THE DISTRIBUTION AND FATE OF CHLORPROPHAM IN COMMERCIAL POTATO STORES.

WAQAR AHMAD KHAN BALOCH
B.Sc (Hons.) Agriculture, M.Sc. (Hons.) Food Science and Technology.

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Agricultural, Food and Environmental Chemistry
University of Glasgow
U.K.

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Summary

The thesis consists of a study of chlorpropham distribution patterns and its behaviour within tuber components in commercial potato stores in the UK. The store examined were both cold (3-4 ^{0}C) and conventional (8-10 ^{0}C) stores.

As a large number of samples from different stores were taken, it was important to have a quick and reliable analytical method for chlorpropham determination. This was developed and compared against the original accepted method. Both methods were more or less similar in their precision of results, with recoveries of 83.80 ± 2.25 and 93.14 ± 3.51 from spiked samples by blending and reflux extracting methods respectively. When the methods were compared for extracting the amount of chlorpropham in commercially treated stored samples, it gave recoveries of 2.49 ± 0.25 and 2.58 ±0.17 for the blending and reflux methods respectively. Overall the reflux extracting method was quicker and easier and it was used for further work with some slight modification.

The present study showed that the level and distribution pattern of chlorpropham was significantly different within the store and as well as between different commercial stores. The overall range of chlorpropham levels in the examined stores was 2.11-236.56 mg/kg. The amount of chlorpropham varied with respect to sampling sites of the examined stores. Each store has its own distribution pattern with some similarities. However there was a common trend in all the stores examined in that the maximum level of chlorpropham was on the top surface and minimum on the lower levels of the stores. In the cold store examined 'A'(3-4 ^{0}C) the level of chlorpropham decreased continuously from the top height

towards the bottom height of the store. However the amount of chlorpropham was excessive throughout the store, even in the middle of the boxes it exceeded the MRL (Maximum Recommended Level). The level of chlorpropham dropped from 128.39 mg/kg in the middle layer samples of the top box to 49.14, 20.15, 19.01, 8.03 3.97 and 4.54 mg/kg towards the bottom boxes of a six high column of boxes in the store respectively. Similarly the other stores have followed more or less the same pattern in that the highest levels were on the top and lower levels on the bottom height of the store. The values were much less, 32.33 to 5.94 and 49.42 to 22.12 in the upper half portion of the top and bottom height in conventional (Store B) and Colsterworth (Store D) respectively compared to the cold store of 236.56 to 9.21 at the same positions. A new sampling technique was adopted by taking individual tubers and dividing them into upper and lower portions. This was to differentiate between chlorpropham fall out on the top surface compared to genuine distribution of the fog throughout the potato pile. Upper portions of the tubers had higher amounts of chlorpropham with respect to the lower portions of the same tubers. The mean range of chlorpropham was 21.60 to 236.56 mg/kg and 8.58 to 32.07 mg/kg on upper and lower half portions of top height box respectively. The levels further decreased on the lower heights of the store with the same trend of 5.94 to 22.12 mg/kg and 2.11 to 10.74 mg/kg on upper and lowers half portions respectively of bottom height of unwashed samples.

The degree of attachment of chlorpropham was also studied by washing the samples taken from the commercial stores on most occasions. The chlorpropham was tightly attached to tubers and significant amounts could not be removed in this way. An exception was, where surface deposition had taken place.

The ratio of unwashed to washed showed highest reduction (0.25: 1) in cold store (Store A) and minimum reduction in conventional store 'B' (1:1) by washing. The maximum reduction was on the upper exposed half portions of top height of the store, which was the result of direct fog falling out and was not tightly attached to the tuber surface. A chlorpropham percentage could be removed on washing, as most of the chlorpropham was not tightly attached to the tuber surface.

Several steps were taken to optimise the chlorpropham distribution in the stores, such as internal air circulation at the time of fogging, adjusting the particle size of the aerosol fog and developing temperature gradients in the stores. The internal air circulation played a significant role in attaining uniform distribution of chlorpropham in the cold stores. The overall range of chlorpropham residues in unwashed samples was 2.59 to 0.27 mg/kg and 31.74 to 0.47 mg/kg from top to bottom height of ventilated and unventilated stores respectively. Difficulties were experienced in attaining different particle sizes. The particle size could be changed slightly by controlling the flow rate in the machine but not by changing the burner temperature in these experiments. The practice of introducing a temperature gradient was good enough to reflect its role in the distribution of chlorpropham. The mean particle size for all treatments was in the range 4.6 to 7.4 um. The patterns of distribution were rather similar for stores 35 and 36 both in terms of deposition and residual chlorpropham after washing. The temperature gradients in these two stores were both positive (store 35 by natural convection, store 36 deliberately imposed) although the gradient in store 36 was greater. If any trend was evident, it was that there was fewer chemicals deposited in the warmer top

box of store 36 compared with that in store 35 where the temperature gradient was minimised.

The study also involved an investigation into the possible presence of bound chemical in the tuber components. Here processing of potatoes by microwave as well as boiling was studied. The results showed that boiling and microwave cooking had reduced the level of the chlorpropham in the potatoes. The higher reduction was the result of microwave cooking of 57% reduction compared to 46% reduction by boiling of peeled potatoes. In case of microwave cooking some additional chlorpropham was shown to be released after a certain time of cooking. The maximum reduction was 78% after 8 minutes and it became 38% after 12 minutes of cooking. The peel of potato remained total unaffected with microwave cooking. On the other hand the unpeeled potatoes were only processed by boiling which showed the highest reduction of 63% in chlorpropham level compared to the peeled tubers of 46% reduction. Further the starch fraction of treated tubers was extracted with methanol a more polar solvent than the normal hexane. A substantial amount of additional chlorpropham was extracted with the methanol (2.13 mg/kg) compared with hexane, the normal solvent used for chlorpropham analysis. The amount extracted in the starch with methanol would amount to a 10% increase based on total fresh weight of potatoes. These findings suggested the presence of bound residues of chlorpropham being presence in potato tubers and associated with the starch fraction. Possible mechanisms involved in binding chlorpropham to starch were discussed as were the overall implication of the findings for food safety etc. This of course did not exclude the possibility of chlorpropham being bound to other potato fractions as well as starch.

Chapter 1:

INTRODUCTION

The thesis consists of a study of chlorpropham distribution in commercial potato stores in the UK, and its interactive behaviour with potato components. Chapter 1 therefore, comprises an introduction to chlorpropham, details of potato storage particularly under UK conditions and general information about bound residues in food.

1.1 CHLORPROPHAM.

Chlorpropham has been used for many years world-wide in potato stores for sprout suppression. At this time there is no substitute to replace chlorpropham therefore the potato processing industry is totally dependent on chlorpropham for sprout control in order to obtain the necessary standards of quality for their products.

Due to its world-wide popularity it is now been investigated for re-registration. Therefore studying the toxicity, environmental effects and other properties are major issues for scientist world-wide (Duncan and Boyd, 1992).: The general information on chlorpropham given below in this chapter is cited verbatim from the Extoxnet profile, (1999) via an Internet file of Oregon State University, US.

"Chlorpropham (isopropyl 3-chlorophenylcarbamate) is a crystalline compound of appreciable volatility at room temperature. It was first introduced as a member of

the phenylcarbamate group of herbicides and thereafter has been used to inhibit sprouting in potatoes. It is regularly used for pre-emergence control of grass weeds in alfalfa, lima and snap beans, blueberries, cane berries, carrots, cranberries, garlic, seed grass, onions, spinach, sugar beets, tomatoes, safflower, soybeans, and woody nursery stock.

1.1.1 Physical properties.

Boiling point	149 ^{0}C at 2mm Hg
Decomposition	150 ^{0}C and above
Water solubility	89 ppm at 25 ^{0}C
Specific gravity	1.180 at 30 ^{0}C
Melting point	41 ^{0}C
Vapour pressure	$7.5x10^{-6}$ mm Hg at 25 ^{0}C

Soluble in solvents

Butyrolactone, benzene, xylene, chloroform, ketones, methanol, ethanol, isopropyl alcohol, esters, aromatic hydrocarbons, most organic solvents and oils etc.

Technically chlorpropham is a white to light brown crystalline solid. It is stable under normal temperatures and pressures, but poses a slight fire hazard if exposed to heat or flame, and a fire and explosion hazard in the presence of strong oxidisers. It

may burn but will not readily ignite. Avoid contact with strong oxidisers, excessive heat, sparks or open flame. Thermal decomposition may release highly toxic fumes of phosgene, toxic and corrosive fumes of chlorides, and oxides of carbon. Workers handling chlorpropham should avoid breathing vapours, should have glasses to prevent eye contact and protective clothing to prevent prolonged skin contact.

1.1.2 Fate of chlorpropham.

1.1.2.1 Fate in soil and water.

Insufficient data are available to permit a reliable prediction of the leaching potential of chlorpropham (Enviromental Protection Agency, 1987). Chlorpropham has some potential to contaminate groundwater because it is soluble in water and it has only a moderate tendency to adsorb to soil particles. Chlorpropham adsorbs strongly to organic matter, so it is unlikely to leach through soils high in organic matter. Chlorpropham does not readily adsorb to montmorillonite or kaolinite clays. A hydrolysis study showed that chlorpropham is relatively stable in sterile water in the dark. After 32 days in aqueous buffered solutions at pH 4, 7 and 9 held in the dark at 40 ^{0}C, about 90% of the applied chlorpropham remained undegraded. Fish accumulation data indicate that chlorpropham bioaccumulated in the skinless fillet of a bluegill sunfish to 100 times the levels in water (Extoxnet profile, 1999).

Degradation rates of chlorpropham are affected by microbial activity and soil moisture levels. Photodegradation and volatilisation do not readily occur,

but increasing temperatures above 35^0C and increasing soil moisture capacity may increase volatilisation. It has been reported that its half- life is from 35 days to 65 days at 15^0C or 30 days at 29 ^{0}C in soil.

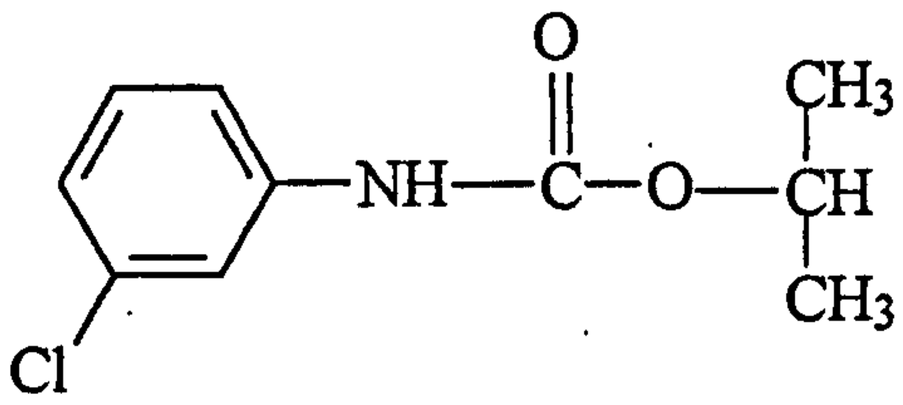
1.1.2.2 Fate in plant.

Chlorpropham is absorbed by the roots of susceptible grass seedlings and transported throughout the plant. Leaves absorb it more slowly. It suppresses plant transpiration and respiration and inhibits root and epicotyl growth. To date, 4-methoxychlorpropham, 3,3 -dichloroazobenzene and 3- chloroaniline are the only reported metabolites of chlorpropham identified in potato tubers in spite of the absence of metabolites reported earlier in radiolabelled chlorpropham studies (Duncan and Boyd, 1992).

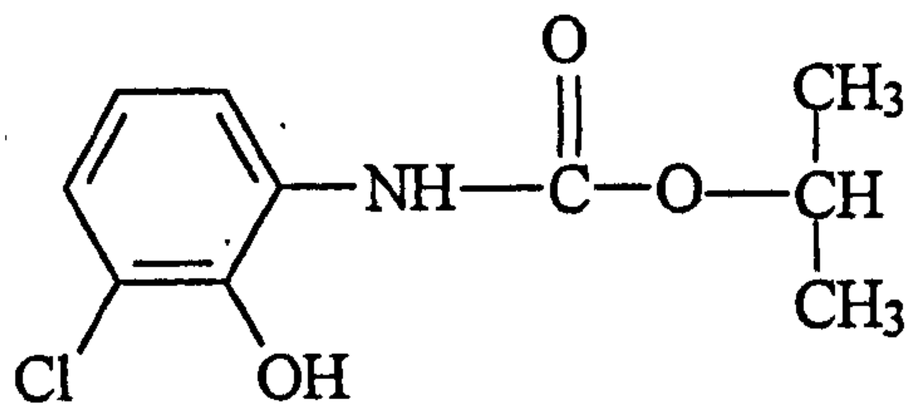
The metabolism of chlorpropham in growing plants has been adequately described. The herbicide is translocated from roots into shoots and residues include chlorpropham, isopropyl 3-chloro-6-hydroxycarbanilate, isopropyl 3-chloro-4-hydroxycarbanilate, 1-hydroxy-2-propyl-3-chlorocarbanilate (isopropyl-OH-CIPC), isopropyl 3-chloro-2-hydroxycarbanilate, and 3-chloroaniline. (Fig. 1.1) (Extoxnet profile, 1999).

1.1.2.3 Fate in atmosphere.

From its estimated vapour pressure of $7.5X10^{-6}$ mm Hg at 25^0C, chlorpropham is expected to be present partially in the vapour phase and partially in the particulate form in air. Based on an estimation method, vapour phase chlorpropham should be



Chlorpropham



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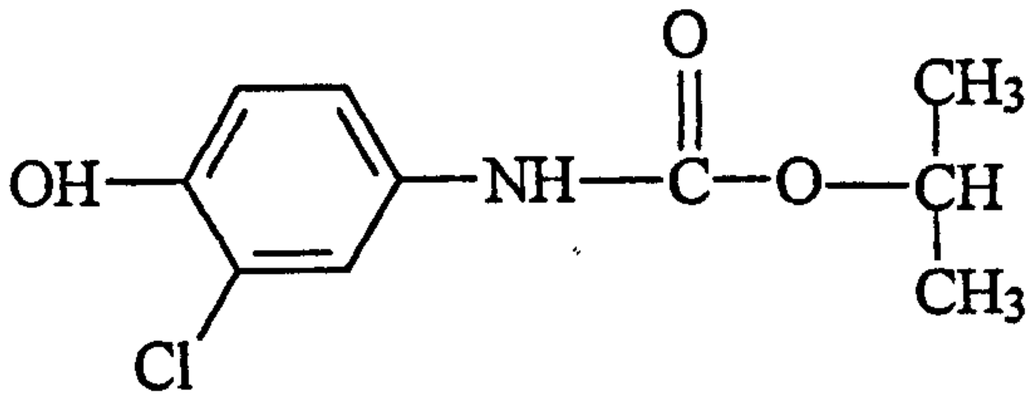

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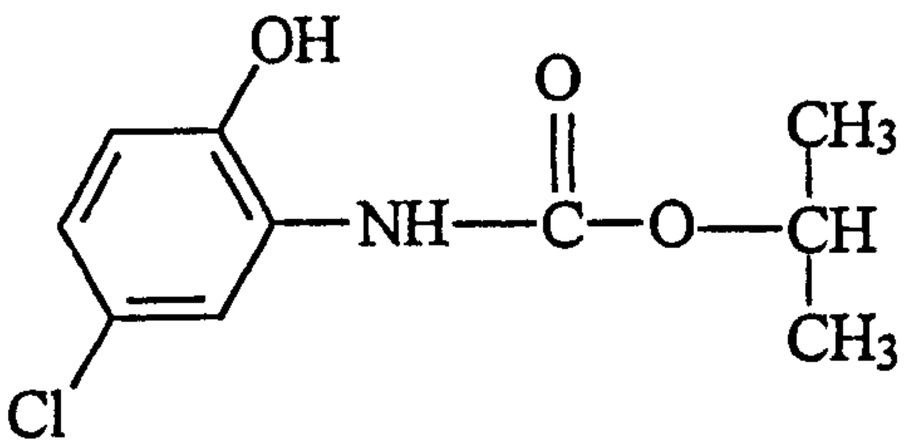
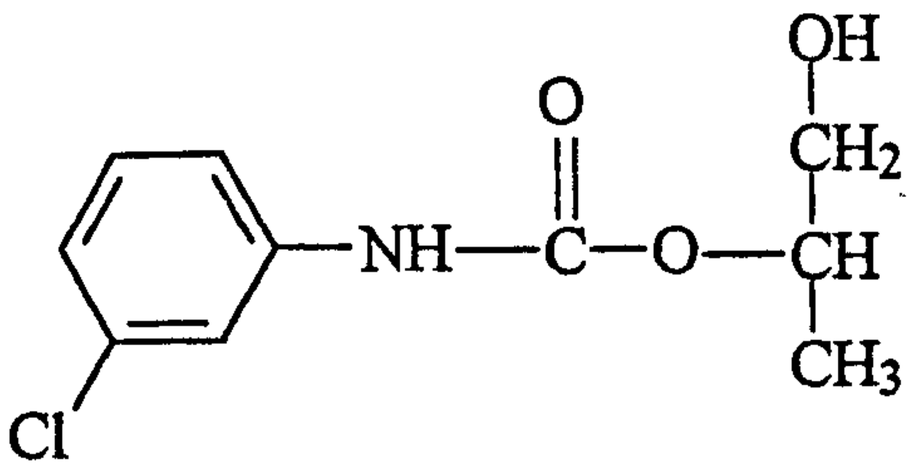


isopropyl 3-chloro-6-hydroxycarbanilate



1-hydroxy-2-propyl-3-chlorocarbanilate

Fig. 1.1. Residues of chlorpropham in plant metabolism.

removed from the atmosphere with an estimated half-life of 5.5 hrs due to reaction with photochemical produced hydroxyl radicals. Partial removal of particulate chlorpropham from the air may occur by dry deposition. Chlorpropham may also be washed out of the air by rain.

1.1.3 Toxicology effect of chlorpropham

Chlorpropham is moderately toxic by ingestion. It may cause irritation of the eyes or skin. The symptoms of poisoning in laboratory animals have included listlessness, noncoordination, nose bleeds, protruding eyes, bloody tears, difficulty in breathing, prostration, inability to urinate, high fevers, and death. Autopsies of animals have shown inflammation of stomach and intestinal lining, congestion of the brain, lungs and other organs, and degenerative changes in the kidneys and liver.

The oral LD50 for chlorpropham in rats ranges from 1,200 mg/kg to 3,800 mg/kg and in rabbits is 5,000 mg/kg. The 4-hour inhalation LC50 in rats is > 32 mg/l. No birth defects occurred in a 3-generation study with rats. Long-term exposure to chlorpropham may cause tumours .

Chlorpropham is practically non-toxic to waterfowl. Its LD50 in mallards is > 2,000 mg/kg. Chlorpropham is moderately toxic to cold and warm fresh water fish. The LC50 for chlorpropham in rainbow trout is 3 to 6 ppm, and 6.3 to 6.8 ppm in bluegill sunfish (David, 1998).

Chronic exposure of laboratory animals has caused retarded growth, increased liver, kidney and spleen weights, congestion of the spleen and death. No deaths or

micropathological abnormalities occurred in rats given diet containing 2% chlorpropham for 90 days" (Extoxnet profile, 1999).

The various studies have been undertaken to see the effects of mixtures of pesticides in daily diet. It was supporting the idea that mixtures of pesticides have more or less effect on the DNA. (Dolara et al., 1994; Lodovici et al., 1997; Dolara et al., 1993). It is therefore important to see the effect of individual pesticides on DNA or any form of animal and human safety.

When the chlorpropham was given in the diet to provide levels 0(control), 0.15, 0.30 and 0.60%, from 5 weeks of age of the F 0 generation to 9 weeks of age of the F 1 generation in mice, and selected reproductive and neurobehavioral parameters were measured it was reported that, chlorpropham produced some significant effects on productive and neurobehavioral parameters. The average body weight of male offspring was significantly affected during the lactation period in the low-dose group of each sex and high-dose group females, and was increased in the middle-dose group of males during the lactation period (Tanaka, 1997).

In another study designed to evaluate the developmental toxicity of chlorpropham in mice, the chlorpropham was administrated to pregnant mice by average on Days 8, 8.3, 9, 9.3, 10 and 11 of gestation at a level of 3000 mg/kg body weight, and females were killed on Day 18 of gestation. The administration on Day 8.3 of gestation induced the highest percentage of external malformations with brachyury occurring among more litters than in other groups. The average fetal body weight of each sex was significantly reduced in the 300 mg/kg treatment group. The total incidence of

external malformations was significantly increased in the two highest dose groups in a dose-related manner (Tanaka et al., 1997).

It was reported by Fujitani et al., (1997) that groups of ten males and ten females were given 750, 1500 or 30 000 ppm of chlorpropham in the diet for 13 weeks. Body weight gain of male and female rats in the 30 000 ppm-group was depressed. Spleen and liver weights of male and female rats in the treated groups were dose-dependently increased. Red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular haemoglobin concentration and platelet count were also decreased in male and female rats of treated groups.

However in most of the above studies the effect of chlorpropham was found on health, when it was fed at high doses. It has therefore less chance of any damaging effects for human health, as the daily intake is much less then the dose given. It is also therefore important to study the distribution of chlorpropham in the commercial stores. The uneven distribution can lead to much higher levels on some tubers, which could encourage the above mentioned problems.

1.2 POTATO CROP.

Although the potato was introduce nearly 400 years ago in England, it was considered till 200 years latter, to have poisonous and aphrodisiac qualities. Once it was accepted and appreciated then it became and has remained a major component of the British diet. "Thus Nature wisely provides for all our wants": so wrote Jerome Cardan about the potato in 1557 (Burton, 1966).

In a survey undertaken on behalf of the Potato Marketing Board, (1995) to obtain information about consumer buying behaviour and attitudes in relation to potatoes, the 74% favoured that "you need potatoes to make a proper meal" and 68% favoured that "you need potatoes for a balanced meal". The potatoes are eaten in one form or another through out the world. Mainly they are eaten as boiled, mashed or chipped potatoes but there is a continuous increase in demand for quantities of crisps, potato snacks, dehydrated instant mash, frozen French fries and canned potatoes (Jilani and Baloch, 1983a and 1983b; Gracimartin et al., 1995).

In fact the consumption of the processed potatoes reduces the consumption of the fresh ones. In UK 40-50% of total stored potatoes are consumed or used either as washed/prepacks or processed products. In USA the current appropriate utilisation of potato is in form of fresh 30%, processed 54%, reviewed by Orr et al., (1994). Potato is a significant source of dietary fibre and there has been increasing trend in dietary fibre (Hampson, 1976). The dietary fibre is getting popular because it gives protection against cardiovascular disease, colonic and diabetes (Thed and Phillips, 1995).

1.2.1 Storing and Management.

The fresh potatoes straight from the field can meet about four months of the ware potato market demand, but the continuing and in fact increased demand throughout the year can be met only either by storing or importing potatoes from very different climatic regions to the time of year. In tropical countries like Pakistan, at the harvest time the total production of the crop in potato producing area is much higher than the consumption (Sharafat et al., 1990).In most countries like UK limitations of climate

mean that a continuous growing season is not possible, therefore the crops must be stored from harvest until they are required. The bulk, bin and box storage systems are the main methods, which are commonly used for storing potato crops. Each of them has its advantages/disadvantages over the others. The main building structure and total capital cost varies depending on type and size of store.

The crops used for storage purposes are grown from high quality seed properly treated with fungicide to control the skin spot disease during the storage. For this purpose the crops are harvested as soon as the skins of the tubers are set and when soil and weather conditions are favourable.

At harvest the crop is lifted at an early stage of maturing early in the main crop lifting season. Early maturity crop can be attained by presprouting (chitting) of seed before cultivation, by early planting or selecting early maturity varieties and proper agricultural practice.

Care should be taken to prevent bruising and damage of the crop at lifting time. The storage loses can be minimised by lifting the dry tubers at 10-15 ^{0}C temperature. If there is rain during this time the crop should be protected by covering with plastic sheets on the top of tubers. As much loose soil as possible should be removed before loading into store as the soil prevents effective control of temperature and the ventilation and ultimately disrupts the distribution of chemical and prevents temperature control.

Traditionally, no management is required for about two weeks after loading into store, if the potatoes are below 15 ^{0}C temperature and the potatoes are reasonably

healthy and dry. Where the temperature rises above 15^0C the ventilation is used to cool down the potatoes back to 12-15°C for at least 10-14 days for curing or wound healing purposes. At temperatures of 12-15°C respiration rates become high which ultimately increases the relative humidity (95% or above), which can help to reduce moisture loss and prevent diseases gaining entry to the internal tissues of the tubers. But if disease is already present in this period then high temperature and high humidity will allow the disease to spread rapidly through the crops before wound healing is completed (Potato Marketing Board, 1996).

It is recommended to apply ventilation during this period to change the air in the store and lower the humidity to reduce the risks of disease spread. This technique is known as dry curing to control blemishing diseases e.g. silver scurf, skin spot and reduces the risk of bacterial soft rot. It is usually done when the humidity of the outside air is low but the temperature high enough to maintain conditions for curing. In case of crop lifted under wet conditions then more intensive drying is required. It can be effectively achieved with a forced ventilation system driven through the potatoes. The forced ventilation is normally at the maximum airflow rate of 0.04m3/s/t and the treatment should be discontinued as soon as the surface moisture has been removed (Potato Marketing Board, 1985). The excessive use of ventilation could lead to heavy moisture evaporation and thus weight loss (Wilson and Hunter, 1965) and softening of the crop.

1.2.2 Types of storage.

1.2.2.1 Bulk stores.

This type of store is very common in UK. Potatoes can be stored to a height of 4.5 m, but more management problems are associated with deeper stores. The ventilation and application of the chemicals is through laterals off a main duct. The bulk store is of only one basic design with various degrees of sophisticated ventilation technology.

The bulks as the name represents, means the tubers are stored in a big room in a heaped form. Therefore the walls of the store should have enough strength to bear the pressure of the tubers. The temperature in these stores is reasonably controlled but the fluctuation can be balanced with proper ventilation. The ventilation could be internal or external. The fans are used to circulate the air of the store in internal circulation and in external ventilation the outside air of lower temperature is brought in to control the temperature to balance the humidity of the store. Nowadays in modern good facilities the temperature can be lowered by the refrigeration in the case of outside air being warm.

The bulk store has a main air duct, which divides further into a number of lateral ducts, depending on height of store. During the application of the chlorpropham the fog first goes into the main duct and then through the lateral ducts towards the potatoes. The chlorpropham fog is then forced by these ducts on an upward direction through the heaps of potatoes, which act like a filter. The fog passing through the bulk of the potatoes then goes to the ceiling of the store where it settles down slowly.

The sampling and management in the bulk store is difficult specially for the deeper potatoes. Therefore mostly the sprouting of the potatoes is discovered by rotting smell in the store. Nowadays the condition of the deep potatoes can be determined by putting in place a mesh pipe, full of potatoes, before loading the store. This pipe can be replaced at any time to know about the condition of the deep potatoes, but it still is not easily accessible to individual sites of the store on different levels.

The time taken in the loading of bulk store is another drawback of this store. The bulk stores take a relatively a long time and allowing the stored potatoes after filling for wound healing to take place can raise the temperature of the store to enable the sprouting to start thereby breaking the dormancy period. Therefore a first tecnazene treatment was developed to overcome this problem.

1.2.2.2 Box stores

The box stores have normally 50-100 tonne tubers capacity. The potatoes are stored in wooden boxes of 0.5-1 tonnes normally in stacks of 5 or 6 in height. As the potatoes are stored in the boxes therefore it does not need any special strengthening of the walls like a bulk store, but the total capital cost due to its lower density of potatoes stored per unit area and the expenses on wooden boxes, is greater than the conventional bulk stores. On the other hand the box storing has considerable advantages over the bulk stores, because it is easily accessible to approach the individual boxes. The boxes can be moved at any time to overcome the diseased problems within the stores. The box stores are flexible, therefore it can be helpful to

keep different varieties of tubers, of even different owners in the same store without any dispute.

The increased interest in box stores has also led to the development of some more novel environmental control methods. 'Wet' cooling, which employs chilled water to cool the store air, and evaporative humidifiers, which can be used to help boost the humidity in potato stores, are two of the more recent systems to come on to the market. 'Suction wall' ventilation is another new system now available

The distribution of the temperature is much better as compared to bulk stores and it can be increased or decreased even within individual bays during the storing. The application of chemical is comparatively easy compared with bulk stores, as the chemical is usually applied between the gap in the box stacks rather then the main ducts under the heaps of tubers. However the main concern in this type of storing is to get even distribution of the chemical. Due to wooden boxes the chemical does not reach equally to each site of the store, therefore exposed surfaces contain higher levels while other sites have remained at the minimum level of chlorpropham. The details of the study about the distribution of chemical is discussed in chapter III of this thesis.

1.2.3 Losses during storage.

After all these management practices the annual loss in weight from the UK potato crop during storage has been estimated to average nearly 600,000 tons, some 13 per cent of the stored crop, due to respiration, evaporation, rotting and sprouting of tubers. In a survey carried out by the Potato Marketing Board, the common storage problems are sprouting (45%) rotting (21%) and softening (13%).One of the principal

contributions to unacceptable storage losses is caused by the sprouting of potatoes. As most of the potato stores are for processing obviously the quality is a major factor. Potato sprouting not only damages tuber quality, but causes loss of weight due to trimming of sprouts. Water loss, and hence weight loss, is greater through sprout tissue than through the potato skin (Burton, 1966). Such losses have led to the development of several methods for controlling sprouting during storage. These methods include the control of storage temperature or storage atmosphere , diffuse light control, refrigeration, irradiation and additionally the application of chemical sprout suppressants. These methods have some merits and demerits over one another, like irradiation is quite successful in developing world (Shirsat et al., 1994) while the level of sugar increased after the irradiation (Muir et al., 1987; Tajima et al., 1988) and therefore is not acceptable for potatoes that are mainly stored for processing purposes. The application of light to control the sprout growth on the other hand is widely practised for seed potatoes in temperate countries (McGee et al., 1987). Here in this study the chemical way of sprout controlling is focused on and will be discussed.

1.3 SPROUT SUPPRESSING CHEMICALS.

The controlled temperature stores are gaining popularity all over the world. The lower temperature below 4 ^{0}C is enough to inhibit sprouting, but this level of temperature is favourable for the conversion of tuber starch into glucose, sugar. The higher sugar level not only gives undesirable sweet taste, but it produces unattractive brown colour of fried products (Talley and Porter, 1968; Nam and Kim, 1984; Jilani and Baloch, 1884; Pokorny et al., 1988; Jiang and Ooraikul, 1988; Kaaber, 1990). As most of the potatoes are stored for the processed food market, therefore the taste and

colour are the quality factors of the products (Fitzpatrick et al.,1965; Karel and Labuza, 1968; Wahid et al., 1986; Leszkowiat et al., 1990; Roe and Faulks, 1991; Khanbari and Thompson, 1993 and 1994) .'To overcome this problem, the temperature is controlled around 7-10 ^{0}C., enough to control sugar levels with the addition of sprout suppressing chemicals to inhibit the sprouting. Growth regulating chemicals remain in contact with the plant and will be absorbed into its tissues in order to bring the desired effects (Anderson et al., 1952). A wide range of chemicals has the ability to inhibit sprouting, however only a few are used commercially (Table. 1.1, Fig. 1.2).

1.3.1 Commercially applied chemicals.

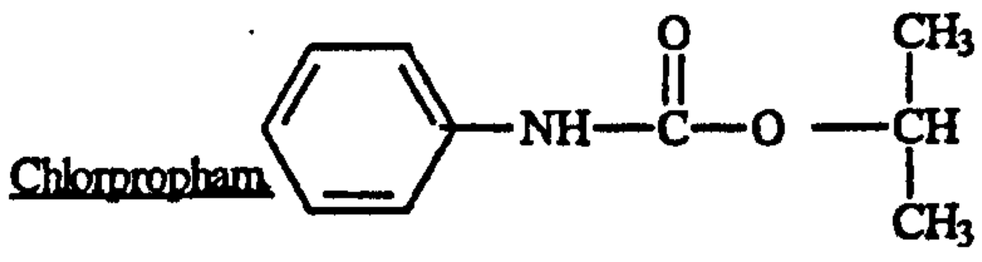
The most common chemicals used for the bulk of potato treatment are tecnazene (TCNB), Maleic hydrazide (MH) and Chlorpropham (CIPC).

1.3.1.1 Tecnazene.

Tecnazene (1,2,3,4,5-tetrachloro-3-nitrobenzene) is colourless crystalline compound, appreciably volatile at room temp. It was initially introduced as a fungicide for the control of Fusarium the agent of dry rot of potatoes . It is active as a vapour and it controls sprouting by prolonging dormancy period. Therefore tecnazene (TCNB) does not control sprouting under the following conditions.

a-If dormancy has already broken or is about to break at the time of application.

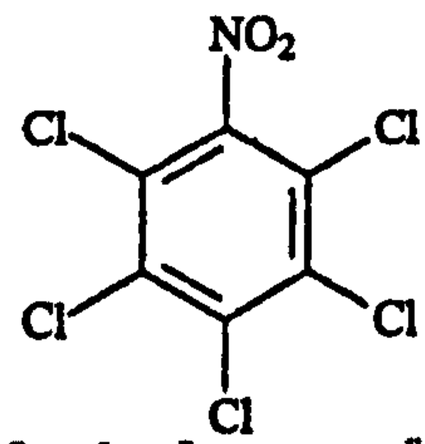
b-If store temperature is too high, the best result could be attained at 15 ^{0}C for two weeks curing and not more than 7 ^{0}C after that. As most of the stores are held at 7-10 ^{0}C therefore tecnazene is not sufficient for this long period.



Chlorpropham

$C_{10}H_{12}ClNO_2$
m.wt. 213.68
m.p. 40.7-41.1 ^{0}C
b.p. 229 ^{0}C.

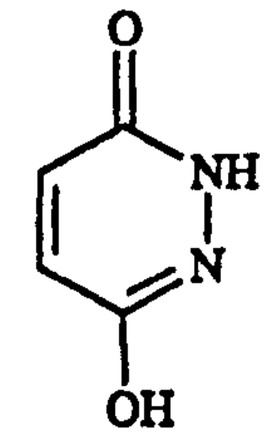
application rate, 10-20 mg/kg., Acute LD 50,5000-7,500 mg/kg (rats).
vapour pressure; 0.039 pa (25 ^{0}C), 0.006 pa (10 ^{0}C).



Tecnazene

$C_6HCl_4NO_2$
m.wt. 260.89
m.p. 98-101 ^{0}C
b.p. 304 ^{0}C.

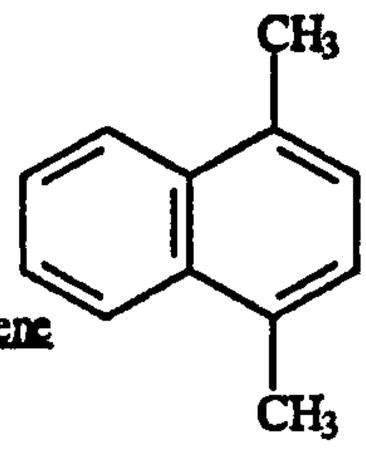
application rate, 135 mg/kg., Low mammalian toxicity; rats fed 57 mg/kg d $^{-1}$ or mice fed 215 mg/kg d ., vapour pressure; 0.06 pa (25 ^{0}C).



Maleic hydrazide

$C_4H_4N_2O_2$
m.wt. 112.1
m.p. 296-298 ^{0}C

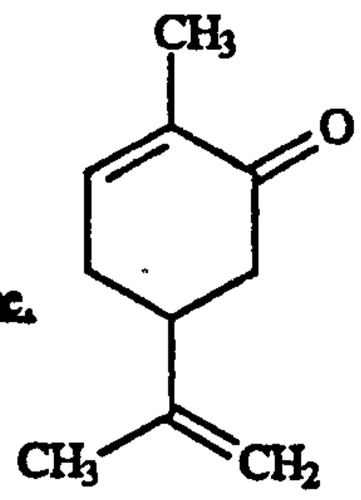
Recomended rate 2-5 kg/ha., The acute oral LD50 , 6950 mg sodium salt/kg (rats).



1,4-Dimethylnaphthalene

$C_{12}H_{12}$
m.wt. 156.23
m.p -18 ^{0}C
b.p. 262-264 ^{0}C

Active volatile potato sprout growth inhibitor. Complete suppression when applied at a headspace level between 0 and 14 mg m^{-3}.
LD $_{50}$, 5 kg^{-1}(rat)., vapour pressure (2,7-isomer); 0.56 pa (25 ^{0}C), 0.09 pa (10 ^{0}C).



Carvone.

$C_{10}H_{14}O$
m.wt 150.21
b.p. 230-231 ^{0}C.

Naturally produced monoterpenoid. Active volatile plant growth inhibitor and sprout growth inhibitor. Complete sprout suppression when applied at a headspace concentration between 9 and 26 mg/kg.

Fig. 1.2. The common spout inhibiting chemicals

Table 1.1 Comparison of potato sprout suppressant chemicals.

	CIPC	TCNB	MH	DMN	S-Carvone
Status	applied in Store	applied in store	applied in field	applied in store	applied in store
Where used	world wide	world wide	popular In USA	under process	under process
Mode of action	mitotic Inhibitor	not known	mitotic inhibitor	mitotic inhibitor	not known
Other uses	herbicide	fungicide	growth regulant		fungicide
Amount needed for sprout suppressing	10-30 (g/tonne)	135 (g/tonne)	3 (kg/hectare)	100 (g/tonne)	50-100 $ml^{-1000kg}$ (repeated every six week)
Retreatment	straight	difficult	not likely	easy	easy
Effect on wound healing	yes	no	not applicable	some	not known

Chlorpropham (CIPC),Tecnazene (TCNB), Maleic hydrazide (MH),

Dimethyle naphathelene (DMN).

Tecnazene was initially applied for seed potatoes as a dust or granule formulation for controlling sprouting but spray and fog formulations are also available on the market. However fog formulations were found to be the least effective formulation. Dust, granular and sprays of tecnazene are applied at store loading and the completion of loading. A vibratory feeder applicator will give an even application of dust or granules although quite satisfactory results can be obtained from application using shovel or hand scoop either to the top of the load before unloading into store and/or to the face of the store. The granular formulation is particularly suitable for use on potatoes stored in pallet boxes (Potato Marketing Board,1985). Airing, chitting regimes removed the majority of the chemical and enabled normal growth processes to ensue (Dalziel, 1978). Application rate of 135 mg/kg potatoes is recommended for sprout control.

In optimum conditions tecnazene could give best sprout control for 150 days. Tecnazene is unusual for store of potatoes as sprout suppressant as it does not inhibit wound healing of potatoes, (Burton, 1966). It can be applied during loading in store before wound healing has taken place. As it is easy to apply as the potatoes are loaded into store therefore it is popular with farmers and other small scale storage systems of potatoes. The mode of action of tecnazene is, as yet, unknown and little has been reported on plant and soil metabolism studies. Uneven germination of previously treated seeds with tecnazene even at much reduced rates has greatly discouraged this practice. Some processors nowadays use tecnazene for sprout control during store

loading prior to the use of first application of chlorpropham which takes place after wound healing. There has been the discovery of significant amounts of tecnazene and its metabolites in river sediment samples down stream of potato washing plants (Whale et al., 1988).

1.3.1.2. Maleic Hydrazide:

Maleic hydrazide (MH), (6-hydroxy - 3-2(H)-pyridazone) is a colourless non-volatile mono-basic acid which is normally formulated as its diethanolamine or potassium salt.

As this has been used for years in the USA therefore most of the information about this chemical came from USA. It is applied via the foliage. It first penetrates the leaf cuticle and after that in the phloem to be distributed evenly amongst the individual tubers present. Although at first MH would appear to have many advantages over chlorpropham as a sprout suppressant. A serious problem is the timing of application to the growing crop. The time of the application is as flowering is completed. The label recommendation for maleic hydrazide is to spray 21-35 days before defoliation. Yield was unaffected by applications within this period but losses, particularly of large ware, were associated with much earlier treatments (Lewis-Rogers, 1990). This time of spraying in UK remains unsuitable for spraying due to wet weather. Unfavourable weather conditions at time of spraying can greatly reduce uptake of chemical by the leaf cuticle giving much reduced effect as reviewed by Duncan et al., (1992). Lack of uniformity of the chemical in the tubers gives uneven sprout control in the store,

which therefore necessitates the addition of another sprout-suppressing chemical at a later stage.

The use of maleic hydrazide is not successful in the store. It was reported that MH proved ineffective in inhibiting sprouting in potatoes although it is a respiratory inhibitor. Maleic hydrazide had some effects on the tuber under the conditions found in this investigation. It might have blocked the apical dominance of potatoes under room temperature resulting in more eyes sprouting, while at the low temperature apical dominance was not affected (Badshah, 1984).One problem associated with this chemical is that 6mg/kg dose of maleic hydrazide is normally enough to retard sprouting for six months but 40 mg/kg can be obtained with commercial formulation. The excessive amount is considered hard on toxicological ground, where the point should be considered that this chemical cannot be reduced by peeling of tubers as it is distributed within the flesh of the tuber. It is unlike other sprout suppressant chemicals by going into the flesh and distributed throughout the tubers (Mackenzie, 1989).

1.3.1.3 Chlorpropham.

Chlorpropham, isopropyl N-(3-chlorophenyl) carbamate, is world wide sprout inhibiting chemical in potatoes (Table 1.2). The chlorpropham was first introduced as a member of the phenylcarbamate group of herbicides and has subsequently been used to inhibit sprouting in potatoes.

Marth and Schultz introduced the chlorpropham in 1950 as a sprout suppressing treatment and it soon gained popularity around the world. In commercial potato stores it is applied with rate of 20 mg/kg in the form of an aerosol fog, dissolved in an

Table 1.2 The treatment of processed potatoes with chlopropham/propham (000's tonnes) in Europe.

Country	snacks/chips	chilled/frozen	dehydrated	total treated
UK	426	350	80	1100
France	129	190	270	1700
Holland	90	1320	220	902
Germany	192	250	270	407

(Duncan and Boyd, 1991).

organic solvent-methanol or dichloromethane, after wound healing. It can also be applied in dry form as a dust and granules for initial application, but the single application throughout storage season in this form is not popular.

The chlorpropham is one of the carbamate class of compounds and is an economical herbicide. It is very effective sprout inhibitor and also influence many other aspects of tuber metabolism. As it has been shown it prevents translocation of nitrogenous constituents into sprouts, so retaining the nutrients within tuber and increasing total nitrogen and protein content of potato (Seetharaman and Mondy, 1991; Mondy, 1993). It is also reported that the internal sprouting of the stored potatoes was also deceased with the increasing of chlorpropham dosage (Sawyer and Dallyn, 1964).

At present the potato processing industry relies heavily on chlorpropham for sprout control in order to obtain the necessary standards of quality for their products. The aerosol fog application can be easily applied and reapplied but its uneven distribution in the stores, particularly in box stores is a big problem.

1.3.2 Developing Chemicals.

There are few other natural components which have proved their ability to inhibit sprouting in potatoes. More work is yet to be done and once they have been fully developed and become available in commercial market, then there popularity will grow up quicker. Among these natural components, substituted naphthalene (DMN) and s-carvone are much more appreciated. The brief comparison of general sprout suppressing chemicals are mentioned earlier (Table 1.1).

1.3.2.1. Substituted naphthalenes.

1,4 dimethylanaphthalene (DMN) is a volatile component produced by potatoes having sprout suppressant characteristics. The application of DMN at 100 mg/kg is successful in controlling sprouting in potatoes for some months at 8-10 0C under commercial conditions.

Increasing public concern regarding the use of chemical additives on food products have compelled researchers to seek new sprout inhibitors that can be classified as natural growth suppressing compounds. Some of these compounds, including several naphthalene products, have been identified as constituents of raw and cooked potatoes. Individual isomers of dimethylnaphthalene (DMN), including 1,4- and 1,6-DMN that occur naturally in the potato peel, were reported to be good sprout suppressants (Lewis et al., 1997). DMN could maintain sprout control over a storage period. It is easily applied and reapplication is possible if necessary and activity and persistence are comparable with chlorpropham. Its presence in the volatile fraction given off by potatoes albeit at low concentration, was an additional point in its favour. The effect of DMN on some major quality factors in tubers are almost similar in comparison even with both tecnazene and chlorpropham, in terms of reducing sugars (glucose and fructose) and sucrose concentrations over a number of storage seasons. There is some evidence of DMN inhibiting wound healing quality, which could suggest its use in stores after a short period curing process after the harvesting of crop.

Numbers of workers have looked at DMN and its analogues or derivatives which could have shown that many other substituted naphthalenes are active as sprout suppressants (O'Hagan et al., 1987). Ten isomers of DMN have been identified of which seven may occur naturally in the potato. The number and differential activity of DMN isomers adds complexity to the process of defining the role of the naphthalene sprout suppressants in tuber tissues. Stephen and Duncan, (1984) demonstrated that 1-ethylnaphthalene, 1-chloro-4-methylnaphthalene, 1-bromo-4-methylnaphthalene, 1-bromo-2-methylnaphthalene and 1,4-di-bromonaphthalene were more effective as sprout suppressants than the 1,4- and 2,3-DMN isomers.

O'Hagan et al., (1987) investigated another naphthalene compound-diisopropylnaphthalene (DIPN), which may have some potential as an alternative to chlorpropham when applied as a thermal fog.

Lewis et al., (1997) have worked on the application methodology and efficacy of dimethylanaphthalene (DMN) and diisopropylnaphthalene for potato sprout control in storage. Two applications of DIPN, at the rate of 300 mg/kg as a thermal aerosol fog, may be a superior potato suppressant compared to DMN applied in the same manner, and equal to chlorpropham. The DMN, when applied at similar rates and with the same methodology, also has the potential to provide adequate sprout suppression but for shorter duration. The results of this research suggest that the current methodology used to commercially apply sprout inhibitors or suppressants is an effective technique for applying DMN and DIPN and can be used by the industry without major equipment changes or adaptations. It was suggested that further investigation of DIPN

and DMN as potential commercial alternative potato sprout suppressants should be continued.

1.3.2.2. Carvone.

Some plants may produce toxic substances that suppress the growth, and thus accounting for severe yield loss (Schumacher et al., 1983; Vaughn and Spencer, 1991). Carvone can be obtained from the essential oil of caraway seed (*Crum Carvil*) (Sovova et al., 1994). Caraway is one of several ancient cultivated species of the Umbelliferae family known for its characteristic aromatic small seeds. The essential oil of caraway seed consists mainly of two monoterpenes: S(+) - Carvone(50-60%) and S(+) - Limonene (35-45%). Carvone has been shown to be the biologically active component to prevent sprouting, whereas limonene did not exhibit sprout suppression.

The carvone is not only an effective fungal growth inhibitor in in-vitro experiments, but had a striking inhibiting effect under semi-practical storage conditions as well (Hartmans et al., 1995). These and other results were used to obtain official registration for the use of carvone (trade name 'Talent') as a sprout inhibitor for potatoes in the Netherlands. 'Talent' was commercially introduced in 1994. Registration procedures have also been started in other European countries.

Oosterhaven, (1993) studied the effect of monoterpene S-Carvone on potato sprout growth. Potato sprout growth was almost completely inhibited within 2 days after the start of the S-Carvone treatment. In plant, HMGR (3-hydroxy-3-methylglutaryl Coenzyme A reductase) is a key enzyme and its activity in plant is crucial for growth and development. It was reported that activity of HMGR

dramatically decreased by S-Carvone treatment. However it is not still clear how S-Carvone regulates the HMGR activity in potato sprout. It was supposed that it interacts with the membrane system of the plant cells, possibly by changing the membrane fluidity.

