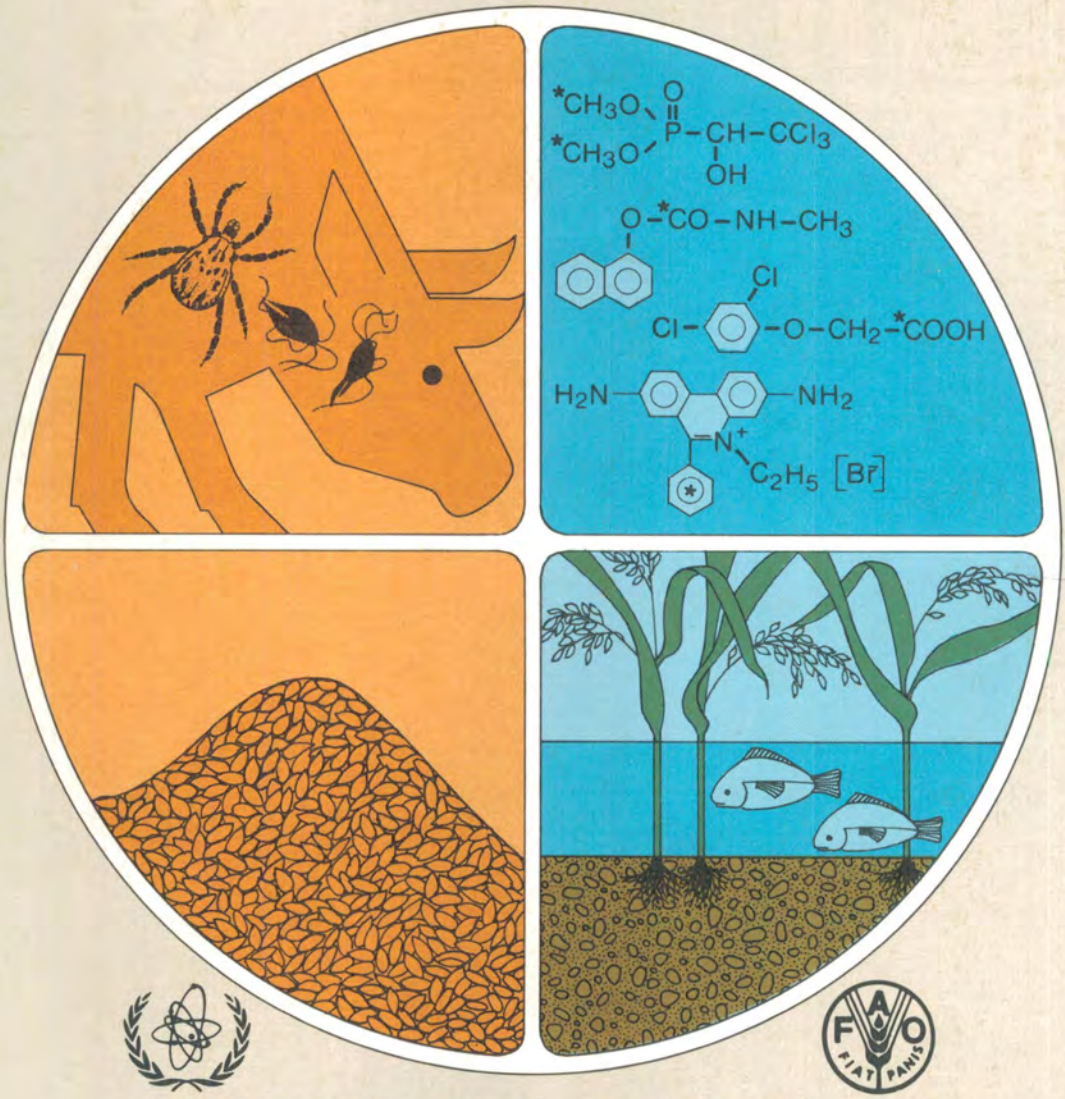

AGROCHEMICALS: FATE IN FOOD AND THE ENVIRONMENT

PROCEEDINGS OF A SYMPOSIUM, ROME, 7–11 JUNE 1982
JOINTLY ORGANIZED BY IAEA AND FAO

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1982

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PROCEEDINGS SERIES

AGROCHEMICALS: FATE IN FOOD AND THE ENVIRONMENT

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM
ON AGROCHEMICALS: FATE IN FOOD
AND THE ENVIRONMENT USING ISOTOPE TECHNIQUES
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
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FOOD AND AGRICULTURE ORGANIZATION
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FOREWORD

Current trends in population dictate intensified agricultural practices with concomitant growing use of agrochemicals, particularly in developing countries. Increased use of pesticides has greatly aided crop production, protected man from diseases such as malaria and filariasis, decreased losses of stored grains, and has generally improved man's welfare. Pesticides are an essential element in agricultural production, and there is little doubt that their use will continue to increase as more food and better health are demanded.

Pesticide usage may lead to the appearance of potentially undesirable residues as trace contaminants of food, the environment and living tissues. The full impact cannot, however, be quantified. In recent years, increasing investment has been made into development of measures to reduce pesticide contamination of food and the environment, while at the same time protecting crops, livestock and people from pest attack. In this context, nuclear technology has played and will continue to play an important role in programmes aimed at understanding the behaviour of pesticide chemicals and at studying their fate. Indeed, for many purposes, such technology is considered a unique tool, and for others a necessary addition to the existing armoury of conventional methodologies.

These technologies and pesticide residue problems were the theme of the International Symposium on Agrochemicals: Fate in Food and the Environment using Isotope Techniques, which was jointly organized by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations, in co-operation with the Comitato Nazionale per la Ricerca e per lo Sviluppo dell'Energia Nucleare e delle Energie Alternative (ENEA). It was held in Rome, Italy, from 7 to 11 June 1982 and was attended by 78 participants from 36 countries and two international organizations. The contributed papers and posters have clearly illustrated the potential value of isotope techniques and have reviewed the advances made in the development and application of these techniques in studying the fate of pesticide chemicals in plants, food and farm animals, and in terrestrial and aquatic ecosystems.

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Chairman

P.C. KEARNEY
United States of America

Invited Paper

AGROCHEMICALS IN INDIA
Impact on agriculture

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Abstract

AGROCHEMICALS IN INDIA: IMPACT ON AGRICULTURE.

Agriculture in India, after years of stagnation, is undergoing a quiet revolution and the country is now self-sufficient in food grains and cash crops. Many modern technological inputs have contributed to this, especially pesticides. India today uses about 61 000 t per annum of pesticides for both agricultural and public health purposes, which is very little compared with some developed countries. The industry now has an installed capacity of 97 000 t and is making 57 basic chemicals, mostly of the older type although four, DDT, BHC, malathion and carbaryl, are major ones. BHC constitutes about 60% of the pesticides used in India. Among the crops, cotton and rice require the most pesticide and wheat the least. The manufacture, sale and use of pesticides is regulated by government agencies in India. The relationship between increased pesticide consumption and food production in the country is shown. To date crop-wise analysis shows that wheat, the main contributor to the 'Green Revolution', did not require very much pesticide; production of rice has not shown a similar growth owing to serious pest problems, which have partially been overcome by pesticides; production of cotton has shown the real impact of pesticides; use of pesticides will improve the production of subsistence crops, such as sorghum, pulses and oil-seeds, if irrigation is assured. To grow more food for the ever-increasing population use of pesticides must increase, but the recent steep increase in price and consequent increase in the cost:benefit ratio are acting as a deterrent.

1. INTRODUCTION

Agriculture plays a dominant role in the Indian economy; more than 80% of India's population is directly dependent on it and 50% of the national income stems from it.

