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PESTICIDE RESIDUES IN THE ENVIRONMENT IN INDIA

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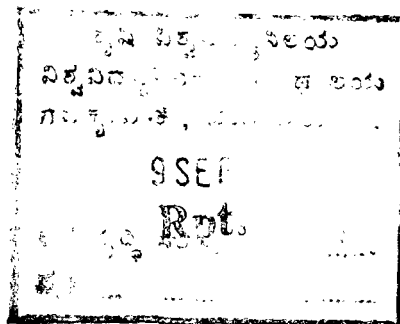
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**PARTICIPANTS OF THE
ALL INDIA SYMPOSIUM ON PESTICIDE RESIDUES IN THE ENVIRONMENT**

held from November 7-10, 1978 at

University of Agricultural Sciences, Hebbal, Bangalore



PREFACE

Effective pest control is essential for two of man's most urgent problems ; the provision of sufficient food for ever-expanding human populations and the maintenance of the health of these people by control of vectors of serious diseases ; these needs apply particularly to tropical countries. In the last four decades, both these problems have been diminished greatly by the continually increasing use of synthetic pesticides. Crop yields have been greatly increased and many millions of lives threatened by insect-borne disease saved by the use of these chemicals. At present, more than 1000 pesticides are in common use around the world and more than a quarter of a million tonnes of insecticides alone are sold annually. Unfortunately, some of these chemicals are very persistent in plant and animal tissues, soils, water and air and cause concern as to future hazards. Others are broad spectrum biocides, very poisonous to animals and man. As a result of the use of pesticides there has been some disturbance of terrestrial and aquatic ecosystems and many 'incidents' of wildlife kills involving animals or man. Moreover, many pests developed resistance to pesticides and the chemicals became less effective. As these problems have become obvious there has been a great deal of research aimed at monitoring residues of pesticides in man, food and the environment in developed countries combined with stringent regulation of pesticide manufacture and use. In India, although pesticides have been quite extensively used for control of mosquitoes and other medical pests, they are only now coming into widespread use for control of crop and stored produce pests. New registration regulations are being introduced to protect food and minimize environmental problems. Because of this the present publication, which aims to bring together the present state of knowledge of pesticide residues in India, is very timely and should provide much-needed data to enable present environmental problems to be lessened and future problems to be minimized.

Our sincere thanks are extended to FAO, UNESCO, UNDP and the Indian Council for Agricultural Research (ICAR) for making the All India Symposium on Pesticide Residues in the Environment possible, by their encouragement and the provision of funds for holding the Symposium and publishing the Proceedings. We also

thank the University of Agricultural Sciences, Bangalore and in particular, the Vice-Chancellor, Dr. H. R. Arakeri and the Senior Professor, Dr. H. C. Govindu, for providing the venue for the Symposium and all possible assistance with its organization. We hope that this volume will have value to all interested in the environmental impact of pesticides and to research committees and regulatory bodies concerned with avoiding environmental problems as pesticide use develops in India.

January, 1980

C. A. Edwards
G. K. Veeresh
H. R. Krueger

ACKNOWLEDGEMENTS

Pesticide residues in the environment has become a matter of concern to both layman and scientists as a result of some startling discovery of their residues in man and animals in places where they have been excessively used. Fortunately in India the pesticides have not been abused to pollutant levels. However use of pesticides is on the increase and it may be inevitable to use more and more to keep pace with increasing agricultural production. Therefore it becomes necessary that we know the real position as to where we stand in regard to pesticides as related to their residues in the environment and the course of action to be envisaged in the country to minimise hazards arising out of their continued use. Work is in progress in several Agricultural Universities to assess the residues of pesticides that are commonly used by our farmers and public health authorities. University of Agricultural Sciences has been alive to this problem and has its own share to contribute to the knowledge of pesticide residues.

The first All India Symposium on pesticide residues in the environment held at U.A.S., Bangalore from Nov. 7 to 10, 1980 helped to bring out the present status of work and knowledge of the subject and to focus attention for future lines of work. Over 150 scientists from all over India took part in 4-day deliberations and presented nearly one hundred papers of which 76 papers have been included in the proceedings. In addition, two appendices, one on the list of insecticides registered and in use in India, and the other – a proforma for registration of pesticides are also given, to facilitate the readers to gain an overall picture of pesticides and their residues.

Several organizations, institutions and individuals have contributed to the successful conduct of the Symposium and to bring out the proceedings. The University gratefully acknowledges the financial support given by the UNDP and ICAR for holding the symposium and publishing the proceedings. Appreciation is due to Dr. C. A. Edwards, Rothamsted Experimental Station, Harpenden U.K., Dr. H. R. Krueger, Professor of Entomology, Ohio Agricultural Research and Development Centre, Wooster, U.S.A. and Dr. G. K. Veeresh, Professor of Entomology, U.A.S.,

Bangalore who helped in organizing the Symposium and editing the proceedings. The interest evinced by Dr. H. C. Govindu, Senior Professor, U.N.D.P., U.A.S. in the organisation of the Symposium is recorded.

The assistance received from Mr. B. Mallik, Department of Entomology in proof reading and the help from M/s Raja Power Press in printing of the proceedings deserves mention.

U.A.S., Hebbal
February 18, 1980

B. V. VENKATA RAO
Editor and Secretary
Communication Council

WELCOME ADDRESS

DR. H. C. GOVINDU

Respected Vice-Chancellor, Dr. Sukhdev Singh, Dr. Krueger, Dr. Bowden, Dr. Edwards, Mr. Walker, Delegates, Invitees and friends :

It gives me great pleasure to welcome you to this Symposium on "Pesticide Residues in the Environment" a subject of topical interest, organized under the auspices of the United Nation Development Programme, the Indian Council of Agricultural Research and the University of Agricultural Sciences, Bangalore.

This Symposium is a continuation of a series of workshops and symposia arranged under the aegis of UNDP/ICAR/UAS since the inception of the UNDP project seven years ago aimed at developing a centre of excellence in plant protection at this institution. So far, ten symposia have been organized in which many experts from India and from outside the country participated. It is our conviction that these activities have benefited many plant protection scientists from the various agricultural universities and agricultural developments in the country. Thus, it is a matter of great satisfaction to us that in addition to fulfilling the primary objectives of developing a Centre of Excellence in Plant Protection at this Institute, we believe we have succeeded in good measure in disseminating the latest technology in Plant Protection Science to fellow scientists in the country. Amongst other activities under this project, we have awarded about 35 doctoral fellowships and two of our Ph.D. students have been honoured by the Jawaharlal Nehru award for their outstanding research. We have had the unique opportunity of getting about 25 foreign consultants which has greatly helped us in reorienting our research programmes. Under the same programme, about 25 UAS scientists visited various research centres in developed countries to acquaint themselves with the latest technology in their respective fields of specialization. This project has also helped greatly in equipping our laboratories and library.

For a gathering of this type, I feel, that it is redundant for me to explain the importance of the present Symposium. I am sure you agree with me that during the last decade or two, awareness of the overall benefits of plant protection measures has percolated to all levels of the farming community particularly in the irrigated areas. Many pesticides are coming into the market and are used at all stages of plant growth ranging from seed treatment to the harvest and marketing stage. Some over zealous farmers have tended to use pesticides indiscriminately, without concern for food contamination and pollution of the environment. This problem will certainly assume serious proportions in the years to come as the usage of pesticides becomes an integral part of raising healthy crops. This situation calls for concerted efforts on the part of plant protection scientists to intensify research in their very important branch of science. It is not enough to produce more food but the food that is produced should also be safe for consumption. Pesticide residue studies will not only throw light on safe dosages but also help in understanding the period of persistence of a pesticide, which in turn can be utilized in developing suitable and economic schedules of pest control. These studies and their popularization are of paramount importance, particularly in developing countries where the majority of the population which uses the pesticides do not possess adequate plant protection expertise. It is in this context, that it was considered most appropriate that a symposium on this subject be organized so that the work already done in the field in India could be reviewed and future programmes of work on a national basis be planned. As a plant pathologist, I feel unhappy that the work on fungicidal residues has not received the same attention as other pesticides. I hope that the participants will discuss this aspect also, so that the studies on these lines can also be accelerated.

I am extremely happy to welcome Dr. H. R. Arakeri, our Vice-Chancellor, who has evinced a keen interest and has been a source of inspiration in the activities of our UNDP project.

Similarly, I welcome with great pleasure our distinguished guest Dr. Sukhdev Singh, Deputy Director General (Crop Sciences) from ICAR, New Delhi who has kindly agreed to accept our invitation to deliver the inaugural address. Dr. Sukhdev Singh whom perhaps most of you know, does not need any formal introduction. He has a very distinguished academic record including a Ph.D. from Louisiana State University, U S.A.

Prior to joining the ICAR in 1974, he was the Director of Research at the Punjab Agricultural University for about 7 years. At that time he guided the research programme of the University in its very crucial formative years with great distinction. Under his leadership, the University evolved 25 new varieties of various crops.

Dr. Sukhdev Singh has played a leading role in the saturation of wheat areas with the new improved varieties in a record time by well-planned production of the nuclear seed at the University's farm and distribution to growers all over the State. At ICAR he has been mainly responsible for developing a strong research programme in crop science in his capacity of Deputy Director General. I am particularly happy that Dr. Sukhdev Singh has readily agreed to inaugurate this Symposium in spite of his busy schedule especially in view of the fact that, he has been Chairman of the Central Insecticide Committee of the Department of Agriculture for the last four years.

Dr. Clive A. Edwards of Rothamsted Experimental Station, Harpenden, Herts. U.K. is an eminent Entomologist who has specialised in soil biology and soil pesticides and their residues. Dr. Edwards who has already been to our University three times under the UNDP programme, has greatly contributed in developing a Soil Biology Unit in the Entomology Department. He is responsible for suggestion that this Symposium be held at U.A.S. and for more than one year past has been closely in touch with us in organising this symposium, for which I wish to offer him my sincere thanks.

Another Scientist who has specialised in pesticide residues, and has been directly involved in organizing the present Symposium is Dr. H. R. Krueger. Earlier, he was a Consultant at the Punjab Agricultural University for two years. He is presently Professor of Entomology at the Ohio Agricultural Research and Development Centre. He is presently with us as UNDP Consultant to set up a residue laboratory and train staff at U.A.S.

Dr. John Bowden, an outstanding Entomologist who has specialised in Diptera and on pest surveying and forecasting has for many years worked in Africa. Presently, he is a Principal Scientific Officer at Rothamsted Experimental Station, U. K. Dr. Bowden has been assigned to our University as a consultant particularly to develop a pest survey and forecasting system.

I am also happy to welcome Mr. Peter Walker who has come on a short visit to our University. He is an eminent Entomologist who has spent several years in Kenya and Tanzania and is at present in the U. K. Tropical Pesticides Unit. He has contributed to the European Plant Protection Organization and has assisted with the FAO Crop Loss Programme since its inception in 1957. He was closely associated with the UAS programme on crop loss assessment and helped in organizing a workshop on "Assessment of Crop Losses due to Pests and Diseases" last year.

A large number of scientists from different parts of the country have come to participate in this Symposium and I extend to them a very hearty welcome and wishes for a profitable and comfortable stay. In addition, a large number of distinguished scientists have responded to our invitation to attend the inaugural session and it is my pleasure to welcome them. I now request our Vice-Chancellor to preside over the Inauguration.

INAUGURAL ADDRESS

DR. SUKHDEV SINGH
Deputy Director-General (Crop Sciences)
Indian Council of Agricultural Research

Vice-Chancellor, Dr. H. R. Arakeri, Dr. C.A. Edwards, Dr. H.R. Krueger, distinguished participants, ladies and gentlemen,

I am thankful to the organisers for the opportunity given to me to participate in the All India Symposium on 'Pesticide Residues in the Environment.' By virtue of my responsibilities in pesticide registration in this country, I have a deep interest in the Symposium and I am keen to learn the latest developments in this field and the consideration of ways and means to tackle the problems that face the industry and users of pesticides.

In recent years, great emphasis on a global basis has been laid for increasing agricultural production. New strategies and technologies have been developed in this country and elsewhere, such as the introduction of new varieties, provision of adequate irrigation facilities and inputs such as fertilizers and pesticides. Intensive agriculture has, in its wake, brought to the fore the problems of pests and diseases which are frequently appearing in epidemic proportions. The Food and Agriculture Organisation has estimated losses of up to one-third of the total world food due to pests and diseases. Estimates about losses may vary, but yet it is an admitted fact that considerable losses occur in our crop production and storage on account of pests and diseases and despite the right emphasis on other methods of control, at the present stage of development of our agriculture, it is inconceivable that we can do without use of certain quantities of pesticides.

With the introduction of DDT in early 1940, a 'Pesticide Revolution' was ushered in and it was believed that the new insecticides would solve the major problems in combating pests. In recent years huge quantities of the chemicals have been used in agriculture in all parts of the world. Although pesticide use in our

country is far less compared to some of the affluent countries, nevertheless an estimated 56,500 tonnes are expected to be used in 1978-79. In advanced countries like Germany, Japan and USA, it is estimated that the pesticide consumption per hectare is about 30 times more than the Indian average. In some parts of our country, however, the use per hectare may be as high as in these countries. Since almost all pesticides are poisonous, they can be hazardous to human beings and animals and their residues in environments can upset the natural biological balance when they hit non-target organisms. The epidemic of cotton mites in Gujarat and *Pyrilla* in sugarcane are some recent examples from our country. Nevertheless, it cannot be denied that in modern agriculture, one of the most efficient, and in some cases most dependable single means of insect control have been pesticide chemicals, therefore, agriculture and public health demands will continue to use these. In fact, chemical control has been instrumental in freeing some parts of the globe from pestilence and famine. In short, the agricultural benefits derived from the chemical control of pests have been incalculable and at present there is no real substitute to pesticides. Although safety in use is essential we need not create anxiety about their use in our country because of excessive use in other parts of the world. Our food and fibres production needs judicious use of this vital input.

One of the most important hazards involved in pesticide use is the persistence of some insecticides and their residues in our food and in the environment. Therefore, UNDP/ICAR and other agencies are giving aid to find solutions to this problem and this Symposium is one such endeavour.

Realising the importance of pesticides in agriculture and public health, the Government of India enacted the Insecticides Act in 1968 to regulate the import, manufacture, sale, transport, distribution and use of insecticides with a view to avoid and also prevent risks to human beings and animals by its indiscriminate use. Under this Act, the Government constituted a Central Insecticides Board to advise the Central and State Governments on technical matters arising out of the administration of this Act and a Registration Committee to undertake the registration of pesticides in the country on the basis of their safety and bio-effectiveness.

Up to date, 110 pesticides have been approved by the Registration Committee for use in agriculture, public health and one namely dieldrin exclusively for the control of locust in scheduled desert areas. Twenty-one pesticides which were either very toxic or were very difficult to ensure precautions by the farmers, have not been approved for registrations. For the registration of pesticides under this Act, the applicant has to submit data on bio-efficacy, toxicology, packaging and residues generated under Indian conditions on its product. Satisfactory data on bioefficacy of pesticides posed for registration is available in most cases, but for toxicology and

residues in environments there is still dependence on foreign data. A sub-committee on Pesticides Toxicology was constituted by the Registration Committee under the Chairmanship of Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay, to draw out protocols for toxicology and pesticide residues. Recommendations of this Committee will be available shortly for the consideration of the Registration Committee. I suggest to the participants of this Symposium that enough pesticides residue data need to be developed in this country so that the Registration Committee can take correct and sound decisions based on scientific considerations, to help the agricultural industry and the farmer in this country.

Considering the health hazards, pesticide residue tolerance limits in food and food commodities against 20 pesticides have been prescribed under the prevention of Food Adulteration Act, 1954 and Rules, 1955, administered by the Ministry of Health and Family Welfare. Efforts are also being made to finalise tolerance limits for 18 more widely used pesticides. Under this Act, if any food commodity in the market contains pesticide residues more than the prescribed tolerance limits, the article can be confiscated and destroyed, and the defaulter is liable for legal action. However, it has, not yet been possible for the Central and State Health Departments to create facilities for testing and monitoring the residues in items of food. To monitor the quality of pesticides and their residues, the Department of Agriculture has already sanctioned the establishment of 17 State Pesticide Laboratories and one Central Insecticide Laboratory by the Directorate of Plant Protection Quarantine and Storage, Faridabad. These laboratories are yet to be established and their construction may take 2-3 years. Eventually this will ensure the supply of good quality pesticides to the farmers and simultaneously safeguard the Indian population from health hazards. It is also suggested that while working out the control schedules of insect pests and diseases with pesticides, residues at the time of harvest should be determined so that the waiting period, *i.e.*, time interval when the residues become lower than the prescribed tolerance limits, can be worked out and recommended as a part of good agricultural practice.

The ICAR has often emphasised the need for research on pesticides and has encouraged specialists with grants to intensify their work in the ICAR Institutes & Agricultural Universities. In addition, the Directorate of Plant Protection, medical research centres and CSIR laboratories are undertaking programmes on pesticide residues. The ICAR has organised review committees and symposia with a view to evolve judicious use of these chemicals. In order to achieve the above-mentioned goals in pesticide residue research our research institutes and agricultural universities must be equipped with very sophisticated and sensitive equipment and manned by trained and dedicated scientists and technicians. The recommendations and follow

up of the Symposium on 'Progress and problems in Pesticide-Residue Analysis' held at Punjab Agricultural University, Ludhiana in November 1971, show that some of our Institutes have already created facilities to undertake research in this field. However, more Institutes would do well to create such facilities and acquire suitable equipment and facilities. Many of the recommendations of this Symposium have been already implemented and certainly proved useful in enhancing knowledge in the field of pesticide residues. With increased use of pesticides, more problems will occur in the field and particularly on residues in food. Thus, it is high time that we should endeavour to take up basic work on the metabolism of these chemicals in plants and animals, toxicities of the metabolites to man and animals and applied work in monitoring pesticide residues in food to make them safe for human consumption. From the broad titles to be covered in this Symposium, I can see that many of the topics I have indicated are on your agenda. I would like to point out that till recently our research workers engaged in pest control research have been concerned primarily with experiments on bioefficacy of the pesticides. In order, however, to make rational recommendations for pest control both in agriculture and public health programmes and also to meet the legal requirements for registration this is not sufficient. Data from indigenous sources on toxicity and residues must be generated and this aspect should be built in to any future programme. Recently, the pesticide industry in this country has shown keen interest in supporting pesticide research particularly with a view to generating data for Registration under the Act. The Tamil Nadu Small Scale Pesticides Manufacturer's Association has signed a protocol with the Tamil Nadu Agricultural University. Similarly the Bombay region formulators are negotiating such a protocol with the Universities in the Maharashtra State. This is indeed a very happy development. The Agricultural Universities would be well advised to give necessary scientific backing to this industry, who in their turn must give financial support for such programmes in these institutions.

I hope that your deliberations will give consideration to these points and prove useful and result in valuable recommendations which will further enhance progress in the field of pesticide research and in making judicious and economical use of pesticides in future. I have great pleasure to be associated with this symposium and wish it all success.

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Pesticides and the biota

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1. Introduction—History of pesticide use

The use of pesticides is not new. As long ago as 70 A.D. Plinius recommended arsenic as an insecticide. In 1763, nicotine was used for the first time, in 1818 extracts of Pyrethrum flowers, in 1865 Paris Green, in 1886 hydrogen cyanide, in 1892 lead arsenate, in 1907 calcium arsenate and in 1927 a root extract, rotenone. The first synthetic insecticide, dithiocyanodiethyl ether was used in 1929. Some of these chemicals were relatively persistent and residues of arsenic are still found in soil from uses prior to the second world war.

The era of synthetic organic pesticides began during the second world war with the discoveries of DDT and BHC followed rapidly by those of organophosphates TEPP and parathion and organochlorines such as chlordane, heptachlor, dieldrin, aldrin and endrin. About this time hormone herbicides such as 2,4-D and 2,4,5-T were discovered. During the 1950's and 1960's many other insecticides such as malathion, diazinon and phorate were developed. The first carbamate insecticide, carbaryl was introduced in 1953, the first formadine insecticide in 1966, the first new synthetic pyrethroid, resmethrin, in 1967, the first insect juvenile inhibitor, altosid in 1973 and the first synthetic chitin inhibitor in 1972.

All of these chemicals have helped to increase yields of crops often several-fold, during the past three decades, and there is little doubt that their use will continue for the foreseeable future in ever-increasing amounts.

The persistent organochlorine insecticides were particularly successful in control of pests both in agriculture and in human health. However, from the early 1950's onwards, anxiety began to grow about their persistence and other characteristics. Reports began to occur of large residues of DDT and other organochlorines in soil, water, plant and animal tissues. Dead birds and small mammals found near treated fields and there were reports of extensive mortality among fish when water was treated with organochlorines, either intentionally or accidentally. It became clear that there was a strong tendency for organochlorine insecticides to be stored in animal tissues, particularly in those in higher trophic levels and reports of appreciable amounts in the tissues of human beings caused anxiety as to ultimate effects on human health.

These fears, and the ever-increasing use of persistent pesticides led eventually to severe restriction on their use in many countries from 1964. DDT was the first to be restricted or banned, because it seemed to accumulate more in animal tissues than other organochlorines. This was followed by similar legislation against aldrin and dieldrin, then heptachlor and chlordane. At the present time, the use of these chemicals is either completely banned or restricted to relatively few uses in most European countries and the U.S.A., Japan and Australia.

However, although the use of organochlorine insecticides has been severely restricted in many of the developed countries, their manufacture has continued and although world production of DDT has fallen off, that of other organochlorine insecticides has not. Moreover, although the use of insecticides is not expanding rapidly in temperate countries, in tropical countries like India the use of insecticides is still outpacing that of herbicides, and fungicides. There is also a strong trend for less pesticides to be imported and more to be manufactured in India. This still includes a very considerable proportion of the effective and cheap organochlorines that are no longer under patent.

There is a vast body of literature relating to the occurrence of persistent pesticide residues in countries such as U.S.A. which have had extensive pesticide monitoring schemes in soil, water and plants, animals and food. However, most of these residue data have been accumulated under temperate conditions and our knowledge of the persistence and accumulation of these pesticides under tropical conditions is still relatively meagre.

2. Hazards of persistent pesticides

Pesticides can be applied as aerial sprays or dusts to foliage or applied directly to soil or water. However, they never remain at the site to which they are applied. They may be carried by wind or volatilize in the atmosphere, from where they can contaminate other areas in precipitation. Eventually they reach soil and are gradually degraded there, or move into aquatic systems by leaching or run-off. More and more evidence is accumulating that even non-volatile organochlorine insecticides are lost from soil into the atmosphere in large amounts under wet and warm conditions when they are no longer held tightly absorbed on to organic matter or clay fractions of soil. Such conditions occur commonly under tropical conditions. In temperate countries, the 'half-life' of most organochlorine insecticides in soil ranges from about one year to several years; in the tropics it seems, this may be much less. Similar considerations apply to pollution of water by pesticides. Residues of DDT and dieldrin are common in rivers and streams in temperate countries; we have little comparative data from the tropics.

There seems to be a strong tendency for animals in higher trophic levels to accumulate more persistent pesticides than lower organisms ; the largest residues tending to occur in fish-eating birds and mammals, predatory birds and man. In each system, there is movement of persistent pesticides from the physical environment into the biota, usually with residues returning to the physical environment when the organism dies. Such movements are seen from soil into plants, from soil into the fauna and microflora, from water into plankton, invertebrates and fish and from animal food into animal tissues.

Although many invertebrates acquire persistent pesticide residues in their tissues only a very small proportion of these animals are killed by pesticides. At particular risk are fish which are sensitive to many pesticides, and predatory birds, which may either accumulate sufficient residues to kill them or can have their reproductive capability affected e.g. laying eggs with thin shells.

Let us attempt to summarize the possible environmental hazards. These can be conveniently divided into effects on fundamental metabolic processes, effects on wildlife and effects on man. There is little evidence of any fundamental processes such as soil organic matter turnover, photosynthesis or elemental nutrient cycles such as the nitrogen, carbon or sulphur cycles being much affected by pesticides ; nearly all the evidence accumulated to date on such effects is negative.

We have already discussed briefly the effects on wildlife. To reiterate : although individual organisms may be killed or affected by pesticide, systems seems to be very resilient and if some kind of organisms is killed, usually other organisms functioning similarly in ecosystems take its place. Only certain animals seems to be at risk and there is no evidence that any species has been put at risk by pesticides.

Finally, to consider pesticide residues in man. It is well-established that the tissues of most human beings contain, at least, small quantities of organochlorine residues and some, particularly individuals involved in spray manufacture or application, contain large amounts. What has not been established is whether these residues cause any long-term hazards. The argument as to whether they act as carcinogens at the levels commonly occurring, still rages, but at present there are no instances of confirmed deaths due to anything other than accidental or suicidal causes.

3. Misconceptions on environmental pesticide hazards

a) *Toxicity to organisms* : It is commonly assumed that if an organism takes up large residues, then these are harmful. This cannot be assumed without experi-

mental evidence. Many organisms carry large residues of different chemicals and exhibit no effect of these residues on their existence or behaviour.

b) *Accumulation in soil* : The concept of accumulation of persistent pesticides in soils and aquatic sediments often leads to the assumption that regular application of recommended doses of such pesticides can lead to massive build-up of residues. It can be shown on theoretical grounds that this is extremely unlikely. If it is assumed that the degradation of pesticides in soil is exponential, then an annual application of 2 kg/ha of the most persistent organochlorine insecticides, DDT, dieldrin and endrin will lead to a maximum residue of only about 8 kg/ha or 4 ppm in an average soil type even in temperate countries. Accumulations of pesticides with shorter 'half-lives' are much less than this.

c) *Bioaccumulation and concentration* : For any individual organism, the residues likely to accumulate in its tissues depends mainly on the regular levels of exposure to a pesticide. Any regular exposure, such as a standard daily dose in the food, leads to an equilibrium state which will not change unless the daily dose is changed. When the exposure ceases there is usually fairly rapid excretion of most of the residues from the organisms tissues.

d) *Food chains* : The concept of gradual increase in residues of persistent pesticides through the different trophic levels of a food chain has become almost a dogma. However, as evidence accumulates, it is becoming clear that many other possible explanations, such as differential rates of excretion, would account for the large residues found in certain organisms in the higher trophic levels. This is particularly true of aquatic food chains, where it has been clearly demonstrated that fish can acquire large residues more readily from the water in which they live than from the organisms upon which they feed.

e) *Dose/effect relationships* : Although it has been established thoroughly for pest insects and organisms used in pesticide bioassay that the dose/effect relationship is a logarithmic one this is often ignored when considering the effects of pesticides in wildlife. Most evidence supports the view that a logarithmic dose/effect relationship applies equally to wildlife hazards. Extending this argument, the dose likely to be needed to exterminate a population of any particular useful animal would be very high.

f) *Persistence and toxicity in assessing hazards* : It is commonly assumed that any chemical that is persistent in organisms and the environment is necessarily hazardous and that transient chemicals are harmless. This is far from the case. For instance, in soil, a nematicide such as D.D. which persists for only a few days but is a broad spectrum biocide, may have much more drastic effects on soil ecosystems

than a persistent organochlorine insecticide that is only moderately toxic to soil organisms. Overall hazards must be assessed by considering body toxicity and persistence.

4. Development in the last decade

During the last decade, there have been many developments relative to environmental hazards due to pesticides.

Patterns of pesticide usage have changed greatly. In temperate countries, there has been a gradual transition from the use of organochlorine insecticides to that of organophosphates, carbamates and pyrethroids and the gradual introduction of sophisticated pesticides such as growth or chitin synthesis inhibitors.

There has been a considerable increase in pesticide use particularly insecticides in tropical countries, particularly India, for control of agricultural and horticultural pests.

In many temperate countries, and in particular the United States, extensive pesticide monitoring programmes have been developed for soil, water and selected biota.

The control of pesticide residue levels in food have been much more rigidly controlled and surveillance of food residues greatly increased. FAO/WHO in collaboration with representatives from different countries have established well-tested tolerances for residues in a wide range of foods and these have been adopted by many countries. This has led to gradually decreasing residues in foods.

Many countries have enacted legislation severely restricting or curtailing the use of organochlorine pesticides. Only lindane is still in general use.

Governmental requirements for pesticide registration have been steadily increasing. This has to some extent led to negative developments. The requirements have increased the cost of developing a new pesticide to more than \$20,000,000 which in turn has meant that it is now too expensive to produce highly-specific or novel pesticides that are environmentally desirable but with a relatively limited market.

A number of groups of workers have developed predictive mathematical models for organisms and ecosystems in attempts to forecast the environmental effects of different pesticides. So far, these have not proved very accurate or reliable but it is expected that, as more data becomes available the accuracy of prediction will improve.

Another concept has been that of a model ecosystem, usually built in an aquarium tank with a terrestrial and aquatic phase and several organisms from different trophic levels. These have been used to assess whether there will be any bioconcentration of a pesticide through organisms. The value of these rather expensive and sophisticated tests, relative to simpler tests, has not been fully established.

There have been considerable developments in what is now called ecotoxicology. There have been extensive investigations, seeking to find key indicator species, processes or suitable biochemical constants that might be used to forecast environmental hazards.

Finally, the whole concept of pesticide usage has been changing so that the use of pesticides has been minimized and associated with biological and cultural techniques in integrated control or pest management programmes.

5. Minimizing Environmental Hazards due to Persistent Pesticides :

Many of the hazards that have led to restrictions in the use of persistent organochlorine insecticides have been due to poor methods of application or careless and unnecessary use. There are many ways in which hazards may be minimized.

a) *Use of alternative pesticides* : The available armoury of alternative pesticides including, organophosphates, carbonates, pyrethroids, biodegradable analogues or organochlorines, antimoultants, juvenile hormones and behavior-affecting pesticides can be used. A carefully planned programme can avoid environmental problems by alternating some of these pesticides with the cheaper organochlorines, also avoiding the rapid development of resistance.

Pesticides can be applied in ways that minimize environmental hazards. These include the use of accurately-placed treatments, seed dressings, microcapsules, ultra-low-volume sprays etc.

Often pesticide use can be minimized by the use of alternative techniques involving cultural control, biological control, pheromones, chemosterilants, biological pesticides or pest management.

Finally, but with less promises, there is some hope of removing undesirable persistent pesticides from water by filtration and from soil by repeated cultivations or absorbents such as carbon.

CONCLUSIONS

1. Pesticide usage will continue to increase for the foreseeable future.

2. Environmental awareness, research programmes and government legislation are avoiding serious environmental hazards.
3. Information is still needed on global movement and the ultimate fate of many pesticides.
4. Data on the persistence of pesticides in soil, water and the biota in the tropics, indicate more rapid breakdown of persistent pesticides, which may minimize environmental hazards and permit their continued use in tropical countries.

2 THE CURRENT STATE OF PESTICIDE USE AND PROJECTION OF FUTURE USE

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INTRODUCTION

If we are to consider pesticide use, particularly for the future, perhaps it would be appropriate to discuss briefly the future needs for food and the development of agriculture. Surely these considerations will have a major influence on the future of pesticides.

World demand for food is expanding more rapidly than ever before. If everyone in the world today sat down to eat at one table, it would stretch around the equator some 30 times. Each day, that table would have to be lengthened 30 miles to make room for new, hungry people.

The United Nations estimates that one-eighth of the world's people are malnourished. Each day, the ravages of malnutrition and starvation kills 15,000. And with the world's population expected to increase from the present four billion people to some seven billion by the year 2,000, the spectre of starvation becomes even more frightening.

Meeting the challenge of feeding seven billion people will take a massive effort using every advantage available. Farming methods must be improved, especially in developing countries. Better distribution systems must be devised to reduce losses to disease, decay and pest infestations that lose from one-third to one-half of the grain during shipment and storage.

The developing countries are now engaged in a desperate race to keep food production growing at least as fast as populations. Viewed in global terms, the food-population equation during the 20 year period, 1951-71, was reasonable. The production of cereal grains, the principal source of world food supply, and that which dominates the world food economy, more than doubled, while there was a less than 50% increase in population. This means that the cereal availability per capita increased by about 40% in 20 years. Because of regional disparities, this increase, however, was very unevenly distributed. More than half the increase was accounted for by the richest 30% of mankind, and the balance, less than 50% of the food supply, was left for the poorest 70%. Even this amount was inequitably distributed, ranging from a high per capita annual increase of 0.9% in Latin America to an actual per capita decline of 1.1% in Africa during the 1953-1971 period. According to FAO estimates, the minimum requirement is to increase the average annual rate of food production in developing countries from 2.6%, over the last decade to at least 3.6% during the next decade. If this minimum increase in production is not made possible, the developing countries as a whole might well face annual deficits of 85 million tons in normal years and over 100 million tons in years of bad harvest.

The urgency of increasing food production should not be underestimated. However, it is vitally important to ensure that the strategies adopted to increase food production with long-term policies. Any strategy to increase food production on a sustained basis that does not consider environmental factors explicitly is doomed to failure, sooner or later. The productive capacity of the biosphere can be sustained and improved only by adopting strategies that are ecologically and environmentally sound.

There are basically two ways of attaining a significant growth in food production :

1. By the expansion of farming to land not now being cultivated.
2. By the intensification of production on land already being cultivated which includes multiple cropping, the use of improved varieties, fertilizers, irrigation, etc., and the reduction in losses from pest attack.

Large areas of the tropics which are not farmed or grazed, constitute huge reservoirs of future production. Before these can be tapped, research on the farming and development technologies suited to their individual economics will have to be undertaken and the political will and political action of individual nations and the world community at large will have to be mobilized to ensure a sustained flow of development resources. Yields per hectare and per farm worker are very low (which accounts not only for the world food shortage but also for the poverty of most of the

world's farmers). It is now clear, however, that where modern plant varieties and farming techniques are introduced, farmers succeed in producing from their land, single-crop yields two or three times as large as the traditional return; multiple cropping of two or three crops on the same piece of land - something that is particularly feasible in the tropics and sub-tropics - gives yields from four to eight times larger than traditional ones. Specialists in agricultural development now believe new farming systems can be specifically designed for various tropical and subtropical conditions that hold immense promise of greatly enhanced output, better year-round utilization of farm labour and a significant opportunity to improve the nutritional and economic well-being of the small cultivator. That is the first step toward not only a new agriculture in the developing countries but also economic health.

As one considers the tropical farming world and the technology now available or soon to be available, there can be no grounds for pessimism about the latent potential of the world to feed increasing numbers of people for a long period ahead. Whether that latent potential will be harnessed to the benefit of man is the question. There has been much talk in both the developed and the developing nations about the need to accelerate agricultural development. The need remains, but the required political actions are slow in coming.

Pesticide Usage

The role of pesticide in attaining a significant growth in food production : Most authorities have placed world-wide pest losses at a minimum of 35 per cent of agricultural production. In developing countries, these losses may exceed 50 per cent, especially when quality deterioration is included. The main reason for this higher incidence is that the greatest majority of these countries are in tropical and sub-tropical areas, where the climate provides long, or often, continuous conditions favourable to pest development without the natural interruption caused (in temperate areas) by winters. In addition, as the agriculture of these developing countries is intensified and modernized with expanded monoculture, large use of fertilizers, multiple cropping, irrigation and higher crop densities, the potential for increased losses from pests is intensified.

The role of pesticides in integrated pest management : Integrated pest management is a systems approach to the reduction of losses from pests and diseases and of the impact of pest control measures on non-target organisms. Many kinds of technologies are applied to support each other. Effective pest control measures are deliberately selected, integrated, and applied with due consideration of economic, ecological and sociological results.

Systems of managing insect pests may involve such components as preventive measures, crop rotation, sanitation, cultivation, use of insect and disease resistant

crop varieties, conservation and the use of natural enemies, insect attractants, and pesticides. Knowledge of the interrelationships between pests, crops and the environment is needed in order to devise the best combination of the pest and produce minimum impact on non target organisms and environment.

Long before today's widespread use of pesticides in the developed countries, ecologically oriented pest-control strategies were being pursued in the United States and other countries. Entomologists working on the boll weevil during the first few years after its invasion into the United States from Mexico in the early 1890's made exceptional contributions. Without insecticides, entomologists employed optimal tactics, including cultural methods, resistant varieties, phytosanitation practices, and biological control. With the advent of petroleum-based insecticides, along with aerial application, ecologically oriented pest control in cotton died down.

But it soon became clear that there are limits to the advances that pesticides have created. And for years we have recognized the need to develop alternative techniques - some new and some old - to control pests and to help agricultural production and profitability.

Many factors suggest that there are sound economic, social, agricultural and public health reasons for exploring alternatives to petrochemical-based pesticides.

- Petroleum-based pesticides have become and will continue to be dramatically more expensive to the farmer ;
- The "energy crisis" is a serious matter, and all segments of our society are going to have to find ways to reduce consumption of petroleum-based products ;
- The ability of pests to develop resistance to chemicals continues to be a serious counter-productive side-effect to conventional pesticide use ;
- We have become increasingly aware of the health and ecological problems that pesticides can pose over long periods of use. Potential for long-term chronic effects such as cancer and birth defects is real. The effects of pesticide use on natural pest enemies and ecosystems in surrounding areas poses another serious problem area.

Research on the combination of pest control technologies and their integration into crop production systems offers the highest potential of any research in crop protection for alleviating world hunger and for improving the human environment. Such systems of integrated pest management are adaptable to all pests on all crops in every region of the globe.

The role of organochlorine insecticides : It would also be appropriate to briefly discuss the question of organochlorine insecticides for use in the developing countries. The importance of these pesticides cannot be measured by the action taken in recent times in a number of countries to severely restrict their availability and use. Their importance should not be measured by the amount currently used in developing countries but, rather, it should be judged by the socio-economic consequences of their use in these countries in the next 10 to 20 years. It is dangerous to generalize and most unwise to consider that all organochlorine pesticides offer the same advantages or present the same problems. Each is a distinct entity as is revealed by their biological performance under differing conditions.

In judging the importance of chlorinated hydrocarbon pesticides the following considerations should be kept in mind :

- As a group they represent the first synthetic insecticides and herbicides with satisfactory biological activity.
- There is a long history associated with their use under a vast variety of conditions. This experience amounts to something akin to a complete understanding of their properties, performance, fate, toxicity and residues.
- They are generally available in most countries or could be were it not for restrictions imposed by some national Governments.
- By comparison with the cost of all other pesticides, chlorinated hydrocarbon chemicals are significantly cheaper. In many developing countries the cost of even these relatively cheap materials is often beyond the means of the individual farmers or their national Government.
- Generally speaking, chlorinated hydrocarbons have a low acute mammalian toxicity with consequent minimal risk to users and bystanders, particularly in countries where literacy, education and medical services are relatively non-existent.
- The broad spectrum of biological activity of the chlorinated hydrocarbon pesticides makes them ideal for use in countries where the plant protection services are inadequate and where users cannot get the benefit of the technical advice required to use the more highly selective pesticides considered so desirable by scientists in industrial countries.
- The residual activity of chlorinated hydrocarbon insecticides makes them ideal for use as soil-applied chemicals. It is impossible to predict with

accuracy when soil insects will attack. The persistent organochlorine insecticides have been in widespread use against a wide variety of soil insects since their early introduction in the late 1940s.

- Under tropical conditions where the loss of biological activity is rapid and where successive generations of pests appear with startling suddenness, it is essential to use materials which will remain effective for at least a number of days, preferably several weeks.

As a class, the chlorinated hydrocarbon insecticides should be evaluated carefully under environmental conditions prevalent in the developing countries, because residue data generated in the temperate zone of the world do not necessarily relate to residue levels found under semi-tropical or tropical conditions.

For example, DDT has a "half-life" of about 30 months in temperate regions. By comparison, a "half-life" calculated from Indian data was between 3-9 months and, in a study in Nigeria, 90 per cent of the amount applied disappeared normally.

In considering world agriculture, one must accept the possibility that the use of specific or otherwise valuable chemicals or methods of application may have to be restricted or forbidden on grounds of unacceptable contamination of the environment. In applying this principle, however, likely gains and losses must be carefully evaluated, bearing in mind that agriculture and the developing world should not be unnecessarily denied the tools known to be required to develop productivity and improve human welfare.

General Comments on Pesticide Usage

The trends and extent of growth for the production and use of pesticides since World War II are documented for the industrial countries, particularly in North America, Western Europe and Japan. The overall amount of pesticides sold to the

TABLE I
Insecticides used in Developing Countries

	Relative use (%)	Annual projected rate of increase (%)
Organochlorines	50	9
Organophosphates	30	14
Carbamates	16	23
Other insecticides	4	—

developing countries is reasonably reliable, but the figures for individual countries, and even more the uses made of the pesticides imported, are not readily and reliably available.

Much pesticide production is limited at least in part to the industrialized countries of Europe, North America and Japan. Although much of the parent pesticidal compounds is formulated into final commercial form in the developing countries – in less frequent cases some steps in the synthesis of the parent compound being carried out in a developing country – there is very little production that includes all steps of synthesis and final formulation.

Although the rate of growth in pesticide use in developing countries has tended to be higher in recent years than in the developed countries, the small base from which this growth started still results in the total tonnage of use by all developing countries to be small in comparison with the countries with highly developed modern agriculture. In 1970, the United States consumed 45 per cent of all pesticide production, Western Europe 23 per cent, Eastern Europe 13 per cent, Japan 8

TABLE II
Relative usage of Pesticides in Developing Countries

Country	Amount 1971-3 (1,000) (metric) (tonnes)	% Total	Amount 1977* (1,000) (metric) (tonnes)	% Total
South America	26.1	30.9	71.8	43.0
Asia & Oceania	42.9	50.8	80.5	48.2
Africa	15.4	18.3	14.6	8.8

*Projected

per cent and the developing countries 7 per cent, with the rest in Australia and other developed countries.

When only the insecticide sector is examined, it is apparent that the trends in insecticide use compared to other pesticides is considerably different between the developing and developed countries. The relative growth rate between insecticides and other pesticides has been much less in the developed world compared with the developing world. In the developed world, insecticides have declined in relative position among pesticides from being the dominant class of pesticide before 1960 to representing about one-third of total pesticides usage currently. This is in spite of large increases in total tonnage of insecticide use. The decline is primarily due to

the rapid growth in the use of herbicides, which now represent the major portion of pesticides used and are still increasing in use at a more rapid rate than the others.

There is an indication that this trend may accelerate because of increased substitution by farmers of herbicides for tillage due to much higher fuel and equipment costs and equipment shortages. Weed control is also of importance in preventing nutrient loss, and the increased prices and scarcity of fertilizer have a stimulating effect on weed control.

In the developing world, insecticides are the dominant class of pesticide used and are increasing at a rate that would appear to maintain their dominant position for some time to come. However, herbicide use is increasing rapidly where the labour supply is depleted in many areas as a result of rapid urbanization.

Pesticide usage at present and projections for the future

During the past 16 years the global pesticide market has grown from 1.1 billion U.S. dollars to 7 billion U.S. dollars. Now, however, there is increasing

TABLE III

*Projected World Demand for Pesticides - Millions of U.S. Dollars**

	1980	1985	1990
Herbicides			
Triazines	900	1240	1710
Carbamates	360	460	600
Phenyl ureas	315	520	860
Phenoxy	260	450	780
Total with others	3450	5140	7700
Fungicides			
Dithio carbamates	580	735	900
Captan	500	580	665
Coppers	186	215	260
Total with others	1345	1600	1880
Insecticides			
Organophosphates	1440	1870	2450
Carbamates	610	800	1050
Organochlorines	330	400	200
Total with others	2390	3070	3700

*From chemicals for Crop Protection and Pest Control, M.B. Green, G.S. Hartley and Tf West Pergamon Press, 1977.

evidence that this rapid growth rate is beginning to slow down. The annual increase in demand has slipped from 10 per cent or more in the 1960s to less than 5 per cent now. And the lower growth rate probably will continue over the next few years.

TABLE IV
Pesticide Sales
Composite Analysis of All Reporting Companies

	1976 (000)	1977 (000)	Per cent change
Domestic Sales of			
Domestic Production	\$ 1,996,391	\$ 2,256,190	+ 13.0
Export Sales of			
Domestic Production	561,367	738,955	+ 31.6
Domestic Sales of			
Foreign Production	98,868	119,638	+ 21.0
Total	\$ 2,656,626	\$ 3,114,785	+ 17.2

For those companies responding to the survey, total sales of pesticides in the U.S. reached \$ 3.11 billion in 1977, an increase of \$458 million (or 17%) over 1976. Most of this growth occurred in domestic sales of domestic production (up \$260 million or 13 per cent) and export sales of domestic production (up \$178 million or 32 per cent). Domestic sales from foreign production increased by 21 per cent, which corresponded to only \$21 million.

*Table IV, V, VI, VII taken from National Agricultural Chemicals Association, 1977, U.S.A.

TABLE 5
Domestic Sales of Domestic Production
Composite Analysis of All Reporting Companies

	1976 (000)	1977 (000)	Per cent change
Herbicides	\$ 1,251,154	\$ 1,348,004	+ 7.7
Insecticides	514,017	634,746	+ 23.5
Fungicides	106,594	134,688	+ 26.4
Plant Growth Regulators	28,065	28,131	+ 0.2
Nematicides	42,426	60,205	+ 41.9
Miscellaneous	54,135	50,416	- 6.9
Total	\$ 1,996,391	\$ 2,256,190	

Number of Participants : 42



Rpt.

Of the 7 billion U.S. dollars worth of pesticides sold last year, herbicides constituted nearly 39 per cent (\$ 2.7 billion) of the market. Insecticides followed a close second at almost 36 per cent of the total (\$ 2.5 billion). Fungicides were the third largest segment at slightly more than 21 per cent (\$ 1.5 billion). Plant growth regulators made up the 4 per cent balance (\$ 300 million).

More than 80 per cent of pesticide sales are in the developed regions of the world. North America is the largest geographical sales area (39 per cent of the 1976 total). Then follow Western Europe (25 per cent), Eastern Europe, including

TABLE VI
Export Sales of Domestic Production
Composite Analysis of All Reporting Companies

	1976 (000)	1977 (000)	Per cent change
Herbicides	\$ 248,456	\$ 327,592	+ 31.8
Insecticides	235,587	315,557	+ 33.9
Fungicides	61,110	70,338	+ 15.1
Plant Growth Regulators	5,515	6,312	+ 14.4
Nematicides	5,784	13,022	+ 125.1
Miscellaneous	4,915	6,134	+ 24.8
Total	\$ 561,367	\$ 738,955	+ 31.6

Number of Participants : 37

TABLE VII
Domestic Sales of Foreign Production
Composite Analysis of All Reporting Companies

	1976 (000)	1977 (000)	Per cent change
Herbicides	\$ 68,588	\$ 91,378	+ 33.2
Insecticides	24,757	24,973	+ 0.8
Fungicides, Plant Growth Regulators, Nematicides and Miscellaneous*	5,523	3,287	- 40.5
Total	\$ 98,868	\$ 119,638	+ 21.0

*Combined to avoid disclosure of individual company data.

Number of Participants : 26

the Soviet Union (10 per cent), the Far East (9 per cent), and Central and South America (7 per cent). The remaining 10 per cent of sales goes to other countries, mainly those in Africa.

Future growth likely will be dominated by expansions in the developed markets rather than in any marked extensions of pesticides use in the less affluent nations.

There are two basic reasons why the less developed countries won't increase their use of herbicides appreciably in the near future. One is that they cannot afford the relatively high cost of the product. The other is that a large proportion of the population in these countries is engaged in agriculture with little alternative employment available. The incentive, therefore, to replace cheap and plentiful labour with costly herbicides is low.

Tables (I-III) give some indication of pesticide use on a world-wide basis. A recent survey of the pesticide industry in the US is documented in Tables IV-V. FAO officials were not able to give me data on pesticide usage in the developing countries at this time but as for the future, a 10 per cent increase per year in overall pesticide usage appears to be a reasonable projection.

3 TYPES AND QUANTITIES OF PESTICIDES USED IN INDIA

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Biocidal agricultural chemicals, collectively called pesticides, which include the insecticides, fungicides, acaricides, nematocides, herbicides, rodenticides and molluscicides, have proved to be the most effective, dependable and economical tool in pest management on crops. Use of the correct pesticides at the proper stages of crop growth, and at the appropriate time in relation to pest appearance, helps in averting or minimising the loss due to pest attack. Chemical methods of control are still the only method for control of many important pests of agriculture and public health as it quickly checks further multiplication of the pest species and helps in keeping pest infestations well below the economic threshold level. Adoption of modern agricultural practices by introduction of new high-yielding and hybrid varieties of crops, use of fertilizers, new kinds of crops etc. has introduced more and more pest and disease problems necessitating increased usage of pesticides. In the

wake of growing concern over environmental hazards, due to large scale and indiscriminate use of pesticides, greater stress has been laid in the recent years on integrated methods of pest control.

Type of Pesticides in use in India

Crop protection in India began in 1947–48 with the introduction of BHC and DDT for control of insect pests of crops. This was soon followed by the introduction of ethyl parathion and HETP among organophosphorus compounds, endrin and endosulfan among organochlorines and carbaryl among carbamates. A large number of organophosphorus insecticides were permitted to be imported subsequently and made available for pest control. A few systemic organophosphorus insecticides found greater acceptance for the control of sucking pests of crops. Research on discovering suitable and safer acaricides for use on tea resulted in dicofol, chlorobenzilate, ethion, tetradifon, etc. being recommended by the Tocklai Experimental Station, Jorhat, Assam and the UPASI Tea Research Station, Valparai. Early fungicides for commercial use in India were based mostly on copper. Later, Bordeaux mixture and Burgundy mixture became prominent as they were more effective and not phytotoxic. From 1940 mercury compounds were introduced from Germany, particularly as seed dressings. Since 1950, organosulphur fungicides such as dithiocarbamates were in large scale use. In recent years, certain antibiotics, organophosphorus compounds and systemic fungicides have found use in control of some specific diseases of crops. A number of compounds specially fumigants, are used to control nematodes, chief among them being DD, ethylene, dibromide and dibromochloropropane. Certain systemic organophosphorus compounds like fensulfthion and fenamiphos have also shown promise. Among herbicides 2,4-D became popular and at present a good number of herbicides are available. With the awareness of damages caused by rodents a few rodenticides have also been introduced. Among fumigants ethylene dichloride and aluminium phosphide are popular in the control of stored grain pests.

In the First Plan period and to a great extent in the Second Plan greater stress was on irrigation and fertilizer usage. Only in the Third Plan period did pesticides receive their due importance as one of the major inputs in crop production.

The different types of pesticides that have been approved for registration (as on Nov. 1, 1978) under the Insecticides Act 1968 are listed in Table I. There are 110 pesticides so far approved for registration in the country.

Production of Pesticides

The spectacular results obtained in crop protection by use of BHC and DDT during 1947–48 led to indigenous manufacture of BHC in 1952 and DDT in 1954 in the country. Since then a number of compounds have been manufactured in the

TABLE I

List of Pesticides that have been approved for Registration by the Registration Committee under the Insecticides Act, 1968

Insecticides

1. Aldicarb
2. Aldrin
3. Allethrin
4. BHC
5. Carbaryl
6. Carbofuran
7. Chlordane
8. Chlorfenvinphos
9. Chlorobenzilate
10. Chlorpyrifos
11. Copper aceto arsenite
12. DDT
13. Diazinon
14. Dichlorvos (DDVP)
15. Dicofol
16. Difenphos
17. Dimethoate
18. Dinocap
19. Endosulfan
20. Ethion
21. Fenitrothion
22. Fenthion
23. Formothion
24. Heptachlor
25. Lindane
26. Malathion
27. Menazon
28. Methyl parathion
29. Monocrotophos
30. Nicotine sulphate
31. Oxydemeton
32. Phenthoate
33. Phorate
34. Phosalone
35. Phosphamidon

36. Propoxur
37. Pyrethrum
38. Quinalphos
39. Tetradifon
40. Thanite
41. Thiometon
42. Toxaphene
43. Trichlorphon

Fungicides

44. Aureofungin
45. Benomyl
46. Calixin
47. Captan
48. Carboxin
49. Carbendazim
50. Copper Oxychloride
51. Copper Sulphate
52. Cuprous Oxide
53. Difolatan
54. Ediphenphos
55. Ferbam
56. Maneb
57. Mencozeb
58. Nickel Chloride
59. PCNB
60. PMA
61. Streptocycline
62. Sulphur
63. Thiram
64. Zineb
65. Ziram

Herbicides

66. Alachlor
67. Atrazine

68. Butachlor	91. D.D. Mixture
69. Dalapon	92. Dibromochloropropane
70. Diuron	93. EDCT
71. DSMA	94. Ethylene Dibromide
72. Fluchloralin	95. Methyl Bromide
73. Glyphosate	96. Paradichlorobenzene
74. Linuron	97. Sodium Cyanide
75. MCPA	
76. Methabenzthiazuron	Rodenticides
77. Metoxuron	98. Barium Carbonate
78. MSMA	99. Calcium Cyanide
79. Nitrofen	100. Coumachlor
80. PCP	101. Warfarin
81. Paraquat dichloride	102. Zinc Phosphide
82. Paraquat Dimethyl Sulphate	
83. Propanil	Growth Regulants
84. Sirmate	103. ANA
85. Simazine	104. Chlormequat Chloride
86. 2,4-D	105. Ethepom
87. Triallate	106. Gibberellic acid
88. Trichloroacetic acid (TCA)	107. Maleic Hydrazide
	Others
Fumigants	108. EMC
89. Aluminium Phosphide	109. Metaldehyde
90. Carbon Tetrachloride	110. MEMC

country. The technical grades of different types of pesticides that have been licensed/letter of intent granted for manufacture in India are listed in Table II.

TABLE II

List of Pesticides that have been Licensed/Letter of Intent Granted for Manufacture in India

(As on June 15, 1978)

Insecticides	6. Dimethoate
1. BHC	7. Endosulfan
2. Carbaryl	8. Ethion
3. DDT	9. Fenitrothion
4. DDVP	10. Fenthion
5. Diazinon	11. Formothion

12. Lindane
13. Malathion
14. Methyl Demeton
15. Monocrotophos
16. Nicotine Sulphate
17. Parathion
18. Phenthoate
19. Phorate
20. Phosalone
21. Phosphamidon
22. Pyrethion insecticides
23. Quinalphos
24. Temephos
25. Tetradifon
26. Thanite
27. Thiometon

Fungicides

28. Car bendazim
29. Carboxin
30. Copper oxychloride
31. Copper sulphate
32. Ferbam
33. Metham sodium (Vapam)
34. Nickel chloride
35. Organo mercurials
36. Sulphur
37. Thiram

38. Zineb
39. Ziram

Rodenticides

40. Coumafuryl (Funarin)
41. Zinc phosphide

Herbicides

42. 2,4-D
43. Dalapon
44. Diuron
45. Fluchloralin
46. MCPA
47. MCPB
48. Nitrofen
49. Paraquat
50. Propanil
51. TCA

Fumigants

52. Aluminium Proshide
53. Ethylene dibromide
54. Methyl bromide

Growth regulants

55. ANA
56. Cycocel

(Source : Pesticides information 4(2) : 46-51, 1978)

The progress of production of pesticides in India from 1954 to 1977 is furnished in Table III. This shows the growth of industry during the period of 20 years. The initial production of 432 tonnes has risen to 40,658 tonnes by 1977. To augment the local demand of pesticide requirements the indigenously manufactured products were supplemented by substantial imports of other registered pesticides. For such of these pesticides which are imported at present, the Government of India is keen to have basic manufacturing programme at the earliest possible time.

TABLE III
Progress of Production of Pesticides in India
(From 1954 to 1977)
(in tonnes)

Year	Insecti- cides	Fungi- cides	Herbi- cides	Rodenti- cides	Others	Total	No of Products manufactured
1954	432	—	—	—	—	432	1
1955	2303	—	—	—	—	2303	2
1956	2836	—	—	—	—	2836	2
1957	2479	—	—	—	13	2492	3
1958	4835	600	—	—	25	5460	4
1959	5549	398	—	—	14	5961	4
1960	6729	598	—	104	11	7442	10
1961	7455	853	—	140	—	8448	12
1962	7334	830	—	39	—	8203	12
1963	8392	1129	—	49	—	9570	15
1964	8913	1567	—	234	—	10714	17
1965	10423	411	60	269	—	12168	20
1966	11391	2274	23	239	24	13951	30
1967	12541	2357	37	404	27	15366	30
1968	14780	800	34	160	55	15829	30
1969	16393	996	12	145	100	18647	39
1970	21722	2191	14	39	135	23501	40
1971	21191	2067	30	195	250	23713	40
1972	23670	2602	63	56	321	26712	42
1973	27319	3156	321	141	—	30937	NA
1974	29745	2505	237	165	—	32652	NA
1975	32898	1906	240	188	15	35247	NA
1976	33016	1193	201	193	1	34644	NA
1977	37772	2342	474	68	12	40658	NA

N.A: Not available

Figures for 1954 to 1972 The Chemical Times, Spl. supplement on Agrochemicals, Sept. 13, 1976.

Figures for 1973 to 1977 - Source : Pesticides Information 4 (1): 45, 1978.

Consumption of Pesticides in India

The trend of consumption of major groups of pesticides during 1954 to 1977-78 is given in Table IV. From a total consumption of 434 tonnes technical

TABLE IV

Trend in Consumption of Major Groups of Pesticides in India (in Tonnes of Technical Material)

Pesticides Groups	1954	1960	1965	1969	1970	1971	1973-74*	1974-75*	1975-76*	1976-77*	1977-78*
Insecticides, Acaricides & Nematicides	434	6,729	10,428	17,393	21,741	22,013	38,970	46,515	46,605	41,250	46,755
Fungicides	—	598	1,480	986	1,774	2,067	10,337	9,600	9,183	8,553	9,685
Herbicides	—	—	—	12	15	30	810	715	890	975	1,425
Rodenticides	—	104	268	145	80	195	150	250	200	290	270
Other	—	—	—	—	—	—	165	150	275	850	845
Grand Total	434	7,341	12,176	18,536	23,160	24,305	50,432	57,230	57,153	51,918	58,980

*Estimated consumption based on import/production (Pesticides Information 4 (2) : 34-36, 1978)

Figures for 1954 to 1971—The Chemical Times—Spl. Supplement on Agrochemicals, Sept. 13, 1976.

material in 1954 it has risen to 24,305 tonnes by 1971, which has shown further increase of 142.7 per cent by 1977-78 reaching a level of 58,980 tonnes. It may be seen that there is a remarkable increase in consumption of insecticides. The growth in the consumption of herbicides is relatively low since 1973 and that of fungicides rather static.

Future requirement of Pesticides and Strategy

The Planning Commissions working group on the pesticides industry has placed the demand for technical grade pesticides at 47,790 tonnes during 1978-79 which is expected to rise to 64,290 tonnes during 1983-84 at an annual growth rate of 6 per cent (Table V).

TABLE V
Estimated requirements of pesticides in Agriculture
(Based on average of 6 per cent growth rate)

Group of Pesticides	(Figures in Tonnes)					
	1978-79	1979-80	1980-81	1981-82*	1982-83	1983-84
Insecticides	34,450	36,855	38,455	41,005	43,320	46,515
Acaricides	170	180	190	200	210	220
Nematicides	30	35	40	45	50	55
Fungicides	10,100	10,624	11,124	11,568	12,013	12,500
Rodenticides	350	450	550	650	750	850
Herbicides	1,630	1,855	9,095	2,320	2,540	2,770
Fumigants	950	1,025	1,100	1,175	1,250	1,325
Plant Growth Regulants	30	35	40	45	50	55
Grand Total	47,790	50,959	53,594	57,008	60,183	64,290
<i>For Public Health</i>						
BHC	17,000	18,000	19,000	20,000	21,000	21,500
DDT	10,200	12,800	1,2800	12,800	12,800	12,800
Malathion	5,500	11,800	12,400	13,000	13,700	13,700

Source : Report of the working Group on Pesticides Industry for the Plan 1978-79 to 1983-84.

(Pesticides Information 4(2) : 31-33, 1978)

According to this working group, the installed capacity of pesticides industry by 1982-83 is likely to rise to 80,000 tonnes with a corresponding level of expected production of 65,000 tonnes which may represent capacity utilisation of 81 per cent.

The central public sectors viz., the Hindustan Insecticides Ltd., and the Hindustan Organic Chemicals Ltd., are presently engaged in pesticide production. HIL produces mainly DDT and BHC and has recently expanded its capacity for these insecticides. It has also a diversification programme for the manufacture of malathion and endosulfan, and also a number of other products.

The Government of India allows import of technical grade material of pesticides approved for registration for developing and sustaining the domestic formulation industry. However, import of such pesticides that are currently manufactured in the country is banned. Furthermore, with a view to ensuring economic and easy availability to farmers, the Government has ruled that 50 per cent of the technical grade pesticides produced in the country is supplied to non-associated formulators. As well as this 50 per cent of the imported technical grade pesticides is allowed to formulators nominated by the State Governments. The Bureau of Industrial Costs and Prices carried out cost study of 10 major pesticides and based on this study Union Ministry of Chemicals and Fertilizers has recently initiated action to bring down the prices.

A suggestion has also been made by the groups that the State Agro-Industries Corporation and State Industrial Development Corporations should provide quality control and marketing facilities to the small scale units. There is also a move to set up a joint sector company in one of the southern states; the public sector HIL and the four southern state agro-industries corporations will be collaborators in the project.

Over the past few years, there has been an increasing use of pesticides for aerial application. Action by the Government of India to build up a strong agro-aviation set up in the country to popularise aerial spraying would help in increasing the consumption of pesticides.

CONCLUSIONS

A critical appraisal of the progress of production of the different types of pesticides, their consumption in the past two decades, and the future projection of the estimated requirement of pesticides for agriculture for the next 5 years bring out some interesting points.

The Registration Committee of the Central Insecticides Board, constituted under the Insecticides Act 1968, plays a vital role in critically evaluating the data on toxicological aspects, bio-efficacy, residues and shelf-life of the different pesticides before according permission for registration. The present policy of the Registration



Committee thus paves the way for proper evaluation and introduction of new products.

It is seen that there were four products manufactured in India during 1959 as against 56 products that have been licenced/letter of intent granted for manufacture in the country as of today. Therefore, for nearly 50 per cent of the approved products there is a basic manufacture programme. It is hoped that there will be substantial progress in that direction aiming at meeting the national demand of pesticides by indigenous production.

Analysis of the trend of consumption of various kinds of insecticides over years indicates that there is steady shift from use of conventional insecticides like BHC, DDT, malathion, etc. to speciality products such as endosulfan, phosalone, monocrotophos, quinalphos, carbaryl, etc. This points out that the production—demand balance for various types of insecticides would be different, and this would call for a careful projection of demand for each individual product to build up necessary production capacities.

Amongst the various group of pesticides, insecticides constitute nearly 73 per cent of the estimated requirement of pesticides for agriculture, whereas fungicides constitute about 20 per cent. The remaining 7 per cent is composed of acaricides, nematicides, rodenticides, fumigants and herbicides. There is lot of scope for development on the usage of herbicides, rodenticides and nematicides. When compared with developed countries Japan, USA and W. Germany the per hectare consumption of herbicides in India is negligible. With increase in cost of labour and awareness on the benefits of chemical weed control good progress should be expected in their usage in the country. Being a fast developing nation it is hoped that in the field of pesticide industry as well as crop protection in agriculture there would be significant progress.

The author is grateful to the management of Voltas Ltd., and to Mr. T. K. Aga, Divisional Manager, Agro-Industrial Products Division for encouragement in the preparation of this paper.

PESTICIDE AVAILABILITY AND FACTORS LIMITING USAGE IN INDIA

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The word availability implies a wider meaning than just the physical availability of the products and concerns also availability in time and place. The pesticide must be available at when and where required. To generate this time and place availability—products must be manufactured depending on market needs *i.e.*, demand, production capacity and physical outlets must be identified and distribution and inventories established of the proper products as per the needs and at the proper place.

So the various aspects of 'Pesticide Availability' and the factors affecting this and limitations for use with special reference to Indian conditions will be discussed here.

Market Demand/Production Capacity

The projected demands for pesticides (insecticides, fungicides and herbicides) in the world, the United States of America and in India are presented in Table I. They indicate that the total estimated demand for pesticides in 1980 will be about

TABLE I
Actual and Projected Market of Pesticides (Million Dollars)

	WORLD		USA		INDIA	
	1974	1980	1974	1980	1977	1980
Insecticides	1822	2413	492	642	122	146
Fungicides	961	1382	116	158	35	50
Herbicides	2190	3422	1058	1523	17	22

Sources : Farm Chemicals, September 1975

*Author's estimate based present trend of market growth at 6%

218 million U.S. dollars. Also, the comparative world and U.S. markets show that the pesticide demand is on the rise. From the available data on pesticide use, the important crops, cotton, rice, vegetables and other horticultural crops consume the major share of pesticides—Table II. In other countries similar trends are observed.

This raises another very pertinent question as to the present and projected capacities of major pesticide production in India? Tables III, IV, V indicate the

TABLE II
Pesticides Market by Crop Group
(In million U.S. Dollars)

CROPS	WORLD—1974			USA—1974			*INDIA—1977		
	Insecti- cides	Fungi- cides	Herbi- cides	Insecti- cides	Fungi- cides	Herbi- cides	Insecti- cides	Fungi- cides	Herbi- cides
Cotton	665	29	240	137	12	97	67.1	—	—
Rice	243	91	181	9	1	23	18.3	3.5	—
Tobacco	39	19	11	14	2	6	—	—	—
Vegetables	129	227	58	40	19	30	12.2	5.25	—
Horticultural									
Crops	491	446	125	160	62	49	—	19.25	—
Maize	174	19	680	85	4	423	—	—	—
Others	81	136	895	47	6	430	24.4	7.0	—
TOTAL	1822	961	2190	492	116	1058	122	35	17

Sources : Farm Chemicals, September 1975

* Based on Author's survey

present installed capacities and also projected demands for the years 1982-83. As regards the projected requirements of insecticides the installed capacities are low for products like malathion and DDT. However, the shortages of these products

TABLE III
Installed Capacity, Actual and Projected Market of Major Insecticides
(in Metric Tons)

Insecticides	Installed Capacity	Actually Market (1976)	Demand (1982-83)
1. B.H.C.	28900	24252	53000
2. D.D.T.	4200	9980	15600
3. Methyl Parathion			
Metasystox, Fenitrothion	2550	1279	2000
4. Malathion	2500	1732	13500
5. Phosphamidon	912	347	950
6. Quinalphos	200	192	1000
7. Dimethoate	1000	352	1000

Communication from Pesticides Association of India

may not be felt as some newer products will be manufactured in the country like fenthion, endosulfan, phosalone and carbaryl. The installed capacity for fungicides and herbicides presently in use seems to be adequate. However, this does not necessarily mean that no more fungicides or herbicides are need for the Indian market. The

TABLE IV
Installed Capacity, Actual and Projected Market of Major Fungicides
(In Metric Tons)

Fungicides	Installed Capacity	Actual Market	Projected Demand (1982-83)
1. Copperoxy Chloride	2460	465	750
2. Zineb/Maneb	984	578	3000
3. Thiram/Ziram	2500	68	
4. Organo Mercurials	47	54	
5. Nickel Chloride	300	30	100

Communication from Pesticides Association of India

present condition of the fungicide and herbicide market may also be due to the fact that these specialised markets are yet to be developed in India and secondly that the control of fungal diseases and weeds in crops may need a more diversified range of products.

TABLE V
Installed Capacity, Actual and Projected Market of Major Herbicides
(in Metric Tons)

Major Herbicides	Installed Capacity	Actual Market (1976)	Projected Demand (1982-83)
1. 2,4-D	1936	202	1000
2. Dalapon	—	100	250
3. Nitrofen/Propanil	1500	80.0	500
4. Diuron/MCPA	—	20.0	510
5. Paraquat	500	125	300

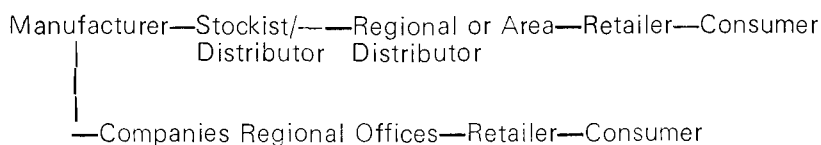
Communication from Pesticides Association of India

In addition to the above products, which are listed in the tables other insecticides/fungicides/herbicides are imported. Although in general, the picture on availability of pesticides looks satisfactory, it may turn into shortages if the average consumption of pesticides increases in India.

Distribution

The channels of pesticide distribution in India are :

1. *Private Trade* :



2. *Semi Government* :



3. *Government supplies* :



This shows that pesticides distribution takes place through various channels and sometimes it is a lengthy process. Although in the recent years the distribution of pesticides has improved considerably it still needs further improvement. Because of various rules and regulation in case of government supplies are there are often inordinate delays in supplies of required products.

The maintenance of equilibrium between production and distribution is a basic necessity. However, this equilibrium is affected by certain factors and these become main limitations for pesticide availability and use.

Factors affecting availability of Pesticides

1. *Seasonal Demands* : Demands for pesticide goods are seasonal and in addition they are of varied nature related to differing crop/pest/disease situations. Sometimes the outbreaks of a disease, pest endemic are predictable but there is much work which remains to be done. This results usually in the occurrence of a peak load in demand of certain pesticides for which neither industry nor government is prepared.

2. *Inadequate Storage Facilities* : The storage facilities available prior to use are deplorably low and are not properly organized. Added to that the investment needed to store the product in advance of the season is not available. This results in a shortage of the desired product at the requisite place.

3. *Lack of well developed means of transportation* : The transport system of our country is not satisfactory because although we are a nation with wide distances

we lack adequate means of transport ; so dispersion of goods takes time.

4. *Lack of timely material supplies* : Raw materials, emulsifiers, and packaging material supplies are not available at the right time.

Factors limiting usage of pesticides in India

1. *Lack of knowledge* : The majority of farmers are not aware of pest disease problems of their crop. Often they do not undertake correct and properly timed control measures. This results in losses in crop yields.

2. *Lack of sufficient extension activities* : Due to the vastness of the problem and changing situation, the extension activities often fall very short. The extension workers are not always properly trained and much of the time are not aware of local pests/disease problems. This ultimately results in poor guidance to farmers about the proper and judicious use of pesticides and also in non-desirable pesticide residues in food/milk etc.

3. *Lack of proper equipment* : Many farmers with small holdings do not have application equipment like sprayers/dusters etc. In a few states through the Plant Protection Dept./Zilla Parishad or local Agricultural Panchayat/Cooperative Societies the equipment is made available, the equipment is often not in full working condition.

4. *Initial high cost of application* : The majority of marginal Indian farmers are poor and lack the resources for an initial investment for plant protection except for cash crops like cotton. They attach least significance to plant protection and feel the initial cost of application of pesticides to be too high.

5. *Reasons for high cost* : One of the reason the prices are high is that of taxes, in particular the importer has to pay excise duty. Secondly the costs of packaging materials, transport, distribution etc. escalate the prices further.

6. *Regulatory control* : Administrative interference from government bodies and delays in grant of manufacturing/import licences are also responsible for such delays and difficulties. Sometimes the products are not distributed to where they are most needed. In some situations a product in stock is recommended for a pest/disease which it does not control.

7. *New products* : Due to the increased safety factors to human beings the cost of development of new pesticides has risen alarmingly. However, due to changes in pest and disease patterns as well as development of resistance to currently used pesticides, a need for newer products is felt. Often, due to varied regula-

tory factors/controls over the manufacturers the new products are not immediately available for Indian farmers.

Thus it is evident that though the present availability of pesticides is satisfactory it needs to be improved in years to come. It is also necessary to eliminate the limitations for use described above by an progressive extension programme for pesticides by the mutual co-operation of Industry and Government Extension Agencies.

5 CURRENT STATE OF PESTICIDE USE IN KERALA FORESTS AND FUTURE PROJECTION UP TO 2000 A.D.

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SUMMARY

Very few pesticides are being used in Kerala Forests currently. About 86 per cent of the 9,400 sq km of forests in the State consist of natural forests where no pesticides are used because there are no pest problems. Even in man-made forests (forest plantations) use of pesticides is an exception rather than rule.

In **Eucalyptus** plantations, aldrin or a similar insecticide is used against termites. As per the method of application standardized by the Kerala Forest Research Institute, only 300 g a.i. of the insecticide need be used per hectare of plantation. Since application of insecticide is necessary only at the time of initial planting (once in 30 years), the rate of use works out to 10 g a.i./ha/year.

Teak, an important forest crop in Kerala which occupies over 66,500 ha, is usually subject to annual defoliation by insects, but there is no justification for resorting to large-scale insecticidal control.

With more intensive utilization of forests, particularly with the projected expansion of area under some of the fast-growing tree species in the next two decades, pest problems will increase both in magnitude and variety. Some of these new pest situations will require the use of insecticides for effective control. However, as a reasonable choice of tree species is available for most uses, the major trend in forestry practice will and should be to select and develop plantations of those species which are susceptible to insects and diseases.

About 24 per cent of the total geographical area of Kerala, that is about 940,000 hectares, is covered by forests. Of this area, about 85 per cent is natural forests which provide the greater part of the forest produce of Kerala. Although they support a rich fauna of insects, there are no pest problems in these natural

forests. The major forest types in Kerala are evergreen, semi-evergreen, moist deciduous and dry deciduous forests. The absence of pest problems in the natural forests is obviously due to minimal interference of the ecosystem by man. Typically, in these natural tropical plant communities, no single species of tree account for more than 3 to 4 per cent of the total number present in a unit area, so that favourable conditions such as in monoculture are not available for pest multiplication.

The remaining 15 per cent of the forest area, that is about 140,000 ha, consists of man-made forests. This is where insects cause some serious economic damage sometimes necessitating the use of pesticides. For most tree species which have already been raised in large plantations, there is at least one major insect problem which appear to be economically significant. The major forest plantations in Kerala and the important pest problems of each are discussed below, with details of the use of pesticides.

The most important man-made forest in which pesticides are used as a routine is in *Eucalyptus* plantations. Two species of *Eucalyptus*, viz. *E. grandis* and *E. tereticornis* are grown in Kerala mainly to meet the requirement for pulpwood. Presently, about 25 per cent of the total man-made forests consist of *Eucalyptus*. Termite attack is the most serious insect problem because Subterranean termites eat the roots of saplings up to 3 years old and kill them. Our experiments showed that, over 70 per cent of the saplings are killed by termites in the first year of planting itself. So it is not possible to grow *Eucalyptus* without controlling termites. To raise a plantation, seedlings are first grown in small polythene bags filled with soil and when the seedlings are about 3-4 months old, they are planted out in field pits along with the soil-cake. Until recently, the planting pits were treated with aldrin or a similar soil insecticide at a rate of 0.5 g a.i. per pit., which amounts to using 1.25 kg a.i. per hectare of plantation. By the method standardised by us, the quantity of insecticide used (aldrin or heptachlor) can be reduced to 0.03 g a.i. per plant by mixing a 5 per cent insecticide dust in the soil used in the polythene bags instead of in field pits. This works out to only 75 g a.i. per hectare of plantation. If a simple application procedure, i.e. drenching the soil in polythene bags with an emulsified preparation of the insecticide is used it will require about 300 g a.i./ha to be effective. It must be emphasised that the application of insecticide is required only at the time of planting and is used only once in 30 years. This is a very meagre amount compared to that necessary in most other situations.

Some fungi also cause damage to *Eucalyptus*. The use of a fungicide such as thiride or dithane in *Eucalyptus* nurseries to prevent damping-off and infection by *Cylindrocladium* has become a routine currently. Since it is used only in nurseries, the total quantity used is meagre. In recent years, a major fungal disease—the pink

disease caused by *Corticium salmonicolor*—has posed a threat to *Eucalyptus* plantations in some parts of Kerala. The current efforts to solve this problem are aimed at developing plantations of other tree species (including alternate species of *Eucalyptus*) which are resistant to *Corticium* and at the same time suitable for pulping (paper pulp), rather than attempting to control the disease by using fungicides. A research unit has been set up in Kerala specifically for this purpose.

Teak which accounts for about 51 per cent of the total man-made forests and more than 100 species of insects are known to attack the living teak tree in India although only two of them are considered serious pests. These are the caterpillars, *Pyrausta machaeralis* and *Hyblaea puera*. In most years, there is severe defoliation by these insects twice a year. An experimental aerial spraying of endrin was carried out by the Forest Research Institute, Dehra Dun in about 76 hectares of teak plantation in Konni Forest Division in the year 1965. However, no control operations are carried out as a routine at present and we have no accurate estimate of the loss of increment, due to defoliation (if any). The Kerala Forest Research Institute is presently attempting to gather this information. There is no justification for chemically controlling these insects until this information is available. Based on the author's judgement, the impact of insect defoliation on increment loss of teak is generally overestimated due to the spectacular visual effect generated by extensive defoliation and control may not be necessary except, perhaps, in very young plantations, during certain years.

The other man-made forests in Kerala at present are mostly small-scale experimental plantations including *Salmalia malabarica* (Elavu), *Swietenia macrophylla* (Mahogany), *Ochroma lagopus* (Balsa), *Dalbergia latifolia* (Rose wood), *Pinus patula* and *Pinus insularis* (tropical pines), *Albizzia falcata* and *Gmelina arborea* (Kumbil). Most of these have insect problems but the full economic impact of these will be known only when large plantations are raised. At present no estimate of the damage is available and no routine insect control measures are practiced. The most important insect problems are the following. *Salmalia malabarica* is attacked by the shoot borer, *Tonica niviferana* (Lep., Oecophoridae). *Swietenia macrophylla* is attacked by the shoot borer, *Hypsipyla robusta* (Lep., Phycitidae). *Pinus* spp. are attacked by the coccid, *Ripersia resinophylla*. A malathion spray was applied to a young plantation of *Pinus* in Devicolam Range in 1973 to control the coccid. *Albizzia falcata* is attacked by a bagworm, *Acanthopsyche (Pteroma)* sp. which has caused extensive total defoliation of trees in some areas in 1977 and 1978. *Gmelina arborea* is attacked by the bug, *Tingis beesoni* which cause defoliation and top-drying in saplings. This year we applied a lindane spray in a small patch of affected area in Kothamangalam Division to check further spread of the infestation.

To summarise, the current state of pesticide use in Kerala forests, except for a small quantity of soil insecticides like aldrin or heptachlor used in *Eucalyptus* plantations and the small quantity of fungicides like thiride and dithane used in *Eucalyptus* nurseries, no insecticide is used as a routine. Experimental applications of some insecticides are made by the Kerala Forest Research Institute when some new pest situations call for control.

The future trend of pesticide use in Kerala forests is linked with the future direction of growth of forestry in Kerala. During the past 25 years, there has been a substantial increase in man-made forests by replacing natural forests. In 1950, only 2.5 per cent of the total forest area consisted of plantations. Today the forest plantations occupy about 15 per cent of the total forest area, a little over 140,000 hectares as compared to about 23,500 hectares in 1950. In 1950, most plantations were of teak; now about 51 per cent are teak, about 25 per cent *Eucalyptus* and the rest consist of miscellaneous hardwood species. Presently a project for planting *Eucalyptus* over an area of 44,500 ha over a period of 10 years is being implemented by the Kerala Forest Development Corporation, to meet the requirements for pulpwood. This in itself will result in substantial increase of the total plantation area in the next 10 years. As no new land is available in Kerala for afforestation, the conversion of natural forests into plantations will progress at a rapid rate in the coming years. This will also mean conversion of pest-free area into pest-prone area. Converting natural forest into plantations is not the only way to increase the output of utilisable timber. Scientific management and tending of the natural forest to promote natural regeneration of the desired tree species is a profitable approach which may cause less ecological disruption. However, plantations offer certain advantages which cannot be ignored. With the establishment of the Kerala Forest Research Institute in 1975, forestry in Kerala is now on the threshold of a new era of scientific growth. The new directions of growth are already clear. Emphasis will be placed on developing techniques of natural regeneration of valuable evergreen forests and in developing scientifically managed plantations of indigenous tree species selected to meet various end use requirements. Attempts will be made to improve the plantation species by genetic selection.

Insecticides may have a new role to play in future plantations, but what the future plantations will be, will become clear only in the next 5 years or so. With the projected expansion of area under some of the fast-growing tree species in the next two decades, pest problems will certainly increase both in magnitude and variety. Although we may wish otherwise, some of the new pest situations will require the use of insecticides for effective control. However, as a reasonable choice of tree species is available to meet most end-uses, the major trend in forestry practice in Kerala will and should be to select and develop plantations of those species which are less susceptible to insects and diseases.

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SUMMARY

The role of pesticides in food production and in the control of vector-borne diseases is unquestionable. However, pesticides do not possess much selective toxicity towards target organisms and pose potential hazards to non-target organisms including man. These obvious hazards are preventable by legislation and the use and availability of pesticides should be controlled in the public interest. To meet this end, the Insecticides Act, 1968 was enacted by the Indian Parliament "to regulate the import, manufacture, sale, transport, distribution and use of insecticides with a view to prevent risk to human-being or animals" and the said Act came into force in the year 1971. The goal in regulating pesticides is to provide society with maximum protection from adverse effects while not denying its access to benefits. The principal regulatory method is by a system of pre-market registration following evaluation by Govt. authorities of properties, performance and hazard of the product and the proposed use as revealed by scientific experimentation and data provided by the importer/manufacturer/formulator. Evaluation involves the mature judgement of experienced professionals using a multi-disciplinary approach and constituted as a registration committee under Section 5 of the Insecticides Act, 1968. In evaluation of pesticides, as in other fields of human endeavour, some degree of risk must be considered acceptable to society. Otherwise there will be a needless prohibition of important benefits of pesticides to economic and aesthetic qualities of life.

Before discussing the basic principles and problems of legislation, two fundamental questions to consider are—(1) Do we need pesticides? (ii) If so, should there be a special legislation on pesticides?

One of the biggest problems today in the world is the population explosion aided by advances in medical knowledge and patient care. Efforts should be made to ensure that enough food is available to feed this increasing population and this can only really be achieved by increasing productivity. Despite improved farming methods, world food production has barely kept pace with the population in this century and half of the world's population today has an inadequate diet. Food supplies will have to be doubled in the next 30 years to achieve even a moderate advance in nutritional standards. The World Food Conference in 1974 recognised that a greatly increased use of pesticides and fertilizers is among the important measures essential for achieving the massive expansion needed in food production.

The pesticides, if responsibly used, are essential adjuvants to the present farming methods and are needed to produce enough food of good quality to feed the world's population. Pests destroy up to 1/3 of the world's food crops during growth, harvest and storage and if pesticides are not used for pest control, farmers' production of crops and live stock might decrease by 25–35 per cent. Careful studies have shown that without pesticide treatment there is a reduction of cotton yields of 25–40 per cent.

The role of pesticides in public health in the control of vector-borne diseases is unquestionable. It should be noted that the registration of DDT for public health purposes in India is in accordance with the spirit of World Health Organisation which states in its 16th Report "The concern that has been expressed in recent years about contamination of the environment by this very stable and persistent insecticide (DDT) should not, in the opinion of the Committee, be considered sufficient reason for substituting other insecticides for indoor residual spraying against mosquitos. The safety record of DDT remains outstanding... those pesticides so far developed as indoor residual sprays are likely to present a greater hazard in use than DDT... The committee urges health authorities to take a realistic view of the hazards presented by these new insecticides. In many public health programmes, the possibility that a few individuals might be adversely affected has to be accepted in order to effect a substantial improvement in the health of the country as a whole."

Need for pesticide control legislation

To turn to the next fundamental question. Should there be a special legislation on pesticides? We have seen that the pesticides are of paramount importance in food production and the control of vector-borne diseases. Although, research on non-chemical methods of pest control is progressing, such methods are not available to replace on broad scale the full use of pesticides and they only offer possibilities in specific cases either alone or in combination with selective pesticides. Further, in the foreseeable future, pesticides will continue to be required in the production, transportation and storage of food, feed, fibre and in the control of vector-borne diseases. However, the uses of pesticides has at the same time resulted in undesirable harmful side effects to man, wild life and environment. These obvious hazards are due to non-specificity and lack of selective toxicity of pesticides to the target organisms. Therefore, there could be no doubt that there is a need for legislation on pesticides to control the availability and use of these hazardous chemicals. Briefly, the reasons for pesticide legislation are –

- (a) the prevention of misuse, since this is how most adverse effects occur ;
- (b) the protection of workers transporting, preparing, and applying the pesticide to ensure that the protection is available to them ;

- (c) the prevention of massive contamination of foodstuffs and other goods during transportation—a not uncommon cause of outbreaks of intoxication ;
- (d) the maintenance of acceptable residues in crops and foods ;
- (e) the protection of domestic and feral sources of meat and fish foods ;
- (f) the protection of other wildlife, and the prevention of land and water pollution.

Current Status of Pesticides Legislation in the Developing Countries

The pesticide regulatory laws have been established with the two main objectives of ensuring quality and safety. Among the developing countries, only a few countries have well developed legislation including well laid registration procedures. These are generally on the lines of pesticide regulations of the developed countries with minor variations to suit technological economic and social development. “There are both mandatory and voluntary types of pesticides registration schemes in the world and I shall not attempt to present a comparative analysis of the existing legislations in different countries because summaries of the most important examples of legislation are presented in two publications namely, “Control of Pesticides” issued by W.H.O. and “Problems of Persistent Chemicals” issued by OECD. However, in the context of this paper I shall quote paragraph 2.4.1 from “A model scheme for the establishment of National Organisations for the official control of pesticides” published by FAO which reads as follows :

“The Working Party studied the voluntary registration and licensing schemes of some countries and concluded that the advantages of schemes of this type were outweighed by the disadvantages, particularly when applied to developing countries. Voluntary registration or licensing does not prevent the sale of relatively ineffective or hazardous products, and does not provide satisfactory protection for the responsible manufacturer and formulator. Accordingly, it is recommended that the legislation should make it an offence to sell, supply or import for use a pesticide or veterinary product which has not been licensed by the appropriate authority.”

Pesticide Legislation in India

In 1958, the Government of India appointed a Commission of Enquiry to suggest *inter-alia* remedial measures following a number of deaths by poisoning through consumption of food which had by accident been shipped together with the pesticide—ethyl parathion. This serious incident, in which over 100 persons died in Kerala after eating food contaminated with ethyl parathion, was found to be due to faulty storage of parathion in polyethylene containers, and of failure to pack these containers with absorbant material, and to provide adequate labelling. It was

also found that a deliberate false description of the consignments was given in the waybills and invoices covering the shipment of the pesticide as the product was classified 'harmless'. The Commission recommended a comprehensive legislation covering registration of pesticides and precautions during manufacture, storage, transport and distribution. Later an Expert Committee of the Indian Council of Agricultural Research, New Delhi headed by Prof, M. S. Thacker, studied in depth, various aspects of pesticide regulation and gave a report on the harmful effects of pesticides in 1967. Based on these recommendations a comprehensive Bill was passed by the Parliament in 1968 known as Insecticides Act, 1968, and all the provisions of the Insecticides Act were brought into force w.e.f. 1st August, 1971.

Prior to the enforcement of the Insecticide Act by the Ministry of Agriculture the uses of nine highly toxic pesticides were being controlled under the State Poisons Rules framed under the Poisons Act, 1919. Four of the pesticides used in Public Health were similarly governed under the Drugs and Cosmetics Act of 1940. The residue aspects of pesticides in items of food were being looked after by the Ministry of Health under the Prevention of Food Adulteration Act, 1954. The manufacturing and formulating units were being partly looked after under the Factories Act of 1948.

All these aspects enumerated above were covered in the comprehensive Insecticides Act of 1968—"An Act to regulate the import, manufacture, sale, transport, distribution and use of insecticides with a view to prevent the risk to human beings or animals, and for matters connected therewith." In addition, the term 'insecticide' has been defined in the Act to include all other chemicals which come under the general category of pesticides such as fungicides, herbicides, rodenticides, nematocides, molluscicides, etc. By enacting such a comprehensive legislation, Government has accepted more or less full responsibility for preventing the hazards to animals, human life and environment and also ensuring the supply of quality pesticides.

Salient features of the Insecticides Act

- (1) Compulsory registration of the product at the Central Level and licences for manufacture, formulation and sale at the State level.
- (2) The Inter-Departmental/Ministerial/organizational coordination is achieved by a high level advisory board "Central Insecticides Board" with, at present 24 members (to be raised to 29 by an amendment) drawn from various fields having expert knowledge of the subject.
- (3) "*Registration Committee*" to look after the Registration aspects of all insecticides.

- (4) Establishment of enforcement machinery like Insecticide Analysts and Insecticide Inspectors by the Central or State Government.
- (5) Establishment of the Central Laboratory.
- (6) Power to prohibit the import, manufacture and sale of pesticides and also confiscate the stocks. The offences are punishable and seizure and other penalties are prescribed.
- (7) Both the Central and State Governments are empowered to make rules, prescribe forms and fees.

Procedure of Registration

The applicant seeking registration applies to the Technical Secretariat of the Registration Committee in the prescribed application form with necessary scientific data as prescribed under Insecticide Rules, 1971. The Secretariat composed of scientists with expert pesticide knowledge includes chemists, toxicologists, entomologists and packaging engineers. The technical secretariat, after thorough scrutiny of the scientific data submitted by the applicant on the quality, stability, bio-efficacy and safety of the product, recommends the application to the Registration Committee for consideration and registration. Final clearance of the product for registration depends on the decisions of the Registration Committee which takes into account purely scientific data for registration of the product. So far in India, the Registration Committee has approved 110 pesticides and their formulations for registration.

Nearly five years of experience of implementation of the Insecticide Act has indicated that for effective enforcement both the Act and the Rules require certain amendments. The Department of Agriculture, Directorate of Plant Protection Quarantine & Storage took steps in this direction by convening the first workshop at Hyderabad in February, 1974 wherein all the probable amendments to the Act and the Rules were discussed and finalised. The amendments to the Act came into force in August, 1977. The amendments provide for the licensing of pest control operators and also provisional registration for a period of 2 years, to allow the applicant to generate the necessary scientific data for issuing commercial registration.

Need for International harmonisation of registration requirements

In view of the mounting costs involved in research and the divergent systems of registration requirements, it is a matter of great urgency to achieve a basic standard of uniformity in pesticides registration. Without such uniformity it is probable that the cost of new pesticides will rise beyond the purchasing capacity of users and the viability of the pesticide industry.

The possibility of harmonising registration requirements needs to be considered in the context of differing technological and economic developments through which different countries are passing. On the one hand are the developed countries where the pesticide industry is firmly established and so are the testing facilities. The pesticide manufacturing units abide by the Governmental environmental rules. There is voluntary discipline in the industry and only approved pesticides are marketed and promoted for registered uses. The field and household application of pesticides is usually in the hands of trained operators.

In contrast to this situation we have the countries, where technological development is either slow or lacking, which still depend on imported pesticides. The formulation industry is in its infancy and the first generation persistent pesticides are still in general use. Facilities for local evaluation, particularly for toxicological and residue investigations are not yet established. There is no trained manpower, nor are the techniques available for evaluating fully the impact upon the environment. The public conscience is rather poor. The farmer applies the chemical himself and with practically no protective clothing or safety equipment. Knowledge of the inherent toxicity of pesticides and their associated hazards is lacking and the extension advice is most inadequate. Suitable application equipment is also not available. Farming is primitive. Nutrition and health standards are equally low.

In between these two extremes fall a great majority of the developing countries where the process of modernization of agriculture has commenced and so has public education, awareness and economic development. A local manufacturing base has been established, but the laboratory facilities for toxicological studies are not adequate. Consciousness of the environment has developed and official agencies and the public are keen to safeguard against the hazards associated with imprudent use of pesticides. Contract spraying work is beginning and so is the availability of protective clothing and safety devices.

The developing countries naturally provide the ideal areas for attempting standardization. In this direction, centralised registration at the national level as is done in India may offer the best alternative, rather than allowing individual States within the country to adopt their own registration. Even several smaller countries within the same agro-climatic region could profit by grouping together to adopt a uniform registration as is being attempted by the Council of Europe. It is in this area that the international organizations like FAO and WHO could play a useful role by establishing laboratories both for toxicological and bio-efficacy tests, including studies of pesticides residues, to satisfy the local evaluation/testing needs in the region.

Simpler items where standardization may be achievable are as follows :

1. Standard application form

The first step to attempt international standardization of pesticide registration requirements in the developing countries will be to have a standard application form for submitting data/information for registration. A cursory examination of available forms/proforma reveals that by and large they are modelled on the same format and aims to seek the same/similar data/information, yet such a simple thing is awaiting standardization. Individual countries could ask through a supplementary form for additional information which may be of importance to that country depending upon its scientific development and particular needs. It is expected that the basic form will serve the purpose in those countries who are yet to make a beginning. Since the majority of developing countries still do not have any kind of regulation they could profitably make a beginning with this approach. The infrastructure does not exist to make local evaluation/verification about registration claims.

2. Standard terminology

The term pesticide may be defined to include all plant protection chemicals, be it insecticide, fungicide, herbicide, molluscicide, rodenticide, nemticide, bactericide, viricide, avicide, piscicide, algicide, defoliants, desiccants, growth regulants, bioinsecticides etc. Some other terms may also need to be defined to have the same connotation.

3. Need for provisional registration

In the developing countries there is urgent need for provisional registration for a period of 2-3 years to generate registration data on bio-efficacy under local conditions as is being insisted by many countries because of variable agro-climatic conditions and pest complexes. Similarly, because of differing use patterns, nutritional and health standards of people and increasing awareness of the hazards to human beings, animals and wild life associated with pesticides, countries require local toxicological evaluation of commonly marketed formulations. Such a procedure will provide ample opportunities for local scientists to assess the hazards to users and to the components of agro-ecosystem.

4. Single agency for official control at the national level

The developing countries may take full advantage of this suggestion because in many developed countries the multiplicity has led to difficulties and procedural delays. The nodal ministry for such a purpose may be agriculture to cover all uses of pesticides *i.e.*, agriculture, horticulture, forestry, home gardening, household pest control, stored products protection, public health and hygiene and veterinary prac-

tice. The inter-ministerial/departmental cooperation to be achieved by a high level coordinating board.

5. Safety labelling including visual symbol of toxicity hazards based on classification

Although the labelling requirements vary a great deal from country to country, there seems to be wide agreement as to what should appear on the label. While safety labelling is not the whole answer to the prevention of poisoning it has useful part to play in the developing countries as long as it is in local language and displays understandable toxicity symbols.

6. Packaging

Packaging is another aspect where standardization not only in the choice of sizes and materials but standard tests and testing procedures is called for. Suitable packaging should protect the quality of formulated pesticides and prolong its life particularly in the humid tropics, besides reducing the risk of exposure to human beings, contamination of commodities and pollution of environment.

7. Specifications for technical grades and formulations

FAO and WHO specifications which are available for commodity types are often not adaptable directly by the developing countries, except for the purposes of import. In those countries, where local manufacturing and formulation industry is developing, raw material sources differ, hence many of the physico-chemical characteristics need modification by a competent body like the national standards institute. Certain of the developing countries have such institutional expertise which could be shared by the neighbouring countries.

8. Methods of analysis

This is one area where standardization has already been achieved to a greater degree by the collaborative International Pesticides Analytical Committee (CIPAC) and the developing countries may further examine them from the angle of suitability for direct adoption/adoption with modification.

9. Simplified requirements of data on bio-efficacy and toxicity giving due consideration to comparable climatic conditions, agro-ecosystem and socio-economic level

In this approach opinions are generally divided and individual countries insist on local testing. However, there is sufficient scope to standardize the methodology of testing to bring much needed uniformity in respect of type of tests, types of crops, number of locations, size of plots and also the duration of tests. Consideration ought to be given to bio-efficacy data applicable to geographical/ecological region

as evidence of performance. Similarly the methodology and required test procedures could be made more uniform in all laboratory toxicological investigations to avoid the necessity for toxicity testing being replicated in different countries, as these investigations are both time consuming and expensive.

10. Standard phrases and pictorials

To prevent misleading statements and overclaims on the label and leaflet, it is desirable to adopt standardized phrases for limitations in use, cautions, precautions, first aid measures and antidote statements. Many developed countries have adopted these phrases. There is great need for standardizing pictorial symbols for labelling of all dangerous pesticides like those of common market regulations.

11. Disposal of left over unused pesticides in the field and containers

In this area there is urgent need for development of guidelines and standard procedures based on the work in the developed countries as the developing countries have practically no experience on how to avoid misuse of used containers, and indiscriminate throwing away of unused pesticides in the field, resulting into pollution of the environment.

12. Standards on safety including equipment

Inside the manufacturing and formulating plants threshold limits of concentration of pesticides which are not injurious to the workers need to be established, together with standardization on safety equipment to protect the workers. There is also urgency in laying standards for manufacturing and formulating equipment together with technical competence of manufacturers and formulators because the chances are that in developing countries, persons with little competence will take up the work and may cause injuries to the workers on one hand or may fail to produce quality products on the other.

The problems of bringing in a degree of uniformity

It is rather unfortunate that there has been no attempt in standardizing a few selected concentrations of active ingredients/strengths in each category of formulations like dust, water dispersible powder, emulsion concentrate, granules etc. which the developing countries could import. The manufacturers have introduced different strength formulations based on the same active material. This not only leads to the problem of variable prices but also adds to the difficulties of bringing out uniform dosage recommendations for the guidance and adoption of farmers. For developing countries which have not yet created the infrastructure for local trials and tests, a declaration on the part of the exporting countries that the formulations conform to some international standards would help developing countries in choosing proper

types/brands and obviate difficulties in arranging pre or post-shipment inspections for quality. If such an approach is possible, there would also be a need for effectively monitoring for such selected pesticides and their formulations on global basis by chosen international agencies, particularly in the interest of developing countries. The shelf life declaration is another problem to be reckoned with. Although there is unanimity that the product to be offered in international trade should be suitable to keep for a minimum of 2 years, there is hardly any international effort to guarantee a shelf life to that extent. Such an approach would further ensure a proper supply of the material to developing countries and their being used in time. It is also for consideration whether a declaration from the exporting countries could be regarded as obligatory, in respect of the registration status in the country of its manufacture, origin *in order to inspire confidence in the developing countries where the knowledge and experience of registration has to be built up.* It is also for consideration whether pesticides/products which are regarded unsafe to be used in the country of origin are not offered to other countries on similar grounds. This could reduce the fears of the developing countries that they may be used as an outlet for products which have either become obsolete or are regarded as unsafe to be used in the manufacturing countries. The developing countries also need simple, less expensive methods of analysis for assessing the quality of the introduced product, because it may take some time for trained man power to be available to handle sophisticated instruments for analytical work.

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7

PESTICIDE RESIDUES IN MAN IN INDIA

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SUMMARY

Widespread use of pesticides to control insect pests of agricultural crops and insect vectors of diseases has resulted in accumulation of some of the persistent chemicals in the environment.

These pesticides have found their way into aquatic systems and food chains. Significant quantities of pesticide residues have been detected in human bodies all over the world. Most of the residues found are of the organochlorine pesticides, particularly DDT and its metabolites. Curiously, the Indian population shows the maximum concentrations of pesticide residues in their bodies with more than 20 ppm of total DDT detected in the adipose tissue in almost every study conducted. A similar trend is detected in the case of blood. These values generally correspond to those of people occupationally involved in the manufacture/formulation of pesticides.

The principal source of these residues in human bodies is believed to be the diet which contains significant quantities of the persistent pesticides. The concentration of these pesticides is somewhat higher in the Indian diet than in others.

DDT residues have been associated with the pathology of the nervous system, carcinomas in different tissues, liver cirrhosis and several other disorders in the body, but these have not been proved to be caused by DDT. Clinical studies on workers engaged in the manufacture of DDT have generally failed to show any adverse clinical effects, even though their bodies contained rather high concentrations of total DDT. While the existing levels of pesticide residues in human bodies may not be the forebodings of impending disaster one cannot be complacent about it and there is an urgent need to investigate all aspects of this problem.

The discovery of the insecticidal properties of DDT heralded a revolution in the control of insect pests and vectors of diseases. Since then the number of synthetic organic pesticides and the quantities used have increased greatly. They have contributed very significantly to the increasing agricultural production necessary for feeding ever growing human populations and in controlling diseases like malaria. However, this large scale release of pesticides in the environment has contributed simultaneously to its pollution, particularly by persistent pesticides such as the organochlorines. DDT which enabled the eradication of malaria from large parts of the world, has caused great concern by its persistence in the environment, so that certain countries have banned its use. It has been considered to be dangerous to certain forms of wildlife and a potential hazard to human beings. However, the recurrence of malaria in countries like India and Ceylon has shown the utility of DDT in controlling insect-borne diseases, particularly malaria. For many countries it is uneconomic to use anything other than DDT. Thus it becomes necessary for the magnitude of the pesticide residue problem be studied thoroughly. Here, this problem will be considered in relation to the occurrence of pesticide residues in man. Human exposure to pesticide could be acute, chronic or incidental. Acute exposure which is due to accidental contamination by pesticides results in poisoning symptoms and even death. Such drastic effects draws the attention of the public and forces the adoption of remedial measures. The other two types of exposure, chronic and incidental do not usually produce symptoms which are easily recognised. Hence,

their effects can remain undetected for long periods. Pesticide residues found in the human body can be used as an index of pesticide exposure.

Magnitude of pesticide residues in man

The rapid progress and increased sophistication in the methods of analysis of pesticides have enabled the detection and estimation of very small quantities of pesticide residues. Many studies have reported significant amounts of these compounds in human populations. Most of the information available is for the organochlorine pesticides which have been used extensively, are highly persistent and easily detected. Human adipose tissue and blood have been analysed for these residues.

Table I summarises DDT residues detected in human adipose tissue in several countries. DDT and its metabolites, particularly DDE, were found in most human populations and usually total DDT residues (including *p,p'*-DDT and various meta-

TABLE I

Country	Year	DDT	DDE	Total as DDT	DDE as % of total	Reference
France	1961	1.7	3.5	5.2	67	Hayes <i>et al.</i> , 1963.
England	1965-67	0.7	2.2	3.0	74	Abbott <i>et al.</i> , 1968.
Belgium	—	1.2	2.1	3.3	64	Maes and Heydrickx, 1966
Holland	—	0.3	1.8	2.2	86	deVlieger <i>et al.</i> , 1968.
Denmark	1965	0.6	2.7	3.3	82	Weihe, 1966.
West Germany	1958-59	1.0	1.3	2.3	57	Maier-Bode, 1960.
Czechoslovakia	1963-64	5.5	4.1	9.6	43	Halacka <i>et al.</i> , 1965.
Italy	1966	4.6	10.6	15.4	68	DelVecchio and Leoni, 1967.
Spain	1966	6.5	9.2	15.7	59	Llinares and Wasserman, 1968
Israel	1965-66	8.2	9.9	18.1	54	Wasserman <i>et al.</i> , 1967.
Australia	1965-66	3.6	6.6	10.2	64	Wasserman <i>et al.</i> , 1968.
Canada	1966	1.0	2.9	4.3	67	Brown, 1967.
United States :						
North-White	1968	—	3.3	4.8	—	Mrak Report, 1969.
North-Negro	1968	—	5.3	7.9	—	„ „ 1969.
South-White	1968	—	6.6	9.2	—	„ „ 1969.
South-Negro	1968	—	9.8	14.4	—	„ „ 1969.

Modified from Davies, J.E., 1973.

bolites) were less than 10 ppm. They were higher in Israel (up to 18 ppm). Residues were comparatively high in Italy, Spain and one group in U.S.A. and DDE accounted for bulk of the total DDT residues. Amounts of organochlorine pesticides detected in adipose tissue are summarized in Table II ; they include dieldrin, HCH, heptachlor epoxide and endrin but their concentration was, usually much less than DDT. Several studies have subsequently found the occurrence of PCB's which have similar analytical consequences to be quite common in the human populations.

TABLE II
Organochlorine pesticides detected in human adipose tissue

Country	Year	HCH	Dieldrin	Endrin	Heptachlor epoxide	Reference
France	1961	1.19	—	—	—	Hayes <i>et al.</i> , 1963.
England	1965-67	0.31	0.21	—	0.04	Abbott <i>et al.</i> , 1968.
Italy	1966	0.08	0.68	—	0.23	DelVecchio and Leoni, 1967
Australia	1965-66	0.68	0.67	0.1	0.02	Wasserman <i>et al.</i> , 1961.
Canada	1966	0.07	0.22	—	0.14	Brown, 1967.
U.S.A. :						
North-White	1968	—	0.11	—	—	Mrak Report, 1969.
North-Negro	1968	—	0.14	—	—	„ „ „
South-White	1968	—	0.13	—	—	„ „ „
South-Negro	1968	—	0.13	—	—	„ „ „

Modified from Davies, 1973.

Data on amounts of organochlorine pesticide residues in human blood are given in Table III. DDT was detected in most of the samples and the total DDT concentration was about 50 $\mu\text{g/litre}$ or less. DDE was the major DDT metabolite and accounted for 50 per cent or more of the total DDT. HCH and dieldrin were also present in many samples, though their concentration was generally less than DDT. In people occupationally exposed to the pesticides (those involved in manufacturing, formulating, packing or application of the pesticides) the level of residues was higher than the general population because these people were exposed to much higher dosages of the pesticides. Long term investigations on such exposures can provide information on the level of storage of the pesticides and also any possible harm caused because of these pesticides.

It has been reported that the body fat and blood of people from India contains higher levels of pesticide residues than those from other countries (Fig. 2). Dale *et al.*, (1965) showed that the adipose tissues of Indians from certain areas contain-

TABLE III
Organochlorine pesticides detected in human blood (ppm x 103)

Exposure group	Year	P,p'-DDT	P,p'-DDE	P,p'-DDD	Total DDT	HCH	Dieldrin	Reference
England								
General population	1964	—	—	—	13.0	—	1.4	Robinson and Hunter, 1966
Occupational	1964	—	—	—	—	—	46.3	" "
U.S.A.								
General population								
Males	1967	25.3	25.7	8.3	59.3	5.7	2.8	Dale <i>et al.</i> , 1967
Females	1967	8.6	13.5	3.7	25.8	0.8	0.9	" "
Israel								
General population	1969	—	—	—	51.5	10.2	0.8	Wasserman <i>et al.</i> , 1970
Occupational	1969	—	—	—	109.5	11.0	1.8	" "
U.S.A. California								
General population	1969	12.0	35.0	—	51.0	—	—	Poland <i>et al.</i> , 1970
Occupational	1969	573.0	506.0	97.0	1359.0	—	—	" "

Modified from Davies, J.E., 1973.

ned more than 20 ppm of total DDT which was the highest concentration detected in populations without a known history of exposure to a rather high concentration of DDT. About 10 years later, another such study by Ramachandran *et al.* (1974) also

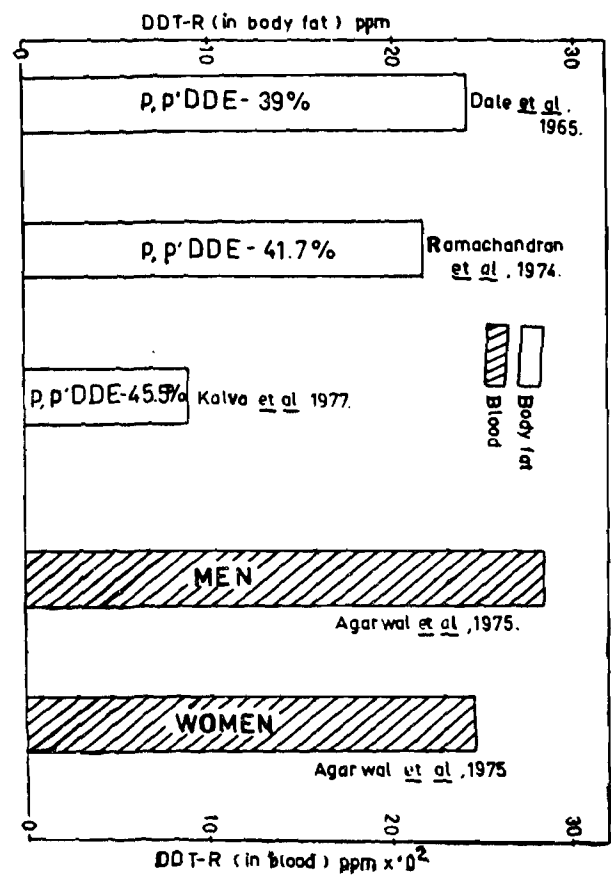


Fig. 1 DDT residues in the body fat and blood of people from India.

found high concentrations. In a more recent study by Kalra *et al.* (1977) the DDT in people from Punjab was less than the earlier two studies. This indicates spatial variations and the need to obtain more such data from different areas ; it is possible that high levels of DDT may be localized only in a few areas. The proportion of p,p' - DDE was about 40 per cent or more of the total DDT residues. A study on pesticide residues in the blood of people from the Delhi area (Agarwal *et al.* 1975) found comparatively high DDT content. The mean value for total DDT in blood of men was 0.284 ppm and for women it was 0.246 ppm. These values are higher than those from other countries. In addition to DDT, residues of HCH particularly β -HCH and dieldrin have also been reported but none of the reports

so far have indicated the presence of PCB's as yet. Possibly they have not been really looked for.

Major sources of pesticide residues

Data on the pesticide residues found in the environment and correlations between these and the residues present in the human body give some indications of the sources of this contamination. The pesticides detected in the human body are present in food, water, air dust and a variety of articles like clothing and bedding.

DDT accumulation in the human body is believed to be due mainly to contamination of food. According to Campbell *et al.* (1965) the total dietary intake accounts for most, if not all, of the DDT and DDE stored in adipose tissues as a result of incidental exposure. Kraybill (1969) also concluded that about 85 per cent

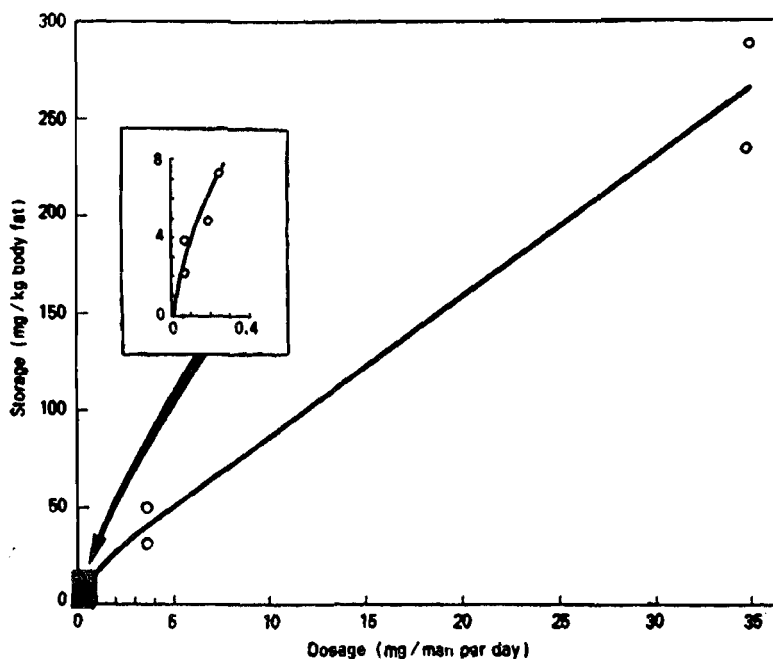


Fig. 2 Concentration of DDT in the body fat plotted against daily dosage (WHO Technical Report Ser. 315 1973)

of the DDT in the human bodies comes from their food. Other sources such as air, water, contact with contaminated surfaces etc. contribute only small proportions of DDT residues. The various estimates for the relative contribution of different sources found in U.S.A. are summarised in the "Report of the Secretary's Commission on Pesticides and their relationship to Environmental Health" (Mrak Report, 1969). The

combined annual intake of DDT and DDE was 0.03 mg from the air, 0.01 mg from the water, 44.8 mg from food and 5.0 mg from other sources including intake from individual household usage.

Some of the reasons for the observed high concentrations of total DDT in the Indian population of certain areas may be evident if the DDT content of various items of food is considered. Studies [Mittal *et al.* (1978) and Pillai *et al.* (1977)] on DDT residues in water from areas around Delhi have reported large amounts of DDT (Table IV), DDT was found in all the samples of water collected from river Jamuna at various places and some lakes. The highest mean values obtained were

TABLE IV
Residues of DDT and its metabolites in water from areas around Delhi

Types of water	Location	Concentration of residues ppm x 10 ⁶ (ng/l)	Reference
<i>River</i>			
Jamuna	Wazirabad upstream	334.8	Mittal <i>et al.</i> , Unpublished work
	Wazirabad downstream	461.4	„
	Okhla upstream	301.4	„
	Okhla downstream	223.8	„
	Majnu ka Tila	960.0	„
	Bela Road	917.5	„
	Nizamuddin	78.2	„
	Hindon	217.4	„
<i>Lakes</i>			
	Suraj Kund	194.6	„
	Damdama	376.7	„
<i>Pond</i>			
	Delhi University Campus	64.0	Pillai, M.K.K., Agarwal, H.C. and Yadav, D.V., 1977

above 900 mg/litre. Many invertebrates such as crustaceans, molluscs, and fish collected from sampling sites contained large amounts of DDT. Milk samples from several places in Punjab were investigated by Kalra *et al.* (1977) and in most of the

samples analysed, the DDT concentration exceeded the practical residue limit and the highest mean value was 0.29 ppm. HCH residues were also found and ranged from 0.002 to 0.055 ppm. There are many reports showing high concentration of DDT in various items of food including eggs, vegetables, butter, etc. An estimate of DDT in various components of the diet of an Indian adult has been made by Tripathi (1966) (Table V). Most of the DDT was obtained from cereals, vegetables

TABLE V
DDT residue obtained with diet of an adult Indian

Item of food	Consumption g/day/person	DDT residue (ppm)	Total DDT residue mg/day
Cereals	465	0.4088	0.1900
Vegetables	75	0.4600	0.0345
Milk and milk products	158	0.0026	0.0410
Meat	20	0.5281	0.0005
Eggs	1.09	0.039	0.0004
Water	2000	0.000	0.000
			0.2664

From Tripathi, 1966.

and milk and he estimated a daily intake of 0.266 mg DDT. However, this estimate is perhaps on the low side considering the concentration of DDT in the various items of food.

Knowledge of the degree of storage and excretion of a pesticide in relation to dosage is very useful. It is generally believed that a dosage-response relationship exists for every effect of a chemical, but this is difficult to demonstrate for human beings, although it has been established for DDT. Known dosages of DDT were administered to volunteers for a period and the DDT concentrations in the body fat were assessed until a steady state of storage was achieved. Results from such studies are presented in Fig. 2 (WHO Technical Report No. 513, 1973). The curve near the origin indicates a considerable change in the ratio between dosage and storage in the low-dosage area. With an increase in dosage, storage also increases though at a progressively declining rate. The ratio between the dosage and storage of DDT is approximately constant above a dosage level of 2.2 mg per man per day. Such a curve is useful in determining the degree of exposure to the pesticide from the level of the pesticide storage in the body. Such estimates are more accurate when

applied to the average storage for a group rather than to an individual. From such a curve it was estimated that workers in one factory absorbed about 35 mg DDT per man per day which was the highest dosage studied in volunteers. In another factory, workers absorbed between 17.5 mg and 18 mg DDT per man per day.

The excretion of DDA also shows a dosage dependent relationship. When the exposure to DDT is excessive, the DDA excretion is also high.

Hazards caused by the pesticide residues

It has already been observed that significant quantities of pesticides, mainly the organochlorine compounds, are found in humans and the major source of these residues has been identified as food. DDT has been shown to be carcinogenic to mice (WHO, Tech. Report, 513). At the highest dietary level (250 mg/kg of feed for the lifetime of the mouse) the incidence of parenchymal liver cell tumours, some of which were malignant, increased in both sexes and life span was shortened. At the lowest level (2 mg DDT/kg of feed or about 0.3 mg/kg body weight per day for the lifetime of the mouse) which on a body weight basis represented a daily intake of DDT similar to the highest known occupational exposure, there was a significant increase of liver tumours in the males only in one survey with no such increase in either sex in another. However, the results from other species cannot be considered to be valid for humans.

Studies in Brazil

This study included periodic clinical examination of 202 spray operators who had been exposed occupationally to DDT for 6 or more years, 77 spray operators exposed to DDT for 13 years from 1947 to 1959 and 78 men who lived in houses sprayed with DDT once every 6 months. The control group consisted of 406 unexposed men whose age distribution and socio-economic level was similar to those of the exposed group. Over a 3 year period, a survey of illnesses requiring medical care showed no differences between the exposed and the control groups. In the first clinical examination carried out in 1971, minor differences in some neurological tests were observed between the two groups, which were, however, not confirmed in the second examination later in the same year. No differences were detected between the people living in houses sprayed with DDT and ones living in unsprayed houses.

Studies in India

In this study, the survey included men who had sprayed DDT indoors for 5 or more years in Gujarat with a similar number not exposed. The mean level of DDT found in the blood of the spray operators was much higher than the controls (1.272 mg/litre compared to 0.170 mg/litre) and the levels were similar to the ones found in

formulators in other countries. Slight differences were found in the neurological tests between the two groups, but on further close follow-up examinations were not substantiated.

In other studies where the occupational exposure of DDT was 35 mg per man per day and 18 mg per man per day for a period of 10–20 years, the body burdens of DDT were high but clinical examination of these men could not detect any specific complaint. Their general health record did not show any specific pattern of ailments which could be distinguished from those experienced by men similar except for high exposure to DDT. Thus, in spite of high DDT residues found in the human body, no specific health problems can be ascribed to these. Similar data for persons occupationally exposed to aldrin, dieldrin, telodrin and endrin have been reported by Jager (1970).

Efforts have been made to lower pesticide residues in food to obtain values within the tolerance limits. Dieldrin residues in milk from a herd of cows accidentally contaminated with aldrin (Reeder, 1969) were reported to have been lowered with phenobarbitone and charcoal. Similar reductions in concentrations of DDT, DDE and dieldrin in the fat of exposed volunteers has been obtained by giving them 100 mg of diphenylhydantoin daily for nine months. Reductions were 75, 61 and 73 per cent respectively. Although the available data indicates an extensive occurrence of pesticide residues in the human tissues, it is reassuring that no specific damage has been associated with these.

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APPROACHES TO STUDIES ON THE OCCUPATIONAL EXPOSURE TO PESTICIDES

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SUMMARY

Population groups exposed to the health hazards from the use of pesticidal chemicals and their residues can be broadly divided into a low risk population group and a high risk population group. The latter includes workers occupationally exposed to pesticides during manufacture, formulation, storage distribution or application of these chemicals. Studies on these workers can be from the view-point of acute and/or chronic exposures. Different aspects of the absorption of pesticidal chemicals, distribution processes and metabolic alterations should be studied. Exposure tests, meant to assess the degree of exposure of people to pesticides before damage results, assume a special significance in studies on occupational exposure to pesticides. These tests may be selective or general. The assessment of the internal pesticide load, the biological responses induced by this load and the exploration of the cytogenetic indicators constitute the epidemiological approaches to studies on occupational exposure to pesticides.

During the last 10 years there has been rapid growth in the production of pesticides. In 1961-62, only nine basic chemicals were produced in India. Currently, 41 pesticides are produced mostly in the organised sector. The total estimated requirement of pesticides in 1978-79 have been put at 85,290 tonnes. The consumption requirement for 1978-79, is estimated to be 570 g/ha. The adverse health effects which might result from the use of these pesticides and subsequent exposures have generated much concern lately.

The population groups exposed to health hazards from the use of pesticides and their residues can be divided broadly into a low risk group and a high risk group. The low risk group includes men exposed to pesticides used for casual spraying, dusting and fumigation at home for the control of household pests or in their food. The high risk group includes workers occupationally exposed to pesticides during manufacture, formulation, storage, distribution or application of these chemicals. The adverse health effects which might result from such occupational exposures have caused much concern and studies are being undertaken for proper, evaluation of these hazards (Sandifer *et al.*, 1979 ; Laws, 1973 ; Menz *et al.*, 1974 ; Levin *et al.*, 1976 ; Gervais, 1976 ; Gupta *et al.*, 1977 ; 1978).

Theoretical Aspects : When an organism is exposed to a chemical, a biological or toxicological effect can occur only after absorption of the substance (with the

exception of radio-active compounds). The uptake of pesticides by human beings from factories depends upon the degree and the rate at which the pesticide in an absorbable form comes into contact with surfaces and organs capable of absorption. For a biological effect to take place, the exposure should permit absorption of the active ingredient of a pesticide. Thus, when man is exposed to pesticides in his occupation, the form in which the pesticide exposure occurs has a marked influence on the absorption and, therefore, on the toxicity. The properties of the insecticide and its formulation such as fineness of distribution and lipid or water solubility, as well as dampness of the skin and intensity of respiration are important. For example, the size of droplets in a spray or the size of dust particles in dusting determine the depth of penetration into the lungs. In occupational poisoning, intake is primarily through the respiratory system and through the skin. Both these organ systems are in direct and continuous contact with the environment.

After absorption, two types of process are important. Firstly, the distribution processes which include transport and binding to tissue components and secondly, metabolic alterations of the parent pesticidal molecule which may mean bioactivation or bioinactivation. These alterations can be accompanied by changes in the chemical properties of the substance, such as hydrophilicity and lipophilicity. These changes result in changes in the distribution, specially storage and excretion. At a certain storage level, effects on the metabolism of normal constituents of the body, on the genetical make-up and on its defence mechanisms (detoxication processes, neuro-endocrine and immunologic processes) may be observed.

Exposure Tests : The detection of the absorption of pesticides before toxic concentrations are reached is important for the prevention of harmful effects and thus assumes a special significance in occupational exposure to pesticides. Exposure tests are meant to determine the exposure of organisms to certain substances before damage results. With these tests, we should distinguish between sampling in the external environment, for instance, air and water, and sampling in the "internal environment" of the body. The degree of exposure is determined from the concentrations of the pesticides in the external environment and time of exposures. More direct and relevant information, however, can be obtained from the concentrations in blood, urine or expired air. These provide information on the degree of absorption, and therefore, the effectiveness of the exposure. Tests based upon the measurement of the concentration of a particular toxic substance or metabolites of such a substance in plasma or urine are selective tests. Such tests are designed for one particular pesticide. For example, the determination of the excretion of paranitrophenol and its conjugates is a selective exposure test for parathion, of which paranitrophenol is a metabolite. In fact, the urinary level of phenolic compounds may be the key for establishing an index of exposure to pesticides containing this as an

easily hydrolyzed or metabolized portion of the molecule. Determination of the urinary excretion of halo and nitrophenol metabolites of biodegradable pesticides is of increasing interest to those involved in pesticide epidemiology. Other tests may be general such as the determination of the activity of acetylcholinesterase in plasma after suspected exposure to organic phosphates.

Epidemiological approaches : The epidemiological approaches to assessing man's exposure to pesticides include :

- a) The assessment of the internal pesticide load constituted by the parent compound and its metabolites ;
- b) The measurement of biological response induced by the internal chemical load. This refers to the impact on the metabolism of normal constituents of the human body and on the activity of defence systems.
- c) The exploration of a cytogenetic indicator, namely the occurrence of chromosomal abnormalities following environmental exposure to certain pesticides.

The estimation of pesticide residues in the biological systems of an occupationally exposed population is particularly useful in the case of organochlorines where the effects are cumulative in nature. A general review of occupational exposure to organochlorines makes it clear that fat is the most important tissue for storage of DDT. This is not surprising in view of the known solubility of the compound in fatty solvents and its insolubility in other solvents. The storage level of DDT in the fat does not increase indefinitely during continuous exposure but reaches a particular plateau characteristic of any particular level of intake. A need to monitoring occupationally exposed workers for pesticide effects has been developed. Such studies are recognised to be essential to stop further exposures once excessive exposures have occurred. The reported literature includes studies wherein occupationally exposed workers have been monitored for specific diseases attributable to the occupational exposures, liver function tests, cholinesterase depression, assessment of behavioral manifestations, incidence of mortality or morbidity etc. Nevertheless, much has been learnt about the effects of storable chlorinated hydrocarbon pesticides on liver cell morphology and function. Under practical conditions of human exposure, effects of organochlorine pesticides on the liver may not be damaging. The absence of severe cell injury from exposure to DDT and dieldrin has been reported by measurements of serum enzyme activities in occupationally exposed workers, who have shown either no changes in serum enzymes or changes so small that they were regarded as indicative of 'adaptation' rather than injury. The evidence, however, to show that workers who have very high tissue levels of DDT by reason of long occupational contact but showing no signs of hepatic cell injury, manifest

some stimulation of the hydroxylating enzymes of the liver. A second issue is the degree of derangement of detoxication mechanisms that can be caused by pesticide inducers. If only one or two strategic enzymes are involved in the response to inducing chemicals, it follows that a single exogenous inducer could affect the hepatic metabolism of a number of substances. Assessment of the impact of occupational pesticide exposure on a wide spectrum of detoxication functions is, therefore, important.

The effects of chronic exposure to phosphate ester pesticides may be cumulative in the sense that repeated exposures closely following each other can reduce cholinesterase activity faster than it can be regenerated. A worker should be removed from any exposure when either plasma or red cell cholinesterase activity drops to below 50 per cent of his normal value and returned to work when it returns to 75 per cent or more of this value.

The evaluation of occupational health hazards could be carried out either by the medical examinations on actually exposed workers or by conducting experimental studies on exposed volunteers. Manifestations of the toxic effects on the nervous system could be evaluated as subjective symptoms e.g. standardized enquiries (or interview) or as objective findings e.g. neurological examination and neurophysiological methods. It must be emphasized that for a correct interpretation of the test results in epidemiological studies, it is important to match populations with respect to education, standard of living etc. It is also important to exclude factors which may give rise to the same type of effects as exposure to pesticides. Another aspect to be considered is that the workers are usually exposed to a number of pesticides at the same time. Thus, men engaged in the formulation industry may be exposed to a number of different pesticides and workers who apply pesticides may be exposed to different pesticides and workers in warehouses may be exposed to fumigants, rodenticides and other pesticides.

To provide proper basic information for future work research in developed countries aims at three issues : (i) mechanisms of absorption, distribution, metabolism and excretion of pesticides in the body, (ii) possible synergistic effects from combinations of pesticides and (iii) development of sensitive methods which allow an early detection of effects on occupationally exposed workers. The same basic issues concern research scientists in developing countries and studies should have following objectives :

- a) to find out evidence of clinical manifestations in occupationally exposed workers,

- b) to study biochemical changes and abnormalities in occupationally exposed workers to assess early detection of exposure and health impairment,
- c) to assess levels of the pesticide residues and their metabolites in blood and urine of occupationally-exposed workers, and
- d) environmental monitoring to assess the atmospheric contamination by the pesticides at the work place.

Conclusions and Recommendations : Occupational exposure to pesticides takes place in situations and ways which make it extremely difficult to provide occupational health services and medical supervision in the classical pattern familiar to large industry, especially, to the large chemical industries. Such services, in fact, require very close supervision and observation and control of the worker. Checks are necessary both for newly employed workers and those who are already exposed. A general health examination should be made concentrating on likely toxic symptoms and hygienic prophylactic measures should ensure that 'no effect' levels are not exceeded. Workers should be informed about toxicity of the pesticides and their possible health risks. Exposed workers should be subjected to quantitative determination of plasma and cellular cholinesterase levels periodically even when they exhibit no clinical symptoms.

Provision of protective equipment, protective garments and respiratory protection to place a barrier between the worker and the environment are necessary though their form and style should be such as to avoid imposing psychological stresses that in our work environment may be unacceptable.

The medical supervision and preventive medical programme include the arrangements necessary to ensure proper treatment of work related illnesses and injury.

