after irradiation.

H.P.L.C. conditions

The H.P.L.C. method used in this study for the analysis of both Chlorpropham and Tecnazene was adapted from the method of Bushway et al. (1984) for the analysis of Tecnazene in potatoes. As the measurements were carried out on irradiated standard solutions no clean-up procedure as described by Bushway et al. (1984) was required.

H.P.L.C. separations were made by the injection of 1 - 10 ul of sample solutions onto a Perkin-Elmer HS-3 C_{18}-phase cartridge column (Perkin-Elmer, U.S.A.) at ambient temperature. Analyses were carried out using a Perkin-Elmer Series 400 Liquid Chromatograph fitted with an ISS-100 Automatic Sampler. Detection of Chlorpropham and Tecnazene was achieved using an LC-90 U.V. Spectrophotometric Detector at a wavelength of 210 nm and at 0.05 AUFS. The mobile phase used was acetonitrile/methanol/water in the ratio of 35:35:30 and at a flow rate of 1.0 cm^3 min^{-1}. All solvents were of H.P.L.C. grade and were degassed by filtration through Millipore 0.45 um pore size, hydrophilic or low-water extractable filters (Millipore, U.K.) before use.

Determination of irradiation dose range

Unreplicated irradiations were carried out on Chlorpropham and Tecnazene solutions in methanol to determine a suitable range of radiation doses for further study, the doses used ranged from 0.1 kGy to 50 kGy.

Preliminary investigations were carried out by irradiating the pesticide solutions in glass sample bottles of approximately 10 cm^3 capacity with either polythene snap-on lids or plastic screw caps with aluminium foil inserts. Samples were placed as near as possible to the centre of the irradiation vessel and irradiated to the appropriate dose as described and calculated in Chapter 2. Concentrations of Chlorpropham orTecnazene remaining in solution after irradiation were measured using the G.L.C. method previously described.

No measurable breakdown occurred at 0.1 kGy, an irradiation dose of the magnitude used to inhibit the sprouting of potatoes, and a more suitable range for further study was found to be between 0 and 50 kGy in 10 kGy divisions. This dose range was therefore chosen for subsequent studies on the sensitivity of both Chlorpropham and Tecnazene to radiolysis.

Variability in the degree of breakdown of pesticides in solution

Large variations in the levels of Chlorpropham and Tecnazene remaining after irradiation were found between replicate samples irradiated at the same radiation dose in the above initial study. Several possible sources of variability in the method used were identified. Firstly inaccuracy in the positioning of samples at the centre of the irradiation vessel. Secondly unequal activity in

individual rods of the circular ^{60}Co source leading to unequal doses received by samples in different positions within the irradiation vessel.

A third source of variation may have resulted from the type of containers used for irradiations. These containers may have provided sites for possible side-reactions of an unknown nature. Plastic and polythene capped containers may have been unsuitable as some plastics have been reported to undergo changes during irradiation treatment (Josephson and Peterson, 1983; Milinchuk and Tupikov, 1989). The presence of aluminium foil and cardboard linings on the inside of some sample bottles may also have introduced potential sites for unwanted reactions.

In order to try to minimise the effects of the irradiation containers on the rate of breakdown of Chlorpropham and Tecnazene solutions and the radiolysis products formed it was decided to use all-glass containers for subsequent irradiations. This should not be taken to imply that glass itself does not provide a surface for unwanted reactions, it does, however, eliminate any side-reactions other materials may take part in and remove the contribution they make to the experimental variability observed.

To provide a means of positioning the sample bottles more consistently within the irradiation vessel a wooden stand was designed in which the samples could be mounted during irradiation. The stand consisted simply of a length of wood with 6 wells of appropriate circumference and depth sunk into it into which samples could be set. The stand was then mounted above an aluminium spacer within the irradiation vessel to position it at a consistent vertical and

horizontal position at each irradiation.

Some variability in the dose received by each set of samples was still present even with the altered experimental procedure. As has been previously discussed, individual rods of the ^{60}Co source have unequal activities which lead to unequal doses being given to samples in different positions within the irradiation vessel. This could not be avoided by careful positioning of the irradiation vessel on each occasion as the vessel spins freely in the water chamber above the source.

To investigate the extent of variability in dose received at positions across the horizontal axis of the source the following unreplicated study was performed.

Eight 10 cm^3 volumetric flasks were filled to the mark with aliquots of a single 100 ppm Tecnazene solution in methanol (Rathburn U.K.), the flasks closed and sealed with glass stoppers. Six of these flasks were then placed in the wooden irradiation stand, their positions noted and the samples irradiated at a dose of 20 kGy. The remaining two samples were retained as non-irradiated controls. After irradiation the Tecnazene concentration of samples was measured by the G.L.C. method previously described.

The resultant concentrations of Tecnazene measured in each sample were expressed as a percentage of that of non-irradiated controls and are shown in table 6.1.

Table 6.1. Percentage Tecnazene in methanol solutions after irradiation at 20 kGy at various positions on the horizontal axis of the S.U.R.R.C. ^{60}Co source.

Distance from centre of axis (cm)	% of control concentration
10.6	20.63
4.4	27.71
2.5	34.13
1.9	49.47
6.1	28.87
10.3	20.75

From these results it can be seen that the positioning of the sample within the source is indeed of critical importance in determining the dose received by the sample. It can also be seen that, as expected, a lower dose will be received in the centre of the source than at the circumference of the irradiation vessel.

As the dose rate used in calculations of doses by staff at S.U.R.R.C. is based on dosimetry at the centre of the source it was decided to use the two central positions of the irradiation stand to position samples in further studies.

Irradiation of solvents

In order to determine whether the irradiation of methanol or hexane could result in the formation of compounds which could interfere with the analysis of Tecnazene or Chlorpropham or their radiolysis products the following experiment was performed.

Two duplicate 10 cm^3 samples of H.P.L.C. grade methanol (Rathburn U.K.) were irradiated using the experimental design previously outlined at doses of 10, 20, 30, 40, and 50 kGy. This procedure was then repeated with glass distilled hexane (Rathburn U.K.) as the solvent irradiated. The irradiated samples were analysed, together with non-irradiated controls, by the G.L.C. method developed for Chlorpropham and Tecnazene analysis.

In irradiated hexane samples a distinct aldehydic/ketonic odour was detectable, the odour became stronger as the dose increased. No such odour was detectable from irradiated methanol samples. G.L.C. analysis revealed no compound peaks in either irradiated hexane or methanol under the conditions used. H.P.L.C. analysis of those samples was also carried out and no interfering compound peaks were found by this method in the analysis of either irradiated solvent.

The odour noted in irradiated hexane samples suggests that some volatile compound is being formed from hexane. The type of odour may indicate that an aldehyde or ketone derived from hexane may be being formed possibly by the reaction in solution of hexane, or free radicals formed from hexane, with dissolved oxygen in solution or with atmospheric oxygen at the solvent/air surface. Such a compound, or mixture of compounds, may not appear on G.L.C chromatograms under the conditions used as they may be volatile enough to be masked by the solvent front.

Milinchuk and Tupikov (1989) have tabulated data on the amounts of various radiolysis products formed from a number of organic solvents. Table 6.2 summarises the data provided by Milinchuck and Tupinov (1989) on the radiolysis products of methanol and hexane.

Table 6.2. G values* for the radiolysis products of methanol and hexane.

Solvent	Radiolysis Product	G value
Hexane	Hydrogen	5.00
	Methane	0.14
	Ethane	0.31
	Ethene	0.24
	Propane	0.34
	Propene	0.18
	iso-Butane	0.05
	n-Butane	0.35
	iso-Butene	0.05
	But-2-ene	0.05
	But-1-ene	0.10
	C_5 products	0.10
	C_7 products	0.06
	C_8 products	0.29
	C_9 products	0.22
	C_{10} products	0.16
	C_{11} products	0.17
Methanol	Hydrogen	4.5 - 4.8
	Methane	0.5 - 0.7
	Water	0.5 - 0.6
	Carbon Monoxide	0.11 - 0.13
	Ethanediol	3.0 - 3.2
	Formaldehyde	1.4 - 1.9
	Dimethylether	0.25

* The G value is the number of molecules of a radiolysis product formed 100 eV^{-1} radiation absorbed.

Without further analysis it is not possible to identify the radiolysis product or products responsible for the odour detected. These results show, however, that under the G.L.C. and H.P.L.C.

conditions used in the analysis of Chlorpropham and Tecnazene, irradiated hexane and methanol do not form products that co-chromatograph with either sprout suppressant. The radiolytic products formed from the solvents should not therefore interfere with the measurement of the amount of intact Chlorpropham or Tecnazene after irradiation.

6.3 Irradiation of Chlorpropham in methanol and hexane

In this section the response of Chlorpropham to irradiation in solutions of hexane and methanol was investigated. A range of radiation doses were used and the degree of breakdown of Chlorpropham and the concentration of some radiolytic products quantified.

6.3.1 Irradiation of Chlorpropham in methanol solution

Experimental

Twelve 10 cm^3 aliquots of a single stock 100 ppm solution of Chlorpropham (Sigma, U.K.) in H.P.L.C. grade methanol (Rathburn, U.K.) were transferred into 10 cm^3 volumetric flasks and sealed with glass stoppers. The flasks were transported to the S.U.R.R.C. where duplicate solutions were irradiated at six different doses on the stand apparatus described in section 6.2. The doses used were 0, 10, 20, 30, 40 and 50 kGy, duplicate samples being positioned centrally as discussed in section 6.2. The concentration of Chlorpropham remaining after irradiation was measured by the G.L.C. method described in that section.

Results

Chromatograms of samples irradiated at a 10 kGy dose had only one peak present, with a retention time corresponding to that of Chlorpropham at approximately 8.5 minutes under the conditions used. At higher doses a second peak was also found to be present which was not observed in the 10 kGy or control samples. The retention time of the second peak was approximately 4.2 minutes. Typical chromatograms of a sample irradiated at 50 kGy and a control are presented as diagrams 6.1 and 6.2. As the irradiation dose increased the peak area under the second peak increased while the area under the Chlorpropham peak decreased. The mean concentrations of Chlorpropham found in solutions at each dose were calculated and expressed as a percentage of Chlorpropham present before irradiation as represented by the control concentrations measured. These values were plotted against dose received in graph 6.1.

Diagram 6.1.

Gas chromatogram of a 100 ppm solution of Chlorpropham in methanol. Chromatographic conditions are given in the text.

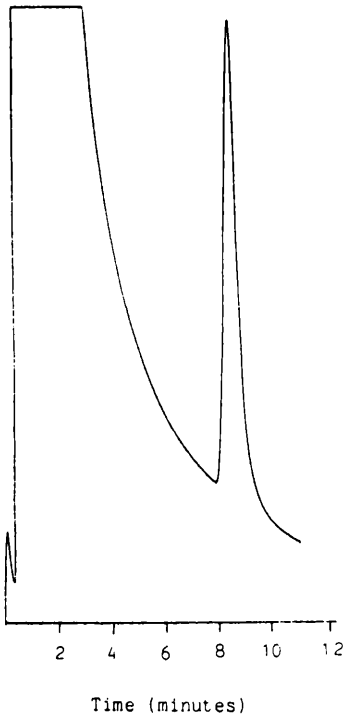
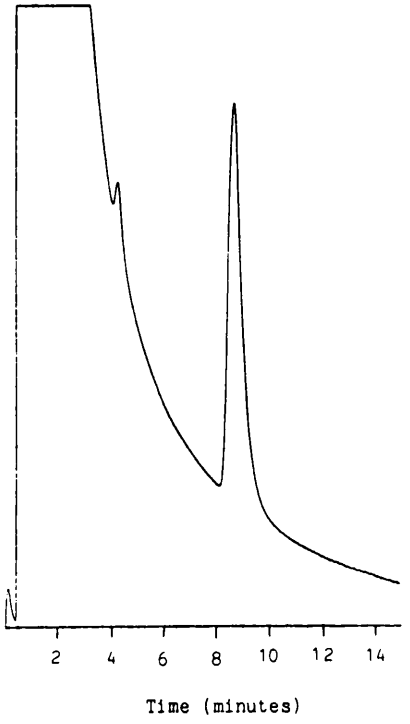
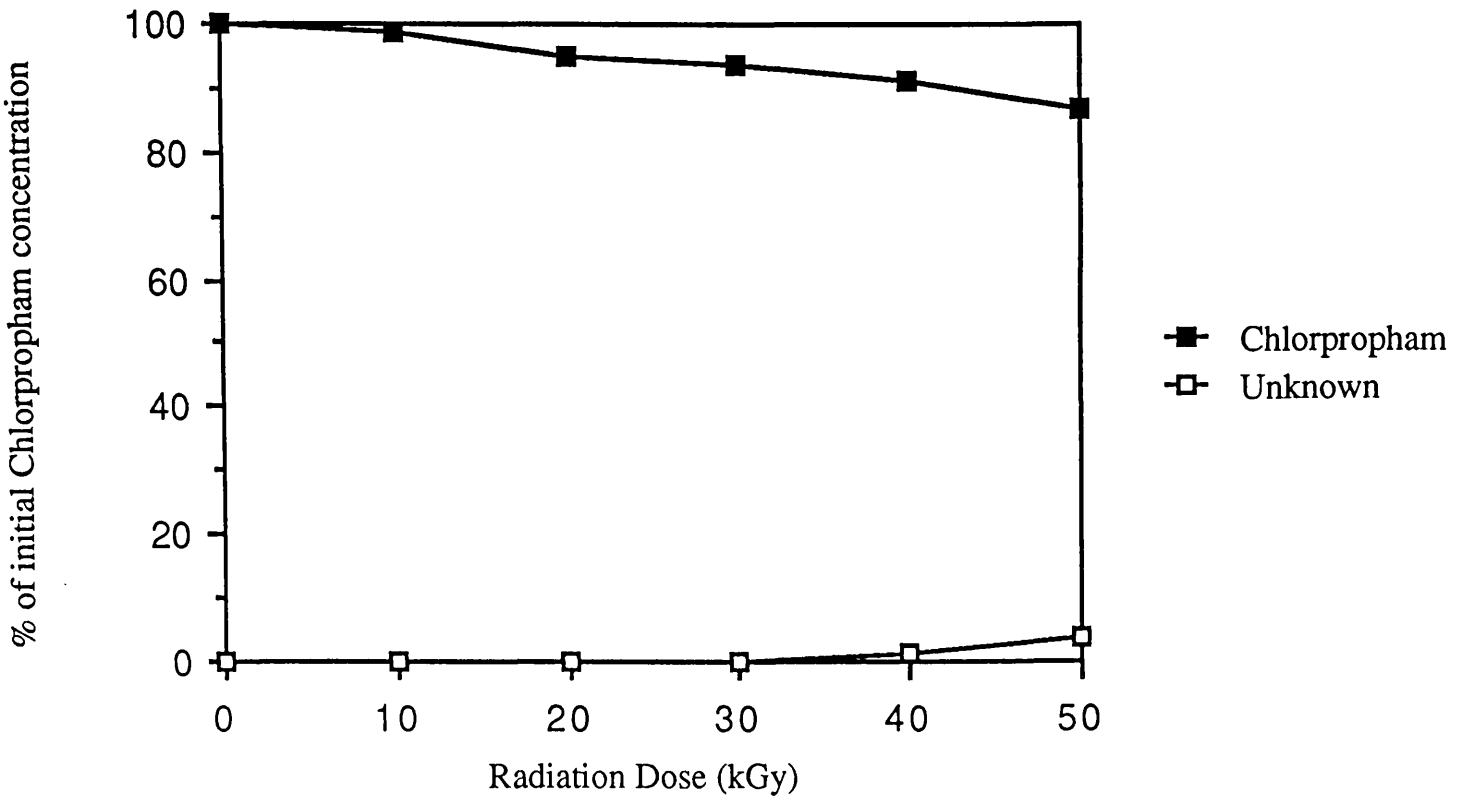


Diagram 6.2.