In recent years agriculture in India has undergone a quiet revolution. After many years struggle with food shortages the country has made good strides in food production. Food grain production in 1981 is expected to be more than 138 million tonnes and similar increases have also been obtained with cash crops. The country is now self-sufficient, at least in cereals. Part of this achievement is certainly due to the good monsoon rains of the last two years, but much of it is

due to the introduction of a new agriculture strategy and the application of modern technologies, such as high-yielding varieties, improved seeds, fertilizers, increased irrigation and, above all, proper plant protection technology by using pesticides. The latter "protection of our produce both in field and storage" is very important for a tropical country such as India. The benefits accrued from all other inputs depend on this because high-yielding varieties and large monoculture technology increase pest problems and the best results are usually obtained under the umbrella of plant protection. In the tropics, therefore, protection is more important than production [1].

2. ESTIMATES OF CROP LOSSES DUE TO PESTS

It has long been known that considerable crop losses occur world-wide as a result of the invasion of insect pests and the infestation of phytopathogens. Several instances of crop epidemics have been recorded in the past, e.g. the ravages of potato blight in Ireland in 1840, coffee rust in Ceylon in 1870, and epiphytotics due to the helminthosporium disease of rice in India in 1918 and 1942. In recent times the red rot attack on sugar-cane in northern India during 1937–1942 and again in 1946–47, wheat stem-rust in 1954, the brown plant hopper attack on rice in 1973–74, and the army worm epidemic on several crops in 1979 have all caused severe losses.

Many complex issues are involved in any attempt to estimate crop losses due to pests and diseases. Hence, there is an urgent expediency to develop a systematic methodology that will ensure determination of the critical value of intensity of attack, taking into account the life cycle of the pest, the variety of the crop, the variables in season and the location of cultivation. Although no systematic attempt has been made to compute field losses, some empirical estimates are, however, available, these being based on limited experimental observations in India.

The National Council of Applied Economics Research (NCAER), New Delhi, on the basis of estimates made in experiments in several states between 1950–51 and 1965–66, recorded a maximum loss of 40.3% in cotton and a minimum of 2.8% in wheat; other crops, namely potato, sugar-cane and paddy, suffered intermediate losses [2].

According to the Programme Evaluation Organisation of the Planning Commission, New Delhi, losses of high-yielding varieties varied from 3 to 4% in wheat to 40% in rice and jowar [3]. These estimates indicate consistently that wheat suffers minimum losses due to pests.

Estimated losses of paddy, on the basis of studies conducted by the Indian Agricultural Statistics Research Institute in 1971 [4], confirm the estimates of the Programme Evaluation Organisation, namely that this crop is very susceptible to pests and diseases, with losses ranging from 3 to 20% depending on the variety, location and season.

TABLE I. AVOIDABLE LOSSES IN INDIAN AGRICULTURE DUE TO PESTS

Type of pest	Percentage of total loss	Financial loss (in crores)[a]
Weeds	33	1980
Plant diseases	26	1560
Insect pests	20	1200
Miscellaneous pests	8	480
Storage pests	7	420
Rodents	6	360
Annual loss		6000

Source: Ref. [5].
[a] 1 crore = 10^7 rupees.

TABLE II. PESTICIDE CONSUMPTION IN DIFFERENT COUNTRIES

Country	Consumption (g/ha)	Percentage share of world consumption
Japan	10 800	7.4
Western Europe	2 000	21
USA and Canada	1 500	35
Latin America	–	10.5
Eastern Europe and USSR	–	9.2
Far East and Australia	–	5.2
Africa	127	5.2
Federal Republic of Germany	–	3.5
India	450	3.0

Source: Ref. [7].

In the absence of a very systematic methodology for estimation, many of the available estimates are based on limited information and they can best be termed as 'felt losses'. Table I gives a recent estimate of some of these avoidable losses due to different types of pests [5]. Other estimates have determined that losses in field and storage cost about US $7 500 million [6].

3. GROWTH OF PESTICIDE USE IN INDIA

The realization that pesticides are essential for achieving India's planned growth has been very slow. Starting with very small quantities (500 t) in the 1950s, use of pesticides has slowly grown and currently stands at about 61 000 t per annum. Even now, by the standards of developed countries, India uses very little pesticide; with 15% of the world's population and almost 4% of the world's cropped area, it has only 3% of the world's total consumption of pesticides (Table II [7]).

A major amount is used for malaria control and other public health programmes. Even this is a recent figure and it has taken many years to reach. However, even with this meagre application of pesticides food production has increased considerably. This figure is also small in comparison with other inputs like fertilizers, the per hectare consumption of which now stands at 27 kg/ha.

4. GROWTH OF THE PESTICIDE INDUSTRY

The pesticide industry in India is relatively young. The manufacture of basic pesticides started in 1952–53 with BHC in Calcutta. In 1955 large-scale manufacture of technical DDT and its formulations started in the public sector in Delhi with the help of UN agencies like UNICEF and WHO. Formulation industries, using imported pesticides, were also started on a large scale at about this time. The initial DDT plant set up in 1955 has now grown into a premier public sector company that has the monopoly on the manufacture of DDT; it also produces other agricultural pesticides such as BHC, malathion and endosulphan.

Manufacture of organophosphorous (OP) compounds started in India around 1965; at present most of the OPs are manufactured by the private sector. There are good deposits of phosphatic rock in India and all the elemental phosphorus needed for pesticides and other products is produced indigenously.

Growth of the industry was very slow up to the mid-sixties and only 14 technical pesticides (chiefly DDT, BHC, malathion, 2,4-D, wettable S and Cu-oxychloride) were being manufactured. It was only during the third planning period (1965–71) that the pesticide industry received the specific attention of planners and started developing properly at an annual growth rate of about 11% [8].

TABLE III. PRESENT POSITION OF PESTICIDES MANUFACTURED IN INDIA

Type of pesticide	Number	Licensed capacity (tonnes per annum)	Installed capacity (tonnes per annum)
Insecticides	28	72 500	57 500
Fungicides	12	12 400	14 100
Herbicides	10	5 900	3 250
Fumigants	3	2 000	2 000
Rodenticides	2	1 200	950
Plant growth regulators	2	220	105
Total	57	93 220	77 905

Source: Ref. [9].

TABLE IV. COMPARATIVE CONSUMPTION OF PESTICIDES IN AGRICULTURE

Type of pesticide	1973–74 (%)	1978–79 (%)
Insecticides	69.4	72.2
Fungicides	27.3	21.3
Weedicides	2.2	3.4
Fumigants	0.4	2.0
Acaricides	0.4	0.4
Rodenticides	0.3	0.7

Source: Ref. [10].

Many new products were added and R and D efforts were increased, especially after 1973. However, the growth rate has slowed down.

The present position regarding licensed capacity and manufacturing capability (installed capacity) is shown in Table III [9]. The current total licensed capacity is about 93 000 t but the total installed capacity is only about 78 000 t.

As will be seen later in this section, even this capacity is not being fully utilized. It is also clear from Table III that in Indian agriculture maximum use is being made of insecticides, followed by fungicides. As yet, not much herbicide has been used and although the installed capacity is 3250 t only about 400 t are being produced. The low consumption of herbicides in India is distressing, since reduction of crop yields through deprivation of nutrients by herbivores is thought to be much more than the loss caused by other pests. Weeds deprive the crops of 30 to 40% of the fertilizer, which is the costliest input in Indian agriculture, and the position is becoming more serious as the amount of fertilizer being used rises. Low use of herbicides may be due to many causes, such as availability of cheap labour, high cost of herbicides especially the newer types, the belief that more use of herbicides would add to the unemployment problem and consequent lack of promotional activity, and lack of extension activity to promote the sophisticated technology needed for herbicide application. At present, the largest users are the tea, coffee and rubber plantations, which are more organized and where the cost:benefit ratio is higher.