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6 A SURVEY OF RESIDUES OF ORGANOCHLORINE INSECTICIDES IN BIOPSY HUMAN FAT SAMPLES

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SUMMARY

Estimates of residues of lipophilic organochlorine insecticides in human beings can provide a reasonable index of their exposure to contaminated food and environment. Fifty-one biopsy human fat samples were collected from the Dayanand Hospital, Ludhiana during 1976-77 to assess the body burden of chlorinated insecticides. These samples were analysed by electron capture gas-liquid chromatography and thin-layer chromatography based on the extraction and clean-up procedure of USEPA 1977).

All samples were found to have DDT residues (DDT-R) while 98 per cent contained measurable level of HCH. DDT-R residues consisted of *p,p'*-DDT, *p,p'*-DDE and a small quantity of *o,p'*-DDT. There was not much difference in the mean DDT-R and HCH levels between males and females. The highest amount of 26.72 ppm of DDT-R was in a rural middle aged non-vegetarian woman. The highest DDT-R in males (31.34 ppm) was in a rural non-vegetarian youth. HCH residues were mainly alpha, beta and gamma isomers. As high as 29 ppm of beta HCH was recorded in a female.

The variation in the present results from those earlier obtained in India suggest the differences in population and environment as well as sampling and analytical techniques.

INTRODUCTION

Since the observations of Howell (1948) that DDT accumulates in the body fat of man, numerous reports have appeared on the storage of DDT and other lipo-

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philic organochlorine insecticides in adipose tissues from all parts of the world (Wassermann *et al.*, 1975). The wide range in kind and magnitude of residues encountered, suggest variations in pesticide use, human exposure and local environment of people whose fatty tissues were sampled. Indians have been reported to carry the highest body burden of DDT residues in their body fat (Dale *et al.*, 1965 ; Ramachandran *et al.*, 1974) but these biopsy human fat samples generally pertained to the population around Delhi except for 16 military personnel who had lower level of residues.

The wide-spread occurrence of pesticide residues in man and lack of information about the situation in the Punjab, inspired this study.

MATERIAL AND METHODS

Biopsy human fat samples from patients undergoing surgery in the hospital of Dayanand Medical College, Ludhiana were collected in 30 ml capacity glass-tubes with aluminium foil-lined lids. Information about the age, sex, food, habits, locality etc. were also recorded. Samples were kept frozen at - 5°C till analysed following the modified method of Thompson (1977). The storage period, however, did not exceed one week.

Appropriate samples weighing 3-5 g. were extracted (3-times) with n-hexane after thorough maceration in admixture with about 20 g. anhydrous sodium sulphate. The fat content ranged from 65 to 86 per cent in the fatty tissues sampled. The n-hexane extract was partitioned three times with an equal volume of acetonitrile containing 10 per cent water. The combined aqueous acetonitrile phase, after dilution with 3 volumes of water and 10 ml of aqueous saturated sodium chloride solution, was partitioned back twice using 100 and 50 ml petroleum ether (B.P. 40-60°C). The combined petroleum ether phase was concentrated to about 5-10 ml and chromatographed on an activated silica gel column prewashed before packing with benzene and methanol. The gradient elution of the column was accomplished using n-hexane and n-hexane + benzene (1 : 1). The two fractions were separately concentrated to small volumes and appropriate aliquots were injected into a Packard Gas-chromatograph (Model 7624) with a tritium foil electron capture detector. Two columns were used i) pyrex glass 1.54 meter long x 2mm i.d. packed with 5 per cent OVLOL on Gas chrom Q and ii) pyrex glass 1.84 meter long x 2mm i.d. packed with 2 per cent DEGS on Gas chrom Q. The temperature of the column and detector was maintained at 190° and 200°C respectively. Nitrogen was used as carrier gas at flow rate of 80 ml./minute. At the operating attenuation of 1×10^{-9} amp., 0.1 ng lindane gave half scale deflection. The polar column was employed mainly to resolve beta-HCH from gamma-HCH, p,p'-DDD from o,p'-DDT and dieldrin

from p,p-DDE. Quantitation of pesticide residues was based on relative peak heights. The recovery of the spiked samples at 0.5 to 1 ppm for TDE and aldrin were 86 and 78 per cent respectively when calculated on peak height basis. The results of the analyses of samples have been expressed on initial weight of fatty tissue without correction for the recovery.

RESULTS AND DISCUSSION

All the 51 human fat samples contained DDT residues (Table I). The highest level of DDT and related compounds (DDT-R) was 31.34 ppm with a mean content of 9.14 ppm. DDE, the major metabolite, constituted 41 per cent of the mean

TABLE I
Residues of Organochlorine insecticides in biopsy human fat samples (51)

Compound	No contaminated	Residues (ppm)	
		Range	Mean
Alpha + gamma HCH	24*	T-11.50	1.21
Beta HCH	23*	T-29.50	5.11
Total HCH	50	T-30.05	4.30
p,p-DDE	51	0.5-21.72	4.16
o,p'-DDT	36	T-6.45	0.65
p,p-TDE	4	T-	T
p,p-DDT	51	T-25.42	4.33
Total DDT	51	1.23-31.34	9.14
Dieldrin	4	T-1.20	0.05

*No. Examined 27.

DDT-R. Among the other isomers, o,p'-DDT was found in 70 per cent of the samples. The high level of DDT in human fat of Indians as observed earlier by Dale *et al.* (1965) and Ramachandran *et al.* (1974) did not occur in the present study indicating comparatively lower exposure of the population sampled. However, the mean level of DDT-R is comparable to the world mean level of 8.6 ppm (Wiese, 1976). Agricultural use of DDT is just a fraction of the total DDT used in the state for mosquito control, so the occurrence of its residues in man could be either the result of transport through food or from the environment. Limited surveys conducted in the Punjab provided evidence of widespread contamination of various food commodities with DDT (Kalra and Chawla, 1978). The corresponding lower level of this insecticide in human fat samples could, therefore, be either due to the occurrence of forms like TDE in milk which are non-cumulative or due to interaction with other compounds.

TABLE II
Distribution of residues of organochlorine insecticides according to sex in biopsy human fat samples

Compound	Males (23)			Females (28)		
	No. contaminated	Residues (ppm)		No. contaminated	Residues (ppm)	
		Range	Mean		Range	Mean
Alpha + gamma - HCH*	14(16)	T- 6.20	0.91	10(11)	T-11.50	1.65
Beta - HCH*	13(16)	T-17.00	4.25	9(11)	T-29.05	6.37
Total HCH	23	T-18.50	4.32	27	T-30.05	4.27
p,p'-DDE	23	0.70-21.72	5.17	28	0.52-19.70	3.32
o,p'-DDT	18	T-3.70	0.52	18	T-6.45	0.80
p,p'-TDE	3	T	T	1	T	T
p,p'-DDT	23	1.20-9.20	4.11	28	T-25.42	4.52
Total DDT	23	2.00-31.37	9.80	28	T-26.72	8.60
Dieldrin	4	T-1.20	0.05	0	0	0

*Figures in parenthesis indicate the number of samples examined

TABLE III

Distribution of residues of organochlorine insecticides according to food habits in biopsy human samples

Compound	Non-vegetarian (23)			Vegetarian (28)		
	No. contaminated	Residues (ppm)		No. contaminated	Residues (ppm)	
		Range	Mean		Range	Mean
Alpha + gamma - HCH	13(15)*	T-11.50	1.62	11(15)*	T- 2.75	0.99
Beta - HCH	13(15)*	T-15.54	4.19	9(15)*	T-29.05	7.18
Total HCH	22	T-15.54	3.93	28	T-30.05	4.84
<i>p,p'</i> -DDE	23	0.52-19.70	5.04	28	0.70-21.72	3.92
<i>o,p'</i> -DDT	16	T-3.70	0.55	20	T-6.45	0.62
<i>p,p'</i> -TDE	4	T	T	0	—	.0
<i>p,p'</i> -DDT	23	T-25.42	4.79	28	1.14-9.20	4.10
Total DDT	23	2.00-26.72	10.38	28	2.22-31.34	8.64
Dieldrin	3	T-1.20	0.05	1	T	T

*Figures in parenthesis indicate the number of samples examined.

TABLE IV

Residues of organochlorine insecticides in biopsy fat samples distributed in the rural and urban population

Compound	Rural (29)			Urban (22)		
	No. contaminated	Residues (ppm)		No. contaminated	Residues	
		Range	Mean		Range	Mean
Alpha + gamma - HCH	15(16)*	T-620	0.68	9(11)	T-11.50	1.83
Beta - HCH	14(6)	T-17.00	5.86	8(11)	T-29.05	5.10
Total HCH	29	T-18.50	4.22	21	T-30.05	4.39
p,p'-DDE	29	0.70-21.72	3.99	22	0.70-19.70	4.48
o,p'-DDT	23	T-6.45	0.72	13	T-2.73	0.55
p,p'-TDE	3	T	T	1	T	T
p,p'-DDT	29	1.20-25.42	5.25	22	T-6.50	3.51
Total DDT	29	2.00-31.34	9.96	22	1.23-25.81	8.19
Dieldrin	4	T-1.20	0.05	0	0	0

*Figures in paranthesis indicate the number of samples examined.

Residues of HCH were found in all but one sample, with the highest level of 30.05 ppm and mean concentration of 4.30 ppm. The major component of its residues was of beta-HCH which is known for its accumulation behaviour with alpha and gamma-isomers in smaller quantities. Such high levels of HCH have been reported from Japan, Rumania, Czechoslovakia. This resulted in a ban on the use of this insecticide in Japan (Ishikura, 1972). In India, HCH comprise 45 per cent of the about 54,000 tons of pesticides used in agriculture and public health. Contamination of various food commodities with this insecticide is also becoming evident through various surveys. The high incidence of HCH confirms the bioconcentration of this compound in fatty tissues, while the high concentration of alpha and gamma isomers in certain samples indicate rather recent exposure.

The occurrence of dieldrin in only 8 per cent of the samples at a mean level of 0.05 ppm indicates its low incidence of contamination of the food and environment, due to probably its limited usage.

The residue data do not show any significant difference in the nature and extent of contamination with reference to differences in sex (Table II), food habits (Table III) or locality (Table IV). However, dieldrin residues were found only in non-vegetarian males from rural areas. Slightly higher amount of residues of DDT-R were recorded in males, and people with non-vegetarian food habits and living in villages while the reverse trend occurred for beta-HCH in females and consumers of vegetarian food. In general, higher levels of DDT-R have been reported in males (Davies, 1973).

The accumulation of lipophilic organochlorine insecticides in fatty tissues of animals is well-documented but the relative contribution through non-vegetarian food such as eggs, meat and related products and vegetarian food such as milk to the body burden of residues do not seem to have been assessed.

The variation in the present results from those earlier obtained in India suggest the need of studying more samples representative of different agro-climatic regions, pesticide use and food habits. This is essential for assessing the status of pesticide residues in the general population of a sub-continent.

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SUMMARY

Milk and feed samples from individual buffaloes from the rural areas and the local dairies have been analysed for DDT residues. Levels of DDT residues ranged from 0.12 to 1.09 ppm in samples of milk collected from rural areas, whereas, they ranged from 0.02 to 0.07 ppm in samples of milk from the local dairies. Feed samples from the rural areas showed comparatively high DDT residues but there was no direct correlation between the levels of DDT in cattle

feed and milk. DDT residues in milk existed in the form of DDE, TDE and DDT of which TDE was predominant. The contribution of ingestion of DDT-contaminated feed by cattle as source of DDT residues in milk is discussed.

INTRODUCTION

The contamination of milk with DDT has been reported from different parts of India (Tripathi, 1966 ; Agnihotri *et al.*, 1974 ; Lakshminarayana and Krishna Menon, 1975 ; Dhaliwal and Kalra, 1977 ; 1978) but no information is available on the success of these residues. There exists a correlation between the level of contamination of animal feed and residues in milk (Leben, 1968 ; Shah, 1969). Dhaliwal and Kalra (1977) reported that the samples of milk collected from rural areas around Ludhiana had higher levels of DDT residues than the samples collected from the University dairy. The present study was undertaken to determine the magnitude of DDT contamination in samples of feed and milk for the individual cattle from these two locations and their relationship.

MATERIAL AND METHODS

Samples of milk and animal feed were collected from twelve individual buffaloes from rural areas (village Harnampura) and the dairy of Punjab Agricultural University, Ludhiana. Two samples were collected at weekly intervals. The animal feed from rural area consisted of composite ready-to-feed material, whereas the separate samples of fodder and concentrate were collected from the University dairy.

Milk was extracted with acetone-hexane (1 : 1, by volume) (Fabubert Maunder *et al.*, 1964). Clean-up was accomplished with silica gel column chromatography (Dhaliwal and Kalra, 1977). Samples of animal feed (consisting of wheat straw and fodder) from rural areas and fodder from the University's dairy were extracted with 65 per cent aqueous methyl cyanide and methyl cyanide respectively. The methyl cyanide extracts, after dilution with aqueous sodium chloride solution, were partitioned into petroleum ether which were further cleaned-up on a silica gel column. Samples of concentrate (fat content <5 per cent) obtained from the University's dairy were extracted by Soxhlet in n-hexane followed by partitioning into methyl cyanide. The methyl cyanide fraction, after dilution with aqueous sodium chloride solution, was partitioned into petroleum ether which was subsequently cleaned-up by silica gel column chromatography. The residual concentrate after Soxhlet extraction was reextracted with aqueous methyl cyanide and cleaned-up by partitioning and column chromatography.

The cleaned-up extracts after concentration to suitable volumes were analysed by thin-layer and gas-liquid chromatography. Thin-layer chromatography was that of Thompson *et al.* (1970) on silver nitrate-incorporated alumina G-coated glass plates using n-hexane as the developing solvent. The Rf values, obtained were : p,p'-DDT, 0.65 ; p,p'-DDE, 0.88 ; p,p'-TDE, 0.35 and o,p'-DDT, 0.77. GLC determinations were made by injecting appropriate aliquots of the sample solution into a Model 7624 Packard gas chromatograph. A pyrex glass column (102 cm long x 0.4 cm ID) packed with 5 per cent DC-200 on Gas Chrom Q was used. The temperature of the column and detector were maintained at 190°C and 200°C respectively. Nitrogen at a flow rate of 70 ml/minute was used as a carrier gas. The retention time of p,p'-DDE, p,p'-TDE, p,p'-DDT and o,p'-DDT was 2, 2.5, 3 and 3.5 minutes respectively. Recoveries of DDT and its metabolites at the fortification levels of 0.1 ppm were found to vary between 70 and 80 per cent. Results were not corrected for recovery.

RESULTS AND DISCUSSION

The results of the analysis of samples of milk and feed are summarised in Table I and II. Levels of DDT residues ranged from 0.12 to 1.09 ppm in samples of milk

TABLE I
DDT residues in milk in buffaloes from the rural area and University's Dairy

Locality	Buffalo Sr. No.	Residues (ppm)							
		1st week				2nd week			
		DDE	DDT	TDE	DDT-R	DDE	DDT	TDE	DDT-R
Village	1	0.12	0.20	0.50	0.82	0.19	0.20	0.60	0.99
Harnampura	2	0.04	0.10	0.12	0.26	0.03	0.05	0.04	0.12
	3	0.08	0.23	0.35	0.66	0.05	0.17	0.19	0.41
	4	0.04	0.04	0.17	0.25	0.08	0.15	0.18	0.41
	5	0.05	0.04	0.22	0.31	0.05	0.07	0.20	0.32
	6	0.13	0.40	0.56	1.09	0.10	0.36	0.42	0.88
Punjab	1	0.01	0.01	0.01	0.03	0.03	0.02	0.02	0.07
Agricultural University	2	0.01	0.01	0.01	0.03	0.03	0.01	0.03	0.07
	3	Tr.	0.01	0.01	0.02	0.02	Tr.	0.02	0.04
	4	Tr.	Tr.	0.01	0.01	0.01	Tr.	0.02	0.03
	5	Tr.	Tr.	0.01	0.01	0.02	0.01	0.02	0.05
	6	0.01	Tr.	0.01	0.02	0.01	0.01	0.01	0.03

Tr – traces (below the limit of determination of 0.01 ppm)

collected from rural areas and from 0.01 to 0.07 ppm in the milk collected from the University dairy. All samples of milk from the rural areas had DDT residues more than the permitted maximum residue limits. Feed samples from the rural area also had comparatively high levels of DDT residues but the results did not indicate a simple relationship between the level of contamination of animal feed and the DDT residues in milk. Mean levels of DDT residues over a 2-week period in the feed and milk for buffalo No. 4 was found to be 0.27 and 0.33 ppm respectively whereas the corresponding value for buffalo No. 6 were found to be 0.21 and 0.98 ppm respectively.

DDT residues in milk occurred in the form of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-TDE with TDE as predominant. The residues of DDT in feed occurred as *p,p'*-DDT and *o,p'*-DDT, but traces of *p,p'*-DDE were also detected. Since TDE is not being used in India either for crop protection or for mosquito control, the TDE residues in

TABLE II
DDT Residues in animal feed

Locality	Buffalo Sr. No.	Residues (ppm)							
		1st week				2nd week			
		<i>p,p'</i> - DDE	<i>o,p'</i> - DDT	<i>p,p'</i> - DDT	DDT-R	<i>p,p'</i> - DDE	<i>o,p'</i> - DDT	<i>p,p'</i> - DDT	DDT-R
Village Harnampura	1	ND	0.04	0.12	0.16	0.06	0.05	0.30	0.41
	2	0.03	0.13	0.25	0.41	ND	Tr.	Tr.	Tr.
	3	ND	0.08	0.16	0.24	ND	ND	0.14	0.14
	4	ND	0.10	0.16	0.26	0.02	0.02	0.24	0.28
	5	Tr.	0.03	0.08	0.11	Tr.	ND	0.09	0.09
	6	Tr.	0.07	0.16	0.23	ND	ND	0.20	0.20
Punjab Agricultural University*	Con- centrate	Tr.	ND	0.03	0.03	Tr.	ND	0.05	0.05
	Fodder	ND	ND	ND	ND	ND	ND	ND	ND

*Cattle feed consisted of an average of 5-10 kg of concentrate and 35-40 kg of green fodder per day per buffalo.

ND - detectable

Tr. - in traces below the limit of determination of 0.01 ppm.

milk must arise as a result of metabolism of DDT. The formation of TDE through metabolism of DDT by rumen microorganisms and its secretion in cow's milk has been reported by various workers (Miskus *et al.*, 1964; Witt *et al.*, 1966; Fries *et al.*,

1969). In buffaloes DDT ingestion in contaminated feed resulted in secretion of DDT, mostly in the form of TDE, in milk. On average, about 75 per cent of DDT residues occurred as TDE. The dermal application of *p,p'*-DDT on buffaloes resulted in its secretion in milk mostly in the form of *p,p'*-DDT (Kalra *et al.*, unpublished). Thus, the ingestion of DDT by cattle in rural areas seems to be a major source of DDT residues in milk. Since levels of TDE in none of the samples examined approached 75 per cent of total DDT-residues, the possibility of the other routes of exposure also exist.

It has been ascertained that no DDT, the fodder or wheat during their growth were not sprayed. But indoor spraying on the walls and roofs at the rate of 1 g/m² was done for malaria control. Massive deposits of DDT present on the walls and roof could be the probable cause of contamination of animal feed stored inside the houses. Nevertheless, the levels of DDT residues as found in animal feed are considered to be too low to cause such a excessive residues (about 1 ppm) in milk. Levels of DDT residues secreted in milk were found to be 0.32, 1.83 and 5.54 ppm as a result of ingestion of feed contaminated with DDT at the levels of 0.5, 2.5 and 10.0 ppm respectively (Kalra *et al.*, unpublished). These results, therefore, emphasise the need for concentrated efforts to identify all the possible sources of DDT residues in milk so that remedial measures may be taken.

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11 METABOLISM OF CARBARYL—NAPHTHYL- C^{14} IN A LACTATING COW WHEN ADMINISTERED ORALLY

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SUMMARY

Carbaryl-naphthyl- C^{14} with total radioactivity of 10.37 mCi (specific activity of 4.0 mCi/mM) was administered orally to a lactating Holstein cow in two doses at an interval of 12 hrs. The cow consumed 0.77 mg/kg body weight/day. Milk, urine and faeces samples were collected at 12 and 24 hrs. after the administration of the first dose of the insecticide. After the completion of the experiment (25 hrs) 58.6, 1.7, 0.21, 0.17 and 0.09 per cent of the total dose of the insecticides applied was recovered in the urine, faeces, liver, kidney and milk, respectively. The fore leg muscle, hind leg muscle and fat contained 0.027, 0.027 and 0.014 ppm of the insecticide residues, respectively. The organo-soluble metabolites in milk were identified

tentatively as metabolite A (3,4-dihydro 3,4-dihydroxy 1-naphthyl N-methylcarbamate), metabolite B (5,6-dihydro 5,6-dihydroxy 1-naphthyl N-methylcarbamate) and carbaryl. The aglycones released from the water soluble conjugates from milk by glucuronidase enzyme were found to be 5-hydroxy 1-naphthyl N-methylcarbamate, 6-hydroxy 1-naphthyl n-methylcarbamate and 1-naphthol. Five aglycones were released from the water soluble conjugates in urine by glucuronidase. These were, metabolite C (1-hydroxy 3,4-dihydro 3,4-dihydroxy naphthalene), metabolite D (1-hydroxy 5,6-dihydro 5,6-dihydroxy naphthalene), carbaryl, 1-naphthol and 1,4-naphthoquinone.

Carbaryl (1-naphthyl n-methylcarbamate) is used extensively on forage crops such as alfalfa, clover and grass (range and pasture) for the control of grasshoppers, alfalfa caterpillar and potato leaf hopper. Due to its persistent nature, it is expected that some might be ingested by the animals, since carbaryl can be present on some forage crops, such as, alfalfa, at levels up to 100 ppm at the time of harvest. The studies of Dorough (1967) indicated that when a lactating cow was given single doses of 0.25 and 3.05 mg per kg of carbaryl-naphthyl- C^{14} , radiolabelled residues were recovered in the milk for 60 hours after treatment. About 0.35 per cent of each dose was detected in the milk. In case of 0.25 mg and 3.05 mg per kg doses, 70 and 58 per cent respectively, were recorded in the urine, and 11 and 15 per cent in faeces. Analysis of various tissues 6 days after the administration of the highest dose revealed that residues were greatest in liver, kidney and ovaries. Studies by Dorough have concentrated on organosoluble metabolites of carbaryl. The aim of the present studies is to investigate the effect of two doses of high specific activity of carbaryl-naphthyl- C^{14} to cows on the distribution of residues in milk, urine and tissues, and to carry out detailed studies on the nature of the water-soluble metabolites.

MATERIAL AND METHODS

Standards : Carbaryl-naphthyl- C^{14} with a specific activity of 4.0 mCi/m mole (total radioactivity 10.37 mCi), analytical samples and metabolites were supplied by Union Carbide Chemical Co., USA. These metabolites were 3,4-dihydro 3,4-dihydroxy 1-naphthyl-N-methylcarbamate (Metabolite A); 5,6-dihydro 5,6-dihydroxy 1-naphthyl N-methylcarbamate (metabolite B); 1-hydroxy 3,4-dihydro 3,4-dihydroxy naphthalene (metabolite C); 1-hydroxy 5,6-dihydro 5,6-dihydroxy naphthalene (metabolite D); 4-hydroxy 1-naphthyl N-methylcarbamate (4-hydroxy carbaryl); 5-hydroxy carbaryl; 6-hydroxy carbaryl; 1-naphthol, and 1,4-naphthoquinone.

Treatment of cow and collection of samples : A lactating cow of Holstein breed, weighing 1485 lbs was placed in a metabolism stall. A rubber tube, 3 inches in diameter, was attached to the vulva to collect the urine and faeces separately.

The rubber tube was taken into a hanging container to collect the urine and the faeces was collected on a polyethylene sheet laid on the floor behind the animal.

Five hundred and eighteen milligrams of carbaryl (total radioactivity 10.37 mCi) was divided into two equal portions and added to No. 10 gelatin capsules, containing crushed grains. One capsule was administered in the morning and the other 12 hours later and the cow consumed 0.77 mg/kg body wt/day.

After treatment, milk, urine and faeces samples were collected after 12 and 24 hours for analysis. After twenty five hours the cow was sacrificed and tissue samples of liver, kidney, fore leg, hind leg muscle and fat were collected. All samples were frozen until analysed.

Measurement of Residues : Milk and urine samples were radioassayed directly by taking 0.2 ml aliquots. Radioactivity in faeces and animal tissues was determined by an oxygen combustion bomb method. For this, 1 gram of a sample was dried in an oven at low temperature to remove moisture and then combusted in the atmosphere of oxygen in an air tight stainless steel container. The $C^{14}O_2$, generated was trapped in 16 millilitres of 1 : 2 solution of ethanolamine-ethylene glycol monomethylether in a special apparatus. The aliquots were removed from the carbon dioxide trap for radioassay.

All samples were radioassayed by liquid scintillation spectrometer (Packed Tricarb model 3380 with 544 analyzer) which gave the radioassay data directly in DPM.

Extraction of carbaryl- C^{14} residues :

- i) *Urine :* Ten millilitres of urine was diluted to 25 ml with distilled water and were extracted three times with 50 ml diethyl ether. Ether portions were combined and treated again with 10 ml of distilled water and aqueous phases combined. The ether extract was dried over anhydrous sodium sulphate before concentrating it to 0.1 ml for spotting on TLC. The aqueous phase was kept for study of water soluble metabolites.
- ii) *Milk :* Fifty millilitres of milk was poured into a 500 ml separatory funnel. To this, 50 ml of saturated sodium chloride solution was added and the contents were thoroughly mixed by shaking the funnel vigorously. 100 ml acetone and 50 ml acetonitrile was added and the ingredients were mixed after the addition of each solvent. After separation of the two phases, the aqueous layer was drained into another funnel and extracted again three times with 100 ml of acetone : acetonitrile (1:1) mixture. The organic phase was com-

bined and saturated with sodium chloride crystals. The water separated out was transferred to the aqueous phase and extracted with 100 ml of acetone : acetonitrile (1:1) mixture. The aqueous phase was discarded because negligible amount of radio-activity was recorded in it. The acetone-acetonitrile extract containing all the radio-activity was condensed to remove the acetone and some of the acetonitrile. After adding 25 ml of extra acetonitrile, the extract was treated twice with 25 ml n-hexane to remove oily-materials without losing residues. The acetonitrile was evaporated and the residue transferred into a centrifuge tube by washing with small quantities of acetonitrile. The volume was reduced to 0.2 ml for spotting on a TLC plate and the chromatogram developed in a 1:1 methylene chloride-ethyl acetate solvent system. By autoradiography, the original material containing the water soluble conjugates and the rest of the chromatogram with organosoluble metabolites were marked and extracted in acetone separately and determined for radioactivity.

Enzymatic cleavage of water-soluble conjugates.

- i) *Urine* : An incubation flask (25 ml Erlenmeyer flask) containing known amounts of watersoluble conjugates, 4 ml of citrate phosphate buffer (pH 4.4) and 0.5 ml *glusulase* enzyme (1 ml = 174,000 units of glucuronidase and 45,800 units of sulfatase, supplied by Endo Laboratories Inc. Garden City, New York) was incubated for 4 hours in a water bath shaker at a constant temperature of 38°C. The contents were poured into 250 ml separatory funnel and flask rinsed twice with 5 ml distilled water and the washings transferred to the same funnel which was also rinsed with a small quantity of ether. The mixture was extracted twice with 50 ml ether by shaking the funnel for 2 minutes and given sufficient time to separate out the two layers before draining out the aqueous phase into another funnel. The two portions of ether were combined and treated with 10 ml distilled water to remove the last traces of water soluble material. The ether extract was dried overnight on anhydrous sodium sulphate, filtered through Whatman Filter Paper No. 1 and concentrated on a rotary evaporator. Radioactivity was determined in the two phases and the percentage cleavage was calculated. Aglycones, obtained in ether were concentrated to 0.1 ml and spotted on a TLC plate. Controls were run simultaneously without the addition of a *glusulase* enzyme to the incubation flask. Three replicates were kept in each case. Different doses of the enzyme and various incubation periods were tested for their effects on the cleavage of watersoluble conjugates.
- ii) *Milk* : The water-soluble conjugates were hydrolysed with *glusulase* under standard conditions, but the incubation period was increased to 24 hours.

The aglycones were extracted by a salt extraction method. 4 ml of saturated sodium chloride solution was added to the incubation mixture and completely saturated by adding salt crystals. The mixture was then extracted with 50 ml acetonitrile followed by two more extractions with 15 ml of the same solvent. After evaporating the acetonitrile extract to dryness, the contents were transferred to the centrifuge tube after rinsing the flask with small amounts of acetone several times. The acetone volume was reduced to 0.1 ml to put on a TLC for developing the chromatogram in suitable solvent system.

Chromatography : 20.32 × 20.32 cm chrom AR 500 sheet and glass plates coated with silica gel G of 0.5 mm thickness were used for thin layer chromatography. Chrom AR sheets were supplied by M/s Mallinckrodt Chemical Works and Laboratory products. For one dimension chromatography, a 4 : 1 ether-hexane system was usually employed and for two-dimensional chromatography, the second solvent system used was 20 : 1 benzene-aceto-nitrile or 7 : 1 and 1 : 1 methylene chloride-ethyl acetate.

The radioactive spots of carbaryl and its metabolites were detected by radio-autography. Kodak no-screen X-ray film was exposed to the developed chromatogram for 3-7 days at -20°C in a freezer. With the help of developed no-screen X-ray film, the residue spots were marked on TLC plates.

RESULTS AND DISCUSSION

Fate of carbaryl - naphthyl - C¹⁴ in cow system : It is clear from Table I, that most of the insecticide was excreted in the urine (58.64 per cent) 24 hours after treatment. During this period small quantities, i.e., 0.09, 1.65, 0.21 and 0.17 per cent of total dose were recorded in milk, faeces, liver and kidney, respectively. The total carbaryl residues in the liver, fore leg and hind leg muscle and fat were less than 0.2 ppm, and in the kidney 3.332 ppm was found which contained only 0.015 ppm of free carbaryl. Milk samples after 12 hours had insecticide residues of 0.025 ppm which increased to 0.048 ppm after 24 hours. Dorrough (1967) demonstrated that carbaryl - naphthyl-C¹⁴ fed to lactating cow at single doses of 0.25 and 3.05 mg per kg resulted in radiolabelled residues in the milk for 60 hrs and approximately 0.35 per cent of each dose was detected. His studies also indicated that major portion of the residues were excreted in the urine, 70 per cent of the first and 58 per cent of the second dose. The corresponding values in faeces were 11 and 15 per cent, respectively.

The detailed investigations carried out on the nature of carbaryl — C¹⁴ residues in urine and milk have been elucidated under separate sub-headings.

- A. *Urine* : The residues extracted in ether from urine after 12 and 24 hours were 1.13 and 4.4 per cent respectively. The ether extractable fractions from urine after 24 hours were separated into 10 different radioactive spots in 4 : 1 ether-hexane. Their composition was 2.91, 20.05, 12.18, 58.84, 1.35, 0.53, 0.50, 1.42, 0.55 and 1.62 per cent of the total organosolubles. No efforts were made to identify these residues.

TABLE I

Distribution of carbaryl- C^{14} residues in cow system, when treated with four hundred and eighteen milligram with total radioactivity of 10.37 mCi in two doses

Sample	PPM	% of single dose	Recovery of total dose %
12 hr milk	0.025	0.07	0.09
24 hr milk	0.048	0.10	
12 hr urine	18.222	45.1	58.64
24 hr urine	26.362	72.3	
12 hr faeces	0.145	0.6	1.65
24 hr faeces	0.932	2.7	
Liver	0.098	—	0.21
Kidney	0.382	—	0.17
			60.76
Foreleg muscle	0.027		
Hindleg muscle	0.027		
Fat	0.014		

Time expressed as hours after first treatment. Animal slaughtered 25 hours after first treatment.

Enzymatic cleavage of water soluble conjugates from urine : The dose effect of glusulase enzyme and different incubation periods were studied on the cleavage of water soluble conjugates. The steep nature of the dose cleavage curve (Fig. 1) indicate that with an increase in enzyme dose from 25 to 400 μ l, a corresponding increase in the per cent cleavage was obtained. Similarly, by hydrolysing the water solubles with 0.5 ml glusulase under standard conditions for 2, 4, 8 and 12 hours, 32.1, 37.4, 38.4 and 42.8 per cent cleavage, respectively, was recorded.

In another experiment, the effect of a second dose of glusulase on the previously cleaved water solubles was investigated. For this, water solubles were hydro-

lysed with 0.5 ml enzyme for 4 hours under standard conditions and to one set of three incubated flasks, another dose of 0.5 ml glusulase was added and incubated the material for further 4 hours, with another set without the addition

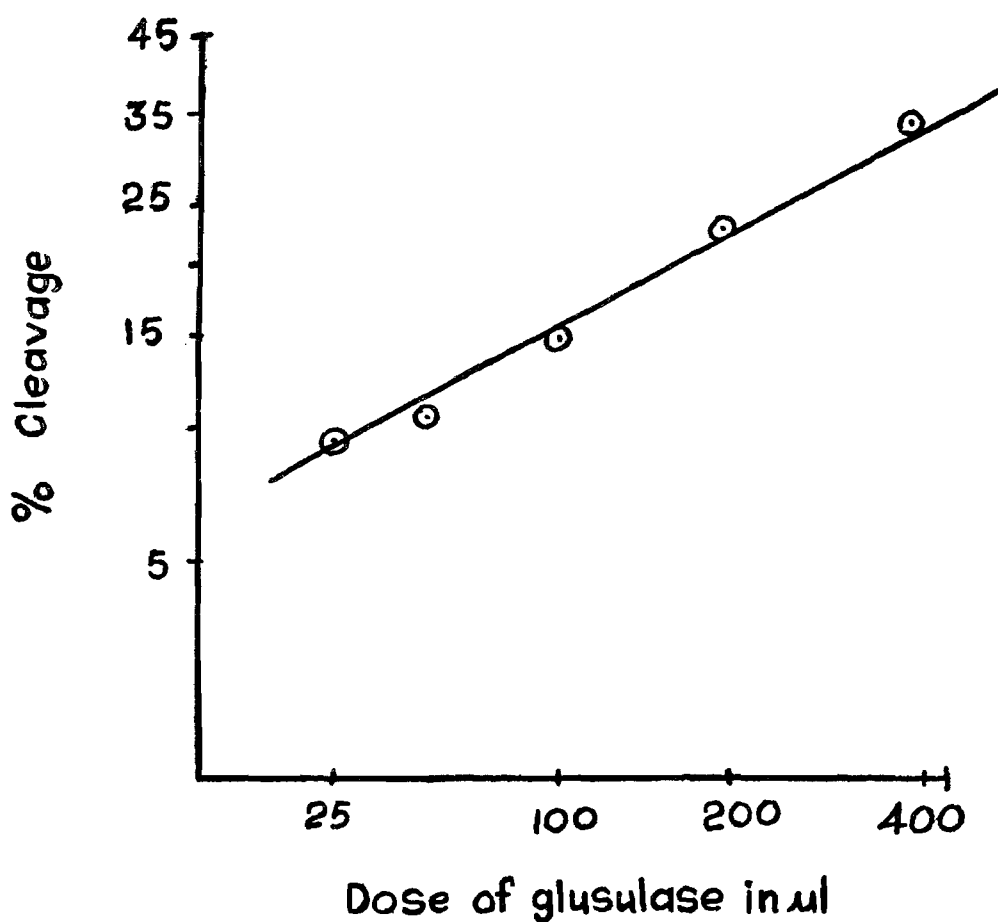


Fig. 1 Dose effect of glusulase enzyme on the cleavage of water soluble from urine when incubated for 4 hours at 38°C.

of second dose of the enzyme. Results in Table II show 7.5 per cent increase in cleavage of water soluble metabolites by the second dose of the enzyme. During the same period 1.8 per cent cleavage in control was enhanced only to 2.2 per cent.

Water-solubles from urine samples after 12 and 24 hours were hydrolysed for 4 and 72 hours with 100 μ l of glusulase to find out the difference in the cleavage pattern of the urine samples and also to achieve the highest percentage of cleavage.

evage of water-solubles. Bacterial growth in the incubated material was checked by adding a drop of toluene. Results in Table III indicate that water solubles from urine after 12 hours was slightly more prone to enzymatic hydrolysis than

TABLE II
Effect of second dose of glusulase on the already cleaved water solubles

S. No.	Treatment	Incubation period in hours	Per cent cleavage
1	0.5 ml glusulase	4	28.3
		8	38.4
2	1 + 0.5 ml glusulase	8	45.9
3	Control	4	1.8
		8	2.2

Amount of W.S. cleaved = 308,772 dpm

24 hr sample. By increasing the incubation period to 72 hours the cleavage percentage of water solubles from the two urine samples was enhanced four fold.

TABLE III
Effect of enzyme on the cleavage of water soluble conjugates from two different samples of urine*

Incubation period in hours	Urine sample	% Cleavage of W.S.	
		Treatement	Conrol
4	12 hr	16.6	1.42
	24 hr	14.8	1.20
72	12 hr	60.4	1.80
	24 hr	55.2	1.61

*The amount of glusulase used was 100 µl.

The effect of a 100 µl dose of glusulase on the two doses of substrates can be observed in Table IV. With a greater amount of the substrate, more than 100 per cent increase in cleavage has been achieved.

From the results of these studies, the most efficient and economical hydrolysis of water soluble conjugates using a suitable combination of enzyme dose, substrate amounts and incubation periods could be selected.

Acid hydrolysis of water soluble conjugates of urine : The water soluble materials obtained from two samples of urine were hydrolysed with 0.38 N HCl

TABLE IV

Effect of glucosylase (100 μ l) on the cleavage of different doses of W.S. when incubated for 72 hours

Dose in dpm	Per cent cleavage of W.S.	
	Treatment	Control
121,708	54.2	1.6
243,416	59.8	1.6

(Table V). By increasing the incubation period from 4 to 42 hours, the percentage cleavage of water solubles was doubled. It is evident that the water

TABLE V

Per cent hydrolysis of water soluble conjugates from urine by 0.38 N HCl

Sample	Incubation period in hours	
	4	42
12 hr	18.40	34.50
Control	1.03	1.00
24 hr	19.80	38.30
Control	1.36	1.40

W.S. used for hydrolysis, 12 hr = 241,324 dpm and 24 hr = 254,191 dpm

soluble materials from urine samples after 24 hours are more acid-labile than 12 hour sample.

Chromatographic characteristics of water soluble conjugates from urine: The water soluble conjugates which were separated out from organosoluble materials in ether from urine after 24 hours were applied on TLC plates as a series of small spots in the form of band along the origin. After drying the water soluble band on the plate, it was developed in 5 : 3 : 1 : 1 ethyl acetate-acetone-formic acid-isopropanol solvent system. The autoradiogram of the plate developed, showed five different bands of water solubles. The C^{14} residues from these bands were extracted in methanol and again with water. The radioactivity was determined in each extract and also what remained in the debris. The two extracts in methanol and water were combined, concentrated and again put on TLC plates in the form of separate small bands. To test that the water solubles from five radioactive bands were characteristically the same, the chromatograms

were developed in a second solvent system of 5:3:1:1 ethyl acetate-acetone-formic-acid-water. Their autoradiograms indicated further that the water solubles from band No. 2 separated into three different regions namely, 2a, b and c, but those from other bands remained as one solid band indicating that they were constituted of homogeneous materials. The water soluble materials from all these bands were extracted in methanol except band No. 1 which remained at the origin and was extracted in water. These water soluble conjugates separated out were cleaved with 0.5 ml glucuronidase for 24 hours and the aglycones, thus liberated, were extracted in ether (Table VI). Results indicate that water-

TABLE VI

Chromatographic and enzyme-cleavage data on water-soluble conjugates in 24 hour urine of a lactating cow treated with carbaryl-naphthyl-C 14

Band No	Rf value	% W S.	% extraction in methanol	% Extraction of the debris in water	% cleavage
1	0.00	6.9	22.1	72.8	3.2
2 **	0.295	6.7	79.7	19.7	
a	—	3.5			28.7
b	—	1.3			46.2
c	—	0.8			39.7
3	0.486	8.5	90.4	9.4	31.4
4	0.754	71.5	93.2	6.8	53.1
5	0.908	6.5	87.1	12.4	49.7

* Solvent system : 5:3:1:1 Ethyl acetate - acetone - formic acid - isopropanol.

** Solvent system : 5:3:1:1 Ethyl acetate - acetone - formic acid-water.

soluble band No. 4 is the major one and constitutes 71.5 per cent of total water soluble materials, which also showed the maximum cleavage by glucuronidase enzyme (53.1 per cent), followed by band 5, 2b, 3, 2a and 1.

Characteristics and composition of aglycones released from the water soluble conjugates by glucuronidase : The total water-soluble conjugates of carbaryl residues in urine (24 hr. sample) when hydrolysed with glucuronidase had seven radioactive spots which were picked up by radioautography and designated from I to VII. All but two aglycones which have 0.00 and 0.578 Rf values, have been tentatively identified (Table 7). The major aglycone was 1-naphthol which constituted about 75 per cent of the total aglycones released from the water soluble conjugates. Other aglycones were metabolite C (1-hydroxy 3,4-dihydro 3,4-dihydroxy naphthalene), metabolite D (1-hydroxy 5,6-dihydro 5,6 -

TABLE VII

Characteristics and distribution of C14- labelled aglycones released by gluculase from the different bands of W.S. conjugates and total W.S. conjugates from 24 hour urine of a lactating cow treated with carbaryl-naphthyl-C14

Aglycone	Rf Value*	Aglycones in total W.S. Conjugates %	Aglycones which are yielded by different bands of W.S. conjugates %						
			1	2a	2b	2c	3	4	5
I Unknown	0.000	5.7	35.0	2.7	4.2	23.1	36.1	2.7	8.2
II Metabolite C	0.152	2.0	16.3	42.6	—	10.9	34.2	2.9	33.9
III Metabolite D	0.225	3.9	17.9	48.4	—	30.0	29.7	1.1	37.7
IV Unknown	0.578	2.6	30.8	1.7	—	10.4	—	1.6	3.1
V Carbaryl	0.672	9.4	—	2.9	2.8	12.5	—	1.5	3.1
VI 1-naphthol	0.864	75.0	—	1.8	90.2	13.1	—	87.5	13.9
VII 1,4 naphthoquinone	0.969	1.4	—	—	2.8	—	—	2.7	—

* Solvent system = 4 : 1 ether - hexane

—No residue was detected.

dihydroxy naphthalene) and 1,4 naphthoquinone. The parent chemical (Carbaryl) also formed a glycoside and was excreted in the urine. The aglycones which were released from the different conjugate bands by enzyme were also characterised tentatively and quantitated. The results indicate that only band No. 4 gave all the seven aglycones in the water-soluble fraction. 1-naphthol was the major constituent. The aglycone unknown I was distributed in all the seven water-soluble bands, metabolites C and D in all but band No. 2 b with maximum concentration in 2a. Unknown IV was not recorded in band No. 2b and 3. Carbaryl and 1-naphthol were missing from bands 1 and 3. The amounts of 1-naphthol varied from 88 to 90 per cent in bands 2 b and 4. Another aglycone was tentatively identified as 1,4 naphthoquinone, from water soluble conjugates of band No. 2 b and 4 only. Since gluculase is a preparation of glucuronidase and sulfatase, it is expected that the water soluble conjugates are glycosides of glucuronic acid and are ethereal sulphate. However, Kuhr and Casida (1967) demonstrated that water soluble conjugates obtained 6 days after injection of carbaryl-C¹⁴ in bean plants were hydrolysed with both B-glucosidase and gluculase. B-glucosidase cleaved water solubles from beans to the extent of 55, 50 and 47 per cent and gluculase to 61, 61 and 47 per cent when carbaryl was labelled at three different positions, Naphthyl-C¹⁴, Carbaryl-C¹⁴ and N-methyl-C¹⁴, respectively. Thus gluculase has also cleaved glucosides of carbaryl metabolites with glucose as sugar molecules, which suggests that the water soluble conjugates separated into different bands by autoradiography in the present investigations are glycosides of not only glucuronic acid, but also of glucose and may be of other sugars. No efforts were made to identify the sugar molecules of the glycosides which are excreted in the urine fraction of a lactating cow. Studies are needed on the formation of glycosides of carbaryl and its metabolites in the mammals.

- B. *Milk* : The materials extracted in an acetone-acetonitrile mixture of solvents from two milk samples were carbaryl and its three metabolites (Table VIII). Of these, two metabolites were tentatively identified as metabolite A (3,4-dihydro 3,4-dihydroxy 1-naphthyl n-methylcarbamate) and metabolite B (5,6-dihydro 5,6-dihydroxy 1-naphthyl N-methylcarbamate). The amounts of metabolite B were the greatest *i.e.*, 77.0 and 81 per cent after 12 and 24 hours respectively. The residues of carbaryl decreased from 7.2 per cent (after 12 hr) to 3.2 per cent in milk sample collected 24 hours after the treatment. The amounts of the other two metabolites remained more or less the same in the two milk samples. The studies of Baron *et al.* (1969) on the characterization of metabolite B, a major organic extractable metabolite of carbaryl in milk and urine of cow, by ultra-violet, infrared, mass, and nuclear magnetic resonance spectroscopy confirmed as that of methylcarbamate of 5,6-dihydro 5,6-dihydroxy 1-naphthol.

TABLE VIII
Organosoluble metabolites obtained from milk by salt extraction procedure

Metabolite	Organosoluble metabolites in per cent in different samples of milk	
	12 hr	24 hr
1 Unknown	5.2	5.6
2 Metabolite A	10.5	10.2
3 Metabolite B	77.0	81.1
4 Carbaryl	7.2	3.2

Characteristics and composition of aglycones released from the water soluble conjugates by glucuronase : Four aglycones were obtained by enzymatic cleavage of water solubles from milk (Table IX)—some of these were tentatively identified as 6-hydroxy carbaryl, 5-hydroxy carbaryl and 1-naphthol. The major aglycone, the unknown, constituted 47.5 and 41.5 per cent after 12 and 24 hours.

TABLE IX
Aglycones released from water soluble conjugates from milk by glucuronase when extracted by salt extraction procedure.

Aglycones	Rf Value*	Salt extraction procedure	
		12 Hr. Sample %	24 Hr. Sample %
1. Unknown	0.00	47.5	41.5
2. 6-hydroxy carbaryl	0.394	16.3	32.7
3. 5-hydroxy carbaryl	0.478	4.4	4.3
4. 1-naphthol	0.864	31.7	21.5

* Solvent system : 4:1 ether – hexane.

respectively. The amount of 5-hydroxy carbaryl was similar in the two samples whereas there was twice as much 6-hydroxy carbaryl after 24 hours (32.7 per cent) as compared after 12 hours as in the unknown aglycone, there was more 1-naphthol after 12 hours (31.7 per cent) than after 24 hours (21.5 per cent)

These results indicate that 0.09 per cent of the total dose of carbaryl was secreted in the milk. Although the amounts of residues were small, the toxicity of carbaryl and its conjugates should be known. Metabolite B is less hazardous than carbaryl as shown by anticholinesterase and fly bioassay tests (Beron *et al.*, 1969). The studies of Carpenter and Weil (1970) indicated that out of four

carbaryl metabolites, *i.e.*, 4-hydroxy carbaryl, 5-hydroxy carbaryl, N-hydroxy methyl carbaryl and 1-naphthol, only 5-hydroxy carbaryl was more toxic than carbaryl to rats. This metabolite has been found to exist in the form of water soluble conjugate in the two samples of milk.

CONCLUSION

Metabolism studies of carbaryl in a lactating cow revealed :

1. Some of the metabolites of carbaryl have been identified but others have not.
2. With various combinations of enzyme dose, substrate and incubation period, maximum cleavage of 60 per cent of water soluble conjugates occurred.

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12 CHRONIC RESIDUAL TOXICITY OF DITHANE R-24 TO RATS : I. EFFECT OF DITHANE R-24 RESIDUES ON FOOD AND WATER INTAKE AND BODY WEIGHT GAIN

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SUMMARY

A chronic residual toxicity study of Dithane R-24 in rats was conducted by feeding rats for 6 months with wheat grains harvested from crops sprayed with the fungicide at the dosage levels of 0.4 kg a.i./ha (X-treatment) and 2.0 kg a.i./ha (5X-treatment). A dry mash with wheat forming the major component (66 per cent) of the feed constituted the diet for the animals. Diet containing wheat from untreated crop was given to the controls. The possible toxic effects which the chemical residues, contained in the wheat grains from the treated crops, would cause to rats were investigated.

Daily consumption of feed and water and weekly body weight gains were recorded. The general health and behaviour of all animals remained unaffected throughout the experimental period. No abnormal clinical symptoms or mortality was noted during the experimental period. No differences were observed between the treated groups of rats and the controls regarding feed and water consumption and there was no detrimental effect on growth rate. All the rats, irrespective of sex and treatment gained weight during the experimental period. Feed and water consumption in males was highly correlated with body weight gain in controls than in X and 5X treated. Whereas, in females, the feed and water consumption was highly correlated with body weight gain in both control and 5X treated than in X treated group. It was inferred that Dithane R-24 treated wheat used as a component of the diet, had no adverse effects on body weight gain.

Systemic fungicides, which rank among the safest pesticides in current use for agricultural purposes (Marsh, 1977) have rapidly increased in number and type. Von Meyer *et al.* (1970) reported that Dithane R-24 (4-n-butyl-1,2,4-triazole also termed as RH-124) was the most selective fungicide and controlled only wheat leaf rust of several rusts studied.

Little is known on the toxicity of Dithane R-24 to animals and this study was conducted for evaluating the toxicity of Dithane R-24 residues in wheat grains to rats.

MATERIAL AND METHODS

Wheat grains sprayed with 0.4 kg (X treatment) and 2.0 kg/ha (5X treatment) of Dithane in field plots were compared with unsprayed grains by preparing feed

mixtures containing these three wheat treatments. The diet was prepared on the lines recommended by ICMR with wheat constituting 66 per cent of the diet. The animals were given the diet at the rate of 20 g/rat per day and water as required.

A total of 156 (78 males + 78 females) Wistar strain, weanling rats weighing from 45-55 g were used for the study. These animals were divided into 3 groups of 52 animals each (26 males + 26 females) with one group as control. The other two groups were 1X and 5X treatment groups, respectively. The study lasted for 6 months.

Daily feed, water consumption and weekly body weight gains were recorded and the uneaten feed was weighed next morning and the amount of water left in the water bottles was measured to determine the average quantity of feed and water consumed by the animals daily.

Statistical Methods

The effect of feed and water intake on body weight gain in the two treatments as compared to the controls was analysed statistically by simple correlation (Ezekiel and Fox, 1966) and the significance between two correlation coefficients was compared using Fisher's Z (Steel and Torrie, 1960) and the multiple correlation coefficient values were determined in the two treated and control groups (Weatherburn, 1962).

RESULTS

General observations

No apparent abnormal clinical symptoms were observed in rats throughout the experimental study.

Body weight

The growth rate in male and female rats during the period of experimentation is given in Fig. 1. Average two-weekly body weight gain by the control and treated groups of male and female rats is presented in Tables I and II, respectively. There was no detrimental effect on growth rate caused by the ingestion of the treated wheat. All the rats, irrespective of sex and treatment gained weight during the experimental period although the rate of gain was less in females compared to the males.

Food consumption

Food consumption was normal in both control and treated animals. Average two-weekly food consumption by control and treated groups of male and female rats is presented in Tables I and II, respectively. The amount of wheat consumed

TABLE I

Average fortnightly feed consumption, water consumption, and body weight gains in male rats fed on diets containing untreated and Dithane R-24 treated wheat

Fort- night	Feed consumption (g)		Water consumption (ml)		Body weight gain (g)	
	Control	X treated	5X treated	Control	X treated	5X treated
1	91.46	89.91	85.84	81.04	82.99	79.36
2	92.78	92.90	92.32	129.29	140.36	139.24
3	95.61	90.57	95.08	140.53	153.18	157.03
4	104.55	100.33	103.85	160.41	167.34	170.42
5	100.26	92.07	100.03	178.28	184.04	187.16
6	99.34	95.37	98.00	203.16	202.57	200.37
7	108.68	103.30	110.00	234.47	227.26	202.50
8	104.79	99.47	108.34	241.25	259.71	193.96
9	88.60	115.09	112.75	306.88	269.59	196.00
10	123.13	123.50	133.44	222.50	305.57	241.26
11	107.09	113.88	118.75	256.25	198.88	199.59
12	114.17	104.38	119.59	231.25	206.67	173.13
13	110.00	107.50	105.00	235.00	169.38	164.00

* Initial body weight of rats

TABLE II

Average fortnightly feed consumption, water consumption and body weight gains in female rats fed on diets containing untreated and Dithane R-24 treated wheat

Fort- night	Feed consumption (g)			Water consumption (ml)			Body weight gain (g)		
	Control	X treated	5X treated	Control	X treated	5X treated	Control	X treated	5X treated
1	83.28	85.67	79.23	76.55	82.92	81.99	51.29*	46.48*	49.07*
2	76.15	79.31	75.26	123.96	116.71	107.93	81.67	83.33	84.81
3	75.63	76.31	76.36	135.90	140.45	123.75	95.20	94.20	94.23
4	81.57	78.27	77.27	149.70	154.74	147.85	110.43	115.43	113.75
5	69.11	63.20	63.13	169.58	168.12	160.50	122.25	125.91	121.14
6	64.06	56.62	57.50	169.53	171.19	168.47	122.50	127.11	124.75
7	68.39	61.26	61.25	211.43	212.32	172.66	127.19	130.29	131.67
8	63.96	58.99	57.50	168.13	157.39	168.57	131.07	136.00	137.50
9	61.67	59.00	61.67	144.69	165.96	188.75	131.25	136.54	141.07
10	82.33	68.82	73.65	206.67	179.96	189.27	140.50	146.36	148.75
11	76.67	66.57	66.57	152.50	143.76	154.07	137.50	143.89	144.00
12	76.88	74.59	76.88	150.63	136.26	135.94	138.33	142.14	146.88
13	58.75	73.13	58.13	115.00	91.25	113.13	140.00	143.00	146.66
							139.00	152.50	150.00

* Initial body weight of rats

TABLE III

Residual amount of Dithane R-24 (PPM) in the total wheat consumed by rats at fortnightly periods

Fort- nightly period	Males				Female			
	X dosage level*		5X dosage level**		X dosage level*		5X dosage level**	
	Wheat consumed (g)	Residue intake (ppm)	Wheat consumed (g)	Residue intake (ppm)	Wheat consumed (g)	Residue intake (ppm)	Wheat consumed (g)	Residue intake (ppm)
1	59.34	0.053	56.65	0.153	56.54	0.051	52.29	0.141
2	120.65	0.109	117.59	0.317	108.88	0.098	101.96	0.275
3	180.42	0.162	180.34	0.487	159.25	0.143	152.35	0.411
4	246.64	0.222	248.88	0.672	210.90	0.190	209.95	0.567
5	307.41	0.277	314.90	0.850	252.61	0.227	251.62	0.679
6	370.35	0.333	379.56	1.025	289.97	0.261	289.57	0.782
7	438.53	0.395	452.16	1.221	330.40	0.297	329.99	0.891
8	504.17	0.454	523.66	1.414	369.33	0.332	367.94	0.993
9	580.13	0.522	598.08	1.615	408.27	0.367	408.64	1.103
10	661.64	0.595	686.15	1.853	453.69	0.408	457.25	1.234
11	736.80	0.663	764.52	2.064	497.62	0.448	501.18	1.353
12	805.68	0.725	856.65	2.313	546.84	0.492	551.92	1.490
13	876.63	0.789	925.95	2.500	595.11	0.536	590.28	1.594

* Contained 0.90 ppm of the chemical residue

** Contained 2.7 ppm of the chemical residue

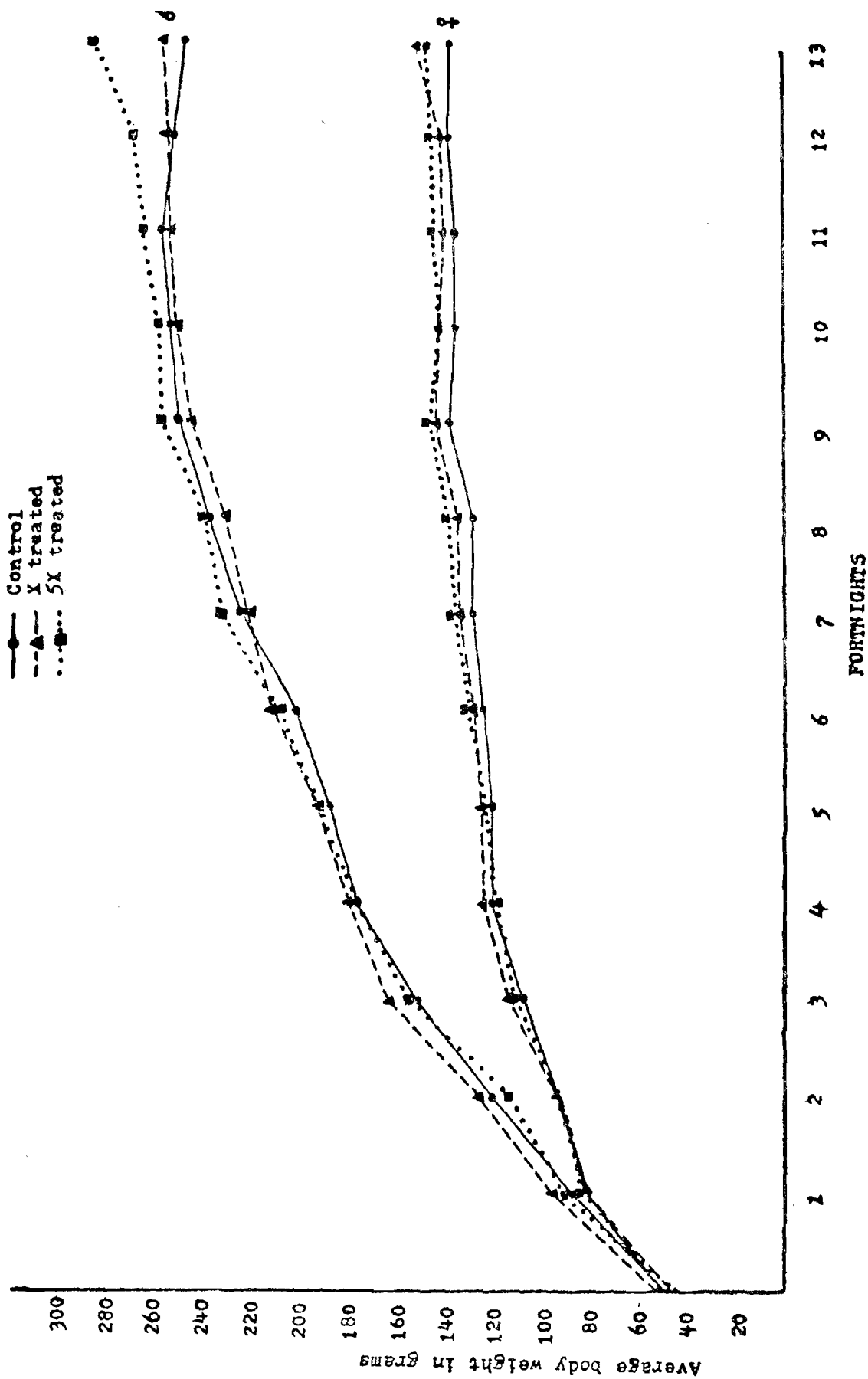


Fig. 1. Growth curves of male and female rats maintained on diets containing untreated and Dithane R-24 treated wheat

and the relative amount of Dithane residues present in the treated grains were 0.90 ppm in 1X treated and 2.70 ppm in the 5X treated wheat samples and ingested by the rats at the two weekly periods is presented in Table III.

Water consumption

Amounts of water consumed by animals did not differ between control and treated groups during the first half of the experimental period. Thereafter the water consumption was variable after the sixth two weeks for all three groups. It neither consistently increased nor decreased (Tables I and II).

The correlations (r) between food and water (r_{fw}) consumption, food and body weight (r_{fb}) and water and body weight (r_{wb}) for male and female rats are presented in Table IV.

The effect of food intake on the water consumption of males, food intake on body weight and water consumption on the body weight were significantly correlated ($P < 0.01$).

TABLE IV

Correlation between feed and water intake (r_{fw}), feed intake and body weight gain (r_{fb}), water intake and body weight gain (r_{wb}) and feed and water intake on body weight gain [$R_{B(FW)}$] in rats fed with untreated and Dithane R-24 treated wheat diet.

	Males			Females		
	Control	X treated	5X treated	Control	X treated	5X treated
r_{fw}	0.81**	0.80**	0.85**	-0.19	-0.74**	-0.50
r_{fb}	0.73**	0.81**	0.86**	-0.42	-0.59*	-0.50
r_{wb}	0.95**	0.84**	0.83**	0.74**	0.61*	0.78**
$R_{B(FW)}$	0.92	0.75	0.78	0.63	0.41	0.62
$R_{B(Fw)}$	0.96	0.87	0.88	0.79	0.64	0.79

* Significant at $P < 0.05$
 ** Significant at $P < 0.01$

For females, a negative correlation was observed of the effects of food intake on water consumption and food intake on body weight gain and a positive correlation on the effects of water consumption on body weight in the control and treated rats. In the control and 5X treated rats, the values of r_{fw} and r_{fb} were not significantly correlated while a significant correlation was seen in the X treated group ($P < 0.05$).

No significant differences were observed with respect to these parameters in the treated groups as compared to control group in both male and female rats.

Food and water consumption was highly correlated with body weight gain, values of the coefficient of determination $R^2_{B(FW)}$ and multiple correlation $R_{B(FW)}$ are presented in Table IV.

DISCUSSION

Dithane R-24 is proposed as a wheat leaf/rust fungicide.

During the study, no deaths of any rats occurred and there were no gross clinical signs of toxicity. All rats gained weight uniformly during the experimental period, irrespective of treatments but females gained less weight than the males.

The amount of the food consumed was similar for control and the treated groups but males consumed relatively more food than females.

Water consumption was variable after 12 weeks for all treatments but this variation could not be attributed to the treatments.

The total residual quantity of Dithane R-24 consumed by the animals during entire period of study ranged from 0.053 to 0.789 ppm in 1X treated males and 0.153 to 2.50 ppm in 5X treated males while it ranged from 0.051 to 0.536 ppm in X treated females and 0.141 to 1.594 ppm in 5X treated females.

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13 **CHRONIC RESIDUAL TOXICITY OF DITHANE R-24 IN RATS :
II. EFFECT OF DITHANE R-24 RESIDUES ON HAEMATOLOGY,
SERUM ELECTROLYTES, BLOOD CHEMISTRY AND SERUM
ENZYME ACTIVITIES**

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SUMMARY

A chronic residual toxicity study of Dithane R-24 in rats was conducted by feeding rats for 6 months with wheat grains harvested from crops sprayed with the fungicide at the dosage levels of 0.4 kg a.i./ha (1X-treatment) and 2.0 kg a.i./ha (5X-treatment). A dry mash, with wheat forming the major component (66 per cent) of the feed, constituted the diet for the animals. Diet containing wheat from untreated crop was given to the controls. The possible toxic effects which the chemical residues, contained in the wheat grains from the treated crops, would cause to rats were investigated.

A total of 156 (78 male plus 78 female) Wistar strain, weanling rats were used for the study divided into 3 groups of 52 animals each (26 male plus 26 female) with one group serving as a control. A total of 12 animals, 4 from each group (2 male plus 2 female) were sacrificed every fortnight and haematology, clinical chemistry, serum electrolytes and serum enzyme activities were estimated. There were no significant changes noticed in haematocrit value, haemoglobin content, total erythrocyte and leukocyte counts and serum potassium and sodium levels between control and treated groups. It was inferred that long term consumption of Dithane R-24 treated wheat in the diet was unlikely to cause abnormalities in the circulating blood cells or haematopoietic tissue and adrenocortical system.

An increase in total serum protein content observed in treated groups of rats, suggested an increased synthesis of protein. A decrease in SGOT and SGPT activities in the treated groups of rats was perhaps due to an unknown toxicological significance. Hypercholesteraemia and decreased alkaline phosphatase activity noticed in the treated groups of animals at intervals after the seventh fortnight period could probably be an indication that thyroid dysfunction was possible due to consumption of the wheat grains harvested from Dithane R-24 treated crop. This aspect of an interference by the chemical residue on thyroid function, in animals which consume wheat grains from Dithane R-24 sprayed crop, may have to be investigated separately.

INTRODUCTION

Von Meyer *et al.*, (1970) reported that among the systemic fungicides, Dithane R-24 (4-n-butyl-1,2,4-triazole also termed as RH-124) exhibited the most selective fungicidal effect since this compound was capable of controlling only wheat leaf rust among the several rusts studied. Little is known on the toxicity of Dithane R-24 to animals and this study was conducted for evaluating the toxicological significance of the Dithane R-24 residues in wheat grains obtained from the fungicide sprayed

wheat crop in rats. It was expected that the results of these studies would serve as an index for assessing the safety and the possible toxic hazards to human and animal health.

MATERIAL AND METHODS

Wheat grains* harvested from plots of wheat crops which were sprayed with 0.4 kg (1X treatment) and 2.0 kg (5X treatment) of Dithane per hectare formed the major part of the feed mixture, with wheat grains harvested from unsprayed plots serving as control. Separate feed mixtures containing the three wheat treatments were prepared. The diet was prepared as recommended by ICMR with wheat constituting 66 per cent of the diet. The animals were given the diet at the rate of 20 g per rat per day and water as required.

A total of 156 (78 males + 78 females) Wistar strain, weanling rats weighing from 45-55 g were used for the study. These animals were divided into 3 groups of 52 animals each (26 males + 26 females). One group was a control and other two groups served as 1X and 5X treatment groups, respectively, and maintained in separate cages under hygienic conditions. The study extended for 6 months.

A total of 12 animals, 4 from each group (2 males + 2 females) were sacrificed every fortnight and two two-weekly values for the parameters studied were combined and used for the statistical analysis.

A. Haematology

Haematocrit and haemoglobin (Hb) values were obtained using the method described by Faulkner and King (1970). Total erythrocyte counts (TEC) and total leukocyte counts (TLC) were made using methods described by Schalm *et al.* (1975).

B. Serum electrolytes

Potassium (K⁺) and sodium (Na⁺) values were estimated using direct flame photometric method described by Annino (1964).

C. Blood chemistry

Total serum protein values were estimated by the Biuret method described by Gornall *et al.* (1949) and total serum cholesterol values estimated using the method described by Carr and Dreker (1956).

D. Estimation of enzyme activities

Serum alkaline phosphatase (SAPase) activity was estimated by the method described by Kind and King (1954) and serum glutamic oxalacetate transaminase

*Supplied by M/s Indofil Chemicals Ltd., Bombay.

TABLE I

Residual amount of Dithiane R-24 (PPM) in the total wheat consumed by rats at fortnightly periods

Two weekly Period	Males				Females			
	1X dosage level*		5X dosage level**		1X dosage level*		5X dosage level**	
	Wheat consumed (g)	Residue intake (ppm)	Wheat consumed (g)	Residue intake (ppm)	Wheat consumed (g)	Residue intake (ppm)	Wheat consumed (g)	Residue intake (ppm)
1	59.34	0.053	56.65	0.153	56.54	0.051	52.29	0.141
2	120.65	0.109	117.59	0.317	108.88	0.098	101.96	0.275
3	180.42	0.162	180.34	0.487	159.25	0.143	152.35	0.411
4	246.64	0.222	248.88	0.672	210.90	0.190	209.95	0.567
5	307.41	0.277	314.90	0.850	252.61	0.227	251.62	0.679
6	370.35	0.333	379.56	1.025	289.97	0.261	289.57	0.782
7	438.53	0.395	452.16	1.221	330.40	0.297	329.19	0.891
8	504.17	0.454	523.66	1.414	369.33	0.332	367.94	0.993
9	580.13	0.522	598.03	1.615	408.27	0.367	408.64	1.103
10	661.64	0.595	686.15	1.853	453.69	0.408	457.55	1.234
11	736.80	0.663	764.52	2.064	497.62	0.448	501.18	1.353
12	805.68	0.725	856.65	2.313	546.84	0.492	551.92	1.490
13	876.63	0.789	925.95	2.500	595.11	0.536	590.28	1.594

*Contained 0.90 ppm of the chemical residue

**Contained 2.7 ppm of the chemical residue

(SGOT) and serum glutamic pyruvic transaminase (SGPT) activities estimated by the method of Reitman and Frankel (1957).

RESULTS

The amount of wheat consumed and the relative amount of the chemical residue in 1X treated (0.90 ppm) and 5X treated sample (2.7 ppm) ingested by the rats at two weekly intervals is presented in Table I.

A. Haematology

No significant differences were observed between treated groups and the control group of male or female rats with respect to haematocrit value, Hb content, TEC and TLC in blood.

B. Serum electrolytes

No significant differences were observed between treated groups and the control group regarding serum potassium and sodium contents in male or female rats

C. Blood chemistry

The results of the analysis of total serum protein and total serum cholesterol values in male and female rats are summarized in Tables II and III, respectively.

TABLE II
Total serum protein content (g/100 ml) of rats fed on diets containing untreated and Dithane R-24 treated wheat

Time in two weekly periods	Mean $\pm$ S.E.			't' values (compared to controls)	
	Control	1X treated	5X treated	1X treated	5X treated
<i>Male rats (4 in each group)</i>					
4 and 5	8.95 $\pm$ 0.84	8.70 $\pm$ 0.69	8.75 $\pm$ 0.59	0.73	0.62
6 and 7	9.88 $\pm$ 0.90	9.48 $\pm$ 0.65	10.38 $\pm$ 0.63	1.14	0.73
8 and 9	9.70 $\pm$ 0.58	10.12 $\pm$ 0.67	10.08 $\pm$ 0.88	1.53	1.13
10 and 11	10.75 $\pm$ 0.84	11.30 $\pm$ 0.69	11.02 $\pm$ 0.79	1.62	0.76
12 and 13	10.65 $\pm$ 1.05	11.82 $\pm$ 0.63	11.40 $\pm$ 0.41	3.05*	2.11
<i>Female rats (4 in each group)</i>					
4 and 5	8.95 $\pm$ 0.53	9.58 $\pm$ 1.16	9.68 $\pm$ 0.88	1.56	2.24
6 and 7	9.80 $\pm$ 0.61	10.05 $\pm$ 0.70	9.60 $\pm$ 0.61	0.85	0.74
8 and 9	9.50 $\pm$ 0.78	9.75 $\pm$ 0.30	9.20 $\pm$ 0.34	0.95	1.12
10 and 11	10.20 $\pm$ 0.41	11.62 $\pm$ 0.67	11.15 $\pm$ 0.28	7.22**	4.80**
12 and 13	10.10 $\pm$ 0.69	11.10 $\pm$ 0.73	12.38 $\pm$ 0.47	3.16*	2.74*

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

TABLE III

Total serum cholesterol content (mg/100 ml) of rats fed on diets containing untreated and Dithane R-24 treated wheat

Time in two weekly periods	Mean $\pm$ S.E.			't' values (compared to controls)	
	Control	1X treated	5X treated	1X treated	5X treated
<i>Male rats (4 in each group)</i>					
4 and 5	35.18 $\pm$ 5.13	34.18 $\pm$ 7.82	32.22 $\pm$ 3.11	0.34	1.56
6 and 7	36.13 $\pm$ 5.94	37.11 $\pm$ 3.58	36.13 $\pm$ 5.94	0.45	0.00
8 and 9	36.13 $\pm$ 5.78	47.85 $\pm$ 7.82	56.64 $\pm$ 6.21	3.79**	7.59**
10 and 11	42.74 $\pm$ 5.13	58.59 $\pm$ 5.07	53.72 $\pm$ 7.82	6.99**	3.73**
12 and 13	47.86 $\pm$ 7.81	62.50 $\pm$ 5.07	56.64 $\pm$ 3.59	5.00**	3.25*
<i>Female rats (4 in each group)</i>					
4 and 5	35.16 $\pm$ 5.07	36.13 $\pm$ 5.94	36.13 $\pm$ 5.94	0.40	0.40
6 and 7	37.11 $\pm$ 3.58	35.16 $\pm$ 5.07	42.00 $\pm$ 7.81	1.00	1.81
8 and 9	41.99 $\pm$ 7.81	57.62 $\pm$ 5.94	71.29 $\pm$ 7.82	5.06**	8.43**
10 and 11	50.79 $\pm$ 7.17	53.71 $\pm$ 7.81	66.41 $\pm$ 5.07	0.88	5.66**
12 and 13	55.67 $\pm$ 3.11	62.50 $\pm$ 10.49	50.78 $\pm$ 7.17	1.99	1.99

* Significant at $P < 0.05$

** Significant at $P < 0.01$

D. Enzyme activities

The results of the analysis of SAPase, SGOT and SGPT activities in male and female rats are summarized in Tables IV, V and VI, respectively.

DISCUSSION

Animals which ingest pesticide-treated grains and crops which contain chemical residues, will be affected.

The data presented in the Tables represent the results recorded from 8th week onwards. No untoward effects occurred in the first six weeks of the feeding trial, no deaths occurred in any of the groups and there were no gross clinical signs of toxicity.

No significant differences were observed between the treated and control groups of male or female rats with respect to haematocrit values, Hb content, TEC and TLC, so that long-term ingestion of Dithane R-24 is unlikely to cause abnormalities in the cellular content of blood or haematopoietic tissues.

TABLE IV

SAPase activity (mU/ml) of rats fed on diets containing untreated and Dithane R-24 treated wheat

Time in two weekly periods	Mean $\pm$ S.E.			't' values (compared to controls)	
	Control	1X treated	5X treated	1X treated	5X treated
<i>Male rats (4 in each group)</i>					
4 and 5	414 $\pm$ 62.76	437 $\pm$ 42.74	564 $\pm$ 82.70	0.97	4.62**
6 and 7	1328 $\pm$ 44.20	1179 $\pm$ 103.87	886 $\pm$ 181.65	4.20**	7.52**
8 and 9	1118 $\pm$ 185.84	612 $\pm$ 126.17	969 $\pm$ 149.34	7.16**	1.99
10 and 11	1083 $\pm$ 124.52	556 $\pm$ 80.02	570 $\pm$ 124.13	11.33**	9.28**
12 and 13	573 $\pm$ 97.72	414 $\pm$ 66.70	540 $\pm$ 112.50	4.29**	0.72
<i>Female rats (4 in each group)</i>					
4 and 5	351 $\pm$ 33.87	586 $\pm$ 45.62	529 $\pm$ 62.17	13.11**	7.97**
6 and 7	462 $\pm$ 69.60	611 $\pm$ 78.75	538 $\pm$ 54.83	4.51**	2.74*
8 and 9	548 $\pm$ 128.18	634 $\pm$ 72.22	170 $\pm$ 27.65	1.84	9.17**
10 and 11	627 $\pm$ 83.65	749 $\pm$ 58.66	318 $\pm$ 76.22	3.81**	8.68**
12 and 13	357 $\pm$ 127.84	414 $\pm$ 93.30	394 $\pm$ 85.73	1.51	0.77

* Significant at $P < 0.05$

** Significant at $P < 0.01$

There were no significant differences in serum electrolytes in the treated rats. So the amount of Dithane R-24 ingested through treated wheat grains had no detrimental effect on adreno-cortical function.

An increase in the total serum protein content observed during 24th-26th weeks in 1X treated males and from the 20th-26th weeks in both 1X and 5X treated females might be due to an increased synthesis of protein and not a resultant leakage of cell content.

An increase in total serum cholesterol levels was observed in both 1X and 5X treated male rats from 16th week up to the end of the study. The total serum cholesterol levels of female rats, increased during the 16th to 18th weeks in 1X treated and from 16th to 22nd weeks in 5X treated rats.

The serum alkaline phosphatase activity decreased in 1X treated males from the 12th week onwards, whereas in 5X treated males the activity decreased only during 12th to 14th weeks and also during the 20th to 22nd weeks. Serum alkaline phosphatase activity of females, decreased from the 16th to 22nd weeks in the 5X treated group.

These results suggest hypothyroidism and are supported by earlier investigations regarding hypercholesteremia (Fredrickson *et al.*, 1967; Chertow *et al.*, 1974; Turner and Bagnara, 1976) and decreased serum alkaline phosphatase levels (Cassar and Joseph, 1969; Wolf *et al.*, 1973). Hypothyroidism was also evidenced by the histopathological changes observed in thyroid glands of treated rats of the present experiment by Gajendragad (1977). Noguchi and Hashimoto (1970) in a short term

TABLE V

SGOT activity (mU/ml) of rats fed on diets containing untreated and Dithane R-24 treated wheat

Time in two weekly periods	Mean $\pm$ S.E.			't' values (compared to controls)	
	Control	1X treated	5X treated	1X treated	5X treated
<i>Male rats (4 in each group)</i>					
4 and 5	104 $\pm$ 6.80	104 $\pm$ 5.27	106 $\pm$ 2.75	0.28	0.54
6 and 7	97 $\pm$ 5.35	97 $\pm$ 1.21	104 $\pm$ 3.31	0.23	3.28
8 and 9	105 $\pm$ 2.75	98 $\pm$ 4.94	108 $\pm$ 2.39	3.93**	2.84*
10 and 11	113 $\pm$ 2.60	99 $\pm$ 2.00	108 $\pm$ 3.53	13.34**	3.45*
12 and 13	107 $\pm$ 1.59	108 $\pm$ 1.84	108 $\pm$ 1.84	0.50	0.65
<i>Female rats (4 in each group)</i>					
4 and 5	102 $\pm$ 3.52	106 $\pm$ 2.0	108 $\pm$ 1.84	2.73*	4.60**
6 and 7	104 $\pm$ 2.45	109 $\pm$ 3.04	111 $\pm$ 2.60	3.78**	6.48**
8 and 9	104 $\pm$ 5.53	100 $\pm$ 3.53	111 $\pm$ 2.60	2.17	3.50*
10 and 11	111 $\pm$ 3.04	107 $\pm$ 2.00	101 $\pm$ 2.60	2.84*	7.55**
12 and 13	111 $\pm$ 7.83	112 $\pm$ 5.43	108 $\pm$ 1.60	0.25	1.29

* Significant at P < 0.05

** Significant at P < 0.01

toxicity study of thiophanate methyl also reported hypercholesteremia accompanied by thyroid changes in rats. Hyperplasia (Nicholas, 1959; Gaines *et al.*, 1973) and adenomatous changes (Jukes and Shafer, 1960; Kroller, 1966) or thyroid gland have been observed in rats which were fed with ATZ at different levels in the diet.

The histopathological changes in thyroid glands of our rats were observed when the residual quantity of Dithane R 24 consumed by male rats ranged from 0.395 to 0.789 ppm and 1.221 to 2.50 ppm and by females 0.297 to 0.536 and 0.891 to 1.59 ppm at 1X and 5X treated levels. These results agree well with the observations of Fregly (1968) who stated that ATZ, a compound closely related to Dithane R-24 affected thyroid activity in rats at a dosage between 0.50 and 2.0 ppm.

An increase in SAPase activity observed in the early stages in 1X and 5X treated females is perhaps related to growth.

SGOT activity increased from the 12th to 18th weeks only in 5X treated males and from the 8th to 14th weeks in both the 1X and 5X treated females. An increase

TABLE VI
SGPT activity (mU/ml) of rats fed on diets containing untreated and Dithane R-24 treated wheat

Time in two weekly periods	Mean $\pm$ S.E.			't' values (compared to controls)	
	Control	1X treated	5X treated	1X treated	5X treated
<i>Male rats (4 in each group)</i>					
4 and 5	63 $\pm$ 4.52	64 $\pm$ 8.75	67 $\pm$ 5.46	0.27	1.87
6 and 7	77 $\pm$ 6.84	76 $\pm$ 2.65	88 $\pm$ 8.80	0.36	3.09*
8 and 9	76 $\pm$ 5.08	66 $\pm$ 6.26	83 $\pm$ 9.43	3.78**	2.27
10 and 11	82 $\pm$ 5.46	65 $\pm$ 7.91	65 $\pm$ 6.62	5.79**	6.17**
12 and 13	63 $\pm$ 5.07	63 $\pm$ 5.07	67 $\pm$ 9.43	0.00	1.24
<i>Female rats (4 in each group)</i>					
4 and 5	53 $\pm$ 7.95	58 $\pm$ 6.67	72 $\pm$ 17.54	1.53	3.29*
6 and 7	58 $\pm$ 6.84	68 $\pm$ 11.20	71 $\pm$ 11.14	2.23	3.04*
8 and 9	71 $\pm$ 7.66	67 $\pm$ 9.43	75 $\pm$ 7.50	1.09	1.24
10 and 11	65 $\pm$ 11.32	74 $\pm$ 5.08	63 $\pm$ 5.07	2.24	0.75
12 and 13	84 $\pm$ 8.24	73 $\pm$ 3.07	73 $\pm$ 2.65	3.92**	4.29**

* Significant at P < 0.05
** Significant at P < 0.01

in SGOT activity occurred during the 16th to 18th weeks in 5X treated females. The SGPT activity was increased during the 12th to 14th weeks in 5X treated males and from the 8th to 14th weeks in 5X treated females. The increased SGOT activity seen in treated rats is in conformity with abnormally high SGOT values reported by Chertow *et al.* (1974) in 15 human hypothyroid patients.

It was also observed that SGOT activity decreased from the 16th to 22nd weeks in 1X treated and during the 20th to 22nd weeks in 5X treated males while the SGOT activity of females decreased during the 20th to 22nd weeks in both 1X and 5X treated females. SGPT activity likewise decreased in the treated males from the 16th to 22nd weeks in 1X treated and during 20th to 22nd weeks in 5X treated, while the activity of females, decreased during the 24th to 26th weeks for both 1X and 5X treated females.

The decrease in SGOT and SGPT activities observed in the treated rats agrees with observations made by Seinen and Willems (1976) on rats fed with Di-*n*-octyl tin chloride who stated it was of an unknown toxicological significance. Noguchi and Hashimoto (1970) in a short-term toxicity study of thiophanate methyl also reported a slight decrease in SGOT activity accompanied by thyroid changes in rats.

Rohm and Haas Co., in an unpublished report on a subchronic, 90 day feed study in rats which were given the diets containing 0, 30, 100 or 300 ppm of Dithane R-24, stated that a 300 ppm dose level was indicated as the possible minimum effect level. At this dosage there were slight increases in weights of the heart, spleen and kidneys of female rats and an increase in the weight of the spleen of male rats. A level of 100 ppm was adjudged to be the no effect level.

The dietary dosage of the chemical given to rats in their experiments appeared to be greater than that in our experiments.

Dithane R-24 is thus apparently non-toxic to rats causing no growth retardation, mortality or any abnormal clinical symptoms but it is probable that the chemical has adverse effect on the activity of the thyroid glands, when fed for a long period.

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14 PRACTICAL CONSIDERATIONS IN THE REGULATION OF PESTICIDE RESIDUES IN FOOD IN INDIA

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SUMMARY

The presence of pesticide residues in food is viewed with serious concern because of the toxic nature of these chemicals. Many countries in the world, either through statutory measures or voluntary registration schemes coupled with monitoring, are regulating the levels of pesticide residues in food to protect the public consuming harmful amounts of such substances. **FAO** and **WHO** also have a comprehensive programme of preparing guidelines to achieve this objective at the International level. The rationale of these procedures has been critically examined.

In India, the levels of pesticide residues in food are regulated by legislative control under 'The Insecticides Act 1968', 'The Insecticides Amendment Act, 1977' and 'Prevention of Food Adulteration Act, 1954'. The rules for registration of pesticides have been framed under the Insecticide Act. The burden of proving that the approved usage of pesticides leaves residues within the safe limits lies with the manufacturer. As the rate and extent of degradation of a pesticide is environment-dependent, such data are required from different agro-climatic zones of the country. It has been estimated that approximately 10 man-years in a well-equipped laboratory is needed for collecting the required residue data. Facilities for residue analysis rarely exist with the manufacturers of pesticides in India. The situation is further complicated by the complexities of the pesticides industry in India which include small-scale formulators, big manufacturers and multinationals. The provisions of prescribing the pesticide residues tolerance in food and their enforcement exist under the Prevention of Food Adulteration Act. Food containing pesticide residues above the established tolerance can be removed from the market. If this law is to be effective, it would require extraordinary efficient machinery, which does not seem to have been provided so far. Data available on monitoring of food commodities in the country indicate that contaminated food with excessive pesticide residues are freely moving in commerce. The arbitrary establishment of tolerance thus become meaningless. It is considered that the failure in the regulation of pesticide residues in food arises from the lack of appreciation of the problem and recognition of the actual situation prevalent in the country. The need for a realistic approach has been stressed and some practical suggestions in this direction have been made for controlling pesticide residues in food.

Accurate estimate of losses in global agricultural production due to pests are not available but the generally accepted figure is 20-35 per cent. The use of pesticides is indispensable to reduce these food losses due to pests. High agricultural yields are usually related to heavy use of pesticides. Trials at the International Rice Research Institute in the Philippines showed that inadequate disease and insect control were the most significant constraints in yield of rice and that the

average yield could be improved by up to 88 per cent from 3.9 tons/ha to 7.3 tons/ha by proper pest control. According to an estimate made by the Pesticide Association of India, a rupee spent on pest control chemicals saves crops worth 10 rupees. Pesticides have also been important in controlling insect transmitted diseases.

Benefits due to pesticides have been recognised, but concern has grown in recent years as to their potentially harmful effects. The problem of major concern has been the contamination of the environment particularly food. Crops treated with pesticides may contain small amounts of these chemicals at harvest. Milk and meat are also contaminated through ingestion of pesticide-treated feed by animals. In order to accomplish their purpose, these compounds are usually toxic to many organisms and the ingestion of residues by man may result in their accumulation in his body tissues and have other undesirable effects. The wide spread occurrence of pesticide residues in soil, water and most of the biota may also cause hazards to the environment.

In the early sixteenth century, Paracelsus in the "Third Defence" said, "Dosis sola facit venenum" or in more popular language, "Dosage alone determines poisoning". This assertion has been borne out for centuries; for example, many people have died from poisoning with arsenic, but people have been eating shrimps or ocean fish containing traces of arsenic which is a natural contaminant of water without any harm to their health. Bean plants contain little cyanide, a well known poison, but we eat beans and consider them a good food. Thus, there exist the possibility of finding a dosage of a pesticide which may not cause any appreciable harm to human health even if consumed throughout life.

In order to regulate pesticide residues to safe levels, FAO and WHO of the United Nations have laid down principles for arriving at the maximum residue limits (tolerances) of pesticides in food commodities. Essentially, this involves a meticulous study of reports of supervised trials designed to determine the maximum residues likely to occur in food by the application of minimum effective dosages of pesticide for pest control. In addition, the results of extensive and detailed toxicological results with the compound and its metabolites administered to laboratory animals continuously, for periods ranging from 2-5 years are evaluated to determine possible effects on animals. From these studies, the level of intake which caused no discernable effect on the most susceptible species (No harmful effect level) is ascertained and this is used to judge the level of intake which could be considered safe for humans if consumed for their whole life. A large safety factor (100) is incorporated as additional safeguard. This 'Acceptable Daily Intake (ADI)' is used to gauge the acceptability of the maximum residue level determined from supervised trials. There is a general agreement among toxicologists and other health experts

that the level of human exposure should be maintained as low as possible. Consequently, restrictions require not only that the residues must be safe, but also that they must not be higher than is actually needed from good agricultural practices in the use of pesticides. ADI and maximum residue limits of large number of pesticides are now available.

The maximum residue limit (tolerance) has statutory authority in many countries like the USA, Japan, Canada, etc., where adequate machinery for their enforcement is also provided. A large number of samples of food commodities are examined every year. If any commodity is found to be contaminated with pesticides exceeding the legally permitted limit, it is prohibited from consumption and trade. In the U.K., however, there is no legal provision for the control of pesticide residues on food other than general regulations on food contamination. There, protection is sought by the voluntary co-operation of the industry with the Government Departments concerned, aimed at the proper usage of a pesticide to ensure that the crops at harvest will bear no harmful residues. It is reassuring to find that in these countries the ingestion of pesticides by man through well-balanced diet has been only a small fraction of the 'ADI' and further, there is no documented evidence that any one has been even temporarily injured from food which has been treated correctly with pesticides for the control of pests in the course of its production.

Pesticide residues have a significant influence in the International trade. When an importing country has established a very low tolerance, it is possible to reject or refuse import of a commodity containing residues more than the limit. In order to establish uniform standards, and to ensure fair practices in food trade, the Codex Alimentarius Commission has been established under WHO/FAO.

In India, there are two laws to regulate the pesticide residues in food. These are 'The Insecticide Act, 1968' and the 'Prevention of Food Adulteration Act, 1954 (PFA rules)'. The registration committee formed under the 'Insecticide Act' is to satisfy that the pesticide allowed for use will not leave residues on the crops above acceptable safe levels. The burden of proving this lies with the manufacturer of a pesticide. The requirement of a pesticide differs from one region to another. In addition, the ultimate amount of a pesticide left on a crop is environmentally dependent. Thus, information on the levels of residues resulting from the recommended usage of pesticide from different agroclimatic zones of the country is necessary before the pesticide can be registered for use. Most of the manufacturers of pesticides in India have not been able to develop facilities for collecting the required information although the 'Insecticide Act' has been in force for about a decade. The situation has been made much difficult by the complexities of the pesticide industry in India, which also includes numerous small-scale formulators

The maximum residue limits (tolerances) on food commodities have been laid under the PFA rules. The marketing of a commodity containing residues more than these limits is an offence. Naturally, the enforcement of these rules would require an effective machinery which is not yet available. We are not aware whether any commodity bearing pesticide residues more than the legally permitted levels has been confiscated during the last 30 years of the operation of these rules. The Ministry of Health responsible for the enforcement of these rules does not seem to have taken any notice of the reports of the contamination of food stuffs with excessive pesticide residues.

Limited surveys carried by the Central Plant Protection Training Institute, Hyderabad; Indian Agricultural Research Institute, New Delhi and the Punjab Agricultural University, Ludhiana have revealed that food commodities containing pesticide residues more than the legally permitted levels are moving freely in commerce. At Hyderabad, a large proportion of the market samples of cereals, vegetables, milk and eggs were found contaminated with pesticides at higher than the permitted levels. Large amounts of DDT were detected in the milk and butter, samples analysed at Delhi. Market samples of vegetables from Delhi showed excessive residues of DDT and HCH. Studies done at the Punjab Agricultural University, revealed that more than 80 per cent of the market samples of wheat flour from different cities in the Punjab and the Union Territory, Chandigarh were contaminated with DDT and HCH. Furthermore, these pesticides present in wheat flour were not found to be lost during *chapati* making which would mean a substantial intake of these pesticides by man through contaminated wheat flour. The contamination of milk presents an even more baffling picture. Most of the milk being marketed in the Punjab was found to be contaminated with DDT at greater than the maximum residue limits. Popular brands of butter obtained from Gujarat, Delhi, Haryana and Rajasthan also showed high DDT residues thereby indicating that the contamination of milk with DDT is quite wide spread in India. HCH residues in milk were also detected. Infant food marketed in India was found to be contaminated with DDT residues.

The enforcement of PFA rules in such situations would mean that most of the Indian population would have to go without milk. The sources of contamination of milk and wheat are still required to be identified. One is, therefore, not sure on whom the onus for this contamination should fall.

As early as 1965, it was reported that the Indians carry the highest load of DDT residues in their bodies. Professor Wayland J. Hayes, Jr. inferred that the ingestion of DDT by the Indians may be 20-40 times higher than in the U.K.

and U.S.A. More than 10 years have elapsed but we do not know precisely the sources and extent of its intake.

Indications have been obtained recently that indoor residual spraying of DDT for malaria control contributes partly towards the contamination of milk and other commodities. However, the WHO still maintain that DDT spraying in the houses is not likely to contribute towards the contamination of the environment. Acceptance of the opinions expressed by the international agencies without proper appraisal, may, therefore, lead to certain unexpected consequences.

The pest control programmes such as the DDT and HCH spraying for malaria control, aerial spraying of insecticides in cotton, etc., wholly financed or subsidized by the Government are rarely critically evaluated with regard to their contribution towards the contamination of the environment and food.

The consumption of pesticides at present is very small as compared to that of developed countries. According to one estimate, about 40 per cent of total world production of pesticide is used in North America, somewhat less in Europe and only 10 per cent in the developing countries. In spite of tremendous use of pesticides in Western countries, they have been able to regulate the usage within the acceptable criteria. The failure in regulating pesticide residues in India arises from a lack of appreciation that pesticides are products of technological society. Laws in such cases can be effective only if there is sufficient technical support a fact to which scant attention has so far been paid. Furthermore, it needs to be realized by the concerned authorities that the pesticide residues are not adulterants in the true sense. As pesticides are costly inputs, the farmer is not likely to use them in quantities more than the required provided the information on the minimum effective dosage of a pesticide for pest control is made available to him. The mechanism for providing this information to the user has not so far been developed in the country. Under the circumstances it will be unrealistic to expect any control on pesticide residue. The PFA rules presently look like 'putting the cart before the horse'.

The need to evolve a practical approach to control pesticide residues in food and in the environment in India needs little emphasis. Mere transplanting of pesticide legislations from other countries may not achieve the desired goals. We do not consider that it would be a wise decision to put the limited resources of man power and facilities available to routine regulatory duties, particularly when the basic requirements for such regulations have not been met. Rather, concerted efforts should be made to have better understanding of the environment-pesticide

problems in India so that the rationale of the safe pesticide usage may be developed. The following suggestions are made in the direction :

- a) It is considered that the formation of an 'Expert Group on Pesticide Residues' is a basic requirement to achieve the desired goal. The Expert Group thus formed could evaluate critically the available information, decide priorities and suggest an action plan for immediate implementation through the co-operation of the government, industry and various research institutions. It could also help the Government in rationalization of the policy with regard to pesticide residues in food and environment.
- b) Nation-wide monitoring for pesticide residues of food commodities which are most important in our diet should be initiated. The laboratories where facilities for such work exist can collaborate in this study. Initially, milk and cereals can be screened for DDT and HCH which are used most extensively in this country. Efforts should also be made to identify the sources of contamination so that corrective measures may be taken. These studies should be gradually expanded to cover the total diet so that the intake of pesticides by the Indian population may be kept below the acceptable daily intakes.
- c) To gain a better perspective of pesticide residues in food, feed and the environment, it is necessary to have complete information on the pattern and quantity of pesticides used both in agriculture and public health. A region-wise inventory of pesticide use is of utmost importance in ascertaining the cause (contamination) and effect (toxicity) relationship.
- d) The contribution of the programme where most extensive usage of pesticide is being made should be evaluated with regard to their contribution towards the contamination of the environment and food.
- e) The region-wise recommended usage of pesticides for the control of pests should be catalogued.
- f) The recommended use of pesticide should be backed by the studies on the residue implications of the compound and its terminal products. It is suggested that at least one regional laboratory be established in each agro-climatic zone of the country to ensure against any hazard accompanying the pesticide usage.
- g) Food commodities meant for export should receive special attention to obviate the problem of unacceptable pesticide residues.

- h) A panel of analysts may be constituted to suggest the methods of analysis for pesticide residues which can be conveniently adapted in our country so that valid data may be obtained.
- i) Numerous brands of the formulation of the same pesticides are available in this country with different compositions with respect to adjuvants and auxillary materials. The comparative effectiveness and residual persistence may, therefore, vary considerably. The auxillary material and adjuvants may also have their own toxicological significance. This problem needs to be critically examined so that a suitable policy is formulated.
- j) Education on the safe and proper usage of pesticides can go a long way to obviate many of the undesirable effects of pesticides. This needs strengthening the required agency.
- k) Labels on the containers of pesticides and the literature issued by the firms should take care of the possible residue implications.
- l) The presence of pesticide residues in food can not be viewed in isolation from the problem of pesticidal contamination of the environment. In the use of pesticides, we must, therefore, be aware of their effects on man's total environment. Determining the effect of pesticides on environmental quality is an extremely complex problem. It is also becoming increasingly apparent that the benefits of using pesticides must be considered in the context of present and potential risks of pesticide usage. Both risks and benefits could vary significantly from country to country and from one period of time to another. Organochlorine pesticide which have been restricted from use in western countries are still used in large quantities in India. However, sound and objective judgements can be made by decision making bodies only if systematic information is available both on the benefits and risks. One could appreciate that it will not be possible to have any assessment of risks in the absence of data on the fate and significance of foreign chemical residues, the susceptibility of the plant or animal species (both target and non-target species) and which food chain is involved under the particular local condition. It is, therefore, important to identify a few centres in India for such work. These should be adequately staffed and equipped (Tracer technique, HPLC, GC-MC, etc) immediately so that the required data and expertise for making objective decision for the country on pesticides usage become available.

The programme as suggested above will, of course, need funds. Substantial monetary gains in the form of increased agricultural production are obtained through the use of pesticides. In India, pesticides worth about 10 million rupees are being used every year.

At least 2 per cent of the net gain should be diverted to research to ensure better safety standards for us, our children and the environment.

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PESTICIDE RESIDUES IN CROPS

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All those connected with agriculture, know that all field crops, suffer heavy losses from insect pests and with increasing human populations such losses must be avoided. If we take a very conservative estimate of 10 per cent, for pest losses to our food crops this amounts to about 13 million tonnes, our annual production being 130 million tonnes or at least Rs. 130 million. Pesticides are still the best weapon to combat insects and diseases and are almost indispensable. Even though their use involves dangers of contamination, possible ill health or even death, progressive agriculture requires their use.

In India, a large number of pesticides are being used and the Central Insecticides Act of 1968 requires that all pesticides must be registered. At present, only 112 pesticides (Table I) are registered, two of which viz., endrin and dieldrin have been registered for a limited period and usage of the 112 compounds, 39 of which have been marked with an asterik (*) fall under the category of insecticides used against field crops; the others are fungicides, herbicides, rodenticides, nematocides, acaricides and fumigants.

This paper is restricted to considering residues of insecticides in/on field crops, including vegetables, plantations and fruit crops.

Work on residues of pesticides in India has been reviewed twice; these are the Proceedings of the Symposium on "Progress and Problems in Pesticide Residue Analysis" held at Punjab Agricultural University, Ludhiana in November, 1971; edited by Dr. O. S. Bindra and Dr. R. L. Kalra; and "Pesticide Residues" by G. Agnihothrudu and V. S. Mithyantha, published by M/s Rallis India Ltd., Bangalore.

TABLE I

*Some cases where washing, cooking, peeling
and dehydration reduce the residue*

S.No.	Insecticide	Commodity	Process	Reference
1	Aldrin	Potato	Washing, cooking, peeling	Misra <i>et al.</i> , 1977
2	BHC	Spinach	Washing	Jaglan and Chopra, 1971
3	Carbaryl	Mustard	Washing	Misra <i>et al.</i> , 1977
		Brinjal	Washing	Deshmukh and Udeaan, 1971
		Cauliflower	Washing	Dewan <i>et al.</i> , 1973
		Tomato	Washing, cooking	Rajukkannu <i>et al.</i> , 1976b
		Bhindi	Washing	Krishnaiah <i>et al.</i> , 1975
4	Carbofuran	Sponge gourd	Washing	Singh <i>et al.</i> , 1976
		Cowpea	Washing	Awasthi <i>et al.</i> , 1977b
		Brinjal	Cooking	Mithyantha <i>et al.</i> , 1977a
		Potato	Cooking	Mithyantha <i>et al.</i> , 1977b
		Cabbage	Cooking	Anon, 1977
5	DDT	Bhindi	Cooking	Anon, 1977
		Spinach	Washing	Jaglan and Chopra, 1971
6	Dieldrin	Potato	Washing, cooking, peeling	Misra <i>et al.</i> , 1977
7	Dimethoate	Cabbage	Washing	Krishnaiah and Lal, 1973
		Cauliflower	Washing	„ „
		French bean	Cooking	Anon, 1977
		Tomato	Washing	„ „
		Chillies	Washing	„ „
8	Endrin	Tomato	Washing, cooking	Rajukkannu <i>et al.</i> , 1976b
9	Endosulfan	Brinjal	Washing	Deshmukh and Udeaan, 1971

Sl.No.	Insecticide	Commodity	Process	References
		Cauliflower	Washing	Awasthi <i>et al.</i> , 1974 and Verma and Lal, 1976
		Bhindi	Washing, cooking dehydration	Nath <i>et al.</i> , 1974
		Mung	Washing	Verma and Pant, 1976a
		Ber	Washing	Kathpal <i>et al.</i> , 1977
10	Fenitrothion	Cauliflower	Washing, cooking	Attri, 1977
11	Heptachlor	Potato	Washing, cooking peeling	Misra <i>et al.</i> , 1977
12	Malathion	Brinjal	Washing	Deshmukh and Udeaan, 1971
		Bhindi	Washing, cooking dehydration	Jat and Srivastava, 1973
		Mustard	Washing	Dixit <i>et al.</i> , 1974
13	Monocrotophos	Cowpea	Washing, cooking	Awasthi <i>et al.</i> , 1977b
14	Oxydemeton methyl	Potato	Washing, peeling, cooking	Awasthi <i>et al.</i> , 1977c
15	Parathion	Cabbage	Washing, rubbing, cooking	Singh <i>et al.</i> , 1971
		Tomato	Washing, cooking	Rajukkannu <i>et al.</i> , 1976b
		Cauliflower	Washing, cooking	Attri and Lal, 1974a
		Cowpea	Washing, cooking	Attri and Lal, 1974b
16	Phorate	Mustard	Washing	Dixit <i>et al.</i> , 1974
		Potato	Washing, boiling, peeling	Awasthi <i>et al.</i> , 1977c

Data on insecticide residues in crops (Table II) is for 36 insecticides, but 11 of these have been studied on only one crop and 2 on only two crops. Residues of only 23 insecticides have been studied on more than 2 crops and of these, BHC, DDT, endosulfan, endrin, dimethoate, disulfoton, malathion, monocrotophos, parathion, phorate, phosphamidon, aldicarb, carbaryl and carbofuran have been studied best. More work has been done on carbamates particularly carbaryl. Most residue work has been on vegetables, particularly, okra, bhindi, brinjal,

TABLE II

Some cases where non-systemic insecticides have been reported to translocate to non-target portions of the plants

Sl.No.	Name of the insecticide	Crop in which it has been reported to translocate	Reference
1	Aldrin	Moong	Agnihotri <i>et al.</i> , 1974
		Cowpea	" "
2	BHC	Cowpea	" "
		Mung	" "
		Sorghum	Kathpal <i>et al.</i> , 1976
		Maize	Yadav <i>et al.</i> , 1977
3	Carbaryl	Brinjal	Kumaresna and Baskaran, 1975
		Paddy	Rajukkannu <i>et al.</i> , 1976a
		Sorghum	Saivaraj <i>et al.</i> , 1976a
		Bhindi	Srivastava <i>et al.</i> , 1976
4	Endrin	Bhindi	Rajukkannu <i>et al.</i> , 1976b
		Paddy	Rajukkannu <i>et al.</i> , 1976a
5	Heptachlor	Sugarcane	Awasthi <i>et al.</i> , 1977a
6	Diazinon	Brinjal	Sathpathy <i>et al.</i> , 1974
		Cowpea	" "
7	Fensulfothion	Cowpea	Rajukkannu <i>et al.</i> , 1977
		Mung	" "
		Gram	" "
8	Fenthion	Paddy	Rajukkannu <i>et al.</i> , 1976a
9	Parathion	Paddy	" "
		Sorghum	Saivaraj <i>et al.</i> , 1976a

cauliflower, cabbage and tomato. Residues in other vegetables such as potato, radish, carrot, spinach, knol-khol, cowpea, chillies, peas etc., have been reported but not in onions, garlic, *methi*, bitter gourd, cucurbits etc. In field crops, most residue work has been on mustard, maize, paddy and sorghum. Although there are some reports on sugarcane, mung, cotton, gram and coconut and only one study each on barley, wheat and none on bajra, sesamum etc.

Most of the work has been in a few laboratories; Divisions of Agricultural Chemicals and Entomology, IARI, New Delhi, University of Udaipur, Udaipur, Punjab Agricultural University, Ludhiana, Haryana Agricultural University, Hissar, Tamil Nadu Agricultural University, Coimbatore, Central Plant Protection Training Institute, Hyderabad, Central Food Technological Research Institute, Mysore and

Central Drugs Research Institute, Lucknow. Other research institutes, agricultural, and traditional universities and industry have also recognised the importance of pesticide residue work and they have started residue work. The pesticide industry is establishing their own research and development laboratories.

Different methods have been used for determining residues with colorimetry, gas liquid chromatography and bioassay more commonly used than others. Radioactive materials for residue studies have been used little. Work on enzymic inhibition, TLC and ultraviolet spectrophotometry has been little.

In most studies, residues fell below the tolerance limit or the detectable level in a reasonable time, and it was concluded that the use of the insecticides was safe. In some studies (Table I) it is stated that washing, cooking, dehydration, rubbing etc., remove much of the residue. Weather, temperature, rainfall, humidity and wind velocity, varieties and location all have a marked influences on residues of insecticides on crops.

Although insecticides are divided into stomach, contact and systemic insecticides, some contact insecticides are also translocated. Such insecticides pose residues are not suspected in the non-target portions of the plants.

Insecticides are metabolised in the plant and animal tissues. Some of the metabolised may be more toxic than the parent compounds but in India, little work on metabolites has been done. Most work shows that some metabolites-dieldrin (from aldrin), heptachlor epoxide (from heptachlor), malaxon (from malathion), alpha-naphthol (from carbaryl) and 3-hydroxy carbofuran (from carbofuran) have been looked for. Metabolites of the commonly used insecticides aldicarb, carbaryl, demeton, lindane, malathion, parathion, fenitrothion have not been worked upon.

Thus, much work remains to be done. Residues of many insecticides have not been assessed. Of those, which have been studied, residues have been reported on only a few crops and most of the work has come from a half dozen laboratories. Many more laboratories need to be set up and equipped, so that minute quantities of residues may be detected. Work on metabolites must be strengthened.

We must also have facilities where scientists working on residues can be trained in use maintenance and repair of equipment. Existing laws demand stringent requirements of data before any pesticide is registered.

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A REVIEW OF WORK DONE IN INDIA ON INSECTICIDE RESIDUES ON/IN VEGETABLES

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SUMMARY

Pesticidal chemicals have become a boon to humanity in that they have helped in protecting agricultural and horticultural produce from insects and diseases and during the past 30 years there has been a great upsurge in the development and usage of pesticides. However, some of the chemicals persist in food and feed and pose a threat to the health of consumers. This is more serious in the case of vegetable crops as these are consumed directly by the consumer within a short period of harvesting and are often eaten without much processing. Hence, it becomes imperative that some sort of monitoring of pesticide residues in vegetables be done in view of the possible risks to human health. Pesticides like DDT, endrin, heptachlor, lindane, aldrin, chlordane, BHC, malathion, parathion and a number of others are being constantly sprayed on vegetables because they have been found to be effective in controlling many pests of vegetables. The analysis of pesticide residues is necessary to ascertain spray schedules and to advocate safe periods or intervals between spray and harvest. Hence dissipation studies of these pesticides on vegetable crops should be made. In this article, the work done in India on pesticide residues in/on vegetables is reviewed and future plans of research in this field to be carried out at the Indian Institute of Horticultural Research, Bangalore have been outlined.

Concern is being expressed by increasing numbers of individuals and government agencies about the possible risks to public health from residues of pesticidal chemicals in foodstuffs. Pesticides have become indispensable to agriculture but some of these chemicals persist in food and feed for long periods of time and pose a threat to the health of the consumers. Often, there is indirect contamination of foodstuffs through food chains, e.g. from feed to grazing animal or poultry and hence to animal products like milk, meat and eggs. Pesticide residue laboratories can study the persistence of pesticides in food and feed and also monitor pesticide residues in market samples of foodstuffs and estimate the time that must elapse before a particular foodstuff which has been sprayed with a pesticide can be consumed safely; they also fix maximum residue limits for different pesticides.

The maximum residue limit (tolerance) is the maximum concentration of a pesticide residue in any commodity for which such a limit is recommended and is expressed in milligrams of the residue per kilogram of the commodity (FRO/WHO 1975). Many Governments have fixed maximum residue limits for different pesticides and at the international level, WHO and FAO have appointed Expert Committees to make recommendations. The Government of India passed the Insecticides Act in 1968 by which a Central Insecticides Board (CIB) was constituted and this Board

has prescribed maximum residue limits for 20 pesticides, which came into force from the 1st July, 1976 (Paharia, 1978). The Registration Committee set up under this Act has approved up to December 1977, 106 insecticides for use in agriculture.

Residue studies on vegetables are important because these are consumed directly without much processing or storage. Moreover, pesticides are being used indiscriminately on vegetables and some monitoring of market samples of vegetables for pesticide residues is essential. An attempt is made here to review the studies in India on monitoring of vegetables for insecticides, dissipation of insecticides on vegetable crops and the effect of processing procedures on the removal, degradation of insecticide residues, and to give an outline of the work done in this field at the Indian Institute of Horticultural Research, Bangalore.

Monitoring of Insecticide Residues in Vegetables:

Such studies have been mainly in Delhi (IARI), Hyderabad (CPPTI), Mysore (CFTRI) and the Punjab (PAU) (Table I). Sixty samples of eight vegetables (bhindi, brinjal, cabbage, cauliflower, cowpea, mustard, spinach and radish top) drawn from eight markets of Delhi were analysed for residues of malathion and organochlorine insecticides (Agnihotri *et al.*, 1974b). Most of the samples analysed were found to contain residues of DDT or BHC or both and about 25 per cent of the samples analysed had residues of DDT or BHC either close to or above the maximum residue limits prescribed by FAO/WHO.

Analyses of market samples for organochlorine insecticide residues were done in Hyderabad (Kakshminarayana and Krishna Menon 1969; and Lakshminarayana and Krishna, 1975). Of 83 samples of vegetables, 58 samples were contaminated with organochlorine insecticides. DDT occurred in 52 samples, endrin in 13, heptachlor in 3, lindane in 5, aldrin in 4, and chlordane in 4. In the second instance, a total of 669 vegetable samples were analysed and 19 of 35 samples were found to be contaminated with organochlorine insecticides, 7 above the USFDA tolerance levels. One hundred and eleven of the 192 samples of starchy vegetables analysed were contaminated, with 52 samples containing residues above the USFDA tolerance levels. Two hundred and eight out of the 442 samples of other vegetables analysed contained detectable residues, 19 of them containing residues above USFDA tolerance levels.

All 300 samples of leafy vegetables analysed (Viswashwaraiah and Jayaram 1962) CFTRI, Mysore, contained excessive residues of BHC. At Ludhiana, 4 of the 10 brinjal samples analysed during 1971 contained DDT (Jaglan and Chopra, 1970). Five of 8 samples of bhindi from different markets in the Punjab contained endrin

TABLE I

Insecticide residues in market samples of vegetables

Commodity	Place	Period of collection	No. of samples examined	No. of samples found contaminated	Insecticide(s) detected	Residue level (ppm)	Method of Analysis	Reference
1	2	3	4	5	6	7	8	9
Beans	H	1968	69	6	DDT	0.4	TLC	Kakshminarayana and Menon (1976)
Bhindi	H	"		5	DDT	1.0	"	"
"	Pb	1967-68		2	DDT, Endrin	0.2, 0.2	"	"
"	D	—	—	6	Endrin	0.06-0.38	GLC	Bhalla <i>et al</i> (1970)
				—	DDT	N.D.-9.00	GLC	Agnihotri <i>et al</i> (1974b)
Brinjal	H	1968	69	21	BHC	N.D.-0.3	"	"
				13	DDT	0.2		
				2	Heptachlor	2,0.2		
					Endrin	0.2		Kakshminarayana and Menon (1976)
				1	DDT	traces		
	L	1964	10	4	Heptachlor	2.0		
				4	DDT	0.08-8.0	C	Jaglan and Chopra (1970)
Bottle gourd	H	1968	69	1	Nil	—	TLC	Kakshminarayana and Menon (1976)
Cabbage	H	1968-69	8	1	Endrin	0.1	TLC	"
				4	Endrin, DDT	0.2, 0.1		
				3	DDT	traces		

1	2	3	4	5	6	7	8	9
Cabbage	Pb	—	33 (for para- thion only)	Nil	—	—	GLC	Jaswant Singh <i>et al</i> (1974)
"	D	—	—	—	DDT BHC	ND-5.00 ND-56.2	GLC	Agnihotri <i>et al</i> (1974b)
Carrot	H	1968-69	8	4	Lindane	2.00	TLC	Kakshminarayana and Menon (1976)
				2	Aldrin, DDT	0.5, 0.1		
				1	Lindane,	0.1, 0.6 & traces		
				1	DDT, endrin	0.1, traces		
				Nil	DDT, endrin	—	TLC	" "
Cauliflower	H	1968-69	1	Nil	Nil	—	GLC	Agnihotri <i>et al</i> (1974b)
"	D	—	—	—	DDT BHC	N.D.-10 N.D.-50	TLC	Kakshminarayana and Menon (1976)
Chillies	H	1968-69	3	Nil	—	—	GLC	Agnihotri <i>et al</i> (1974b)
Cowpea	D	—	—	—	DDT BHC	N.D.-5.5 N.D.-0.3	TLC	Kakshminarayana and Menon (1976)
Mustard	D	—	—	—	DDT BHC	N.D.-2.5 N.D.-2.5	GLC	" "
Potato	H	1968-69	15	3	DDT, endrin	1.0, traces	TLC	Kakshminarayana and Menon (1976)
				2	aldrin, DDT	2.0, 3.0		
				4	Chlordane, DDT	2.0, 3.0		
"	H	"	10	4	DDT	0.5	TLC	" "
"	L	1971	10 (aldrin, dieltrin only)	Nil	—	—	GLC	Tegindar Singh and Kalra (1973)
"	M	—	10	10	DDT	0.1-169.0	CI	Majumdar (1973)
Radish top	D	—	—	—	DDT BHC	N.D.-1.00 N.D.-50.00	GLC	Agnihotri <i>et al</i> (1974b)

1	2	3	4	5	6	7	8	9
Spinach	D	—	—	—	DDT BHC	N.D.-35.00 N.D.-60.00	GLC	Agnihotri <i>et al</i> (1974b)
Tomato	H	1968-69	10	4	DDT	0.05	TLC	Kakshminarayana and Menon (1976)
"	L	1964	10	1	DDT	0.08	C	Jaglan & Chopra (1970)
Leafy vegetables	M	—	300	300	BHC	10.5-20.00 (Average)	TLC	Viswashwaraiiah & Jayaram (1972)
"	H	—	35	19	BHC	tr-2		
					DDT	tr-2	TLC	Lakshminarayana and Menon (1975)
					Endrin	tr-0.2		
					heptachlor	traces		
Starchy vege- tables	H	—	192	111	Aldrin	tr-2	TLC	Lakshminarayana and Menon (1975)
					BHC	tr-6.6		
					DDT	tr-5		
					dieldrin, endrin	traces		
					heptachlor	"		
					lindane	0.1-2		
Other vegetables (beans, bhindi, brinjal, cucurbits and tomato)	H	—	442	208	BHC	tr-0.5	TLC	"
					DDT	tr-7		
					endosulfan	traces		
					endrin	tr-0.8		
					heptachlor	tr-2		
					lindane	tr-1		

In column 2 : D — Delhi, H — Hyderabad, L — Ludhiana, M — Mysore, Pb — Punjab.

In column 8 : C — Colorimetry, Ci — total chlorine method, TLC — thin layer chromatography, GLC — Gas liquid chromatography.

In column 7 : N.D. — not detectable; tr — traces

(Bhalla *et al.*, 1970). However, none of the 33 samples of cabbage examined for parathion were found to be contaminated (Singh *et al.*, 1971).

Studies on the Dissipation of Insecticides on Vegetable Crops

Dissipation studies are very important from the point of view of defining waiting periods before consumption. Spraying frequently continues up to the harvest of the crop. The waiting period is governed by several factors like initial deposit, rate of dissipation, and the maximum residue limit. The initial deposit, in turn, is found to be determined by other factors like the dose of insecticide, size of fruits during spraying (Attri and Lal, 1974b, and Rajukkannu *et al.*, 1976). The rate of dissipation varies with crop variety and weather conditions.

Available data from investigations made on vegetable crops show that carbaryl, endosulfan, ethyl parathion, methyl parathion and parathion dissipate to the safety limits on almost all the vegetables within 3-10 days on an average (Table II). However, endrin cannot be recommended (Bhalla *et al.*, 1970 and Rajukkannu *et al.*, 1976) and brinjal and tomato (Rajukkannu *et al.*, 1976a) for it is prescribed on a 'zero tolerance' basis, which is not achieved even after a long interval of time.

Harvest time Residues

The concentration of insecticide residues present in vegetables at the time of harvest determines the dietary intake of the insecticide to a large degree (Table III).

Harvest time residue data are available for only 6 vegetables. Disulfoton and aldicarb applied at 0.5 kg ai/ha at the time of sowing of bhindi resulted in residues below the maximum residue limit at harvest time 55 days after sowing (Asaf Ali *et al.*, 1976 and Rajukkannu *et al.*, 1973 and 1977). Residues of phorate and disulfoton applied to the soil under brinjal at 2 kg ai/ha, were below the maximum residue limit between 3 and 4 weeks after application, while those of diazinon were below the maximum residue limit 10 days after application (Satpathy *et al.*, 1974). Carbaryl sprays on cauliflower 10 days before harvest resulted in residues above the maximum residue limit (Deshmukh and Saramma, 1971). However, lindane sprays 15 days before harvest, left residues below the maximum residue limit (Yadava and Sachan, 1973). On phorate, disulfoton, diazinon and dimethoate, residues on cowpea were below maximum residue level on pods and leaves 3 weeks after application. Dimethoate residues were below maximum residue limit 10 days after application. Residues 8.7 ppm, 11.47 ppm, and 1.15 ppm of phorate were found on mustard green 15, 50 and 90 days respectively after soil application (Dixit *et al.*, 1974). Aldicarb applied to the soil at 1.5 kg ai/ha left non-detectable residues on pea pods at harvest, 127 days after sowing (Dixit *et al.*, 1976).

TABLE
Dissipation of insecticides

Insecticide	Period of application	Age of crop (days)	Dose of ai/ha (g)	Formulation
1	2	3	4	5
<i>Bhindi</i>				
Carbaryl	—	—	900	WP
„	July-Sept 1972	60	1500 L/ha	„
„	May-June 1973	Early formation of fruits	1000 L/ha	„
„	July-Sept 1973	—	0.1%	EC
„	Aug-Nov 1974	3 weeks after germination	1000	Suspension
DDT	—	222	360	WP
„	—	20 DAS to 35	1750	„
„	—	20-51 DAS	2625	„
Endosulfan	July-Sept 1972	60	1500 L/ha	EC
„	July-Sept 1973	—	0.07%	EC
„	—	—	150	EC
„	—	20 DAS	250	EC
Endrin	1969	23-46	150-375	EC
„	July-Sept 1973	—	0.02%	EC
Fenitrothion	—	—	150	EC
Fenthion	—	—	100	EC
Gamma-BHC	—	—	360	WP
Malathion	March 1971	—	850 L/ha	EC
Monocrotophos	Nov. 1973	—	500	—
„	Sept. 1976	—	„	—
„	Nov. 1976	—	„	—
Parathion	July-Sept 1973	—	0.025%	EC
Phosphomidon	—	—	850 L/ha	—
„	—	—	225	EC
„	—	—	425	—
<i>Brinjal</i>				
Carbaryl	May-June 1966	110-180	2250-3375	Suspension
„	Sept-Nov 1966	„	„	„
„	Dec-Jan 1966-67	„	„	„

II
on vegetable crops

No. of appli- cations	Deposit (ppm)	Waiting period (days)	WHO/FAO maximum residue limit (ppm)	Method of analysis	Reference
6	7	8	9	10	11
1	1.5	—	10	C	Dewan <i>et al.</i> (1967)
1	17.53	1	„	C	Srivastava <i>et al</i> (1975)
—	14.40	2	„	C	Ragwaj <i>et al</i> (1973)
—	15.92	3	„	C	Rajukkannu <i>et at</i> (1976)
—	7.63	—	„	C	Mann and Chopra (1969)
1	8.9	3	7	C	Dewan <i>et al</i> (1967)
2	4.02	—	7	GLC	Jaswant Singh & Deshmukh (1974)
3	6.80	—	7	„	„ „ „
—	6.98	NR	2	C	Nath <i>et at</i> (1974)
—	2.74	3	„	TLC	Rajukkannu <i>et al</i> (1976)
—	0.16	—	„	C	Joia and Kalra (1974)
1	0.83 (one day)	—	„	GLC	Jaswant Singh and Deshmukh (1974)
2	3.22	NR	0	GLC	Bhalla <i>et al</i> (1970)
—	2.74	NR	0	TLC	Rajukkannu <i>et al</i> (1963)
1	0.14	—	0.2	GLC	Joia and Kalra (1974)
1	0.22	—	0.5	GLC	„ „
1	5.1	4	3	C	Dewan <i>et al</i> (1967)
—	2.58	2	3	C	Jat and Srivastava (1973)
—	0.23	4	0.2	C	Krishnaiah & Prasad (1978)
—	0.49	4	0.2	C	„ „ „
—	0.03	—	0.2	C	„ „ „
—	1.72	3	1	C	Rajukkannu <i>et al</i> (1976)
—	6.60*	After	0.5	B,C	Ramasubbaiah & Lal (1974)
—	6.64§	10 days	9 0.5	—	Vevai (1974)
—	—	12	0.5	—	„
—	65.5	15	10	B,C	Deshmukh and Lal (1963)
—	„	21	„	„	„ „ „
—	„	30	„	„	„ „ „

1	2	3	4	5
Carbaryl	Sept-Oct 1966	„	2250	Suspension
„	July-Sept 1973	—	0.1%	EC
DDT	—	Ready fruits	1.125	EC
Endosulfan	July-Sept 1973	—	0.07%	EC
Endrin	„	—	„	„
Gamma-BHC	—	Ready fruits	1,125	EC
Malathion	—	From 1 week after planting to fruiting	2,000	EC
Parathion	July-Sept 1973	—	0.025%	EC
<i>Cabbage (W-Wrapper leaves ; H-Heart)</i>				
Carbaryl	—	60	1250	WP
„	—	„	2500	„
„	Sept-Nov 1965	—	1250	„
„	—	—	2500	„
Dimethoate	—	60	1050	EC
„	1968-69	—	900	„
Malathion	—	30	600	EC
„	Winter crop 1968	116	600	EC
	„	„	600	WP
	„	„	600	dust
„	Spacing crop 1968	„	600	EC
	„	„	„	WP
	„	„	„	dust
Monocrotophos		10 days after planting	0.10%	EC
Parathion	1968	114-135	43-93	EC
Quinalphos	July, 1975	10 days after planting	0.05%	EC
	„	„	0.1	„
<i>Cauliflower (C-Curds, L-Leaves)</i>				
Carbaryl	—	90	2125	WP
Endosulfan	Oct 1972	27 days after transplanting	0.05%	EC
„	—	90	1100 L/ha	EC
Endrin	Jan-Feb 1970	93	1700	EC

6	7	8	9	10	11
—	40,82*,39,59§	13	16	B,C	Deshmukh & Lal (1970)
—	18.20	3	„	C	Rajukkannu <i>et al</i> (1976)
—	3.9±0.24	—	7	C	Dewan and Beri (1965)
—	3.50	3	2	TLC	Rajukkannu <i>et al</i> (1976)
—	3.00	NR	0	TLC	„ „ „
—	4.0±0.18	4	3	C	Dewan and Beri (1965)
—	4.00	3	3	TLC	Lakshminarayana & Menon (1973)
—	1.60	3	1	C	Rajukkannu <i>et al</i> (1976)
—	37.2	5	10	B	Kaushik and Gupta (1973)
—	63.2	10	„	B	„ „ „
—	23.8	3	„	C	Majumdar (1973)
—	33.4	5	„	C	„ „
—	15.4	10	2	B	Kaushik and Gupta (1973)
—	18.2 (W)	14 (W)	2	B,C	Krishnaiah and Lal (1973)
—	9.7 (H)	10 (H)	2		
5	9.49	1	8	GLC	Deshmukh & Saramma(1973)
—	79.922*; 79.04§	9	8	B,C	Hamaad and Lal (1971)
—	79.73*; 72.80§	12	8	B,C	„ „
—	59.52*; 59.84§	5	8	B,C	„ „
—	67.95*; 70.16§	7	8	B,C	„ „
—	67.15*; 66.08§	7	8	B,C	„ „
—	55.672*; 53.36§	7	8	B,C	„ „
—	1.570	9	0.2	C	Prasada Rao (1976)
6	1.1–1.7	1	1	GLC	Jaswant Singh (1971)
—	0.97	—	NA	C	Prasada Rao (1976)
—	1.40	—	„	C	„ „
—	36.5 (C)	22	5	C	Dewan <i>et al</i> (1973)
—	58.5 (L)	22	5	C	„ „
2	13.652 (L)	10	2.0	Spectro photometric	Kavadia <i>et al</i> (1974)
2	16.40 (L)	8	„	B	Verma and Lal (1976)
—	10.00 (C)	4	„	B	
—	12.3	15	0	B	Yadava and Sachan (1973)

1	2	3	4	5
Ethyl parathion	Jan-Feb 1971	15 days before harvest	325	EC
"	"	"	520	"
"	—	Bearing Stage	325	"
Fenitrothion	—	—	550	EC
Lindane	Jan-Feb 1970	93	850	EC
Malathion	Dec 67-March 68	—	424	EC
"	"	—	720	EC
"	"	—	960	"
"	Jan-Feb 1970	93	340	"
Methyl parathion	Jan-Feb 1971	15 days before harvest	320	EC
"	"	"	520	"
"	—	Bearing Stage	325	"
Monocrotophos	Sept 1975	10 days after planting	0.05% 0.1%	EC EC
Quinalphos	July 1975	10 days after planting	0.05% 0.1%	EC "
<i>Chilli</i>				
Dimethoate	—	—	0.06%	—
Monocrotophos	Sept 1976	Fruiting stage	0.08%	—
<i>Knol-Khol (Outer Portion)</i>				
Malathion	Spring Crop	116	600	EC
"	"	"	"	WP
"	"	"	"	dust
"	Winter crop	"	"	EC
"	"	"	"	WP
"	"	"	"	dust
Monocrotophos	Sept 1975	10 days after planting	0.05% 0.1%	EC "
Quinalphos	July 1975	10 days after planting	0.05% 0.1%	EC "
<i>Mustard Greens</i>				
Lindane	Jan 1965	—	1,123	EC
Malathion	—	90	725	—

6	7	8	9	10	11
1	4.28*; 5.29§ (C)	6	1	B,C	Attri and Lal (1974)
	10.80*; 11.97§(L)	9	1	„	„ „
	11.67*; 12.00§(C)	8	1	„	„ „
	21.77*; 22.60§(L)	11	1	„	„ „
1	4.99	6	1	C	Attri and Lal (1974)
1	4.68*; 4.93§ (C)	—	0.3	B,C	Attri (1977)
	9.55*; 8.89§ (L)	—	0.3	„	„ „
—	11.6	7	3	B	Yadava and Sachan (1973)
1	18.0	7	3	B	Gupta and Kapoor (1972)
1	26.2	9	3	B	„ „
1	30.8	10	3	B	„ „
—	22.9	7	3	B	Yadava and Sachan (1973)
1	5.41*; 5.82§(C)	4	1	B,C	Attri and Lal (1974)
	10.37*; 12.85§ (L)	7	1	„	„ „
1	12.18*; 11.08§ (C)	7	1	„	„ „
	19.81*; 19.57§ (L)	8	1	„	„ „
1	5.01	4	1	C	Attri and Lal (1974)
—	1.63	10	0.2	C	Prasada Rao (1976)
	2.22	18	0.2	C	„ „
—	1.92	—	NA	C	„ „
	3.08	—	NA	C	„ „
Once in 10 days	7.89	—	2.0	C	Agnihotrudu & Mithyantha (1978)
1	11.79	5	0.2	C	Nath <i>et al</i> (1975)
1	20.163*; 18.34§	3	8	B,C	Hamaad and Lal (1971)
1	18.601*; 17.83§	3	8	„	„ „
1	15.278*; 14.96§	3	8	„	„ „
1	21.357*; 21.21§	3	8	„	„ „
1	19.749*; 19.46§	4	8	„	„ „
1	19.862*; 19.72§	1	8	„	„ „
—	1.49	6	0.2	C	Prasad Rao (1976)
—	1.89	14	0.2	C	„ „
—	0.63	—	NA	C	„ „
—	1.33	—	NA	C	„ „
1	11.6–13.6	7	3	B,C	Kavadia and Lal (1967)
—	3.61	—	8	C, TLC	Dixit <i>et al</i> (1974)

1	2	3	4	5
Malathion	Oct 1976	117	310	EC
Parathion	Feb 1965	—	560	..
<i>Peas (P - Pods)</i>				
Endosulfan	—	Pod formation	350	EC
Malathion	Jan-Feb 1972	—	560	EC
	..	—	1,120	EC
	..	—	..	WP
<i>Ridge Gourd</i>				
Carbaryl	July 1974	30-45	750 and 1000	dust and WP
<i>Spinach</i>				
BHC	Aug 1974	—	5,380	WP
DDT	..	..	..	..
<i>Tomato</i>				
Carbaryl	Jan 1970	Flowering stage	800	WP
..	July-Sept 1973	—	0.1%	EC
DDT	Jan 1970	Flowering stage	800	WP
Endosulfan	..	..	400	EC
..	July-Sept 1973	..	0.07%	EC
Endrin	..	—	0.02%	..
Malathion	April 1962	—	560	EC
Malathion	—	One week after planting	2000	EC
Parathion	April 1962	—	560	EC
..	July-Sept 1963	—	0.025%	..