Gas chromatogram of a 100 ppm solution of Chlorpropham in methanol irradiated at a dose 50 kGy. Chromatographic conditions are given in the text.



Graph 6.1. The effect of irradiation dose on the percentage retention of Chlorpropham in methanol solution. *



* each point is the mean of two replicate samples.

The increasing concentration of the second unknown compound could not be quantified readily at lower doses as, in comparison to the Chlorpropham peak, its area was too small for accurate measurement. A further difficulty encountered in quantifying the unknown compound was that the response of the F.I.D. to it was unknown. As a first approximation, however, it was decided to express the measurable peaks, that is those at higher irradiation doses, as a percentage of the non-irradiated Chlorpropham peak area. By using this method it was calculated that the mean concentration of the unknown compound was 3.9 ppm in solutions irradiated at 50 kGy and 1.3 ppm in those irradiated at 40 kGy. Concentrations in solutions irradiated at 30 and 20 kGy were too low to be determined.

6.3.2 Irradiation of Chlorpropham in hexane solution

Experimental

The procedure described in section 6.3.1 was repeated using glass distilled grade hexane (Rathburn, U.K.) as the solvent for Chlorpropham.

Results

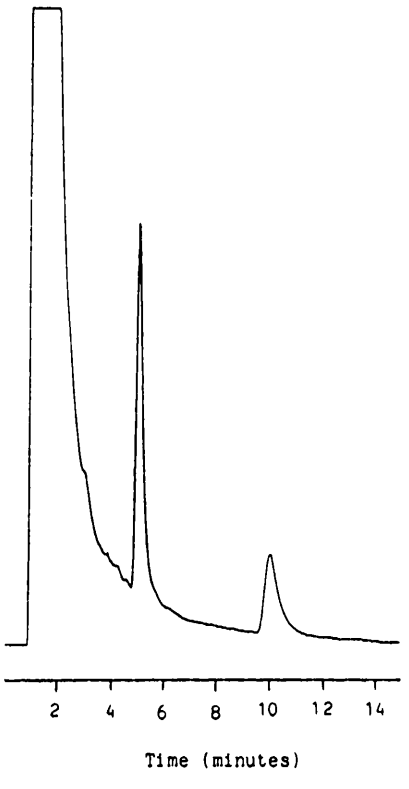
Two peaks appeared in the chromatograms of irradiated solutions at all doses used. One peak had a retention time consistent with that of Chlorpropham. The second peak of an unknown compound was found to increase with radiation dose received. Typical chromatograms of a control Chlorpropham solution and a solution irradiated at 50 kGy are presented as diagrams 6.3 and 6.4 respectively.

Diagram 6.3.

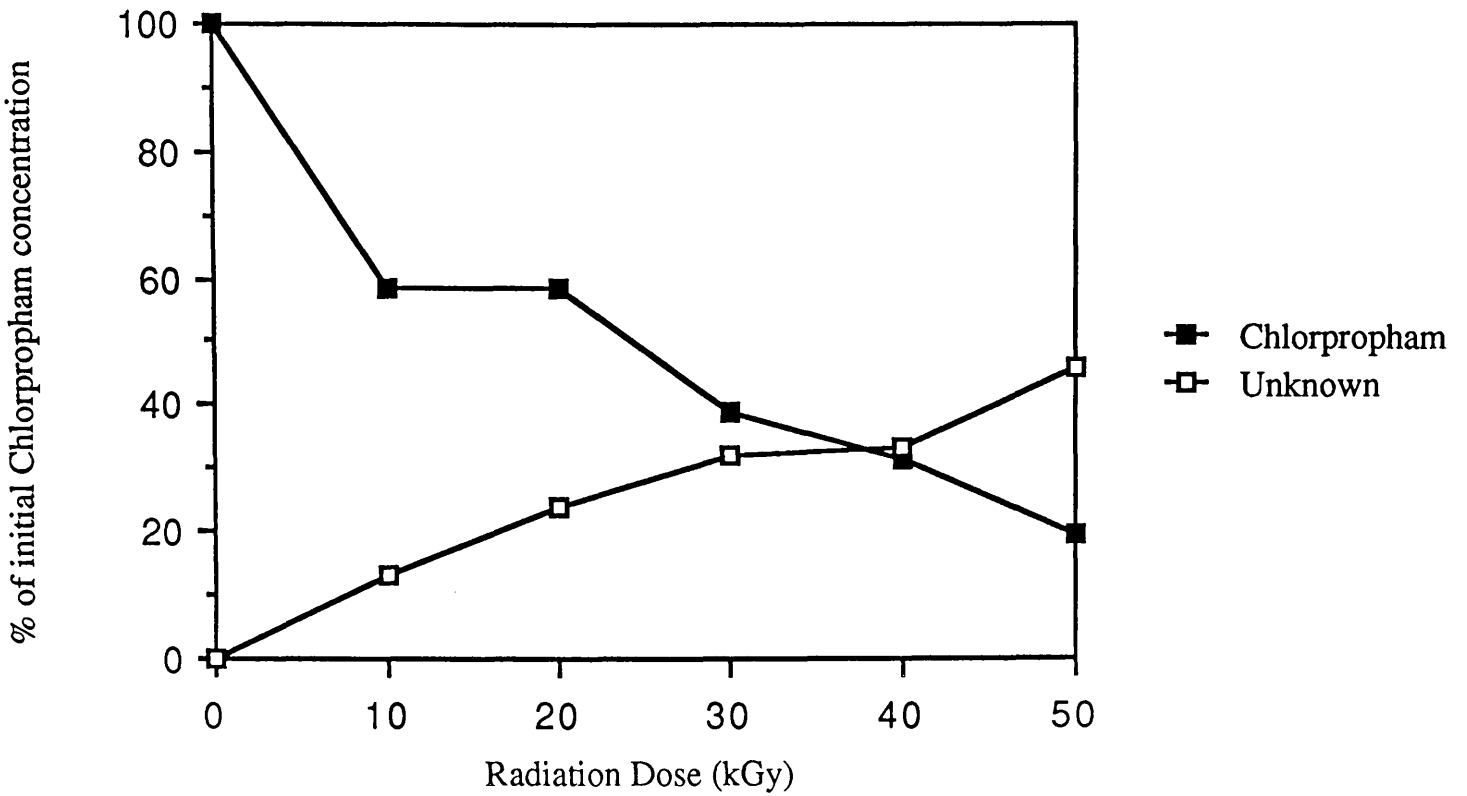
Gas chromatogram of a 100 ppm solution of Chlorpropham in hexane. Chromatographic conditions are given in the text.

Diagram 6.4.

Gas chromatogram of a 100 ppm solution of Chlorpropham in hexane irradiated at a dose 50 kGy. Chromatographic conditions are given in the text.



Graph 6.2. The effect of irradiation dose on the percentage retention of Chlorpropham in hexane solution. *



* each point is the mean of two replicate samples.

The concentration of Chlorpropham remaining in irradiated solutions was calculated and expressed relative to that of Chlorpropham in non-irradiated solutions. Nominal concentrations of the unknown compound were calculated and expressed in the manner described in section 6.3.1. The percentage of Chlorpropham remaining after irradiation and of the unknown compound were plotted against dose received in graph 6.2. The nominal concentrations of the unknown compound calculated are tabulated in table 6.3.

Table 6.3. Nominal concentrations* of unknown compound formed by irradiation of Chlorpropham in hexane solution.

Radiation Dose (kGy)	0	10	20	30	40	50
Concentration of Unknown Compound (ppm)	0	13.24	24.02	32.03	33.06	45.92

* Each value is the mean of two replicate samples.

6.3.3 Conclusions

As can be seen from graphs 6.1 and 6.2 the sensitivity of Chlorpropham to radiolysis is highly dependent on the solvent in which it is irradiated. Chlorpropham irradiated in methanol solution is not degraded greatly at radiation doses of up to 50 kGy, some 87% is retained at that dose. Chlorpropham irradiated in hexane solution is much more sensitive to radiolytic breakdown, 41% is degraded by a dose of 10 kGy and only 20% is retained intact at a dose of 50 kGy.

The combined percentages of Chlorpropham and products do not account for 100% of the initial Chlorpropham concentration. This may

imply that other, unresolved, components of radiolysis are being formed. Possible mechanisms by which they may be formed include the coupling or polymerisation of more than one Chlorpropham molecule, as observed by Tanaka et al. (1984) in the U.V. radiolysis of Monuron, or the formation of other monomeric products at concentrations too low to detect by the method employed.

In order to explain the difference in sensitivity of Chlorpropham to irradiation in methanol and hexane some knowledge of the nature of the products formed from Chlorpropham is required. This subject is dealt with in more detail in section 6.6 and a discussion of the mechanisms of Chlorpropham and Tecnazene radiolysis is to be found in section 6.7.

6.4 Irradiation of Tecnazene in methanol and hexane

The experimental procedure used to investigate the radiolysis of Chlorpropham in section 6.3 was used in this section to study the effect of irradiation on Tecnazene solutions.

6.4.1 Irradiation of Tecnazene in methanol solution

Experimental

The procedure described in section 6.3.1 was repeated for Tecnazene radiolysis in methanol solution. The Tecnazene used was technical grade, manufactured by Aldrich (U.K.).

Results

A single peak was present in the chromatograms of each solution, the area under which decreased with dose. Typical chromatograms of a non-irradiated solution and a solution irradiated at 50 kGy are presented in diagrams 6.5 and 6.6.

The concentration of Tecnazene remaining in irradiated solutions was calculated and expressed relative to that of Tecnazene in non-irradiated solutions as described in section 6.3.2 for Chlorpropham. The percentage of Tecnazene remaining after irradiation was plotted against dose received (graph 6.3).

Diagram 6.5.

Gas chromatogram of a 100 ppm solution of Tecnazene in methanol. Chromatographic conditions are given in the text.