The consumption pattern and trend of increase or decrease are shown in Table IV [10]. Even though there was a rise of about 22% in the overall consumption of pesticides, the percentage-wise use of various groups of pesticides has not changed very much. Only weedicides and fumigants have shown an appreciable increase in recent years.

The pesticide industry in India now produces 57 basic chemicals [11]. Of these, 28 are insecticides, although only four are major ones, i.e. BHC, DDT, carbaryl and malathion. Of the four, only three are important for agriculture. DDT is used mainly for malaria control by government agencies. The largest single chemical used in agriculture is BHC, which contains 13% active isomer. It is also used for public health purposes and in locust control and constitutes about 60% of the total pesticide produced in the country.

The production volume of carbaryl and malathion is second to BHC. Although the installed capacity for carbaryl is about 5000 t, actual production is only about 3000 t. The capacity for malathion production has recently been increased greatly as it has been selected as an alternate chemical for public health uses. The manufacture of endosulphan has recently been increased and it is expected to be the most important insecticide for agricultural uses. The remaining insecticides consist of a large number of small volume OP compounds. Besides carbaryl, the only other insecticidal carbamate produced is aldicarb or Temik.

About 12 important fungicides are now being made, but again older types, based on sulphur and copper, predominate. The newer systemic ones are used only in small volumes and are mostly imported.

Although several herbicides are now being used in Indian agriculture only two, 2,4-D and paraquat, deserve mention and adequate amounts are being manufactured. Propanil and nitrofen have recently been added to this list. Others, used in small volumes, are being imported.

In the recent past preservation of stored grains posed some problems and there was a need to produce more fumigants. Three of these, aluminium phosphide, EDB and methylbromide, are now being produced in adequate quantities, especially for use in the government storage programme.

Plant growth regulators are being made only in small quantities, but their use is increasing.

In India, the pesticides in use are mainly of the older type. These have many defects and have been abandoned elsewhere; their continuance in India is mostly because of cost considerations. Small quantities of organomercurials are still being used, mainly for seed treatment.

Further, it should be pointed out that it is planned to increase the production and consumption of DDT in India. It is the cheapest chemical available for public health purposes and its consumption has remained steady for the past decade; part of it is produced and an almost equal amount is imported. Production of this chemical is about to be doubled.

Pesticides, produced or imported and formulated, are not used evenly throughout the country. The maximum amount is consumed in southern India which, being warmer, is more prone to pest attack; the eastern zone, comprising Assam, West Bengal, Bihar and Orissa, is next in line, followed by the western zone. The lowest pesticide consumption is in the northern part, which has a relatively colder climate.

Andhra Pradesh heads the list in pesticide consumption presumably because of the requirements of cash crops like cotton and tobacco and cereals like rice which are grown there.

Crop-wise consumption of pesticides in India is shown in Table V [12]. Among crops, cotton uses the largest amount of pesticide as it is susceptible to a large variety of insect pests. Use of synthetic pyrethroids (imported) has just begun and is showing spectacular yield increases. Among cereals, rice is the largest consumer of pesticides. It is pertinent to note that not much pesticide has been used on maize which has a very high photosynthetic efficiency, soy beans which are the best source of vegetable proteins, and groundnuts which are the most important source of edible oil and hence these crops have not kept pace with production.

A comparison between the amount of pesticide used in agriculture and for public health purposes is shown in Table VI [13, 14]; production figures are also

TABLE V. CROP-WISE CONSUMPTION OF PESTICIDES IN INDIA

Crop	Percentage share of pesticide consumption	Percentage share of cropped area
Cotton	52–55	5
Rice	17–18	24
Vegetables, chillies, fruits	13–14	3.4
Plantations	7–8	2
Cereals, oil-seeds, pulses	6–7	58
Sugar-cane	2–3	2
Others	1–2	6

Source: Ref. [12].

TABLE VI. TRENDS IN PRODUCTION, CONSUMPTION AND COMPARISON OF USES OF PESTICIDES

Year	Production (10^3 t)	Import (10^3 t)	Consumption (10^3 t)	Public health use (10^3 t)	Agricultural use (10^3 t)	Area under plant protection[a] (10^6 ha)	Gross cropped area (10^6 ha)
1960	–	–	–	10.6	5.5	6.5	153
1966	14.0	0.6	14.6	8.0	6.6	16.6	157
1968	15.8	4.8	20.6	9.7	11.0	36.0	164
1970	27.4	0.6	28.0	14.0	12.0	44.0	164
1974	33.0	11.0	45.0	13.0	32.0	64.0	–
1976	34.7	24.1	58.8	15.4	43.4	77.0	171
1978	50.0	9.0	59.0	19.0	40.0	81.0	–
1979	50.0	–	43.0	18.0	25.0	78.0	–
1980	40.0	–	59.0	19.0	41.0	84.0	173
1981	52.6	8.4	61.0	–	–	–	–
2000	–	–	–	–	–	100.0[b]	200[b]

Source: Ref. [13].
[a] Taken from Ref. [14].
[b] Projected.

given. Public health consumption has approximately doubled in one decade, but in agriculture there has been a manifold increase and it is rising steadily. Part of this increase is certainly due to the increase in area under plant protection, but the real increase is due to intensification of pesticide use, which has risen from 77 g/ha in 1965 to a current 450 g/ha.

Although pesticide consumption is rising very steadily in India, it is only a fraction of the total estimated demand. Calculating the pesticide demand and forecasting consumption is a very difficult task and is often inaccurate owing to the uncertainties of the monsoon. For example, as seen in Table VI [13, 14], after a steady rise in consumption during the good monsoon periods of 1977 and 1978, there was a steep fall in 1979, the year in which India suffered the worst drought of this century. The pesticide demands envisaged by the Planning Commission by the end of the sixth planning period in 1985 are 81 000 t. Total production figures for pesticides are much lower than the installed capacity of 78 000 t and for the last three years they have remained stagnant, i.e. around 50 000 t. This, in spite of the fact that the area under plant protection is being increased, albeit slowly. Several causes can be ascribed to this stagnancy, the main one being the steep increase in the price of pesticides without a proportionate rise in production, resulting in a decrease in the cost:benefit radio, making this input uneconomic for farmers. Vagaries of monsoon and drought could be other reasons.

The pesticide industry in India is a good example of 'mixed economy' as far as the manufacture of basic chemicals is concerned. Although the industry was started by the multinationals, there are now participants from both the public and private sectors and at national as well as multinational level. The manufacture and use of DDT is a government monopoly.

5. FORMULATION INDUSTRY

The pesticide formulation industry in India consists of two categories: large-scale units as a part of basic manufactured goods and small-scale formulations that enjoy certain benefits from the government. The latter accounts for nearly 70% of the total production of pesticide formulations (about 90 000 t). India uses mostly conventional types of formulations, such as dust and water dispersable powders, and less emulsifiable concentrates and granular formulations. Modern formulations of aerosols, ULVs and recent types, such as micro-encapsulated and flowable concentrates, are not as yet greatly used in India.

6. HAZARDS THAT HAVE ARISEN FROM PESTICIDE USE

The environmental impact of pesticides and the associated questions of hazards were raised in India almost at the same time as the use of pesticides was

begun, unlike in developed countries where the polluting effects of pesticides were discovered long after their initial use. The manufacture, sale and use of pesticides are strictly controlled by the Central Insecticides Board of the Ministry of Agriculture. Any new pesticide or formulation must pass through very rigorous toxicological and environmental tests before it is allowed to be used.

India now has reasonably good R and D facilities to sustain the pesticide industry. The focus, however, is on application and development and not on the discovery of new chemicals.