In Column 2 : DAS = days after sowing.

In Column 7 : 1 = by bioassay ; 2 = by chemical assay.

In Column 8 : NA = Not available ; NR = Not recommended.

Effect of processing on removal of insecticide residues : The effects of washing and cooking and dehydration on the removal of insecticide residues from vegetables have been investigated. Washing bhindi fruits with water reduced residues of endosulfan by 34.67 per cent (Nath *et al.* 1974), malathion by 89.15 per cent (Nath *et al.* 1975) and carbaryl by 66.12 per cent (Deshmukh and Lal, 1969 and Nath *et al.*, 1975). A reduction of 95.1 per cent in the initial deposits of carbaryl on brinjal occurred on washing (Deshmukh and Lal, 1969). Initial deposits of endosulfan, ethyl parathion

6	7	8	9	10	11
5	7.15	1	1	GLC, TLC-EI	Kalra and Kalra (1976)
1	27.2	14	8	B	Kavadia and Lal (1968)
1	10.72	—	2.0	C	Satpathy <i>et al</i> (1974)
—	3.2 (P)	1	3	B	Pritam Singh & Lal (1967)
—	6.8–7.4 (P)	2	3	B	„ „
—	7.3 (P)	2	3	B	„ „
3	27.725	6	3	Spectro photometer & equipment	Kavadia <i>et al</i> (1975)
—	44.4	NA	NA	C	Jaglan and Chopra (1971)
2	60	7	7	C	„ „
5	5.4	1	5	C	Deshmukh <i>et al</i> (1972)
—	17.32	3	5	C	Rajukkannu <i>et al</i> (1976)
5	2.7	—	7	GLC	Deshmukh <i>et al</i> (1972)
5	1.4	—	2	„	„ „
—	4.35	3	2	TLC	Rajukkannu <i>et al</i> (1976)
—	2.25	NR	0	„	„ „
—	2.7–3.0	—	3	B	Pritam Singh & Lal (1966)
—	8.0	4	3	TLC	Lakshminarayana & Menon (1973)
—	2.1	3	1	B	Pritam Singh & Lal (1967)
—	1.90	3	1	C	Rajukkannu <i>et al</i> (1976)

In Column 10 : B = bioassay ; C=colorimetric ; GLC=Gas liquid chromatography ; TLC=thin layer chromatography
TLC—EI=thin layer chromatography enzyme inhibition technique.

and methyl parathion on cauliflower curbs were reduced by 38.67 per cent (Awasti *et al.*, 1974) and 42.3 per cent (Verma and Lal, 1976), 42.01 per cent and 44.31 per cent respectively (Attri and Lal, 1974b). Malathion deposits on mustard greens were reduced by 76.4 per cent on washing (Dixit *et al.*, 1974). Deposits of ethyl parathion and methyl parathion on cowpea were reduced by 47.15 per cent and 42.7 per cent respectively (Attri and Lal, 1974b) and malathion deposits were completely removed on washing (Dewan *et al.*, 1969). One day old residues of carbaryl,

TABLE III
Harvest time residues of insecticides

Insecticide	1	2	3	4	5	6	7	8	9
	Dose of ai/ha (kg)	Formulation & method of application	No. of application/ age of birth	Interval between application & harvest (days)	Harvest-time residues (ppm)	Maximum residue limit (ppm)	Method of analysis	Reference	
<i>Bhendi</i>									
Aldicarb	0.5	Granules applied at sowing	1	50	N.D.*, 0.120§	0.1	B,C	Asaf Ali <i>et al</i> (1976)	
„	0.5	„	1	55	N.D.*, 0.075§	0.1	B,C	„	
„	1.0	„	1	50	0.17*; 0.165§	0.1	B,C	„	
„	1.0	„	1	55	N.D.*, 0.09§	0.1	B,C	„	
„	0.5	„	1	35-39	N.D.	0.1	C	Navaneethan <i>et al</i> (1973)	
„	1.0	„	1	35-39	0.18	0.1	C	„	
„	0.5	10% granules-soil application	1	50	0.12	0.1	C	Rajukkannu <i>et al</i> (1977)	
„	0.5	„	1	55	0.08	0.1	C	„	
„	1.0	„	1	50	0.17	0.1	C	„	
„	1.0	„	1	65	0.13	0.1	C	„	
Disulfoton	0.5	Granules in furrow application at the time of sowing	1	50	0.63*; 0.57§	0.75	B,C	Rajukkannu <i>et al</i> (1973)	
Disulfoton	0.5	„	1	55	0.52*; 0.325§	0.75	B,C	„	
„	1.0	„	1	50	0.85*; 0.76. §	0.75	B,C	„	
„	1.0	„	1	55	0.67; 0.537§	0.75	B,C	„	

1	2	3	4	5	6	7	8	9
Diculfoton	1.0	"	1	35-39	0.75	0.75	C	Navaneethan <i>et al</i> (1973)
"	1.5	"	1	35-39	1.92	0.75	C	"
Parathion	0.45-0.675	Emulsion spray	1; (On appearance of fruits)	5	0.65-2.0	1	C	Manda <i>et al</i> (1969)
<i>Brinjal</i>								
Carbofuran	0.65	3% granules around base of plants	1	7	0.088	0.2	C	Agnihotru and Mithyantha (1978)
				21	0.055	0.2	C	
Diazinon	2	Granules soil application	2; 1 & 4 weeks after transplanting of seedlings	51	0.020	0.2	C	Satpathy <i>et al</i> (1974)
				10	0.578	0.75	C	
				20	0.395	0.75	C	
Disulfoton	2	"	"	30	0.025	0.75	C	
				10	1.288	0.75	C	
				20	1.876	0.75	C	"
				30	0.624	0.75	C	"
Phorate	2	Granules soil application	2; 1 & 4 weeks after transplantation of seedlings	10	1.270	1	C	
				20	1.013	1	C	"
				30	0.692	1	C	"
<i>Cauliflower (C - Curds, L - Leaves)</i>								
Carbaryl	2.0	Suspension spray	2	10	3.0-10.0	5	C	Deshmukh and Saramma (1971)
Endosulfan	0.5	Emulsion spray	2	0	13.652 (L)	2.0	Spectro-photometric	Kavadia <i>et al</i> (1974)
					5.705 (C)			
<i>Cowpea (L - Leaves, P - Pods)</i>								
Aldrin	10	5% dust	Soil application in furrows before sowing	20	0.27	—	GLC	Agnihotri <i>et al</i> (1974a)
				40	0.37			
					at harvest 0.125			

1	2	3	4	5	6	7	8	9
Diazinon	2	Granules in soil	1st application 2 days before seed was sown 2nd application just before initiation of flowering	10	1.65 (L) 1.212 (P) 1.012 (L) 0.525 (L) 0.275 (L) 0.200 (P)	0.75 0.75 0.75	C	Satpathy <i>et al</i> / (1974)
Dimethoate	2	Granules in soil	1st application 2 days before the seed was sown 2nd application just before initiation of flowering	10 20 30	1.437 (L) 1.275 (P) 0.920 (L) 0.712 (P) 0.575 (L) 0.300 (P)	2 2 2 2 2	C C C C	Satpathy <i>et al</i> / (1974)
Disulfoton	2	Granules in soil	„	10 20 30	1.100 (L) 1.035 (P) 0.912 (L) 0.787 (P) 0.425 (L) 0.350 (P)	1 1 1 1 1 1	C C C C C C	Satpathy <i>et al</i> / (1974)
Phorate	2	Granules in soil	„	10 20 30	1.462 (L) 1.463 (P) 1.087 (L) 1.050 (P) 0.558 (L) 0.525 (P)	1 1 1 1 1 1	C C C C C C	Satpathy <i>et al</i> / (1974)

1	2	3	4	5	6	7	8	9
<i>Mustard Greens</i>								
Phorate	1.15	10% granules furrow g/m application	1	15 50 90	8.77 11.47 1.15	NA NA NA	C C C	Dixit <i>et al</i> (1976)
<i>Peas</i>								
Aldicarb	1.5	Granules at the time of sowing	1	127	N.D.	0.2	C	Dixit <i>et al</i> (1976)
Disulfoton	1.5	Soil application of 5% granules at the time of sowing	1	15 90	2.48 N.D.	6.75 0.75	C C	Pandey <i>et al</i> (1975)

In Column 6 : 1-by bioassay ; 2-by chemical assay.
 In Column 7 : NA-not available ; N.D.-not detectable.
 In Column 8 : B-bioassay ; C-colorimetric assay.

endrin and parathion on tomato were reduced by 50, 11 and 11 per cent respectively on washing (Rajukkannu, 1976). Endrin deposits on bhindi were reduced by 60.21 per cent by the combined processes of washing, cooking and dehydration (Bhalla *et al.*, 1970). The percentage reduction of endosulfan, malathion and carbaryl (Nath *et al.*, 1974 and 1975) by dehydration was 57.59, 91.86 and 79.24 respectively. Jaswant Singh *et al.*, (1971) found that parathion deposits on cabbage were reduced by 83.8 per cent by a combination of rubbing, washing and cooking. One hundred per cent reduction of malathion deposits on cowpea was observed on cooking (Dewan *et al.*, 1969). Reductions of 77 per cent, 96 per cent and 100 per cent in phorate residues on cooking mustard greens occurred 15, 25 and 90 days after application (Dixit *et al.*, 1974). 64 per cent, 22.22 per cent and 37.0 per cent reduction of one-day old residues of carbaryl, endrin and parathion occurred respectively on cooking of tomatoes (Rajukkannu *et al.*, 1976) (Table IV).

Residue Analysis work done on Vegetables at the Indian Institute of Horticultural Research, Bangalore.

The project entitled 'Analysis of insecticide residues on fruits and vegetables,' sponsored by the Department of Science and Technology, was started at the Indian Institute of Horticultural Research, Bangalore in 1976. Studies have so far been carried out on the dissipation of carbaryl residues in/on brinjal and peas, monocrotophos in okra and capsicum (sweet pepper) and carbofuran in peas.

1) *Carbaryl Residues on brinjal*: Carbaryl at 2 and 2 kg ai/ha was sprayed on brinjal variety 'Pusa Purple Cluster'. Marketable fruits were harvested at '0' (i.e., half an hour after spraying) 1, 4 and 7 days after spraying and residue estimations were made according to the method of A.O.A.C. (P.A.I., 1977). Effect of washing with water on removal of residues was also studied. Results indicate that it is safe to harvest brinjal after 1 day of carbaryl spray. Carbaryl residues dissipated with 1.9 – 2.3 days half-life.

2) *Carbaryl residues on peas*: 0.2 per cent carbaryl suspension was sprayed on peas variety 'Bonneville'. Estimations on whole pods and seeds were made half an hour (0 day), 3, 5 and 8 days after spraying using the method of A.O.A.C. (1965). The extent of deposition of carbaryl on whole pods was 4.85 ppm and residues on the 3rd, 5th and 8th day after spraying were 2.38, 0.2 and 0.1 ppm respectively. Carbaryl residues in seeds exceeded 0.1 ppm at any time up to 8 days following its spray.

3) *Monocrotophos residues in okra variety 'Pusa Sawani'*: Monocrotophos supplied as Nuvacron was used at 0.5 kg ai/ha. Samples collected after half an hour ('0' day), 1, 4, 7 and 10 days of spraying were analysed by the cholinesterase

TABLE IV

Effect of processing on removal of insecticide residues

Insecticide	Days after spraying	Concentration (%)	Average deposits/residues (ppm)	Per cent reduction of insecticide due to washing	Per cent reduction of insecticide due to cooking	Per cent reduction of insecticide due to dehydration	Reference
1	2	3	4	5	6	7	8
<i>Bhindi</i>							
Endosulfan	0	0.1	6.98	34.67	26.22 (O.C.) 36.93 (S.C.)	57.59	Nath <i>et al</i> (1974 and 1975)
Endrin	0	0.25	2.89	total per cent reduction :		60.21	Bhalla <i>et al</i> (1970)
Malathion	0	0.05	2.58	89.15	86.82 (O.C.) 75.97 (S.C.)	91.86	Nath <i>et al</i> (1974 and 1975)
<i>139</i>							
Carbaryl	0	0.25	17.53	66.12	41.81 (O.C.) 81.75 (S.C.)	79.24	"
Malathion	0	0.05	2.58	89.15	86.82 (O.C.) 75.90 (S.C.)	91.86	Jat and Srivastava (1973)
<i>Brinjal</i>							
Carbaryl	0	0.25	65.49	95.1	—	—	Deshmukh and Lal (1969)
"	5	0.25	44.38	86.9	—	—	"
<i>Cabbage</i>							
Parathion	0	0.025	1.149	Total reduction due to washing, rubbing and cooking = 83.8%		—	Singh <i>et al</i> (1971)
<i>Cauliflower (C = curds, L = leaves)</i>							
Endosulfan	0	0.05	11.25	38.67	50.7	—	Awasti <i>et al</i> (1974)
		0.10	18.60	43.50	65.3	—	"

1	2	3	4	5	6	7	8
„	0	0.05	10.4 (L) 10.0 (C)	42.3 (L) 53.8 (C)	— 65.0 (C)	—	Verma and Lal (1976) „ Aftri and Lal (1974b)
Ethyl parathion	0	0.325 kg/ha	4.99	42.01	55.91	—	
Methyl parathion	0	„	5.01	44.31	58.28	—	„
Ethyl parathion	0	0.325 kg/ha	1.93	47.15	65.80	—	„
Malathion	0	0.03	2.04	100	—	—	„
Mythyl parathion	0	0.325 kg/ha	1.85	42.70	69.72	—	„
Malathion	0	0.05	3.61	76.4	—	—	Dixit <i>et al</i> (1974)
Phorate	15	1.25 g/m	8.77	45.0	77.0	—	„
„	25	„	10.12	67.5	96.0	—	„
„	90	„	1.15	100	100	—	„
Carbaryl	1	0.1	17.32	50.00	64.00	—	Rajukkannu <i>et al</i> (1976)
Endrin	1	0.02	2.25	11.00	22.22	—	„
Parathion	1	0.025	1.90	11.00	37.00	—	„

In column 6 : O.C.—Open cooking, S.C.—Steam cooking.

inhibition method of Lal (1966) and Colorimetric determination of Ellman *et al* (1961). A four day gap between spraying of monocrotophos and harvesting okra fruits appeared to be safe. Washing the fruits harvested half an hour, 2 and 3 days after spraying removed 61, 50 and 25 per cent monocrotophos residues respectively. Residues level in '0' day and 1 day samples were less than 0.2 ppm.

4) *Monocrotophos residues in Capsicum (sweet pepper)* : Fruits of the sweet pepper variety 'California Wonder' were sprayed with 0.05 per cent monocrotophos samples collected 0, 1, 4, 6, 10 and 14 days after spraying were analysed by the same method as for (Ellman *et al* (1961) and Lal, (1966). An eleven day gap between spraying and harvesting capsicum fruits was safe. Washing did not reduce the residue levels. Rates of dissipation of monocrotophos were quicker for okra than capsicum. This may be attributed to the differences in growth pattern of both.

5) *Carbofuran residues in peas* : Carbofuran granules as applied to the soil at 1.0 kg/ha at the time of sowing peas of the variety 'Sonneville'. Analysis was by the colorimetric method of Gupta and Dewan (1973). Green pods were harvested after 65 days and analysed. An average value of 0.28 ppm was found in pea seeds, so granular application of carbofuran in peas appears to be safe.

Current research : Studies on the dissipation of endosulfan in okra, endosulfan, carboaryl, carbofuran and quinalphos on tomato and endosulfan, carbofuran and quinalphos on brinjal and cabbage are under way.

CONCLUSION

Much work remains to be done in India on residues in vegetables. The monitoring of vegetables for insecticide residues is rare. The few analyses done show gross contamination of vegetables by insecticides. Hence, regular monitoring of vegetables for these residues should be carried out.

Standard procedures of sampling and analysis must be developed to ensure uniformity in results. Studies on the dissipation of any pesticide should be made in different parts of the country the metabolites which, in some cases, are more harmful than the parent compound, should be studied. The nature and extent of the interaction between the different insecticides on crops need to be studied because interactions might modify the degradation and persistence of an insecticide.

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17 RESIDUES OF CHLORINATED HYDROCARBONS IN MARKET VEGETABLES AT HYDERABAD

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SUMMARY

Samples of vegetables collected from different markets at Hyderabad were analysed by a thin layer chromatography for residues of organochlorines. Of 350 samples tested, 145 samples (41.4 per cent) contained detectable levels of insecticide residues, among which 20 samples (5.7 per cent) exceeded the tolerance limits prescribed by United States Food and Drug Administration. BHC was detected in 52 samples and DDT in 65 samples. Endrin, endosulfan and lindane were detected in 8, 6 and 14 samples respectively.

Thirty-two of 75 bhindi samples contained insecticide residues and six exceeded the tolerance limits. BHC and DDT were detected in 14 and 12 samples respectively, lindane three times, endosulfan once and endrin twice.

Of 70 brinjal samples, 27 contained insecticide residues with three above tolerance. BHC and DDT were detected 14 and 11 times respectively, endrin and endosulfan once each.

Of 80 tomato samples analysed, 42 contained residues and five exceeded the tolerance limit. DDT, BHC and lindane were detected in 21, 11 and 6 samples respectively and endrin and endosulfan in 2.

Of 75 potato samples, 6 contained residues above tolerance and 30 below. BHC was detected in 10 samples and DDT in 16. Endrin, endosulfan and lindane were recorded in 3, 2 and 5 samples respectively.

None of the coccinia samples contained residues above tolerance limits although 8 of 50 contained residues of BHC or DDT.

It is an accepted practice in modern agriculture to use pesticides for producing good quality vegetables. High yielding varieties which are susceptible to pest damage necessitate adoption of plant protection measures to control pests and when a chemical is used it usually is deposited on plant produce. Residues of organo-chlorine insecticides persist for a long time and may pose a health hazard to the consumer. Information about the existence of insecticide residues on food products will help extension staff to advise cultivators in the proper usage of chemicals.

MATERIAL AND METHODS

Samples of bhendi, brinjal, tomato, coccinia and potato were collected from different markets of Hyderabad-Secunderabad twin cities and analysed for residues of chlorinated hydrocarbons. A chemical assay by thin layer chromatography was used to detect residues.

Extraction : A simple and single step extraction method was adopted (Laxminarayana and Menon (1975b). Five hundred grams of vegetables were grated and/or cut to small pieces, out of which 25 g were weighed and transferred to 250 ml glass stoppered conical flask. These were extracted by shaking with 100 ml of 3 : 1 hexane acetone mixture for 1 hr on a shaker. The contents were filtered into a stoppered bottle through a cotton plug containing anhydrous sodium sulphate.

Preparation of TLC plates : Glass plates 20 × 20 or 20 × 10 cm of uniform thickness were used. To coat 5 plates of 20 × 20 cm size with a 250 micron layer, 20 ml of water containing 60 mg silver nitrate solution was added to 20 mg silica gel G prior to coating of plates. After coating, the plates were air dried and later on activated in a hot air oven at 110°C for 10 min. and kept ready for further use. A standard solution was prepared – a small quantity (10-15 mg) of pure insecticide was weighed and an equal quantity of ethyl acetate was added to dissolve it in a glass-stoppered test tube. One microlitre of this solution contained 1 microgram of the insecticide. Other solutions were also prepared similarly and stored in a refrigerator. For spotting development and identification, an aliquot of 4 ml of the sample equivalent to 1 g vegetable was pipetted into a 10 ml beaker and evaporated to near dryness. Standards and sample spots were applied by a 10 µl microsyringe taking care to see that the diameter of the spot was less than 5 mm. Samples were transferred quantitatively to the plate by rinsing three times using 0.2 ml hexane. The plates were developed in cyclohexane solvent. After development the plate was exposed to steam and UV light of short wave length. Insecticides appeared as brownish-black spots against a white background. Samples were identified by comparing the RF values with those of the standards. Quantitative estimation was

made by visual comparison of the area and intensity of the spot in the sample and the standard.

RESULTS AND DISCUSSION

A total of 350 vegetable samples were analysed (Table I) to determine the residues of organochlorines. Forty one point four per cent samples contained detectable residues and 5.7 per cent samples exceeded the tolerance level prescribed by the United States Food and Drug Administration.

Bhendi : Of 75 samples analysed, 32 (42.6 per cent) contained residues of BHC, DDT, endosulfan, endrin and lindane. Residues in eight per cent of the

TABLE I
Residues of chlorinated hydrocarbons detected in vegetables

Vegetable	Total samples analysed	No. of samples		No. of occasions various insecticides detected				
		With detectable residues	With residues above tolerance level	DDT	BHC	Endrin	Endosulfan	Lindane
Bhendi	75	32	6	12	14	2	1	3
Brinjal	70	27	3	11	14	1	1	—
Coccinia	50	8	—	5	3	—	—	—
Potato	75	36	6	16	10	3	2	5
Tomato	80	42	5	21	11	2	2	6

samples exceeded the tolerance level. BHC and DDT were detected in 14 and 12 samples respectively. Lindane was detected three times, endosulfan once and endrin twice.

Brinjal : Of the 70 samples analysed, 27 contained organochlorines, BHC and DDT occurred in 14 and 11 samples, endrin and endosulfan once each. Only in three samples did insecticide residues exceed the tolerance limits. The smooth surface of the fruit and the thick foliage might have prevented the insecticide from reaching the fruits. The occurrence of BHC and DDT residues on brinjal has been reported earlier (Dewan and Beri, 1965 ; Jaglan and Chopra, 1970 ; Laxminarayana and Menon, 1975).

Coccinia : Of 50 samples none contained residues above the tolerance limits, although eight contained residues of BHC and DDT. The value of this crop probably restricts the farmer from using insecticides on it.

Potato : Six of 75 samples analysed contained residues above the tolerance limit. In 10 samples BHC was detected and DDT in 16 samples. Residues of BHC on potato tubers have been detected by Greenwood and Tice (1949).

Tomato : Of 80 samples analysed, 41 samples contained residues which in seven samples exceeded the tolerance limit. DDT and BHC were detected in 21 and 11 samples respectively, while endrin and endosulfan occurred in two samples and lindane in six. These results agree with those of Laxminarayana and Menon (1975).

From Table I it is clear that out of the 145 samples that contained pesticides BHC occurred in 52 samples (35.9 per cent), DDT in 65. Hence BHC and DDT are popular with the farmers for insect control on vegetables because they are cheap and protect plants longer. Endrin, endosulfan and lindane were detected in 8.6 and 14 samples respectively out of a total of 145.

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INSECTICIDE RESIDUES IN VEGETABLES FOLLOWING EFFECTIVE CHEMICAL CONTROL SCHEDULES

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SUMMARY

Review of literature on the subject reveals that in most cases the initial deposit of the insecticides at the time of application is generally always higher than the tolerance limit fixed for them. The time taken by the deposit to return to a safe level depends upon the insecticide, crop, weather conditions, etc., emphasizing the need to establish separate waiting periods in different regions of the country differing widely in agro-climatic conditions.

Taking into consideration practical conditions, the following insecticide applications may be reckoned as hazardous: 1) Cabbage: carbaryl and phosphamidon; 2) Cauliflower: endosulfan and fenitrothion; 3) Bhindi: DDT, lindane and phosphamidon as well as granules of phorate and disulfoton; 4) Tomato: BHC and phenthoate; 5) Brinjal: lindane, phorate, disulfoton and diazinon; 6) Spinach: DDT and BHC; 7) Mustard (sag): BHC, phorate and disulfoton; 8) Potato: Aldrin; 9) Cowpea: dimethoate, monocrotophos, phosphamidon and disulfoton granules.

The use of pesticides on agricultural crops is essential in modern agricultural practices. The protection of vegetables from pests poses special problems because any insecticidal treatment may cause residual hazards because there is often little time between treatment and harvest. Screening of the recommended chemical control schedules for safety to consumer is essential.

The literature on this subject has been reviewed by Bindra and Kalra (1971) and Agnihothrudu and Mithyantha (1978). This paper is largely a summary from these reviews with some addition of more recent references.

A summary is presented in Table I which shows that in most instances the initial deposit is higher than the tolerance limit fixed for the insecticide. The time taken by the deposit to dissipate to an acceptable tolerance level depends on a variety of factors such as the insecticide, crop, weather conditions, insecticide formulation, dose applied, soil type and agricultural practices followed. Wide variations in residue data are observed due to different methods of estimation. Colorimetric methods seem to give inflated residues because they estimate all non-toxic metabolites, as compared to bioassay which determines only toxic residues. On the other hand, bioassay has the limitation that it fails to identify different compounds. Their uses as complementary methods is recommended. Ultra low volume sprays

TABLE I

Residues of various insecticides on vegetable crops

Insecticide	Locality	Dose (Kg a.i./ha)	Formulation	No. of applications	Deposit (ppm)	WHO/FAO tolerance limit (ppm)	Waiting period (days)	Method of analysis	Reference
1	2	3	4	5	6	7	8	9	10
<i>Bhindi</i> (<i>Abelmoschus esculentus</i>)									
Aldicarb	Coimbatore	0.5	Gr	1 (AST)	BDL	0.1	Safe	C	Navneethan <i>et al</i> (1973)
		1.0	Gr	1 (AST)	0.3	0.1	NR	C	"
BHC	Delhi	1.5	WP	1	4.6	NA	NR	GLC	Sarode (1978)
		3.0	"	1	6.9	NA	NR	"	"
Carbaryl	Ludhiana	1.25	"	3	6.7	10	0	C	Deshmukh and Udean (1971)
			"	4	6.4	10	0	C	"
	Delhi	0.90	"	1	1.5	10	0	C	Dewan <i>et al</i> (1967)
	Coimbatore	1.00	"	1	12.1	10	0	C	Raghuraj <i>et al</i> (1973)
	Bangalore	1.00	"	1	7.6	10	0	C	Krishnaiah <i>et al</i> (1975)
		2.00	"	1	11.7	10	1	C	"
Carbofuran	Bangalore	Seed soaked in 10% suspension for 6 hrs.	"	1	0.10	0.2	0	C	Anon (1977)
DDT	Delhi	0.360	"	1	8.9	7	3	GLC	Dewan <i>et al</i> (1967)
	Ludhiana	0.875	"	2	4.0	7	0	"	Singh and Deshmukh (1974)
			"	4	4.8	7	0	"	"

1	2	3	4	5	6	7	8	9	10
Disulfoton	Coimbatore	0.5	Gr	1(AST)	0.6 (AFH)	1	NR	C	Rajukkannu <i>et al</i> (1973)
		0.5	Gr	1	0.9 (AFH)	1	NR	C	Navneethan <i>et al</i> (1973)
Endosulfan	Udaipur	1.5	EC	1	4.2(1)	2	NR	C	Nath <i>et al</i> (1974)
	Ludhiana	0.15	EC(ULV)	1	0.16	2	0	C	Joia and Kalra (1974)
		0.25	EC	1	0.83(1)	2	1	GLC	Singh and Deshmukh (1974)
Ethyl parathion	Coimbatore	0.20	EC	1	1.72(1)	1	3	C	Rajukkannu <i>et al</i> (1976)
Fenitrothion	Ludhiana	0.1	EC(ULV)	1	0.14	0.2	0	GLC	Joia and Kalra (1974)
Fenthion	"	0.1	"	1	0.22	0.5	0	GLC	" "
Lindane	Delhi	0.36	WP	1	5.1	3	4	C	Dewan <i>et al</i> (1967)
	Delhi	1.5	EC	1	5.64	3	2	GLC	Sarode (1978)
		3.0	EC	1	6.78	3	3	GLC	" "
Malathion	Ludhiana	0.25	EC(ULV)	1	0.22	3	0	GLC	Joia and Kalra (1974)
		0.50	EC	1	2.13	3	0	C	Dixit <i>et al</i> (1975)
		0.625	EC	1	5.7	3	1	C	Deshmukh and Singh (1975)
Phorate	Coimbatore	0.5	Gr.	1(AST)	0.2-0.5	0.1	NR	BGE	Navneethan <i>et al</i> (1973)
	Delhi	1.5	Gr.	"	1.38(AFH)	0.1	NR	B and Sarode	(1978)
								GLC	
Phosphamidon	Delhi	0.25	WSC	1	6.6	0.5	NR	C,B	Ramasubbaiah and Lal (1974)
Carbaryl	Ludhiana	0.70	WP	4	6.9	5	3	C	Deshmukh and Udean (1971)
		0.65	WP	1	14.8	5	5	C	Mann and Chopra (1969)
		1.25	WP	1	23.8	5	7	C	" "
DDT	Delhi	1.125	WP	4	3.9	7	0	C	Dewan and Beri (1965)

Brinjal

1	2	3	4	5	6	7	8	9	10
	Ludhiana	5.38	WP	1	10.2	7	3	C	Jaglan & Chopra (1975)
Disulfoton	Bhubneshwar	2.00	Gr.	2	0.58	0.1	NR	C	Satpathy <i>et al</i> (1974)
Endosulfan	Ludhiana	0.35	EC	4	0.4	2	0	C	Deshmukh and Udean (1971)
	Coimbatore	0.35	EC	1	2.74(1)	2	3	C	Rajukkannu <i>et al</i> (1976)
Lindane	Delhi	1.125	EC	1	4.00	3	4	C	Dewan and Beri (1965)
Malathion	Ludhiana	0.50	EC	4	3.4	3	1	C	Deshmukh and Udean (1971)
	Hyderabad	2.00	EC	1	4.5	3	3	TLC	Lakshminarayana and Menon (1974)
Phorate	Bhubneshwar	2.00	Gr.	2	1.27(AFH)	0.1	NR	C	Satpathy <i>et al</i> (1974)
<i>Cabbage</i>									
Carbaryl	Hissar	1.25	WP	1	37.2	5	10	B	Kaushik & Gupta (1973)
		2.50	WP	1	63.2	5	15	B	"
	Ludhiana	1.25	WP	1	14.8	5	8	C	Mann and Chopra(1969)
		2.50	WP	1	23.8	5	10	C	"
	Udaipur	1.8	WP	1	8.09	5	2	C	Sachan and Srivastava (1975)
Dimethoate	Hissar	0.25	EC	1	6.8	2	3	B	Kaushik & Gupta (1973)
		0.50	EC	1	13.0	2	7	B	"
	Delhi	0.42	EC	1	0.7	2	0	B,C	Krishnaiah & Lal (1973)
		0.56	EC	1	0.7	2	0	B,C	"
Fenitrothion	Delhi	0.50	EC	1	1.0	0.3	3	B,C	Attri (1978)
Malathion	Ludhiana	0.68	EC	5	9.5	8	1	GLC	Deshmukh and Bhalla (1973)
	Delhi	0.60	EC	1	79.0	8	7	C,B	Hameed and Lal (1971)

1	2	3	4	5	6	7	8	9	10
	Udaipur	1.20	EC	1	127.3	8	14	C,B	Hameed & Lal (1971)
		0.60	WP	1	72.7	8	14	C,B	"
		0.435	EC	1	11.06	8	3	C	Sachan and Srivastava (1975)
Methyl parathion	Delhi	0.250	EC	1	0.36	1	0	B,C	Attri and Lal (1972)
Parathion	Ludhiana	0.93	EC	6	1.1-1.7	1	1	GLC	Singh <i>et al</i> (1971)
Phosphamidon	Delhi	0.250	WSC	1	7.90	0.5	10	B,C	Ramasubbaiah and Lal (1978a)
		0.400	WSC	1	11.80	0.5	11	B,C	"
<i>Cauliflower</i>									
BHC	Delhi	1.5	WP	1	8.74	NA	ND(15)	GLC	Sarode (1978)
		3.0	WP	1	12.00	NA	ND(15)	GLC	"
Carbaryl	Delhi	1.06	WP	1	28.7	5	22	C	Dewan <i>et al</i> (1973)
		2.125	WP	1	36.5	5	22	C	"
Dimethoate	Delhi	0.42	EC	1	6.37	2	7	C,B	Krishnaiah & Lal (1973)
Endosulfan	Delhi	0.55	EC	1	11.25	0.5	23	C	Awasthi <i>et al</i> (1974)
		0.55	EC	1	10.00	0.5	4	B	Verma and Lal (1976)
Ethyl parathion	Delhi	0.325	EC	1	4.5	1.0	6	C,B	Attri and Lal (1974)
		0.520	EC	1	11.6	1.0	8	C,B	"
Fenitrothion	Delhi	0.55	EC	1	4.68	0.3	9.5	C,B	Attri (1977)
Lindane	Udaipur	0.85	WP	1	11.6	3	7	B	Yadav & Sachan (1973)
	Delhi	1.5	EC	1	9.5	3	5	GLC	Sarode (1978)
		3.0	EC	1	13.7	3	7	GLC	"
Malathion	Udaipur	0.34	EC	1	22.9	3	7	B	Yadav & Sachan (1973)
	Haryana	0.96	EC	1	33.0	3	10	B	Gupta & Kapoor (1972)
	Delhi	1.2	EC	1	28.2	3	7	C,B	Hameed & Lal (1971)

1	2	3	4	5	6	7	8	9	10
Methyl parathion	Delhi	0.325	EC	1	5.5	1	4.0	C,B	Attri & Lal (1974)
Phorate	Delhi	0.520	EC	1	11.5	1	7	C,B	"
		1.5	Gr.	1	0.78(50)	0.1	NR	C,B	Sarode (1978)
					<i>Cowpea</i>				
Aldicarb	Coimbatore	1.0	Gr.	1(AST)	0.09(35), ND(45)	0.2	0	C	Rajukkannu <i>et al</i> (1977)
BHC	Delhi	1.5	Dust	1(AST)	3.95(seeds)	3	NR	GLC	Agnihotri <i>et al</i> (1974)
Carbaryl	Delhi	1.56	WP	1	24.16	5.0	10	C	Awasthi <i>et al</i> (1977)
							(ND in seeds)		
Disulfoton	Coimbatore	3.12	WP	1	26.46	5.0	"	C	"
Ethyl parathion	Delhi	1.00	Gr.	1(AST)	ND in seeds	0.75	"	C	Rajukkannu <i>et al</i> (1977)
		0.350	EC	1	1.93	1	3	C,B	Attri & Lal (1974)
		0.520	EC	1	3.92	1	6	C,B	"
Malathion	Udaipur	0.500	EC	2	32.5	8	1.5	C	Fotedar <i>et al</i> (1976)
Methyl parathion	Delhi	0.350	EC	1	1.5	1	2	C,B	Attri & Lal (1974)
		0.520	EC	1	3.2	1	3	C,B	"
Monocrotophos	Delhi	0.375	EC	1	3.7	0.2	3	C	Awasthi <i>et al</i> (1977)
		0.625	EC	1	5.5	0.2	3	C	"
Phosphamidon	Delhi	0.260	WSC	1	7.7	0.2	NR	C,B	Ramasubbaiah & Lal (1975)
					<i>Peas</i>				
Aldicarb	Delhi	1.5	Gr.	1(AST)	0.4 (green pods) ND (seeds at harvest)	0.2	NR (Green pods)	C	Dixit <i>et al</i> (1976)
Disulfoton	Delhi	1.5	Gr.	1(AST)	ND (seeds at harvest)	0.75	—	C	Pandey <i>et al</i> (1975)

1	2	3	4	5	6	7	8	9	10
Indosulfan	Delhi	0.35	EC	1	3.2(7) 3.3(15)	2	NR	C	Pandey <i>et al</i> (1977)
Lindane	Delhi	0.50	EC	2	0.2(3) 4.3(7) 6.0(15) 0.112 (seeds at harvest)	3	NR	GLC	"
Malathion	Delhi	0.560 1.120	EC EC	1 1	3.2 7.0	3	1 2	B B	Pritam & Lal (1966) "
Aldrin Heptachlor Phorate	Delhi Simla Jullundur	1.25 1.50 1.50	Dust Dust Gr.	1(AST) 1 1	0.8(RHT) 1.6(RHT) 1.6(RHT)	0.1 0.5 0.5	NR NR —	B, GLC GLC C	Attari <i>et al</i> (1976) Misra <i>et al</i> (1977) Awasthi <i>et al</i> (1977)
ND (if boiled and peeled)									
<i>Sarson (leaves)</i>									
BHC	Delhi	5.9	WP	1	79.5	NA	NR	C, B	Kavadia & Lal (1967)
Dimethoate	Delhi	0.66	EC	1	8.5	2.00	8	B	Krishnaiah & Lal (1973)
(seeds ND)									
Disulfoton	Delhi	1.5	Gr.	1	Residues > T.L.	0.75	NR	C	Agnihotri <i>et al</i> (1975)
for 90 days (NR in seeds)									
	Delhi	1.5	Gr.	1	Res. > T.L.	0.75	NR	C	Jain <i>et al</i> (1974)
for 60 days ND after 75 days									
Lindane	Delhi	1.125	EC	1	12	3	7	C, B	Kavadia & Lal (1967)
Malathion	Delhi	0.700	EC	1	33-37	3	5	C, B	Kavadia & Lal (1968a)

1	2	3	4	5	6	7	8	9	10
Parathion	Delhi	0.560	EC	1	37.5	1	21	B	Kavardia & Lal (1968a)
Phorate	Delhi	1.5	Gr.	1	> T.L. for 60 days	0.5	NR if crop to be used (seeds ND)	C	Jain <i>et al</i> (1974) and Agnihotri <i>et al</i> (1975)
Phosphemidon	Jobner	0.200	WSC	1	0.81	0.5	8	CHE	Yadav & Gupta (1975)
	Delhi	0.250	WSC	1	5.6	0.5	10	C, B	Ramasubbaiah & Lal (1978a)
BHC	Ludhiana	5.38	WP	1	44.4	NA	—	C	Jaglan & Chopra (1971)
DDT	..	5.38	WP	2	60.0	7	NR	C	..
				<i>Sweet Potato</i>					
Carbofuran	Coimbatore	1.00	Gr.	1	0.1(RHT)	0.2	—	4	Rajukkannu <i>et al</i> (1976)
				<i>Tomato</i>					
Carbaryl	Ludhiana	0.800	WP	5	5.4	5	1	4	..
Dimethoate	Delhi	0.44	EC	1	3.9	2	7	B, C	Krishnaiah & Lal (1973)
DDT	Ludhiana	0.80	WP	5	2.7	7	0	GLC	Deshmukh <i>et al</i> (1972)
Endosulfan	..	0.40	EC	5	1.4	2	0	GLC	..
	Coimbatore	0.07	EC	NA	4.3(1)2.1(2)	2	3	C	Rajukkannu <i>et al</i> (1976)
Malathion	Hyderabad	2.00	EC	1	8.9	8	3	TLC	Lakshminarayana & Menon (1974)
Ethyl parathion	Coimbatore	1.00	EC	NA	1.9(1)	1	3	C	Rajukkannu <i>et al</i> (1976)
	Delhi	0.28	EC	1	1.6	1	3	B	Singh & Lal (1967a)
Phenthoate	Delhi	0.5	EC	1	2.6	NA	NR	C, E	Attri <i>et al</i> (1978)

AST—at sowing time, NR—not recommended, NA—not available, AFH—at first harvest, C—colorimetric, B—bioassay, E—enzymatic, ND—not detectable, RHT—Residue at harvest time, T.L.—tolerance limit, Figures in brackets in column 6 & 8 indicate days.

formulations seems to reduce residue hazards considerably because the initial, is usually less than the tolerance limit. Many soil insecticides can occur at hazardous levels in the first harvest of vegetables.

Insecticide applications given in Table II may be reckoned as hazardous and should be discontinued. Any treatment requiring a waiting period of more than 4 days on bhindi, tomato and cowpea and more than 7 days on cabbage, cauliflower, brinjals, sarson and spinach can be considered as hazardous.

TABLE II
Insecticide applications reckoned as hazardous

Crop	Insecticide	Dosage (Kg a.i./ha)
Bhindi	Aldicarb	1.00 (soil)
	BHC	1.5
	Disulfoton	0.5
	Phorate	0.5 (soil)
	Phosphamidon	0.5 (soil)
Brinjal	Disulfoton	2.0 (soil)
	Phorate	2.0 (soil)
	Carbaryl	2.5
	Phosphamidon	0.25
Cauliflower	BHC	1.5
	Carbaryl	1.1
	Fenitrothion	0.55
	Phorate	1.5
Cowpea	BHC	1.5
	Phosphamidon	0.26
Pea	Aldicarb	1.5 (soil)
	Endosulfan	0.35
	Lindane	0.5
Potato	Aldrin	1.25 (soil)
	Heptachlor	1.5 (soil)
Sarson	BHC	5.9
	Disulfoton	1.5 (soil)
	Parathion	0.56
	Phorate	1.5 (soil)
	Phosphamidon	0.2
Spinach	DDT	5.3
Tomato	Phenthoate	0.5

TABLE III

Effect of processing on dissipation of insecticide residues

Crop	Compound	Plant part	Residue in raw produce (ppm)	Process involved	Residue after processing (ppm)	Tolerance limit (ppm)	Reference
1	2	3	4	5	6	7	8
Bhindi	Endosulfan	Fruits	15.1	Washing	7.7	2.0	Nath <i>et al</i> (1974)
			15.9	Cooking	11.5	"	"
		Fruits	16.9	Washing	4.5	"	"
			16.9	Cooking	5.1	"	"
	Carbaryl	Fruits	11.1	Washing	3.2	10.0	Krishnaiah <i>et al</i> (1975)
			17.3	Washing	8.6	10.0	Rajukkannu <i>et al</i> (1970)
Cabbage	Dimethoate	Heads	17.3	Cooking	5.2	10.0	"
			5.1	Washing	2.2	2.0	Krishnaiah and Lal (1973)
		Heads	3.2	"	2.1	2.0	"
			1.3	"	1.0	2.0	"
	Parathion	Heads	1.2	Washing & cooking	0.2	1.0	Singh <i>et al</i> (1971)
			0.2	"	0.1	1.0	"
Cauliflower	Endosulfan	Curds	18.6	"	6.5	0.5	Awasthi <i>et al</i> (1974)
			11.3	"	5.5	0.5	"
		Curds	3.4	"	1.1	0.5	"
			1.2	"	ND	0.5	"
	Dimethoate	Curds	4.4	Washing	2.3	2.0	Krishnaiah and Lal (1973)
			2.8	"	2.0	2.0	"
Fenitrothion	Fenitrothion	Curds	4.7	Washing & cooking	1.8	0.3	Attri (1977)
							"

1	2	3	4	5	6	7	8
			1.9	"	1.1	0.3	Attri (1977)
			0.8	"	0.5	0.3	"
			0.4	"	0.2	0.3	"
		Curds	5.0	Washing & cooking	2.1	1.0	Attri and Lal (1974)
	Methyl parathion		2.9	"	1.2	1.0	"
			1.1	"	0.6	1.0	"
	Monocrotophos	Pods	5.8	Cooking	0.7	0.2	Awasthi <i>et al</i> (1977)
			3.7	"	0.7	0.2	"
	Methyl parathion	Pods	1.8	Washing & cooking	0.5	0.75	Attri and Lal (1974)
			0.8	"	0.3	0.75	"
	Carbaryl	Pods	24.1	Washing	2.2	5.0	Awasthi <i>et al</i> (1977)
			33.1	"	3.4	5.0	"
			24.1	Cooking	3.7	5.0	"
	Carbaryl	Fruits	17.3	Washing	8.6	10.0	Rajukkannu <i>et al</i> (1976)
			11.5	"	6.2	10.0	"
			8.6	Washing & cooking	5.2	10.0	"
	Parathion	Fruits	1.7	Washing, cooking & peeling	1.2	1.0	"
			1.1	"	0.6	1.0	"
	Aldrin pulp	Pulp	0.2	"	0.3	0.1	Attri <i>et al</i> (1976)
	Heptachlor	"	0.2	"	0.1	0.05	Misra <i>et al</i> (1977)
	Phorate	"	2.1	"	ND	0.5	"
			1.9	"	ND	0.5	"
			1.0	"	ND	0.5	"

ND = not detected

Processes such as water-washing and cooking reduce residues considerably, but do not always bring residues below the tolerance limit. The amount of reduction depends upon factors such as the type of insecticide, formulation, period of sampling, severity of the treatment, etc. In general, processing undoubtedly reduces the total risk, though each case has to be considered separately. Some important findings in this context are presented in Table III.

How far have we gone in achieving the ultimate aim of preventing health hazards to consumers. There is a big communication gap between residue specialists and plant protection staffs. The latter are often poorly informed and go on distributing and using insecticides with little regard to hazards. The implementation of research recommendations and monitoring of pesticides on agricultural produce is becoming difficult due mainly to—(i) Registration of more than 100 insecticides in their different formulations for use on agricultural crops by the Central Insecticide Board (CIB), (ii) absence of tolerance limits for insecticides under Indian conditions, (iii) lack of facilities in the country to handle residue problem. Taking into consideration our capacity and facilities available, the CIB should review its policies and restrict the number of insecticides to a minimum possible. This would enable the government to enforce legal provisions and safeguard the health of consumers.

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19 RESIDUE IMPLICATIONS OF INSECTICIDE SPRAY SCHEDULE IN A VINE-YARD

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SUMMARY

The residues resulting from a recommended spray schedule of 250 ml of malathion, 750 g of carbaryl and 175 ml of endosulfan per 100 vines, on soil, leaves and grape berries in a vineyard at Ludhiana have been estimated using gas-liquid and thin-layer chromatography.

No detectable residues of malathion and carbaryl were found after 2 and 6 months of application. Residues of endosulfan estimated as its A and B isomers degraded rapidly. Only a small quantity of endosulfan B (0.17 to 0.64 ppm) was detectable after 15 days. The results suggest no problem of persistence of these insecticides in vine-yards.

Grapevines in the Punjab are attacked by a number of insect pests such as thrips, jassids, leaf-rollers, hairy caterpillars and chafer beetles. To avoid losses in yield and quality of grapes, a spray schedule based on application of 500 ml of malathion 50 per cent e.c., 1.5 kg of carbaryl 50 per cent w.p. and 500 ml of malathion endosulfan 35 per cent e.c. per 100 vines is recommended (PAU, 1975). Because grapes are consumed fresh it is useful to ascertain the extent of toxic residues left after spraying. The application of endosulfan coincides with fruit maturation stage.

MATERIAL AND METHODS

Thirty grapevines, Cultivar *Khalili* planted 3 x 3 metres apart and trained on vertical trellis at the Horticultural Experimental area of the Punjab Agricultural University, were sprayed with a manually operated knapsack sprayer according to the recommended schedule as detailed below :

Insecticide	Trade Name Formulation	Dosage	Date of spraying	Stage
Carbaryl	Sevin 50% W.P.	1.5 kg	17.11.74	Dormant
Malathion	Cythion 50 e.c.	0.5 lit.	18.4.75	Fruit setting
Endosulfan	Thiodan 35% e.c.	0.5 lit.	25.5.75	Fruit maturation

Six control vines were sprayed with only water.

Samples weighing about 1/2 kg each of grape berries, leaves and surface soil (0-10 cm) were collected on 25th May, 1975 at random immediately before the final spray of endosulfan and also at 2 hours and 1, 3, 5, 7 and 15 days after it. These were transported in double-walled polyethylene bags to the laboratory for residue analyses. The samples were kept frozen (grape-berries 0°C ; others-18°C), until analysed within one week :

Sample preparation and analytical methodology are given below :

	Insecticides	
	Endosulfan	Carbaryl & malathion
*Extraction	<i>n</i> -hexane + Isopropanol (2 : 1)	Acetonitrile
Clean-up	Partitioning-into <i>n</i> hexane Column chromatography alumina-elution with <i>n</i> -hexane + diethyl ether (1 + 1)	<i>n</i> -Hexane —
Estimation & Identification	TLC Silica gel-G. Ag No ₃ UV light GLC-E.C. on 5% OV 101 gas Chrom Q Column Colorimetry ethanol NaOH-pyridine (maitlen <i>et al.</i> , 1963)	TLC-E.I. goat liver enzyme- indoxyl acetate on silica gel-G
Minimum detectability limit	1 ug or 0.1 ppm	500 ng ² and 300 ng ³ or 0.05 and 0.03 ppm
Maier-Bode (1968)		Mendoza 2, 3 <i>et al</i> (1968)

* Soil samples were moistened with water before extraction. The recoveries of the insecticides were about 90 per cent.

RESULTS AND DISCUSSION

No detectable residues of carbaryl and malathion were found in grape berries, leaves and soil sampled prior to the application of endosulfan indicating the fast degradation, of these insecticides in the vineyard as also reported by Gupta (1972), Koivistoinen, (1961), Tew and Sillibourne (1960).

The residues of endosulfan are summarised in Table I and Figure 1. Initial deposits of endosulfan on grape berries, leaves and soil were 10.7, 19.7 and 14.2 ppm respectively and after one day more than 80 per cent of the insecticide had lost.

Within two weeks, more than 95 per cent of the insecticide had disappeared. The initial high rate of degradation could be attributed to high temperatures, strong

TABLE I
Endosulfan residues in/on grape berries, leaves and soil estimated by Gas-Liquid Chromatography

Substrate	Days after treatment	Residues (ppm)			Per cent degradation
		Endosulfan			
		A	B	Total	
Grape berries	0	6.11	4.62	10.73	—
	1	1.16	0.84	2.00	81.4
	3	0.73	0.42	1.15	89.3
	5	0.38	0.56	0.94	91.3
	7	0.13	0.56	0.64	93.6
	15	N.D	0.17	0.17	98.4
Leaves	0	11.27	8.40	19.67	—
	1	1.38	0.98	2.36	88.0
	3	1.07	0.84	1.91	90.3
	5	0.51	0.84	1.35	93.1
	7	0.13	0.84	0.97	95.1
	15	N.D.	0.64	0.64	96.0
Soil	0	8.68	5.49	14.17	—
	1	1.28	0.84	2.12	85.0
	3	1.16	0.70	1.86	86.9
	5	0.52	0.70	1.22	91.4
	7	0.19	0.70	0.89	93.7
	15	N.D.	0.44	0.44	96.0

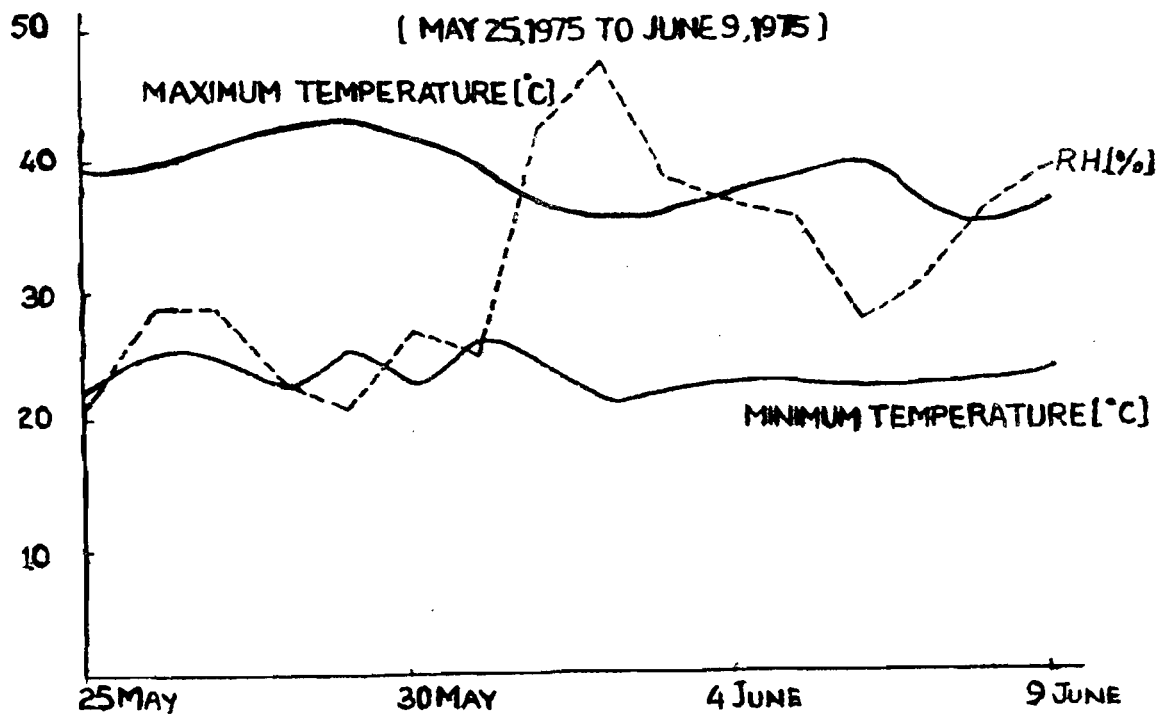
Range of maximum—35.6 to 43.2°C, Range of minimum temperature—21.8 to 26.7°C
Relative humidity—21 to 48%, Total rainfall in mm—0 mm

winds and bright sunlight. The influence of light in accelerating degradation has been observed by Archer (1973).

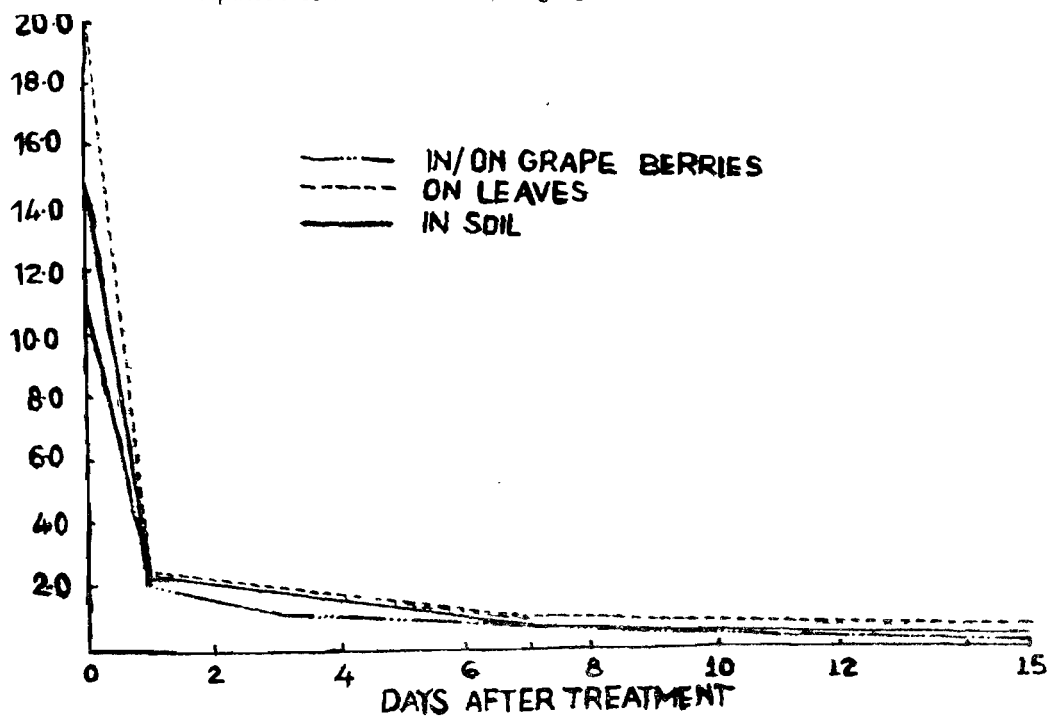
The higher level of endosulfan residues on grape leaves than on berries could be ascribed to the greater surface area/mass ratio of the former as well as greater interception of the spray (Hoeringer and Kenanga 1972). The proportion of the endosulfan missing the plant and reaching the soil is large. Initially, the residues of endosulfan A were higher than those of endosulfan B but the trend reversed after 5 days and finally the proportion of endosulfan B was only just detectable.

Temperature, Humidity and Rainfall (mm) during the experimental period

(MAY 25, 1975 TO JUNE 9, 1975)



Dissipation of endosulfan in/on grape berries, leaves and soil (GLC)



Similar trends for endosulfan A and B residues was observed by Harrison *et al* (1967) on black currents. Thin-layer chromatographic identification of the samples (Archer, 1977) confirmed these results.

No measurable peak on GLC or identification spot by TLC corresponding to endosulfan sulfate was found although a small amount of endosulfan diol was detectable.

These results suggest that the recommended spray schedule can be used without any residue problems in vine-yards. However, a waiting period of one day after the final application of endosulfan is recommended so that residues degrade below the prescribed tolerance limit of 2 ppm (FAO, 1972).

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20 RESIDUES OF FENVALERATE (SUMICIDIN) ON VEGETABLES

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SUMMARY

Residues of fenvalerate (sumicidin) were studied under field conditions on the vegetable crops cauliflower, okra, spinach and tomatoes using electron capture gas-liquid chromatography. Effects of washing and cooking on residues were also studied.

The vegetable crops were sprayed with 0.05 per cent ee fenvalerate at 500 l/ha. The initial deposits on cauliflower heads, okra, tomatoes and spinach were 0.86, 4.0, 0.85 and 9.5 ppm respectively. The residues on cauliflower and okra persisted for 15 days and for 30 days on spinach and tomatoes.

Washing with cold water was effective only on the initial day of application, when it removed about 30-50 per cent of the deposited insecticide but thereafter, it removed only 3-13 per cent insecticide.

Cooking removed 71-88 per cent of the residues on the initial day but on later samplings it removed 68-70 per cent from spinach and tomatoes and 38-40 per cent from okra and cauliflower.

INTRODUCTION

Fenvalerate (Sumicidin), α -cyano-m-phenoxybenzyl α -isopropyl-p chlorophenyl acetate, is a synthetic pyrethroid. It is more stable than natural pyrethrins and is required in only small concentrations (0.05-0.2 kg ai/ha) for insect control so the risk of environmental contamination is low. It is especially recommended for control of pests of vegetables and cash crops. There is little information available on residues that may be left on vegetable crops. The present investigation was, therefore, conducted to study the dissipation of residues of fenvalerate on vegetable crops such as cauliflower, tomatoes, okra and spinach. Effects of washing and cooking on the residues were also studied.

MATERIAL AND METHODS

Experiments were in 1.5 m² plots at the Indian Agricultural Research Institute, New Delhi. Each treatment, and a control, was replicated three times. Okra variety 'Pusa Sawani', Spinach variety 'Pusa Jyoti', cauliflower variety 'Snowball-16' and tomato variety 'Pusa Ruby' were used. The okra and tomato plants were sprayed at the time of fruiting, the cauliflower when the heads had just started developing

but were still covered with leaves, the spinach after one months of sowing when the crop was in the 6-8 leaf stage. Insecticide was applied as 0.05 per cent ec at 500 l/ha. Samples for assessing initial deposits were taken 1 hr and 3, 5, 7, 15 and 30 days after spraying.

Effects of washing were studied by determining residues after samples were washed in running water passing through a sieve for 15 min. Some water-washed samples were cooked in water for another 15 min. in an open vessel.

For residue analysis, 50 g samples were extracted three times with 150 ml acetone after blending in a Waring blender for 3 min. Acetone was evaporated off using a Kuderna Danish evaporator and the concentrate was diluted with water and re-extracted in *n*-hexane using three successive washings of 25 ml each. The hexane extract was dried with anhydrous sodium sulphate, concentrated to 1 ml and cleaned-up by passing through a neutral alumina column prewashed with about 100 ml hexane. Fenvalerate was eluted with a 100 ml mixture of hexane and acetone (9 : 1). The light yellow coloured eluate obtained was used for GLC analysis. A Tracor MT 220 model gas chromatograph equipped with an electron capture detector was used. Operating parameters were ; column 230°C, inlet 250°C and detector 275°C ; glass column 1.8 m X 6.3 mm (I.D.) packed with 3 per cent OV-1 on Chromosorb-W 60-80 mesh, and nitrogen 100 ml/min

Recovery from fortified samples of vegetables varied from 90-100 per cent.

RESULTS AND DISCUSSION

Gas liquid chromatography with a 3 per cent OV-1 column gave two peaks with retention times 180 and 204 sec. A peak height of 180 sec was used to compute the results.

The residues at different time intervals on cauliflower, okra, tomatoes and spinach fresh, washed and cooked samples are presented in Table I, II, III & IV.

The initial deposits on spinach, okra, cauliflower head and tomatoes were 9.5, 4.0, 0.84 and 0.85 ppm respectively. Higher residues on spinach and okra resulted because insecticide was received as a direct spray. Cauliflower heads and tomatoes were covered with leaves, so, the deposits were low. Talekar (1977) reported a deposit of 0.6 ppm on cabbage applied at the rate of 0.1 kg ai/ha, which is comparable with our results.

Residues persisted for 15 days on okra and cauliflower and 30 days on tomatoes and spinach. The rate of dissipation was faster on cauliflower and okra than on spinach and tomatoes (Figure I). By the third day residues were reduced by

60 per cent on cauliflower and okra and the corresponding loss on tomatoes and spinach was only 11 per cent and 15 per cent. Similarly, on the 7th day the reduction in insecticide on okra and cauliflower was 80 per cent and 65 per cent and on spinach and tomatoes 47 per cent and 39 per cent. Thus, the dissipation rate was

TABLE I
Residues of fenvalerate in/on cauliflower head

Days	Unprocessed	After washing		After cooking*	
	Residues (ppm)	Residues (ppm)	% loss	Residues (ppm)	% loss
0 (1 hr)	0.86	0.62	28	0.25	71
3	0.60	0.56	7	0.27	55
5	0.44	0.40	9	0.21	52
7	0.30	0.27	10	0.18	40
15	N.D.	N.D.	—	N.D.	—

* Cooking : After washing the sample was boiled for 15 min. in water.

definitely faster on okra and cauliflower than tomatoes and spinach, but, if we take into consideration the growth of plant we find that the loss of insecticide was in order of their growth rates : okra > cauliflower > spinach > tomatoes. Thus, it

TABLE II
Residues of fenvalerate in/on okra fruits

Days	Unprocessed	After washing		After cooking*	
	Residues (ppm)	Residues (ppm)	% loss	Residues (ppm)	% loss
0 (1 hr)	4.0	1.6	60	0.6	85
3	2.8	2.4	14	1.6	43
5	1.6	1.4	13	1.0	38
7	0.8	0.7	13	0.5	38
15	N.D.	N.D.	—	N.D.	—

* Cooking : After washing the sample was boiled for 15 min. in water.

could be concluded that reduction of residues in these vegetables was due primarily to dilution by increase in weight and not by degradation.

Effect of Washing

Washing was found to be very effective in reducing residues in all vegetable samples collected within 1 hr of spraying. It removed 28, 52, 53 and 60 per cent residues from cauliflower, tomatoes, spinach and okra. Apparently, these residues

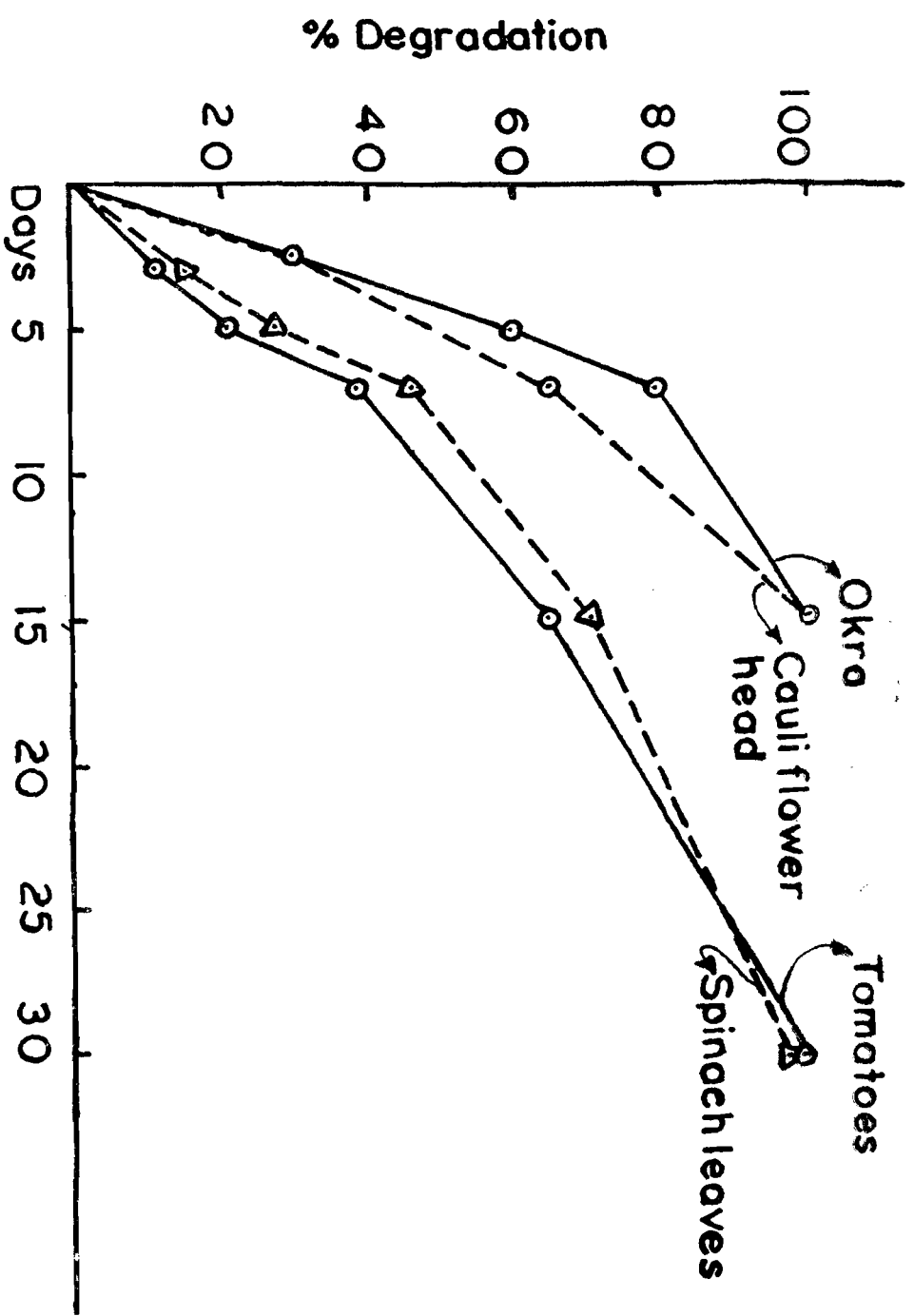


Fig. Degradation of fenvalerate in different vegetables.

were deposited on the upper surface of vegetables and were easily washed off by ordinary water washing. However, as the time progressed, the residues penetrated into the vegetables and washing became less effective. Only 3-13 per cent residues were removed from vegetable samples collected 3 days after application.

Effect of Cooking : The effect of cooking was very effective on all vegetables collected within 1 hr of spraying removing 71-88 per cent of the residues. However

TABLE III
Residues of fenvalerate in/on tomatoes

Days	Unprocessed	After washing		After cooking*	
	Residues (ppm)	Residues (ppm)	% loss	Residues (ppm)	% loss
0 (1 hr)	0.85	0.41	52	0.1	88
3	0.76	0.71	7	0.18	76
5	0.67	0.63	6	0.15	77
7	0.52	0.49	6	0.13	75
15	0.30	0.29	3	0.09	70
30	N.D.	N.D.	—	N.D.	—

* Cooking : After washing the sample was boiled for 15 min. in water.

in subsequent samplings on the 3rd, 7th and 15th days, the effect of cooking in removing residue was still good in tomatoes and spinach but less so on okra and cauliflower. It removed 68-70 per cent residues from tomatoes and spinach but for okra and cauliflower only 38-40 per cent. It appears that residues of fenvalerate form some sort of conjugate in cauliflower and tomatoes which is not susceptible to ordinary boiling.

TABLE IV
Residues of fenvalerate in/on spinach leaves

Days	Unprocessed	After washing		After cooking	
	Residues (ppm)	Residues (ppm)	% loss	Residues (ppm)	% loss
0 (1 hr)	9.5	4.5	53	1.2	87
3	8.1	7.5	7	2.1	74
5	6.8	6.2	9	1.7	75
7	5.0	4.6	8	1.5	70
15	2.8	2.6	7	0.90	68
30	N.D.	N.D.	—	N.D.	—

* Cooking : After washing the sample was boiled for 15 min. in water.

Results of these studies show that the use of fenvalerate is safe on cauliflower and tomatoes but for okra a waiting period of 7 days would be required for residues to fall within the safe limits of 1 ppm. Residues in spinach were high up to 15 days (2.8 ppm), so its use should be restricted.

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21 PERSISTENCE OF CARBOFURAN IN SOME VEGETABLES

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SUMMARY

Studies were carried out at the Horticultural Experimental Station, Hessaraghatta of the Indian Institute of the Horticultural Research, Bangalore during 1977-78 on the persistence of carbofuran residues in peas, sweet pepper and okra following its application as preplanting treatments i.e., soil application of granules at 1 kg ai/ha in peas at sowing at 0.5 and 1.0 kg ai/ha in sweet pepper at planting and seed dressing with carbofuran S.P. 5 per cent and soil application of granules at 0.5 and 1.0 kg ai/ha at sowing in okra. Carbofuran residues were estimated by a colorimetric method in pea seeds and fruits of okra and sweet pepper harvested after 65, 50 and 70 days of application. Amounts of residues were : 0.28 ppm in pea seeds, 0.032 ppm in sweet pepper fruits, 0.031 ppm in okra fruits from seed treatment, 0.249 and 0.327 ppm in okra fruits from soil treatments at 0.5 and 1.0 kg ai/ha respectively. The residue tolerance for carbofuran has been fixed at 1.00 ppm on capsicum and potato by the US, EPA and FAO/WHO.

Carbofuran, (2,2-dimethyl-2,3-dihydro-7-benzofurenyl, *n*-methylcarbamate) a systemic carbamate insecticide, is very popular for controlling pests of vegetable crops. Soil applications of carbofuran (1.0 kg ai/ha) controlled pea aphid (Mathur *et al.*, 1974) and thrips on sweet pepper (Jagan Mohan *et al.*, 1978). Shoot borer and leaf hoppers on okra were suppressed effectively by carbofuran seed treatment and soil application (Krishnaiah *et al.*, 1976). This study aims to determine the persistence in pea seeds, capsicum and okra fruits.

MATERIAL AND METHODS

Crops of sweet pea variety 'Bonneville' and capsicum variety 'California Wonder' (winter 1977-78) (December-February) and okra variety 'Pusa Sawani' (summer 1978) were raised at the Horticultural Experiment Station, Hesaraghatta, of the Indian Institute of Horticultural Research, Bangalore. Carbofuran granules were applied in furrows at the rate of 1.0 kg ai/ha to peas and 0.5 kg and 1.0 kg

TABLE I
Persistence of carbofuran in Peas, Capsicum and Okra

Crops	Dosage a.i kg/ha	Days after application	Residue in ppm	Tolerance limit ppm
Peas	1.0	65	0.28	—
Capsicum	0.5	70	0.032	1
	1.0	70	0.032	
Okra (Seed Treat.) Soil application		50	0.031	
	0.5	50	0.249	
	1.0	50	0.327	

* Average of three replications

Weather conditions during the experimental season :

Crops	Peas	Capsicum	Okra
Max. Temp.	31.2°C	32.0°C	36.3°C
Min. Temp.	12.0°C	12.0°C	18.1°C
Rainfall	1.70 mm	1.70 mm	23.59 mm
No. of rainy days	One	One	25

ai/ha to capsicum and okra at the time of sowing. Okra had a second treatment of 5.0 per cent seed dressing with carbofuran 50 SP. All treatments and the check were replicated three times in randomised blocks in plots 5×3 m.

Matured green pods of peas, fruits of capsicum and okra were collected from the middle rows of the plots after 65 days, 70 days and 50 days of sowing or planting. The carbofuran residues in pea seeds and capsicum fruits were estimated following a slightly modified method of Gupta and Dewan (1973). For bhendi fruits the method described by Baskaran and Jotwani (1976) was followed. The modification for capsicums was made at the clean up stage and consisted of the addition of a coagulating solution immediately after evaporating the extract to dryness and then partitioning with dichloromethane.

The standard curve was prepared using analytical grade carbofuran in methanol. The recovery of fortified carbofuran (1 ppm) was 70–75 per cent in pea seeds, 65–70 per cent in capsicum and 80–90 per cent in okra.

RESULTS AND DISCUSSION

Carbofuran residues in pea seeds were of the order of 0.28 ppm. In capsicum fruits treated at 0.5 kg and 1.0 kg ai/ha the residues were 0.032 ppm after 65 days and 70 days of its application to the soil. Bhendi fruits contained 0.031 ppm carbofuran residues after seed treatment and 0.249 ppm and 0.327 ppm carbofuran residues at 50 days after sowing with soil application at 0.5 kg and 1.0 kg ai/ha respectively. The tolerance limit of carbofuran for pepper has been fixed at 1.0 ppm by EPA of USA (Anon, 1971). Thus, the carbofuran residues in pea seeds, capsicum and okra fruits were well below the tolerance limit of 1.0 ppm. Hence, the application of granules of carbofuran at sowing/planting of peas, okra and sweet pepper and seed dressing with carbofuran at 5.0 per cent was safe to avoid residues in fruits. Similar results were obtained by Rajukkannu *et al.*, (1975) in sweet potato tubers and Rajukkannu *et al.*, (1976) in paddy when carbofuran was applied at the rate of 1.25 kg/ha.

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22

RESIDUES OF QUINALPHOS IN VEGETABLES

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SUMMARY

Laboratory studies were conducted to evaluate the effects of quinalphos at concentrations of 0.05 and 0.01 per cent a.i. in spray fluid. The pesticide residues were assayed at different concentrations using pig-liver enzyme. The recovery of quinalphos from fortified samples of crop produce were estimated by comparison with standards spotted simultaneously along with extracts. The recovery was 100 per cent. Extractions with either acetone or chloroform did not make any difference in the recovery. The use of charcoal-alumina mixture for clean-up of chloroform extracts of fortified samples of the produce did not result in any loss of pesticide. The residues of quinalphos in cabbage, cauliflower and knol-khol were also assayed by TLC method. The residues of quinalphos on cabbage, cauliflower and knol-khol were 9.91-1.4 : 1.9-3.08 and 0.63-1.33 ppm at 0.05 and 0.01 of pesticide applied respectively.

INTRODUCTION

Many pesticides have been tested against brassica crops but only limited information is available under Indian conditions. Furthermore, the persistence on the treated crop(s) has not been studied in many instances. There is little information on quinalphos, so a study was undertaken to determine its residues in cabbage, cauliflower and knol-khol.

MATERIAL AND METHODS

Cabbage, cauliflower and knol-khol crops were planted at the UAS, Hebbal.

The nurseries of all the three crops—cabbage (Pride of India), Cauliflower (snow ball) and knol-khol (White Vienna) were sown on raised beds of 1 m width and 2 m length.

Fifteen rows of 5 m length rows spaced 0.6 m from crest to crest of the ridges were used for each treatment in each crop. During the nursery stage and again at the time of crop establishment, 0.05 per cent quinalphos spray was applied at rates of 0.05 and 0.1 per cent a.i., when the crop was close to the ripening stage and to analyse the residues.

Determination of quinalphos residues : The method of determining residues of quinalphos, (Ekalux 25 EC) and its metabolites, in plant material was based on a TLC–EI method and by spectrophotometry. The plant material was extracted either with chloroform or acetone and the clean-up, separation of the insecticide from the co-extractives was achieved in by TLC. The crude extract was spotted in μ l quantities on silica gel TLC plates activated at 110° C for 45 min. Hexane and acetone (5 : 1) was used to develop plates and separate the insecticide and the plant pigments. The plates were exposed to bromine fumes for about 15 seconds and the excess of bromine settling on the silica plates was driven away by keeping them under fan for half an hour. Then the plates were sprayed with pig liver extract in 0.05 m solution. After an incubation for 20 min. at room temperature (20-28° C) indoxyl acetate was sprayed on them. The pesticide and its metabolite appeared as white spots on a permanent blue background. Each time standard quinalphos solution in acetone was also spotted for comparison with the plant samples. Quantitation was achieved in one of the following two ways :

a) by measurement of the area of the spots developed for the standard and the plant extracts (using a planimeter).

b) the plates were developed in duplicate under identical conditions. One plate was used for the development of the spots by spraying enzyme and a substrate. The corresponding strip of silica in the second plates was scraped and the insecticide was eluted with acetone. The solvent was evaporated by blowing air and a known quantity of distilled water was pipetted into a dry beaker containing the insecticide. The contents were stirred well using a magnetic stirrer. A suitable aliquot was taken and cholinesterase inhibition was followed using methods for monocrotophos. The aqueous extract of the brominated compound used as above would enable the detection of the pesticide at sub-nanogram levels.

Cabbage, knol-khol and cauliflower were sprayed with quinalphos and samples were collected in replicate 0, 1, 4, 7, 10 and 14 days after spraying.

Samples of about 500 g were shredded into small pieces using sharp stainless steel knife and mixed thoroughly. Twentyfive grams of the material was placed in the blender jar and 100 ml of chloroform (for cauliflower) or acetone (for cabbage and knol-khol) was added and macerated thoroughly for 3 min. at high speed. The contents of the blender were filtered with suction and the filtrate was washed twice with 25 ml portions of the solvent and transferred into a 500 ml conical flask. Thirty grams of anhydrous sodium sulphate was added to remove moisture. For the chloroform extracts, 30 g of mixture containing 3 parts of activated charcoal, 8 parts of celite 545, 6 parts of aluminium oxide, 33 parts of sodium sulphate anhydrous were added and the contents thoroughly shaken and left for few minutes. The dried extract was filtered through a fluted Whatman No. 4 filter paper collecting the contents into a EM flask. The sodium sulphate from the mixture was washed twice with 25 ml of the solvent and the washings were also filtered. The filtrate and washings were pooled and condensed in a rotary evaporator to about 15 ml (for chloroform) or about 30 ml (for acetone) and transferred to a volumetric flask. The contents were made up to volume with the respective solvent (to 25 or 50 ml).

*Standardization—*a) *TLC*: The TLC apparatus model No. 8 CR, QRA was employed to prepare the plates. An anodized aluminium spreader with four accurately measured slots of 0.25, 0.5, 0.75, 1.0 mm thickness was used to spreading the silica gel on plates. Glass plates of 20 × 20 cm size were cleaned thoroughly and dried in an oven. Five plates were placed on the apparatus leveller and fixed tightly, cleaned and spread with the silica gel of 0.5 mm thickness.

A known aliquot of standard quinalphos solution in acetone was spotted on each strip of the plate and plates were developed with 5:1 hexane and acetone. When the solvent front reached about 15 cm, the plates were removed from the tank and dried in air.

The plates after development and drying, were exposed to bromine vapour for 15 sec. After treatment, the plates were kept in open air and excess bromine driven out in air blast for half an hour.

About 30 g of chopped pig liver was homogenized in 10 ml distilled water for each gram of tissue and separation was carried out at 0-4°C. The homogenate was centrifuged at 10,000 rpm for 30 min. in a refrigerated centrifuge and supernatant liquid transferred into 20 × 160 cm corning rimless test tubes, sealed with parafilm and stored in a deep freeze at -20°C.

The developed plates containing activated quinalphos at different concentrations were sprayed uniformly with diluted pig liver extract using a glass automizer and plates were kept in horizontal position on a table for 15 min. for the inhibitor to act.

The method used by Mendoza *et al.* (1968) was followed for preparation of spray reagents.

Solution A : Fifteen mg of indoxyl acetate was dissolved in 5 ml of absolute ethanol, just before use.

Solution B : Tris (hydroxy methyl aminomethane) 0.5 M solution-4 ml was pipetted into a 50 ml beaker. Five ml of 2.00 M sodium chloride solution, 0.2 ml of 1.00 M calcium chloride solution and 3.8 ml of glass distilled water were added to the Tris to make 13 ml of the solution *i.e.*, salt solution.

Solution C : Solutions of 0.5 M potassium hexacyanoferrate II and 0.05 M potassium hexacyanoferrate III were also prepared and kept refrigerated. One ml of each of the two solutions were mixed and kept for use.

Substrate spray solution : Reagents B and C were mixed and the resulting solution was thoroughly mixed with solution A giving 20 ml of spray solution which was sufficient for three plates. The spray reagent was used immediately after mixing the ingredients, to spray the TLC plates pre-treated with liver extract. The pesticide appeared as white spots on a uniform blue background because the aryl esterase of liver was inhibited by the pesticide.

The spots were traced and area of each spot was measured with a planimeter. The area of the spot was roughly proportional to the concentration and a standard curve was prepared with known concentrations of pesticide applied to TLC plates.

Suitable aliquots of the extracts were spotted, on the plates in duplicate. One set of TLC plates was developed as detailed above and quantitation done by measurement of areas of spots of inhibition and compared with standards run simultaneously.

Spectrophotometry : The other set of developed plates was used for determination of pesticide residues by spectrophotometry after elution. The area on the second set of plates corresponding to the spot developed in the first set was scraped and the scrapings were transferred into a sintered funnel and eluted with about 10 ml of acetone. The eluent was allowed to dry and the residue was taken up in distilled water and stirred with a magnetic stirrer. A known aliquot of this solution

was used for assay spectrophotometrically. Quinalphos inhibits cholinesterases of human plasma and the inhibition is a measure of the amount of quinalphos present.

One ml of standard quinalphos solution (1 ppm in acetone) was pipetted into a clean dry beaker and evaporated, then 10 ml of distilled water added. For brominated quinalphos, a known quantity of the pesticide in acetone was dropped on a silica plate and the solvent evaporated. The insecticide on the plate was exposed to bromine vapours for 15 seconds and excess of bromine was driven off. The compound was made into an aqueous solution and a standard solution of 0.0002 ppm was prepared. The procedure for the assay of monocrotophos was used to prepare standard curves using aqueous solutions of 0.1 ppm quinalphos and 0.0002 ppm brominated quinalphos.

The absorbance values were plotted taking the concentration of the pesticide on the abscissa and the absorbance on ordinate. The curves were linear for quinalphos (10 to 100 ng) and for brominated quinalphos (0.02 to 0.2 ng). The brominated compound was tested at different concentrations and 0.002 ppm solution caused nearly as much inhibition as 0.1 ppm of untreated quinalphos.

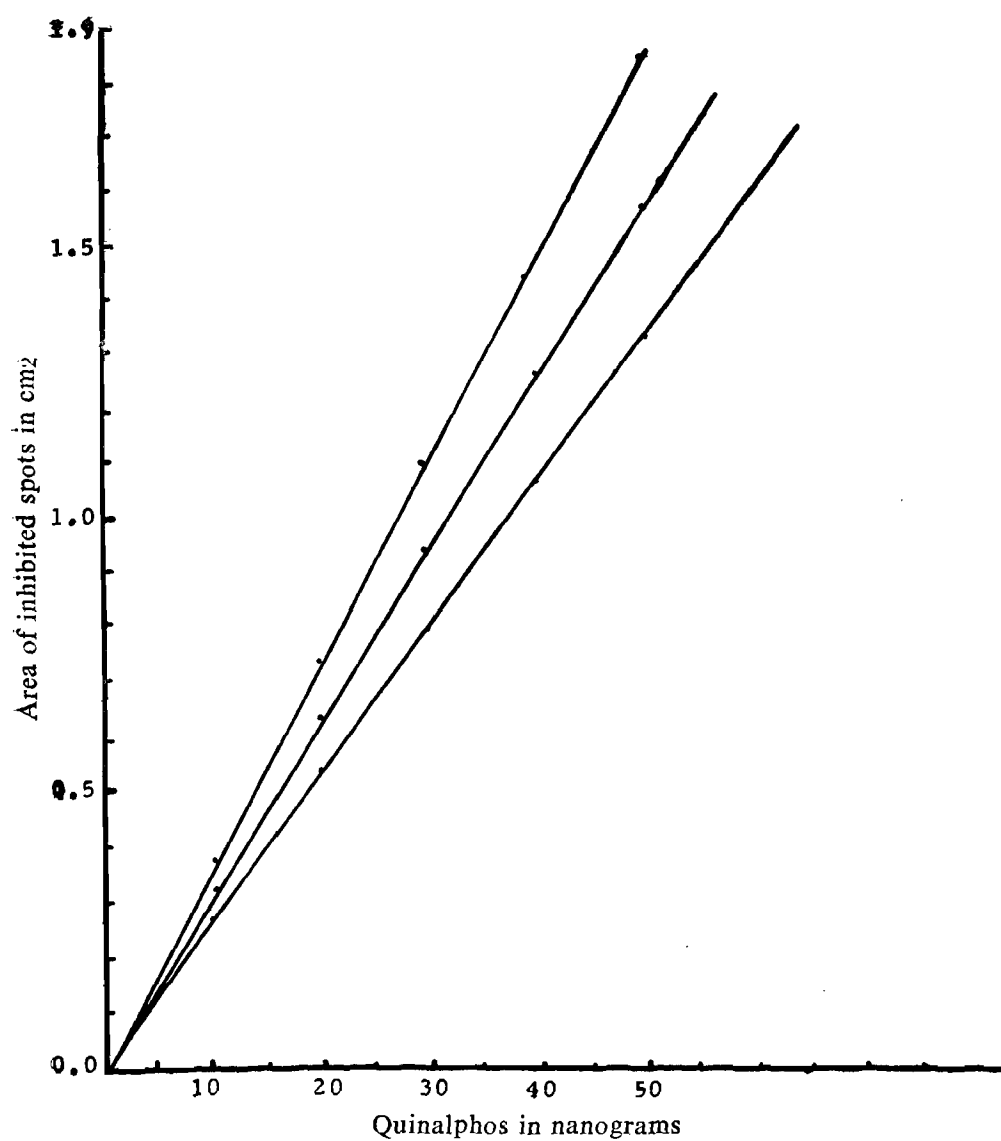
The crop extracts were also spotted, developed and eluted either with or without bromination. Aqueous extracts were prepared and a known aliquot used for assaying the degree of inhibition. Samples from untreated plants were also extracted and assayed. The TLC separation was so efficient that the corresponding zone in the untreated crop sample did not show any inhibition.

RESULTS AND DISCUSSION

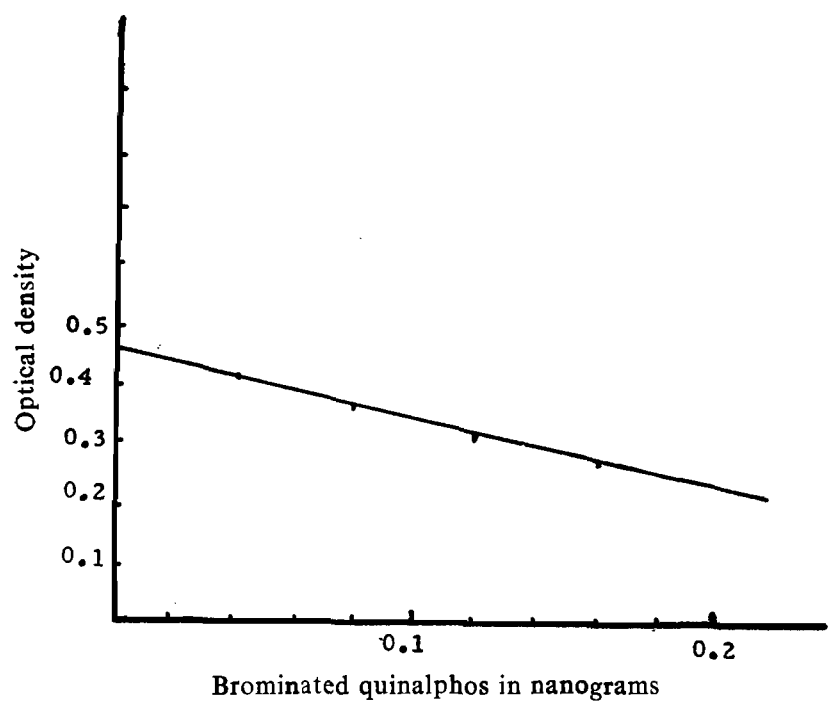
The standard curve for assaying the pesticide residues using pig liver enzymes is in Fig. 1 and those showing the relationship between the quantity of the inhibitor (in nanograms) and enzyme activity of human plasma for quinalphos and brominated quinalphos in Fig. 2 and 3.

The recovery of the quinalphos from fortified samples of crop as estimated by comparison with standards spotted with extracts was 100 per cent. The extraction with acetone or chloroform did not alter the recovery. The use of a charcoal/alumina mixture for clean up of chloroform extracts of fortified samples of cauliflower did not result in loss of pesticide.

The initial deposit and the residues of quinalphos in cabbage, cauliflower and knol-khol assayed by TLC are given in Tables I to III. The deposits of quinalphos in cabbage, cauliflower and knol-khol were 0.91, 1.40, 1.92, 3.08, 0.63 and 1.33 ppm at 0.5 and 0.1 per cent of pesticide spray, respectively.



Legend : Dilution of Pig Liver enzyme. A=1 : 30; B=1 : 40; C=1 : 50
 Fig 1. Standard Curve for Quinalphos (TLC method)



Brominated quinalphos in nanograms

Human plasm dilution 1 : 500

Fig. 2 Standard curve for brominated quinalphos

In certain instances, substances inhibitory to the enzyme were detected on the plates and were identified as metabolite I and II with Rf values of 0.27 and 0.17, respectively. The concentrations reported are based on standard curves prepared for quinalphos. The results obtained on the analysis of residues of quinalphos in

TABLE I
Initial deposits and residues of quinalphos in cabbage (TLC Method) 1976

Particulars	No. of days after spray	Concentrations of quinalphos in ppm			
		Spray concentration at 0.05% a.i.		Spray concentration at 0.1% a.i.	
		RI	RII	RI	RII
Cabbage head washed*	0	0.67*	—	1.04*	—
Cabbage whole head	0	1.08	0.74	1.46	1.33
Cabbage outer leaves*	0	2.74*	—	3.15*	—
a) Metabolite I	0	0.13*	—	0.25*	—
b) Metabolite II	0	0.40*	—	0.30*	—
Cabbage whole head	1	0.87	0.60	1.40	1.43
Cabbage whole head	4	0.52	0.61	0.68	0.80
Cabbage whole head	7	0.17	0.18	0.80	1.35
Cabbage whole head	10	0.41	0.55	0.41	0.38
Cabbage whole head	14	0.00	0.40	0.46	0.48
Cabbage whole head	21	0.00	0.00	0.00	0.00

* Samples pooled and reassayed

Weather data for the period from 15.9.1976 to 10.10.1976

Mean maximum temperature	—	29.1°C	
Mean minimum temperature	—	19.6°C	
Relative humidity (average)	—	At 7.20 a.m.	91%
		At 2.20 p.m.	56%
Total rainfall during the period	—	32.5 mm	

the above crops by spectrophotometry are presented in Tables IVa, b and c, respectively. Residues of quinalphos in cabbage levels at 0.05 and 0.1 per cent a.i. and 14 days after spraying are given in Table V.

Data on residues of quinalphos in cabbage, cauliflower and knol-khol obtained with TLC were taken into account to calculate half-lives. The results obtained for the same sample extracts by spectrophotometric assay agreed closely with those obtained with TLC when the samples were brominated. But, if the samples were used directly, wide disparity between the two methods was observed. The assay

of quinalphos residues by the TLC method was convenient and gave consistent results.

The effect of quinalphos on the activity of human plasma as an enzyme source and L-naphthyl acetate as substrate is given Fig. 4. The curve shows a biphasic

TABLE II
Initial deposits and residues of quinalphos in cauliflower (TLC Method) 1976

Particulars	No. of days after spray	Concentration of quinalphos in ppm			
		Spray concentration at 0.05% a.i.		Spray concentration at 0.1% a.i.	
		RI	RII	RI	RII
Whole flower	0	1.92	1.92	3.17	3.00
a) Metabolite I	0	nd	nd	0.30	0.27
Whole flower	1	1.50	1.40	2.4	2.0
a) Metabolite I		nd	nd	0.24	0.11
Whole flower	4	0.58	0.58	0.62	0.46
a) Metabolite I		nd	nd	0.11	0.08
Whole flower	7	nd	0.22	0.72	0.48
Whole flower	10	0.222	0.036	0.15	0.09
Whole flower	14	0.06	0.11	0.17	0.16
Whole flower	21	nd	nd	nd	nd

nd : Not detectable

TABLE III
Initial deposits and residues of quinalphos in knol-khol (1976) (TLC Method)

Particulars	No. of days after spraying	Concentration of quinalphos in ppm			
		Spray concentration at 0.05% a.i.		Spray concentration at 0.1% a.i.	
		RI	RII	RI	RII
Modified stem of knol-khol in whole	0	0.53	0.73	1.27	1.40
	1	0.40	0.56	0.72	0.76
	4	0.36	0.50	0.52	0.54
	7	0.21	0.31	0.33	0.36
	10	0.38	0.40	0.44	0.18
	14	nd	0.20	0.24	0.20
	21	nd	nd	nd	nd

nd : Not detectable

pattern which may be due to the presence of more than one esterase, each being differentially susceptible to the inhibitor.

With naphthyl acetate as a substrate linearity was not obtained with either enzyme sources. Experience showed that following the Ellman procedure (1061) with DTNB and Bu Tch with human plasma was more satisfactory.

TABLE IVa

Initial deposits and residues of quinalphos in cabbage, cauliflower and knol-khol (Spectrophotometric method of analysis) 1976

(Cabbage)					
Days after spray	Type of sample	Concentration of quinalphos (ppm)			
		0.05% a.i.		0.1% a.i.	
		RI	RII	RI	RII
0	+ Washed head*	1.67	—	3.32	—
0	+ Outer leaves*	5.81	—	6.66	—
	a) Metabolite	12.45	—	15.77	—
0	Whole head	1.67	1.27	5.6	3.32
1	Whole head	0.67	1.00	1.66	1.66
4	Whole head	0.40	0.60	0.40	0.70
7	Whole head	0.40	0.50	0.60	1.0
10	Whole head	0.40	0.42	0.50	0.16
14	Whole head	nd	0.18	0.48	0.38

TABLE IVb

(Cauliflower)					
Days after spray	Type of sample	Concentration of quinalphos (ppm)			
		0.05% a.i.		0.1% a.i.	
		RI	RII	RI	RII
0	Whole flower	1.88	6.25	6.85	6.85
1	Whole flower	2.56	2.56	6.85	5.00
4	Whole flower	1.00	1.00	0.80	0.75
7	Whole flower	0.09	0.08	0.32	0.24
10	Whole flower	0.032	0.022	0.13	0.06
14	Whole flower	0.10	0.10	0.25	0.24

Residues of quinalphos were analysed by TLC-EI and spectrophotometric methods using pig liver enzyme and human plasma, respectively. The insecticide

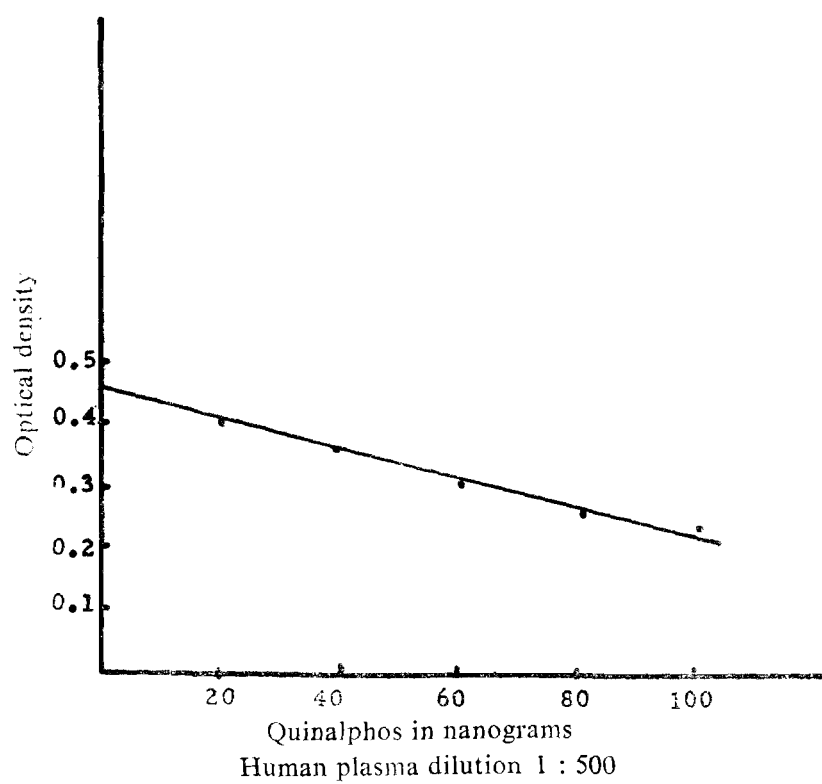


Fig. 3. Standard Curve for Quinalphos (Spectrophotometry)

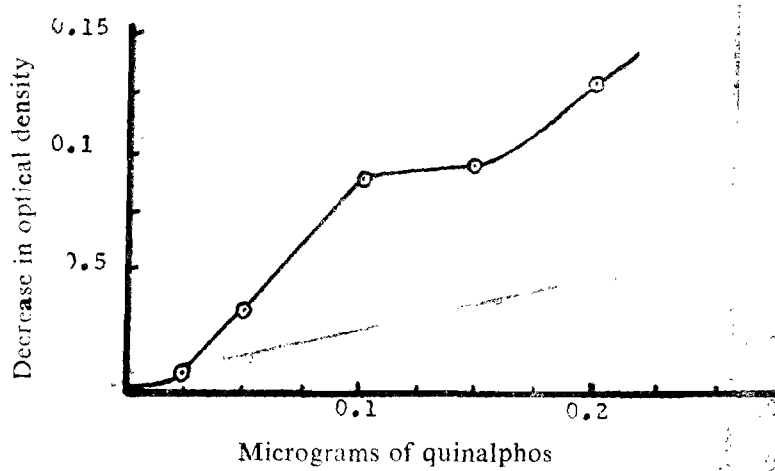


Fig. 4. Relationship between Quinalphos and Cholinesterase of Human Plasma with Naphthyl Acetate as substrate.

did not cause inhibition of the liver esterase on TLC plates. Visual estimation of the compound on TLC plates with UV light was unsuccessful without treatment with bromine vapour because bromine converts it into a potential inhibitor of pig liver enzymes.

TABLE IVc
(Knol-Khol)

Days after spray	Type of sample	Concentration of quinalphos (ppm)			
		0.05% a.i.		0.1% a.i.	
		RI	RII	RI	RII
0	Whole stem+	1.55	2.20	4.00	4.40
1	Whole stem	0.40	0.40	0.30	0.50
4	Whole stem	0.40	0.60	0.60	0.65
7	Whole stem	0.14	0.34	0.48	0.33
10	Whole stem	0.40	0.24	0.46	0.10
14	Whole stem	nd	0.10	0.13	0.15

* The replicates were pooled and the average assayed
 + On these days samples were not brominated and the standard curve prepared for unbrominated sample was used. On all other days the samples were brominated and the corresponding standard curve was used
 nd = not detectable.

The substrate used in these studies was indoxyl acetate. In principle, the substrate is hydrolyzed by the pig liver enzyme and forms indoxal which will be oxidized, by the oxidizing agent present (Fe⁺⁺/Fe⁺⁺⁺/) in the substrate solution itself, to form indigo blue. Wherever, the pesticide is present on TLC plates, the enzyme is inhibited and does not react with the substrate and the pesticide appears as white spots on a blue background of indigo blue.

Linearity was observed between enzyme activity and incubation period with the inhibitor up to 30 min. The area of the spot was proportional to the enzyme

TABLE V
Residues of quinalphos in cabbage leaves after 7 and 14 days after spraying

Number of days after spraying	Residues of quinalphos (ppm)	
	0.05% spray	0.1% spray
7	0.86	1.08
14	0.54	0.67

dilution from 1 : 20 to 1 : 50. The area of the spot was proportional to the quantity of the pesticide up to 50 ng.

The sensitivity of the TLC-EI method employed in the studies was high with a lower limit of detection of pesticide of 2 ng.

Residues of quinalphos in samples of cabbage, cauliflower and knol-khol were determined by TLC-EI method at periodical intervals from 0 to 14 days. Quantities were measured as the area of the spots in comparison with the standards spotted simultaneously. A second set of plates developed simultaneously were employed for spectrophotometric assay. As perfect linearity was observed up to 50 ng of pesticide, quantitation of the pesticide by measurement of the area of the inhibition spots was valid.

Recovery tests indicated 100 percent recovery of the pesticide at 1 ppm fortification of crop samples. The results obtained by spectrophotometry agreed with those by TLC. Whenever an assay by spectrophotometry was made using the standard curve for quinalphos, higher values were recorded and this anomaly could not be explained. However, it is surmised that the higher values were due to oxidation of a part of quinalphos on TLC plate, which would have caused more inhibition, due to either : (i) the purified compound on the TLC plate was exposed over an increased surface area and (ii) the compound contained unsaturated bonds in the quinoxaliny ring.

The reason for better agreement when assayed using the standard curve for brominated quinalphos by spectrophotometry with that obtained by TLC method might be that the exposure to bromine vapour would have caused more stability in the compound.

Brominated quinalphos is nearly 500 times more inhibitory to human plasma enzyme than quinalphos. Perfect linearity in inhibition of the plasma by the brominated quinalphos was observed from 0.02 to 0.2 ng (Fig. 2).

On the basis of data on residues of quinalphos determined by TLC-EI method, in cabbage, cauliflower and knol-khol crops, dissipation curves were worked out.

Deposits of quinalphos in cabbage, cauliflower and knol-khol were 0.91 and 1.0, 1.92 and 3.08, 0.63 and 1.33 ppm at 0.05 and at 0.1 per cent of the pesticide spray, respectively.

The residues after 2 week period in cabbage, cauliflower and knol-khol were 0.2 and 0.47, 0.08 and 0.16, 0.1 and 0.22 at lower and higher concentrations of pesticide, respectively.

The safety interval for the harvest and consumption of produce of the crops treated with quinalphos could not be assessed due to lack of information on tolerance limits for this pesticide. However, from the present studies, it was found that quinalphos was five times more inhibitory to the human plasma than monocrotophos. As 0.2 ppm is a fixed tolerance limit for monocrotophos in cole crops, the tolerance limit for quinalphos may be fixed at 0.04 ppm with respect to cole crops.

From the residue data on these crops it is evident that quinalphos will take about 20 days to dissipate to the level of 0.04 ppm in cabbage and knol-khol. So it is not advisable to use quinalphos at the time of crop maturity.

Deposits : The magnitude of deposits of monocrotophos and quinalphos was greater in cauliflower than in cabbage and knol-khol. This can be attributed to differences in the plant surfaces, cauliflower with a flat head and interfloral spaces in the curd can retain more insecticides than the round and upright cabbage and knol-khol. Ebeling (1963) stated that factors like plant form (erect or prostrates), shape of plant part (broad or narrow or linear), position (horizontal or upright) etc., influence the distribution, retention and ultimate deposition of insecticides on plant surfaces.

According to Renwall and Akerblem (1971) most pesticides like ethion and parathion were stable for one year when extracted with dichloromethane and stored in brown bottles at room temperature. In the present studies, quinalphos in dichloromethane extracts of cabbage, stored at 5°C during March 1976 and analysed by TLC during September 1976, showed some transformation. Four different compounds inhibitory to pig liver enzyme were noticed on TLC plates. Thus, quinalphos is not stable in crude crop extracts of dichloromethane. So these samples were discarded and fresh ones were used for extraction and assay of residues.

Quinalphos showed similar trends of dissipation irrespective of concentrations of spray, but they varied with crop and was faster on cauliflower than cabbage and knol-khol.

The tolerance limits of this pesticide on different crops have not been prescribed so far. However, based on the information available with regard to its toxicity to human plasma enzyme when compared to monocrotophos, it may be tentatively fixed that quinalphos would require about 20 days to dissipate to harmless level. Nevertheless, it is advisable not to use it at the time of crop maturity.

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23 EFFECT OF TEMPERATURE AND HUMIDITY ON DISSIPATION OF PHOSPHAMIDON IN SOME VEGETABLES

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SUMMARY

Following foliar application of phosphamidon to brinjal (*Solanum melongena* L.), chillies (*Capsicum annuum* L.) and peas (*Pisum sativum*) in different seasons under varied conditions of temperature and humidity, the total phosphamidon residue toxins in fruits and foliage were analysed using an enzyme inhibition method. Comparisons of rates of degradation of residues from foliage and fruits of the same crop under different temperature and humidity level, revealed that temperature was a major factor influencing the rate of phosphamidon dissipation. At higher temperatures, degradation of residues was rapid. Humidity has been observed to have little effect on dissipation rates within the ranges that prevailed under field conditions.

Studies on the analysis of phosphamidon residues in a wide range of crops such as cereals, vegetables and fruit plants were reported by Menzer and Ditman (1963a). Voss and Geisbuhler (1971), Orphanides *et al.* (1959), Brewerton (1963), Saini and Khurana (1969), Gupta (1970), Ramasubbiah (1971), Mishra and Baksh (1973), Westlake *et al.* (1973), Shinde (1977) and these workers indicated that weather exerted a great influence on dissipation of phosphamidon residues.

Menzer and Ditman (1963b) investigated the effect of temperature and photo-period on the degradation of phosphamidon on snap beans and potatoes under controlled conditions in the laboratory. The present investigations were conducted in the field to study the effect of temperature and humidity on the rate of dissipation of phosphamidon residues in brinjal, chillies and peas.

MATERIAL AND METHODS

Experiments were made on fullgrown plants of local varieties of brinjal (*Solanum melongena* L.) ; Chillies (*Capsicum annum* L.) and peas (*Pisum sativum* L.) at the fruit-bearing stage. In all, nine experiments each were conducted with brinjal and chillies and eight with peas over two years. Plants were thoroughly and uniformly treated with 0.05 per cent phosphamidon solution using Sandovit as sticker at the rate of 0.5 ml per litre. Sprays were applied with a garden sprayer of one litre capacity. Foliage and fruit samples were picked from the plants at 2 hr. and 1, 4, 7, 10, 13 and 16 day intervals after treatment. Foliage samples consisted of leaves and succulent twigs. Fruits of only harvestable size were selected. Samples were pooled and sub divided according to Gunther and Blinn (1955). Phosphamidon residues in and on the foliage and fruits were analysed by a cholinesterase inhibition method using acetylcholine as substrate. With this method recoveries were above 90 per cent at 0.1 ppm and higher fortification levels. On foliage samples, recoveries for brinjal, chillies and peas varied between 83.00 and 95.40, 81.00 and 95.70 and 92.10 and 101.40 per cent respectively. For fruit samples the corresponding values were 85.90 to 96.68, 84.00 to 101.00 and 87.35 to 95.40 per cent, respectively. At lower levels of fortification (0.05 ppm) recoveries were almost halved. The method was adjudged sensitive up to 0.1 ppm level.

Dissipation curves were drawn for phosphamidon residues on foliage and fruits separately and 'half-life' values were calculated as described by Gunther and Blinn (1955) and Hoskins (1961).

During each experiment maximum and minimum temperatures, relative humidity and rainfall were recorded daily. The residue 'half-life' values obtained and the corresponding average minimum and maximum daily temperatures and relative humidity are given in Tables I, II and III.

To show the effect of temperature and relative humidity on the rate of dissipation, the data have been arranged in the descending order of 'half-life' values.

RESULTS AND DISCUSSION

Initial deposits on the foliage ranged between 8.37 and 10.52, 9.58 and 14.21 and 15.78 and 19.93 ppm, respectively for brinjal, chillies and peas. Depending

upon the weather it needed 5.56 to 9.40, 9.18 to 22.42 and 10.92 to 17.43 days for the initial deposits of 0.87 to 1.81, 2.42 to 3.16 and 2.02 to 3.08 ppm phosphamidon, on fruits of brinjal, chillies and peas to reach the tolerance level of 0.1 ppm prescribed by FAO for vegetables.

Phosphamidon degradation was slower on chillies than on brinjal and for peas it was slowest. Residues on foliage dissipated faster than those on fruits. The

TABLE I
Rate of dissipation of phosphamidon residues on brinjal at varied levels of temperature and humidity

Experiment No.	Date of spraying	Meteorological data during Experimentation				'Half-life' values of phosphamidon residues (in days)	
		Average temperature in °C		Average daily relative humidity (per cent)	Precipitation in mm	Foliage	Fruits
		Minimum	Maximum				
1	17-12-1972	12.22	28.42	74.18	Less than 1 on 8th and 10th day.	2.308	2.874
2	2-3-1972	14.98	31.12	57.05	NIL	2.025	2.673
3	15-9-1972	19.26	36.84	53.18	NIL	1.895	2.182
4	13-7-1972	25.52	36.87	60.47	Traces on 3rd day	1.652	1.978
5	17-4-1973	24.30	41.22	31.25	NIL	1.638	1.900
6	5-4-1972	20.07	36.62	41.00	NIL	1.632	1.865
7	11-6-1973	27.50	37.17	66.00	NIL	1.602	1.785
8	8-5-1973	26.06	40.19	43.93	1.4 on 9th day	1.464	1.705
9	2-5-1973	26.85	41.87	39.56	NIL	1.424	1.675

'half-life' values for residues on foliage and fruits of brinjal ranged between 1.424 and 2.308 and 1.675 and 2.874 days, respectively. Corresponding values for chillies were 1.567 to 3.031 and 1.826 to 4.645 days and for peas 2.029 to 2.670 and 2.242 to 3.516 days.

When 'half-life' values for any one crop were compared with average temperatures and humidities a marked increase in the rate of dissipation was invariably coupled with a rise in average temperatures, either minimum or maximum or both.

TABLE II
Rate of dissipation of phosphamidon residues on chillies at varied levels of temperature and humidity

Experiment No.	Date of spraying	Meteorological data during Experimentation				'Half-life' values of phosphamidon resi- dues (in days)	
		Average tempe- rature in °C		Average daily relative humidity (per cent)	Precipitation in mm	Foliage	Fruits
		Mini- mum	Maxi- mum				
1	17-11-1972	12.22	28.42	74.18	0.7 on 8th day & 0.9 on 10th day	3.031	4.645
2	18-8-1972	23.95	34.14	84.66	NIL	1.987	2.454
3	13-7-1972	25.52	36.87	60.47	Traces on 3rd day	1.903	2.413
4	8-5-1973	26.06	40.19	43.93	1.4 mm on 9th day	1.668	1.976
5	2-5-1973	26.85	41.87	39.56	NIL	1.614	1.946
6	11-6-1973	27.50	37.17	66.00	NIL	1.608	1.905
7	2-5-1972	24.80	41.02	29.17	NIL	1.592	1.854
8	17-4-1973	24.30	41.22	31.25	NIL	1.577	1.839
9	21-5-1973	27.88	41.83	37.43	NIL	1.567	1.826

TABLE III
Rate of dissipation of phosphamidon residues on peas at varied levels of temperature and humidity

Experiment No.	Date of spraying	Meteorological data during Experimentation				'Half-life' values of phosphamidon residues (in days)	
		Average temperature in °C		Average daily relative humidity (per cent)	Precipitation in mm	Foliage	Fruits
		Minimum	Maximum				
1	22-1-1973	6.63	20.60	70.83	NIL	2.670	3.516
2	9-2-1973	8.12	24.25	63.62	NIL	2.542	3.266
3	9-2-1973	8.12	24.25	63.62	NIL	2.505	3.219
4	4-1-1973	9.09	23.78	71.31	NIL	2.431	3.076
5	4-1-1973	9.09	23.78	71.31	NIL	2.396	3.016
6	7-12-1971	8.15	25.71	79.76	NIL	2.335	2.776
7	19-1-1972	11.98	25.23	76.06	NIL	2.163	2.618
8	17-11-1972	12.22	28.42	74.18	NIL	2.029	2.242

When changes in the relative humidities were compared with the changes in the 'half-life' values, the rates of dissipation of phosphamidon residues from fruits and foliage were negatively correlated with relative humidities, but the increases were not always coupled with the reductions in the relative humidities.

Thus, the rate of dissipation of phosphamidon from treated plants was influenced mainly by temperature and less by relative humidity. These findings confirm those of Menzer and Ditman (1963b) in laboratory experimentation under controlled conditions with exceptionally very high initial deposits and extreme ranges of temperatures. However, some of the present data does not agree indicating that as well as temperature and humidity, some other unknown factors might also influence the dissipation of phosphamidon residues from plants.

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RESIDUES OF CARBOFURAN IN SOME CROPS

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SUMMARY

In a number of field trials conducted all over India for evaluating the insecticidal efficacy of carbofuran on rice, sorghum, maize, sugarcane, banana and cotton, residues were estimated after harvest in the edible portions of the plants. Three formulations : 3 per cent granules (3G), 50 per cent soluble powder (50 SP) and a 40 per cent flowable paste (40 F) were used in these studies.

For rice, different methods and times of application of doses ranging from 0.21 to 1.73 kg a.i./ha as 3 G were studied. In general, with increase in dose applied there was an increase in the residue level, and also as the time lag between application and harvest decreased, residues in the grain and straw increased. The maximum residues were 0.097 ppm in the grain and 0.095 ppm in the straw. These are much below the EPA tolerance limit of 0.2 ppm. Soil incorporation of the granules produced lower residues than broadcast applications.

For sorghum, seeds were treated with either 50 SP or 40 F at the rate of 5 per cent a.i. on seed weight basis. At harvest, carbofuran residues were in the range of 0.013 to 0.033 ppm in the grain and 0.035 to 0.094 ppm in the straw. For maize, seed treatment or whorl application produced low residues in the grain and stover at harvest. Seed treatment of cotton gave only traces of residues in the seed. In general, seed treatment with 40 F produced lower residues than 50 SP in all these crops.

Sugarcane juice from a crop treated with 3 G at 0.90 to 1.0 kg a.i./ha contained less than 0.010 ppm carbofuran.

Banana fruits, from plants raised where carbofuran had been used for treatment of suckers for the control of nematodes, contained less than 0.036 ppm residue. The peel of the fruit contained up to 0.136 and the pseudostem up to 0.077 ppm. All the residues were less than the tolerance limits.

Carbofuran, a broad spectrum systemic and contact insecticide and nematicide, is used extensively for soil or seed treatment on a number of crops including rice, sorghum, maize, sugarcane, banana, cotton, etc. Generally it gives effective protection of the crop against many pests for 4 to 6 weeks after application (Kuhr and Dorrough, 1976). Two formulations, 3 per cent granules (Furadan 3G) and 50 per cent soluble powder (Furadan 50 SP) are commercially available, and a newer formulation of 40 per cent flowable (Furadan 40 F) is being tested. While 3G is used mainly for soil application, the other two formulations are used for seed treatment. Although considerable data on the biological efficacy of carbofuran is available, little is known about its residues on various crops. In this investigation the harvest residues of carbofuran in rice, sorghum, maize, cotton, sugarcane and banana, grown under different agro-climatic regions of the country were assessed.

MATERIAL AND METHODS

A number of field trials were made in Maharashtra around Poona and in Tamil Nadu around Coimbatore on rice, sorghum, maize and cotton, in Shahajanpur (U.P.) on sugarcane and in Satyamangalam (Tamil Nadu) on banana.

Carbofuran was applied to rice as Furadan 3G at dosages ranging from 0.38 to 1.73 kg a.i./ha. These levels were applied as one to three applications on a number of varieties. After harvest grain and straw samples were collected from randomly selected plants, dried and analysed for carbofuran residues. In one experiment at Karjat, Maharashtra, broadcast application of Furadan 3G was compared with a soil incorporated where the insecticide granules were broadcast before the last puddling operation prior to transplanting.

Carbofuran seed treatment is effective against shootfly infesting sorghum (Jotwani *et al.*, 1971) and maize (Sharma & Chaudhary, 1974). Cotton seeds treated with carbofuran prevent the attack of early pests for a month after sowing (Rao *et al.*, 1974). In the present investigation, sorghum, maize and cotton seeds were treated with Furadan 50 SP and Furadan 40 F at the rate of 5 per cent a.i. After harvest, samples of grain and straw of sorghum and maize, lint and seed of cotton were analysed. Residues were also determined from maize plants receiving whorl application of Furadan 3G.

Preinage treatment of banana suckers is effective against banana nematodes (Anon., 1976, 1978). Banana suckers were treated with Furadan 3G at 20, 40 and 60 g or Furadan 40 F at 1.5, 3.0 and 6.0 g followed by another application of 3G at 40 g/sucker five months later. Fruits were collected from bunches in a spiral manner from each finger and carbofuran was estimated in the peel and the pulp separately and in the pseudostem as well.

Furadan 3G is effective in controlling top shoot borer (Anon., 1977 and Tambekar and Patel, 1977). In the present study residues were estimated. A sugarcane crop was side-dressed with Furadan 3G at 1.0 kg a.i./ha at different intervals and canes were harvested and the juice extracted was analysed for carbofuran residues.

For analysis, after acid hydrolysis carbofuran and its metabolites were extracted using methylene chloride. For clean up, the extract was then passed through silica-gel, activated alumina and activated carbon column (Cook, 1973). After clean up, carbofuran was determined colorimetrically by the method described by Gupta and Dewan (1973).

RESULTS AND DISCUSSION

Carbofuran residues in rice : Residues of carbofuran on rice are summarised in Table I. Several common varieties were raised on different soil types treated with different levels of the insecticide. The number of applications ranged from one to three in the main field after transplanting in addition to one treatment in the nursery, 5 to 7 days prior to uprooting. As the number of applications increased, the residues in the grain and straw also increased. As the interval between application and harvest decreased, residues were little higher. Carbofuran content ranged from 0.020 ppm to 0.097 ppm in the grain and up to 0.095 ppm in straw. The values did not differ much in samples collected from various places, viz., Tamil Nadu and Maharashtra. Residues of carbofuran in the grain and straw did not seem to differ in various rice varieties. Carbofuran residues resulting from soil incorporation prior to transplanting were lower than the broadcast application. When incorporated into soil, there would have been greater adsorption of the insecticide on the soil colloids resulting in decreased absorption by the plants. Thus, there is a reduction in residue levels when the insecticide is incorporated in the soil. Earlier work (Mithyantha, 1973) has shown that soils have great capacity to retain carbofuran. On the other hand, absorption of the toxicant by the plants may be enhanced by broadcast application or root zone placement, as observed by Aquino and Pathak (1976). Results obtained in the present study, corroborate those of Rajukkannu *et al.* (1976, 1977). All residues were less than the WHO tolerance limit of 0.2 ppm (Table I).

TABLE I
Carbofuran residues in rice

Location	Variety	Dosage (kg a.i./ha)	Method and time of application	Interval bet- ween last application and harvest (days)	Part of plant	Residues of Carbofuran (ppm)
Mannuthy	IR-5	1.00	Soil application of Furadan 3G	30	Grain	0.033
Palghat	Masuri	0.38	Soil application of Furadan 3G at 38 DAT	85	Grain	0.076
Erode	IR-20	1.58	3 applications of Furadan 3G at 15, 39 and 65 DAT	18	Grain	0.024
		0.63	2 applications of Furadan 3G at 16 and 48 DAT	41	Grain	0.041
					Straw	0.030
Palghat	Masuri	1.05	2 applications of 3G at 16 and 48 DAT	74	Grain	0.041
Satyaman- galam	Ponni	1.73	4 applications of 3G at 36, 58, 80 & 123 DAT	40	Grain	0.097
Poona		1.25	1 application of 3G at 20 DAT		Grain	0.050
					Straw	0.008
Karjat	Ratna	0.675	Broadcast application of Furadan 3G in nursery at 25 DAT	68	Grain	0.046
					Straw	0.053
		1.20	Broadcast application of Furadan 3G in nursery at 25 and 55 DAT	38	Grain	0.061
					Straw	0.059
		1.56	Broadcast application of 3G in nursery at 25 and at 55 DAT	38	Grain	0.081
					Straw	0.095
		0.675	Soil incorporation of 3G, in nursery and main field	93	Grain	0.020
					Straw	0.008
		0.90	Soil incorporation of 3G, in nursery and main field	93	Grain	0.030
					Straw	0.037
Gobichetty- palyam	IR-20	0.75	2 applications of 3G, at 30 and 60 DAT	25	Grain	0.021
Nanjaikala- mangalam	IR-20	1.05	2 applications of 3G, at 44 and 56 DAT	32	Grain	0.027

Sorghum, maize and cotton : Seeds of these crops were treated with carbofuran formulations viz., 50 SP or as 40 F at the rate of 5 per cent a.i. These residues of the toxicant at harvest in sorghum grain ranged from 0.012 to 0.033 ppm and that in the straw from 0.035 to 0.094 ppm (Table II). Maize grains had 0.007 ppm and the straw had 0.022 ppm carbofuran. Cotton seeds contained 0.071 to 0.077 ppm of the toxicant. Residue levels in the crops at the time of harvest was very low (less

TABLE II
Carbofuran residues in jowar, maize and cotton

Crop	Variety	Trial Local	Method and time of application	Time lag between treatment and harvest (days)	Plant part	Residues of carbofuran (ppm)
1	2	3	4	5	6	7
Jowar	CSH-1	Bangalore	Seed treatment with Furadan 50 SP at 5% a.i. on seed		Grain	0.033
					Leaves	0.030
	CSH-1	Parbhani	Seed treatment with Furadan 50 SP at 5% a.i. on seed		Grain	0.012
					Straw	0.065
			Soil application of Furadan 3G at 7 kg/acre at sowing		Grain	0.013
					Straw	0.035
	CSH-1	Ahmed-nagar	Seed treatment with Furadan 50 SP at 5% a.i. on seed	116	Grain	0.023
					Straw	0.077
			Seed treatment with Furadan 40 F, at 5% a.i. on seed	116	Grain	0.017
					Straw	0.041
	CSH-5	Mundwa	Seed treatment with Furadan 50 SP at 5% a.i. on seed	110	Grain	0.023
					Straw	0.094
			Seed treatment with Furadan 40 F at 5% a.i. on seed	110	Grain	0.017
					Straw	0.069
Maize	Ganga-5	Palghat	Whorl application of 3G at 0.06 kg a.i./acre one month after sowing.	59	Grain	0.006
					Stover	0.016

1	2	3	4	5	6	7
	Ganga-5	Satyaman-galam	Seed treatment with Furadan 50 SP at 5% seed+whorl application of 3G at 3 kg/acre	108	Grain Stover	0.007 0.022
Cotton	H-4	Poona	Seed treatment with Furadan 50 SP at 5% a.i. on the seed	180	Lint Seed	N.D. 0.077
			Seed treatment with Furadan 40F at 5% a.i. on seed	180	Lint Seed	N.D. 0.071

N.D. = Not detected

than the 0.2 ppm WHO tolerance limit), because of the long interval between application and harvest. Gupta and Dewan (1974) and Kapoor and Kalra (1975) found that residues in jowar and maize grain and straw at the time of harvesting resulting from seed treatments were very low. Whorl applications of Furadan 3G to maize left only 0.006 ppm carbofuran in the grain. The use of newer formulation of Furadan 40 F left lower residues in the plants than the 50 SP treatment.

Sugarcane and Banana : Side-dressing with Furadan 3G, for the control of top shoot borer left less than 0.01 ppm of residues in the juice (Table III). This is much lower than WHO tolerance limit of 0.10 ppm. Awasthi *et al.* (1977) also observed that soil application of carbofuran granules even at 2 kg a.i./ha to sugarcane failed to leave any residue in the juice.

TABLE III
Residues of carbofuran in sugarcane

Resulting from the application of Furadan 3G at 1.0 kg a.i./ha at different intervals.

Sample No.	Details of the sample	Plant Part	Residue of carbofuran (ppm)
1	2	3	4
Soil Samples			
1	3rd brood	Soil	0.039
2	4th brood	Soil	0.033
3	3rd and 4th brood	Soil	0.022
4	Control	Soil	0.010
Plant Sample			
1	3rd brood, bottom	Juice	0.010
2	3rd brood, middle	Juice	N.D.

1	2	3	4
3	3rd brood, top	Juice	0.003
4	3rd brood, leaves	Leaves	0.084
5	4th brood, bottom	Juice	0.005
6	4th brood, middle	Juice	N.D.
7	4th brood, top	Juice	N.D.
8	4th brood, leaves	Leaves	0.068
9	3rd and 4th brood, bottom	Juice	N.D.
10	3rd and 4th brood, middle	Juice	0.004
11	3rd and 4th brood, top	Juice	N.D.
12	3rd and 4th brood, leaves	Leaves	0.084
13	Control cane, bottom	Juice	0.004
14	Control cane, middle	Juice	N.D.
15	Control cane, top	Juice	N.D.
16	Control cane, leaves	Leaves	0.024

N.D. = Not detected

Banana fruits from plants treated with carbofuran for the control of nematodes contained 0.015 to 0.036 ppm of its residue in the peeled fruits, 0.045 to 0.136 ppm

TABLE IV
Results of analysis of banana samples for carbofuran residues

Quantity of Furadan 3G applied at planting (g/sucker)	Quantity of Furadan 40F applied at planting (g/sucker)	Quantity of Furadan 3G added 5 months after planting (g/sucker)	Residues of carbofuran+ 3 hydroxy carbofuran (ppm)		
			Peeled fruit	Peel of the fruit	Pseudo- stem
20	—	40	0.036	0.077	0.045
40	—	40	0.030	0.119	0.077
60	—	40	0.036	0.136	0.077
—	1.5	40	0.015	0.036	0.030
—	3.0	40	0.015	0.060	0.036
—	6.0	40	0.015	0.094	0.060
—	—	40	0.015	0.045	0.053
— control —	—	Nil	N.D.	0.021	0.015

N.D. = Not detected

in the fruit and 0.030 to 0.077 ppm in the pseudostem (Table IV). These residues are less than WHO tolerance limit of 0.1 ppm in the fruit and the pseudostem.