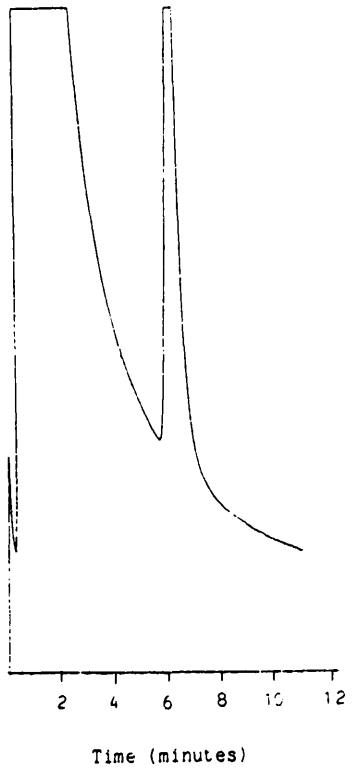
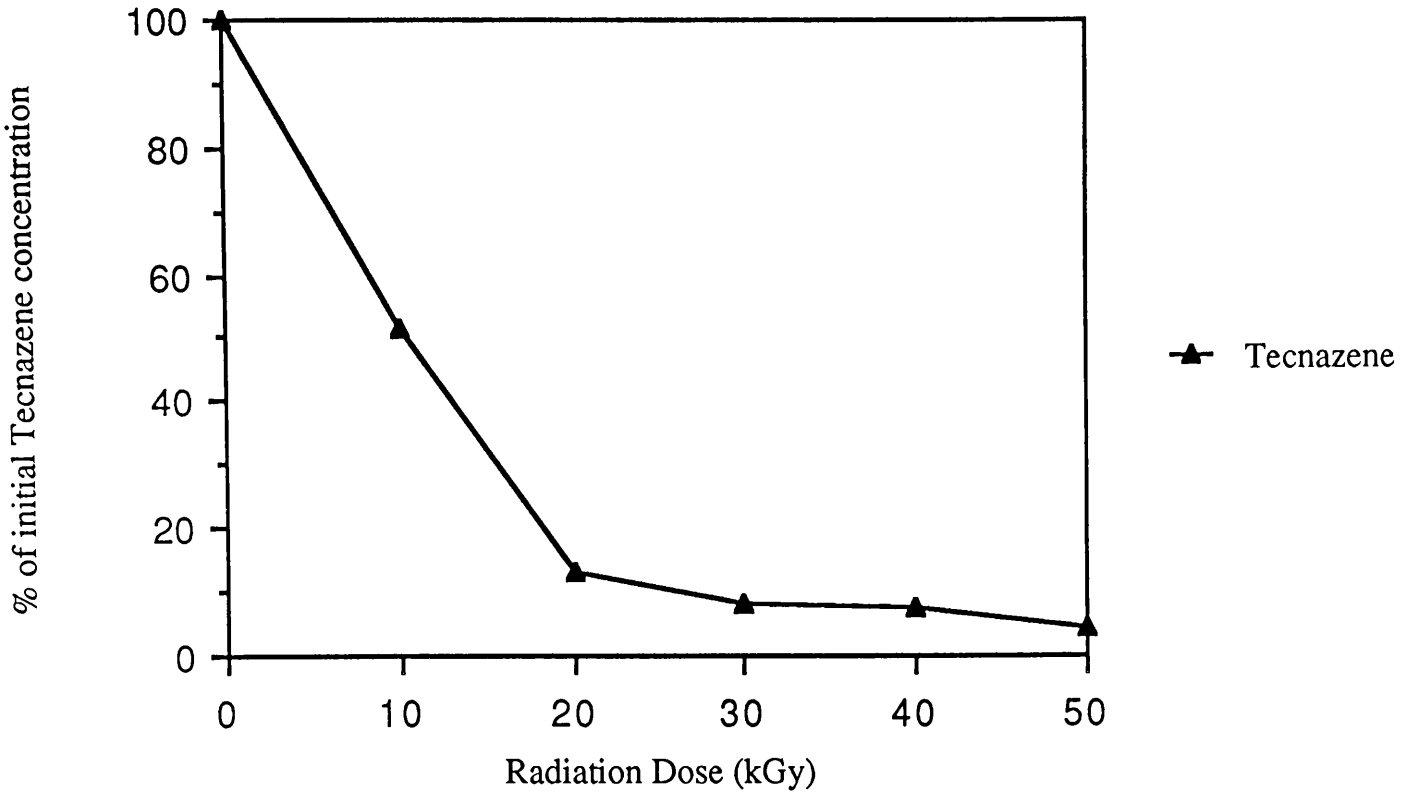


Diagram 6.6.

Gas chromatogram of a 100 ppm solution of Tecnazene in methanol irradiated at a dose 50 kGy. Chromatographic conditions are given in the text.

Graph 6.3. The effect of irradiation dose on the percentage retention of Tecnazene in methanol solution. *



* each point is the mean of two replicate samples.

6.4.2 Irradiation of Tecnazene in hexane solution

Experimental

The procedure described in section 6.3.1 was repeated for Tecnazene radiolysis in hexane solution. The Tecnazene used was technical grade, manufactured by Aldrich (U.K.).

Results

A single peak was present on the chromatograms of each solution, the area of which decreased with dose. By comparison with standards this was found to represent Tecnazene. Typical chromatograms of a non-irradiated solution and a solution irradiated at 50 kGy are presented in diagrams 6.7 and 6.8.

The concentration of Tecnazene remaining in irradiated solutions was calculated and expressed relative to that of Tecnazene in non-irradiated solutions as described in section 6.3.2 for Chlorpropham. The percentage of Tecnazene remaining after irradiation was plotted against dose received in graph 6.4.

Diagram 6.7.

Gas chromatogram of a 100 ppm solution of Tecnazene in hexane. Chromatographic conditions are given in the text.

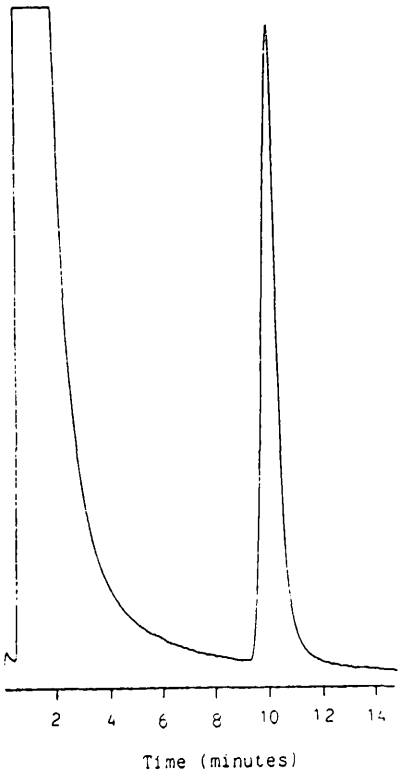
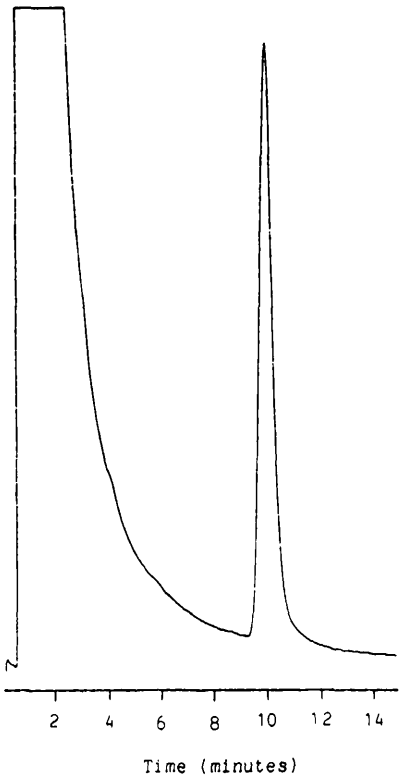
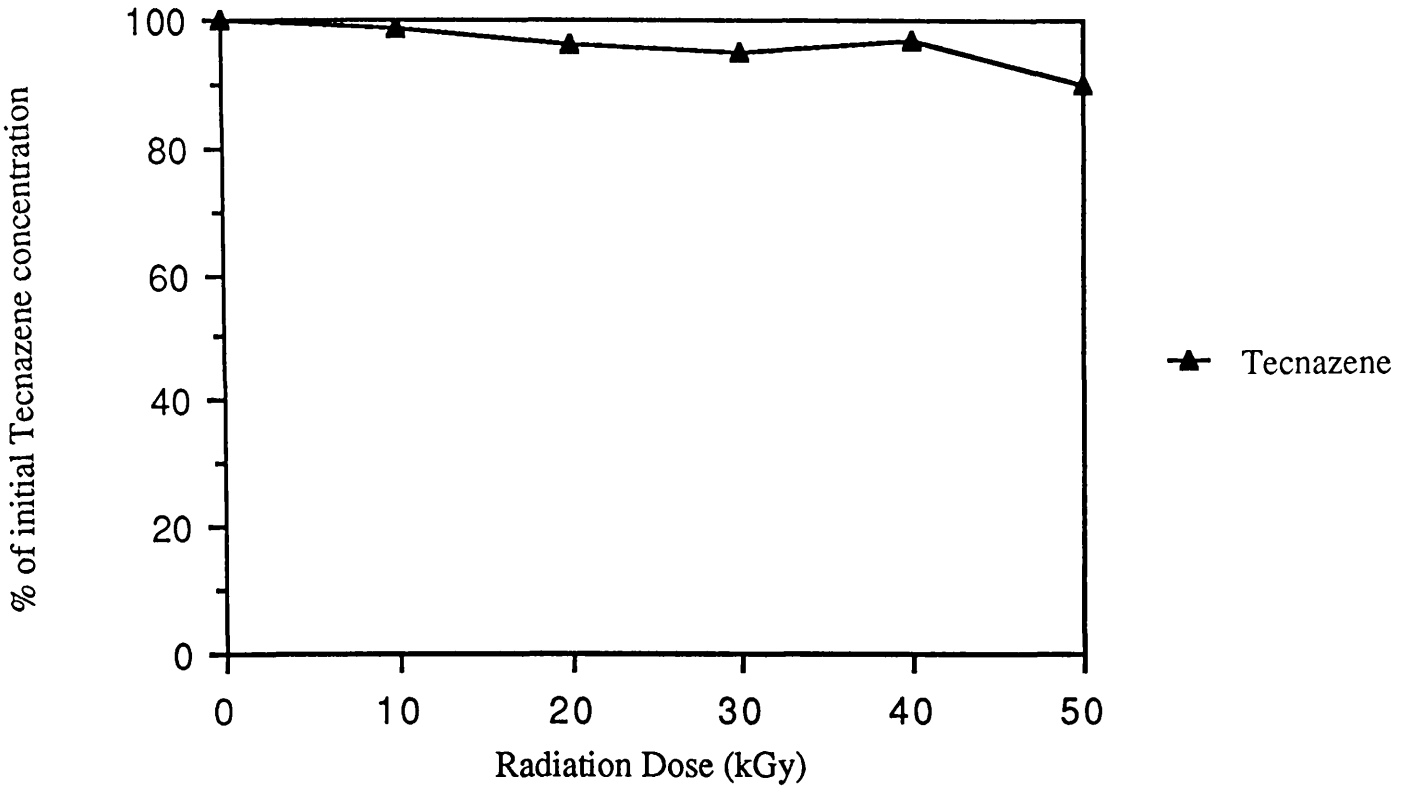


Diagram 6.8.

Gas chromatogram of a 100 ppm solution of Tecnazene in hexane irradiated at a dose of 50 kGy. Chromatographic conditions are given in the text.



Graph 6.4. The effect of irradiation dose on the percentage retention of Tecnazene in hexane solution. *



* each point is the mean of two replicate samples.

6.4.3 Conclusions

The sensitivity of Tecnazene to radiolytic breakdown is greater in methanol solution than in hexane solution as can be seen from graphs 6.3 and 6.4. This is in contrast to the results obtained in the study of Chlorpropham radiolysis where greater breakdown was observed when Chlorpropham was dissolved in hexane. The different sensitivities of the two compounds to irradiation in methanol and hexane solution are more fully discussed in section 6.7.

6.5 Irradiation of Tecnazene formulations

6.5.1 Extraction of Tecnazene from formulations

As a first step in determining the sensitivity of Tecnazene in commercial formulations to radiolysis a method for the extraction of Tecnazene from formulations was developed. Two main extraction methods were compared; Soxhlet extraction and shaking the formulation with a known volume of extracting solvent. Each type of extraction was carried out for varying lengths of time and in all cases a single formulation, Fusarex, was extracted for the comparisons.

Soxhlet extraction of Fusarex

Experimental

Approximately 0.5 g of Fusarex (I.C.I., U.K.) was weighed accurately into eight 30 mm X 80 mm Soxhlet thimbles (Whatman, U.K.) and a plug of cotton wool inserted into the thimble. Each thimble and two empty control thimbles with cotton wool plugs were placed into the thimble chamber of a $60cm^3$ Soxhlet extractor. The Soxhlet extractors were filled with H.P.L.C. grade methanol (Rathburn, U.K.), drained

into 150 cm^3 flat bottomed flasks and refilled with methanol until 50 cm^3 of methanol had been added to each.

The Soxhlet extractors were mounted on heating units and Tecnazene extracted. Half of the Fusarex samples, and one of the controls, were extracted for 4 hours, the remainder for a further four hours. At the end of the appropriate extraction time the extractors were removed from the heat for 1 hour to cool. The extractors were then tilted to drain any solvent remaining in the thimble chambers into the flasks which were then stoppered and left to cool to room temperature. The contents of each flask was then transferred with washings into a 100 cm^3 volumetric flask and made up to the mark with methanol.

The concentration of Tecnazene present in each solution was determined by the G.L.C. method described in section 6.2.

Results

The percentage Tecnazene extracted from each sample of Fusarex was calculated (g Tecnazene 100 g^{-1} Fusarex) and is displayed in table 6.4. Tecnazene was not detected in control solutions.

Table 6.4. Percentage Tecnazene extracted from Fusarex formulation by Soxhlet method.

Time extracted (hours)	% extractable Tecnazene*	Standard Deviation
4	3.07	0.199
8	3.07	0.059

* each value is the mean of 4 replicate extractions.

An analysis of variance procedure was carried out to determine whether time of extraction affected the percentage extractable Tecnazene. The F-statistic of 0.00058 is very much smaller than the tabulated F-statistic of 5.987 for the appropriate degrees of freedom at the 5% confidence level. This indicates that the percentage extractable Tecnazene from Fusarex is not significantly increased by extending the Soxhlet extraction time from 4 to 8 hours.

Extraction of Fusarex by shaking with methanol

Experimental

Approximately 0.2 g of Fusarex was weighed accurately into 16, 120 cm^3 glass jars with plastic screw caps. A 50 cm^3 aliquot of H.P.L.C. grade methanol was transferred into each jar and into each of 8 empty control jars. The jars were then closed and shaken on an end-over-end shaker. Four durations of shaking were compared; 6, 12, 24 and 48 hours. After each time interval 4 jars containing formulation and extract and 2 control jars were removed from the shaker. Samples and controls were filtered through Whatman No.1 filter paper (Whatman, U.K.) with washings into 100 cm^3 volumetric flasks and made up to 100 cm^3 with methanol.

The concentration of Tecnazene present in each solution was determined by the G.L.C. method described in section 6.2.

Results

The percentage Tecnazene extracted from each sample of Fusarex was calculated (g Tecnazene 100 g^{-1} Fusarex) relative to the same primary standard used for Soxhlet extract measurement and is displayed in table 6.5. Tecnazene was not detected in the control solutions.

Table 6.5. Percentage Tecnazene extracted from Fusarex formulation by shaking with methanol.

Time extracted (hours)	% extractable Tecnazene*	Standard Deviation
6	2.42	0.044
12	2.44	0.005
24	2.42	0.119
48	2.37	0.020

* each value is the mean of 4 replicate extractions.