7. IMPACT ON CROP PRODUCTION

After years of stagnancy and chronic food shortages, the phenomenal growth in crop output in more recent years has been perhaps the most outstanding achievement of Indian agriculture. This is the result of embarking upon the path of planned economic development. The absolute increase in food grain production (76 million tonnes) in the past 30 years of development (1950–1980) has itself far exceeded the absolute output (57.1 million tonnes) of the initial triennium. The increase in wheat output has indeed been most spectacular, being nearly four times that of the average output in 1949–52. Besides wheat, there has been an all-round improvement in other crops like potatoes, cotton, sugar-cane, tea and coffee. Between 1960–61 and 1978–79 crop output recorded a compound rate of growth of 2.45%. In the period after 1967–68 the growth of production accelerated to 2.8% per annum [15]. The major cause of this overall growth has been mainly due to the increase in productivity and not to the increase in area under cultivation, which has probably reached a limit and can only be further increased with great difficulty. The increase in productivity is linked to the increase of several modern inputs. Thus, the irrigation potential was raised from 22 million hectares in 1951–52 to more than 55 million hectares in 1980. While earlier Indian agriculture hardly used any chemical fertilizer, by 1980 the consumption of NPK nutrients had reached 5.6 million tonnes.

In 1965–66 new high-yielding varieties of wheat were introduced, ushering in what is known as the 'Green Revolution' and within 15 years the area under these varieties reached 45 million hectares. Production and distribution of quality seeds have been organized practically from nothing. Agricultural chemicals also form part of these essential inputs, especially for the high-yielding varieties. As can be seen in Table VI, the area under plant protection has risen today to about 84 million hectares, which is 49% of the total cultivated area.

It is difficult, however, to quantify the gains from the use of pesticides alone. After all, pesticides do not grow crops directly but help in protecting the gains from other inputs. The relation between total food grain production and pesticide consumption is shown in Fig. 1 which clearly indicates the impact of

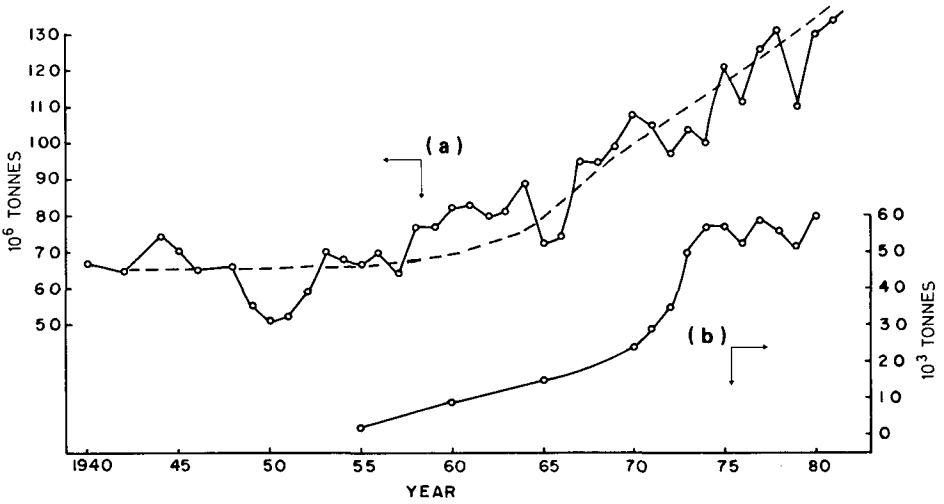

FIG.1(a). Total food grain production; (b) pesticide consumption.

pesticides on the total production of food grains. The steep rise in food grain production between 1966 and 1975 also corresponds to a massive increase in the use of pesticides.

A better concept of the impact of pesticides may be seen in Fig. 2, which shows the increase in average productivity of food grains in conjunction with the rise in intensity of application of chemicals. Again, it can be seen that there is a close correspondence between the rise in productivity and the per hectare consumption of pesticides.

Crop-wise analysis of the impact of agricultural chemicals on yields reveals not only the main areas of gain but also where pesticides have not made much impact.

7.1. Wheat: needed no impact

The annual increase in wheat production is shown in Fig. 3, which also depicts separately the rise in pesticide consumption. It will be seen that the wheat yield increased almost three-fold (10.4 to 36.5 million tonnes) within the short span of 15 years. It is the main contributor to the 'Green Revolution'. But it is doubtful whether pesticides have played any key role in this growth. As mentioned earlier, wheat does not suffer much from pest attack and losses have been computed to be only about 2.8%. Increased production of wheat, with the introduction of high-yielding varieties from 1966 onwards, needed fertilizers and irrigation but not much plant protection.

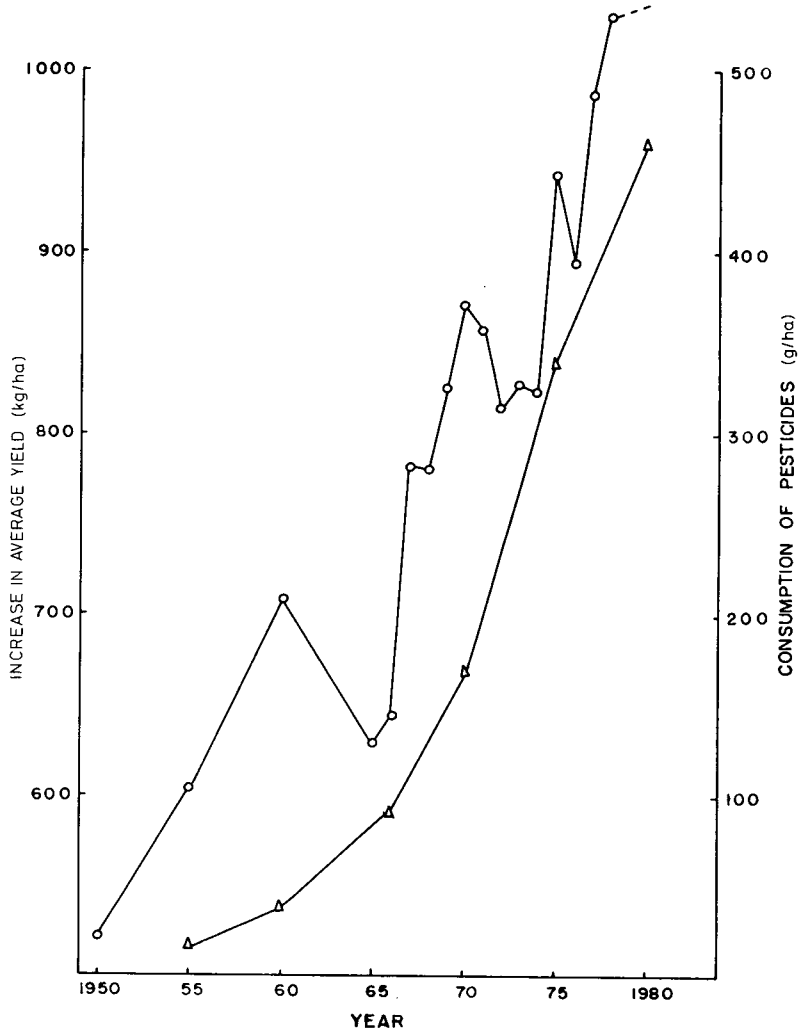

FIG. 2. Impact of pesticides on productivity.