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25 DISSIPATION OF PHORATE, DISULFOTON, ALDICARB AND CARBOFURAN IN POTATO TUBERS

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SUMMARY

Insecticide residues following single and split soil treatments with phorate, aldicarb, disulfoton and carbofuran to potato crops were assessed in potato tubers. All the insecticides involved in the experiment resulted in excessive residues of their tolerances on potato tubers in unprocessed tubers for 2 months after application from either of the treatments but processing of tubers through washing, peeling and boiling greatly reduced residues to a safe level. However after another month, residues, though detectable only from carbamate compounds, were below safe limits from all treatments. Split doses of insecticides resulted in slightly higher residues.

Potatoes are now grown in most climatic and topographical conditions in India. Many insects, diseases and nematodes attack this crop causing serious damage. Granular applications of phorate, disulfoton, aldicarb and carbofuran at planting time were found to be successful in controlling these pests in the field. Since these compounds are toxic and leave residues in crops like potato they may cause health hazards. In the present investigations residues in potato tubers were determined to find the safe waiting period before use.

MATERIAL AND METHODS

A potato crop was raised at Central Potato Research Station, Jullunder (Punjab) during the winter season of 1976 in 4.8 x 4.0 m plots. Each plot contained 8 rows of 16 plants at a plant to plant distance of 25 cm within the rows and 60 cm between the rows. Four systemic granular insecticides viz., phorate 10G, disulfoton

Contribution from Division of Agricultural Chemicals, I.A.R.I., New Delhi.

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5G, aldicarb 10G and carbofuran 3 G were applied and mixed in the soil of the experimental plots in two different ways of application *i.e.*, single and split dosages. In the first set a single dose of 1.5 kg a.i. per hectare, was applied in the furrows at planting before seed tuber placement and to the second set 0.75 kg a.i. per hectare was applied before seed tuber placement and the other 0.75 kg a.i. per hectare was applied near the base of plants at first earthing time. All 9 treatments including a control were replicated 3 times in a randomised block design. Normal agronomic practices were followed during the crop in the field.

Potato tuber samples from each of the replicate of different treatments were collected 60, 70, 80 and 90 days after planting for determination of residues of the insecticides.

Extraction, clean up and estimation of residues of phorate, disulfoton and aldicarb : Residues were extracted from raw potato tubers *i.e.*, unpeeled and un-boiled tubers and processed tubers. Two types of processing, peeling and boiling, were used on the treated tubers to see any reduction of residues. A representative 100 g sample from each of the sample was taken for extraction. Tubers were cut into small pieces and stripped with 200 ml of distilled chloroform for one hour. The extract was filtered and the residue extracted again with a like quantity of solvent for another hour. The two aliquots were combined and concentrated to a known volume in a Kuderna Danish evaporator. The extract was dried over anhydrous sodium sulphate and stored at low temperature.

An aliquot from the extracts of the samples was then passed through a 2 cm diameter column containing a 1.25 cm layer of anhydrous sodium sulphate overlaid with 5 g of adsorbent mixture (2:2:1, activated charcoal : magnesium oxide : celite 545) for clean up of phorate and disulfoton samples. The eluate was then concentrated to 1 ml in a Kuderna Danish evaporator and insecticide residues of phorate and disulfoton were determined with the colorimetric method of Getz and Watt (1964) as modified by Jain *et al.* (1974). The samples of aldicarb extract were cleaned up with a coagulation method and residues determined by Johnson and Stansbury (1966) method.

Extraction, clean up and estimation of carbofuran residues : A 100 g representative sample of potato tubers was transferred into a round bottom flask and 500 ml of 0.25 N hydrochloric acid was added. The contents were refluxed for an hour with occasional shaking throughout the refluxing period. After completion of refluxing, the contents were filtered through the glass wool into a 500 ml storage bottle. A 250 ml supernatant liquid representing 50 g sample was transferred into a 500 ml separatory funnel and was extracted with 50 ml methylene chloride three times. The combined extract was concentrated to less than 100 ml and the volume

made up to 100 ml. Residues resulting from carbofuran were determined by the colorimetric method as reported by Gupta and Dewan (1973).

RESULTS AND DISCUSSION

Analytical data on residues of phorate, disulfoton, aldicarb and carbofuran in or on unprocessed tubers and processed tubers are in Tables I and II.

Residues of phorate and disulfoton : Table I gives average residues of phorate and disulfoton following single and split dosage applications to potato tubers. The average residues of phorate and disulfoton on potato tubers after a single dosage of 1.5 kg a.i. per hectare at planting were 0.90 and 0.85 ppm respectively and the two treatments with 0.75 kg left slightly higher residues of 1.20 ppm 60 days after

TABLE I
Residues of phorate and disulfoton in potato tubers

Days after planting	Mode of application	(Residues in ppm)					
		Phorate			Disulfoton		
		Unpeeled	Peeled	Boiled	Unpeeled	Peeled	Boiled
60	Single	0.90	0.30	0.08	0.85	0.32	0.08
	Split	1.20	0.51	0.08	1.20	0.47	0.12
70	Single	0.66	0.22	0.07	0.45	0.18	ND
	Split	0.75	0.32	0.07	0.63	0.22	ND
80	Single	ND	ND	ND	ND	ND	ND
	Split	0.12	ND	ND	ND	ND	ND
90	Single	ND	ND	ND	ND	ND	ND
	Split	ND	ND	ND	ND	ND	ND

ND - Not detectable

planting the tubers. These residues were in excess of their tolerances (0.5 ppm for phorate and disulfoton fixed by WHO/FAO (1975). After 10 days, residues reduced to 0.66 ppm of phorate and 0.45 ppm of disulfoton. Residues of phorate and disulfoton from split application were 0.75 ppm and 0.63 ppm respectively. This reduction in residues could be due to dilution factor as the tubers grew in size and simultaneous reduction due to enzymic activity. There was an insignificant difference in residues following the two different methods of application. Residues were more than their tolerances, but after another 10 days the residues reduced to undetectable limits from both single and split applications. Therefore a waiting period of 80 to 90 days after application of these insecticides on potato tubers should be observed

for safe use. However this waiting period could be further decreased if the treated tubers were peeled. Peeling removed more than 50 per cent of residues of either chemical. Suett and Wheatley (1970) reported 80 per cent residues of fonofos in carrot peel. Jacob *et al.* (1970) found 0.5 ppm of arsenic in the flesh of potato tubers and up to 84 ppm in peel. Phorate and disulfoton residues in the potato pulp ranged between 0.30 and 0.51 ppm. So peeling could reduce residues of these

TABLE II
Residues of aldicarb and carbofuran in potato tubers

Days after planting	Mode of application	(Residues in ppm)					
		Aldicarb			Carbofuran		
		Unpeeled	Peeled	Boiled	Unpeeled	Peeled	Boiled
60	Single	1.52	0.47	0.15	1.35	0.41	0.11
	Split	2.05	0.83	0.18	1.48	0.68	0.11
70	Single	0.95	0.31	0.15	0.80	0.27	0.08
	Split	1.30	0.57	0.15	1.11	0.47	0.08
80	Single	0.43	0.11	ND	0.13	0.06	ND
	Split	0.55	0.17	ND	0.15	0.11	ND
90	Single	0.07	ND	ND	ND	ND	ND
	Split	0.07	ND	ND	0.03	ND	ND

ND – Not detectable

insecticides to safe limits. Processing treated tubers in boiling water reduced residues of phorate and disulfoton. Boiling tubers reduced the insecticide residues by 90 per cent from both the insecticide treatments. Awasthi *et al.* (1977) found that phorate and oxydemeton methyl residues reduced to non-detectable limits after processing of tubers with washing followed by boiling.

Residues of aldicarb and carbofuran : Residues of aldicarb and carbofuran on unprocessed and processed tubers following single and split dosage application are summarised in Table II. The residues of aldicarb and carbofuran 60 days after planting were in the range of 1.52 and 1.35 ppm respectively after a single treatment. However split dosage application resulted in slightly higher residues of the order of 1.05 and 1.48 ppm respectively. A progressive reduction in residues after 10 days could be attributed to dilution factors and enzymic degradation. After another 10 days, that is 80 days after planting, residues fall below the tolerance limits, 1 ppm for aldicarb on potatoes and 0.2 ppm for carbofuran on rice grains. Residues were also found in the peelings of the potatoes. The pulp of potato tubers treated with aldicarb and carbofuran contained between 0.41 and 0.83 ppm,

from either treatment. Processing of tubers by boiling reduced residues of aldicarb and carbofuran.

Carbamate compounds left higher residues than organophosphate compounds, perhaps due to their slower rate of decomposition.

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DISSIPATION OF CARBARYL RESIDUES IN AND
ON CAULIFLOWER

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SUMMARY

Field trials were conducted for two consecutive years (1977 and 1978) to find out the persistence of carbaryl (Sevin) in and on cauliflower and its leaves, after application of dusts and wettable. In the first trial, an 89 day old crop was treated with carbaryl 10 per cent dust at 1.5-2.0 kg ai/ha, 40 per cent low volume at 1.0 kg ai/ha and 50 per cent wettable powder at 0.75-1.0 kg ai/ha. The extent of carbaryl residue in and on cauliflowers, 1 day after application varied between 12.67 and 34.57 ppm, and declined to 3.64-9.59 ppm within 8 days. In the other trial, the crop was sprayed twice when 44 and 59 days old with carbaryl 40 per cent low volume and 50 per cent wettable powder at recommended and excessive rates. The cumulative carbaryl deposits in and on cauliflowers after the second application varied from 4.35-27.43 ppm and dissipated to 2.65-7.35 ppm within 3 days. The cumulative carbaryl deposits in and on leaves were between 19.45 and 42.08 ppm, far less than the WHO/FAO tolerance limit of 100 ppm.

Water washing diminished carbaryl residues by 36-95 per cent, so, for cauliflower a waiting period of 8 days is recommended, but for leaves, no waiting period need be observed and it can be fed to cattle immediately after application.

Carbaryl has a broad spectrum of insecticidal activity and a low mammalian toxicity (Haynes *et al.*, 1957). It is neither stored in the fat, nor is secreted in the milk of dairy cattle (Gyrisco *et al.*, 1960), which permits its application on edible crops and fodder. Carbaryl has been found to be very effective for controlling cauliflower insects, *viz.*, diamond back moth caterpillar, cabbage caterpillar, mustard sawfly, etc. (Verma *et al.*, 1971, 1972). Experiments were carried out for two consecutive years (1977-78), to find out the persistence of carbaryl in and on cauliflower heads and leaves.

MATERIAL AND METHODS

In 1976-77, a cauliflower crop was grown at the Union Carbide India Limited Experimental Farm, Bhopal (MP). Treatments were carbaryl 10 per cent dust at 1.5 and 2.0 kg ai/ha, 40 per cent low volume at 1.0 kg ai/ha and 50 per cent wettable powder at 0.75 and 1.0 kg ai/ha. A cauliflower crop was treated 89 days after transplanting with three replications in randomized blocks. Samples of cauliflower were taken at random from each replicate at intervals of 1, 2, 3, 8 and 11 days

and analyzed by the diazonium-salt-colorimetric procedure (Benson and Finocchiaro, 1965 and Johnson, 1964).

In the other field trial, a cauliflower crop was grown at Asha Niketan Farm, Bhopal (MP) during the *rabi* season of 1977-78. The first spray of carbaryl 40 per cent low volume at 1.0 and 1.5 kg ai/ha and carbaryl 50 per cent wettable powder at 0.75 and 1.25 kg ai/ha was applied when the crop was 44 days old. A second spray was applied when the crop was 59 days old. Each treatment was replicated three times in a randomized block design. Samples of cauliflower heads and leaves were taken before the second application and after the second application at intervals of 0, 1, 3, 7 and 15 days and analyzed. Cauliflower samples were washed under tap water and analyzed.

RESULTS AND DISCUSSION

The results of the field trial conducted in 1976-77 are summarized in Table I. Carbaryl residues due to the application of 10 per cent dust after 1 day were 19.72 and 34.57 ppm, respectively, and 6.92 and 9.59 ppm within 8 days. Carbaryl 40

TABLE I
Carbaryl Residues in/on Cauliflower (October, 1976—February, 1977)

Formulation	Dose (kg ai/ ha)	% Conc.	Age of the plant (Days)	Carbaryl Residues (ppm*)				
				1 day	2 days	3 days	8 days	11 days
Carbaryl	1.50	—	89	19.72	20.25	17.40	6.92	2.83
10%	2.00	—	89	34.57	34.57	33.50	9.59	3.67
40% LV	1.00	0.25	89	13.62	13.85	12.86	6.11	3.55
50% WP	0.75	0.15	89	12.67	11.91	11.74	3.64	2.76
	1.00	0.20	89	28.99	26.41	26.59	7.93	7.78

* Average of three replicates.

per cent low volume applied left residues of 13.62 ppm, which declined to 6.11 ppm in 8 days. Similarly, the carbaryl residues from 50 per cent wettable powder were 12.67 and 28.99 ppm and 3.64 and 7.93 ppm, respectively, after 8 days. All carbaryl residues were less than the WHO/FAO tolerance limit of 10 ppm by the 8th day after application.

Table II and III present the analytical data for samples of cauliflower and leaves in 1977-78. Carry over of carbaryl residues in and on cauliflowers due to the first application was less than 0.5 ppm. The residues of carbaryl from 40 per cent low

TABLE II
Carbaryl Residues in/on Cauliflower (October, 1977—February, 1978)

Formulation	Dose (kg ai/ ha)	% Conc	Age of the plant (days)	Carry over Residue (ppm)	Carbaryl residues after II appln.(ppm)*				
					0 day	1 day	3 days	7 days	15 days
Carbaryl	1.00	0.20	59	0.16	10.08	6.30	5.39	0.73	0.19
40% LV	1.50	0.30	59	0.28	27.43	11.56	7.35	1.14	<0.16
50% WP	0.75	0.15	59	0.50	4.35	5.01	2.65	0.60	<0.16
	1.25	0.25	59	0.37	—	10.89	7.25	0.75	<0.16

* Average of three replicates.

volume were 10.08 ppm, which declined to 6.3 ppm within 24 hours. The residues of carbaryl from 50 per cent wettable powder were 4.35 ppm, less than the WHO/FAO tolerance limit of 10 ppm, so a waiting period of 24 hours should be observed. Where the crop was sprayed with carbaryl at excessive rates, the residues of carbaryl after the second spraying were higher than the WHO/FAO tolerance level of 10 ppm.

TABLE III
Carbaryl Residues in/on Cauliflower Leaves (October, 1977—February, 1978)

Formulation	Dose (kg ai/ ha)	% Conc	Age of the plant (days)	Carry over Residues (ppm)	Carbaryl residues after II appln.(ppm)*				
					0 day	1 days	4 days	7 days	15 days
Carbaryl	1.00	0.20	59	0.83	19.45	13.75	6.48	3.93	0.67
40% LV	1.50	0.30	59	1.48	29.88	23.57	12.97	3.24	0.59
50% WP	0.75	0.15	59	1.36	27.74	17.78	9.32	5.44	0.89
	1.25	0.25	59	1.78	42.04	34.48	23.14	3.50	0.88

* Average of three replicates.

Residues on leaves were less than 2.0 ppm. Residues due to the second application of 40 per cent low volume were 19.45 and 29.88 ppm, respectively. Residues due to 50 per cent wettable powder were 27.74 and 42.04 ppm, respectively.

Table IV summarizes the effect of water washing on residues. Cauliflower samples from both trials were washed under tap water which reduced residue by 36–95 per cent.

Different dissipation rates of residue observed in the two trials were probably due to the difference in the age of the crop when it was sprayed.

TABLE IV
Effect of Water Washing on Carbaryl Residue in/on Cauliflower

Formulation	Year	Dose (kg ai/ ha)	Carbaryl Residue		% Reduction
			Before	After	
Carbaryl 10% D	1977	1.5	19.72	0.94	95.24
		2.0	34.57	5.58	83.86
40% LV	1977	1.0	13.62	5.05	62.93
	1978	1.0	10.08	2.89	71.33
		1.5	27.43	8.94	67.41
50% WP	1977	0.75	12.67	8.10	36.07
	1978	0.75	4.35	1.76	59.54
	1977	1.00	28.99	11.50	60.34
	1978	1.25	5.44	0.71	85.95

A waiting period of 8 days is recommended for cauliflowers crop after spraying with carbaryl. For the consumption of leaves by cattle, no waiting period is required.

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27 **PERSISTENCE OF TOXICITIES OF CERTAIN APHICIDES
IN COMBINATION WITH UREA AGAINST *BREVICORYNE*
BRASSICAE LINN. ON CAULIFLOWER**

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SUMMARY

Brevicoryne brassicae Linn., is a serious pest of cauliflowers and reduces the yield as well as head quality. The foliar application of urea in conjunction with insecticides on many crops has proved useful and also economical. Persistence of the toxicity of 0.05 per cent croneton, 0.025 per cent dimecron, 0.025 per cent metasystox and 0.05 per cent tamaron alone and in combination with urea (0.02 per cent N₂) was investigated against **B. brassicae** Linn. by recording its mortality at different intervals after treatment on Snow Ball variety of cauliflower.

The toxicity of 7 and 10 day old spray deposits of metasystox alone and with urea did not differ significantly, but significant reduction in the toxicity of 15 and 21 day old spray deposits of metasystox mixed with urea was observed. Urea has a detrimental effect on dimecron and reduced its toxicity considerably 15 and 21 days after the treatment, but caused a synergistic effect when mixed with tamaron and the toxicity of 10 and 15 day old spray deposits increased significantly as compared to the toxicity of tamaron alone. However, the toxicity of 21 and 27 day old spray deposits of tamaron was equal to that of tamaron used in combination with urea. A slight reduction in the toxicity of croneton when mixed with urea was observed but the reduction was not statistically significant. It could be concluded from the observations that tamaron is fully compatible with urea though less persistent than other insecticides. Croneton mixed with urea was more persistent, giving a 37 per cent mortality of aphids after 27 days. Metasystox did not appear to be safely compatible with urea, where its longer persistence is required and dimecron may also not be mixed with urea.

The 15 day old spray deposits of croneton, metasystox and dimecron and with urea alone(*) gave 63.18 (53.76*), 60.74 (40.20*) and 29.31 (19.38*) per cent mortality, respectively, in the first year, when temperature and humidity varied from 19.4°C to 21.1°C & 43-57 % respectively.

Foliar application of insecticides and urea together has been found to be useful in increasing the yield of crops (Sekhawat *et al.*, 1971 ; Srivastava and Singh, 1974 and Krishna Murthy, 1975). The observations of Sexena *et al.* (1972) and Israel *et al.* (1969) indicated that toxicity of various contact and systemic insecticides when used in combination with urea did not differ significantly, but did influence the duration of effectiveness of the insecticide. The present investigations were undertaken to evaluate the persistence of toxicities of certain aphicides when used with urea against *Brevicoryne brassicae* Linn. on Snow Ball variety of cauliflower for two consecutive years.

MATERIAL AND METHODS

Cauliflower plants having almost equal amount of foliage were selected for treatments. Commercial formulations of metasystox, croneton, tamaron and dimecron were used for foliar application. Each insecticide was diluted (i) directly with water and (ii) solution of 0.2 per cent N₂ in the form of urea to obtain desired concentrations. The plants were sprayed before appearance of heads with a hand sprayer to the drain off stage. Water solutions of 0.2 per cent N₂ in the form of urea and water were also sprayed separately.

Leaves were collected at random from plants under each treatment at different intervals and brought to the laboratory. Twenty mature nymphs of almost equal size were released on treated leaves in glass petri dishes. The mortality of nymphs in each treatment was recorded after 24 hours, with moribund nymphs counted as dead. Nymphs were also released on the leaves collected from water sprayed plants which served as untreated control. Each treatment including the control was replicated three times.

The data obtained were analysed statistically after angular transformation and persistence of toxicity based on the mortality of aphids was calculated for all the insecticides used with urea and alone at intervals of 1, 7, 10, 15, 21 and 27 days after treatments during the years 1977 and 1978. Meteorological data, viz., temperature and humidity prevailing during the experiment were also recorded.

RESULTS AND DISCUSSION

The toxicity of 7 and 10 days old spray deposits of metasystox alone and with urea did not differ significantly. Urea appeared to exert adverse effect on dimecron resulting in a slight reduction in toxicity initially, but considerable reductions 15 and 21 days after the treatment. The observations of Saxena *et al.* (1972) supported these findings where a slight reduction in the toxicity of dimecron was reported

TABLE I

Persistence toxicity of aphicides in combination with urea and alone against Brevicoryne brassicae Linn.

Treatments	Average percentage mortality* of aphids at various intervals during 1977 and 1978													
	1 day		7 days		10 days		15 days		21 days		27 days			
	77	78	77	78	77	78	77	78	78	78	78	78		
1	2	3	4	5	6	7	8	9	10	11				
1. Urea alone	2.24 (8.61)	00	0.56 (4.31)	00	4.54 (12.29)	00	2.24 (8.61)	00	00	00	00	00		
2. Metasystox .025	100.00 (90.00)	100.00 (90.00)	90.00 (71.65)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	76.05 (60.74)	100.00 (90.00)	98.87 (83.86)	14.77 (22.60)				
3. Metasystox .025 + Urea	99.44 (85.69)	100.00 (90.00)	100.00 (90.00)	95.46 (77.71)	99.44 (85.69)	100.00 (90.00)	41.66 (40.20)	95.23 (67.40)	71.76 (57.86)	11.59 (19.89)				
4. Dimecron .025	100.00 (90.00)	100.00 (90.00)	57.80 (49.49)	100.00 (90.00)	41.49 (40.11)	96.68 (79.55)	23.95 (29.31)	85.23 (67.40)	66.77 (54.84)	13.20 (21.34)				
5. Dimecron .025 + Urea	100.00 (90.00)	100.00 (90.00)	48.52 (44.15)	100.00 (90.00)	44.77 (42.04)	94.79 (76.84)	10.92 (19.38)	51.74 (45.96)	26.37 (30.95)	13.08 (21.07)				
6. Croneton' .025	96.68 (79.55)	100.00 (90.00)	78.16 (62.14)	100.00 (90.00)	65.28 (53.87)	88.41 (70.12)	24.10 (29.42)	83.59 (66.14)	71.56 (57.86)	29.98 (33.16)				
7. Croneton' .025 + Urea	85.85 (67.91)	100.00 (90.00)	53.40 (46.95)	98.83 (83.86)	62.43 (52.16)	83.83 (65.95)	52.79 (46.65)	76.64 (61.14)	58.34 (49.81)	14.40 (22.29)				
8. Croneton' .05	100.00 (90.00)	100.00 (90.00)	85.70 (67.78)	100.00 (90.00)	85.85 (67.91)	100.00 (90.00)	79.67 (63.18)	100.00 (90.00)	91.84 (73.41)	53.31 (45.93)				
9. Croneton' .05 + Urea	80.23 (63.60)	100.00 (90.00)	81.72 (64.69)	99.44 (85.69)	92.03 (73.55)	100.00 (90.00)	65.12 (53.76)	97.76 (81.39)	99.44 (85.70)	36.72 (37.26)				

	1	2	3	4	5	6	7	8	9	10	11
10. Tamaron "		N.T.	100.00	N.T.	96.68	N.T.	78.34	N.T.	13.20	3.16	4.98
.05			(90.00)		(79.55)		(62.40)		(21.34)	(16.60)	(12.92)
11. Tamaron "		N.T.	100.00	N.T.	97.76	N.T.	90.04	N.T.	43.21	2.39	1.13
.05+Urea			(90.00)		(81.39)		(71.57)		(41.15)	(8.86)	(6.14)
12. Control		18.13	00	0.56	00	2.24	00	1.13	00	00	00
(Water spray)		(25.19)	00	(4.31)	00	(8.61)	00	(6.15)	00	00	00
C.D. at 5%		15.65	N.S.	22.80	10.08	18.33	8.36	19.68	5.65	10.67	7.17
Av. Temp. °C		19.5	14.5	19.4	16.0	18.83	12.66	21.1	15.0	16.25	14.83
Av. Humidity %		57.0	79.0	52.0	70.25	48.83	74.2	43.2	72.0	73.9	77.4

* Average of three replications.

Croneton 500EC is (2-ethylthiomethyl-Phenyl) N-methyl carbamate & " Tamaron 600 LC is (O, S-dimethyl ester amide of thiophosphoric acid), were obtained from Bayer (India) Limited.

N.T.—Not tested.

N.S.—Non-significant.

Figs. in paranthesis indicate the mean data after angular transformation.

Figs. of .025 and .05 under treatment denote per cent concentration.

when used with urea. Urea seems to have some additive effect when mixed with tamaron because the toxicity of 10 and 15 day old spray deposits increased significantly over the toxicity of tamaron when used alone. However, the toxicity of 21 and 27 day old spray deposits of tamaron did differ significantly from that of tamaron used with urea. Initially, a reduction, though not statistically significant, in the toxicity of croneton when mixed with urea was noticed but subsequently, there was no adverse effect of urea on its toxicity. Croneton 0.05 per cent mixed with urea was more persistent than other insecticides causing a 37 per cent mortality of aphids 27 days after treatment.

It was observed that 15 day-old spray deposits of croneton, metasystox and dimecron alone and with urea gave 63.18 (53.76*), 60.74 (40.20*) and 29.31 (19.31*) per cent mortality, respectively in the first year, when temperature and humidity varied from 19.4°C to 21.1°C and 43-57 per cent, respectively, whereas, the aforesaid insecticides and tamaron gave 90.00 (81.39*), 90.00 (67.40*), 67.40 (45.96*) and 21.34 (41.15*) per cent mortality, respectively, at temperature varying from 14.5°C to 15.0°C and humidity 72 to 79 per cent, during the second year.

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* Figures in paranthesis denote mean per cent mortality caused by insecticides when mixed with urea.

RESIDUES OF MONOCROTOPHOS IN CABBAGE, CAULIFLOWER AND KNOL-KHOL

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SUMMARY

Residues of monocrotophos from crop samples of cabbage, cauliflower and knol-khol were assayed utilising an enzyme inhibition technique. The recoveries of monocrotophos in fortified samples of the cole crops were tested. The recovery of the chemical was 100, 90 and 70 per cent from cabbage, cauliflower and knol-khol, respectively. The initial deposits and the residues of monocrotophos in the cole crop produce were also determined spectrophotometrically. Deposits of 1.63 and 1.82 ppm of monocrotophos resulting from 0.05 and 0.01 per cent concentrations of sprays dissipated to 0.05 ppm after 14 days in cabbage. The deposits of lower and higher concentration of sprays on cauliflower in the order of 1.83 and 2.22 ppm attenuated to 0.07 and 0.27 ppm, respectively, in two weeks. In knol-khol, the magnitude of residues at the end of a two week period were 0.09 and 0.50 ppm respectively. The safety intervals for monocrotophos on cabbage, cauliflower and knol-khol were found to be 8.1, 8.5 and 10.4 in lower and 17.8, 6.32 and 13.7 days respectively, at the higher concentrations.

INTRODUCTION

Insecticides are a source of potential danger to human health, if ingested with food. This work is an attempt to study the dissipation of a promising insecticide - monocrotophos, which is in indiscriminate use on brassica crops around Bangalore.

MATERIAL AND METHODS

Cabbage, cauliflower and knol-khol crops were planted at the Research Station, Hebbal.

All three crops - cabbage (Pride of India), cauliflower (Snow ball) and knol-khol (White Vienna) were sown on raised beds of 1 m width and 2 m length and transplanted in plots, each with fifteen rows of 5 m length, rows spaced at 0.6 m from crest to crest of the ridges.

Monocrotophos 0.05 and 0.1 per cent sprays, replicated twice on each of the crops and an untreated check of the same dimension were used.

A general and uniform spray of monocrotophos 0.05 per cent was given to the rest of the field. 100 kg N, 100 kg P₂O₅ and 50 kg K₂O/ha fertilizers were applied

in three split doses *i.e.*, one basal and two topical applications. When the crops nearly matured, plots were sprayed with monocrotophos and plants sampled periodically for analysis. There were no rains during the period of sampling except one small shower during which the plants were protected with polythene sheets.

An aqueous solution of the insecticide was added to a reaction mixture containing an appropriate type of cholinesterase in a buffer at pH 8.0. After incubation, a substrate containing butyryl thiocholine iodide (Bu Tch I) and 5,5'-dithiobis-2-

TABLE I
*Deposits and residues of monocrotophos in cabbage**

Type of sample	No. of days after spraying	Monocrotophos in ppm	
		Concentration of spray fluid 0.05%	Concentration of spray fluid 0.1%
Washed cabbage head	0	1.570	1.600
Outer leaves of cabbage head	0	3.325	3.375
Cabbage head whole	0	1.630	1.820
Cabbage head whole	1	1.500	1.600
Cabbage head whole	4	0.550	1.300
Cabbage head whole	7	0.370	0.535
Cabbage head whole	10	0.100	0.138
Cabbage head whole	14	0.050	0.055

* Average of two replications

Weather data for the period from 9.11.1975 to 5.12.1975

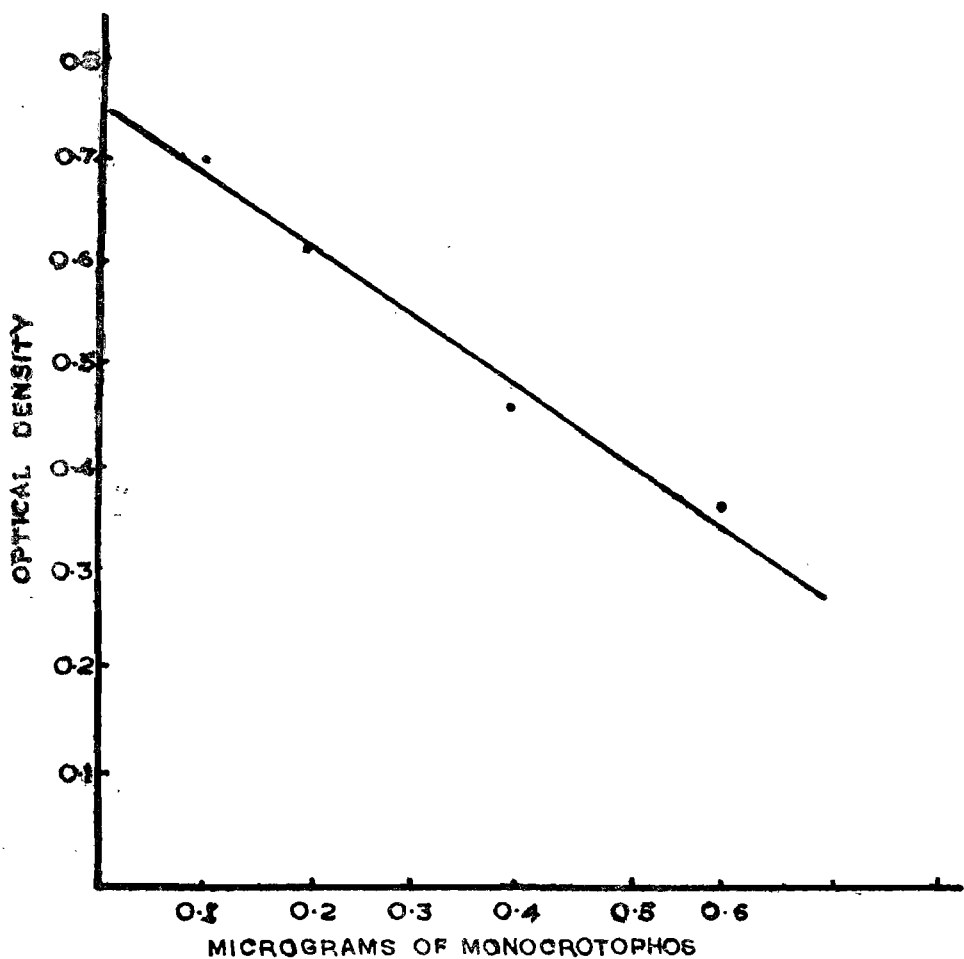
Mean maximum temperature	—	25.3°C	
Mean minimum temperature	—	15.3°C	
Relative humidity (average)	—	at 7.20 a.m.	82%
		at 2.20 p.m.	52%
Total rainfall during the period	—	9.3 mm	

nitrobenzoic acid (DTNB) was added. The thiocholine formed upon substrate hydrolysis by non-inhibited cholinesterase, reduces DTNB to its yellow anion *i.e.*, 5-thio-2-nitro benzoic acid the absorbance of which was colorimetrically determined at 420 μ .

The plant materials were processed on the day of sampling. A sample of about 500 g was chopped into small pieces, divided into quarters and the opposite quarters mixed again and divided twice and finally 25 g of shredded material were transferred to a Waring Blender and macerated at high speed for 2-3 min. in 100 ml of chloroform. The suspension was filtered through a Buchner funnel using What

No. 1 filter paper into a 500 ml Erlen Meyer flask under suction. The filter cake in the Buchner funnel was washed with chloroform. The contents of the Erlen Meyer flask were transferred to a conical flask of 500 ml. The chloroform extract was dried with 30-40 g of anhydrous sodium sulphate until the extract became clear. The extract was decanted through a filter paper into a 500 ml Erlen Meyer flask and the sodium sulphate washed twice with 25-30 ml portions of chloroform which were

FIG. 1. STANDARD CURVE FOR MONOCROTOPHOS



Human Plasma Dilution 1 : 500

also added to the extract through the filter paper. The extract was concentrated to approximately 10 ml on a rotary evaporator at 40°-45°C. About 25 ml of hexane was added and the volume reduced to 10 ml. This was repeated three times and

the last concentrate after the 3rd evaporation was transferred to a 25 ml volumetric flask and the rinsings of the evaporation vessel were also washed into a volumetric flask and the volume made up with hexane.

An aliquot 5 ml of the hexane solution was pipetted into a 15 ml centrifuge tube and 5 ml distilled water and 30-40 mg of celite 545 added. The centrifuge

TABLE II
*Initial deposits and residues of monocrotophos in cauliflower**

Number of days after spraying the crop	Type	Monocrotophos in flowers as ppm	
		Concentration of spray fluid at 0.05% a.i.	Concentration of spray fluid at 0.1% a.i.
0	Washed flower	1.10	1.46
0	Flower	1.83	2.22
1	Flower	1.27	1.92
4	Flower	1.17	1.74
7	Flower	0.60	1.05
10	Flower	0.35	0.85
14	Flower	0.07	0.27

* Average of two replications

TABLE III
*Initial deposits and residues of monocrotophos in knol-khol**

Number of days after spraying the crop	Type of sample	Monocrotophos in modified stem of knol-khol as ppm	
		Concentration of spray fluid at 0.05% a.i.	Concentration of spray fluid at 0.1% a.i.
0	Washed stem	0.34	0.50
0	Whole stem	1.49	1.89
1	Whole stem	1.36	1.57
4	Whole stem	0.59	1.24
7	Whole stem	0.46	0.96
10	Whole stem	0.37	0.56
14	Whole stem	0.085	0.15

* Average of two replications

tube was shaken vigorously for about half a minute and centrifuged at 3000 rpm for 15 to 20 min. to separate the two phases. The upper organic layer of hexane

containing the plant pigments and other contents was removed using a propipette. The aqueous phase was filtered through filter paper to remove the sediment of celite.

Human blood plasma was brought from the blood bank of Victoria Hospital, Bangalore, as an enzyme working solution and stored in a refrigerator in a sealed sterile bottle. The optimum dilution of the plasma with buffer 'C' for estimation

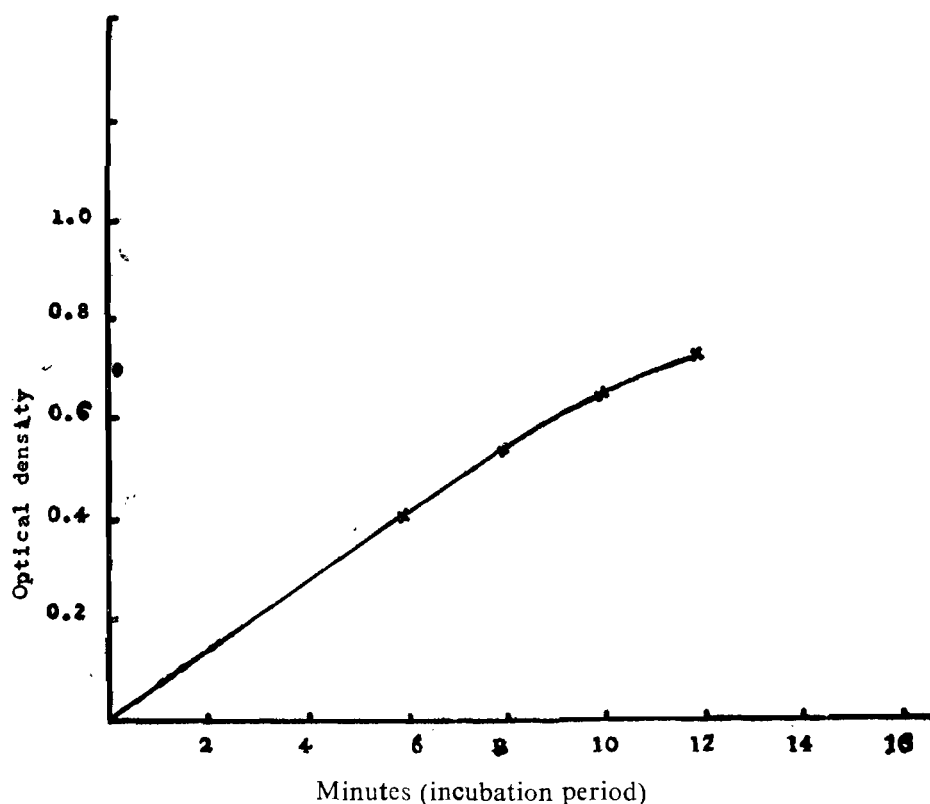


Fig. 2 Relationship between time and cholinesterase activity of human plasma

of cholinesterase activity in plasma was 1 : 500. A fresh enzyme working solution was prepared as and when required.

One hundred mg of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) was dissolved in 50 ml of 'D' pH 7.0 phosphate buffer as a stock solution and stored in a refrigerator. The DTNB was obtained from M/s Koch Light Laboratories, England.

For a substrate-reagent working solution, 20 mg of butyryl thio-cholineiodide (Bu Tch) obtained from Fluka (Switzerland) was dissolved in 18 ml of distilled water. Two ml of DTNB reagent stock solution was added using a pipette. This

solution was stored at 0°C until use during that day and fresh solution was prepared each time.

A standard solution of monocrotophos was prepared. 100mg of monocrotophos a.i., was accurately weighed and dissolved in acetone, made up to 10 ml in a volumetric flask and stored under refrigeration. The monocrotophos used for the standard curve was the same as that used for spraying of the crops under test. An appropriate volume of this standard stock solution was diluted in water to the final concentrations, for the standard curve.

The assay mixture contained different quantities of monocrotophos in 2 ml water. Two ml of diluted human plasma was added to it. The pre-incubation period was 30 min. at 30°C. One ml of DTNB, Bu Tch I substrate solution was

TABLE IV
Residues of monocrotophos in cole crops

Particulars	Cabbage Monocrotophos		Cauliflower Monocrotophos		Khol-khol Monocrotophos	
	0.05% a.i.	0.1% a.i.	0.05% a.i.	0.1% a.i.	0.05% a.i.	0.1% a.i.
	(days)					
Half life	2.67	2.64	3.26	5.1	2.18	4.22
Tolerance time/ Safety interval	8.08	8.44	10.4	17.8	6.32	13.67
Time sensitivity of the method	11.4	11.8	16.6	27.5	12.3	25.2

TABLE V
Residues of monocrotophos in cabbage leaves after 7 and 14 days after spraying

Number of days after spraying	Residues of monocrotophos* (ppm)	
	0.05% spray	0.1% spray
7	3.00	5.50
14	1.50	2.50

* Average of two replications.

added, incubated for 6 min. at 30°C and the optical density was read at 420 nanometres against a blank (boiled enzyme). The percentage of residual enzyme activity in the four inhibited samples was calculated by taking the non-inhibited control (tube Nos. 2 and 3) as 100 per cent active. These figures were plotted on a logarithmic scale against inhibitor concentration on linear scale. Since the activity in the

stored plasma seemed to change, a fresh standard curve was prepared every time an assay was conducted.

To take into account that the substances naturally present in the crop might inhibit cholinesterase activity, crop samples not treated with any pesticide were

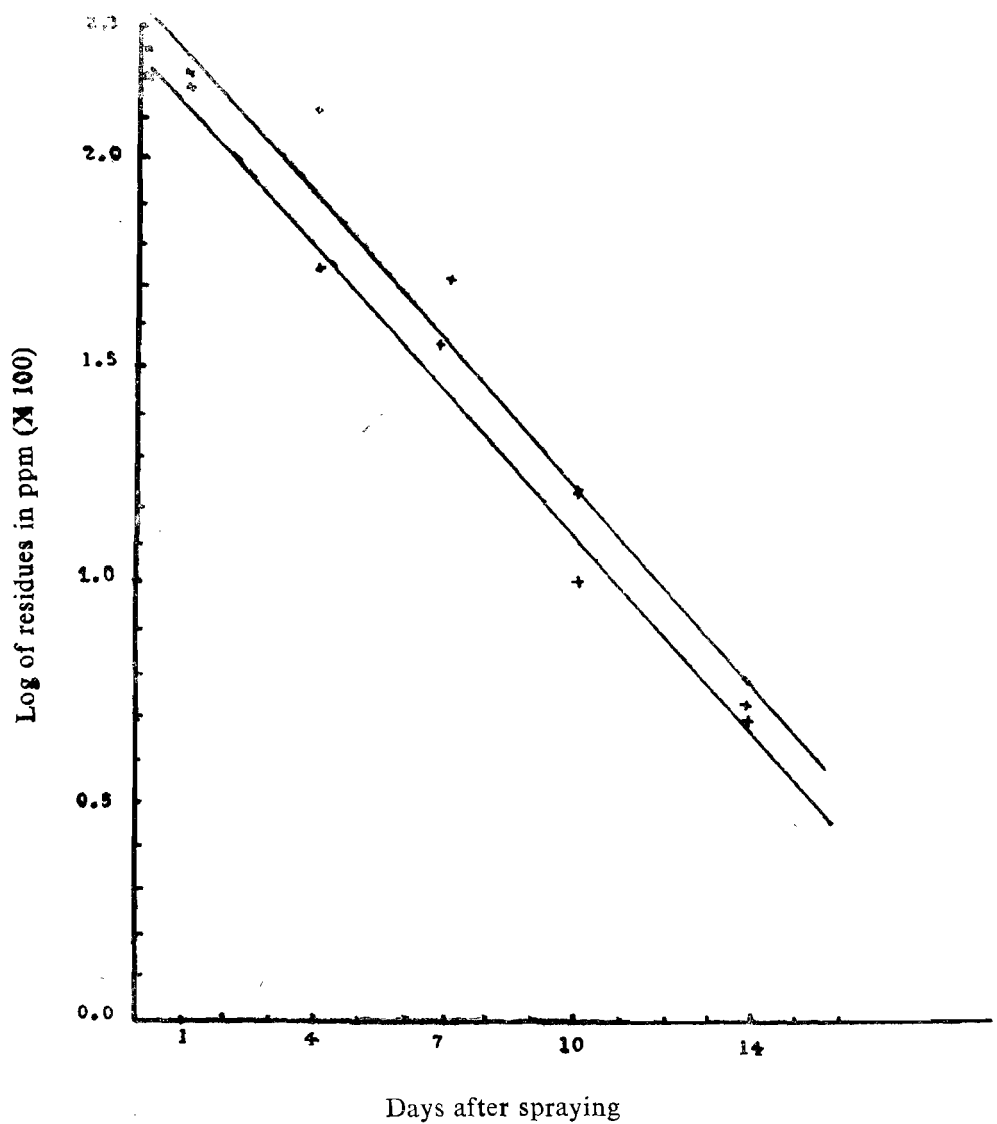


Fig. 3 Residues of monocrotophos in cabbage [A (lower line) : 0.05% spray ; B (upper line) : 0.10% spray ; * : 0.05% spray; + : 0.10% spray]

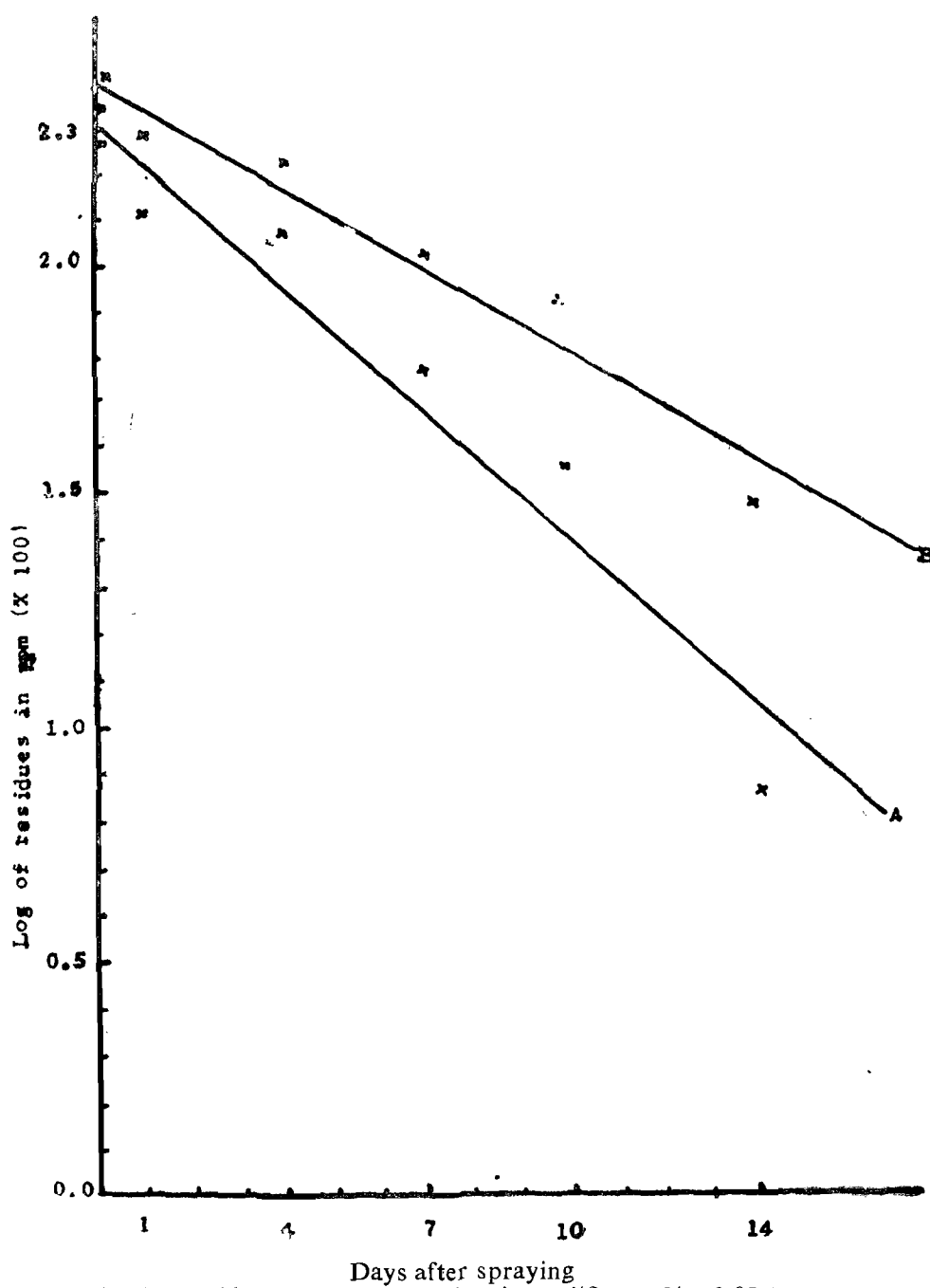


Fig. 4 Residues of monocrotophos in cauliflower [A : 0.05% spray ;
B : 0.10% spray ; * : 0.05% spray ; + : 0.10% spray]

analysed, read with the treated samples and due corrections were made. For determining monocrotophos residues, the crops were extracted from the day of spraying at periodical intervals until the residues were below the tolerance limit. The aliquot

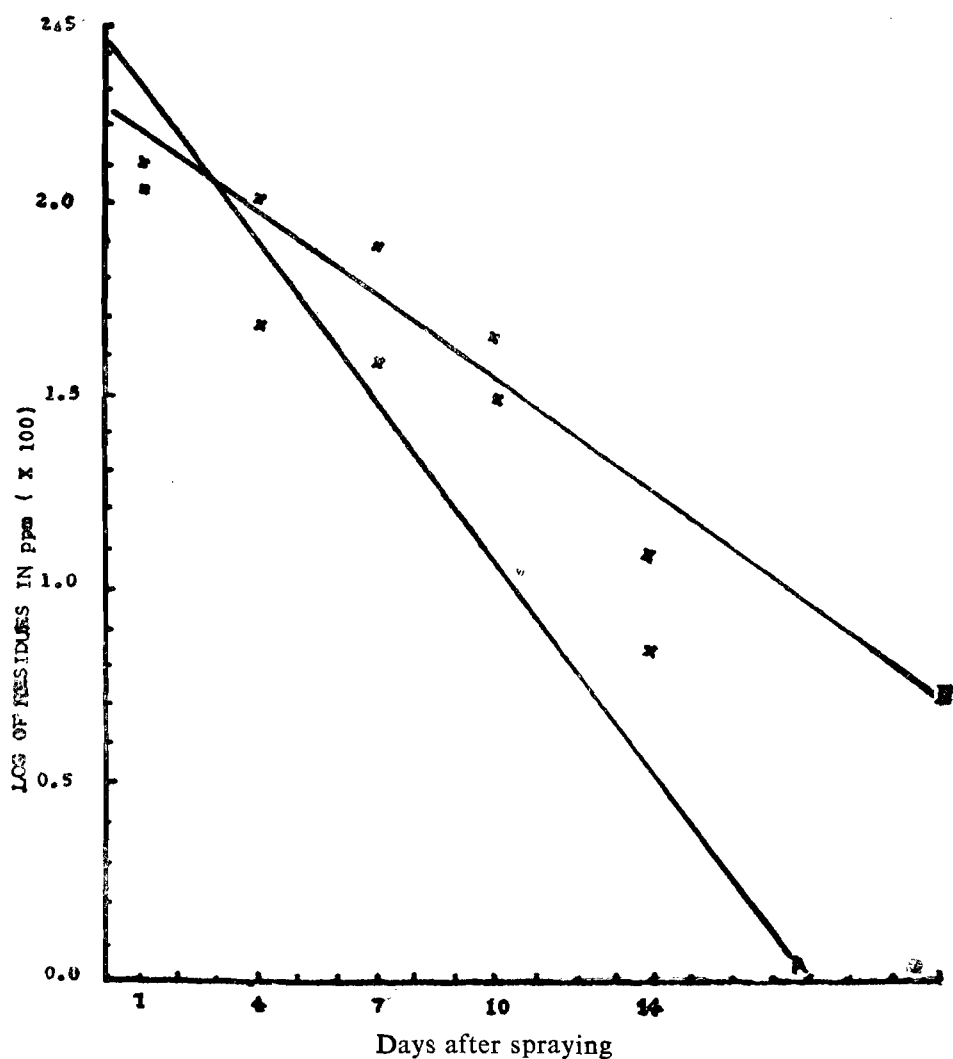


Fig. 5 Residues of monocrotophos in knol-knol [A : 0.05% spray ; B : 0.10% spray ; * : 0.05% spray ; + : 0.10% spray]

of aqueous plant extract (*i.e.*, after centrifugation with water and celite 545) used for the estimation was adjusted as to give a value between 30-80 per cent transmittance. The ppm figure obtained from the reference curve was multiplied by the dilution factor.

The data on monocrotophos residues were statistically analysed. Regression coefficients for the degradation of the residues with the lapse of time, were worked out for each concentration of active ingredient in the spray fluid. The 'half-life' of monocrotophos at different spray concentrations for different crops was estimated. The tolerance time was determined based on the permissible daily intake as per the standard determined by the FAO. The tolerance limit of monocrotophos on cole crops is 0.2 ppm.

RESULTS AND DISCUSSION

Typical standard curves showing the concentration of the inhibitor (monocrotophos) in micrograms on the abscissa and the enzyme activity (human plasma in 1 : 500 dilution with phosphate buffer, pH 8.0 : 0.667 M) on the ordinate are presented in Fig. 1 and 2. The recovery of monocrotophos was 100, 90 and 70 per cent from cabbage, cauliflower and knol-khol, respectively. The initial deposits and the residues of monocrotophos are presented in Tables I to V.

Deposits of 1.63 and 1.82 ppm of monocrotophos resulting from 0.05 and 0.1 per cent concentration sprays dissipated to 0.05 ppm on cabbage in 14 days. The deposits of sprays on cauliflower of the order of 1.83 and 2.22 ppm, attenuated to 0.07 and 0.27 ppm, respectively in 2 weeks. In knol-khol, the residues at the end of a 2 week period were 0.09 and 0.15 ppm. The safe intervals for monocrotophos on cabbage, cauliflower and knol-khol were - 8.1, 8.3 and 10.4 in lower and 17.8, 6.32 and 13.7 days respectively for the higher concentrations. The safety intervals (*i.e.*, minimum gap between spraying and harvesting of the marketable produce) were calculated on the basis of the tolerance limits prescribed by FAO/WHO committee on pesticide residues (Anon, 1973) and the regression equation worked out for residue dissipation. The time sensitivity of the method employed for the determination of the pesticides are also incorporated. The dissipation curves of monocrotophos on cabbage, cauliflower and knol-khol are presented in Figs. 3 to 5.

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29 CARBARYL RESIDUES ON BRINJAL AND PEAS

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SUMMARY

Studies on the persistence of carbaryl on/in brinjal and peas were carried out at Horticultural Experiment Station, Hesaraghatta of the Indian Institute of Horticultural Research, Bangalore during 1976-77. Residue estimations were made colorimetrically. The deposits of sprays of carbaryl on brinjal at 1 and 2 kg a.i./ha were 3.42 and 5.03 ppm respectively. Deposits of carbaryl 1 kg/ha were less than the residue tolerance (5 ppm) while the residues of carbaryl 2 kg/ha attenuated to below the tolerance after a day. Washing the fruits with water within 1 hour and after a day of spray reduced the residues to about 1 ppm level.

Deposits of carbaryl (2 kg/ha) spray on whole pea pods were of the order of 4.85 ppm. Residues dropped to 0.20 ppm in 5 days. In pea seeds the carbaryl residues ranged from 0.03 to 0.09 ppm from 0 to 8 days after spraying.

Field trails to determine residues of carbaryl on peas var. 'Bonneville' and brinjal var. 'Pusa Purple Cluster' were laid out in randomised block design at the Horticultural Experiment Station, Hesaraghatta of the Indian Institute of Horticultural Research, Bangalore. Treatments were replicated three times with a control. Plots were 5 X 3 m and carbaryl suspension at 1.0 kg and 2.0 kg a.i. ha was sprayed on peas and brinjal. Spraying was a full coverage during the fruiting season of the crops. Marketable fruits of brinjal and pods of peas were sampled at 0 (within one hour of spraying), 1, 4 and 7 days after spraying. Samples for residues determination were 50 grams.

Residues in the fruits of both crops were determined by a colorimetric method (A.O.A.C., 1965). The possibility of removing carbaryl residues by washing brinjal fruits (0 and 1 day samples) under tap water for 30 seconds was also determined. Analytical recoveries of added carbaryl at 1 ppm level was about 80-85 per cent for brinjal and 92-95 per cent for peas.

RESULTS AND DISCUSSION

Brinjal: The magnitude of carbaryl deposits after spraying at 1 and 2 kg ai/ha were 3.42 and 5.03 ppm. Deposits from 1 kg ai/ha were less than the tolerance limit of carbaryl on brinjal (FAO/WHO, 1973) and deposits of carbaryl at 2 kg

TABLE I
Carbaryl Residues in Brinjal and Peas §

Brinjal

Dosage of carbaryl	Days after spraying	Residues in fruits (ppm)	% Reduction due to washing
1.0 kg a.i./ha	0	3.42	75.7
		0.38*	
	1	1.70	63.5
		0.62*	
	4	0.48	—
2.0 kg a.i./ha	7	0.26	—
	0	5.03	78.1
		1.10*	
	1	2.68	68.7
		0.84*	
	4	0.87	—
	7	0.61	—

* Residue after washing

Peas

Dosage	Days after spraying	Residues in whole pods (ppm)	Residue in seeds (ppm)
2.0 kg a.i./ha	0	4.85	0.03
	3	2.38	0.03
	5	0.20	0.06
	8	0.10	0.09

§ Average of three replications

Weather conditions :—

	Brinjal (12.3.76—19.7.76)	Peas (1.10.76—18.1.77)
Min. Temp.	18.5°C	9.6°C
Max. Temp.	30.1°C	29.9°C
Relative humidity	84.5%	86.1%
Rainfall	4.0 mm	Nil

ai/ha reached the residue tolerance in one day. Washing with water after spraying and 1 day after spraying removed 63.5 to 78.1 per cent of residues.

Peas : On whole pods the deposits of spray carbaryl at 2 kg ai/ha were 4.85 ppm with a 5 ppm tolerance limit for carbaryl on peas (FAO/WHO, 1973). The residues 3, 5 and 8 days after spraying were 2.38, 0.20 and 0.10 ppm respectively. In seeds the residues of carbaryl ranged from 0.03 to 0.09 ppm between 0 and 8 days.

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30 TOXICITY AND RESIDUES OF PHOSPHAMIDON AGAINST PESTS OF BRINJAL AND BHENDI

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SUMMARY

The toxicity of phosphamidon to some important pests of brinjal and bhendi during 1977-78 at Rajendranagar and persistence of residues were assessed. The results indicated that 0.05%

phosphamidon gave effective control of bhendi fruit borer, **Earias insulana**, brinjal fruit borer, **Lucinodes orbonalis**, jassids, **Amrasca biguttula biguttula**. Residue studies on fruit samples collected 0, 1, 4, 7, 10 and 15 days from spraying showed that phosphamidon fell below the tolerance limit of 0.5 ppm in 10 days.

INTRODUCTION

Phosphamidon is a powerful insecticide characterised by high degree of solubility in water, greater mobility and translocation from the point of application to other parts of the plant, possession of systemic and contact toxicity on a broad spectrum basis, killing many major pests including borers, grass-hoppers, jassids, aphids, psyllids, bugs and beetles in low concentrations. Technical phosphamidon consists of 89 per cent of alpha and beta isomers of phosphamidon, 1 per cent of deschlorophosphamidon and 2 per cent of gamma-chlorophosphamidon with the remaining 8 per cent inert material. Most of the insecticidal property of the technical product is due to beta isomer of phosphamidon (CIBA, 1967).

MATERIAL AND METHODS

Bhendi

The experiment was made on the variety Pusa sawani in a randomized block design with four treatments replicated four times. The plot size was 9 m x 5 m. Phosphamidon was used at two levels, 0.03 per cent and 0.05 per cent and quinalphos at 0.10 per cent. Sprays were applied on 16-8-1977, 30-8-1977, 13-9-1977 and 27-9-1977 with a knapsack compressed air sprayer at constant pressure.

Efficacy against the bhendi fruit borer was assessed (*Earias insulana*) and jassid (*Amrasca biguttula biguttula*). For estimation of residues of phosphamidon, bhendi fruits were collected on 0, 1, 4, 7, 10 and 15 days after treatment.

Phosphamidon was detected by paper chromatography spotting of the extracts and developing in a selective solvent system (petroleum ether 250 ml; toluene 250 ml, methanol 350 ml and distilled water 150 ml) and spraying with blue tetrazolium dye, which made the spot of phosphamidon quite distinct. Presence of phosphamidon in the extracts was confirmed by comparing against the R.F. values of the standard (R.F. value of phosphamidon 0.4). A quantitative estimate was made by visual comparison of the spot area and intensity in the samples and the standard solution.

Brinjal

The same procedure was adopted except that the pests assessed were the

shoot and fruit borer (*Leucinodes orbonalis*) and the jassid (*Amrasca biguttula biguttula*).

RESULTS AND DISCUSSION

Bhendi Pests

Data on the bhendi fruit borer collected over a period of three months are aggregated and presented in Table I.

TABLE I
Aggregate percentage of infestation in bhendi fruits by the borers

Treatments	Replication				Mean
	I	II	III	IV	
Phosphamidon 0.03%	16.91	19.60	20.28	22.58	19.84
Phosphamidon 0.05%	12.85	13.94	16.23	16.08	14.28
Quinalphos 0.10%	17.14	16.54	17.91	18.51	17.53
Control	27.56	28.16	30.93	32.04	29.67
Mean	18.62	19.56	21.09	22.05	

STATISTICAL ANALYSIS

Source of Variation	'F' test (5%)	S.Ed.	C.D. at 5%
Treatments	Significant	0.44	0.99
Ranking :	1. Phosphamidon (0.05%) 2. Quinalphos (0.10%) 3. Phosphamidon (0.03%).		

From Table I, it is seen that phosphamidon 0.05 per cent recorded the highest efficacy against the bhendi fruit borer followed by quinalphos 0.10 per cent and phosphamidon 0.03 per cent. Similar results were reported with fortnightly applications of phosphamidon 0.05 per cent (Yazdani, 1971).

From Table II, it can be seen that all the treatments including phosphamidon 0.03 per cent and 0.05 per cent and 0.10 per cent quinalphos reduced jassids to almost insignificant levels 2 days after spraying. However, the rise of numbers after 7 days was quite considerable, although still much below the level in the control indicating a persistence of phosphamidon 0.05 per cent up to about 7 days.

Bhendi Residues

Data on the residues of phosphamidon in bhendi fruit extracts are presented in Table III.

TABLE II
Effect of insecticides on the bhendi jassid

Treatments	Population			
	Before spray	2 days	4 days	7 days
Phosphamidon 0.03%	140.50	9.00	18.50	84.75
Phosphamidon 0.05%	110.25	4.75	15.50	73.75
Quinalphos 0.10%	170.25	9.00	22.00	100.50
Control	224.50	209.50	187.75	165.75
'F' test at 5%	Sig.	Sig.	Sig.	Sig.
S.E.	13.86	3.69	3.18	4.66
C.D. at 5%	31.35	8.35	7.19	10.55

N.B. :- 1. Population is total of 10 plants
 2. Count is average of 4 replications
 3. Days = Days after spraying

TABLE III
Residues of phosphamidon in bhendi fruit

Days after spraying	Phosphamidon 0.03% ppm	Phosphamidon 0.05% ppm
0	6.5	8.5
1	4.5	6.0
4	2.5	3.0
7	1.0	1.5
10	0.5	0.5
15	0.1	0.1

From Table III, it is seen that the initial deposits of phosphamidon 0.03 per cent and 0.05 per cent were 6.5 and 8.5 ppm respectively. In both the concentrations the tolerance limit of 0.5 ppm was reached in 10 days after spraying indicating thereby that the bhendi fruits are safe for human consumption 10 days after spraying.

Brinjal Pests

The data on the effect of jassids and shoot and fruit borer are presented in the following tables.

From Table IV, it can be seen that phosphamidon 0.05 per cent killed jassids up to 4 days after spraying, followed by phosphamidon 0.03 per cent and quinalphos

0.10 per cent. Even after 7 days phosphamidon 0.05 per cent was keeping down the jassid population more effectively than the other treatments.

TABLE IV
Effect of insecticides on brinjal jassids

Treatments	Population			
	Before spray	2 days	4 days	7 days
Phosphamidon 0.03%	94.00	7.00	11.75	81.00
Phosphamidon 0.05%	76.00	2.75	6.75	44.50
Quinalphos 0.10%	115.25	9.75	14.00	89.25
Control	163.50	170.00	137.75	123.75
'F' test	Sig.	Sig.	Sig.	Sig.
S.E	5.35	1.84	1.20	10.58
C.D. at 5%	12.10	4.17	2.75	23.94

TABLE V
Aggregate percentage infestation in brinjal fruits by the borer.

Treatments	Replications				Mean
	I	II	III	IV	
Phosphamidon 0.03%	27.17	42.16	33.56	29.92	33.20
Phosphamidon 0.05%	16.78	16.89	20.93	17.19	17.95
Quinalphos 0.10%	24.21	22.47	31.81	26.14	26.16
Control	47.81	47.25	60.29	45.59	50.24
Mean	28.99	32.19	36.65	29.71	
Statistical Analysis					
Source of variation	'F' test (5%)		S.Ed.	C.D. at 5%	
Treatments	Sig.		1.88	4.26	

Ranking of insecticidal treatments : 1. Phosphamidon 0.05%
2. Quinalphos 0.10%
3. Phosphamidon 0.03%

From Table V, it is seen that phosphamidon 0.05 per cent showed the greatest efficacy against the shoot and fruit borer of brinjal followed by quinalphos 0.10 per cent and phosphamidon 0.03 per cent.

Brinjal residues

Data on the brinjal residues are presented in Table VI.

TABLE VI
Residues of phosphamidon in brinjal fruits

Days after spraying	Phosphamidon 0.03% ppm	Phosphamidon 0.05% ppm
0	6.0	8.0
1	4.0	5.5
4	2.0	3.0
7	1.0	1.5
10	0.5	0.5
15	0.1	0.1

From Table VI, it is seen that the initial deposits of phosphamidon in brinjal fruit extracts were 6.0 and 8.0 ppm. In both concentrations the residues dissipated to 0.5 ppm in 10 days after spraying which means that 10 days after spraying it is safer for human consumption.

CONCLUSIONS

To control bhendi fruit borer (*Earias insulana*) phosphamidon 0.05 per cent was the most effective followed by quinalphos 0.10 per cent and phosphamidon 0.03 per cent.

The jassids (*Amrasca biguttula biguttula*) in bhendi and brinjal were controlled with phosphamidon 0.05 per cent closely followed by phosphamidon 0.03 per cent.

In the control of brinjal fruit borers (*Leucinodes orbonalis*) phosphamidon 0.05 per cent was best, with quinalphos 0.10 per cent and phosphamidon 0.03 per cent rated as second and third, respectively.

Data on the chemical analysis indicated that the initial deposit of phosphamidon was somewhat higher in bhendi than in brinjal fruits possibly because of the spiny pericarp.

The residues of phosphamidon in both concentrations reached the tolerance limit of 0.5 ppm in 10 days after spraying in bhendi and brinjal.

It is a great pleasure to acknowledge the field and laboratory facilities provided by Dr. B. H. Krishnamurthy Rao, Professor and University Head, Entomology Division, College of Agriculture, Rajendranagar for this work.

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**31 RESIDUES OF DIMETHOATE AND CAPTAFOL IN AND ON
FRENCH BEANS, CHILLIES AND TOMATO**

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SUMMARY

Green fruits from seven varieties of French beans, and local varieties of tomatoes and chillies, sprayed at 10 days intervals with 0.06 per cent dimethoate and 0.2 per cent captafol were analysed for residues at intervals.

Residues of dimethoate on chilli fruits were 7.89 ppm after one day and 0.25 ppm in 15 days. On tomato it was 1.78 ppm after one day and reached 0.20 ppm in 25 days. On French beans the residues were above the tolerance limit of 2 ppm upto 2 days after spraying. There was not much difference in residue levels in different varieties of French beans.

On chilli fruits, captafol residue was 6.74 ppm after one day, which reduced to 0.23 ppm in 10 days. In tomato, the deposit was 1.23 ppm after one day. On French beans residues were in range of 0.627 to 0.995 ppm in 2 days.

Boiling the fruits of French beans in salt water reduced residues of dimethoate and captafol 50 to 70 per cent.

Dimethoate has been used extensively for the control of a number of sucking pests of chillies, tomato and French beans at concentrations ranging from 0.03 to 0.06 per cent. Although considerable data on the biological efficacy of this insecticide has accumulated on these crops (Mote, 1976, 1977; Rangarajan and Mahadevan, 1974; Kareem *et al.*, 1977; Rataul and Butter, 1976; Sastry *et al.*, 1976), there is no information on its residues on these crops.

Similarly, with captafol, a contact fungicide widely used to control a large number of fungal diseases of crops at concentrations of 0.2 per cent (Anon., 1976, 1978), there is a need to establish the residue levels of this fungicide on a number of crops. The present investigation was carried out in order to determine the residues of dimethoate and captafol on French beans, chillies and tomato.

MATERIAL AND METHODS

A field experiment was conducted with seven varieties of French beans, and local variety of chilli and tomato, at Bangalore during August-December, 1977. The crops were sprayed with 0.06 per cent dimethoate and 0.2 per cent captafol at the

rate of 800-1000 litres per hectare using a high volume sprayer, regularly at 10 days intervals. In all, dimethoate was sprayed six times on all crops and captafol was sprayed six times on chillies and three times on tomato and French beans. Fruits were harvested at intervals after the last spray and analysed for residues of dimethoate and captafol.

Dimethoate residues were extracted from fruits using CH_2Cl_2 and the extract after concentration to about 5 ml, was passed through an activated alumina-activated carbon column. Residues were eluted with water and partitioned with methylene chloride. The CH_2Cl_2 extract was evaporated to dryness and redissolved in alcohol mixtures. Dimethoate was estimated colorimetrically by the methylamine method using chlorodinitrobenzene (Sutherland, 1964).

Captafol residues were estimated by the procedure described by Mithyantha *et al.* (1977). Finely chopped fruits were extracted with benzene and the extract was evaporated to dryness. The residues were dissolved in acetonitrile, washed with *n*-hexane and passed through a silicic acid column. Captafol was eluted from the column with a 1 : 1 pentane-ether mixture and the elute was evaporated to dryness. The residue was treated with alkaline *N, N*-dimethyl *p*-phenylene diamine solution and after making up the volume to 10 ml, the absorbance was measured at 440 nm.

To study the effect of cooking the chopped fruits on residues, they were boiled with water containing about one per cent salt for 30 minutes and after cooling, residues were estimated as earlier.

RESULTS AND DISCUSSION

Data on residues of dimethoate on the three crops are given in Table I. The initial deposit of dimethoate on chilli fruits was 7.89 ppm 24 hours after the last spray. The residues after 15 days came to levels of 0.25 ppm. The residues were higher than the WHO tolerance limit of 2 ppm in the initial stages. In tomato, residues on the fruits were less than those on chillies and were 1.78 ppm after one day. Dimethoate residue level dissipated fast on tomato fruits reaching 0.62 ppm on the 9th day and 0.20 ppm on 25th day after last spray. On the several varieties of French beans initial residues of dimethoate were high and were in the range of 2.24 to 2.67 ppm after 2 days. Further dissipation was fast, reaching very low levels by the 10th day. A waiting period of only two days is suggested after dimethoate spray on French beans fruits. Residues on fruits of different varieties were almost the same.

In the literature, there is no information on dimethoate residues on these crops. Work on several other crops such as cabbage, cauliflower, sarson, grapes,

TABLE I

Residues of Dimethoate in Crops

Crop	Variety	Locality	Dosage used	No. of applications	Part of plant	Dissipation rate (ppm after days)*
Chillies	Local	Bangalore	0.06% spray	Once in 10 days	Green fruit	7.89 (1), 0.25 (15)
Tomato	"	"	"	Three	Fruit	1.78 (1), 0.62 (9), 0.55 (11), 0.20 (25)
French beans	Prochessor	"	"	Once in 10 days	Green fruit	2.35 (2), 1.27 (4), 0.48 (10)
	Blue crop	"	"	"	"	2.35 (2), 0.91 (4), 0.57 (10)
	Ozark	"	"	"	"	2.24 (2), 1.09 (4), 0.39 (10)
	Var-60	"	"	"	"	2.46 (2), 1.00 (4), 0.65 (10)
	Canyon	"	"	"	"	2.56 (2), 1.00 (4), 1.00 (10)
	Royalty purple	"	"	"	"	2.67 (2), 1.09 (4), 0.65 (10)
	Silver	"	"	"	"	2.56 (2), 1.27 (4), 0.65 (10)

* The numbers in the parenthesis indicate days after application.

TABLE II
Residues of Captafol in/on Chillies, Tomato and French Beans

Crop	Variety	Method of application	Plant part	Dissipation rate (ppm after days*)	Residues at harvest (ppm)
Chillies	Local	Six sprays of 0.2% captafol	Green fruits	6.745 (1), 0.237 (10), 0.099 (26)	
			Dry fruits		0.022
Tomato	Local	Three sprays of 0.2% captafol	Fruit	1.23 (1), 0.234 (10), 0.214 (12), 0.066 (26)	
French beans	Prochessor	Three sprays of 0.2% captafol	Green fruits	0.885 (2), 0.995 (4)	
	Blue crop	"	"	0.957 (2), 1.067 (4), 0.049 (10)	
	Ozark	"	"	0.957 (2), 1.144 (4), 0.071 (10)	
	Var-60	"	"	0.924 (2), 0.995 (4), 0.099 (10)	
	Royalty purple	"	"	0.995 (2), 0.786 (4), 0.099 (10)	
	Canyan	"	"	0.852 (2), 0.786 (4), 0.049 (10)	
	Silvert	"	"	0.627 (2), 0.852 (4), N.D. (10)	

* Numbers in the parenthesis indicate the number of days after last spray.

peach, guava etc indicate a fast dissipation of the insecticide residue on different crops (Agnihotrudu and Mithyantha, 1978), which conforms with the results obtained in this investigation.

Residues of captafol on the three crops are given in Table II. The initial deposits of the fungicide on chilli fruits were 6.74 ppm one day after the 6th spray. Further dissipation of the residue was fast and reached 0.23 ppm in 10 days, and 0.10 ppm in 26 days. At the time of harvest, the red chillies had only 0.02 ppm captafol residue. The EPA tolerance limit suggested for various crops was 5 ppm and the residue was higher than the tolerance limit for only a short duration after spray, so it is safe to consume captafol sprayed chilli fruits after 8-10 days.

On tomato the initial deposit which was less than the WHO tolerance limit of 2 ppm was 1.23 ppm after one day and 0.23 ppm after 10 days. On French beans captafol residues were 0.627 to 0.995 ppm after 2 days and fell to low levels in 10 days. The residue levels did not differ by any considerable extent among the different varieties of French beans.

Although there is no information on captafol residues on these crops, a fairly fast rate of its disappearance on apple fruits was observed by Agnihotrudu *et al.* (1978).

Cooking French beans in salt water reduced residues of dimethoate and captafol to varying extents (Table III). In many cases the reduction was to the

TABLE III
Effect of Cooking on the Residues of Dimethoate and Captafol on French Beans

Compound	Residue in the raw fruit	Residue after Cooking
1	2	3
Dimethoate	1.27	0.24
	0.91	0.20
	1.09	0.20
	1.00	0.24
	1.27	0.16
	2.35	0.11
	2.24	0.16
	2.46	0.16
	2.67	0.12
	2.56	0.16
	2.56	0.12

1	2	3
	0.48	0.12
	0.57	0.08
	0.65	0.16
Average	1.58	0.16
Captafol	1.00	0.13
	1.07	0.06
	0.89	0.03
	0.96	0.06
	0.63	0.13
	0.79	0.06
	0.85	0.09
Average	0.88	0.08

extent of more than 70 per cent. Bognan (1977) also noticed that cooking could reduce dimethoate residue on French beans up to 82 per cent.

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RESIDUES OF METHYL PARATHION, MALATHION AND FENITROTHION

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SUMMARY

Investigations were made on residues of methyl parathion, malathion and fenitrothion on tomato. These insecticides were sprayed at recommended doses. Fruits were analysed on specified intervals and residues of parent compounds were determined on GLC.

The residues of the above pesticides weathered to less than tolerance limits after two to three days suggesting their transient nature in the tropical environment. Washing the fruit

did not remove all the pesticides and a waiting period of three days is recommended for the above pesticides.

MATERIAL AND METHODS

i) Crop and application of insecticides

The experiments were conducted under field conditions on tomato plants. No pesticides were applied to the field prior to our study.

Metacid 50 EC (methyl parathion), Malathion 50 EC (malathion) and Folithion 50 EC (fenitrothion) were sprayed at recommended doses (Table I). Each treatment

TABLE I

Pesticide	No. of applications	Applied dosage of fomulations	Applied dosage of acitive ingredient	Applied vol. of spray	Applied con. of spray
Methyl parathion	Once before analysis	225 ml/ac	112.5 g/ac	400 l/ac	0.028%
Fenitrothion	„	350 ml/ac	175.0 g/ac	„	0.043%
Malathion	„	500 ml/ac	250 0 g/ac	„	0.062%

with a plot size of 4 x 10 m was replicated three times in a randomized block design A backpack hand operating sprayer was used.

ii) Sampling

Composite, representative, fruit samples of 200 g were taken for the analysis. Collected fruits were covered in polythene bags and brought into the laboratory at specified intervals after the initial spray.

iii) Extraction and clean up

The extraction and clean up methods described by Mollhaff (1967) were followed. 200 g of tomatoes were washed with tap water and then cut up for maceration in a fruit mixer with 300 ml of acetone. The filtered extract was shaken three times with chloroform and finally with sodium sulfate (anhydrous) to remove any water and evaporated to dryness.

Eight grams of deactivated florisil was packed in a column and the residues from the above extraction loaded and eluted with benzene. The eluted residue samples are then concentrated and used for Gas-liquid chromatography determination(GLC 7).

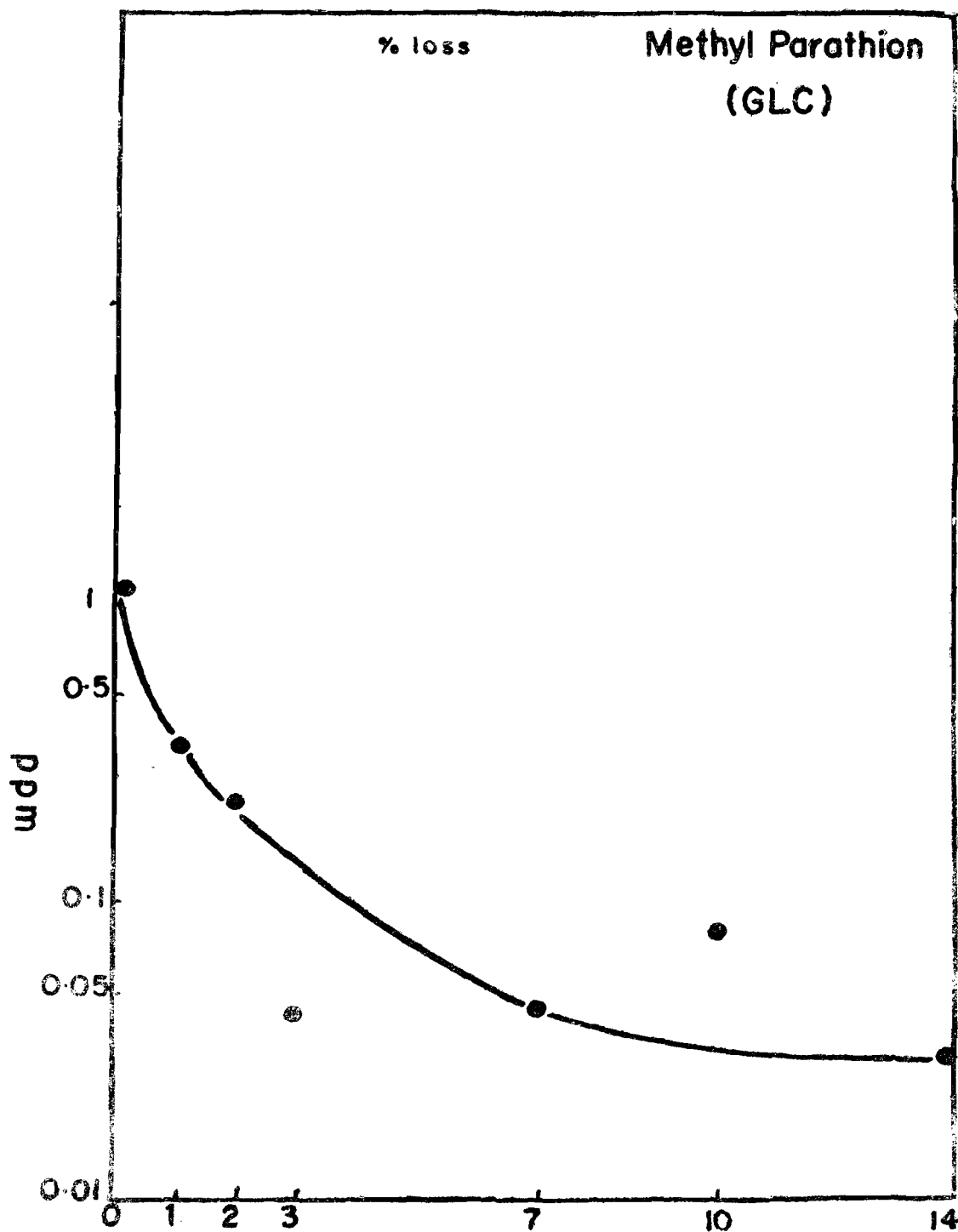


Fig. 1 Degradation curve for tomato sprayed with methyl parathion.

RESULTS AND DISCUSSION

The decline of residues was followed for 14 days. The average temperature during that period was 26.83°C and relative humidity was 86.47 per cent. Rainfall of 16.2 cm and 2.6 cm occurred on the 5th and 6th day of spraying.

Methyl parathion

The initial deposit of methyl parathion on the fruits was 1.12 ppm. This weathered off by 71.4 per cent in the second day and 81.2 per cent in the third day. Thereafter, the degradation was rapid with almost nil level on the 14th day (Table II).

The residue levels in the fruit after second day was well below the EPA tolerance limit for methyl parathion on tomato (1 ppm).

Similar conclusions were made at the Institute für Pflanzenschutz, Germany (FAO/WHO 1965). Their residue level after three days (0.04 ppm) agrees tentatively with our results of 0.06 ppm.

Malathion

Malathion had an initial deposit of 1.58 ppm. The disappearance was slower than for methyl parathion. To attain a safe limit of 0.5 ppm (Chojivo Tomizawa, 1977), it took more than three days.

This may be due to the higher dosage sprayed, compared to the other pesticides.

Fenitrothion

Fenitrothion had the highest dissipation among the pesticides studied. A deposit of 1.32 ppm dropped to 0.07 ppm on the second day. This is well below the Japanese tolerance limit of 0.2 ppm (Chojivo Tomijawa, 1977) and Swiss tolerance of 0.75 ppm (FAO/WHO 1965).

These results suggest the following :

- 1) These organophosphorus insecticides are not persistent. They agree with the low values of organophosphorus residues reported in food in an extensive survey (Abbott *et al.* 1970, and Corneliussen, 1970).

- 2) Contrary to popular opinion that washing may remove most of the chemicals sprayed, significant amounts of residues persisted even after washing (Rajukannu *et al.*, 1976 and Stobwasser 1967). Due to their high solubility in lipid media, these organophosphorus pesticides penetrated the cuticle via the waxy outer

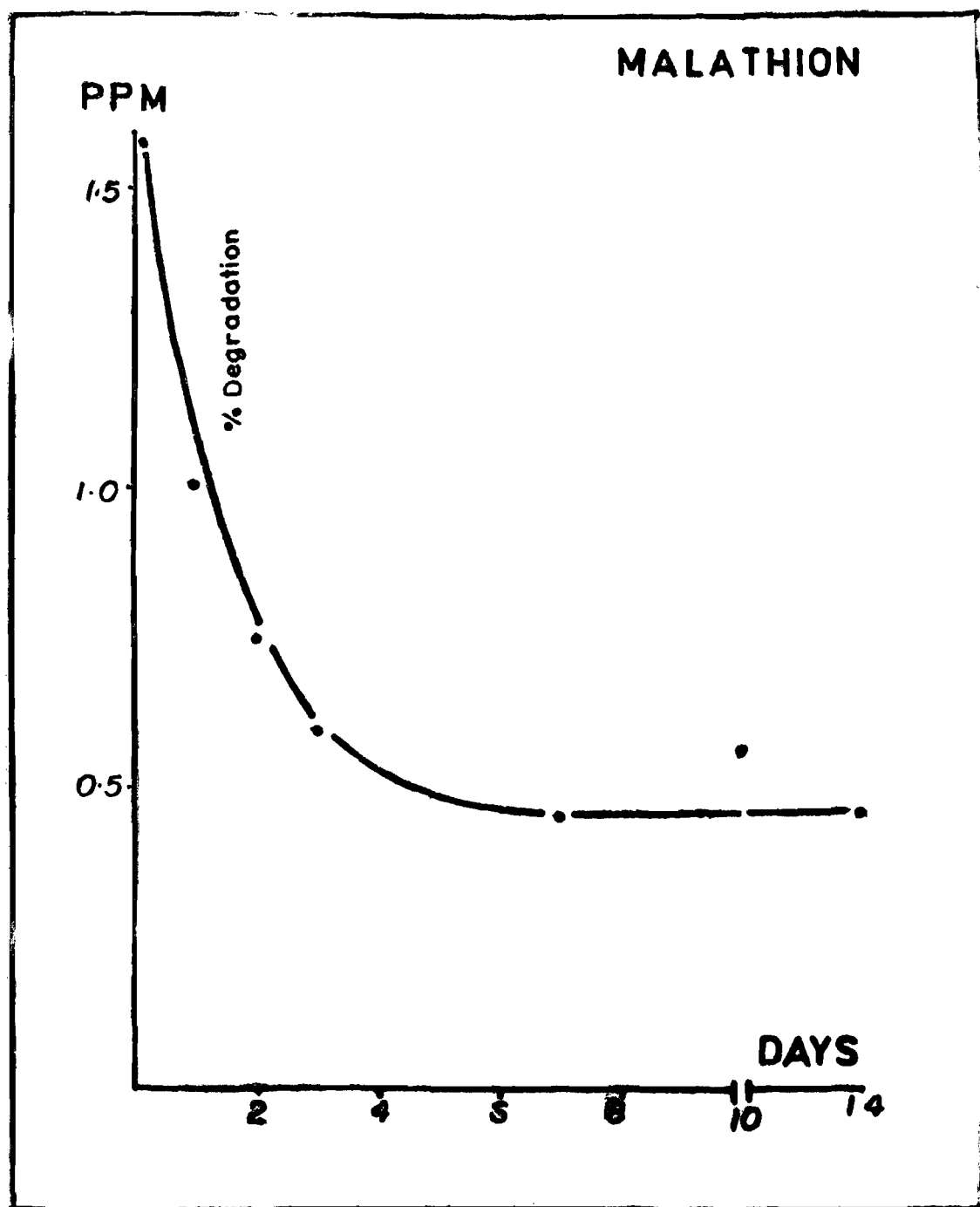


Fig. 2 Degradation curve for tomato sprayed with malathion.

layers of fruit (Van Middelem, 1963). Fenitrothion and methyl parathion can behave in a similar way in rice, where the residues ranging from 1-7 ppm were detected in dehusked grains suggesting their penetrating capacity.

3) There was a consistent increase in residue levels until the 10th day for all pesticides studied. This may not be due to the accumulation of metabolites—at least

TABLE II
Residues of Pesticides in Tomato

Pesticide	Interval between treatment and sampling in days	Residue in ppm	% reduction of residue
Methyl parathion	00	1.12	—
	01	0.32	71.42
	02	0.21	81.25
	03	0.04	96.42
	07	0.04	96.42
	10	0.08	92.85
	14	0.03	99.09
Malathion	00	1.58	—
	01	1.00	36.30
	02	0.75	52.22
	03	0.59	62.42
	07	0.45	71.33
	10	0.56	64.33
	14	0.45	71.33
Fenitrothion	00	1.32	—
	01	0.07	94.69
	02	0.05	96.21
	03	0.05	96.21
	07	0.05	96.21
	10	0.06	95.45
	14	0.06	95.45

Average weather conditions :

Maximum Temperature — 31.96°C

Minimum Temperature — 21.71°C

Relative humidity — 86.47 %

Rain fall (i) 18th Feb. 77 — 16.20 cm

(ii) 19th Feb. 77 — 02.60 cm

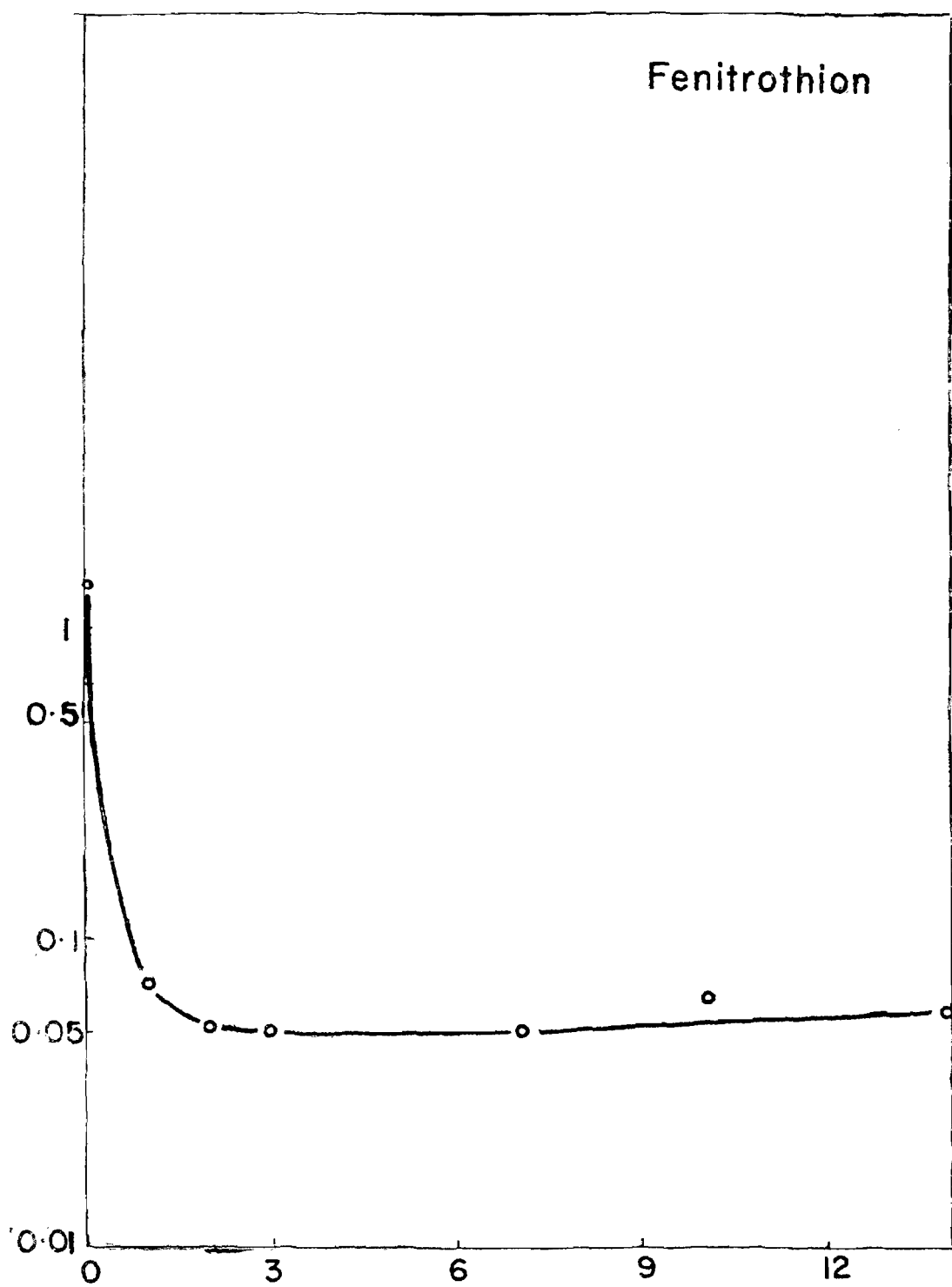


Fig. 3 Degradation curve for tomato spraped with Fenitrothion.

for methyl parathion and fenitrothion—because Mollhoff (1968) clearly established that metabolites exist in a very insignificant amount that they would not interfere in the routine analysis of the parent compounds.

So, it may be that the pesticides washed off during rain may be translocated up via the root system. However, Smith *et al.* (1952) showed that very little, if any, translocation occurred from the leaves to the fruits or from the soil to the fruit of tomatoes. Nevertheless, we must not rule out this possible explanation for an increase in the residue level on that day.

4) Based on the results obtained, a waiting period of three days can be suggested before consumption of tomato fruits after sprays of either methyl parathion, malathion or fenitrothion.

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DISSIPATION OF QUINALPHOS ON TOMATO

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SUMMARY

The dissipation of quinalphos on tomatoes was studied following its application at a dosage of 250 g ai/ha in a field experiment. Samples of marketable fruits taken at various intervals were extracted with acetone followed by partitioning with methylene chloride. The methylene chloride was vacuum evaporated and the residue dissolved in suitable aliquots of acetone and directly analysed by gas-liquid-chromatography without further clean up. Quinalphos spray resulted in a mean deposit of 1.77 ppm which dissipated to levels lower than the proposed maximum tolerance limit of 0.25 ppm in 4 days. The extracts on analysis by TLC-enzymatic inhibition technique showed the presence of 'oxon' of quinalphos in negligible amounts. Results indicated that the use of polyethylene bags for the collection of samples caused significant errors in the estimation of quinalphos residues. Washing under tap water removed 70.7 and 21.4 per cent of the residues from tomato samples collected immediately and 1 day after spraying.

Tomato crops are attacked by many insect pests such as jassids, white fly, tobacco caterpillar and fruit borer. The fruit borer (*Heliothis armigera* Hubner) is difficult to control. Recently, Singh *et al.* (1976) found that repeated application of quinalphos (0,0-diethyl-0-(2-quinoxaliny) phosphorothionate) at weekly intervals at the dosage of 0.25 kg a.i./ha effectively controlled this insect pest. Pesticides

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applied to food crops leave residues which may be hazardous to the consumers. Although dissipation of quinalphos on tomato has been studied by Heenan (1974) in New Zealand, there is no information available from India. The present study attempts to provide information on the dissipation of quinalphos on tomato under North Indian climatic conditions. The effect of washing on the removal of residues was also studied.

MATERIAL AND METHODS

Tomatoes (Var. Punjab Tropic 210) were raised at the University Farm. Seedlings were transplanted during the third week of November and quinalphos emulsion, (0.3 per cent) prepared from Emulphos 20 per cent was sprayed at a dosage of 200 g a.i./ha with a knapsack sprayer. The plants were completely drenched with the spray.

Three samples of $\frac{1}{2}$ kg of marketable size tomatoes were taken from each plot 0, 1, 2 and 4 days after spraying. One set of samples was brought to the laboratory in polyethylene bags and an other set in glass beakers. After cutting into small pieces these were thoroughly mixed. From these samples, representative samples of 50 g were taken for analysis.

Extraction was done the same day as the samples were received to prevent losses during storage. The method described by Luke *et al.* (1975) for the extraction of organophosphorus insecticides was followed, with slight modifications as suggested by Singh and Kalra (1978). The samples were extracted with acetone and partitioned with methylene chloride. The methylene chloride was vacuum evaporated and residue dissolved in suitable aliquots of acetone which was analysed directly by gas-liquid-chromatography on 3 Packard gas-chromatograph (Model 7624) using KCl-coated thermionic detector. The glass-chromatographic column (1.5 m long x 2 mm i.d.) packed with 3 per cent DC-200 on Gas chrom Q (50-100 mesh) was used. The column and detector temperatures were 180 and 200°C respectively. The gases used were nitrogen as carrier (40 ml/minute), air (400 ml/min.) and hydrogen (40 ml/minute). The quantity of the insecticide was estimated by comparing the peak height of the unknown with standards injected under similar conditions. Recovery was tested by adding standard solutions of quinalphos to untreated tomato fruits at concentrations of 0.1, 0.25, 0.5, 1.0 and 2.0 ppm before extraction. To study the effect of washing, the fruits collected 0 and 1 day after spraying were washed thoroughly with tap water. These were then processed by the method described above.

A thin-layer chromatography-enzyme inhibition technique described by Mendoza (1972) was used to detect the oxygen analogue of quinalphos. Before TLC,

extracts obtained as for analysis by GLC were cleaned up further by column chromatography. An absorbent mixture of charcoal (Darco-60), celite 545 and magnesium oxide in the ratio of 2:2:1 by weight was packed in the glass column (2.25 cm dia) to a height of 10 cm. The concentrated extract in chloroform was poured over the column and the insecticide was eluted with 150 ml of chloroform. The eluate after concentration to small volume was spotted on a silica gel-G (0.25 mm thickness) coated plates and was developed using hexane-acetone (19:1 v/v) as the solvent system. Goat-liver esterases as enzyme source and indoxyl acetate as substrate were used for visualising the spots.

RESULTS AND DISCUSSION

The retention time of quinalphos on GLC under the conditions used was 6 minutes. The linear response was obtained in the range of 1 to 10 ng of quinalphos. Typical gas-chromatograms obtained after injection of standard quinalphos and extracts of control and treated plants showed clearly that plant extracts as such did not give any interfering peak. Extracts of the 50 g fruit sample could be concentrated to 2 ml, out of which 2 μ l, if injected, did not cause any interference. Based on twice the noise level, the minimum limit of estimation of quinalphos was found to be 0.01 ppm in 50 mg of the sample injected. The percentage recovery varied between 78.0 and 96.0 per cent with a mean value of 87.7. Results were not corrected for recovery.

Amounts of quinalphos on tomato fruits are given in Table I. The initial deposit was 1.77 ppm and in samples collected after 1, 2 and 4 days, the residues were 1.24, 0.50 and 0.22 ppm respectively. About 29.9 per cent of the insecticide dissipated in 1 day and the degradation at the end of 2 and 4 days was 71.8 and 87.6 per cent respectively. So a fairly high rate of degradation occurred. These results agree with those of Chawla *et al.* (1978) and Singh and Kalra (1978) who reported about 70 per cent degradation of quinalphos in 4 days on cauliflower and cabbage respectively.

The samples of tomatoes collected in polyethylene bags had significantly lower residues of quinalphos than samples collected in glass beakers (Table I), indicating possible loss of quinalphos residues from tomato fruits through contact with polyethylene bags. In two earlier experiments, when only polyethylene bags were used or the collection of the samples, the residues of quinalphos were low (Table II). The use of polyethylene bags for the collection of samples therefore, caused significant errors in the estimates of quinalphos residues. Also, an insignificant quantity of quinalphos (0.005 ppm) was detected in the glass beakers after the transference

TABLE I

Residues of quinalphos on tomato fruits

Days after spraying	Replicate	Samples taken in glass beakers			Samples taken in polyethylene bags		
		Residue level (ppm)	Mean residue level (ppm)	Per cent degradation	Residue level (ppm)	Mean residue level (ppm)	Per cent degradation
0 (30 minutes after spray)	1	1.82			0.52		
	2	1.69	1.77	—	0.51	0.54	—
	3	1.81			0.59		
1	1	1.26			0.30		
	2	1.21	1.24	29.9	0.29	0.32	40.7
	3	1.26			0.38		
2	1	0.46			0.28		51.9
	2	0.56	0.50	71.8	0.26	0.26	
	3	0.48			0.23		
4	1	0.20			0.12		
	2	0.22	0.22	87.6	0.13	0.13	75.9
	3	0.24			0.13		

Meteorological data for the experimental period

Temperature (°C)			Relative humidity (%)			Rainfall (mm)
Maximum	Mean	Minimum	Maximum	Mean	Minimum	
Range	Mean	Range	Range	Mean	Range	Mean
43.5-41.3	42.4	25.8-19.3	53-37	42.6	18-10	13.6
						0

TABLE II
Residues of quinalphos on tomato fruits (samples taken in polyethylene bags)

Days after spraying	Replicate	Fifth spray		Eighth spray	
		Residue level (ppm)	Mean residue level (ppm)	Residue level (ppm)	Mean residue level (ppm)
0 (immediately before spray)	1	0.14	0.15	0.15	0.13
	2	0.15		0.11	
0 (30 minutes after spray)	1	0.30	0.30	0.27	0.28
	2	0.29		0.28	
1	1	0.21	0.17	0.17	0.15
	2	0.13		0.13	
2	1	0.11	0.12	0.07	0.07
	2	0.12		0.07	
4	1	0.08	0.07	0.03	0.03
	2	0.05		0.03	
8	1	0.04	0.03	0.02	0.02
	2	0.02		0.02	

of the tomato samples. Thus, the use of glass beakers for sampling of the tomato fruits sprayed with quinalphos seems appropriate.

Amounts of quinalphos on tomatoes before the 5th and 8th sprays were 0.15 and 0.13 ppm respectively (Table II), which suggested that repeated application of quinalphos did not cause any significant accumulation of residues of the insecticide. The extracts of the samples 2 and 4 days after spraying gave a distinct spot with Rf value of 0.16 by TLC even without exposure to bromine vapours. This corresponded to the spot obtained with the reference sample of the oxygen analogue of quinalphos. On the basis of the visual comparison of the intensity of the spot, the levels of oxon were found to be around 0.01 ppm in the samples. When the TLC plate was sprayed with enzyme and substrate solutions after exposure to bromine vapour the intense spots corresponding to quinalphos having Rf value of 0.70 were also indicated in the samples.

A tolerance of 0.25 ppm has been tentatively proposed for quinalphos. Our results suggest that a waiting period of 4 days may be necessary for the residues of the insecticide on tomato to be within the prescribed maximum residue limit. Washing removed 70.7 and 21.4 per cent of residues from samples collected immediately and 1 day after spraying respectively (Table III). However, residues were not reduced below the proposed tolerance of 0.25 ppm, by washing.

TABLE III
Effect of washing on removal of quinalphos residues from tomato

Age of residues (days)	Replicate	Residue level (ppm)	Residue level (ppm) after washing	Per cent reduction
0*	1	2.06	0.67	70.7
	2	2.28	0.48	
	3	1.91	0.67	
		(2.08)	(0.61)	
1**	1	0.49	0.33	21.4
	2	0.62	0.49	
	3	0.56	0.50	
		(0.56)	(0.44)	

* Samples taken in glass beakers

** Samples taken in polyethylene bags

Figures in parentheses indicate the mean residue levels.

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34 RESIDUES OF INSECTICIDES IN AND ON TOMATO

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SUMMARY

A field experiment was conducted during 1978 to determine the residues of monocrotophos, dichlorvos, quinalphos and endosulfan in tomato fruits at harvest. Monocrotophos and dichlorvos were tested at 0.3 and 0.45 kg a.i/ha and quinalphos and endosulfan at 0.3 a.i/ha only. The insecticides were sprayed at the time of fruiting using a high volume sprayer. Fruit samples were collected one, two and six days after spraying and the residues assessed both by chemical

and bioassay techniques. The insecticide residues estimated one day after spraying ranged from 0.07 to 0.09 ppm for monocrotophos, 0.05 to 0.07 ppm for dichlorvos, 1 ppm for endosulfan and 0.48 ppm for quinalphos indicating that none of the insecticides required a waiting period based on the EPA tolerance limit.

INTRODUCTION

Heliothis armigera Hubner is the major insect pest damaging tomatoes. Control of this pest has been by spraying with endrin or parathion. The restriction or banning of these insecticides in recent years necessitated the use of monocrotophos, dichlorvos, endosulfan and quinalphos for the control of this pest. In an investigation testing the above insecticides against the fruit borer, an attempt was made to assess the residues of these insecticides on tomato.

MATERIAL AND METHODS

A field experiment was set up in August, 1978 at Perur, Coimbatore. The insecticides tested were monocrotophos at 0.3 and 0.45, dichlorvos 0.3 and 0.6, endosulfan and quinalphos at 0.3 kg a.i./ha. The tomatoes were sprayed once at the time of fruiting with a high volume sprayer using 600 litres of spray fluid per hectare. Samples of uniform sized fruits were collected one, three and six days after spraying and the residues were assessed both by chemical and bioassay techniques. Residues of monocrotophos and dichlorvos were determined following the methods of Getz and Watts (1964), quinalphos by the modified method of Mac Dougall *et al.* (1963) and endosulfan by the method of Maitlen *et al.* (1963). The bioassay techniques of Sun *et al.* (1965) were used using *Drosophila melanogaster* Meign. The mean percentage recoveries ranged from 75 to 80.

RESULTS AND DISCUSSION

Residues are summarized in Table I. The residues of all these insecticides from chemical analysis were well below tolerance limit prescribed by EPA for the different insecticides even for fruits harvested one day after spraying. The same trend was also observed in respect of bioassay estimation. Monocrotophos applied at 0.3 kg a.i./ha had residues of 0.064, 0.06 and 0.03 ppm one, three and six days after spraying and at 0.45 kg a.i./ha the corresponding values were 0.074, 0.07 and 0.04 ppm. For endosulfan, the residues after one day were 0.5 ppm and fell to 0.04 ppm within six days. A reduction of 50 per cent in quinalphos residue was observed within 5 days. These data agree with Shankar and Kavadia (1976) who reported that the residues of endosulfan fell below tolerance level within 7 days when applied at 0.1 per cent concentration. The residues of dichlorvos and quinalphos were the

TABLE I
Insecticide Residues (in ppm) in/on Tomato fruits harvested at different periods

Sl. No.	Insecticide	Dose a.i./ha	Chemical assay			Bio assay			EPA tolerance
			—Days after application—			—			
			1	3	6	1	3	6	
1.	Monocrotophos (Nuvacron 40 E)	0.5	0.054	0.062	0.03	0.04	0.03	ND	0.2
2.	Monocrotophos (Nuvacron 40 E)	0.45	0.074	0.07	0.04	0.08	0.06	0.02	
3.	Dichlorvos (Nuvan 100 E)	0.3	0.05	0.04	0.03	0.02	ND	ND	0.1
4.	Dichlorvos (Nuvan 100 E)	0.6	0.06	0.05	0.02	0.03	ND	ND	
5.	Endosulfan 33 E	0.5	0.56	0.4	0.11	0.22	0.23	ND	2.0
6.	Endosulfan 33 E (Systex 25 E)	0.5	0.42	0.21	0.15	0.25	0.13	0.0	2.0

* As given by the manufacturer
ND---below detectable level.

form estimated for this crop. There were no detectable residues after 10 days for all the insecticides tested.

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35 RESIDUES OF PHENTHOATE APPLIED FOR CONTROL OF TOMATO FRUIT BORER, *HELIOTHIS ARMIGERA* HUBNER

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SUMMARY

Phenthoate (0.05 per cent) applied at 0.5 kg a.i./ha for the control of tomato fruit borer *Heliothis armigera* H. was found to be comparable in efficacy with malathion applied at the same dosage but was significantly inferior to carbaryl applied at 1 kg a.i./ha. The average per cent infestations were 12.3, 10.6 and 3.2 respectively. Residue studies on phenthoate indicated that an initial deposit of 2.62 ppm dissipated to 14 ppm in 10 days and to non-detectable limit in 14 days. Washing of tomatoes with water reduced 75 per cent of the insecticide deposit and washing followed by cooking removed 82 per cent of the deposit. In view of the absence of tolerance limit for phenthoate and the long time it takes to completely dissipate, its use on tomato should be limited.

MATERIAL AND METHODS

A tomato crop was raised in 5x8 m plots separated by 1 m paths. All treatments and control were replicated 3 times in a random block. The crop was sprayed with phenthoate (0.05 per cent), carbaryl (0.1 per cent), malathion (0.05 per cent) at the rate of 1000 l/ha (equivalent to 0.5, 1.0 and 0.5 kg a.i./ha respectively), when fruit formation had just started a second spray was given 20 days thereafter when fruit ripening had started. Efficacy of control of the pest was based on the percentage of fruits infested compared with control. For this all the ripe fruits were picked daily for 15 days and examined for infestation. Residues of phenthoate were determined by a cholinesterase inhibition method (Porter, 1964) and also by a colorimetric method (Getz and Watts, 1964) based on 4-p'-nitrobenzyl pyridine as chromogenic reagent. Residues were determined on unwashed, washed and cooked tomatoes. For washing, the tomatoes were taken in double their weight of water in a tray and rubbed gently with hands for 2 minutes. For cooking, washed tomatoes were taken in water half the weight of the tomatoes and boiled for 20 minutes in a metallic pan covered with a loose cover on medium heat till they became soft. For all the estimates a representative homogenous sample of 50 g from a bulk sample of 2 kg for each treatment was taken and analysed.

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RESULTS AND DISCUSSION

a) Efficacy of insecticides for the control of tomato fruit borer

Carbaryl was the most effective insecticide and significantly superior to malathion and phenthoate. The average per cent infestation was 3.2, 10.6 and 12.3 for the three insecticides respectively. Although phenthoate was slightly inferior to malathion, however, statistical analysis did not support any difference between these two treatments. All treatments produced significantly smaller infestations than the check plots where average infestation were 24.6 per cent.

TABLE I

Efficacy of different insecticides for the control of tomato fruit borer H. armigera

Insecticide	Concentration	Dosage kg a.i./ha	Commercial formulation used	Average fruit infestation	
				per cent	angular value
Carbaryl	0.1	1.0	Sevin 50 WP	3.2	10.2
Malathion	0.05	0.5	Malathion 50 EC	10.6	19.0
Phenthoate	0.05	0.5	Phendal 50 EC	12.3	20.5
Control	—	—	—	24.6	29.7
SE					1.08
CD at 5%					4.02

b) Residues of phenthoate

Analysis of samples by an enzyme inhibition method and the colorimetric method of Getz and Watts (1964) gave comparable results except that the sensitivity of cholinesterase inhibition method could detect up to 0.12 ppm residues after

TABLE II

Residues of Phenthoate on tomato when used at 0.5 kg a.i./ha

Days after treatment	Residues ppm					
	Enzyme inhibition method			Colorimetric method		
	Unwashed	Washed	Washed & cooked	Unwashed	Washed	Washed & cooked
0	2.62	0.62	0.39	2.35	0.68	0.30
1	1.23	0.57	0.35	0.96	0.50	0.26
4	0.58	0.44	0.25	0.40	0.33	0.24
7	0.42	0.40	0.37	0.35	0.40	0.19
10	0.14	0.18	0.12	ND	ND	ND
11	ND	ND	ND	ND	ND	ND

10 days whereas the other method did not detect any residues (Table II). Therefore, only the enzyme inhibition method was considered. The initial deposit of phenthoate on tomatoes was 2.62 ppm which dissipated to 0.14 ppm in 10 days. Washing the fruits reduced residues by about 75 per cent on the day of spraying but the percentage removal dropped with time so that on 10th day there was no reduction due to washing. Washing coupled with cooking removed about 82 per cent residues on the day of spraying but as with washing the effect declined with time. No tolerance limit has been fixed for phenthoate in India or abroad on agricultural commodities, although 0.5 ppm has been suggested on grains by Rowlands (1975). In the absence of a tolerance limit for this insecticide, it is concluded that a waiting period of 14 days should be observed on tomatoes but this is impracticable because picking has to be done after every 2-3 days. Use of phenthoate on tomato may, therefore, be restricted.

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PERSISTENCE OF MALATHION IN TOMATO, BHENDI AND BRINJAL CROPS

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SUMMARY

Studies on the persistence of malathion on tomato, brinjal and bhendi vegetables were made. Chemical assay by thin layer chromatography was used to determine residues of malathion. The crops were raised in summer and monsoon seasons and sprayed with four concentrations i.e., 0.05, 0.1, 0.2 and 0.4 per cent malathion. The vegetables were analysed daily until the residues disappeared.

A total of 315 vegetable samples were analysed with 57, 74, 87 and 97 samples for 0.05, 0.1, 0.2 and 0.4 per cent concentrations respectively. The initial residues were largest and varied according to levels of concentration. At 0.1 per cent concentration, residues ranged from 4 to 9.5, 5.5 to 6.5 and 5 to 11 ppm on tomato, brinjal and bhendi respectively. Eventhough the initial deposit was high, the residues fall below the tolerance limit of 8 ppm fixed by U.S.F.D.A. within 72 hours. Hence malathion can safely be applied to vegetables at 0.1 per cent concentration and the produce can be consumed 3 days after spray, without any health hazard to the consumer.

The rate of dissipation of malathion was more in summer than in the monsoon season. Residues were more on bhendi fruits than on tomato or brinjal, which may be attributed to the direct exposure to spray in the case of bhendi fruits. Even at high concentrations like 0.2 and 0.4 per cent there was no phytotoxicity noticed on the plants.

The hazards involved in the use of organophosphorus insecticides are well known and the use of pesticides on crops like tomato, bhendi and brinjal which are frequently harvested and marketed for consumption demand attention to residues. The persistence of malathion on tomato, bhendi and brinjal fruits was studied at the College of Agriculture, Hyderabad.

MATERIAL AND METHODS

Tomato variety 'Pusa ruby', bhendi variety 'Pusa savani' and brinjal variety 'Pusa kranti' were grown at the Insectory Farm in plots of 10 x 20 m. Two plots were planted with 21 day old seedlings of tomato or brinjal at 60 x 45 cm and 50 x 30 cm spacing, respectively. Seeds of bhendi were sown with a spacing of 30 x 10 cm. For each vegetable one plot was used as control and the other plot was sprayed with 0.05, 0.1, 0.2 and 0.4 per cent concentrations of malathion at 1 kg a.i./ha leaving two rows between each of the different concentrations. The plants were sprayed at fortnightly intervals one week after planting.

When the vegetables became ready for picking, samples were taken immediately after spraying and at intervals of 24 hours up to 144 hours. From each plot 2 kg of vegetables were picked at random, bulked and divided into three portions for determining residues.

EXTRACTION

Five hundred grams of each of the sample was grated, mixed together and 25 g transferred to a 250 ml ground glass stoppered conical flask and shaken on a flask shaker for one hour, after adding 50 ml methylene chloride and 25 g of sodium sulphate. Addition of anhydrous sodium sulphate helped to reduce the moisture content of the samples. The extract was filtered through cotton into a ground glass stoppered flask.

Preparation of Standard Solution : Analytically pure malathion obtained from C.P.P.T.I. was used for a standard solution. A known weight of the pesticide was taken in a ground glass stoppered tube and diluted with ethyl acetate to give a concentration equivalent to 1 mg/ml. One microlitre of this solution contained 1 microgram of malathion. The standard solution was stored in a refrigerator.

Spotting and Development : An aliquot of the sample equivalent to one gram of the vegetable (2 ml) was pipetted into a 10 ml beaker and its volume reduced to about 0.5 ml. The extract was taken in a 10 μ l microsyringe and spotted on the TLC plate. Care was taken to see that the diameter of the spot was not allowed to exceed 5 mm. The sample was transferred quantitatively by rinsing the beaker three times with small volumes of ethyl acetate and also spotting the rinsings. After spotting the samples, a standard insecticide solution containing 1 μ g/ μ l was spotted on the plate. Six samples and three spots of standard insecticide were accommodated on each plate.

The plate was developed in a glass tank (25 × 15.5 × 13 cm) by ascending chromatography with 95 : 5 benzene and acetone. The solvent front was allowed to rise to a height of 15 cm from the origin which took about 45 minutes and the plate was exposed to bromine vapours for half a minute and was sprayed with bromocresol green indicator. Malathion appeared as yellow spots on a bluish green background.

Confirmation and Quantitation : Malathion was confirmed by the RF values of the standard spot with those in the samples. A quantitative estimate was made by visual comparison of the spot area and intensity in the sample and the standard. The smallest quantity detected by this method was 0.5 μ g malathion. The quantity

of malathion was estimated as described by Lakshminarayana and Krishna Menon (1974).

RESULTS AND DISCUSSION

A total of 97 samples for 0.4 per cent conc. of malathion, 87 samples for 0.2 per cent conc., 74 samples for 0.1 per cent conc. and 57 samples for 0.05 per cent conc. of malathion were analysed. In all 315 samples were analysed for four different concentrations of malathion on brinjal, bhendi and tomato crops and the results are given in Tables I and II.

Tomato analysis : The results of analysis of the samples for residue of malathion on tomato showed that the residues fell to 3 ppm within 48 hrs of spraying during summer, although the initial deposit was as high as 6 ppm when sprayed at

TABLE I
Residue analysis of malathion during summer season

% Conc. of malathion	Residue in ppm after hours						
	1	24	48	72	96	120	144
Tomato							
0.4	7.0	5.5	4.0	2.5	1.5	—	—
0.2	5.5	4.0	2.5	1.0	—	—	—
0.1	4.0	3.0	2.0	0.5	—	—	—
0.05	3.0	2.0	1.0	—	—	—	—
Bhendi							
0.4	8.0	6.5	5.0	3.5	1.5	0.5	—
0.2	6.5	5.0	3.0	1.5	0.5	—	—
0.1	5.0	3.5	2.5	1.5	—	—	—
0.05	3.0	2.0	0.5	—	—	—	—
Brinjal							
0.4	8.5	7.0	6.0	4.5	2.5	1.0	—
0.2	7.0	6.0	4.0	3.0	1.5	—	—
0.1	6.0	4.5	3.0	1.0	—	—	—
0.05	4.0	3.0	1.0	—	—	—	—

0.1 per cent conc. (1 kg a.i./ha). Similar results were obtained by Singh and Rattanlal (1965) with 0.1 per cent and 0.2 per cent concentrations of malathion on tomato fruits where residues fell below the tolerance limit within 72 hours. The dissipation of malathion on tomato fruits was faster during March and April than during Nov. Dec. (Singh and Rattanlal, 1971).

According to the U.S.F.D.A., the tolerance of malathion on vegetables is 8 ppm and the recommendation in India is 3 ppm. It is very unlikely that the treated crop will reach a kitchen in less than 48 hours. Hence, a spray giving a residue less than 3 ppm of malathion within 48 hours can be considered very safe to the consumer.

When the crop was sprayed later, in the year *i.e.* in the monsoon season the initial deposit increased from 8 to 9.5 ppm. This fell to an average of less than 3

TABLE II
Residue analysis of malathion during monsoon season

% Conc. of malathion	Residue in ppm after hours						
	1	24	48	72	96	120	144
Tomato							
0.4	11.5	9.0	7.5	5.0	2.0	0.5	—
0.2	10.0	8.0	6.0	3.5	1.5	—	—
0.1	9.0	7.0	4.5	2.0	0.5	—	—
0.05	7.0	5.0	3.0	1.5	—	—	—
Bhendi							
0.4	13.5	11.0	9.0	7.0	4.5	2.5	1.0
0.2	11.5	9.5	6.0	4.0	2.0	0.5	—
0.1	10.0	7.5	5.5	4.0	2.0	0.5	—
0.05	8.0	6.0	4.0	2.0	0.5	—	—
Brinjal							
0.4	10.0	7.5	5.5	3.0	1.5	—	—
0.2	8.0	5.0	3.0	1.5	—	—	—
0.1	6.0	4.0	2.5	1.0	—	—	—
0.05	4.0	2.0	0.5	—	—	—	—

ppm within 72 hours. These results agree with those of Lakshminarayana and Menon (1974) and Smith *et al.* (1954).

The degree of insect control obtained from the different dosages applied did not differ much. There was little change in mortality when higher concentrations were used. Hence, 0.1 per cent spray applied at a rate of 1000 litres per hectare (1 kg a.i./ha) is satisfactory and does not pose any health hazard for the consumer.

Bhendi analysis: Bhendi samples showed that residues fell to 3 ppm or less within 48 hours after spraying at 0.1 per cent conc. (1 kg a.i./ha). Under normal conditions, vegetables such as bhendi do not reach the consumer within 48 hours. Similar analysis were obtained by Gupta and Mishra (1971) who used 0.1, 0.2 and

0.4 per cent conc. of malathion on cauliflower heads and the residues diminished by 50-70 per cent within three days of spraying.

When the monsoon crop was sprayed later in the year, initial deposits with 0.1 per cent conc. sprayed at the rate of 1 kg a.i./ha were 9.5 to 11 ppm. This fell to an average of 3 ppm within 72 hours except in one instance when it took 96 hrs. These results agree with Waites and Middlelem (1955) for tomatoes and bhendi. In bhendi insect control obtained by the different dosages applied did not vary much. At higher concentrations there was no marked increase in mortality and to obtain good control of the insects a 0.1 per cent spray on 1 kg a.i./ha was satisfactory.

Brinjal analysis : The residues of malathion on brinjal fell to 3 ppm or less within 48 hours after spraying when sprayed at 0.1 per cent conc. 48 hours was sufficient to diminish the higher deposit to 3 ppm which is considered to be very safe to consumer. These results are in agreement with those of Lakshminarayana and Menon (1974) who recommended a withholding period of three days after spraying for tomato and brinjal.

Later in the year when malathion was sprayed at 1 kg a.i./ha of 0.1 per cent conc. on the monsoon crop, the initial deposit of 6.0 to 6.5 ppm fell to 3 ppm or below within 72 hours. These results agree with those reported by Singh and Rattanlal (1965, 1971). The mortality of insects did not increase with higher concentrations and the insect control did not vary much from the different dosages applied. Hence 0.1 per cent spray of malathion at 1000 litres per ha can be adopted safely by farmers for insect control on brinjal.

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37 PERSISTENCE OF RESIDUES OF SOME ORGANOPHOSPHORUS INSECTICIDES IN CUCUMBER FRUITS

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SUMMARY

Five organophosphorus insecticides were tested for toxicity as residues in cucumber fruits (*Cucumis sativus* L.) to the neonatal larve of fruit fly (*Dacus cucurbitae*) in the laboratory. Fenitrothion and methyl-parathion were highly toxic; malathion and trichlorfon were intermediate and fenthion was least toxic compound. The persistence of effective toxicity of these insecticides at recommended rates of application close to picking was examined under field conditions on cucumber cv. Pusa Sanyog. Fenitrothion and methyl-parathion provided reasonable protection to the fruits but the others were less persistent and effective against the pest. The safety intervals for consumers following treatment were minimum with malathion and maximum (12 days) with trichlorfon, about 7 days for methyl parathion and fenitrothion and 4 days for fenthion.

The damage by *Dacus cucurbitae* (Coquillett) becomes very serious after the first shower of the monsoon (which normally enters the valley in the third week of June) when the infestation reaches 100 per cent within a fortnight at a time when farmers expect maximum return from this crop. Amongst organophosphorus (OP) insecticides reported giving effective control of the pest are fenthion, fenitrothion, malathion and trichlorfon (Dale and Nair, 1966; Keiser, 1968; Tang and Chang, 1971 and Sharma *et al.*, 1973). None of these workers, however, give sufficient informa-

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tion on the toxicity of residues to the pest on cucumber close to picking and there is yet no indication on the field persistence of either of these insecticides in India. The present investigation, involving both field and laboratory studies on the toxicity and persistence of residues of five insecticides was, therefore, considered necessary to obtain more detailed information on the biological performance of insecticides for close-to-harvest application, than is normally available from either field trials or laboratory assessment of their relative toxicity.

MATERIAL AND METHODS

Persistence of insecticides

Commercial formulations of each of the 5 insecticides, listed in Table I, were sprayed to 'run-off' at 0.05 per cent on 10 vines, bearing cucumber fruits, per treatment (cv Pusa sanyog) using a double barrel pump, on 17th June, 1978.

Sampling and extraction

Samples were taken immediately the spray was dry (0 day) and after 1, 3, 5, 7, and 15 days. On each occasion, 12 cucumber fruits were taken at random from each treatment and divided into three replicates. Fruits of each replicate was peeled

TABLE I
*Toxicity of insecticides to the neonatal larvae of D. cucurbitae
in 5 mm layer of cucumber fruits*

Insecticides	LD ₅₀ (ppm)	95% Confi- dence limits (ppm)	LE ₉₀ (ppm)	95% Confi- dence limits (ppm)	Dosage-mortality regression equations
Malathion	0.1569	0.1086-0.2267	1.0640	0.4531-2.5000	$Y=1.5412x+0.0750$
Trichlorfon	0.4166	0.3257-0.5328	1.5180	0.9237-2.4970	$Y=2.2816x-3.2586$
Fenitrothion	0.1454	0.1299-0.1628	0.2717	0.2161-0.3416	$Y=4.7201x-9.9276$
Fenthion	0.6628	0.5174-0.8492	2.4070	1.4530-5.1740	$Y=2.2877x-3.7422$
Methyl- parathion	0.0819	0.0525-0.1280	0.7338	0.3253-1.6560	$Y=1.3463x+1.0774$

Heterogeneity : X^2 (3 df) : Non-significant ($P=0.05$) in all insecticides.

Y = Probit kill, x = log concentration $\times 10^4$ (ppm).

from all sides and one end to the other to a depth of 5 mm with an ordinary kitchen peeler. This removed a uniform peel of 0.82 ± 0.2 mm thickness except for the top layer where the peel was 0.98 ± 0.2 mm thick. With five such peelings, an epidermal layer of 5 mm depth could be obtained. The peels were weighed and transferred to a Waring blender to which equivalent amount of anhydrous sodium sulphate was

added. The insecticides were extracted in suitable solvents by blending them for 1-2 minutes until a slurry was produced which was decanted, filtered and cleaned up with activated charcoal. Samples were processed the same day but if not possible, stored in a refrigerator with 8-10 g anhydrous sodium sulphate per 200 ml reagent bottle.

Analytical method

Residues were determined by a colorimetric method using a Spectronic-20. Fenthion and trichlorfon residues were estimated by the method of Jain *et al.* (1974) based on Getz and Watts (1964). Malathion residues were determined by Sutherland's (1964) method; while fenitrothion and methyl-parathion by Averell and Norris' (1948) method. Standard calibration curves for each insecticide, dissolved in respective technical grade solvents (for malathion CCl_4 and for the rest benzene), were used to determine insecticide residues in the unknown field sample extracts. Recoveries of malathion and methyl-parathion were 95 and 93 per cent (± 5 per cent) respectively, and those of fenitrothion, fenthion and trichlorfon 85 per cent (± 5 per cent).

Bioassay

Toxicity of the residues was determined by bioassay using neonatal larvae of the fly (*D. cucurbitae*) from a laboratory culture. Three ml of spray solutions of the commercial formulations of the insecticides (Table I) were sprayed in a Potter tower on a portion of cucumber fruit at a rate of 2 mg cm^{-2} (± 5 per cent). Deposits were allowed to dry and the sprayed fruits were stored overnight under laboratory conditions (Temperature $25 \pm 5^\circ\text{C}$, RH 80 ± 5 per cent) to allow the insecticide to penetrate the epidermal layer of the fruits. A metallic ring ($17 \times 9 \text{ mm}$) was inserted over the sprayed surface of the fruit to cover the epidermal layer to a depth of 5 mm. The fruit surface inside the ring was punctured superficially with a fine needle to facilitate the entry of larvae in the pulp before releasing them. In a preliminary experiment, it was observed that the larvae rarely penetrated more than 5 mm deep inside the fruit 24 hours after their release; the ring facilitated location of the released larvae inside the pulp. Five newly-hatched larvae were placed within each ring and the test material was stored for 24 hours at $25 \pm 1^\circ\text{C}$, and 70 per cent RH before mortality counts were made. On a separate occasion, similar disks ($16 \times 5 \text{ mm}$) from sprayed fruits (*i.e.*, sprayed a day earlier) were taken out, weighed, blended in a Waring blender, using equivalent amount of anhydrous sodium sulphate and extracted in the respective solvents to determine the actual amount of insecticide residue (using colorimetric methods as mentioned earlier) in the epidermal layers, for each concentration of each of the five insecticides causing mortality of the larvae from about 20-80 per cent. Data on the mortality of the larvae corresponding to the actual

amount of insecticides were corrected by Abbott's (1925) formula and subjected to probit analysis (Finney, 1952).

Calculations

The 'half-life' values were calculated on the basis of formula : $t_{\frac{1}{2}} = 2/K_i$ as suggested by Gunther and Blinn (1955) and Hoskins (1961), and effective life and safety intervals were worked out by respective regression equations taking $\log LD_{90}$ values and the recommended tolerance levels (ppm) of the insecticides as 'Y' and days as 'X'.