Until now sprout suppression effects of carvone were tested in small-scale experiments with different application rates for short storage periods (max., 3 to 4 months) at constant temperature (10 ^{0}C) In practice, however, most potatoes are stored in large stores cooled with an outside air cooling system. With this system the potatoes can only be cooled (with high ventilation rates of +- 100 m^3 air per 1000 kg potatoes per hour), when the outside temperature is lower than the temperature of the potatoes, reviewed by Ooterhaven, et al., (1993).

It is found that in semi-practical condition (15 tons) the carvone concentration rapidly decreased to low levels within weeks. Carvone was highly effective in preventing sprout growth when the atmospheric concentration was continually replenished. This is necessary because of the high volatility of carvone and the use of an outside air cooling system with high ventilation rates. Repeated applications every six weeks of 50 to 100ml per 100 kg gave adequate sprout suppression. The total amount applied during the long-term storage period was between 450 and 600ml per 100 kg (Hartmans et al., 1995).

The Carvone residues in the whole, unpeeled potatoes are more or less related to the dosage applied. The increase and decrease also depends on outside air ventilation. Most Carvone was found in the peelings and only a very small amount appeared to be

in the peeled tubers. Carvone treatment does not have a positive nor a negative effect on the sensory quality or fry colour of the potatoes (Hartmans et al., 1995).

1.4 BOUND RESIDUES.

Agriculture can be considered a victim of its own success having attained an increase in production by using different chemicals. Approximately 10% of chemical treated (sprout suppressing) potato material is lost during processing in the form of peel, starch and waste water. The load of chemical used for this production and the fate of chemical residues associated with these fractions has not been fully investigated (Duncan and Boyd, 1991). After the recognition of that a portion of the chemical remained bound to the plant even when the pesticide was extracted from crops. It therefore became more important to fully investigate the fate of the chemical used. These residues although in bound form can become potentially toxic for human health.

The pesticide residues which are normally unextractable by the conventional methods are known as bound residues, but the term bound residues is debatable and not fully defined for all situations. It became clear among pesticide chemists they had to standardise the definition of a bound plant pesticide residue. In plant metabolism studies using C14 labelled pesticides the terms "bound", "insoluble", "residual" and "unextractable" have been employed interchangeably to identify the fraction of radioactivity present in the plant residlum. Bound pesticide residues in plants are defined as chemical species originating from pesticides, used according to good agriculture practice and which cannot be extracted by methods that do not

significantly alter the chemical nature of these residues. The detail review on the bound residues by Kacew et al., (1996) is summarised here.

1.4.1 Significance of bound residues.

With the establishment that bound pesticide residues are indeed present in plants and subsequently ingested by mammals, the environmental consequences and human health significance have been the subject of numerous debates. It become vital to evaluate the significance of bound pesticide residues by studying their distribution in plants, the amount present, the structure of bound residues, and finally its toxicological importance.

1.4.1.1 Distribution of bound residues.

It is well known that binding of pesticide residues into lignin creates an insoluble material which is usually not digestible in animals. It would serve as an inactivation mechanism and basically not be potentially harmful. However, in recent studies it is clearly demonstrated that administration of lignin-bound pesticide to rats resulted in pesticide release within the animal, which may possess significant ecotoxicological impact. Although the majority of studies favour the view that distribution of bound pesticide residues, particular binding to lignin of the plants, can act as a buffer against potential mammalian toxicity, it should not be assumed any more that pesticides bind solely to lignin. Therefore the knowledge of distribution of bound residues in the plant is an important consideration.

1.4.1.2 The amount of bound residues.

At the time of harvest the degree of bound residue formation varies with plant species and type of pesticide involved, in general, the percentage of unextractable residue increases in relation to duration after the treatment with chemicals.

The measurement of toxicity relevant to the amount of bound residues is still a difficult task, as the most direct route to examining the potential of bound residues would be to feed animals tissues containing the bound residues. The major drawback to this approach is the limited concentrations of bound residues in the tissues. Even when the tissues containing the bound residues are used as the only dietary source in order to reach the highest concentration possible, the total concentration of the bound residue given is still orders of magnitude lower than the lowest concentration of the parent compound (David, 1998).

The high concentration of postharvest treated corn fed to lactating cows resulted in body weight gain and decreased milk production, although this phenomenon wasn't apparent with low pesticide amounts (Kacew et al., 1996).Therefore the amount of bound residues is a concern for human health.

1.4.1.3 Toxicological importance.

Bound residues are of toxicological significance if these compounds become systematically bioavailable to the organism that ingests the material. Attempts have recently been made to quantify and identify the grain protectant insecticide residues which remain nonextractable (bound) after solvent extraction. The potential biological

and toxicological consequences of such residues have been evaluated. During the studies of the bioavailability to rats of bound residues of deltamethrin in stored wheat it was shown that stored wheat containing bound residues of pyrimiphos-methyl and melathion when fed to rats were bioavailable and produced toxicity (reviewed by Singh et al., 1993).

Any pesticide residues that are not extractable and detected by recommended methods of residue analysis but remain bound with the constituents of food under practical agricultural conditions may become a potential hazard to consumers, and therefore both the quantity and the nature of these residues must be determined. Therefore in this regard a basic work was carried out and described in the chapter 4 to know whether there are any bound form of chlorpropham in the potatoes. This was a basic work, which concentrated on the relationship of chlorpropham with treated potato starch.

1.5 OBJECTIVES OF THESIS.

The detailed study of chlorpropham distribution in commercial stores and its interaction with the tuber components is presented in the thesis. The thesis is therefore divided accordingly with respect to its objectives as follows.

Chapter 1.

The up to date review of the literature on chlorpropham and potato storage is completed in this chapter. The detail survey of literature has given an idea and understanding about current situation of the existed problem.

Chapter 2.

The survey of literature and other relevant information about the developing of the methods of chlorpropham determination is carried out here. In the experimental section of the chapter the trial was carried out for the development of a method by comparing two most commonly used methods of chlorpropham determination. The objective was to choose quicker and more precise method of chlorpropham analysis for a large number of tuber samples.

Chapter 3.

This chapter involves a major part of the study. The objective was to find out the trends of chlorpropham distribution in commercial stores. The trial was also carried out to optimise its level in commercial stores. Therefore large number of samples from UK commercial potato stores were taken for the analysis of chlorpropham level. The

samples were taken from bulk and box stores, which were maintained at ambient and low level temperature*.

The effect of washing on chlorpropham level of stored tubers is also a part of the work to find out its attachment behaviour with tubers taken from different sites of the sampled stores.

Chapter 4.

In this chapter some basic trails was carried out to find out about any bound residues of chlorpropham with the tuber components. The experiments therefore were set up to understand the attachment behaviour of chlorpropham with tuber and its starch. Here the experiment was also done to know the effect of processing on chlorpropham level in tubers. In connection of bound residues the work was specially concentrated on starch of the tubers.

Chapter 5.

It contains a general summary of the work carried out and presented in the thesis.

*A part of this work has been published in the Abstracts of 13th Triennial Conference EAPR (Holland). Pp 591-92. Khan et al., (1996). Chlorpropham residues in box stored potatoes held at 3-4 ^{0}C.

Chapter 2:

CHLORPROPHAM DETERMINATION METHOD.

2.1 INTRODUCTION.

The selection of the method of analysis is a vital step in the solution of an analytical problem. A choice cannot be made until the overall problem is defined. Inevitably, in the method selected, a compromise has to be reached between the sensitivity, precision and accuracy desired of the results and the costs involved.

Any analytical method used to determine chlorpropham must be extremely sensitive because chlorpropham can affect plant growth at very low levels. The methods previously used for chlorpropham extraction, gas-liquid chromatography, infrared spectroscopy and colorimetry require either lengthy extraction steps or varying degrees of cleanup, often both reviewed by Corsini et al., (1978). The main work in this thesis was to study the detail of the chlorpropham distribution in the various kinds of commercial stores. The even distribution of the chlorpropham is not only concerned with the damage caused to the potato crop in the different parts of the store due to sprouting but also is very important from the health point of view. Another problem with chlorpropham use in this country has been the occurrence of contamination of seed tubers, which obviously effects their growth and development. Therefore the method should be capable of determining the

difference of the low amount of chlorpropham in the different parts of the store and the method chosen for routine analysis of tubers has to be capable of determining chlorpropham at <0.05 mg kg^{-1} fr. wt .tubers.

Part of this chapter is concerned with a survey of methods available for chlorpropham determination under a wide range of conditions i.e. some developed for routine screening of freshly treated tubers and others for extremely sensitive but time consuming measurements. Before going to the detail of the methods available it is useful to describe a section covering general principles and procedures in pesticide determination. The basic procedure involved in the determination of a substance includes four basic steps, Sampling, Extraction ,Purification (clean-up) and determination of the compound, using one or a combination of techniques available to the analytical chemist.

In these basic steps the statistical procedures for handling the results are known also to play a deciding role. Checks of the methods using blanks, spiked samples and replication are all included in deciding whether a particular technique would be suitable or not.

The first and most important step in any analytical procedure is sampling. It is axiomatic that unless the sample is correctly selected, taken and handled then there is no point in carrying out an analysis. A sample must provide a meaningful measure of the analyte, and only careful consideration of the overall problem by the analyst will ensure correct sampling (Lehotay et al., 1995). For a completely homogenous system any part of the whole is suitable for analysis. Field collection of sample- always over sample if at all possible particularly if little is known of

variability. Often, the laboratory chemist has no control over this stage and once samples reach the laboratory, they must be further sampled to allow residue determination to take place. Procedures for this are already available which include, coring, quartering and mincing, macerating or homogenising.

Generally, in studies reported in this thesis, samples were chopped and then re-sampled after mixing to obtain representative samples of tuber materials. The decision to wash or peel the tubers obviously must be taken before this stage.

Before going further to the chlorpropham determination method it would be helpful to review the existing literature on chlorpropharm determination. The survey cannot claim to be a comprehensive review but as many as possible are given as examples of methods for the determination of the chlorpropham.

2.2EXISTING TECHNIQUES FOR CHLOPROPHAM DETERMINATION.

As chlorpropham has been commonly used as a herbicide world wide since its introduction in 1951, therefore many methods have been developed for its analysis since its introduction. In most early cases hydrolysis was followed by column chromatography as a cleanup procedure. The residue in the purified extracts are determined with colorimetry, thin layer chromatography, gas chromatography, high liquid chromatography, gas chromatogrophy-mass spetrometry or infrared spectrophy (reviewed by Beernaert and Hucorne, 1991). Simply these techniques can be roughly classified according to the final technique used to determine chlorpropham as, spectrophotometric methods of analysis and direct techniques of measurement. The direct measurement techniques include gas chromatography (GC), high pressure liquid chromatography (HPLC) and HPTLC/HPLC.

The literature reviewed in this work is related with the above techniques particularly reference to HPLC and GC. There are many other methods such as used for multiresidues and the method used for routine monitoring of pesticides (Bernal et al., 1992; Cairns et al., 1993 and Association of Official Analytical Chemistry, 1992) are not included because it was felt that as they were developed to include as many pesticides as possible, the efficiency of extraction and/or method of determination of any particular pesticide could be compromised.

2.2.1 Spectrophotometric Methods.

The methods available untill 1988 were reviewed by Boyd, (1988) in detail therefore the summary concluded by the author is only included here.

The spectrophotometric methods of analysis for chlorpropham can be divided into 3 separate stages, such as the extraction of chlopropham from plant materials using a convenient solvent, hydrolysis (either alkaline or acidic) of chlorpropham to produce 3-chloroanaline, isopropanol and CO_2 and finally quantitative determination of the 3-chloroaniline produced on hydrolysis, by the addition of a reagent causing the development of a colour.

The methods of chlorpropham analysis based on colorimetric determination, have been changed and/or modified over the years in attempts to overcome problems that have appeared. The drawbacks of these colorimetric methods of analysis is the need for untreated control samples. These are of the utmost importance because they act as blanks for the analytical method. Obtaining untreated samples grown or stored under the same conditions as the suspect plant material may prove difficult if not impossible.

The colorimetric methods of analysis are based on the hydrolysis of chlorpropham to 3-chloroaniline and its subsequent quantification. Any other aniline based pesticide susceptible to hydrolysis may interfere. This interference is not confined to pesticides containing the 3-chloroaniline, 4-chloroaniline and aniline itself are sufficiency close to that of 3-chloroaniline to cause problems.

2.2.2 Direct Measurement Methods.

2.2.2.1 High performance liquid chromatography (HPLC)

The use of high performance liquid chromatography (HPLC) in pesticide analysis has increased especially in the determination of compounds difficult to analyse by GC methods e.g. polar, non-volatile or thermally labile compounds. HPLC has been used in plant and animal metabolite studies.

Wilson et al., (1981) reported a high performance liquid chromatographic method for determination of chlorpropham residues in potatos, beans, peas, and blueberries fortified at 0.25 ppm. Chlorpropham was extracted with methanol, and the extract was cleaned up by an acid alumina column. The average recoveries from all four commodities ranged form 64 to 103%. The lower limit of detection by using this method for beans, peas, potatoes and blueberries was 0.12 ppm. It indicates that for a higher concentration, above 1ppm of chlorpropham, the recovery was 100% or better while at lower residue levels, 1ppm or below recovery was less.

Selective detection techniques are beginning to be used in HPLC for improving the qualitative aspect of the determination of organic compounds. One of these detection techniques, fluoresence, offers increased selectivity and in many cases increased sensitivity. A number of pesticides have been reported to fluoresce naturally.

A high performance liquid chromatograph equipped with a C 18 reversed-phase column and fluorescence detector was investigated for the selective

determination of toxic chemicals at (ng) residue levels (Krause, 1983).

The purpose of the work was to determine which pesticide could be chromatographed and detected at low residue levels with the reversed-phase HPLC fluorometric technique. Seventy-five compounds were selected for study based on their known fluroesence or chemical structures indicating possible fluorescence. Initially, studies were conducted to determine the fluorescence in neutral and basic media under static conditions. Those compounds found to fluorese were selected for HPLC-fluorometric study.

Among the selected compounds of pesticides included propham, metabolites and a few industrial chemicals initially studied, 35 compounds were successfully chromatographed and detected at the low residue levels. The relative fluorescent intensity was medium fluorescence (instrument sensitivity rang 1-3) in both neutral media (acetonitrile-water 1:1) and base media (acetonitrile-aqueous -0.4 M sodium hydroxide 1:1).

Miles and Moye, (1987) used a high performance liquid chromatograph with post-column photolysis of pesticides for generation of fluorophores of water and vegetable samples. Several classes of pesticides have been found to respond to a high performance liquid chromatograph post-column reaction detector that employs UV photolysis with an optional reaction with o-phthalicdicarboxaldehyde-2-mercaptoethanol (OPA-MERC) reagent followed by fluorescence detection. Photolysis of most of the N-methylcarbamates, and carbamoyl oximes, carbamothioic acids,dithicarbamates, and phenylureas tested produced primary amines which react with OPA-METC to form the respective

derivatives. In some cases, substituted aromatic pesticides photolysed to chemical species which had native fluorescence. The limits of detection for propham and chlorpropham were found to be 5.0 ng.

Two simple, rapid reversed-phase high performance chromatographic procedures, external and internal standard methods were developed by Heras and Sanchez, (1982) to determine the chlorpropham. They have developed this type of method for nearly five carbamic herbicides (Heras and Sanchez, 1986). For the determination of chlorpropham the samples were diluted with methanol and 4-nitro-diphenyl ether was added as the internal standard. Calibration and quantitation is made with the use of pure chlorpropham, and results obtained in absolute or relative amounts. It was reported that internal standard method improves slightly the confidence limit and the relative standard deviation with regard to the external standard method.

Voyksner et al., (1984) analysed the selected 25 pesticides, including carbamates (propham) by high performance liquid chromatography-mass spectrometry (DLA-HPLC-MS). The HPLC system consisted of UV detector and C18 reversed phase column to perform the separation by using mobile phase of acetonitrile-water at 254 nm.

The carbamate pesticides displayed a range of sensitivity in positive ion mode. The detection limits ranged from 40 ug to 0.02 ug injected on-column. Such variability in detection limits are common in chemical ionisation processes. Moreover more interferences were detected when using lower mass fragment ions to determine the presence of particular pesticide.

In recent years, electrochemical detectors have been developed and applied to many of the analytes. For some classes of analytes, electrochemical detecter has proved to be more sensitive that UV detectors. The UV detector is not as sensitive as desired for carbamates (Thomas and Sturrock, 1986). Previous attempts to apply electrochemical detection to carbamates has been successful in a few cases in which the specific carbamate pesticide also contains functional groups such as aromatic amines which can be oxidised readily.

Thomas and Sturrock, (1986). determined the carbamates by high-performance liquid chromatography with electrochemical detection using pulsed-potential cleaning.

The mobile phase was acetonitrile-citrate buffer, pH 5.50 (1:1). The capacity factor of chlorpropham was 6.48.The limit of detection for the carbamates varies with the compound, the potential and the retention time. The primary phase thrust of this work was the evaluation of the detector, and optimum chromatographic separation was considered to be of secondary importance. No attempt was made to estimate optimum limits of detection except for amino carbamate.

Beside HPLC methods for chlorpropham determination by using different kinds of detectors several workers recently also tried to combine the HPLC with HPTLC. The commercial availability of thin layer chromatographic (TLC) plates with layers of very different polarity and high performance (HP) plates were two good reasons for reconsidering the use of TLC methods for qualitative and quantitative analysis. If the considerable advantages of thin layer chromatography are also considered (simplicity, economy, speed, recovery of single components,

direct visualisation in most cases of all components of the sample), it is logical to think of a re-evaluation of this method .

Corti et al., (1991) compared the HPTLC and HPLC procedure for the determination of chlorpropham, propham and thiabendazole residues in potatoes. The minced potatoes were extracted with dichloromethane with the presence of saturated aqueous solution of NaCl in a separating funnel. The pooled organic phase was dried over $Na_2 SO_4$ and evaporated. The residue was spotted on silica 60 TLC plates and developed spots of compounds were scraped off, eluted and then separated by centrifuging. The solution was spotted directly on HPTLC for quantitative analysis.

The HPLC analysis was also carried out with detection at 248 nm and mobile phase was methanol/ 0.1, phosphate buffer PH 5.5 (3:2). It was described that although HPTLC is more limited than column chromatography, it is still quite sensitive enough for detecting residues of chlorpropham, propham and TBZ in potatoes down to approximately 1/10th of the maximum permissible residues which is 0.5 mg/kg in peeled potatoes according to Italian Law.

Reuke and Hauck et al., (1995) studied the transferability of chromatographic conditions from HPTLC precoated plates to HPLC columns using 62 pesticides including chlorpropham, as standard substances and one real sample (lettuce). He described that frequently TLC and HPLC are unjustly looked on as competitive methods, but each of those separation methods has its advantages and disadvantages. Using advantages of both methods, combination of TLC and HPLC leads to a considerable saving of time and expense for the analysis.

2.2.2.2 Gas Chromatography (GC).

The chlorpropham determination methods with GC were reviewed by Boyd, (1988). Despite difficulties in the gas chromatographic determination of chlorpropham because of the instability of the compound at temperatures greater than 230 ^{0}C, this technique has since proved popular with other workers However, problems with the GC of the intact molecule resulted in many workers attempting derivatisation of some kinds. But as hydrolysis of chlorpropham is involved , this method of analysis suffers from some of the drawbacks mentioned in connection with colorimetric analysis.

The other methods developed were direct determination of chlorpropham by gas chromatography using flame ionisation detectors (FID), although the sensitivity of the method was no greater than that of existing colorimetric methods. The analytical method consisted of a soxhlet extraction of potato tubers using chloroform in the presence of sufficient anhydrous conditions.

The review afterwards of methods for the determination of chlorpropham by using GC compared and discussed the merits of steam distillation (not following hydrolysis) with the methods of extraction of the chemical using suitable solvents. It is concluded that the lack of sensitivity in the direct determination of the chlorpropham was due to the fact that the solvent chloroform could extract many other components of the tubers. To improve sensitivity it was suggested that the use of hexane in the presence of anhydrous sodium sulphate would result in extracts which could be more easily purified using an alumina column. Following this procedure they found that 0.02 mg chlorpropham kg^{-1} could be detected by

GC using nitrogen specific detectors Boyd, (1988).

A simplified method for chlorpropham extraction of large numbers of potatoes was developed by Corsini et al., (1978). The frozen ground potato peel was extracted with petroleum ether for 20 hours at room temperature. The solvent was evaporated to near dryness under minimum vacuum. The concentrated extract was then ready for analysis by GC equipped with FID. This method was straight forward but not particularly sensitive. The minimum detectable amount was not less than 1 mg/kg.

A method for the determination of chlorpropham residues in crisps and crisp frying oil was developed by Ritchie et al., (1983). Here the crisps were blended with hexane and filtered. The solid was then blended with a mixture of hexane and diethyl ether and filtered. The residue was finally blended with hexane and diethyle ether and filtered. The filtrates were combined and then evaporated. The chlorpropham was separated from the oil and passed through alumina column. The final values of chlorpropham was determined by GC, FID. It was reported that this method gave overall recovery of 93.2% and the minimum detectable amount of 0.035 mg/kg.

The determination of atrazine, chlorpropham and triallate in soil was developed by Voznakova et al., (1987). The herbicides were extracted from soil by shaking the sample with extractant on a laboratory shaking machine. Two solvent extractants, acetone and methanol, and two extraction procedures, single and multiple extraction, were tested. The extract was concentrated by vacuum evaporation and purified on a column packed with Separon SI C 18. The column

was eluted with the solvent and effluent was collected in a volume chosen with respect to the character of the experiment. Methanol and acetone were again used as solvents. The substances were determined gas chromatographically. From the recovery point of view, acetone appears to suit better than methanol. The results obtained with acetone were virtually unaffected by wetting whereas those obtained with methanol were improved by it. On the other hand, however, wetting has a negative effect on the evaporation of the extract and calls for the use of additional operations in the procedure, and therefore its use was abandoned. Therefore the final method suggested using the acetone as a solvent. This method enables the three herbicides in soil to be determined at tenths of ppm level.

Boyd, (1988) developed a method for chlorpropham determination in potatoes using GC, FID. Sub sample of minced potatoes was extracted by blending with hexane in the presence of anhydrous sodium sulphate. The mixture was then transferred to an aluminium bottle and shaken, filtered and concentrated. The filtrate was passed through the alumina column for purification. The purified extract was injected into GC equipped with FID. The concentration of chlorpropham was determined by comparison with standard solutions. The recovery of spiked potatoes was 91.2% +- 4.9 sd (standard deviation).

Beernaert and Hucorne, (1991). developed a simple method for fresh potatoes for routine checking the residues of chlorpropham and propham. The residue was extracted with methylene chloride added to a fresh cut potato slurry in demineralized water. The mixture was centrifuged and the organic layer separated and dried through a filter containing an anhydrous sodium sulphate layer. The concentrated extract with internal standard was then injected to GC for analysis.

The method was quick and accurate for higher levels over 5 mg/kg but was not suitable for analytical purposes for less amounts of chlorpropham and propham.

Recently a simple and quick on-line derivation method was developed for carbamate pesticides , including chlorpropham, in water (Farber and Scholer, 1993). Sample preparation consisted of solid-phase extraction with RP C18 cartridges. Trimethylsulfonium hydroxide solution was added to the concentrated eluate, and an aliquot of the mixture was injected in a temperature vaporizer, where carbamate pesticides underwent methylation and the derivatives were separated, detected and quantitated using high-resolution gas chromatography/mass spectrometry. The average recoveries at four concentration levels in water (25, 50, 100, and 200 ng/l) ranged from 46 to 104 % with determination limits of 25 and 50 ng/l.

2.3 DEVELOPMENT OF ANALYTICAL METHOD.

Based on the studies reported above, an accurate, sensitive analytical method for chlorpropham quantification was needed, particularly in this study where it was necessary to analyse a large number of samples from the commercial potato stores. Therefore the project objectives demanded a quick and precise method. The laboratory was equipped with GC, FID and there was no HPLC available at the commencement of the work. The standard method of chlorpropham extraction at that time was by blending, using a procedure previously developed in this department. The final determination of chlorpropham was by GC with FID, and these facilities were available in the laboratory. Therefore the blending extraction method was compared with a reflux extraction method as commonly used in industry for pesticide determination. The aim of the comparative trial was accurate measurement of chlorpropham within the shortest possible time. In the following section these methods were compared and advantages of one over the other for accuracy and sensitivity are discussed. The two detailed procedures are described in the relevant section (section 2.3.2).

2.3.1 Material and Method.

The blending method was developed by Boyd, (1988) in this department. Whole chopped, washed tubers were used for both methods for chlorpropham determination. The sub sample of 50 gm was minced by the electric mincer and blended for 1 min with 150ml hexane (glass-distilled grade, Rathburn Chem. Co., Scotland) in the presence of 80 gm anhydrous sodium sulphate (A.R. grade Hopkin

and Williams).

The mixture was transferred with hexane washing to an aluminium bottle, shaken on a wrist shaker for at least 30 minutes. It was then left for 24 hours to extract chlorpropham from the potatoes .The mixture was then passed through filter paper on a Buchner assembly to get a clear extract of sample. The residues were washed three time with 50 ml hexane and the filtrate obtained was concentrated to 2 m1 on a rotary evaporator keeping the temperature below 40 ^{0}C to prevent chlorpropham losses. The extracted sample of 0.5 ul was then injected into a standardised GC for chlorpropham measurement. The values were compared with the standard solution values.

The second method was a reflux extraction method and hexane solvent was used to extract chlorpropham from potatoes. The procedure followed here was a modification of the original method commonly applied in the crisp industry.

The detailed procedure of the reflux extraction method is described in stepwise fashion as below.

1.The sub sample of 250 gm of potato cubes were taken from the prepared sample.

2. The tuber samples were extracted for chlorpropham in a reflux unit for two hours by n-hexane (150 ml) as solvent.

3. The extract was then collected in a flask and then passed through the Buchner assembly containing Whatman no.1 filter paper with a layer of anhydrous sodium sulphate (100 gm).

4.The filter paper was washed with n-hexane (50 ml) three times to get a

maximum amount into the collecting flask.

5. The collected filtrate was evaporated by using rotary evaporator by keeping the temperature below 40 ^{0}C, to prevent chlorpropham losses during evaporation.

7. The 0.5 ul of extracted sample was finally injected into GC, FID, for chlorpropham determination.

8. The chlorpropham values of the peaks in the GC were finally compared with standard solution values used for standardising the machine.

All the reflux extraction was done under the laboratory safety rules in the fume cupboard, although there were few chances for the evaporation of the solvent

All the glassware used during these methods were washed with water and detergents and rinsed with acetone. The washed glassware was kept in the oven to evaporate traces of possible contamination.

2.3.1.1. Typical Gas Chromatography conditions.

Column Glass	2mm I.D.
Stationary Phase	3% OV17 (80-100 mesh) on WHP.
Detector temperature	F.I.D 230 ^{0}C
Injector temperature	220 ^{0}C
Oven temperature	174 ^{0}C

Carrier Gas Nitrogen 25 cm^3/minutes.

Sample volume injected 0.5ul.

After setting the conditions of GC and standardising it, standard solutions of chlorpropham in hexane were run through the system for a few days to assess the accuracy in injection technique and output of the GC. In all the rest of the experimental work the standard solution was injected a few times at the start, middle and at the end of daily work. The values from the daily work were confirmed with standard solution values.

2.3.1.2. Preparation of Sample.

The potato samples were prepared for the method comparison. Potato samples used as blank samples were purchased from local supermarket (Safeway), two kg of potato sample were taken and washed with cold tap running water to remove the dust/soil stuck on the surface of the potatoes. The diseased and damaged potatoes were discarded and each individual potato was cut into two halves lengthways with a stainless steel kitchen knife on chopping board. Each half of individual potato was cut again into two lengthways pieces and then sliced into small cubes (2-4 cm). The sample was then homogenised by mixing by hand to get a uniform sample. The prepared sample was then divided into two lots for two methods. The sample was kept in pre- labelled polyethylene bags and stored immediately in the freezer (-18 ^{0}C) for further experimental work. These samples were used for recovery determination a sub sample was also taken to find any chlorpropham traces and it was found to contain no trace of chlorpropham.

The standard solution of 100ug/ml of chlorpropham/hexane was prepared for spiking of blank sample to compare their recoveries.

The sample for the blending method was spiked with 1ml of 100 ug/ml standard solution. The 5 separate batches of diced sample were spiked with standard solution prior to blending in an electric blender. While in reflux extraction method, the weighed potato samples were put into thimbles in the reflux unit and spiked with 1ml of 100 ug/ml standard solution before extraction for two hours. Here five replications were run together with an average weight of 250 gm of potato sample. The values were then compared with the standard solution values and compared by statistics.

For the efficiency of these two methods, they were compared for minimum detectable level of chlorpropham in potatoes. Therefore blank potato sample was extracted by these two methods with hexane, dried and concentrated up to 2ml. The samples were then spiked with different known concentrations of standard solution (0.01 to 10.0 mg/kg range). The 0.5 ul of spiked samples were then injected into GC and chromatogram were compared.

The potato samples were also taken from a conventional potato box store, maintained at 8-10 ^{0}C. The tubers were taken randomly from different boxes. The tubers were washed with cold running water, to remove loose soil. The bruised and diseased ones were also removed. The potatoes were then cut into two halves longitudinally and each one was again cut into small pieces of 0.5 cm^2.The cubes were then mixed prior to being homogenised. The whole sample was divided into two lots for the two methods. Five replications were run for each method.The procedure for each of the methods was the same as described before. 50 and 250 gm weights were taken for the blending and the reflux method respectively. The chlorpropham values were compared with the standard solution values in GC.

2.3.1.3 Statistical analysis.

All the results were compared statistically by oneway analysis of variance using the computer Minitab 10.1 version

2.3.2 Results and Discussion.

The result of recoveries is presented in Table 2.1. The average percent recoveries were 83.80 and 93.14 in the blending and reflux methods. The recovered percentage value of chlorpropham was higher by reflux method as compared to the blending extraction method. The recoveries by the blending method of five replications were 85.96, 88.27, 82.27, 80.16 and 77.17 % and 96.00, 87.05, 94.42, 93.65 and 94.6 by reflux extracting method. The standard deviation among the reflux samples was slightly greater than the blending, 3.51 and 2.25 in reflux and blending methods respectively.

It was noticed in the reflux extracting method, that the reflux siphon tube became stuck several times during extraction. The higher standard deviation in reflux extraction replication could be due to blocking of the reflux siphon tube during extraction. The water in the sample formed another layer which was separated from the hexane, and as water is more dense than the hexane therefore it blocked the way of the hexane to pass down to the flask from the reflux tube. During the extraction therefore the reflux system was stopped and shaken many times to allow the water to pass through the reflux tube. However adding anhydrous sodium sulphate on the filter paper dried up the extraction when passing through the Buchner funnel. Therefore it was obvious that there would be some loss in precision of results, depending on amount of water present in the sample. In

Table 2.1 Percentage recovery of chlorpropham from spiked potato samples with two extracting methods.

	Method of extraction	
Replication	Blending	Reflux
1	85.96	96.00
2	88.27	87.05
3	82.27	94.42
4	80.16	94.60
5	77.17	93.65
Ave.recovery.	83.80	93.14
Stdev.	±2.25	±3.51

Table 2.2 Residues (mg/kg) of stored, treated samples (8-10 ^{0}C).

	Method of extraction	
Replication	Blending	Reflux
1	2.36	2.75
2	2.62	2.74
3	2.12	2.57
4	2.56	2.45
5	2.77	2.37
Mean ±Stdev	2.49±0.25	2.58±0.17

the next section therefore it was tried to overcome this problem by adding the anhydrous sodium sulphate in the thimble of the reflux unit. The reason for lower recovery in the blending method could be due to there being more steps involved and lower temperature extraction during blending.

After recovery measuring and comparison, the methods were applied to the samples of potatoes which were treated in the conventional box stores. Five replicates were run in both the blending and the reflux methods, with average weights of 50 and 250 gm. respectively. The average chlorpropham residues recovered from these methods was 2.49 $\pm$ 0.25 mg/kg and 2.58 $\pm$ 0.17 from blending and reflux extraction method respectively (Table 2.2). Although the recovered values from these methods were very close and was not statistically significantly different, more chlorpropham was recovered by the reflux extraction method and its precision was greater than the blending extraction method. This confirmed the superiority of the reflux method over the traditional blending method.

Comparison of these two methods showed that the reflux extraction method was easier and quicker compared to the blending method. In the blending method many steps were involved, therefore it takes a long time. There were greater chances of chlorpropham losses especially during the transferring step from blender to aluminium bottle and passing through the Buchner funnel. Extra care had to be taken at two stages in the procedure to maximise recovery of chlorpropham. Firstly, shaking the contents of the blender for 30 minutes to one hour was important and secondly, careful washing of the residues on the filter pad was essential for high recovery of chlorpropham (Boyd, 1988).

Therefore further analysis was recommended by the reflux extraction method. A problem that arose with almost every replicate in the reflux extraction method was that the tube of the reflux condensers blocked many times during the extraction of the samples due to the water present in the sample. The water in the sample was then cleaned or absorbed after the extraction by adding anhydrous sodium sulphate and during filtering through the Buchner assembly, otherwise there was no need for a cleaning step after the extraction. The reflux method was very quick and less time consuming than the blending method.

2.3.3. Improvement of reflux extracting Method.

After getting the recovery factor for the reflux method some modifications were tried to improve the reflux method as there was always a problem of blocking in reflux tube due to emulsion. There are two ways to prevent emulsificaton, by keeping the tuber pieces for 3-10 days in organic solvent, or homogenising the tissue with sufficient anhydrous sodium sulphate (Van Vliet and Hertog, 1966). Therefore a trial was set up to introduce the anhydrous sodium sulphate in to the thimble before adding the sample. The second trial was to check its low weight sample efficiency. As the method needed a lot of solvent and large sample sizes (roughly 250 gm) it was not always convenient to prepare this amount of sample and to use large amounts of solvent.

To see the effect of anhydrous sodium sulphate addition in the thimble before extraction a trial was carried out. The sample taken was frozen and already chopped, taken from the store where the potatoes were treated with the sprout suppressant chlorpropham. The prepared sample was divided into two lots. In one

lot (no. 5) each of 30 gm anhydrous sodium sulphate was added into the thimble and the other series was left without anhydrous sodium sulphate. The extract that had not been dried in the thimble with anhydrous sodium sulphate was subsequently filtered through a pad of anhydrous sodium sulphate on a Buchner funnel. The dried extracts were then evaporated by rotary evaporation under vacuum and made up to 2 ml. 0.5 ul of sample extract was then injected into the GC and the chlorpropham concentration calculated.

To determine the optimum weight of potato sample for future analytical work a further experiment was set up. Three different weights of 5, 10 and 30 gm were extracted as above with three replicates. Anhydrous sodium sulphate was added into the thimble before adding the potato sample. Each sample was spiked with a standard solution of 100 ug/ml (chlorpropham in hexane) before running the extraction procedure.

2.3.3.1. Results and Discussion.

The effect of anhydrous sodium sulphate on the recovery of chlorpropham from potatoes is shown in Table 2.3. The values were compared statistically. The sample with anhydrous sodium sulphate added to the thimble before adding the sample gave 2.92, 2.92 and 2.90 mg/kg of chlorpropham respectively for the three replicates with an average value of 2.91 mg/kg. The other sample extracted with the same procedure but without adding anhydrous sodium sulphate until the extract passed through the Bucher funnel gave 2.93, 2.74 and 2.94 mg/kg of chlorpropham respectively for the three replicates with an average value of 2.87 mg/kg.

The values recovered in both were close, whether anhydrous sodium sulphate was added to the thimble or not. Where the anhydrous sodium sulphate was not added in thimble the extract was passed through filter paper having a pad of anhydrous sodium sulphate over it. There was no significant difference between them for recovering the chlorpropham, however the standard deviation was greater in samples, which were extracted without adding the anhydrous sodium sulphate in the thimble (Table 2.3). The extracted values were more precise in anhydrous sodium sulphate added samples. This shows that the addition of anhydrous sodium sulphate into the thimble did not interfere with the amount of chlorpropham recovered but also gave more reproducible values as compared to the other. Addition of anhydrous sodium sulphate reduced the cleanup steps and reduced the time taken for sample extraction.

Another trial was carried out to check the efficiency of this method for the determination of chlorpropham from small sized samples. Three different weights of roughly 30, 20, 10 and 5 gm of homogenised chopped potato were used. The mean chlorpropham values were 2.9, 3.16, 2.14 and 3.07 mg/kg for the 30, 20, 10 and 5 grams respectively with standard deviation of 0.01, 0.43, 0.45 and 0.83 respectively (Table 2.4). There was no significant difference among the values obtained from these different weights of sample even as low as 5 gm. Therefore by this method the minimum 5 g weight of sample can be determined. However the values were more precise from the 30 gm weight sample as compared with other weights of sample. The least precision was in the 5g-sample weight with higher standard deviation, therefore best results can be achieved from 30g of sample weight.

Table 2.3 Effect of anhydrous sodium sulphate on extracting amount of chlorpropham in treated stored potato samples (8-10 ^{0}C).

	Anhydrous sodium sulphate	
Replication	added	not added
1	2.92	2.93
2	2.92	2.74
3	2.90	2.94
Mean+Stdev	2.91±0.01	2.87±0.11

Table 2.4 The chlorpropham residues (mg/kg) in different weights samples of stored treated samples.

	Sample weight taken			
Replication	5gm	10gm	20gm	30gm
1	3.79	2.04	3.10	2.92
2	2.16	1.76	2.79	2.92
3	3.28	2.64	3.64	2.90
Mean±stdev.	3.08±0.83	2.15±0.45	3.18±0.43	2.91±0.01

2.3.4 Conclusion.

As it was planned to take large quantity of samples, therefore a faster and more reliable method was needed for chlorpropham analysis. The two methods were compared for this purposes, blending and reflux extraction method, to finding out the most suitable analytical method for chlorpropham extraction in treated potato stored samples.

It was concluded after several trials of method comparison, that reflux extracting method has more advantages over the blending extracting method. Although the both methods were precise enough, but reflux extracting method was much faster. Atleast 20-25 samples can be completed in a day by reflux method comparing to 5-10 samples in 24 hours with blending method. The reflux method was more precise with a standard deviation of 0.17 comparing to 0.25 of blending method for treated tuber samples (Table 2.2). It was noticed that the reflux method was straightforward and therefore it was more reliable. In the blending method, on the other hand, too many steps were involved. The reflux extraction method removes the need for an aluminium bottle and shaking of sample for at least 24 hours, which required for blending method, and there was no need for clean up steps at the final extraction of samples. It was also found that in reflux method 30 gm of sample weight could give values of high precision results and furthermore it has the potential to extract the chlorpropham from as little as 5g of sample. It is however suggested that this method could be further developed by comparing the efficiency of extraction with solvents of different polarity like acetone and methanol. To maximise the recovery of chlorpropham it would also be possible to use different solvent ratios or combinations of different extracting solvents.

2.3.5 Recommended Method

The final method developed during this trial is summarised below.

The washed or unwashed samples of treated potatoes from commercial stores were chopped with an electric food processor. Sub-samples of 30 g were taken with 3-5 replicates from the original homogenised chopped sample. The sub-sample of 30g was placed into a pre-weighed thimble with 10 g of anhydrous sodium sulphate added. The sample was then extracted with 150 ml of hexane for two hours in a reflux extracting unit.

The collected extract from each replicate was then separately evaporated to dryness in a rotary evaporator under reduced pressure, keeping the temperature below 40 ^{0}C. The evaporated sample was then redissolved in 2 ml of hexane, rinsing the flask several times with solvent. The final sample was made to exactly 2 ml volume and any excess solvent was evaporated with dry nitrogen. The sample (0.5 ul) was then injected into the GC. The GC conditions were as fallow.

Column Glass	2mm I.D.
Stationary Phase	3% OV17 (80-100 mesh) on WHP.
Detector temperature	F.I.D 230 ^{0}C
Injector temperature	220 ^{0}C
Oven temperature	174 ^{0}C

Carrier Gas Nitrogen 25 cm^3/minutes.

Sample volume injected 0.5ul.

The amount of chlorpropham was calculated by comparing them with GC values for a standard solution. The average of 3-5 replicates was calculated and considered as the mean CIPC residue level in the tubers.

This final method was used for all further analytical work. In the next chapter, samples were taken from commercial potato stores and chlorpropham was extracted with the developed reflux method. This method have extracted most of the unbound chlorpropham from the treated potatoes stored in commercial stores. However the recommended method was not tested on this stage for the bound chemical in the tubers. The detail and the further recommendation of this method regarding the bound residues are described in the chapter 4 of the theses.

Chapter 3.

CHLORPROPHAM DISTRIBUTION IN STORES.

3.1 INTRODUCTION.

Chlorpropham has been used for sprout suppressing for a long time in potato stores due to its world wide popularity. Chlorpropham is normally applied to potatoes at a rate of 10-20 mg/kg in stores after wound healing. Chlorpropham is normally applied three times in a storage season. It can be reapplied easily but big challenge is to attain even distribution of the chlorpropham in the stores as on many occasions variability in that much has been recorded in residue levels in the range of 0.5-80 mg/kg (Duncan and Boyd, 1991). The uneven distribution of the chlorpropham means the higher level of deposition of chlorpropham chemical on one part and its minimum level on other sites of the store. The uneven distribution not only causes management problems and wastage of chemical but equally considerable threat for environment and health safety aspects. Nowadays much more interest exists in the total environment rather than just the narrow aspects of crop and animal production.

3.1.1 Application of Chlorpropham.

The chlorpropham can be applied in the commercial potato stores as granules, spray and or as a fog . In aerosol fog form the chlorpropham is dissolved in a particular solvent like methanol as a carrier, the mixture is then heated and pressurised to an exit pipe, resulting in a misty thermal fog. While the dry form of

chlorpropham can be applied as granules or as an air chlorpropham mixture. It can be applied either by, rotary atomiser, where a fine mist is produced by the chemical being thrown off a rapidly rotating wheel, typhoon applicator, where a stream of air is blown over the chemical, and a compressed air atomiser, where compressed air is used to blow the material into an air inlet leading to the potato store.

Granular applications of chlorpropham, is usually applied at the beginning of the storage period to delay and slow release the chlorpropham to avoid wound healing problems during the 'curing period'. The application of granular and specially only one application is not very popular, but in bulk stores it is spread over the top potatoes to avoid higher sprouting by condensation.(Boyd, 1988). The granular application has considerable disadvantage, it can clump together on the tubers, specially when the potatoes are wet. Therefore chlorpropham gives higher levels on certain parts of tubers, which can produce skin spot (Polyscytalum pustulans) on tubers by high levels of chlorpropham (Ives, 1955).

Aerosol applications of chlorpropham are far the more popular method of application available, due to a number of reasons. The application can be delayed until after the tubers have cured and also the re-application is easy. Therefore the flexibility in deciding which potatoes can be used for long term storage is possible. In aerosol fog the active ingredient is dispersed by the carrying agent like methanol. To form an aerosol of chlorpropham a fine stream of the chemical is dissolved in a suitable solvent which is then exposed to a heat source, resulting in a chemical 'fog'. In U.K commonly, CIPC 500M, supplied by Mirfield Sales Services, is used in commercial stores. Each litre of product contains 500 grams of

the active ingredient, the balance comprising methanol, used as a carrying agent. Analysis of a sample of the product showed that it contained about 55.6% by volume of methanol at 20 ^{0}C (Duncan and Boyd, 1992). The aerosol fog is distributed by fans forcing air through ducts installed before the potatoes are placed in storage . Box, bulk stores or bins can all be treated by aerosol chemical application although specialised machinery is needed and slightly different practices must be followed in each case. As there is a fire hazard, these machines are operated only by well trained person, who mostly own this equipment. The detailed information about these store types is mentioned in chapter one of this thesis.

There are two main types of machine available to produce these aerosols these are the Swing fog or pulsejet and the TIFA (Todd Insecticidal Fog Applicator).

3.1.1.1 Swing Fog or Pulsejet.

A thermal fogging machine , 'Swing Fog Vaporiser' or pulsejet is used commonly in the stores. The machine consists of a fuel tank, pesticide tank, blow pump, spark plug, carburettor and long exhaust pipe. To start the machine both tanks are pressurised by using pump. An initial mixture of fuel and air supplied through non-return valves into a combustion chamber, which is then ignited by a high-tension spark. The resulting hot gases escape as a pressure wave passing through a 'flame trap' to a fogging head where the treatment chemical is pumped in. A mist or fog of fine droplets is ejected and passes along a flexible tube connected to an inlet port on the store. This fog then rises throughout the store before settling on surfaces within the store. Droplet sizes are typically of the order

of 1-2 micrometers. The droplet size and the exposed liquid temperature can be changed by controlling the flow rate of solution.(Mathews, 1979).

3.1.1.2 TIFA (Todd Insecticidal Fog Applicator).

The TIFA is a modification of the smoke generator used in World War 2. After the war was over these generators were reused and they were adopted for application of pesticides. The machine consists of a petrol engine which operates an air blower and two pumps. The hot air of 500-600 ^{0}C produced and passed to a distributor head. At the point where the fog is created the temperature has been measured as 265 ^{0}C with a flow rate of 95 litres /hour.

Working on these high temperature fogging machines the breakdown of chlorpropham can be expected because of its thermal instability over 230 ^{0}C, but up till now breakdown in fogging machine has not been reported . The chances of breakdown in fogging machine can be reduced as the presence of chlorpropham is only for a short period in the fogging machine and as well all the processes are carried out in the absence of oxygen therefore no chance for the oxidation of chlorpropham. Like chlorpropham a similar thermal sensitive compound pyrerhroid, has been reported to give no or little breakdown around 230 ^{0}C temperature in fogging machines (Boyd, 1988).

3.1.2 Distribution of Chlorpropham.

Although the chlorpropham is a registered pesticide used for post-harvest sprout inhibiting in potato stores nearly for forty years all over the world, records of residues remaining on the potatoes after various forms of treatment have not .

been readily available. Residue data are important for understanding the mode of action, decay or metabolism of the chemical and also for safety issues (Duncan and Boyd, 1992). A tolerance of 5 mg/kg in or on potatoes has been established for residues of chlorpropham in most E.U countries. Existing residue data are, however, insufficient to evaluate current residue levels of chlorpropham and its distribution throughout bulk and box potato stores.

Several workers have studied the effect of air flow and its velocity on the movement of chlorpropham. The air flow and the volatilisation could lead to the redistribution of the chlorpropham in the stores after its application. The pile ventilation is a common practice for potatoes stored in bulk. Potato storage ventilation systems have regionally been introduced throughout the world since the introduction of through-the-pile ventilation. Reasons for these variations include local climate, market requirements, and the condition of the potato going into storage. In West Germany and The Netherlands where the requirement to dry potatoes coming from the field is commonly cited, recommended ventilation rates are 100-150 m^3/hr/tonne. In the mid-Western United States where fall harvest conditions vary, ventilation rate recommendations are 38-56 m^3/hr/tonne (Forbush and Brook, 1993). The ventilation rates differ markedly with geographic area (Bertolini and Guarnieri, 1990).

Wilson and Hunter, (1965) studied effect of airflow rates, during application of chlorpropham, on the distribution of sprout inhibitor within the bin. The residue on samples from the various locations within the bins indicates that, the distribution of chlorpropham was uniform at all of the airflow rates. However,

because of the limitations of the analytical method it was not possible to determine the actual distribution in the different areas of the bins. Only at the highest airflow rate was the residue on samples within the pile greater than 0.5 ppm. They suggested it was not necessary to operate the fans after completion of the application. They also pointed out that the droplet size is as equally important as the airflow.