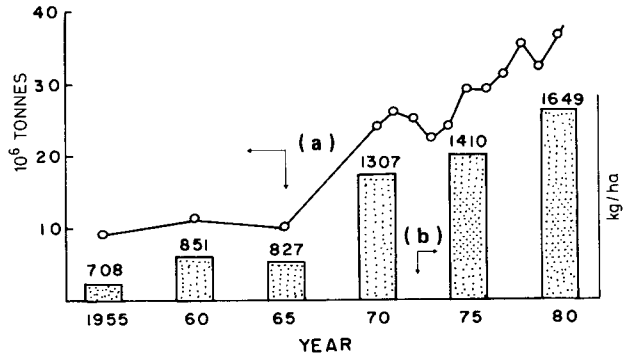

FIG.3. Wheat: (a) production; (b) productivity.

However, if this level of production is to be maintained and further increased some plant protection measures will become necessary in the near future. Cultivation of high-yielding varieties was first started in the Punjab, which in many ways had ideal conditions for wheat growing. Gradually cultivation was extended to the entire country and even non-traditional areas like West Bengal and Tamilnadu are now cultivating these varieties, as well as the neighbouring countries of Pakistan and Bangladesh. It is now feared that if an epidemic of rust or smut occurred it would spread like wild fire and production of this monoculture in the entire sub-continent would be threatened. The country lost about 1 million tonnes of wheat last year due to loose smut intensification arising from the free exchange of seed materials and the susceptbility of new high-yielding varieties. Smut control is possible with new systemic chemicals like vitavax and it is advisable to start seed treatment with these chemicals as a short-term measure to avoid this epidemic. Gene deployment in different zones would be the only effective barrier to avoid the threat of rust, but it is a long-term process and needs constant attention. Meanwhile, fear will continue since there are no effective, economical chemical control measures.

Earlier, wheat did not have very much of a problem with weeds and, as they are broad leaved, what little there was could easily be controlled by 2,4-D. Intensive cultivation and rice/wheat rotations have, however, introduced new weeds like *Phallaris minor* and *Avena fatua* which, being monocots, require very selective herbicides that have to be imported. Future intensive wheat growing and storage will certainly require more use of chemicals.

7.2. Rice: beginning to have an impact

Rice is the major cereal crop of India, constituting about 40% of the total food grain production. Production of rice increased from 21 million tonnes in

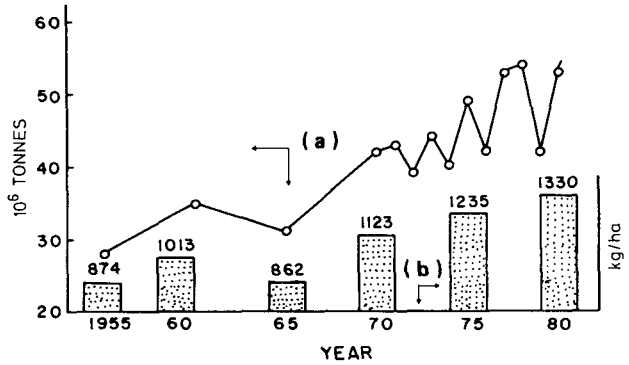

FIG.4. Rice: (a) production; (b) productivity.

1952 to nearly 44 million tonnes in 1973–74, i.e. it doubled in 20 years, but from then on further increase seemed to be very difficult. The recent increase from 53 to 54 million tonnes from 1977 is mainly because of starting cultivation of upland rice in non-traditional areas like the Punjab and Haryana (Fig. 4). It would thus seem that the increase in production of rice, although substantial, is not as spectacular as that of wheat. Despite introduction of HYVs and other inputs like fertilizers, the average yield of rice per hectare in the country has risen marginally from 1080 kg/ha in 1964–65 with traditional varieties to 1330 kg/ha in 1978–81 with HYVs (Fig. 4). This is only a very modest improvement. The reasons for the lower productivity increases in rice are many but the main one is that, being a kharif crop, it is highly susceptible to insect pests, diseases and weeds. Even applied nitrogen cannot be efficiently utilized under conditions that are necessary for rice growing. Also, normal plant protection measures are not efficient under high rainfall and temperature conditions.

Among the rice diseases that severely limit production in epidemic years are blast disease (*P. oryzae*), bacterial blight (*X. oryzae*) and virus diseases of various types. Only a few of these can be controlled economically by fungicidal chemicals.

Among the insect pests, stem borer, gall midge and rice bug cause great damage, but they can be controlled by timely application of chemicals.

Introduction of HYVs has actually increased the pest problem in rice. An example is brown plant hoppers, which previously only caused damage in Japan but which have now become a problem wherever new varieties have been introduced. The attractive factors are probably the increased number of tillers and denser plantings, creating a favourable environment for this insect near the base of the plant. India suffered heavily from BPH attack in 1973–74 and periodically since then in smaller areas. In many ways the new HYVs are also responsible for aggravating the weed problem, which used to be suppressed by tall varieties.

The potential for increasing rice production is very high as the highest yield so far obtained under the adequate plant protection umbrella in national demonstration plots is well over 8000 kg/ha, but whether such inputs will be economical or not is another consideration. Existing chemicals, especially the more recent very potent ones like synthetic pyrethroids, disturb the rice ecosystem, in particular the rice/fish culture. Possibly new chemicals designed especially for rice will launch another 'Green Revolution'.

Plant protection measures have been more successful under the upland conditions of the Punjab and Haryana where water management is easy and where there are no pests.

An area of great interest in rice growing is the efficiency of utilization of nitrogenous fertilizers; under tropical conditions it is only 30 to 40%. Efforts to increase this efficiency by using chemicals like N-serve and AM are well known, but excellent progress has been made by using indigenous materials like neem (*Azadirachta indica*) cake [16], which has been found to be comparable to N-serve. Large-scale application will improve the nitrogen economy in rice.

7.3. Cotton: registered real impact

India has the largest area in the world under cotton (8 million hectares) and production in 1982 is also expected to be a record of 8.5 million bales.

Figure 5 shows how production has increased during the last 15 years. It is mainly due to the rise in productivity. While other inputs like fertilizers, new hybrid varieties and irrigation have all contributed handsomely to achieve this, the major contribution has undoubtedly come from applying pesticides. This crop alone consumes more than 50 to 52% of all pesticides and requires every possible pest control measure to achieve a really good yield. Being a cash crop, application of pesticides is not uneconomic.

Cotton suffers damage from many pests and diseases, about 12 of which are important, but two deserve special mention as they cause about 80% of the total losses: jassids (early stage) and bollworms (bud, flowering, boll stage). Other pests cause damage sporadically and are space bound, but there is a distinct possibility that some of them may become major pests because pest-resistant, poor yielders like *Arboreum* and *Herbaceum* are being replaced by *Hirsutum* varieties that produce high yields of quality fibre but require protection from pests, and because monoculture extends in a continuous belt from north to south. A large number of chemicals are used on cotton but the newly discovered synthetic pyrethroids seem to be wonder chemicals in this respect.

After the initial success with chemicals, problems have now arisen. Indiscriminate use of large amounts of chemicals is causing resistance development and ecological imbalance. Pest control costs are rising as more sprays are required and

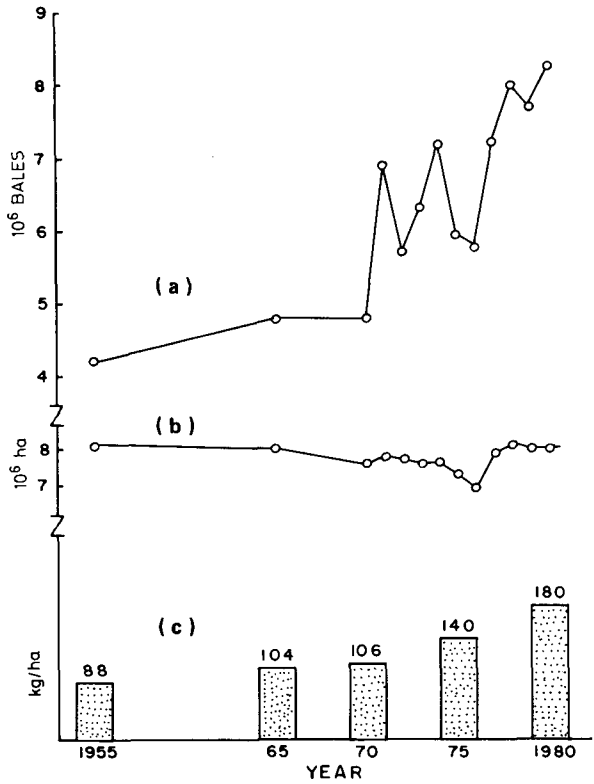

FIG.5. Cotton: (a) production; (b) area; (c) productivity.

the only way to derive full advantage from the chemicals will be to adopt integrated pest management techniques, using cultural, sanitary and other components [17].