An analysis of variance procedure was carried out to determine whether duration of shaking affected the percentage extractable Tecnazene. The calculated F-statistic of 0.911 is smaller than the tabulated F-statistic of 3.885 for the appropriate degrees of freedom at the 5% significance level. This indicates that the percentage extractable Tecnazene from Fusarex is not significantly increased by extending the shaking time from 6 to 48 hours.

Choice of extraction method

The results of the investigation of methods for the extraction of Tecnazene from Fusarex demonstrate that extraction time was of little consequence in determining the percentage Tecnazene extractable by either Soxhlet extraction or by shaking. Soxhlet extraction was more effective in extracting Tecnazene from Fusarex than shaking. This may be because fresh solvent was constantly circulated in the Soxhlet extraction system while shaken samples may have reached an equilibrium with the solvent. If it is assumed that Soxhlet extraction exhaustively extracts Tecnazene, shaking, by comparison, extracts approximately 80% of the total extractable Tecnazene. Although shaking is thus less effective at extracting Tecnazene it can be seen from the standard deviations in table 6.5 that, as with Soxhlet extraction, a consistent percentage of the total Tecnazene was extracted.

A requirement of the extraction method to be employed in studying Tecnazene formulations in this study is that the method should extract a consistent percentage, and if possible all, of the Tecnazene in a sample of irradiated formulation. A further requirement is that the method should be able to efficiently cope with the number of samples required for the necessary comparisons in subsequent experiments.

Although Soxhlet extraction fulfills the first requirement it is a time consuming process when compared to the shaking procedure used. In order to measure the response of irradiated formulations over the dose range envisaged with proper replication 28 samples were to be extracted simultaneously. The equipment necessary for the number of samples to be run was not available and additionally such a large

number of samples would have been difficult to extract simultaneously. Thus it was decided to use the shaking method in further formulation studies.

It is worth noting that since the degree of breakdown in irradiated formulations was to be measured in relation to non-irradiated formulations the absolute percentage Tecnazene extracted is not of critical importance if the percentage of the total Tecnazene present extracted is consistent.

6.5.2 Irradiation of Fusarex, Hortag and Bygran S

Experimental

Approximately 10 - 15 g of Fusarex was weighed into each of twelve glass weighing bottles and the lids sealed on with teflon tape (James Walker, U.K.). These bottles were transported to the S.U.R.R.C. and two replicates of each dose were irradiated in the central two positions of the wooden irradiation stand as previously described in section 6.2. The doses used were 0, 10, 20, 30, 40 and 50 kGy.

One 0.2 g sub-sample of each of these Fusarex samples was then extracted by shaking with methanol for 6 hours in the manner described in section 6.5.1. The concentration of Tecnazene present in each solution was determined by the G.L.C. method described in section 6.2.

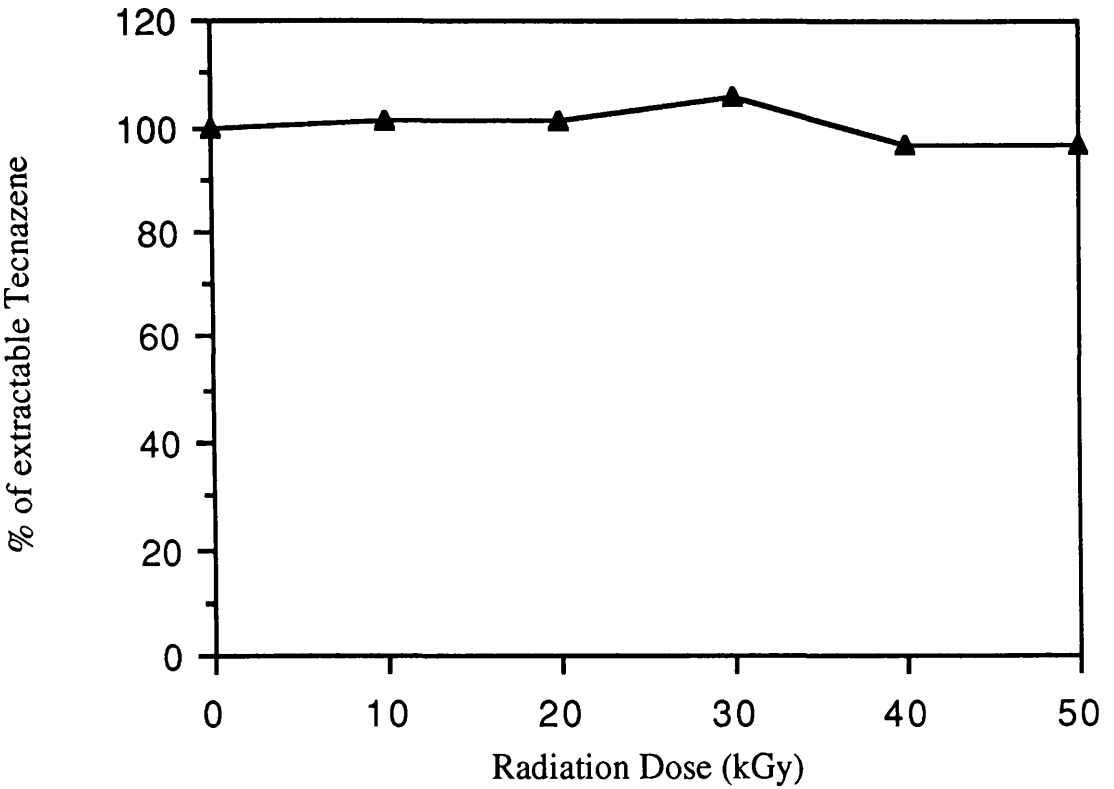
This procedure was repeated using the Tecnazene formulations Hortag (Avon Packers, U.K.) and Bygran S (Wheatly Chemicals, U.K.). Fusarex dust was nominally 3% Tecnazene, Hortag 5% and Bygran S 7.5% The only amendment made to the procedure used was in the filtration of Hortag extracts which had appeared cloudy after filtration through Whatman No.1 filter papers in preliminary experiments. Hortag extracts were

instead filtered through Whatman No.40 filter papers (Whatman, U.K.).

Results

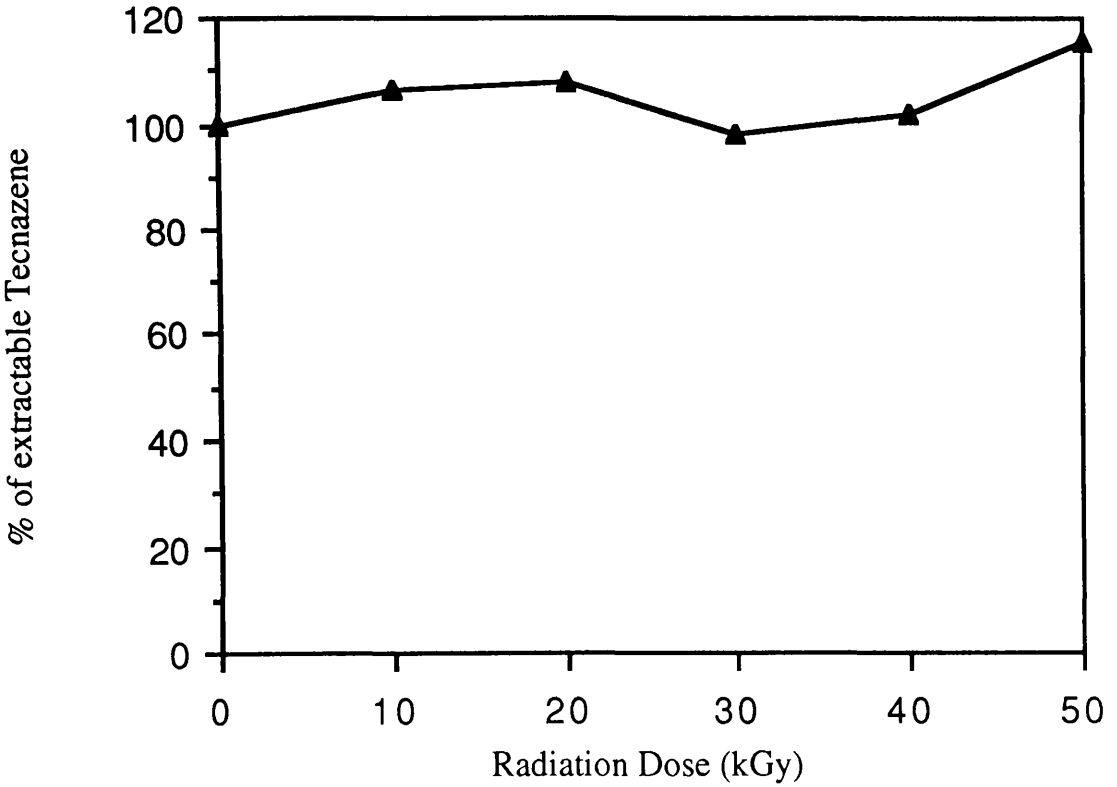
A single peak was present in the chromatograms of each extract, which, by comparison with standards, was found to represent Tecnazene. The concentration of Tecnazene remaining in extracts of irradiated formulations was calculated and expressed relative to that of Tecnazene in the extracts of non-irradiated formulations as described in section 6.3.2. The percentage of the initial Tecnazene content remaining after irradiation was plotted against the dose received for each formulation in graphs 6.5 to 6.7.

Graph 6.5. The effect of irradiation dose on the percentage extractable Tecnazene from Fusarex. *



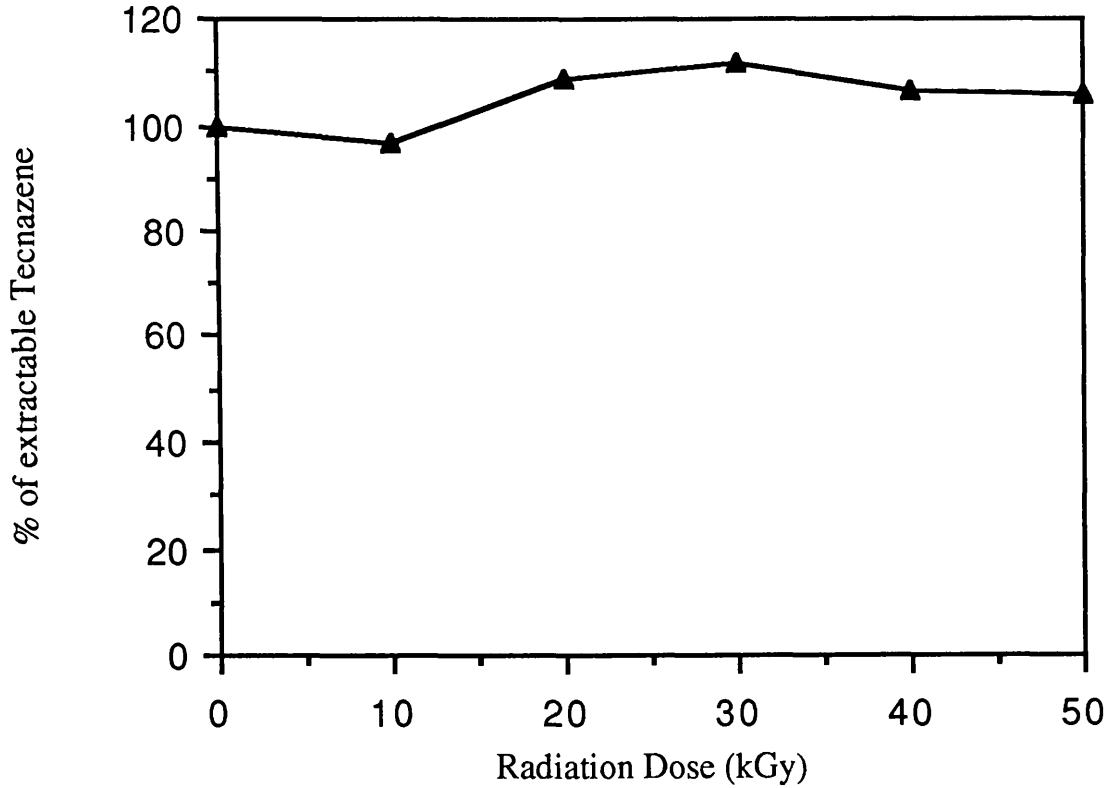
* each point is the mean of four replicate samples.

Graph 6.6. The effect of irradiation dose on the percentage extractable Tecnazene from Hortag.*



* each point is the mean of four replicate samples.

Graph 6.7. The effect of irradiation dose on the percentage extractable Tecnazene from Bygran S.*



* each point is the mean of four replicate samples.

Conclusions

From graphs 6.5 to 6.7 it can be seen that the percentages of Tecnazene extracted from irradiated Fusarex, Bygran S and Hortag were not appreciably lower than the percentages extracted from non-irradiated controls over the dose range considered. It should be noted, however, that a significant degree of variability still exists within replicate treatments and in some cases percentage extractable Tecnazene values from irradiated samples exceed 100% of the appropriate control values. This variability is particularly noticeable in Hortag extracts. Hortag extracts were filtered through Whatman No.40 filter papers rather than No.1 filter papers and this may have introduced variability by an unknown means.

Therefore in drawing conclusions from these results the degree of variability observed must be borne in mind. Differences in the percentage degradation of Tecnazene of less than 5% in most cases and 10 - 15% in the case of Hortag extracts cannot be demonstrated with any real confidence.

Some important inferences about the breakdown of Tecnazene in formulations can still be made. Firstly there appeared to be no large differences in the sensitivity of Tecnazene to radiolysis between the formulations irradiated. Secondly any breakdown of Tecnazene which did occur was small in comparison to that observed in irradiating Tecnazene in solution, particularly in methanol solution.