Corsini et al., (1979) measured chlorpropham concentration in the peel of tuber samples taken from large commercial potato storage and from test bins after aerosol application. Tuber samples were taken at different levels within the pile and at numerous sites near the surface. A differential in chlorpropham concentration was found within the pile soon after the application. Residue levels were highest near the bottom level of the store and at the top of the pile and the interior of the pile had an intermediate level.

It was concluded that, the high levels at the top of the pile were a result of fallout from the aerosol mist circulation above the pile, as it was higher residues in surface samples taken from open bins as compared with covered ones. The bottom of the pile apparently functions to some extent as a filtering surface since the aerosol is introduced via the ventilation system, which circulates from the bottom to the top of the pile. Residue levels at most sites within the pile decreased during the storage season. The bottom or lower sites in a pile maintained relatively high residue levels. The steady state rate of loss versus rate of gain established in any given storage apparently favoured loss from the top and gain at the bottom of the pile.

Boyd, (1988) studied the distribution of the chlorpropham immediately after the application of fog aerosol in the commercial potato stores. The filter papers were used as adsorbent material for analysis instead of tuber sampling to get maximum sample in a short time without disturbing the store. It was also easy to position them on particular points before the application of aerosol fog and take them out immediately after the application. The filter papers were then analysed for chlorpropham like the tubers.

The chlorpropham amount on filter papers varied throughout the store. The higher values were on the top while the lower values were in the middle box samples. It was concluded by this study that it is possible to know the trend of distribution just after the application, but the filter papers can not take the place of the real tuber samples, due to their flat surface which definitely cannot disrupt and absorb the air passing over them like the potatoes in the store.

Very recently Kleinkopf et al., (1997) studied the residues in the bins after thermal aerosol treatment with maximum labelled chlorpropham in a Russet Burbank potato store: Five bins were set where one was controlled and the remainder were treated with chlorpropham. Among the treated bins standard conditions for storage of potatoes for processing (7.2 ^{0}C, 95% RH) and fresh market utilisation (10 ^{0}C, 95% RH) were maintained throughout the storing period. After the chlorpropham treatment , all air circulation was shut off to allow the aerosol to settle down.

The average chlorpropham residue concentrations on potatoes from various parts of the bulk pile after one or two aerosol applications stored at both

temperatures were found greater near the bottom than at the top of the pile. Approximately one-third of the chlorpropham applied was recovered and the remainder of the applied chlorpropham was non-recoverable through the tuber sample analysis. Changes in chlorpropham residue concentrations during the storage season most likely result from a combination of microbial decomposition, volatilisation or particle movement in the circulating air and metabolism of the observed chemical.

At present the potato processing industry relies heavily on chlorpropham for sprout control in order to obtain the necessary standards of quality for their products. If chemical does not make sufficient contact with each individual tuber then some sprouting will result. In the past more chlorpropham has been applied to overcome this problem, but an overdose could lead to values higher than the MRL level. Therefore uniformity in distribution of chemical has become vital in the present time.

The consumer behaviour is constantly changing toward the consumption of individual potatoes as a food. Therefore the amount of chemical on individual basis is gaining importance. The present study therefore is not only concerned about the amount of chemical on individual whole tubers, but further the level of chlorpropham on various parts of single tubers is also being investigated. The reduction of chlorpropham by common washing with tap water is also a part of this study for finding the exposed level of chlorpropham for the consumers as this is the basis of MRL measurements.

The study may not only be beneficial in improving the application practice in future by finding certain areas of stores of uneven distribution, it will be a step further toward consumer health safety by considering MRL levels. In this study a vast scale of samples are taken, with new improved sampling method, from various bulk and box commercial potatoes stores throughout the UK.

The application of chlorpropham in cold stores has recently started and trend is rapidly growing. The potatoes stored at low level temperature are for the consumption as a fresh whole individual potato. The purposes of chlorpropham application is to control any chances of sprouting during the period of lifting from store into the market onto the consumer table. At the time of this study no one has worked on the distribution behaviour of chlorpropham in these cold stores (4 ^{0}C), therefore the study will be an important contribution toward the understanding of the distribution behaviour of chlorpropham in these environments.

This chapter is divided into three sections. Section one describes the analysis of the chlorpropham residues in commercial potato stores in UK. Section two consists of a study to optimise chlorpropham distribution in commercial potato cold stores. The intention was to optimise the distribution of chlorpropham by applying a single dose of chlorpropham instead of three doses as in current practice and by manipulating internal air circulation during aerosol fog application. In the last section (section three) of this chapter, an attempt was made to find out the effect of particle size during aerosol fogging, and of the temperature gradient in model box stores, on the distribution of the chlorpropham residues.

Section 1:

3.2 DISTRIBUTION IN COMMERCIAL STORES

3.2.1 Introduction

The study of this section was to investigate the distribution pattern of chlorpropham after the aerosol application in commercial potato stores. For this purpose the different stores were sampled which were stored at various temperatures. The stores which were studied including both box store and bulk store, were commercially used for storing of potatoes in UK. The main object was to understand differences in the distribution of the chlorpropham and to find any pattern of distribution of chlorpropham.

A new technique of sampling is developed by taking the individual tuber section for the chlorpropham determination rather than the bulk of tubers. One of the objectives of taking the individual tuber was to establish the degree of tuber to tuber variability in chlorpropham residues to know sample techniques and validity of representative sample for chlorpropham residue determination. In previous residue work the values of the chlorpropham was the average value of at least 1 kg tubers which were mixed and further sub-sampled to provide a sample for analysis. This meant that most residue work was based on an average figure obtained from at least 6 and in many cases more tubers. Working on an individual tuber basis for chlorpropham analysis meant that variables such as tuber size and weight and state of sprouting could be associated with the residue.

These samples were also subjected to washing to know its effect in removing the chlorpropham on tubers. The washing will give an idea how firmly the chlorpropham will stick with the tubers and how much is to be consumed by ordinary consumers. The washing can also lead to an understanding of the form of chlorpropham by which the tubers were exposed during storage.

The study will be helpful to improve the practice of chlorpropham application in future in stores. Therefore samples were taken from different sites of the stores to know the over all distribution of the chlorpropham in the stores.

3.2.2 Material and Method.

3.2.2.1 Sampling.

In general the tubers were taken from the box and bulk commercial stores used for the storing of the potatoes. In case of the box store the tubers were taken individually from the different sites and height of the stores. The individual tubers were then analysed separately and the average values were represented in the table. In most of the cases the tubers were cut into two halves, upper and lower half of the tubers. These tubers were cut horizontally as they lay on the surface of the stores. The sampling site for individual store is little varied and it is mentioned in detail in their respective sections.

In most of the stores the tubers were not only taken from the surface of the boxes of different heights but also tubers were sampled from the different levels in the boxes, such as they were taken from the top surface of the box and also middle

and the bottom layers of the box in the stores. However the individual store is also described separately in their respective section in detail.

3.2.2.2 Washing.

The tubers were washed to find out the effect of any reduction on the level of the chlorpropham from the tubers. The washing was done by simple tap cold water and some time with slightly brushed to remove apparent soil on the surface of the tubers. The sample of the tubers washed were the portion of the individual tubers taken for the chlorpropham determination. In case of the half tubers for upper and lower portions of the tubers, the half sections were again cut into quarter longitudinally. The quarter of the tuber were then washed and kept in the separate bags in the refrigerator for the analysis whenever needed.

In case of the tubers taken as a whole and studied for washing they were cut into two equal parts and the one half was washed and other kept as it is. They were then placed in the refrigerator in separate polyethylene bags for the further analysis when required. The five tubers, samples from all sites were taken and the average of the five tubers were then presented in the form of the table or graph. The statistics were also applied to know the significance of the distribution or the effect of the washing on the level of the chlorpropham.

3.2.2.3 Residues determination.

The individual tuber or the section of the tuber was chopped in the food processor. The sample was individually put into the thimble containing the nearly 10 gm of the anhydrous sodium sulphate. The weight of the sample was then

recorded and thimble was then placed in the reflux unit for the extraction. The extraction of the chlorpropham was done by the developed method of the reflux extraction for two hours with n-hexane as a solvent. The extract was then evaporated to 2 ml volume by keeping the temperature below 40 ^{0}C. The 0.5 ug of the sample was then injected into the GC for the reading of the chlorpropham. All values were calculated with reference to the standard solution used for standardising of the GC.

3.2.2.4 Statistical Analysis

The statistic package of Minitab 10 was used for the significance of the data. ANOVA is applied to the comparison of the values of the chlorpropham.

3.2.3 Store A (cold store at 3-4 ^{0}C).

This was a cold store maintained at temperature 3-4 °C. Low temperature has played an important part in the development of potato storage. By using temperatures up to 4 ^{0}C it is possible to delay sprouting for considerable time (Van Vliet and Schriemer, 1963). The application of sprout suppressant chlorpropham has become a regular practice to an estimated one third of the cold stores potatoes in the UK. In response to this recent trend of applying chlorpropham to cold stores in UK, information has been gathered on the efficiency of uptake and the distribution of the chemical on an individual tuber basis.

The main reason for its use is to minimise sprouting when the potatoes are removed from store to market. The distribution of chlorpropham in the cold box stores has not been reported previously. This study will show the distribution pattern of chlorpropham in potatoes from the cold box storage. The store was a modern cold store with refrigeration facilities and environmental control units to closely monitor humidity and temperature. Full, the store held approximately 2500 tonnes. The potatoes were sampled at the 14-15 June (8 weeks after the last chlorpropham application)

1995/96 storage season	History of chlorpropham application to store.
1. Early November.	30ml/ton (roughly 18g/ton)
2. Late February.	30ml/ton. (roughly 18g/ton)
3. Late April.	25ml/ton.(roughly 15g/ton).

All formulation were 60% chlorpropham or 600g/L.(w/v) in methanol, Warefog (MS)

3.2.3.1 Sampling

The plan of the store is shown in the diagram with boxes where tubers samples were taken also shown. Basically the store contains two massive blocks of the boxes. Each of the block which is 14 x 13 and seven high. There was a passage way between the blocks, which is also filled with boxes which contained one ton of potatoes. The samples were taken at the end of the storage season after three applications of chlorpropham aerosol fog. Five tubers were selected from each sampling site of the store (Fig 3.1). The tubers were sampled from top, middle and the bottom height of the stack in the store. The tubers were taken from surface, middle and bottom layers of potatoes in each selected box of the stack. The detail of sampling is described in Fig 3.1.

The tubers taken from surfaces of the boxes were cut into two portions horizontally as they lay, immediately after sampling into upper and lower portion. The upper portions were those which were directly exposed to the air, while the lower portions were the other side of the potato. All others samples which were taken from middle and bottom layers were whole tubers. The tubers were then immediately put individually into pre-labelled polythene bags and quickly stored in the $-18\ ^{0}C$ refrigerator for further analysis in the laboratory.

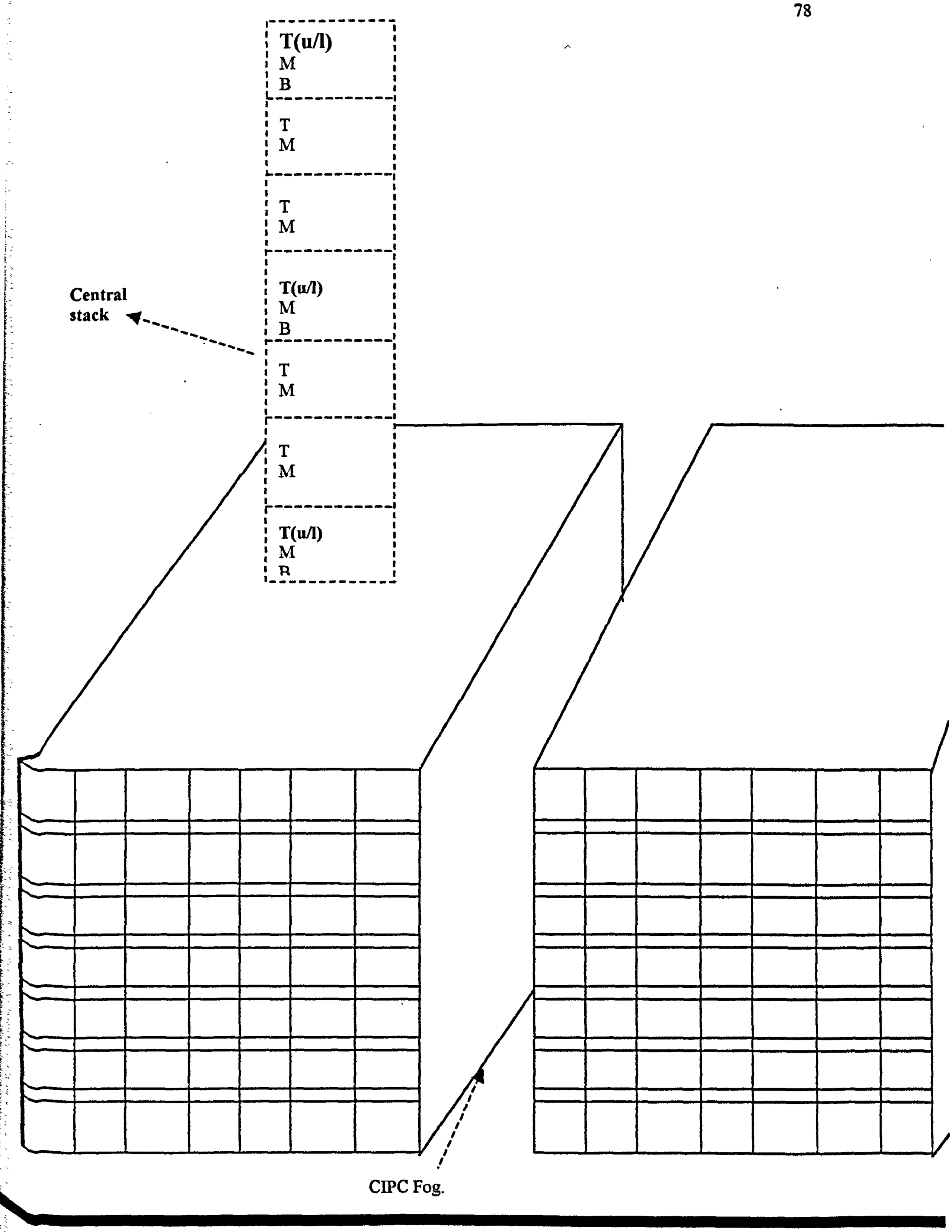


Fig-3.1 The cold box store(3-4 ^{0}C), sampling point showed by letters.

3.2.3.2. Results and Discussion.

3.2.3.2.1Unwashed Samples.

The distribution of chlorpropham found throughout the store is presented in Table 3.1. The level of chlorpropham varies from site to site in the bay. The amount of chlorpropham was even different within the same box, taken from different depths in the box. However there was a trend of decreasing pattern of chlorpropham from top to bottom of the selected bay.

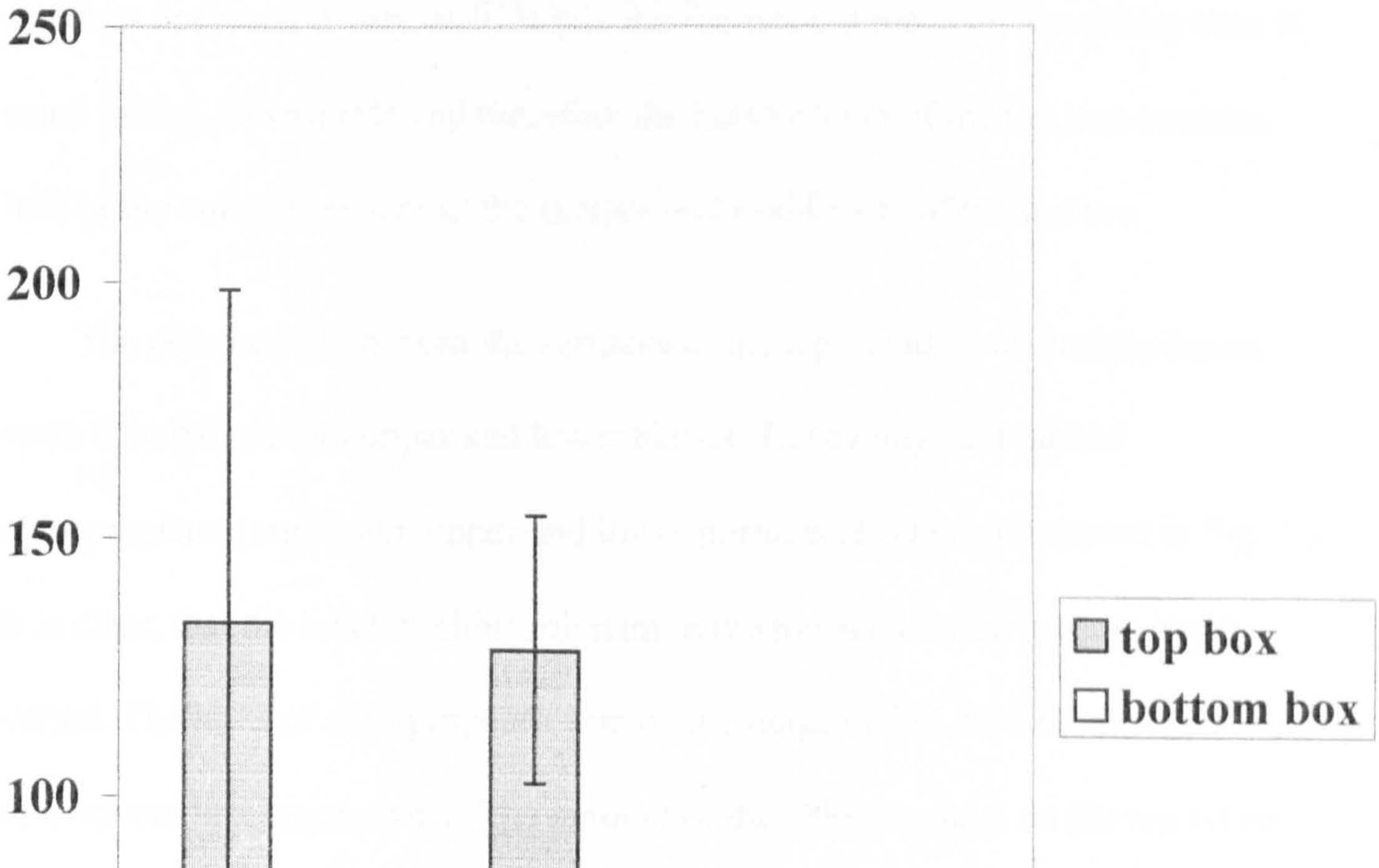
The surface tubers of the top box in the store, which was supposed to be directly exposed to the fog had the highest amount of chlorpropham as compared to the rest of the store. The surface of the top box contained 137.47 mg/kg, while the minimum amounts were in middle tubers of lower height boxes particularly in ground level two boxes of 4.54 and 3.97mg/kg respectively. The top surface potatoes were not significantly different when compared to the middle samples of the top two height boxes. These findings show that up to middle height of bay the chlorpropham was at highest level and relatively evenly distributed.

The top and bottom were compared by plotting a graph (Fig. 3.2). Both boxes were sampled from the surface, middle and bottom of the boxes. It is clear from the Fig. 3.2, that the level of the chlorpropham is much different in these boxes. The bottom box has very low level of chlorpropham as compared to the higher level of chlorpropham in the top box. It is also clear from that bottom layer of the top box contains nearly half of the amount present in the surface and middle of the box. The surface and middle tubers contain the same level, while considering the bottom height of the bay, it is found that the bottom layer contains

Table 3.1 Chlorpropham residues (mg/kg) in unwashed tubers samples of box store 'A'(3-4 ^{0}C).

	Position of tubers in the box			
	surface (halves)		middle	bottom
Box height	upper	lower	whole	whole
Top				
7	236.56±20.32	30.96±7.94	128.39±26	62.93±5.96
6			49.14±6.71	
5			20.15±4.86	
4			19.01±1.00	8.91±1.08
3			8.03±1.90	
2			3.97±0.38	
1	9.21±1.55	7.63±1.37	4.54±0.37	7.63±0.69
Bottom				

Fig-3.2 The chlorpropham residues in varied depth of top and bottom height box of the cold store'A' (3-5 ^{0}C).



higher levels of chlorpropham than their middle samples and bottom layer is equal to the surface tubers.

This shows that chlorpropham passes through the potatoes to move upward in the bottom height and because this bay was in the centre of the store, therefore fog does not reach it very well. While the fog when it reach on the ceiling then it starts falling downwards and therefore the bottom layer of the top box contains half of the amount present in the surface and middle site of the top box.

The potatoes taken from the surfaces of the top, middle and bottom boxes, were then halved into upper and lower halves. The average amount of chlorpropham (mg/kg) on upper and lower portions of tubers are shown in Fig. 3.3. It is clear, that the level of chlorpropham in the top and bottom box are highly varied. The level of chlorpropham was in the range of 236.56-7.63 mg/kg in top and bottom box respectively. The amount of the chlorpropham on the top boxes was much higher as compared to the bottom box within the same bay.

The level of chlorpropham is even significantly different in the same box on their upper and lower portions of tubers. The highest amount of chlorpropham was found on the upper half portion of the top box compared with rest of the store. The average amount of chlorpropham of five tubers of the upper portion in the top box was 236.57 mg/kg, while it was much lower (30.96 mg/kg) on the lower side of the same top box tubers. It was noticed during the analysis that there was a prominent layer of chlorpropham on the upper portion of top box tubers. The highest amount of the chlorpropham on the top half portion could be the result of falling of the fog

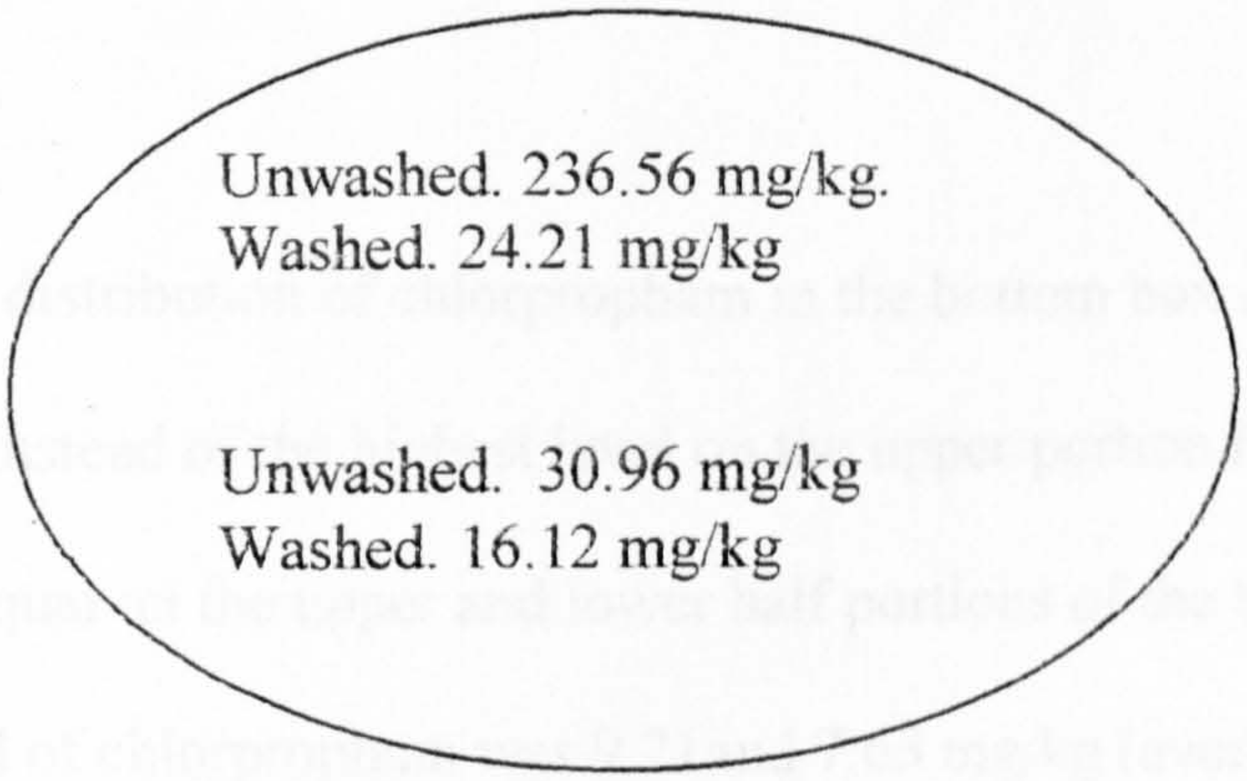


Fig. 3.3. Chlorpropham residues in cold store, store 'A'. on its top surface tubers of both top and bottom box of the store.

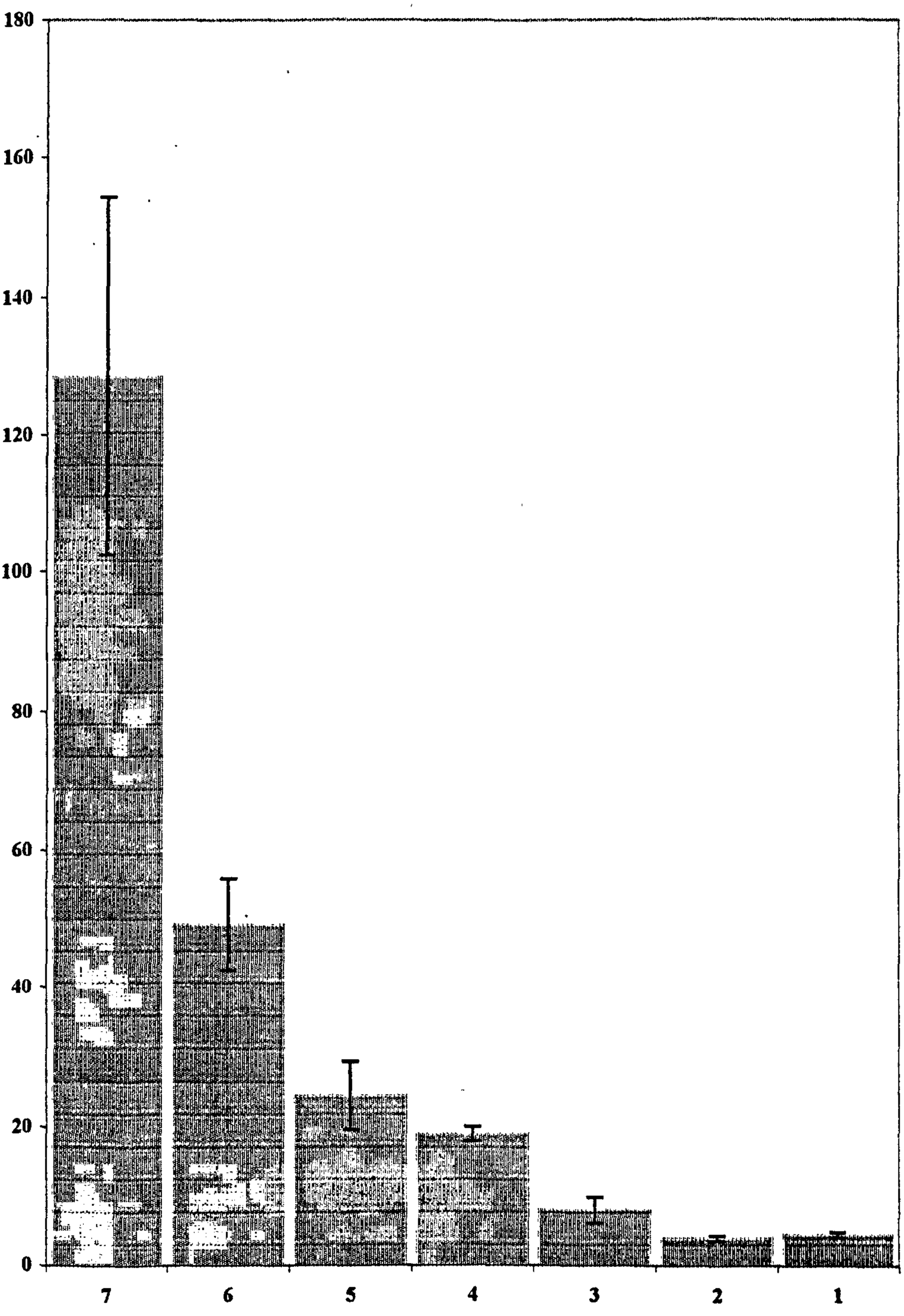
directly on the surfaces. As these tubers were directly exposed to the contact of fog therefore a thick layer of the chlorpropham deposited over them. The level on the top portion was nearly eight time greater compared to the lower portions of the tubers.

The distribution of chlorpropham in the bottom box does not follow the same pattern. Instead of the highest level on the upper portion in the top box it was almost equal on the upper and lower half portions of the tubers in the bottom box. The level of chlorpropham was 9.21and 7.63 mg/kg (average of five individual potatoes) on the upper and lower portions of bottom box tubers respectively.

In terms of chlorpropham distribution or uptake it was equally distributed in the bottom box on both surfaces. This also shows that fog remained in contact at the same level in the ground while it started falling downwards directly on the tubers in the top height. The studying of upper and lower halves was found important, as these halves of tubers represent the direction of flow of fog during application or after it.

The distribution of the chlorpropham among the tubers taken from the middle portion of the box is shown in the Fig. 3.4. The level of chlorpropham continuously decreased from the top height box towards the bottom height of the store. The range of chlorpropham among middle samples from top to bottom height was 128.39-4.54 mg/kg. The amount of chlorpropham was highest in the middle samples of the top box. It was nearly 32 times greater than the same sampling point of bottom box tubers. The chlorpropham level rapidly dropped from 128.39 mg/kg, in top box to 49.14 mg/kg in the second top height box of the

Fig.-3.4 Chlorpropham residues in unwashed tubers taken from the middle of boxes from the cold store'A'(3-5 ^{0}C).



store. The level of the chlorpropham from second top height box to the bottom box was dropping, while the level in the middle height of store, box 5, 4, was almost equal i.e. 24.51 and 19.01 mg/kg respectively. Similarly the lowest height boxes (boxes 2 and 1) contained the equal distribution of chlorpropham i.e. 3.97 and 4.54 mg/kg respectively. The bottom boxes contained the minimum amount of chlorpropham as compared to the rest of the store height in the middle tuber samples.

This very interesting trend in chlorpropham distribution, points towards the contact behaviour of fog to the tubers. Here the middle sample contains higher levels of chlorpropham, which normally is not expected, as it is in the middle of the box covered from all sides. The decreasing trend towards the bottom height in the stack shows that fog starts to settle down from the top height to ground. This will suggest that further chlorpropham application will not increase the level of certain areas where it will deposit on other sites. Therefore top height will be targeted every time it is applied and lower ground level boxes will not get much increase in chlorpropham level. The level on the ground level will not increase even if many fog applications are made until its direction is changed. Here the level of chlorpropham was high throughout the store and minimum level sites have higher levels than the MRL, while other sites have higher amounts still, which is not suitable for consumption at all.

The samples from the bottom layer of boxes were taken after down loading of the box. The samples were taken from three different heights top, middle and lower boxes of the store. Although there was a trend of decreasing chlorpropham from top height box to the bottom height box of the store, it was almost equal 8.91

and 7.63 mg/kg in the middle and lower height of the store in bottom layer tubers respectively (Table 3.1).

As the bottom samples were taken from the top, middle and bottom boxes so it can be predicted from this trend of two lower boxes that the chlorpropham from middle to bottom boxes in their bottom layers could be almost the same. Bottom layer of top box is roughly 8 time greater than the middle and bottom box samples. However their stdev. in the five potatoes in the top box samples are much higher 13.36 than the other two boxes for the bottom layer samples, which shows that this could be due to the direct contact of the chlorpropham fog during application.

The samples taken from the bottom layer of the boxes were also lowered as compared to top height, yet these lower height boxes contained double the amount that was found in middle samples of respective height box. The chlorpropham in the bottom layer became almost equal to the surface potatoes of the same height. This trend shows that either the chlorpropham was redistributed after fogging by moving upward or it was due to the fog, which was supposed to be applied from the ground level. The application of fog on ground level means it is passing through the potato layers which act as a filter and may cause the higher values in the bottom layer of the bottom box.

3.2.3.2.2 Washing.

The washing of these samples were also carried out to study its effect and to under stand its contact behaviour with tubers. The samples were selected from the top surfaces of top and bottom box, the middle of the box from top to bottom box in the stack. The tubers, which were taken from the surfaces of the top and bottom

box, as described earlier were cut into upper and lower portions. Therefore these halves were again cut longitudinally into two pieces for washing. One of each half portion is washed and compared with the other piece of unwashed.

The potatoes taken from middle of the boxes in the stack were whole potatoes, of five tubers from each box. These whole potatoes were cut into two halves. One of the halves of the whole potato was washed and the other one was kept as such. The mean of five replicates of washed portion is compared with the other unwashed portion of tubers. The tubers were washed simply by cold running tap water. The washed and unwashed samples were placed separately in pre-labelled polythene bags and stored at -18 ^{0}C for further analysis .

The overall washed values from different sites of the stack are presented in Table 3.2.The effect of washing on the chlorpropham reduction in the upper and lower surfaces of top and bottom box are shown in Fig 3.3. Washing reduces more or less the level of chlorpropham in almost every site of these samples. The effect of washing was clearly different in top and bottom box, surface tubers.

The amount of chlorpropham washed was looked at relative to the total amount present on the tubers. The highest reduction in chlorpropham level was achieved on the upper portions of the top box tubers. The washing reduces from the original unwashed level of 236.57 to 24.20 mg/kg, almost 90% reduction. The washing reduces the thick layer deposited on the surfaces of the tubers therefore the reduction was much higher on this position in the box. In other hand washing was not so effective in reducing chlorpropham amount from the lower half portions of the top box tubers as it was on upper half portions.

Table 3.2 Chlorpropham residues (mg/kg) in washed tubers of box store 'A' (3-4 ^{0}C).

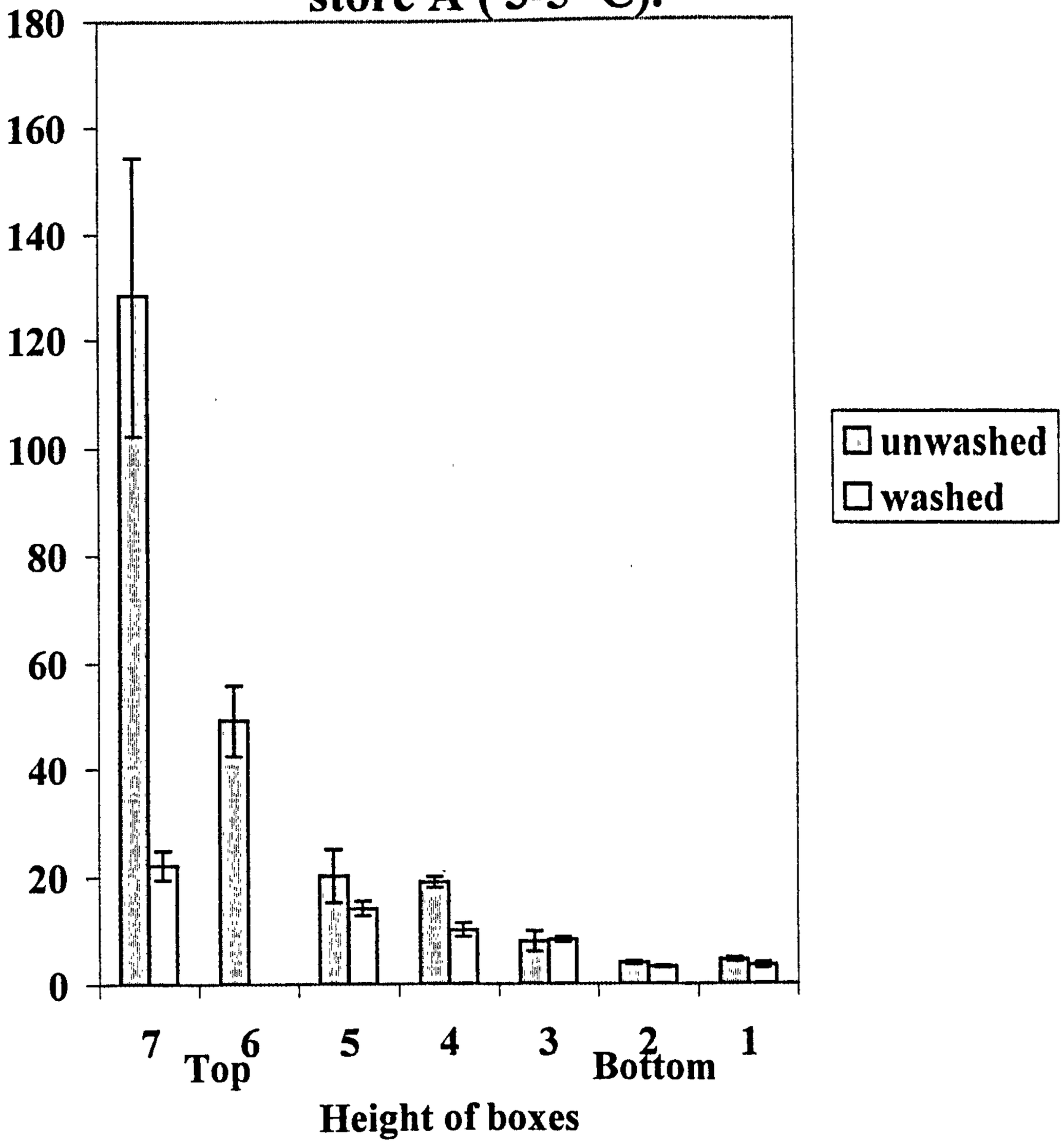
	Position of tuber in the box		
	surface tubers (halves)		Middle of the box
Box height	upper	lower	whole
Top			
7	24.21±1.97	16.12±2.33	22.15±2.67
6			*
5			14.15±1.33
4			10.12±1.22
3			8.33±0.51
2			3.21±0.19
1	6.50±0.79	5.24±0.29	3.48±0.53
Bottom			

The washing reduces from the original unwashed tuber level of 30.96 mg/kg to 16.12 mg/kg in lower portions of top box tubers. Although there was 50% reduction but it was non-significant on this side of the tubers.

The trend of washing in reduction of chlorpropham level in bottom box tubers was not the same as it was found in the top box tubers. The level of chlorpropham reduces by washing from 9.21 and 7.63 mg/kg in unwashed tubers on upper and lower portions to 6.50 and 5.24 mg/kg respectively (Fig.3.3) and this reduction by washing was totally non significant difference statistically. Here the reduction of chlorpropham level was much less comparing to the top box tubers of their upper and lower portion of tubers. The less reduction could be due to sticking of fine particle sizes on these positions. The fine particles became tightly attached with the tuber components. Although it is 40% reduction statistically this reduction in the bottom box is non-significant.

The effect of washing on the chlorpropham amount on the middle of the box sample in the stack from top to bottom box was plotted as shown in the Fig. 3.5. It can be seen from the graph that washing reduces the level of chlorpropham up to a certain height of the stack of the middle samples from top to bottom height boxes of the store. The reduction was higher from the top four height boxes, nearly 82 %-50 % in the box height up to box four, while it was maximum reduction from the top height of the stack. It was 128.39, 24.51 and 19.10 in the top three height, which reduces to 22.15, 14.15 and 10.12 with the washing in top boxes no 7, 5 and

Fig. 3.5 The effect of washing on the chlorpropham residues, of tubers taken from the middle of the boxes from cold store'A'(3-5 ^{0}C).



4 respectively. However comparing to the top four height boxes, the washing does not effect the level of lower three heights of the stack.

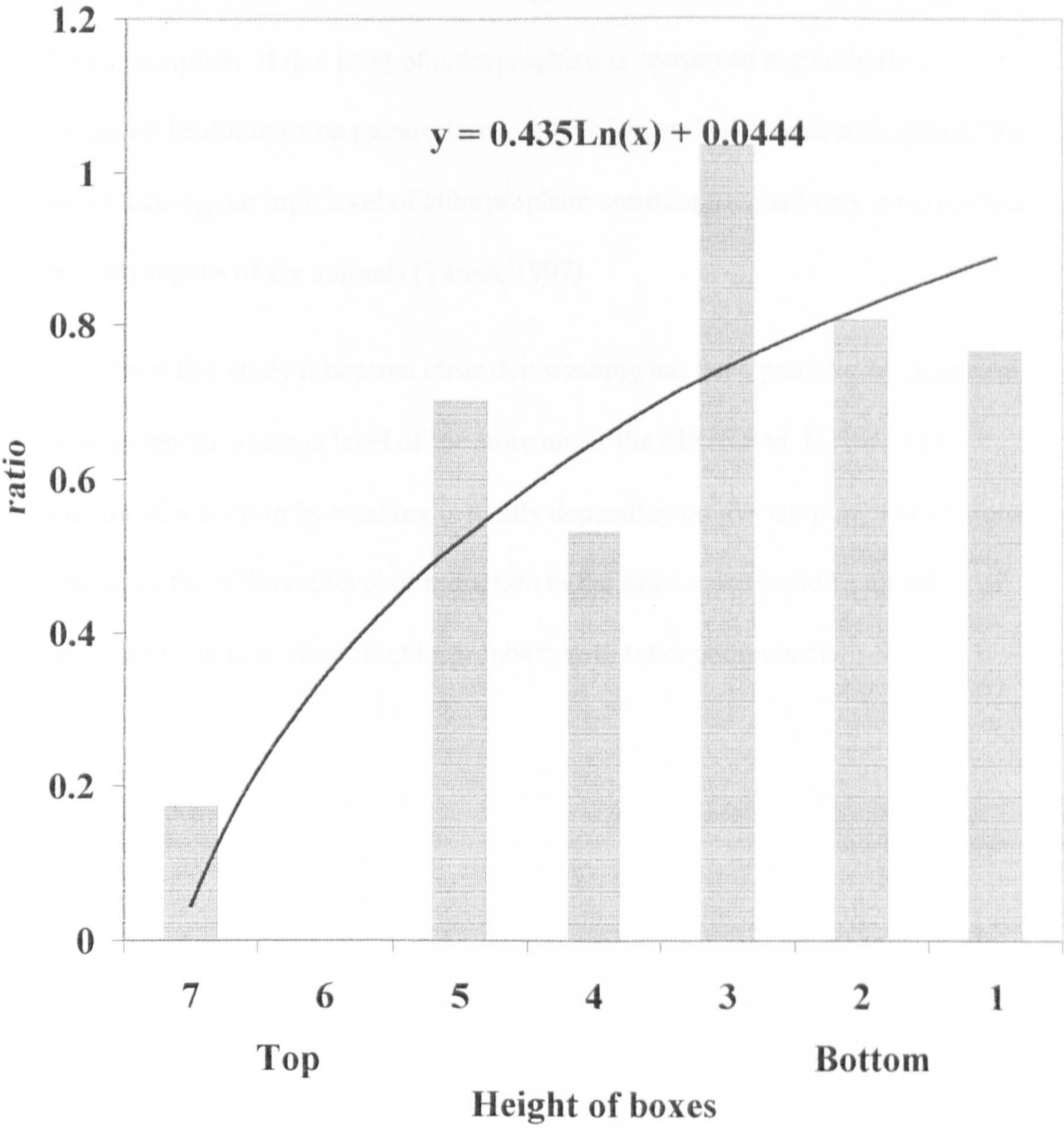
The chlorpropham level was in the range of 22.15-3.48 mg/kg after washing the tubers taken from the middle site of the boxes. Overall the statistical reduction by washing remains ineffective and non-significant amount of chlorpropham was reduced from the middle tubers of the boxes. However the only significant reduction was from the top most height box of the store on their middle samples.

Perhaps the highest significant reduction by washing in this box could be the result of their being directly exposed to the aerosol fog for a relatively greater period than to the other boxes. Therefore larger particles of chlorpropham loaded on the top box, could be easily removed as they are loosely attached, while the other heights of the store contained fine or small particle sizes that remain attached to the tuber components.

The overall ratio of washed to their unwashed in the samples in the middle layers was plotted as a graph (Fig.3.6). It is apparent from the fig, that reduction of chlorpropham by washing was related to the original amount present on the unwashed tubers. A trend line was drawn on washed/unwashed, which shows that the ratio increased towards the bottom height of the stack.

It explained that up to certain level of chlorpropham it cannot be washed with water. If the level on unwashed samples increased then that could be washed easily because it will be loosely attached to the tuber surface. As the chlorpropham level dropped towards the bottom height, reduction by washing was also lowered.

Fig. 3.6 The ratio of washed samples with respect their unwashed in the cold store'A' of their middle layers of tubers in boxes.



Therefore a higher amount means greater reduction in washing. However even after washing, the chlorpropham level was still greater than the MRL, in Europe. Because these tubers were supposed to be consumed individually so this trend and the level of chlorpropham after washing is not appropriate. Therefore efforts must be put to lower down the chlorpropham level on the tubers.

The top tubers, which contain the highest amount, were totally unacceptable for consumption. If this level of chlorpropham is consumed regularly then consumer health must be reconsidered. It has been reviewed in first chapter of the thesis that regular high level of chlorpropham consumption had very severe effects on body organs of the animals (Tanka, 1997).

From this study it became clear that washing has been potentially successful in reducing the average level of the store under the MRL level. However the amount of reduction by washing is totally depending on the sampling site of store. Therefore the different level of reduction in the same store could be an effect of the attachment behaviour of chlorpropham with tuber components.

3.2.4 Store B (conventional store at 8-10 ^{0}C).

This store was a commercial store. The potatoes were in wooden boxes of 0.5 tonne. The sampling was carried out on June '95; following 3 applications of chlorpropham to the store. All formulation were 60% chlorpropham or 600g/L.(w/v) in methanol, Warefog (MS). This was the normal store temperature of 8-10 ^{0}C with six boxes high. This was the normal potato store where the tubers were stored for further processing.

3.2.4.1 Sampling.

The diagram of sampling in the store is shown (Fig. 3.7), the samples were taken only from the top and bottom boxes of the store. Five potatoes were taken from each box, and halved immediately into two equal portions on their axes as they were lying into upper and lower surfaces . The samples taken were placed in a deep freeze (-18 ^{0}C) in pre-labelled polythene bags for further analysis. These halves were again cut into two equal parts for washing purposes. One of the quarters is then washed and compared with the unwashed portions.

3.2.4.2 Results and Discussion

3.2.4.2.1 Unwashed samples

The overall distribution in this store is presented in Table 3.3. The level of the chlorpropham varied with respect to the height of the box in the stack. The level of chlorpropham was different in the tubers of top and lower height box in the stack.

ged by lateral box
ment.