RESULTS AND DISCUSSION

Bioassay of the intrinsic toxicity (Table I), based on LD_{50} values, of the insecticides showed that methyl-parathion was highly toxic to *D. cucurbitae*. Fenitrothion and malathion were intermediate and trichlorfon and fenthion were less toxic compounds. A better guide to likely field performance is the minimum effective level at LD_{50} (Gratwick *et al.*, 1965 and Gratwick and Tew, 1966). Of the compounds tested and compared, fenitrothion surpassed methyl-parathion while malathion and trichlorfon were the next better compounds. There was, however, no change in fenthion.

When the insecticides were sprayed on cucumber at the same concentration the magnitude of the initial deposits (Table II) varied slightly. The rate of dissipation was rapid in malathion and trichlorfon and residues could not be detected after 7 days, whereas methyl-parathion, fenthion and fenitrothion were slightly more persistent ('Half-life' = 2 days).

'Half-lives' and effective life of insecticides (Table III) provide useful and interesting information, to enable the performance of insecticides under field conditions to be judged. Malathion and trichlorfon provided least protection to the cucumber against *D. cucurbitae* (c. 3-5 days) due to low persistence and lower toxicity, whereas fenitrothion followed by methyl-parathion were highly effective against the pest because of their relatively high persistence and high toxicity. There was little variation in temperature and RH conditions during the experimental period (averages in Table II) and also negligible rainfall up to 21st June, and thereafter. Except for the slight rain during last few days of the sampling period it is unlikely that the weather conditions played any significant role in the dissipation of insecticide residues.

The safety period of trichlorfon on cucumber for consumers was unusually very high (11.7 days) when calculated on the 0.1 ppm basis fixed for pumpkin (Anon., 1976). The minimum period of safety was involved in the use of malathion (Table

TABLE II
Persistence of OP-insecticide residues on cucumber fruits at Palampur

Insecticides	0-day	Deposits (ppm) 1-day	3 day	following 5-day	7-day	spray 15-day
Methyl parathion	10.634 ± 0.438	4.817 ± 0.449	2.101 ± 0.325	1.191 ± 0.131	0.557 ± 0.111	0.032 ± 0.014
Fenitrothion	9.890 ± 0.798	3.772 ± 0.546	1.450 ± 0.241	0.867 ± 0.242	0.303 ± 0.009	0.065 ± 0.002
Malathion	8.220 ± 0.630	3.395 ± 0.631	1.190 ± 0.337	0.594 ± 0.107	0.224 ± 0.105	BDL
Trichlorfon	10.092 ± 1.785	5.981 ± 0.802	3.608 ± 0.525	1.587 ± 0.285	0.563 ± 0.169	BDL
Fenthion	10.480 ± 1.341	5.579 ± 1.332	2.716 ± 1.294	1.172 ± 0.882	0.448 ± 0.094	0.056 ± 0.002

BDL = Below detectable limit

Weather condition during the experimental period : Temp. : Max. 26.8°C, min. 20.5°C

RH = 79.6 per cent, Rainfall = negligible up to 21 June '78 and slightly more till the period of sampling.

TABLE III

Biological performance of OP-insecticides on cucumber (fruits) at Palampur (HP)

Insecticides	Dosage ¹ % a.i.	Time-deposit regression equations ²	me l log depo-sit X10 ³ (vide table I)	Effective life (days)	Half life ³ (days)	Safety interval ³ (days)
Methyl parathion	0.05	$Y = 3.9397 - 0.1389x$	2.8656	7.7	2.17	6.77
Fenitrothion	0.05	$Y = 3.7062 - 0.1378x$	2.4341	9.2	2.18	7.31
Malathion	0.05	$Y = 3.8102 - 0.2129x$	3.0269	3.7	1.41	0.48
Trichlorfon	0.05	$Y = 4.0063 - 0.1713x$	3.1784	4.8	1.76	11.71
Fenthion	0.05	$Y = 3.8857 - 0.1498x$	3.3815	3.4	2.01	3.90

¹sprayed on 17th June 1978.² x = days corresponding to log deposit (Y) $\times 10^3$.³worked out on the basis of respective time-deposit regression equations of insecticides (col. 3).

III) followed by fenthion (c. 4 days, based on 2 ppm tolerance fixed by FAO/WHO, Anon. 1971) and c. 7 days for methyl-parathion and fenitrothion worked out on the basis of 1 and 0.5 ppm tolerances of FDA/USA, Anon. (1967) and Codex, FAO, Anon. (1976) respectively.

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38 INSECTICIDAL RESIDUE PROBLEMS IN CONTROLLING PESTS OF OKRA

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SUMMARY

Okra is an important source of vegetable throughout the year. The crop suffers from the heavy attack by insect pests such as jassids (*Amrasca biguttula biguttula* Ishida), fruit borers

(*Earias vitella* Fab.) aphids (*Aphis gossypii* Glor.) etc. The use of pesticides is essential to protect the crop from insect pests. The persistence of toxic residues is serious for the consumers which needs attention.

The paper deals with the residues of different insecticides left on the fruits after application; the observance of the required waiting period for the harvest of the fruits; the effect of home processing to bring down the level of the residues and the safe time of application etc.

Okra suffers from heavy attack by two pests, jassids (*Amrasca biguttula biguttula* Ishida) and fruit borers (*Earias vitella* Stoll.), which are major limiting factors in its profitable cultivation. The use of pesticides is essential not only to protect the crop, but also for good quality of fruits and for better harvest of crop.

Fenitrothion is now being used in India to control various agricultural pests. Fenitrothion controls insect pests of vegetables, viz., mustard aphid on mustard (Sharma and Saxena, 1972); fruit and shoot borer on 'bhendi' (Mote and Pokharkar, 1974); jassid on potato (Mavi and Singh, 1975) and cabbage aphid on cauliflower (Attri, 1977). Lindane controls a number of insect pests of vegetables viz., *Earias fabia* Stoll. on bhendi (Dhall and Rattan Lal, 1965); mustard aphid on brown sarson (Gupta, 1971) and fruit and shoot borer on brinjal (Ganguli *et al.*, 1971).

Information on the persistence of residues of these insecticides on okra under different agro-climatic conditions of India is lacking. The present studies were undertaken to investigate their residues on 'bhendi' a widely grown and commonly used vegetable. The effects of home processing such as washing and cooking were also studied to bring down the level of the residues.

MATERIAL AND METHODS

Okra (Var. Pusa Sawani) was raised at the farms of IARI, New Delhi. A randomised block design was used for the experiment and the treatments and the control were replicated three times in 7m x 6m plots separated from each other by a 1.2 m buffer area. Fenitrothion and lindane (0.1 per cent emulsion) at 1500 l/ha (1.5 kg a.i./ha) were sprayed at the fruit setting stage of the crop. Average temperature during the investigation was 26°C. No rain was recorded.

Samples of fruit (marketable size 3—4") were pooled by treatment and chopped finely with knife. A representative sample of 40 g was taken for each analysis. Samples treated with fenitrothion were extracted in redistilled chloroform and lindane in hexane + isopropyl alcohol (3 : 1) then this was removed by distilled water before analysis. The extracts were cleaned with hyflosupercel + anhydrous sodium sulphate + activated charcoal (5 : 5 : 1) by column chromatography. Bioassay was used as

the principal method of residue analysis and the results were checked by a colorimetric method described by Getz and Watts (1964) for fenitrothion and a GLC method for lindane. Red cotton bug, *Dysdercus koenigii* Fab. (48-72 hr old-nymphs) and vinegar fly, *Drosophila melanogaster* Meigen (one day old) were used as test insects for bioassay of fenitrothion and lindane respectively.

To study the effect of washing on the reduction of residues a 50 g sample was taken in a small tray in 500 ml of water. The material was gently rubbed by hand for 30 seconds and the water discarded. This was repeated twice and samples analysed. Studies on the combined effect of washing and cooking, on the reduction of residues were made on samples which were washed as above and chopped and then transferred to 500 ml glass beaker. About 50 ml water was added and the beaker covered with a petri-dish. It was allowed to boil on a hot plate of medium heat for 20 minutes. The samples were cooled at room temperature; extracted and residues determined.

RESULTS AND DISCUSSION

(i) Persistence of residues

Okra is a fast growing crop, and fruits reach a marketable size within 3-4 days. An initial deposit of 4.72 ppm from 0.1 per cent fenitrothion spray required about 6 days to fall to the tolerance limit of 0.3 ppm. No residues were detected after 10 days.

TABLE I
Residues of fenitrothion and lindane on marketable size fruits

Days after treatment	Residues (ppm)	
	Fenitrothion	Lindane
0 (1 hr)	4.72	5.64
1	1.98	4.82
3	0.56	3.06
7	0.21	0.48
10	BDL	BDL
Total	5.62 days	2.26 days

The percentage loss of insecticide was high being about 58 to 64 per cent within the first 24 hrs. Similar results were reported by Joia and Kalra (1977) with a reduction of fenitrothion residues of 71 per cent after one day. The residues present in the fruits from the foliar application of 0.1 per cent lindane reached the

tolerance limit of 3.0 ppm within three days. The initial deposit of 5.64 ppm dissipated quickly and there was reduction of nearly 90 per cent in 7 days. The residues of fenitrothion and lindane dissipated at a faster rate on 'bhendi' fruits than the other vegetable crops. Fenitrothion fell to the tolerance level in about 10 days after application on cauliflower curds (Attri, 1977), and lindane after 14 days on cabbage (Floru and Alexandri, 1973). The reduction of residues of these insecticides could be due to two factors, either dilution of the insecticide by the fast growth of the fruits or the effect of weathering. These results agree with those made by Van Middlelem and Baranowski (1962) on a tomato crop. In terms of the waiting period required for the use of the commodity lindane seems to be better than fenitrothion. The dissipation of fenitrothion was faster than lindane but, the waiting periods were different, due to the low tolerance limit of fenitrothion. It is suggested that the marketable fruits should be removed before treatment. A waiting period of 6 and 3 days should be observed for fenitrothion and lindane respectively after the application.

(ii) **Effect of water washing on residues :**

Washing the fruits for reduction of insecticides residues were very effective and reduction was more in earlier samples and decreased in later samples. Washing on the treatment day in case of lindane and after 3 days in case of fenitrothion brought residues to safety limit recommended for these insecticides. Reductions of 23.81 to 48.73 for fenitrothion and 12.50 to 48.40 per cent for lindane were recorded depending upon the samples interval and quantity of insecticide applied.

TABLE II
Effect of home processing (washing and combined effect of washing and cooking) on the reduction of residues

Treatment	Days after application	Residues on unprocessed fruits (ppm)	Home processing			
			After washing (ppm)	Percent reduction	After cooking (ppm)	Percent reduction
Fenitrothion 0.1%	0	4.72	2.42	48.73	1.95	58.80
	1	1.98	1.18	40.40	0.95	52.02
	3	0.56	0.39	30.36	0.30	46.66
	7	0.21	0.16	23.81	—	—
Lindane	0	5.64	2.91	48.40	2.45	56.56
	1	4.82	2.85	40.87	2.32	51.86
	3	3.06	2.36	22.88	1.63	46.73
	7	0.48	0.42	12.50	0.34	29.13

(iii) **Effect of combined washing and cooking on the reduction of residues :**

Cooking after washing caused a reduction of 58.80 to 46.66 per cent residues on the fruits from 0 to 3 days after spraying the crop with 0.1 per cent fenitrothion. A similar trend was observed for lindane with a reduction from 56.56 per cent to 29.13 per cent so that washing and cooking was more effective than washing alone in reducing residues.

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39 STUDIES ON THE RESIDUES OF SYSTEMIC INSECTICIDES INJECTED INTO THE MANGO PLANTS

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SUMMARY

Six systemic insecticides, phosphamidon, monocrotophos, dimethoate, formothion, oxydemeton methyl and vamidothion were injected into mango trees at rates 0.1, 0.2 and 0.3 ml a.i./cm girth of the plant. To assess residues in the leaves, flowers and fruits bioassay method was adopted.

Analysis of the leaves one week after injection showed a maximum of 8.26 ppm of phosphamidon 0.3. A minimum of 5.2 ppm of monocrotophos was recorded. The lowest residue recorded at the end of the sixth week was 0.3 ppm and the maximum was 0.86 ppm for phosphamidon (0.1) and vamidothion (0.3) respectively. After this period no residues were found.

Similar conditions were observed for flowers. Maximum residues at the end of the first week were 8.00 ppm of phosphamidon (0.3) and the lowest 4.96 ppm of dimethoate (0.1) which reduced to 1.2 ppm and 1.0 ppm respectively at the end of the sixth week. After this period no insecticide residues were detected.

Bioassay of residues, in the young fruits revealed no residues.

INTRODUCTION

Mango is grown in many countries such as the Philippines, Indonesia, Jawa, Thailand, Burma, Malaya and Ceylon, in addition to India. It is gaining importance in Hawaii and West Indies. Over fifty per cent of the area under fruit crops in India, is occupied by mango, but, the yield is very low, compared to other fruit crops. Among the factors that have been considered responsible for such a low yield are damage by insect pests and diseases. Of the insect pests, the mango hoppers, *Amritodus atkinsoni* Leth., *Idioscopus niveosparsus* Leth., and *Idioscopus clypealis* Leth. have been found to cause severe losses by sucking the sap from the tender leaves and inflorescences. Losses are from 25 to 60 per cent (Vasanthraj David and Kumaraswamy, 1975).

Although insecticides are used to control these hoppers they have not given satisfactory control probably due to difficulties experienced in the application.

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Thontadarya *et al.* (1978) attempted to control these hoppers by altogether a new technique of stem injection of systemic insecticides, but their studies were restricted to off season mango and they did not attempt to determine residues. The present investigation, tests different insecticides and attempts to assess residues in leaves, flowers and fruits of mango during the flowering season.

MATERIAL AND METHODS

Grafted mango trees of about 20 years age were selected for the experiment at Dharwar, Karnataka State in 1977-78. Six systemic insecticides, dimethoate, formothion, monocrotophos, oxydemeton-methyl, phosphamidon and vamidothion were injected at concentrations of 0.1, 0.2 and 0.3 ml a.i. per running cm of tree girth. The insecticides were injected. Holes (1.25 cm dia.) were bored into the stems to a depth of 15 cm at an height of 60 cm above the ground level in late December 1977. Two holes were bored at opposite side of each tree at an angle of 45° to the main axis of the tree. Insecticides were applied using a funnel and holes were plugged with a piece of fresh mango twig. The effect of the insecticides was estimated by assessing reductions in population of hoppers at weekly interval up to March 1978.

Residues in leaves, flowers and fruits of the trees were assessed at weekly intervals. To assess the residues a 'bioassay method' described by Sun *et al.* (1965) was used with different solvents for different insecticides. Acetone was used to extract phosphamidon, monocrotophos, formothion and vomidothion and chloroform for dimethoate and oxydemeton methyl. Extraction was made with both the solvents separately on control samples. Two-day old unsexed *Drosoohila melanogaster* Meig., reared on an artificial diet, were used to estimate residues. Mortality of the flies was recorded after 12 hours and corrected for the control by Abbott's formula. The corrected mortality percentages were converted to probit values and residues estimated on a standard curve. Each treatment was replicated three times and twenty flies were introduced into each replicate. The technical insecticide was used for constructing the standard curve.

RESULTS AND DISCUSSION

All six insecticides reduced populations by 90 per cent for seven weeks after application and by more than 70 per cent even after ninth week. Results for dime-thoate agreed with those of Thontadarya *et al.* (1978).

Residues in leaves, flowers and fruits are presented in Tables I and II.

Residues in leaves

All insecticides at the highest concentrations (0.3 ml) gave maximum residues in leaves up to 8.26 ppm for phosphamidon. The lowest residue was 5.2 ppm for dimethoate at 0.1 ml at the end of first week after application. In all the treatments no residue of any insecticide was detectable after seven weeks.

Even at the lowest residue level of 1.5 ppm, found at the end of six weeks, more than 95 per cent reduction in the hopper populations was observed in all the treatments. The reduction in the population was more than 85 per cent even nine weeks after treatment, and was still 50 per cent or more at the end of eleventh week, after application. These results indicate that even the lowest amount of residue, at the end of six weeks did not permit hopper population to build up.

TABLE I
*Residues of insecticides injected for the control of mango hoppers
(in ppm) in leaves*

Insecticides	Concen- trations	1st week	2nd week	3rd week	4th week	5th week	6th week
Phosphamidon	0.1	6.0	5.01	4.3	3.1	2.3	0.3
	0.2	7.1	6.3	5.1	3.6	2.4	0.5
	0.3	8.26	6.8	5.3	4.1	2.7	1.0
Monocrotophos	0.1	6.1	5.2	4.5	2.9	1.5	0.8
	0.2	6.9	5.4	4.56	3.1	1.6	0.85
	0.3	8.1	6.9	5.2	4.2	1.7	0.9
Dimethoate	0.1	5.2	4.5	3.3	2.7	1.3	0.6
	0.2	7.5	6.8	5.1	4.0	2.1	0.9
	0.3	8.1	6.98	5.4	3.6	2.4	1.1
Formothion	0.1	5.2	4.8	3.5	2.6	1.25	0.56
	0.2	5.95	4.85	3.6	2.5	1.2	0.59
	0.3	7.3	6.4	4.1	2.9	1.9	0.62
Oxydemeton methyl	0.1	5.3	5.0	3.5	2.8	1.6	0.7
	0.2	6.0	4.91	3.89	2.87	1.68	0.8
	0.3	7.3	6.1	4.25	2.98	1.99	0.85
Vomidothion	0.1	5.2	4.98	3.6	2.5	1.5	0.44
	0.2	5.3	4.9	3.8	2.56	1.6	0.56
	0.3	7.3	6.0	5.03	3.6	2.1	0.86

Residues in flowers

All six insecticides at the 0.3 ml level gave maximum amounts of residues in flowers, of up to 8.0 ppm of phosphamidon with the lowest residue 4.96 ppm of

dimethoate treatment with 0.1 ml, after the first week of application. None of the insecticides were detectable in flowers six weeks after application. There was not much difference in levels of residues between leaves and flowers.

Residues in fruits

Residues in the young fruits of pea size were zero. This may be due to nonentry of the insecticides into these fruits or to nonpersistence of residues. Stelzer (1970), who analysed the residues of monocrotophos after injection in samples of

TABLE II
*Residues of insecticides injected for the control of mango hoppers (in ppm)
in flowers and fruits*

Insecticides	Concentration	1st week	2nd week	3rd week	4th week	5th week	6th week	In fruits
Phosphmidon	0.1	5.12	4.8	4.2	4.0	2.75	0.5	nil
	0.2	6.44	4.96	4.58	4.2	3.8	0.5	nil
	0.3	8.00	7.2	6.44	5.68	4.08	1.2	nil
Monocrotophos	0.1	5.6	5.12	4.20	3.6	2.5	1.6	nil
	0.2	5.8	5.2	4.8	3.7	2.7	1.7	nil
	0.3	7.9	6.93	4.8	3.75	2.88	1.8	nil
Dimethoate	0.1	4.96	4.7	4.1	3.6	3.4	1.0	—
	0.2	7.2	6.31	4.96	3.8	2.8	1.0	—
	0.3	7.98	7.3	5.8	5.1	4.0	1.5	—
Formothion	0.1	5.1	4.9	4.0	3.3	2.15	1.6	—
	0.2	5.8	5.19	4.8	3.5	2.91	1.1	—
	0.3	7.0	6.18	5.8	5.21	4.1	1.9	—
Oxydemeton methyl	0.1	5.01	4.2	3.5	2.51	1.9	0.5	—
	0.2	5.8	5.0	4.1	2.6	1.8	0.42	—
	0.3	7.1	6.5	4.3	3.1	1.9	0.6	—
Vamidothion	0.1	4.98	3.86	2.6	1.6	1.0	0.3	—
	0.2	5.2	4.8	3.7	2.5	1.2	0.44	—
	0.3	7.0	6.34	4.4	3.0	1.8	0.51	—

nut water, nut meat from the immature coconuts and the meat from mature nuts at different intervals, reported that the nut water contained less than 0.01 ppm and no residues could be detected in the nut meat.

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SUMMARY

Mango fruits were sprayed twice with B.H.C., D.D.T., endosulfan, fenitrothion, fenthion, dimethoate, monocrotophos, phosalone, phosphamidon, methyl parathion, malathion, dichlorvos and carbaryl at 0.1 per cent concentration, using a low volume sprayer. The first spraying was immediately after fruit formation and the second 30 days later. Fruit samples were collected at harvest (55 days after the final spraying) and analysed for insecticide residues by colorimetry and bioassay. Sprays of endosulfan, B.H.C. and malathion left residues below detectable level when determined colorimetrically, but residues of 0.12, 0.46 and 0.31 ppm respectively were determined by bioassay. Residues of other insecticides were recorded by both the methods and but were all below tolerance limits.

The nut weevil, *Sternuchus mangiferae* F. is the most common pest found in all the districts of Tamil Nadu and damages all varieties. Both adults and larvae feed on the endocarp and hasten the maturity of the fruits resulting in fruit fall. Control of this pest is by insecticides and during March-April, 1978, 13 insecticides were evaluated at the Horticultural Research Station, Periakulam, Madurai district with the object of identifying the best insecticide and to determine residues in the fruits. B.H.C., D.D.T., endosulfan, fenitrothion, fenthion, dimethoate, monocrotophos, dichlorvos, phosalone, phosphamidon, methyl parathion, malathion and carbaryl were sprayed twice at 0.1 per cent concentration using a low volume sprayer, the first application immediately after fruit formation and the second 30 days later. Fruit samples were collected at harvest and analysed for residues by colorimetry and bioassay.

For chemical analyses, methods were those from the Analyst (1960, 1963, 1968) for residues of B.H.C., D.D.T. and dimethoate, Gets and Watts (1964) for monocrotophos and dichlorvos, Maitlen *et al.* (1965) for endosulfan, MacDougall *et al.* (1964) for fenthion, fenitrothion, phosphamidon, Johnson (1964) for carbaryl, Wisenberg *et al.* (1968) for malathion, Donald (1963) for methyl parathion and the Centre de Recherches Nicholas Grillet for phosalone. The bioassay method of Sun *et al.* (1965) using Meigu was employed.

The results of the residues of insecticides are given in the Table. Residues in fruit samples harvested 55 days after the final spraying were within the safety limits

TABLE I
Harvest Residues of Insecticides in Mango Fruits

Insecticide	Residue in ppm		EPA tolerance limit
	Chemical assay	Bioassay	
B.H.C. 50 W.P. 0.1%	ND	0.46	1.0
Carbaryl (Sevin 50 WP) 0.1%	0.42	0.36	1.0
D.D.T. 50% W.P. 0.1%	0.15	0.16	3.5
Dichlorvos (Nuvan 100) 0.1%	0.05	ND	0.1
Endosulfan 35E 0.1%	ND	0.12	2.0
Fenitrothion (Sumithion 50E) 0.1%	0.45	0.35	0.5
Fenthion (Lebaycid 1000 E) 0.1%	0.45	0.08	2.0
Phosalone (Zolone 35 E) 0.1%	0.35	0.32	3.0
Malathion 50 EC 0.1%	ND	0.31	8.0
Phosphamidon (Dimecron 100 E) 0.1%	0.45	0.14	1.0
Monocrotophos (Nuvacron 40 E) 0.1%	0.14	0.13	0.2
Dimethoate /Rogor 30 E) 0.1%	0.07	0.12	2.0
Methyl parathion (Metacid 50 E) 0.1%	0.34	0.33	1.0

ND = Below detectable level

prescribed for the respective insecticides. Endosulfan, B.H.C. and malathion left residues below the detectable level when determined colorimetrically but by bioassay residues of 0.12, 0.46 and 0.31 ppm respectively occurred. Fenitrothion residues were high 0.45 by chemical assay and 0.35 ppm by bioassay and only just below the tolerance limit of 0.5 ppm. Residues of carbaryl, D.D.T., methyl parathion, phosalone, monocrotophos, phosphamidon, dimethoate and dichlorvos were well below the tolerance level. Thus all these insecticides at this concentration and time of application should be quite safe.

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SUMMARY

During 1977-78, the cutworm, *Prodenia litura* F. caused severe damage to the leaves and fruits of grapevine. A trial on Muscat variety evaluated certain new insecticides and determin-

ed residues in berries at harvest. Carbaryl 0.125 per cent, fenitrothion 0.125 per cent, malathion 0.125 per cent, fenthion 0.2 per cent, endosulfan 0.1 per cent and chlorpyrifos 0.06 per cent and 0.08 per cent were sprayed once, using a high volume sprayer 80 days after pruning. Composite berry samples were collected 30 days after spraying (at harvest) and analysed for insecticide residues by colorimetry and bioassay.

There was no residue in the berries due to malathion and those of carbaryl, endosulfan, chlorpyrifos and fenitrothion, were well below the tolerance limit. Although fenthion residues of 0.65 ppm, were below the tolerance level of 0.75 ppm they were high compared to other insecticides.

INTRODUCTION

The berry thrips *Scirtothrips dorsalis* Hood is a serious pest on grapevine in Tamil Nadu causing scabs and warts on the developing fruits. During 1977-78, a large scale incidence of the cutworm, *Spodoptera litura* F. also damaged the leaves and fruits. Asaf Ali *et al.* (1973) tested the application of four sprays of either fenthion 0.1 per cent, dimethoate 0.03 per cent or carbaryl 0.1 per cent to control thrips and Saivaraj *et al.* (1976) studied the residues of these insecticides in the fruits at harvest. In the present experiment, six insecticides were evaluated for efficacy and to determine the extent of residues in the fruits at harvest.

MATERIAL AND METHODS

A field trial was conducted during 1977-78 on Muscat grapevines at Dasainaickenpalayam in Coimbatore district using carbaryl, fenthion, fenitrothion, malathion, endosulfan and chlorpyrifos (Table). The insecticides were sprayed

TABLE I
Insecticide Residues in Grape Berries

Insecticide	Residue in ppm		EPA tolerance level (ppm)
	Chemical assay	Bioassay	
Malathion 50E 0.125%	ND	ND	4.0
Carbaryl 50 WP 0.125%	0.21	0.22	5.0
Endosulfan 35 EC 0.1%	0.14	ND	2.0
Fenitrothion (Sumithion 50 E) 0.125%	0.25	0.33	0.5
Fenthion (Lebaycid) 0.2%	0.65	0.7	0.75
Chlorpyrifos 0.06% (Dursban 20 E)	0.45	Not done	2.0
Chlorpyrifos 0.08%	0.90	Not done	2.0

ND = below detectable level

once 80 days after pruning (bearing stage) with a high volume sprayer using 1000 litres of spray fluid per hectare. Composite berry samples, at harvest, were collected 30 days after spraying and analysed by chemical and bioassay methods.

Residues of fenthion, fenitrothion and chlorpyrifos were estimated by the total phosphorus method in which the organic phosphorus present in the extract was digested to inorganic phosphate and estimated colorimetrically (Mac Dougall *et al.*, 1964). The colorimetric method of Johnson (1964) was adopted for estimating carbaryl residues while that of Wiisenberg *et al.* (1968) for malathion. Residues of endosulfan were determined by the method of Maitlen *et al.* (1963). The above methods yielded a recovery of 70 to 80 per cent for the respective insecticides.

RESULTS AND DISCUSSION

The results of the residue analyses of the insecticides are furnished in Table I. The residues of all the insecticides in the berries at harvest were well below the tolerance limit fixed by the Environmental Protection Agency of U.S.A. (1971). Spraying of Malathion at 0.125 per cent left no detectable residues by both the methods but fenthion at 0.2 per cent gave higher residues, although they were within the permissible level. Saivaraj *et al.* (1976) reported that four applications of fenthion at 0.1 per cent one month after pruning, left residues of 0.27 and 0.24 ppm estimated by chemical and bioassay methods respectively, in the berries fifteen days after the final spraying. The higher residues in the present study are probably due to the greater concentrations of insecticide applied. Residues of carbaryl and fenitrothion ranged from 0.21 to 0.22 and 0.25 to 0.33 ppm by chemical assay and bioassay respectively. A residue of 0.14 ppm was recorded in berries treated with endosulfan 0.1 per cent by chemical assay while it was below detectable level by the bioassay. Spraying of chlorpyrifos at 0.06 per cent left a residue of 0.45 ppm while at 0.08 per cent the residue was 0.90 ppm which are below the permissible level of 2 ppm.

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42 STUDIES ON RESIDUES OF CARBOFURAN IN BANANA

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SUMMARY

Studies on residues of carbofuran (granule and flowable), applied at two doses as 'pralinage' and post-planting treatments, in raw and ripe fruits were made. The experiment was conducted during 1977-78 at Sathiamangalam in Coimbatore district on Cavendish (Dwarf) variety. Carbofuran 3 per cent g were applied at 40 and 80 g per sucker and the flowable at 3 and 6 g at planting. This was followed by an uniform application of 40 g carbofuran 3 per cent g five months after planting. Fruit samples were collected from the respective treatments at harvest (7 months after final application) and residues determined by colorimetry and bioassay. For colorimetric determination, the method of Gupta and Dewan (1973) was adopted and for bioassay, that of Sun et al. (1965) using *Drosophila melanogaster* Meig.

The results revealed that the lower doses of carbofuran in either of the formulations left no detectable residue in the raw fruit, a residue of 0.03 ppm was estimated in the ripe fruits (peel removed). At higher doses, the residue was 0.06 ppm in the raw fruit but the ripe fruits contained 0.15 and 0.13 ppm for granular and flowable treatments respectively which are above the permissible level of 0.1 ppm.

INTRODUCTION

Banana is one of the chief fruit crops grown extensively in Tamil Nadu. Recently, the prevalence of the nematodes *Radopholus similis*, *Helicotylenchus multicinctus* and *Pratylenchus* sp. have been reported in two popular varieties,

Robusta and Dwarf cavendish causing heavy damage in all the varieties, particularly, (Rajagopal *et al.*, 1977). Among the insecticides evaluated, 'pralinage' treatment with carbofuran 3 per cent G proved to be not only effective in decreasing the population of the nematodes but also in substantially increasing the yield (Rajagopal *et al.*, *loc. cit.*; Rajendran *et al.*, 1978). Since there is no information available on the accumulation of residues of carbofuran in fruits, an experiment was conducted during 1977-78 with carbofuran 3 per cent granules and 40 per cent flowable.

MATERIAL AND METHODS

The experiment was conducted during 1977-78 at Sathiamangalam in Coimbatore district on Dwarf Cavendish. The 'pralinage' of suckers was made by dipping them in clay slurry (40 kg of clay in 50 l water) followed by a sprinkling of granules/flowable uniformly over the suckers before planting. Carbofuran 3 per cent G was applied at 40 and 80 g and the flowable at 3 and 6 g per sucker. A uniform appli-

TABLE I
Estimation of Residues of Carbofuran in Banana
Variety : Dwarf Cavendish

Treatment	Residues (ppm)			
	Raw Fruit		Ripe fruit (peel removed)	
	Colorimetry	Bioassay	Colorimetry	Bioassay
Carbofuran 3 per cent G at 40 g at planting followed by 40 g (post planting)	ND	ND	0.03	ND
Carbofuran 3 per cent G at 80 g at planting followed by 40 g	0.06	ND	0.15	0.12
Carbofuran 40 per cent flowable at 3 g at planting followed by 40 g	ND	ND	0.03	ND
Carbofuran 40 per cent flowable at 6 g at planting by 40 g	0.06	ND	0.13	0.12

ND = Non detectable

EPA tolerance limit—0.1 ppm

cation of 40 g of carbofuran 3 per cent G five months after planting was also made. Fruit (raw) samples were collected from the respective treatments at harvest (7 months after final application) and analysed for residues. Some fruits were kept for natural

ripening and were also processed for residue estimation. Determination of residues was by colorimetry and bioassay. The method suggested by Gupta and Dewan (1973) was used for colorimetric estimation and for bioassay that of Sun *et al.* (1965) using *Drosophila melanogaster* Meig.

RESULTS AND DISCUSSION

Data on the insecticide residues determined in raw and ripe fruits for the two formulations at two dosages are given in Table I. It was observed that the lower doses of carbofuran in either of the formulations left no detectable residues in the raw fruit but residues of 0.03 ppm were recorded in the ripe fruit (peel removed) by colorimetry. At higher doses the residue was 0.06 ppm in the raw fruit and the ripe fruits contained 0.15 and 0.13 ppm for granular and flowable treatments respectively which are both above the permissible level of 0.1 ppm. In bioassay residues were below detectable level in raw fruits and 0.12 ppm in ripe fruits for both formulations. It is evident that there was no influence of insecticide formulations on residues and they are largely dependent on the dose. Considering the residues recorded for both the doses, it is suggested that treatment of rhizome at 40 g/sucker is safe.

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43 INSECTICIDE RESIDUE PROBLEMS IN COCONUT GARDENS

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SUMMARY

Coconut palm is infested by a large number of insect pests, although few are serious or economically important because of their sporadic outbreaks. Recommendations for the control of these pests include insecticides to be applied as spray, soil applications, injection into the palm trunk, filling leaf axils, and treatment of breeding places including cattle dung/compost pits. Parasites and predators are employed to control these pests naturally or artificially. There are problems of adverse effects of insecticides on pollinators, parasites and predators and soil microflora and fauna, cause poor results of biological control measures and deposition of insecticide residues in soil, coconut kernels and tubers of intercultivated crops.

Coconut palms are infested by insect pests at various stages of growth. Several of these cause serious damage to the crop. The crop loss ranges from death of young palms infested by the red weevil (*Rhynchophorus ferrugineus* Fabr.) to only minor injury. Adult Rhinoceros Beetle (*Oryctes rhinoceros* L.) has its immature stages in cattle dung pits, compost heaps and decaying organic debris and they bore into unopened leaves and spathes and thereby cause reduction in yield. Black headed caterpillar (*Nephantis serinopa* Meyr.) feeds on chlorophyll and may completely denude the coconut crown. White grubs (*Leucopholis coneophora* Burm.) infest not only coconut roots but also the inter crops of coconut gardens in sandy loam soils resulting in serious loss in yield. The lace bug (*Stephanitis typica* (Dist.) is important as a vector of coconut root (wilt) disease. The coreid bug (*Paradasynus rostratus* Dist.) has recently turned out to be a serious threat to coconut cultivation in Kerala because it causes extensive shedding of immature or mature nuts. Slug caterpillars (*Conthyla rotunda* Hist., *Latoia lepida* Cram.) which are endemic in certain localities, some times cause complete defoliation of coconut palms in vast areas.

The damage caused by these pests demands effective control measures. Although promising biological control has been achieved for several of these pests and a few mechanical and cultural methods of controlling pests of coconut are in practice, insecticidal control has been advocated when an immediate check is desired. The recommendations of chemicals for the control of the various pests include :

- 1) Filling leaf axils with 250 g of 5 per cent BHC/chlordane dust + equal volume of sand, three times a year before and after the monsoons, to keep off black beetles and red weevils,

- 2) Treating the breeding places of Rhinoceros beetles with BHC/carbaryl at 0.01 per cent (W/W) to check *Oryctes* by killing the grubs. Beetles are trapped and killed in trap pits filled with cow dung treated with BHC or aldrin at 0.1 per cent (W/W).
- 3) *Nephantis* at its peak periods of infestation are controlled by spraying BHC 0.1 per cent or malathion 0.05 per cent at monthly intervals. Implantation of 18 ml monocrotophos 40 EC into the holes in the stem is another method of control.
- 4) *Rhynchophorus* infested palms are treated and cured by injecting insecticides viz., pyrethrins piperonyl butoxide/carbaryl at 1 per cent or trichlorphon 0.2 per cent into the infested part of the palm.
- 5) *Leucopholis* is checked by broadcasting and ploughing BHC/aldrin/chlordane 5 per cent or heptachlor 3 per cent at the rate of 120 kg/ha twice a year, and
- 6) Outbreaks of *Contheyla* and *Parasa* are checked by spraying BHC/carbaryl 0.1 per cent. Spraying the coconut bunches at monthly intervals with 0.1 per cent BHC/carbaryl/endosulfan is recommended against *Paradasynus*. *Stephanitis* is controlled by spraying phosphamidon or carbaryl at a very low concentration. Mealy bugs and scales are controlled by spraying dimethoate at 0.05 per cent.

Hazardous side effects of insecticidal application

Thus many insecticides find a place in the schedule of plant protection in the case of coconut. Immediate and residual toxicity of these insecticides which are effective in controlling the coconut pests, however, creates other problems of concern.

(a) Pollinators and agents of biological control

One ill effect is the deleterious influence of insecticidal spray on pollinators and agents used for biological control. Spraying against the coreid bug is recommended with utmost caution to be restricted to bunches in which the fruits have set. How much insecticidal sprays against this pest and leaf caterpillars adversely affect pollination through mortality of honey bees is a problem demanding immediate investigation. Filling of leaf axils with BHC/carbaryl against Rhinoceros beetle and Red palm weevil may repel the pollinators. Several predators are recorded as feeding on immature stages of *Oryctes* in breeding materials. A mirid bug *Stethoconus praefectus* helps control field populations of lace bug. The natural population of red palm weevil is partially reduced by the predator earwig *Chelisoches moris* and similarly the white grubs by the scoliid parasite *Campsomeris collaris*. There are many indigenous and effective larval and pupal parasites of *Nephantis*. Four among these viz., *Trichospilus pupivora* Fer., *Elasmus nephantidis* Roh., *Bracon brevicornis*

Wesm. and *Perisierola nephantidis* Mues. are bred in parasite breeding centres spread all over the infested tracts and liberated in the infested gardens as biological agents. Ramachandran and Ponnammma (Personal communication) reported BHC and malathion at the recommended doses were toxic to all the laboratory bred parasites (except BHC to bethylids). Malathion was more toxic than BHC to parasites. Kurian (1969) reported that BHC, aldrin, isobenzan and carbaryl used for killing larvae of *Oryctes* destroyed the predator *Agrypnus*. Much more work seems necessary to determine the effect of insecticides recommended against pests of coconut on the beneficial fauna. For example, the leaf axil filling may adversely affect the earwigs, the spraying against *Stephanitis* may reduce mirid predator, soil application against white grubs may destroy parasitic scoliid. The residual toxicity studies gained additional significance in the present day trend to intergrate the chemical and biological methods of control with the other effective ones.

The deleterious effect of insecticides on beneficial soil fauna/flora and micro-organisms of cattle dung/decaying organic debris is another aspect which warrants detailed study. Farmers have reported on the poor manurial quality of dung-heaps sprayed repeatedly with BHC/carbaryl for over a period of six months for controlling immature stages of Rhinoceros beetle. Basic information on the deterioration of manure with the incorporation of insecticides is lacking.

(b) Resurgence of pests

Resurgence of pests and the appearance of alternate pests such as mites may occur.

(c) Residue in plants

Another concern of great importance is the cumulative effect of insecticides used repeatedly. No information is available on residues left by insecticides on coconut and coconut products. The accumulation of insecticides (used as foliar spray or when incorporated into soil) in kernel, the edible part or in nut water of tender fruits needs to be determined. Residues in the kernel may go into the oil. Residues in copra in storage is also a problem inviting study.

Similarly, leaf axil filling and soil application with insecticides are likely to leave in soil insecticides residues. Even at the dose of 120 kg/ha of insecticide recommended to control soil grubs, cultivators have registered complaints of insecticidal odour and undesirable taste of cooked tuber crops raised as intercultivated crops in BHC treated coconut gardens. Terriere and Ingalsbe (1953) found heptachlor in potato tubers grown in soil treated the same year. BHC, chlordane and dieldrin were found in potatoes three years after the soil was treated.

There are many published works showing persistence of aldrin, DDT, BHC etc. in loam soils for prolonged years. 3-6 lb aldrin/acre in loam soil has resulted in the detection of dieldrin in 0-4'' soil in 12.3 years. (Stewart and Fox, 1971). Kushwaha (1975) reported 0.188 ppm aldrin in sandy soils after 15 years of application of 15 kg/ha. Terriere and Ingalsbe (1953) reported residues of DDT, BHC, toxaphene, chlordane, aldrin and dieldrin were shown to persist in sandy loam soil for at least 3 years.

Thus the after effects of wide use of insecticides in coconut gardens can be (1) adverse effect on pollinators, (2) on parasites and predators, (3) poor results of biological control measures, (4) adverse effect of soil microflora/fauna and (5) deposition of insecticide residues in coconut kernel, water, oil and intercultivated tuber crops. So far no study has been out to assess these problems. It is necessary to work out details of an economically sound, less hazardous and more practicable control schedule.

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44 STUDIES ON RESIDUES OF SOME SYSTEMIC INSECTICIDES IN COCONUT WATER AND COPRA

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SUMMARY

Systemic insecticides such as dimethoate, monocrotophos, phosphamidon, formothion and demeton methyl were injected into the stems of 15 years old coconut palms at 2.5 and 5.0 g

a.i./palm for the control of the black headed caterpillar and the red mite. It was observed that there was no detectable quantities of these insecticides after three weeks in coconut water. The insecticide residues detected in coconut water a week after stem injection were 23.6, 35.9, 192.2, 152.5 and 63.0 u/ g/litre respectively for dimethoate, monocrotophos, phosphamidon, formothion and demeton methyl applied at the rate of 5 g/palm. These residues were below the tolerance limits prescribed for these compounds by FAO/WHO.

The residues in coconut meat (copra) of these systemic insecticides applied at the rate of 5 g/palm three weeks after stem injection were below the tolerance limits prescribed by FAO/WHO. This investigation suggests that there is no harmful effect in using the coconuts three weeks after injecting the toxicants.

A field experiment was conducted to evaluate the efficacy of certain selected systemic insecticides in the control of the scarlet mite (*Raoiella indica* Hirst) infesting coconut. The insecticides were applied by stem injection (Stelzer, 1970 and Nadarajan and ChannaBasavanna, 1978) for the control of the coconut stick insect, *Graeffea crouanii* (Le Guillou) and *Nephantis serinopa* Meyrick, a caterpillar of coconut.

A coconut orchard with 15 year old palms in the Agricultural College Farm, Hebbal, Bangalore, was used for this study. Five systemic insecticides (emulsifiable concentrates), namely, dimethoate (Rogor 30 per cent EC), phosphamidon (Dimecron 100 per cent EC), formothion (Anthio 25 per cent EC), monocrotophos (Nuva-cron 40 per cent EC) and demeton methyl (Metasystox 25 per cent EC) were used.

The stem was bored by using a carpenter's auger (2 cm dia.). The auger was inserted into the stem at a height of 100 cm above ground at 45° angle by rotating it in a clockwise direction to a sufficient depth to hold the amount of the insecticide applied. After injection with a propipette, the entrance of the hole was plugged with wet soil mixed with a small quantity of a copper fungicide.

Each chemical was tested at two levels, namely, 2.5 and 5.0 ml a.i./palm. The treatments were replicated three times.

Two nuts from each of the treated and control palms were picked at weekly intervals starting one week after treatment for analysis of residues of the insecticides in coconut water and copra. The method of analysis was that described by Lawes and Webley (1961) based on the estimation of total phosphorus.

All the systemic insecticides tried in this experiment were toxic to adults, nymphs and larvae of *R. indica* and reduced populations considerably.

The analysis for residues at the end of the first week revealed that residue levels in coconut water were less than the tolerance limits of the chemicals set by

TABLE I

Residues of systemic organophosphate insecticides in coconut water (in ppm)

Treatments	Quantity a.i./palm	I week	II week	III week
Dimethoate	2.5 g	0.0191	0.0036	N.D.
	5.0 g	0.0236	0.0044	N.D.
Monocrotophos	2.5 g	0.0143	0.0036	N.D.
	5.0 g	0.0359	0.0115	N.D.
Phosphamidon	2.5 g	0.0270	0.0106	N.D.
	5.0 g	0.1922	0.0454	N.D.
Formothion	2.5 g	0.0231	0.0198	N.D.
	5.0 g	0.1525	0.0555	0.0116
Demeton methyl	2.5 g	0.0178	0.0103	N.D.
	5.0 g	0.0630	0.0385	N.D.

TABLE II

Residues of systemic organophosphate insecticides in coconut copra

Treatment	Time lag after stem injection	Residue (ppm)
Monocrotophos 5 g/palm	One week	0.2342
Formothion 5 g/palm	One week	0.1724
Dimethoate 5 g/palm	Two weeks	0.0428
Phosphamidon 5 g/palm	Two weeks	0.0840
Formothion 2.5 g/palm	Two weeks	0.0480
Demeton methyl 5 g/palm	Two weeks	0.0645
Monocrotophos 2.5 g/palm	Three weeks	0.0208
Formothion 5 g/palm	Three weeks	0.0240

TABLE III

Residue tolerance limits set by WHO/FAO

Insecticides	ppm
Dimethoate	2.0
Monocrotophos	0.2
Phosphamidon	0.2
Formothion	0.3
Demeton methyl	0.3

WHO/FAO (Table I, III). However, in copra the residues of monocrotophos were slightly higher (0.23 ppm) than the tolerance limit (0.2 ppm) but reduced to a much lower level (0.02 ppm) at the end of three weeks. For all other compounds, residues were much less than the tolerance limits after two weeks of treatment (Table II).

Analysis of residues of different insecticides in coconut water and coconut copra are within the tolerance limits set by WHO/FAO, three weeks after treatment, indicating that the nuts were quite safe to eat.

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45 RESIDUES AND RESIDUAL TOXICITY OF MONOCROTOPHOS ON COWPEA

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SUMMARY

Residue of monocrotophos applied at 1100 l/ha to cowpea using 0.03 and 0.05 per cent emulsion were assessed at the pod formation stage of the crop. Dissipation of monocrotophos on cowpea leaves were from 3.90 ppm from 0.03 per cent and 7.34 ppm from 0.05 per cent. In cowpea pods residues were 2.73 ppm and 3.21 ppm respectively. Residues on leaves were below the tolerance limit after about 6.9 days for the lower dosage and 5.2 days for the higher dosage. The time for the residues to fall to the level of tolerance was estimated to be 4.5 and 5.8 days on pods. Washing leaves and pods removed monocrotophos residues to the extent of 33.77 to 75.16 per cent from foliage and 12.50-100 per cent from the pods, and cooking removed 18.61 to 100

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per cent residues. Studies on residual toxicity to *Amrasca biguttula biguttula* Fab. revealed that the insecticide was effective in controlling this pest for about 10 days. The residues analyzed by bioassay and chemical assay were closely correlated.

Monocrotophos is a popular and widely used insecticide recommended to control insect pests attacking vegetables and other agricultural crops (Mavi and Singh, 1971 ; Gopalan *et al.*, 1974 ; Ayyanna *et al.*, 1976 ; Verma and Rattan Lal, 1976). Assessment of residues is important from the safety of humans and animals. There is little information, so the residual toxicity and dissipation of residues of monocrotophos on cowpea crop was studied in the present investigation.

MATERIAL AND METHODS

Cowpeas (Pusa Falguni) were raised at the farm of the Indian Agricultural Research Institute, New Delhi, in a randomized block design. The crop was sprayed with the insecticide when the pest population in the field built up.

Nuvacron 40 E.C., the commercial formulation of monocrotophos, was used in the field experiment. Two concentrations – one the recommended and one higher dosage consisting of 0.03 and 0.05 per cent were sprayed to the run-off stage at a rate of 1100 lit/ha (0.33 and 0.55 kg a.i./ha). Each plot was 7m x 7m and replicated three times with plots separated from each other by a buffer zone of 1.2 metres.

Sampling : Samples of the plant parts, i.e., leaves and pods, were collected periodically. Sufficient leaves and pods (about 1½–2 kg) were picked up randomly for each treatment in equal quantities from all the three replications. They were mixed thoroughly, chopped finely and representative samples of 40–50 g were taken for extraction and analysis.

Extraction and clean up : Both for bioassay and chemical assay, the monocrotophos treated samples were extracted in chloroform at the rate of 3 ml per gram of sample.

In bioassay, the cowpea leaves and pods samples were non-toxic and required no clean up. For chemical assay, the plant samples were cleaned-up by adsorption chromatography as used by Attri and Rattan Lal (1972).

Estimation of residues : Residues were estimated by bioassay and colorimetric method as given below :

A. Bioassay

- (i) *Test insect :* Red cotton bug, *Dysdercus keonigii* Fab. which was used

as the test insect was reared on soaked cotton seeds. Young nymphs 48–72 hrs. old were selected to determine residues of monocrotophos.

(ii) *Preparation of standard dosage mortality curves and determination of residues in the samples* : Stock solutions of the insecticide containing 1 μ g per ml of solvent were prepared from technical grade monocrotophos (69.34 per cent) obtained from CIBA-GEIGY of India Ltd., Bombay. The subsequent working standards were prepared by diluting with chloroform, extracts of respective plant parts from untreated plots.

The dry film method developed by Kavadia and Rattan Lal (1967) was used to estimate residues. One ml of the extract was used per pair of petridishes and a dried film prepared by gentle swirling. No food was given to the red cotton bug nymphs during the period of exposure. Ten red cotton bug nymphs were exposed to the film for 24 hrs after which mortality counts were made. Recovery of the method was found to be 82.3 per cent.

Standard dosage mortality curves were prepared for different plant parts. Regression equations for these curves were worked out and the amount of residues in 1 ml of the sample extract was calculated from the regression equation and calculated as parts per million (ppm). The sample extracts were diluted when too toxic and concentrated when too dilute to give concentrations causing 20–80 per cent mortality.

The calculations for residue (ppm), T_{tol} (Time taken by the deposit to reach the level of tolerance), and T_{sen} (Time taken by the deposit to degrade to the level of sensitivity or detectability) were based on Hoskins' (1961) method.

B. Chemical analysis :

Residues of monocrotophos on cowpea were also estimated by a chemical method. For this purpose, the method of Getz and Watts (1964) with modifications by Jain *et al.* (1974) was used. Recovery ranged from 71.4 to 81.5 with an average of 76.0 per cent.

Persistence of residual toxicity to jassids

The residual toxicity was determined by exposing the jassids, collected from untreated cowpea field, to the leaves of treated plots for 24 hrs and making mortality counts at the end of the exposure period. Ten adults (starved) per replicate were released inside a petridish containing treated leaves and pods over a blotting paper moistened with water. The comparison of residual toxicity of the insecticide was made on PT values (Pradhan and Venkatraman, 1962 ; Saini, 1959) and LT₅₀ values (Finney, 1952).

RESULTS AND DISCUSSION

Bioassay and chemical assay methods proved to be sensitive and were highly correlated. In the present investigation, bioassay was used as the principal method and results were checked by chemical analysis.

(i) *Residues*: A comparison of the residues obtained is made in Table I.

TABLE I
Residues of monocrotophos on cowpea by bioassay and colorimetric methods

Dosage (% emulsion sprayed to run-off stage)	Days after appli- cation	Residue (ppm)		Co-efficient of correla- tion	Characteristics of residue dissipation from bioassay data
		Bioassay	Colorimetric		
Pods					
0.03	0	2.73	2.73	0.999**	Regression equation :
	1	1.00	1.01		$Y = 2.3509 - 0.2335x$
	3	0.42	0.35		$T_{tol} = 4.50$ days
	5	0.17	0.15		$T_{sen} = 10.07$ days
	10	BDL	BDL		$RL_{50} = 1.28$ days
0.05	0	3.21	3.18	0.99**	Regression equation :
	1	1.90	1.93		$Y = 2.5530 - 0.2141x$
	3	0.84	0.85		$T_{tol} = 5.85$ days
	5	0.37	0.37		$T_{sen} = 11.92$ days
	10	BDL	BDL		$RL_{50} = 1.40$ days
Leaves					
0.03	0	3.90	3.69	0.999**	Regression equation :
	1	2.24	2.25		$Y = 2.4540 - 0.1663x$
	3	0.79	0.83		$T_{tol} = 6.93$ days
	5	0.22	0.27		$T_{sen} = 14.76$ days
	10	0.07	0.01		$RL_{50} = 1.80$ days
	15	BDL	BDL		
0.05	0	7.34	7.30	0.999**	Regression equation :
	1	4.24	4.72		$Y = 2.7446 - 0.1744x$
	3	1.09	1.13		$T_{tol} = 8.29$ days
	5	0.45	0.45		$T_{sen} = 15.74$ days
	10	0.22	0.21		$RL_{50} = 1.72$ days
	15	BDL	BDL		

**Significant at 1% level. Duration of sampling = 29.6.76 to 19.7.76 average weather conditions : Max. temp. = 37°C, Min. temp. = 27°C, Mean temp. = 32°C, Rainfall 7 mm ; BDL = Below detectable limit.

(a) *Cowpea pods*: Cowpea is a fast growing crop and pods reached a marketable size within 7–8 days and diluted insecticide residues very quickly. An initial deposit of 2.73 to 3.21 ppm required less than five days for the lower dosage and slightly more than five days for the higher one to fall below the tolerance limit of 0.2 ppm. Both doses failed to result in any residues on the 10th day after spray application as assessed by bioassay and colorimetric method.

Awasthi *et al.* (1976) reported that residues from 0.03 and 0.05 per cent monocrotophos sprayed at the flowering stage of the cowpea crop persisted as 0.2 and 0.8 ppm on green pods after 10 days. However, in the present investigation the residue level on 5th day after treatment was 0.17 and 0.37 ppm for 0.03 and 0.05 per cent monocrotophos spray applications. Thus a waiting period of five days should be satisfactory for 0.03 monocrotophos sprays but a higher dosage would require at least ten days before the pods were free from insecticide poison.

(b) *Cowpea leaves*: There was an initial deposit of residues of 3.69 to 3.90 ppm from lower dose and 7.30 ppm from the higher dose. Residues on leaves were below the tolerance limit after 6.93 days for the lower dosage and 8.29 days for the higher dose to dissipate to the tolerance level. T_{sen} value was estimated to be about 15 days for both the dosages. Residues were below detectable level on 15th day onwards for both doses.

(ii) **Residual toxicity to the jassid *Amrasca biguttula biguttula* Fab.**

The data on residual toxicity of monocrotophos to *Amrasca biguttula biguttula* Fab. are presented in Table II. Monocrotophos at 0.03 and 0.05 per cent concent-

TABLE II
Residual toxicity of monocrotophos to the jassid, Amrasca biguttula biguttula Fab. on cowpea leaves

Days after treatment	*Corrected percentage mortality at two dosages	
	0.03	0.05
0 to 5	100.00	100.00
10	56.66	83.33
15	3.33	6.66
21	—	—
LT ₅₀	11.10	12.21
PT value	1001.44	1142.04

Duration of sampling and meteorological details as under Table I.

*Average of three replications and mortality counts taken after 24 hours.

rations remained toxic to the test insects up to 5 days after spray application. Both the doses remained effective for about 10 days causing 56.6 and 83.3 per cent mortality of test insects and residues of 0.07 and 0.22 ppm respectively. Gopalon *et al.* (1974) indicated that two foliar applications of 0.1 per cent monocrotophos gave good control of aphid and leaf-hopper up to 60 days after sowing 'bhendi'. Similarly, Sharma and Saxena (1972) reported that 0.02 per cent monocrotophos caused 57.8 per cent mortality of *Lipaphis erysimi* Kalt. after 15 days.

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46 RESIDUES AND TOXICITY OF PHORATE TO *APHIS CRACCIVORA* KOCH ON COWPEA PLANTS

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SUMMARY

Residues of phorate in cowpea plants treated with granules at dosages of 1, 2 and 3 kg ai/ha were determined at weekly intervals after application, using an enzymatic method. Determinations were made on the vegetative parts after application of the insecticide with seeds as a basal dressing and in the vegetative parts and pods after application of the insecticide 47 days after sowing as a top dressing. Residues of the basal-dressed insecticide could be detected in the vegetative parts up to 7 weeks but residues of the top-dressed insecticide could be detected in shoots for four weeks and in pods for 3 weeks only.

Toxicity of phorate residues in cowpea plants to adults of *A. craccivora* was assessed by exposing the insects to the treated plants at the same time as residue determinations. This relationship could be best expressed by transforming the per cent mortality to probits and residues to log dose and fitting a linear model. The regression equation for the residue-mortality relationships and the corresponding co-efficients of determination (r^2) were $y+0.8118x + 5.0068$ and $r^2 = 0.7791$ for basal dressing, $y = 1.5324x + 4.9831$ and $r^2 = 0.9361$ for top dressing (shoots) and $y = 1.0635x + 4.9821$ and $r^2 = 0.9938$ for top dressing (pods). The co-efficient was significant in each case. The three residue-mortality relationships approximated to each other and the LD 50 values in all these cases approximated to 1 ppm of the residue.

Cowpea (*Vigna sinensis* L.) is often heavily infested by the pea aphid *Aphis craccivora* Koch throughout its growth. An application of contact insecticides can destroy natural enemies which control the aphid, the use of systemic insecticides has been considered to control the pest. Phorate was reported to be effective in controlling pests of pulses (Naresh and Thakur, 1972 ; Mathur *et al.*, 1974 ; Shashiverma, 1975 ; Bhattacharjee *et al.*, 1975). The studies presented here were undertaken to assess the effect of phorate granules applied in soil in the control of the aphid and the persistence of the toxicant in the plant.

* Part of a Ph.D. thesis submitted to the Kerala Agricultural University, Mannuthy, Kerala

MATERIAL AND METHODS

Cowpea seeds of variety 'Philippines' was sown in pots (30 x 30 cm) containing 20 kg of soil. Phorate was applied at the rate of 1, 2 and 3 kg a.i./ha around the seed at the time of sowing. To study the translocation of phorate to pods the granules were top dressed to the soil around 47 days old plants.

Phorate residues within plants and pods were determined by the enzymatic method of Sutherland *et al.* (1964). The residual toxicity of phorate to aphids was determined by exposing aphids to 5 cm of the apical parts of the plants or pods collected at different intervals after application, inside specimen tubes (7 cm x 1.5 cm) with mortality counts of the aphids made 24 hours after exposure.

RESULTS AND DISCUSSION

Data presented in Table I show that the peak phorate residues were in the seedlings 14 days after application of the granules to the seeds. Residue levels increased with larger dose of the insecticide, the highest residues being 27.0, 33.0 and 43.0 ppm for the 3 doses of 1, 2 and 3 kg a.i./ha, respectively. From the

TABLE I
Residues of phorate in cowpea (ppm) at different intervals after application

Treatment	Intervals in days	Residues at different dosages (kg ai/ha)		
		1	2	3
Phorate granules applied with seeds (residues in leaves)	7	24.4	25.04	26.20
	14	27.0	33.00	43.00
	21	16.4	24.40	32.00
	28	8.2	16.20	21.90
	35	0.36	0.56	0.65
	42	0.30	0.36	0.54
	49	0.17	0.23	0.30
	56	ND	ND	ND
Phorate granules top dressed on 47th day of sowing (residues in leaves)	54	0.22	0.78	1.80
	61	0.91	2.12	2.40
	68	0.36	0.93	1.32
	75	0.20	0.50	0.67
Phorate granules top dressed on 47th day of sowing (residues in pods)	54	0.90	1.40	2.20
	61	0.06	0.18	0.36
	68	0.03	0.11	0.18

3rd week onwards there was a gradual decrease in residues to below 1 ppm at the end of 5 weeks and to non-detectable levels after 8 weeks.

The maximum residues of phorate in plants top-dressed with insecticide granules also were 14 days after application residues increasing with the dose applied : 0.91, 2.12 and 2.4 ppm. In the pods the maximum residues were 7 days after granule application. There was an increase in the residues in pods corresponding with dosage. At 1 kg a.i./ha residues in the pods were above tolerance limit (0.1 ppm) until one week after application but at the higher doses the residues remained above the tolerance limit even after 3 weeks of application.

Mortality of adult *Aphis craccivora* is summarised in Table II. When phorate was applied with seeds there was 100 per cent mortality with internal residues of

TABLE II
*Residue-mortality relations between phorate residues in
cowpea and adults of A. craccivora*

Phorate applied with seed			Phorate applied to plant 47 days after sowing			
Residue ppm	shoots		Residue ppm	shoots Aphid mortality (%)	Residue ppm	pods Aphid mortality (%)
24.40 to 43.40	100		2.40	73.3	2.20	6.33
24.40	83		2.12	63.3	1.40	50.0
21.90	96		1.80	60.0	0.90	46.7
16.40	80		1.32	56.7	0.36	30.0
16.20	83		0.93	50.0	0.18	20.0
8.20	60		0.91	53.0	0.18	20.0
0.65	60		0.78	43.3	0.11	13.3
0.56	50		0.67	40.0	0.06	1.0
0.54	37		0.50	33.3	0.03	0.33
0.36	46		0.36	30.0		
0.36	37		0.22	16.0		
0.30	17		0.20	6.7		
0.30	1					
0.23	0.3					
0.17	0					

Regression equation $y = 0.8118x + 5.0068$ $y = 1.5324x + 4.983$ $y = 1.0635 + 4.9321$
(y = probit mortality x = log-residue)

Coefficient of determination $r^2 = 0.7791^{**}$ $r^2 = 0.9361^{**}$ $r^2 = 0.9938^{**}$

** Significant at 1 per cent level

24.4 ppm and above. A gradual decline in mortality corresponded to the decline in the residue of the insecticide in the plant tissues. The relationship was best expressed by transforming the per cent mortality to probits and dosage to log-dose and fitting a linear model to this. This is the same as fitting a sigmoidal curve to the mortality dosage relations.

The regression equation for the residue-mortality relationships and the corresponding coefficient of determinations (r^2) are given in Table II. The coefficient was significant in each case.

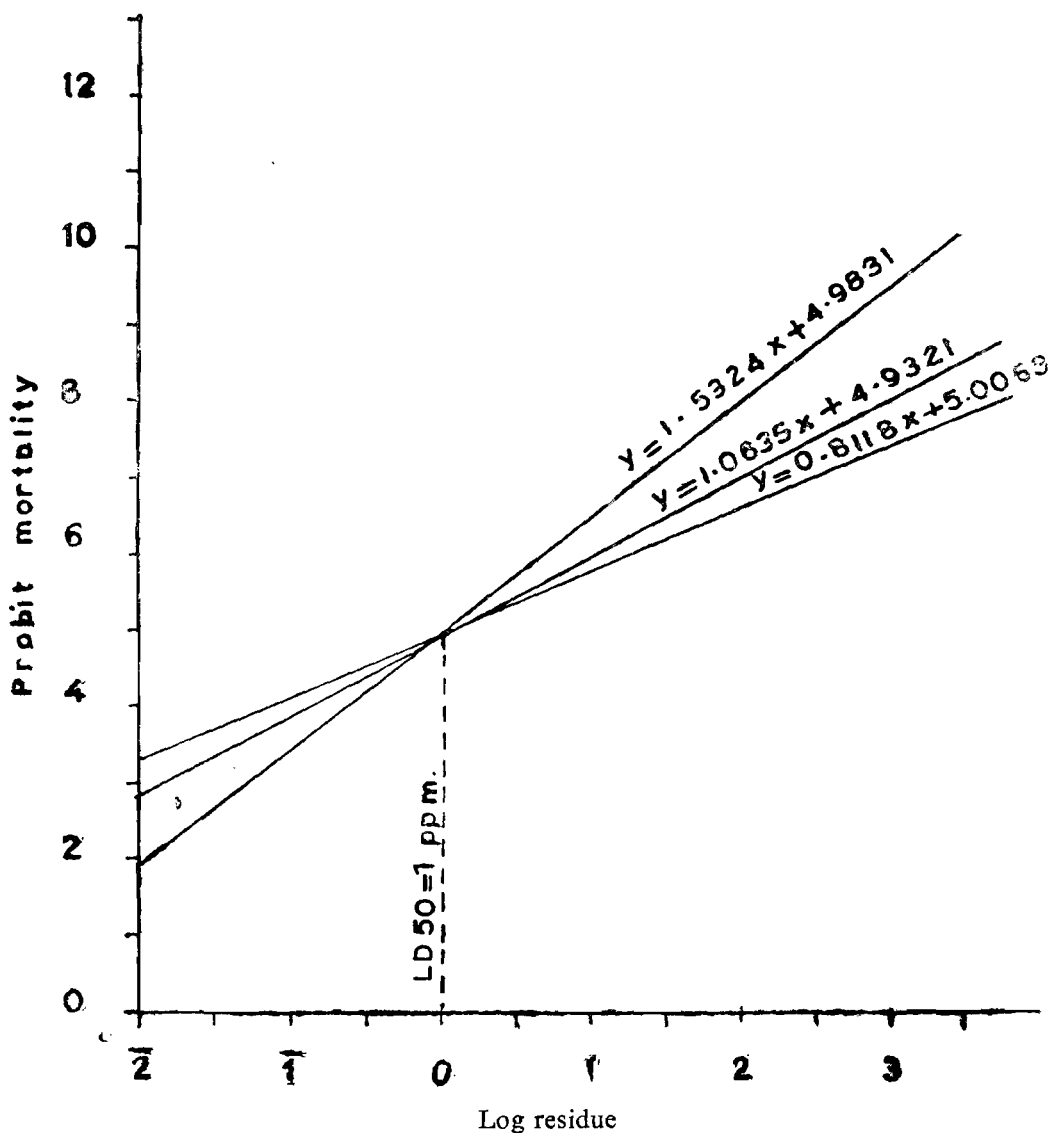


Fig. 1 Log residue—probit mortality relation between phorate and *A. craccivora*

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47 RESIDUES OF MONOCROTOPHOS IN GRAIN AND HUSKS OF BLACKGRAM

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SUMMARY

Residues of monocrotophos in pods (husk) and grains of blackgram were assessed by harvesting at two different periods after spraying (12 and 20 days) using both chemical assay and bioassay methods. Residues were not detectable in pods 12 days after spraying. The residues in grains were below tolerance limit when assayed chemically but slightly above the tolerance limit 12 and 20 days after spraying when determined by bio-assay. Thus, a minimum waiting period of 20 days is necessary before eating treated grain.

Damage to blackgram by stem fly and pod borers is very severe and monocrotophos 0.03 per cent in 800 litres/ha has been recommended for the control of stem fly in legume vegetables (Jotwani and Butani, 1977). Chellaiah *et al.* (1977) recommended application of aldicarb, phorate or disulfoton granules at 1.0 kg a.i./ha at the seedling stage followed by spraying with monocrotophos at 0.04 per cent at the flowering stage for blackgram pod borers. Because some blackgram varieties are harvested in 65-70 days, there is the possibility of the presence of toxic residues of monocrotophos in grains, so studies of residue were conducted under field conditions.

MATERIAL AND METHODS

A field experiment at Tamil Nadu Agricultural University, Coimbatore using a randomised block design with ten treatments replicated three times to test the efficacy

of certain insecticides in the control of pests of blackgram. Monocrotophos (Nuvacron) was sprayed on blackgram plants at a rate of 0.4 kg a.i./ha 7, 21, 30 and 50 days after sowing. It was also sprayed after 55 days to control pod borers. Composite samples of pods 12 days and 20 days after spraying were harvested and taken for analysis. Residues were analysed, in pods (husk) and grain by both chemical assay and bio-assay.

Chemical assay : The chemical assay of monocrotophos residue in the pods and grains was estimated by the method of Getz and Watts (1964).

Extraction and clean up : Fifty grams of pods and grains were blended with acetone for three minutes and filtered through a Buchner funnel and residues extracted with chloroform. Clean up was with an absorption mixture of activated charcoal, magnesium oxide and celite in the ratio of 1:1:1 respectively in a chromatographic column. The colourless filtrate was collected and made up to 50 ml with chloroform.

Estimation

The colour of the cleaned up extract was developed by adding 0.4 ml of two per cent solution of (p-nitrobenzyl) pyridine in acetone and 0.4 ml of two per cent solution of cyclohexylamine solution in acetone and heating in an oil bath for three minutes at 170°-180°C. Then the tubes were dipped in an ice bath, three ml of ethyl acetate added and the absorption read at 540 μ .

A standard curve was prepared using known quantities of monocrotophos by plotting a graph with absorbance vs concentration. The concentration in the sample was determined from the standard curve.

Bio-assay

The method suggested by Sun *et al.* (1965) was used to construct a standard mortality curve. One day old unsexed *Drosophila melongaster* Meig., flies were used as test insects. The curve was based on log concentrations and probit values determined by exposing flies to known quantities of monocrotophos for 24 hrs.

Residue determination

One ml of the residue extract was used as a deposit per beaker and the mortality percentage of *Drosophila* flies determined. The equivalent quantity of monocrotophos was calculated from the standard curve and converted to ppm.

Recovery percentage

Samples of pods and grains were analysed for residues by chemical assay to calculate the percentage of recovery.

RESULTS AND DISCUSSION

The recovery percentage and monocrotophos residues in pods and grains of blackgram determined by chemical and bio-assay methods different times after harvest are given in Tables I and II.

TABLE I
Recovery percentage of monocrotophos (Chemical assay)

Quantity (ppm)	Pods		Grains	
	Recovery (ppm)	Recovery (percentage)	Recovery (ppm)	Recovery (percentage)
0	0	0	0	0
2	1.62	81.00	1.70	85.00
5	4.25	85.00	4.55	91.00
10	8.80	88.00	8.90	89.00

The recovery percentage by chemical assay was 84.6 per cent and 88.3 per cent in pods and grains respectively.

Residues were not detectable in pods when assayed on 12 and 20 days after spraying by both methods. In grains, residues were 0.18 and 0.05 ppm by chemical assay and 0.33 and 0.27 ppm by bio-assay 12 and 20 days after spraying. For cowpea pods, Awasthi *et al.* (1977) reported that residues from 0.03 and 0.05 per cent monocrotophos treatments ten days after the spray were 0.22 and 0.58 ppm respectively. After a second application, residues were either not detectable or 0.09 ppm respectively 15 days after application. They also reported that the safety period for monocrotophos used at 0.75, 1.25 and 3.12 kg/ha on cowpea was 11-15 days.

TABLE II
Monocrotophos residue in blackgram pods and grain

Sample No.	Days after spraying	Monocrotophos 0.4 kg a.i./ha				FAO/WHO tolerance limits for beans in ppm
		Residue in ppm				
		Chemical assay		Bio-assay		
		Pods	Grains	Pods	Grains	
1	12	N.D.	0.18	N.D.	0.33	0.2
2	20	N.D.	0.05	N.D.	0.27	

The tolerance limit for this chemical for beans is 0.2 ppm (Anon, 1976) and a tolerance limit for blackgram is not available. Taking beans as a possible standard, residues were below the tolerance limit even 12 days after spraying, but the residues were slightly above the tolerance limit 20 days after spraying when assessed by bio-assay indicating that a waiting period of 20 days has to be allowed.

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48 EFFECT OF CONVERSION METHODS ON THE DEGRADATION OF MALATHION RESIDUES ON MOONG SEED (*PHASEOLUS AUREUS*)*

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SUMMARY

Studies on the effect of washing and rubbing treatments on the persistence of malathion residues on moong (*Phaseolus aureus*) seed indicate that at all dosage levels (i.e., 10, 20, 30, 40 and 50 ppm) one minute of washing reduced residues by 73 to 81 per cent, two minutes of washing reduced them by 78 to 89 per cent, three minutes of washing reduced them by 86 to 93 per cent and four minutes of washing reduced them by 91 to 97 per cent.

* Part of a thesis submitted for the award of Ph.D. degree of the University of Udaipur, Udaipur.

Investigations on the local method of conversion of moong seed into 'dal' indicate that no residues were present either on seed coats or cotyledons at a 10 ppm dosage, but from 20, 30, 40 and 50 ppm dosages of malathion, 0.68, 0.73, 0.91 and 0.95 ppm residues, could be detected from the seed coat. The cotyledons (i.e. dal) from all the treatments were free from even traces of residues.

Cooking the whole grain at all treatment dosages reduced residues by 23.75 to 35.33 per cent and 28.94 to 74.42 per cent immediately and six months after treatment respectively.

There seems little likelihood of harmful residues in 'dal'.

Out of many methods of prevention and control of stored pulse pests, the use of grain protectants is gaining rapid popularity. In the simplest form this means the application of a thin, protective residual film of an insecticide to cover as much of the surface of as many grains as possible.

Little work has been done on the evaluation of insecticides as grain protectants for stored pulses. Godavari *et al.* (1960) and Mookherjee *et al.* (1965) studied the effectiveness of malathion stored product insect control of wheat, and Teotia and Singh (1966) studied the effectiveness of malathion, BHC and sevin against *Sitophilus oryzae*.

Malathion has been in extensive use in other countries like U. S. A., U. K. and Canada and other developing countries in the world and has proved to be a most effective and suitable insecticide. These investigations were conducted to examine the effect of different conversion treatments such as washing and rubbing and conversion of moong seed into dal and cooking of the treated grain on the persistence of residues, on moong seed.

MATERIAL AND METHODS

Fresh seeds of greengram (Moong) was obtained during 1975 from the University of Udaipur Farm. The germination and moisture percentages recorded to be as 96.0 and 10.68 respectively. These seeds were treated with malathion at 10, 20, 30, 40 and 50 ppm dosages. For 10 kg of seed, 50 ml of spray solution was used. Thus 200, 400, 600, 800 and 1000 mg of 50 per cent EC malathion was used. The treated seed was stored in 15 kg capacity 600 gauge polythene bags, closed with rubber bands and kept in a room maintained at constant $30 \pm 2^\circ\text{C}$

A standard dosage/mortality curve for 95 per cent malathion was prepared using one day old *Drosophila melanogaster* Meig. for microbioassay, as suggested by Sun *et al.*, (1965).

Two ml of stripping (extract) was spread as a film on petridishes and the mortality of vinegar flies due to 2 ml of extract was corrected for mortality in control dishes. The corrected percentage mortality was converted into probits and the following regression obtained :

$$\text{Regression equation} = Y = 5.3878 x + 3.4904.$$

a. Effect of washing and rubbing on the persistence of malathion residues on seed :

500 grams of treated grains were taken in a beaker of 1000 ml and washed with tap water four times. Each treatment consisted of rubbing and washing the grain for 1, 2, 3 and 4 min. respectively and the water utilised for washing was allowed to decant freely from the grain. Then 50 grams of the washed grain was taken from each concentration and analysed immediately for malathion residues. Three replicates were used for each observation.

b. Persistence of malathion residues on 'dal' (i.e., cotyledons) and husk (seed coat) :

500 grams of treated seeds were washed in the laboratory with tap water for one minute. Then about 1000 ml of water was used to soak 500 grams of moong seeds for 3 hours. After soaking, the seeds were washed again with tap water and dried on blotters in the laboratory for 12 hours. After drying, the seed was broken into pieces with the a country grinder. The seed coat adheres to the cotyledons so it was necessary to separate them for final utilisation. The raw 'dal' was soaked in water for 2 hours and then washed with tap water. The seed coat was separated from cotyledons by decantation technique, because the seed coat floated in water. After about 6 washings all the seed coat separated from cotyledons. About 50 grams each of seed coat and cotyledons were taken separately from each concentration and analysed for malathion residues by chemical assay and microbioassay techniques with three replicates for each observation.

c. Effect of cooking whole grains on the persistence of malathion residues :

Samples were taken of treated moong grains, soon after treatment and six months after treatment, from each, treatment. To a 50 grams sample of moong, in a 250 ml beaker, 150 ml of water was added and kept on an electric hot plate and boiled for one hour until thoroughly cooked. The cooked grain was analysed for malathion residues both by microbioassay and chemical assay. Three replicates were maintained of each treatment.

RESULTS

1. Effect of washing :

The results indicate that the residues recovered by chemical assay and bioassay were identical for all application dosages i.e. 10, 20, 30, 40 and 50 ppm

malathion and washing and rubbing treatments for one, two, three, four minutes respectively. In almost all application dosages one minute of washing and rubbing reduced residue by 73 to 81 per cent, two minutes of washing reduced them by 78 to 79 per cent, three minutes of washing reduced 86 to 93 per cent and four minutes of washing and rubbing reduced by 91 to 97 per cent of residue.

Thus, by few minutes of washing and rubbing the grain, the residue falls considerably to bring it below the tolerance level of 3 ppm, even for an application rate 50 ppm. It indicates clearly that however at high application dose, a few minutes of rubbing and washing treatment reduces the residues to below tolerance level. It

TABLE I

Effect of washing and rubbing treatments (in minutes) on the reduction of malathion residues on moong grain immediately after treatment

Concentration applied (ppm)	Control not washed	One minute		Two minutes		Three minutes		Four minutes	
		Residues recovered (ppm)	Percentage reduction	Residues recovered (ppm)	Percentage reduction	Residues recovered (ppm)	Percentage reduction	Residues recovered (ppm)	Percentage reduction
by chemical assay									
10	8.365	2.229	73.35	1.704	79.62	0.771	90.78	0.449	94.63
20	17.896	4.182	76.63	2.287	87.22	1.733	90.31	0.630	96.47
30	26.989	5.144	80.94	2.978	88.96	1.937	92.82	0.946	96.49
40	36.984	7.664	79.27	4.235	88.54	2.238	93.89	1.003	97.21
50	45.352	11.105	75.51	7.418	83.64	2.870	93.67	1.396	96.92
by microbioassay									
10	8.500	2.222	73.85	1.893	77.72	1.208	85.78	0.723	91.49
20	18.169	4.440	75.56	2.589	85.75	1.956	89.23	0.946	94.79
30	27.732	5.398	80.53	3.204	88.44	2.141	92.27	1.040	96.24
40	37.058	8.031	77.08	4.467	87.94	2.634	92.89	1.221	96.70
50	46.852	11.342	75.79	7.674	83.62	3.298	92.96	1.766	96.23

was also found in the studies that high rates of application dosage, i.e. 50 ppm of malathion can provided protection to the grain for more than nine months. Such a high rate cannot be used because of toxic hazards of the residues to the consumer of the treated products. But by washing and rubbing for a few minutes only, the residue levels could be brought down to below tolerance limit and would thus be rendered safe for human consumption.

2. Effect of conversion of moong seeds (greengram) into 'dal'.

The effect of local method of conversion of whole grain of moong on the reduction of malathion residues on treated grain was studied at 'zero' and 'six' months intervals. After conversion into dal the residues were analysed in seed coat and cotyledons separately both by bioassay and chemical assay. The results obtained presented in Table II, indicate that immediately after the treatment, when the whole grains was converted to 'dal', no residue could be detected either from seed coat or in cotyledons at 10, 20 and 30 ppm concentrations by chemical assay and 10 ppm by microbioassay. However an average of 0.68, 0.73, 0.91 and 0.95 ppm of malathion residues were detected from seed coat by microbioassay for 20, 30, 40 and 50 ppm dosages respectively. An average of 0.25 ppm and 0.40 ppm of residues were obtained by chemical assay at 40 and 50 ppm respectively from seed coat. It is interesting to note that from the cotyledons for all treatment dosages no residue could be detected either by chemical assay or by microbioassay.

Residue analysis six months after treatment indicated no residues which could be detected indicating the absence of even microquantities of malathion or its metabolite 'malaxa' either on seed coat or cotyledons.

3. Effect of cooking

In India it is customary to cook 'dal' in boiling water before use for human consumption.

The microbioassay method was able to detect larger quantities of residues than the chemical assay method. Table III shows that moong treated with as high a dosage as 50 ppm and cooked immediately after had residues of only 34.6 per cent. For application dosages of 10, 20, 30 and 40 ppm the percentage decrease was between 23 and 25 only, compared with 35 per cent for 50 ppm.

Cooking reduced the residue by about 29, 48, 32, 55 and 75 per cent, for application dosages of 10, 20, 30, 40 and 50 ppm respectively, six months after treatment.

DISCUSSION

One minute washing and rubbing reduced the malathion residue by about 73.0 to 81 per cent for all treatment dosages and four minutes of washing and rubbing of the grain reduced the residues by 91 to 97 per cent. Thus even few minutes of washing and rubbing the grain removes the residues considerably to bring it below the tolerance level of 3 ppm even for as high an application rate as 50 ppm. 50 ppm provided absolute protection against pulse beetle for more than nine months, but the

TABLE II

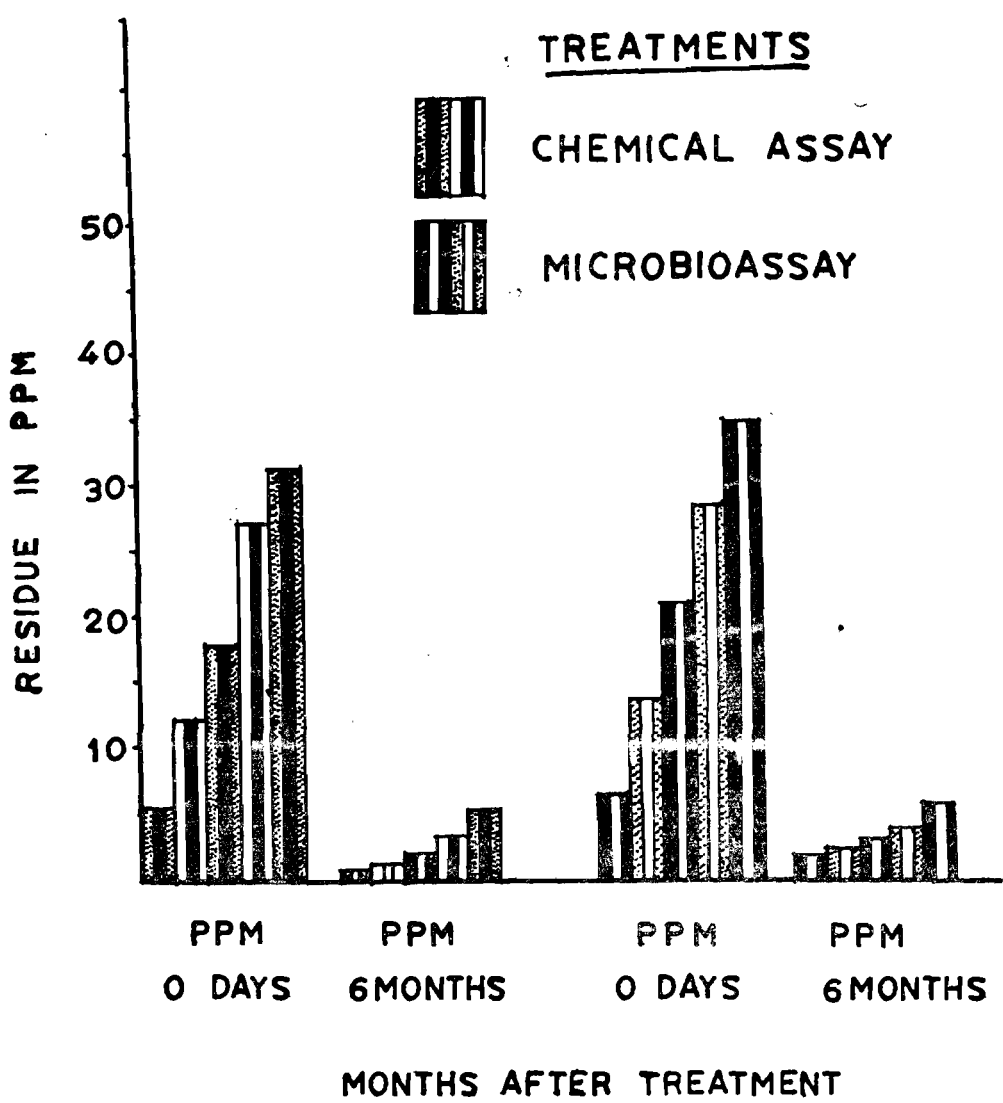
*Reduction of malathion residues (ppm) on seed coat and cotyledons of moong
(when local conversion of seed into 'dal' was practised).*

Interval	Chemical concentration (ppm)	Chemical assay of residues (ppm)				Microbioassay of residues (ppm)			
		Seed coat		Cotyledons		Seed coat		Cotyledons	
		I	II	III	Ave- rage	I	II	III	Ave- I II III range
Immediately after treatment	10	*	*	*	—	*	*	*	—
	20	*	*	*	—	0.68	0.68	0.68	0.68
	30	*	*	*	—	0.73	0.73	0.73	0.73
	40	0.25	0.25	0.25	*	0.91	0.91	0.91	0.91
	50	0.40	0.40	0.40	*	0.95	0.90	0.95	0.90
6 months	10	*	*	*	—	*	*	*	—
	20	*	*	*	—	*	*	*	—
	30	*	*	*	—	*	*	*	—

* undetectable

I, II, III—replications

Reduction of Malathion Residues in Cooked Moong (*P. Aureus*)



Effect of Washing on the Reduction of Malathion Residues on Moong

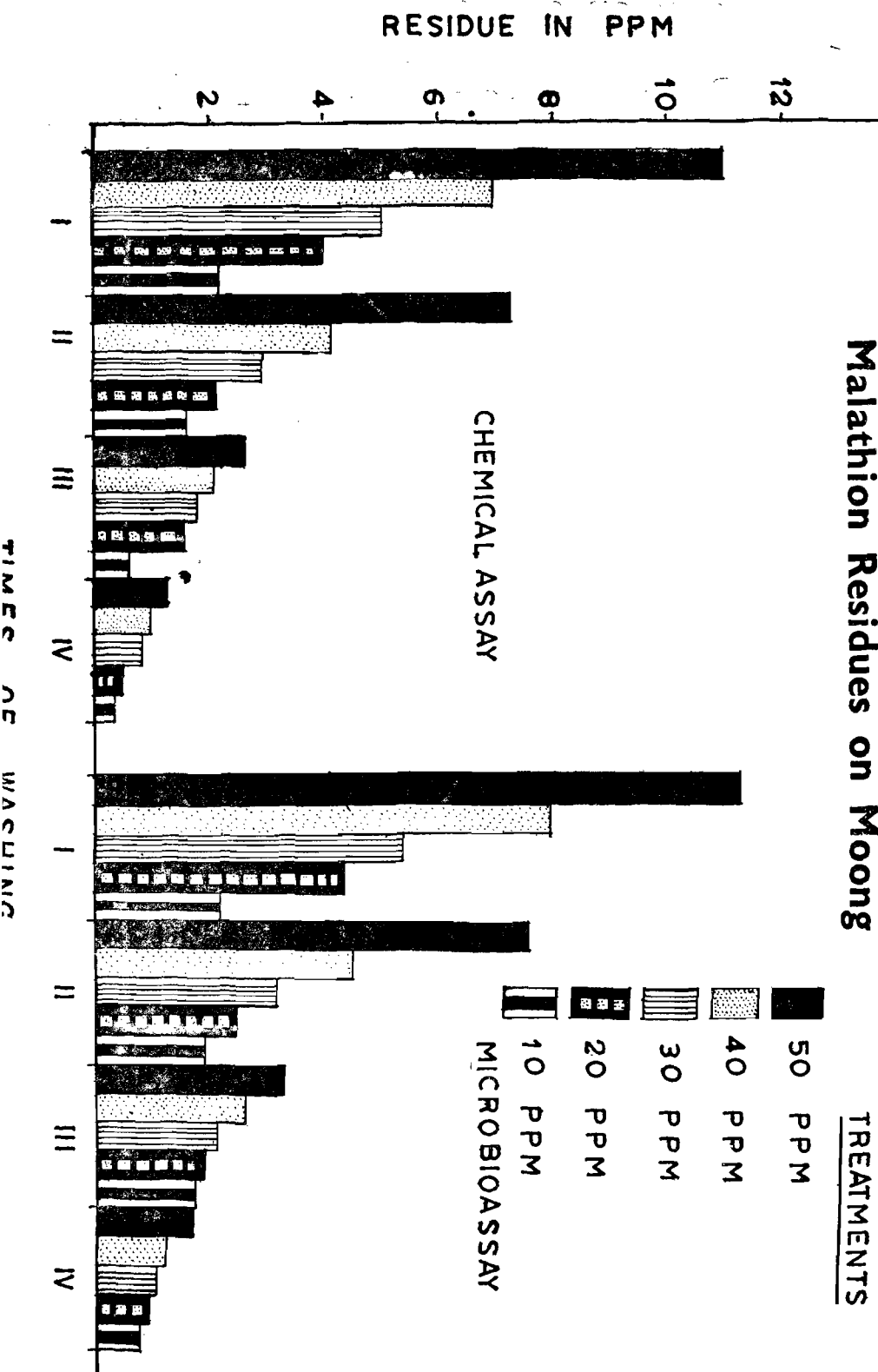


TABLE III
Reduction of malathion residues in case of cooked mung (*Phaseolus aureus*) (by chemical and microbioassay),
0 day and 6 months after treatment

Chemical concent- ration	Chemical assay of residues (ppm)				Average (ppm)	Percentage Reduction	Microbioassay of residues (ppm)				Average (ppm)	Percentage Reduction
	Without cooking		Replications				Without cooking		Replications			
	I	II	I	III			I	II	I	III		
0 days												
10 ppm	8.365	5.669	5.669	5.581	5.639	32.59	8.500	6.481	6.481	6.481	6.481	23.75
20 ppm	17.896	12.738	12.738	12.912	12.796	28.50	18.169	13.433	13.433	13.433	13.686	24.67
30 ppm	26.989	18.128	18.128	18.128	18.128	30.75	20.478	20.855	20.855	20.855	20.729	24.80
40 ppm	36.984	27.224	27.224	27.924	27.691	25.13	37.058	28.588	28.086	28.086	28.253	24.21
50 ppm	45.352	31.772	31.772	31.772	31.772	29.94	46.852	34.620	34.620	34.620	34.620	35.33
6 months												
10 ppm	1.718	0.946	1.018	1.018	0.994	42.14	2.173	1.524	1.554	1.544	1.544	28.94
20 ppm	3.292	1.717	0.844	1.717	1.426	48.17	3.832	2.085	1.839	2.085	2.003	47.72
30 ppm	3.874	1.892	2.592	2.592	2.358	39.13	4.682	2.192	3.678	3.678	3.182	32.03
40 ppm	8.015	3.292	3.466	3.466	3.408	57.48	8.378	3.809	3.809	3.678	3.765	55.06
50 ppm	20.460	5.632	5.632	5.043	5.336	73.92	22.066	6.481	5.224	5.224	5.643	74.42

use of such a high dosage as 50 ppm might cause toxic hazards to the consumers but by giving a thorough washing and rubbing of the treated grains, this residue level can be brought down below the tolerance limit.

b) Effect of conversion of moong seed into 'dal'

It is interesting to note that there was considerable degradation of malathion residues during the process of conversion of whole grain into 'dal' even without washing.

c) Effect of cooking

The results of cooking the treated whole grain at all treatment dosages reduced malathion residues by 23.75 to 35.33 per cent and 28.94 to 74.42 per cent on zero day and six months after treatment respectively.

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DEGRADATION OF PIRIMIPHOS METHYL (ACTELIC) IN STORED FOOD GRAINS

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SUMMARY

Pirimiphos methyl, (0-[2-diethyl amino-6 methyl pyrimidin 4-yl] 0,0 dimethyl phosphorothioate) (trade name Actellic) is a promising insecticide having both contact and fumigant action widely used as an insecticide against stored grain insect pests. In the present study, degradation of residues in treated food grains was studied. Actellic 50 per cent EC was sprayed on wheat and triticale in jute bags to give a uniform deposit of 0.15 g/m² of the insecticide. Residue of pirimiphos methyl were estimated by TLC 24 hours, 7, 15, 30, 45 and 60 days after spraying. The bags were stored at 30 C and 70 per cent r.h. Residues were 0.50, 0.40, 0.30, 0.20, 0.16 and 0.12 ppm in wheat and 0.50, 0.45, 0.40, 0.25, 0.15 and 0.10 ppm in triticale after 24 hours, 7, 15, 30, 45 and 60 days of treatment respectively. Thus, the disappearance of residues is rapid in both the commodities and were below the WHO/FAO tolerance limit of 10 ppm for wheat grains. Under the conditions studied, there appears to be no toxicological hazard with the use of this new insecticide for prophylactic treatment in grain storage to control stored grain insect pests.

Pirimiphos methyl, 0-1 (2-diethylamino - 6 methyl pyrimidin 4 - yl) 0, 0, dimethyl phosphorodithioate (Actellic) is a fast-acting broad spectrum, organophosphorous insecticide with both contact and fumigant action. Active against a wide spectrum of insect pests, including ants, aphids, beetles, caterpillars, cockroaches, fleas, flies, mites, mosquitoes, moths and thrips. The effectiveness of this insecticide against stored grain insect pests has been tested by a number of workers (Girish *et al.*, 1973, McCallum, 1974 and Lahue, 1975a). Wildey, 1977 reported this insecticide to be effective against malathion-resistant stored grain insects. Chawla *et al.* (1976) stated that pirimiphos methyl was a promising insecticide against stored grain insect pests. Majule (1977), Seth (1974) and Lahue (1977) reported that pirimiphos methyl was a successful insecticide against major stored grain insect pests. However, little work has been done on the fate of its residues in treated food grains. Lahue (1975b) reported that degradation of pirimiphos methyl residue takes place gradually. The present study was to find out the rate of degradation of residues of pirimiphos methyl in wheat and other Graminae.

MATERIAL AND METHODS

Wheat and other grains were put in small jute bags each containing 5 kg of grain. Pirimiphos methyl 50 per cent e.c. was sprayed on all sides of the bags to

give a deposit of 0.15 g/m² on each bag which were stored in a controlled chamber at 30°C and 70 per cent R.H. for two months. Samples of grain for residue analysis were taken in air tight clean glass vials after 24 hours, 7 days, 15 days, 30 days, 45 days and 60 days of storage. The residues of pirimiphos methyl were estimated by TLC technique using hexane + acetone (3 : 1) as solvent and bromocresol green as the chromogenic reagent. The procedure was similar to the analysis of malathion residues. Clean up of the extract to remove fat was done using methyl cyanide and hexane. The recovery test was also done by spotting the pirimiphos on methyl with a micro pipette.

RESULTS AND DISCUSSION

As is evident from the table, the dissipation of pirimiphos methyl was rapid in all grains. However, residues were much less than the WHO/FAO tolerance limit for pirimiphos methyl which is 10 ppm for wheat grain. Thus, there seems to be no toxicological hazard associated with the use of this insecticide for prophylactic treatment for bagged grain against stored grain insect pests.

TABLE I
Degradation of pirimiphos methyl in wheat and other grains

Sl. No.	Dosage g/m ²	Interval during treatment and estimation	Residue in ppm	
			Wheat	Other grains
1	0.15	24 hours	0.50	0.50
2	„	7 days	0.40	0.45
3	„	15 days	0.30	0.40
4	„	30 days	0.20	0.25
5	„	45 days	0.16	0.15
6	„	60 days	0.12	0.10

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INORGANIC BROMIDE RESIDUES IN COMMODITIES FUMIGATED WITH ETHYLENE DIBROMIDE

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SUMMARY

Commodities such as wheat grain and flour, soybean grain and flour and "protein plus" were fumigated with ethylene dibromide at 3, 6 and 12 ml/quintal. Residue analyses after 7 days of aeration revealed that the highest inorganic bromide residue was observed in protein plus followed by soybean grain, soybean flour, wheat grain and least in wheat flour. Inorganic bromide residues fell below the tolerance limit of 50 ppm after 7 days in wheat grain and flour and soybean grain and flour at all dosages tested except the dose of 12 ml/quintal applied to soybean grain. The "protein plus" was found to contain residues above the tolerance limit with all dosages after 7 days of aeration but after 15 days of aeration it was below tolerance limit at the dose of 3 ml/quintal and after 45 days of aeration the residue was below the tolerance limit at all dosages tested in "protein plus". There was a significant positive correlation between the increase in moisture contents and increases in inorganic bromide residues in all commodities tested. The immediate recovery of ethylene dibromide has been reported in all commodities. The possible mechanism of inorganic bromide residues in different commodities is discussed.

INTRODUCTION

Ethylene dibromide (EDB) is widely used as fumigant against the control of insect pests of stored grain (Muthu and Pingale, 1965 and Girish *et al.*, 1972). Girish and Kumar (1975) investigated residues of EDB in wheat and its products and concluded that an increase in dosage of EDB resulted in an increase in inorganic bromide residues of EDB which dissipated after 22 days in grain and flour treated with EDB at 3 ml/q. However, there seems to be no record of inorganic bromide residues in soybean, particularly "protein plus" which is a new product developed by Soya Production and Research Association, Bareilly. Therefore, the present investigation was carried out to determine inorganic bromide residues in wheat grain and flour, soybean grain and flour and "protein plus" fumigated with EDB at different dosages and stored for various periods.

MATERIAL AND METHODS

Wheat variety RR-21 and soybean variety U.P.S.E., 22 and "protein plus" were used for the present investigation. None of the commodities were fumigated earlier and they were sterilized at 60°C for 5 hrs in Uday hot air oven. After sterilization, all commodities were kept in an incubator at 30°C $\pm$ 1 with 70 $\pm$ 5 per cent relative humidity. Before fumigation the moisture content of all commodities was determined by an Osaw universal electrically operated moisture meter except for "protein plus" which was determined by a hot air oven method. The moisture contents of all commodities were kept at 10 per cent.

Fumigation: Small glass jars measuring 20 x 10 cm were taken for fumigation. The jars were filled with 1 kg of each commodity (kept in incubator at 30°C $\pm$ 1, 10 per cent moisture content). For fumigation, EDB of 2.17 specific gravity at 20°C was used. Known amounts of EDB (*i.e.* at 3, 6 and 12 ml/q) were placed in jars using a micropipette and the jars were sealed immediately with wax to avoid leakage of air and kept at room temperature (28-32°C) for two months. Samples were taken 7 days, 15 days, 30 days, 45 days and 60 days after fumigation and were sealed again and kept at room temperature. Fumigant dilution during sampling does not seem to have affected the observations recorded because immediately after sampling the container is sealed. However a slight variation could not be recorded.

Determination of inorganic bromide residues: Inorganic bromide residues were determined in samples by the A.A.C.C. 60-10 method. Organic bromide was removed by 4-5 washings of dichloromethane and the rest of the inorganic bromide was determined by the above method. The inorganic bromide was calculated by the formula :

$$\text{Inorganic bromide} = \frac{V \times 0.1332 \times 1000}{W} \text{ ppm}$$

V = Volume of 0.01 N Sodium thiosulphate used in titration

W = Weight in gms of commodity taken

In determining the residues in grain, the grain (10 g) was crushed with a pestle and mortar and sieved to avoid loss of residues. Flour was prepared from grain by milling the grain in an electrically operated small grinding mill. The "protein plus" was fumigated directly and residues analysed without any further processing. A single sample was run simultaneously without fumigant to determine the amount of inorganic bromide already present in the commodity. The whole experiment was replicated three times and residues were analysed 7, 15, 30, 45 and 60 days after fumigation.

Effect of moisture content on bromide residues: Relative humidities were maintained in desiccators by means of saturated salt solutions (NaCl and K₂SO₄) (Hubbard *et al.*, 1957). Moisture contents and relative humidity equilibrium was maintained and the moisture contents determined by an electrically operated Osaw universal moisture meter except for "protein plus" which was determined by the hot air oven method. The moisture contents of all commodities were maintained at 8, 10, 12, 14 and 16 per cent and these commodities were fumigated with EDB at 3 ml/quintal and stored for 7 days, after which they were fully aerated and samples were taken for residue analysis. The experiment was replicated three times. A correlation coefficient was calculated between moisture contents and bromide residues.

Recovery of EDB: To determine the immediate recovery of EDB in different commodities, a known amount of EDB was added to different commodities and the amount of EDB determined. The total amount of bromide converted into EDB and the amount of EDB recovered was estimated by the earlier formula.

RESULTS AND DISCUSSION

Inorganic bromide residues in wheat grain and flour: The inorganic bromide residues were only 14.98 ppm after 7 days of fumigation and reached 1.12 ppm in 60 days when EDB was applied at 3 ml/quintal to grain. At the doses of 6 ml and 12 ml/quintal residues were 29.60, 41.48 ppm after 7 days and 3.28 and 5.35 ppm after 60 days of fumigation (Table I).

In wheat flour residues were 8.24, 12.26 and 20.08 ppm after 7 days and fell below the detectable limit, 0.57 and 1.27 ppm in 60 days, when EDB was applied at 3, 6 and 12 ml/quintal respectively.

Thus, an increase in dosages of EDB resulted in an increase in residues (Table I) and residues were below the tolerance limit after 7 days of fumigation with all dosages. Girish and Kumar (1975) also reported of residue below tolerance limit 7 days after fumigation with EDB at 3, 6 and 12 ml/quintal in wheat grain and flour. The lower residues in flour than grain may be due to loss of some residues during milling from grain into flour. Smaller amounts of residues persisted up to 60 days with dosages of 6 ml and 12 ml/quintal. Bondi *et al.* (1955) reported that EDB may take 45 days to disappear from grain on aeration.

TABLE I
Inorganic bromide residues in different commodities fumigated with ethylene dibromide

Sl. No.	Name of commodity	Doses of EDB (per qtl.)	ppm inorganic bromide residue (average of three replications)				
			After 7 days	15 days	30 days	45 days	60 days
1.	Wheat	3 ml.	14.98	9.18	7.34	4.22	1.12
		6 ml.	29.60	20.77	15.22	9.57	3.28
		12 ml.	41.48	35.15	20.37	12.44	5.35
2.	Wheat flour	3 ml.	8.24	5.71	3.44	1.07	N.D.
		6 ml.	12.26	8.75	5.30	2.76	0.57
		12 ml.	20.08	15.58	10.11	5.46	1.27
3.	Soybean grain	3 ml.	20.64	14.65	10.34	5.31	3.75
		6 ml.	39.88	30.69	18.92	9.26	5.56
		12 ml.	56.67	47.70	27.88	14.17	10.36
4.	Soybean flour	3 ml.	10.24	7.60	4.67	3.16	0.81
		6 ml.	19.49	14.07	8.32	6.34	3.04
		12 ml.	28.97	20.43	11.53	11.07	5.92
5.	'Protein plus'	3 ml.	49.45	39.13	27.65	15.24	10.21
		6 ml.	98.20	63.08	55.76	28.25	20.43
		12 ml.	147.24	91.47	80.39	44.44	31.62

N.D. Not detectable residue.

Inorganic bromide residues in soybean grain and flour: The inorganic bromide residues of 20.64, 39.88 and 56.67 ppm 7 days after fumigation fell to 3.75, 5.56 and 10.36 ppm after 60 days in soybean grain fumigated with EDB at 3, 6 and 12 ml/quintal respectively (Table I). In flour, residues were less 10.24, 19.49 and 28.97 ppm after 7 days and 0.81, 3.04 and 5.92 ppm after 60 days when EDB was applied at the same dose.

The inorganic bromide residues were below the tolerance limit after 7 days for EDB dosages of 3ml/quintal, but at a dose of 12 ml/quintal the residues exceeded the tolerance limit. Residues were comparatively less in flour than grain which may be due to loss of some residue in processing (*i.e.*, milling from grain into flour).

Soybean contained slightly more residues than wheat as grain or flour. This may be due to a higher percentage of protein which absorbed EDB, in soybeans than wheat. Rao *et al.* (1977) also reported higher bromide residue in soybean flour than wheat flour and concluded that the protein fraction was mainly responsible for decomposition of methyl bromide and formation of inorganic bromide and a series of methylated derivatives.

Inorganic bromide residues in 'protein plus': "Protein plus" contained 49.45, 98.20 and 147.24 ppm as inorganic bromide 7 days after fumigation and fell to 10.21, 20.43 and 31.62 ppm 60 days after fumigation with EDB at 3 ml, 6 ml and 12 ml/quintal respectively (Table I). 7 days after aeration "protein plus" contained residues equal to tolerance limit of 50 ppm at all dosages tested but after 15 days of aeration it fell below the tolerance limit after treatment with 3 ml/quintal. After 45 days residues fell below the tolerance limit at all dosages.

When all commodities were compared, the "protein plus" had more residues than other commodities. Olumucki and Bondi (1955) reported that higher amount of EDB was sorbed by milled products than whole grain in wheat.

Effect of moisture contents on bromide residues: There was a significant positive correlation between different moisture contents and inorganic bromide residues in all commodities used (Table II). Increases in moisture contents from 8 to 16 per cent resulted in increases in bromide residues (Table II). Getzendaner

TABLE II

Effect of moisture contents on inorganic bromide residues of different commodities fumigated with EDB

Commodity	ppm inorganic bromide residue (based on 13 replications)					Co-efficient of correlation **
	8% moisture	10%	12%	14%	16%	
Wheat grain	14.08	15.31	18.49	20.74	24.47	0.99
Wheat flour	7.33	8.93	12.02	14.95	17.93	0.99
Soybean grain	18.49	20.02	22.32	26.56	29.95	0.98
Soybean flour	9.18	10.64	12.24	15.29	19.73	0.97
"Protein plus"	46.41	48.04	52.00	54.36	59.50	0.99

Dose – at 3 ml/quintal

Exposure period – 7 days.

** Significant at 1% level.

et al. (1968) and Berk (1965) reported that bromide residues increased with the moisture contents of milled products. However, a linear relationship existed between moisture content and bromide residues in the present findings.

Recovery of EDB : The immediate recovery per cent of EDB in different commodities is given in Table III. The recovery of EDB was higher (80 per cent) from

TABLE III
Recovery of inorganic bromide in different commodities fumigated with EDB.

Commodity	Moisture content %	Average % recovery
Wheat grain	10	79.00
Wheat flour	10	67.00
Soybean grain	10	72.00
Soybean flour	10	63.00
“Protein plus”	10	80.00

Specific gravity of EDB 2.182 at 20°C or 2.172 at 25°C based on three replicates.

„protein plus“ followed by wheat grain, soybean grain, wheat flour and least from flour. Girish and Kumar (1975) recorded 70–75 per cent recovery of EDB from starch. The slight variation may be due to different commodities used.

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52 BIOASSAY OF INSECTICIDE RESIDUES ON PADDY STRAW

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SUMMARY

Insecticides are being used indiscriminately by cultivators to control the brown plant hopper ***Nilaparvata lugens*** on rice, often leaving harmful residues on straw, which is fed to cattle. The persistence of residues of selected insecticides were studied by bioassay using the vinegar fly, ***Drosophila melanogaster*** Meig., as a test insect.

Carbaryl 4.0 oil applied at 0.75 kg a.i./ha gave an initial deposit of 31.67 ppm and a cumulative residue of 21.05 ppm after 4 applications in 60 days. The straw at harvest contained 6.18 ppm, and 25 days after harvest fell to less than 1 ppm.

Carbaryl 50 WP (0.75 kg a.i./ha) gave an initial deposit of 32.15 ppm and when applied 4 times fell to 1.14 ppm in 60 days and no traces of residues could be detected at harvest.

Carbaryl, 10 per cent dust, (1.5 kg a.i./ha) gave an initial deposit of 28.93 ppm which after 4 applications fell to 0.83 ppm after 60 days and no residue could be detected at harvest.

Malathion (0.5 kg a.i./ha spray) gave deposit of 23.01 ppm which fell to less than 1 ppm in 10 days and gave no residues after 15 days.

BHC 50 per cent WP (1.25 kg a.i./ha spray) produced a deposit of 31.50 ppm from a single spray with a cumulative residue from 4 sprays after 60 days of 33.14 ppm. This degraded to 16.95 ppm at harvest and 11.0 ppm on straw 25 days after harvest.

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Methyl parathion 50 per cent EC (0.375 kg a.i./ha) gave a deposit of 16.57 ppm and after 4 applications the cumulative residue after 60 days was 28.28 ppm, which degraded to 23.45 ppm at harvest, and 10.73 ppm on straw 25 days after harvest.

With the introduction of high yielding dwarf varieties of rice, some insects so far of minor importance, gained the status of major pests. One is the brown plant hopper, *Nilaparvata lugens* Stal., which is causing havoc to paddy crops for the last five years.

Insecticides are often being utilised indiscriminately and excessively by cultivators to control brown plant hoppers, harmful residues are left on paddy straw, which is fed to cattle. The persistence of residues of some selected insecticides were studied by bioassay utilising vinegar fly *Drosophila melanogaster* Meig. as a test insect.

MATERIAL AND METHODS

a) *Sowing details*: The trial was laid out at the Rice Research Unit, Agricultural College Farm, Bapatla, (A.P.) during *kharif* 1975. The variety was MTU 8089. The nursery was protected with a methyl parathion spray (0.375 kg a.i./ha.) Plants were transplanted after 27 days.

The size of plots was 7.05 x 3.6 m in a randomised block design with three replicates.

The crop was sown on 23.7.75 at 20 x 15 cm spacing. Chemicals were applied four times at 15 days interval starting from 60 days after sowing of the crop (Table I).

Standardization of microbioassay: For microbioassay method one day old adult vinegar flies, *Drosophila melanogaster* Meig. were used. They were reared on a mass scale under a constant temperature of $25 \pm 1^\circ\text{C}$ in incubators. The method of rearing vinegar flies is essentially the same as that of Lewis (1960) and their diet consisted of maize in distilled water. For each gram of grain sample 2 ml of solvent was used. Standard dosage/mortality curves were prepared as suggested by Sun and Sun (1952). The extract was spread as films in 10 cm petridishes at a rate of 2 ml per pair (*i.e.*, 1 ml per dish) and allowed to dry at $25 \pm 1^\circ\text{C}$ for one hour. 20 anaesthetized one day old male vinegar flies were used for each concentration. Mortality counts were made 24 hours after exposure to the residue film.

The standard dosage/mortality curves for estimation of carbaryl, malathion, BHC, methyl parathion were prepared and regression equations were calculated.

Carbaryl	$Y = 1.7600 - 0.5100 X$
Malathion	$Y = 5.3878x + 3.4904$
BHC	$Y = 4.1517x + 0.3537$
Methyl Parathion	$Y = 5.7765x - 3.0474.$

Estimation of the persistence of insecticide residues on paddy: Samples of a green paddy crop were collected at application time 5 days, 10 days and 15 days after spraying of the chemical and at the time of harvest and also 25 days after harvest. From each treatment 100 g sample was collected and the residue extract obtained using redistilled acetone.

RESULTS AND DISCUSSION

a) *Degradation of carbaryl:* Carbaryl applied at a dosage of 0.75 kg a.i./ha produced a deposit of 31.67 ppm which degraded by 79.69 per cent. However when

TABLE I

Sl. No.	Chemical & concentration	Technical name	Source of supply	Dates of application of the chemical
1.	Carbaryl 50 % WP at 0.75 kg a.i./ha.	1-napthyl n-methyl Carbamate	Union Carbide Ltd., Bhopal	Ist spray - 60 DAS 22.9.75
2.	-do- 10% dust at 1.5 kg a.i./ha.	-do-	-do-	2nd spray -75 DAS 7.10.75.
3.	-do 4 oil (48% ULV oil) 0.75 kg a.i./ha	-do-	-do-	3rd spray -90 DAS 22.10.75.
4.	Malathion 50% EC at 0.5 kg a.i./ha	S-(1,2 bis (Ethoxy Carbomyl ethyl) phosphoro thioate	Cynamide India Ltd., Bombay.1.	4th spray - 105 DAS 6.11.75
5.	BHC 50% WP at 1.25 kg a.i./ha	1,2,3,4,5,6, Hexachloro- Cyclohexane	Alkali & Chemical Co., Culcutta.	
6.	Metacid 50% EC at 0.375 kg a.i./ha	2,2 - bisdime- thyl (4 nitro mtolyl) Phos- phorothioate	Bayer India Limited, Bombay-1.	

applied for the second time the amount of initial deposit increased to 37 ppm which reduced to 10.98 ppm in 15 days. The rate of degradation of the second spray was slower than the first one. After the third spraying the initial deposit further increased to 41.57 ppm which degraded to 21.75. The final spray after 105 days gave an initial deposit of 52.03 ppm which degraded to 21.05.

The chemical degradation was faster for the first application, and slower for subsequent sprays.

b) *Degradation of carbaryl 50 per cent WP*: Carbaryl 50 per cent WP, applied at 0.75 kg a.i./ha, gave an initial deposit of 32.15 ppm, which degraded to 3.37 ppm in 15 days. However, with 2nd spraying, the initial deposit increased by 2.73 ppm. The third spraying gave an initial deposit of 33.61 ppm, higher by 1.46 ppm which degraded to 0.80 ppm in 15 days. The fourth spraying gave a deposit of 0.25 ppm less than the first spraying which degraded to 1.14 ppm in 15 days.

The rate of degradation was much faster and the residues were less than 3 ppm. These results agree with those of Deshmukh and Saramma (1971) and Dewan *et al.* (1971).

c) *Degradation of carbaryl 10 per cent dust*: Carbaryl 10 per cent dust applied at 1.5 kg a.i./ha gave an initial deposit of 28.93 ppm, which degraded to 1.89 ppm in 15 days, for all sprays the rate of reduction was faster. The reduction percentage arranged in the descending order for 1, 2, 3 and 4 times of spraying was 93.46, 95.26, 97.74 and 98.12 per cent respectively.

d) *Degradation of malathion 50 per cent EC*: Malathion 0.5 kg a.i./ha spray gave an initial deposit of 23.01 ppm which reduced to 0.89 ppm in 10 days. The residues of all four sprays reduced to 96 per cent (less than 1 ppm).

These results agree with those of Dixit *et al.* (1975), Yadav and Sachat (1971) and Kavadia and Gupta (1977).

e) *Degradation of BHC 50 per cent WP*: BHC 50 per cent WP at 1.25 kg a.i./ha spray gave an initial deposit of 31.5 ppm which degraded to 11.4 ppm in 15 days. A second spraying gave an initial deposit of 42.2 ppm. A similar accumulation occurred for the 3rd and 4th sprays which finally degraded to 33.1 ppm. For all sprays the percentage degradation was low (less than 50 per cent) and after 120 days of crop growth the degradation was 39.6 per cent. These results are at variance with those obtained by Kavadia and Gupta (1977) and Kavadia and Rattan Lal (1967). The main reason for this variance could be that crops like rape and onion will have more moisture than paddy.

TABLE I
Days after spraying - Residue in ppm

	0 days	% reduc- tion	5 days	% reduc- tion	10 days	% reduc- tion	15 days	% reduc- tion
<i>Carbaryl Oil at 0.75 kg. a.i./ha</i>								
I Spray	31.67	0	17.53	44.64	9.41	69.97	6.43	79.69
II Spray	37.00	0	23.39	36.78	14.64	60.43	10.98	70.33
III Spray	41.57	0	27.01	35.84	25.84	38.73	21.75	48.79
IV Spray	52.03	0	32.08	38.50	27.08	47.96	21.05	59.57
<i>Carbaryl 50 per cent WP (0.75 kg a.i./ha)</i>								
I Spray	32.15	0	16.40	48.99	9.35	70.91	3.37	89.52
II Spray	34.68	0	17.98	48.15	9.81	71.71	2.14	93.83
III Spray	33.61	0	16.94	49.46	7.81	76.56	0.80	99.02
IV Spray	31.90	0	14.61	54.19	5.41	83.05	12.14	92.42
<i>Carbaryl 10 per cent Dust (1.5 kg a.i./ha.)</i>								
I Spray	28.93	0	13.45	53.51	6.34	78.07	1.89	93.46
II Spray	30.14	0	14.16	53.04	8.14	72.98	1.43	95.26
III Spray	29.69	0	13.93	53.09	6.43	78.34	0.67	97.74
IV Spray	28.95	0	14.61	49.52	7.34	74.64	0.83	98.12
<i>Malathion 50 per cent EC (0.5 kg a.i./ha)</i>								
I Spray	23.01	0	5.20	77.41	0.89	96.14	nil	100.00
II Spray	22.95	0	5.30	77.07	0.91	96.25	nil	100.00
III Spray	23.10	0	5.41	76.58	0.85	96.34	nil	100.00
IV Spray	23.05	0	5.38	76.67	0.89	96.16	nil	100.00
<i>B.H.C. 50 per cent WP (1.25 kg a.i./ha)</i>								
I Spray	31.50	0	25.98	17.50	19.81	37.13	11.40	63.81
II Spray	42.21	0	34.93	17.25	28.48	32.53	21.01	50.23
III Spray	50.23	0	42.68	15.03	34.93	30.46	26.45	51.33
IV Spray	54.90	0	49.83	9.23	41.25	24.86	33.14	39.62
<i>Parathion 50 per cent EC (0.375 kg a.i./ha)</i>								
I Spray	16.57	0	14.81	10.62	11.43	31.02	9.61	42.01
II Spray	26.48	0	23.93	9.63	20.15	23.91	17.17	35.16
III Spray	34.15	0	31.41	8.24	27.68	18.94	23.95	29.86
IV Spray	39.65	0	35.89	9.48	32.01	19.27	28.28	28.64

f) *Degradation of methyl parathion 50 per cent EC*: Methyl parathion applied at 0.375 kg a.i./ha gave an initial deposit of 16.57 ppm, which decreased to 9.61 ppm after 15 days. The percentage degradation gradually decreased and was very slow. The cumulative residue after 4 sprays after 120 days of crop growth was still 28.28 ppm.

These results are at variance with those reported by Kavadia and Gupta(1977), this may be because onions have more moisture.

TABLE III

Cumulative residues of different insecticides on paddy straw at harvest and post harvest

Name of the Chemical and dosage/ha.	Cumulative residue after 120 days of crop (in ppm)	Residue recorded at harvest i.e. 165 days of crop growth (in ppm)	Residue recorded on paddy straw kept in open for 25 days after harvest (in ppm)
Carbaryl 4 oil at 0.75 kg a.i./ha	21.05	6.18	1.0
Carbaryl 50 per cent WP at 0.75 kg a.i./ha	1.14	nil	nil
Carbaryl 10 per cent dust at 1.5 kg a.i./ha	0.83	nil	nil
Malathion 50 per cent EC at 0.5 kg a.i./ha	nil	nil	nil
BHC 50 per cent WP at 1.25 kg a.i./ha	33.14	16.95	11.00
Parathion 50 per cent EC at 0.375 kg a.i./ha	20.28	23.45	10.73

CONCLUSIONS

The cumulative residues of carbaryl on paddy straw at harvest from the oil treatment was 6.18 ppm, but the residues 25 days after harvest were less than 1 ppm, which is very safe so the straw can be fed to the cattle.

However, carbaryl 50 per cent WP, carbaryl 10 per cent dust and malathion 50 percent EC did not persist even in negligible quantities until harvest of the crop. This shows that these chemicals could be used for the control of brown plant hopper without leaving straw residues.

However, BHC 50 per cent WP, and methyl parathion 50 per cent EC gave residues which persisted 25 days after harvest of paddy crop and at levels of 11.00 ppm and 10.73 ppm so when these two chemicals are used, there should be some waiting period before feeding the straw to cattle; this should exceed, 2 to 3 months.

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52 RESIDUAL TOXICITY OF TRIAZINE, PHENOXY AND AMIDE HERBICIDES TO SUCCESSIVE CROPS IN ROTATION

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SUMMARY

The soil carryover of the herbicides atrazine, simazine, ametryne, prometryne, terbutryn, 2,4-D-Na salt and alachlor was studied by the bioassay of crop germination after harvest of main crop. In the first experiment atrazine and simazine were applied as pre-emergence herbicides and 2,4-D-Na salt as post-emergence herbicides to sorghum crop during the rabi season. There were no significant effects of treatments on groundnut and cowpea as following crops. The germination percentage of blackgram varied significantly for some treatments. Atrazine and simazine at 2.0 kg/ha persisted in soil and affected the germination of blackgram.

In the second experiment, ametryne, prometryne, terbutryn, atrazine and alachlor were applied as pre-emergence herbicides to sorghum grown with redgram, bengalgram and safflower as mixed crops. The residual toxicity studies indicated no significant difference between treatments on wheat and maize as following crops. Cowpea germination was affected greatly by residues of prometryne, ametryne, terbutryn and atrazine.

In the third experiment, 0, 1, 2 and 4.0 kg a.i./ha of atrazine was used, and residual toxicity was noticed on succeeding crops of wheat and gram at 2.0 and 4.0 kg/ha.

INTRODUCTION

Pre-emergence herbicides make the production of sorghum without cultivation a feasible practice (Wiese and Rea, 1962). Crops such as sorghum and maize can overcome the phytotoxic effects of triazines.

The loss of herbicides from soil may be due to breakdown by biological and non-biological means, or because their availability may be altered by adsorption, volatilization, leaching and plant uptake. Carryover of herbicides in soil is important in crop-rotation programme, where legumes usually follow the sorghum crop. Many workers have reported phytotoxic effects of herbicides on the succeeding crops in rotation (Wicks and Fenster, 1969 ; Hugar, 1974 ; Kurdikeri, 1978 ; Srikant *et al.*, 1978). The amounts of herbicides remaining in soil influences their phytotoxic effects on succeeding crops and depends on soil type, soil organic matter, rainfall and type of cultivation, irrigation and climate. Thus residual toxicity studies varies according to local conditions. Prometryne, ametryne, terbutryn are now used on sorghum, redgram and bengal gram. We studied the residual toxicity of various herbicides at various concentrations on the germination of crops in rotation.

MATERIAL AND METHODS

Three experiments were conducted at the Agricultural College Farm, Dharwad. The soil was of black clay with 51.95 per cent clay, 28.00 per cent silt and pH 7.5. Dharwad comes under transition tract of Karnataka. The experiments were laid out in randomized block designs with three replicates.

In the first experiment during 1975-76 (Rabi) sorghum was grown under irrigation with pre-emergence herbicide treatments of atrazine, simazine at 0.4, 0.8, 1.2 and 1.6 kg a.i./ha., (one day after sowing), 2,4-D-Na salt post-emergence (30 days after sowing) at 0.8, 1.2, 1.6 and 2.0 kg a.i./ha. Other treatments were hoeing 25 and 40 days after sowing, a weed free control and unweeded control. After the harvest of the sorghum crop, the residual toxicity was studied by sowing groundnut, blackgram and cowpea and taking the survival of plants 25 days after sowing as a criterion.

In the second experiment during 1976-77 (Rabi) sorghum was grown with redgram, bengalgram and safflower as mixed crops. The treatment were sprays

of prometryne, ametryne, terbutryn at 0.50 and 1.0 kg a.i./ha, atrazine at 0.40 and 0.8 kg a.i./ha and alachlor at 0.48 and 0.96 l a.i./ha, a weed free control and unweeded control. All herbicides were applied one day after sowing. After harvest the residual toxicity of the herbicides was studied by germination percentage 25 days after sowing wheat or maize.

In the third experiment during 1976-77 (*khari*) 24 sorghum genotypes were screened for tolerance to atrazine as a pre-emergence spray applied at 0, 1, 2.0 and 4.0 kg a.i./ha one day after sowing. After harvest residual toxicity was studied by sowing wheat, safflower, redgram, Bengalgram and counting the number of plants 30 days after sowing. The data were analysed statistically after transformation in angular form.

RESULTS AND DISCUSSION

1975-77 Experiment (Rabi) : The residual toxicity of atrazine, simazine and 2,4-D-Na salt on the bioassay crops is summarised in Table I which shows that groundnut and cowpea were tolerant to residues but blackgram was susceptible.

Groundnut : The percentage survival of plants per treatment indicated no significant differences between the various treatments. In general there was reduction in per cent survival of plants on the plots treated with atrazine and simazine.

Blackgram : The percentage survival of plants between treatments differed significantly at the 5 per cent level. The lowest survival was due to simazine at 1.6 kg a.i. per ha. The relationship between percentage survival of plants and the dosage of herbicides is proportional. The higher doses of 2, 4-D-Na salt were also phytotoxic to blackgram.

Cowpea : The treatments did not differ significantly at 5 per cent level. The trend of per cent plants surviving was similar to that of groundnut,

1976-77 Experiment (Rabi) : The data regarding percentage germination 25 days after sowing is given in Table II.

Wheat : The percentage germination of wheat did not differ significantly. The germination was due to ametryne at 1.0 kg a.i. per ha and atrazine at 0.8 kg a.i. per ha (66.67 per cent). This confirms that wheat can tolerate residues of these herbicides. Wicks and Fenster (1969) and Gupta (1973) reported that yield of wheat, was unaffected by atrazine residues and Srikant and Hosmani (1978) reported a reasonable tolerance of wheat to residues of atrazine, simazine and nitrofen.

Maize : The percentage germination of maize was unaffected by residues of any herbicide due to the plants ability to degrade the absorbed parent toxic material into a nontoxic form.

Cowpea : The percentage germination of cowpea differed significantly between treatments. Residues of ametryne, prometryne, terbutryn and atrazine affected this crop.

1976-77 (Kharif) Experiment : Toxicity of atrazine was in the order : Sesfflower > wheat > redgram > Bengalgram. Dry weight studies indicated a redu-

TABLE I
Residual toxicity of various herbicides (1975-77) on percentage survival of crops 25 days after sowing

Treatment detail	Groundnut		Blackgram		Cowpea	
	Original	Angular Trans-formation	Original	Angular Trans-formation	Original	Angular Trans-formation
Atrazine (6.4 kg a.i. per ha) pre-emergence	72.85	59.17	61.15	52.88	69.77	57.13
" (0.60 " " "	62.22	52.32	59.01	50.37	67.77	55.53
" (1.20 " " "	61.11	51.45	57.55	49.33	63.51	51.08
" (1.60 " " "	60.98	51.40	58.00	49.61	61.19	51.67
Simazine (0.1 " " "	68.22	55.79	63.11	53.81	78.50	61.44
" (0.8 " " "	66.88	52.29	62.80	52.31	75.55	61.72
" (1.20 " " "	63.77	53.07	62.67	52.42	72.89	58.13
" (1.60 " " "	62.00	51.94	45.05	43.85	72.00	59.08
2,4-D-Na Sat (0.80 " " Post-emergence	70.22	57.06	71.33	57.67	78.00	62.89
" (1.20 " " "	67.10	55.04	64.67	53.53	75.53	60.37
" (1.60 " " "	64.88	53.74	63.55	52.88	65.53	51.30
" (2.00 " " "	63.77	53.03	57.33	49.24	66.22	54.75
Hoeing 25 days after sowing	70.99	57.42	72.21	58.20	79.11	62.81
Weed free control	71.77	58.43	75.11	60.14	80.66	64.35
Unweeded control	73.77	59.22	72.66	58.50	84.00	66.45
SEm ±		2.77		1.91		3.66
C.D. at 5 %		—		5.61		—

tion in weight with the increase in the levels of atrazine applied (Table IV) and residual toxicity of atrazine remained for five months after application if used above 2.0 kg a.i./ha.

TABLE II
Residual toxicity of various herbicides (1976-77) on the per cent survival of crops 25 days after sowing

Treatment detail	Wheat		Maize		Cowpea	
	Original	Angular Transformation	Original	Angular Transformation	Original	Angular Transformation
Prometryne (0.50 kg a.i. per/ha)						
pre-emergence	76.67	61.22	88.67	70.52	86.67	68.67
" (1.0 ")	73.33	59.01	85.53	67.55	81.33	64.41
Ametryne (0.50 ")	73.33	59.71	90.67	72.37	87.33	67.11
" (1.00 ")	66.67	54.75	82.00	64.92	81.33	64.50
Terbutryn (0.50 ")	73.33	59.01	88.00	69.85	88.00	69.67
" (1.00 ")	70.00	57.00	88.00	69.77	84.00	66.65
Atrazine (0.40 ")	76.67	63.33	90.00	71.62	86.00	68.20
" (0.80 ")	66.67	54.39	88.00	69.77	80.00	63.46
Alachlor (0.43 l a.i./ha)	80.00	63.93	86.67	70.44	90.66	72.23
" (0.86 ")	76.67	61.22	86.67	69.75	86.33	70.05
Weed free control	80.00	63.93	90.00	71.62	92.33	73.10
Unweeded control	80.00	63.93	86.33	71.48	94.00	74.25
SEm+		5.8		1.00		1.11
C.D. at 5%						1.72

TABLE III
Residual toxicity of various levels of atrazine on plant populations

Treatments/Crops	Safflower	Redgram	Wheat	Bengal gram
Hand weeding	21	15	33	14
Atrazine (1.0 kg a.i./ha)	16	14	28	13
Atrazine (2.0 kg a.i./ha)	15	12	11	13
Atrazine (4.0 kg a.i./ha)	7	11	10	12

The bio-availability of soil-applied herbicides for plants is governed essentially by the dynamic equilibrium existing between the adsorbability of the compounds to the soil colloids and their solubility in the soil/water phase. Consequently an

TABLE IV

Residual toxicity of various levels of atrazine as dry weight of plants/meter row (g)
Mean of 3 replicates

Treatments/Crops	Safflower	Redgram	Wheat	Bengalgram
Hand weeding	39.82	10.11	20.03	19.37
Atrazine (1.0 kg a.i/ha)	17.09	8.40	19.73	17.46
Atrazine (2.0 kg a.i/ha)	10.30	4.52	4.93	12.60
Atrazine (4.0 kg a.i/ha)	8.04	2.47	4.23	11.67

appreciable influence of these parameters on the selective action of S-triazines must be expected. Because of the relatively strong adsorption of the S-triazines, selective phytocidal behaviour is shown.