Pesticides have also registered similar impacts on other cash crops, such as sugar-cane (60 million tonnes in 1955 to 150 million tonnes in 1980), tobacco (0.3 million tonnes in 1955 to 0.45 million tonnes in 1977), tea (0.3 million tonnes in 1955 to 0.6 million tonnes in 1977) and coffee. In fact, all give a better return with pesticides.

7.4. Sorghum, pulses and oil-seeds: with some irrigation pesticides could have an impact

These crops are grown mostly under subsistence, semi-dry conditions and are traditionally known as the 'poor man's' crop. Of these, sorghum has long received attention and high yield potentials of 5000 kg/ha, with new technologies

TABLE VII. PRODUCTION OF SORGHUM, PULSES AND OIL-SEEDS

Year	Sorghum		Pulses		Oil-seeds	
	Production (10^6 t)	Yield (kg/ha)	Production (10^6 t)	Yield (kg/ha)	Production (10^6 t)	Yield (kg/ha)
1955	6.7	387	11.0	476	5.7	474
1965	7.6	429	9.9	438	6.4	419
1970	8.1	466	11.8	524	9.3	601
1972	7.0	449	9.9	474	6.7	465
1974	10.4	643	10.0	455	8.5	545
1975	9.5	591	13.0	533	9.9	651
1976	10.5	667	11.4	494	7.8	528
1977	12.1	740	12.0	509	8.9	576
1978	11.4	708	12.2	515	10.0	–
1979	11.6	698	8.6	385	–	–
1980	10.5	673	11.2	493	–	–

Source: Ref. [18].

and improved hybrid varieties, have been demonstrated. However, as can be seen in Table VII [18], productivity and production have shown little improvement over the years. As with rice, the heavy incidence of pests like shootfly, stemborer, midge, etc., especially on new hybrid varieties, is the main yield-limiting factor. Chemical control with carbofuran has been successfully demonstrated but it is practised only in limited areas and farmers are not willing to invest in chemicals as there is an economic risk involved due to uncertain rains. The pesticide umbrella will work best in these areas provided irrigation is assured.

Only recently have pulses and oil-seeds started receiving attention. Although the area under pulses is about 25.7 million hectares, only 2.92 million hectares are irrigated. Production has actually decreased and now stands at about 12 million tonnes, providing per capita protein of only 10 g, which is very meagre. Pests are again a limiting factor, but available protection technology with a variety of chemicals can easily raise the production to 15 to 18 million tonnes. Again, farmers are unwilling to invest in chemicals and fertilizers as these crops do not provide sufficient return unless they are at least partially irrigated.

TABLE VIII. AVERAGE AND RECORD YIELD OF SOME CROPS IN INDIA

Crop	Average yield 1979–80 (kg/ha)	Record yield (kg/ha)
Wheat	1568	7 700
Rice	1330	14 000
Maize	1076	11 500
Sorghum	708	8 500
Mustard	525	3 800
Pigeon pea	715	3 500

Source: Ref. [19].

Pest control in oil-seed crops is a much more difficult problem. Two decades ago India exported substantial quantities of edible oils but currently the country is compelled to import large quantities to meet internal demands. From 1950 to 1978 production only increased from 5.5 million tonnes to about 10.0 million tonnes, but the area under cultivation has virtually remained static at about 17.4 million hectares and the productivity is not only low but fluctuates violently. Groundnut, the major oil-seed crop, accounting for more than 50% of the total production, suffers seriously from white grubs, especially in the new areas of Rajasthan. Chemical control for this pest is not yet economical. Mustard, the second most important oil-seed crop (production 2 million tonnes), suffers seriously from aphid attack from time to time, resulting in fluctuations of yield. Chemical control and good varieties are available, but unless much larger areas are brought under irrigation investments in fertilizers and pesticides will not be economic.

All these crops are now receiving serious attention and hopefully the impact of pesticides will soon be visible, but at the moment pesticide use alone has not resulted in much improvement in yield.

8. UNTAPPED POTENTIAL

The average and record yields of some crops are given in Table VIII [19]. Record yields have been obtained under a package of technology, of which an important component is the optimum use of agrochemicals. Data show that there is a high, untapped yield potential for all crops.

The estimated requirement for food in India in the year 2000 is 220 million tonnes [20]. It should be possible to meet this figure if the existing potential is largely tapped. For a healthy growth of agriculture there is a need for harmonious use of all inputs. So far the primary attention of planners and scientists has been on varietal development and fertilizer. Pesticides, being highly complex, did not receive due attention and actually suffered from the unmerited emphasis placed on hazards and pollution. It is hoped that pesticide use will continue to expand in order to maintain agricultural productivity and to derive fuller benefit from other developments.

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Invited Paper

PROBLEMS CAUSED BY PESTICIDES WITH PARTICULAR REFERENCE TO THE IMPACT ON THE AGRICULTURAL ENVIRONMENT

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Abstract

PROBLEMS CAUSED BY PESTICIDES WITH PARTICULAR REFERENCE TO THE IMPACT ON THE AGRICULTURAL ENVIRONMENT.

Pesticides play an important role in agriculture in the United States of America and are a major economic entity in the US production of synthetic organic chemicals. Herbicides are the leading class of pesticides from both an annual production (680 000 t) and sales (US $2.7 billion) standpoint. Many of the early perceived environmental problems caused by pesticides were due to persistent chlorinated hydrocarbon insecticides. These were usually outside the agricultural environment and primarily due to their ubiquitous detection in certain fish and wildlife species. Subsequent to the ban of many chlorinated hydrocarbon insecticides in the USA, most of the environmental problems can be sub-divided under two major categories: (a) environmental processes and (b) specific compounds and/or their by-products. While economic data are available on sales and production of pesticides, no overall economic assessment exists on the problems posed by these processes and by-products. The major environmental processes that have received extensive study are movement, persistence and plant uptake, adaptation and exposure. The specific by-products of pesticides that stimulated wide environmental interest were the dioxins associated with the phenoxy herbicides, ethylenethioureas associated with ethylenebis(dithiocarbamate) fungicides and the nitrosamines associated with certain dinitroaniline and some acid herbicides. Use of ^{14}C-labelled samples of pesticides and their by-product has provided much valuable information in laboratory and model ecosystem studies on these two categories of environmental problems. If the user of pesticide is considered a major component of the agricultural ecosystem, then some data do exist on the short-term economic impact of potential loss of certain pesticides undergoing review by the RPAR process.

1. INTRODUCTION

We have been asked to discuss the problems caused by pesticides with particular reference to the impact on the agricultural environment. Since this presentation is under the general session entitled "Pesticides: Benefits and Costs", we will try to provide some economic data with reference to the impact on the agricultural environment. It is extremely difficult, however, to obtain accurate information on the economic costs of environmental effects of pesticides. Before we examine what these effects might be, it would be informative to provide a background on the economic situation on pesticide usage in the United States. This background is necessary to put the subsequent environmental costs in their proper perspective.