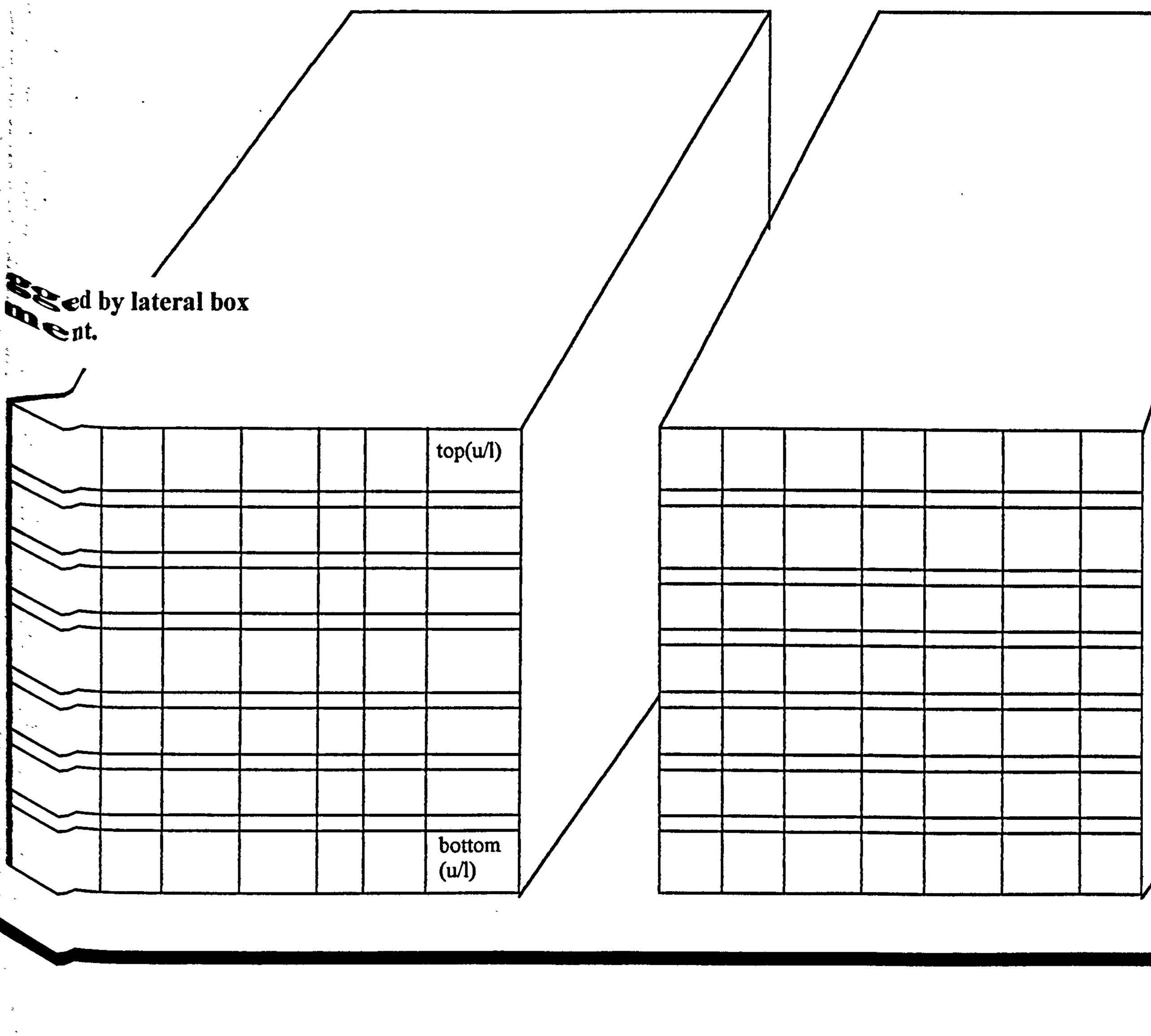


Fig. 3.7 The layout of Conventional store, store' B'. Held at 8-10 ^{0}C. Sampling points are represented in the stack.

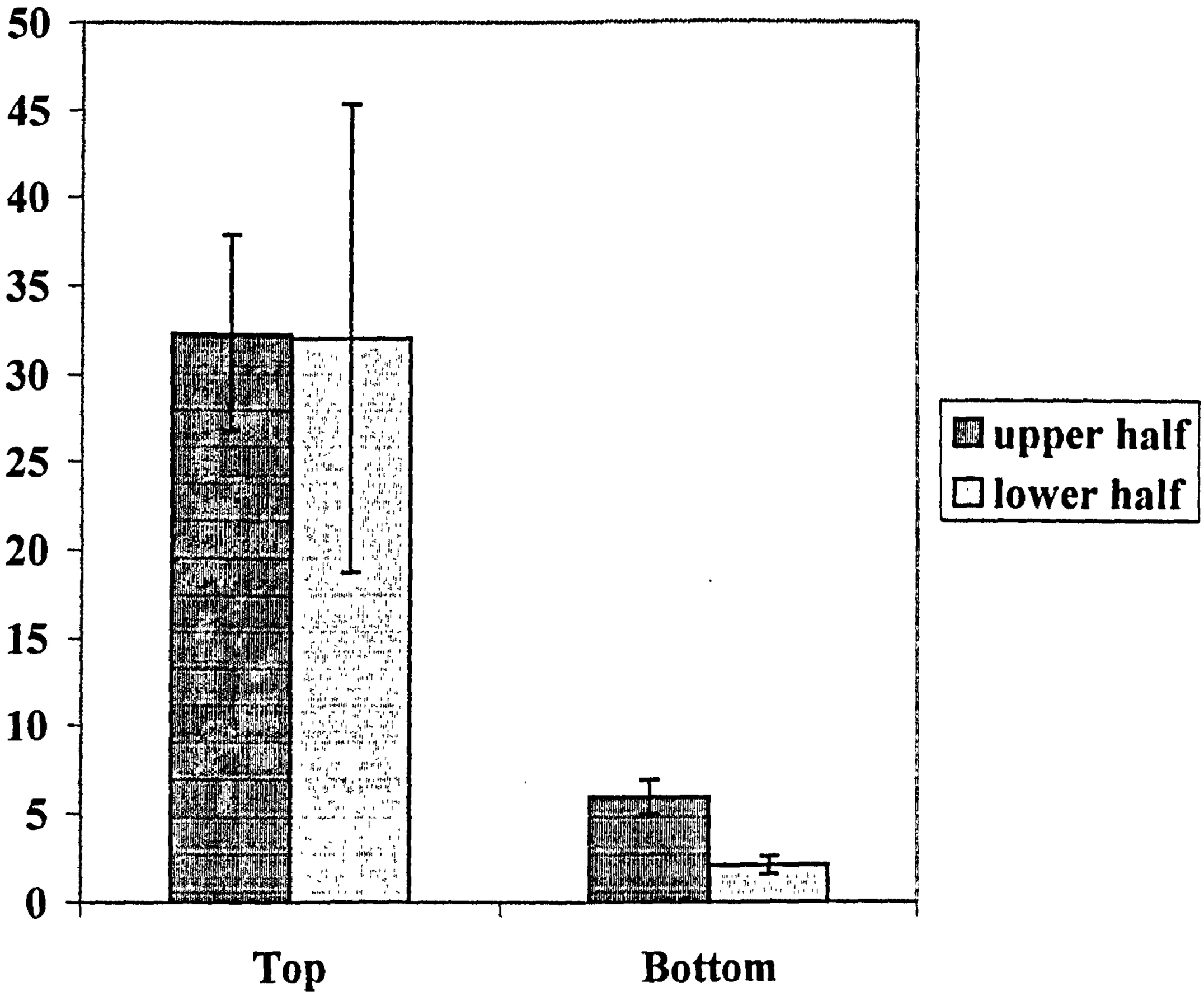
Table 3.3 Chlorpropham residues (mg/kg) in conventional store 'B' (8-10 ^{0}C).

	Position of tuber in the box	
	Surface tubers (halves)	
Box height	upper	lower
Unwashed		
Top	32.33±5.51	32.07±13.30
Bottom	5.94±0.95	2.11±0.50
Washed		
Top	39.76±10.01	26.43±6.42
Bottom	6.44±1.08	1.81±0.25

The unwashed samples in the stack were plotted as a graph (Fig. 3.8). The level of chlorpropham was highest on the surface tubers of the top height box of the store when compared to the bottom height box. It was 32.33 and 32.07 mg/kg in the top boxes and , was least in the bottom box with the level of 5.94 and 2.11mg/kg in their respective upper and lower halves of the tubers. The reasons for the higher level of the chlorpropham on the top box could be the falling down of the fog directly on the surface of the tubers, while the bottom box remained comparatively unexposed to direct contact with the falling fog . Although top and bottom boxes had different levels of chlorpropham like the cold store, this difference in trend was not the same as it was in the cold store. Here the difference in the top box to bottom box was almost 7:1, but it was much greater in the cold store (16:1). Surprisingly the value in the lower portion of the top box in the cold store was the same as it was here in the conventional store.

Therefore the main difference would be the heavy deposit of chlorpropham on the surface tubers of the top box in the cold store. Here as the chlorpropham was applied through lateral ducts therefore the top box, surface tubers on both sides of the tubers have the same level of chlorpropham. So the difference between cold and conventional store would be in total amount of chlorpropham on the surface of the top tubers on the upper half. By considering the washing it is clear that chlorpropham acts differently in attachment with the tuber components. In this conventional store the chlorpropham was stuck more firmly to the tubers.

Fig. 3.8 The chlorpropham residues in unwashed tubers of conventional store'B' (8-10 ^{0}C).



As the tubers are living organisms therefore the temperature and other conditions can effect on its physiology. The higher temperature in the conventional store increases the respiration/evaporation rate of tubers and it also increases the relative humidity in the store (Potato Marketing Board, 1996). Therefore CIPC in the vapour phase could have different behaviour at different temperatures. During the low temperature storage respiration rate could be reduced to a minimum level.

Unlike the cold stores the level of the chlorpropham was almost same in upper and lower halves of the tubers in both boxes of the store. Overall the range of the chlorpropham in this store was from 2.11-32 mg/kg, it was much lower as it was in the cold store.

3.2.4.2.2 Washing.

The graph plotted for washed and unwashed samples is represented in the Fig. 3.9. it is clear that washing remains completely ineffective on both height samples and the sides of the tubers. Unlike the cold store where a big amount of the chlorpropham was removed at least from the top surface of the top height box, it was completely different behaviour in this store after washing. Although there was a great amount of the chlorpropham present on the top surfaces of tubers, this remained almost the same after washing of these tubers.

The washing was ineffective on lower height tubers as it was noticed in the cold store. The range of chlorpropham after washing was almost same as it was in unwashed samples (range of washed tubers 1.81-39.76 mg/kg, Table 3.3, Fig. 3.9).

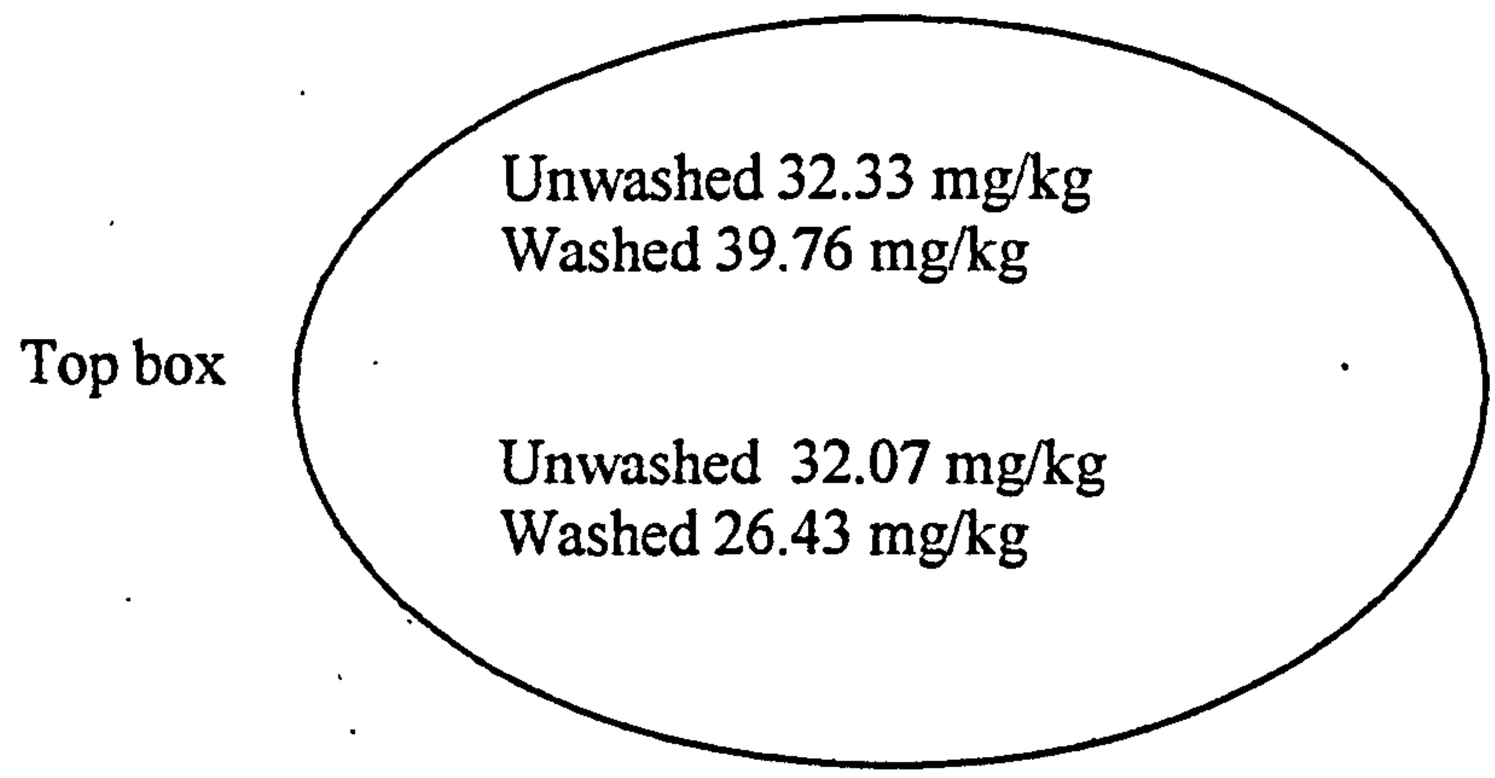


Fig. 3.9. Chlorpropham residues in conventional store, store 'B'. on its top surface tubers of top box.

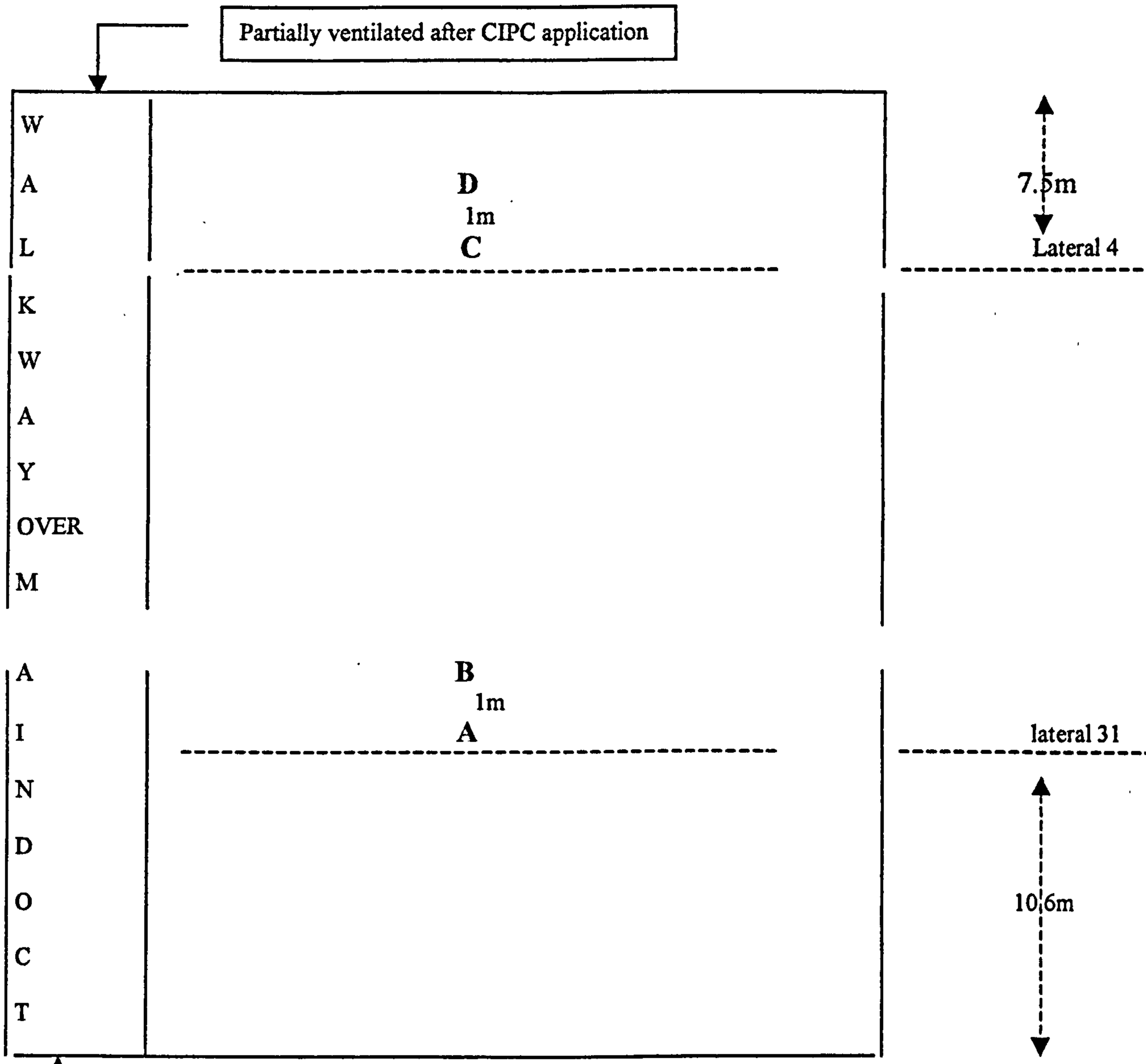
3.2.5 Store C (bulk store at 8-10 ^{0}C).

The bulk store, McCain Whittlesey Store number 1, used commercially for potato storage, was sampled on December 19th , 1995. The store was maintained at 8-10 ^{0}C for long time storage. The store consists of 35 lateral ducts, the 33-35 lateral ducts were not covered with potatoes to the full height. The chlorpropham is usually applied into the main duct at the front of the store, but under adverse (wind) conditions it may be applied at the back Fig. 3.10.

All formulations were 60% chlorpropham or 600g/L.(w/v) in methanol, Warefog (MS).The diagram of the store is shown in Fig. 3.10.The chlorpropham is usually introduced as shown from the one end of the main duct, but under unusual weather conditions (wind) it may be introduced at the opposite end of the main duct. A rot problem developed in this store, and the back end (including the area around lateral 4) was selectively ventilated. The chlorpropham comes from the main duct to the lateral ducts of the store.

3.2.5.1 Sampling.

The potato samples were taken on 19/12/95 roughly eight weeks after application of chlorpropham in the store. The tubers were sampled from four sites (A-D) in the store Fig. 3.10. The two sites, A and C, were directly over laterals 31 and 4 respectively. While the sites B and D, were 1 metre towards the back of the store, from areas A and C sites respectively. From each of the selected sites, the individual tubers were obtained from a narrow band of length 1 meter half way across the store (Fig. 3.10). This was not an attempt to show the effect of distance along lateral duct on the chlorpropham residues, but an attempt for the sampling of



A B C D are the sampling points.

Fig. 3.10. Store 'C', McCain Bulk store, Held at 8-10 ^{0}C.

one tuber not to affect the result of the next. At each location (A-D) five tubers were obtained from the surface of the bulk store and cut in half along the horizontal axis of the tuber as it lay. Halves were placed separately in pre-labelled polythene bags and stored in a deep freezer for further analysis. The five individual potatoes were analysed separately for their upper and lower portions.

3.2.5.2 Results and Discussion.

3.2.5.2.1 Unwashed samples.

A graph is plotted for the average values of chlorpropham for its upper and lower portions on the four sites of the store (Fig. 3.11). The mean range of chlorpropham on upper and lower half portions of the unwashed tubers of four sampling sites in the store was 14.38-25.16 mg/kg and 6.74-11.32 mg/kg respectively (Table 3.4).

Overall the store can be divided into two symmetrical parts, the site A, B and site C, D, with respect to the level of chlorpropham distribution. The site A, B contains higher levels of chlorpropham as compared to the site C, D. The site C, D was almost half of the average amount as compared to the site A, B. However by taking the average values (upper and lower halves) of each site showed non-significant difference from one to other site of the store.

From the Fig 3.11, it is clear that the upper portion of the tubers is higher in amount of chlorpropham from the lower portion of the tubers. It was 25.76 and 28.96, 17.4 and 14.38 mg/kg in upper half portion of the sites A, B, C and D compared to 11.32, 10.53, 5.74 and 6.74 mg/kg in respective site of lower halves

Fig. 3.11 The chlorpropham residues on four sites of bulk store'C'. (8-10 ^{0}C).

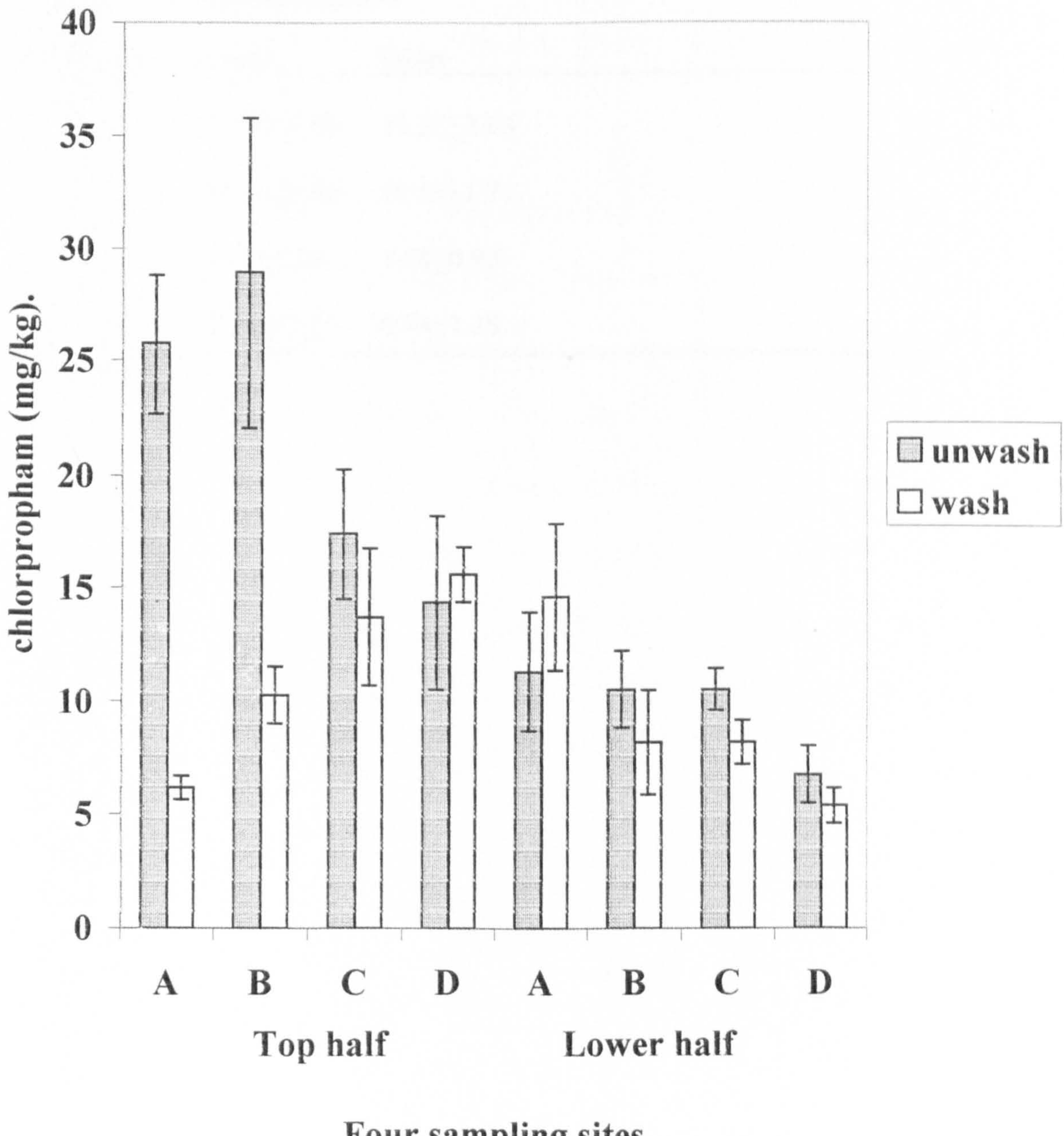


Table 3.4 Chlorpropham residues (mg/kg) in unwashed tubers of bulk store 'C' (8-10 ^{0}C).

	surface (halves)	
Site	upper	lower
A	25.76±3.06	11.32±2.63
B	28.96±6.86	10.53±1.71
C	17.4±2.88	5.74±0.92
D	14.38±3.83	6.74±1.28

(Table 3.4). The site A, B was almost double the amount on the upper halves to the respective lower sides, and similarly it was the same trend in upper and lower sides of site C, D. By comparing the two sides of tubers on different sites, it was noticed that the upper portions were not significantly different in chlorpropham level from one site to the other, while the lower sides were significantly different to each other site in the store.

The total chlorpropham in unwashed tubers, upper and lower portions, are represented in the Fig. 3.12, which shows that overall the both sides have much different level of chlorpropham. The lower halves contain nearly half of total the amount present in the upper halves of the tubers. This again explained the way of fog settling. The aerosol fog could directly fall on the exposed surface, upper halves. The trend of chlorpropham was again different as compared to the other two stores. Here the surface tubers have not got a heavy deposit layer of chlorpropham as it was in cold store, and both sides of the tubers were nearly 1:0.5 ratio which was 1:1 in the conventional stores.

As no sampling could be taken from various depth of the store, therefore the over all chlorpropham level in the store could not be explained. But by keeping points from previous stores it would be explained again by considering the application way of chlorpropham in the stores. Here the fog was applied through the main ducts, underneath the bulk of tubers, which comes up by passing through the layers of tubers and settling down on the top of the store. Therefore the lower level height of the store, near to the main ducts, and the top most layers would

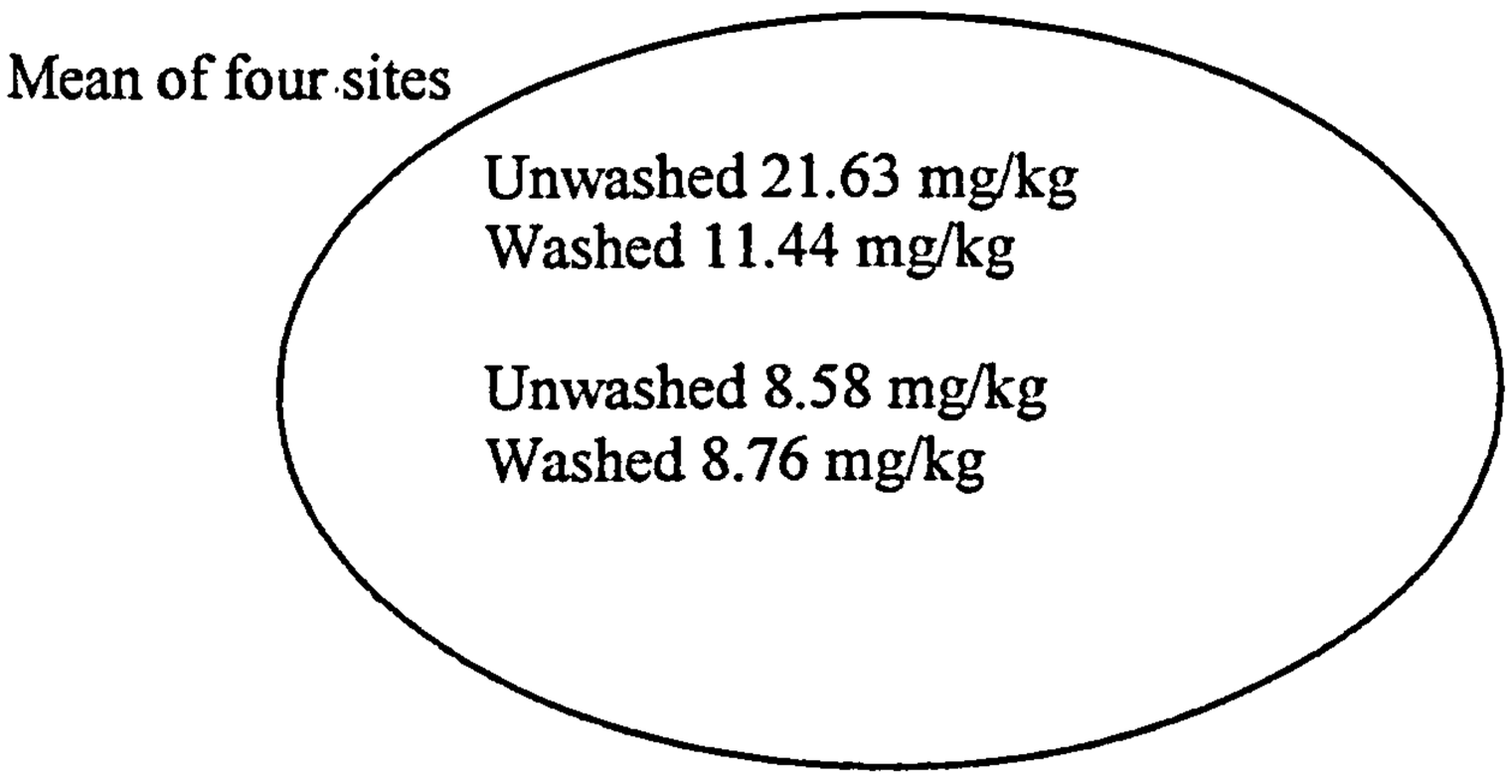


Fig 3.12 Chlorpropham residues in Bulk store 'C'. Mean of four sites of surface top tubers in the store.

have higher level of chlorpropham compare with middle height layers of the store. It is also expected that the average value of lower half side of the top most tubers could be equal to the overall average level of the whole store.

3.2.5.2.2 Washing.

It is clear from Fig. 3.11 that washing reduces the chlorpropham on certain areas, while it remains completely ineffective on other sites of the store. The greater amount is washed off only from the site A, B on the upper portions of the tubers, while although there was some reduction in the lower halves of the same sites but this reduction was statistically nonsignificant.

The washing behaviour among the upper portions of the tubers can be divided into two sections, the site A, B and site C, D. There is much reduction on the site A and B i.e. from 25.76, 28.96 to 14.62, 8.17 mg/kg respectively, while this pattern was not followed on the site C, D on their upper halves, which was 17.4, 14.38 mg/kg in unwashed that became 13.72 and 15.6mg/kg after washing respectively. It can be seen that the effect of washing decreases with respect to the original amount present on the unwashed tubers (Table 3.5).

The effect of washing towards the reduction of the chlorpropham amount was totally ineffective on the lower halves of the tubers nearly in all sites of the store, except for a little reduction in site A, which was however non-significant statistically. The interesting trend among the washed values can be seen, that there can be a limited amount of the chlorpropham up to certain higher values were removed, but when the amount becomes lower below certain limits then it can not be washed and remains stuck to the tuber component. This trend can be due to the

Table 3.5 Chlorpropham residues (mg/kg) in washed tubers of bulk store 'C' (8-10 ^{0}C).

	Surface tubers (halves)	
Site	upper	lower
A	6.16±0.52	14.62±3.25
B	10.29±1.27	8.17±2.33
C	13.72±3.01	6.89±0.99
D	15.6±1.20	5.36±0.77

behaviour of particle sizes of the fog applied and also can suggest the binding of the chlorpropham with the tuber components.

The total mean of the four sites of the upper and lower halves are shown in the Fig. 3.12. It is clearly seen that the overall chlorpropham was reduced by washing in the upper halves of the tubers. The reduction was nearly 50%, from 21.63 mg/kg to 11.44 mg/kg. Even after this higher reduction the level in the tubers was still higher than the MRL of the European Commission (5 ppm). These tubers were also supposed to be consumed individually, therefore high levels in any individual parts can be a risk for health safety. Within the lower halves the washing was totally ineffective. The unwashed tubers contain 8.58 mg/kg, which was 8.76 mg/kg after washing. The slightly higher level in the washed samples does not mean any increase in the level, but could be result of the variation in individual tubers.

Here it shows that chlorpropham is tightly stuck with the potato components and this trend of chlorpropham could be unsafe for consumption. It is also clear that taking of average value chlorpropham in bulk of tubers would not be a total representation of chlorpropham level. The studying of the individual tubers and their section can provide enough detail and true picture of chlorpropham level and behaviour of its attachment to the tubers. Here for health safety it would be necessary to consider the position of tubers in the stores and the conditions during the storing period.

3.2.6 Store D .

The store 'Colsterworth' was chosen for the study of chlorpropham distribution in the boxes of the store. The potatoes were stored at 8-10 ^{0}C. It was a modern box store, designed for long term storage of potatoes with refrigerator facilities and environment control units closely monitoring humidity and temperature.

The potatoes were stored in wooden 0.5 tonne boxes. The sampling was carried out on 10/1/96 after three applications of aerosol fog of chlorpropham. All formulations were 60% chlorpropham in methanol, Warefog (MS).

3.2.6.1 Sampling.

A plan of the store is shown in the diagram Fig. 3.13, with the boxes where the tubers samples were taken marked with an asterisk. The samples were taken at different positions i.e. top, middle and bottom boxes. All these tubers were taken from the top surface of the box in the stack. However from the top box, besides a sample from the top surfaces of the box , central potatoes, at a depth of a few potatoes, were taken as well for chlorpropham analysis.

Five tubers were taken from each position of the store. Each potato taken was cut into two halves horizontally as they lay on the surface of the box, immediately after the sampling, into upper and lower portions. Each half of the tuber was then placed in pre-labelled polythene bags, at – 18 ^{0}C in a deep freeze.

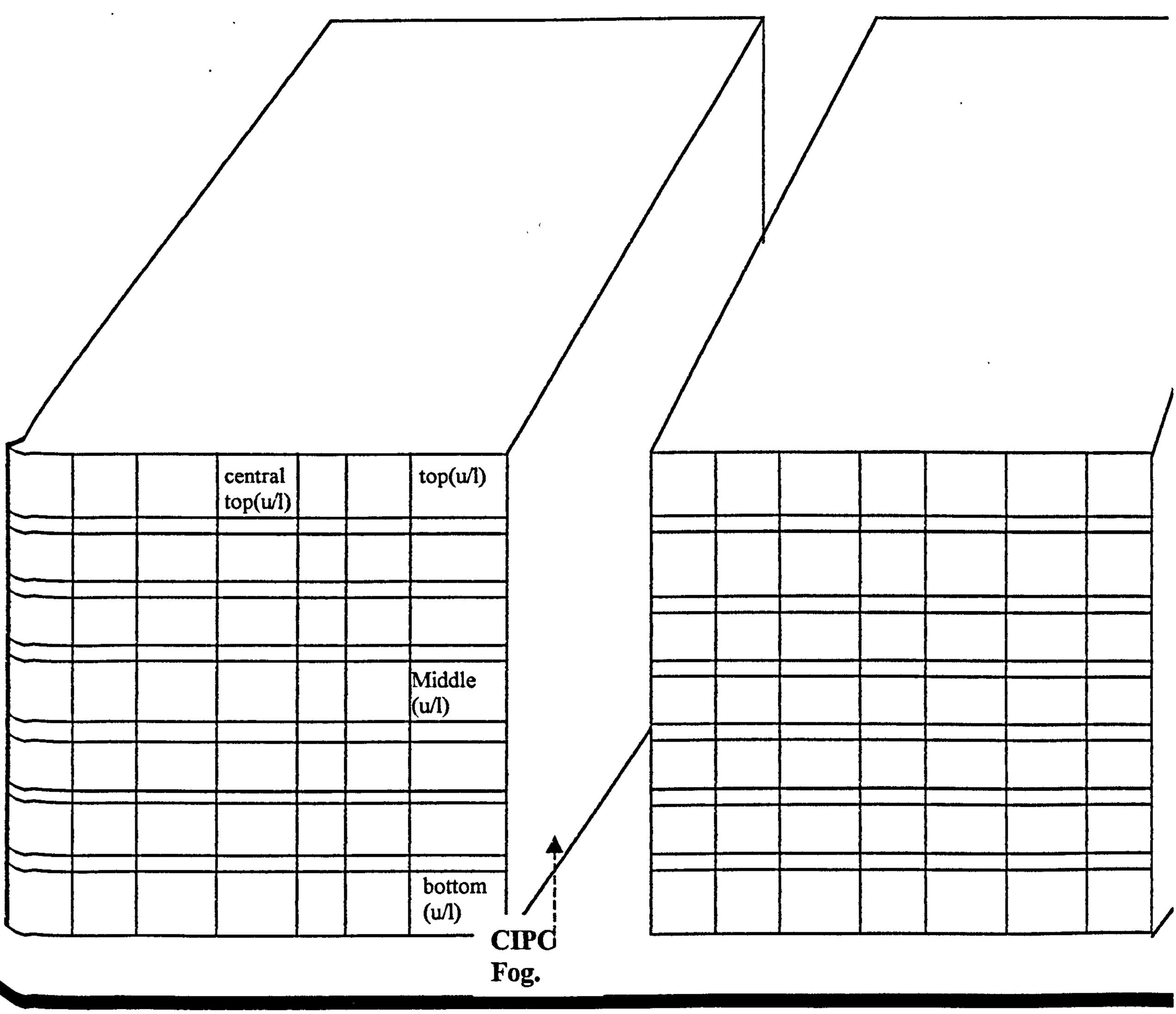


Fig. 3.13 The layout of Colsterworth store, store 'D'. Held at 8-10 ^{0}C. Sampling point are represented in the stack.

3.2.6.2 Results and Discussion.

It is clear form Table 3.6 and Fig. 3.14, that chlorpropham varied within the various parts of the store. The chlorpropham continuously reduced in decreasing trend from top height to the lower height of the store. This trend of decreasing concentration was on both sides of the tubers. The highest level of the chlorpropham 49.42 mg/kg was found on the upper portions of the top height box , while the least was 10.74 mg/kg on the lower portion of the bottom box height of the store. Overall the bottom and middle heights were almost half of the amount present on the top height of the boxes in this store.

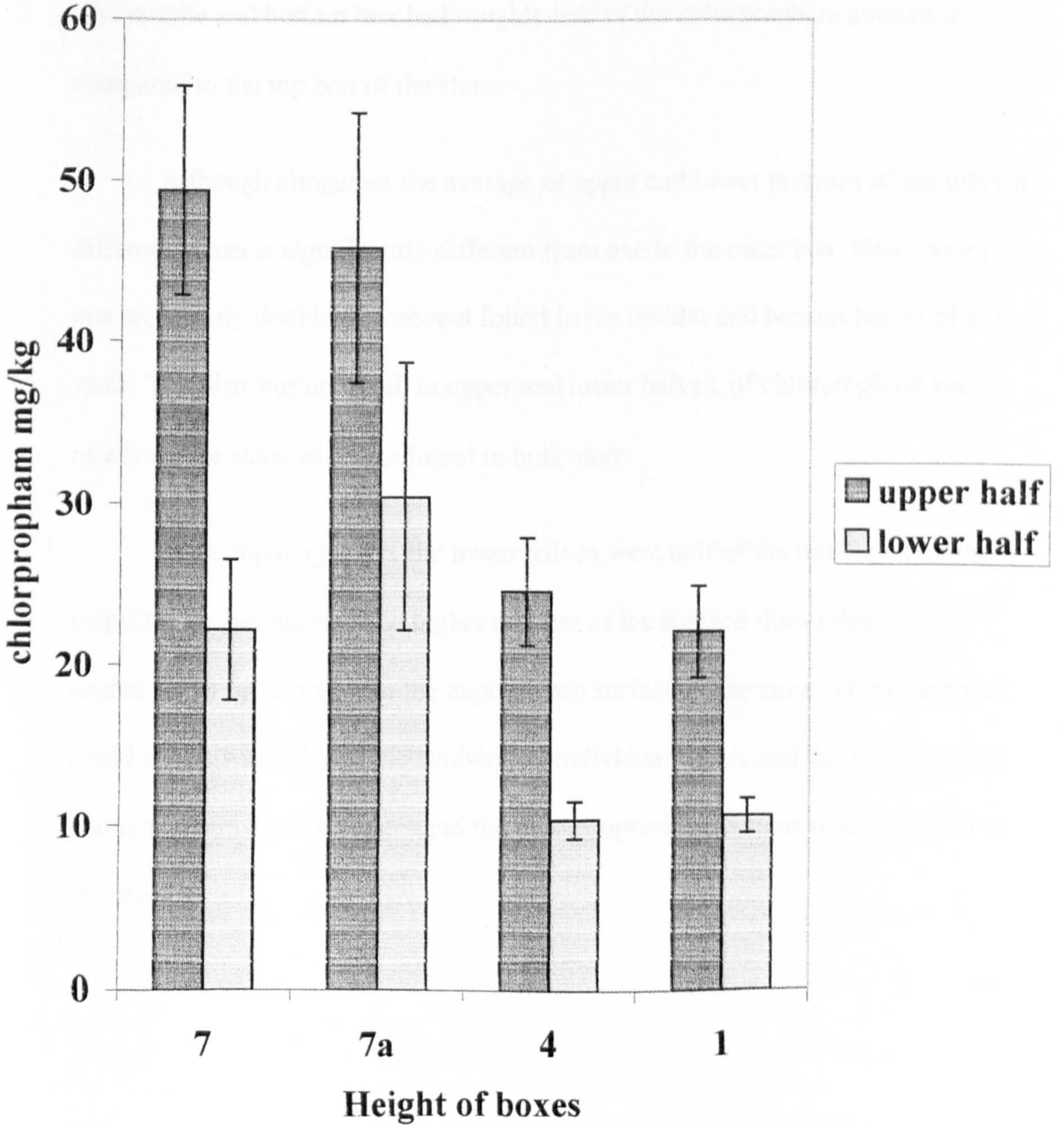
The distribution of the chlorpropham also showed that the top two height boxes of the store, top and central top were almost equal, i.e. 49.42 and 45.92mg/kg respectively, while the middle height and lower/bottom height box were having equal amounts of the chlorpropham i.e. 24.60 and 22.12mg/kg respectively in their respective upper portions of the tubers. A similar trend was also achieved from their lower half sides of the tubers. It was 22.22, 30.47 mg/kg and 10.41 and 10.74 mg/kg in the top two boxes and lower two boxes respectively (Table 3.6).

Comparing the chlorpropham distribution behaviour with respect to upper portions to the lower portions of the store, it became clear that on each height the upper halves contained higher almost double the amount of the chlorpropham compared with their respective lower halves of respective height of the store. However this difference between upper and lower portions was not statistically significant, due to greater stdev. in the upper portions of the tubers, except only the

Table 3.6 Chlorpropham residues (mg/kg) in unwashed tubers of box store, Colsterworth 'D' (8-10 ^{0}C).

	surface tubers (halves)	
Box height	upper	lower
Top	49.42±6.54	22.22±4.35
Central top	45.92±8.40	30.447±8.26
Middle	24.60±3.30	10.41±1.13
Bottom	22.12±2.80	10.74±1.05

Fig. 3.14 The chlorpropham residues of unwashed tubers in store' D' (8-10 ^{0}C).



top box. The average of the upper and lower portions of the different sites of the store showed that overall the chlorpropham varied from top of the store to the bottom of the store. However the middle and bottom boxes apparently contained the same amount of the chlorpropham while the top box surface potatoes and the below surfaces potatoes of top box was similar in the amount of chlorpropham. The middle and bottom box had roughly half of the chlorpropham amount as compared to the top box of the store.

Although altogether the average of upper and lower portions of the tuber in different boxes is significantly different from one to the other box. Here the top box was nearly double the amount found in the middle and bottom height of the stack. The distribution trend, in upper and lower halves, of chlorpropham was relatively the same as it was found in bulk store.

In the top height box the lower halves were half of the total level on their respective upper halves. The higher amount of the surface shows that fog was settled down by falling onto the exposed top surface of the store. These samples could not be washed, and the studying of individual tubers and their halves gave enough information to understand the chlorpropham behaviour of distribution in this store.

3.2.7 Summary

The amount of chlorpropham was found to be significantly different among the different commercial stores. Each store has its own distribution pattern with some similarities. In each store the level of chlorpropham varied with respect to sites of the stores. Maximum chlorpropham was on top height samples of the stores and it was minimum in lower height of respective stores.

The maximum amount in the sense of the total is recovered from the cold store, where it seems that hardly any chlorpropham does not go out to the target

and do not evaporate. On other hand the maximum amount of the chlorpropham was in the cold store which shows its efficiency of take-up by the tubers. Inspite of cold stores other stores have nearly similar amounts of chlorpropham. Their lower halves are definitely different from their upper halves, which can lead to the idea of how the chlorpropham contacted the tubers.

All four examined stores were compared to their upper and lower halves from their top and bottom height in the store (Table. 3.7). By studying the upper and lower halves of the tubers, it became clear that they have an important role in representing the pattern of the chlorpropham distribution within the store. The amount of the chlorpropham was significantly lower in the lower halves of tubers with respect to their upper halves. In case of conventional stores the amount was equally distributed in the tubers on both sides of the tuber (upper and lower half).

Table 3.7 Chlorpropham residues (mg/kg) in unwashed tubers of different commercially treated stores.

	Surface tubers (halves)	
Store	upper	lower
Top height		
A	236.56	30.96
B	32.33	32.07
C	21.60	8.58
D	49.42	22.22
Bottom		
A	9.21	7.63
B	5.94	2.11
C	–	–
D	22.12	10.74

Where. A(cold store), B(conventional store), C(bulk store), D(coulworth store).

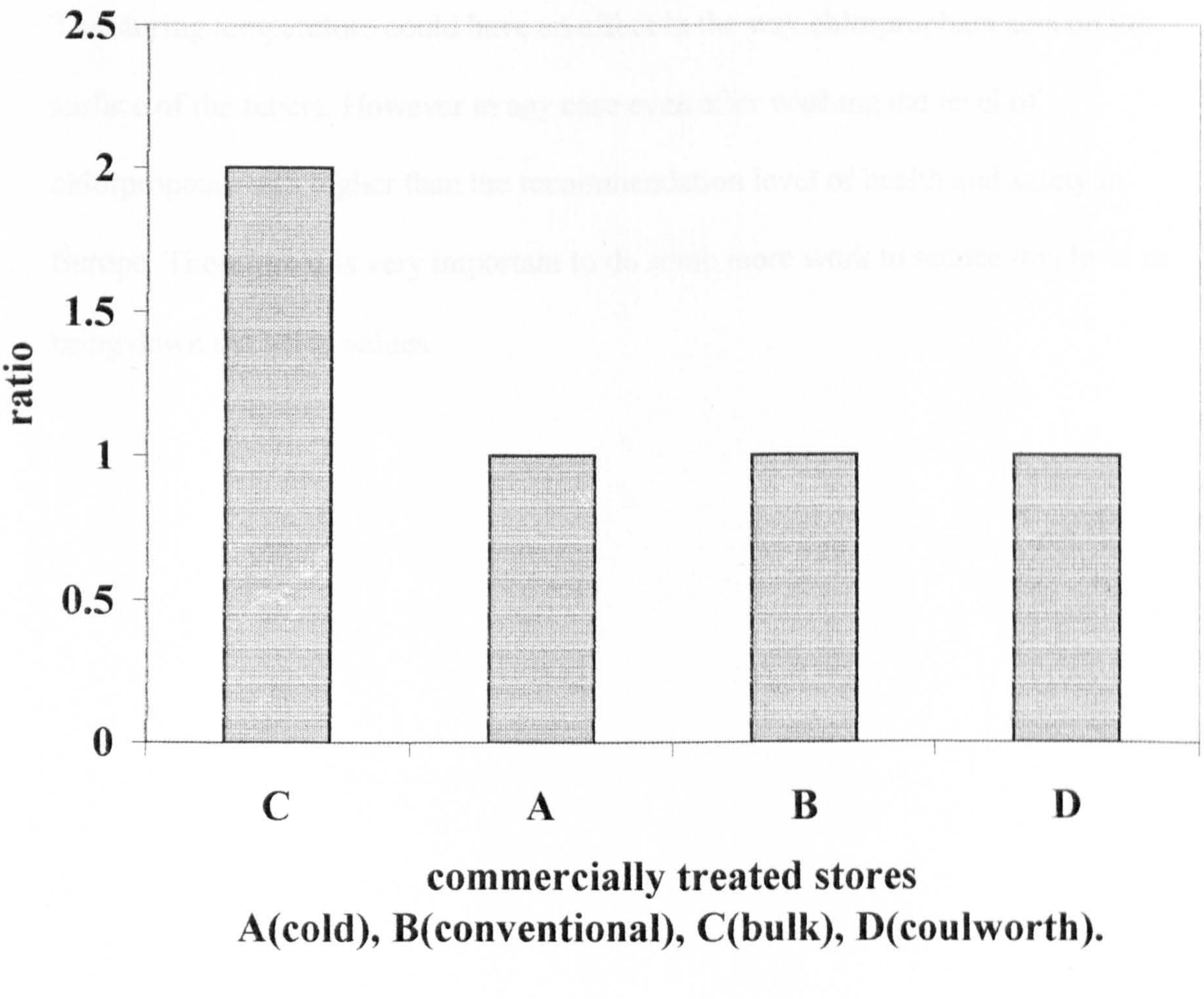
It was later found that the chlorpropham was applied by lateral arrangement in the store.