The ability of plants to detoxify S-triazines by specific metabolic reactions is an additional factor necessary to fully explain their selectivity because susceptibility to S-triazines can be correlated with the amount of unchanged triazine available for action at sensitive sites (Negi *et al.*, 1964).

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53 STUDIES ON PESTICIDAL DRIFT AND INCIDENTAL DEPOSIT IN USING DIFFERENT SPRAY SYSTEMS

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SUMMARY

A field experiment with paddy was conducted to measure the extent of pesticidal drift while operating HV, LV and battery operated ULV sprays. Three commercial formulations of quinalphos, phosphamidon and dichlorvos were used through knapsack and power sprayers and for the ULV sprayer, suitable modification of the formulation was made. The fall of pesticides on an inert surface (lower and upper), target plant, standing water and on the operator was measured by a colorimetric technique. The results indicated no significant difference in deposition on target plants between sprayers. Deposit on an inert surface was significantly more with the power sprayer than in the rest. Pesticidal drift in water was significantly different between sprayers, the maximum being in the case of knapsack sprayer followed by powder and ULV sprayers. The accidental deposition (both in number of droplets and in amount) on the operator during the spray was maximum with ULV followed by power and knapsack sprayers.

INTRODUCTION

The drift of pesticidal chemicals while spraying contributes immensely to problems of environmental pollution and not only make control operations inefficient and uneconomical but endangers people. Although the importance of the problem was realised in 1942 (Brooks, 1947) not much has been done to evaluate relative drift in respect of various spray equipments. With the advent of the hand-held battery operated ULV spray system, the problem assumed greater importance because minute invisible particles of concentrated spray solutions are produced and be carried

to the target by wind. Under such circumstances the spray operator is in danger of being exposed to the spray droplets. Studies on the extent of such exposure and the drift of the pesticides with different sprayers are lacking. The present paper reports the results of a field spray experiment in which drift and incidental deposits of pesticides were evaluated for HV, LV and ULV spray systems.

MATERIAL AND METHODS

A field trial was laid out with a split plot design to accommodate three spraying methods as main treatments and three insecticides as sub-treatments, replicated five times. The treatments were laid in three different fields which were well separated from each other but in the same block. The following three sprayers were used representing high volume (HV), low volume (LV) and ultra low volume (ULV) spray system.

1. H.V. Hand compression knapsack sprayer, Aspee, Bombay.
2. L.V. Motorised knapsack sprayer, Aspee, Bombay.
3. U.L.V. Battery operated rotary disc ULV sprayer, Entokil, Ludhiana.

Commercial emulsifiable concentrates of quinalphos, phosphamidon and dichlorvos were used in the above three systems with suitable standardisation. Calculated amount of insecticides were sprayed 20 days after planting (DAP) and the second and third sprays were applied after 40 and 60 days respectively. The weather was taken into consideration when strating each spraying.

Estimation of spray deposit on target plants : Samples of treated leaves weighing about 5 g were collected carefully immediately after spraying and were soaked in an excess of acetone to extract chemical. The solution obtained was passed through a funnel packed with activated charcoal and celite 545 and the filtrate was collected after additional washing with acetone. The filtrate wash then concentrated in a Kuderna-Danish evaporatory condenser and taken in a 25 ml volumetric flask and made up to volume by adding acetone. Paired untreated paddy leaves weighing 5 g were measured for area subjected to the above treatments and the filtrate collected for a control.

From the volumetric flask, a known sample was taken and the insecticide present estimated as total phosphorus by the method described by Jackson (1962). The amount of deposit present/unit area was calculated and given as $\mu\text{g}/\text{cm}^2$.

Estimation of spray-drift into paddy water : To estimate the quantity of pesticides that drifted into paddy water, water samples were collected from each plot

soon after the completion of spraying. About 50 ml of water was taken and centrifuged for 15 minutes to remove dirt. Then the clear water was taken with an equal amount of hexane in a 250 ml separatory funnel and the insecticide extracted repeatedly with further additions of solvent. The extracts were combined and passed through a column of anhydrous sodium sulphate to remove the last traces of water. Then it was concentrated in a Kuderna-Danish evaporatory condenser of a known capacity and this was subjected to total phosphorous analysis as before.

Estimation of spray droplet number and deposition on operator : A one sq. ft. sheet of tin was fixed on the chest of the operator during spraying and after the completion of spraying for each plot, the tin sheet was washed with excess acetone and the solution was collected in a 250 ml beaker and then passed through a funnel containing celite 545 and anhydrous sodium sulphate, the clear filtrate was collected and concentrated in a 25 ml volumetric flask. A known portion was taken from this and subjected to total phosphorous analysis.

To estimate the number of droplets falling on the operator, pieces of sensitive paper were fixed on the apron of the operator on the chest portion. These paper pieces having a coating of a chemical produce coloured spots as the spray droplets strike the surface. After the completion of spraying the bits were collected and the number of coloured spots were counted and density was expressed as number/sq inch.

RESULTS AND DISCUSSION

Results obtained in the estimation of drifted pesticide into paddy water as against the amount that are deposited on the target are given in Table I. Among the three sprayers, ULV caused the least amount of drift, whereas the other two systems were on par with each other. There was no significant difference between insecticides. It is evident that the maximum wastage of pesticidal material is possible for both H V and L V sprayers irrespective of the nature of insecticides.

The pesticides in the form of drift go unused and may harm non-target organisms. There is little information on the cost of and consequences of this drift. The present data line indicate clearly that such losses could be greatest, both in HV and LV systems. Such losses could be minimal in the case of the ULV spray system because there is little scope for the minute particles of spray to fall immediately into water as they are air-borne and thus carried along and through the canopy of plants so increasing the chances for deposition on the target.

Table II shows the incidental deposition of pesticides on the operator during spraying for various spray systems. It is evident that maximum number of droplets

TABLE I

Extent of drifted insecticides as against actual deposits on paddy leaves in various spray systems

Treatments		Age of the crop					
Sprayer	Sub Tr.	20 DAP		40 DAP		60 DAP	
		Leaf ($\mu\text{g}/\text{cm}^2$)	Drift (ppm)	Leaf ($\mu\text{g}/\text{cm}^2$)	Drift (ppm)	Leaf ($\mu\text{g}/\text{cm}^2$)	Drift (ppm)
ULV	Quinalphos	2.94	0.66	14.60	1.26	13.00	0.50
	Phosphamidon	2.38	0.40	12.38	1.00	11.49	0.50
	Dichlorvos	3.96	1.08	12.68	1.45	10.76	0.45
LV	Quinalphos	10.30	1.62	9.88	4.00	9.35	3.00
	Phosphamidon	9.54	3.06	7.64	3.94	10.00	2.75
	Dichlorvos	10.18	2.58	11.12	3.46	10.20	3.10
HV	Quinalphos	9.68	3.36	10.50	2.62	11.30	2.00
	Phosphamidon	12.38	3.00	11.66	3.74	9.90	2.21
	Dichlorvos	9.42	3.42	9.26	4.56	8.70	2.63

ULV differed significantly from LV & HV. $P=0.05$

TABLE II

Deposition of spray droplets on the operator while using various spray systems

		Age of the crop					
Sprayer	Sub tr.	20 DAP		40 DAP		60 DAP	
		No./Sq in	$\mu\text{g}/\text{cm}^2$	No./Sq in	$\mu\text{g}/\text{cm}^2$	No./Sq in	$\mu\text{g}/\text{cm}^2$
ULV	Quinalphos	38	4.0	72	5.2	52	12.8
	Phosphamidon	27	2.7	68	3.1	83	10.0
	Dichlorvos	48	3.8	53	1.0	171	11.0
LV	Quinalphos	21	1.6	4	0.8	56	4.5
	Phosphamidon	2	0.8	7	0.0	21	1.6
	Dichlorvos	3	0.0	9	1.2	43	4.4
HV	Quinalphos	1	0.8	1	0.0	15	1.9
	Phosphamidon	1	0.0	3	0.2	7	0.5
	Dichlorvos	1	0.0	1	0.0	18	0.8

Main Tr. Sig : ULV LV HV

Sub Tr. N.S.

and amounts deposited are for the ULV sprayer followed by LV and HV. Since the minute droplets normally drift in the windward direction the deposition on the operator is influenced greatly by existing wind velocity and direction. Reed (1975) reported that in ULV spraying sudden wind shifts could give a heavy coverage on the most careful operator and in one test in Swaziland, 1600 droplets/sq cm were counted on a paper pinned to the back of the operator only after 10 minutes spraying. Bourke *et al.* (1971) reported similarly that ULV spraying with methyl parathion resulted in a reduction of CLE level in the blood of persons checking cotton leaves soon after spraying.

The above reports and the present results emphasise the intricate problem of safety of the operator in using a hand held disc type ULV sprayer loaded with concentrated formulation.

Grateful thanks are due to Dr. C. N. Sambandam, Dean, Faculty of Agriculture, Annamalai University for the facilities provided for the study.

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SUMMARY

Degradation of several soil-applied organochlorine, organophosphorus and carbamate insecticides have been studied under laboratory and field conditions.

Degradation of organochlorine insecticides such as DDT, dieldrin, aldrin, heptachlor and lindane was more rapid than reported in the literature.

Among the organophosphorus and carbamate insecticides the degradation of monocrotophos, dicrotophos, disulfoton, phorate, fensulfothion, chlorpyrifos, chlorfenvinphos, aldicarb and carbofuran has been studied under laboratory and field conditions. In general, the degradation of all these insecticides followed the first-order reaction. It was also observed that degradation of pesticides proceeded both biologically by soil microbes and non-biologically or chemically by interaction with clays, organic matter and soil solution.

The degradation of disulfoton and phorate was found to be faster under irrigated than unirrigated conditions. Under flooded conditions in rice fields, the degradation of these insecticides was much slower as compared to non-flooded fields of greengram or cowpea.

Fertilizers enhanced the degradation of monocrotophos, dicrotophos, disulfoton and phorate under field and laboratory conditions by 1-7 per cent.

The soil is an unequivocal dynamic system responsible for the break down of most of the pesticides used for pest control. Irrespective of the method or target of the spray, most of the applied-pesticide ultimately finds its way into the soil. Surprisingly, 20-80 per cent of the foliar sprays fall on to the soil depending on the crop and the applicator. Even in aquatic systems, pesticide molecules have a tendency to become attached to sediments and settle in the bottom. Soil serves not only as a reservoir for these chemicals but also as a viable medium where these molecules are subjected to different physiological and biochemical processes which help to eliminate these pesticides from our ecosystem. Understanding of these processes is of utmost importance in evaluating the hazards or the extent of damage that can be caused from the use of these chemicals.

The nature of degradation of these pesticides is not very clear. It is well-established that soil microorganisms such as actinomycetes, fungi & bacteria can degrade pesticides both under aerobic and anaerobic conditions by β -oxidation, ether cleavage, hydrolysis of ether and amide bonds, dealkylation of C-R and N-R bonds, dechlorination, epoxidation, oxidation of alcohols and aldehydes, reductive dehalogenation, aromatic ring cleavage etc. The chemical or non-biological degradation of these pesticides is equally important (Griffiths, 1965 ; Sun and Johnson, 1965 ; Scriber and Munkle, 1972 ; Brown *et al.*, 1966 ; Eichelberger and Lichtenberg, 1971) and has been suggested as a major pathway in the degradation of non-biodegradable pesticides. Certain pesticide molecules are inherently resistant to microbial degradation, but chemical degradation can convert these to non-toxic biodegradable metabolites which can be attacked easily by soil microbes. For example, atrazine is converted chemically to hydroxyatrazine which is metabolised to CO₂ and other products by microbes (Skipper *et al.*, 1967).

Chemical degradation is facilitated by organic matter and clay minerals. Soil properties such as soil pH, surface acidity and cation saturation are considered to be the principal controlling factors in degradation of pesticides in soil.

This paper presents some of the recent work in our Division of Agricultural Chemicals on the degradation of pesticides in soil under field and laboratory conditions.

Degradation of organochlorine insecticides in soil: Earlier work (Agnihotri *et al.*, 1974, 1977) on the persistence of several organochlorine insecticides in IARI soil (pH 8.2, C 0.32 per cent, clay 19.0 per cent and CEC 2.1 meq/100g) has shown that these pesticides are quickly lost under our Indian tropical conditions (Table I). In 180 days, 99.4 per cent lindane, 96.4 per cent dieldrin, 96.3 per cent aldrin and

TABLE I
Persistence of organochlorine insecticides

Insecticide	Rate of application kg/ha	Crop	% loss	
			100 days	180 days
BHC	10	Mung, Lobia	97.5	—
Lindane	10	Fallow	94.0	99.4
Aldrin	10	Mung, Lobia	87.9	—
„	10	Fallow	87.0	96.3
Dieldrin	10	Fallow	79.0	96.4
Heptachlor	10	Fallow	68.0	86.0
DDT	10	Fallow	74.0	94.8

86.0 per cent heptachlor was lost when applied at the rate of 10 kg a.i./ha. Similar observations have been made by Srivastava and Yadav (1977) who have reported 77-90 per cent loss of BHC in three months when applied at the rate of 5-30 kg a.i./ha. The rapid loss of these insecticides from soil was attributed to the quick volatilization which removed insecticide molecules left on or near the surface of soil. Chemical hydrolysis was also considered to be important since the pH of IARI soil was very high (8.2) and organochlorine insecticides especially BHC, DDT and lindane are known to be susceptible to alkaline hydrolysis.

Further experiments were done to differentiate the roles of degradation and volatilization in dissipation of these insecticides in soil. Five per cent dusts of aldrin, dieldrin, heptachlor and lindane were applied at a rate of 10 kg ai/ha and mixed with top 10 cm soil with a spade. Concrete rings of 60 cm I. D. and 20 cm height were inserted vertically into the soil, covered with glass and sealed with adhesive clay.

Samples were taken at a depth of 0-10 cm at different time intervals, extracted with Soxhlet apparatus and analysed by gas-liquid chromatography.

TABLE II
Chemical and physical properties of soils used for incubation study

Soil	pH (1 : 2.5) soil : water	% c organic carbon	Field capacity	CEC me q/ 100 g	Mechanic al analysis		
					% sand	% silt	% clay
IARI	8.0	0.44	18.1	4.3	76	10	14
Assam	5.4	0.94	20.1	6.5	67	21	12

The dissipation of aldrin and heptachlor was higher under uncovered than covered conditions (Figure 1) although the differences were much greater for heptachlor than aldrin. In 120 days, 62 per cent of the heptachlor was lost under covered and 83 per cent under uncovered conditions ; corresponding values for aldrin were 70 and 75 per cent.

For dieldrin and lindane the dissipation was faster under covered than uncovered conditions (Figure 2). In 120 days, 50 per cent dieldrin was lost under uncovered and 74 per cent under covered conditions ; corresponding values for lindane were 80 and 94 per cent.

These data clearly suggest that volatilization had a very small role in the dissipation of these pesticides in soil and the loss is primarily by degradation under Indian condition.

A faster degradation of dieldrin and lindane under covered conditions could be explained on the basis that covering the cylinder with glass kept the soil hot and moist for much longer time than under open conditions thus making it more conducive for anaerobic degradation by microbes (Yashida and Castro, 1970). Similar observations have been made by Matsumura (1973) and Siddaramappa and Sethunathan (1975) who reported a rapid degradation of α and β -isomers of BHC under rice cultivation. The degradation was correlated with the negative redox potential. Other organochlorine insecticides such as DDT, heptachlor and methoxychlor (Guenzi and Beard, 1968 ; Castro and Yoshida, 1971 and Ko and Lockwood, 1968) have also been reported to breakdown rapidly under anaerobic conditions.

From these experiments, however, it is not certain whether the degradation was biological or non-biological in nature. Further experiments are in progress to elucidate this.

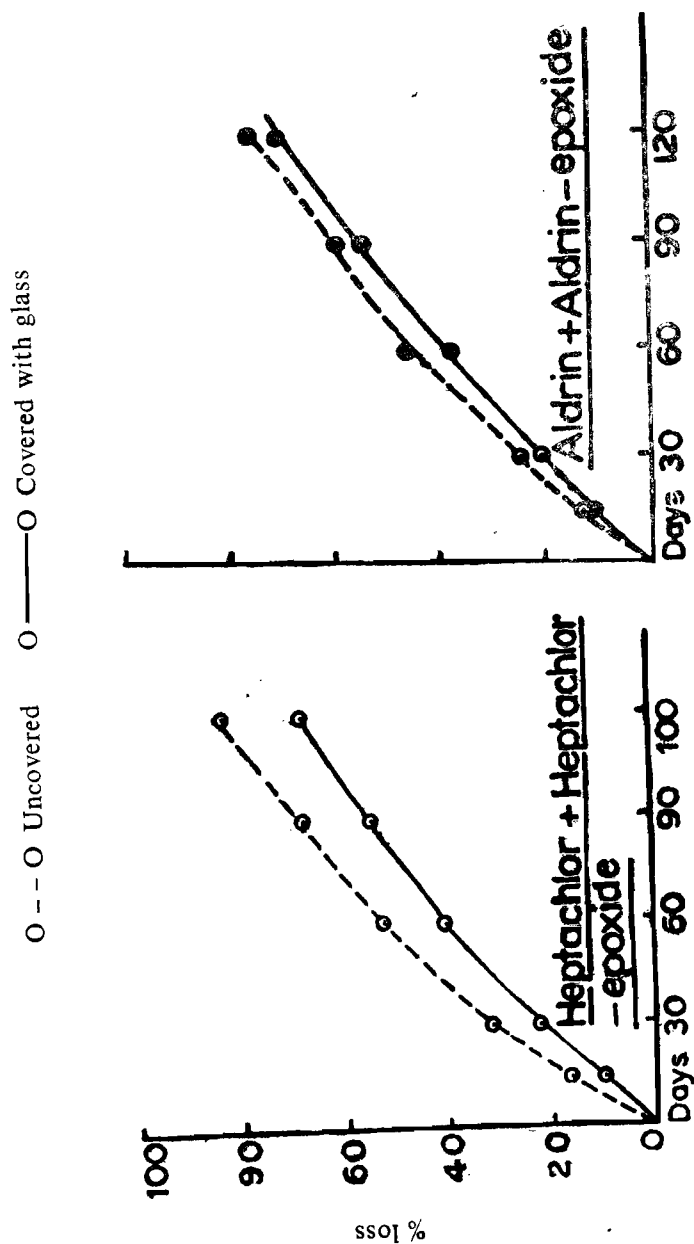


Fig. 1 Degradation of aldrin and heptachlor in soil under covered and uncovered conditions

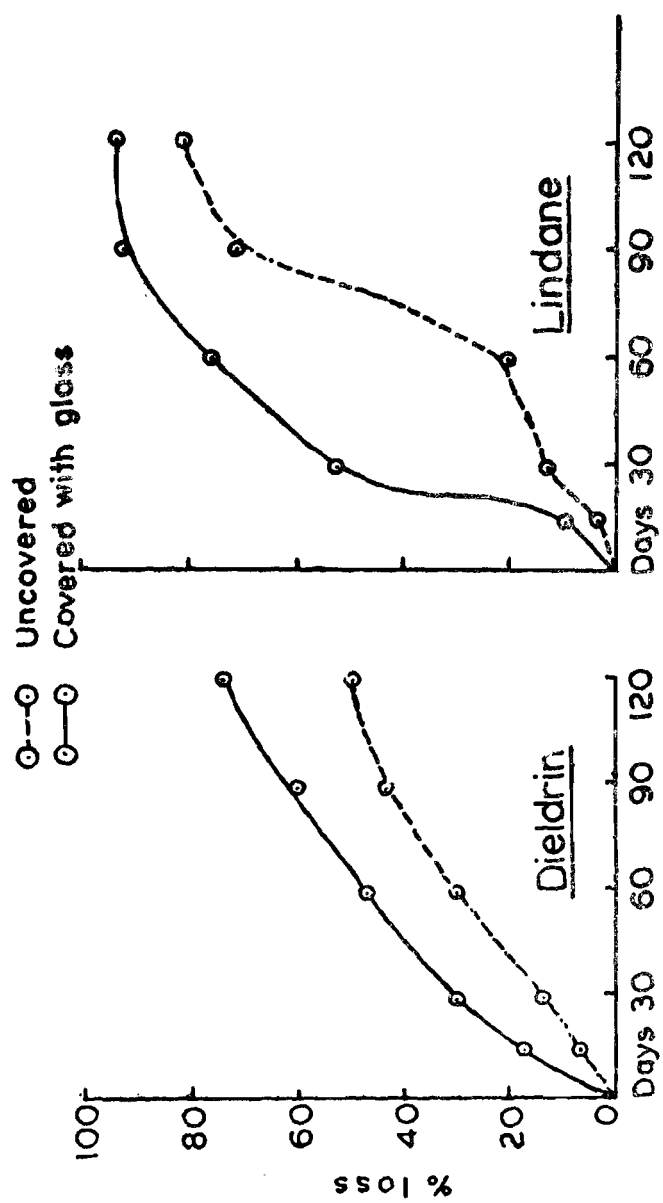


Fig. 2 Degradation of dieldrin and lindane in soil under covered and uncovered conditions

Degradation of organophosphorous and carbamate insecticides in soil : The degradation of monocrotophos, dicrotophos, disulfoton, phorate, fensulfothion (systemic type) ; chlorpyriphos, chlorfenvinphos (contact type) ; aldicarb and carbofuran (carbamates) was studied under field condition by applying granules in furrows at 1.5 kg a.i./ha. Degradation of all the insecticides in soil followed a first-order reaction. 'Half lives' under fallow conditions were monocrotophos 10.3 days ; tetrachlorovinphos 14.0 days ; disulfoton 28.6 days ; chlorfenvinphos 33.1 days ; fensulfothion 37.0 days ; and chlorpyriphos 40.6 days. Under crops, the 'half-life' of disulfoton was 10.3 in *kharif* (rainy) season and 14.5 days in *rabi* (winter) season (Pandey *et. al.*, 1975 and 1976). The half-life of aldicarb under a pea crop was 15 days (Dikshit *et al.*, 1975). For carbofuran under sorghum and maize crop it varied from 7-9 days (Sahu, 1977).

Factors affecting degradation of insecticides in soil—1) Moisture—The degradation of disulfoton and phorate was studied under irrigated and nonirrigated conditions under a mustard crop. Both insecticides dissipated faster under irrigated conditions, although the differences were marginal ranging from 1 to 2 per cent only (Agnihotri *et al.*, 1975) (Figure 3). However, in rice, under flooded conditions the residues of phorate and disulfoton persisted for 60 days as compared to 50 days in mustard, probably because of the low microbial activity (Figure 4).

2) *Fertilizers* : Effects of N.P.K. fertilizers on the degradation of disulfoton and phorate were studied under field and laboratory conditions at 30°C. Fertilizers were applied at the rate of 110 kg N, 30 kg P and 15 kg K per hectare. The rates of degradation of these insecticides were 1-6 per cent faster in soils which did not receive any fertilizer application (Figure 5) (Pandey and Agnihotri, 1976), however, under field conditions, dissipation of these insecticides was 10-20 per cent faster in soil which received the fertilizers because of better plant growth in the treated plots.

Biological vs. chemical degradation : To differentiate between biological and chemical degradation one set of soil samples was sterilized by autoclaving. Both sterilized and non-sterilized soil samples were treated with monocrotophos, dicrotophos, disulfoton, phorate, cytolane and carbofuran at 5 ppm and incubated at 30°C for 60 days.

About 30 to 35 per cent of the applied disulfoton and phorate degraded chemically (Figure 6), whereas the relative degradation of monocrotophos, dicrotophos and cytolane was 50 to 60 per cent. For carbofuran, 80 to 90 per cent degraded chemically (Pandey, 1976, Sahu, 1977).

It can be concluded from these studies that degradation of pesticides in soil is by both chemical and biological means. The chemical structure is the most important single factor in determining the stability which is influenced by the soil type

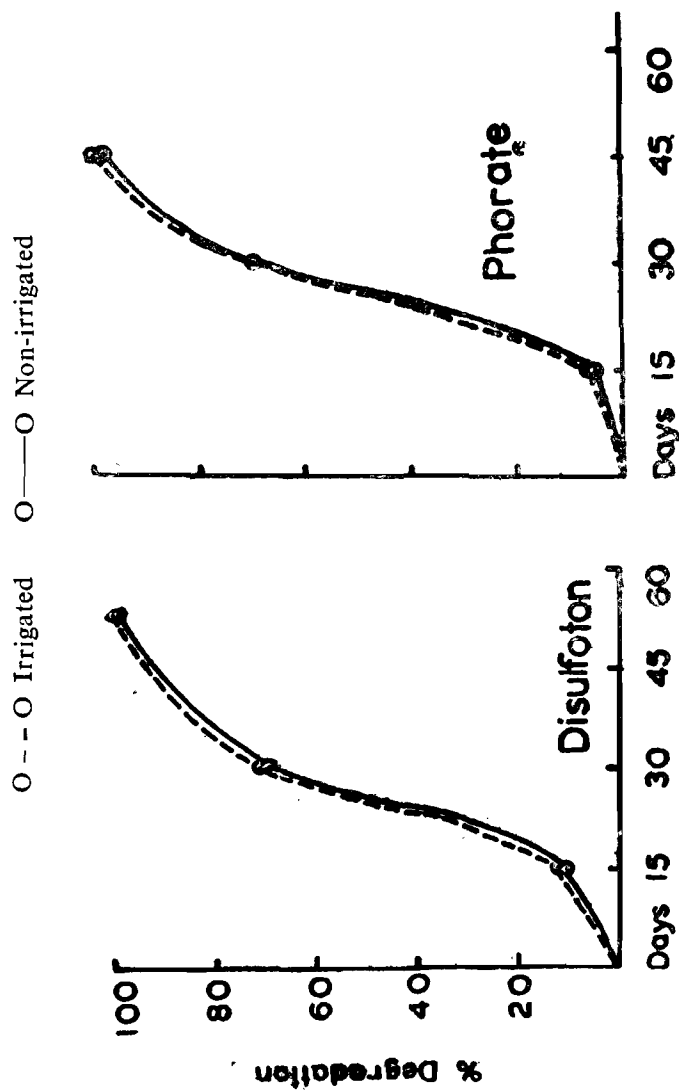


Fig. 3 Degradation of disulfoton and phorate in soil under irrigated and non-irrigated conditions

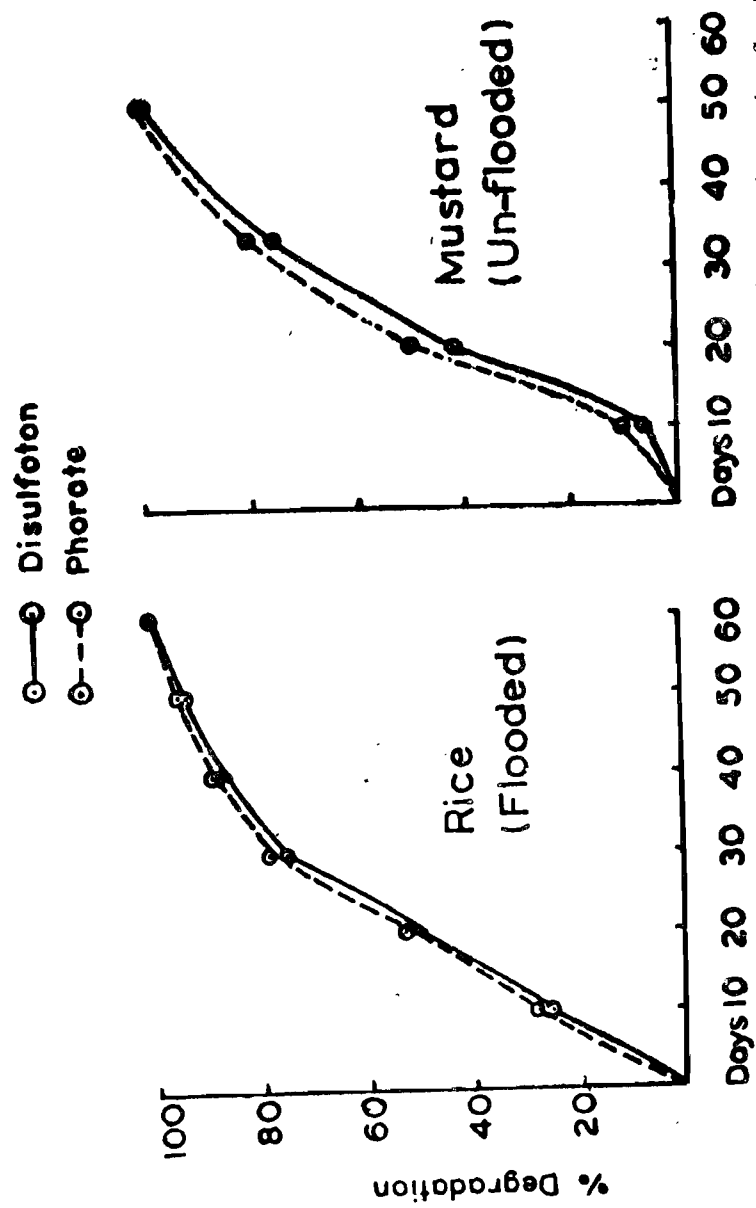


Fig. 4 Degradation of disulfoton and phorate in soil under rice (flooded) and mustard crop (un-flooded)

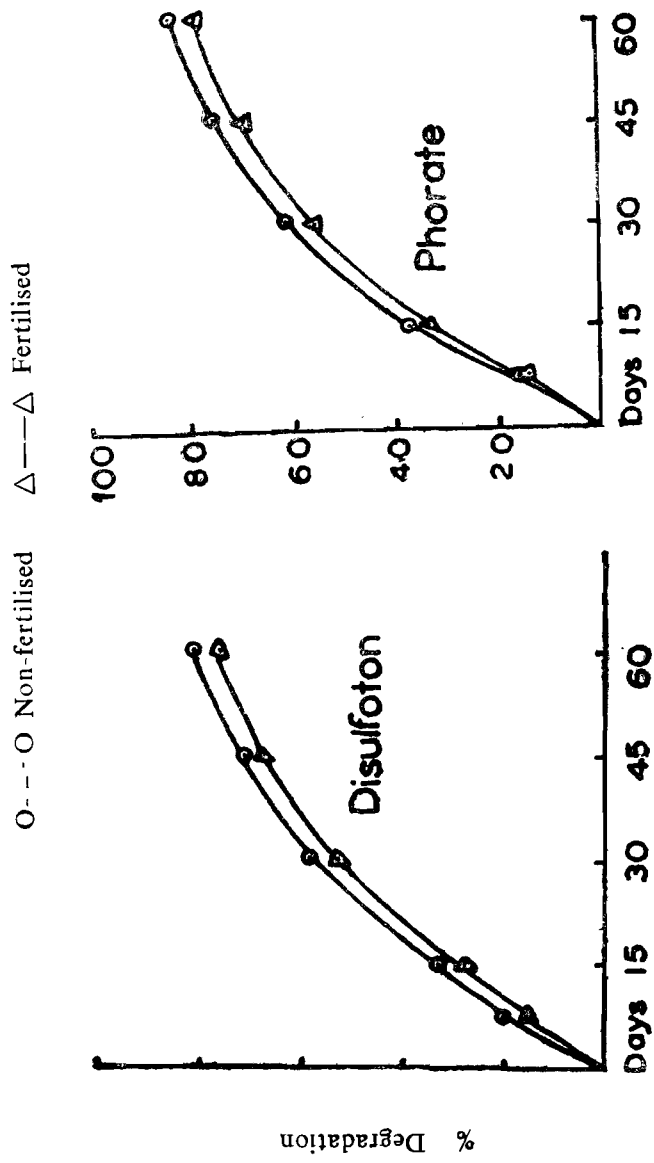


Fig. 5 Effect of fertilisation on degradation of disulfoton and phorate in soil at 30°C

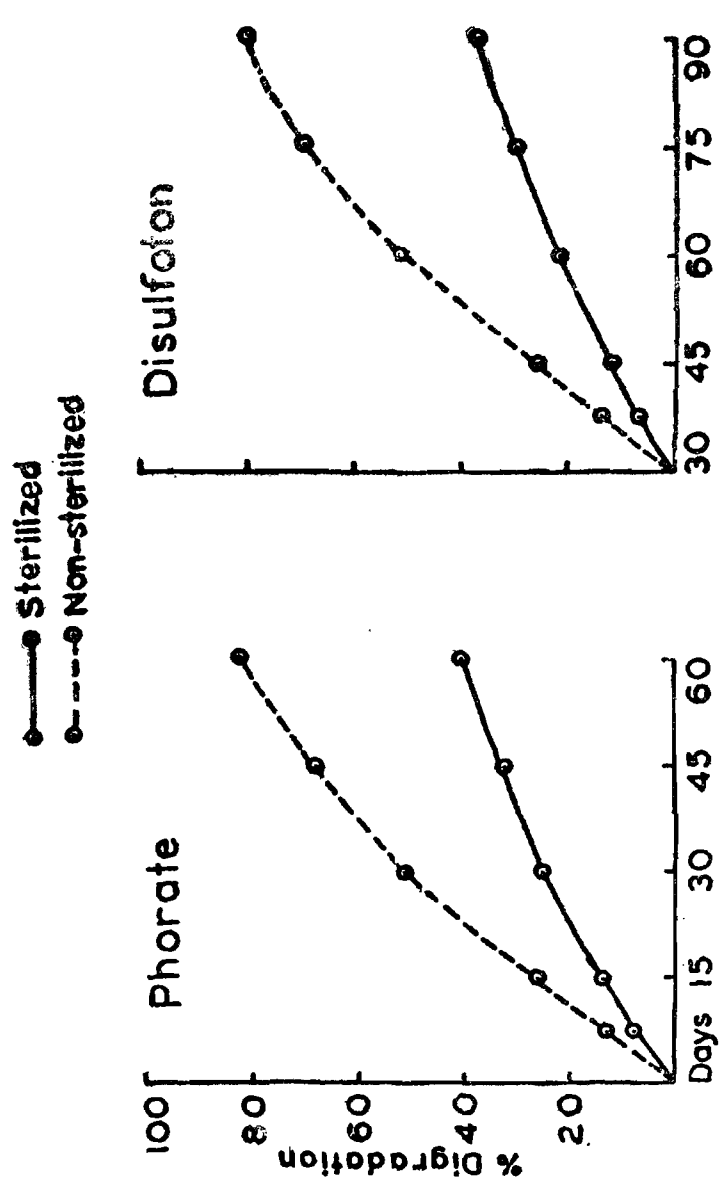


Fig. 6 Degradation of disulfoton and phorate in IARI soil under sterile and non-sterile conditions

used. To study the effect of soil type, the degradation of cytolane and carbofuran were studied under two different soil types, IARI soil (pH 8.0 ; C, 0.44 ; clay, 14 per cent ; CEC 4.3 meq/100 g) and Assam soil (pH, 5.4 ; C, 0.94 ; clay, 12 per cent ; CEC 6.5 meq/100 g) for 60 days. The degradation of cytolane was almost identical in IARI soil (51.8 per cent) and Assam soil (57.8 per cent) but chemical degradation was more pronounced in IARI soil and biological degradation in Assam soil. The degradation of carbofuran in IARI soil was non-biological and more chemical in nature. Biological degradation was dominant in Assam soil. The degradation depended primarily on the alkalinity of the soil and organic matter had very little importance in the degradation of carbofuran in the IARI soil.

From these studies it can be inferred that pesticides are broken down in soil both biologically by anaerobic and aerobic microorganisms and chemically by soil particulates. The rate of degradation depends primarily on the structure of the compound and soil chemical properties such as soil pH, surface acidity and cation saturation.

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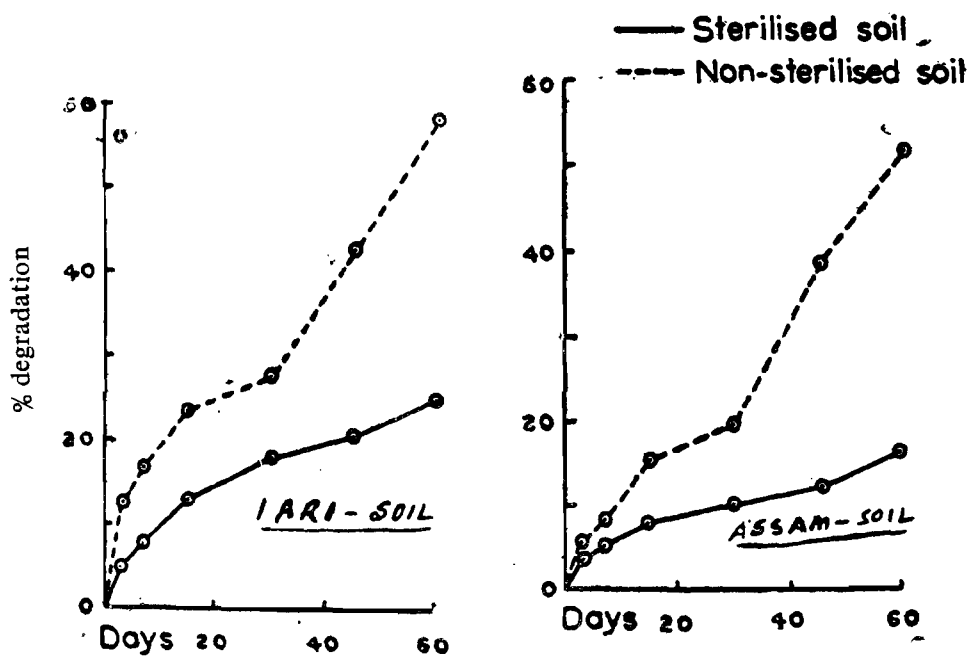


Fig. 7 Persistence of cytolane in IARI and Assam soil under sterile and non-sterile conditions

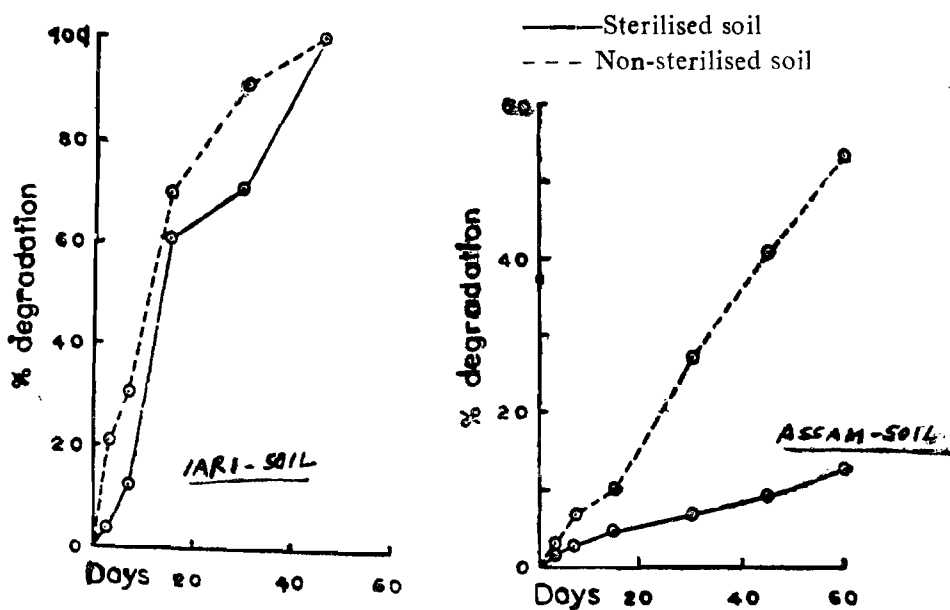


Fig. 8 Persistence of carbofuran in IARI and Assam soil under sterile and non-sterile conditions

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CONTAMINATION OF PUNJAB SOILS WITH RESIDUES OF COMMONLY USED INSECTICIDES

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SUMMARY

A preliminary survey to assess the status of insecticide residues in Punjab and Chandigarh soils was undertaken during 1976-77. Surface (0-10 cm) soil samples were collected at random, representing the total area of the constituent administrative units and land use. Extraction with methanol:water (2 plus 1) by overnight immersion and partitioning with n-hexane followed by column chromatography on activated alumina was standardized with satisfactory recovery and minimum interference of the co-extractives.

Of the 106 soil samples analysed using gas-liquid chromatography and thin-layer chromatography, 91 (85 per cent) were contaminated with insecticide residues. Of these, 90 contained DDT and related compounds (DDT-R) constituted by p,p'-DDT, p,p'-DDE, p,p'-TDE and a very small proportion of o,p'-DDT and dicofol. DDT-R level in the samples ranged from 0.02 to 1.63 ppm with geometric mean of 0.017 ppm. Other organochlorine insecticides identified were BHC, endrin and lindane at one site each at levels of 0.5, 0.2 and 0.1 ppm respectively. Of 21 soil samples showing a positive response by cholinesterase inhibition, only 7 could be identified tentatively as phorate sulfone and methyl parathion.

The wide-spread contamination of soils with DDT may be the result of dispersion and re-distribution of the pesticides employed mainly for mosquito control in public health rather than to its limited use in agricultural pest control. Further results do not suggest any accumulation of insecticides in the soils under the present pattern of usage.

INTRODUCTION

The introduction of a pesticide into the environment results invariably in the contamination of soils. The pesticide may persist in soil for different lengths of time depending upon the characteristics of pesticide and formulation, method, dose and frequency of application, soil and weather conditions, cultural practices, plant cover, etc. The persisting pesticide residues may give rise to several adverse effects and have been the subject matter of numerous studies as reviewed by Edwards (1970; 1973). Substantial progress has been made in several countries in monitoring the insecticidal contamination of soils (Holden, 1975). The most commonly occurring insecticide residues in soils are DDT and related compounds (DDT-R), aldrin and dieldrin, heptachlor and its epoxide, endrin, BHC, etc. Their regular use in certain temperate environments may have resulted in their accumulation in orchard soils (Stevens *et al.*, 1970). The organochlorines are now being substituted by less persistent organophosphorus and carbamate insecticide. The literature review did not reveal any

significant extent of contamination with residues of such insecticides except for the reports of Stevens *et al.* (1970) ; Harris and Sans (1971) and Wiersima *et al.* (1972) where extensive use of phosphatic insecticides was involved.

In India, only limited surveys have been conducted of DDT residues in Panthnagar and Delhi soils by Tripathi (1966) and Dharamvir (1977) respectively and of BHC in Karnataka soils by Venkataramiah and Singh (1973). No published reports of the situation prevalent in other parts of the country are available. The present investigations attempt to provide results of a preliminary survey of Punjab and Chandigarh soils for the occurrence of residues of commonly-used insecticides.

MATERIAL AND METHODS

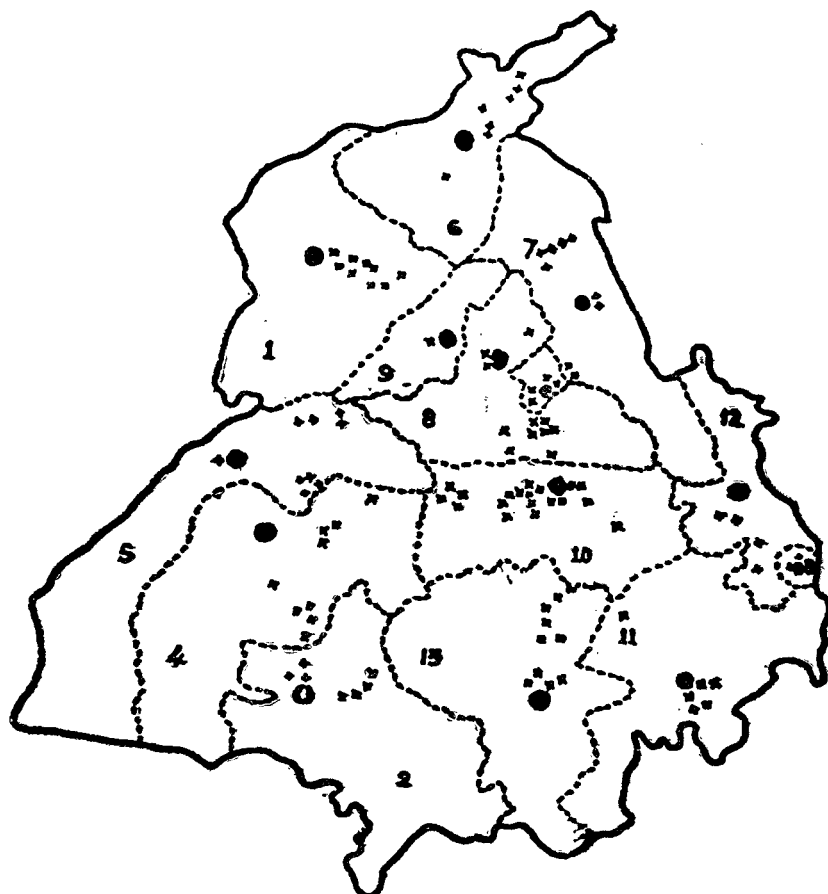
Representative surface soil samples were collected at random during October, 1976 to March, 1977 from 106 sites in all the districts of the Punjab and Union territory of Chandigarh taking into consideration the land use and soil properties (Figure 1). Each surface (0-10 cm) soil sample comprised of a minimum of 12 cores drawn from an acre unit with the help of a U-shaped tube of 2.8 cm internal diameter. These cores were pooled and collected in double walled polyethylene bags, transported to the laboratory on the same day stored in a deep freezer at -18°C until analysed.

The samples were analysed for residues of commonly used insecticides following the extraction and clean-up procedure of Drager (1969) after suitable modifications. A 50 g subsample of soil was extracted twice by dipping in a 100 ml methanol-water (2+1) solvent mixture for a day with occasional shaking. After filtration the extract was partitioned twice with 100 and 50 ml portion of n-hexane. The aqueous layer was also partitioned with dichloromethane before discarding. The combined organic fraction of n-hexane and dichloromethane was concentrated to about 5 ml and chromatographed on an activated column of alumina using n-hexane and n-hexane acetone (4+1) as the elutants. The two fractions were analysed separately by thin-layer (TLC) and gas-liquid chromatography (GLC).

Screening of organochlorine insecticides was carried out by TLC on Alumina-G impregnated with silver nitrate (Thompson *et al.*, 1970). The cleaned-up samples were co-chromatographed with standard compounds and visualization was accomplished by exposure to UV lamp without a filter.

The residues of organophosphorus and carbamate insecticides were screened by enzyme-inhibition-TLC on silica gel-G plates using goat liver homogenate as the enzyme source and 2-naphthyl acetate as the substrate and p-nitrobenzene diazonium fluoborate as the visualizing reagent (Kumar *et al.*, 1976).

The samples showing a positive response were estimated on a Packard gas liquid chromatograph (Model 7264) using a tritium source electron capture detector for chlorinated hydrocarbons and KCl coated platinum helix thermionic detector for organophosphorus compounds. These compounds were resolved on two glass



1 Amritsar 2 Bhatinda 3 Chandigarh (U.T) 4 Faridkot 6 Ferozepur
 6 Gurdaspur 7 Hoshiarpur 8 Jullundur 9 Kapurthla 10 Ludhiana
 11 Patiala 12 Roopnagar 13 Sangrur
 x Sample sites ● District Headquarters

Fig. 1 Location of the soil sample sites in different district of Punjab and Chandigarh

columns- i) 130 cm packed with 5 per cent OV 101 and, ii) 165 cm packed with 5 per cent OV210 on Gas chrom Q. Recoveries of the spiked samples were above 90 per cent for p,p'-DDT, dieldrin and methyl parathion and compared favourably with the

methods of Chiba and Morley (1968) and Woolson (1974), when checked for incurred residues in soils.

RESULTS AND DISCUSSION

Analyses of the 106 samples revealed 85 per cent of the soils were contaminated with insecticide residues (Table I). Residues of organochlorine insecticides were the most common ; occurring mainly as DDT-R consisting mainly of p,p'-DDT

TABLE I
*Nature and magnitude of insecticidal residues found in soils of
Punjab and Chandigarh*

Nature of insecticide residue	Frequency (No. of samples found contaminated)	Geometric mean (ppm)	Residues (Dry weight basis)	
			Arithmetic mean (ppm)	Maximum level (ppm)
p,p'-DDT	76	0.007	0.03	0.54
p,p'-DDE	69	0.006	0.03	0.54
p,p'-TDE	21	0.001	0.01	0.54
o,p'-DDT	1	—	—	—
Dicofol	2	—	—	0.50
DDT-R	89	0.017	0.07	1.63
BHC	1	—	—	0.50
Lindane	1	—	—	0.20
Endrin	1	—	—	0.10
Methyl parathion	2	—	—	—
Phorate sulfone	5	—	—	—
Unidentified	15	—	—	—

and its transformation products as p,p'-DDE and p,p'-TDE and a small quantity of o,p'-DDT and dicofol. The maximum residues of DDT-R found were 1.63 ppm with a geometric mean of 0.017 ppm. Residues of other organochlorine insecticides-BHC endrin and lindane were detected in a few samples. The predominant occurrence of residues of DDT-R is in contrast to its agricultural usage in the state which was only 1.5 tons out of the total pesticide use of 3800 tons during 1976-77. However if the quantity of DDT used for mosquito control by public health authorities is also included which amounted to 750 tons for 1976-77 and has been a regular feature under the National Malaria Eradication Programme since 1962, the widespread contamination of soil could be attributed to the dispersion and redistribution of this into the environment. Tripathi (1966) reported that 87% of the Panthagar soil samples were

contaminated and Dharamvir (1977) observed 97 per cent of Delhi soils to be contaminated with DDT. The maximum level encountered by these workers was 15 and 4.24 ppm respectively.

Residues of DDT have been widely distributed in the soils of Canada, U.K. and U.S.A. (Harris and Sans, 1971 ; Wheatley *et al.*, 1962 ; Wiersima *et al.* 1972). These residues have been directly associated with the agricultural use of DDT in these countries resulting in much higher build-up than that observed in India. The general lower level of DDT in soils of this country could be ascribed to the faster rate of degradation (Chawla and Chopra, 1967 ; Agnihotri *et al.*, 1977).

The low frequency of occurrence of residues of other organochlorine insecticides in the present investigations can be attributed to the small quantities used. This however cannot account for BHC the use of which is the maximum in India. Perhaps it may also be due to its high rate of degradation in soils as observed earlier by Chawla and Chopra (1967). The presence of cholinesterase inhibitors in 19 per cent of the soil samples indicate contamination with organophosphorus and carbamate insecticides. Since many of the metabolites of these insecticides are also inhibitors of this enzyme and occurred in small quantities, it has been rather difficult to estimate their nature. Only methyl parathion and phorate sulfone could be identified tentatively in 7 out of 21 samples. The occurrence of these residues, despite a faster rate of degradation, supports the changing emphasis from organochlorines to organophosphorus and carbamate insecticides which are often applied at shorter intervals during the growing season of the crop which may compensate for their lower persistence. It is rather difficult to ascertain whether the occurrence of residues of methyl parathion and phorate sulfone encountered in the present investigations were the result of recent applications or to aged residues because very few farmers had the necessary information regarding the use of insecticides and formulation, dose and frequency of application, etc.

The increased persistence of phorate sulfone as observed by Getzin and Shanks (1970) may also be an explanation for the present situation.

The present results, suggest that there is no indication of an accumulation of residues of insecticides in the soils of Punjab and Chandigarh as a result of their agricultural or public health usage. However their contribution to the secondary contamination of various agricultural commodities through the food chain remains an important problem.

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56 TRANSPORTATION OF ENDOSULFAN RESIDUES FROM ARABLE SOILS

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SUMMARY

Transportation of endosulfan-A, endosulfan-B and endosulfan sulfate from arable soils to adjoining low lying areas is reported. Endosulfan-A seems to be bound to the original soil to a lesser extent than endosulfan-B and hence is transported first. The possible hazards to aquatic organisms from such contamination are discussed.

INTRODUCTION

Endosulfan is a broad spectrum, non-systemic insecticide belonging to the cyclodiene series of organochlorine group. During the recent past it is being used widely in India, due to the ban on endrin. Technical endosulfan is a mixture of two isomers, endosulfan-A and endosulfan-B in the ratio 4 : 1 respectively (Lindquist and Dahm, 1957). Of the various metabolites reported in the literature viz., endosulfan alcohol, endosulfan ether, endosulfan hydroxyether, endosulfan lactone and endosulfan sulfate, the last one, like the technical material is toxic to mammals (Maier-Bode, 1968).

Endosulfan, when compared to other dienes like aldrin, dieldrin and endrin, is less toxic to mammals (for rats acute oral LD₅₀ is 100 mg/kg body weight and acute dermal LD₅₀ is 681 mg/kg body weight); but like endrin, it is highly toxic to fishes (LC₅₀ values range between 1 and 10 ppb for various species of fishes depending on their sensitivity (Gorbasch and Knauf, 1971), hence Maier-Bode (1968) stressed that endosulfan residues should not enter into aquatic systems where fishes live. However, several instances of aquatic pollution with endosulfan residues, either due to acci-

dental contamination (Sievers *et al.*, 1972) or due to agricultural practices (Gorbasch *et al.*, 1971 and Van Dyk and Greeff, 1977) were reported. It is also known that residues from agricultural lands enter ponds (Rao *et al.*, 1978), irrigation canals and channels through runoff or seepage (Edwards, 1973).

The work reported here is study of the transportation of endosulfan following heavy rains from arable soils to adjoining low-lying areas.

MATERIAL AND METHODS

Endosulfan (35 per cent E.C.) was sprayed on to a paddy field at a rate of 87.5 g active ingredient/acre. The soil was of clay loamy type. Soil from the paddy field, as well as from adjoining low lying areas were analysed for residues in samples collected at 5 day intervals after spraying down to a depth of 5 cm. 1 kg of sample soil was extracted with acetonitrile (Maier-Bode, 1968 and Lindquist and Bat, 1954) on a flask shaker for 2 hrs. The aqueous acetonitrile was filtered from the slurry under partial vacuum, and the slurry repeatedly washed with fresh quantities of solvent. All the acetonitrile fractions were combined and concentrated to about 100 ml. 700 ml of 2 per cent sodium sulfate solution was added to the acetonitrile and the residues were partitioned serially into hexane (USA HEW). The hexane extract was dried by passing through an anhydrous sodium sulfate column (4'') and cleaned up with activated carbon (Nuchar C-190N) and concentrated to 1 ml using first a Kuderna-Danish evaporator and then with a modified microsnyder column. Fortified wet soil samples gave an average recovery of 95 per cent (S.D. $\pm$ 2.915). The results reported are an average of replicates corrected for percentage recovery and reagent blank with untreated soil samples.

Thin layer chromatography : Silver nitrate impregnated silica gel (Silica gel was prewashed) plates (20 x 20 cm, 250 μ thick), were spotted with the extract, technical endosulfan and products of degradation prepared according to the suggested methods (Lindquist, Dahm, 1957 ; Barnes and Warer, 1965). The plates were developed in 4 per cent acetone in n-heptane and the spots made visible by exposing the plates to UV light. The sensitivity of this method was 0.5 μ g.

Colorimetric analysis : The method of Maitlen *et al.* (1973) was followed. They observed that captan, chlordane, heptachlor and ovex caused some interference in the method, but 45 other pesticides did not interfere. The method is applicable in the range of 5-100 μ g. None of the interfering pesticides was ever used in the fields under study. The values herein reported were corrected for values of untreated sample as well as the recovery values obtained after fortification of the untreated sample.

RESULTS AND DISCUSSION

Although endosulfan residues were absent initially in the adjoining low lying area, they appeared a few days after heavy rains. Table I shows the results of the investigation.

TABLE I
Endosulfan residues in low-lying areas adjacent to a paddy field

Days after spraying	Components* present	Quantity of both isomers (ppm)
0	—	—
5	—	—
10	endosulfan A only	0.01
15	endosulfan A, endosulfan B and endosulfan sulfate	0.04
20	endosulfan B and endosulfan sulfate	0.016

* On the basis of thin layer chromatograms.

Absence of both isomers in the adjoining low lying area on the treatment day as well as the 5th day after spraying indicates clearly that there was no detectable amount of aerial transportation of the pesticide during spraying. Between 5 and 15 days after spraying there were heavy rains followed by a considerable seepage of water from the paddy field into the adjoining low lying area. Soil samples collected on the 10th day from the latter showed endosulfan-A only whereas endosulfan-B and endosulfan sulfate appeared for the first time in the 15th day sample. By the 20th day, endosulfan-A had disappeared and only traces of endosulfan-B and endosulfan sulfate remained. Thus, transportation of endosulfan-A and endosulfan-B and the principal metabolite, *i.e.*, endosulfan sulfate from arable soils to adjoining low lying area was clearly observed which is contrary to the earlier report of Maier-Bode (1968). Endosulfan-A has been reported to be less persistent than endosulfan-B as the latter is more strongly adsorbed to soil; consequently a lower rate of leaching and volatilization of endosulfan-B than endosulfan-A has been suggested Byers *et al.* (1965). Our work confirms this observation as endosulfan-A is transported first; with further run off, endosulfan-B also is transported.

After the 20th day, there was a heavy downpour. Further analyses did not show any residues in the soil samples of the low lying area. The total quantity of endosulfan that had been transported is rather on the high side of the median tolerance limits reported for fish. In nature, there is every possibility that fish may be affected because of transportation of endosulfan through run off.

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57 PERSISTENCE OF PHORATE IN SOIL AND ITS UPTAKE AND METABOLISM IN MUSTARD CROPS

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SUMMARY

The persistence of phorate in soil and its uptake and metabolism in *Brassica campestris* var. *sarson* was studied in pot-culture experiment, following its application to the soil at the rate of 1 and 4 kg a.i./ha at two stages of crop growth i.e., 22 days and 72 days after sowing. Analyses for phorate and its metabolites were done by gas-liquid chromatography and TLC-FI technique. Phorate residues in soil were found mainly in the form of its sulfoxide and sulfone. The parent compound was not detected more than 15 days after its application to the soil. Half of the phorate residues disappeared from the soil in about 7 days. In the plants, traces of phoratoxon sulfoxide and phoratoxon sulfone were found in addition to phorate sulfoxide and phorate sulfone. The proportion of phorate sulfoxide decreased with time while that of phorate sulfone increased. Pot-culture experiments did not represent quantitatively the field conditions, although the nature of the metabolites in *Brassica* plants under these two conditions were identical.

INTRODUCTION

The application of granules of phorate to the soil at the rate of 1 kg a.i./ha has been recommended for the effective control of mustard aphid, the major insect pest of mustard crop (Anon., 1974). The need to assess the residue hazards emanating from the official recommendation on the use of pesticides hardly needs any emphasis. Although, the metabolism of phorate in soil and its translocation and further metabolism in plants has been well-documented (Menn, 1972; Edwards, 1975), there is as yet no information available on its metabolism in the mustard crop. This paper describes pot-culture experiments undertaken to determine the persistence of phorate in soil and its uptake and metabolism by a mustard crop following broadcast application of the recommended dosage of 1 kg a.i./ha and four times the recommended dosage.

MATERIAL AND METHODS

Application of Insecticides: The Sarson, *Brassica campestris* var. *Sarson* (Yellow Sarson Punjab 24) were grown in plastic buckets (30 cm x 22.5 cm), in sandy soil with a low-organic matter content (sand 92 per cent, salt 6 per cent, clay 2 per cent, pH 8.75, organic matter 0.54). Phorate (Thimet 10 G, Cyanamid India Ltd) was applied at 1.0 kg and 4.0 kg a.i./ha at two stages of the crop growth (22 days and 72 days after sowing) in separate pots on area basis. The number of plants

per pot differed at different growth stages, depending on the capacity of soil to support plant growth. The number of plants were 45-55 in early stages and 11-20 in later stages. The pots were placed in a glass house without temperature control and watered as necessary.

Sampling: Plant and soil samples were collected at intervals. The plant material was chopped and after thorough mixing a sub-sample of 50 g was taken for analysis. Soil was sampled with a shovel down to a depth of 15 cm at two points in each bucket, mixed and sub sampled to a convenient size before analysis.

Extraction and clean up: The plant samples were extracted and cleaned up essentially by the method described by Lichtenstein *et al.* (1973). However, the soil samples were extracted by the method described by Lichtenstein *et al.* (1973) with some modifications. Fifty grams of wet soil in a stoppered conical flask was shaken for half an hour in a horizontal shaker, twice with 100 ml of methanol-acetone (1:1, v/v) and then with 100 ml of benzene-methanol-acetone (1:1:1, by volume). The combined extract after drying over anhydrous sodium sulfate was concentrated to about 10 ml under *vacuum* at 45°C. If necessary, further concentration was done under gentle stream of nitrogen. The concentrated extract thus obtained was used for analysis by GLC. For TLC analysis, the concentrated extract was cleaned up by diluting it with aqueous sodium sulfate solution followed by extraction with benzene. The benzene extract was concentrated to suitable volume.

Gas-liquid chromatography: A Packard gas chromatograph (Model 7624) equipped with a hydrogen flame-ionization detector modified as a thermionic detector (kCl-coated) was used. Other gas-chromatographic parameters were:

- Columns: a) Glass, 200 cm x 3 mm i.d., packed with 5 per cent by weight DC-200 on 80-100 mesh Gas chrom Q.
- b) Glass, 105 cm x 3 mm i.d., packed with 2 per cent by weight DEGS on 80-100 mesh Gas chrom Q.
- Temperatures: Injection port 210°C, detector oven 190°C, and column oven 200°C for column (a); Injection port 210°C, detector oven 195°C and column oven 195°C for column (b).
- Gas flows: Nitrogen (carrier) 70 ml/min, hydrogen 40 ml/min, air 250 ml/min.

The columns were conditioned before use by repeated injections of the reference materials until a consistent response was obtained. Quantitative estimates were made by comparing the peak heights of the unknown with those of standards injected under parallel conditions.

Thin-layer chromatography: The nature of metabolites in the plant/soil was confirmed by TLC-EI method (Mendoza, 1972).

Accuracy and sensitivity of the analytical techniques: The retention time and sensitivity of phorate and its metabolites on the two column used are given in Table I. The column packed with 5 per cent DC-200 on Gas Chrom Q separated phorate from phoratoxon and other metabolites. However, the separation of phorate sulfoxide from phorate sulfone and phoratoxon sulfoxide from phoratoxon sulfone could not be obtained on this column. On the column packed with 2 per cent DEGS on Gas Chrom Q, the distinct separation of phorate sulfoxide from phorate sulfone was obtained, but phoratoxon sulfoxide did not give any response. Phorate and phoratoxon eluted almost along with the solvent peak on column (b). Each sample was, therefore, analysed on both the columns for the quantitative estimation of phorate and its metabolites. Suett (1971, 1974) and Maskell *et al.* (1974) also made use of dual columns for the individual estimation of phorate and its metabolites. The GLC-method was found to be quite sensitive for the estimation of phorate, phoratoxon, phorate sulfoxide and phorate sulfone as these compounds at the level of 1-5ng gave half-scale deflection. However, the oxygen analogues of phorate sulfoxide and phorate sulfone had poor sensitivity.

The cleanedup extracts of 50 g plant/soil samples could be concentrated to 5 ml, out of which 3 μ l when injected did not give any interference. Based on twice the noise level, the limit of estimation for phoratoxon sulfoxide and phoratoxon sulfone were found to be 0.2 - 0.3 ppm as compared to 0.005 - 0.02 ppm for other compounds (Table I).

TABLE I

Retention time, sensitivity and limits of determination of phorate and its metabolites

Compound	Retention time (min)		Sensitivity (ng)		Limit of determination (ppm)	
	DC-200 column	DEGS column	DC-200 column	DEGS column	DC-200 column	DEGS column
Phorate	2.17	—	1.0	—	0.005	—
Phorate sulfoxide	5.66	4.82	4.0	5.0	0.02	0.02
Phorate sulfone	5.66	7.17	4.0	5.0	0.02	0.02
Phoratoxon	1.47	—	1.0	—	0.005	—
Phoratoxon	3.96	Not	60.0	Not	0.3	—
sulfoxide		resolved		resolved		
Phoratoxon sulfone	3.96	8.00	30.0	100.0	0.2	0.4

— Not done.

The efficacy of the analytical method used was checked by a recovery experiment. Samples of soil and plants as obtained from untreated plots were fortified with known amounts of phorate and its metabolites immediately before extraction. The mean recovery of phorate, phorate sulfoxide and phorate sulfone was 88.5, 85.7 and 77.0 per cent respectively. The results in the experimental samples have been expressed as such and not corrected for recovery.

RESULTS AND DISCUSSION

Residues in soil: Phorate disappeared almost completely from soil within 15 days of application even when applied at the rate of 4 kg a.i./ha (Table II). Phorate sulfoxide and phorate sulfone were the only metabolites detected in the soil. Pho-

TABLE II
Residues of phorate and its metabolites in soil

Time of application	Days after application	1.0 kg a.i./ha				4.0 kg a.i./ha			
		Percentage of total residue				Percentage of total residue			
		Phorate	Phorate & sulfoxide	Phorate sulfone	% residue remaining	Phorate	Phorate sulfoxide	Phorate sulfone	% of residue remaining
22 days	30	BDL	42	58	20.0	BDL	58	42	12.5
after sowing	50	BDL	Traces	Traces	1.8	BDL	20	80	4.6
72 days	0*	85	15	BDL	100.0	87	13	BDL	100
after sowing	3	9	73	18	80.0	10	82	8	87.3
	7	6	56	38	56.4	8	72	20	46.8
	15	BDL	33	67	30.9	1	33	66	20.0
	30	BDL	25	75	12.7	BDL	11	89	7.3
	50	BDL	33	67	10.0	BDL	1	99	4.1

0* 2 hrs after application.

Temperature during the 15 days following application

Time of application	Max. Temperature °C		Min Temperature °C	
	Range	Mean	Range	Mean
22 days after sowing	21.2-23.5	22.9	0.1-7.0	4.0
72 days after sowing	15.0-21.5	18.7	3.8-12.0	7.2

ratoxon, phoratoxon sulfoxide and phoratoxon sulfone were not detected in any soil samples. The nature of the metabolites found in the present investigations was the same as observed by Getzin and Shanks Jr. (1970), Suett (1971) and Lichtenstein *et al.* (1973). Phorate sulfoxide constituted about 14 and 78 per cent of the total residues at 1 and 3 days after treatment. With the lapse of time, the proportion of phorate sulfoxide decreased with a corresponding increase in phorate sulfone. Lichtenstein *et al.* (1973) also reported the rapid formation of oxidative metabolites of phorate in the soil.

To facilitate the direct comparison of the loss of the insecticide from soil, the results are shown as the percentage of the applied amount of the parent compound remaining on each sampling occasion. The initial application of 1 kg a.i./ha and 4 kg a.i./ha incorporated to the depth of 150 mm would have given concentrations of approximately 0.55 and 2.20 ppm respectively. By 2 hrs after application, the actual residues were 1.21 and 2.33 ppm respectively. The variations from the aimed dosage may be due to inadequacy of the sampling method used. The results indicated a rapid decline of phorate residues in soil and negligible amounts of cumulative phorate residues were detected at the end of 50 days (Table II). Half of the applied amount had disappeared after about a week. The degradation of phorate under actual field conditions was still fast (Krishnaiah, 1978), Singh and Gulati (1973) reported 50 per cent of phorate degradation in soil in about 15 days. On the other hand, Suett (1974) reported that approximately 35 per cent of the applied phorate still persisted as phorate sulfone after 35 weeks under field conditions in UK after incorporation in sandy loam soil. According to Edwards (1975), the persistence of an insecticide in soil depends upon various physical forces such as, temperature, relative humidity, the type of the soil and biological forces, such as soil microflora and plant species grown in the soil. The influence of these factors is modified further by the method and dosage of application. The rapid degradation as observed in the present study might be due to the surface application of phorate granules to sandy soil with low organic matter content and the prevailing high temperature.

Residues in Brassica: Phorate was detected in the plants only up to 15 days after application and its level did not exceed 3 per cent of the total residues. Most of the residues existed in the form of phorate sulfoxide and phorate sulfone (Table III). In addition to these metabolites, phoratoxon sulfoxide and phoratoxon sulfone were also detected. However, the levels of these metabolites in most samples were negligible. The maximum of 0.26 ppm of combined level of phoratoxon sulfoxide and phoratoxon sulfone was detected 15 days after application of phorate at the rate of 4 kg a.i./ha. None of the samples showed the presence of phoratoxon, Bowman and Casida (1957) and Lichtenstein *et al.* (1974) also reported the presence of phorate, phorate sulfoxide, phorate sulfone, phoratoxon sulfoxide and phoratoxon

sulfone in beans and corn. In contrast, Menzer and Ditman (1968) observed that Spinach foliage contained most phorate residues as phorate sulfone and phoratoxon sulfone. According to Suett (1974), carrots grown in phorate-treated soil contained the parent compounds, its sulfoxide and sulfone only.

The cumulative residues of phorate and its metabolites were 5.23 ppm 15 days after application of the insecticide at 1 kg a.i./ha 22 days after sowing. The same level was maintained up to 30 days and was followed by decline to a level of 0.95 ppm at 50 days. At the rate of 4 kg a.i./ha, however, the residues in the plants steadily increased up to 30 days and the same level was maintained up to 50 days. The level of the soil-applied systemic insecticide in plant depends upon its level in the soil, its rate of uptake, its rate of degradation in/on plant system, etc. It is, therefore, quite likely that the losses due to enzymatic/chemical degradation, dilution through plant growth, etc. had been amply compensated by the considerable rate of absorption from the soil at the high application rate thus enabling the plants to maintain a steady level for a relatively long period. The results, further, indicated that the proportion of phorate sulfone at 1 kg a.i./ha was more than the corresponding value at 4 kg a.i./ha, thereby suggesting that the metabolism at low rates of

TABLE III
Residues of phorate and its metabolites in Brassica plants

Time of applica- tion	Days after applica- tion	1.0 kg a.i/ha			Total residue (ppm)	4.0 kg a.i/ha			Total residue (ppm)
		Percentage of total residue				Percentage of total residue			
		Phorate	Phorate sulfoxide	Phorate sulfone		Phorate	Phorate sulfoxide	Phorate sulfone	
22 days after sowing	15	1	40	59	5.23	1	54	45	8.92
	30	BDL	19	81	4.58	BDL	39	61	23.18
	50	BDL	3	97	0.95	BDL	14	86	21.40
72 days after sowing	3	3	54	43	2.06	1	59	40	3.32
	7	1	36	63	1.76	1	53	46	9.39
	15	1	9	90	0.46	1	15	84	4.32
	30	BDL	1	99	1.42	BDL	4	96	2.18

application was fast. The enhanced degradation of phorate at low rates of application has been reported already in alfalfa (Dohson *et al.*, 1960) and carrot (Maskell

et al., 1974). The low level of phorate residues found in the old crop appears to be due to the dilution effect through growth (Table III).

The amounts of phorate residues from plant growth in pots were different from those obtained under field conditions (Krishnaiah, 1978). However, the pattern of uptake and the nature of metabolites was identical. The present study, therefore, indicated that the pot-culture experiment does not represent the field conditions quantitatively.

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58 PERSISTENCE OF DIMETHOATE AND FENITROTHION IN SOME SOILS OF ORISSA

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SUMMARY

The persistence of dimethoate and fenitrothion in three cultivated soils of Orissa was studied under sterile and non-sterile soil conditions at the laboratories of the Department of Soils and Agricultural Chemistry, OUAT, Bhubaneswar. Soils were treated with 5 ppm insecticide and the residues in the chloroform extract were estimated by the total phosphorus spectrophotometric method. Under non-sterile soil conditions dimethoate and fenitrothion persisted for 45 days, whereas under sterile soil conditions fenitrothion persisted for 60 days and dimethoate for 75 days. Chemical degradation was greater than biological degradation in all three soils. The rate of degradation for both chemicals followed the first order reaction with 'half-lives' ranging from 9 to 13 days under non-sterile and 12.5 to 17.5 days under sterile soil conditions.

Dimethoate (O, O-dimethyl S-(methyl carbomoyl methyl) dithiophosphate) and fenitrothion (O, O-dimethyl-O-(3-methyl-4-nitrophate) are used extensively for insect control. Novozhilov and Razova (1974) detected dimethoate for 40-60 days after application in upper 30 cm soil and the degradation in soil increased with increases in moisture content from 5 to 20 per cent. Yule and Duffy (1972) found that concentration of fenitrothion in the top 15 cm of forest soil decreased from about 0.04 ppm

in samples taken immediately after spraying to an undetectable level between 32 and 64 days later. Another worker (Anon., 1973) reported that, in flasks containing silty loam soils flooded with water, fenitrothion had a half life of 3 to 6 days at 23°C. Since no published literature pertaining to the persistence of these pesticides in Orissa soils or in the tropics is available, the present investigation was undertaken to study their persistence in three cultivated soils of Orissa.

MATERIAL AND METHODS

Three cultivated soils having different physico-chemical properties (Table I) were collected from different parts of Orissa, dried and ground to pass through a 2 mm sieve. Duplicate soil samples of 20 g were placed in separate 100 ml conical flasks and moistened to field capacity. One set of soil samples was sterilised by autoclaving twice at 15 lb pressure for 30 minutes. A 5 ml aqueous solution of each insecticide of 20 ppm concentration was added to each flask and incubated at $30 \pm 1^\circ\text{C}$ in a BOD incubator. The samples were taken for analysis at 0, 5, 10, 15, 20, 30, 45, 60 and 75 days. The moisture loss in the incubated samples was compensated by adding sterile water every 7th day.

TABLE I

Chemical and physical properties of soils used for persistence study

Soil	pH	% organic carbon	Field capacity in %	CEC me/100 g	% sand	% Silt	% Clay
Bhubaneswar	5.2	1.08	11.5	4.46	76.24	8.0	15.76
Nayagara	7.4	0.90	13.87	2.74	62.24	10.0	27.76
Nimapara	6.3	0.84	21.17	11.19	78.24	12.0	9.76

The pesticides were extracted from the samples by a tumbling method with chloroform and the residues in the extract were determined by the modified total phosphorus method of Manuel (1968). Recoveries of dimethoate and fenitrothion from fortified samples ranged from 80 to 89 per cent.

RESULTS AND DISCUSSION

Persistence of dimethoate: Residues that were initially 4.5 to 4.7 ppm persisted for 45 days under non-sterile soil conditions and for 75 days under sterile soil conditions (Table II). For both soil conditions the loss of the toxicant was higher in Nayagar soil than in Nimapara and Bhubaneswar soil. This might be due to the higher pH (7.4) of Nayagar soil compared to the other two soils. Chemical degra-

TABLE II
Persistence of dimethoate in soil

Days	ppm under non sterilized condition			ppm under sterile condition		
	Bhubane- swar soil	Nayagara soil	Nimapara soil	Bhubane- swar soil	Nayagara soil	Nimapara soil
0	4.50	4.60	4.70	4.5	4.60	4.70
5	2.47	2.31	2.53	3.44	3.40	3.52
10	1.75	1.61	1.79	2.43	2.30	2.53
15	1.47	1.33	1.43	2.04	1.97	2.09
20	0.76	0.69	0.77	1.57	1.56	1.59
30	0.45	0.36	0.43	1.08	0.96	1.05
45	N.D.	N.D.	N.D.	0.67	0.55	0.65
60	N.D.	N.D.	N.D.	0.36	0.32	0.32
75	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

ation was the major pathway of degradation. Biological degradation was more pronounced from the 20th to the 45th day of incubation. The rate of degradation, under both non-sterile and sterile soil conditions, seemed to have followed a first order reaction. Under non-sterile conditions the 'half-life' of dimethoate varied from 9.1 days in Nayagar soil to 10.6 days in Bhubaneswar soil, whereas under sterile conditions it was from 16.2 days in Nimapara soil to 17.5 days in Bhubaneswar soil.

Persistence of fenitrothion: Residues that were initially 4.4 to 4.6 ppm persisted for 45 days under non-sterile conditions in all three soils (Table III)

TABLE III
Persistence of fenitrothion in Soil

Days	ppm under non sterile condition			ppm under sterile condition		
	Bhubane- swar soil	Nayagara soil	Nimapara soil	Bhubane- swar soil	Nayagara soil	Nimapara soil
0	4.40	4.50	4.60	4.40	4.50	4.60
5	2.42	2.25	2.30	3.30	3.15	3.31
10	1.84	1.71	1.84	2.86	2.65	2.92
15	1.40	1.21	1.38	2.42	2.36	2.48
20	0.72	0.63	0.69	1.49	1.35	1.44
30	0.52	0.27	0.46	0.96	0.81	0.92
45	N.D.	N.D.	N.D.	0.55	0.45	0.55
60	N.D.	N.D.	N.D.	0.17	N.D.	N.D.
75	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

studied. Under sterile conditions, residues persisted for 60 days in Nayagar and Nimpara soil and for 75 days in Bhubaneswar soil, probably due to its low pH (5.2) because fenitrothion is more susceptible to alkaline hydrolysis. Both chemical and biological modes of degradation were responsible for detoxifying this chemical. The rates of degradation under sterile and non-sterile soil conditions both followed 1st order reactions. The 'half-life' of fenitrothion ranged from 9 to 13 days under non-sterile soil conditions and from 12.5 to 15.5 days under sterile soil conditions.

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59 PERSISTENCE OF MEPHOSFOLAN IN RICE SOILS OF TAMIL NADU

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SUMMARY

Mephosfolan persisted more in clay soil containing a higher organic matter than in sandy soil. Moisture played an important role in its persistence which was more in soil at field capacity than under flooding. The 'half-lives' under field capacity were 1.48, 1.43 and 1.33 weeks for Aduthurai, Lower Bhavani and Coimbatore soils whereas the corresponding values under flooding were 1.04, 1.02 and 1.03 weeks respectively.

INTRODUCTION

Application of granules to the water surface is one of the current developments in rice pest control. The biological efficacy of a soil-applied insecticide is dependent on the nature of the insecticide and the physico-chemical properties of the soils whether submerged or not. The degree of sorption and desorption of the

pesticide on the soil colloids and the resultant persistence of the pesticide is governed by the above factors.

MATERIAL AND METHODS

The persistence of mephosfolan (Cytrolane 5 G) was studied in three types of soil representing three major rice-growing tracts of Tamil Nadu viz., Thanjavur, Coimbatore and Lower Bhavani. The physico-chemical properties of the soils were studied (Table I). The method of Mythyantha and Perur (1974) was used to study the persistence of mephosfolan. Twenty g of each soil was transferred to a series of

TABLE I
Analysis of the Soils

Properties	Aduthurai	Lower Bhavani	Coimbatore
<i>Mechanical analysis</i>			
(per cent moisture free basis)			
Clay	38.80	10.0	45.0
Silt	25.00	5.0	10.0
Fine sand	13.9	33.0	21.0
Coarse sand	18.9	46.1	22.3
<i>Moisture content (per cent)</i>			
Maximum water holding capacity	45.25	23.57	62.05
<i>Chemical analysis (per cent)</i>			
Organic matter	1.45	1.18	1.10
<i>Other properties</i>			
pH	7.2	7.2	7.1
Electrical conductivity (Millimhos/cm)	0.20	<0.20	0.20
Total cation exchange capacity (m.e./100 gm)	48.5	17.0	33.0

100 ml capacity test tubes and the soil treated with 200 μ g of mephosfolan (100 ppm). After mixing the insecticide with the soil, the tubes were separated into two batches and the soils in the first batch were maintained at field capacity (1/3 bar tension) and the other at flooded moisture level (3 cm standing water level). The tubes were then covered tightly with polythene to prevent loss of water by evaporation and kept in a incubation chamber, where the temperature fluctuation was low ($25 \pm 2^\circ\text{C}$). Each treatment was replicated three times. Periodical analysis of soil samples was done at weekly intervals up to a period of 10 weeks. Each set of

samples was extracted separately with benzene and the mephosfolan content estimated. 'Half-life' values (RL-50) of mephosfolan were calculated by the method of Hoskins (1961).

Estimation of Mephosfolan residues in soils

In the absence of a specific method for estimating mephosfolan residues in the soils, different methods were tried and the following method gave about 80 per cent recovery (Table II). To 100 g of soil sample taken in an Erlenmeyer flask, 75 ml of isopropyl alcohol and 200 ml of benzene were added and shaken thoroughly for

TABLE II
Recoveries and background values for mephosfolan from soils

ppm fortified	ppm recovered	per cent recovery
0.0	0.00	0.00
1.0	0.812	81.20
2.0	1.553	77.65
3.0	2.482	82.75
5.0	4.091	81.82
Mean	—	80.85

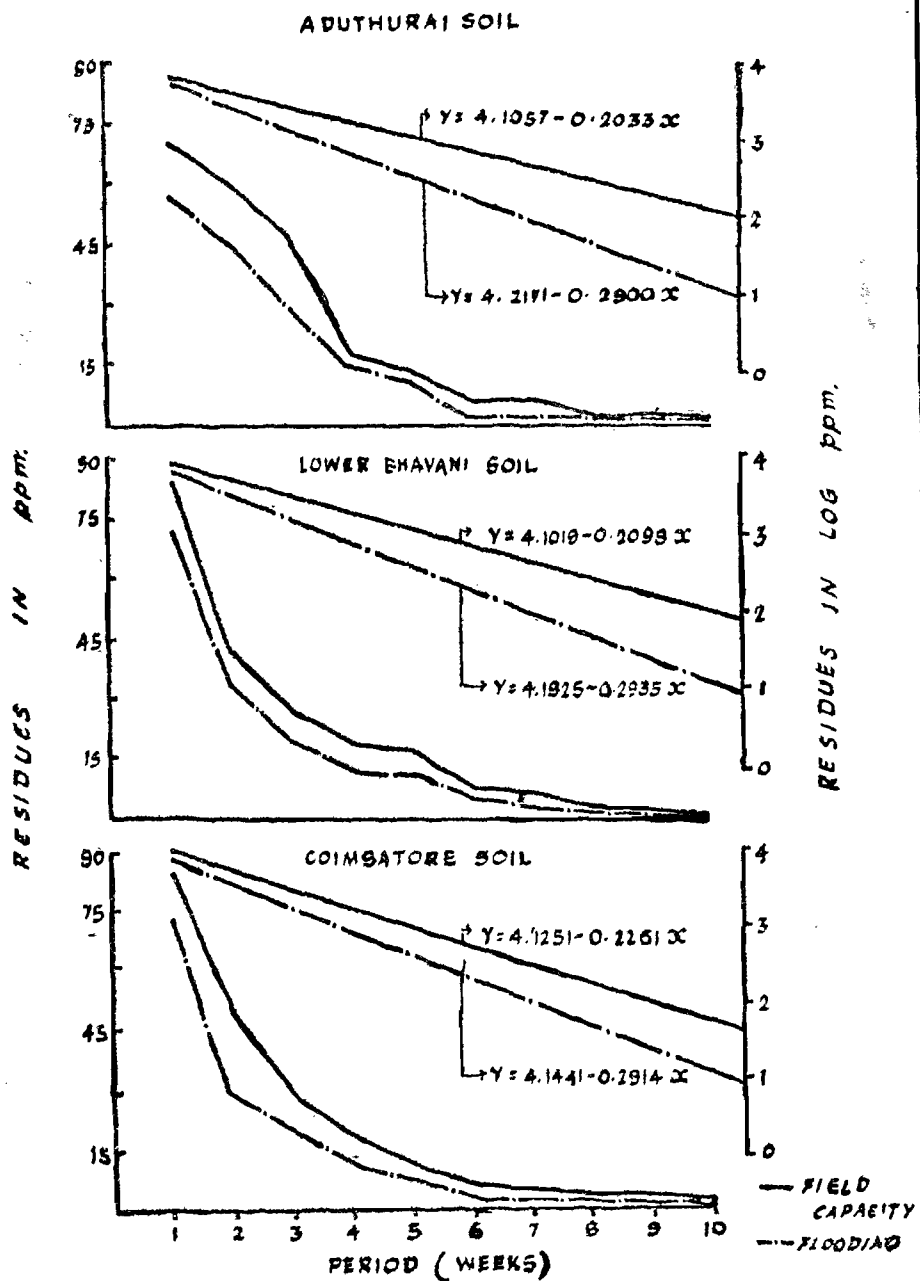
thirty minutes. The contents were filtered through a Buchner funnel. 50 ml of benzene was added to the flask and filtered. The filtrate was passed through a column containing celite and charcoal at 1 : 1. The benzene layer was separated in a separating funnel. The volume of the filtrate was measured and a known aliquot was taken and evaporated to dryness in a Kuderna-Danish evaporative concentrator. The residue was dissolved in 0.5 ml acetone, then 200 ml of distilled water was added and transferred to a 250 ml separating funnel. It was extracted with two 25 ml portions of dichloromethane. The extract was dried over anhydrous sodium sulphate and used for mephosfolan estimation calorimetrically.

RESULTS AND DISCUSSION

The data on the persistence of mephosfolan in three soil types under different moisture conditions are given in Table III and Fig. 1. A significant difference in persistence between field capacity and flooded moisture conditions existed. The differences between the different soil types with respect to mephosfolan residue were significant.

The 'half-life' values were 1.33, 1.43 and 1.48 weeks for Coimbatore, Lower Bhavani and Aduthurai soils respectively. It is evident that soil properties contributed

FIGURE-4
PERSISTENCE OF CYTOLANE IN DIFFERENT SOIL TYPES AT
DIFFERENT MOISTURE LEVELS



to such differences in persistence. Persistence was more in Aduthurai soil followed by Lower Bhavani and Coimbatore soils. The possible reason for the greater persistence in the Aduthurai soil might be due to higher organic content of 1.45 per cent compared to 1.18 and 1.10 per cent in the latter two soils respectively. Although the Coimbatore soil contained 45.0 per cent of clay, the persistence of mephosfolan did not equal that in the Aduthurai soil which was only 38.8 per cent. Hence, the presence of organic matter alone could have attributed for such a higher persistence in Aduthurai and Lower Bhavani soils. Harris and Mazurek (1964) attributed the

TABLE III
Persistence of mephosfolan at different moisture levels
(Residues expressed in ppm)

Periods (Weeks)	Moisture levels		Mean
	Field capacity	Flooding	
1	79.59	67.03	73.31
2	51.38	36.81	44.10
3	34.80	22.86	28.83
4	18.05	12.70	15.38
5	13.31	9.65	11.48
6	6.83	2.70	4.76
7	4.89	1.86	3.38
8	2.43	0.99	1.71
9	1.67	0.32	0.99
10	0.82	0.09	0.46
Mean	21.38	15.50	—

Comparison of significant effects

	Level of significance	C.D. (P=0.05)
1. Between moisture levels	0.01	0.27
2. Between periods	0.01	0.604
3. Interaction between periods and moisture levels	0.01	0.855

greater persistence of phorate and disulfoton in soils to high organic matter by the adsorption of these chemicals on the soil organic matter. Similar results were also reported by several workers (Abdel Latif *et al.*, 1967 and Harris, 1970 ; 1972).

The next important factor which influenced the degradation and persistence of the mephosfolan was the moisture content of the soils. The present study revealed that, with time, there was an exponential decrease in mephosfolan residues and

the persistence was significantly higher in soils at field capacity (1/3 bar tension) than those flooded. Irrespective of the soil types, this factor acted independently and reduced the 'half-life' values from 1.48 to 1.04 weeks in Aduthurai soil, 1.43 to 1.02 weeks in Lower Bhavani soil and 1.33 to 1.03 weeks in Coimbatore soil. Such a phenomenon has been reported by other researchers in recent years (Mythyantha and Perur, 1974 ; Gowda and Sethunathan, 1976 ; Kandaswamy *et al.*, 1975 and Rajukkannu *et al.*, 1977).

TABLE IV
Persistence of mephosfolan in different soil types (Residues expressed in ppm)

Periods (in weeks)	Aduthurai			Lower Bhavani			Coimbatore		
	Moisture levels			Moisture levels			Moisture levels		
	Field capacity	Flooding	Mean	Field capacity	Flooding	Mean	Field capacity	Flooding	Mean
1	70.73	57.30	64.02	84.02	72.03	78.02	84.02	71.77	77.89
2	59.83	44.41	52.12	44.47	36.04	40.26	49.85	29.99	39.92
3	47.10	30.27	38.69	28.43	18.93	23.68	28.86	19.38	24.12
4	17.33	14.62	15.98	19.23	11.96	15.60	17.60	11.53	14.56
5	13.42	10.66	12.04	16.69	10.79	13.74	9.83	7.49	8.66
6	6.92	3.13	5.03	7.47	2.57	5.02	6.09	2.39	4.24
7	5.72	2.20	3.96	4.52	1.83	3.17	4.42	1.56	2.99
8	2.40	1.01	1.70	2.53	1.01	1.77	2.35	0.96	1.66
9	1.70	0.36	1.03	1.68	0.27	0.98	1.61	0.31	0.96
10	1.28	0.09	0.69	0.78	0.09	0.44	0.41	0.09	0.25
Mean	22.64	16.41	19.53	20.98	15.55	18.27	20.50	14.55	17.53
Half life value in weeks	1.48	1.04	—	1.43	1.02	—	1.33	1.03	—

Comparison of significant effects

	Levels of significance	C.D. (P=0.05)
1. Between soils	0.01	0.331
2. Soils × moisture levels	0.01	0.468
3. Periods × soils	0.01	1.047

It is reasonable to conclude that the decrease in mephosfolan concentration with time may be due to its increased hydrolysis at higher moisture levels leading to degradation to simple non-toxic compounds. Furthermore, soil microorganisms help the degradation of mephosfolan. This has been confirmed by other research workers (Kandaswamy *et al.*, 1975 ; and Sidaramappa and Sethunathan, 1975). The

microorganisms utilise the pesticide as a source of carbon, nitrogen and phosphorus. Naturally, an increase in populations of microbes would lead to decreased persistence of the insecticide. Faster degradation and dissipation of mephosfolan in flooded soils might be due to increased hydrolysis and utilization of mephosfolan by more efficient anaerobes that were present under flooded conditions.

The Lower Bhavani soil containing only 10 per cent of clay retained the mephosfolan from degradation and its persistence was higher than in the Coimbatore soil which contained 45 per cent of clay. Both the soils had equal quantities of organic matter so the persistence might be [due to silt and sand fractions. This agrees with Getzin and Rosefield (1966) who observed that thionazin persisted longer in coarse-textured soil than in fine-textured soils. Harris (1966) also suggested that insecticides such as diazinon and parathion were deactivated to a greater extent by sand silt fractions.

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60 THE VERTICAL DISTRIBUTION OF CARBARYL IN SOIL

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SUMMARY

The vertical distribution of carbaryl (1 naphthyl, n-methyl-carbamate), in soil was studied. It was noted that the pesticide persisted for 40 days after single spraying on brinjal and that it moved to a depth of eighteen cm after 20 days. It would be sufficient for analytical purposes if samples were collected to a depth of twenty cm.

Carbaryl (1-naphthyl n-methyl carbamate) is a broad-spectrum insecticide used to control insect pests on a variety of crops. Analyses of the residues of carbaryl in soil have been reported by Johnson and Stansburry (1965) ; Kazano *et al.* (1972) ; Deshmuk and Lal (1969) ; Dhal and Lal (1971) ; Kuhr *et al.* (1974); Lafleur (1976) ; and Mulla (1960). No other information is available on the vertical distribution of the pesticide in the soil which is essential for obtaining a representative sample of the soil before any meaningful analysis of the residue can be attempted (Chiba, 1969).

MATERIAL AND METHODS

A plot of 0.35 ha was chosen on which a brinjal crop (*Solanum melongena*) was being grown by local farmers. The field was hand-sprayed with 0.5 kg of 50 per cent wettable carbaryl in 70 l of water.

The soil samples were collected at 10 days intervals from three randomly selected points. At each sampling point the soil was collected down to a depth of eight inches in one inch strata. The preliminary analysis of the soil showed that it was free of carbaryl residues. The soil was of clay-loam type. Soil samples were air dried at room temperature and sieved before extraction.

The residues were extracted in a wrist action flask shaker for two hrs with acetonitrile. The substance to solvent ratio was 1 : 2. The soil extract was treated with 50 ml of coagulating solution (Benson and Finocchiaro, 1965) and was filtered through 1'' column of celite 545. The column was eluted with another 50 ml of coagulating solution and the pesticide was partitioned into methylene chloride. The extract was treated with C-190N Nuchar (Westavaco, Virginia, USA) and passed through a column containing a two-inch layer of silica-gel (60-120 mesh) and four inch layer of sodium sulphate. The column was eluted with 10 ml volumes of methylene chloride. Any interference from 1-Naphthol was eliminated by treatment with alkali (Meghalona, 1975). The cleaned-up extract was concentrated in a Kuderna-Danish evaporator with few drops of ethylene glycol as the retaining solution at a temperature of $40 \pm 1^\circ\text{C}$.

The colorimetric method of Benson and Finocchiaro (1965) was used. The preparation of the plates was as described by El-Dib (1970). The spots were visualised by heating the acid plates in at 120°C for 15 minutes (Finocchiaro and Benson, 1967).

RESULTS AND DISCUSSION

Table I shows the vertical distribution of the pesticide in 1'' horizons. Obviously the surface values were greater in the vertical distribution and less than 10

TABLE I
*Vertical distribution of carbaryl in soil**

Depth (inches)	0 day (in ppm)	10th day (in ppm)	20th day (in ppm)	30th day (in ppm)	40th day (in ppm)	50th day (in ppm)
0-1	50.0	10.3	NIL	NIL	NIL	NIL
1-2	5.0	2.4**	NIL	NIL	NIL	NIL
2-3	4.2	—	3.0	0.6	NIL	NIL
3-4	3.3	—	3.8	1.2	0.4	NIL
4-5	1.2	—	2.7	0.8	0.4	NIL
5-6	0.8	—	2.0	0.5	0.3	NIL
6-7	NIL	—	NIL	0.4	0.6	NIL
7-8	NIL	—	NIL	NIL	NIL	NIL

* Values corrected for recovery and blank studies

** Pooled value at depth 1'' to 7''

per cent of the pesticide was present in the deeper layers. The pesticide occurred only to a 5 to 6'' depth. The surface of the soil was free of residues by the 20th day. Even in the subsurface samples the residues did not persist beyond 50 days.

Thus it appears that while studying residues of carbaryl, it is adequate if the sample is collected up to a depth of seven inches (18 cm).

The authors thank the University Grants Commission for providing financial assistance and the authorities of Nagarjuna University for providing facilities.

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THE PERSISTENCE OF CARBOFURAN IN BLACK SOIL OF VADAPALANCHI (MADURAI) AND ITS EFFECT ON THE MICROFLORA

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SUMMARY

The effects of carbofuran in black soil of Vadapalanchi on the soil microflora was studied at different concentrations (1, 10, 100 ppm) and no significant change was noted. Treated soil was extracted with acetone and residues were determined by microbioassay using *Drosophila melanogaster* as the test insect. Residue levels were compared with the microbial population and their interrelationships studied.

INTRODUCTION

Soil tends to become contaminated from all pesticide use and the persistence and stability of pesticides in soil depends on factors such as the type of formulation, soil type, soil structure, soil pH, soil organic matter, soil temperature, soil water, humidity and the microbial population (Chopra, 1971) chlorinated hydrocarbons are persistent whereas organophosphates and carbamates are more quickly decomposed and their persistence in soil is much less (Junshi Miyamoto, 1975).

An attempt was made to study the fate of carbofuran in and around the area of Madurai Kamaraj University. Studies were also made on the interactions of carbofuran with the soil microflora.

MATERIAL AND METHODS

A black soil in the village Vadapalanchi (Adopted by Madurai Kamaraj University) was collected for this study and its physical and chemical characters analysed (Table I.)

Carbofuran 75 per cent technical grade, an easily biodegradable methyl carbamate was applied to the soil at rates of 1, 10, 100 ppm, mixed well and distributed in polyethylene bags (100 g/bag). A control soil was kept and the moisture content was about field capacity. The incubation temperature ranged between 25° and 30°C.

Microbial analyses : Changes in the microflora of the treated soil were determined by periodical sampling (every ten days). A soil dilution plate technique

was followed with Martin's (1950) medium, soil extract agar and Kuster's agar. All dilutions were plated in triplicate. The numbers of bacteria, fungi and actinomycetes per gram of soil were calculated (Table II) and the total microflora of the treated soil estimated (Table III).

Residue analyses : Soil samples were agitated with distilled acetone for 15 min. in a shaker and the contents were filtered and the filtrate evaporated. The residue dissolved in acetone which was filtered and evaporated and redissolved.

TABLE I
Physical and chemical properties of Vadapalanchi soil

Characters	Soil
Clay	39.92%
Silt	50.30%
Sand	15.30%
pH	7.5
Temperature	28.5°C
Free carbonates	3.22%
Organic matter	1.3%

This extract was analysed for its carbofuran by a residue film bioassay method (Sun, 1963) using *Drosophila melanogaster* Meig. as the test insect. The amount of carbofuran recovered (Table IV) were compared with the microbial population and their interrelationships studied.

RESULTS AND DISCUSSION

The results of the microbial analyses (Table II) shows that there was a ten-fold increase in bacterial population over the control, in all the three concentrations. There was a significant increase in actinomycetes in the 1 and 10 ppm concentrations. However, after the 100 ppm concentration there was a reduction of actinomycetes compared with that of the control. The fungal population, remained almost the same as that of the control during the early periods of incubation, gradually increased as the period of incubation was prolonged and almost double after 30 days. In general there was no harmful effect on the total microbial flora due to carbofuran.

The percentages of carbofuran recovered from the soil is given in Table IV. Residues disappear faster in the beginning and slower later. There was an inverse correlation between the total microbial flora and the percentage of carbofuran

TABLE II
Microbial populations in carboturan treated soil

Organism	Incubation days	Carbofuran concentration			
		Control	1 ppm	10 ppm	100 ppm
Bacteria (10^8 cells/g)	10	1.42	3.6	3.9	2.8
	20	2.01	23.24	25.86	22.5
	30	2.6	27.03	28.33	23.2
Actinomycetes (10^8 cells/g)	10	0.08	0.22	0.21	0.05
	20	0.18	1.81	1.52	0.02
	30	0.48	2.3	1.8	—
Fungi (10^6 cells/g)	10	0.73	0.96	0.8	0.72
	20	2.13	3.84	3.5	2.22
	30	2.42	4.77	3.9	3.4

TABLE III
Total microbial populations in treated soil (10^8 cells/gm soil)

Incubation days	Carbofuran concentration			
	Control	1 ppm	10 ppm	100 ppm
10	1.51	3.83	4.11	2.86
20	2.211	25.01	27.42	22.54
30	3.004	29.37	30.17	23.24

recovered. So possibly the carbofuran was utilised for the growth of microbes. The effect of pesticides on micro-organisms has been reported by several investigators (Edwards, 1973; Alexander, 1972). Gupta and Sud (1971) reported that soil bacteria utilise carbamates for their growth. During microbial interactions

TABLE IV
Percentage recovery of carbofuran added to black soil of Vadapalanchi

Incubation days	Percentage Carbofuran recovered		
	1 ppm	10 ppm	100 ppm
10	63	69	78
20	44	46	60
30	32	37	47

carbofuran is hydrolyzed to form the parent phenol and 2, 3-dihydro-7 hydroxy-2, 2-dimethyl benzofuran (Miyamoto, 1975). Intermediate metabolites formed depends on the types of organisms present in the soil.

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62 THE RAPID COLORIMETRIC ESTIMATION OF CARBOFURAN AND ITS RELATIVE PERSISTENCE IN COMPARISON WITH BAYGON AND CARBARYL

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SUMMARY

Carbofuran (2 : 3 dihydro-2,2 dimethyl-7 benzofuranyl N-methyl carbamate) is widely used as an agricultural insecticide. In the presence of alkali it hydrolyses into dihydro dimethyl furanyl phenol. The phenol is coupled with a diazonium salt solution of para-nitroaniline in an alkaline medium. Its colour is purple and it is stable and has λ max at 540 nm. It obeys Beer's law for the concentration range of 0-35 μ g of carbofuran. The chromogenic reagent is cheap and indigenously available.

This method has also been applied in the estimation of baygon and carbaryl which give purple and blue colours having λ max at 540 and 600 nm respectively. The relative persistence

of carbofuran, baygon and carbaryl under laboratory conditions in daylight and dark have also been studied using the same chromogenic reagent.

PART I

INTRODUCTION

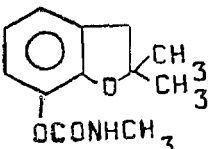
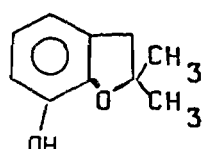
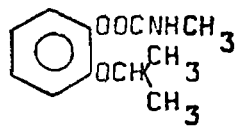
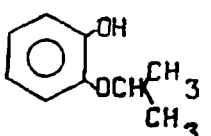
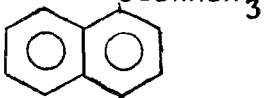
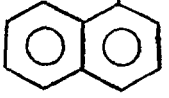
Carbamate insecticides are gaining wide popularity both for household and agricultural use presumably due to their broad spectrum, biological activity, high 'knock down', relatively low persistence and biologically harmless degradation products. In India, three such carbamates, namely, carbofuran, baygon and carbaryl, are in use.

Their quantitative estimation is required largely in order to ensure purity of samples, evaluation of formulation and their residual persistence in water, soil, vegetables and fruits. Classical methods of titrating the alkyl amine after hydrolysis and distillation are still in vogue for ascertaining purity and evaluation of formulations when large samples are available. By contrast residue determinations demand highly sensitive methods because the available quantity is in only microgram level. Gas chromatography (Bulter and Mc Donough, 1968) is one such tool but it has yet to become routine laboratory equipment in india. Enzymatic (Dale, 1975) and fluorometric (Lawrence, 1972) methods offer the desired sensitivity but have been rarely used for quantitative estimation. The last alternative is colorimetry and in fact, few such methods have been reported in literature for the estimation of baygon, carbaryl and carbofuran. Moreover the chromogenic reagents used in these methods are costly and require to be imported. Moreover some give high blank colours. One method utilizes paranitrobenzene diazonium fluoroborate (Benson, *et al.*, 1965 and 1968 and Gupta and Dewan, 1971) as the chromogenic reagent which produces a final yellow colour for estimation in acidic medium. We replaced this reagent with paranitroaniline which is cheap and available indigenously. We further changed the reaction medium from acidic to alkaline where the blank is nil and sensitivity is greater. Baygon and carbaryl (Maiti and Sethi, in press) give purple and blue colours respectively with this reagent and in this paper we have extended the use of this reagent in the estimation of carbofuran. It consists of first hydrolysing carbofuran with alcoholic alkali at room temperature followed by coupling of the liberated phenol with diazotised p-nitroaniline. A purple colour which can be estimated develops.

A comparison of the various shades of colour, their stability, sensitivity, wave-lengths of maximum absorption and ranges over which Beer's law is followed for baygon, carbaryl and carbofuran is given in Table I.

By using the suggested reagent the relative persistence of above carbamates on filter paper surfaces and in soil have been studied individually both in dark and in daylight.

TABLE I
Azo dyes from different carbamate insecticides

Trade Name and structure	Liberated phenol	colour	λ Max	Baer's law obedience	Sensitivity	Stability
CARBOFURAN (FURADON)						
		Pur- ple	540 nm	0-35 μ g	1 μ g/ml	Up to 24 hr
BAYGON (ISOPROPOXUR)						
		Pur- ple	540 nm	0-25 μ g	0.5 μ g/ml	Up to 24 hr
CARBARYL (SEVIN)						
		Blue	600 nm	0-20 μ g	0.3 μ g/ml	Up to 24 hr

MATERIAL AND METHODS

Apparatus : Visible colorimeter - Spectronic 20 (Bausch and Lomb, U.S.A.)

Reagents—

- (1) Stock solution of p-nitroaniline 0.05% -
50 mg/100 ml of 1 N hydrochloric acid.
- (2) Sodium hydroxide solution—2% in distilled water.
- (3) Sodium nitrite — 0.1% in distilled water.

- (4) Rectified spirit — (aldehyde-free).
- (5) Stock solution of carbofuran :- 10 mg/100 ml acetone.
- (6) Starch iodide paper.
- (7) Diazotised p-nitroaniline solution, 2 ml of stock solution of p-nitroaniline (0.05%) is diluted to 10 ml using 1 N hydrochloric acid and cooled in ice. The sodium nitrite solution is added dropwise to the above ice cold solution just sufficient for complete diazotisation as judged by dipping a starch iodide paper, which turns blue when sodium nitrite is in excess.

Procedure : A standard solution of carbofuran 10 μ g/ml was prepared by diluting 10 ml of stock solution (10 mg/100 ml) to 100 ml using acetone. Measured solutions representing 5, 10, 15, 20, 25, 30 and 40 μ g of carbofuran were transferred to seven 10 ml graduated stoppered test tubes separately. A blank with acetone alone was run simultaneously. Acetone from each tube was evaporated in presence of a drop of glycerol on a water bath. After evaporation and cooling, 5 ml of rectified spirit and 2 ml of 2 per cent sodium hydroxide solution were added to each and mixed well. After 10 minutes, 1.5 ml of ice cooled diazotised reagent was added followed by 1 ml of 2 per cent sodium hydroxide solution and the final volume was made to 10 ml using alcohol. A purple colour developed immediately in all except the blank which remained colourless. The colours were measured in a spectronic 20 calorimeter at 540 nm.

RESULTS AND DISCUSSION

It will be apparent from Fig. 1 and Fig. 2 that carbofuran can be estimated quantitatively at a low concentration of 0–35 μ g, using diazotized paranitroaniline as the chromogenic reagent. There is no need to import the costlier and unstable reagent paranitrobenzene diazonium fluoroborate for this and similar estimations. The hydrolysis takes place almost instantaneously (Fig. 3). We used a longer time of hydrolysis for convenience.

PART II

Study of Relative Persistence of carbofuran, carbaryl and baygon

The surfaces for study were Whatman filter papers (No. 1) and soil. The soil was slightly alkaline (pH 8). Filter paper treated with different carbamates and soil mixed with these were either (a) inside a room exposed to open light and (b) inside a closed cupboard. Six replicates were prepared for each carbamate separately to study their persistence.

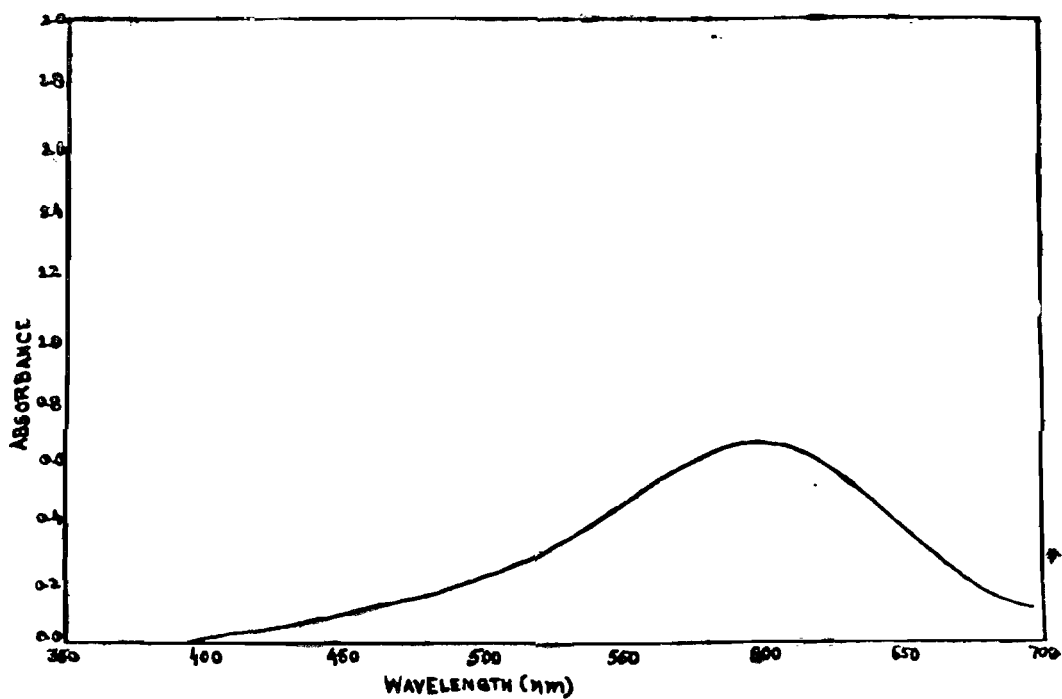


FIG.1 ABSORPTION SPECTRA OF BLUE AZODYE FOR CARBARYL

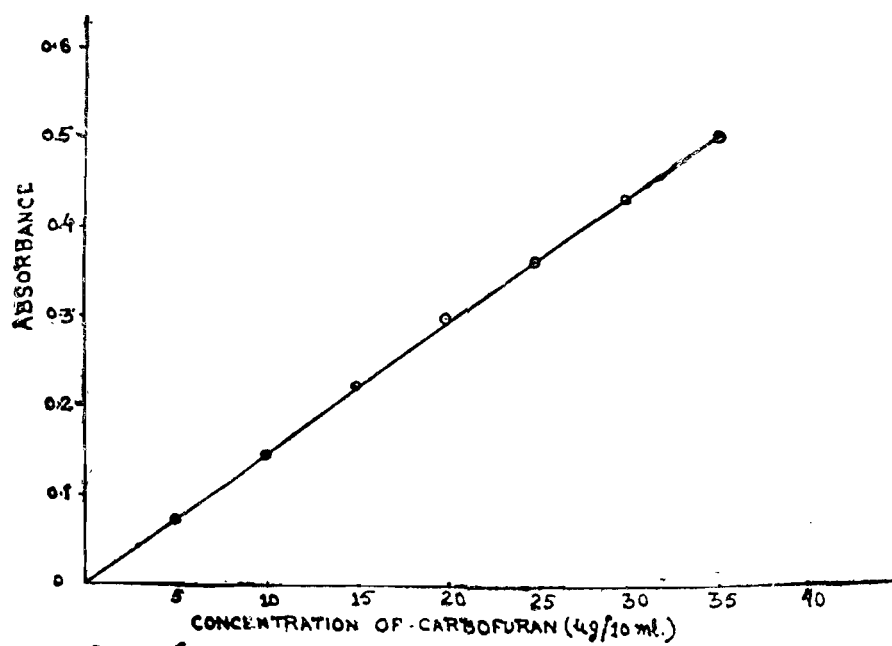


FIG.2 CALIBRATION CURVE FOR CARBOFURAN

Commercial samples of carbofuran and carbaryl were purified by recrystallisation from acetone and a baygon spray formulation obtained from the market was used for the experiment.

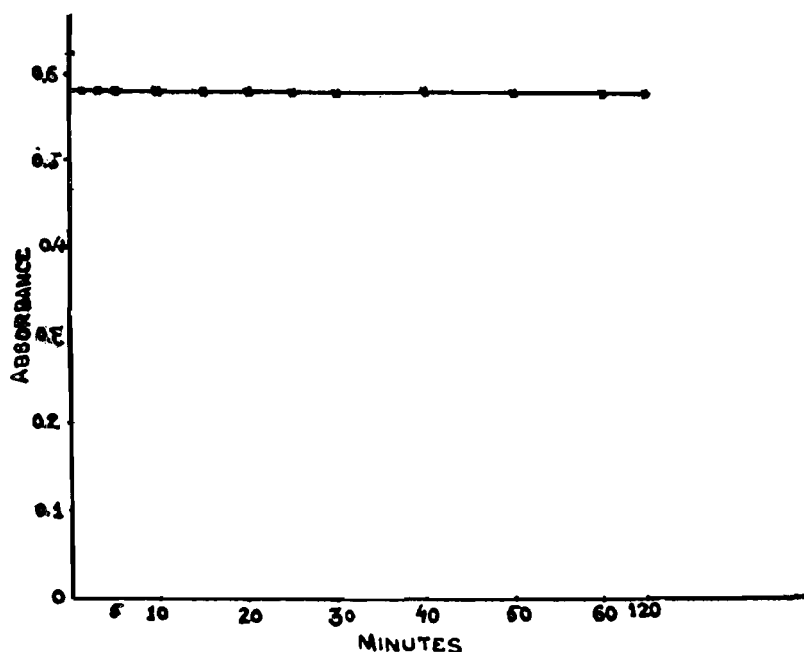


FIG. 3. TIME REQUIRED FOR HYDROLYSIS

Standard solutions : Carbofuran — 10 mg/ml in acetone.
 Carbaryl — 10 mg/ml in acetone.
 Baygon spary formulation — 10 mg/ml without dilution.

1 ml of each standard solution representing 10 mg was transferred slowly with pipette on to a circular filter paper No. 1 and a layer of soil having 144 sq. cm. area and approximately of 2.5 mm thickness. After the solvent had evaporated, the treated filter paper and soil were placed in dark or light.

Method of Extraction :

Treated filter papers and soil layers were removed after the required period and inserted directly into the thimble of 60 ml soxhlet extractor and extracted using rectified spirit for 4 hrs. and 7 hrs. for the filter paper and soil samples respectively. The longer time needed for extraction from soil may be due to its high absorption as compared to filter paper. The recovery of carbamates in each case was 100 per cent. After complete extraction the solution was concentrated to about 15 ml by distilling off the solvent in the presence of 1 ml glycerol, and transferred to a 100 ml

volumetric flask. Volume was made up to 100 ml using alcohol. An aliquot representing 20 μ g of carbamate was taken and diluted to 5 ml using alcohol and the colour was developed. The same procedure was adopted for all samples and optical densities recorded at their λ max as indicated for each carbamate in Table I.

RESULTS AND DISCUSSION

Data in Tables IIa and IIb represent percentage persistence for three carbamates exposed to light or kept in dark.

The three insecticides on a soil surface exposed to light (Fig. 4) showed a slow and gradual loss up to ten days. For the first three days there was little difference in persistence but between six and ten days baygon was nearly 7 per cent and 16 per cent, respectively, more persistent than carbaryl and carbofuran. In the

TABLE IIa
Persistence (%) of various carbamates in soil

Carbamates	Days									
	3		6		10		13		17	
	L	D	L	D	L	D	L	D	L	D
Baygon	91.7	96.7	88.7	91.7	86.2	88.8	62.5	66.7	35.9	40.8
Carbofuran	87.5	92.6	72.5	75.8	69.2	70.4	56.7	63.3	30.8	36.7
Carbaryl	91.0	98.3	81.6	91.9	76.3	83.3	51.6	60.0	45.0	53.3

next seven days, 50 per cent of baygon, 38 per cent of carbofuran and 31 per cent of carbaryl dissipated. After twelve days a parallel degradation pattern was exhibited for baygon and carbofuran but carbaryl was more persistent.

TABLE IIb
Persistence (%) of various carbamates on filter paper

Carbamates	Days									
	3		8		12		14		17	
	L	D	L	D	L	D	L	D	L	D
Baygon	48.7	90.4	38.4	77.4	29.6	73.9	27.8	73.9	14.8	67.8
Carbofuran	91.4	91.4	50.0	87.9	37.9	86.2	37.9	81.9	37.9	81.9
Carbaryl	88.0	89.0	76.0	76.0	50.0	65.0	49.0	64.0	44.0	64.0

L — Sample exposed to light

D — Sample kept in dark.

The experiment on the soil surface kept in dark (Fig. 5) showed that baygon and carbaryl remained unaffected (approx. 92 per cent) for the first week but carbofuran reduced to approx. 75 per cent. Carbofuran and baygon followed parallel degradation pathways but carbaryl behaved similarly only after 10-12 days.

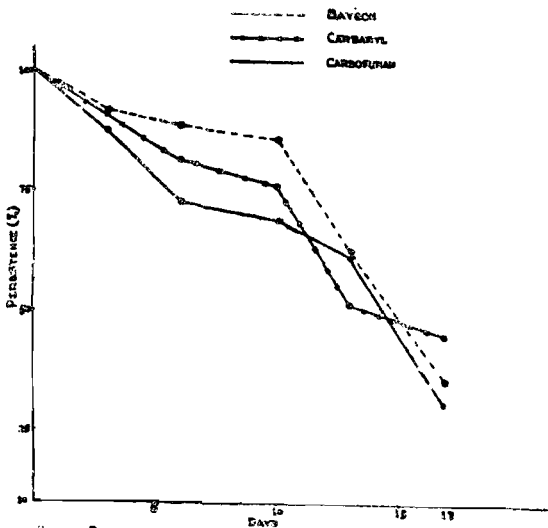


FIG. 4. PERSISTENCE OF CARBAMATES IN SOIL EXPOSED TO DAY LIGHT

Carbaryl was more persistent than the other two carbamates. Despite different patterns of degradation, both carbofuran and carbaryl showed a loss of approx. 38-40

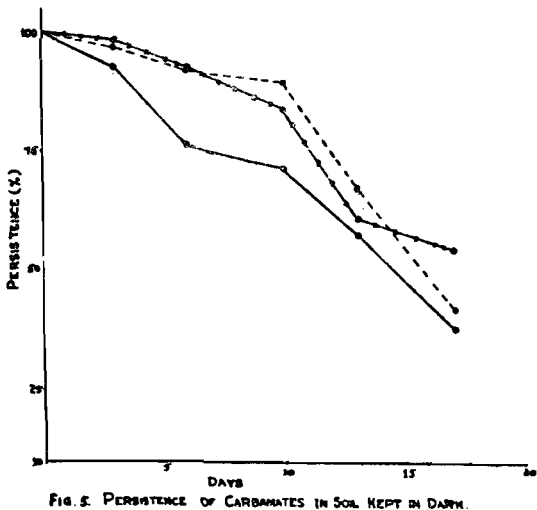
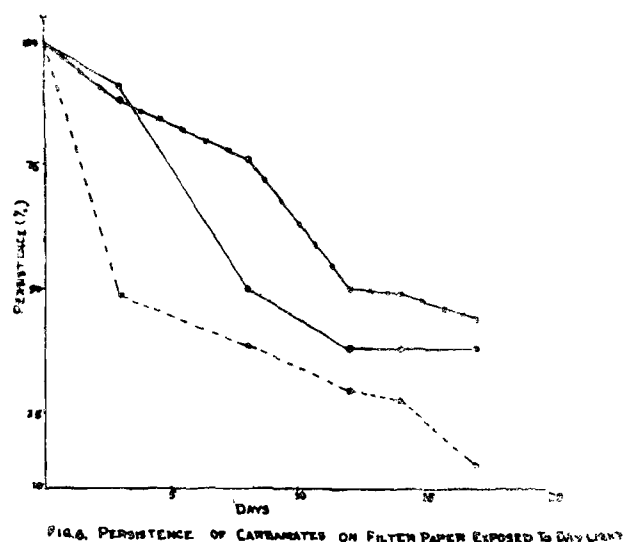


FIG. 5. PERSISTENCE OF CARBAMATES IN SOIL KEPT IN DARK.

per cent between the sixth day and the seventh day while 51 per cent of baygon disappeared during the same period.

Baygon disappeared fastest to a 15 per cent residue with 50 per cent loss by the end of three days. Both carbofuran and carbaryl underwent only 9-12 per cent degradation compared to 50 per cent for baygon during the first three days. However,



another 34 per cent dissipation took place during the next fourteen days. Carbofuran and carbaryl also degraded rapidly and continuously for 12 days to a extent of 62 per cent and 50 per cent respectively but thereafter showed little or no loss.

Studies on filter paper kept in the dark : (Fig. 7)

For the first three days the three carbamates had roughly same persistence (90 per cent loss). Thereafter, only 8 per cent of carbofuran degraded in 14 days, and during last three days there was no change. Carbaryl residues fell to 65 per cent in 12 days but thereafter was constant up to 17 days. Baygon had the same tendency as carbaryl for 8 days but in the following days only a 7 per cent loss occurred. Baygon was most stable in light and dark whereas carbofuran was least stable. Degradation pattern for these carbamates in light and dark were similar rising only 5-10 per cent more in dark than in light (Table III a). Baygon was least stable both in light and dark on filter paper contrary to soil. Surprisingly, the same sequence of degradation was not observed for the same substance on the same paper surface exposed to light and dark. More persistence was observed in dark than in light. The 'half-life' (50 per cent degradation) derived from figures 4 to 7 is another convincing parameter to compare relative persistence (Table IV).

Similar 'half-life' values of 14 - 15 days for different carbamates in soil under light and dark was due to their same degradation pattern. For samples on paper

and exposed to light, 50 per cent degradation was achieved only after three days for baygon whereas 8 and 12 days were required for carbofuran and carbaryl. However, with paper kept in the dark 50 per cent degradation could not be achieved during the period of study. Previous studies (Getzin, 1973) in soil revealed that at least 3 weeks to 50 weeks were required for 50 per cent degradation of carbofuran whereas 8 to 15 days were needed in our experimental studies. Ramaswamy (1974) investigated the degradation of baygon in mud blocks and cheese cloth for a period of 29 weeks and reported remarkable differences in degradation on these two surfaces *i.e.* 15 per cent and 63 per cent on mud and cheese cloth respectively.

TABLE IIIa
Persistence (%) in soil

Carbamate	Light			Dark		
	Days			Days		
	5	10	15	5	10	15
B	90	86	47	92	90	53
F	77	70	45	80	70	50
C	85	76	47	93	83	57

TABLE IIIb
Persistence (%) on paper

B	45	34	23	85	33	23
F	75	44	37	90	87	82
C	83	63	47	84	70	64

B — Baygon
F — Carbofuran
C — Carbaryl

<i>Conclusion of Table III-a Soil</i>				<i>Conclusion of Table III-b Paper</i>			
Days	5	10	15	Days	5	10	15
Light	B	>	C	>	F	>	B
Dark	B	=	C	>	F	>	B

TABLE IV
'Half-life' (days)

		Soil			Paper
<i>Light</i>					
	B	15			3
	F	14			8
	C	14			12
<i>Dark</i>					
	B	15.5	B	>	15 (67%)
	F	15	F	>	15 (82%)
	C	> 15 (53%)	C	>	15 (64%)

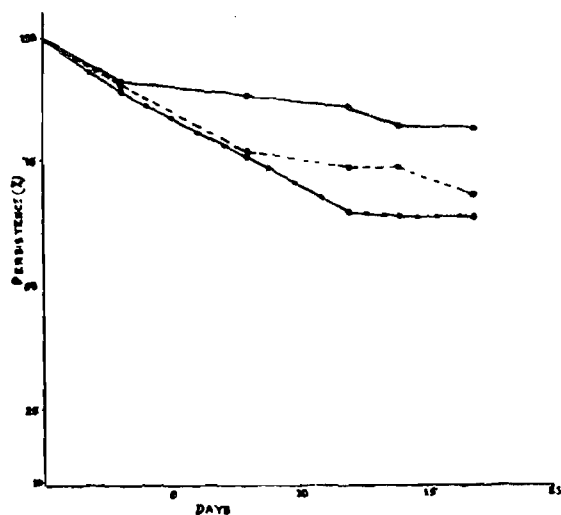


FIG. 7. PERSISTENCE OF CARBAMATES ON FILTER PAPER KEPT IN DARK

CONCLUSIONS

A colorimetric method for the estimation of carbofuran and other phenolic carbamates was simple, rapid and utilised a reagent (p-nitroaniline) which is cheap and indigenously available.

The study of relative persistence of three carbamates under experimental conditions show that :

1) Baygon in soil and exposed to light was more persistent than carbaryl and carbofuran. In the dark baygon and carbaryl had equal stability but both were greater than carbofuran.

2) *Study on Whatman filter paper*: Baygon kept in light and dark showed maximum losses compared to other two carbamates. Carbofuran was more stable than carbaryl when kept in the dark but less so when exposed to light.

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63 THE EFFECT OF PHYSICO-CHEMICAL PROPERTIES OF SOILS ON LEACHING AND DOWNWARD MOVEMENT OF CARBOFURAN AND METHOMYL

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SUMMARY

The leaching of carbofuran in red, laterite and black soils 0-6, 6-18, 18-24, 24-42 and 42-90 hours after treatment was studied in soil columns. Significantly greater amounts of carbofuran were lost by leaching in red soil, than in laterite soil and least of all in black soil. The important factors influencing leaching were adsorption, water solubility, soil type, amount of moisture and percolation velocity. The water solubility and adsorption of carbofuran were inversely related. The solubility of carbofuran in water was low so the chemical was adsorbed tightly and its movement through soil was slow.

The amounts of methomyl leached from red, laterite and black soil was in the order red > laterite > black soils.

Downward movement of carbofuran in red, laterite and black soils indicated that most of the insecticide was adsorbed to the top 0-6 cm of the soil columns. The low overall content of carbofuran in the red soil column was due to the faster diffusion of the insecticide through column. The mobility of non-ionic compound was related inversely to the clay and organic matter contents and the cation exchange capacity of soils. As these properties were greater in the black soil the movement of the insecticide through the column was lowest in this soil.

The amounts of methomyl in the red, laterite and black soil columns was in the order of black > laterite > red soils. Methomyl is more soluble in water and the downward movement through the soil columns was faster than that of carbofuran. There was no significant difference in distribution of methomyl in the red and laterite soil but more methomyl occurred from 0-12" in the black soil column.

INTRODUCTION

The systemic carbamate insecticides are becoming popular for control of crop pests because these insecticides are relatively persistent in soils. The uptake of these insecticides by the plant roots depends upon the soil on which the crop is grown and soil physico-chemical properties are important in the absorption of insecticides by the crop plants. The movement of insecticides in soil depends on the surface properties of both the adsorbate and adsorbent. Furthermore, the chemical property of the adsorbate, such as solubility in water facilitates greater movement in soil. Conversely, less soluble compounds move very slowly in the soil because these compounds are strongly sorbed by the soil colloids.

During the movement of chemicals through the soil, there is always a dynamic equilibrium between the free chemical and its adsorbed state. Consequently, a tightly bound chemical should leach slowly and vice-versa (Freed and Haque, 1973). Bailey and White (1964) postulated that the water solubility of an insecticide and its adsorption on to soil fractions are often inversely related. Swason *et al.* (1954) and Roberts (1963) reported that the adsorption of pesticides was more in heavy textured soils and that the diffusion coefficient of pesticides in light soil was high. According to Helling (1971) pesticide mobility in soil depends on the soil properties and the mobility of nonionic compounds was inversely related to the clay and organic matter content and cation exchange capacity of soils. High diffusion coefficients of pesticides in light soils was reported by Lindstrom *et al.* (1968). In the present investigation, the influence of soil physico-chemical properties on the leaching loss and downward movement in three soils from Karnataka are reported.

MATERIAL AND METHODS

Collection of soil samples

Red sandy loam (Rhodustalf) from Hebbal, Bangalore; laterite sandy clay loam (Haplustox) from Mangalore and black clay soil (Grumaquert) from Dharwad were used. Samples were collected from areas which had no known history of pesticide application, brought to the laboratory, dried in the shade, powdered, passed through a 2 mm sieve and stored in polythene boxes. The general characteristics of the soils are given in Table I.

Methods of soil analysis

The chemical properties of the soils such as pH, EC, organic carbon, CEC and exchangeable hydrogen were determined as described by Jackson (1958).

The physical properties of soils such as maximum water holding capacity, field capacity, and mechanical analysis were determined as described by Piper (1950).