There are several possible explanations of the latter observation. It has been shown that radiolytic breakdown shows a marked concentration effect. If different concentrations of a solute are irradiated to the same dose a greater percentage of the more dilute

solution will be altered. This occurs because a given radiation dose produces the same number of primary radiolytic species in the two solutions. As most molecules present in a 100 ppm solution of Tecnazene are solvent molecules most primary radiolytic species will be derived from the solvent. The same number of solute molecules react with primary radiolytic species in each solution but that number represents a larger percentage of the number of solute molecules in the more dilute solution. At high concentrations the concentration effect is less applicable as a greater number of the solute molecules are directly affected by radiation. A 100 ppm solution of Tecnazene contains only 0.1% Tecnazene compared to 3 - 7.5% Tecnazene in formulations.

An alternative explanation of the differences in degree of breakdown is that the adsorbants used in Tecnazene formulations provide a much more inert medium than free solution. In solution Tecnazene molecules are free to react with any species produced in solution by irradiation, the importance of the nature of the solvent used has already been demonstrated. Tecnazene adsorbed in a formulation is not as free to diffuse and react with solvent derived species and therefore less breakdown may be observed.

The level of breakdown of Tecnazene in formulations and in solution may not therefore be readily comparable. The design of experiments which are capable of measuring small degrees of breakdown of Tecnazene within formulations is required, although this may be difficult while the degree of variability found in measurements in this study remains. Alternatively, a valid comparison could be made by irradiating Tecnazene formulations of much lower Tecnazene content. This study

has shown, however, that there is no measurable large scale radiolysis of Tecnazene in irradiated commercial formulations at radiation doses up to 50 kGy.

6.6 Identification of radiolytic products

6.6.1 Introduction

The identification of the compounds produced by the irradiation of Chlorpropham and Tecnazene demonstrated in sections 6.3 to 6.4 present two quite different problems which can be overcome using different chromatographic techniques. Irradiation of Chlorpropham in methanol or hexane was shown to produce compounds some of which are detectable by G.L.C. using an F.I.D.. Some steps were taken in their identification using that technique. Attempts were then made to identify such radiolytic products unequivocally by combining the G.L.C. separations of solutes with mass spectrometric detection, that is by G.C.M.S..

The irradiation of Tecnazene, however, resulted in its reaction to form products none of which could be detected by G.L.C. under the conditions used. Longer duration temperature gradient separations were carried out in order to determine whether compounds of lesser or greater volatility than those detectable under the standard G.L.C. conditions were formed. No such compounds were observed. (A similar investigation was carried out on irradiated Chlorpropham solutions with equally negative results.) Analytical techniques utilising U.V. absorbance as a detection method, such as H.P.L.C., were therefore employed in further attempts to identify the radiolysis products of Tecnazene as G.L.C. analysis had proven unproductive.

6.6.2 Radiolytic products of Chlorpropham in methanol solution

G.L.C. analysis

In section 6.3 the results of the irradiation of Chlorpropham in methanol solution showed that two peaks were visible in chromatograms of irradiated solutions. The first steps in identifying those peaks were made using G.L.C. analysis. Irradiated samples were subjected to G.L.C. analysis a second time and the average retention time found for Chlorpropham in non-irradiated solutions under the conditions used was calculated to be 3.61 minutes. In irradiated solutions the average retention times of the two peaks present were 1.84 and 3.62 minutes and from these times, and later confirming evidence, it was concluded that the peak at 3.62 in irradiated solutions was Chlorpropham. These retention times were quite different from those found in the first G.L.C. analyses indicating a change in conditions between the two analyses. From the retention times obtained from the second analysis the R_{CIPC} of the peak with retention time 1.84 minutes was calculated using the following formula.

$$R_{CIPC} = \frac{\text{Mean retention time of radiolysis peak}}{\text{Mean retention time of Chlorpropham}}$$

The R_{CIPC} of the radiolytic product was found to be 0.51. This value is similar to the R_{CIPC} of Propham observed in other studies. As radiation-induced dechlorination of other pesticides has been observed by other workers it was hypothesised that the radiolysis product with retention time of 1.84 minutes was Propham.

To more directly compare the R_{CIPC} of the radiolysis product and Propham a 180 ppm solution of Propham (Sigma, U.S.A.) was prepared and subjected to G.L.C. analysis concurrently with 100 ppm solutions of irradiated and non-irradiated Chlorpropham. This comparison was carried out using the G.L.C. conditions outlined in section 6.2 but at column temperatures of 150°C and 140°C to resolve the Propham and radiolysis peaks further from the solvent front. The R_{CIPC} values for Propham and the radiolysis peak were both 0.43 at 150°C and both 0.41 at 140°C. (The fact that these R_{CIPC} values are different from those obtained in initial experiments is due to the different chromatographic conditions used on the two occasions.)

These G.L.C. results show that the radiolytic product has the same retention time as Propham at two different column temperatures providing some evidence that the original hypothesis may be correct.

H.P.L.C. analysis

Using the H.P.L.C. method described in section 6.2 it was found that Chlorpropham had a retention time of 1.16 minutes and Propham a retention time of 0.81 minutes. The capacity factor k' of Propham was calculated by the formula below to be 1.00.

$$k' = \frac{t_r - t_o}{t_o}$$

where t_r is the distance from the point of injection to the peak maximum of Propham (or other compound of interest) and t_o is the distance between the point of injection and the peak maximum of an unretained compound, both in mm.

H.P.L.C. analyses of Chlorpropham solutions prepared in section 6.3 were carried out according to the method described in section 6.2. Two peaks appeared on the chromatograms of irradiated solutions with retention times of 0.74 and 1.02 minutes. By comparison with standards it was concluded that the peak with retention time 1.02 minutes represented Chlorpropham. The k' of the radiolysis peak was calculated to be 1.00. The k' of the radiolytic product is consistent with that compound being Propham but H.P.L.C. analyses were unable to provide conclusive identification of the radiolytic product.

G.C.M.S. analysis

Both G.L.C. and H.P.L.C. techniques provided good evidence to suggest that the radiolysis product observed may have been Propham. To confirm this unequivocally it was decided to submit an irradiated Chlorpropham solution to G.C.M.S. analysis.

In order to facilitate the G.C.M.S. identification of the Chlorpropham radiolysis product several preparative steps had to be carried out. Due to the relatively low concentration of the radiolysis product in more dilute solutions it was decided to irradiate a much more concentrated solution of Chlorpropham in methanol and to use a higher dose.

A 100 mg cm^{-3} solution of Chlorpropham in H.P.L.C. grade methanol was prepared in a 10 cm^3 volumetric flask and irradiated at the S.U.R.R.C. as described in section 6.3 at a dose of 100 kGy. Two serial 1:10 dilutions of that solution were made and 1 cm^3 aliquots of all three solutions were evaporated to dryness using a Buchi Rotavapor-R rotary evaporator (Orme Scientific, U.K.) and re-dissolved in 1 cm^3 of glass distilled grade hexane. The transfer of solutions

from methanol to hexane was necessary as the G.C.M.S. system used for identification used capillary G.L.C. columns, the stationary phase of which is degraded by methanol.

G.C.M.S. analysis of the irradiated solutions was undertaken using facilities kindly made available at the Hannah Institute by Dr.W. Christie. The G.C.M.S. instrument consisted of a Hewlett Packard 5890A gas chromatograph fitted with a 5970 series mass selective detector. The gas chromatograph was fitted with a Hewlett Packard Ultra 2 capillary column of 25 m length and 0.2 mm internal diameter. The Ultra 2 column has a bound stationary phase of 5% methyl silicone, a non-polar phase.

On-column injections of 1 ul of were made of each of the three dilutions of irradiated Chlorpropham using a Hamilton 700 series 5ul syringe with fused silica needle (Hamilton Bonaduz, Switzerland). A temperature gradient program was used for the separations as follows. Injections were made at a column temperature of $60^{o}C$, followed by a rise in column temperature to $170^{o}C$ at a rate of $60^{o}C\ min^{-1}$ and a $1^{o}C\ min^{-1}$ rise to $210^{o}C$. Under these conditions a compound, later identified as Chlorpropham, had a retention time of 34.1 minutes and the radiolysis product had a retention time of 28.7 minutes. Spectra of Chlorpropham and Propham standards were also obtained. Mass spectra of both compounds present in irradiated solutions are presented as diagrams 6.9 and 6.10.

Diagram 6.9. Mass spectrum of Chlorpropham from G.C.M.S. analysis of a 1000 ppm solution of Chlorpropham irradiated at 100 kGy.

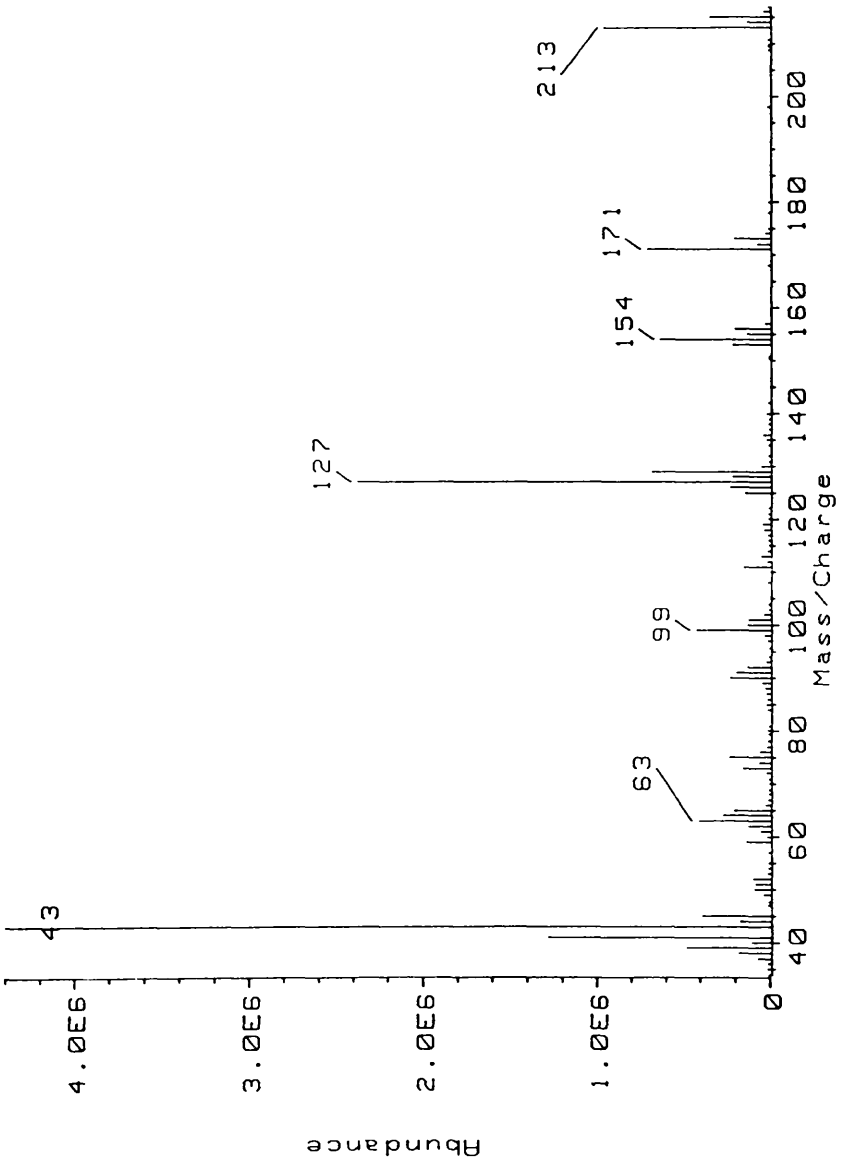
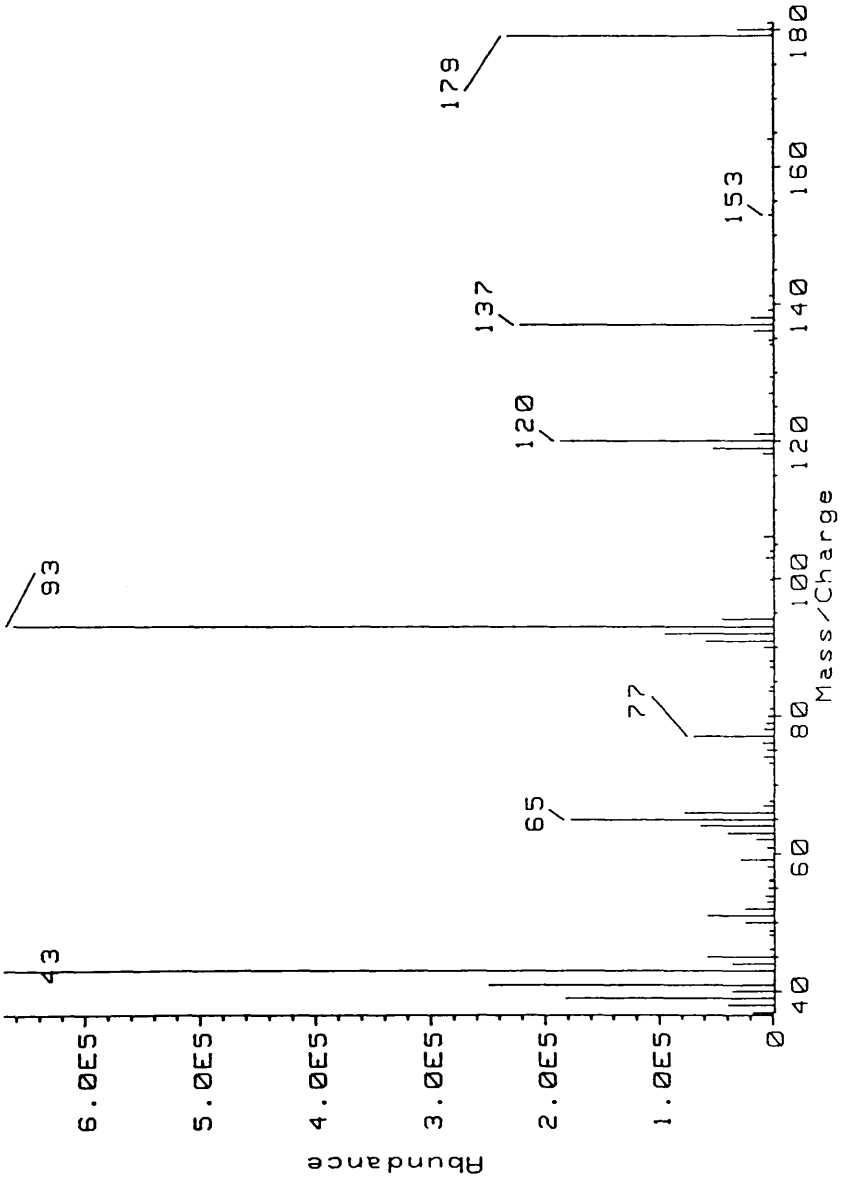


Diagram 6.10. Mass spectrum of a radiolysis product of Chlorpropham from G.C.M.S. analysis of a 1000 ppm solution of Chlorpropham irradiated at 100 kGy.