It can also be seen that although the upper halves of the top most surface of the tuber contained much different amounts from each other in different stores the lower halves contained relatively equal amounts of the chlorpropham in all stores. This shows that there is a significant advantage to divide the tuber into two halves, the lower portion can lead to the same conclusive points for the predicting of the chemical in the stores. The ratio of lower half portion of top layer tubers to mean of the store was found 1:1 in most examined stores except from bulk (store 'C) (Fig.3.15). Therefore it can be concluded that the average amount of the chlorpropham in the store could be easily determined by knowing chlorpropham level on lower halves portions of the top surface tubers of the selected store.

The determination of chlorpropham level in upper and lower half would definitely be better than taking the average values of 6-10 tubers, as was common practice in past. The upper and lower halves also show how the movement of the chlorpropham in the store has taken place and how the chemical could make contact with the tubers during the fogging of the chlorpropham (Fig 3.15). There was no significant difference in their upper and lower halves from the tubers sampled in the lower height boxes of the stores

The washing overall remained ineffective in removing significant amounts of chlorpropham in all stores. The only significant amount of chlorpropham was washed from the tubers on the top box top surface of the tubers in cold store and bulk store. The ratio of washed to unwashed samples of all examined stores

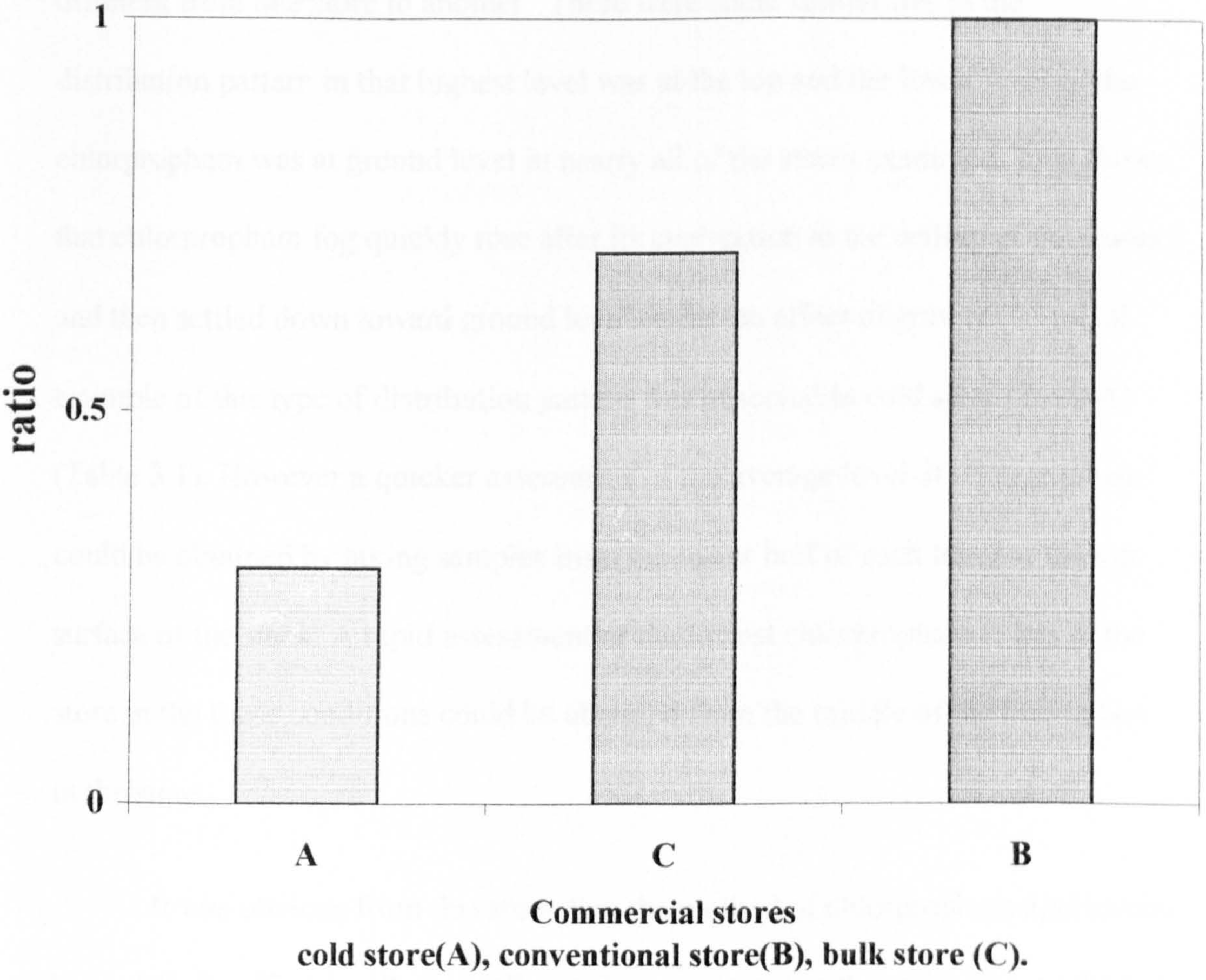
Fig. 3.15 Ratio of lower surface of top tuber to mean of stores (unwashed).



showed (Fig. 3.16) that only the highest level of chlorpropham reduction by washing was in cold store whereas the level of chlorpropham in bulk (Store 'C') and conventional store (Store 'B') remained totally unaffected. These results agreed with Wilson et al., (1981).

In the cold store the chlorpropham was loosely attached to the surface where as in the rest of the stores (8-10 ^{0}C) it was attached firmly to the tubers. It might be the difference of temperature between these stores during the storage. The storing temperature could have an effect in the way chlorpropham acts on the surface of the tubers. However in any case even after washing the level of chlorpropham was higher than the recommendation level of health and safety in Europe. Therefore it is very important to do some more work to reduce this level to bring down the MRL values.

Fig. 3.16 Ratio of mean of washed to unwashed in different commercial stores.



3.2.8 Conclusions.

It is concluded after intensive study of chlorpropham distribution behaviour in commercial potato stores, that:

The distribution of chlorpropham was not uniform in any of the stores examined. The store design and storage conditions were obviously different in each store, therefore the pattern of chlorpropham distribution was significantly different from one store to another. There were some similarities in the distribution pattern in that highest level was at the top and the lower level of the chlorpropham was at ground level in nearly all of the stores examined. This shows that chlorpropham fog quickly rose after its application to the ceiling of the stores, and then settled down toward ground level under the effect of gravity. A typical example of this type of distribution pattern was observed in cold store (Store A) (Table 3.1). However a quicker assessment of the average level of chlorpropham could be obtained by taking samples from the lower half of each tuber at the top surface of the stack. A rapid assessment of the lowest chlorpropham levels in the store in the these conditions could be obtained from the middle of the lowest box in the store.

It was obvious from this study that the method of chlorpropham application has a definite effects on the overall distribution pattern in the store. The method of aerosol fog application should be designed individually with respect to the store design and the storage conditions. The detailed procedure for chlorpropham application and the conditions in the store, i.e. temperature, humidity and ventilation, would play an important role in optimising chlorpropham distribution

in commercial stores. It would be helpful to apply the aerosol fog through lateral ducts with internal air circulation in all box stores, or at least in cold stores. The application of chlorpropham fog from different directions and heights in the store would also play an important part in attaining uniform distribution of chlorpropham.

It was also noticed that level of the chlorpropham on the tubers was much higher than the MRL in most of the commercial stores. The amount of chlorpropham in the cold store (Store A) was nearly 50 times greater in unwashed tubers than the MRL in Europe and hardly any chlorpropham was lost during the storage period. As on most occasions the chlorpropham was firmly stuck to the tubers, washing could not bring down the level of the CIPC below the MRL. On some occasions in the cold store, visibly obvious layers of chlorpropham were attached. These were the only sites at which the residue level was reduced by washing. If this huge amount is washed off then economic and environmental issues are badly affected. On the other hand if it remained stuck to the tubers then health risks to consumers could not be avoided. In either case this trend is not positive. For further study it becomes vital to find out the effects of this higher amount of the chlorpropham on soil, air and water and their relevant living organisms.

It is matter of great importance how to deal with the tubers having such a high level of chlorpropham. The best possibility would be always washing, possibly hot water washing, before eating. However further study is needed to know the level of CIPC reduction, which could be up to 80% with some possible washing procedures.

The work carried out in this section revealed that the chlorpropham distribution is different in different kinds of store and temperatures, therefore further work needs to the carried out to optimise the distribution of chlorpropham in the stores. The particle size of the aerosol fog, and the temperature in the stores are the two common factors which could effect the chlorpropham distribution. The next section therefore is focused on the effect of these factors have on the distribution of chlorpropham in model stores.

As there were much higher chlorpropham levels on the tubers in the cold store, this indicated that the uptake of the chlorpropham was efficient. Therefore the objective of further work in the cold store is to minimise the amount of the chlorpropham on the tubers, which should be enough to control sprouting but not in excess of the MRL.

Section 2.

3.3 OPTIMISING CHLORPROPHAM DISTRIBUTION IN COLD STORES.

3.3.1 Introduction.

After studying the commercially treated cold store it was found that the chlorpropham was much higher in amount on the tubers throughout the store. The value of chlorpropham was much higher specially on the top surface tubers of the store, which highly exceeded than the MRL of the chlorpropham in Europe. It was also realised that nearly all of the chlorpropham applied in the store remained there at the end of the storage season. Almost all of the chlorpropham applied was found on the tubers, therefore uptake of chlorpropham by the tubers was appreciably higher than other high temperature stores.

In the light of the previous study it was suggested to optimise the level of chlorpropham in the cold stores by reducing the application dose of chemical. Therefore in the following stores chlorpropham as an aerosol fog was applied only once in a storage season instead of 3-5 times application as was common practice in past.

The second parameter in these cold stores was the comparison of two kinds of stores. One with the internal air circulation and other without any circulation during the application of aerosol fog of chlorpropham, to know the effect on the distribution of chlorpropham in the commercial cold stores. Where there was internal cross ventilation the chlorpropham was the Luxan formulation(60% of chlorpropham in dichloromethanol) and Mirfield CIPC in the other stores. The

proposed fogging treatments at Branston could form the basis of a useful comparison of the distribution of the chlorpropham residues using alternative products and application techniques. The supply of fresh potatoes to consumers is in many ways more sensitive than that of processed products where most residues have been removed.

These stores were box stores. Each store (Stores 1-4) were 1100 tonnes capacity of storage with six box height. Each store has 2 fridge units, one on each was

outside the store walls. Stores 1 and 2 were ambient air mixture units, while the stores 3 and 4 had cross flow ambient cooling facilities.

It was suggested that these stores be used to compare the distribution of the chlorpropham following applications of Luxan Grostop in stores 1 and 2, and Mirfield chlorpropham formulation in stores 3 and 4. The ambient air-mixer was used to recalculate during fogging of Grostop in stores 1and 2, no ventilation was applied during fogging with Mirfield chlorpropham in stores 3 and 4. These stores were fogged only one time about 5 weeks post filling on the following dates.

Store 1, 2nd October, 1995.

Store 2, 4th November, 1995

Store 3, 15th December, 1995

Store 4, 1st December, 1995

Two other stores were sampled on December 1995. One of them was treated, like Grostop stores, and the other store was untreated at the time of sampling. These stores (3.5–4 ^{0}C) contain pre-pack potatoes of a mixture of varieties, Cara, Estima, Desiree and King Edward.

3.3.2 Sampling.

3.3.2.1 Grostop treated and untreated store.

These stores were sampled for analysis on December 6, 1995. Two stores were sampled at the same time one of them was treated as detailed above and the other was untreated at the time of sampling. Five tubers were taken from each position of the store. The potatoes taken from the top surface in the treated store were halved by cutting horizontally, as they were laid, into upper and lower portions of the tubers immediately after being taken. Each half was placed in pre-labelled polythene bags. The samples were then placed in the deep freeze for further analysis (-18 ^{0}C). All other tubers taken from other sites were whole tubers.

3.3.2.2 Grostop store1 and Mirfield store no. 3.

These two stores were sampled on1st April 1996. Samples were taken from surface of the boxes in four different positions of the store i.e. wall top, central top, wall middle and wall bottom. All these samples except the central top were taken from close to the wall. Central top were top samples in the centre of the store. Five potatoes tubers were taken from each position, each one of them was halved into upper and lower half portions immediately as they were laid in boxes. All the samples were kept in a deep freezer (- 18 ^{0}C) for further analysis.

3.3.2.3 Store. 2 Grostop and Mirfield store no. 4

These stores were sampled on 11th April 1996. The tubers were sampled from top box, middle box and bottom box of the store. The surface potatoes taken from the top box in the stores were halved into upper and lower half portions. While the samples which were taken from middle and bottom height boxes were whole tubers. All samples were kept in the deep freeze (- 18 ^{0}C) for further analysis.

3.3.3 Results and Discussion.

3.3.3.1 Treated and untreated store.

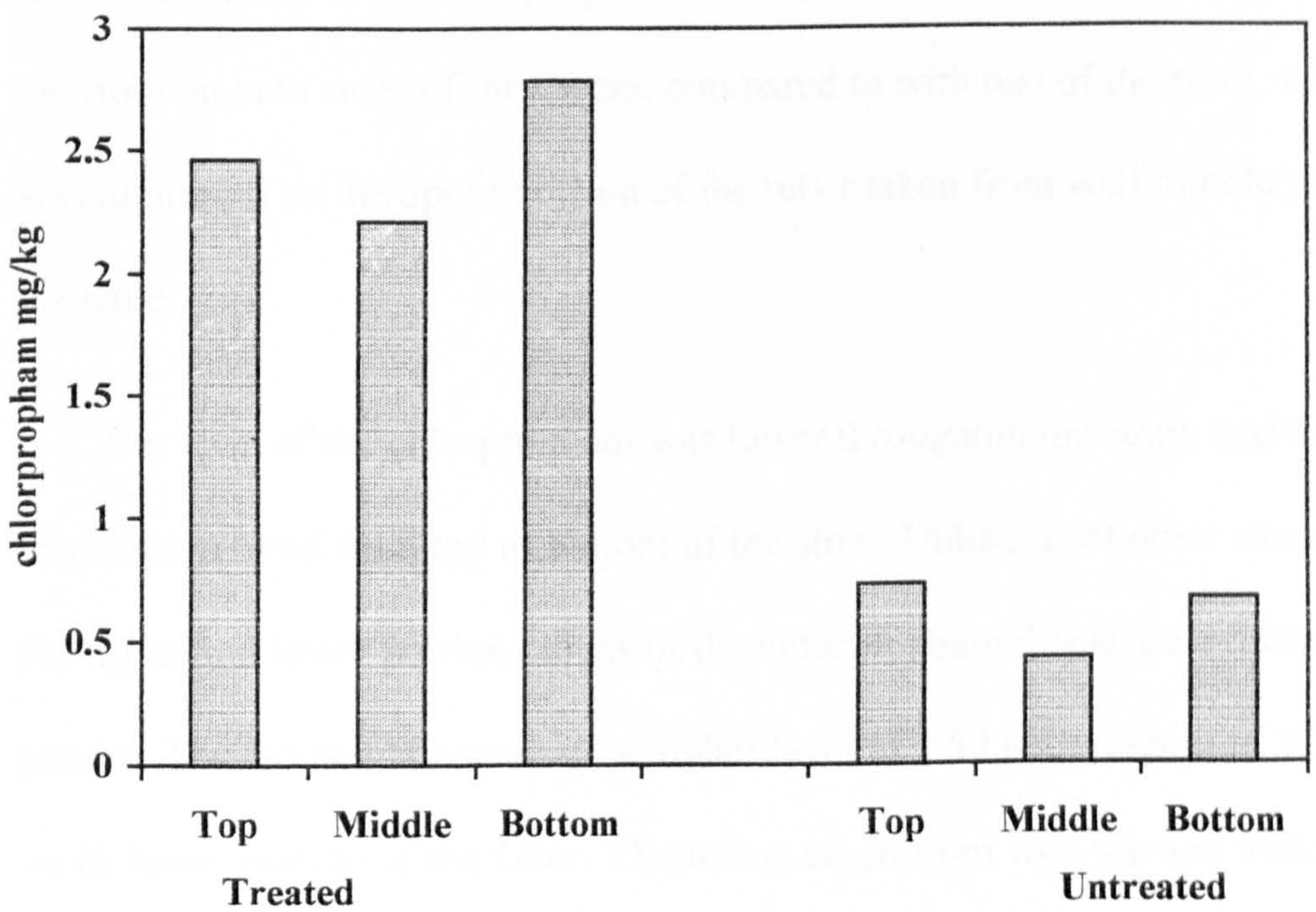
The distribution of the chlorpropham is presented in Table 3.8 and Fig. 3.17. The samples were taken from the three heights of the store, top, middle and bottom box. The level of the chlorpropham was much less as compared to the other cold store studied before, in section one. Here not only the level of chlorpropham was low throughout the store, but the distribution was relatively equal throughout the store, compared with the previous section cold store 'A'. The highest amount of the chlorpropham was 3.22 mg/kg in the store, which was on the upper portion of the top box tubers, while it was 236.56 mg/kg in the cold store 'A' of section one. The level of chlorpropham on the upper and lower portions of the top box were much different, the lower portion containing nearly half of the upper portion level, 3.22 and 1.70 mg/kg in upper and lower tuber sides respectively. The average value of chlorpropham was uniform throughout the store.

The store which was not treated at the same time of the sampling, was side by side with the treated store. Although it was an untreated store still it has found

Table 3.8 Chlorpropham residues (mg/kg) in unwashed tubers of Branston series of stores, sampled at 6th December 1995, (3-4 ^{0}C).

	surface tubers in box		
Box height	upper	lower	whole
Grostop			
Top	3.22±1.04	1.70±0.29	
Middle			2.21±0.34
Bottom			2.78±0.48
Untreated			
Top			0.73±0.14
Middle			0.43±0.16
Bottom			0.67±0.27

Fig.3.17. Chlorpropham distribution in Branston box stores (3-4 ^{0}C).



to contain some chlorpropham in the different positions in the store. The values were much less compared to the treated store, less than 1 mg/kg. This little amount of the chlorpropham was nearly equal throughout its different heights of the store, except a little less was found in the middle height of the store (Table 3.8). This shows how the fog can be transferred from one place to other. Therefore there are a greater chances that if the stores has been heavily fogged or been previously used for chlorpropham treatment then the distribution level of chlorpropham would be effected.

3.3.3.2 Grostop store1 and Mirfield store no. 3.

The chlorpropham values found in these stores are given in the form of Table 3.9. All tubers taken from the different heights were half tubers. In case of Grostop store 1, the level of the chlorpropham was maximum on the surface top tubers of the store on both sides of the tubers, compared to with rest of the store, while it was minimum on the upper portion of the tuber taken from wall middle position of the store.

The level of the chlorpropham was lower throughout the store, and there was a distribution trend from top to bottom of the store. Unlike most other stores, here the upper and lower portion values of the chlorpropham displayed a different pattern. The top height contained a higher level of 2.59 with respect to 2.01 mg/kg on its lower portion of the tuber. The tubers taken from wall top and middle top heights of the store have, almost double on the lower sides of the tubers as compared to the upper portions of the tubers. It was 0.45 and 0.27 mg/kg on the upper halves, and 0.67 and 0.69 mg/kg on the lower half portions of the tubers. It

Table 3.9 Chlorpropham residues (mg/kg) in unwashed tubers of Branston series of stores, sampled at Ist April, 1996 (3-4 ^{0}C).

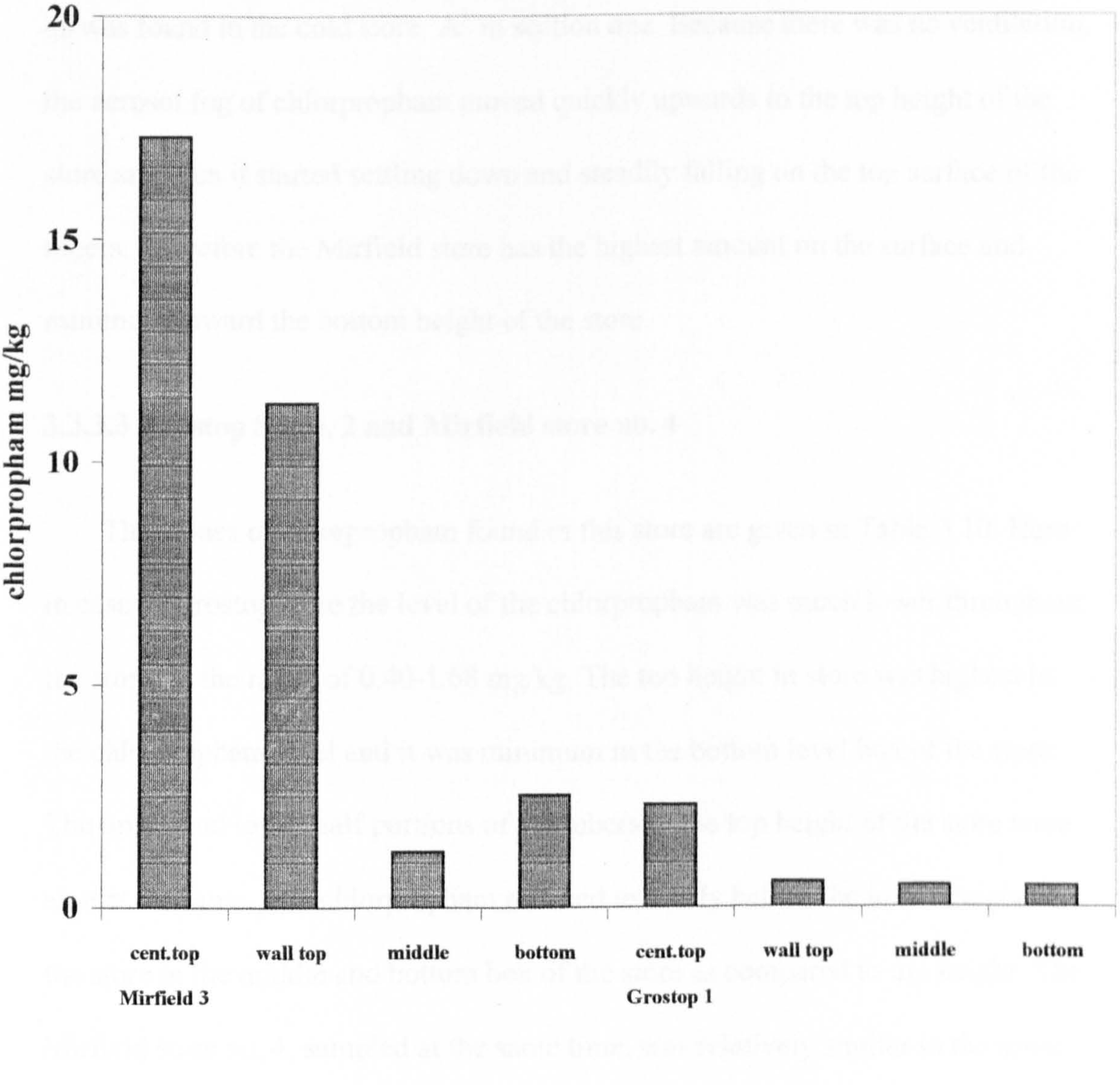
	Section of surface tubers in the box			
Box height	upper	lower	upper	lower
	Grostop		Mirfield	
Central top	2.59±0.35	2.01±0.33	31.74±5.04	2.87±0.38
Wall top	0.45±0.17	0.67±0.16	21.60±4.05	0.97±0.31
Wall middle	0.27±0.12	0.69±0.28	1.58±0.28	0.89±0.29
Wall bottom	0.58±0.12	0.37±0.17	3.46±1.07	1.58±0.39

was again low in the lower halves on the wall bottom height of the store compared to respective upper halves, it was 0.58 and 0.37 mg/kg on upper and lower portion of the tubers. In case of Mirfield store, which was sampled at the same time and same manner as was described in the above store, the distribution of the chlorpropham was different. Here the amount of the chlorpropham varied throughout the store. The over all range of chlorpropham in this store was 0.89 to 31.74 mg/kg. The top two heights, central top and wall top, contain highest amounts on their upper portions of the tubers, of 31.74 and 21.60 mg/kg compared to the rest of the store. The minimum chlorpropham was found in the wall middle height of the store on their both upper and lower half portions of tubers.

The levels of the chlorpropham in the upper portions of the tubers on all the sites were greater than with respect to their lower half portions of the tubers. The difference was much greater between central and wall top positions of the store. The chlorpropham level varied in great deal in the upper portions of the store throughout the store, while the lower half portion of the tubers contained almost the same amount of chlorpropham.

The mean values of these stores, at different heights in the store were compared and presented as a graph (Fig. 3.18). It is clear, that Mirfield store has much higher levels of chlorpropham in the top height, central top and wall top as compared to Grostop store 1. In this store the level was above 10 mg/kg while it was below 4 mg/kg in the Grostop store. In lower height such as wall middle and wall bottom, the levels were less as compared to top heights of these stores. In the case of Mirfield store, the level of chlorpropham was nearly double the middle values, and in the Grostop store these sites contained nearly equal amounts of

Fig. 3.18 Chlorpropham distribution in Branston box stores, (3-4 0C).



chlorpropham upto the bottom height of the store. Therefore it can be concluded that Grostop has much more uniformity of chlorpropham distribution as compared to Mirfield store. The more uniformity in the Grostop store is the result of internal air ventilation during fogging. In the case of Mirfield store where there was no internal air circulation, shows nearly the same pattern of chlorpropham distribution as was found in the cold store 'A' in section one. Because there was no ventilation, the aerosol fog of chlorpropham moved quickly upwards to the top height of the store and then it started settling down and steadily falling on the top surface of the tubers. Therefore the Mirfield store has the highest amount on the surface and minimum toward the bottom height of the store.

3.3.3.3 Grostop Store. 2 and Mirfield store no. 4

The values of chlorpropham found in this store are given in Table 3.10. Here in case of Grostop store the level of the chlorpropham was much lower throughout the store, in the range of 0.40-1.68 mg/kg. The top height in store was highest in the chlorpropham level and it was minimum in the bottom level box of the store. The upper and lower half portions of the tubers in the top height of the store were almost the same. The chlorpropham reduced to nearly half in the lower height of the store in the middle and bottom box of the store as compared to top height. The Mirfield store no. 4, sampled at the same time, was relatively similar in the sense of total level of chlorpropham as it was in the above store 2. Top height of the store contained the highest level of chlorpropham compared to the rest of the store. It was minimum in middle and bottom height box of the store. The upper and lower half portions of top box were much different unlike the Grostop store 2. The upper portion contained almost double the amount of the chlorpropham compared

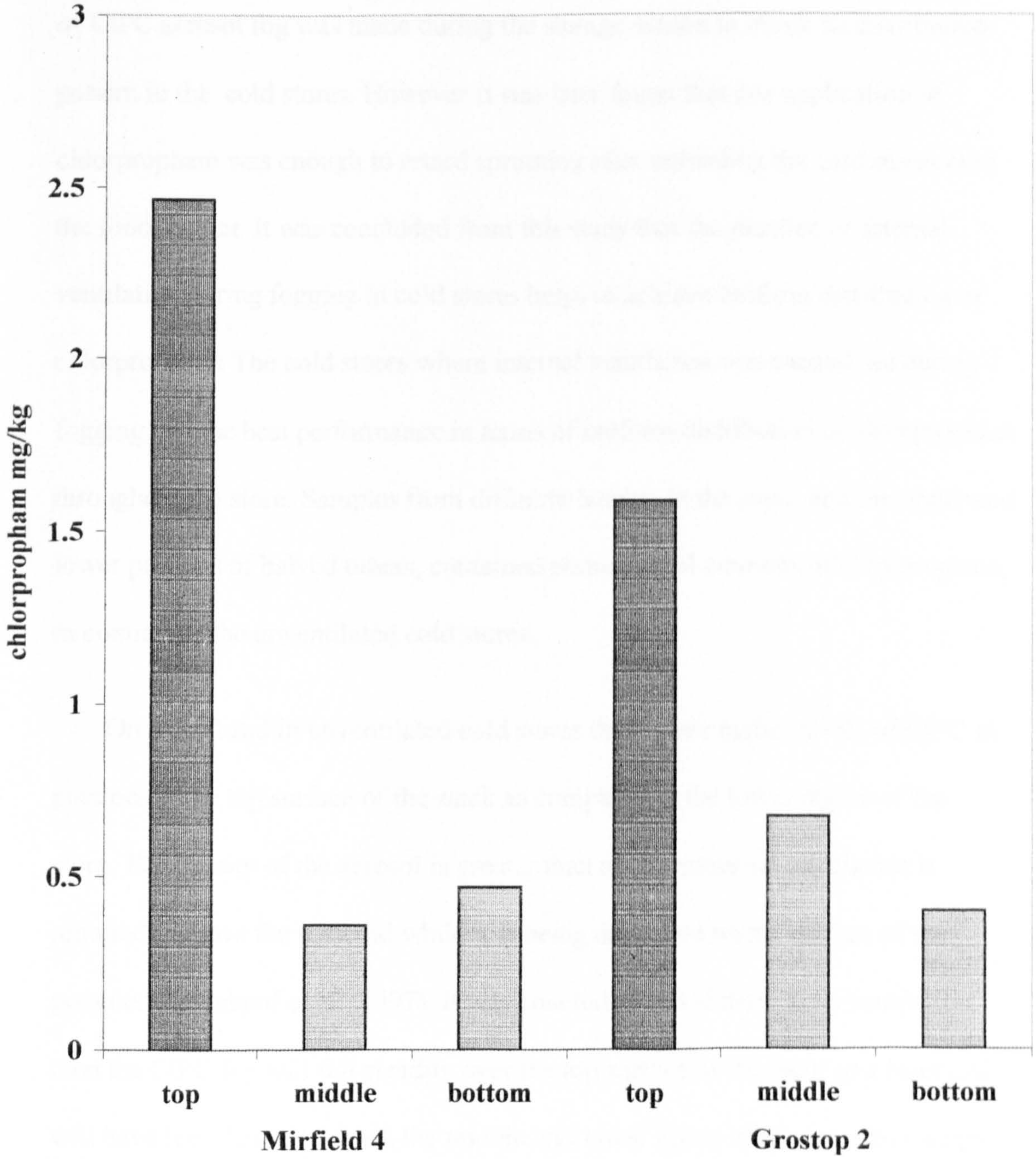
Table 3.10 Chlorpropham residues (mg/kg) in unwashed tubers of Branston series of stores, sampled at 11Th April, 1996 (3-4 ^{0}C).

	Section of surface tubers in box		
Box height	upper	lower	whole
Grostop			
Top	1.50±0.28	1.68±0.31	
Middle			0.67±0.21
Bottom			0.40±0.18
Mirfield			
Top	3.13±1.05	1.80±0.32	
Middle			0.36±0.27
Bottom			0.47±0.16

with the lower half portion of tubers. It was 3.13 and 1.68 mg/kg in upper and lower portions of the tubers respectively.

The mean values of these stores, at different heights of the store were compared and presented as a graph (Fig. 3.19). The over all chlorpropham distribution trend was similar as it was found in their respective stores, Grostop 1 and Mirfield 3. Here the top height of the stores contained higher levels as compared to the lower heights of the store. The difference in this store, specially in Mirfield store was a lower amount of chlorpropham compared to the first Mirfield store 3. Here the Grostop store was again more uniformly distributed than the Mirfield store. In considering the lower heights of the store it was noticed that the level was nearly equal from middle to bottom heights of the respective stores.

Fig. 3.19 Chlorpropham distribution in Branston box stores (3-4 0C).



3.3.4 Conclusion.

In this study internal air circulation in the cold stores during the application of CIPC aerosol fog was compared with unventilated cold stores. A single application of CIPC aerosol fog was made during the storage season to check its distribution pattern in the cold stores. However it was later found that one application of chlorpropham was enough to retard sprouting after unloading the cold stores onto the food market. It was concluded from this study that the practice of internal ventilation during fogging in cold stores helps to achieve uniform distribution of chlorpropham. The cold stores where internal ventilation was carried out during fogging had the best performance in terms of uniform distribution of chlorpropham throughout the store. Samples from different heights in the store, and the upper and lower portions of halved tubers, contained almost equal amounts of chlorpropham, in contrast to the unventilated cold stores.

On other hand in unventilated cold stores there were higher levels of CIPC in potatoes at the top surface of the stack as compared to the lower region of the store. The density of the aerosol is greater than air, so some air circulation is required to move the material while it is being deposited on the surface of the potatoes (Kleinkpof et al., 1997). It was concluded that if there is no ventilation then the CIPC fog will fall steadily over the top surface of the store as a layer and will have less chance to reach the middle and lower layers of tubers in box stores. Therefore it is recommended to apply ventilation during fogging to get a better effect on the distribution of chlorpropham.

The next section is on the effect of the particle size of the chlorpropham fog, and the temperature gradient in the store, on uniformity of chlorpropham distribution in box stores.

Section 3.

3.4 EFFECT OF PARTICLE SIZE AND TEMPERATURE GRADIENT ON THE DISTRIBUTION OF CHLORPROPHAM IN MODEL STORES.

3.4.1 Introduction.

The main way applying of sprout suppressant chemicals to potatoes in store is by using fog treatments and specialist thermal fogging equipment. The chlorpropham is used as the main chemical for inhibiting sprouting in the store and is applied as a fog. The uneven distribution of the chemical is a worry for the industry.

Market and environmental pressure is increasing on the processing sector to further improve the efficiency of its use of chlorpropham and maximum residue limit (MRL) will be imposed by European legislation shortly. A major programme of work has been funded by the British Potato council (BPC), formerly the Potato Marketing Board (PMB), in conjunction with the Potato Processors Association (PPA) to assist in optimising the use of chlorpropham.

To date, studies have successfully been undertaken by the University of Glasgow (GU) to develop techniques to accurately measure the deposition of chlorpropham in potato stores (Duncan and Boyd, 1994); by Sutton Bridge Experimental Unit (SBEU) and Glasgow University to identify the minimum amounts of chlorpropham required by tubers to suppress sprouting by Silsoe Research Institute (SRI) to model distribution of fogged chemical within a store

(Burfoot et al., 1994; Burfoot et al., 1996) and to develop a suitable technique for measurement of particle size within the fog (Miller et al., 1997a).

This study was conducted at Sutton Bridge Experimental Unit to validate the effect of parameters that would influence the performance of chemical treatment applied as fogs. The studies have looked at the effect of chlorpropham particle size and temperature gradient on the distribution of chlorpropham. The work was carried out with the collaboration of Sutton Bridge Experimental Unit and Silsoe Research Institute.

In this collaborative experiment, the responsibilities for the different components of the work can be summarised as follows:

Sutton Bridge Experiment Unit-provision of the stores, loading boxes and stores, operation of the stores and sampling the tubers within boxes;

Silsoe Research Institute-monitoring the size distribution in the applied fog and the temperature differences between the air space above potatoes in a box and in the plenum chamber;University of Glasgow-measurement of chlorpropham deposits on sampled potatoes.

3.4.2 Material and Method.

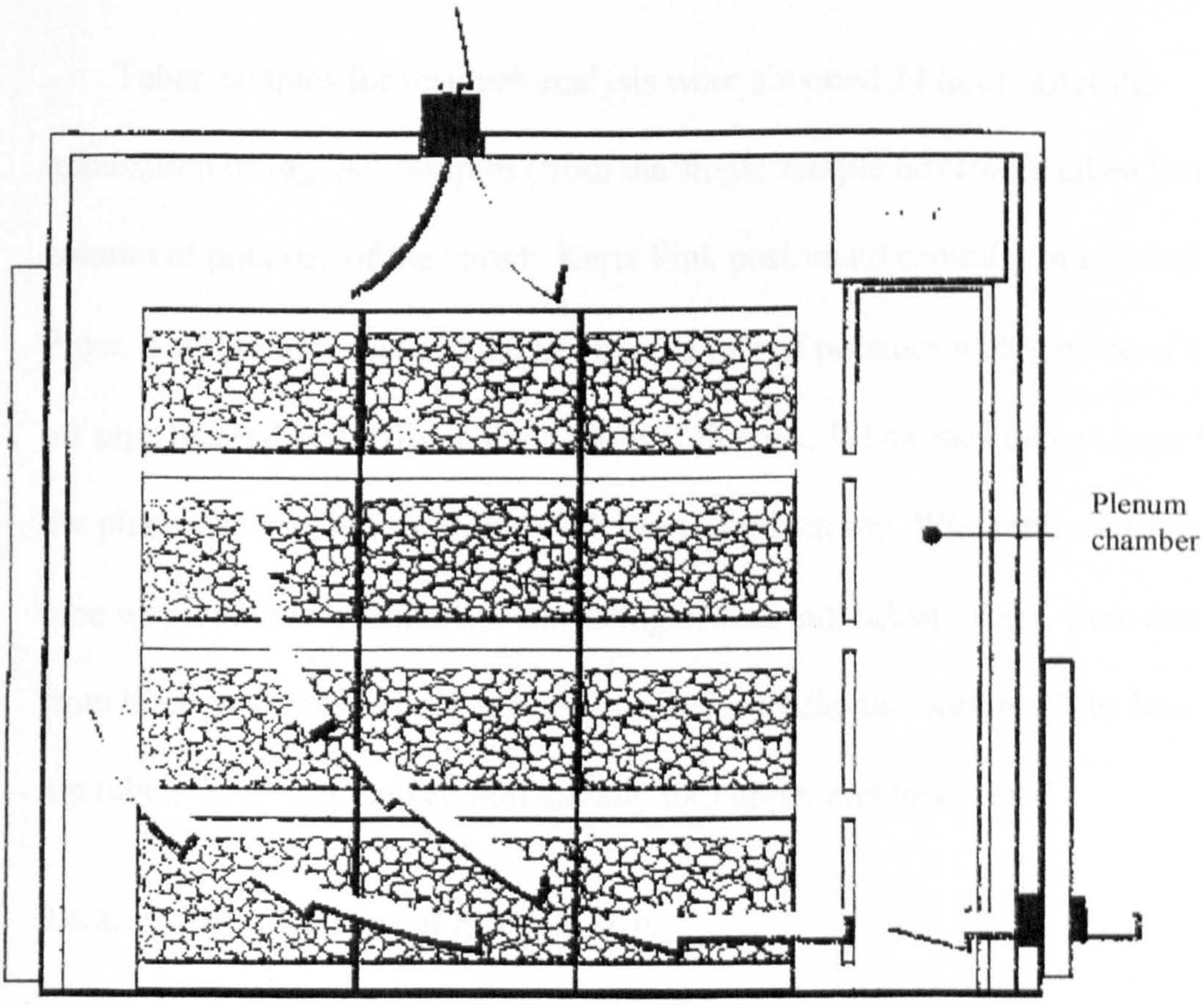
3.4.2.1 Particle size: Experiment 1.

The experimental work was conducted at Sutton Bridge Experimental Unit using three 12-tonne capacity experimental stores.

The layout of the stores used for this experiment is shown in Figure 3.20. Boxes were stacked in three columns of four boxes forming a solid 'block', with gaps between boxes minimised. Boxes were solid-sided with slatted bases. The entire 'block' of boxes was stacked away from the plenum chamber ensuring that chlorpropham fog had access to boxes via pallet apertures at both ends of the block and via the surface boxes. Samples for residue analysis were obtained from a single box. This was located in the middle column- the third box up. The walls of the sample box were also covered in polyethylene film to ensure that chlorpropham fog could only enter the box by horizontal movement via pallet apertures, and vertical movements through the crop. The store was set up 6 days prior to chemical application, to allow conditions to stabilise.

Chlorpropham fog of three 'qualities' was applied to different stores. The applicator used was a Superfog, applying the formulation MSS CIPC 50 M (containing 500g CIPC per litre). Fog was introduced at low level (500 mm above ground) by a port in the store access door, with excess fog being allowed to escape via an exhaust system in the store roof. To obtain differences in chlorpropham fog 'quality' in each store the applicator burner temperature setting was adjusted by the operator. Temperatures of 400 ^{0}C, 460 ^{0}C (superfog optimum) and 520 ^{0}C were employed. Stores were switched off prior to chemical application, and remained

Figure 3.20. Store stacking pattern: Experiment 1 (different particle size of fog).



The diagram is provided by Sutton Bridge Experimental Unit, UK.

off until all residue samples had been obtained. Residue samples were stored at 3.5 ^{0}C before taken for analysis.

Applications were carried out in the following order:

1. Store 36- burner temperature 400 ^{0}C. Application poor due to leakage.

2. Store 35- burner temperature 460 ^{0}C. Application acceptable.

3. Store 34- burner temperature 520 ^{0}C. Application acceptable.

Tuber samples for residues analysis were obtained 24 hours after the application of fog. All samples (from the single sample box) were taken from a column of potatoes of the variety Kerrs Pink positioned centrally in a box of Maris Piper. This column was created by filling a box of potatoes with a piece of blanked off pipe located vertically in the middle of the box. When the box had been filled the pipe was unsealed and filled with the second variety. When this was full the pipe was withdrawn. Samples, consisting of five individual tubers, were obtained from three positions in each sample box, top, middle and bottom of the box. The top tubers were then halved horizontally into upper and lower half.

3.4.2.1.1 Measurement of Particle size.

The method developed at SRI for particle size measurement by Miller et al., (1997b) uses an instrumentation system designed for monitoring airborne particle sizes in clean air environments with dilution stages to bring airborne concentrations into a range that can be measured by the instrument. This system has been used to monitor the size distributions in commercial box and bulk potato

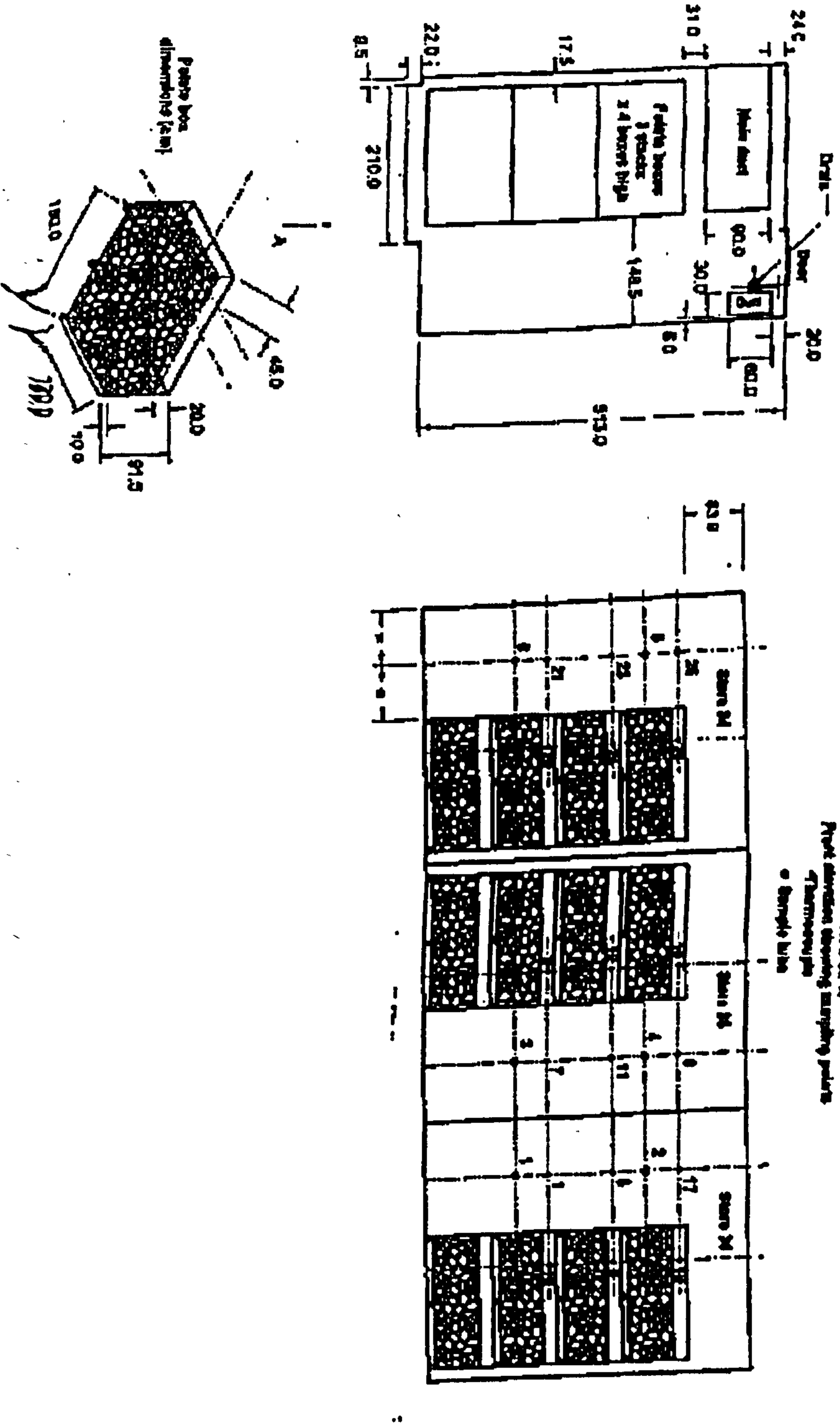
stores during a fogging treatment and in the period immediately following the application of the fog so as to obtain direct measures of chemical movement within a treated store Miller et al., (1997a).

Results from this work showed that in typical commercial fog treatments, between 60 and 70 % of the airborne material applied to a store was in particle sizes of between 5.0 and 10.0 um. Measurements in a loaded box store confirmed that the input fog rose rapidly to the top of the store and became reasonably well mixed through a monitored section of the store after a period of some 2.5 hours after treatment and the time the airborne fog levels had declined to levels approaching those of a store prior to treatment.

While the stores were being loaded, paired thermocouples were installed to measure the temperature difference in the air immediately above potatoes in the box and at an equivalent position within the free air of the corridor to the side of the boxes. Sampling tubes were also installed to enable samples of air laden with fog particles to be drawn from two levels within a treated store such that size distribution measurements could be made using established techniques (Miller et al., 1997b).

The arrangement of the instrumentation in the store is shown in Figure 3.21. All of the instrumentation systems for measuring fogging temperature differences and the particle size distributions were mounted on the roof of the stores to be monitored, enabling sampling pipes to be arranged with an almost vertical path. This is close to an optimum arrangement for minimising the impaction of particles on the walls of the sampling tubing.