TABLE I
Physical and chemical characteristics of the soils

Property/Constituent	Soils		
	Red	Laterite	Black
Coarse sand	44.31	23.70	10.57
Fine sand	30.20	16.36	13.30
Silt	6.40	16.10	15.21
Clay	20.00	43.30	65.23
Maximum water holding capacity	20.37	40.00	51.00
C.E.C.me/100 g	13.50	18.00	67.50
Field capacity (%)	12.00	30.00	42.00
pH	7.10	4.50	8.10
E.C. m.mhos/cm (25°C)	0.21	0.22	0.37
Organic carbon (%)	0.34	0.62	0.52
<i>Exchangeable cations me/100 g</i>			
Calcium	6.25	2.50	40.00
Magnesium	0.31	0.62	20.00
Potassium	0.53	0.62	3.21
Sodium	0.63	0.65	4.20
Hydrogen	1.30	6.50	0.00
Bulk density (g/cm ₃)	1.61	1.54	1.30
Hydraulic conductivity (cm/hr)	14.10	7.90	2.40

The bulk density and hydraulic conductivity of the soil were determined as described by U.S.D.A. (1954).

Insecticide residue analysis in soil

Carbofuran residues in soil

Air dried soil samples were extracted with 20 ml chloroform twice and filtered into an evaporating basin. After evaporation of the chloroform to dryness the residue was dissolved in a known volume of methyl cyanide. A control sample

(without carbofuran) was prepared similarly. An one ml aliquot (without hexane extraction) was employed for colour development. Carbofuran residues in soil was determined as described by Rangaswami *et al.* (1970) whose method is based on coupling the diazotized aniline with carbofuran so that a yellow coloured compound with maximum absorption at 460 nm is formed.

Methomyl residues in soil

A soil sample was taken in a beaker chloroform added and stirred well for 10 minutes. Then the chloroform layer was transferred to a separate beaker. Extraction was repeated twice and the coloured solution centrifuged before taking a reading. The pooled chloroform extract was used for colour development as described by Rangaswami *et al.* (1977). The method consisted of alkaline hydrolysis of methomyl, S-methyl-N [(methyl carbamoyl)Oxy] thio-acetamide. The latter an oxime of methomyl, which when heated with hydrochloric acid yields hydroxylamine, and on oxidation with iodine in the presence of sulphanilic acid, p-diazoniumbenzene sulfonic acid. The diazonium compound after reduction of the excess of iodine when coupled with naphthylamine forms a crimson-red coloured compound, p-benzene sulfonic acid-azo-naphthylamine with an absorption maximum at 520 nm.

Leaching studies

Leaching losses of carbofuran and methomyl from red, laterite and black soils were studied by the use of soil columns as described by Austin and Briggs (1976).

Plastic tubes 25 cm long and 3 cm diam. were used with their bottoms fixed to plastic funnels. Glass wool plugged the opening of stem of the funnel. The columns were filled with red, laterite and black soils to a height of 18 cm with 3 replicates for each soil. A constant head of 2 cm of water was maintained above the tubes. Thirty mg of carbofuran or methomyl was applied to the top of the respective tubes. The leachate of carbofuran was collected at intervals of 0-6, 6-18, 18-24, 24-42 and 42-90 hours. Methomyl was collected at intervals of 0.0-0.5, 0.5-1.0, 1.0-1.45, 1.45-2.15, 2.15-24 and 24-44 hours. The leachates were analysed for carbofuran and methomyl.

Downward movement of carbofuran and methomyl in soils

Soil columns were prepared as described above with red, laterite and black soils (Austin and Briggs, 1976) with 3 replicates for each soil. Thirty mg of carbofuran or methomyl was applied to the top of the columns. The carbofuran was allowed to diffuse through the column for 90 hours and methomyl for 44 hours. After these periods the columns were cut into 6 equal parts of 3 cm and the residues in each segment were analysed.

RESULTS

Leaching losses of carbofuran and methomyl from soils :

The leaching losses of carbofuran from red, laterite and black soils indicate that greater amounts of insecticide were lost from red soil than from laterite soil and least from black soil (Figure 2). The total amount of carbofuran leached from red, laterite and black soil was 16,091.5, 12,620.34 and 8119.1 μg respectively.

The amounts of methomyl leached from the red, laterite and black soil columns were in the order of red > laterite > black soil, respectively (Figure 3). Methomyl is water soluble, so most was lost in the early period.

Downward movement of carbofuran and methomyl

The rate of downward movement of carbofuran in red, laterite and black soils indicated that most of the insecticide was adsorbed to the top 0–6 cm of soil (Figure 4). Carbofuran moved fastest in red soil followed by laterite and black soil. The amounts of carbofuran retained at 0–3 cm, 3–6, 6–9, 9–12, 12–15 and 15–18 cm depths in the soil columns were 4700, 2766, 1000, 900, 900 and 900 μg in red soil ; 6811, 2115, 1601, 1302, 1302 and 1302 in laterite soil ; 10,300, 1300, 1300, 1200, 1000 and 1000 μg in black soil.

The downward movement of methomyl was faster in red soil, compared to laterite and black soils (Figure 5). The quantity of methomyl retained at the 0–3, 3–6, 6–9, 9–12, 12–15 and 15–18 cms depth of the soil column in red soil was 18.0, 14.0, 20.0, 18.0, 22.0 and 22.0 μg . For the laterite soil it was 54.0, 18.0, 99.0, 99.0, 84.0 and 117 μg in 0–3, 3–6, 6–9, 9–12, 12–15 and 15–18 cms depth of soil column, respectively. The quantity of methomyl retained in the black soil was 174.0, 270.0, 258.0, 300.0, 150.0, 48.0 μg at 0–3, 3–6, 6–9, 9–12, 12–15 and 15–18 cms depth of soil column respectively. Significantly more methomyl was found in black soil than in laterite and red soil.

DISCUSSION

Downward movement and leaching of carbofuran and methomyl from soil :

The movement of carbofuran through soil columns was slow. The reason for low leachability of carbofuran from black soil and laterite soil was their higher clay content and organic matter.

The quantity of methomyl leached from the red, laterite and black soil columns at different time intervals was in the order of red > laterite > black soils,

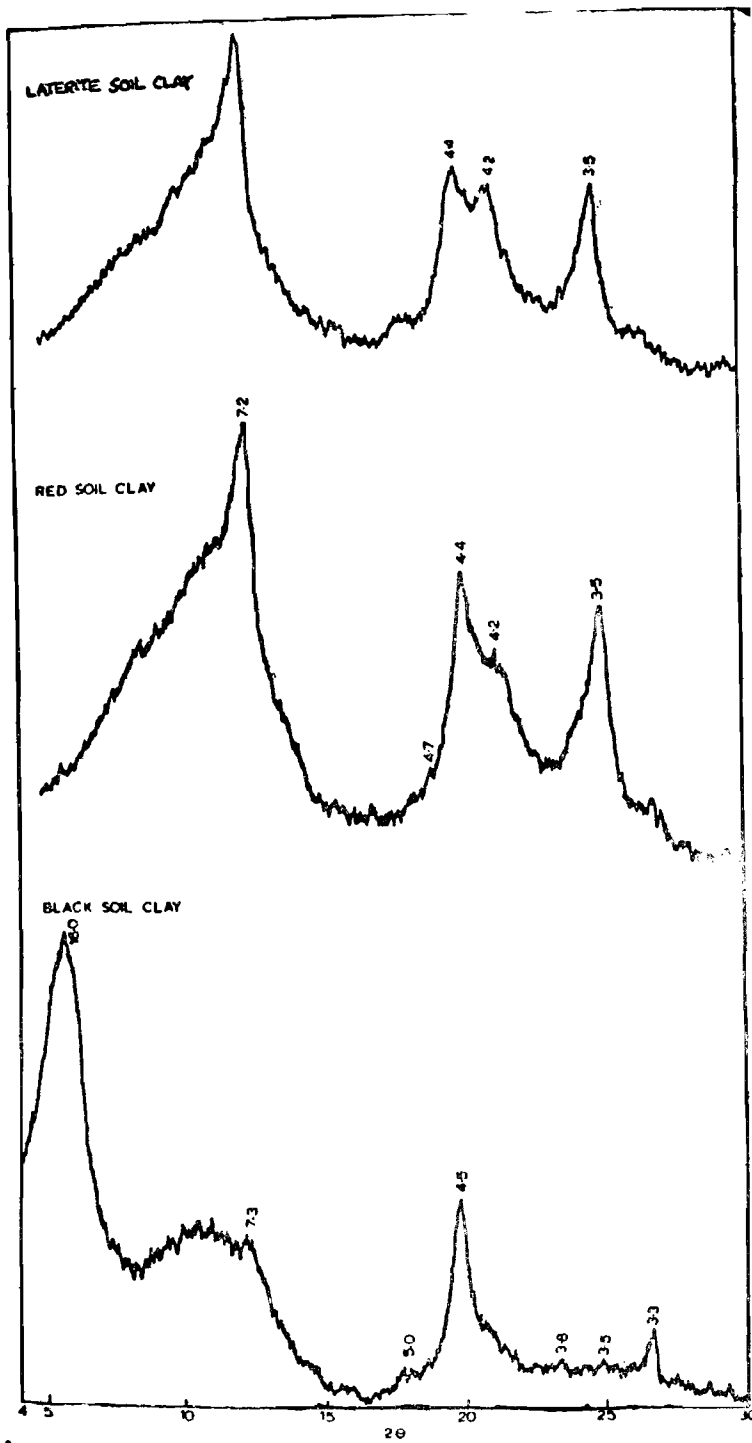


FIG. 1. X-RAY DIFFRACTION PATTERNS OF THE CLAY FRACTIONS OF THREE SOILS OF KAWIATAMA

respectively (Fig. 2). The high rate of leaching from red soil may be due to high diffusion coefficient of methomyl in this soil due to its loose texture.

In the black soil the clay content is high, so more is adsorbed and a small amount was lost from leaching.

Downward movement of carbofuran and methomyl in soils :

The downward movement of carbofuran in red, laterite and black soils indicated that most of the insecticide was adsorbed to the top 0-6 cm of soil column (Figure 4).

The small amount of carbofuran in the red soil column was due to the faster diffusion of the insecticide through the column. According to Helling (1971) pesticide mobility in soil depends on soil properties and the mobility of nonionic compounds was inversely related to clay, organic matter and cation exchange capacity of soils. A high diffusion coefficient of pesticides in light soils was reported by Lindstrom *et al.* (1968). The higher rate of diffusion in red soil may be due to its low adsorption capacity for carbofuran.

The high content of carbofuran in black and laterite soil may be due to their high adsorption capacity for carbofuran compared to red soil. In soils of high clay content the molecular diffusion is restricted due to the presence of pores with small diameters (Goring, 1957). Scott and Weber (1967) observed that high clay content is responsible for higher adsorption of pesticide by soils.

The quantity of methomyl in the red, laterite and black soil columns was in the order of black > laterite > red soils respectively (Figure 5). Bailey and White (1964) reported that water solubility of an insecticide and its adsorption on to soil fractions are frequently inversely related. As methomyl is soluble in water the quantity in red soil was low compared to laterite and black soils.

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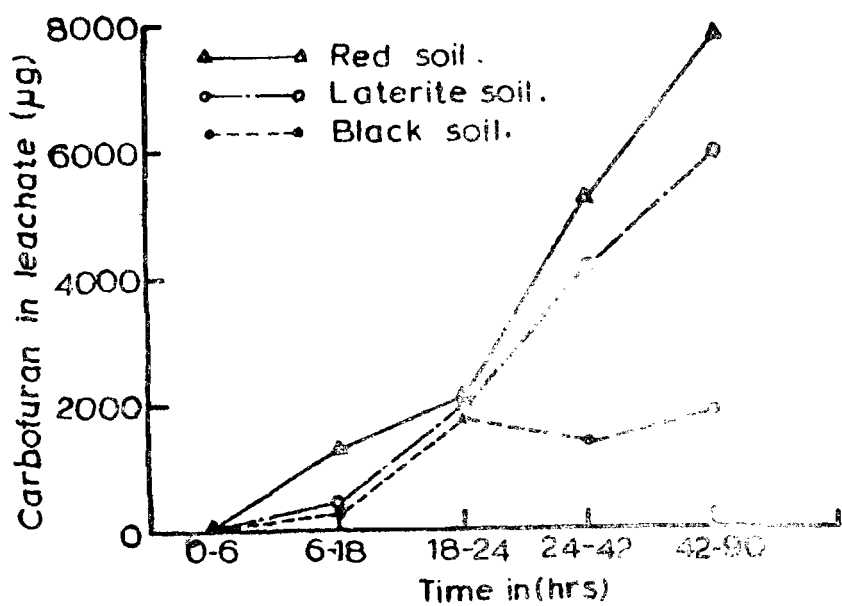


Fig. 2. Leaching of Carbofuran in Red, Laterite and Black Soils

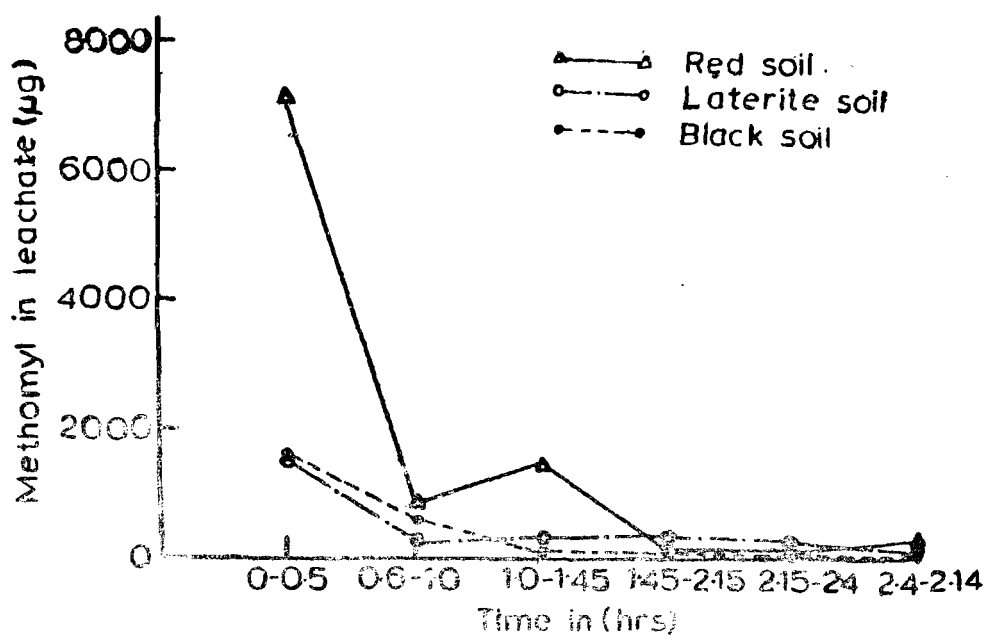


Fig. 3. Leaching of Methomyl in Red, Laterite and Black Soils

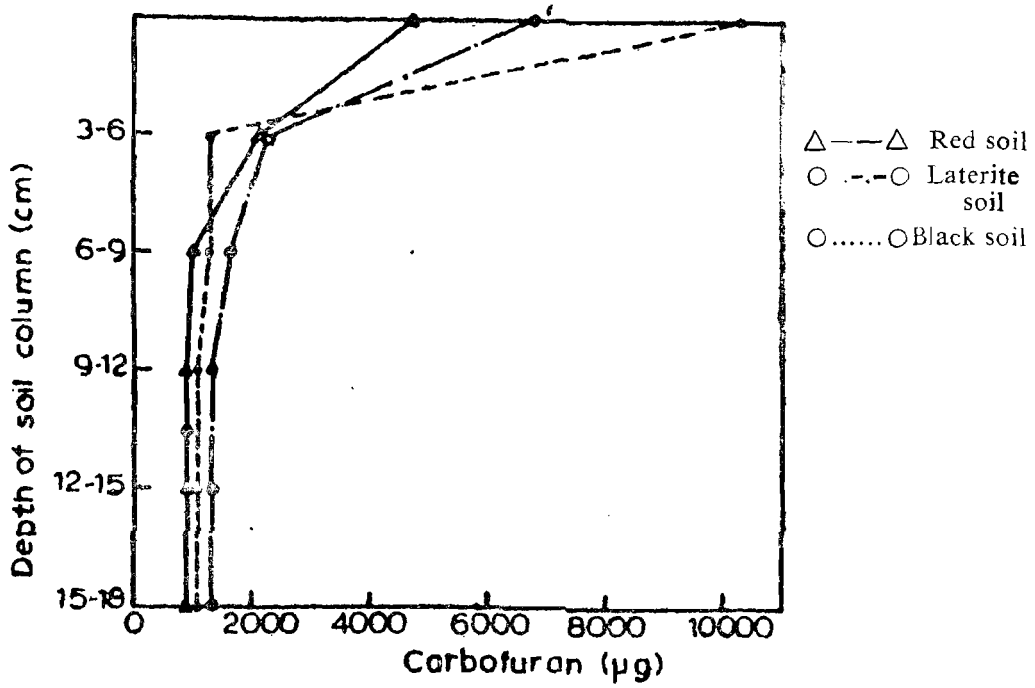


Fig. 4. Downward movement of Carbofuran in Red, Laterite and Black Soils

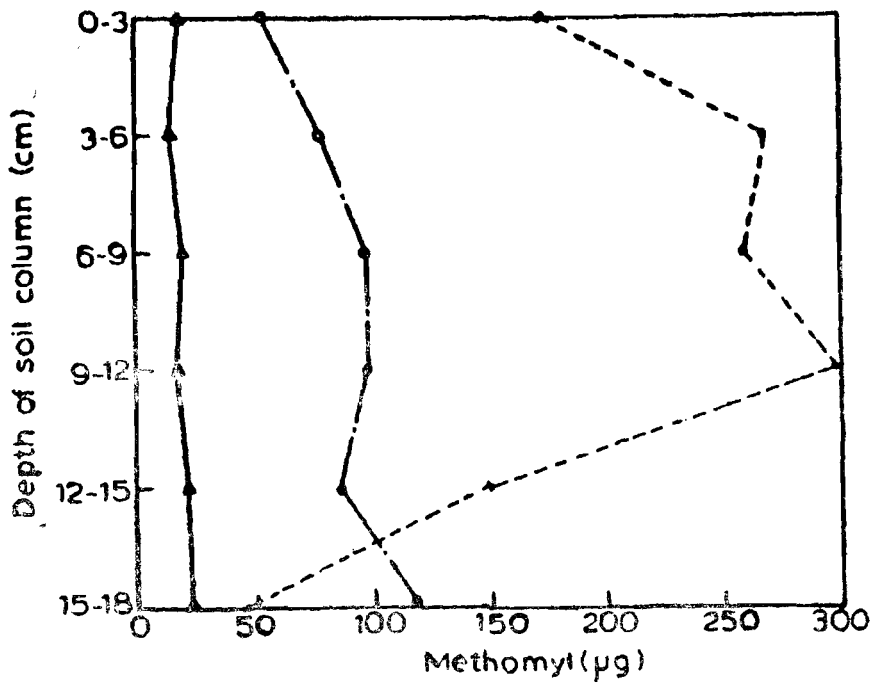


Fig. 5. Downward movement of Methomyl in Red, Laterite and Black Soils

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STUDIES ON THE EFFECT OF SOIL PHYSICO-CHEMICAL PROPERTIES ON THE UPTAKE AND PERSISTENCE OF CARBOFURAN AND METHOMYL IN VEGETABLE CROPS

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SUMMARY

Carbofuran residues in the roots of cowpea, bhendi and tomato plants was greater when grown in black soil than in red and laterite soils. Carbofuran residues in the roots of crops grown in red soil was very low due to its weak sorption in this soil. Methomyl residue in the roots of cowpea, bhendi and tomato plants were most when grown in black soil. Carbofuran residues in the shoots of crops grown in red soil were larger than in laterite and black soils. A similar trend was observed in the methomyl residue in the shoots of these crops. Carbofuran residues were largest in the leaves of cowpea, bhendi and tomato grown in red soil than those grown in laterite soil and least in plants grown in black soil. The order of carbofuran absorption by plants in was tomato > bhendi > cowpea.

The transpiration rate in these vegetable crops was in the decreasing order of tomato > bhendi > cowpea respectively. The higher absorption of carbofuran by tomato plants was attributed to their higher transpiration rate.

Methomyl residues in the leaves of cowpea, bhendi and tomato plants grown in red soil were greater than in laterite and black soil. The higher absorption of methomyl by the tomato plants was attributed to its high transpiration rate.

Carbofuran residues occurred in higher amounts in plants grown in laterite soil for 30 days, Significantly higher amounts of methomyl were detected in plants grown in laterite soil for 240 hrs than plants grown in red and black soils.

The absorption of carbofuran and methomyl by cowpea, bhendi and tomato plants increased with increase in temperature. The absorption of these insecticides by these vegetable crops was in the decreasing order 32°C > 22°C > 5°C.

With an increase in soil moisture, the absorption of these insecticides by cowpea, bhendi and tomato plants also increased. The absorption of these insecticides by these vegetable crops was in the order of 2 times field capacity > field capacity > 50 per cent field capacity.

INTRODUCTION

In recent years, systemic carbamate insecticides are becoming popular because of their efficiency in controlling pests, less contribution to air, water and soil

pollution and low mammalian toxicity. A systemic insecticide is considered good if it is absorbed by the plant roots, stems or leaves and translocated to the target of control in concentrations effective to kill the pest in question. The physico-chemical properties of the soil play an important role on uptake and persistence of systemic insecticides by plants. Soil pH, soil texture, organic matter content, soil moisture and temperature all influence the uptake of systemic pesticides by plants. In the present investigation, the effect of soil physico-chemical properties on uptake and persistence in some vegetable crops were studied.

Bailey and White (1964) reported that an increase in temperature decreased adsorption of pesticides and increases in temperature also increased solubility of the compounds which may reduce adsorption (Goring, 1967).

The chemical hydrolysis and break down of pesticides in soils are dependant upon the moisture content, the rate of break down or hydrolysis being faster at higher moisture content (Hamaker, 1972).

The translocation of systemic insecticides depends on transpiration of plants (HacsKaylo *et al.*, 1961), plants with a higher transpiration rate having a higher absorption capacity for systemic insecticides. Bhatnagar *et al.* (1974) reported that the temperature, humidity of surrounding and physiology of the plant are important in translocating insecticides.

MATERIAL AND METHODS

Collection of soil sample : Three soils, red sandy loam (Rhodustalf) from Hebbal, Bangalore; laterite sandy, clay loam (Haplustox) from Mangalore; black clay soil (Grumaquert) from Dharwad were selected. Samples were collected from areas with no known history of pesticide application. Samples were brought to the laboratory, dried in the shade, powdered, passed through a 2 mm sieve and stored in polythene boxes. The general characteristics of the soils are given by Appaiah and Deshpande (p—).

Standard methods of soil analysis were used as described by Piper (1950), Jackson (1958) and U.S.D.A. (1954).

Laboratory and pot experiments were used to study the persistence and loss of carbofuran and methomyl from soils of Karnataka and their uptake and persistence in cowpea (*Vigna sinensis* L., var : C-152), bhendi (*Hibiscus esculentum* Mill., var: Pusa sawani) and tomato plants (*Lycopersicon esculentum* Mill., var: Pusa ruby).

Insecticides

1. Carbofuran (2, 3 Dihydro-2, 2-dimethyl 7-benzofuranyl methyl carbamate)
2. Methomyl (S-methyl-N [(methylcarbamoyl) oxy] thioacetimidate).

Pot culture experiments

One kg of red, laterite and black soils were put into polythene bags, and watered daily to the respective field capacities of the soils. Seeds of cowpea, bhendi and tomato were sown in each of these soils. Only one plant was allowed to grow in each bag until they were harvested for residue analysis. The experiments were on a completely randomised block design with 3 replicates. The plants grown as above were used for the pot culture experiments mentioned below.

Uptake and distribution in roots, shoots and leaves

Two sets of plants of one month old (cowpea, bhendi and tomato) grown in red, laterite and black soils were used for this experiment. One set was used for the carbofuran experiment, and the other was used for the methomyl experiment. There were 3 replicates for each crop and for each soil. Carbofuran was applied to each of the soil bags at the rate of 10 mg and the residues in root, shoot and leaves were analysed after 24 and 72 hours. Plants were pulled out after 24 hours or 72 hours, washed with water, air dried at room temperature and the fresh weight of root, shoot, and leaves recorded. The residues were assessed and expressed in $\mu\text{g/g}$ of fresh weight of plant sample.

Methomyl was added to the second batch of soil at the rate of 10 mg/plant. Residues in plants were analysed after 24 and 72 hours of treatment to soil. The residues were assessed and expressed in $\mu\text{g/g}$ fresh weight of plant sample.

Persistence in plants

One month old plants of cowpea, bhendi and tomato grown in red, laterite and black soils were used for this experiment. One set for carbofuran and the other for methomyl treatments. The residues were analysed 1, 3, 7, 15 and 30 days after application of carbofuran or methomyl to soil.

Effects of temperature

Two sets of one month old plants of cowpea, bhendi and tomato plants grown in red, laterite and black soils were used for this experiment. 10 mg of carbofuran was applied to one set of plants and they were kept in incubators at 10°C, 22°C or 32°C. Then the residues were analysed.

Methomyl was applied at the rate of 10 mg/plant to the second set of plants which were kept in incubators at 10°C, 22°C or 32°C. After 72 hours, the residues were analysed.

Effect of soil moisture on absorption

Two sets of one month old plants grown in red, laterite and black soils were taken. The soil moisture level was maintained either at 50 per cent of field capacity, field capacity or twice field capacity. Carbofuran and methomyl were added at 10 mg/plant to respective sets of plants. The residues were analysed after 24 hours.

Insecticide analysis : A simple colorimetric method developed by Rangaswamy *et al.* (1976) was used for the residue analysis of carbofuran. The colorimetric method of Rangaswamy *et al.* (1977) was used for the residue analysis of methomyl.

RESULTS

Effect of physico-chemical properties of soils on carbofuran and methomyl content in the plants

Carbofuran residues in the roots of cowpea, bhendi and tomato plants grown in black soil were significantly greater than in plants grown in red and laterite soil (Table I). Further, more of this insecticide occurred in tomato roots grown in black soil.

TABLE I

Carbofuran residues in the roots of cowpea, bhendi and tomato plants grown in red laterite and black soils

Soils	Carbofuran added to soil at 10 mg/plant					
	Cowpea roots		Bhendi roots		Tomato roots	
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
	μg/g of fresh weight of roots					
Red soil	16.0	16.3	18.3	15.0	22.3	23.6
Laterite soil	20.0	26.0	17.0	16.0	20.6	18.0
Black soil	52.0	12.0	54.6	21.0	58.3	24.0
S. Em crops, soils ...	±	0.79	S. Em time		...	± 0.66
C. D. ,, ,, ...		2.29	C. D. ,,		...	1.91
S. Em. interactions :						
Crops X soils ...	±	0.40	Soils X time		...	± 1.12
C. D.		1.17	C. D.		...	3.24
Crops X time ...	±	1.12	Crops X soils			
C. D.		3.24	X periods		...	± 1.95
			C. D.		...	5.42

A similar trend in the content of methomyl in the roots of these plants grown in black soil was observed (Table III)

TABLE II

Methomyl residues in the roots of cowpea, bhendi and tomato plants grown in red, laterite and black soils

Soils	Methomyl added to soil 10 mg/plant					
	Cowpea roots		Bhendi roots		Tomato roots	
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
	—µg/g fresh weight of roots—					
Red soil	7.0	3.0	9.0	4.3	15.0	9.6
Laterite soil	7.0	5.0	11.0	6.0	17.0	11.0
Black soil	10.0	5.0	14.6	7.0	21.0	12.0

S. Em soils, crops	...	±	0.26	S. Em time	...	±	0.22
C. D. „ „	...		0.76	C. D. „	...		0.63
S. Em. interactions :							
Soils X crops	...	±	0.46	Soils X time	...	±	0.38
C. D.	...		1.34	C. D.	...		1.10
Crops X time	...	±	0.38	Soils X crops			
C. D.	...		1.10	X time	...	±	0.66
				C. D.	...		1.90

Carbofuran and methomyl residues in the shoots of cowpea, bhendi and tomato plants grown in red soil were significantly greater than in plants grown in black and laterite soil (Table IV and V).

Carbofuran and methomyl residues in the leaves of cowpea, bhendi and tomato plants grown in red soil was significantly greater than in those from black and laterite soil (Table VI and VII).

Carbofuran persisted longer in plants grown in laterite soil (Figure 1). Further more carbofuran was detected in cowpea and bhendi plants than in tomato plants.

Methomyl persisted longer in plants grown in laterite soil. However, low residues of methomyl occurred in tomato plants grown in laterite soil (Figure 2).

An increase in temperature from 5°C to 32°C, resulted in an increase in uptake of carbofuran and methomyl by plants (Figure 3 and 4). Further, the absorption of these insecticides was greater in tomato plants both at low and higher temperatures.

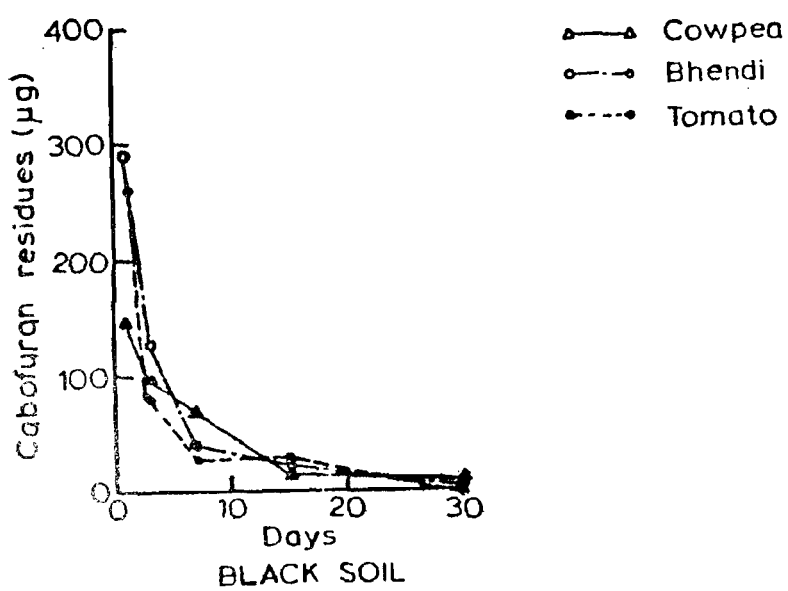
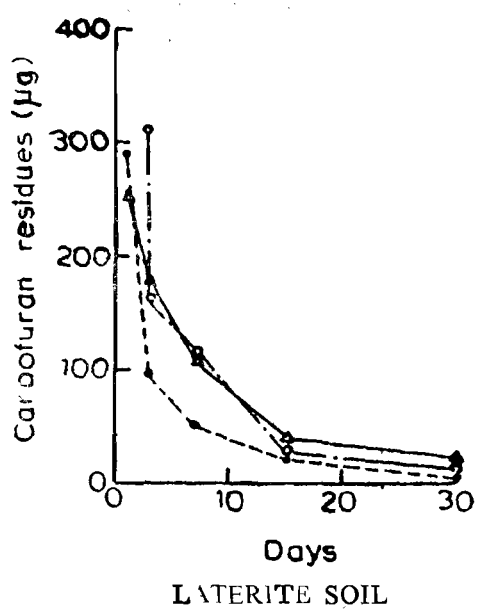
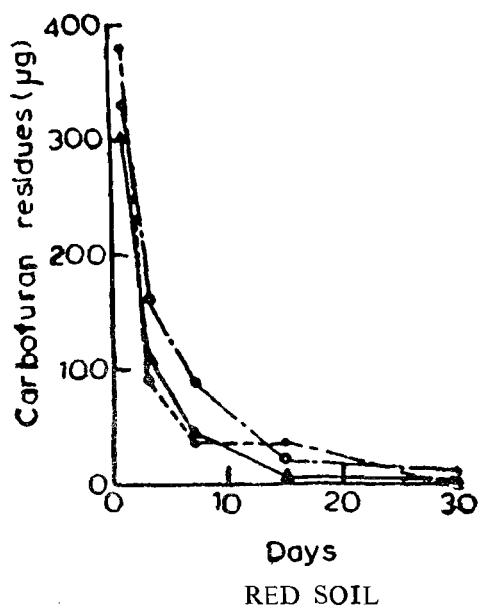


Fig 1 Influence of soil types on persistence of carbofuran in cowpea, bhendi and tomato plants.

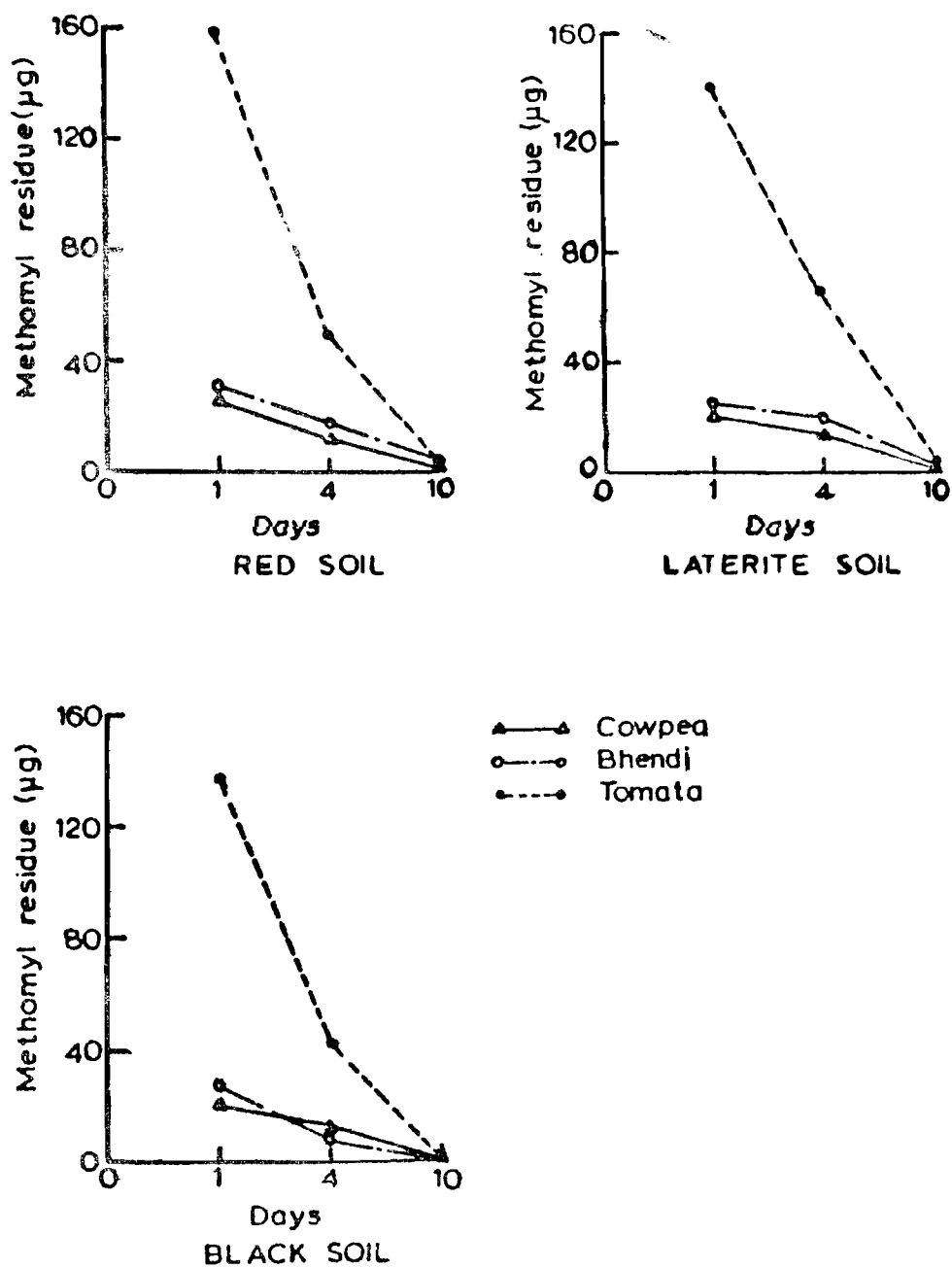


Fig. 2 Influence of soil types on persistence of methomyl in cowpea, bhendi and tomato plants.

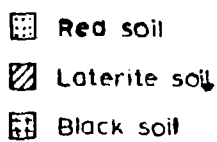
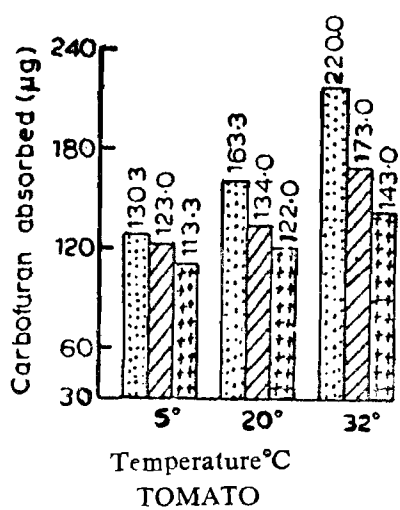
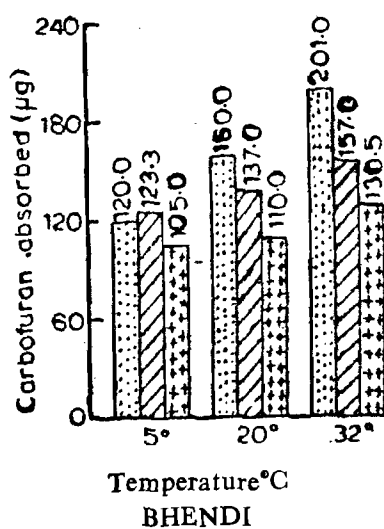
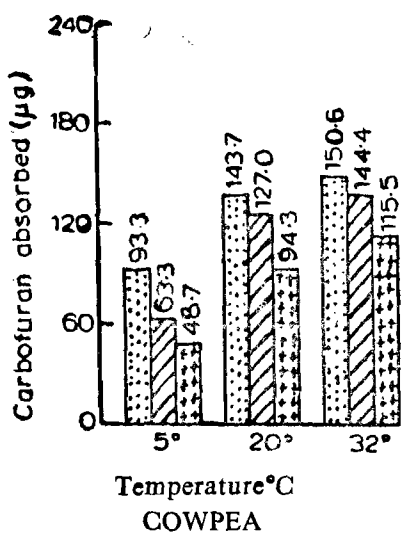


Fig. 3 Effect of temperature on absorption of carbofuran by cowpea, bhendi and tomato plants grown in red, laterite and black soils.

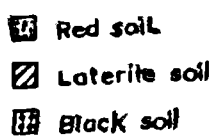
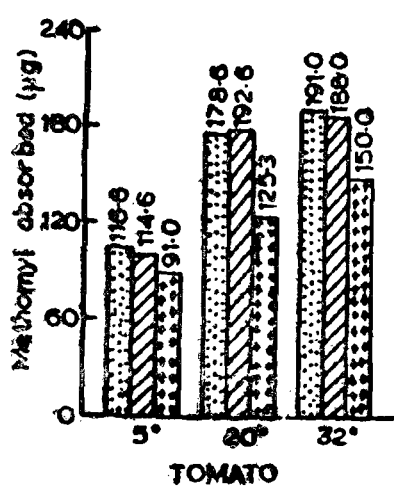
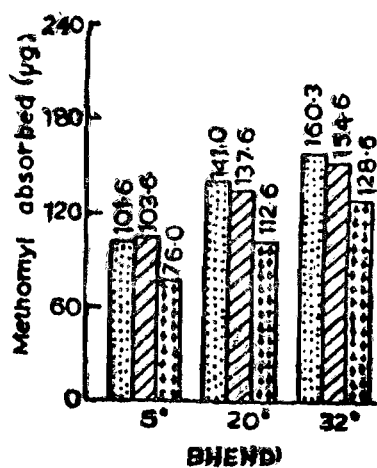
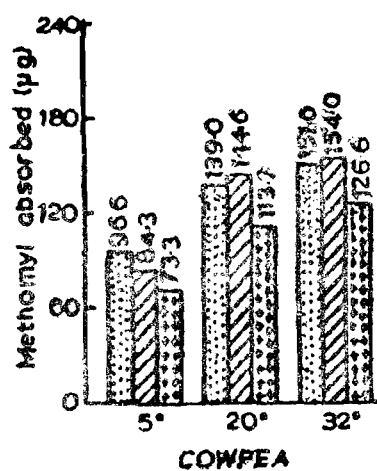


Fig. 4 Effect of temperature on absorption of methomyl by cowpea, bhendi and tomato plants grown in red, laterite and black soils.

TABLE III

Carbofuran residues in the shoots of cowpea, bhendi and tomato plants grown in red, laterite and black soils

Carbofuran applied to soil 10 mg/plant							
Soils		Cowpea shoots		Bhendi shoots		Tomato shoots	
		24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
		————Carbofuran residue $\mu\text{g/g}$ fresh tissue————					
Red soil		50.00	34.50	65.00	47.60	77.60	67.30
Laterite soil		19.80	35.30	42.00	54.00	71.00	61.60
Black soil		5.00	21.60	24.60	20.00	32.30	25.30
S. Em crops, soils ...		$\pm$	0.99	S. Em time		...	$\pm$ 0.82
C. D. „ „ ...			2.86	C. D. „		...	2.38
S. Em interactions :							
Crops X soils ...		$\pm$	1.72	Soils X periods ...		$\pm$	1.41
C. D. ...			4.97	C. D.		...	4.05
Crops X time		$\pm$	1.41	Crops X soils			
C. D. ...			4.05	X periods		...	$\pm$ 2.44
				C. D.		...	7.03

TABLE IV

Methomyl residues in the shoots of cowpea, bhendi and tomato plants grown in red, laterite and black soils

		Methomyl applied to soil 10 mg/plant					
Soils		Cowpea shoots		Bhendi shoots		Tomato shoots	
		24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
		—Methomyl in $\mu\text{g/g}$ fresh tissue—					
Red soil		30.0	23.0	40.0	31.0	53.00	41.0
Laterite soil		27.0	25.0	43.0	35.0	55.00	47.0
Black soil		17.0	19.0	37.0	31.0	45.00	39.0
S.Em	soils, crops	...	$\pm$ 1.01	S.Em	time	...	$\pm$ 0.82
C.D.	„ „	...	2.89	C.D.	„	...	2.37
S.Em interactions :							
Soils X crops		...	$\pm$ 1.75	Soils X time		...	$\pm$ 1.42
C.D.		...	5.02	C.D.		...	4.09
Crops X time		...	$\pm$ 1.42	Soils X crops X time		...	$\pm$ 2.47
C.D.		...	4.09	C.D.		...	7.08

TABLE V

Carbofuran residues in the leaves of cowpea, bhendi and tomato plants grown in red laterite and black soils

Soils	Carbofuran added to soil 10 mg/plant					
	Cowpea leaves		Bhendi leaves		Tomato leaves	
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
	Carbofuran residue $\mu\text{g/g}$ fresh tissue					
Red soil	300.0	160.0	332.0	236.0	351.0	270.6
Laterite soil	255.0	165.0	300.0	226.0	329.0	263.0
Black soil	145.0	120.0	160.6	137.3	216.3	177.0
S.Em varieties, soils	...	$\pm$ 3.45	S.Em. periods		...	$\pm$ 2.87
C.D. „ „	...	9.95	C.D. „		...	8.28
S.Em. interactions :						
Varieties X soils	...	$\pm$ 5.98	Varieties X periods		...	$\pm$ 4.88
C.D.	...	17.23	C.D.		...	14.07
Soils X periods	...	$\pm$ 4.88	Varieties X period		...	$\pm$ 8.46
C.D.	...	14.07	X soils		...	24.38
			C.D.		...	

TABLE VI

Methomyl residues in the leaves of cowpea, bhendi and tomato plants grown in red, laterite and black soils

Soils	Methomyl applied to soil 10 mg/plant					
	Cowpea leaves		Bhendi leaves		Tomato leaves	
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
	—Methomyl residue $\mu\text{g/g}$ fresh tissue—					
Red soil	60.0	55.0	73.0	65.0	83.0	69.0
Laterite soil	70.0	59.0	79.0	70.0	72.3	60.0
Black soil	51.0	51.0	63.0	55.0	65.0	60.0
S.Em. soils, crops	...	$\pm$ 1.38	S.Em. time		...	$\pm$ 1.64
C.D.	...	3.97	C.D.		...	3.33
S.Em interactions :						
Soils X crops	...	$\pm$ 2.47	Soils X time		...	$\pm$ 2.00
C.D.	...	7.08	C.D.		...	5.73
Crops X time	...	$\pm$ 2.00	Soils X crops X time		...	$\pm$ 3.49
C.D.	...	5.73	C.D.		...	10.02

The absorption of insecticides by cowpea, bhendi and tomato plants increased with an increase in the soil moisture content (Figure 5 and 6).

DISCUSSION

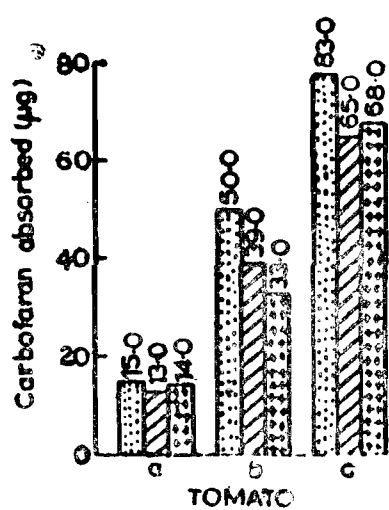
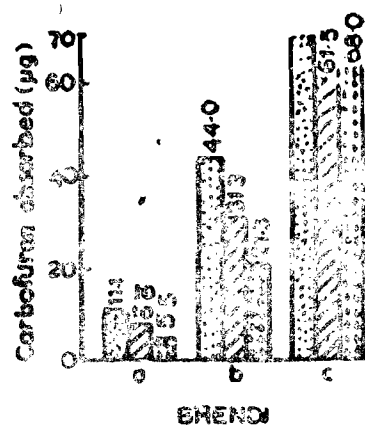
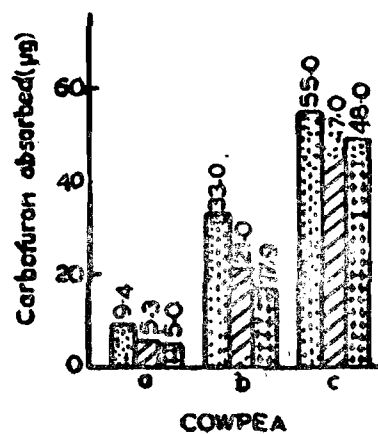
The content of carbofuran and methomyl in the roots of cowpea, bhendi and tomato plants grown in black soil was significantly greater than the plants grown in red and laterite soil. These results are in accordance with the findings of Goodman (1962) and Wain and Carter (1967). The occurrence of higher amounts of carbofuran in the roots of cowpea, bhendi and tomato plants grown in black soil are probably due to greater adsorption of carbofuran and methomyl than in red and laterite soils.

Carbofuran and methomyl content in the shoots of cowpea, bhendi and tomato plants grown in red soil was highest compared to laterite and black soils (Table III). The low content of these insecticides in the shoots of cowpea, bhendi and tomato plants grown in black soil was due to its higher adsorption on this soil.

Wain and Carter (1967) reported that highest concentrations of a chemical occurred in the roots and least in the plant tops if the chemical is adsorbed strongly by the soil, persistent in the soil and nonpersistent in the plant. The low content of carbofuran and methomyl in the shoots of these crop plants grown in black soil may be also due to their degradation under alkaline condition of this soil (Harvey and Reiser, 1973).

The carbofuran and methomyl residues in the leaves of cowpea, bhendi and tomato plants grown in red soil were highest, followed by those in laterite soil and were least in plants grown in black soil. Getzin and Chapman (1959) reported that soil type is the important factor affecting uptake of systemic insecticides and postulated that systemic insecticides are absorbed and translocated by plants more effectively from light textured soils than heavy soils.

Carbofuran was more persistent in cowpea plants grown in laterite soil, followed by black soil and it was least in red soil (Fig. 1). The reason for longer persistence of carbofuran in cowpea plants grown in laterite soil may be due to its stable nature in the acidic condition (Caro *et al.*, 1973). Read (1969) showed that carbofuran applied to acidic mineral soil persisted for 3 months. The low content of carbofuran in these vegetable crops grown in black soil may have been due to its faster degradation in alkaline condition and heavy soil texture (Cargo *et al.*, 1973 ; Read, 1969 ; Morishita and Jafferson, 1969).



-  Red soil
-  Laterite soil
-  Black soil
- a. 50% Field capacity
- b. Field capacity
- c. 2 times field capacity

Fig. 5 Effect of soil moisture on absorption of carbofuran by cowpea, bhendi and tomato plants grown in red, laterite and black soils.

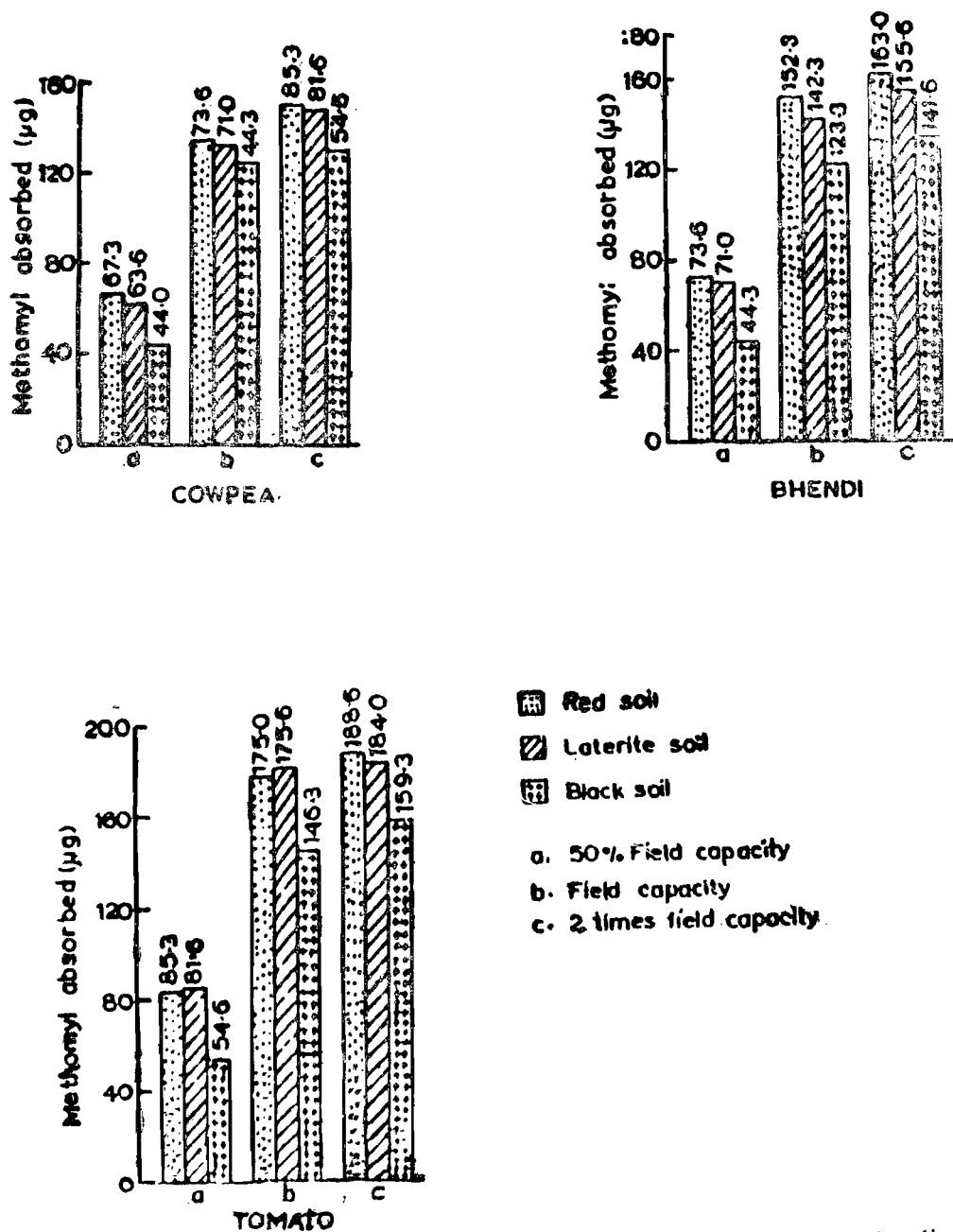


Fig. 6 Effect of soil moisture on absorption of methomyl by cowpea, bhendi and tomato plants grown in red, laterite and black soils.

Methomyl absorption was highest in tomato plants, less in bhendi and least in cowpea plants (Figure 2). The larger amount of methomyl residues in bhendi and tomato plants may be due to their high absorption capacity.

The absorption of carbofuran by cowpea, bhendi and tomato plants grown on red, laterite and black soils was more at 32°C than at 22°C and 5°C (Fig. 3). An increase in the temperature of the soil would reduce adsorption of pesticide on soil and favour the desorption process (Bailey and White, 1964; Harris and Warren, 1964; Goring, 1967, McGlamery and Slife, 1966). Increase in temperature also increases the solubility of the compounds and this in turn may reduce adsorption (Goring, 1967). As a consequence of lower adsorption and higher solubility of carbofuran at higher temperatures there was increase in the absorption of carbofuran by the crop plants. Another reason for higher absorption of carbofuran by crop plants at higher temperatures (Meyer and Anderson, 1952) may be the higher transpiration rate. Methomyl absorption by cowpea, bhendi and tomato plants grown in red, laterite and black soils increased with increase in temperatures (Figure 4). The higher rate of absorption in plants grown in red soil may be due to low absorption in this soil. Swanson *et al.* (1954) and Roberts (1963) believed that adsorption of pesticide was lower in light textured soils and amounts of methomyl in soil solution in turn influence the absorption by crop plants. Because methomyl is water soluble the quantity adsorbed on the soil particle may be low. Bailey and White (1964) reported that water solubility of an insecticide and its adsorption on to soil fractions are frequently inversely related. Further, an increase in temperature of the soil would reduce adsorption of pesticide in soil and favour the desorption process (Bailey and White, 1964; Harris and Warren, 1964; Goring, 1967; McGlamery and Slife, 1966). The increase in absorption of methomyl by these plants with increase in temperatures may be due to an increase in transpiration rate. An increase in leaf temperature promotes transpiration in plants (Salisbury and Ross, 1969).

Carbofuran and methomyl absorption by cowpea, bhendi and tomato plants grown in red, laterite and black soils increased with increase in moisture in the soil (Figure 5 and 6). The increase in absorption due to higher moisture levels was greatest in red soil, less in laterite soil and least in black soil. The low absorption of these insecticides at 50 per cent field capacity may be due to moisture stress. Bhatnagar *et al.* (1974) reported that translocation rates of systemic insecticides were low under stress. Lindquist *et al.* (1965) stated that a low moisture content in soil affected the uptake of systemic insecticides in soil. At low moisture contents the adsorption of carbofuran and methomyl by soil was high and very little insecticide was in a free state. Water can compete for adsorption sites on soil particles with insecticides because it is a very polar molecule; so in drier soils, there are

fewer water molecules to compete with the insecticide molecule for adsorption sites (Goring and Hamaker, 1973). Harris and Mazurek (1966) reported that insecticides were strongly adsorbed in dry mineral soil but were released when moistened. Thus, under conditions of higher moisture levels in soil, the insecticide absorption by these vegetable crops also increased.

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65 DEGRADATION AND PERSISTENCE OF CARBOFURAN IN SOIL

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SUMMARY

The adsorption of carbofuran by the 16 soils (natural and organic matter oxidised) and their clay fractions were determined by the batch equilibrium method. The data were plotted as per Freundlich adsorption equation and the constants 'k' and 'n' obtained. The distribution coefficients ('kd') were also derived from the carbofuran adsorption isotherms. Simple correla-

tion coefficients and multiple linear regression analysis were worked out between 'kd' values and soil properties like pH, EC, CEC, contents of clay, organic matter and sesquioxides. The adsorbed carbofuran on representative soils (both natural and organic matter oxidised) and their clay fractions were desorbed with deionised water as well as 1N CaCl₂ solution and the total amount desorbed by each extractant was then calculated.

The adsorption of carbofuran was highest in laterite soils and decreased sequentially in black, alluvial and red soils. Soil organic matter was a more potent adsorber of carbofuran than the mineral fractions and over 98 per cent of the total adsorption of carbofuran in soils was by soil organic matter alone. pH and soluble salt contents were negatively related with carbofuran absorption.

Although the adsorption of carbofuran by soil organic matter was greater in magnitude, clay contributed significantly in red, black and alluvial soils for its adsorption. The significant relationships obtained between carbofuran adsorption in soils excluding laterites and their clay fractions have clearly established the importance of clay in the inactivation of carbofuran in red, black and alluvial soils.

Studies on the desorption of carbofuran from soils with deionised water disclosed that the adsorption was reversible. It was inferred that carbofuran was bound on the mineral colloids loosely while it was held strongly on organic colloids. Carbofuran adsorption on soil and clay surfaces might be due to physical adsorbate-adsorbent attractions and not due to cation exchange mechanism since the amount of carbofuran desorbed by deionised water and 1 N CaCl₂ were almost the same.

INTRODUCTION

Once a pesticide finds its way into the environment, much of it ultimately reaches soil, where it is either totally deactivated or its biocidal efficacy becomes reduced by adsorption, leaching, volatilisation, decomposition. Although information is available on the behaviour of some herbicides in soils, data on many newer insecticides, particularly the carbamates such as carbofuran is very meagre in soils of this country. The present investigation aims to study the adsorption and desorption pattern of carbofuran in the four major soil groups of Tamil Nadu.

EXPERIMENTAL RESULTS

Sixteen surface soils (0-25 cm depth) representing the major soil groups of Tamil Nadu, namely, red, black, alluvial and laterite were collected from widely scattered locations in Tamil Nadu. The general characteristics of the soils are given in Table I. For the adsorption and desorption studies, the clay fractions of sieved soils samples (100 mesh) without organic matter were used.

The method of determining the adsorption capacity was that described by Talbert and Fletchall (1965) and Adams and Li (1971) with slight modifications.

TABLE
General physical and chemical properties

Property/ constituent	S1	Red Soils		S4	S5	Black Soils	
		S2	S3			S6	S7
Clay%	16.95	13.30	8.87	23.10	40.90	53.00	43.00
Textural class	Sandy loam	Sandy loam	Loamy sand	Sandy clay loam	Sandy clay	Clay	Sandy clay
Field capacity %	15.60	14.80	13.16	21.42	30.60	38.30	33.27
pH	7.9	7.8	7.7	7.5	8.2	8.2	8.1
E.C. (millimhos cm)	0.35	0.20	0.25	0.28	0.60	0.85	0.65
Organic matter %	0.55	0.26	0.48	0.42	0.76	0.57	0.72
Sesquioxides %	10.10	10.75	9.30	11.20	12.97	10.20	10.90
CEC-Soils %	11.80	5.40	8.75	10.30	46.10	61.40	38.60
CEC-Clay %	24.15	15.60	18.40	28.25	88.00	95.00	66.55

Preliminary studies indicated that estimates of the adsorption were reproducible with a ratio of 1 g soil of 0.5 g of clay in 25 ml of solution. Equilibrium was essentially complete between the quantities of carbofuran in solution and that on the adsorbent in 4 hours and increasing the contact time did not increase adsorption beyond this values. Hence the following procedure was adopted. Aliquots of 1 g of soil (natural and organic matter oxidised) or 0.5 g clay samples of each soil were transferred to a series of stoppered centrifuge tubes to which were added different aliquots of stock solution to give a convenient range of 20, 40, 60, 80 and 100 μ g of carbofuran/g of adsorbent and the final volume of the suspension was made up to 25 ml with distilled water. The tubes were stoppered tightly and shaken for 6 hours in a shaker and centrifuged for 15 min at 2000 rpm. The concentration of carbofuran in a suitable aliquot of the supernatant was estimated by the method of Gupta and Dewan (1971).

The quantity of carbofuran adsorbed was calculated from the difference between the amounts initially added and the quantity remaining in the equilibrium solution. The data were plotted as per the Freundlich adsorption equation, since this could be applied to heterogenous systems such as soil-pesticide-water suspension as

$$x/m = kC^{1/n} \quad \text{where } x/m = \text{Quantity of carbofuran adsorbed per unit weight of the soil or clay } (\mu\text{g/g})$$

I

of the soils used for the study

S8	S9	Alluvial Soils		S12	S13	Laterite Soils		S16
		S10	S11			S14	S15	
32.80	20.87	18.10	38.20	15.80	25.25	25.80	14.60	22.43
Sandy clay	Sandy clay loam	Sandy loam	Clay loam	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy loam	Sandy clay loam
30.55	19.20	20.40	28.50	20.75	20.21	21.50	17.40	22.00
8.20	7.8	7.1	7.2	7.4	5.2	5.1	5.3	4.8
0.80	0.36	0.30	0.60	0.35	0.20	0.18	0.20	0.12
0.72	0.83	0.55	0.83	0.83	3.63	2.72	1.48	4.29
10.22	9.72	12.22	10.95	5.95	10.66	14.99	13.16	11.51
50.22	22.35	21.35	35.30	10.90	19.15	21.31	9.15	21.41
90.12	40.30	28.10	46.25	24.91	20.25	25.20	22.30	28.50

C—equilibrium concentration (ppm). 'k' and 'n' are constants.

The constants 'k' and 'n' were obtained from the intercept and reciprocal of the slope of the straight line graph obtained from plotting the data on a log-log scale i.e., $\log x/m = \log k + 1/n \log c$.

The desorption study was conducted with soils S1, S8, S11 and S16 representing red, black, alluvial and laterite soils. Deionised water and 1 N CaCl₂ solution were used for desorbing carbofuran from soils and clays. The soils (natural and organic matter oxidised) and their clay fractions of the four soils were used as adsorbents. Quadruplicate samples of 1 g of each adsorbent (only 0.5 g for clay) were transferred to the stoppered centrifuge tubes. Aqueous carbofuran solution at a rate of 100 µg/g of adsorbent (150 µg/g of natural and organic matter oxidised laterite soil) was added and the volume made up to 25 ml with distilled water. After shaking 6 hours in a shaker, it was centrifuged and the concentration of carbofuran in the supernatant was determined, by the method of Gupta and Dewan (1971). The quantities of carbofuran adsorbed on soils and clays was then calculated. There were four replicates for each adsorbent and to the first two replicates, water was added and made up to 25 ml, shaken for 6 hours in a shaker, centrifuged and the aqueous phase decanted and the concentration of carbofuran in the equilibrium solution was estimated. The process of desorption of carbofuran with deionised water was repeated 5 times. A similar desorption study was done using 1 N CaCl₂

solution with the remaining 2 replications of each adsorbent. From this, the percentage of carbofuran desorbed by water and 1 N CaCl₂ solution was calculated.

RESULTS AND DISCUSSION

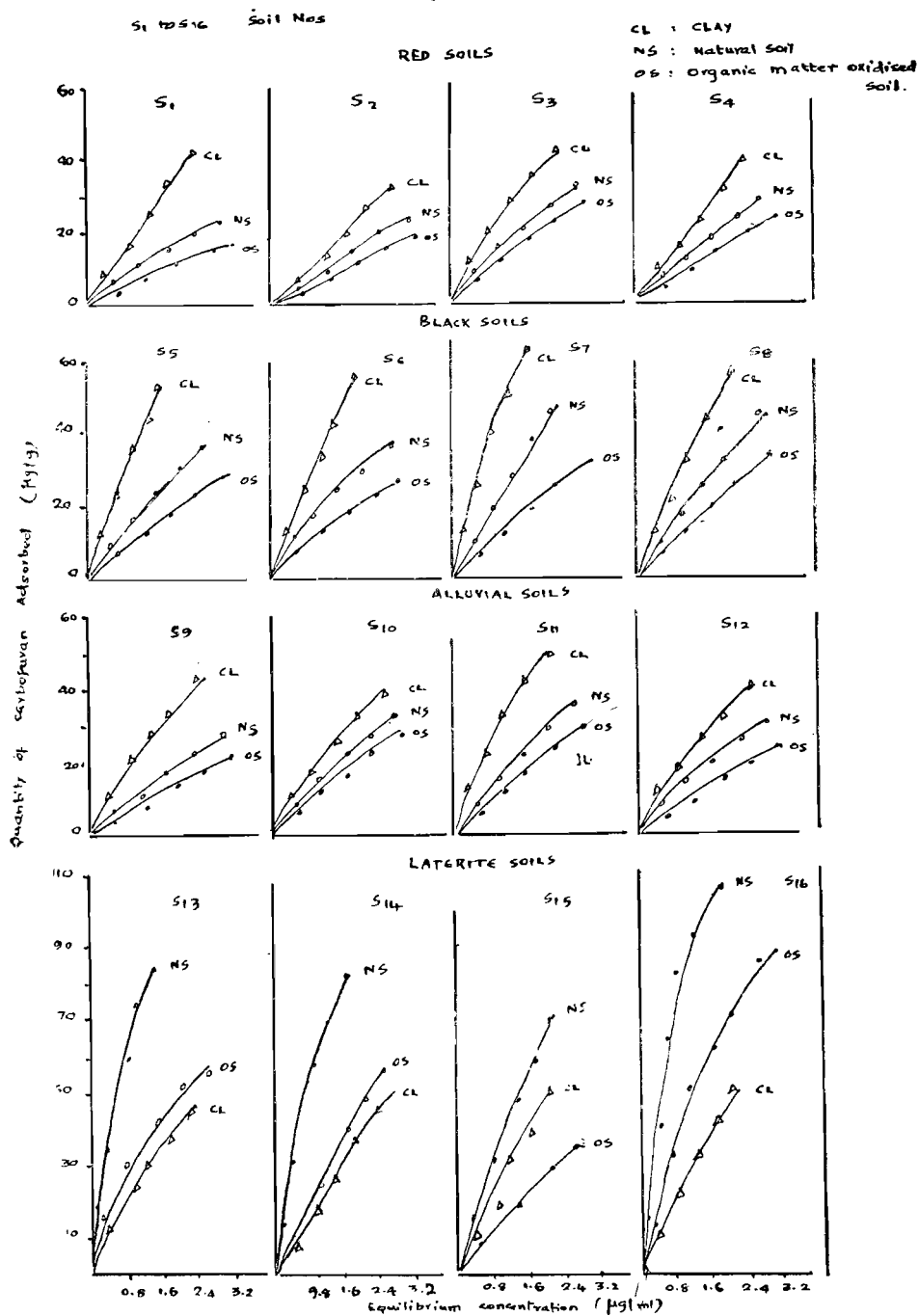
The quantities of carbofuran adsorbed on the 16 soils (natural as well as organic matter oxidised) and their clay fractions subsequent to the addition of different quantities of this compound are given in Fig 1. The amount of carbofuran adsorbed increased gradually with increase in the amount of added toxicant. The adsorption of carbofuran on natural soils was higher than on the oxidised soils. Adsorption was highest in laterite soils followed by black, alluvial and red soils. The percentage adsorption of carbofuran ranged from 60.94 to 77.52 for laterite, 37.76 to 44.79 for black, 27.07 to 39.92 for alluvial and 20.04 to 34.85 for red soils.

The data on the extent of adsorption of carbofuran on natural soils, organic matter oxidised soils and their clay fractions (Fig 1) show that a large portion is adsorbed. The adsorption data were best represented as adsorption isotherms. The Freundlich equation was used to fit the isotherms. Furmidge and Osgerby (1967), Chopra *et al.* (1970), Mithyantha (1973) and several others used the Freundlich equation to explain the adsorption pattern of various groups of pesticides on soils and clays.

According to the system of classification of adsorption isotherms given by Gils *et al.* (1960), the isotherm obtained in the present study in almost all of the soils were of L-type. This indicated that the soils as well as their clay fractions possessed a high affinity for carbofuran. The L-type isotherms have been considered to be the most common ones occurring with pesticides (Weber, 1970; Mithyantha, 1973).

The Freundlich's 'k' constant was the highest for laterite soils and lowest for red soils (Table II). The distribution coefficient 'kd' was also high for laterite soils, followed by black, alluvial and red soils (Table III). Variations in the contents of organic matter and clay, and pH of the various types of soils were partly responsible for the observed differences. Laterite soils with a low pH and containing 1.55 to 4.29 per cent of organic matter adsorbed the highest amounts of carbofuran; the order of carbofuran adsorption by the four soil groups was the same as that of their organic matter content. Although the clay content and surface area of black and alluvial soils were higher than those of laterite soils, the extent of carbofuran adsorption did not vary with these properties. The contents of clay and organic matter were low in red soils and least adsorption of carbofuran was observed in

Fig: 1



ADSORPTION ISOTHERMS OF CARBOSULFAN BY SOILS AND THEIR CLAY FRACTIONS.

these soils. Jamet and Piedallu (1975) observed that the adsorption of carbofuran in 9 types of soils increased with increase in organic matter content and the isotherms of carbofuran adsorption and desorption satisfied the Freundlich equation.

The adsorption of carbofuran on organic matter oxidised soils was much less compared to that on the corresponding natural soils. The reduction was highly pronounced in laterite soils wherein the organic matter oxidation was higher than the other groups of soils. The decrease in adsorption capacity of soils following

TABLE II
Adsorption of carbofuran by soils and their clay fractions
(Freundlich 'K' and 'n' values)

Soil number	Freundlich 'k'			'n'		
	NS	OS	Cl	NS	OS	Cl
S ₁	9.09	4.52	18.41	0.89	0.67	1.05
S ₂	6.26	4.57	10.88	0.94	0.98	0.79
S ₃	14.13	10.57	23.46	1.25	1.11	1.41
S ₄	9.12	5.88	15.49	0.91	0.81	1.05
S ₅	15.14	7.41	32.14	0.95	0.80	1.02
S ₆	17.78	11.48	30.20	1.38	1.28	1.23
S ₇	19.86	10.02	42.66	0.86	0.84	1.17
S ₈	16.97	11.19	29.65	0.93	0.95	1.03
S ₉	9.56	5.31	18.41	1.08	0.79	1.16
S ₁₀	15.17	9.49	11.08	1.26	0.93	1.38
S ₁₁	15.85	10.59	30.20	1.21	1.02	1.39
S ₁₂	13.18	5.63	20.63	1.21	0.84	1.34
S ₁₃	70.79	32.59	18.62	0.69	0.77	0.92
S ₁₄	63.10	23.99	19.95	1.11	0.99	1.38
S ₁₅	38.02	15.14	23.99	1.23	1.13	0.89
S ₁₆	87.10	41.69	28.84	1.00	1.16	1.13

N.S. - Natural soil, O.S. - Organic matter oxidised soil, Cl - Clay

oxidation of organic matter indicated that organic adsorptive surfaces had a greater capacity to retain the toxicant than mineral ones. The calculated adsorption capacity per unit weight of the organic matter expressed as distribution coefficient (kd) (Table IV) was very high and 'kd' values ranged from 1322 to 1480 $\mu\text{g/g}$ for laterite, 641 to 1250 $\mu\text{g/g}$ for black, 361.44 to 783.13 $\mu\text{g/g}$ for alluvial and 520 to 729 $\mu\text{g/g}$ for red soils. But the 'kd' values for clays separated from the soils were only 30 to 42 $\mu\text{g/g}$ for black, 19 to 30 $\mu\text{g/g}$ for alluvial, 20 to 27 $\mu\text{g/g}$ for laterite and 9.50 to 23 $\mu\text{g/g}$ for red soils. This explained why the adsorption of carbofuran was higher

in laterite soils than others. Further, the organic matter of the soils was correlated highly with the 'kd' value of natural soils($r=0.980^*$). Multiple linear regression analysis showed that only organic matter and pH were significantly related with 'kd' values of carbofuran, adsorption, the former was positively related while the latter was negatively correlated. The affinity of organic matter towards the carbofuran was so great that the organic matter of soils contributed over 98 per cent to the total adsorption. These findings are in accordance with earlier reports indicating that soil

TABLE III
*Carbofuran adsorption by soils and their clay fractions :
 Distribution coefficients (kd)*

Soil number	'kd' values ($\mu\text{g/g}$)		
	Natural Soil	Organic Matter oxidised soil	Clay
S1	9.00	4.50	16.50
S2	6.00	4.00	9.50
S3	14.00	10.50	23.00
S4	9.00	6.00	15.00
S5	15.00	6.50	35.00
S6	18.00	12.00	32.00
S7	22.00	12.00	42.00
S8	16.50	12.00	30.00
S9	10.00	6.00	19.00
S10	14.50	10.00	20.00
S11	16.00	11.00	30.00
S12	14.00	8.00	21.00
S13	72.00	32.00	25.00
S14	63.00	26.00	20.00
S15	39.00	16.00	28.00
S16	85.00	44.00	27.00

organic matter of a soil is most important factor in describing adsorption of pesticides in soils (Edwards *et al.*, 1957; Talbert and Fletchall, 1965; Harris, 1970; Campbell *et al.*, 1971; Adams and Li, 1971; Leenher and Ahlrichs, 1971; Mithyantha, 1973).

The soil pH and soluble salt contents of black, alluvial and red soils were high. But the laterite soils had a very low pH and also a low soluble salt content. Further the contents of free CaCO₃ in most of the black and alluvial soils were high, whereas laterite soils did not contain CaCO₃. Thus, the higher adsorption of car-

bofuran in laterite soils could be attributed to the low pH and low soluble salt content that favoured the adsorption of carbofuran on soil colloids without much competition. Conversely, the comparatively lower adsorption in black, alluvial and red soils might be due to the competition of soluble salts with insecticide for the

TABLE IV
Contribution of soil organic matter and mineral fractions towards carbofuran adsorption : Distribution coefficients (*kd*) (calculated values)

Soil number	μg of carbofuran adsorbed at the equilibrium concentration of 1 $\mu\text{g}/\text{ml}$ (<i>kd</i>)	
	Organic matter	Mineral fraction
S1	3.50 (636.36)	5.00
S2	1.50 (576.06)	4.50
S3	3.50 (729.00)	9.50
S4	2.50 (520.00)	5.50
S5	5.50 (705.12)	9.50
S6	7.00 (1228.06)	11.50
S7	9.00 (1250.00)	11.00
S8	5.00 (641.00)	11.00
S9	3.00 (361.44)	5.50
S10	4.00 (689.65)	11.00
S11	4.00 (481.92)	11.00
S12	6.50 (783.13)	6.50
S13	48.00 (1322.00)	20.00
S14	40.25 (1480.00)	22.75
S15	22.00 (1419.00)	16.00
S16	57.19 (1333.00)	26.81

Figures in parantheses indicate the adsorption capacity of soil organic matter per unit weight (calculated values).

adsorption sites and due to the instability of carbofuran molecules at a higher pH. The negative correlation which existed between pH and soluble salts with the '*kd*' values support the above conclusion.

The adsorption of carbofuran by fractions separated from the soils showed that clay too possessed a considerable affinity for carbofuran as evidenced by the higher Freundlich's '*k*' constants. Clays separated from black soils showed the highest adsorption followed by laterite, alluvial and red soils. The higher adsorption of carbofuran by black soil clays was due to the presence of large quantity of 2:1 type of clay minerals such as montmorillonite and illite. This agrees with Beri-

gari (1967) and Bowman *et al.* (1970). The lower sorption of carbofuran by laterite and soil clays might be due to the dominance of kaolinite and illite minerals and are in agreement with Weber *et al.* (1965) and weber and Scott (1966).

Although the adsorption of carbofuran on the soil organic matter was much greater in magnitude than the adsorption on to clay, the role of clay is probably important because most soils contained more clay than organic matter. The calculated values of carbofuran adsorption in soils as contributed by the clay and organic matter fractions showed that in red, black and alluvial soils, the total contribution made by the clay was more than the organic matter because these soils contained higher quantities of clay and low amounts of organic matter. Conversely, the contribution made by the organic matter in laterite soils was high because the content of organic matter was high in these soils. Hence simple correlations were worked out between 'kd' values of red, black, alluvial soils and soil properties. Clay was highly correlated with 'kd' values ($r=0.686^{**}$). However, when the laterite soils were also included, the relationship of clay with 'kd' values was not significant. This indicated the overriding effect of organic matter when the soils contained higher amounts of organic matter. The significantly high correlation between the 'kd' values of organic matter oxidised soils (red, black and alluvial) and clay, strongly supports the reasoning that clay is mainly responsible for retention of carbofuran in soils containing low amounts of organic matter. The high correlation of CEC of soils with 'kd' values adds further strength to the present findings. The findings of Abdel latif *et al.* (1967). Scott and Weber (1967) and Bowling (1970) also revealed the importance of clay in the inactivation of pesticides in soils and lend support to the present findings.

The desorption of carbofuran by deionised water showed, that, in general, the desorption from organic matter oxidised soils was higher than in the natural soils. The laterite soil (S16) retained higher quantities of carbofuran even after 5 extractions and the desorption was 45.16 percent. However, the percentage desorption was increased to 70.40 per cent when a portion of the organic matter of the soil was oxidised. The fact that the oxidation of organic matter increased the desorption of carbofuran, suggests that the organic matter of a soil is important in desorption. The higher retention of carbofuran in laterite soil was due to higher content of organic matter (4.29 percent). It was observed that the desorption was very high in all the other types of soils and could be attributed to the low content of organic matter. This agrees with the findings of Yuen and Hilton (1962) and Adams (1966).

The desorption of carbofuran from clay fractions separated from soils indicated that, in general, higher amounts were desorbed by all the clays although the black

TABLE V

Desorption of carbofuran by soils and their clay fractions with deionised water and 1N CaCl₂ solution

Desorption of carbofuran by soils and their clay fractions with deionised water																	
Soil No.	Adsorbent	Amount adsorbed (ppm)	Quantities extracted with deionised water (ppm)					% desorbed	Amount adsorbed (ppm)	Quantities extracted with 1N CaCl ₂ (ppm)					% desorbed		
			I	II	III	IV	V			Total	I	II	III	IV		V	Total
S ₁	Natural soil	29.85	14.80	8.10	3.42	2.00	ND	28.32	94.50	29.85	18.45	3.60	4.10	0.35	ND	26.40	88.50
	O.M. Oxidised soil	22.60	14.26	3.20	2.60	0.56	ND	20.62	91.23	22.60	15.50	3.20	1.60	1.40	ND	20.70	94.90
	Clay	45.27	20.15	12.66	5.29	2.10	0.40	40.60	89.68	45.27	18.31	7.27	7.36	5.60	3.00	41.54	91.76
	Natural soil	45.00	17.35	10.12	3.25	3.50	1.15	35.37	78.30	45.00	18.55	6.27	5.60	1.42	1.60	33.44	74.31
S ₈	O.M. Oxidised soil	33.00	17.47	5.20	2.55	2.40	1.66	29.65	89.00	33.00	15.27	6.40	4.06	1.20	1.40	25.33	88.00
	Clay	55.00	20.50	8.47	6.31	4.25	2.00	51.03	72.00	55.00	20.29	10.75	4.31	4.05	2.60	42.00	76.40
	Natural soil	35.00	16.35	7.00	2.12	5.00	0.30	28.77	82.20	35.00	13.27	6.23	3.60	2.10	1.80	27.00	77.14
	O.M. Oxidised soil	30.00	15.00	5.29	2.85	1.42	2.65	27.21	89.00	30.00	16.21	4.32	2.41	1.50	1.36	25.80	86.00
S ₁₁	Clay	49.00	18.30	10.65	7.51	5.08	1.17	42.71	87.13	49.00	17.50	15.48	5.66	3.05	1.88	43.60	89.02
	Natural soil	105.00	15.55	10.20	8.20	8.26	5.16	47.37	45.16	105.00	12.41	16.92	9.46	7.25	7.61	47.65	41.60
	O.M. Oxidised soil	70.00	17.20	12.31	8.45	6.20	5.12	49.28	70.40	70.00	19.25	11.72	8.29	4.80	4.60	48.66	69.51
	Clay	52.00	15.59	13.10	8.11	4.20	4.50	45.50	87.51	52.00	16.20	12.30	9.41	6.20	3.26	47.37	91.99

ND: Not detectable

soil clays was a little lower than the others; presumably the abundance of montmorillonite present in the black soil accounted for such higher retention.

Desorption studies with 1N CaCl_2 indicated the same trend of carbofuran desorption as that of water desorption and suggested that the adsorption of carbofuran might be due to physical adsorbate-adsorbent attraction and not due to cation exchange mechanism. The EC of the soils and 'kd' values were negatively related which also confirmed the hypothesis that carbofuran adsorption is not ionic in nature. These findings closely agree with those of Leanheer and Ahlrichs (1971), and Carringer *et al.* (1975).

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66

DISSIPATION OF CARBOFURAN IN RICE SOILS

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SUMMARY

The dissipation of carbofuran in an acidic rice soil (pH 4.5, O.M. 3.85) was assessed in a field experiment. The effect of soil amelioration with lime on the rate of dissipation of carbofuran was studied. Carbofuran applied at the rate of 0.54 kg a.i/ha dissipated to undetectable levels 21 days after application. In plants treated with carbofuran at intervals of 1 to 7 days after lime application, considerable amounts of the insecticide persisted even after three weeks.

INTRODUCTION

Carbofuran (2, 3 dihydro 2, 2, dimethyl-7-benzofuranyl methyl carbamate) at the rate of 18.5 kg, 3 per cent granules/ha is used extensively for the control of paddy pests such as brown planthopper, stem borer, gall fly etc. (Anon., 1978). Rice is grown in Kerala mainly under flooded conditions and the soil type is acidic in most cases. Because, the pH of these soils is generally below 5.5, liming has been recommended at the rate of 350 kg/ha as a basal application and 250 kg/ha as a top dressing one month after transplanting. There is a possibility of interaction between carbofuran and the lime used as top dressing. Carbofuran is reported to persist longer under acidic soil conditions (Venkateswaren *et al.*, 1977) and degrade faster in neutral and alkaline soils (Getzin, 1977). Hence the present study is undertaken to investigate the effect of liming acidic rice soil on the rate of dissipation of carbofuran.

MATERIAL AND METHODS

A field trial at the Instructional Farm, College of Agriculture, Vellayani, Kerala during March-July, 1978 used the Triveni variety of rice in a silty loam soil of medium

fertility of pH=4.5 and O.M.=3.85 per cent was laid out as a randomised block design with plots, 3 m x 3 m. Separate irrigation and drainage channels were provided for the plots to avoid inter plot contamination. The treatments were :

- 1) T₁ — Carbofuran alone 30 days after planting.
- 2) T₂ — Lime and carbofuran 30 days after planting.
- 3) T₃ — Lime on 30th day + carbofuran on 31st day.
- 4) T₄ — Lime on 30th day + carbofuran on 32nd day.
- 5) T₅ — Lime on 30th day + carbofuran on 33rd day.
- 6) T₆ — Lime on 30th day + carbofuran on 37th day.

Carbofuran (furadan 3G) was applied at the rate of 0.54 kg a.i./ha and lime at the rate of 300 kg/ha. The treatments were replicated three times. Soil samples were taken from various plots 0, 1, 3, 7, 14 and 21 days after the carbofuran treatment. The samples collected from each plot were mixed thoroughly and a 25 g portion of the wet soil was transferred to a 250 ml Erlenmeyer flask. The pesticide residue was extracted, separated on TLC and eluted with methanol following the procedure adopted by Venkateswarlu *et al.* (1977). The colorimetric determination of carbofuran residues were done by the method developed by Gupta and Dewan (1971). The pink colour developed was read against the appropriate blank in a spectronic 20 (Bausch and Lomb, U.S.A.) at 550 nm.

RESULTS AND DISCUSSION

The data on the dissipation of carbofuran in lime treated and untreated plots are given in Table I. The initial recovery of carbofuran in plots treated with lime was lower than that of the unlimed plots. This may be due to the lime providing additional sites of adsorption for the insecticide. One day after the application of carbofuran the recovery of residues was uniformly higher in all the treatments, values ranging from a maximum of 10.73 ppm in plots which received the pesticide 7 days after liming to 8.46 ppm from the plot treated with carbofuran 2 days after liming. The recovery of residues dropped to low levels within 14 days in unlimed plots and plots where carbofuran was applied with lime. In these plots, carbofuran residues were undetectable 21 days after its application, whereas in other plots in which carbofuran was applied one day after liming, there were appreciable residues of carbofuran.

The difference in the rates of dissipation of carbofuran under different treatments may be attributed to the effect of lime on (i) the general microbial activity and (ii) the chemical degradation of carbofuran. Liming of an acidic soil alters not only the pH of the soil but also stimulates general microbial activity (Rajaram, 1964).

This may result in the inactivation of the group of soil microflora involved in biodegradation (Venkateswarlu *et al.*, 1977) and active detoxification (Kandaswami *et al.*, 1977) of carbofuran, particularly if their competitive ability is very low. Similar inhibitory effect was reported on parathion hydrolysing microorganisms after addition of lime and other oxidising materials (Rajaram *et al.*, 1978). Under such conditions carbofuran persists longer in lime-treated plots. But addition of alkaline

TABLE I
Persistence of carbofuran in soil when applied at different intervals after liming
(ppm - moisture free soil basis)

Treatments	Period after application of carbofuran (days)					
	0	1	3	7	14	21
Carbofuran alone without lime	7.09	9.33	6.38	5.36	1.89	ND
Carbofuran along with lime	5.12	8.71	4.50	2.63	1.12	ND
Carbofuran applied one day after liming	3.28	10.56	5.19	4.13	5.75	1.60
Carbofuran applied two days after liming	6.88	8.46	4.77	2.76	2.07	1.48
Carbofuran applied three days after liming	5.22	8.71	6.15	4.25	2.78	2.51
Carbofuran applied seven days after liming	5.75	10.73	6.97	5.96	6.12	2.12

materials to carbofuran can decompose it by alkaline hydrolysis. Hence, when it is applied with lime the alkaline hydrolysis could have resulted in enhanced dissipation of the pesticide.

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67 PERSISTENCE OF CARBARYL RESIDUES IN CLAY LOAM SOIL AND THEIR UPTAKE AND TRANSLOCATION INTO CROPS

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SUMMARY

Residues of 389.4, 692.0 and 1036.9 ppm of carbaryl in soil from 20, 40 and 60 kg a.i./ha and 77.9 to 232.2, 152.5 to 408.9 and 242.3 to 545.5 ppm in soil from 15, 30 and 45 kg a.i./ha respectively were recorded. These residues decreased by 73-98 per cent after 15 days of treatment. The persistence of carbaryl in soil was between 15 and 100 days. The 'half-life' values ranged from 3.54 to 13.87 days. Residues of carbaryl in maize (plants and grains), beetroot, carrot and radish were in detectable level (BDL) at harvest time. The germination and growth of all these crops were not affected adversely by carbaryl when applied to soil at 15-60 kg a.i./ha.

Carbaryl (1. naphthyl N methyl carbamate), a synthetic organic carbamate insecticide has been used as a soil insecticide against termites (Bhatnagar, 1960); eye-gnats *Hippelates collusor* (Mulla, 1960 a); black cut worms, *Agrotis ypsilon* (Begg *et al.*, 1963); mites (Stegeman, 1964); green June beetles, *Cotinis nitida* (Campbell *et al.*, 1966), larvae of fruit flies, *Dacus cucurbitae* (Dale *et al.* 1966) and wire worms (Edwards and Thompson, 1971). Dhali and Lal (1971) reported that carbaryl was mildly systemic and might translocate to crop plants when applied to soil. The persistence of insecticide in the soil could cause hazards in the form of absorbed residues in edible portions of the crop plants grown in treated soils. The present programme of investigations was a part of approved plan of work by USDA and GOI for a PL-480 project entitled "Studies on the phytotoxicity and persistence of some soil insecticides and their residues in the crops grown on treated soils".

MATERIAL AND METHODS

Residues of carbaryl in soil and the crops, maize, beetroot, carrot and radish were determined by a spectrophotometric method (Benson and Finocchiaro, 1965) and by an enzymatic method (Zweig and Archer, 1958).

Carbaryl was extracted from soil, roots, leaves, stems and grains using distilled methylene chloride as a solvent at a rate of 3 to 5 ml per gram of sample.

Clean up of the extracts was required only for the estimation of residue by the spectrophotometric method suggested by Benson and Finocchiaro (1965) with slight change.

The experiments were at the farm of the College of Agriculture, Udaipur from kharif 1972 to 1974 in randomized block design.

In preliminary experiments with 5 per cent carbaryl dust applied at 5, 10 and 20 kg a.i./ha, the average deposits were 3.3, 9.03 and 14.15 ppm respectively. In another experiment, the granular formulation persisted longer than dust. So 4 per cent carbaryl granules were selected for the study. The dosages were also increased to 20, 40 and 60 kg a.i./ha which gave very high deposits, so the dosages were lowered to 15, 30 and 45 kg a.i./ha in the subsequent years of the experiment.

Furrows were made to the requisite depth and 4 per cent carbaryl granules drilled uniformly by hand to about 10 cm deep. The furrows were half-filled with soil and sown with maize in Kharif followed by beetroot, carrot and radish in season.

Soil and plant samples were collected and quartered until the required amount of 50-100 g of soil and 100-300 g of plant samples were obtained.

RESULTS AND DISCUSSION

The average recoveries obtained spectrophotometrically from clay loam soil, maize plant, maize grain, beetroot, carrot, radish leaves were 82.776, 88.906, 91.720, 92.687, 88.834, 91.049, 88.448, 87.340, 88.183 and 90.332 per cent respectively. The average recoveries by the enzymatic method were 92.881, 96.142, 94.352, 93.332, 91.030, 93.297, 87.397, 89.621, 90.318 and 90.885 per cent respectively.

In the kharif season average residues of 389.4, 692.0 and 1026.9 ppm in soil from applications of 20, 40 and 60 kg a.i./ha (Table II a) and 208.7, 408.9 and

545.5 ppm in 1973 (Table 2 b) and 232.2, 397.5 and 525.9 ppm in 1974 (Table 2 c) from applications of 15, 30 and 45 Kg a.i./ha were obtained respectively. The variation in residues was due to the amount of 4 per cent carbaryl granules which varied according to the length and number of furrows per plot every year (Table I).

TABLE I
Amount of 4% carbaryl granules required for application in different plots

Year	Treatment a. i./ha (kg)	Plot size (m ²)	Amount of granules per plot (g)	No. of furrows per plot	Amount of granules per furrow (g)
Kharif	20	10 x 4	2000	14	142.8
1972	40	10 x 4	4000	14	285.6
	60	10 x 4	6000	14	428.4
Rabj					
1972-73 and	15	3 x 1	112.5	5	22.5
1973-74	30	3 x 1	225.0	5	45.0
	45	3 x 1	337.5	5	67.5
Kharif	15	4 x 3	450.0	5	90.0
1973 and	30	4 x 3	900.0	5	180.0
1974	45	4 x 3	1350	5	270.0

TABLE IIa
*Carbaryl residues in clay loam soil and in maize grown on treated soil
(Treatment on 6-8-1972)*

Days after treatment	20 Kg a.i./ha		40 Kg a.i./ha		60 Kg a.i./ha		Maize
	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	Average residues (ppm)
0	389.498	—	692.055	—	1026.953	—	
5	197.033	49.41	389.498	43.72	651.589	36.55	
10	166.032	57.37	290.115	58.08	436.118	57.53	
15	104.738	73.11	139.230	79.88	211.050	79.45	
31	19.908	94.89	28.602	95.87	41.643	95.94	
46	0.185	99.95	0.360	99.95	2.691	99.74	BDL
61	0.145	99.96	0.204	99.97	1.164	99.89	BDL
92	BDL	100.00	0.062	99.99	0.337	99.97	BDL
Half-life (days)	8.03	—	6.06	—	7.24	—	

About 94-96 per cent of the carbaryl was lost in 31 days but traces persisted upto 92-108 days, except in 1973 when 80-86 per cent of the insecticide was lost in 5 days and all by 31 days (Table 2 b). The 'half-life' values ranged from 6.06 to 8.03 days in 1972, 3.54 to 4.10 days in 1973 and 12.81 to 13.26 days in 1974.

In the rabi season average deposits in 1972-73 ranged from 77.9 to 110.0, 152.5 to 172.4 and 242.3 to 293.7 ppm and in 1973-74 ranged from 103.5 to 123.5, 167.5 to 216.5 and 303.7 to 421.8 ppm from the application of 15, 30 and 45 Kg

TABLE IIb
Carbaryl residues in clay loam soil and in maize grown on treated soil
(Treatment on 30-6-1973)

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Maize plant
	Average residues (ppm)	% reduction	Average residues	% reduction	Average residues	% reduction	Average residues (ppm)
0	208.785 (207.600)	—	408.929 (407.250)	—	543.555 (507.750)		
5	28.844 (33.201)	86.18	64.593 (65.700)	84.20	111.219 (119.133)	79.61	
10	2.034 (1.343)	99.63	5.676 (5.210)	98.61	13.790 (13.980)	97.47	
15	0.170 (0.023)	99.92	1.590 (2.520)	99.61	7.435 (6.411)	98.64	
31	BDL (BDL)	100.00	BDL (0.61)	100.00	BDL (0.271)	100.00	BDL
47	—	—	BDL (BDL)	100.00	BDL	100.00	BDL
75	—	—	—	—	—	—	BDL
95	—	—	—	—	—	—	BDL
Half-life (days)	4.10	—	3.71	—	3.54	—	
Coefficient of correlation	0.999	—	0.999	—	0.999		

a.i./ha respectively in beetroot, carrot and radish (Tables 3, 4 & 5). 90-98 per cent of the insecticide disappeared in 15 days and some residues persisted 61-100 days. The 'half-life' values ranged from 11.45 to 13.87 and 7.92 to 12.34 days in 1972-73 and 1973-74 respectively.

It was evident that the carbaryl was lost rapidly for the first 15 days and then slowly indicating that when the amount of carbaryl reached a minimum level the degradation slowed down markedly.

TABLE IIc
Carbaryl residues in clay loam soil and in maize grown on treated soil
(Treatment on 15.7.1974)

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Maize plant
	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	Average residues (ppm)
0	232.250 (230.468)	—	397.593 (400.387)	—	525.924 (536.556)	—	
5	133.776 (133.367)	42.40	187.620 (185.520)	52.81	229.677 (232.715)	56.33	
10	74.008 (74.786)	68.13	112.833 (113.635)	71.62	142.903 (144.170)	72.83	
15	40.554 (39.535)	82.54	61.389 (61.090)	84.56	73.762 (75.403)	85.97	
31	8.340 (8.550)	96.41	19.100 (18.732)	95.20	33.750 (33.506)	93.58	
63	0.853 (0.833)	99.63	3.385 (3.562)	99.15	6.674 (6.997)	98.73	BDL
108	BDL (BDL)	100.00	BDL (BDL)	100.00	1.309 (1.266)	99.75	BDL
Half-life (days)	12.97		12.81		13.26		
Coefficient of correlation	0.999		0.999		0.999		

Note : The residues of carbaryl in maize grain were below detectable level in 1972, 1973 and 1974.

These results agree with those obtained by other workers. The persistence of carbaryl was found to be more than one month with 50 per cent dissipation in about a month (Lichtenstein, 1965), 'half-life' of 8 days (Johnson and Stansbury, 1965), 'half-life' of 9 days (Burkhardt, 1966), 'half-life' of 6-9 days (Mishra and Gupta, 1971). Caro *et al.* (1974) also reported the loss of carbaryl to be 95 per cent 135 days after application.

TABLE IIIa
Carbaryl residues in clay loam soil and in beet root grown on treated soil
(Treatment on 12.12.1972)

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Beet root
	Average residues (ppm)	% reduction	Average residues	% reduction	Average residues	% reduction	Average residues (ppm)
0	110.040	—	152.555	—	242.393	—	
5	45.625	58.54	69.439	54.48	112.354	53.65	
10	27.584	74.93	45.009	71.15	64.542	73.37	
15	2.742	97.51	7.176	95.30	25.288	89.57	
31	1.716	94.44	2.742	98.20	7.643	96.85	
46	0.397	99.62	0.652	99.57	2.279	99.06	
62	0.093	99.92	0.199	99.87	0.622	99.74	
98	BDL	100.00	BDL	100.00	BDL	100.00	BDL
Half-life (days)	12.44	—	11.80	—	11.45	—	

TABLE IIIb
Carbaryl residues in clay loam soil and in beet root grown on treated soil
(Treatment on 11.11.1973)

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Beetroot
	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	
1	2	3	4	5	6	7	8
0	103.597 (95.007)	—	172.196 (187.736)	—	421.856 (387.120)	—	
2	61.971 (60.680)	40.18	103.551 (113.940)	39.86	248.077 (251.310)	41.19	
5	46.108 (43.745)	55.49	80.451 (76.688)	53.28	133.837 (125.575)	68.27	
7	24.633 (26.425)	76.22	50.770 (53.906)	70.52	76.398 (78.858)	81.89	
10	12.957 (14.212)	87.49	97.608 (30.850)	83.97	50.026 (47.488)	88.14	
15	5.073 (6.364)	95.10	11.621 (14.096)	93.25	22.588 (23.136)	94.65	

1	2	3	4	5	6	7	8
30	1.690 (2.459)	98.37	4.943 (6.825)	97.13	6.763 (8.718)	98.39	
46	0.634 (0.915)	99.39	0.949 (1.705)	99.45	2.822 (2.668)	99.33	
61	BDL (BDL)	100.00	0.468 (0.601)	99.73	1.676 (1.326)	99.60	
100	BDL (BDL)	—	BDL (0.0608)	100.00	0.508 (0.600)	99.88	BDL
Half-life (days)	8.60	—	11.94	—	10.60		
Coefficient of correlation	0.998		0.998		0.997		

TABLE IVa
Carbaryl residues in clay loam soil and in carrot grown on treated soil
(Treatment on 12.12.1972)

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Carrot
	Average residues (ppm)	% reduction	Average residues	% reduction	Average residues	% reduction	Average residues (ppm)
0	84.893	—	172.431	—	293.706	—	
5	34.889	58.90	75.205	56.39	107.680	63.24	
10	20.306	76.08	39.720	76.96	57.073	80.57	
15	4.793	94.35	8.527	95.05	16.670	94.32	
31	1.249	98.53	3.303	98.08	8.201	97.21	
46	0.397	99.53	0.630	99.63	2.217	99.25	
62	0.206	99.76	0.380	99.78	0.532	99.82	
98	BDL	100.00	BDL	100.00	BDL	100.00	BDL
Half-life (days)	13.62	—	12.09	—	11.45	—	

Carbaryl was lost more in *kharif* 1973 from clay loam soil than in other normal years. This unexpected loss was influenced by a heavy rainfall of 125 cm in 1973 compared with 25 cm in 1972 and 34 cm in 1974. Caro *et al.* (1974) also reported a loss of 90 per cent carbaryl from watershed alone due to a single rainfall. Carbaryl is soluble to an extent of 0.1 per cent in water (Stansbury and Miskus, 1964).

Cover crops like maize, beetroot, carrot and radish had no effect on the persistence of carbaryl in soil. Gupta (1975) also reported that the dissipation of aldrin in clay loam was not affected by cover crops, viz., maize, beetroot, carrot and radish grown on treated soil.

TABLE IVb

*Carbaryl residues in clay loam soil in carrot grown on treated soil
(Treatment on 11.11.1973)*

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Carrot
	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	Average residues (ppm)	% reduction	
0	123.567 (121.783)	—	167.529 (163.650)	—	303.799 (295.513)	—	
2	65.704 (66.482)	46.83	104.217 (106.900)	37.79	199.009 (181.990)	34.49	
5	38.638 (39.047)	68.73	58.523 (57.903)	65.07	124.312 (109.900)	59.08	
7	23.241 (19.915)	81.19	37.806 (35.645)	77.43	66.926 (62.898)	77.97	
10	15.759 (12.712)	87.25	22.293 (23.471)	86.69	43.303 (37.699)	85.75	
15	3.503 (4.784)	97.17	8.353 (7.975)	95.01	19.967 (19.414)	93.43	
30	1.326 (1.541)	98.93	3.218 (2.973)	98.08	10.155 (8.802)	96.66	
46	0.298 (0.347)	99.76	0.843 (1.040)	99.50	3.100 (3.522)	98.98	
61	BDL (BDL)	100.00	0.446 (0.384)	99.73	0.953 (1.015)	99.69	
400	BDL (BDL)	—	BDL (BDL)	100.00	0.684 (0.656)	99.77	BDL
Half-life (days)	7.98		12.34		11.07		
Co-efficient of correlation	0.999		0.996		0.999		

Absorption of carbaryl in plants/root crops

There was no translocation of carbaryl from treated soil into maize. Caro *et al.* (1974) reported no residues of carbaryl in harvested crop samples raised in

TABLE Va
Carbaryl residues in clay loam soil and in radish grown on treated soil
(treatment on 12.12.1972)

Days after treat- ment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Radish	
	Average residues (ppm)	% reduc- tion	Average residues	% reduc- tion	Average residues	% reduc- tion	Stem	Leaves
0	77.931		161.879		269.199			
5	34.903	55.21	65.243	59.70	100.221	62.77		
10	20.861	73.23	38.969	75.93	51.101	81.02		
15	3.487	95.53	8.913	94.49	15.211	94.35		
31	1.390	98.22	2.555	98.42	7.549	97.20		
46	0.503	99.35	0.777	99.52	1.962	99.27		
62	0.185	99.76	0.338	99.79	0.511	99.81	BDL	BDL
98	BDL	100.00	BDL	100.00	BDL	100.00	BDL	BDL
Half- life (days)	13.87		12.24		11.62			

TABLE Vb
Carbaryl residues in clay loam soil and in radish grown on treated soil
(Treatment on 11-11-1973)

Days after treatment	15 kg a.i./ha		30 kg a.i./ha		45 kg a.i./ha		Radish	
	Average residues (ppm)	% reduction	Average residues	% reduction	Average residues	% reduction	Stem	Leaves
1	2	3	4	5	6	7	8	9
0	109.217 (112.337)	—	216.535 (210.847)	—	347.181 (317.760)	—		
2	64.768 (64.995)	40.70	108.580 (111.667)	49.86	170.621 (144.715)	50.86		
5	40.971 (34.294)	62.49	67.380 (65.627)	68.88	119.276 (97.665)	65.65		
7	26.327 (25.195)	75.90	47.044 (43.330)	78.27	74.848 (62.069)	78.44		
10	13.423 (11.405)	87.71	24.784 (22.508)	88.55	45.637 (44.376)	86.86		

1	2	3	4	5	6	7	8	9
15	6.590 (6.867)	93.97	13.902 (12.780)	93.58	23.701 (18.657)	93.17		
30	1.640 (2.753)	98.51	4.145 (5.702)	98.09	8.384 (8.683)	97.59		
46	0.261 (0.631)	99.76	0.815 (1.420)	99.62	3.562 (3.756)	98.97		
61	BDL	100.00	0.227 (0.099)	99.90	1.373 (0.764)	99.60	BDL	BDL
100	BDL	100.00	BDL (BDL)	100.00	0.378 (0.199)	99.09	BDL	BDL
Half-life								
(days)	7.92	—	11.36	—	10.52	—		
Coefficient of								
correlation	0.998		0.999		0.999			

Figures in parentheses are the average residues of carbaryl by enzymatic method
BDL = Below detectable level.

treated soils. Dhali and Lal (1971) found that maize plants could absorb carbaryl residues through root system and translocate them to the aerial parts of the plant, however, the insecticide could not be detected in grain, stover or silk. Carbaryl was not absorbed by beetroot, carrot and radish grown on treated soil and residues were below the detectable level (BDL) in the edible parts of root crops. Stobwasser (1963) reported only traces of carbaryl residues in carrot.

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68 EFFECTS OF DIFFERENT LEVELS OF IRRIGATION ON THE UPTAKE AND PERSISTENCE OF PHORATE IN RICE

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SUMMARY

In a field experiment and a field cage study the effects of applying 10 per cent phorate granules at 12.5 kg a.i./ha. (T₁) to plots maintained by irrigation at field capacity, (T₂) to plots with water to a level of 8 cm and (T₃) to plots with water at field capacity and subsequently let in and maintained at 8 cm level, were studied. The residues in plants collected from (T₁) plots were larger than those collected from T₃ and the values from T₂ did not significantly differ from those of T₁. This showed that the maintenance of irrigation water at field capacity and to 8 cm (standard recommendation) did not adversely affect the uptake of Phorate into rice in paddy fields.

The degradation of granular insecticides applied to soil depends to a great extent on field moisture (Harris, 1964 ; Harris and Mazurck, 1967 ; Agnihotri *et al.*, 1975). Rice is now grown with controlled irrigation and the water level in fields treated with granular insecticides should be maintained at the optimum for uptake and dissipation of the insecticides. No information is available on this aspect and preliminary studies were done in the College of Agriculture, Kerala during 1977-78, using phorate.

EXPERIMENTAL RESULTS

An experiment on the college Farm with a randomised block of 3 x 3 m plots with each treatment replicated three times had separate irrigation and drainage

channels for each plot, with buffer plots to avoid interplot interference. A second experiment was done in field cages (3x2m) because it was difficult to maintain the exact level of irrigation in field plots. The three treatments included in the experiments were (1) granules applied to plots irrigated to field capacity level (2) granules applied to plots retaining 8 cm water level (3) granules applied to drained plots and then water let in up to a height of 8 cm.

Samples of plants were collected from plots at random at 2, 4, 7, 14 and 23 days after application of phorate in the field experiment and 1, 2, 4, 7, 10, 15 and 23 days after treatment in the field cage experiment. The insecticide residues in each sample were estimated by the method of Getz and Watts (1964) modified by Jain *et al.* (1974).

RESULTS AND DISCUSSION

The data from the two experiments are given in Tables I and II. Residues in plant tissues reached a maximum 2 days after application and decreased subsequently. In the field experiment the residue of 1.20 to 3.70 ppm on the 7th day decreased

TABLE I

Thimet residue (ppm) in plants collected from fields treated with granules and maintained with irrigation at different levels

Treatment	Intervals after treatment (days)					Mean
	2	4	7	14	23	
T1- Irrigation to field capacity level	5.70	4.13	3.70	1.86	0.57	3.19
T2- Irrigation to 8 cm height	5.63	4.00	2.10	1.04	0.35	2.62
T3- Irrigation to 8 cm after broadcasting of granules	4.60	3.60	1.20	0.60	0.19	2.04

Significant at 0.05 per cent level

C. D. for comparing means 1.65

rapidly to 0.6 to 1.86 ppm after 14 days and 0.19 to 0.57 after 23 days. In the field cage experiment, the residue also dropped drastically after 10 days and reached insignificant levels by the 23rd day.

As is seen from the marginal means in Tables I and II and the C. D. values, three treatments show significant variations. The residue in T₁ is significantly higher than that of T₃ but remains on par with that of T₂. This shows that the absorption

of insecticides applied to soil is not affected by irrigating the field to field capacity alone or to the standard method of keeping water to a height of 8 cm. The application of insecticide in field and subsequent irrigation adversely affect the quantity of the toxicant available in the plant tissue. This may be due to the dislodging and

TABLE II

Thimet residue (ppm) in plants collected from fields treated with granules and maintained with irrigation at different levels

Treatment	Intervals after treatment (days)							Mean
	1	2	4	7	10	15	23	
T1- Irrigation to field capacity level	6.45	6.78	5.98	5.23	4.42	2.1	1.24	4.60
T2- Irrigation to 8 cm height	5.40	6.20	5.10	4.92	4.26	1.76	0.92	3.95
T3- Irrigation to 8 cm after broadcasting of granules	4.57	5.20	4.10	3.80	3.71	1.50	0.46	3.28

Significant at 0.05 per cent level.

C. D. for comparing combinations 2.36

C. D. for comparing means 0.89

transferring of granules unevenly by the flowing in of irrigation water immediately after insecticidal application.

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FACTORS AFFECTING RESIDUES IN FRUITS OF SOME VEGETABLES AFTER SOIL APPLICATION OF PHORATE

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SUMMARY

Total phorate residues in harvestable fruits of brinjal (*Solanum melongena* L.), chillies (*Capsicum annum* L.) and peas (*Pisum sativum* L.) were analysed by a potentiometric enzymic inhibition method following soil application of granular phorate at the time of sowing or transplantation, with varied doses, methods of application, soil moisture regimes, soil types, applications of animal fertilizer and seasons.

The residues in fruits were maximum when granules were applied at the higher dosage, placed in furrow or above the seed, in loamy sand soils, following application of animal fertilizer at the rate of 50 q/ha or when crop was irrigated at 90 per cent available soil moisture. In brinjal and chillies, higher residues were obtained during the monsoon season. Similarly, higher residues were detected in the October-sown pea crop than in the November-sown crop.

Phorate has given promising results against insect pests of brinjal (Uthamasamy *et al.* 1973; Sohi *et al.*, 1974; Kumareson and Baskaran, 1975); chillies (Anbarasan, 1972) and peas (Sarup *et al.*, 1974). In Rajasthan, white grub *Holotrichia consanguinea* Blanch attacks all *kharif* crops including brinjal, chillies and early sown pea crops. Phorate has been found effective against this pest (Sharma and Shinde, 1970; Yadava and Yadava, 1973, and Sharma and Shinde, 1973). The present investigations were aimed at studying the extent of phorate residues in fruits of harvestable size following soil application of phorate granules to brinjal, chillies and peas at different dosages and the effects of some of the extrinsic factors such as soil type, method of application soil moisture, season and application of farm yard manure on residues in the fruits.

MATERIAL AND METHODS

Separate experiments were conducted with brinjal (*Solanum melongena* L.), chillies (*Capsicum annum* L.) and peas (*Pisum sativum* L.) to study the effects of individual factors on phorate residues in the fruits. All experiments were under field conditions on replicated plots (3m x 2m) except in the studies of the effects of soil type. 10 per cent phorate granules were used in all experiments. The granules were placed in the transplantation furrows for brinjal and chillies at the time of transplantation and in seed furrows for peas, except when the effects of application methods were studied. Similarly except in experiments where the effects of dosages

were tested the granules were applied at the rate of 2 kg a.i. per ha. Brinjal and chilli seedlings were transplanted 40 cm apart in 60 cm wide furrows. Pea seeds were sown in furrows 50 cm apart at 10 cm distances. The treatments were :

1. *Effect of dose* : The three doses tested were 1, 2 and 4 kg a.i. per ha. Insecticide was applied on 29-6-1971 to brinjal and chillies and on 11-10-1971 to peas.

2. *Effect or method of application of granules* : For brinjal and chillies, the methods used were (a) broadcasting of granules followed by hoeing prior to transplantation, (b) application in furrows before transplantation and (c) application around seedlings immediately after transplantation.

For peas the methods were broadcasting before sowing, placement of granules in seed furrows below the seeds and placement in seed furrow above the seeds. The treatments were given to brinjal and chillies on 29-6-1971 and to peas on 11-10-1971.

3. *Effect of soil type* : Three soil types : loamy sand, sandy loam and sandy clay loam were used in the studies. Sieved soil was placed in 30 cm diam. earthen pots. For brinjal and chillies 360 mgs and for peas 300 mgs of 10 per cent granules were applied to each pot and mixed in the soil. Brinjal and chilli seedlings were transplanted (one per pot) and pea seeds were sown (3 per pot) in a furrow drawn in the centre. Brinjal and chillies were treated on 15-2-1973 and peas on 28-10-72.

4. *Effect of farm yard manure* :- The three treatments of farm manure at three doses : 50, 100 and 200 q. per ha thoroughly worked into the soil prior to transplantation or sown with granules. The treatments to brinjal and chillies were on 15-2-1973 and in peas on 28-10-1972.

5. *Effect of soil moisture* :- Following soil application of granules at transplantation or sowing, the crops were raised under three different soil moisture regimes i.e. irrigating when the soil moisture level reached 90, 60 or 30 per cent available soil moisture. The experiments were in fields with a field capacity of 12 per cent soil moisture. The treatment dates were 15-2-1973 for brinjal and chillies and 28-10-1972 for peas.

6. *Effect of season* : Phorate granules were applied to brinjal and chillies sown during monsoon (on 29-6-1971), summer (on 2-5-1972) and winter (on 15-2-1973) and to pea crops sown on 11-10-1971 (early sown) and on 27-11-1972 (late sown). Fruit samples were taken randomly at periodical intervals. Samples from all the replicates were pooled and subdivided. The total phorate residues in fruits were analysed by a cholinesterase inhibition potentiometric method described

by Archer *et al.* (1963). For brinjal, chillies and peas the recovery values obtained were 75 to 112, 78 to 96 and 80 to 103 per cent respectively at 0.04 and 0.10 ppm fortification levels. The method was sensitive to 0.01 ppm level.

RESULTS AND DISCUSSION

1. Phorate residues in fruits :

The analysis of fruits from the first picking of brinjal, chillies and peas indicated phorate residues in significant amount. The Environmental Protection Agency of U. S. A. has prescribed very low tolerance levels for phorate and its metabolites in different commodities. In India, tolerance limits for phorate in vegetables have not yet been prescribed. The phorate residues in the first harvests of the vegetables were mostly well above the tolerance limits of 0.1 ppm prescribed for U. S. A. Thus, the use of phorate on brinjal, chillies and pea grown for pods is questionable. However, the residue data indicate that phorate can be applied safely to peas grown for seeds. Navaneethan *et al.* (1973) detected 0.31 to 2.12 ppm residues in okra fruits following application of phorate granules as a basal treatment or side dressing and questioned the use of phorate in crops fruiting before 50 days. Jain *et al.* (1974) found 1.45 ppm phorate residues in green pea pods following application of 1.5 kg a.i./ha granular phorate to furrows.

2. Effect of extrinsic factors on residues in fruits :

Increased phorate residues in fruits were obtained (Table I) with the increased dosages applied. Longer residual action against soil insects and higher residues in plants at higher dosages of phorate have been reported by Reynolds *et al.* (1957); Hopkins *et al.* (1959); Getzin and Chapman (1960) and Rao (1964).

Broadcasting of granules gave lower residues in fruits (Table II) because the insecticide was distributed throughout the field and remained in the top few inches of soil resulting in reduced uptake by the plants due to relatively lower concentration of insecticide near the root zone. Furrow application to brinjal and chillies gave the highest residues in fruits. For peas the placement of granules below the seeds gave higher residues as compared to when placed above the seeds.

The highest residues were in loamy sand soils (Table III) followed by sandy loam and sandy clay loam. In heavy soils, due to the higher clay fractions, more insecticide remained bound to the soil, resulting in decreased availability to kill soil insects and uptake by plants (Getzin and Chapman, 1959, 1960; Zaki and Reynold, 1961 and Patterson and Rawlins, 1968 a,b).

TABLE I

Residues (ppm) in fruits following soil application of phorate granules at three different dosages

Crop	Days Post treatment	Dose a.i. per ha		
		1 kg	2 kg	4 kg
Brinjal	45	0.81	1.12	2.98
	60	0.14	0.14	0.30
	75	0.03	0.08	0.14
	90	BDL	0.02	0.04
Chillies	60	0.04	0.11	0.24
	75	0.02	0.05	0.10
	90	BDL	BDL	0.02
Peas	70	0.21	0.20	0.59
	85	0.03	0.16	0.11
	100	0.02	0.02	0.04

BDL = Below detectable limits

TABLE II

Residues (ppm) in fruits following soil application of phorate granules by different methods

Crop	Days post treat- ment	Broadcast before transplan- tation or sowing	Furrow application at trans- plantation	Application around plants immediately after trans- plantation	Furrow place- ment below seed	Furrow place- ment above seed
Brinjal	45	0.25	1.41	0.99	—	—
	60	0.02	0.24	0.16	—	—
	75	BDL	0.06	0.04	—	—
	90	—	0.03	BDL	—	—
Chillies	45	0.32	1.09	1.00	—	—
	60	0.06	0.15	0.15	—	—
	75	BDL	0.06	0.05	—	—
	90	—	BDL	BDL	—	—
Peas	70	0.11	—	—	0.18	0.16
	85	BDL	—	—	0.11	0.08
	100	—	—	—	0.14	BDL
	120	—	—	—	BDL	—

Phorate residues in fruits were inversely proportional to the amount of farm yard manure added to the soil (Table IV). Probably because soil organic matter plays an important role in binding of the soil applied insecticides (Getzin and Chapman, 1959 and Patterson and Rawlins, 1968 a,b).

TABLE III
Residues (ppm) in fruits following application of phorate granules to three different soil types

Crop	Days post treatment	Soil type		
		Loamy Sand	Sandy loam	Sandy clay loam
Brinjal	45	3.00	2.11	0.64
	60	0.44	0.32	0.08
	75	0.28	0.24	0.02
	90	0.06	BDL	BDL
Chillies	60	0.31	0.20	0.06
	75	0.18	0.06	BDL
	90	0.03	BDL	—
Peas	70	0.81	0.21	0.06
	85	0.14	0.10	BDL
	100	0.06	0.02	—
	120	BDL	BDL	—

TABLE IV
Residues (ppm) in fruits following application of phorate granules to soils receiving three levels of farm yard manure

Crop	Days post treatment	Dose of farm yard manure (q/ha)		
		50	100	200
Brinjal	60	0.24	0.21	0.14
	75	0.31	0.10	BDL
	90	0.02	BDL	—
Chillies	60	0.51	0.12	0.03
	75	0.06	0.04	BDL
	90	0.04	BDL	—
Peas	70	0.28	0.30	0.02
	85	0.24	0.05	BDL
	100	0.08	0.04	—

When plants were grown under adequate moisture (irrigation at 90 per cent available soil moisture), the uptake of phorate into them was greater and the fruits had higher residues (Table V). When irrigations were delayed (irrigation at 60 and 30 per cent available soil moisture), the uptake was affected adversely. Adequate moisture replaces the insecticides at the adsorption sites on soil particles and hence

TABLE V
Residues (ppm) in fruits following soil application of phorate granules under three soil moisture regimes

Crop	Days post treatment	Soil moisture level at which irrigations were given (per cent field capacity)		
		90	60	30
Brinjal	60	0.31	0.18	0.20
	75	0.20	0.10	BDL
	90	BDL	BDL	—
Chillies	60	0.28	0.14	0.04
	75	0.08	0.04	BDL
	90	0.04	BDL	—
Peas	70	0.21	0.08	0.02
	85	0.10	0.06	BDL
	100	0.04	BDL	—

more insecticide becomes available to the plant roots. Thus, the soil moisture and availability of soil applied insecticide to kill insects or be taken up by plant roots have been found to be positively correlated (Harris, 1964 a, b; Harris and Svec. 1968). However, Harris (1964 b) found that organophosphorus insecticides are adsorbed strongly in dry soils and the influence of soil moisture is related to soil type.

When residues in fruits of brinjal and chillies are compared (Table VI) the maximum residues occurred in summer crops and the minimum in winter crops. Verma and Pant(1976) also reported higher residues of phorate in greengram and red gram during the monsoon which dissipated at a faster rate compared to the summer crop. During the present studies higher initial residues and their rapid degradation in peas in the early sown crops, may be explained by early sown peas growing faster resulting in dilution of the absorbed phorate and its metabolites. Slow growth of late sown crops and the lower temperatures result in slow degradation of phorate taken up from the soil. Thus, the rate and method of application of phorate granules together with season, soil type, soil moisture and organic matter content

TABLE VI
Residues (ppm) in fruits following soil application of phorate granules
in different seasons

Crop	Days post treatment	Crop season				
		Winter	Summer	Monsoon	Early sown crop	Late sown crop
Brinjal	45	—	2.41	1.12	—	—
	60	0.11	0.32	0.14	—	—
	75	0.04	0.20	0.08	—	—
	90	BDL	BDL	0.02	—	—
Chillies	45	—	0.89	—	—	—
	60	0.08	0.25	0.11	—	—
	75	0.02	0.11	0.05	—	—
	90	BDL	BDL	BDL	—	—
Peas	70	—	—	—	0.20	0.18
	85	—	—	—	0.16	0.12
	100	—	—	—	0.02	0.08

of the soil greatly affects the quantum of residues in fruits of brinjal, chillies and peas.

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70 EFFECTS OF HERBICIDE RESIDUES ON SOIL MICROFLORA*

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SUMMARY

Effects of herbicide residues (diuron, pebulate and nitrofen) on the soil microflora (bacteria, fungi and actinomycetes) were studied in 1972 under laboratory conditions. Diuron, pebulate and nitrofen were applied to air dry soil collected from the 0-30 cm depth at 1, 2, 5, 10, 25 and 50 kg a.i./ha respectively. Effects of different doses of these three herbicides on the development of fungi, bacteria and actinomycetes colonies were studied 15 days after treatment. The results of this study indicate that there was no significant reduction in the microbial population by the insecticides up to 5 kg a.i./ha, although higher doses significantly reduced the microbial population. Application of diuron increased fungal populations, but bacterial population were not

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affected. The application of pebulate had no effect on fungal and actinomycetes populations but reduced bacterial populations. After nitrofen, the fungal population was decreased due to increase in the dose, but it increased both actinomycetes and bacterial populations.

Soil fertility depends often on a very delicate balance between the various types of microorganisms involved in the nitrogen, carbon and other mineral cycles. Addition of any herbicide to the soil constitutes a serious threat to this equilibrium and hence the future fertility of the soil. The effect of applied herbicides on soil microflora depends upon the kind of the chemical, their concentration and or environmental conditions (Fletcher, 1960 and Kruetzer, 1963). Reductions in soil microorganisms after the application of Simazine and Atrazine was reported by Milkowska and Grozelak (1966). The effects of diuron, nitrofen and pebulate on soil microorganisms viz., bacteria, actinomycetes, fungi were investigated.

MATERIAL AND METHODS

An experiment at Dharwad in 1972 investigated the effects of diuron, nitrofen and pebulate on populations of bacteria, actinomycetes and fungi under laboratory conditions. The black clay loam soil possessed the following properties (Table I).

TABLE I
Physical and chemical properties of the soil

Depth cm	Mechanical Analysis			Bulk density	Field capacity	Permanent wetting point	pH	Total N	Available P205
	Sand	Silt	Clay						
	(Percentage on oven dry basis)							(Percentage on oven dry basis)	
0-15	13.74	53.46	32.90	1.11	26.50	14.50	7.4	0.051	0.009
15-30	11.31	35.17	43.52	1.14	28.50	15.25	7.5	0.043	0.003

Two kg of air dried, finely-ground soil was treated with one of the herbicides diuron, nitrofen or pebulate at rates of 0, 1, 2, 5, 10, 25 or 50 kg a. i. per ha in the laboratory to study the effect of each herbicide on the development of bacteria, actinomycetes and fungal colonies.

Soil samples were taken from treated soil after 15 days and transferred into sterile water blanks in flasks shaken thoroughly for 2 minutes. Serial ten-fold dilutions were prepared by pipetting out 10 ml of the original suspension with a sterile

pipette into 90 ml sterile blanks to the required dilutions. When suitable dilutions were prepared, 1 ml of the suspension was transferred to a sterile petri dish to which 10-15 ml of appropriate agar media were added. The suspension was mixed thoroughly with the agar medium and allowed to set. The dishes were incubated at room temperature for a specified time before counting the microbial colonies.

The remaining portions of the original suspension in the flask were transferred to a weighed petri dish and evaporated to dryness over a hot water bath. The dishes were dried further in an oven at 150°C for 8-12 hours, and the samples weighed dry. The microbial populations per gram of soil on a dry weight basis were estimated by the procedure given by Allen (1957). The bacterial colonies developing on soil extract agar were counted after 7 days. The fungal colonies developing on Martins rose Bengal agar medium were counted after 4 days of incubation. The actinomycetes developing on Kuster's agar were counted after 10 days of incubation. The data on effect of herbicides on soil microflora were statistically analysed following the procedures described by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The microbial populations were estimated 15 days after the application of herbicides to the soil (Table II) (Figures 1, 2 and 3).

Effect of herbicides on bacteria

The three herbicides had different effects on the bacterial population. The bacterial population was considerably increased by 50 kg a.i. per ha of nitrofen (28.0×10^6). Nitrofen had almost equally influenced microorganisms at 25 kg a.i./ha which corresponds to a bacterial population of 23.6×10^6 . Significant differences in the bacterial populations were observed from one dose to the other. Pebulate had considerable influence at higher doses (10 and 20 kg a.i./ha) and the resulting population was 15.4×10^6 . However, 50 kg a.i. per ha appeared to have an adverse effect. Diuron had increasing effect on bacterial populations up to 5 kg a.i. per ha with a population of 19.0×10^6 , but the population decreased steadily at higher doses. Of the three herbicides, the larger bacterial population occurred after nitrofen followed by diuron and pebulate. Increases in bacterial populations may be attributed to the proliferation of certain group of bacteria which use these herbicides as substrates (Audus, 1960). The results confirm the studies of Sriramajulu and Rangaswami (1971) and Kaufman (1964) who studied the effects of diuron and nitrofen on bacterial populations.