The mass spectrum of the compound with the retention time of 34.1, diagram 6.9, shows that it has a molecular ion of m/e of 213.05 and a fragmentation pattern which was found to be identical to that of the Chlorpropham standard. The theoretical mass of Chlorpropham containing ^{35}Cl was calculated to be 213.0556. The most abundant masses of fragmentation species formed from Chlorpropham in the clearer high m/e range of its spectrum were at m/e values of 171, 154 and 127. These fragments can also be seen in the spectrum in diagram 6.9. Accurate mass measurements made using high resolution mass spectrometry identified those fragments to represent the stepwise loss from the molecular ion of C_3H_6 to m/e = 171, OH to m/e = 154 and C=O to m/e = 127. The presence of (m/e + 2) peaks at m/e values of 215, 173, 157 and 129 at a ratio of approximately 1:3 of the m/e peak is indicative of the presence of a single chlorine atom on the fragment remaining at each stage. The (m/e + 2) peaks are produced by the fragmentation of Chlorpropham containing the ^{37}Cl isotope rather than the more abundant ^{35}Cl isotope. This fragmentation pattern indicates a stepwise degradation of the isopropylcarbamate substituent of the aromatic ring, although possible direct loss from e.g. m/e 213 to 154 or 127 of a larger fragment cannot be completely excluded.

The fragmentation pattern observed in the mass spectrum of the radiolysis product of Chlorpropham with a retention time of 28.7 minutes seen in diagram 6.10 was found to be identical to that of the Propham standard. The theoretical mass of Propham was calculated to be 179.0946 - only one mass of major abundance occurs as none of the elements present in Propham have other isotopes of high percentage abundance. The major m/e peaks in the high m/e range of the spectrum of the radiolysis product (and of Propham) as can be seen from diagram

6.10 are at m/e values of 179 (molecular ion), 137, 120 and 93. The molecular ion is 34 mass units smaller than that of Chlorpropham indicating a molecule with the chlorine atom (atomic mass = 35) replaced by a hydrogen atom (atomic mass = 1). The lack of appropriate (m/e + 2) signals of ratio 1:3 of the m/e signals at m/e 181, 139, 122, and 95 strongly support the inference that no chlorine atom is present in the radiolysis product. The major signals found in the spectrum of Propham were identified by the use of high resolution mass spectrometry to be due to the stepwise loss from the molecule of C_3H_6 (to m/e = 137), O (to m/e = 120) and C=O (to m/e = 93).

From the above results it can be stated with confidence that the radiolysis of Chlorpropham in methanol solution results in the formation of Propham by the replacement of the chlorine atom on the aromatic ring by a hydrogen atom. The possible mechanism by which this occurs is discussed in section 6.7.

6.6.3 Radiolytic products of Chlorpropham in hexane solution

G.L.C. analysis

G.L.C. analysis of irradiated hexane solutions of Chlorpropham showed the presence of two compounds with average retention times of 5.18 and 9.53 minutes respectively. By comparison with standards the compound with retention time 9.53 minutes was found to be Chlorpropham. The R_{CIPC} calculated for the radiolytic product by the method outlined above was 0.54.

H.P.L.C. analysis

The analysis of irradiated hexane solutions of Chlorpropham produced similar results to that in methanol solution. The two compounds observed had retention times of 0.74 and 1.00 minutes, a k' value of 1.00 was calculated for the radiolytic product.

G.C.M.S. analysis

The radiolysis product was found in much greater concentration in samples irradiated in hexane than in methanol and therefore no concentration step was required for hexane solutions. Transfer of solutes from methanol solution was, of course, also unnecessary. A 100 ppm Chlorpropham solution in hexane irradiated at a dose of 20 kGy was submitted to G.C.M.S. analysis by the method used in section 6.6.2. The only modification to that method used in this case was that the column was an polar BPX 70 capillary column (S.G.E., Australia) of length 36 m and internal diameter 0.22 mm. Under the same chromatographic conditions as used in section 6.6.2 using the BPX 70 column the retention time of Chlorpropham was 18.6 minutes and the retention time of the radiolysis product was 11.7 minutes. Mass spectra of Chlorpropham and the radiolysis product were obtained and were found to be identical to those obtained in the radiolysis of Chlorpropham in methanol solution. The above evidence demonstrates that the irradiation of Chlorpropham in hexane solution results in the production of Propham but in a higher yield than is found when Chlorpropham is irradiated in methanol.

6.6.4 Radiolytic products of Tecnazene in methanol solution

From the results of section 6.4 it can be seen that there is a large degree of breakdown of Tecnazene when irradiated in methanol solution to doses of 10 kGy and greater. As has been previously established no radiolysis compounds were detected by either isothermal or temperature gradient G.L.C. techniques.

Several possible radiolytic reactions of Tecnazene were considered. Firstly the removal or substitution of the aromatic ring substituents from the Tecnazene ring has been reported by Hamadmad (1967) in photolysis of Tecnazene using U.V. light. It is possible that this could occur in the gamma irradiation of Tecnazene. If the resultant products were involatile, too volatile or produced in small yields of possibly mixed products the G.L.C. analysis carried out may not have detected them. Secondly the species produced from Tecnazene by irradiation may have undergone polymerisation resulting in involatile products. Finally irradiation could lead to the opening of the aromatic ring and the open chain products from ring opening may not have been detectable by the G.L.C. method used.

U.V. spectrometry

In order to discover if the aromatic ring was intact after irradiation the U.V. absorbance of a non-irradiated solution of Tecnazene and a solution irradiated at a dose of 50 kGy in section 6.4 were scanned between the wavelengths of 190 nm and 500 nm. Two absorbance maxima were observed in the spectrum of the non-irradiated Tecnazene solution at wavelengths of 217 nm and 240 nm. These absorbances are the result of the excitation of electrons from pi to pi^* orbitals in the aromatic ring of Tecnazene by the absorbance of

energy from U.V. light. Unsubstituted benzene has absorbance bands at 204 nm (E band) and 230 - 270 nm (B band) which are red-shifted by the substituents attached in the Tecnazene molecule to give the absorbances measured.

The absorbance spectrum of the irradiated solution was also found to have two absorbance maxima at 217 nm and 240 nm. The magnitude of the absorbances appeared to be little affected by irradiation when compared to the spectrum of the non-irradiated solution. This indicated that the radiolysis of Tecnazene probably did not result in the destruction of the aromatic ring. It also indicated that H.P.L.C. analysis of irradiated solutions using U.V. detection could detect products formed that were not detected using G.L.C. analysis techniques.

H.P.L.C. analysis

H.P.L.C. analyses of the solutions of Tecnazene prepared in section 6.4 were carried out by the method described in section 6.2. In non-irradiated solutions only one peak was present excluding the solvent front and by comparison with standards it was identified as Tecnazene. Tecnazene had a retention time under the conditions used of 2.48 minutes.

In samples irradiated at 10 kGy a third peak was also present and had a retention time of 1.27 minutes. In solutions irradiated at doses of 20 - 50 kGy eight peaks, including the solvent front and Tecnazene, were visible. A typical chromatogram of a non-irradiated sample and a sample irradiated at a dose of 20 kGy are shown in diagrams 6.11 and 6.12.

Diagram 6.11.

H.P.L.C. chromatogram of a 100 ppm solution of Tecnazene in methanol. Chromatographic conditions are given in the text.

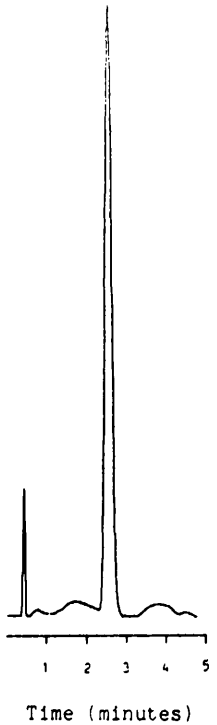


Diagram 6.12.

H.P.L.C. chromatogram of a 100 ppm solution of Tecnazene in methanol irradiated at a dose of 20 kGy. Chromatographic conditions are given in the text.

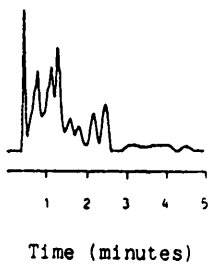


Table 6.6 displays the retention times and k' values of peaks present in a solution of Tecnazene irradiated at a dose of 20 kGy.

Table 6.6. Retention times and k' values of the H.P.L.C. peaks present in the chromatogram of a 100 ppm solution of Tecnazene in methanol irradiated at 20 kGy.

	Peak Number							
	1	2	3	4	5	6	7	8
Mean retention times*	0.44	0.76	1.10	1.27	1.57	1.79	2.16	2.48
k' (capacity factor)	Solvent	0.78	1.55	1.89	2.56	3.00	3.89	4.78 (TCNB)

* mean retention times are the means of 16 replicate injections.

The peaks representing radiolysis products, that is peaks 2 - 7 were not fully resolved and therefore quantification of their nominal concentrations was not possible.

Attempts were made to identify the radiolysis products formed by isolating fractions as they were eluted from the H.P.L.C. column, timed to represent particular radiolysis peaks, and to analyse the fractions by mass spectrometry (M.S.). Fractions obtained were dried to remove water, which interferes with M.S. analysis, by passing them through a narrow column of anhydrous Na_2SO_4 (B.D.H., U.K.) The eluent was blown down using N_2 gas to increase the concentration of solutes and the samples submitted to M.S. analysis.

These attempts to identify radiolysis products failed as the signal from the products was masked by a signal from a contaminant present in

the fractions. It was thought likely by inspection of the spectra obtained that the contaminant was a plasticiser compound commonly used in the manufacture of plastic tubing. This contaminant was thought to have been introduced into the fractions by the action of the mobile phase solvents on the plastic solvent lines of the H.P.L.C. itself. This problem could not be overcome using the H.P.L.C. instrumentation available for this study. Alternative strategies for identification such as Thin Layer Chromatography (T.L.C.) or H.P.L.C. interfaced with mass spectral identification (H.P.M.S.) could not be pursued in this study.

6.6.5 Radiolytic products of Tecnazene in hexane solution

Much less degradation of Tecnazene took place when it was irradiated in hexane solution than in methanol solution. Both G.L.C. and H.P.L.C. analysis failed to detect the presence of compounds other than Tecnazene in irradiated hexane solutions. These techniques were therefore unsuitable for investigating the formation of radiolysis products in those solutions at the low concentrations at which they are formed under the conditions used in this study.

6.7 Conclusions

Irradiation of Chlorpropham in methanol and hexane solution

From the results of sections 6.6.2 and 6.6.3 it can be seen that the major product of the gamma radiolysis of Chlorpropham in methanol and hexane solutions is Propham. The replacement of the ring Cl by H, as has been previously discussed in section 6.1, is a well documented photolytic and radiolytic reaction. The reaction mechanism by which Chlorpropham is dechlorinated cannot be fully understood without further experimentation, only tentative explanations of how dechlorination may have occurred can be made. The following discussion, therefore, can only be speculative.

U.V. photolysis studies have shown that in solvents with labile H the homolysis of the Cl bond of chlorinated aromatics to form radicals can be followed by the attachment of H by combination with $H^{\bullet}$ radicals produced by the irradiation of the solvent (Tanaka et al., 1984; Kamrin and Rodgers, 1985). How comparable then are the reactions which take place in photolysis and radiolysis?

The formation of radicals by U.V. irradiation occurs by the absorption of photons by the electrons of the irradiated substance. Such electrons are thus promoted to higher energy orbitals resulting in an excited molecule able to undergo chemical change. The direct effect of the absorption of a gamma photon by a target molecule may also result in that molecule undergoing chemical change but another consequence is the emission of an energetic electron from the absorbing atom. The emitted electron can cause many subsequent chemical changes by losing a proportion of its kinetic energy to other molecules by colliding with them. These collisions form molecules in

excited states which can then undergo chemical reactions such as bond breaking and reactions with other substances in solution (most of the original energy of the gamma photon is transferred into the kinetic of the electron). Alternatively after several collisions the energy of the electron may be reduced sufficiently for it to be absorbed directly by the target molecule. Therefore the way in which U.V. photolysis and gamma radiolysis have their effect is different. They may, however, produce similar end products.

The collision of an electron with a Chlorpropham molecule could result in the formation of an excited molecule in which homolysis of the bond between the aromatic ring and the Cl substituent could take place, forming two radicals which could further react.