Figure 3.21. Arrangement of instrumentation in model stores.



The diagram is provided by Sutton Bridge Experimental Unit, UK.

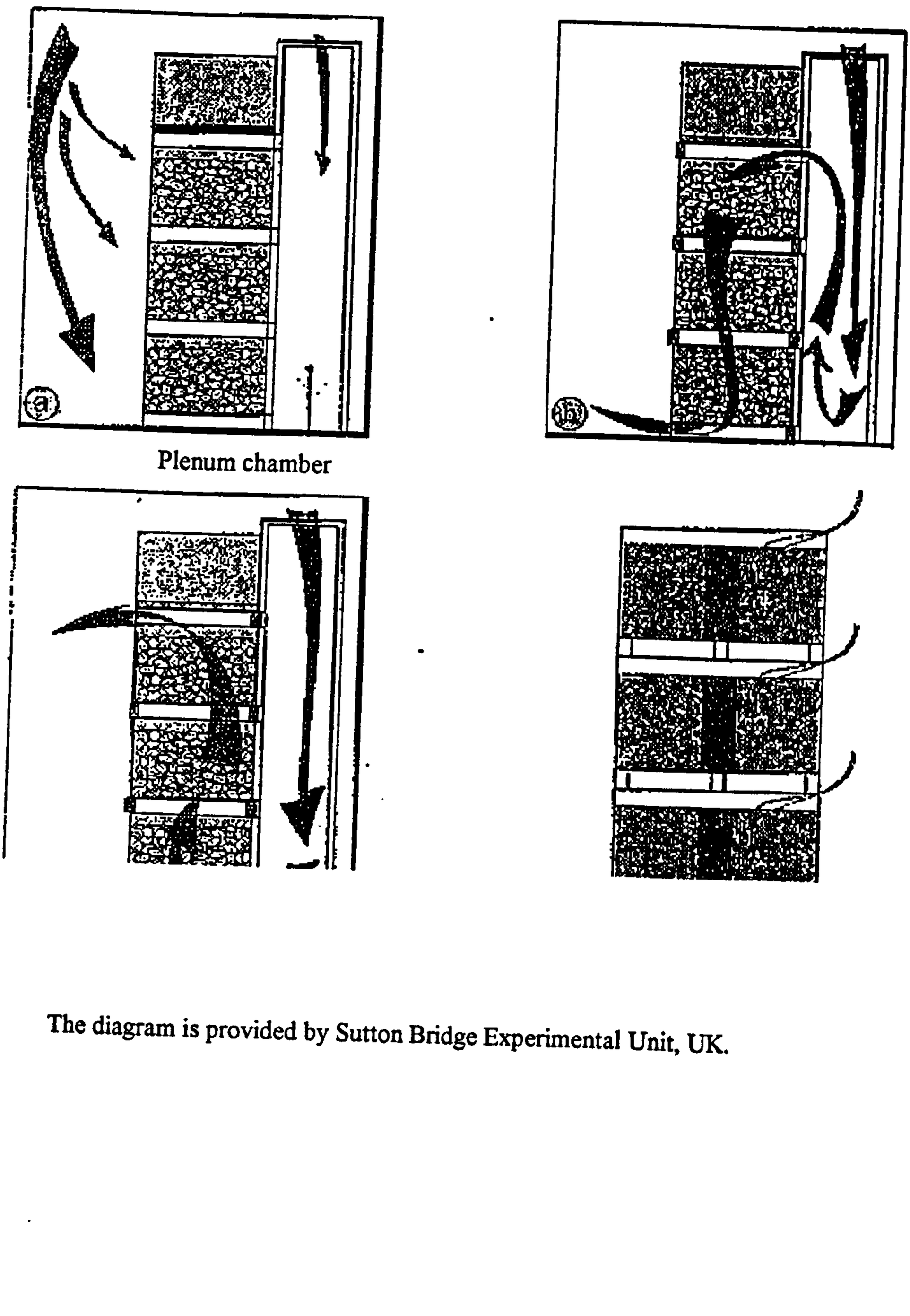
Prior to the fogging treatment, all stores were subject to the same air flow and temperature treatments and the conditions in each store were the same at the time of treatment. Due to the problems of leakage and co-ordination when the first store (store 36) was treated, reliable measurements of the airborne fog distribution could only be made after the stores were sealed at the end of the whole treatment process. The applied dose of the chlorpropham formulation was considerably in excess of normal treatment levels and this led to very high levels of airborne particle concentration in the treated stores which influenced the accuracy within which size distributions could be measured.

3.4.2.2 Temperature gradient: Experiment 2.

Experiment 2 was also carried out in three 12-tonnes stores. Each store contained four boxes in a single column stacked against the plenum chamber. The top box in each store was not filled, but had a sealed base, allowing the pallet aperture of this box to be used as a manifold controlling air movement (Fig. 3.22). Air distribution was controlled in such a way as to give a) minimal gradient, b) positive gradient [increasing temperature with increasing height within stack] and c) negative gradient [decreasing temperature with increasing height within stack].

Air movements were controlled by selectively sealing pallet apertures with foam blocks. Prior to chemical application the crop temperature was 7 ^{0}C and the relative humidity 95%. Approximately two hours before chemical application, the store heaters were switched on in the two stores where a temperature gradient was required. Once a satisfactory temperature gradient had been established, stores were switched off, foam sealing blocks removed and chlorpropham was applied.

Figure 3.22. Air flow pattern in model stores.



The diagram is provided by Sutton Bridge Experimental Unit, UK.

For all three stores the formulation used was MSS CIPC 50 M applied using a superfog. A flow rate of 1 litre per minute was used, with a burner temperature of 460 ^{0}C. The fog was applied for 30 seconds to each store. As previously, fog was introduced at low level, and allowed to escape from an exhaust in the roof. Stores remained switched off until all samples had been obtained.

Applications were made in the following order:

1. Store 36- positive gradient (cold bottom/warm top).

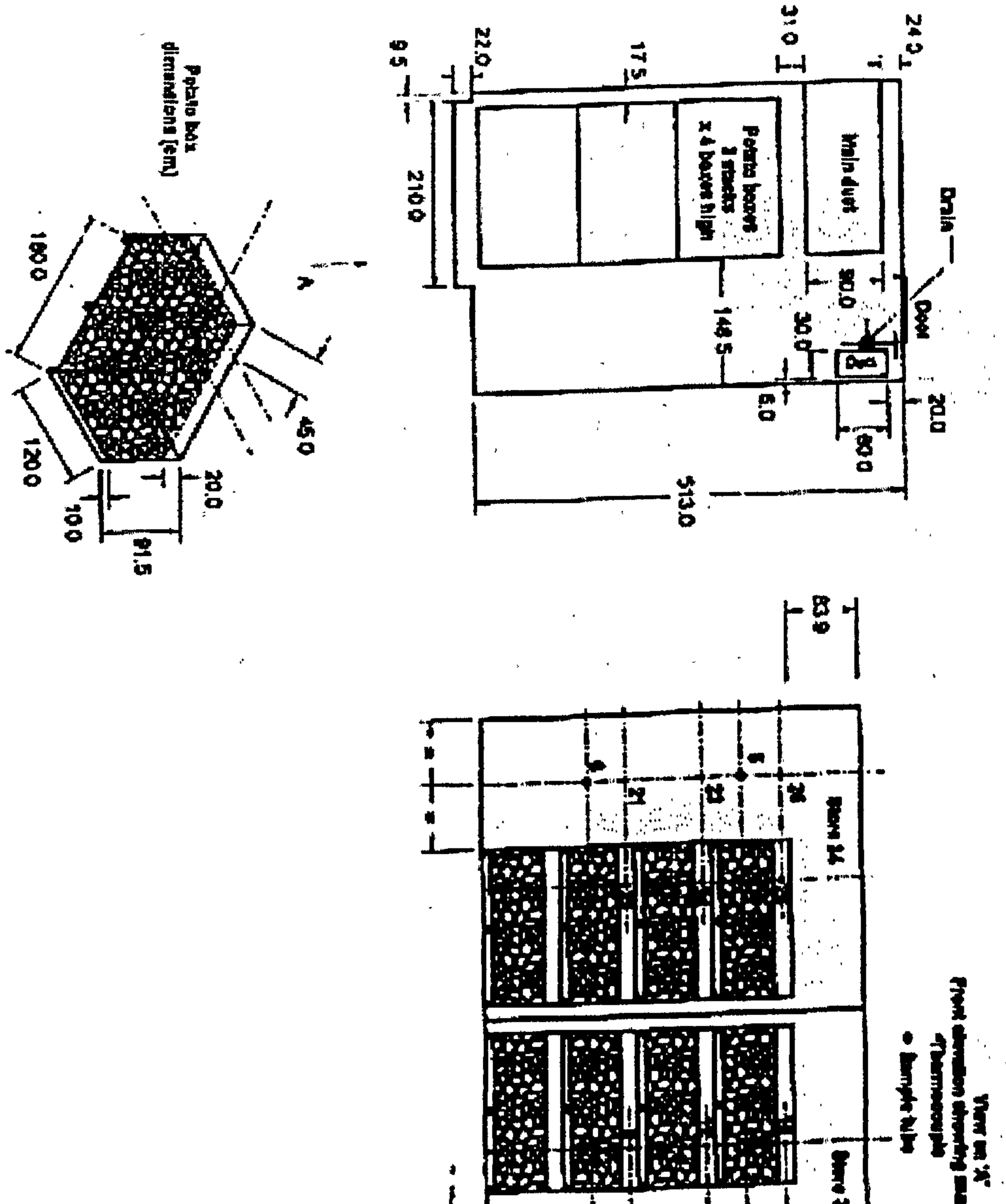
2. Store 35- minimal gradient.

3. Store 34- negative gradient)cold top/warm bottom).

Tuber samples for residue analysis were obtained, from each box, from a central, vertical column of potatoes, constructed similarly to those in experiment 1. As previously, samples were taken from the surface of boxes, and from depths of 300 mm and 600 mm. Surface tubers were halved horizontally in situ. Samples were stored at 3.5 ^{0}C prior to despatch for analysis.

Stores were, as in Experiment 1, fully instrumented by Silsoe Research Institute to monitor temperatures and chlorpropham particle sizes. The layout of the store instrumentation system for this second experiment is shown in Fig. 3.23. The application of fog for the shorter duration of 30 second (compared with 3 minutes for Experiment 1) gave a lower airborne concentration system. However, a failure in the power supply for the data logger at the start of this experiment meant that only absolute temperature measurements in the centre of the store air space were recorded.

Figure 3.23. Arrangement of instrumentation in model stores.



The diagram is provided by Sutton Bridge Experimental Unit, UK.

3.4.3 Results and Discussion.

3.4.3.1 Particle size

The measured size distributions for the three stores were relatively small differences in the treatments applied to the stores with volume median diameters of 11.3, 7.7 and 7.3 um for stores numbered 34, 35 and 36 respectively.

This means that the store that should have been treated with the smallest mean particle size was measured as being treated with the largest. High percentage of the measured particles were assessed as being in the size range 10.0 to 25.0 um. This was probably due to a very high level of airborne particles in this store at the time of measurement (from the high dose of chlorpropham applied) resulting in the instrument recording the high concentrations of small particles as a single larger particle. The leakage during the treatment of store 36 (treated with a temperature setting of 400 ^{0}C) resulted in a lower airborne concentration of fog particles, and hence a more reliable measure of the particle size distribution.

The similarity between the size distributions measured at the two lower fogger temperatures suggests that variation in temperature may not have been the most effective way of changing the particle size distribution in this experiment. It was chosen because it enabled treatment times to be kept constant for the three treated stores. However, there is now unpublished evidence to suggest that varying the flow rate of the liquid formulation through the fogger may be a better way to achieve a difference in the size distribution of the applied fog. This would then

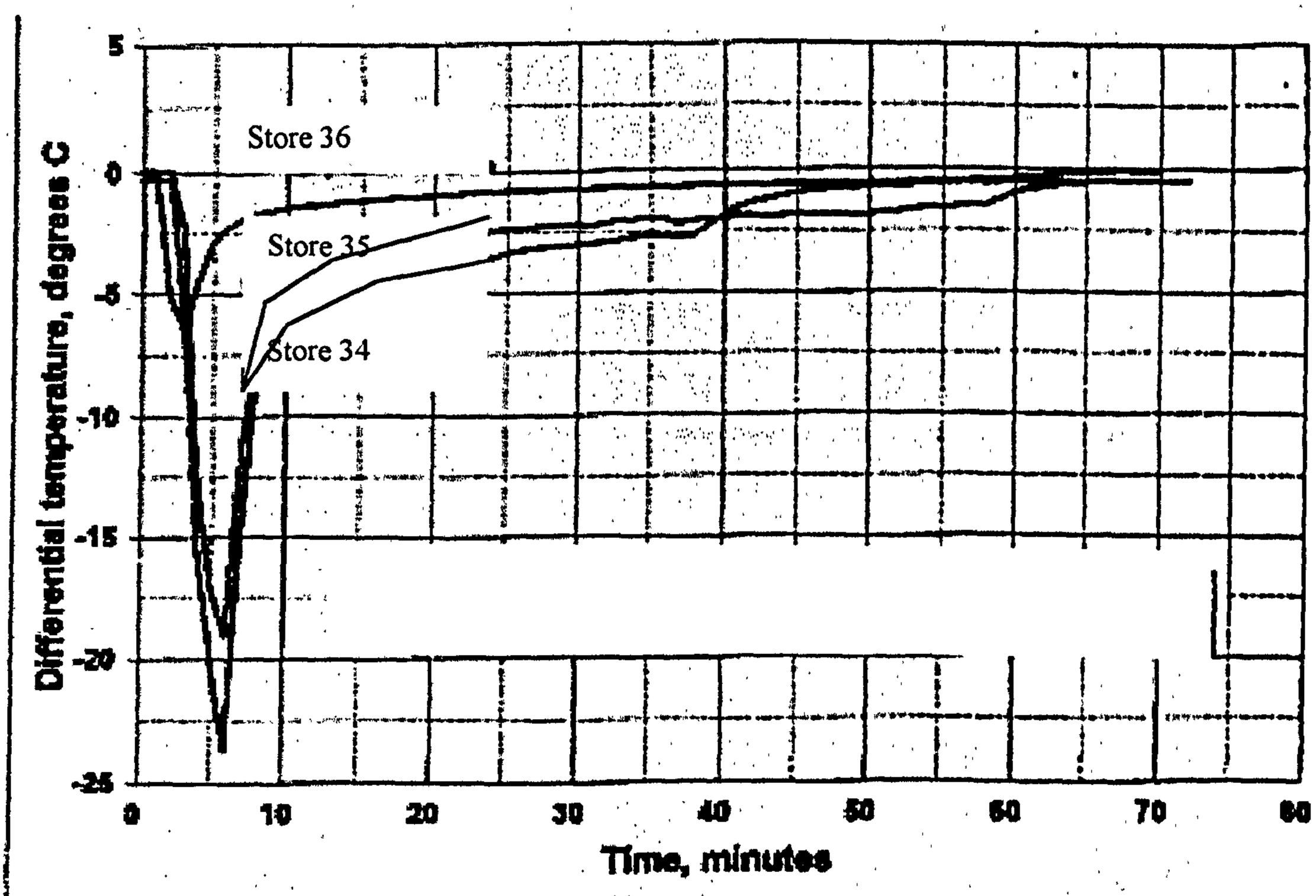
mean that treatment times would need to be varied to keep the applied dose to each store constant.

The change in temperature difference between the air in the potato boxes immediately above the crop can be seen from the results plotted in Figure 3.24. Store 36 which was treated first and from which there was considerable loss, showed a much smaller temperature difference at the time of treatment than was the case for stores 34 and 35. This is consistent with substantial losses of hot particle laden gas from this store. In the case of stores 34 and 35, temperature differences between air in the box and in the plenum chamber at the time of applying the fogging treatment were up to 20 ^{0}C.

It should be noted that, because of the relatively high applied dose rate and the small size of the test stores, this temperature difference is higher than would be expected and has been measured in full scale store treatment conditions.

The chlorpropham deposit levels measured in the samples taken from this experiment are summarised in Table 3.11. The ratio of top/lower halves to mean of unwashed samples of stores were plotted in the Figure 3.25. It shows that store 36 was significantly different in distribution pattern as compared to the other two stores. The ratio increased from store 36 to store 34. The chlorpropham was in minimum amount throughout the store 36 as compared to the other two stores. The range of chlorpropham was 3.63-15.27 mg/kg, while it was 42.92-138.73 and 39.54-146.37 mg/kg in stores 35 and 34 respectively (Table 3.11). This is most likely, when considered with the particle size data, to be a result of the leakage of

Figure 3.24. Air/crop differential temperatures

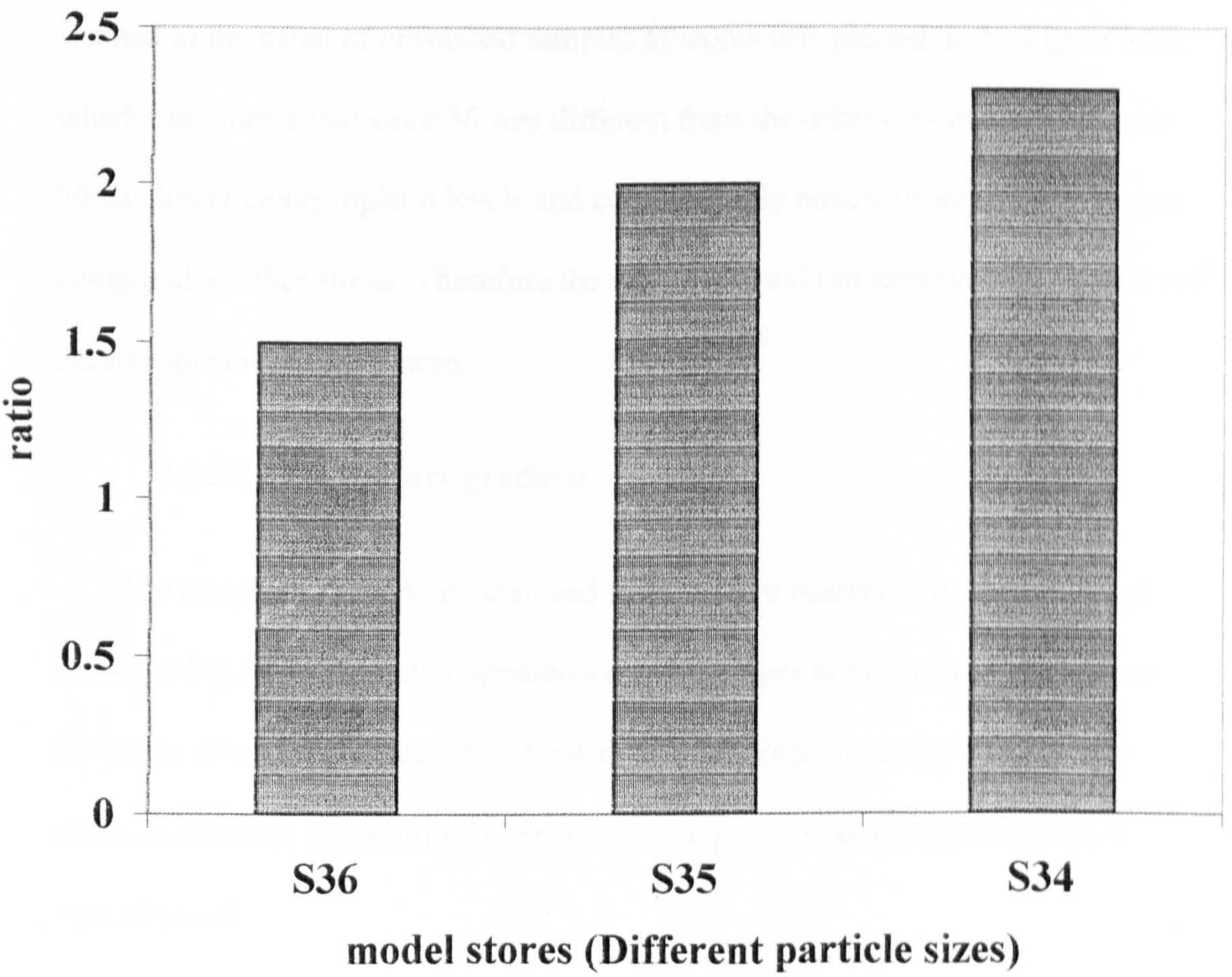


The diagram is provided by Sutton Bridge Experimental Unit, UK.

Table.3.11 Chlorpropham residues (mg/kg) of model box stores of varied particle sizes.

		Store 36 (400 ^{0}C, 7.3 um)	Store 35 (460 ^{0}C, 7.7 um)	Store 34 (520 ^{0}C, 11.3 um)
Sampling Box				
		unwashed		
Top	upper half	15.27 ± 5	138.73 ± 82	146.37 ± 57
	lower half	4.40 ± 2	42.92 ± 14	39.54 ± 26
Middle	whole	3.63 ± 1	79.42 ± 35	86.29 ± 38
Bottom	whole	4.42 ± 2	64.34 ± 42	72.84 ± 34
		washed		
Top				
	upper half	10.89 ± 4	98.29 ± 30	76.95 ± 27
	lower half	1.53 ± 1	7.30 ± 3	4.35 ± 2
Middle	whole	3.75 ± 2	33.84 ± 15	32.64 ± 16
Bottom	whole	2.49 ± 1	30.85 ± 15	38.51 ± 23.82

Fig. 3.25 Ratio of top/lower half to mean of unwashed of model stores.



chlorpropham on application to this store rather than as a direct consequence of burner temperature or particle size.

The similarity in deposits from stores 34 and 35 (on both unwashed tubers and after washing) suggest that particle sizes are similar in these two cases. This supports the measurement made by SRI and the case that, on this occasion, changing the temperature setting on the applicator did not make a significant difference to the particle size patterns or chlorpropham deposition. The ratio of washed to the mean of unwashed samples of stores was plotted in the Figure 3.26, which also shows that store 36 was different from the other two stores. The store 36 has lower chlorpropham levels and comparatively adhere more to the tubers as compared to other stores. Therefore the washing could not remove high amounts of chlorpropham from this store.

3.4.3.2 Temperature gradient

The temperature gradient achieved in each store measured by the probes is shown in Fig 3.27. The actual gradients achieved were not exactly as planned as the boxes receiving the heated air tended to be disproportionately warmer than others in the store but minimal (less than 2 ^{0}C), positive and negative gradients were attained.

The measured size distributions in the fogs applied to the three stores treated in this experiment showed relatively good agreement as expected. The mean particle size for all treatments was in the range 4.6 to 7.4 um. There was some tendency to measure higher concentrations of the smaller particle sizes at the lower

Fig. 3.26 Ratio of washed to their mean of unwashed of model stores.

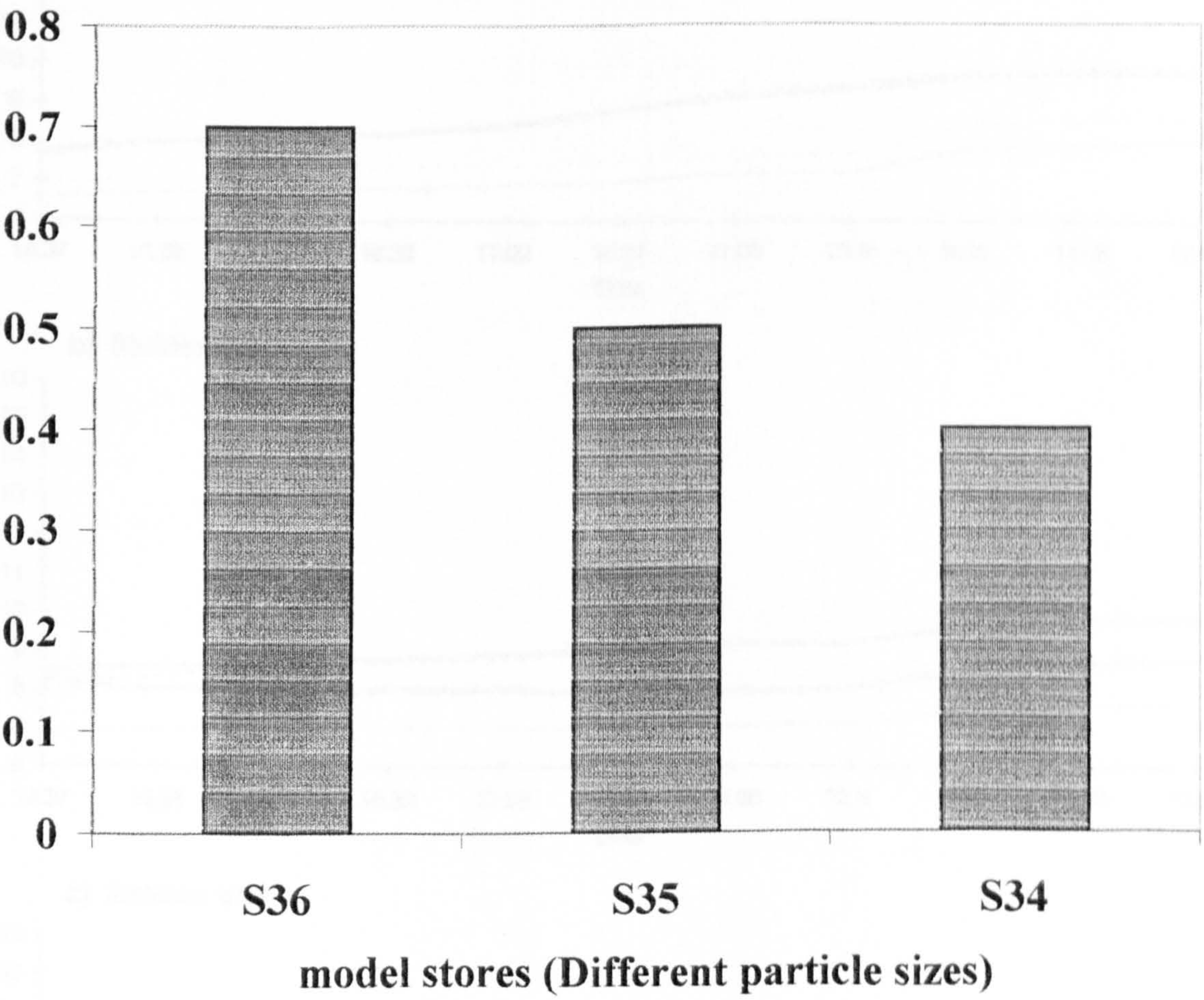
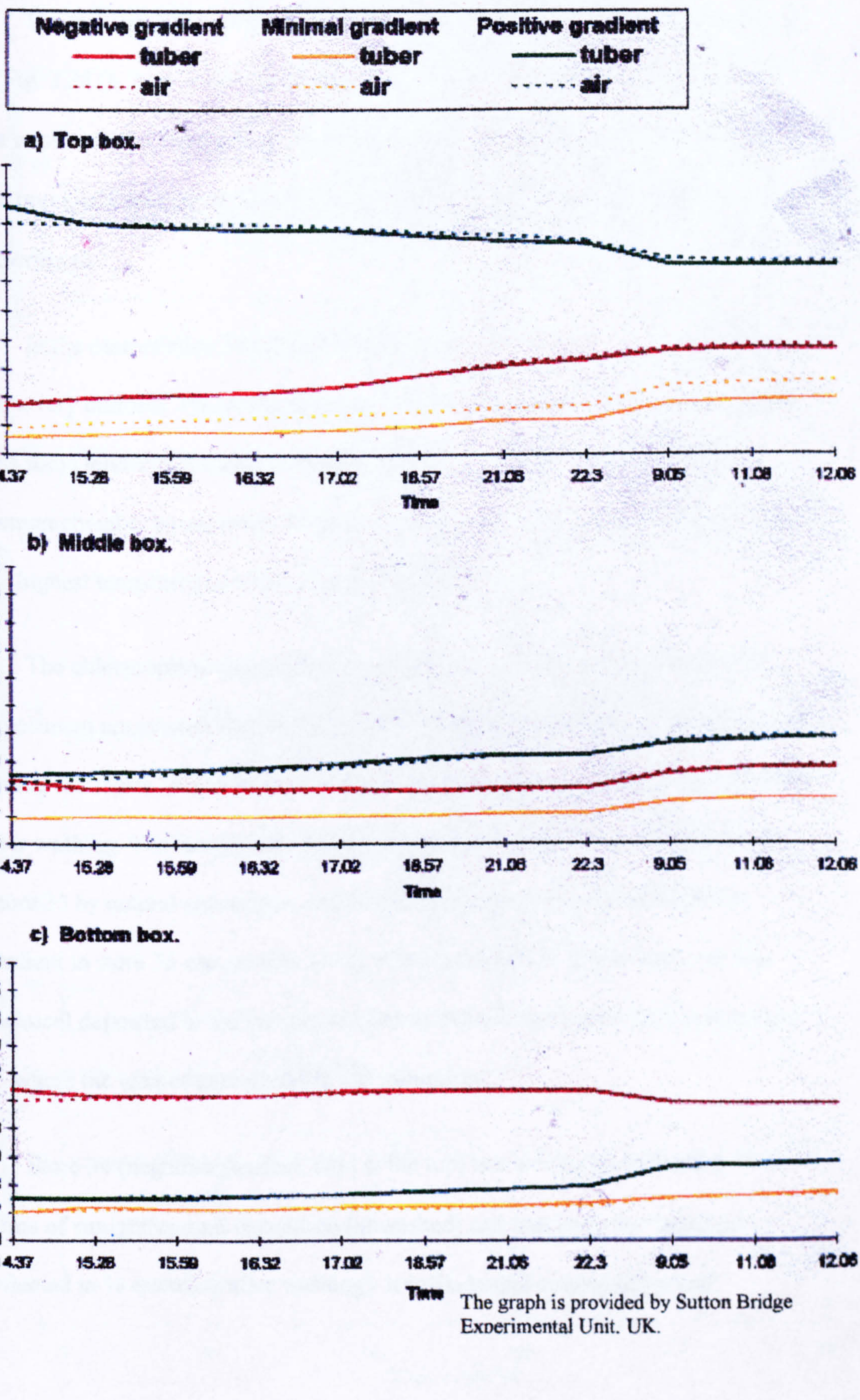


Figure 3.27. Temperature reading after fogging of chlorpropham: Experiment 2.



The graph is provided by Sutton Bridge Experimental Unit. UK.

sampling points which was not as expected and may be due to localised flow patterns in the plenum as the fog was introduced.

The measured temperatures in each of the stores during application is shown in Fig. 3.28 (a, b, c). In all cases there is a steep rise in temperature at the time of the application, although at approximately 10 ^{0}C, this rise is less than that noted in the previous experiment. This is consistent with the shorter fogging time used for experiment 2.

In the case of store 35 (Fig. 3.28(b)), temperatures during the period of control were very uniform. When the control system was turned off prior to fogging there was some tendency for higher temperatures to be measured in the top of the plenum chamber as expected. In both stores 34 (Fig. 3.28 (a)) and 36 (Fig 3.28 (c)) the highest temperatures were recorded in store 36.

The chlorpropham deposit levels measured in the samples taken from this experiment are summarised in Table 3.12. The patterns of distribution were rather similar for stores 35 and 36 both in terms of deposition and residual chlorpropham after washing. The temperature gradients in these two stores were both positive (store 35 by natural convection, store 36 deliberately imposed) although the gradient in store 36 was greater. If any trend is evident, it is that there was less chemical deposited in the warmer top box of store 36 compared with that in store 35 where the temperature gradient was minimised.

Store 34 (negative gradient, cold at the top) was markedly different both in terms of straightforward deposition (unwashed) and residual attachment (as reflected in % retention after washing). It is likely that condensation and

Figure. 3.28. Temperature variation in the stores: Experiment 2.

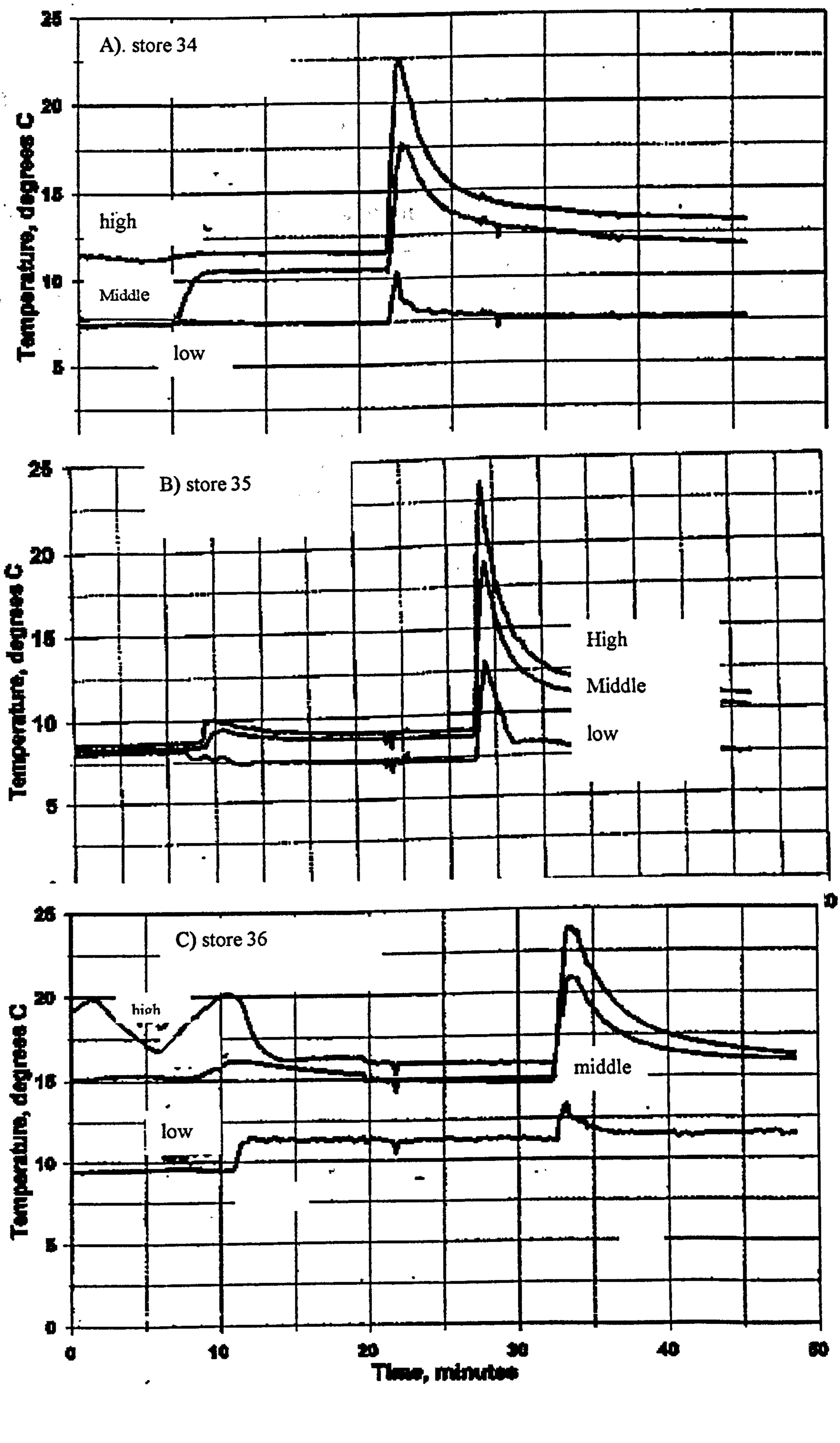


Table.3.12 Chlorpropham residues (mg/kg) in unwashed tubers in model box stores of varied temperature gradient.

Box	position	section	Chlorpropham (mg/kg). Store 34	Store 35	Store 36
			Negative**	Minimal	Positive*
Top	top	upper	344.38	159.14	57.72
		lower	13.38	16.87	9.73
	middle		38.99	45.53	9.22
	bottom		34.20	40.54	19.67
Middle	top	upper	728.82	91.63	109.16
		lower	11.94	9.27	14.59
	middle		18.93	32.02	42.74
	bottom		24.02	37.12	34.08
Bottom	top	upper	37.80	73.04	78.73
		lower	21.77	9.88	6.19
	middle		18.26	29.53	34.55
	bottom		11.62	16.29	24.80

** negative = cold at top of store; * positive = warm at top

deposition of the chemical has occurred on the cold surfaces at the top as the amount of chlorpropham which readily washed off in the top boxes of store 34 would suggest that the attachment of the majority of the chlorpropham to the tubers was slight under these conditions (Table 3.13). The ratio of top/lower halves to the mean of unwashed samples of the stores was plotted as a graph (Fig. 3.29). Here store 34 was very different from the other two stores . The overall ratio of washed to unwashed shows that store 34 responded differently than the other stores. Here the chlorpropham was much reduced by washing as compared to the other stores. This shows that the distribution pattern in this store is much different as compared to the other two stores. Similarly the ratio of the mean of washed samples to mean of unwashed in respective sites was plotted. Stores 35 and 36 have similar trends (Fig. 3.30). The chlorpropham adheres more to potatoes in the bottom height of the stores as compared to the top heights.

The measurement conditions in this experiment were such that good quality estimates of the droplet size distributions applied to the stores were obtained. The deposit assessments also exhibits a good degree of consistency (once allowance for the variation between single tuber assessments is made).

Table 3.13 Chlorpropham residues (mg/kg) in washed tubers of model box stores of varied temperature gradient.

Box	position	section	Chlorpropham (mg/kg).		
			Store 34	Store 35	Store 36
			Negative **	Minimal	Positive *
Top	top	upper	60.99	72.30	47.62
		lower	5.78	10.79	4.91
	middle		17.59	27.79	10.86
	bottom		19.70	23.88	13.10
Middle	top	upper	36.46	46.91	78.51
		lower	10.82	9.91	8.87
	middle		13.93	23.08	16.83
	bottom		18.95	25.83	20.30
Bottom	top	upper	23.41	52.54	53.64
		lower	5.16	8.62	7.43
	middle		7.45	15.91	15.11
	bottom		9.91	12.47	16.50

**Negative = cold at top of store; * Positive = warm at top.

Fig. 3.29 Ratio of lower half of top tubers to the mean of stackin model stores.

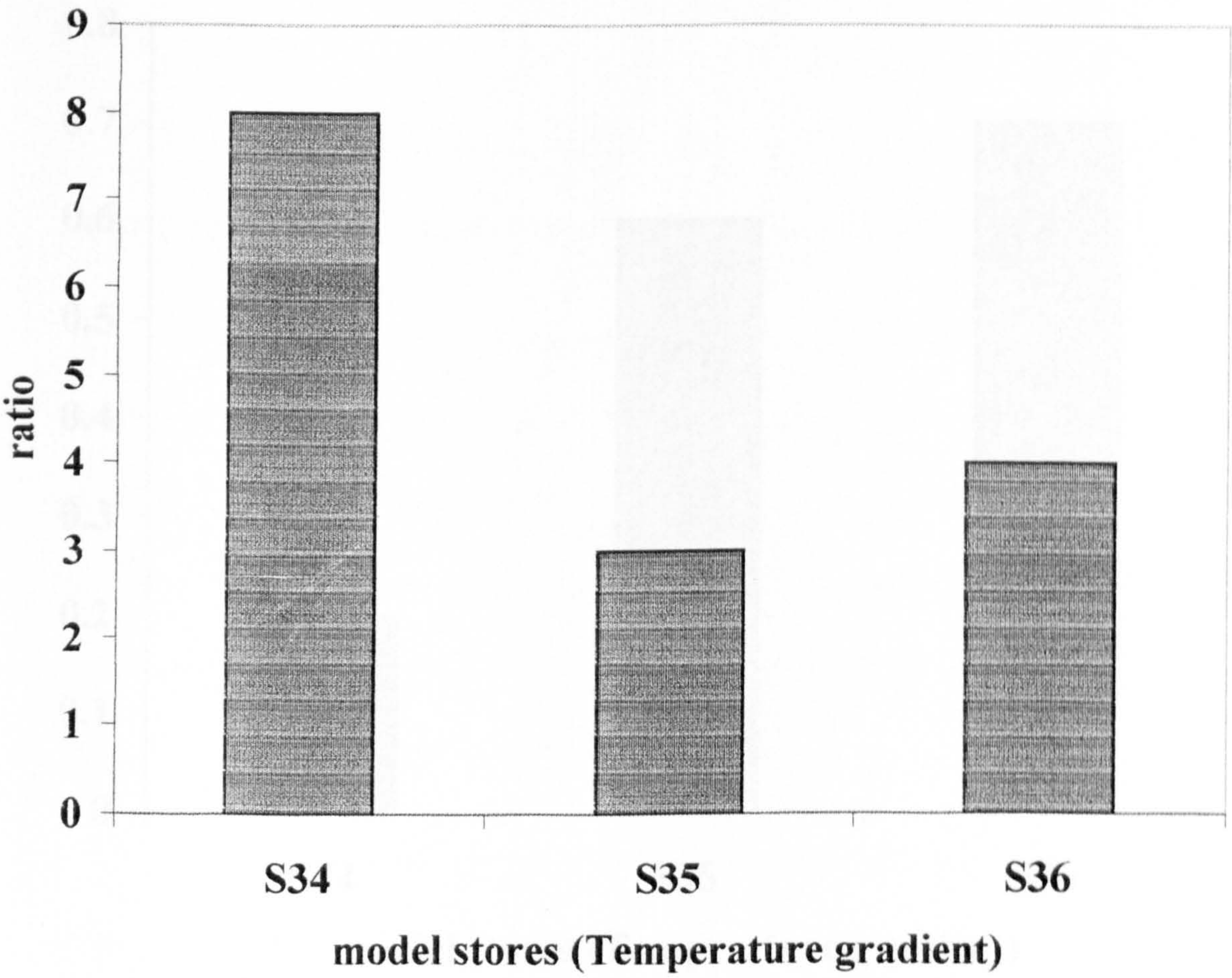
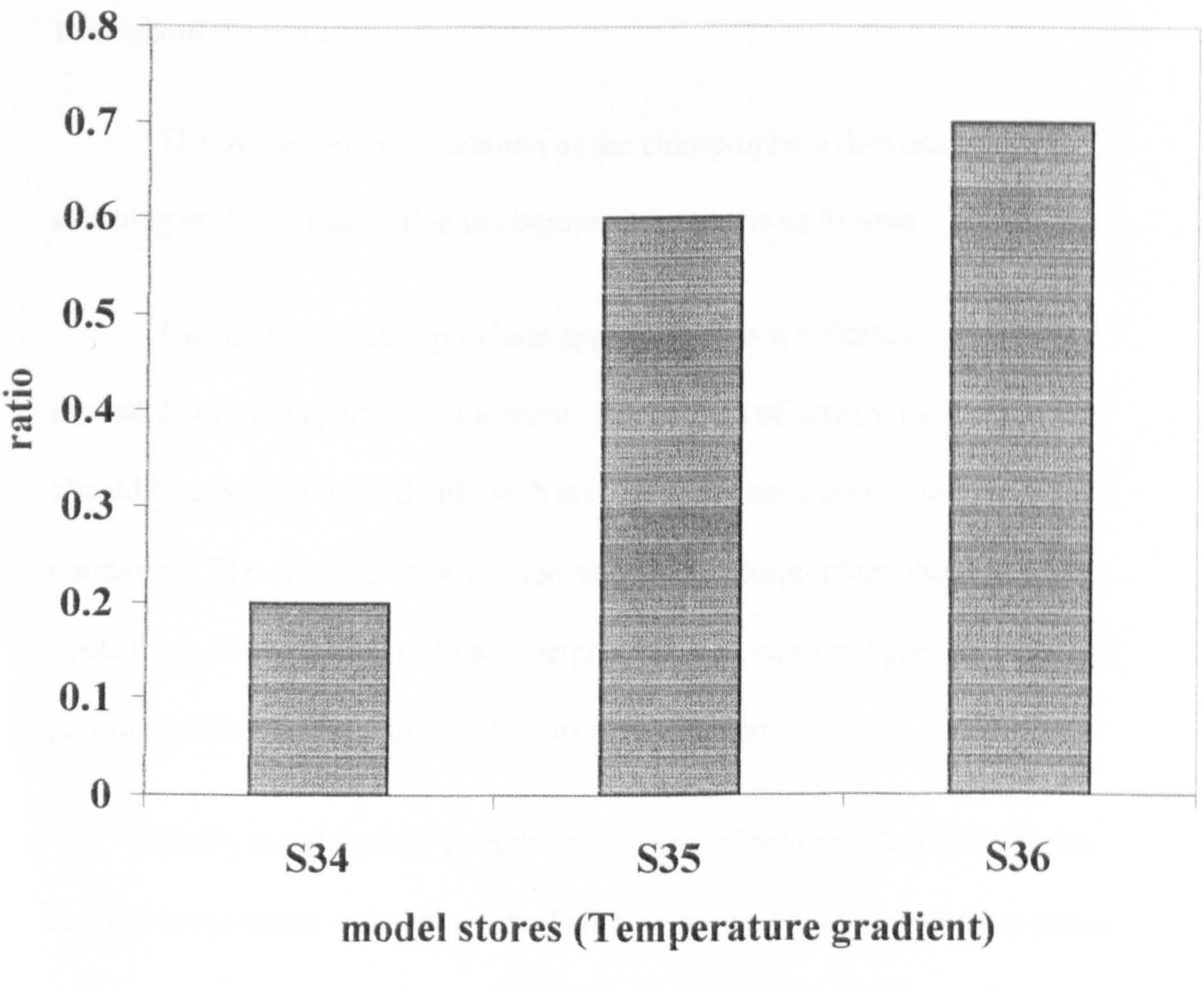


Fig. 3.30 Ratio of mean washed to mean of unwashed of stack in model stores.



3.4.3.3 Conclusion.

It was concluded from this experiment that the method of controlling particle size should be modified. The desired particle size should be achieved by changing the flow rate rather than by applying a different temperature during fogging. However the temperature gradient showed that the colder sites receive more chlorpropham. Therefore in cold stores at least, higher temperature during fogging could have a better effect on the uniformity of chlorpropham distribution throughout the store.

The overall recommendation of the chlorpropham distribution study for attaining uniform distribution in commercial stores is as follows.

The method of chlorpropham application has a definite effect on the overall distribution pattern in the store. The method of aerosol fog application should be designed individually with respect to the store design and the storage conditions. The application of the aerosol fog of chlorpropham through lateral ducts from different directions and heights in the store would play an important part in attaining uniform distribution of chlorpropham.

Finally in cold stores a single application of chlorpropham with higher fogging temperature and with internal air circulation would be helpful to attain uniform distribution of chlorpropham. However a further study is needed to apply all the above factors with the combination of temperature, humidity, ventilation and particle sizes of fog during the application of chlorpropham and storage.

Chapter 4.

BOUND RESIDUES IN POTATOES

4.1 INTRODUCTION.

Postharvest application of pesticides to agricultural products has become a subject of great interest all over the world and potatoes are no exception. Chlorpropham has been used for years in potato stores but there is inadequate information available on its attachment behaviour with the tuber components.

In the previous Chapter 3, the study carried out on the distribution of chlorpropham in commercial stores and the effects of washing on the level of chlorpropham in tubers revealed some sort of attachment behaviour. It was concluded from Chapter 3 that the chlorpropham was firmly attached to the tubers and its level was higher than the MRL in European countries even after washing the tubers. Therefore this chapter was initiated to discover and to understand any possible attachment of chlorpropham to tuber components.