The volume of synthetic organic pesticides by class sold in the United States from 1979-1981 is shown in Table I [1]. The total volume equaled almost 680 000 metric tons (1.5 billion pounds) in 1981, of which the herbicides occupy slightly over 363 000 metric tons (800 million pounds) or 54% of the volume.[1] For the three-year period 1979-1981, the volume of herbicide sales has remained slightly above 50% of the total market. The value (in U.S. dollars) of sales of synthetic organic pesticides by class in the United States from 1979-1981 is shown in Table II. Total domestic sales were approximately $4.5 billion in 1981 of which the herbicides contributed about 60% to the total market. The projected growth in domestic herbicide production for the years 1980-1985 is shown in Table III. Industry surveys have estimated a 13.7% growth in volume, accompanied by a 46.4% increase in sales revenue, between 1980 and 1985. Finally, the use of herbicides on various crops at the user level in 1980 is shown in Table IV. Over 2/3 of herbicides sales are for use on two crops, corn and soybeans. The leading herbicides in corn production are atrazine, alachlor, 2,4-D, EPTC and pendimethalin; for soybeans, the principal herbicides are trifluralin, metribuzin, chloramben and bentazon.

The significant growth of the U.S. pesticide industry in both volume and sales has taken place during a time in history when tremendous environmental pressures and regulatory restrictions were placed on the producer. This has resulted in substantially increased research and development cost by the producer to register new pesticides. Their cost is now estimated at $20-25 million per compound and a development time of 7-9 years. This, then, constitutes one cost incurred by the producers due to potential environmental problems and is passed along to the user.

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[1] US billion = a thousand millions; 1 lb = 0.4536 kg.

TABLE I. Production of Synthetic Organic Pesticides: Volume of Sales by Class in the United States, 1979-1981

Class	1979		1980		1981	
	Amount, metric tons	% of total	Amount, metric tons	% of total	Amount, metric tons	% of total
Fungicides	65 230	10.5	66 520	10.4	67 180	9.8
Herbicides	319 720	51.4	348 980	54.6	366 420	54.0
Insecticides	237 360	38.1	223 740	35.0	246 120	36.2
Total	622 310	100.0	639 240	100.0	679 720	100.0

TABLE II. Sales of Synthetic Organic Pesticides: Value of Sales by Class in the United States, 1979-1981

Class	1979		1980		1981	
	Value, U.S. $ (millions)	% of total	Value, U.S. $ (millions)	% of total	Value, U.S. $ (millions)	% of total
Fungicides	253	7.0	290	7.1	322	7.1
Herbicides	2 165	59.6	2 588	62.7	2 716	60.0
Insecticides	1 211	33.4	1 230	30.2	1 489	32.9
Total	3 630	100.0	4 078	100.0	4 527	100.0

TABLE III. Growth in Domestic Herbicide Production in United States, 1980-1985

Year	Quantity, metric tons	Value, U.S. $ (thousands)
1980	348 980	2 588 290
1981	366 420	2 716 670
1982	373 750	2 950 300
1983	381 230	3 204 020
1984	388 850	3 479 570
1985	396 630	3 788 810

TABLE IV. The U.S. Herbicide Market by Crop for 1980 (User's Level)

Crop	Value, U.S. $ (millions)	% of total	Crop	Value, U.S. $ (millions)	% of total
Corn	889	34.3	Sorghum	70	2.7
Soybeans	884	34.0	Fruits and nuts	66	2.5
Cotton	168	6.5	Rice	62	2.4
Wheat	97	3.7	Peanuts	37	1.4
Potatoes and vegetables	71	2.7	Pasture and rangeland	30	1.2
			Total	2,396	92.4
			Percent of total U.S. use		92.5

Pesticides play an important role in food production and our export markets. Food exports for fiscal 1981 was projected to be $44.7 billion, up $4.5 billion from 1980. Deducting imports of $17 billion gave the U.S. a $27.7 billion surplus to our balance of trade. The gross farm income projected for American agriculture in 1981 was $164 billion, up 14 billion from 1980. The net farm income for 1980 was in the range of $23-25 billion. Besides being an important component in our balance of payments, agriculture is an important part our total economy.

2. ENVIRONMENTAL PROBLEMS

The perception of environmental problems associated with pesticide usage has intensified over the last two decades. One result of these concerns was the establishment of the U.S. Environmental Protection Agency (EPA) in 1970. The Agency was granted broad administrative power, under the Federal Environmental Pesticide Control Act of 1972, to regulate and control pesticides. Early environmental concerns in the United States were primarily focused on the the chlorinated hydrocarbon insecticides, and specifically, on DDT. In 1971, EPA canceled the registration of DDT for most uses in the United States, although this insecticide still plays a key role in crop protection and public health programs on a global basis. Since its cancellation over a decade ago the ubiquitous environmental residues of DDT have been steadily declining [2]. Most other chlorinated hydrocarbon insecticides have had certain uses canceled, or otherwise restricted, and only toxaphene remains a major compound still used in the United States. As studies of other compounds were pursued, some problems fell into certain fairly well defined categories. These may be considered with respect to: (a) environmental processes, and (b) specific compounds and/or their byproducts. The following subcategories of environmental processes will, under certain circumstances, be associated with pesticide problems. Each will, therefore, be briefly discussed:

Movement
- drift
- leaching
- runoff

Persistence and Plant Uptake

Adaptation
- pests
- soils

Exposure
- user
- consumer.

2.1. Movement

Movement of the pesticide away from its intended site of application (target area) into other components of the environment has been a recognized environmental problem. The three forms of pesticide movement of primary concern have been drift and volatilization, leaching, and runoff. Drift problems usually occur during ground or aerial spray application of a pesticide and are influenced by the pesticide's formulation; application parameters such as nozzle design, spray pressure, air velocity past the nozzle, and fluid properties; prevailing meterological conditions and height of release [3]. A high percent (30% or more) of a spray application can move 15 m or more away from a target area if conditions are ideal for drift. The major contribution to the problem in drift control is small droplet size. Small droplets tend to move further than the larger droplets. For example, a droplet 20 μm in diameter can drift over 900 meters under normal spray conditions. The amount transported by drift depends on the proportion of small drops. Bowers [4] noted a situation in which more than half of the droplets were less than 63 μm in diameter, yet of the volume was contained in the larger droplets, particularily those ranging in size from 64 to 210 μm. Nevertheless, drift has resulted in the movement of a pesticide into wildlife habitat and water, causing undesirable residues. In the case of herbicides, phytotoxicity to nearby sensitive crop species has occurred. The extent of damage due to drift off of the target area is difficult to document.

Movement of pesticides by wind erosion is much less studied, although a wind storm shortly after application of several herbicides to a fine sandy loam soil did remove enough pesticide to decrease weed control in treated plots and cause crop damage in adjacent areas [5]. Trace residues of pesticides in dust may undergo transcontinental movement, though the significance of such residues is not known [6].

Leaching, or mass transport of pesticides within the soil, can have a variety of consequences. Some are favorable, such as moving the pesticide away from the surface, thereby reducing volatilization loss. In some cases, such movement may also improve pest control. Leaching can also have unfavorable consequences, typically evidenced by reduced weed control when a herbicide has leached too far. A less likely, but more serious, consequence of leaching is the potential contamination of potable and other groundwater supplies. Concerns are then two-fold: those related to human health and the agronomic aspects of possible crop damage or illegal residues.