Effect of herbicides on fungi

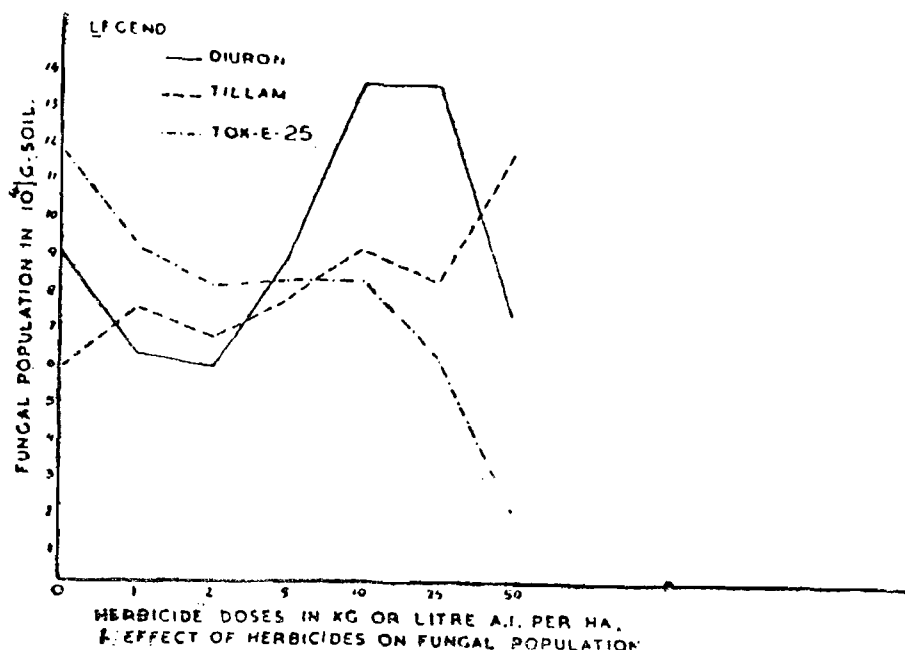
At doses tested, the herbicides had no adverse effect on the fungal populations. Diuron at 10 and 25 kg a.i. per ha appeared to favour the fungal population

TABLE II

Effects of herbicides on soil microflora (expressed as population per gram of moisture free soil)

Treatments		Organisms		
		Fungi 10 ⁴	Actino- mycetes 10 ⁵	Bacteria 10 ⁶
Control		8.856	5.686	12.586
diuron	1 kg a.i. per ha	6.183	4.163	19.500
diuron	2 kg a.i. per ha	5.840	2.666	16.716
diuron	5 kg a.i. per ha	8.676	2.883	19.016
diuron	10 kg a.i. per ha	13.363	4.413	14.220
diuron	25 kg a.i. per ha	13.346	4.880	9.940
diuron	50 kg a.i. per ha	7.186	2.280	9.220
	C.D. at 5%	3.7043	1.93	4.554
	C.D. at 1%	5.1935	NS	6.385
Control		5.716	3.593	13.490
Pebulate	1 lit a.i. per ha	7.396	5.586	15.620
„	2 lit a.i. per ha	6.553	5.253	10.923
„	5 lit a.i. per ha	7.610	4.673	13.370
„	10 lit a.i. per ha	8.876	4.440	15.400
„	25 lit a.i. per ha	8.136	3.120	17.873
„	50 lit a.i. per ha	11.616	2.890	10.883
	C.D. at 5%	NS	NS	3.726
	C.D. at 1%	NS	NS	NS
Control		11.616	2.890	10.883
Nitrofen	1 lit a.i. per ha	9.033	4.363	19.313
„	2 lit a.i. per ha	7.976	3.493	12.233
„	5 lit a.i. per ha	8.143	5.603	12.710
„	10 lit a.i. per ha	8.116	3.893	13.960
„	25 lit a.i. per ha	6.126	3.843	23.620
„	50 lit a.i. per ha	1.940	8.170	28.010
	C.D. at 5%	2.375	2.7237	2.8763
	C.D. at 1%	3.330	3.8187	4.0326

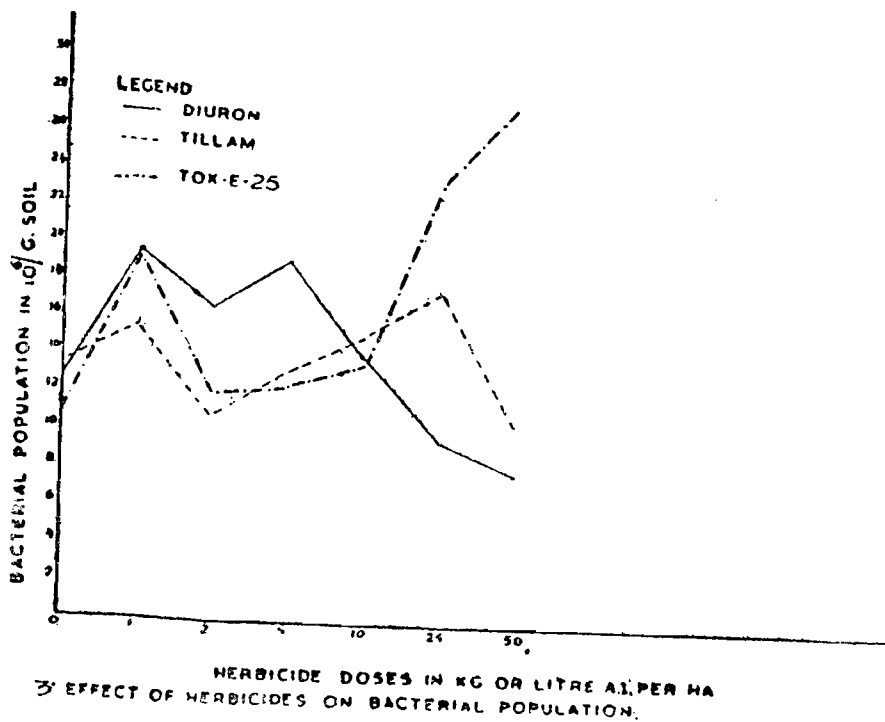
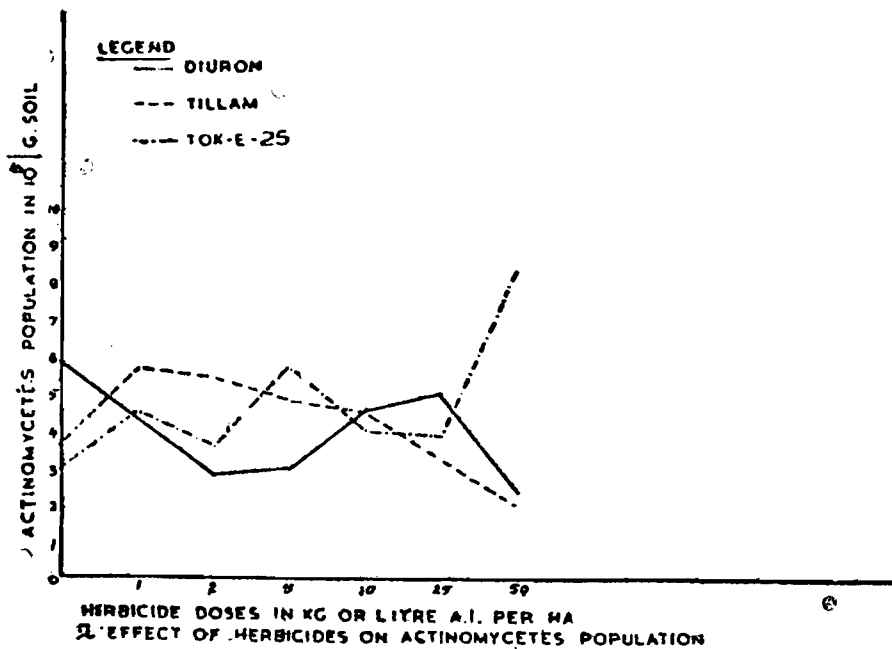
up to 13.4×10^4 and 13.3×10^4 , respectively. Pebulate had no influence on fungal populations and there was no significant difference between treated and control soils. Nitrofen reduced fungal populations as the dosage increased. Although the fungal population of the control soil was significantly higher than that of the treated soil



increased fungal populations were reported by nitrofen (Eno, 1962 ; Srirammaraju and Rangaswamy, 1971), which is contrary to the present work. This can be explained because increases or decreases in fungal population may be due to the stimulation of some group of fungi and inhibition of other group. It is possible that the herbicide had an inhibitory effect on sprouting mycelia of particular group.

Effects of herbicides on actinomycetes

Actinomycetes are tolerant to most groups of herbicides. Diuron had decreasing effect over actinomycetes population in comparison to population in treated soil. The actinomycetes populations differed significantly after different doses of herbicides. With pebulate, there was no significant difference in actinomycete populations in control and treated soil. Applications of nitrofen appeared to increase the actinomycete populations compared to those in control soil. The highest population was caused by nitrofen (50 kg a.i. per ha) which corresponded to 8.17×10^5 . The present results confirm those of Srirammaraju and Rangaswamy (1971), but there was no significant difference within the different dosages. Increases in the actinomycete populations can be explained because the herbicides were utilised as sources of carbon or nitrogen.



However, variations in the effects of the herbicides on the soil microflora either confirm or contradict. The reports of earlier workers, and can be attributed to variations in the concentrations of the herbicides used, soil properties, environmental conditions and time and method of assessment of the effect.

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71 MICROBIAL DEGRADATION OF PESTICIDES IN TROPICAL SOILS - A REVIEW OF WORK IN INDIA

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Pesticides applied to the environment are acted upon by several forces, physical, chemical and biological, independently and/or in combination. Ever since the classical demonstration of microbial breakdown of hormone herbicide 2, 4-D

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(Audus, 1951), it has now become well established that the major or frequently the only means of degradation for several pesticides in the environment is microbial. In biological reactions, essentially involving constitutive and adaptive enzyme systems, stepwise degradation of pesticide molecule is accomplished with the formation of inorganic end products while in non-biological reactions, the degradation is generally less extensive and far from complete.

Microbial involvement in pesticide degradation is generally established by :

- (i) comparing the relative rates of degradation in sterile and nonsterile samples ;
- (ii) evolution of $^{14}\text{CO}_2$ from ^{14}C - labelled pesticides applied to biological systems;
- (iii) an initial lag followed by a more rapid rate of degradation attributed to the adaptation of microorganisms ; (iv) the initial lag in degradation becoming shorter with successive application of pesticides due to enrichment of pesticide-degrading microorganisms ; and (v) finally and conclusively demonstrating the degradation of pesticides in isolated cultures of microorganisms.

The microbial degradation of pesticides in soil and aquatic environments has been studied extensively, especially in developed countries as excellently reviewed by several workers (Helling *et al.*, 1971 ; Tu and Miles, 1976). According to these studies, restricted mostly to aerobic and temperate environments, the pesticides in terms of their stability followed the order : chlorinated hydrocarbons > organophosphates = carbamates. The use of certain chlorinated hydrocarbon pesticides have been banned or restricted in most developed countries because of their recalcitrance to degradation and subsequent biomagnification through the food chain. Price consideration and non-availability of substitutes would delay the replacement of relatively cheap and effective chlorinated hydrocarbons in developing countries although there are recent instances of banning or restricting the use of certain insecticides, for instance endrin, in Indian agriculture. The mineralisation of organic pesticides, primarily by microbial action, is expected to be more rapid in tropical and subtropical environments as exist in India. It is therefore, important that a realistic assessment of the persistence of pesticides and their impact on the ecosystem should be made under actual Indian conditions before restricting the use of specific pesticides based on foreign literature of no relevance to Indian conditions. Also, persistence of pesticides is largely influenced by the conditions and cultural practices used for specific crops. Thus, lindane (gamma-hexachlorocyclohexane, gamma-HCH), known to persist for several years in aerobic upland soils, undergoes fairly rapid decomposition in tropical soils under flooding, the most widely used cultural practice for the major food crop, rice (Raghu and MacRae, 1966). Likewise, several other pesticides undergo rapid decomposition in soils upon flooding (Table I, Sethunathan *et al.*, 1976). The studies in India on microbial degradation of pesticides are more recent and are summarized below :

(i) *Benzene hexachloride (HCH) and endrin*

HCH is extensively used in Indian agriculture. Commercial formulations of HCH generally contain the alpha, gamma, beta and delta isomers of which the gamma isomer is the most insecticidal and beta isomer is the most persistent. Entry of beta isomer into the food chain following intensive use of commercial formulations of HCH has caused great concern in Japan. Isotope studies using Indian rice

TABLE I
Stability of certain insecticides in flooded soils (Sethunathan et al., 1976)

Insecticides	period of persistence* (days)
<i>Chlorinated hydrocarbons</i>	
α -HCH	30-90
β -HCH	30-90
δ -HCH	30-90
γ -HCH	30-90
DDT	30-90
Methoxychlor	30-60
Heptachlor	30-90
Endrin	55
<i>Organophosphates</i>	
Diazinon	70
Parathion	20-60

*In nonflooded soils, chlorinated hydrocarbon insecticides included in this group persist for several years and organophosphates only for six months.

soils revealed that both gamma and beta isomers of HCH decomposed rapidly in alluvial and laterite soils and in the acid sulphate saline soil, Pokkali from Kerala, under flooded conditions (Siddaramappa and Sethunathan, 1975). The degradation of these isomers was considerably retarded in autoclaved samples of these soils indicating microbial participation. Degradation was slow, however, in a sandy soil with low organic matter content and in another acid sulphate saline soil from Kerala, Kari with high organic matter content and extremely low pH even after 6 weeks of flooding. The redox potentials of alluvial, laterite and Pokkali soils exhibiting a high capacity to decompose HCH isomers declined to -50 to -145 mV within 20 days of flooding while sandy (+45 mV) and Kari (+165 mV) soils with low HCH-degrading activity were characterized by high potentials even after 6 weeks of flooding (Table II). The low redox potential associated with the degradation of HCH isomers supports the reported role of anaerobic microorganisms in the degra-

dation. The addition of rice straw, a common cultural practice in rice culture, to flooded soils further enhanced the microbial activity and the drop in redox potential, thereby the anaerobic degradation of HCH isomers.

The fate of ^{14}C -endrin in eight Indian rice soils was studied under flooded conditions. Endrin decomposed to six metabolites in most soils except in sandy and Kari soils (Gowda and Sethunathan, 1976). The degradation of endrin was retarded in soils by autoclaving indicating microbial participation. Radioautography revealed that endrin was converted to six metabolites in nonsterile soil samples as compared

TABLE II
HCH degradation and changes in redox potentials in Indian rice soils under flooded conditions (Siddaramappa and Sethunathan, 1975)

Incubation (days)	Acid Sulphate, Saline				
	Alluvial	Laterite	Pokkali	Kari	Sandy
Gamma-HCH recovered, cpm x 10 ⁴ /20g soil					
0	51.7	52.2	44.9	43.3	49.4
20	2.3	19.4	25.3	31.5	47.2
41	0.8	1.3	2.1	24.8	42.4
Beta-HCH recovered, cpm x 10 ⁴ /20g soil					
0	71.8	75.6	63.4	49.6	79.9
20	16.2	45.2	58.9	42.0	73.5
41	1.9	2.9	20.6	36.0	65.5
Redox potential (Eh), mV					
0	+235	+285	+250	+380	+220
20	-120	-50	-80	+160	+150
41	-145	-75	-100	+165	+45

to three compounds in sterile samples. Evidently, the degradation of endrin in flooded soils is both biological and chemical, but biological degradation is more extensive. The breakdown products of endrin formed by chemical and biological processes persisted under continued flooding and under alternate aerobic and anaerobic conditions (Gowda and Sethunathan, 1977) necessitating further studies on the aspect of minimizing residue problems from endrin metabolites.

(ii) *Parathion and fensuliothion*

Nitro group reduction and hydrolysis are essentially detoxication reactions in the degradation of parathion. Contrary to the common belief that nitro group reduction is the major pathway of microbial metabolism of parathion in soil, water and

pure cultures, rapid hydrolysis of parathion by biological action occurred in several soils under flooded conditions, especially after its repeated additions (Sethunathan, 1973). Recent studies demonstrated the widespread occurrence of biological hydrolysis of parathion also in water samples from diverse aquatic ecosystems such as ponds, rivers, lakes and flooded rice fields (Sudhakar Barik and Sethunathan, 1978). Bacteria capable of hydrolyzing parathion to *p*-nitrophenol and then metabolizing the *p*-nitrophenol to nitrite and carbon dioxide as end products have been isolated from parathion-amended flooded soil and characterized (Sudhakar Barik *et al.*, 1976). Even *m*-nitrophenol, known for its resistance to biodegradation, was decomposed by a bacterium isolated from parathion-amended soil. The effect of organic sources on the degradation of parathion in flooded soils depended on the pathway involved. Thus, the addition of organic sources enhanced the nitro group reduction of parathion, but inhibited the hydrolysis of parathion in soils inoculated with parathion-hydrolysing enrichment culture (Rajaram and Sethunathan, 1975). Certain cultures capable of reducing parathion to amino parathion in pure culture have been isolated from soils (Roa and Sethunathan, 1974; Parasnis and Dhala, 1977).

Parathion degradation in flooded soils proceeded by nitro-group reduction and hydrolysis, both mediated by microorganisms. The major pathway of degradation after first addition of parathion was nitro-group reduction, but after two or three additions, the pathway shifted to hydrolysis as a consequence of build-up of parathion-hydrolyzing microorganisms during *p*-nitrophenol metabolism. Again, parathion was converted to aminoparathion in flooded soil never treated with *p*-nitrophenol; but in flooded soil pre-treated with *p*-nitrophenol, parathion was readily hydrolyzed (Sudhakar Barik, 1978).

In a study with pesticide combinations, benomyl at a concentration as low as 5 ppm inhibited the nitro-group reduction of parathion to aminoparathion in flooded soil; while 2, 4-D and HCH had no effect on parathion reduction even at 200 ppm level. Although parathion concentration decreased in the presence of atrazine, no aminoparathion was detected. Rice straw hastened the nitro-group reduction of parathion and the drop in redox potential in soils upon flooding. Even in the presence of rice straw, the inhibitory effect of benomyl on nitro-group reduction was well pronounced. Interestingly, benomyl retarded the drop in redox potentials upon flooding both in unamended and rice straw-amended soils. This would explain, at least in part, the inhibitory action of benomyl on nitro-group reduction (Sudhakar Barik, 1978).

Benomyl at 200 ppm level effected significant inhibition of the hydrolysis of parathion and the metabolism of *p*-nitrophenol in flooded soils inoculated with para-

thion-hydrolyzing enrichment culture; but 2, 4-D, citrazine and HCH even at a concentration of 1000 ppm were almost innocuous in influencing parathion hydrolysis and further metabolism of *p*-nitrophenol (Sudhakar Barik, 1978).

Clearly, the data demonstrated long-term persistence of generally short lived parathion in a flooded soil even in the presence of added rice straw when it is applied in combination with benomyl.

More recently, Sheela and Vasantharajan (1977) studied the persistence of generally more persistent organophosphorus pesticide, fensulfothion in soils employing bio-assay and choline esterase inhibition techniques. Fensulfothion applied to soils at 50 to 175 ppm persisted for about 50 to 60 days.

(iii) *Carbofuran, carbaryl and baygon*

In recent years, there has been a steady increase in the use of carbofuran, the most effective insecticide against the major pest of rice, brown planthoppers. Carbofuran decomposed rapidly in most soils except Kari soil upon flooding between 20 to 40 days. Degradation was more rapid in non-sterile soils than in sterile soils presumably due to microbial action (Venkateswarlu *et al.*, 1977). A bacterium isolated from flooded soil decomposed carbofuran in pure cultures. Carbofuran is relatively more water soluble than several commonly used insecticides. Its water solubility and resistance to degradation for 20 days after application raise the problems of contamination of water resources and inland fisheries through carbofuran residues in surface run-off waters from treated rice fields.

Recent reports from the International Rice Research Institute, Philippines show that carbofuran is no longer effective against brown planthoppers after its repeated applications for 2-3 years continuously. Degradation of carbofuran during incubation with paddy-water samples from the carbofuran-treated and untreated plots was studied to find out whether the decreased efficiency of carbofuran was in part due to the build-up of carbofuran-degrading microorganisms upon its repeated applications. After an initial lag of about 20 days, degradation of carbofuran proceeded almost equally rapidly in water and soil samples of both untreated and carbofuran-treated plots. Applications of carbofuran to flooded rice soils close to field application rates did not lead to accelerated rates of its degradation after its successive applications (Venkateswarlu and Sethunathan, 1978; Siddaramappa *et al.*, 1978).

An isotope study showed that degradation of carbofuran in flooded soils was more rapid under undisturbed conditions than under aerobic conditions provided by shaking. Under continued anaerobiosis of undisturbed flooded soils, the hydrolysis products, carbofuran phenol in particular, accumulated, but when the undisturbed

soil was returned to aerobic conditions, the hydrolysis products decreased rapidly (Venkateswarlu and Sethunathan, 1978).

The addition of rice straw accelerated the hydrolysis of carbofuran phenol in predominantly anaerobic flooded soil. Carbofuran phenol appeared to accumulate under continued anaerobiosis and was readily decomposed with the significant formation of unextractable ('soil bound') residues when the system was returned to aerobic conditions. $^{14}\text{CO}_2$ evolution from the aromatic ring in carbofuran was almost negligible accounting for less than 0.9 per cent of ring - ^{14}C even after 40 days of flooding as compared to 27 per cent released from carbonyl - ^{14}C (Venkateswarlu and Sethunathan, 1979).

A bacterium capable of degrading carbofuran and carbaryl was isolated from flooded alluvial soil. A study with ring labelled ^{14}C -carbofuran showed that in the presence of soil extract and yeast extract in the medium, the bacterium after 6 transfers effected more than 70 per cent degradation of carbofuran with the concomitant formation of an unidentified product and a water soluble metabolite within 20 days, as compared to negligible degradation in inoculated medium devoid of soil extract and yeast extract.

A strain of *Achromobacter* sp. isolated from nonflooded soil converted carbaryl in a mineral salts medium to hydroxyquinone, 1-naphthol, catechol and pyruvate (Sud *et al.*, 1972). A *Pseudomonas* sp. isolated from Baygon-treated nonflooded soil degraded Baygon to 2-isopropoxy phenol (Gupta *et al.*, 1975).

(iv) *Dexon*

Dexon applied to a nonflooded soil disappeared from the soil within 60 days, presumably by microbial action (Karanth and Vasantharajan, 1973). A soil bacterium *Pseudomonas fragi* BK9, isolated by enrichment culture reduced dexon to *N, N*-dimethyl-*p*-phenylene diamine by a cometabolic process (Karanth *et al.*, 1974).

(v) *Thiram and ziram*

Raghu and coworkers studied the fate of fungicides thiram and ziram in non-flooded soils. In aerobic soils thiram was converted to dimethylamine, carbondisulphide and an unidentified divalent sulfur compound (Raghu *et al.*, 1974, 1975). This unidentified compound was subsequently characterized as copper dimethyl dithiocarbamate (CuDDC_2) using ^{64}Cu labelled compounds and thin-layer chromatography (Kumarasamy and Raghu, 1976). The breakdown products of thiram, particularly CuDDC_2 were highly fungitoxic and this would explain, at least in part, the prolonged fungitoxicity of thiram despite its instability in soil.

Ziram was decomposed by a soil bacterium in pure cultures essentially to water-soluble metabolites which were not identified (Raghu *et al.*, 1976).

(vi) *Carboxin and oxycarboxin*

A strain of *Pseudomonas* isolated from red sandy loam soil of Bangalore was reported to degrade carboxin and oxycarboxin (Balasubramanya and Patil, 1976). The bacterium hydrolyzed oxycarboxin to aniline and oxidized carboxin to its sulphoxide and to the sulphone. The sulphone derivative was hydrolyzed to aniline. Aniline was further degraded to ammonium which in part was oxidized to nitrite leading to the accumulation of both ammonium and nitrite.

Nitrate pollution

For providing adequate food to the fast increasing population of India, high yielding, nitrogen-responsive crop varieties had been introduced and consequently the nitrogen input had increased. This fast increasing nitrogen input may become a health hazard through nitrate pollution of water resources in coming decades, unless timely steps are taken. In India there is a feeling that the problem of nitrate pollution of water is causing an unnecessary scare. However, the recent reports of accumulation of high levels of nitrate in ground waters of Punjab and Haryana and other reports available on nitrate concentration in well, tank and irrigation waters (Table III) do indicate that it will not be wise to ignore this aspect of pollution altogether (Shinde and Vamadevan, 1974). The extent of nitrate pollution due to

TABLE III
Nitrate-N in water resources of India (Shinde and Vamadevan, 1974)

Location	Water source	Range of NO ₃ concentration expressed as nitrogen (ppm)
Kutch (Gujarat)	Ground-water	0—43
Unnao district (Uttar Pradesh)	Drainage water	0—641
—	Tank water	0—403
Gurgaon district (Haryana)	Village well water	2—21

steadily increasing use of nitrogenous fertilizers in agriculture has not been fully assessed. Micro organisms may assist in preventing the accumulation of undesirable levels of nitrate through denitrification under favourable conditions of anaerobiosis and adequate organic matter supply.

Perspectives and Conclusions

Developing countries differ in their awareness of the environmental hazards associated with over increasing use of agro-chemicals. India took a lead in recognising the implications of pesticide residue problems as early as 1967, as exemplified by Thacker reports (ICAR, 1967). Of immediate concern to us are the more recent reports on the accumulation, at astonishingly high levels, of pesticides, particularly the persistent chlorinated hydrocarbons in humans, food and grains and of nitrate in ground waters of Punjab and Haryana. It would be devastating to ban these persistent pesticides in developing countries, because their substitutes are more costly and not easily available. Moreover, conclusions on the pesticide persistence and bio-magnification characteristics based on researches under temperate environments may not be directly applicable to tropical or sub-tropical environments of almost all developing countries. Admittedly, various aspects of complex problems related to steadily increasing use of pesticides in tropical countries including India are far from clear. It is, therefore, important and necessary that systematic studies under Indian conditions, both in the laboratory and field should be initiated towards :

- (i) identifying areas of intensive use of pesticides and monitoring for residues and ecological disturbances due to intensive use of pesticides in such ecosystems ;
- (ii) a realistic assessment of side-effects of pesticides on organisms and biochemical transformations of importance to soil and aquatic fertility;
- (iii) a better understanding of physical, chemical and bio-chemical fate of pesticides in the environment;
- (iv) minimizing pesticides and nitrate pollution problems in the environment by maximizing the indigenous microbial activity through cultural practices such as organic matter amendements, liming of acid soils etc. ;
- (v) developing techniques or criteria utilizing certain sensitive biological reactions as indicators of environmental pollution, for eg. $^{14}\text{CO}_2$ evolution from ^{14}C -labelled substrates, nitrification, specific enzyme activity etc.

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72 PERSISTENCE OF PHORATE IN KERALA SOILS IN RELATION TO ITS EFFECT ON SOIL MICROBES*

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SUMMARY

Colorimetric and bioassay estimate of residues of phorate applied at 100 ppm approximated closely to each other. The rate of dissipation of the residue was slow in forest and alluvial soils ('half-life' of 48.5 and 30.2 days) and rapid in red, laterite and sandy soils ('half-life' of 16.8, 15.7 and 13.4 days respectively).

Residues stimulated fungal populations throughout the 11 weeks of observation. Actinomycetes were suppressed in populations by the higher levels of residues and stimulated at reduced levels. Populations of bacteria were stimulated at higher residues and suppressed at reduced levels of the residues in soil. The insecticide residues in soil were negatively correlated with populations of fungi and actinomycetes and positively with population of bacteria.

Residues of insecticides in soil and the soil microorganism appear to react mutually. Excessive concentrations of residues in the edaphic environment may affect microbial populations by temporarily favouring them and over populating soil or by suppressing them and leading to new and more complex problems. On the other hand the persistence of insecticides in soil is influenced greatly by the micro-

* Part of Ph.D. thesis of the senior author submitted to Kerala Agricultural University, Kerala.

bial flora which degrade the residues to toxic and/or to non-toxic metabolites (Tu and Miles, 1976).

Phorate is used widely in soils in Kerala as granules for pest control. Because no information was available on its impact on the soil microorganisms in these soils the present studies were undertaken to observe the effect of phorate residues on the microflora of the major five types of soils of Kerala.

MATERIAL AND METHODS

The types of soils studied were forest, alluvial, red, laterite and sandy soils. Samples were collected from a depth of 15 cm, air-dried and sieved through a 2 mm sieve, then filled in pots (30 x 15 cm) at 1 kg per pot with two replicate for each soil type and observation. Phorate (0,0-diethyl 5-(ethyl thiomethyl) phosphorodithioate) 10 per cent granules were incorporated into the soil in each pot to give a dosage of 100 ppm. The moisture level was maintained at 40 per cent of the maximum water holding capacity throughout the period of the experiment. Phorate residues in the soils were determined 1,3,5,7,9 and 11 weeks after application, using the colorimetric method of Getz and Watts (1964) as modified by Jain *et al.* (1974). Simultaneously residues were estimated biologically using adults of *Drosophila melanogaster* as the test insect.

Populations of soil microbes were assessed at intervals same as those of insecticide residues using the standard serial dilution plate method.

RESULTS AND DISCUSSION

The results of the colorimetric and bio-assay methods closely approximated each other (Table I) and the discussion is based on the colorimetric assay results.

In forest soil, the rate of dissipation was steady but slow, the initial residue of 83.2 ppm in the first week falling down to 30.2 ppm by the end of 11 weeks with a 'half-life' of 48.5 days. A similar trend was observed in alluvial soil, the residue reaching a level of 26 ppm by the end of 11 weeks, with a 'half-life' of 30.2 days. The rate of dissipation in red, laterite and sandy soils was very rapid reaching levels of 2.4, 1.9 and 1.4 ppm respectively by the end of 11 weeks with 'half-life' estimates of 16.8, 15.7 and 13.4 days respectively. Fungal populations showed stimulation throughout the 11 weeks of observation during which residues in the different soils depreciated from 83.2 ppm (in forest soil) to 1.4 ppm (in sandy soil) (Table II) Tewari *et al.* (1972) recorded a similar stimulatory response in fungi. Actinomycetes were suppressed by the higher levels of residues and stimulated by the reduced

TABLE I

Residues from 100 ppm phorate in different types of soils at different intervals after application

Type of Soil	Phorate residues after different periods (weeks)						'Half life' in days
	1	3	5 (ppm)	7	9	11	
Forest	83.2 (85.10)	65.0 (66.00)	51.5 (53.0)	45.5 (51.2)	35.6 (35.0)	30.2 (31.6)	48.5 (50.8)
Alluvial	73.5 (79.43)	52.8 (53.70)	41.4 (45.71)	35.9 (31.6)	31.1 (26.3)	26.0 (23.4)	30.2 (39.9)
Red	65.4 (68.00)	29.1 (26.18)	14.3 (16.2)	7.7 (9.3)	4.5 (7.1)	2.4 (4.3)	16.8 (18.0)
Laterite	61.1 (60.15)	26.2 (25.13)	11.4 (11.2)	6.1 (6.5)	3.4 (4.2)	1.9 (2.9)	15.7 (15.1)
Sandy	52.2 (47.86)	22.3 (37.15)	9.4 (4.2)	5.1 (3.0)	2.7 (2.5)	1.4 (1.6)	18.4 (13.5)

Note : The values in the parenthesis are for microbioassay

levels. In forest soil, there was a suppression of populations during the entire period of 11 weeks which may be attributed to the slow rate at which the insecticide residue was lost from this soil. In all other soils ('half-life' estimates of 30.2 to

TABLE II
Effect of phorate on microbial populations in different types of soil at different intervals after application

Soil type	Per cent increase (+) or decrease (—) in the population over control at different intervals (weeks)						
	0	1	3	5	7	9	11
<i>Forest</i>							
Fungus	+32.29	+15.63	+54.11	+0.35	+9.56	+35.93	+39.48
Actinomycetes	+20.92	—78.03	+89.33	—42.48	—1.60	—5.98	—4.77
Bacteria	—81.54	+536.22	+30.82	+459.09	—94.38	—0.63	—37.19
<i>Alluvial</i>							
Fungus	+43.04	+18.18	+5.77	+27.63	+5.23	+85.50	+59.88
Actinomycetes	+94.58	—1.53	+6.51	—66.46	—18.33	+52.13	+17.24
Bacteria	+81.67	+12.26	+341.97	+200.00	—15.88	+40.04	—24.80
<i>Laterite</i>							
Fungus	+22.00	+41.45	+3.56	+59.28	+15.09	+39.42	+52.93
Actinomycetes	—32.14	—35.69	—91.22	—14.45	—66.72	+83.52	+29.32
Bacteria	—34.33	+22.91	—94.56	—63.27	—1.53	—52.86	—67.95
<i>Red</i>							
Fungus	+4.96	+53.26	+88.76	+2.41	+11.03	+70.40	+3.99
Actinomycetes	—24.57	—85.00	—38.91	—64.59	+50.23	+14.53	+91.19
Bacteria	—36.36	—11.13	—88.76	—96.42	—83.74	—79.14	—7.90
<i>Sandy</i>							
Fungus	+89.77	+35.57	+35.71	+13.47	+12.68	+13.52	+19.82
Actinomycetes	—46.56	—99.45	—94.81	+19.35	+37.19	+12.85	+33.55
Bacteria	—96.19	—88.88	—84.87	—92.10	—75.40	—36.83	—20.93

13.4 days) the shift from suppression to stimulation of the population was manifested in alluvial soil conversely, a stimulatory effect of phorate residues on actinomycete populations was reported by Kandasamy *et al.* (1975) and Pandian (1975).

With the bacterial population, in the forest and alluvial soils up to 5 weeks the insecticide residues stimulated the bacterial populations and towards the later part it suppressed them. In laterite, red and sandy, soils suppression occurred all the time. Stimulation was associated with higher residues and suppression with

lower residues. The suppression manifested in the laterite, red and sandy soils may be due to the more rapid decrease of the insecticide residues ('half-life' estimates 15.7, 16.8 and 13.4 days) and the more prolonged stimulation in the forest and alluvial soils due to the slower deterioration of the phorate residues in these soils (half-life period 48.5 and 30.2 days respectively). Both stimulatory and suppressive effects of phorate on bacteria, were reported earlier (Pandian, 1975 ; Murthy *et al.*, 1976).

From all these observations it is evident that one important factor governing the response of microbial flora to phorate applied in different types of soils was the fate of the residues in the different soils. The correlation studies between the phorate residues in soils and population of soil microbes (Table III) showed that in

TABLE III
Correlations between phorate residues in different types of soils and microbial populations

Types of soil	Fungi	r values Actinomycetes	Bacteria
Forest	-0.556	-0.797*	0.617
Alluvial	0.245	-0.802*	0.852*
Laterite	-0.752*	-0.656	0.296
Red	-0.105	-0.618	0.641
Sandy	-0.643	-0.712	-0.470

*Significant at 0.1% level

forest soil the fungal and actinomycetes populations were negatively correlated with the insecticide residues while bacterial population was positively correlated. The negative correlation of actinomycetes with the residues was significant. In the case of alluvial soil, the bacterial and fungal populations were positively correlated with the insecticide residues and was highly significant with bacteria and insignificant with fungi. The actinomycetes however was negatively correlated with the residues the correlations being highly significant.

With red, laterite and sandy soils the fungal and actinomycetes population were negatively correlated with the residues, while the bacterial population was positively correlated with the residues in laterite and red soils and negatively correlated in the sandy soil.

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72 INFLUENCE OF A PESTICIDE ON THE GROWTH AND METABOLISM OF A BENEFICIAL NON-TARGET SOIL BACTERIUM

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SUMMARY

The influence of aldicarb (2-methyl-2 (methylthio) propionaldehyde-O-methyl carbamoyl oxime), on the growth, pigmentation, respiration and ³²P-phosphate incorporation by the free-living, nitrogen fixing soil bacterium *Azotobacter chroococcum* Beij. was studied *in vitro*. The growth was affected adversely by 5 ppm of aldicarb but not at the lower levels. Not only pigment formation in the cells was delayed but the quantity of the colouring matter was also reduced drastically by aldicarb at 5 ppm. The respiratory rate was inhibited significantly by the 5 ppm level of aldicarb. Enhanced inhibition of ³²P-phosphate incorporation by the higher

concentration of aldicarb was also observed. The results indicated that aldicarb, particularly at 5 ppm and higher concentrations inhibited certain biosynthetic pathways of *A. chroococcum* by affecting the respiratory process of the microorganism resulting in reduced growth.

Several pesticides applied to soil and crop plants to control pests and diseases persist in soil for a considerable period. The influence of such residues on the metabolic activities on non-target soil microorganisms of agricultural importance is understood little. MacKenzie and MacRae (1972) observed no significant effect of lindane and DDT on nitrogen fixation by *Azotobacter vinelandii* at normal and 50 times field rates of application. MacRae and Celo (1974) found that several organophosphorus pesticides decreased the rate of respiration of *A. vinelandii* at 100 ppm concentration whereas, a stimulation was observed at both 2 ppm and 100 ppm levels of diazinon. Whereas, diazinon, DDT and malathion stimulated the growth of *A. vinelandii*, its nitrogenase activity was reported to be greatly reduced by diazinon and malathion. Balasubramanian and Gita Nilakantan (1975) found a reduction in cell yield of *A. chroococcum* by 1 and 5 ppm of aldicarb and also reported that certain assimilatory processes of the cells were altered by the insecticide. The results of further studies on the effect of aldicarb on growth, pigmentation, respiration and ^{32}P -phosphate incorporation by the cells of *A. chroococcum* Beij. are reported in this paper.

MATERIAL AND METHODS

The inoculum of *A. chroococcum* (an isolate from soil) was multiplied in nitrogen-free Waksman's medium No. 77, under aerated conditions. One ml of the inoculum undergoing the active growth phase (log phase) was inoculated into 99 ml of the same medium. Calculated quantities of aldicarb solution (2-methyl-2 (methyl thio) propionaldehyde-O-methyl carbamoyl oxime) or the crystalline technical material, were added aseptically into the flasks to arrive at the final concentrations of the active ingredient of the chemical and incubated on a wrist-action shaker. Three replicate of each treatment and appropriate controls were maintained throughout the experiment. Growth was measured as optical density (O.D) of the culture at intervals for 72 hr and total cell yield (dry weight) at the end of 5 days incubation period.

To determine the effect of the insecticide on pigmentation of *A. chroococcum* cells, not only the time taken for the appearance of dark pigmentation in the growth medium was noted, but the pigment was also extracted and estimated as follows: The cells from 50 ml of a 5 day old culture were harvested by centrifugation and suspended in 50 ml of 1 M KOH solution. After boiling the suspension for 1 hr the

cell debris was centrifuged off. The dark coloured supernatant solution was adjusted to pH 3 with 1 : 1 HCl and the OD of the pigment was recorded with a colorimeter at 470 nm.

The respiratory studies with *A. chroococcum* cells were conducted in a Warburg's respirometer using the procedure described by Umbreit *et al.* (1964). From the nitrogen-free culture medium, 36 hr old cells were harvested and after repeated washings with 0.5 M phosphate buffer they were starved for 72 hr to reduce their endogenous respiration to a minimum before use. The volume of oxygen consumed per mg dry weight of the cells under the different treatments was calculated.

For the ^{32}P -phosphate incorporation study, *A. chroococcum* cells were grown in a nitrogen-free medium supplemented with 0, 1, 5 and 10 ppm of aldicarb for 48 hr. To each flask 10 microcurie of radio-active ^{32}P -dipotassium hydrogen phosphate solution (carrier-free, with specific activity 5 mCi/mM of K_2HPO_4) was injected and incubated again for 6 hr. After incubation the cells were harvested by centrifugation at 27,000 g at 5°C for 10 min with repeated saline washings. The cell pellet was transferred to a stainless steel planchet, demounted under infra-red heat and the radio-activity monitored with an end-window GM counter and its specific activity computed.

RESULTS

Significant reductions in the growth of *A. chroococcum* cells treated with 5 ppm of aldicarb in the growth medium were observed both after 72 hrs (Fig. 1) and at the end of 3 days growth as measured by total cell yield (dry weight) (Table 1). Although some stimulation in the growth of the 2 ppm aldicarb-treated cells was noticed at 72 hrs, this was not reflected in terms of total cell yield after 5 days growth.

Appearance of dark pigmentation was very delayed (95 hr) in cells treated with 5 ppm aldicarb, as compared with untreated control cells (72 hr). The relative quantity of the colouring matter of the cells was also reduced drastically in the 5 ppm aldicarb-treated *A. chroococcum* cells (Table I).

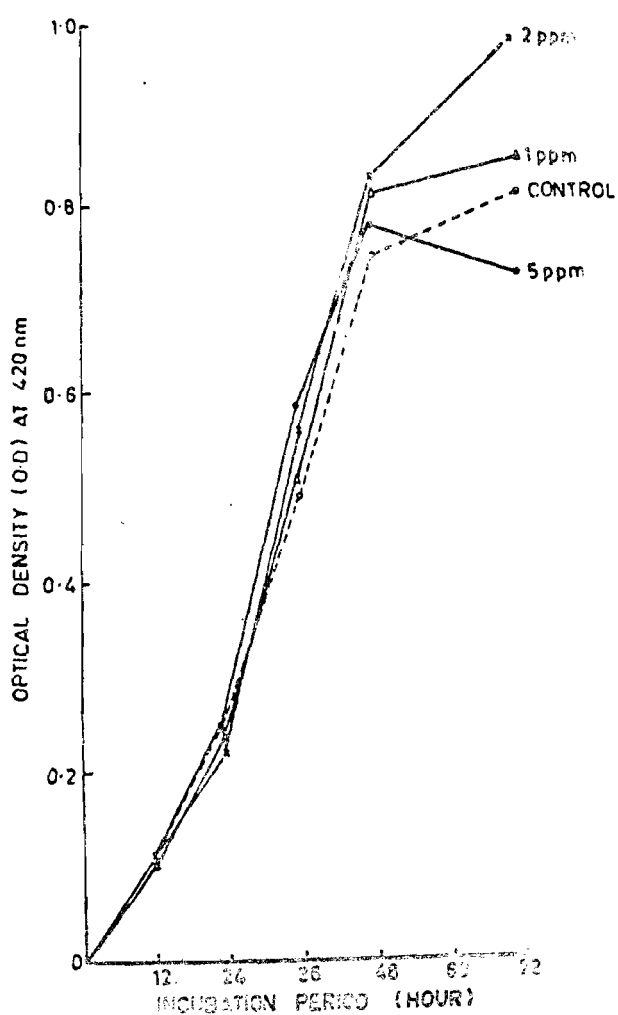
The rate of respiration decreased with increased concentrations of the insecticide in the reaction medium and the inhibition of respiration was more pronounced in the presence of 5 ppm aldicarb than the other treatments (Fig. 2).

The incorporation of ^{32}P -phosphate by *A. chroococcum* cells decreased with increasing concentrations of aldicarb and the reduction was a maximum in 5 and 10 ppm aldicarb treated cells (Table II).

DISCUSSION

The results indicated clearly that the growth of *A. chroococcum* was reduced in the presence of 5 ppm of aldicarb which conforms an earlier report (Balasubramanian and Gita Nilakantan, 1975). The delayed formation of the dark pig-

EFFECT OF ALDICARB ON THE GROWTH OF *Azotobacter chroococcum*



ments as well as the drastic reduction in the quantity of the colouring matter in the cells treated with 5 ppm aldicarb indicated that the insecticide had interfered with

the biosynthesis of the melanin pigment by the cells and the inhibition of this synthetic process was maximum at the higher concentration of the chemical. Although the role of the pigments in the cellular functions of *A. chroococcum* is not yet clear, the results show that aldicarb was able to alter the metabolism of this bacterium. Balasubramanian and Gita Nilakantan (1975) showed that ^{14}C -glucose incorporation by *A. chroococcum* cells was affected by aldicarb.

TABLE I
Effects of aldicarb on the total cell yield and pigmentation of
Azotobacter chroococcum

Treatment	Cell yield ¹ (dry weight)	Pigmentation	
		Formulation time ²	Quantity (O.D) ³
No aldicarb (control)	128	72	0.30
1 ppm ,,	118	72	0.28
2 ppm ,,	120	60	0.21
5 ppm ,,	89	96	0.02

- ¹ Cells harvested after 5 days growth, expressed in mg per 100 ml culture.
- ² Number of hr after inoculating the medium with the organism.
- ³ Pigment was extracted from the cells harvested from 50 ml of the culture medium after 5 days growth.

The drastic reduction in the respiratory rate of *A. chroococcum* cells by 5 ppm of aldicarb indicates its probable mode of action. This organism, well known for its high respiratory quotient, has a low efficiency for carbon utilization. When the respiration was inhibited by aldicarb, carbon assimilation was also affected. Both reduction as well as stimulation of respiration in *A. vinelandii* in presence of several

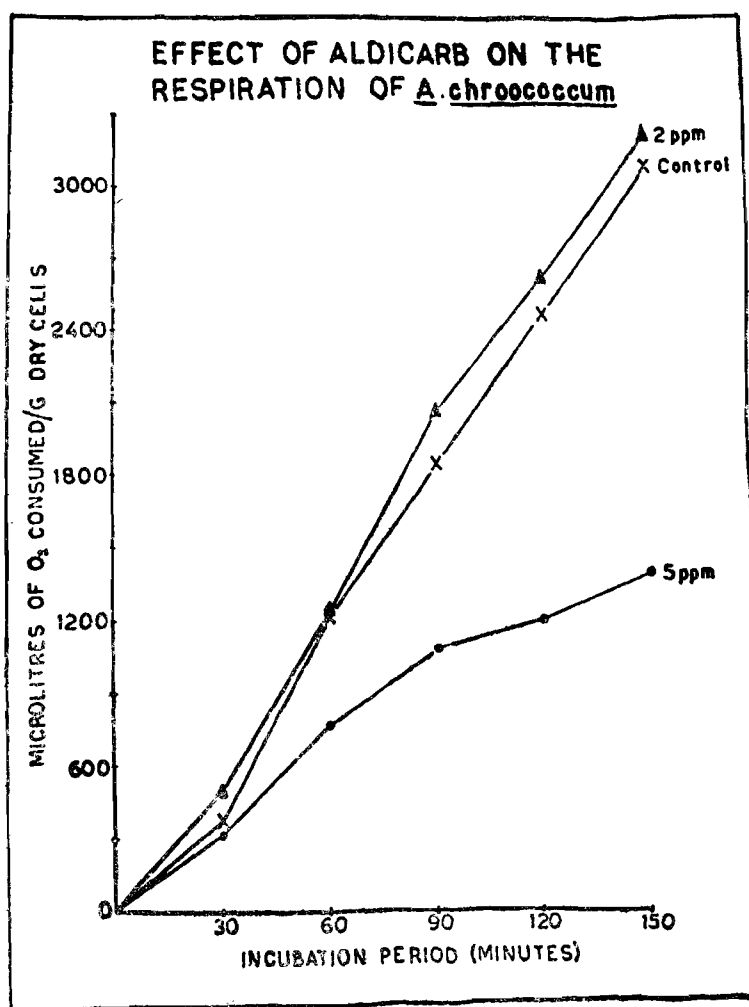
TABLE II
Influences of aldicarb on the incorporation of ^{32}P -dipotassium hydrogen phosphate by
Azotobacter chroococcum cells

Treatment	*Incorporation of ^{32}P -activity (cpm/100 mg dry cells)
No aldicarb (control)	1,76,820 $\pm$ 360
1 ppm aldicarb	1,42,976 $\pm$ 322
5 ppm aldicarb	86,642 $\pm$ 187
10 ppm aldicarb	55,214 $\pm$ 166

*Radio-activity was determined 6 hr after injection of the radio-chemical into the growth medium.

other insecticides have been reported (MacRae and Celo, 1974). Inhibition of respiration and induction of an adaptive mechanism by aldicarb has been reported in *Pseudomonas solanacearum* (Balasubramanian and Gita Nilakantan, 1976). A similar mode of action of aldicarb is possible in *A. chroococcum*.

The reduction in incorporation of ^{32}P -phosphate by the aldicarb treated cells, especially at 5 and 10 ppm, provides additional evidence to explain the mode of action of the insecticide on the metabolic activities of this organism. It is quite possible that the pesticide adversely affected the electron transport system of



A. chroococcum resulting in a reduced uptake of P with a consequent reduction in the oxidative phosphorylation and ATP synthesis, which is essential for the cellular

metabolism and growth of the organism. Thus, it can be postulated that aldicarb, especially at 5 ppm and higher concentrations can inhibit certain biosynthetic processes of *A. chroococcum* by interfering with the respiration of the organism resulting in reduced phosphorus uptake and growth. The implications of such adverse effects of aldicarb, at the normal recommended levels (2 to 5 ppm), on the nitrogen fixation by *A. chroococcum* in soil is of practical concern.

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74 EFFECT OF CERTAIN INSECTICIDES ON THE GROWTH OF BLUE GREEN ALGAE

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SUMMARY

The granular insecticides fenthion, oftanol, Garvox, carbofuran, diazinon, sevidol, phosphate, cytolane, ekalux and MIPC were incorporated in culture media at 50, 100, 200 and 400 ppm. The blue green alga *Aphanothece pallida* was inoculated and the growth *in vitro* which was studied was not adversely affected by the pesticide applications.

In recent years, blue green algae have been shown to be agriculturally important particularly in tropical rice field soils chiefly because of their capacity to synthesize organic substances and to fix atmospheric nitrogen. Our soils are deficient in nitrogen and a supply of nitrogen by the application of blue green algae

would enrich soil nitrogen and increase rice production. Extensive field trials have already indicated clearly the positive effects of these algae on the growth and yield of rice plants under different agroclimatic conditions. In submerged rice field soils, biological nitrogen fixation can contribute 20-30 kg N/ha.

The application of granular pesticides to rice can have a direct effect on the soil microflora. The degradation of pesticides by the soil microflora under water-logged soil conditions is well known (Alexander, 1969). Very little information is available on the effects of pesticides on the growth and survival of blue green algae (Shtina, 1957), although some strains of algae have been used to assay soil-applied herbicides (Pillay and Tehan, 1970). A study of the effects of certain granular insecticides on the growth of a blue-green alga *Aphanathece pallida* is presented here.

MATERIAL AND METHODS

The granular insecticides fenthion, oftanol, garvox, carbofuran, diazinon, sevidol, phorate, cytolane, ekalux and MIPC were tested at 50, 100, 200 and 400 ppm levels. To 100 ml of Fogg's nitrogen free medium in 250 ml conical flasks appropriate concentrations of the insecticides were introduced. The flasks were inoculated with a pure culture of *Aphanathece pallida* and incubated at 30°C for 30 days under fluorescent lamps. The growth of the algae was measured at the end of 30 days as dry weight. The difference in growth of insecticide treated and untreated alga was used to assess the effect of insecticides under test.

RESULTS AND DISCUSSION

The growth of *Aphanathece pallida* was little affected by the incorporation of granular pesticides in the medium (Table I). There was not much difference in the growth of the alga due to different concentrations of the pesticides. Although growth was slightly inhibited at all the concentrations tested.

Singh (1972) reported that lindane, endrin and diazinon in nitrogen-free as well as nitrate-nitrogen media had no adverse effect on the blue green algae viz., *Cylindrospermum* sp. and *Aulosira fertilissima*. Gamma BHC application to rice fields has been reported to stimulate the algae populations by inhibiting the predators which feed on algae (anonymous, 1965). Both 2, 4-D and MCPA affected nitrogen fixation by *Nostoc muscorum*, *N. punctiforme* and *Cylindrospermum* sp. at concentrations recommended for field application but stimulated nitrogen fixation at 10^{-4} and 10^{-5} M concentrations. However, it has been shown that many blue green algae strains could tolerate high levels of some of these pesticides, although the

TABLE I
Effects of granular pesticides on the growth of blue green algae,
Aphanothece pallida

S. No.	Treatment	Mean dry weight of algae (g/50 ml)			
		Concentrations of pesticides (ppm)			
		50	100	200	400
1	Fenthion	0.0291	0.0287	0.0283	0.0280
2	Oftanol	0.0295	0.0292	0.0290	0.0286
3	Garvox	0.0301	0.0297	0.0293	0.0291
4	Carbofuran	0.0303	0.0303	0.0298	0.0295
5	Diazinon	0.0300	0.0297	0.0293	0.0289
6	Sevidol	0.0304	0.0300	0.0295	0.0200
7	Phorate	0.0236	0.0277	0.0280	0.0274
8	Cytrolane	0.0290	0.0285	0.0281	0.0276
9	Ekalux	0.0287	0.0285	0.0282	0.0280
10	MIPC	0.0303	0.0298	0.0284	0.0288
11	Control		0.0306		

algae exhibit a wide range of variation in their tolerance (Venkataraman and Rajalakshmi, 1972). Venkataraman (1972) attributed the growth of algae in the presence of the pesticides to their active metabolism of pesticide or to adaptation. The present results agree with the above findings. The results show that *Aphanothece pallida* could tolerate the granular pesticides at 50, 100, 200 and 400 ppm levels.

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75 THE BIOCONCENTRATION OF P,P'-DDT AND ITS METABOLITES IN A CILIATE PROTOZOAN, *TETRAHYMENA PYRIFORMIS* AND ITS RELATIONSHIP WITH THEIR N-OCTANOL/HEXANE : WATER PARTITION COEFFICIENTS

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The accumulation, metabolism and excretion of DDT and its metabolites was studied in a ciliate protozoan *Tetrahymena pyriformis* which accumulated p,p'-DDD, p,p'-DDE, o,p'-DDT, DDMU and p,p'-DDT in a decreasing order of magnitude. To account for these variations in accumulation, partition coefficients of these compounds in n-octanol/hexane: water were determined. The details of these studies and bioconcentration potential of DDT and its metabolites in relation to n-octanol/hexane : water partition coefficients are discussed.

p,p'-DDT can be degraded by micro-organisms and p,p'-DDE, p,p'-DDD and DDMU are the common end products of such conversion (Cox, 1972 ; Johnson, 1975 ; Butler, 1977). When such compounds are lipophilic or have high biological affinities, they become potential environmental pollutants. DDT metabolites are toxic to certain avian and mammalian systems (Lincer, 1975 ; Wilkinson, 1976), but limited attention has been paid to the study of their accumulation and biomagnification in fresh water food chains.

Ciliate protozoans are among the most common micro-organisms at the base of aquatic food chains (Borror, 1963 ; Fenchel, 1967) and have been reported to accumulate very high levels of p,p'-DDT (Gregory *et al.*, 1969). The ciliate protozoan, *Tetrahymena pyriformis* which occurs world-wide in a variety of fresh water habitats, was used to study the accumulation and elimination of p,p'-DDT ; p,p'-DDD ; o,p'-DDT ; p,p'-DDE and DDMU. The magnitude of accumulation of some of the organochlorine compounds in aquatic organisms has been related to their partition-

ing in *n*-octanol and water (Kenaga, 1975). The validity of such a system with reference to ciliate protozoans was also investigated.

MATERIAL AND METHODS

T. pyriformis was grown at 28°C in sterilized 1 percent (w/v) proteose peptone supplemented with 0.3 per cent (w/v) yeast extract and 0.5 per cent (w/v) sodium chloride. Stock cultures were maintained in 15 ml test tubes containing 2 ml of the culture medium and were subcultured once in 2 weeks. All inoculations were made under aseptic conditions.

Stock solutions of *p,p'*-DDT and its metabolites were prepared in acetone. Appropriate quantities were added to the culture media to obtain the desired concentration of each compound. The purity of *p,p'*-DDT and its metabolites was more than 99 per cent as estimated by gas liquid chromatography (GLC). Concentrations of acetone up to 0.5 per cent had no detrimental effects on the growth of the organisms, concentrations of acetone were kept at 0.5 per cent or less.

Uptake of p,p'-DDT and its metabolites

For these studies, cultures were set up as follows: Two ml of a 72 hr old culture was added to 98 ml of sterilized proteose peptone (under aseptic conditions) in 250 ml flasks. Cultures were grown for 24 hrs at 28°C and the number of cells were counted in each flask before treatment. Each flask received 0.1 ml of the acetone solution of the toxicant which gave a final concentration of 1 ppm. Three separate controls using: a) solvent, medium and organisms; b) solvent, DDT and medium and c) medium and organisms were set up simultaneously. The last control was to examine any possibility of contamination due to DDT or its metabolites in the organisms and in the medium. Treated cultures were shaken periodically to keep the cells in suspension, and after the appropriate time intervals, cultures were harvested by centrifuging at 5000 rpm for 10 minutes and the resulting pellet was washed three times with a toxicant free medium and the washings added to the supernatant which was collected in a separate flask. Both pellet and supernatant were analysed by GLC. Cell counts were taken in the experimental as well as the control cultures. All treatments were replicated three times. For dry weight estimation, separate cultures (in triplicate) were treated with 1 ppm of each compound, pelleted by centrifugation and washed pellet suspended in 0.5 ml of distilled water, transferred to boat-shaped aluminium pots and dried to a constant weight at 80-90°C. The weight of the dried residue was thus determined.

Elimination of p,p'-DDT and its metabolites

The animals were treated with 1 ppm *p,p'*-DDT under standard experimental

conditions for four days, transferred to a toxicant free medium (The medium consisted of 100 ml of sterilised Chalkley's medium supplemented with 1 per cent of yeast extract) and allowed to grow for 24 hrs. During this period, samples were collected at 3, 12 and 24 hrs for estimation of the levels of DDT and its metabolites.

The pellets and supernatant were extracted three times for DDT and its metabolites using hexane. The combined hexane extract either from the pellet or from the supernatant was concentrated to approximately 1 ml under vacuum and cleaned up by a Florisil column (Dharam Vir, 1976). The cleaned-up extracts were evaporated to dryness and dissolved in a known volume of hexane for analysis by GLC on 1.5 per cent OV-17 on Supelcon AW DMCS, 100-120 mesh using a Packard A 7400 gas liquid chromatograph equipped with an electron capture detector. The operating parameters were : Inlet temperature 210°C, column temperature 190°C, detector temperature 210°C and nitrogen gas flow was 75 ml/min. The recovery of standards from the spiked samples from the medium was : *p,p'*-DDT 80 per cent ; *p,p'*-DDD 83 per cent ; *o,p'*-DDT 78 per cent ; *p,p'*-DDE 89 per cent ; DDMU 76 per cent and from the organisms was : *p,p'*-DDT 95 per cent ; *p,p'* DDD 97 per cent ; *o,p'*-DDT 98 per cent ; *p,p'*-DDE 95 per cent ; and DDMU 99%. All residues were calculated on a dry weight basis but not corrected for recovery.

Partition coefficients of each compound in n-octanol/hexane over water were determined and calculated according to the method described by Voerman (1969). Solutions of each compound were prepared in n-octanol/hexane at room temperature to obtain a concentration of 500 µg/ml. Each estimation was repeated five times.

RESULTS

One ppm of *p,p'*-DDT had no detrimental effect on the cell population of *T. pyriformis* (Table 1). The acetone control at a concentration corresponding to that used for *p,p'*-DDT or its metabolites did not alter the morphology and had no effect on the growth of the organisms.

The amounts of *p,p'*-DDT adsorbed/absorbed by *T. pyriformis* are given in Fig. 1. Pick up was rapid in the beginning and total DDT residues increased from 6.30 ppm after on hr. to 23.8 ppm after 12 hrs. However, the total DDT residues declined to 4.6 ppm after 48 hrs and did not change thereafter. Bio-concentration factors for *p,p'*-DDT varied from 2 to 24.

The rates of accumulation of DDMU, *p,p'*-DDE and *o,p'*-DDT were higher in the beginning (Fig. 2). The time for maximum adsorption/absorption was 6 hrs for *p,p'*-DDE, one hr for *o,p'*-DDT and 24 hrs for DDMU, *p,p'*-DDE was concentrated by 485 times, *o,p'*-DDT by 417 times and DDMU by 148 times. Accumu-

TABLE I
Growth response of *Tetrahymena pyriformis* to 1 ppm of p,p'-DDT

Exposure time in days	cell number x 10 ⁴ / ml		Treated
	Control ^a	Control ^b	
1	13.65	13.50	13.50
2	22.36	22.35	21.50
3	25.50	24.62	25.40
4	23.40	23.00	24.00
5	22.30	21.00	22.50
6	21.50	22.00	22.00
7	21.50	21.50	21.00
8	20.20	20.00	20.50
9	20.00	20.30	20.00
10	20.00	19.80	20.00

(a) Control lacking both acetone and DDT.

(b) Control lacking DDT but having corresponding concentration of acetone.

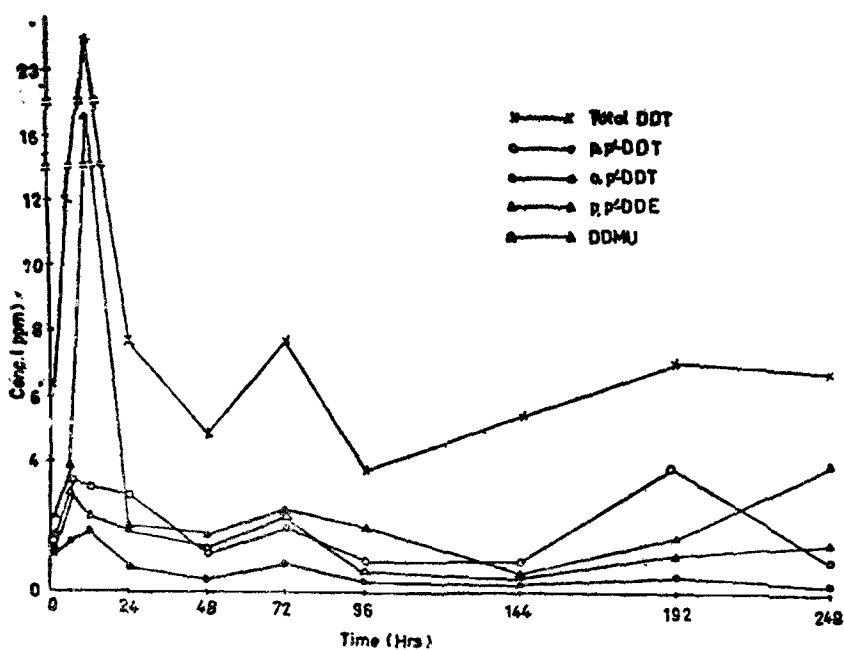


Fig 1 Uptake and metabolism of p,p'-DDT at different time intervals by *T. pyriformis* exposed to one ppm of p,p'-DDT for ten days.

lation of *p,p'*-DDD was rapid during first six hrs and after this period the animals continued accumulating *p,p'*-DDD, but at a slower rate (Fig. 3). The plateau concentration was not reached during the 10 days of the experiment and organisms accumulated 777 ppm of *p,p'*-DDD by the end of the experiment.

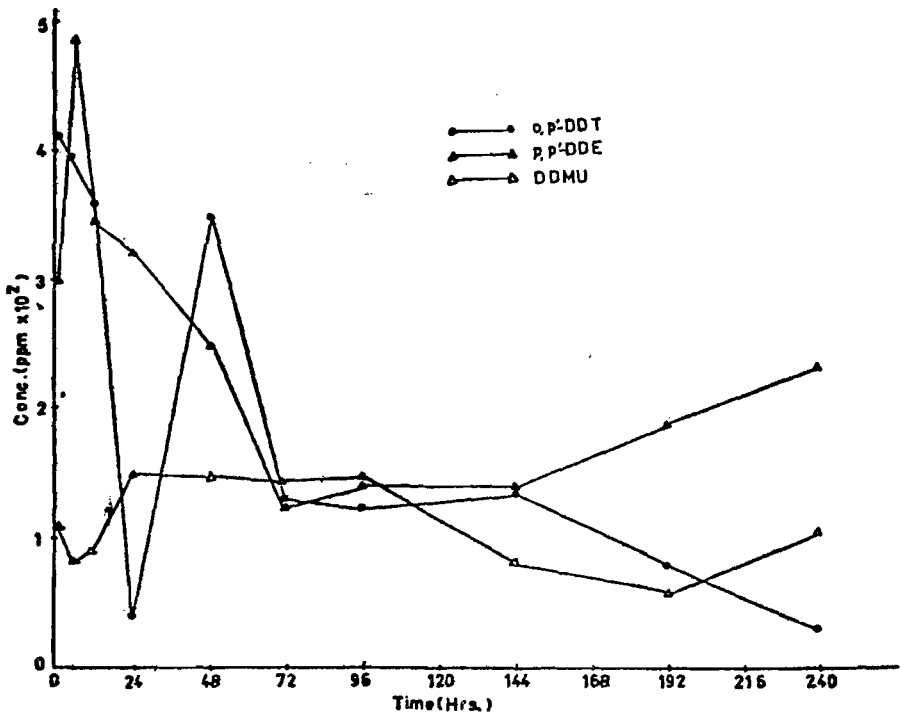


Fig. 2 Uptake of *p,p'*-DDE, *o,p'*-DDT and DDMU at different time intervals by *T. pyriformis* exposed to one ppm of each compound separately for 10 days.

Elimination of *p,p'*-DDT when organisms were transferred to a toxicant free medium occurred in two phases (Fig. 4). a) A rapid phase of elimination which was completed during the first three hrs and b) A slow phase of elimination which continued for another 21 hrs. During the rapid phase of elimination, more than 50 per cent of DDT residues were excreted by the organisms but during the second phase only 35 per cent of DDT residue was lost from the organisms. Elimination of DDT metabolites followed a similar pattern as that observed for DDT elimination (Fig. 5). However, during the rapid phase of elimination about 80 per cent of the metabolite residue was released to the medium and during next 21 hrs only 10 per cent *o,p'*-DDT ; 3.5 per cent *p,p'*-DDD ; 0.75 per cent *p,p'*-DDE and 0.5 per cent DDMU was retained by the organisms.

There was a direct correlation between the bioconcentration factors and partition coefficients of DDT and metabolites in octanol over water. However, values for partition coefficient for DDMU deviated from the normal pattern. Such a relationship could not be established between bioconcentration factors and partition coefficients for DDT and its metabolites in hexane over water.

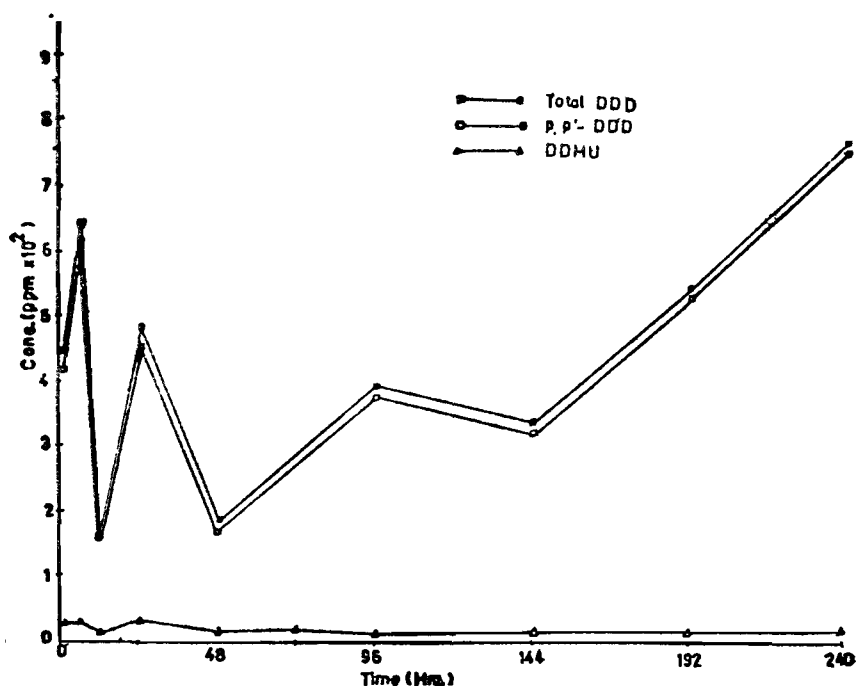


Fig. 3 Uptake metabolism of *p,p'*-DDD at different time intervals by *T. pyriformis* exposed to one ppm *p,p'*-DDT for 10 days.

DISCUSSION

T. pyriformis accumulated *p,p'*-DDT and its metabolites in the order *p,p'*-DDD > *p,p'*-DDE > *o,p'*-DDT > DDMU > *p,p'*-DDT. A common feature in accumulation of these compounds was their higher rates of accumulation in the beginning regardless of whether accumulation continued till the end of the experiment or not. A rapid accumulation of *p,p'*-DDT and its metabolites in the initial stages of treatment is of importance since physical adsorption to surfaces which occurs immediately and long term penetration (absorption) are involved in residue accumulation (Kenaga, 1975). It is therefore, possible that the first period of residue accumulation is due mainly to adsorption and may result in high rates of residue accumulation.

Slow but continuous accumulation of p,p' -DDD after this period suggests that such an accumulation is mainly due to absorption.

The time for maximum adsorption/absorption of a particular compound varies from organism to organism and depends upon the chemical nature of the pesticide to which the organism is exposed. The cells of the marine alga, *Tetraselmis chuii*

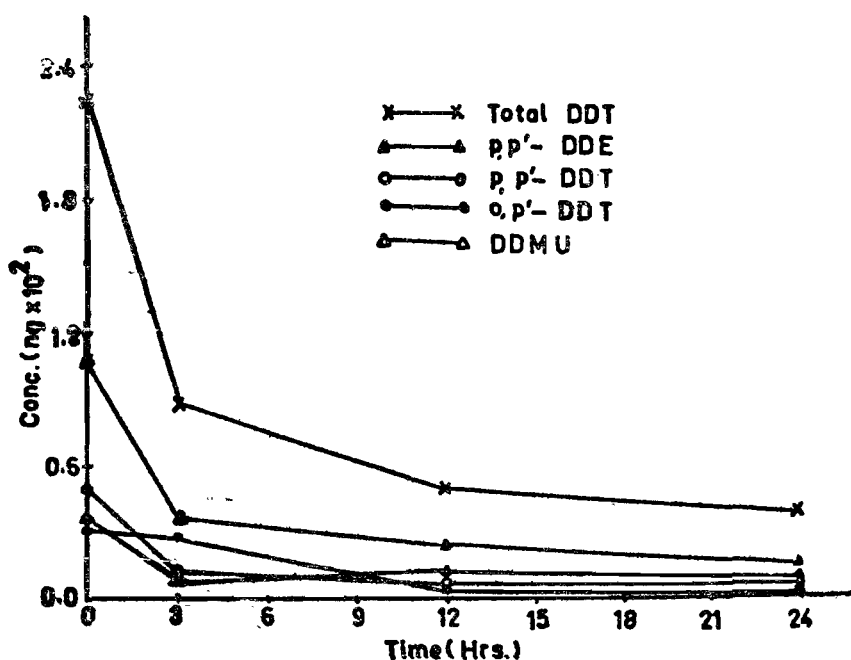


Fig. 4 Elimination of p,p' -DDT and its metabolites from *T. pyriformis* when they were transferred to toxicant free medium after an initial exposure to one ppm of p,p' -DDT for four days. The data is expressed in terms of total ng DDT/metabolite retained by the organisms. The number of animals used in each replicate was same.

accumulates maximum amounts of p,p' -DDT within one hr. while the maximum amount of dieldrin by *Cyclotella nana* is accumulated in less than one hr. (Rice and Sikka, 1972). However, the fresh water algae, *Chlorella pyrenoidosa* absorbed the maximum dieldrin after 48 hrs. (Wheeler, 1970) and another species of *Chlorella* accumulated the maximum amount of p,p' -DDT in one minute (Sodergren, 1968). For another algae, *Ankistrodesmus amalloides* the time for maximum accumulation was 120 min. for DDT, 30 to 60 min. for dieldrin and 30 min. for photodieldrin (Neudorf and Khan, 1975). The kinetics of adsorption/absorption of insecticides in micro-organisms are not known and quick accumulation and differences in the levels of accumulation of organochlorine insecticides have been related to their

water solubility (Neudorf and Khan, 1975) and surface adsorption (Keil and Priester, 1969 and Cox, 1970). Factors such as vapor pressure, water solubility, molecular weight of the compound and surface area of the organisms, chemical constituents of the cell membrane and cell constituents of the organisms may be responsible for differences in accumulation of insecticides.

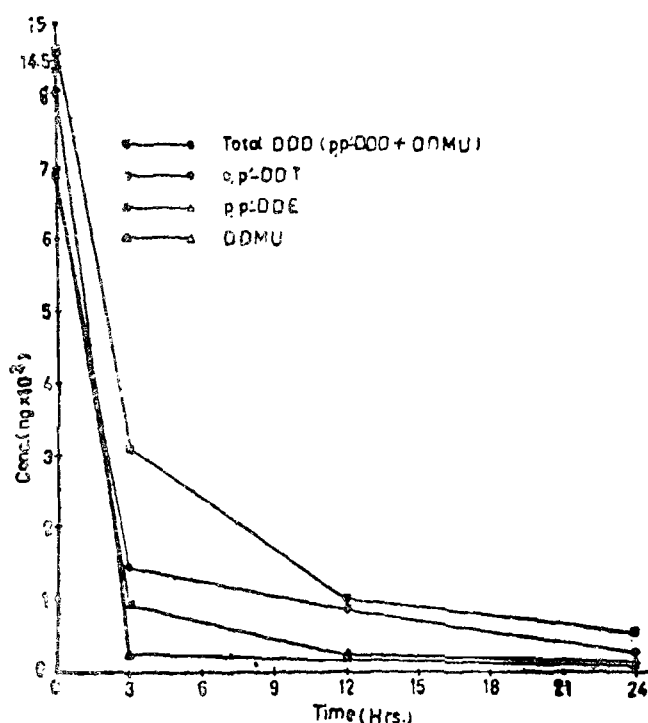


Fig. 5 Elimination of *p,p*-DDD, *o,p*'-DDT, *p,p*'-DDE and DDMU from *T. pyriformis* when they were transferred to toxicant free medium after an initial exposure to one ppm toxicant separately for four days. The data is expressed in terms of total ng toxicant retained by the organisms. The number of animals used in each experiment was same.

The accumulation of *p,p*'-DDT and its metabolites did not follow any set pattern in *T. pyriformis* and such changes in the levels of toxicants have been attributed to factors such as the time of exposure, rate of uptake, chemical nature of the compound, metabolism of the compound within the organism, rate of excretion and physiological state of the organism (Kenaga, 1975 ; Hamlink *et al.*, 1977). The last factor is more important in *T. pyriformis* because the physiological state of the organism is under continuous change.

Cox (1972) suggested that uptake of *p,p'*-DDT was dependent on the phase partitioning of DDT between the medium and the lipid portion of the cell. Thus, the greater uptake of DDT metabolites and much less uptake of *p,p'*-DDT by *T. pyriformis* indicate that *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT and DDMU partitioning is more in favour of the organisms than that of *p,p'*-DDT. Non-polar compounds such as *p,p'*-DDT partition in octanol and water in a ratio similar to the partition coefficient of *p,p'*-DDT between the lipid of aquatic organisms and that of water containing DDT (Kenaga, 1975). During the present investigation it was seen that such a relationship did exist between the bioconcentration potential and n-octanol partitioning between DDT metabolites and water, but it could not be established in hexane : water partitioning. Therefore, n-octanol can perhaps be used as a solvent for determining representative partition of DDT metabolites between lipid and water. It also suggests that in addition to many other factors, partitioning of a particular compound between the medium and the lipid portion of the cell play a significant role in determining the bioconcentration potential of pesticide in organisms. However, more work is needed on the kinetics of partitioning of organochlorine compound in different solvent systems, using standard methods before valid comparisons can be made.

Elimination of *p,p'*-DDT or its metabolites from *T. pyriformis* when the organisms were kept in a toxicant-free medium was in two phases, an initial rapid phase of loss during which most of the accumulated residue was eliminated and a slow phase of loss during which elimination continued at a much retarded rate. After 24 hrs. about 80-95 per cent of the residue was eliminated from *T. pyriformis* and subsequent analysis revealed no further loss indicating a long term binding of the remaining residue. Johnson and Kennedy (1973) found that microbial cells exposed to DDT when washed with a DDT-free medium, lost DDT residues by 55 per cent for *Aerobacter aerogenes* and 70 per cent for *Bacillus subtilis*. Elimination of gamma-HCH was rapid and completed within 30 minutes in *Chlamydomonas* (Canton *et al.*, 1977). However, DDT was held without being eliminated in *Chlorella* (Sodergren, 1968) and *Euglena* (Dekoning and Mortimer, 1971).

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76 CHANGES IN THE ABUNDANCE OF SOIL INHABITING ACARI OF A PADDY FIELD IN RESPONSE TO THE APPLICATION OF TWO HERBICIDES

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SUMMARY

In the present investigation, effects of two herbicides, nitrofen (Tok, E-25) and Propanil, (Stam E-34) on the Acari in the soil of a highland paddy field with a laterite soil were evaluated. Farm yard manure significantly increased the abundance of Acari in all plots (except for Cryptostigmata in the untreated and propanil treated plot). Propanil had no adverse effect on the abundance of Prostigmata, but decreased those of Mesostigmata significantly. Cryptostigmatid mites, increased significantly in numbers after the application of this herbicide. Nitrofen had a more detrimental influence on the Acari than on the Prostigmata and Mesostigmata, which were significantly reduced in number within 45 days of its use. Populations of Cryptostigmata remained rather unaffected. Mesostigmata seems to be the group most affected by both herbicides. The Influences of the herbicides, on the various species of Cryptostigmata are discussed in the paper.

INTRODUCTION

The influence of various herbicides on non-target arthropods in soil has gained much importance in recent years. Edwards (1970) and Eijasckers and Van der Drift (1975) reviewed the problem, Bhattacharya and Joy (1977 a & b) and Joy and Bhattacharya (1977) showed that herbicides such as Banvel-D and Tok, E-25 have more effects on the abundance of soil microarthropods as a whole, than herbicides such as Stam, F-34 and 2, 4-D, at least under tropical conditions. In the present study, an attempt has been made to evaluate the effects of herbicides such as nitrofen and Propanil upon the populations of different Acari groups inhabiting a paddy field with a laterite soil at Santiniketan.

MATERIAL AND METHODS

The present investigation was during June to October, 1976 in three small high land plots under a dry living variety of paddy Pusa-2-21. The plots had no previous agricultural manipulations or pesticide contamination. Abundance of the Acari were estimated before and after agricultural activities such as ploughing, manuring and herbicide treatments by taking random soil samples at fortnightly intervals. Of the three plots, in one nitrofen was used and in the other Propanil was used at the usual agricultural rate. The third plot was an untreated control. Dry farm yard manure was the only fertilizer used and no mechanical weeding was done during the entire period of investigation. All the plots were watered regularly. Nitrofen (2,4-dichlorophenyl-4-nitrophenyl ether) was sprayed at the rate of 2 kg. a.i./ha., before the sprouting of paddy seeds and propanil (3,4-dichloro propion anilide) was sprayed at a rate of 3 kg. a.i./ha., after the complete emergence of weeds in the plot. The temperature and moisture content of the soils were recorded at every sampling in all the plots. Nine soil samples, each 5x5x10 cms., were taken at a time from each plot between 08.00 to 09.00 hours, and the Acari were extracted together with all other actively moving soil microarthropods with a battery of "Tullgren" funnels. The data obtained were analysed statistically with the help of tw test (Lord, 1947).

RESULTS AND DISCUSSION

The main findings of the present investigation are summarized in Tables I-III and Figures 1-3. The mean dry weight percentage of soil moisture and mean soil temperature differed little between treated and untreated pots throughout the investigation (Table-I). The application of dry farmyard manure significantly increased the abundance of Acari as a whole (and all groups of Acari except for Cryptostigmata) in untreated and post-emergence herbicide treated plots (Table II).

The pre-emergence herbicide nitrofen stopped the increase in abundance of Acari, so there was a significant difference in numbers between the treated and untreated plots before 45 days after application. Further analysis shows that among the Acari the Mesostigmata and Prostigmata were most affected and by the end of the investigation abundance of these two groups in the treated plot was almost equal to that of the untreated plot. Notwithstanding the gradual decrease in the abundance of Cryptostigmata, this group seems to be the least affected group. (Table I & III and Fig. 1).

Propanil had little or no adverse effects on the Acari. An analysis revealed that Propanil which had no adverse effect on the abundance of Prostigmata decreased

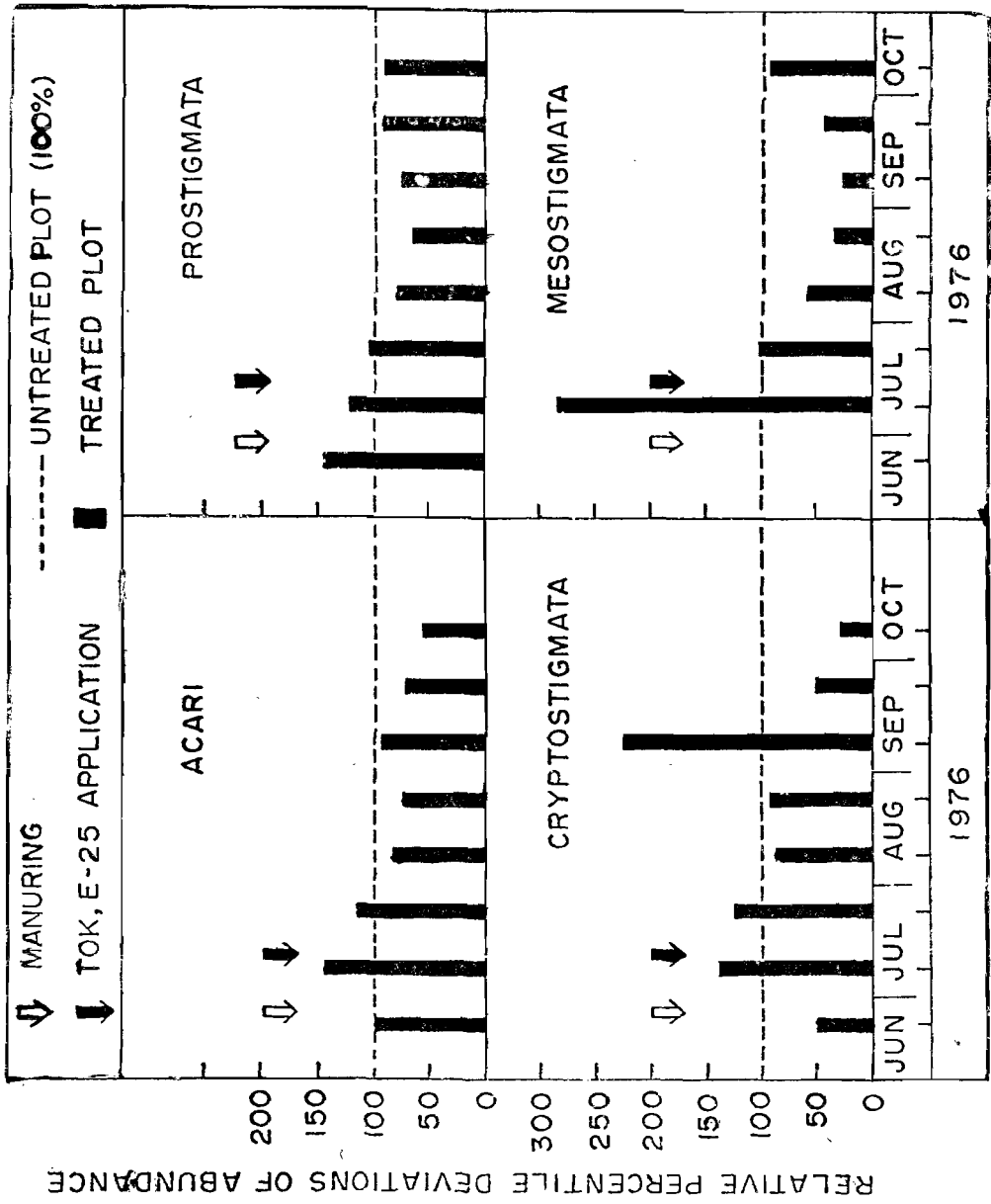


Fig. 1 Relative per cent deviations between untreated and nitrofen treated plots.

numbers of Mesostigmata significantly. Cryptostigmatid mites, increased in number after the application of this herbicide (Fig. 2 Table I & III).

Cryptostigmata were identified to the species level. (Fig. 3). It seems that nitrofen had an adverse effect at least on *Daemeobelba* sp. and *Cosmochthonius*

TABLE I
Abundance of Acari in soil in relation to moisture content and temperature

Groups	Variant	Sampling occasions							
		1	2	3	4	5	6	7	8
Acari	U	5911	15689	21511	28089	31644	23644	25022	16089
	T ₁	5911	22622	24889	23200	23066	21955	17822	9022
	T ₂	8355	14489	16889	25822	46933	24000	24889	14178
Cryptostigmata	U	3022	5466	9866	14889	11378	3333	11289	7333
	T ₁	1511	7644	12266	12933	10444	7466	5866	2178
	T ₂	4355	5289	2178	12933	19155	7289	11511	4622
Prostigmata	U	2000	6311	5822	7600	11466	9289	6889	4666
	T ₁	2933	7778	6044	6044	7422	6978	6222	4178
	T ₂	3333	5511	6933	7378	11733	7955	7066	5511
Mesostigmata	U	89	755	3155	2844	2044	4533	3422	755
	T ₁	—	2133	3200	1733	711	1200	1511	711
	T ₂	89	1289	4089	2044	1378	1155	755	978
Moisture %	U	2.77	10.38	11.61	10.86	7.41	13.51	9.77	8.81
	T ₁	3.41	12.61	11.73	10.99	6.16	11.98	9.89	8.11
	T ₂	3.95	12.11	15.47	12.61	7.99	10.74	6.95	12.23
Soil temp. (C)	U	32.25	30.00	29.25	29.50	29.25	27.00	28.75	25.75
	T ₁	32.25	29.00	29.75	29.25	29.75	27.00	28.75	24.75
	T ₂	32.75	28.75	27.75	28.75	28.75	28.00	28.75	24.25

U=Untreated plot; T₁ = nitrofen treated plot; T₂ = propanil treated plot.

emmae, whereas Propanil appeared to increase numbers of *Archegozetes longisetosus*, *Oppia* sp. and *Scheloribates albialatus*. The other species remained rather unaffected.

Adverse effects of various herbicides upon the soil acarofauna have been shown by a number of workers (Eijsackers & Van der Drift, 1976). A few such as Fox (1964), Davis (1965), Stringer *et al.*, (1970 b), Muller (1972) have also pointed out the increase of Acari as a result of herbicide application.

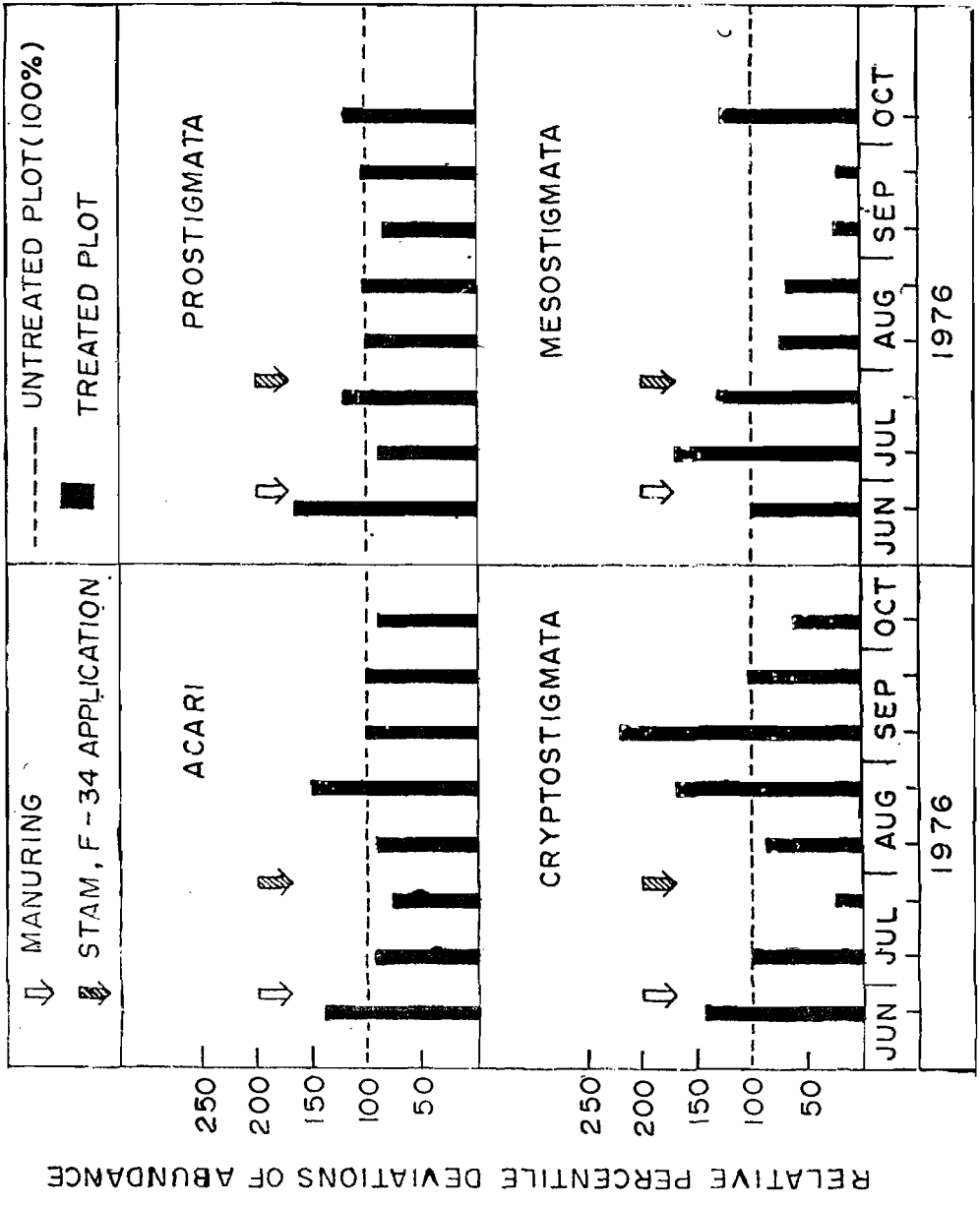


Fig. 2 Relative per cent deviations between untreated and propanil treated plots

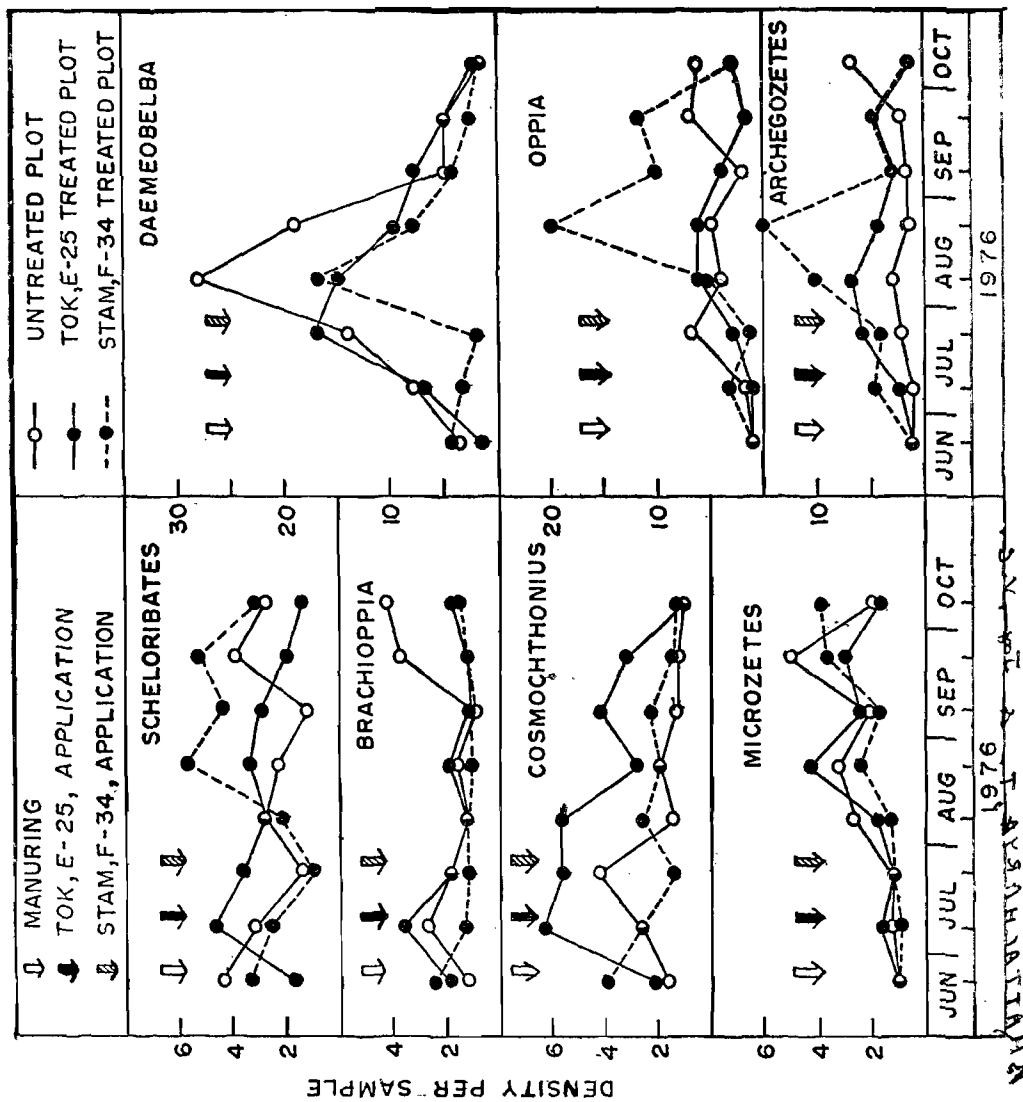


Fig. 3 Changes in the abundance of different cryptostigmatid species.

TABLE II

Values of tw test between treated and untreated plots at different sampling intervals

		Acari	Cryptostigmata	Prostigmata	Mesostigmata
<i>Untreated Plot</i>					
I	II	0.543 **	0.249	0.634 **	0.371 *
II	III	0.213	0.319	0.044	0.316
III	IV	0.184	0.185	0.207	0.031
IV	V	0.103	0.121	0.450 *	0.125
V	VI	0.388 *	0.718 **	0.232	0.226
VI	VII	0.053	0.565 **	0.273	0.091
VII	VIII	0.315	0.170	0.278	0.325
<i>Nitrofen Treated Plot</i>					
I	II	0.836 **	0.529 **	0.621 **	0.969 **
II	—→ III	0.061	0.210	0.188	0.148
III	IV	0.047	0.028	0.000	0.171
IV	V	0.005	0.154	0.223	0.243
V	VI	0.049	0.298	0.070	0.271
VI	VII	0.246	0.136	0.171	0.078
VII	VIII	0.677 **	0.486 **	0.330	0.267
<i>Propanil Treated Plot</i>					
I	II	0.557 **	0.100	0.376 *	0.429 *
II	III	0.119	0.472 **	0.158	0.206
III	—→ IV	0.319	0.618 **	0.022	0.140
IV	V	0.580 **	0.296	0.473 **	0.119
V	VI	0.603 **	1.211 **	0.270	0.058
VI	VII	0.029	0.213	0.668	0.143
VII	VIII	0.454 *	0.308	0.211	0.073

* Significant at $P < 0.05$; ** Significant at < 0.01 .

I—VIII Samplings at fortnightly intervals.

The arrows indicate the stages at which herbicides were sprayed.

In conclusion the effect of herbicides on the mites in soil differ from group to group and depends upon the chemical used. Mesostigmatid mites are most adversely affected and the Cryptostigmata seems to be the least affected group.

TABLE III

Values of tw test between different sampling intervals in different plots

Sampling Occasions		Acari	Cryptostigmata	Prostigmata	Mesostigmata
Untreated plot	Nitrofen Treated plot				
	I	0.000	0.315	0.311	0.000
	II	0.221	0.131	0.126	0.362 *
	—> III	0.101	0.125	0.025	0.004
	IV	0.127	0.062	0.311	0.143
	V	0.346 *	0.070	0.413 *	0.444 *
	VI	0.092	0.530 **	0.385 *	0.340 *
	VII	0.298	0.299	0.092	0.341 *
	VIII	0.411 *	0.409 *	0.070	0.028
Untreated plot	Propanil Treated plot				
	I	0.260	0.153	0.256	0.000
	II	0.061	0.017	0.108	0.133
	III	0.165	0.801 **	0.087	0.054
	—> IV	0.063	0.056	0.037	0.108
	V	0.437 *	0.518 **	0.022	0.145
	VI	0.015	0.659 **	0.115	0.331
	VII	0.004	0.009	0.018	0.556 **
	VIII	0.100	0.154	0.162	0.092

* Significant at $P < 0.05$; ** Significant at < 0.01 .

I—VIII Samplings at fortnightly intervals.

The arrows indicate the stages at which herbicides were sprayed.

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77 EFFECTS OF ORGANOCHLORINE INSECTICIDES ON THE LIFE AND ACTIVITY OF NON-TARGET MARINE ANIMALS

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SUMMARY

Heptachlor and heptachlor epoxide have been found in estuarine and marine systems. Marine animals seem to have a capacity to accumulate very high quantities of heptachlor. Since, the organochlorine pesticides are lipophilic, it is likely that tissues which contain more fat can accumulate them. The toxicity of heptachlor to *Perna viridis* gives a 96 h-LC 50 of

3.3 $\mu\text{l/l}$. In the bivalves, the path of entry is probably the gills due to adsorption of pesticides on the gill surface. Even 0.1 $\mu\text{l/l}$ has resulted in a 50 per cent reduction of byssus secretion and at 1 $\mu\text{l/l}$ a cessation of secretion occurred. Probably, concentrations above 1 $\mu\text{l/l}$ keep *Perna viridis* in a state of suspended animation or kills it. Total cessation of filtration occurs at 1.0 $\mu\text{l/l}$. Considerable quantities of mucus secreted on the gill surfaces could result in impairing the gill irrigation and hence the rate of filtration.

INTRODUCTION

Considerable attention has been paid to the effects of pesticide residues in the marine environment. Edwards (1973), in a well annotated review on the pesticide residues in soil and water has, stated that very little information is available on pesticide residues in sea water. The majority of these insecticides are hazardous because they kill or immobilize aquatic organisms at extremely low concentration. According to Tarzwell (1965), Cope (1965) and Eisler (1969), these levels are substantially below $10\mu\text{g l}^{-1}$. Because of the high resistance of pesticides to physical and microbial degradation and the possibilities of their being carried to very great distances by physical processes, pesticides are likely to become a menace to marine life. The accumulation of non-degradable pesticides in lipid deposits of useful aquatic species is also possible (Johnson, 1968). To evaluate stress profiles induced by pesticides, Eisler (1972) gave an account of the effects of methyl parathion, endrin and methoxychlor on the organs of fishes and bivalves. The effects of pesticides on marine and estuarine organisms have been documented by several authors. Schimmel *et al.* (1976a) investigated the effects of heptachlor on several estuarine organisms. The uptake, depuration, retention and metabolism of heptachlor by an estuarine fish (*Leiostomus xanthurus*) was worked out by Schimmel *et al.* (1976b). Roberts (1975) discussed the effect of endosulfan on rates of byssus thread formation by *Mytilus edulis*. Cook and Moore (1976) found insecticides in the tissues of shrimps, oysters and fishes. Holden (1973) stated that organochlorines could be concentrated by fish rapidly from very low doses by factor of 10^3 or 10^4 , which will make them highly vulnerable if not rapidly and easily degraded.

MATERIAL AND METHODS

The test animals were collected from the low tide belts of Someswara rocky shore ($12^\circ 47'$; $74^\circ 51'$ E). Large numbers of these animals were transported to the laboratory in polythene buckets and kept aerated for 24 hrs before the experiment. Animals of a size range 20–23 mm shell length were used in the experiments.

The purpose of the investigation was to delineate acute and sub-acute toxicity levels, rate of mortality, rate of activity by way of byssogenesis (Roberts,

1975), rate of filtration (dye-clearance technique) and oxygen consumption (Winkler's method) of *Perna viridis*. Concentrations ranging from 1.0 to 10.0 $\mu\text{l l}^{-1}$ for acute toxicity and 0.1 to 1.0 $\mu\text{l l}^{-1}$, for sub-acute toxicity studies were used. The durations of mortality and byssogenesis tests were 96 hr each. Oxygen consumption and rate of filtration tests usually lasted 12 hrs.

RESULTS

Of seven heptachlor concentrations tested ranging from 1 μl to 10 $\mu\text{l l}^{-1}$ death occurred after 84 hr of exposure at 3 $\mu\text{l l}^{-1}$ and 4 $\mu\text{l l}^{-1}$ whereas, *P. viridis*, started dying at 5, 7.5 and 10 $\mu\text{l l}^{-1}$ from 60 hr onwards. The LT50 (lethal time) ranged from 85.8 hr at 4 $\mu\text{l l}^{-1}$ to 58.8 hr at 10 $\mu\text{l l}^{-1}$. The LT50 was never below 4 $\mu\text{l l}^{-1}$ (Fig. 2). The LC50 values showed a drastic reduction between 60 and 96 hr; this was 7.8 $\mu\text{l l}^{-1}$ at 60 hr, and the LC50 is 3.3 $\mu\text{l l}^{-1}$ at 96 hr.

The capacity of *P. viridis* to produce byssus when exposed to concentrations ranging from 0.1 to 1.0 $\mu\text{l l}^{-1}$ is summarized in Fig. 3. Even, 0.1 $\mu\text{l l}^{-1}$ resulted in a 50 per cent reduction of byssus production. Further increases in concentration produced similar results. 1 $\mu\text{l l}^{-1}$ led almost to a complete cessation of pedal gland activity (Table I).

TABLE I

a) Total numbers of byssus threads secreted during 96 hr by ten individuals each of *P. viridis* exposed to different concentrations of heptachlor

Concentration ($\mu\text{l l}^{-1}$)	Control	0.1	0.2	0.3	0.5	0.75	1.00
Number	3372	1753	950	834	387	270	80

b) Percentages of individuals of *P. viridis* secreting byssus threads at 12 hr intervals in various concentrations of heptachlor

Concentrations ($\mu\text{l l}^{-1}$)/Time(h)	L 12	D 24	L 36	D 48	L 60	D 72	L 84	D 96
Control	100	100	100	100	100	100	100	100
0.1	80	100	100	100	100	100	100	100
0.2	60	90	100	80	100	100	100	100
0.3	50	90	90	90	80	100	100	100
0.5	50	90	60	100	80	50	50	10
0.75	40	90	60	70	50	40	40	10
1.00	50	30	30	20	30	10	10	10

D—Dark ; L—Light

TABLE II
Oxygen consumption ($\mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$) of individuals of *Perna viridis* exposed to different concentrations of heptachlor

Period (h)/ Concentration $\mu\text{l/l}$	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	Mean	Standard deviation
Control	2.2140	1.4625	1.4250	0.3750	2.0625	1.5750	1.3875	0.4125	1.1625	1.3275	2.5320	1.4487	0.6692
0.033	1.7880	1.2720	1.1100	0.6900	1.7400	0.8700	0.3300	2.8800	2.0940	1.0080	1.4580	1.3855	0.7158
0.066	1.7400	1.1400	0.4200	0.0600	2.1900	1.5900	0.9300	1.4700	0.4800	0.9000	2.1480	1.1880	0.7062
0.1	1.5250	0.7000	1.1250	0.1500	2.4750	0.9500	0.2500	1.6500	0.8500	1.1650	1.8100	1.1500	0.6874
0.16	1.9875	0.6750	1.5000	1.1625	0.4875	2.2025	2.1750	1.3725	0.1875	0.0750	3.3000	1.3750	0.9922
0.25	1.9350	1.0000	0.4750	0.0250	1.5750	0.6250	0.7100	1.7400	0.1000	0.4500	1.1250	0.8873	0.6474
0.33	1.4100	1.2300	0.4800	1.8300	0.4200	1.4700	0.1800	2.6700	0.8100	1.2000	1.5900	1.2082	0.7174
0.66	2.6100	0.2100	0.2700	1.6800	0.2580	1.6320	2.7000	1.3260	1.8540	1.4400	0.6000	1.3255	0.8986

Fig.1. Heptachlor-Cumulative % mortality

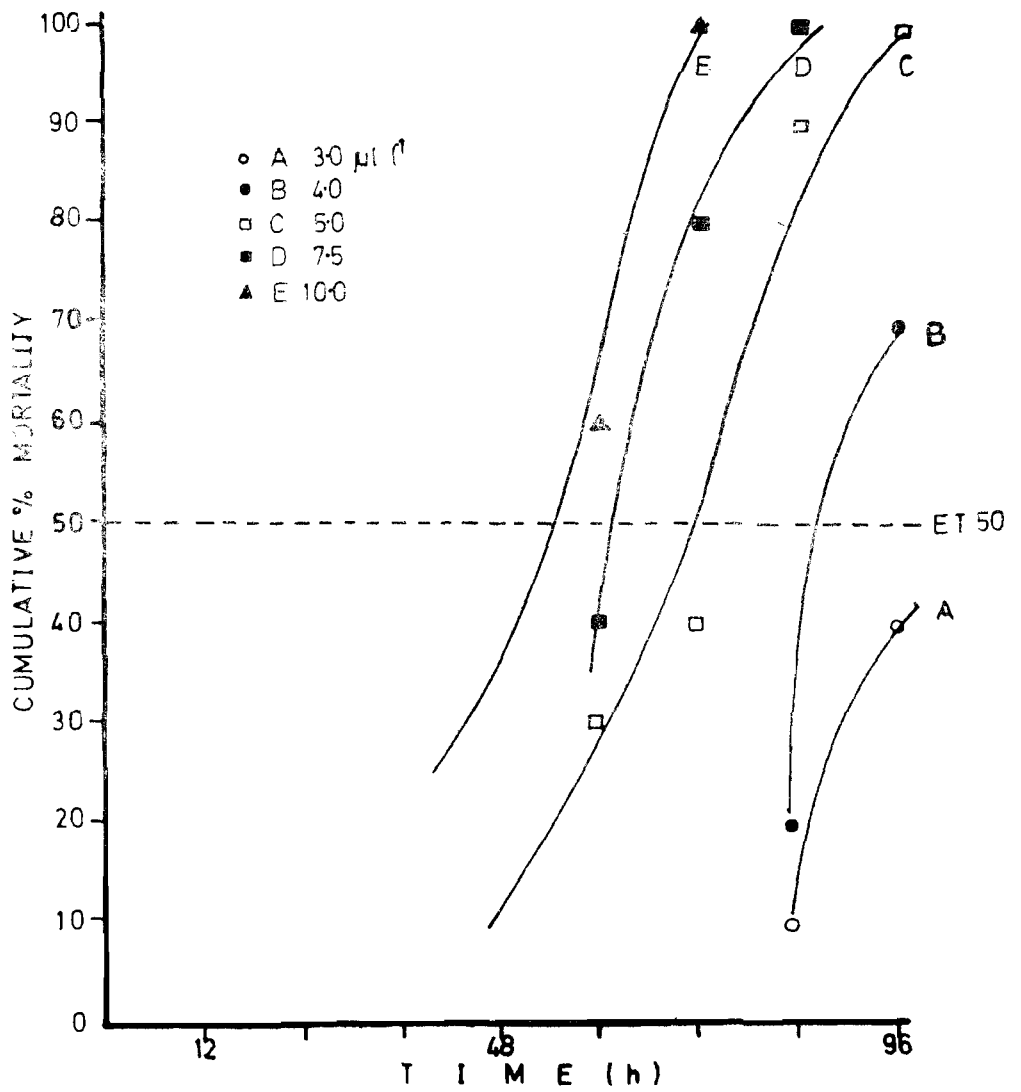


Fig. 2. HEPTACHLOR

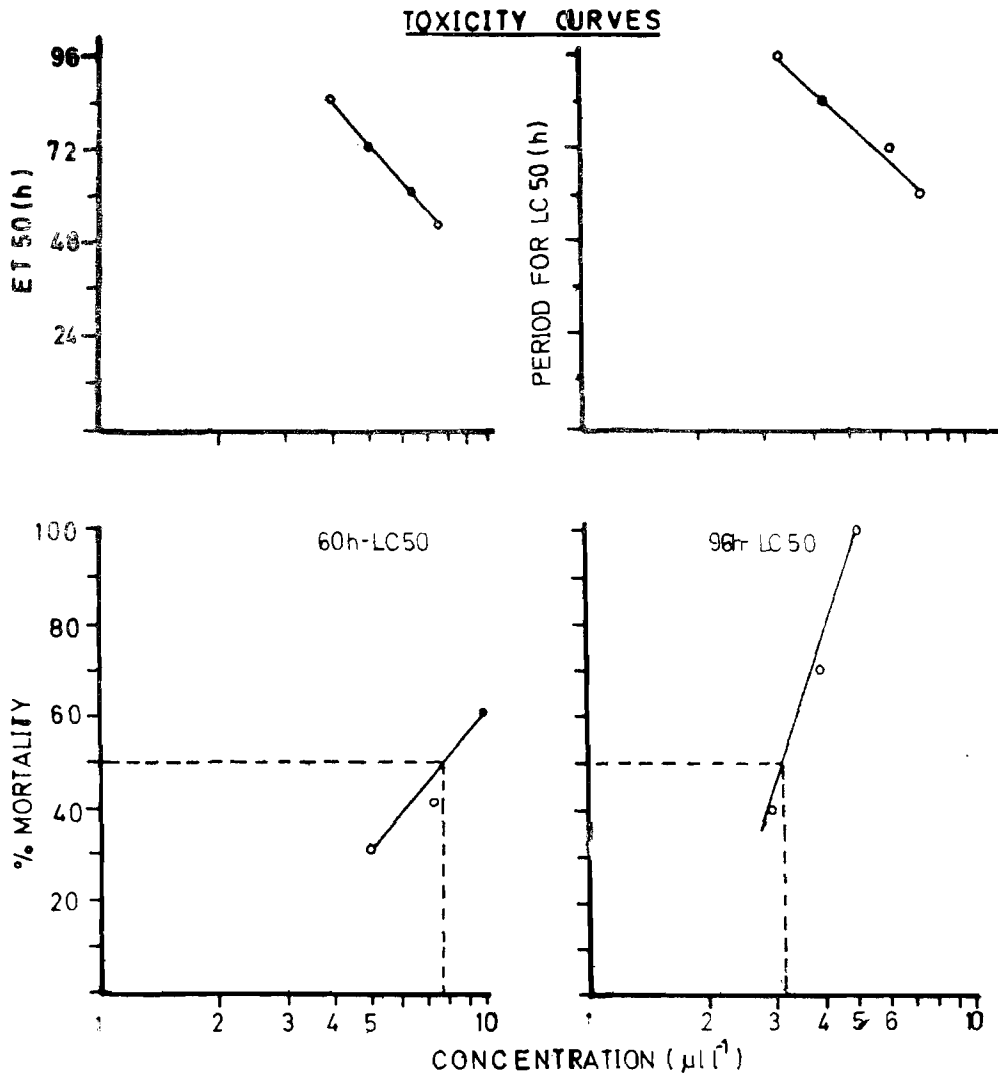
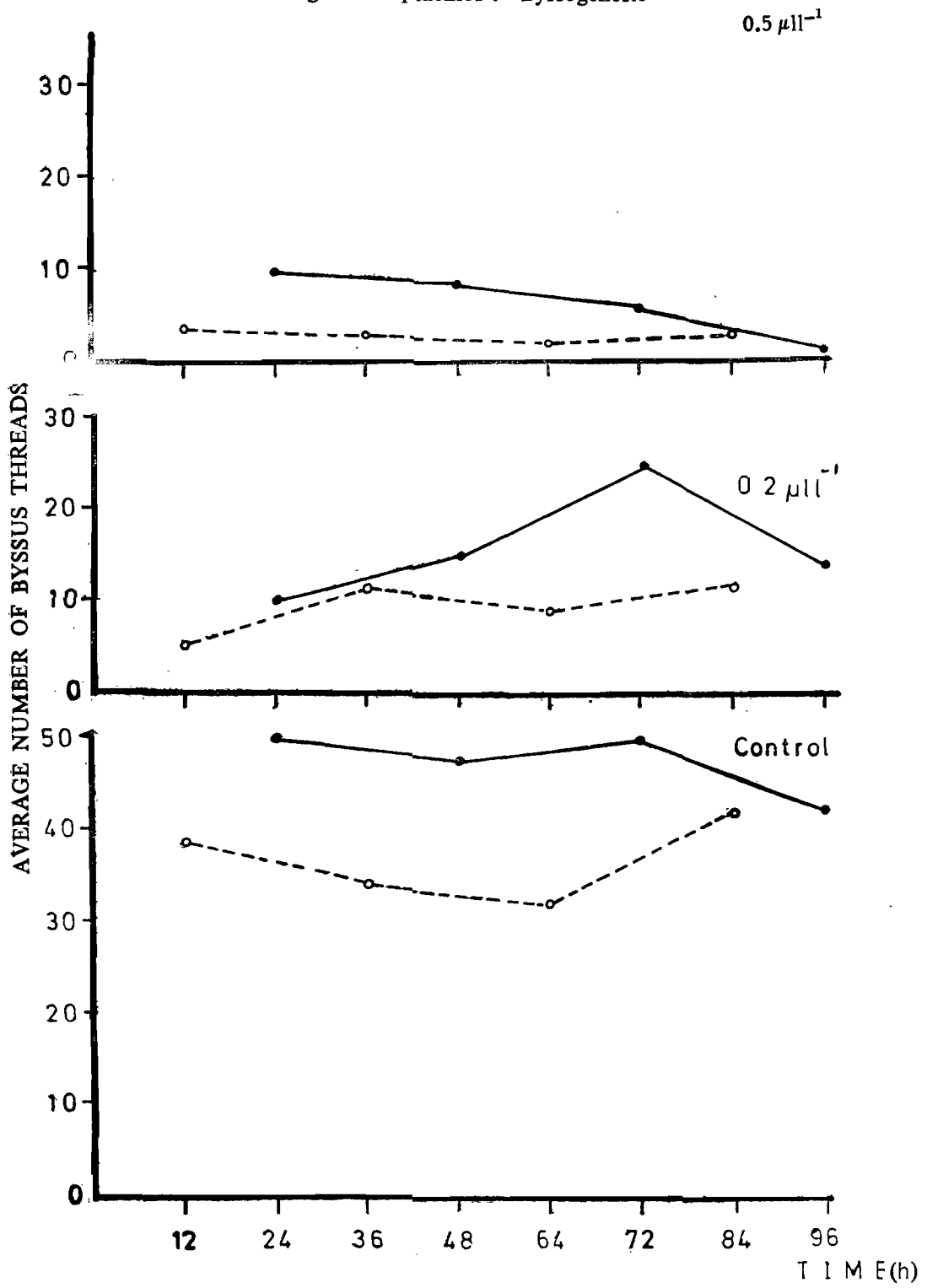
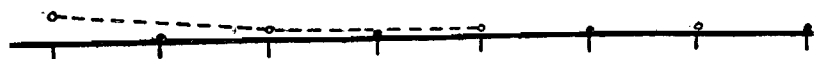


Fig. 3 Heptachlor : Byssogenesis

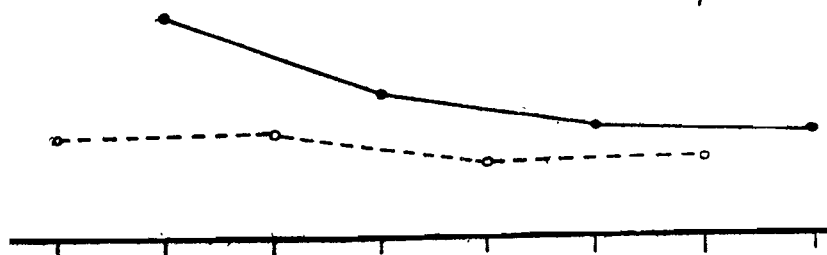


○ — — — ○ light ● — — — ● dark

$1.0 \mu\text{l l}^{-1}$



$0.3 \mu\text{l l}^{-1}$



$0.1 \mu\text{l l}^{-1}$

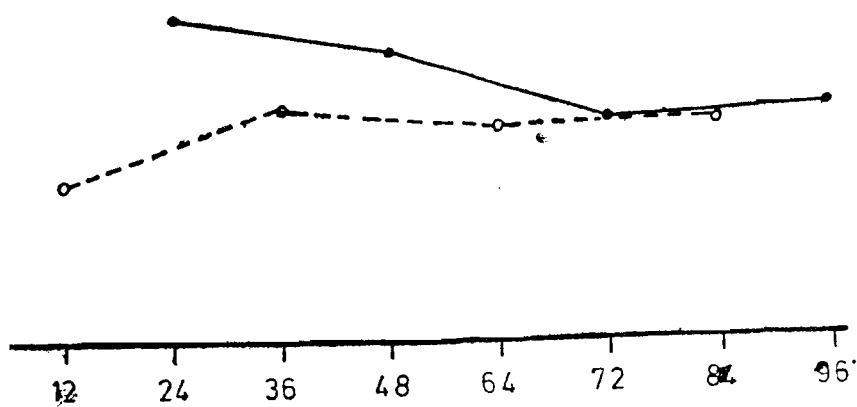
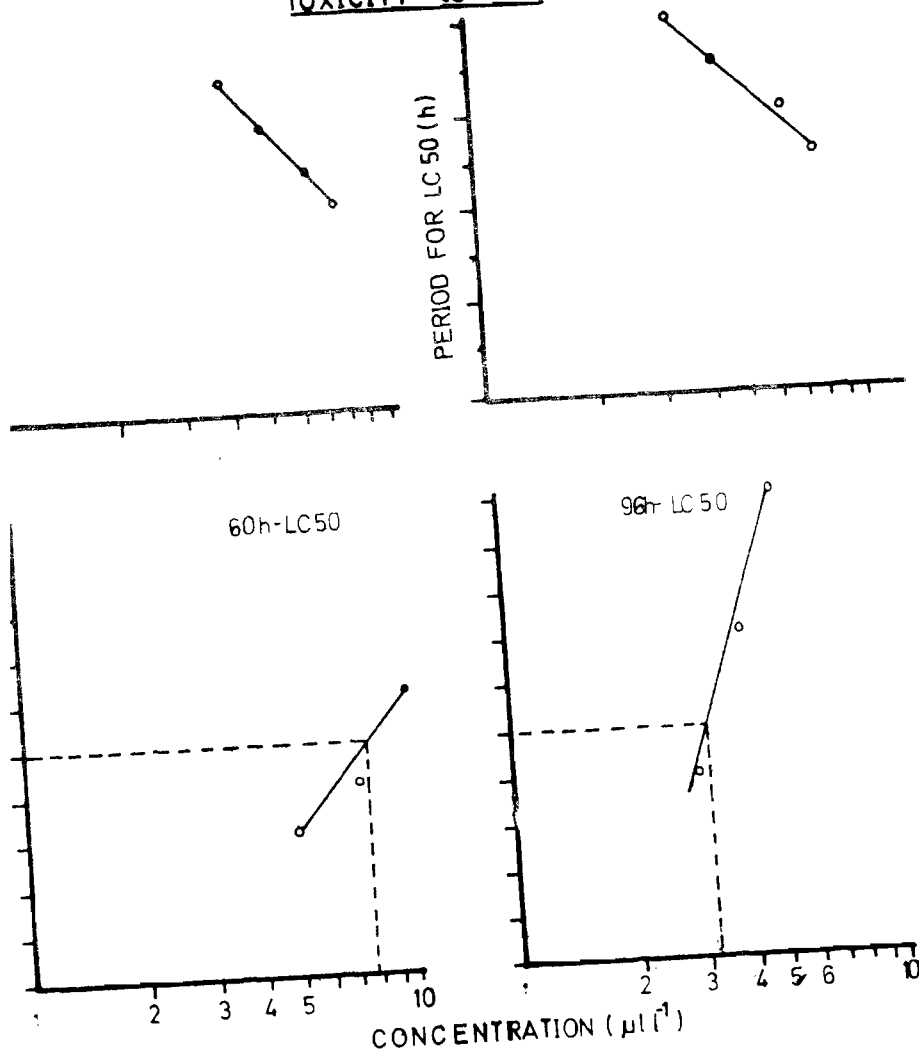


Fig. 2. HEPTACHLOR

TOXICITY CURVES



The observed variations in byssus thread production between concentrations was highly significant ($P < 0.01$). However, the differences between time intervals was significant only at 5% ($P < 0.05$).

The numbers of animals capable of byssal attachment did not vary much upto $0.3 \mu\text{l l}^{-1}$ but fell from 0.75 and $1.0 \mu\text{l l}^{-1}$. Clear-cut incapacitation occurred after 48 hr exposure.

The rate of oxygen uptake did not show conspicuous differences between concentrations (Table II). When, the oxygen uptake between the time intervals employed (2 hr) were compared, it was apparent that the animal had varying degrees of consumption.

The rate of filtration was more or less uniform in the control and $0.1 \mu\text{l l}^{-1}$. Although during the initial phase of the experiments, the rate of filtration was fairly slow in the higher concentrations of 0.3 , 0.5 and $0.75 \mu\text{l l}^{-1}$, the rate was very high between 9-12 h. A real reduction in filtration rate occurred only at $1 \mu\text{l l}^{-1}$ (Table III).

TABLE III
Rate of filtration by Perna viridis when exposed to different concentrations of heptachlor

Concentration ($\mu\text{l l}^{-1}$)/Period (h)	0-2.5	2.5-5.0	5.0-7.0	7-9	9-12	0-12	Mean	Standard deviation
Control	12.7	19.6	18.3	48.1	C	94.0	24.7	15.9
0.1	17.1	12.8	26.8	39.8	C	88.9	24.1	12.0
0.2	9.4	12.4	6.4	7.4	—	25.3	8.9	2.7
0.3	4.4	14.0	10.8	42.1	C	50.7	17.8	16.7
0.5	14.2	28.7	—	9.6	70.4	33.3	24.6	27.6
0.75	5.2	20.4	5.3	23.1	40.8	37.2	19.0	14.8
1.00	1.9	5.3	9.4	12.7	19.6	20.7	9.8	6.8

— No observation ; C — Cleared suspension.

DISCUSSION

In the present investigation it was found that the 96 h-LC₅₀ heptachlor *P. viridis* was $3.3 \mu\text{l l}^{-1}$. Probably a 96 hr exposure would have resulted in a high build up of heptachlor in the vital tissues. Although, the addition of low quantities of this pesticide did not change the colour of the test medium, the brownish hue of the mucus secreted by the gill indicated a high rate of adsorption. It is likely

that these adsorbed matter could find entry into the gill directly and into the digestive gland with particulate matter. Butler (1971) showed differences in the rate of assimilation of pesticides by different species of molluscs. Particularly in relation to the area of body surface available for contact with the pesticide.

Roberts (1975) tested endosulfan and trichlorphon for effects of byssogenesis. He found a 50 per cent reduction in byssal attachment in the presence of 0.45 mg l^{-1} of endosulfan, and the byssal attachment was not affected even at 30 mg l^{-1} by trichlorphon. The byssus secretion is controlled by at least four factors, including collection of secretions from the pedal gland, rapid molting of thread, subsequent tanning and the mechanical action of the foot (Bairati and Vitellaro, 1974). Any pollutant that could influence any of these would influence the rate of byssogenesis and possibly the structure of the threads. Since even $0.1 \mu\text{l l}^{-1}$ of heptachlor reduced the byssus secretion by 50 per cent and $1 \mu\text{l l}^{-1}$ cause secretion to cease it may be assumed that heptachlor even at $1 \mu\text{l l}^{-1}$ leads to a cessation of pedal gland activity.

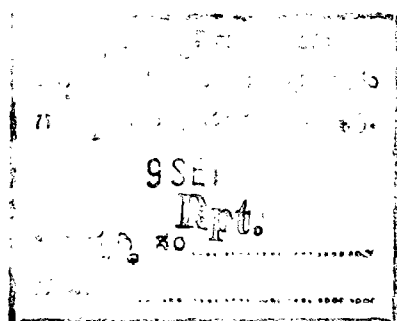
The rate of oxygen consumption of *Perna viridis* did not show any clear-cut differences due to heptachlor. So it may be that the respiratory enzymes are not affected by the concentrations employed. The rate of filtration did decrease at concentrations ranging from $0.3 \mu\text{l l}^{-1}$ and above. Considerable quantities of mucus secreted on the gill surface, when *P. viridis* was exposed to heptachlor would have impaired gill irrigation and this would have affected the rate of filtration.

Thanks are due to Prof. H. P. C. Shetty, Director of Instruction, College of Fisheries (UAS) for facilities provided and constant encouragement. A. E. Eknath is thankful to the Indian Council of Agricultural Research for the award of a Junior Research Fellowship, during the tenure of which the work was carried out.

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APPENDIX I

List of Insecticides which have been considered by the Registration Committee under the Insecticides Act, 1968

Approved for Registration

*1. B. H. C.	32. Ethylene Dibromide
*2. D. D. T.	33. EDCT
*3. Malathion	*34. Pyrethrum
4. Copper Oxychloride	35. Warfarin
5. Cuprous oxide	36. Ferbam
6. Copper Sulphate	37. MCPA
*7. Lindane (Gama B.H.C.)	*38. Trichlorophon (Dipterex)
*8. Carbaryl	39. P C N B
*9. Dimethoate	40. Tetradifon
10. Zineb	*41. Fenthion
11. Ziram	*42. Allothrin
12. Thiram	43. Carbon Tetrachloride
*13. Aldrin	44. DD Mixture
*14. Chlordane	45. Sodium Cyanide
*15. Heptachlor	46. Calcium Cyanide
*16. Toxaphene	47. Para dichloro benzene
17. PMA	48. Meleic hydrazide
18. EMC	49. Pentachlorophenol (PCP)
19. MEMC	50. Trichloro acetic acid (TCA)
20. Sulphur	51. Chlorebenzilate
*21. Diazinon	52. Metaldehyde
*22. Oxydemeton	53. Maneb
23. Zinc Phosphide	54. Coumachlor
24. Captan	*55. Thanito (Isobarnyl thiocynoacetate)
25. 2, 4-D	*56. Endosulfan
26. Dalapon	*57. Fenitrothion
*27. Nicotine Sulphate	*58. Phosphamidon
*28. Thiometon	*59. Quinalphos
29. Aluminium Phosphide	60. Diuron
*30. Dichlorvos (DDVP)	61. Triallate
31. Methyl Bromide	

62. Alachlor	*88. Chlorfenvinphos
63. Alpha Naphthyl Acetic Acid	89. Aureofungin
64. Chlormaquat chloride	*90. Formothion
65. Nitrofan	*91. Chloropyriphos
66. Butachlor	92. DSMA
*67. Phorate	93. MSMA
*68. Monocrotophos	94. Propanil
*69. Phenthoate	95. Calixin
*70 Phosalone	96. Difolaton
*71. Ethion	97. Dinocap
72. Fluchloralin (Basalin)	98. Copper Aceto Arsenite (Paris green)
73. Carbendazim (Bavastin)	99. Dibromochloropropane
74. Benomyl	*100. Methyl Parathion
75. Dicofol	101. Nickel chloride
76. Mancozeb	102. Methabenzthiazuron (Tribunil)
77. Paraquat dichloride	*103. Aldicarb
78. Difonphos	104. Streptocycline
*79. Propoxur	105. Ethepon
*80. Carbofuran	106. Barium Carbonate
81. Carboxin	107. Paraquat Dimethyl Sulphate
82. Sirmate	108. Metoxuron
83. Simazine	*109. Menazon
84. Atrazine	110. Glyphosate (Round-up)
85. Linuron	**111. Endrin
86. Ediphenphos (Hinosan)	**112. Dieldrin
87. Gibberellic acid	

* Insecticides

** Registered for limited time/use

APPENDIX II

*Data required under Annexure III by the Central Insecticides Board
for the Registration of Pesticides*

- I. Experimental data on safety :
- a) Acute toxicity studies in mammals (on at least two laboratory animals like rat and rabbit).
 - 1) Oral
 - 2) Dermal
 - 3) Inhalation
 - 4) Eye and Skin
 - b) Sub-acute studies in mammals (on at least 2 laboratory animals like rat and rabbit).
 - 1) Oral 90 days
 - 2) Dermal 21 days
 - 3) Inhalation 14 days
 - c) Supplementary toxicological studies in mammals (on at least 2 laboratory animals like rat and rabbit).
 - 1) Neurotoxicity
 - 2) Teratogeniety
 - 3) Effects on reproduction
 - 4) Carcinogenicity
 - 5) Synergism and potentiation
 - 6) Metabolism
 - 7) Toxic effects of metabolites and residues from treated plants.
 - d) Information on wild life hazards
 - 1) Toxicity to birds (Acute toxicity)
 - 2) Toxicity to fish (Acute toxicity)
 - 3) Toxicity to bees
 - 4) Toxicity to other beneficial insects (field trials and Observation)
 - 5) Toxicity to livestock (Field trials and observation)
 - 6) Toxicity to wild animals (field trials and observation)
 - e) Observation on man
 - 1) Direct observations (clinical cases)
 - 2) Health records both on Industrial and Agriculture use.

f) Medical data

- 1) Diagnosis, poisoning, specific signs of poisoning, chemical test
- 2) Treatment of poisoning
- 3) First-aid measures
- 4) Medical treatment

II. Experimental data on efficacy

Biological test on proposed formulation must be conducted under Indian conditions in the laboratory and field to determine if the product will control the plant diseases, insects, noxious animals or weeds named on the label without causing significant adverse effects on the crop or property being treated. The data to be submitted within 3 years.

- 1) Effectiveness
- 2) Phytotoxicity
- 3) Translocation within the plant or animal being treated.
- 4) Persistence in soils, water or plants.
- 5) Compatibility with other chemicals.
- 6) Direction concerning the dosage (concentration)
- 7) Time of application, type of application equipment and the manner in which the insecticide is to be used at the time of application.

III. Experimental data on residue

The residue test must be conducted under Indian conditions and data submitted within a period of 4 years.

- 1) Methods of sampling and residue analysis in food and feeding stuffs, water and soils and wild life.
- 2) Expected residue levels in edible crops and soil *i.e.*, amount of residue to be left following good agricultural practices and the residue disappearance curve.
- 3) Worker hazard after treatment.

IV. Stability of the proposed formulation

The test must be conducted under Indian conditions and data submitted within a period of 3 years.

- 1) Stability under different agro-climatic conditions.
- 2) Rate of decomposition.
- 3) Identity of breakdown products (only critical metabolites)

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