The chlorpropham is mostly found in the upper layer or peel and roughly accounts for 80% of the total extractable amount in the tuber. The labelled chlorpropham penetrates little beyond the peel layer and no identifiable degradation product of chlorpropham was detected in the extracts (Coxon and Filmer, 1985). However in the past, information on the fate of chemical on

storage was obtained only from the chemical analysis of the solvent-extractable residues from the substrate. The analysis of extractable residues alone does not provide a clear understanding of total pesticide residues, as it does not include any information on nonextractable residues which are generally referred to as bound residues. These formally seen as non extractable (bound) residues can amount to a significant proportion of the applied dose after an extended storage period.(Singh et al., 1993).

The meaning of nonextractable or bound residues may remain open to interpretation depending on the extraction system employed to remove bound pesticide or metabolite constituents in the food. However, in this study, the term "bound residues" is used according to the most widely accepted IUPAC definition , the residues which cannot be extracted by methods commonly used in residue analysis studies.

Attempts have been made here to study and quantify the sprout suppressant chemical, chlorpropham, which remains nonextractable from the treated tuber components after normal solvent extraction. It is obvious that any pesticide residues which are not extractable and detected by recommended methods of residue analysis but remain bound with the constituents of food under practical agriculture conditions may become a potential hazard in future.

The following study was divided into two major parts. The first experiment was set up to find out the effects of two common processing methods, microwave cooking and boiling, on the residues level of chlorpropham in treated tubers. In the second, efforts were made to extract any nonextracable amount of

chlorpropham in the treated potatoes with highly polar solvents to find out the possibilities of bound 'unextractable' chlorpropham residues in the stored potatoes. One of the possibilities regarding bound residues in the potato is that they can be associated with the starch of the commercially treated stored tubers.

The largest component of the dry matter of potatoes is starch. Starch is not normally considered to bind pesticides, but some of its characteristics suggest that it could do so. Starch is a well-established adsorption agent (Westgate and Ladisch, (1993) and is widely applied in pharmacy as binders in tablets, or as excipients in controlled release formulations. Starch has also been used for slow release of chemicals (Wierik et al., 1996).

4.2 STARCH.

Starch is the chief reserve carbohydrate of plants. It is therefore one of the most widely distributed substances in the vegetable kingdom. It occurs in seeds and embryos when the seeds or fruits are planted and in the vegetative parts of plants. The starch granules appear to be built up by deposition of layers around a central nucleus or hilum. In some plants shell formation of the starch granules is controlled by an endogenous rhythm as in potato starch. Starch is usually found accompanied by other reserves, such as protein, fat, and organic phosphates.

4.2.1 Structure of Starch

Within the past few years, new and improved methods of isolating starch and isolating starch components have induced a considerable renewal of interest

in research on the structure of starch. Previously, there had been a considerable diversity of opinion about starch fractions such as that starch could be separated into its components by making use of differences in solubility and the relative presence or absence of phosphorus or other inorganic constituents influenced the properties of the basic starch substance.

These older views have been eliminated, and at the present time an attempt is being made to ascribe the properties of starch or any of its fractions to the basic starch chain or its ramifications. On the whole, two extremes of starch types are recognised: amylose, simple straight chain starch, and amylopectin, in which the regularities of the fundamental starch chain are interrupted by frequent branching.

4.2.1.1 Amylose

In the linear fraction the glucose units are joined exclusively by a- 1- 4 glucosidic bonds. The number of glucose units may range in various starches from a few hundred to several thousand units. In the most common starches, such as corn, rice and potato, the linear fraction is the minor component and represents about 17-30% of total (Whistler and Paschall, 1965).

The multiple amylose theory of starch composition rests in the main on efficient methods of fractionating starch into its ultimate components. Quite recently new and improved methods of fractionation have been developed which allow the isolation of undegraded starch components which are basically different in structure and have quite different physical properties such as, hot

water fractions, selective alcohol precipitation and cellulose adsorption. Since the discovery of iodine, it has been known that starch is capable of reacting with iodine to give a deep blue colour, and there have been many investigations and speculations on the nature of this reaction. The characteristic blue colour of starch produced with iodine relates exclusively to the linear reaction.

Amylopectin is branched because of the occurrence of a-1,6 linkages at certain points in the molecules and gives a purple red colour with iodine (Whistler, 1964b)

The inclusion of iodine is due to an induced dipole effect and consequent resonance along the helix. Each turn of the helix is made up of six glucose units and encloses one molecule of iodine. The potential at which iodine is taken up during the titration of an amylose or other starch fraction is a haracteristic of the sample and is apparently a function of the chain length.

In amylose-iodine solutions oriented flow and X-ray diffraction indicated that asymmetric amylose molecules oriented roughly parallel to the flow lines and that in amylose-iodine solutions as well as amylose-iodine crystals the iodine molecules are tightly held in the centre of a helical amylose coil. During flow, the helices orient themselves roughly parallel to the flow lines and thereby orient the iodine molecules. In a kinetic study of the complex formation between amylose and iodine it was found that the rate constant of complexing was practically temperature-independent. This meant that complex formation is a diffusion-controlled process, and therefore it is reasonable to assume that helical segments do exist before complex formation.

One of the fundamental experiments supporting the helical-segment theory was a study of the viscosity of amylose solutions as a function of saturation by iodine. The viscosity remained unchanged until amylose became saturated with iodine, but increased thereafter. From this observation it was concluded that as long as the helical segments do not become filled up with iodine, the conformation of the molecule does not undergo significant changes. Since it was shown that the iodine complex had a helical structure, in aqueous solution amylose must be similar (Szejtli, 1982).

4.2.1.2 Cycloamyloses.

Some of the cyclodextrins can be considered as models for the helical conformation of amylose and that they bind other molecules in a similar, but not dentical, way. The cyclodextrine can be represented as a single unit of the long chain of amylose and its structure is similar to the amylose therefore its study is also relevant.

The cyclodextrins are macrocyclic nonreducing D-glucosyl polymers containing six or more residues bonded by a-(1-4) links. They are produced by the action of the amylase of Bacillus macerans on starch and related compounds. Two systems of nomenclature are in current use. The first of these indicates the number of residues in the cyclic polymer by prefixing Greek letter to the series name. Since the smallest known cycloamylose is a hexamer, it is assigned the prefix a. The cyclic heptaose, octaose, etc., are referred to respectively, as b, y, etc. dextrins. In the alternative system, the homologous are designated by the name cyclohexa-, cyclohepta-, and cyclooctaamylose, etc., the Greek prefix

corresponding to the degree of polymerization. The latter scheme is preferred because it is more descriptive of the structures. The cycloamyloses display a large number of "anomalous" chemical properties when compared to the flexible open-chain polymers. These unexpected properties must arise from the cyclic nature of the molecules.

Cyclodextrins have doughnut-shapes with all glucose units in substantially undistorted C1 (D) (chair) conformations. The secondary hydroxyl groups (on the C-2 and C-3 atoms of the glucose units) are located on one side of the taurus, whereas the primary hydroxyl groups are located on the opposite side of the taurus. The secondary hydroxyl side is more open than the primary hydroxyl side. The primary hydroxy groups can rotate so as to partially block the cavity, while the secondary hydroxyl groups on the relatively rigid chain cannot. The interior of the taurus consists only of a ring of C-H groups, a ring of glucosidic oxygen and another ring of C-H groups. Therefore, the interior of the taurus of cyclodextrins are relatively apolar compared to water (Szejtli, 1982).

One of the most important characteristics of cyclodextrins is the formation of inclusion complexes with various compounds (guests), in which guest compounds are included in the cavity of cyclodextrins (host). Guest compounds range from polar reagents such as acids, amines, small ions such as CLO-4, SCN-, and halogen anions to highly polar aliphatic and aromatic hydrocarbons and even rare gases.

Cyclodextrins are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity. The extent of

complex formation depends, however, also on the polarity of the guest molecule. Complex formation with molecules significantly larger than the cavity may also be possible in such a way that only certain groups or side chains penetrate into the carbohydrate channel (Klause and Lehn, 1977).

The rings of the higher cyclodextrins are highly flexible; as the cyclodextrine cavity diameter increases, the apolar cavity can accommodate a greater number of water molecules, therefore in aqueous solution these "complexed" water molecules will differ energetically less and less from the bulk of the solvent. Furthermore, the too wide cyclodextrine cavities do not enclose the guest molecules tight enough to prevent slip-out.

Other scientists believe that geometrical, rather than chemical, factors are decisive in determining the kind of guest molecules which can penetrate into the cycodextrin cavity to form an inclusion complex. Some others have pointed out that the structures of cyclodextrine inclusion compounds significantly differ in the crystalline state and in solution. In solution the guest molecule resides in the cavity, and the whole complex is surrounded by a solvate shell of water molecules. In the crystalline state, however, the guest molecules can be accommodated not only in the cavities of the cyclodextrine molecule, but also in the intermolecular cavity formed by the crystal lattice, while some of the cyclodextrine molecules remain unoccupied, or they include water (Szejtli, 1982).

The nature of the binding force still remains controversial. The interaction force for inclusion complex formation can not be classical apolar binding such

as that for enzyme-substrate complex formation, since inclusion complex formation is associated with a favourable enthalpy change and an unfavourable (or slightly favourable) entropy change. Usually apolar binding is characterised by a very favourable entropy change.

Several proposals were made to interpret this sizeably favourable enthalpy change.

a) Van der Waals interactions between guest and host

b) hydrogen bonding between the guest and the hydroxyl groups in the cyclodextrine.

c) release of high energy water molecules in complex formation

d) release of strain energy in the macromolecular ring of the cyclodextrine

In practice, the preparation of cyclodextrine complexes is always effected in aqueous solution, although it is reported that inclusion complex formation was feasible also in dimethyl sulfoxide or dimethyl formamide. However, it has been found so far that only water is suitable for the preparation of complexes, because from other solvents partially or exclusively solvent complexes will crystallise, or a ternary complex is formed. Water has a crucial role in the complex formation, since hydration of the cyclodextrine complex is energetically favoured as compared with the separate hydration of the components. This phenomenon is called hydrophobic interaction, and as a matter of fact primarily it is not due to the mutual attraction of the two components, but rather to the intrinsic cohesion of the water. There is namely a

decrease in the energy of the system caused by the increase of the solvent-solvent interaction, since the contracting surfaces between the solvent and guest molecule, as well as between the solvent and cyclodextrine cavity are reduced.

4.2.1.3 Fat in starch.

Small quantities of fatty substances are present in starch (less than 1%). Its presence significantly alters such properties as granule swelling, enzyme conversion and iodine affinity (Whistler, 1964a). This is the only case where extraneous substances are found naturally in close association with starch inside starch grains. The chlorpropham may behave like fats due to having similar polarity. The conditions used to defat starch are therefore relevant to the desorption of any other bound substances, especially those of low polarity.

The recognition of their presence entails extraction in solvents, and their identification usually involves the use of solvent systems before their degradation products can be determined. The major portion of the fatty material could not be removed by common fat solvents before acid hydrolysis. The extraction of corn starch in the soxhlet apparatus with nine different solvents showed that the polar solvents yielded generally larger amounts of fatty materials. Corn, rice, sago, cassava, potato, and horse chestnut starch contain 0.61, 0.83, 0.11, 0.12, 0.04, and 0.56 % respectively by acid hydrolysis. The lipids percentage in potato, corn, sorghum, wheat, and rice starches could be extracted as 0.20, 0.41, 0.32, 0.24, and 0.18 % respectively by methanol, while acid hydrolysis yielded 0.12, 0.57, 0.72, 0.42 and 0.67% fat content for the respective starches (Whistler and Paschall, 1965).

The small samples may be efficiently defatted in extractor with ethanol or methanol and the completely removal of the fatty acids from corn, wheat, and rice starches could be achieved by the water-miscible solvents. The alcohol extraction of fat is essentially a desorption, therefore several extractions will be needed to reduce the fat content of corn starch from 0.65 to 0.04%. Hot extraction either by digestion or boiling in solvent is the most effective means of desorbing fatty material, however it may cause mechanical damage within the granule or produce some slight change in the physical organisation. %(Whistler, 1964a).

4.2.2 Potato Starch.

The starch in potatoes is normally 10-15% of the tuber weight depending on varieties and other factors. The distribution of the starch in the tuber follows that of the dry matter; in other words, the percentage of starch rises from the skin inwards as far as the vascular system, and decreases from the vascular ring inwards to the central medullary region, while the heel end tends always to contain more starch than the rose end (Burton, 1966).

The grains of potato starch are usually simple and compound grains rarely occur. In polarised light there is a well-defined cross, centring on the hilum. It is stained golden yellow by Metachrom Red G. Agfa; blue by a mixture of Methyl orange, Fuchsin and Methyl Breen; pink by Natural Red and it gives a more intense blue-black coloration with iodine/potassium iodide solution than does, for example, wheat starch (Burton, 1966).

Potato starch is an extremely hygroscopic substance, the commercial product is delivered with 20% moisture. Air drying in a heated laboratory usually lowers the moisture content to 12-20%. More intensive drying procedures have a tendency to produce fractures in the starch granules which, in turn, affect some properties. Dewatering with agents such as ethanol, ether, and acetone produces similar effects (Whistler, 1964b).

From the above literature survey and discussion it is concluded that the starch has the capability to hold some components in it. Obviously the holding capacity of starch is dependable on different factors and the desorption of components from the starch could be varied in different situations. This chapter is actually an effort to find out if any amount of chlorpropham, can remain or be bound to the starch structure. Therefore a series of experiments were developed to find out the amount of chlorpropham, that could be bound to the starch of potato. For this purposes the effect of microwave cooking and boiling on the level of chlorpropham in treated potatoes were studied. Beside this pure starch of treated potatoes was also extracted with polar solvents to remove any bound chlorpropham which could not be extracted with normal solvents. The next part of this chapter is the description of the techniques used and the results which were noticed during this trial will be discussed.

Section 1:

4.3 PROCESSING OF POTATOES.

4.3.1 Introduction.

The study was carried out to find out any effect of processing on chlorpropham level of treated potatoes. Microwave and boiling are two common techniques that have been used for processing of food. The processing of potatoes with a microwave is getting popular. New products like micro wave chips have great potential value to the chip industry and are a valuable outlet for chip processing. The use of microwave processing of potato chips enables processors to produce desirable colour that would be useless when fried conventionally. Similarly boiling is very common and daily used in homes for potato products. Due to the unique characteristic of microwave processing, it penetrates very quickly into the centre of food. In conventional methods heat passes slowly into the food through conduction. Therefore this study will be a useful step in comparing the effects of these two different kinds of processing.

Recently microwave techniques have also been used for analytical purposes, to extract pesticides from soil and food samples. The microwave extracting methods are very popular for extracting pesticides due to its unique properties, faster and better recoveries. The penetration of the microwave energy travels through the moisture distributed in the food. Therefore water plays a very

important role in microwave techniques and its distribution pattern could effect differently. Microwave heating produces pronounced non-uniform temperature distribution in certain geometries. This leads to large temperature gradients inside the food material. Therefore the contribution of temperature gradient to overall moisture transfer during microwave heating is expected to be larger than in conventional heating methods. (Zhou et al., 1994).

In this experiment the microwave cooking method was used to process the tubers to find out any effect on chlorpropham level of treated tubers and compared with conventional methods of cooking like boiling. These are popular methods of cooking, therefore to see their effect would give vital information about chlorpropham levels being consumed after cooking of treated tubers. The comparison will also be anticipated to understand the behaviour of chlorpropham in potatoes.

4.3.2 Materials and Methods.

4.3.2.1 Sample Preparation for Microwave Cooking.

The peeled potatoes were subjected to microwave cooking. These potatoes were taken from the commercial store where they had been treated with the sprout suppressing chemical chlorpropham and stored at 8-10 ^{0}C. The sample was taken at the end of the storage season. The potato sample of relatively uniform size, of 4kg weight, was selected and then washed with cold tap water. The washed tubers were then peeled manually with a stainless steel knife. The peel was removed with care to have uniformity in thickness in all samples. The total peel found was 15 % of total weight of sample taken.

A few potatoes were randomly taken, before cooking, to determine chlorpropham level. Each tuber was considered as a replicate to assess the variation of chlorpropham level between them. The rest of the peeled potatoes were divided into five groups, containing each of three whole peeled potato with average weights of 500 gm (Table 4.1, section 4.3.3.1).

The peel of potatoes was also subjected to microwave cooking. The peel was cut into small pieces to get a homogenised sample and some of the peel was taken before cooking for measuring chlorpropham level on fresh basis. The rest of the peel was divided into two equal parts for cooking in the microwave.

The whole peeled washed potatoes were put on a glass plate with a small amount of water (20 ml). The sample was loosely covered with another glass plate. The tubers were cooked for 4, 8, 10, 12 and 16 minutes on maximum power of 850 W, Matsui M184S, microwave. The tubers were turned over during cooking half way through the processing time to uniformly expose all areas. After cooking, the tubers were placed outside openly to cool down the tissues before the extraction for chlorpropham. Each tuber was chopped separately, and detail of extraction method of analysis is given in chapter two.

Similarly the peel of the tubers was also cooked in the microwave, for three and six minutes on the same microwave power. The sample was placed on a plate containing some water (20 ml) covered with another loose glass plate. At half way of the cooking time, the sample was turned over for uniform heat to all sides. The cooked peel was cooled down before extraction by reflux method.

4.3.2.2 Sample Preparation for Boiling of Potatoes.

These tubers were taken from commercial potato store, stored at 8-10 ^{0}C, where tubers were treated with chlorpropham at normal rates (20mg/kg). The samples were taken at the end of the storage season. The tubers of relatively uniform size were chosen and washed with cold tap water. Each sample was then divided into two equal lots for peeled and unpeeled sample, and chlorpropham was also determined before boiling.

The peeled and unpeeled washed potatoes were placed into two separate beakers with distilled water. The water was enough to cover the potatoes completely and boiled for 30 minutes. After boiling the potato pieces were placed on a glass dish to cool down before chopping and extraction with hexane.

4.3.3 Results and Discussion.

4.3.3.1 Microwave Cooking.

The effect of microwave cooking with time is presented in Table 4.1 and 4.2. It is clear from the Table 4.1 that the microwave cooking has an effect on total extractlability of chlorpropham in treated potato. The total extractable chlorpropham in the fresh treated potato decreased overall during cooking. The decrease in the amount of the chlorpropham by microwave cooking alsohas been reported earlier by Mondy et al., (1993). The level of chlorpropham decreased from 1.09 mg/kg in fresh to 0.59 mg/kg after cooking in microwave. However by looking closely at the results presented in Table 4.1, it can be noticed that the extractable amount of the chlorpropham varied during cooking time and has not

Table 4.1 Effect of microwave processing duration on chlorpropham residues level of washed, peeled commercially treated tubers.

	Processing time (minutes)					
Chlorpropham (mg/kg)	0	4	8	10	12	16
extractable	1.09	0.52	0.24	0.33	0.68	0.59
extractable ratio	1.00	0.48	0.22	0.31	0.63	0.54
Stdve		0.028^{ADE}	0.049^{B}	0.113^{E}	0.014^{D}	0.079^{AB}
loss %	nil	52	78	65	38	46
moisture loss %	nil	7	11	12	17	22

Where similar letters shows non-significant difference.

Table 4.2 Effect of microwave processing on chlorpropham residues level in the peel of washed, commercially treated tubers.

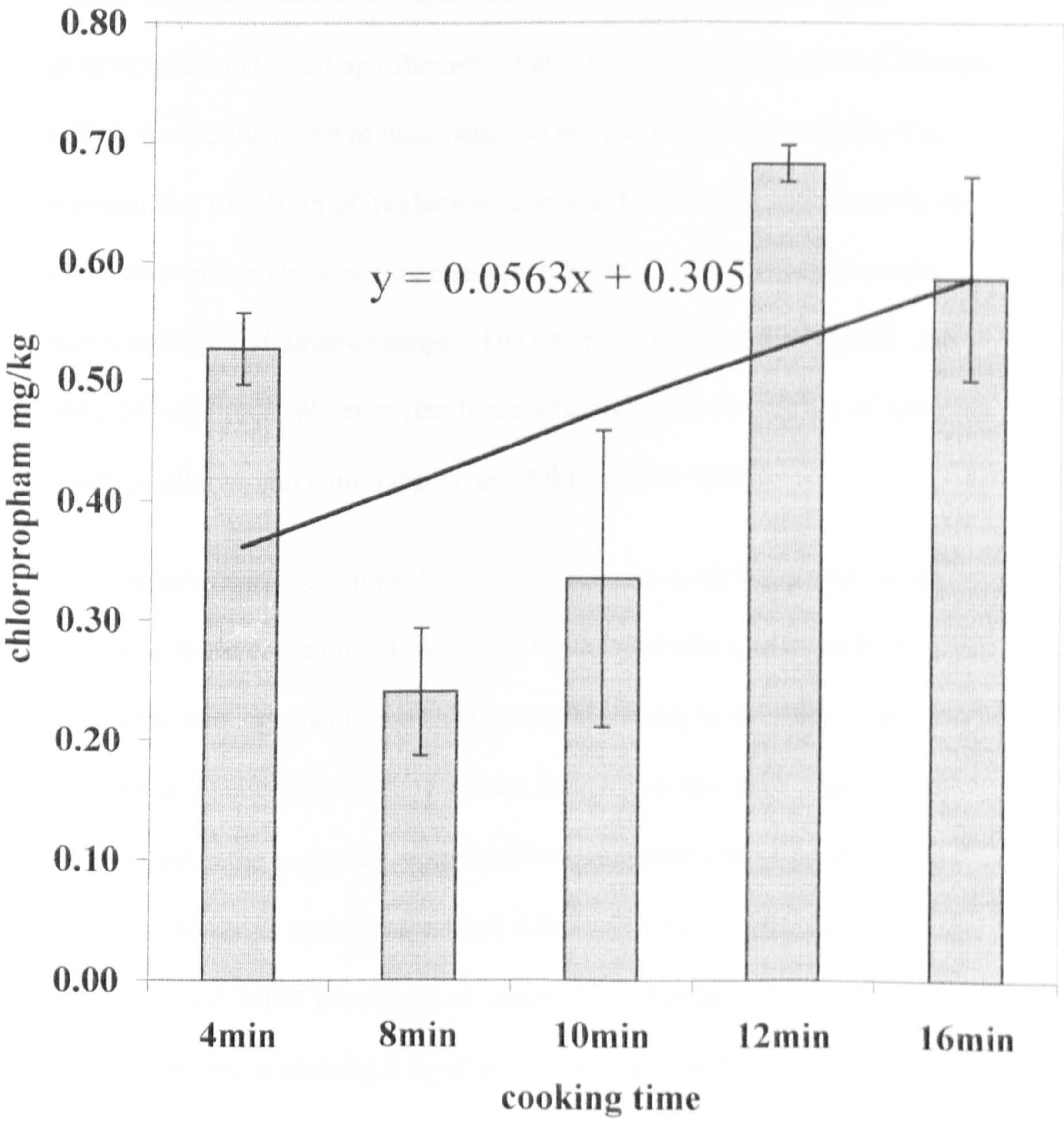
	Processing time(minutes)		
Chlorpropham (mg/kg)	0	3	6
extractable	18.74	15.5	21.26
extractable ratio	1.00	0.83	1.13
loss (%)	nil	17.29	-13.45
moisture loss %	nil	1.19	2.15

decreased continuously throughout the period. The extractability of chlorpropham over the total cooking period can be separated into different parts with respect to time interval. There was rapid decrease in extractable chlorpropham amount in the first 8 min of cooking, here it decreased from 1.09 mg/kg to 0.48 and 0.22 mg/kg after 4 and 8 minutes of cooking respectively. In second phase, up to 12 minutes, the extractability of chlorpropham started increasing (or less reduction) as compared to the first 8 minutes of cooking. Here the maximum chlorpropham level was found after 12 minutes as compared to all processed samples. In third phase, after 16 minutes, although the amount was lower than from second phase, it was higher than it was in first phase of processing.

The ratio of extractable chlorpropham with respect to fresh sample was plotted in a graph as is shown in Fig 4.1. The trend line was dropped on the ratio, which shows an increase in chlorpropham ratio with respect to cooking time. The increasing level in extractable chlorpropham by microwave cooking has not been reported earlier. The increased trend in extractability of the chlorpropham indicates bound chlorpropham in potato components. As cooking time increases, the tuber components start to release bound chlorpropham.

The level of chlorpropham was much higher, on fresh basis, in the peel of potato compared to flesh (Mondy, 1993). Due to short time only two time intervals could be done which shows that the chlorpropham reduced from 18.14 mg/kg in fresh peel to 15.5 mg/kg after 3 minutes of cooking, while the level of chlorpropham apparently increased after 6 minutes of cooking time which was 21.36 mg/kg as compared to the fresh base level (Table 4.2).

Fig. 4.1 Effect of duration of microwave cooking on chlorpropham level of washed peeled, treated potatoes.



Overall there was a non-significant effect on the available amount of chlorpropham in the peel after cooking in microwave but the heat was the same as noted above.

Cell size, cell wall polysaccharides, non-starch polysaccharides and pectic substances are textural governing factors (Jaswal, 1991).The texture has been related to two dramatic changes occurring during heat treatment, starch gelatinization and swelling (Shomer, et al., 1994). Muzimbaranda and Tomasik, (1994) studied the effect of microwave on the potato and maize starch. It is reported that first 5min of irradiation of potato starch brings no changes in its granules expressed by viscosity changes. After that induction period potato starch undergoes dramatic changes. The viscosity of its gel decreases by one order of magnitude. Water in starch can be adsorbed on the surface of granules, inside capillaries and within the cavity of the amylose helix.

The microwave technique has recently been used for the extraction of pesticides. Most commonly it been used for the pesticide extraction from sediments, like organochlorine and organophosphorus in the onion were determined by microwave where a home microwave was used. The samples were placed in the beaker by wrapping the beaker with clear film then it was heated for 60 sec in a microwave oven (Okaihashi et al., 1996). Another study was carried out where the effects of microwave exposure to pesticides like Iso-octane solutions, containing 2 ug of both endrin and dieldrin, were studied. Here no evidence of any breakdown or alteration in the pesticides (Onuska and Terry, 1993) were noticed. In the present study the total chlorpropham in the peel of treated potatoes remained unchanged even after maximum microwave cooking

time. Therefore the reduction in the chlorpropham level in the peeled tubers could not be just due to evaporation of chlorpropham. It could be penetration toward the central portion of the tubers or it could be formation of a CIPC complex with potato components. However it would require a detailed study to properly understand the fate of chlorpropham in potatoes after cooking by microwave.

However the moisture content of potato in the flesh and in the peel continuously decreased with respect to the time of cooking. The reduction of moisture was directly related to the cooking time of the tubers. The moisture percent reduced by 6.86, 11.39, 12.02, 17.35 and 22.12 % after 4, 8, 10, 12, 16 minutes of cooking in peeled samples. The samples became slightly brownish due high temperature, which could cause nonenzymic browning (Mudahar et al., 1990).The water content in the peel was also decreased with respect to the time interval. The moisture loss was 1.19% and 2.15% after 3 and 6 minutes of cooking respectively. The moisture loss was actually a reduction in the weight of sample during cooking (Tables 4.1, 4.2).

4.3.3.2 Boiling of Potatoes.

The boiling of treated tubers, taken from commercial stores was also carried out. Peeled and unpeeled potato were boiled in water to study its effect on chlorpropham level. The results of boiling on chlorpropham level is presented in Table 4.3. It was noticed that the level of chlorpropham has significantly decreased by boiling in both unpeeled and peeled tuber samples. However the effect of boiling was much greater in unpeeled tuber samples than

Table. 4.3 Effect of processing on chlorpropham residues level of washed, treated tubers.

Processing Method	sample	replication	Chlorpropham mg/kg raw sample	processed sample	ratio[a] & Stdve.
Microwave					
	Peeled	15	1.09	0.47	0.43^{A}+0.165
	Peel	6	18.74	18.38	0.98^{B}+0.172
Boiling					
	unpeeled	3	2.29	0.85	0.37^{A}+0.131
	Peeled	3	0.61	0.33	0.54^{A}+0.117

a- average chlorpropham before processing/average chlorpropham after processing. Where the different letter shows significance different.

it was found in peeled tubers. The raw unpeeled tubers contain 2.29 mg/kg, which decreased to a level of 0.92 mg/kg after boiling in water (Table 4.3). While in the case of peeled tuber samples, which contain much less chlorpropham, the level of total available as compared to unpeeled raw potatoes, has reduced from 0.61 to 0.36 mg/kg after boiling. The reduction of chlorpropham was less in peeled as compared to unpeeled tubers. Almost 41% reduction in total chlorpropham was recorded as compared to 61% in unpeeled tubers.(Table 4.3).

The total chlorpropham was much higher in fresh tuber peel. The distribution ratio of chlorpropham in peel and flesh was 1: 0.27. Therefore peeling of tubers reduced the higher amount to 73% of total available, of the chlorpropham.. The reduction by peeling has been reported by many other authors (Boyd, 1988 and Mondy et al., 1992). Working on the fate and distribution of labelled chlorpropham, it was reported that more than 70% of the applied chlorpropham was recovered in the peel extract, 5% was recovered from the 10-mm layer beneath the peel, and 0.2-0.4% was in the extract from the interior tissues (Coxon and Filmer, 1985).

The reduction of the chlorpropham level by boiling has been reported (Mondy et al.,1992, 1993). In their study the tubers were dipped in chlorpropham solution, while here the tubers were taken from the commercial treated stores after six month of the treatment with aerosol fogging of chlorpropham. The aerosol fogging would have different impact on tubers than by simply dipping in chlorpropham solution. The aerosol fogs contain very fine particles which would have the tendency to penetrate more deeply into the skin

and become tightly bound with tuber components. As it was described in the previous chapter the washing does not effect significantly the reduction of chlorpropham level, while in their study the washing was effective and reduced 60-70% of the chlorpropham.

The chlorpropham level in peeled tuber remained less effected by boiling as compared to unpeeled tubers. The ratio of decreased chlorpropham, from the total amount before boiling, was 0.85 and 0.33 in unpeeled and peeled tubers. The different effect in both samples could be due to many reasons. The higher amount of chlorpropham in unpeeled tubers as compared to peeled, before boiling, so its loss was greater in unpeeled than in the peeled sample. The chlorpropham might be not bound so effectively in peel as compared to flesh of the samples.

Otherwise the other possibility could be that chlorpropham was in a complex with starch in peeled sample, the boiling of unpeeled tubers causing the chlorpropham to transfer from peel to the central part of the tuber where it could be bound with the starch of the tubers. Therefore the less available amount was not actually the reduction of the chlorpropham by boiling but was the transfer from the peel to starch and the formation of a complex.

It can be observed from Mondy et al., (1992) that the total amount in water plus tubers was 15% after boiling in respect to total amount applied to the tubers. This meant that the rest of nearly 85% of chlorpropham was missing that possibly could be in complex form and was not easily extractable. Therefore the loss by the boiling in the water is not actually total leaching into water, but a

possibility of transfer of chlorpropham to a more deep position into the centre section of tubers after boiling in water.

In the boiling in water swelling of cell wall is obvious and in heated potato tuber tissue, the cell swelling has been suggested to be dependent on two main factors, attractive and repulsive forces acting in the diffuse electrical double layer at the cell wall (CW) fibril surfaces and inflation of the cell by heat-gelatinised starch. Diffusion of starch from potato tissue during cooking had been ascribed to cell rupture.

However, starch leakage has been found to occur during boiling of potato tissue even when the cell wall (CW) remained intact. It was reported that the leakage was higher from the cortex than from the pith and higher from stored than from fresh potatoes, and it increased with boiling time. The heat-gelatinised starch is found in various sol-gel states, but only the soluble molecules have been found to leak into the boiling water: therefore, the leakage of starch may depend on the CW porosity of the cell (Shomer et al., 1994).

A study was carried out (Mondy and Ponnampalam, 1983) on the effect of baking on the major macrominerals present in potatoes. Conventional baking of potatoes resulted in significant losses of potassium and phosphorus contents from the cortex tissues and a significant increase in potassium and phosphorus in the pith tissues. Such compositional changes suggest that potassium and phosphorus transfer from cortex to pith tissues occurs. The higher potassium concentration in the pith tissues is probably due to dehydration and cellular damage of cortex tissues on prolonged heating which could cause a diffusion

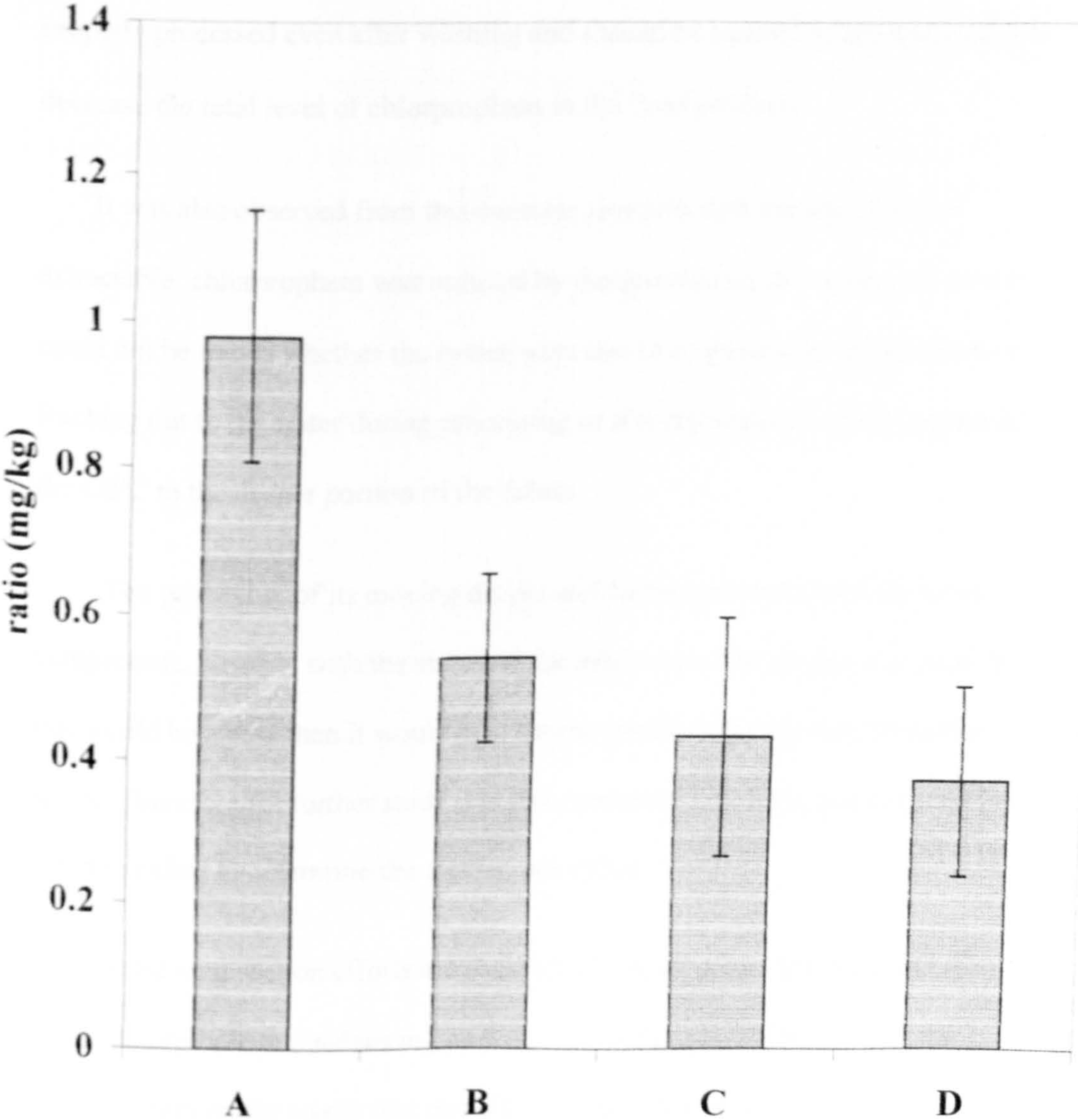
gradient for the movement of potassium from cortex to pith. The same trend was found for the iron and aluminium. The iron, copper and zinc are present in potatoes as protein complexes and the increased iron in the pith area following baking may have resulted from protein degradation in the cortex area. The reduction of the chlorpropham by the two processing methods in this study could be a transfer from one site of the tuber to other or it may a formation of CIPC complex with tubers components.

The overall effect of processing techniques on chlorpropham level was plotted in Fig. 4.2. The Figure showed the ratio of chlorpropham present after processing of potatoes. It was clear from Fig. 4.2 that boiling has a greater effect in reduction of chlorpropham in unpeeled tubers, while on the other hand microwave heat remained totally ineffective on peel of the tubers. The effect on the peeled tubers regarding chlorpropham ratio was relatively the same both in boiled and microwave samples. The higher effect of boiling in reduction of chlorpropham in the unpeeled tubers compared with peeled showed that boiling has heated up the surface of the tubers and could not penetrate into the centre of the tubers hence it might be a chlorpropham transfer from the surface to the centre of the tubers.

4.3.3.3 Conclusion

It was concluded from these experiments that the amount of extractable chlorpropham was significantly reduced after processing the treated tubers by two different processing methods. The total percent reduction of the chlorpropham was higher after microwave cooking for 16 minutes (57%) as

Fig. 4.2 The effect of method of processing on chlorpropham level.



A(microwaved peel), B(boiled peeled), C(microwaved peeled tubers), D(boiled unpeeled).

compared to 20 minutes boiling of peeled treated potatoes (46%). It was also concluded that duration and the power of the microwave cooking can differently affect the level of chlorpropham. The microwave cooking of washed potatoes can reduce the amount of extractable chlorpropham by about 50% or over. However it is suggested that for home consumption the treated tubers should be properly processed even after washing and should be peeled before processing to decrease the total level of chlorpropham in the final products.

It was also observed from this exercise that although the total level of extractable chlorpropham was reduced by the processing, due to lack of time it could not be traced whether the losses were due to evaporation, degradation or leaching out to the water during processing or if it represented actual transfer of the CIPC to the deeper portion of the tubers.

The possibility of its moving deeper and forming complexes with tuber components, possibly with the starch of the tubers could be proved occurred. If this would be a case then it would be a serious problem for consumer's health safety. Therefore for further study it is recommended that these points should be clearly studied to determine the mechanism of loss.

In the next section efforts are focused on starch to find out any relation to bound chlorpropham residues in potatoes. The adsorption/desorption of the chlorpropham on the starch was carried out, where the treated tuber starch was also subjected to extraction with polar solvents. It was an expectation that any bound chlorpropham in the starch would be extracted in greater amount with polar solvents as compared with ordinary extracting solvents.

Section 2:

4.4 CHLORPROPHAM EXTRACTION FROM STARCH

4.4.1 Introduction.

From the previous experiment it was realised that chlorpropham in the tubers could be tightly held by the tuber components. The starch is one of the most important components, which can be studied in relation to the bound chemicals. One of the most important characteristics of both starch and cyclodextrins (hosts) is the formation of inclusion complexes with various compounds (guests). The guest compounds are included in the cavity of cyclodextrins.

The extent of complex formation depends, however, also on the polarity of the guest molecule. Complex formation with molecules significantly larger than the cavity may also be possible in such a way that only certain groups or side chains penetrate into the carbohydrate channel. The starch products are being proved suitable candidates for application both as filler-binders in tablet formulations for direct compression in controlled release systems (Wierik et al., 1996). The starch can adsorb dyes, the extent of adsorption on starch related to the ratio of amylopectin to amylose (reviewed by Boki et al., 1991). Therefore there is a chance that the chlorpropham in the tubers could make a complex with the starch which could not be extracted with normal solvents.

In the following experiment an attempt was made to find out if there was chance of the chlorpropham being bound to the starch of the tubers. An attempt was also made to understand this bonding behaviour and whether or not it could be extracted from the tubers.

4.4.2 Chlorpropham Extraction from Treated Potato Starch.

There is no information on chemical residues in starch manufactured from treated potatoes. A stepwise study has been made on this matter. The potato sample was extracted after washing with cold water before blending, and then the filtrate was extracted after separating the starch, and lastly the pure starch of the potato separated from this procedure was extracted with methanol and hexane solvents.

4.4.2.1 Materials and Methods.

4.4.2.1.1 Extraction of Starch.

The potatoes were taken from the commercially treated potato store, McCainScarborough, from top front position. These potatoes basically were sent to the laboratory for chlorpropham residues on 20th May, 1996. Two kg were taken as sample for the extraction of the starch. The tubers were then washed with cold tap water with slight brushing to remove the soil on the surface of tubers. These tubers were then cut into small pieces, placed immediately in the water containing a small amount of sodium bisulphite, to inhibit any browning.

These potato pieces were then minced in the high-speed electric mincer with the addition of aqueous sodium bisulphite to get a homogeneous sample.

This slurry was then screened through muslin cloth. During screening it was washed again and again with water containing sodium bisulphite. The screened material was the mixture of starch, water and pieces of potatoes. The starch in the beaker then started to settle down in the bottom of the beaker. It was allowed to settle for 24 hours. All the supernatant and liquid was decanted. After decanting the supernatant liquid the starch layer was reslurried in water and rescreened on a much finer sieve. The residue on the screen was discarded; filtrate was collected and was dried in the open air.

4.4.2.1.2 Chlorpropham Extraction.

The tubers were taken from a commercially treated store for starch extraction. These tubers were washed with cold tap water. The tubers were extracted before separating the starch by using hexane and methanol solvents for two hours for estimating chlorpropham levels in them. The extracting method for the chlorpropham was the same as described before in Chapter Two.

The 30 gm of clean dry starch, with three replication, was extracted with two different polarity solvents hexane and methanol for any amount of the chlorpropham. The extract was collected and evaporated to inject in to the GC. The methanol and hexane extracted samples were again reextracted for another two hour to find out if any more chlorpropham remained in these starch samples. The extraction procedure was followed as described in detail in Chapter Two. Similarly the filtrate of the tubers was also extracted with hexane and methanol for two hours. These samples were tested for starch by the iodine method and were significant amounts of the starch were still present.

4.4.2.2 Results and Discussion.

The pure starch, separated from the treated potatoes stored at *8-10* ^0C *in* commercial potato stores was subjected to extraction with hexane and methanol. The starch was extracted with a higher polar solvent to find out any amount of the chlorpropham which is non extractable with hexane that could be removed with methanol. The results of the extraction with the two solvents are given in the Table 4.4.

The Table 4.4 shows that hexane was unable to extract any amount of chlorpropham in the treated potato starch, while methanol gave substantially amounts of chlorpropham. The results indicate that the amount of chlorpropham was negligible with hexane while methanol extracted 1.37 mg/kg of chlorpropham. The findings revealed that some of the amount of chlorpropham was bound in the starch which was unextractable with normal solvent, like hexane, and when it was subjected to a more polar solvent, methanol, this released significant amounts of chlorpropham. Any pesticide residue not extractable and detected by recommended methods of residue analysis but remaining bound with theconstituents may become a potential hazard to consumers, and, therefore both quantity and nature of these residues must be determined (Singh et al., 1993).

The methanol solvent, has been used for defattning of starch and therefore potentially it has a capability to extract bound chlorpropham from potato starch. There is no earlier study regarding the chlorpropham residues in treated starch.

Table 4.4 The chlorpropham residues (mg/kg) of treated tubers, and starch by hexane and methanol.

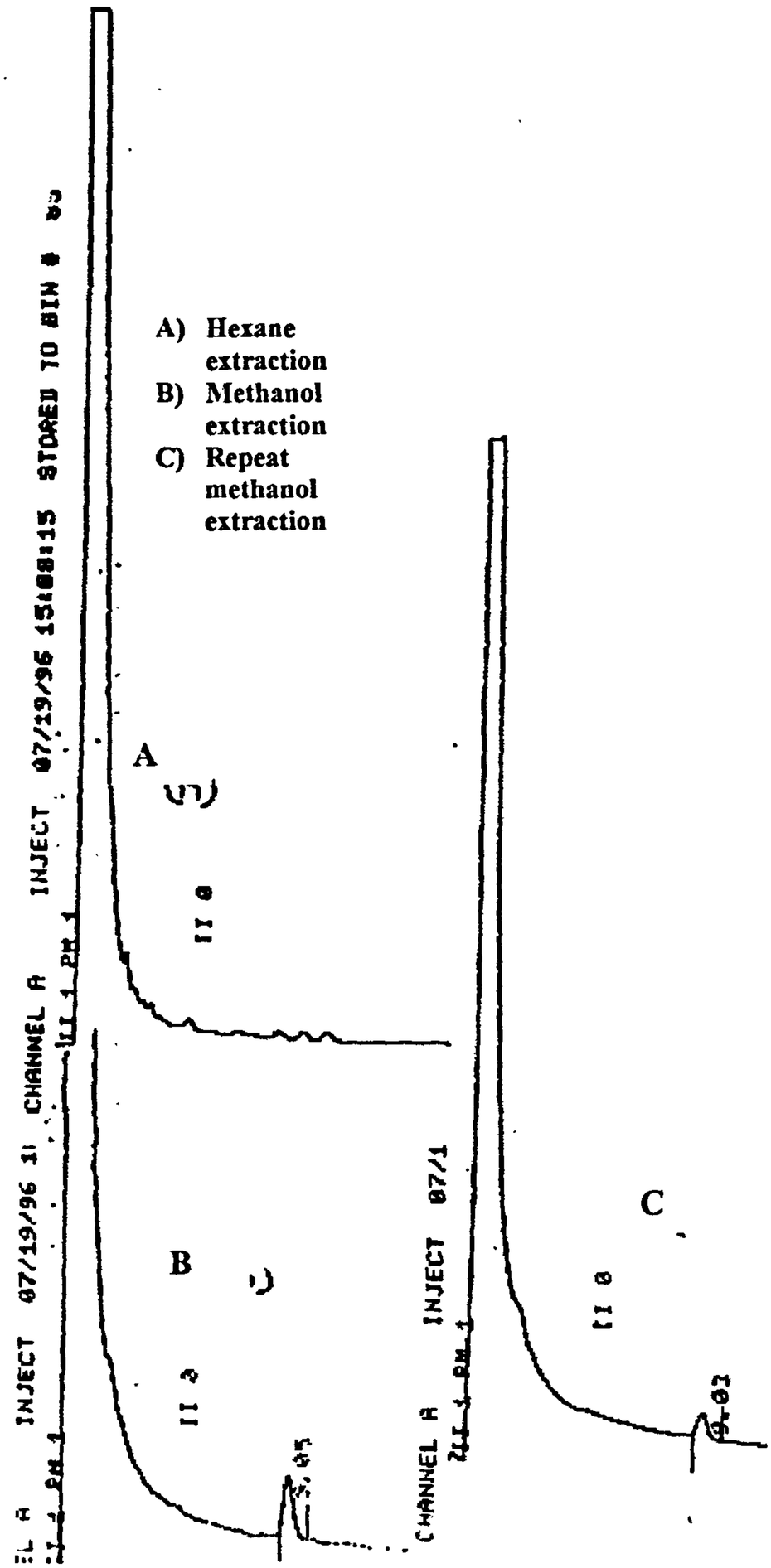
Sample	Hexane	Methanol
washed tubers		
A	0.56	0.59
B	1.16	0.48
C	1.35	0.49
Mean	1.02	0.52[a]
blending		
before	2.12	2.64
filtrate	2.29	2.66
starch	nil	1.37
		0.57[b]
		0.19[c]

a-Re-extraction with methanol after hexane extraction.
b-Re-extraction (second attempt).
c-Re-extraction (third attempt).

However Hasegawa et al., (1992) did a step wise extraction of treated tubers which also includes starch. It is reported that washing has removed highest amount of chlorpropham, almost 88% of the total chlorpropham applied on potatoes. It was also reported that below 1% of total chlorpropham was in the starch of treated tubers. However their extraction procedure was with a normal solvent hexane and the method of chlorpropham application was quite different as from the samples studied here.