Another reaction of Chlorpropham which may be caused by irradiation can be highlighted by a discussion of the radiolysis of chlorobenzene. In the irradiation of chlorobenzene it was found that electrons are absorbed into an unoccupied pi^* orbital of the aromatic ring (Lazar et al., 1989). This absorption is followed by the production of the chloride anion to give the overall reaction shown in equation 6.1.

$$Ph\text{-}Cl + e^- \longrightarrow Ph^\bullet + Cl^- \qquad \text{(equation 6.1)}$$

A similar reaction mechanism for the radiolysis of Chlorpropham is shown in equation 6.2 where R represents the isopropylcarbamate substituent on the aromatic ring.

$$R\text{-}Ph\text{-}Cl + e^- \longrightarrow R\text{-}Ph^\bullet + Cl^- \qquad \text{(equation 6.2)}$$

One product of this reaction would also be formed by homolysis of Chlorpropham, the $R\text{-}Ph^\bullet$ radical. Reaction of the resultant $R\text{-}Ph^\bullet$

radicals, formed by either mechanism, with H$^{\bullet}$ radicals produced by the radiolysis of solvent molecules would result in the formation of Propham, as described in equation 6.3.

$$R\text{-}Ph^{\bullet} + H^{\bullet} \longrightarrow R\text{-}Ph \qquad \text{(equation 6.3)}$$

This proposed mechanism for the formation of Propham would in part explain the relative sensitivities of Chlorpropham to irradiation in hexane and methanol. Hexane has 14 H atoms per molecule compared to only 4 in the methanol molecule resulting in higher G values for H$^{\bullet}$ in hexane than in methanol (G values of >4.1 and 2.5 respectively (Milinchuk and Tupikov, 1989)). If this is indeed how Chlorpropham radiolysis occurs the presence of other radiolysis products of Chlorpropham can be predicted. Free radicals produced in irradiated hexane include; $^{\bullet}CH_3$, $^{\bullet}C_2H_5$, $^{\bullet}C_3H_7$, $^{\bullet}C_4H_9$, $^{\bullet}C_5H_{11}$, $^{\bullet}C_6H_{13}$ (Milinchuk and Tupikov, 1989). Such free radicals, although present in irradiated solutions in lower concentrations than H$^{\bullet}$ radicals, could also combine with R-Ph$^{\bullet}$ radicals to form alkyl derivatives of Chlorpropham. Such derivatives have been detected in the U.V. photolysis of Chlorpropham in hexane in which it is proposed that the R-Ph$^{\bullet}$ radical is also formed, although by a different mechanistic route (Zaater, 1989). Other reactions such as two R-Ph$^{\bullet}$ radicals combining to form R-Ph-Ph-R are also possible and have been observed in photolysis studies (Tanaka et al., 1984; Zaater, 1989). Once formed the R-Ph$^{\bullet}$ radical could also adopt other resonance forms which could also react with other species in solution further increasing the number of possible radiolysis products. The formation of other products from the interaction of electrons with Chlorpropham molecules add further to the possible radiolytic products, they may also provide alternative mechanisms for the formation of Propham from Chlorpropham.

The quantities of Propham produced by the irradiation of Chlorpropham, particularly when irradiated in hexane, seem quite large in comparison to the yields found in the radiolysis of other compounds. This may reflect the fact that Cl is a good leaving group and that the most abundant radical in solution available for combination with the R-Ph$^{\bullet}$ radical is H$^{\bullet}$. Once formed Propham is open to radiolysis itself, the fact that it accumulates with dose implies that it is much less sensitive to radiolysis than Chlorpropham. This may be due to the unsuitability of the H substituent as a leaving group when compared to Cl. Even if the R-Ph$^{\bullet}$ were to be formed from Propham a proportion of it could recombine with H$^{\bullet}$ radicals to form Propham.

More evidence to confirm the free radical nature of the reaction mechanism could be obtained by irradiating solutions of Chlorpropham containing substances which are known to either promote free radical reactions, such as riboflavin or acetone, or inhibit them, such as butylated hydroxy anisole (BHA) or butylated hydroxy toluene (BHT). Other techniques which may be of some use in elucidating the reaction mechanism include Electron Spin Resonance (E.S.R.) and Pulse Radiolysis by which the presence of free radicals can be measured directly.

The production of Propham from Chlorpropham by irradiation treatment is not of concern with regard to the toxicity of that product. However, the combined contents of Propham and Chlorpropham in irradiated solutions do not account for 100% of the reaction products and other possible products have been suggested earlier in the text. It would seem unlikely that in a solution containing so

many possible reactive species Propham should be the sole product. Further investigations in this field are required.

The concentrations of Chlorpropham solution irradiated and the doses used in this study may seem unrealistically high. This is a necessary consequence of the need for clear identification of the products being formed. The effect of concentration on the % retention of an irradiated substance has been discussed in section 6.5.2. The proposed maximum permitted dose for the irradiation of food in the U.K. is 10 kGy, although the effective sprout inhibition dose is of the order of 0.1 kGy. Therefore if potatoes are irradiated at the sprout inhibition dose less breakdown of Chlorpropham than was observed here would occur. If higher doses are applied to second generation products for another purpose greater breakdown may be possible. These arguments can also be applied when discussing the degree of breakdown of Tecnazene in food.

Irradiation of Tecnazene

The failure of these experiments to identify the products of Tecnazene degradation leave the question of whether a health hazard is presented by them unanswered. This study does, however, show that breakdown of Tecnazene can occur and that the degree to which it takes place depends on the chemical environment in which it is irradiated. Without a knowledge of the radiolysis products, however, the mechanism by which such degradation takes place cannot be properly discussed. Further studies are required to refine the experimental methods used to identify the radiolysis products of Tecnazene.

If Tecnazene is degraded by gamma radiation in the same way as it is when exposed to U.V. light, the methods used to detect the products identified by Hamadmad (1967) may provide a starting point for further work. The observation that greater degradation of Tecnazene takes place when it is dissolved in methanol than in hexane solution, in contrast to the effect of the solvent on Chlorpropham radiolysis, may suggest that at least some of the radiolysis products of Tecnazene are formed by different mechanisms to those proposed for Chlorpropham radiolysis.

Irradiation of Chlorpropham and Tecnazene with potatoes

One clear implication of this study is that any discussion of how Chlorpropham and Tecnazene applied to potatoes will be affected by irradiation needs to take account of the nature of the chemical environment in which they are found in potatoes. Sprout suppressants can be found within the flesh of the potatoes, in the potato periderm or in contact with the soil adhering to the potatoes. Each of these environments provides different conditions for radiolysis and each environment is also heterogeneous. Potato flesh is essentially an aqueous environment, but lipid membranes may also provide sinks for semi-polar molecules such as Chlorpropham and Tecnazene. The potato surface, on which sprout suppressants will be adsorbed after application, is largely lipophilic in character but more polar aqueous conditions are also to be found where water is evaporating out through the periderm. Soil is a very heterogeneous medium consisting of organic, inorganic and aqueous fractions in contact with each other. The moisture content of soil associated with the potatoes in the store will also influence the breakdown of the sprout suppressants. The impact of soil micro-organisms on the degradation of sprout

suppressants should not be overlooked.

The comparisons made by irradiating Chlorpropham and Tecnazene in a non-polar solvent, hexane, and a semi-polar solvent, methanol, may be of some help in understanding how they may behave when they are irradiated with potatoes. This study has shown that Chlorpropham is broken down more rapidly in hexane than in methanol. It has also shown that the converse is true of Tecnazene. This may indicate that Chlorpropham is more sensitive to radiolysis when it is associated with the less polar environments of the periderm, with the membranes of the potato flesh and in the organic fraction of the soil. In contrast to this, Tecnazene associated with the more polar environments in the potato or soil may be more susceptible to breakdown. The radiolysis of Chlorpropham and Tecnazene in aqueous solution would be of great interest in broadening the possible scope of such discussions. Further studies of how Chlorpropham and Tecnazene behave in chemical conditions which more closely resemble those found in potatoes are required for a better understanding of how Chlorpropham and Tecnazene will behave when irradiated with potatoes.

A distinction must also be drawn between the methods by which Chlorpropham and Tecnazene are applied to potatoes. Chlorpropham is normally fogged onto the potato surface dissolved in an appropriate organic solvent while Tecnazene is dusted on as powder or granular formulation. Tecnazene irradiated in the highly viscous environment of an inorganic adsorbant is likely to behave quite differently to Tecnazene which has vapourised and been re-adsorbed by the potato or soil adhering to it. Kamrin and Rodgers (1985) state that the recombination of radicals formed in the photolysis of chloro-aromatics

on inorganic surfaces is more likely when compared to photolysis in less viscous liquids in which reactive species can migrate. This may also apply to the radiolysis of Tecnazene.

This study has only really made a beginning to the investigation of this topic. Much work has still to be carried out to gain a fuller understanding of the reactions taking place in model systems and the translation of that understanding into an interpretation of what may occur in a potato store. Only Chlorpropham and Tecnazene have been discussed here, other compounds merit similar investigation.

Chapter 7

Conclusions

7.1 The effect of irradiation on sprouting

The purpose of this study was to investigate various aspects associated with the inhibition of the natural sprouting of potatoes by irradiation. In the course of that study it was found that regression, and more particularly logistic regression, was a useful statistical technique to model the effect of increasing radiation dose on the degree of sprouting developed. Logistic regression is particularly suited to describing relationships in which, like sprouting, one of the parameters measured can be negative or affirmative as well as having magnitude when affirmative. For this reason it is recommended as a more informative technique than the more commonly used methods applied to describe the way in which some chemical sprout suppressants affect sprouting.

The degree of sprouting found ordinarily in potatoes is dependent on a number of factors, as discussed in Chapters 1 and 2, amongst which are seasonal variation and cultivar dependence. In order to more fully understand how effectively sprouting is controlled by irradiation further work is necessary to assess the dependence of the degree of sprout control by irradiation on seasonal fluctuations. Although the number of growing seasons able to be considered in this study was small, cultivar dependence effects were observed. From this evidence and the evidence of other studies on cultivar dependence effects it would seem sensible to assess the sensitivity to irradiation of each cultivar proposed for irradiation treatment.

The practical irradiation of a large volume of potatoes necessitates the use of large scale commercial irradiators. The spread of doses received by potatoes in experiments carried out in this study was significantly wide, especially at dose levels at the critical boundary between inadequate sprout control and complete sprout inhibition. In the report of the ACINF (Anon., 1986a) it was stated that for a food receiving an average dose of 10 kGy, the maximum dose recommended for use on food in the U.K., a typical range of doses received by different parts of the food could be between 8 and 12 kGy or in some cases as wide as 6.5 and 13 kGy depending on the design of the irradiation plant. This non-uniformity of dose received will also be found at the lower doses required for sprout inhibition. The spread of doses obtained and dose levels required for the necessary degree of sprout inhibition should therefore be assessed for each irradiation plant.

7.2 The effect of sprout suppressant treatments on sugar contents

Experiments carried out in this study on the response of sugar levels in potatoes to sprout inhibition treatments have demonstrated a number of features of that response. Firstly the initially very high sugar levels noted in control potatoes in comparison to those observed by previous workers emphasise the variability present in the sugar levels of potatoes found from crop to crop. This illustrates that no simple formula can be applied to predict the effect of a given radiation dose on potato sugar levels. The initial sugar levels of potatoes under study must be determined prior to irradiation and those levels can then be used to make estimates of how large the radiation induced sweetening effect will be. Work carried out already may be of some use in this respect but further studies on the response of tubers

of varying degrees of initial sweetness and physiological age to irradiation treatment would provide a clearer understanding of this effect.

After the immediate post-irradiation sweetening has reached a peak it again falls to a level similar to controls. In potatoes which had initially suitable sugar contents this reduction may allow irradiated potatoes to be used commercially after a suitable storage interval has elapsed. Immediate use of irradiated potatoes is inadvisable as the quality of the final potato product will be disappointingly low. The rate of fall in the sugar contents in these experiments was found to be slower than that observed by Burton et al. (1959). This may be a consequence of the different experimental conditions and cultivars used in the two studies. It may, however, also be as a consequence of the markedly different initial sugar contents prior to irradiation. The identification of the controlling influences on the rate of fall in sugar contents after radiation induced sweetening would be of great interest to processors using irradiated potatoes as it would facilitate the development of good store management practices and cultivar selection to minimise the desweetening period.

Conflicting results have been obtained in studies, including this one, investigating the effect of irradiation on the timing of senescent sweetening. Burton et al. (1959) reported that senescence occurred earlier in irradiated potatoes than in controls. In this study only cv. Desiree senesced by the end of the storage period of the experiment. Further work is required in this area, particularly as it has been suggested that a desweetening interval should be maintained between irradiation and use. If irradiated potatoes are

stored to desweeten them for too long senescence could intervene.

The link between sprouting, its inhibition and sugar contents has in part been illustrated by the comparison of the degrees of sprouting and sweetness of potatoes irradiated or treated with Chlorpropham or Tecnazene towards the end of the storage period studied. Treatments which completely inhibited sprouting, Chlorpropham and irradiation, did not give rise to an accumulation of sugars in tubers as previously reported over the storage period measured. Treatments in which sprouting was evident did however exhibit small sugar content increases as sprouting progressed. More specific studies of the effect of sprout inhibition on sugars are required to fully understand the way in which the initiation and growth of sprouts affects free sugar contents.

With further work to increase the knowledge of how sugar contents are affected by irradiation and the application of appropriate radiation treatment and potato storage practices based on that knowledge, it is unlikely that sweetening induced by irradiation will stand in the way of the introduction of the irradiation of potatoes. Such sweetening does, however, limit the flexibility of use of irradiation by placing constraints on the timing of the processing of products from irradiated potatoes.

7.3 The effect of sprout suppressant treatments on water loss

The rapid deterioration of potatoes with wounds after irradiation found in this and other studies underlines the need for a pre-irradiation curing period. This is in agreement with the advice of Metlitsky et al. (1967) and the general advice on limiting rotting discussed in Chapter 4. Rates of water loss from non-wounded irradiated potatoes were found to be no greater, and in the case of the 0.10 kGy treatment slightly lower, than those from controls.

Due to external factors it was not possible to include potatoes irradiated directly after harvest in this study, a fully balanced experiment would produce interesting information complementary to that provided by this study. It is, however, in part possible to predict the likely outcome of such an experiment from the results of water loss studies carried out here. A comparison of wounded and non-wounded potatoes irradiated after curing revealed much greater loss from wounded tubers. It would seem likely that uncured potatoes whose ability to form normal periderm was inhibited by irradiation would lose water at a rate comparable to that of wounded tubers.