Therefore higher amounts of chlorpropham in the starch could be expected in this situation. Although this level of chlorpropham was the reflection of one sample, the chromatograph (Fig. 4.3) clearly showed a significant difference. The method of extraction applied here was able to detect the chlorpropham level below 0.02 mg/kg, therefore this amount in the chromatograph could not by any means represent the noise of GC. It is clear from the chromatogram that the peak was at the proper retention time and was reproducible (Fig 4.3). When the extracted sample of treated starch with methanol was re-extracted with methanol for a longer time, more chlorpropham was recovered. The amount extracted was 0.572 and 0.188 mg/kg at the second and third attempt respectively after extracting 1.37 mg/kg at the first extraction stage. This means that chlorpropham was in a tight enough complex with the starch structure, to ensure that it could not be released with a single attempt and needed more severe conditions or more re-cycling of extractant to completely remove it from the starch. The traditional solvent based methods, often do not remove all the residues. Therefore it is likely that the residues may have been underestimated (Khan, 1995). As is reviewed above, in amylose-iodine solutions as well as amylose-iodine crystals

Figure 4.3. Extraction of chlorpropham from treated potato starch.



the iodine molecules are tightly held in the centre of the helical amylose coil. Therefore the chlorpropham in the starch could be bound in centre of helical amylose coil. It is therefore difficult to remove the chlorpropham with ordinary solvents from the amylose coil. On other hand complexes of fats with starch would give higher recovery of fat with more polar solvents and with longer extraction times (Whistler, 1964a).

The major portion of the fatty material could not be removed by common fat solvents before hydrolysis. It is reported that after the removal of extraneous fat from starch with petroleum ether, subsequent acid hydrolysis liberated an additional 0.5-0.6% of fatty acids. Higher recovery of fatty materials could be ielded with highly polar solvents, and addition of some water (15 and 20 %) to polar solvents such as methanol and dioxan is reported to be most effective in the removal of fatty substances (Whistler, 1964a). This means that complete extraction of the chlorpropham from the starch would need acid hydrolysis or extraction with a combination of different solvents. Therefore the present reflux method for the extraction of CIPC should be modified to yield maximum extraction of bound residues of chlorpropham in the treated tubers.

The extraction of chlorpropham from the filtrate and the whole washed potatoes were also carried out with hexane and methanol extracting solvents. The methanol solvent has extracted a higher amount of chlorpropham than hexane (Table 4.4, page 202). Extractable levels were 2.64, 2.66 mg/kg and 2.12, 2.29 mg/kg of the chlorpropham residues by methanol and hexane in whole washed tubers and blended filtrate respectively. Here it waş also noticed that the filtrate which was blended at high speed for separation of starch had

higher amounts of CIPC than were found in the whole washed tubers before blending. This trend has been already described i.e. the level of chlorpropham was surprisingly found greater in sliced potato samples than in the whole, unsliced potatoes (Lewis et al., 1996).

Apart from this stepwise extraction, a few more washed whole tuber samples, selected from different sites of the commercial store, were also extracted with hexane and then reextracted with methanol solvent (Table 4.4, page 202). Each sample was considered as one replication as they were selected from different sites of the treated store. Here again methanol extracted additional amount of the chlorpropham from the samples which were already extracted with hexane solvent. Another interesting point emerging from these results was a fixed amount of chlorpropham extraction on reextracting with methanol. The chlorpropham residues which were 0.56, 1.35 and 1.16 mg/kg from hexane extraction, after re-extraction with methanol gave an additional 0.59, 0.49 and 0.48 mg/kg respectively. It might be thought that this fixed amount of chlorpropham may a representation of any bound chlorpropham residues, which was not extractable with hexane alone.

In these samples as compared with starch the evaporation of the extract particularly with methanol was much more difficult. A lot of water was extracted along with other material in case of methanol extraction, and it was difficult to evaporate down the sample enough for GC injection. It was tried to separate the extraction into two phases, hexane and methanol, by using separating funnel. The two resulting layers of different solvents were then analysed separately, but this approach was not successful. The amount of

chlorpropham was small in the hexane layer so again a lot of chlorpropham remained in the methanol. It was also considered to pass the extracted sample through a C18 cartridge but, again, as the extraction contains a large amount of methanol this approach was also not applicable.

Therefore the methanol extract remained unevaporated and it was as such injected into GC. The total volume of sample was measured before injecting into GC, and the total amount was calculated on the basis of total volume of sample. The chromatograph peaks of chlorpropham were distinct which was not expected. It could be due to the smaller amount of water in sample as compared to methanol amount in the extraction sample. However the peaks were not always good and reproducible depending upon the water to methanol ratio in the sample, therefore many samples were rejected.

However extraction of additional chlorpropham by methanol particularly from treated starch have lead toward a new theory concerning the total amount of chlorpropham in the potatoes. If the starch content is considered as 10% of fresh weight of the tuber then total amount of chlorpropham extracted from starch would be an additional 0.213 mg/kg, above the total amount in washed tubers extracted by the hexane. Thus the additional chlorpropham extracted by methanol is almost 6% of the total amount extractable with the hexane by the reflux extraction method. The manner and amount of chlorpropham attachment may depend on storage period, storage environment and application method of chlorpropham in commercial stores.

4.4.3 Adsorption/desorption of Chlorpropham in starch.

From the previous experiment it was a feeling of some bound nextractable residues of chlorpropham in treated tubers. When treated potato starch was extracted it gave clearly higher residues of CIPC by methanol comparing to hexane. Therefore a further experiment was set up, which was focused on the starch isolated from potatoes. In the following experiment an effort was made to form a potato starch :chlorpropham complex. The purpose of the experiment was to understand whether there is any chance of chlorpropham-starch complex formation by eluting it with solvents of different polarity with a quantitative approach. The following method was developed for this study, and the details of the experimental set-up are given in the following section.

4.4.3.1 Material and Method

4.4.3.1.1 Spiking of Starch.

The most common procedure for preparation of inclusion complexes is to stir or shake an aqueous solution of cyclodextrine with the guest molecule or its solution. Using a common solvent, different but miscible solvents, different immiscible solvents, or no solvents at all may carry this out. The driving force of complex formation is either a decrease of the ring strain resulting from complex formation or the removal of the high energy water molecules from the cavity; in any case, there must occur a decrease in the free energy of the system (Szejtli, 1982). A similar approach was used for addition to starch.

The potato starch was spiked with different standard solutions added with chlorpropham. The standard solutions in different solvents were made, details are given in the Table 4.5. The starch sample was taken from the laboratory. The potato starch was clean and white in colour. The starch was weighed, detail given in the Table, and put in the standard solutions in the beaker. The beaker was covered with aluminium foil, placed in the automatic electric shaker for 24 hours. The shaker was set on the 120-rev/min speed at temperature 25 0C. After shaking, the beakers were taken out and the starch allowed to settle down for another 24 hours.

The starch was then separated by passing through the Buchner funnel with filter paper on it. The separated starch became almost dry but it was put into the open glass plate for another 24 hours at room temperature to get enough dry uniform starch. However the weight of starch increased compared with the dry starch before adding to solution. The weight increase was due to higher water content in treated starch.

The remaining solution after separating the starch, was measured by volume, cleaned solution injected to GC for determination of chlorpropham remained. The values were then compared with the fresh standard solution. In case of water spiking media the water was first passed through the C18 cartridge. The cartridge was standardised and conditioned with 3 ml of methanol and 3 ml of deionised water. The sample water passed through the cartridge, at the end the cartridge was washed with 5ml of deionised water. The compound (chlorpropham) was then eluted with 2 ml methanol. The eluted compound in methanol was injected into the GC for determination of chlorpropham.

Table 4.5 Preparation of starch/chlorpropham complexes in various solvent media.

Media	Solvent (ml)	Added starch (gm)	Concentration of solution (ppm)
water	500	250	100
acetone	200	100	100
hexane	200	100	100
a/water	100+100	100	100
m/water	100+100	100	100
methanol	200	100	100

a/water, m/water are the solution of water in acetone and methanol (1:1 v/v).

The separated dry spiked starch was then extracted with hexane, acetone and methanol solvents. Only three spiked-starches were selected from a total of six samples of prepared starch. The starches spiked with chlorpropham in water, hexane and acetone were taken for desorption. Three replications of 30 gm were extracted with each solvent. The extraction was collected and evaporated to a suitable volume for GC injection. The GC value was calculated and compared with standard solution.

4.4.3.2 Result and Discussion.

The sorption hysteresis is the phenomenon in which different paths exist for adsorption and desorption isotherms, and it has important theoretical and practical implications. In foods Sorption isotherms are important in considering drying, packaging and storing process for foods (Hubinger et al., 1992). The sorption hysteresis could be effected by different factors. In the present study the spiked-starches in different solvents were made containing chlorpropham. The spiking media was made in water, hexane, acetone, methanol, acetone/water and methanol/water. The weight of starch and other details for spiking of starch are given in Table 4.5, (section 4.4.3.1).

The amount of chlorpropham adsorbed (mg/kg) into the starch was determined by the following formula:

The amount of chlorpropham adsorbed on starch (mg/kg)

=DC*V/W

where DC= the difference in concentration (mg/ml) of solution, before and

after shaking in starch for 24 hours, used for spiking.

V= The total volume of solvent taken.

W= The total weight of starch taken.

The adsorption of chlorpropham (mg/kg) on the starch is given in Table 4.6. It showed that starch adsorb maximum amounts of chlorpropham from the water media, with amount of 167.65 mg/kg, as compared to other solvent media used for spiking. The minimum or not at all amount of adsorbent goes into starch spiked in methanol solvent media, while from acetone media was slightly higher comparing to hexane. In case of water/solvent spiked-starch, the starch adsorbed very small amounts of chlorpropham.

It has been found so far that only water is suitable for the preparation of the complexes, because from other solvents complexes will crystallise, or a ternary complex is formed. Water has a crucial role in the complex formation, since hydration of the cyclodextrine complex is energetically favoured as compared with the separate hydration of the components. There is namely a decrease in the energy of the system caused by the increase of the solvent-solvent interaction, since the contacting surface between the solvent and guest molecule, as well as between the solvent and cyclodextrine cavity are reduced, (Szejtli, 1982).

Because the potato starch is an extremely hygroscopic substance the commercial product is delivered with 20% moisture (Whistler, 1964a). It was found that increase in the weight of starch after shaking with the solutions for 24

Table 4.6 The adsorption/ desorption of chlorpropham (mg/g) in potato starch.

Solvent	Adsorption (mg/g)	Desorption (mg/g) by different solvents. Acetone	Hexane	Methanol
water	167.65	32.09[a]	60.83[b]	98.85[c]
acetone	34.97	25.53[c]	36.35[cdb]	48.65[dc]
hexane	32.33	42.31[e]	61.59[e]	54.01[ec]
a/water	11.67			
m/water	8.42			
methanol	-9.81			

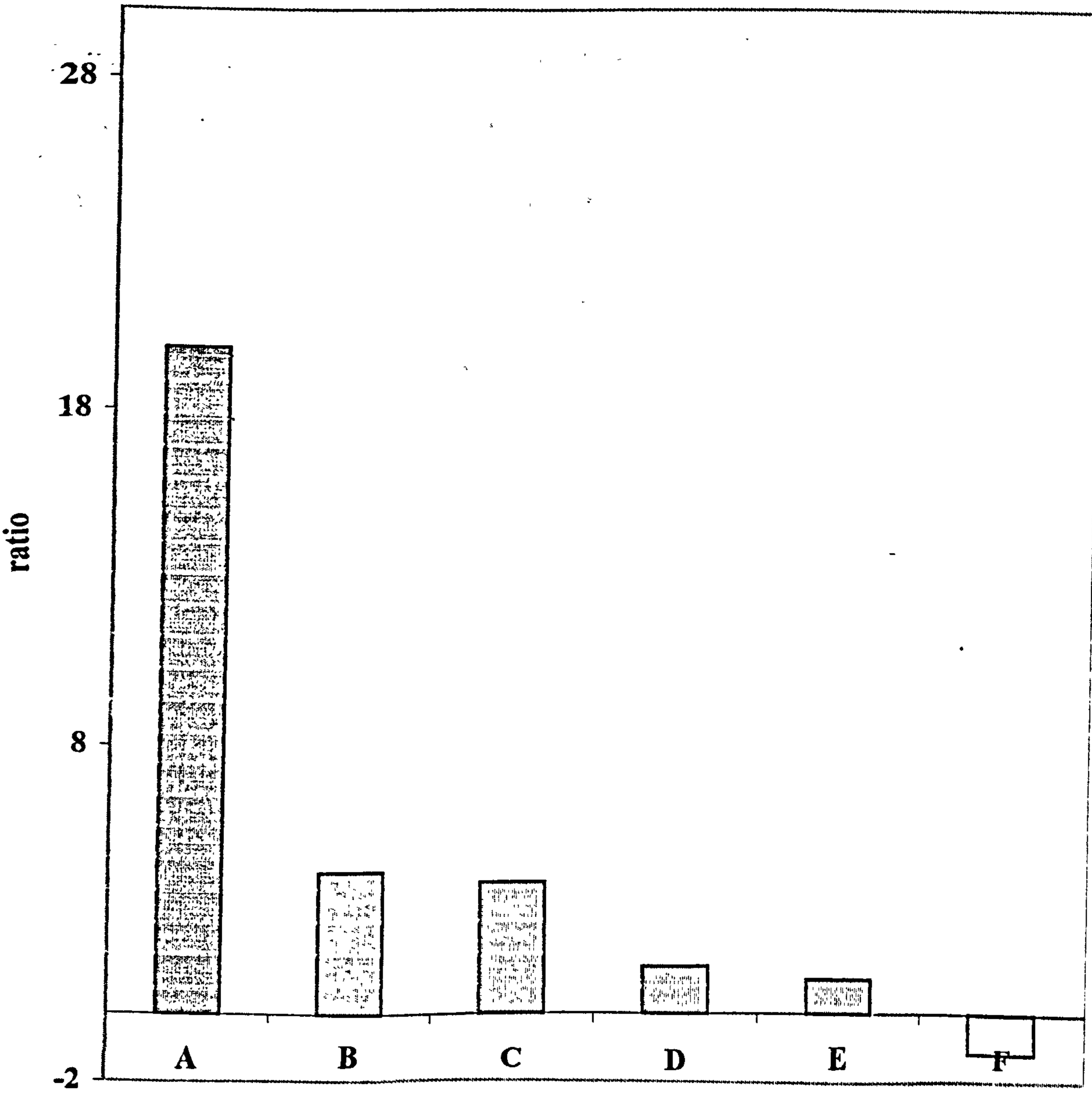
a/water, m/water are the solution of acetone and methanol in water (1:1 v/v).
The small letters shows the statistical difference, the same letters shows non-significant different. The each value is mean of three replicant.

hours, was maximum for water spiked starch media. While in the case of acetone and hexane, just after the shaking for 24 hours, the starch and solution were two separate layers, and in the rest of the samples the starch solution media was like a slurry, for example, methanol, methanol/water and acetone/water solution.

When the ratio of the amount of chlorpropham adsorbed from different solvent media into the starch was plotted, by keeping the methanol/water constant (Fig.4.4). It showed that the ratio of adsorption into the starch was highest in water spiking media which was decreased toward the methanol spiked media. Methanol has been used for defatting starch and chlorpropham is highly dissolvable in methanol, therefore it remained in methanol solvent rather than going into starch. Iodine in the solid complex could be extracted with hydrophilic solvents such as methanol (Murdoch, 1992).

The starch could not absorb significant amounts of chlorpropham from aqueous solvents like acetone/water and methanol/water media. It shows the higher polarity of water added solvents which could retain the chlorpropham in them. The water-miscible solvents, such as methanol or 80% dioxane, have completely removed the fatty acids from corn, wheat, and rice starches (Whistler, 1964b). The corn starch extraction in the Soxhlet apparatus with nine different solvents yielded generally larger amounts of fatty materials with polar solvents and 15-20 % water in methanol and dioxan, respectively, were most effective in the removal of fatty substances. Therefore retention of chlorpropham in these media increased with their polarity with the adding of water.

Fig. 4.4 Adsorption ratio of chlorpropham into starch from various solvent medias.

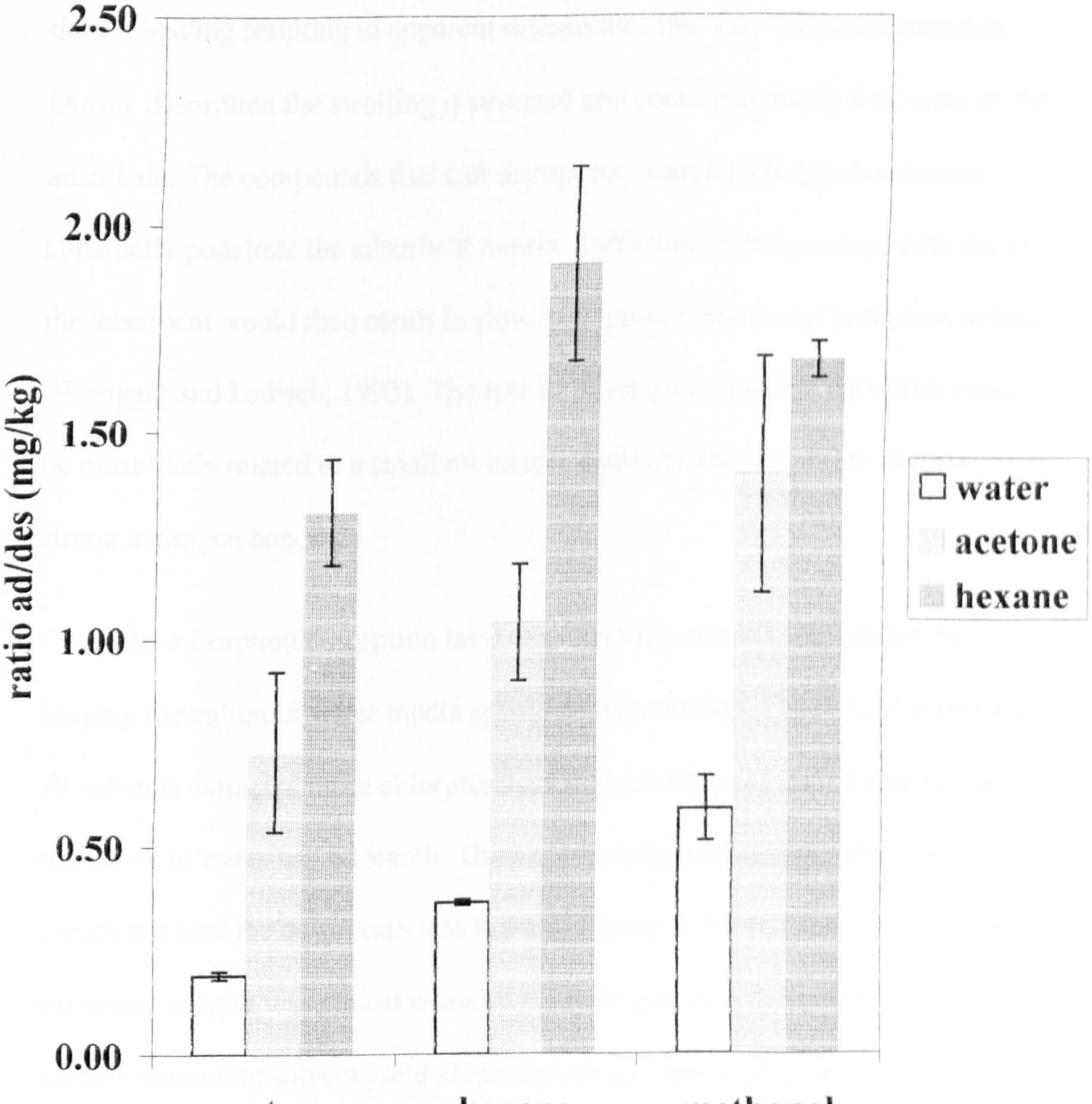


The desorbtion of these spiked starches were afterward carried out with three solvents of different polarity (Table 4.6, page 213). Due to time limitation only three types of spiked-starches out of six were used for the desorption purposes. The desorption was only completed with acetone, hexane and methanol solvents by using the same reflux extracting method. The maximum amount (mg/kg) of chlorpropham was desorbed from spiked-starch in water media. It was 32.09, 60.83 and 98.85 from water spiked-starch by acetone, hexane and methanol extracting solvents respectively.

However the ratio of adsorption/desorption was minimum in water media spiked-starch and was highest from hexane-spiked starch (Fig. 4.5). It was less from acetone-spiked starch than hexane and was higher from water-spiked starch. The maximum adsorption/desorption ratio was in hexane media spiked-starch suggests that chlorpropham does not go deeply into the starch or that it was only partially included in the amylose helix and thus was loosely attached on surface of the starch.

It shows that water was the most important factor for the formation of complexes between chlorpropham and potato starch. The highest amount of chlorpropham was extracted by methanol from water-spiked starch, indicating that during the storage of treated tubers if the starch in tuber formed any complex with chlorpropham then it could be hard to extract with a normal solvent like hexane and the possibility of chlorpropham to remain in a bound form in the starch of treated tubers would exist.

Fig. 4.5 Adsorbtion /desorption ratio of spiked-starch in different media.

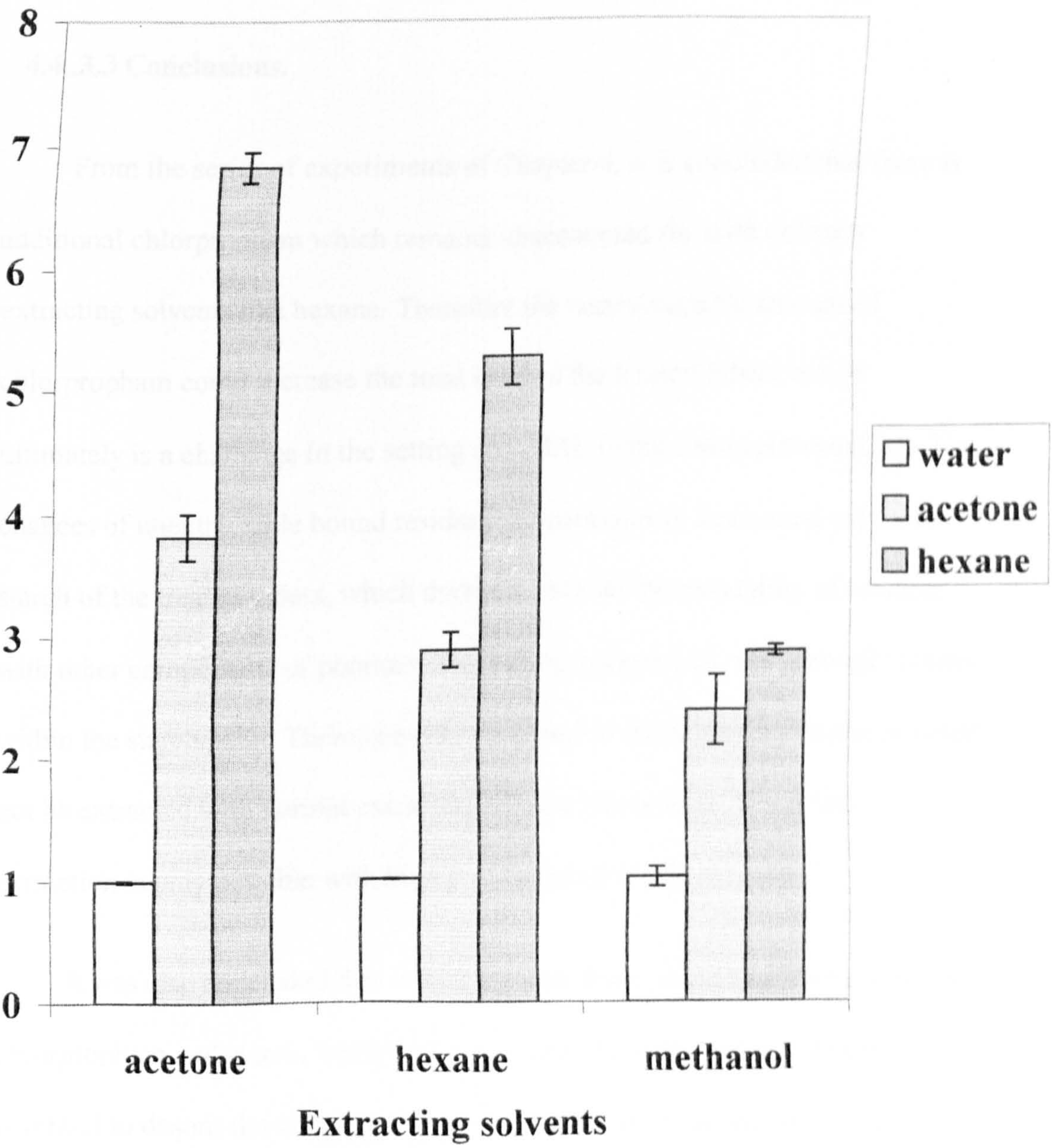


The acetone also permitted a strong complex of chlorpropham with the starch. Although the amount adsorbed into the starch was not much higher than hexane media spiked- starch, the adsorption/ desorption ratio was certainly less than the hexane spiked starch which shows the strength of CIPC complex formation. Penetration is particularly complex since the starch matrix is swollen during loading resulting in apparent diffusivities that vary with concentration. During desorption the swelling is reversed and could potentially trap some of the adsorbate. The compounds that can disrupt intrastarch hydrogen bonds then apparently penetrate the adsorbent matrix. Diffusion of these compounds out of the adsorbent would then result in slow desorption kinetics and extensive tailing. (Westgate and Ladisch, 1993). The rate of penetration through the starch could be most likely related to a small molecular diameter and the ability to form strong hydrogen bonds.

The adsorption/desorption ratio of extracting solvents was plotted by keeping the values of water media spiked-starch constant (Fig. 4.6). It shows that all solvents extracted more chlorpropham from all kinds of spiked starches as compared to water-spiked starch. The hexane-spiked media gave the weakest complex where the desorption was highest. However the efficiency of methanol extracting solvent was almost constant from all kind of spiked medias. The acetone extracting solvent yielded the highest amount of chlorpropham from hexane spiked starch.

This comparison shows the strength of the chlorpropham complex with starch and the extracting efficiency of different kinds of extracting solvents. Here it become clear that the water spiked starch had the highest complex

Fig. 4.6 The desorption ratio of water spiked starch to other spiked starches in different solvents.



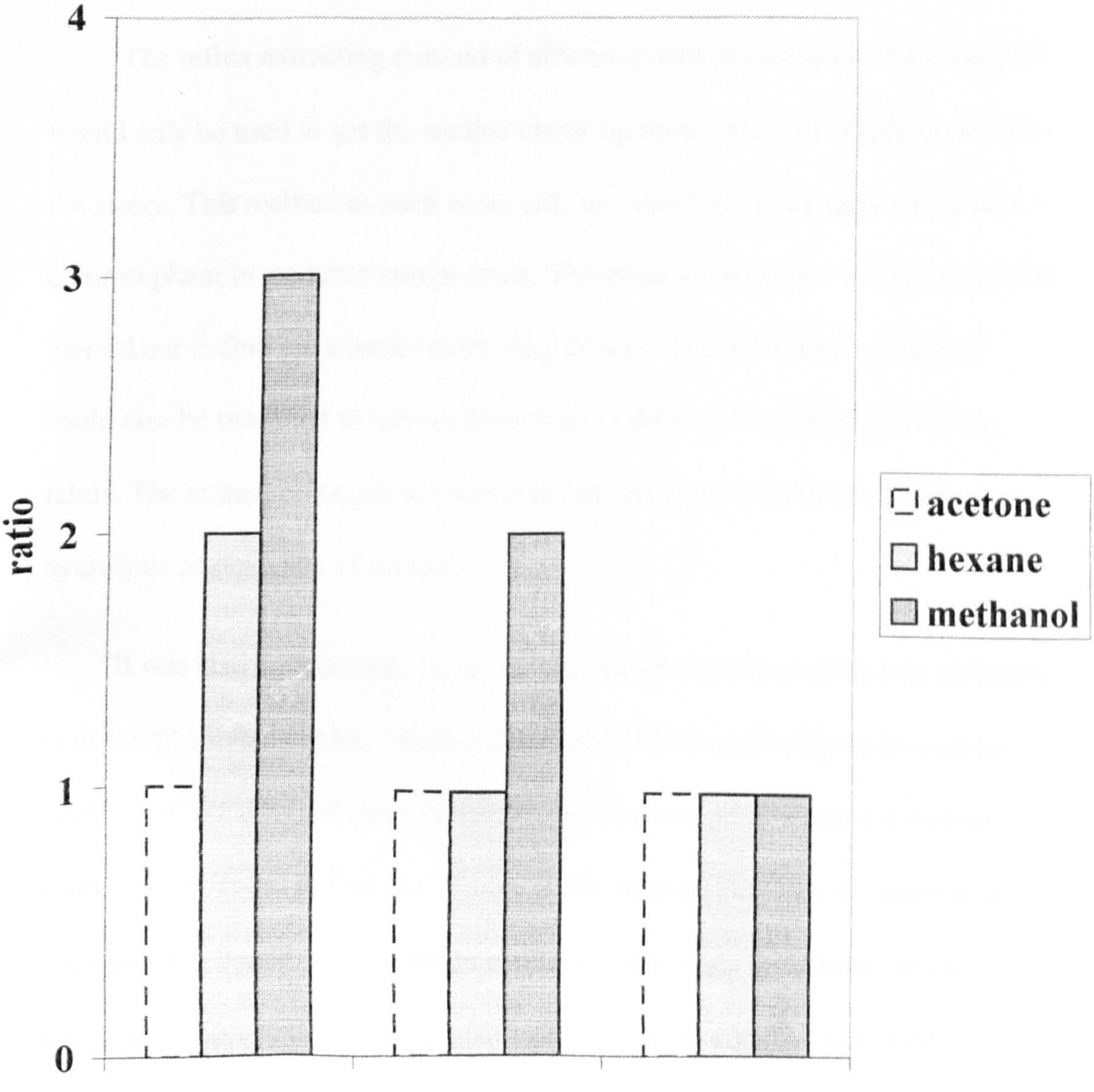
strength and hexane media has least. The methanol had the highest extracting power to desorb any kind of chlorpropham complex from the starch. Similarly, when adsorption/desorption of acetone was kept constant (Fig.4.7) showed that all extracting solvents act similarly starting from hexane spiked starch. This combination gave the highest recovery of chlorpropham observed in these experiments.

4.4..3.3 Conclusions.

From the series of experiments of Chapter 4, it is concluded that there is additional chlorpropham which remains unaccounted for with ordinary extracting solvents like hexane. Therefore the nonextractable amount of chlorpropham could increase the total level in the treated tubers which ultimately is a challenge in the setting of a MRL in the European countries. The chances of unextractable bound residues are particularly associated with the starch of the treated tubers, which does not exclude the possibility of binding with other components of potatoes. The chlorpropham was most strongly bound within the starch helix. Therefore chlorpropham in the treated tuber starch could not be extracted with normal extracting solvent like hexane. Complete extraction is only possible with highly polar solvents like methanol.

It was also concluded that where for complexes made artificially between chlorpropham and starch, highly polar solvents like methanol had greater potential to desorb the chlorpropham from the starch. This unextracted amount could be in a bound form in the helix of the starch like its fatty acid components. Methanol dissolved these fatty acids and penetrated deeply into the helix of the

Fig. 4.7 The desorption ratio of extracting solvents compared to acetone from spiked staches.



starch as compared to other extracting solvents. The nature of the CIPC-starch complex depends on different factors. The CIPC could be partially or fully attached in the centre of the starch structure. One thing which is obvious is that the extraction method for chlorpropham, especially for bound chlorpropham, should therefore be improved .

The reflux extracting method of chlorpropham developed in the Chapter 2, would only be used to get the routine check-up information of chlorpropham in the stores. This method as such is not able to extract the more tightly attached chlorpropham in the tuber components. Therefore a comparative study should be carried out to find out a better extracting solvent. The extraction procedure could also be modified to use combinations of different solvents in different ratios. The extraction could be carried out in methanol solvents with partial hydrolysis or digestion of samples.

It was also noticed that the starch-chlorpropham complexion was different in different solvent media, which shows that CIPC-starch complex formation could be effected by different factors during the storage. Therefore a further study is also needed to find out if there is any relation between the amount of unextractable bound chlorpropham residues and storage conditions, storage period and temperature in the treated stores. It is also equally important to include the amount of the CIPC residues in the starch fraction with total amount in the whole washed treated tubers.

Chapter 5:

GENERAL DISCUSSION

The thesis consists of the study of chlorpropham distribution patterns in commercial potato stores and its attachment behaviour with tuber components.

Potato, being a rich source of carbohydrates not only decreases the burden on our cereal crops to great extent but also provides a cheap source of energy (Jilani and Baloch, 1983a: 1983b). However storing tubers under ambient conditions results in high losses due to sprouting, which results in moisture losses and rotting (Metha and Kaul, 1991). Therefore chlorpropham is a chemical used world-wide for years to controle sprouting of tubers in storage period.

The chlorpropham nowadays is mainly applied three to five time a season, in the stores as an aerosol fog with a dose of 20 mg/tonne. Most of the work carried out so far has been done in laboratory samples and there are not enough information about the actual pattern of chlorpropham distribution in the commercial stores. Therefore the huge amount of chemical in stores and its distribution are main concern for environmental scientist. In recently unpublished results of the of chlorpropham distribution in commercial stores, showed that chlorpropham residues were highly different in various sites of the commercial stores. It was reported that literally there was no chlorpropham in the centre of the lower box and the residue level on the top height of the boxes was very high. The corner samples contained higher residues than the respective central samples. (personal communication with Boyd).

This work was carried out on a vast scale covering almost all types of commercial stores in the UK in an attempt to understand its distribution pattern, and to minimise variation of residue levels in the stores. As large samples taken from different stores, therefore it was vital to have a quicker and more reliable analytical method for chlorpropharm determination. For this purpose the literature was surveyed about analytical methods of chlorpropham determination, which showed that till now there is no such analytical method which could determine chlorpropham in such a large number of samples. The commonly used methods for chlorpropham determination were either slow or much complicated.

It is demanded in the present study to have a better and quicker analytical method. In this regard two methods were selected for the comparison, which have been commonly used for pesticide residue determination. The blending extraction method was recently been developed in this department by Boyd, 1988. This method was compared with the reflux extracting method, which was being used in industry for routine pesticide determination.

Although the extraction procedure for the samples was different in these methods, the final steps like evaporation of extracts by vacuum evaporator and measurement of chlorpropham by GC. FID, were the same in both cases. It was found during their comparison that both methods were more or less similar in their precision of results with some merits and demerits over one another. The blending method was precise enough, if carried out with proper attention, but it was a slow and time consuming method. There are higher risks of sample losses from sample extraction to final evaporation stage. On the other hand the reflux extraction method was quicker and easier than the blending method. Therefore it was decided

to use this method for further sample extraction work. From this study it was hoped that we would be able to predict in future that how well a fog had penetrated into boxes with out extensive sampling. If this approach can be validated for box stores then the possibility exists of extending the method to bulk stores, where particular difficulties are experienced in sample taking. The samples were taken from the stores which were maintained at different temperatures, cold store (3-4 ^{0}C) and conventional stores (8-10 ^{0}C).

The present study shows that the level of chlorpropham was significantly different among different commercial stores. Each store has its own distribution pattern with some common similarities. In each store the amount of chlorpropham varied with respect to sampling sites within the stores. However this was a common trend in all stores that maximum level of chlorpropham was on top and minimum on the lower height of the stores.

In cold store 'A'(3-4 ^{0}C), there was a white thick layer of chlorpropham on the top tubers of top height in the store. However overall distribution of chlorpropham was in a specific pattern. The level of chlorpropham decreased continuously from the top height towards the bottom height of the store. In this store extensive sampling was carried out from top, middle and bottom height of a selected bay in the store of six boxes high. Besides this each box of the bay was again sampled on its top, middle and bottom level of the box. It was noticed that the level of the chlorpropham was higher throughout the store, even in the middle of the boxes where the value exceeded MRL level. However the highest amount was on the top height of the store, which gradually decreased toward the bottom height of the bay. It was found that the chlorpropham not only varied in different

heights in the stores, but it was also highly different within the same box of the bay.

In respect to the cold store 'A', other stores have followed more or less the same pattern in the way of the highest on the top and lowest on the bottom height. However the overall level of the chlorpropham was much less in other stores as compared to the cold store. Therfore it was realised that the application of chlorpropham fog, three to five times in a storage time, a common practice in the past, would only increase the existing higher level on the top of the store unless the direction of the fog application is changed.

It was also a common practice to take the average value of CIPC residues from 8-10 selected tubers samples. It was believed that average values of numbers of potatoes would not represent a true picture of chlorpropham residues in the particular stores. Therefore a new technique was adopted by taking the individual tuber. These tubers were then divided into two, upper and lower half portions. This technique has played a significantly important role in truly understanding the pattern of chlorpropham residues in the stores.

It was noticed that the upper portions of the tubers contained the higher amounts of CIPC with respect to their respective lower portions of the tubers. The difference was much higher in the halves of the top most height of the stores as compared to bottom height half tubers of the stores. The study of upper and lower portions has contributed an important role in understanding the attachment behaviour of aerosol chlorpropham fog with tubers and its movement direction during the application.

In conventional store 'B'the amount of CIPC was equal on its both sides of the tubers, which was latter on found that the chlorpropham was applied through a lateral box arrangement. While in the cold store a thick layer on the upper half shows the direct falling out of the fog on the surface of the tubers, whereas respective lower halves contained much less level as they were not directly exposed to the falling fog. Therefore it shows that application of aerosol fogs from different directions would have a better effect on the distribution of chlorpropham in box stores.

Another interesting point of the upper/ lower halves study was clear that the upper halves of the topmost surface of the tubers in different stores has big differences in CIPC amounts while their lower halves were relatively have same amounts. Therefore by considering the lower portion only can lead to some interesting conclusive points to understand the overall distrubution behaviour in the stores. It was found that in most of the stores the ratio of over all mean of the store was equal to the mean value of lower half of the top height boxes in the store.

It was realized in distribution study that ventilation at fogging time could play an important role in attaining the even distribution of chlorpropham in the stores. Therefore an experiment was set up to prove the effect of internal air circulation on the distribution of the chlorpropham in cold stores. The findings revealed that the internal air circulation was highly effective to attain even distribution of CIPC from top bottom of the store. While on the other hand in control stores, higher amounts of chlorpropham were found on the top height samples compared to the bottom height samples of the store.

The effect of the particle size of CIPC fog and the temperature gradient in the store were also examined to know their effects on the distribution pattern of the chlorpropham in commercial stores. This work was carried out collaboratively between the three organizations involved in work to date, ie Sutton Bridge Experimental Unit, University of Glasgow and Silsoe Research Institute.

Chlorpropham fog of three 'qualities' was applied to different stores using a Superfog machine. To obtain differences in chlorpropham fog 'quality' in each store the applicator burner temperature setting was adjusted by the operator.

Prior to the fogging treatment, all stores were subject to the same air flow and temperature treatments and the conditions in each store were the same at the time of treatment. Due to the problems of leakage and co-ordination when the first store (store 36) was treated, reliable measurements of the airborne fog distribution could only be made after the stores were sealed at the end of the whole treatment process. The applied dose of the chlorpropham formulation was considerably in excess of normal treatment levels and this led to very high levels of airborne particle concentrations in the treated stores which influenced the accuracy within which size distributions could be measured.

The measured size distributions for the three stores showed relatively small differences in the treatment applied to the stores. In fact the store that should have been treated with the smallest mean particle size was measured as being treated with the largest. The leakage during the treatment of store 36 resulted in a lower airborne concentration of fog particles, and hence a more reliable measure of the particle size distribution.

The similarity between the size distributions measured at the two lower fogger temperatures suggests that variation in temperature may not have been the most effective way of changing the particle size distribution. The results highlight that the levels of chlorpropham deposited in one store (store 36) were very different from those measured in the other two (stores 34 and 35). This was most likely, when considered with the particle size data, to be a result of the leakage of chlorpropham on application to this store rather as a direct consequence of particle size. This similarity in deposits from stores 34 and 35 suggested that particle sizes are similar in these two cases. It means that changing the temperature setting on the applicator did not make a significant difference to particle size patterns or chlorpropham deposition.

Besides this the effect of temperature gradient on the distribution of the chlorpropham was also *studied.*

The temperature gradients achieved were not exactly as planned as the boxes receiving the heated air tended to be disproportionately warmer than others in the store but minimal, positive and negative were attained. The measured size distributions in the fogs applied to the three stores treated in this experiment showed relatively good agreement as expected. The temperatures in each of the stores during application showed a steep rise at the time of the application. This rise is less than that noted in the previous experiment. This is consistent with the shorter fogging time used for experiment 2. In the case of store 35, temperatures during the period of control were very uniform, while in stores 34 and 36 the highest temperatures were recorded in store 36.

The chlorpropham deposit levels were rather similar for stores 35 and 36 both in terms of deposition and residual chlorpropham after washing. The temperature gradients in these two stores were both positive, although the gradient in store 36 was greater. Store 34 (negative gradient, cold at the top) was markedly different both in terms of straightforward deposition (unwashed) and residual attachment. It is likely that condensation and deposition of the chemical has occurred on the cold surfaces at the top of the stores. The amount of chlorpropham, which readily washed off in the top boxes of store 34, would suggest that the attachment of the majority of the chlorpropham to the tubers was slight under these conditions. In a further study it would be important to combine these two parameters to get an optimum condition.

The study of the chlorpropham attachment behaviour with tubers is as equally important as the study of its distribution in the stores. The study of chlorpropham attachment behaviour is important for several reasons, i.e. to understand the fog movement in the stores and to know its losses during and after storing period. This study was carried out by simple washing and processing of the selected tubers.

The washing has shown different effects on the different sides of the selected tubers and on different sites in the store. Overall it was ineffective in removing a significant amount of chlorpropham. These findings are not in agreement with the results of Mondy, et al. 1993, where washing has reduced about 80% of the total CIPC on the tubers. This could be due to the difference between of the aerosol fog to simple dipping of the tubers in the chlorpropham solution. The aerosol fog contains very fine and coarse particles which could have

impact differently on tuber surface as compared to dipping of the tubers in the chlorpropham solution.

However the washing has reduced higher amount in the cold store and its effect was decreased as one goes towards the bottom height of the bay. The less effect of washing on the lower level of tubers showed that fog particles, which come with contact to the lower tubers, become firmly attached and therefore could not be reduced. However washing reduced almost 90% of the chlorpropham from the upper halves of the cold store from its top box tubers. Here it was noticed a thick layer of chlorpropham deposited on the top tubers surfaces potatoes. This layer was loose enough and therefore was easily removed by washing of the tubers.

In other stores the washing was totally unable to remove any amount of the chlorpropham. Therefore it was noticed that even after washing the level of chlorpropham was still higher than the MRL proposed for Europe. As these tubers were consumed individually therefore the chlorpropham level on each tuber is important. The chlorpropham was tightly attached to the tubers and it might have transfer to inner cells of the tubers. If that happend then it would be a higher health risk problem. This study has also indicated that the application of internal air circulation should be carried out during the application of the aerosol fog otherwise during the storage period it would not be helpful for the redistribution of the chemical as it is firmly attached with the tubers.

After noticing that washing had no effect in reducing the chlorpropham level from the surface of the tubers therefore it was thought that some of the chlorpropham may remained bound with the tubers and could not be extracted

with a normal extracting solvent. Therefore in this chapter (Chapter 4) some basic work was carried out towards its attaching behavior with tuber components. This chapter was divided into two sections. The first section contained the study of the effect of processing on chlorpropham level. Where as in the second section the starch of the tubers was concentrated on to find out if there was any bound chlorpropham within the starch.

Microwave cooking and boiling both have an effect in reducing the chlorpropham level in the tubers. The overall effect of processing techniques on chlorpropham level was plotted, which showed that the highest loses occurred in unpeeled samples by boiling. The higher effect of boiling in reduction of chlorpropham in the unpeeled tubers compared with peeled showed that, the chlorpropham on the surface in the peel may either leached into the water or have transferred to the center of the tubers. In the case of microwave cooking it was found that although the chlorpropham level was reduced over all during cooking in the case of peeled potatoes, however there was an increasing trend in the extractable amount of the chlorpropham after certain periods of cooking.

The increase trend in chlorpropham level during microwave cooking could a result of some bound chlorpropham which released during cooking. Further work is needed to quantify the total amount of chlorpropham released during the microwave processing. It is also important to investigate if any degradation of chlorpropham occurs during microwave cooking of potatoes.

Regarding the bound residues in the starch a further experiment was set up where the starch was separated from the treated, stored tubers. This starch was then

extracted with methanol and compared with the normal solvent hexane. Here it was found that substantial amounts of chlorpropham were extracted by the methanol, whereas hexane remained unable to extract any amount of chlorpropham. Not only this, when a few starch samples which were already extracted with methanol for two hours, were again reextracted for a longer time gave some more chlorpropham. This showed that chlorpropham was tightly held within the starch structure probably within the fatty material in the starch helix, which was not easily removable with the normal solvent hexane. It was felt that more chlorpropham could be extracted with still more polar solvents.

The blank potato starch was also spiked with different solvent medias, like water, hexane, acetone, methanol, acetone/water and methanol/water for adsorbtion/desorbtion purposes. It was found that the starch has absorbed the maximum amount of chlorpropham from the water media and minimum or none at all from methanol-spiked media. The absorbing behaviour from the water media was totally different than with other spiking media, which showed its suitability for making a complex with starch. The acetone solvent media was the second most successful after water media for making a chlorpropham complex with starch. The acetone may have penetrated deep into starch and therefore chlorpropham got better chances to make a complex with the starch structure.

The spiked-starches were also subjected to desorption by different polarity solvents. It was noticed that absorption/desorption ratio was minimum from water media spiked-starch and it was highest from hexane-spiked starch. The ratio of absorb/desorb from acetone-spiked starch was less compared to hexane but it was higher from water-spiked starch. Penetration is particularly complex since the

starch matrix is swollen during loading resulting in apparent diffusivities that vary with concentration. During desorption the swelling is reversed and could potentially trap some of the adsorbate. (Westgate and Ladisch, 1993).

These preliminary experiments showed that there was a possibility of chlorpropham penetration into the deeper cells of the tubers. Once it reached there then it would become difficult to extract. The residues of the chlorpropham in peeled potatoes depend on the amount applied to the tubers and it is obvious that chlorpropham residues to some extent depend on the method of application (Coxon and Filmer, 1985).

However the amount of chlorpropham extracted from the starch is not much, especially when the starch is considered as a 10% of the total weight of tubers. The total amount of chlorpropham in the washed tubers was 2.64 mg/kg, where in the starch the amount determined was 2.00 mg/kg. Here there is a possibility that this amount can increase with the passage of storage time or other factors can also effect penetration down quickly into the internal cells. Therefore a further intensive study is required to find out chlorpropham behaviour specially when it is applied as an aerosol fog form in the commercial stores. i.e if of chlorpropham was determined as 4.5 mg/kg by conventional methods and 2.0 mg was found to be present in the starch then the latter would enhance the residue value of 0.2 mg bearing in mind the starch content accounts for some 10% of the weight of a fresh potato. Starch was extracted in this study as being a suitable risks for chlorpropham but this does not exclude possibilities such as binding to cell wall or skin of potatoes. Such possibilities need to be investigated in full scale in further work.

It is concluded from this study that the pattern of the chlorpropham distribution varies from store to store. Therfore a fix and hard rule can not be applied for the pridiction of its distribution pattern. However there are some common possibilities which can be generalised. The work here on distribution would suggest that more effort should be made to improve the methods of chlorpropham application to individual potato stores.

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