Chlorpropham has been shown by this study to adversely affect the rate of water loss from wounded tubers but not non-wounded tubers and by implication affects the ability of potatoes to wound heal properly. Further experiments are required to adequately determine how this occurs, although it seems likely that it may be due to the inhibition of cell division in periderm tissue. The effect of methanol on the rates of water loss suggests that the combination of methanol and Chlorpropham in fog applications may deleteriously affect water losses from uncured potatoes by combining the adverse effects of both

chemicals. Alternative solvents for Chlorpropham application should be investigated.

DMN was shown to have little discernible adverse effect on wound healing. It should be noted, however, that only one dose of DMN could be included in this study and it was found to be ineffective in controlling sprouting. Further studies using other experimental techniques and a wider range of DMN doses are required to demonstrate its efficacy conclusively.

Tecnazene, in contrast to all of the other treatments, appeared to slightly reduce the rate of water loss in comparison with controls. The reduction was, however, at a level which was not statistically significant. This sub-significant effect has previously been observed, as is discussed in Chapter 4, and is worthy of further interest. Tecnazene may afford an improvement over water losses from controls by reducing fungal infection and inhibiting sprouting, but without further work this explanation can be only speculative.

The rate of water loss at various points in the storage season is influenced to varying degrees by many factors including sprouting, periderm formation, bacterial and fungal attack. It would be of great interest if the magnitude of the contribution of each of these factors to water losses at any given point in the storage season could be identified. For example it may be possible to use Tecnazene in further studies as a non-sprouting "control" to eliminate contributions of sprouting and some fungal spoilage to water loss as it does not seem to inhibit wound healing.

The rate of water loss found in cured, non-wounded irradiated potatoes was not significantly greater than that from controls. Thus

the effect of irradiation on the ability of potatoes to heal wounds can be overcome by the use of appropriate store management practices, primarily by the observance of a pre-irradiation curing period.

7.4 Isolated periderm studies

Measurements of the weights of periderm isolated from a given area from untreated potatoes over a storage season indicate that a rapid development takes place in the first 20 days of storage. This was followed by a slower but constant period of periderm weight increase. These observations are in agreement with the way in which periderm has been shown to develop after harvest or after wounding.

No significant effects of the sprout suppressant chemicals Chlorpropham and Tecnazene or 0.10 and 0.15 kGy irradiation treatments were found on the periderm permeabilities of treated tubers. There was, however, a large degree of variability in the measurements of permeability coefficients. Further experimentation involving the development of the method used to determine permeabilities may lead to a sufficient refinement of that method to enable the effects of treatment to be seen above random variability.

The studies carried out in Chapter 5 were carried out on two points in the storage season, after 20 days and after 123 days. No significant difference was found between the permeabilities of periderms isolated at the two points in storage. Periderms were isolated from several other points in the storage season and further information on the effects of treatment with storage time may be provided by measuring their permeabilities.

It may also be fruitful to measure the permeabilities of periderms

isolated at the earlier storage sample points in subsequent studies to detect differences when periderm development is more rapid. Periderms isolated from group 1 potatoes may provide information about the permeabilities of periderms isolated at the earlier stages of storage as more samples were taken during that period from group 1 potatoes. Further work is required if the potential of this technique is to be realised.

7.5 Irradiation of pesticides

The effect of irradiation on pesticides that are applied to food is as yet incompletely understood. Much work is required to fill any gaps in our knowledge of the potential risks posed by the possible products of such combined treatments. This study has shown that two commonly applied potato sprout suppressant chemicals, Chlorpropham and Tecnazene, can in some chemical environments be broken down to other products by irradiation.

The major breakdown product of Chlorpropham was identified as Propham but other products of Chlorpropham radiolysis and several products of Tecnazene radiolysis observed in the course of this study have not yet been identified. The continuing progress of investigations on this topic would be of relevance in deciding whether irradiation treatment is safe to use in conjunction with chemical treatments. The mechanism by which the radiolysis of Chlorpropham and Tecnazene takes place has yet to be elucidated and could provide an insight into how these chemicals would behave if irradiated in a potato store. Subsequent studies of the effect of a wider range of chemical environments on the radiolytic breakdown of those chemicals would also be of practical interest.

Other chemicals commonly applied to stored potatoes, and therefore which may also be irradiated, such as Thiabendazole (TBZ) and 2-aminobutane (2-AB) are also worthy of further research. In a wider context the scope of such investigations can be broadened to include food contaminants or additives likely to be irradiated with food. If priorities are to be set as to which chemicals merit most immediate interest it would be sensible to place a high priority on chemicals which are applied after harvest and will therefore definitely be present in food in significant quantities. Other factors which may be weighed in deciding priorities for further study are the toxicity of the chemicals under consideration, the toxicity of their likely products, where reasonably predictable, and the amount of treated food likely to be ingested in an average diet.

Investigations of the breakdown of food additives and contaminants should be seen as a prerequisite to consent for irradiating food containing them, although little consideration has been given to them in the decision to approve the use of irradiation in the U.K.. For example no reference to the radiolysis of these substances could be found in the report of the ACINF (Anon., 1986a). The approval of irradiation for use on such foods should be withheld until these investigations are completed.

7.6 Viability of food irradiation

Siting of the irradiation facility

If irradiation is to be used as a method of sprout suppression there are several logistical problems inherent in its use which must first be solved. The nature of these problems depends on the requirements of the producers, potato processors and consumers.

An important consideration in irradiating agricultural produce of low value per weight is the siting of the irradiation facility, as transportation to a remote facility may result in unacceptable increases in cost. The choice of a suitable site will depend on the ultimate use to which the irradiated potatoes will be put. If they are to be used for processed potato products, and therefore to be stored for a substantial period of time before use, a centralised facility at the site of storage is perhaps the most efficient answer. However, if potatoes are to be transported after irradiation directly to the consumer, transportation to a centralised facility may not make economic sense. Instead, smaller local facilities or mobile irradiators may be the methods of choice. Mobile facilities may also raise the question of the safety of transporting a radiation source of the necessary size on the public networks.

In the U.K. it would appear likely that processors with a few central storage facilities may prefer to have irradiation facilities at those few sites (depending on the cost of a facility). The type of potato store itself is an important factor in determining the suitability of the application of the irradiation process. Bulk storage or box stores are the two most common types used by processors, irradiation treatment would be made easier if potatoes

were stored in boxes as whole boxes could then be irradiated. Bulk stored potatoes would have to be handled more resulting in greater damage and loss.

In a purely Scottish context, the option of irradiation as a sprout suppressant technique is less easy to fit into the market structure than in the U.K. as a whole. Scottish potato production falls into two main categories; production for domestic ware consumption and production for seed. The latter is often transported for use in the rest of the U.K. or exported.

Sprout suppressants are not used on ware potatoes for direct domestic consumption at present but irradiation could be used to prolong their shelf life. If irradiation is to be used it would require a coordinated approach amongst producers who would be unable to bear the high capital cost of an irradiation facility individually. Increased transport costs would be incurred as potatoes would have to be transported to the irradiator and then to the market place or retailer.

The use of sprout suppressants on seed potatoes is banned, although Tecnazene is often applied to them, under the guise of a fungicidal treatment, to inhibit sprouting until the following growing season. Tecnazene treated potatoes are aired before planting to remove the chemical enabling them to sprout. However, sprout inhibition by irradiation is not a reversible sprout inhibiting process and once treated the potatoes cannot be used as seed. The dose required for adequate sprout control by irradiation, 0.05 - 0.15 kGy, would be too great to allow any subsequent sprouting. A 0.05 kGy dose was found to result in reduced emergence by Sparrow and Christensen (1956),

resulting in a yield of only 4% of the yield of controls at that dose. Low doses of irradiation have been reported to stimulate sprouting (Fishchnich et al., 1962) and have been suggested as a method of chitting potatoes prior to planting. However, a very close control of low doses would have to be kept which would not be practicable due to the non-uniformity of dose received by irradiated foods. Any small increase in dose above that required for stimulation would result in reduced emergence.

The choice of site and type of irradiation facility most suitable will therefore be made on largely economic grounds. Secondary or shared uses of the irradiation facility for other foods and other purposes may make it a more attractive proposition.

It is outwith the scope of this thesis to discuss the economic arguments concerning irradiation in any detail. It must, however, be understood that irradiation treatment will only be adopted if it is economically advantageous.

The irradiation debate

One requirement for the successful introduction of irradiated food in the U.K. is an adequate level of consumer acceptance of irradiated food. From market surveys of public opinion it appears that there is a substantial anti-irradiation feeling amongst consumers (Anon., 1987c; Anon., 1989c). It is worth commenting in connection with this subject that a great deal of the material written on the subject of food irradiation in newspapers and magazines has been inaccurate, lacking in balance, and in some cases, sensationalist. These contentions can be illustrated by a brief perusal of the headlines of

some newspaper and magazine articles on the subject; "Plans to radiate our food must be scrapped right now" (Anon., 1986b), "Irradiation - who needs it?" (Anon., 1986c), "Policing the food Zapper" (Coghlan, 1986). This poor level of journalism is not universal, some articles have provided essential facts for the readers to make their own judgements (Highfield, 1986; McGregor, 1989). However, in a consumer survey in 1987 the majority of people questioned failed to correctly answer more than half of 13 true or false questions on the subject of food irradiation (Anon., 1987c). It is to be hoped that some improvement in the public's understanding of the process has taken place since.

It is not this author's intention to support the approval or otherwise of irradiation on any but scientific grounds, but rather to observe that a well informed debate on the subject is desirable, and is more likely to result in a sensible, considered decision being reached on this matter than is the present level of public discussion. Some popular misconceptions about food irradiation, such as those outlined in Chapter 1, need to be corrected by the provision of information which is both available to and assimilable by the layman. For example the much repeated misconceived connection of irradiation with "radioactive food" can be corrected by a suitable educational response. The report of the government working party on food irradiation (Anon., 1989d) indeed recommends such a course. It is to be hoped that the publicity generated as the Food Bill passes through parliament will result in more information being made available to the public on the subject of food irradiation.

The views of food retailers toward selling irradiated food have been reportedly quite negative, many insist they will not stock

irradiated food (Anon., 1989c). This can be seen as a reflection of their customers desire not to pick up irradiated food accidentally from the shelves of their local supermarket or to purchase it in an otherwise non-irradiated product on sale in a supermarket which does sell irradiated food. Distinctive labelling of irradiated food may go some way to assuaging those fears.

Identification of irradiated food

At present there is no direct universal method of determining whether food has been irradiated or not. Some methods presently under investigation in the U.K. include radioimmunoassay of DNA radiolysis products, E.S.R. of hydroxyl radicals produced by radiolysis and the detection of the lipid, protein and sugar degradation products in irradiated food (Anon., 1987b; Coghlan, 1990). The Consumer Association, through their publication Which?, have stated that they regard the introduction of irradiated food without a method for its identification as premature (Anon., 1989c). The government working party report (Anon., 1989d) argues that the labelling of irradiated food with batch numbers which can be traced back to the original manufacturer or processor is sufficient to ensure only the proper use of food irradiation. However, without some form of identification test, even with labelling regulations, some abuse of this system may occur. At this time the technical problems associated with developing such a universal method may be very difficult to overcome due to the large number of differently constituted foods to be assessed and the inclusion of small amounts of irradiated ingredients in otherwise non-irradiated products (2nd generation products).

One answer to this dilemma may be to demonstrate by research that

irradiated food is safe and does not require special identification. If irradiation is found to be unsafe for particular foods or products they should not be approved for irradiation although this may once more raise the problem of the monitoring of such regulations.

Irradiation legislation in the U.K.

The proposals contained in the report of the government working party on the introduction of irradiated food to the U.K. (Anon., 1989d) are likely to be largely included in the legislation if passed by parliament. The report suggests that approval should be given for all foods to be irradiated to a maximum dose of 10 kGy subject to good manufacturing practice. This recommendation is in line with the recommendations of the ACINF (Anon., 1986a) and the Codex Alimentarius Commission, the joint W.H.O/F.A.O body which sets standards on practices in the agriculture and food industries (these recommendations are reviewed by Urbain (1986)). The blanket acceptance of the irradiation of all foods is in marked contrast to the form of approval envisaged by the E.C. and which some European countries, such as France, Belgium and The Netherlands, already apply. The E.C. proposals specify that irradiation will only be allowed if it can offer a demonstrable benefit to consumers or technological advantage. The U.K. proposals treat all foods alike and place the initiative in deciding whether a food should be irradiated or not largely in the hands of processors. It is perhaps inadvisable to expect the food industry to both decide which procedures make good commercial sense and which are in the best interests of consumers. Such decisions are more properly the responsibility of government through the departments of Agriculture and Health.

The process of irradiation is to be introduced in the U.K. at a time when, with increasing public concern about the wholesomeness or otherwise of food, the food industry is under close scrutiny. To some extent the public attitude to irradiated food can be seen as a victim of our increasing interest in what we eat. As public awareness and knowledge on food issues has increased the standards required for introducing new food additives and processes have been, quite correctly, raised, making it more difficult to introduce them than in the past. This is particularly the case when such a potentially emotive process as irradiation is involved. The association of irradiation with radioactivity has also linked the process with the nuclear industry at a time when questions are being raised as to its safety. These factors may have influenced the public's attitude to the introduction of food irradiation.

In order to be approved irradiation should be shown either to be safe, or to offer an improvement over a less safe practice. The safety of irradiation has been demonstrated with the exceptions already discussed in this and other chapters but while these exceptions remain limited approval of irradiation would appear to be a more sensible course than the comprehensive approval proposed. Where irradiation can offer a positive safety or technological advantage provided it offers no potential harm, approval should not be resisted. However, blanket approval is not yet justified.

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