Most cases of groundwater contamination by agricultural use of pesticides are probably undocumented or undetected. Because of the low rates applied, under edaphic conditions that favor degradation and other attenuation processes, movement of pesticides to groundwater is much less likely to be a problem than is movement of hazardous materials from isolated landfill disposal sites Nevertheless, in 1979, a survey of 119 wells in California's agriculturally rich San Joaquin Valley revealed the nematicide DBCP to be present in 59 instances, at residue levels averaging 5 ppb [7]. DBCP had been used for nearly 20 years on these irrigated sandy soils, and was now present in some wells at great depth. This finding was a significant factor in suspending DBCP's use in the U.S.; loss of DBCP has dealt a serious economic blow to American farmers. There is evidence that under other agronomic and soil conditions, groundwater contamination is unlikely. Atrazine, a widely used herbicide in the U.S., leached through the upper soil profile, into drainage tile, and subsequently, into the drainage tailwater pit [8]. At the least, this represents a waste of the herbicide; at worst -- potential damage to nontarget crops or other ecosystem components.

Through extensive testing schemes using soil thin-layer chromatograms, soil columns, lysimeters, or field plots, the vertical mobility of many pesticides has been classified. Radioisotopes, as tracers within the pesticide molecule, have been used to facilitate many of these tests. Generally, polar pesticides with low soil-binding potential are most subject to leaching, and persistent compounds in this category pose the greatest threat to groundwater contamination.

Runoff is the surface movement of a pesticide due to rainfall. A number of varables influence the amount of pesticide transported by this process, including slope, vegetation, formulation, soil type, cultivation, amount of rainfall and a host of other varables. Many of these have been studied and the chemicals subject to surface runoff are probably reasonably well understood. In an extensive literature survey, Waucope [9] estimated that for the majority of commerical pesticides, total runoff losses are <0.5% of the amount applied, unless severe rainfall conditions occur within 1-2 weeks after application. Exceptions to this general estimate would include (a) organochlorine insecticides, which may lose about 10% regardless of weather conditions because of their long persistence, and (b) soil surface-applied, wettable-powder formulations of certain herbicides, which may lose up to 5%, depending on weather and slope. In the case of the pesticides formulated as wettable powders, usually herbicides, losses up to 5% are estimated from fields of moderate slope (10-15%) and losses of up to 2% on fields of low slope (3% or less).

The primary problem arises when persistent, nonpolar pesticide, such as the organochlorine insecticides, move into the aquatic environment. These pesticides are subject to bioconcentration in aquatic food chains. The routes and amounts of pesticide accumulation are fairly well characterized, primarily due to the improvement in aquatic ecosystem technology over the last 10 years. The use of labeled compounds, especially ^{14}C, has greatly facilitated residue measurement in the various ecosystems systems. Pesticide residues have occasionally produced restrictions on commercial and sport fishing when such residues were deemed unsafe for human consumption. This has had an adverse economic impact on those industries.

2.2. Persistence and Plant Uptake

Persistence or carryover can be a problem when the longevity of the compound is such that it may be taken up by subsequent crops, resulting in phytotoxic or undesirable residues. There is considerable literature on persistence of pesticides in soils. Highly chlorinated, nonpolar compounds with no readily metabolizable groups tend to be the most persistence compounds in soils.

Plant uptake of residual pesticides used in a current or previous cropping system has been the subject of considerable study [10]. It represents one possible avenue of direct human exposure to pesticide in the diet. One of the major early obstacles in determining the degree of risk from such residues was lack of selective and sensitive methods. The advent of gas chromatography for field samples and high specific activity labeled pesticides for laboratory studies have largely overcome this obstacle. A number of plant, soil and pesticide properties are known to affect the uptake process. The plant variables include soil residues, plant species, growth patterns (root crops vs. aerial crops), oil content, and growth stage. As a general rule, plant residues are directly related to soil residues and oil content. The most important soil factor influencing uptake is organic matter content. As this organic matter increases, plant uptake decreases, particularily for nonpolar pesticides. This suggests that pesticide adsorption to soil organic matter has reduced uptake. The most important chemical property of a pesticide influencing plant uptake is water solubility. Water soluble pesticides move to the roots, pass through the epidermis, and translocate more readily than nonpolar compounds.

2.3. Adaptation

There are mechanisms in nature for adaptation to continued use of certain classes of pesticides. One such mechanism is the development of resistance by organisms to the applied pesticides.

The ability of insects to develop resistance is widely recognized. Since the last survey on resistance in 1976, the number of species of insects and acarines in which resistant strains have evolved has increased by 62.5% to a total of 367 [11]. Of these, 225 are of agricultural importance and 139 of medical or veterinary importance. With regard to chemical pesticides involved, relatively large percentage increases in resistance have occurred to the organophosphates (172%), DDT (107%) and the carbamates. Nearly all cases of carbamate resistance (33) are new.

Control of cotton insects in the United States is becoming more difficult according to Ridgway [12] because of:

(1) Increasing development of insecticide resistance;
(2) Decreased availability of insecticides; and
(3) Rapidly increasing costs of insecticides and their applications.

These three factors are probably interrelated. At least 57 commercial insecticides and acaricides have been affected by resistance, and cross resistance may adversely affect the performance of many new products. If the rate of buildup of resistance to insecticides in certain crops exceeds the rate of introduction of new chemicals, then serious economic losses could arise in the near future.

A second aspect of adaptation that could have economic consequences is the ability of soil microorganisms to metabolize certain pesticides due to repeated usage. Results primarily from laboratory studies demonstrate that repeated application of herbicides to soils accelerate the microbial degradation process [13-14]. Until recently this was an interesting laboratory phenomenon that could be observed in soil perfusion columns or other devices designed to select an effective soil population responsible for degrading certain biodegradable pesticides. There is now evidence of such adaptation in the field. For example, the herbicidal efficacy of EPTC was rapidly lost following nine annual applications [15]. The authors postulate that accelerated microbial degradation may be responsible, resulting in reduced EPTC efficacy following its previous use.

The insecticide carbofuran may be similarily subjected to the same adaptation process. Kaufman et al. [16] have shown that [carbonyl-^{14}C]carbofuran is metabolized more rapidly to $^{14}CO_2$ in all carbofuran-history soils than from nonhistory soils. There appears to be a buildup of actinonycetes in "carbofuran problem" soils, which may be responsible for the insecticide's metabolism.

The first economic consequence of these examples of effective soil populations adapting to important pesticides is obvious: loss of efficacy resulting in lower production or greater costs to the user. A second, less apparent consequence has been increased research efforts to find effective and environmentally acceptable inhibitors to protect the vulnerable pesticide. This cost must be absorbed by the producer, and ultimately the consumer.

2.4. Exposure

Exposure of the user or consumer is a potential environmental problem if there is an associated risk with a specific pesticide use. Since exposure data is an important component in developing risk assessments, there is considerable research underway to understand the magnitude and routes of human exposure to agricultural pesticides.

From a number of recent studies on various applications, using the phenoxy herbicides, certain trends are emerging [17-20]. Factors that influence the degree of human exposure, as measured by residues in urine, are related to the type of job, hours of exposure, amount of pesticide applied, and care in application. Exposure could be considerably reduced if the worker wore gloves and other protective clothing, particularly for the mixers during the preparation of the spray. To reduce the risk associated with some pesticides, ground application equipment has had to be modified to include air-conditioned cabs on the tractor. Although the cost of protective clothing is nominal, the cost associated with improved protection afforded by installing or purchasing a tractor with a cab is substantial from the user/grower standpoint.

To determine consumer exposure to residues in agricultural commodities, the U.S. Food and Drug Administration (FDA) monitors pesticide residues in raw agricultural products and the U.S. Department of Agriculture (USDA) is responsible for monitoring residues in red meat and poultry. On an annual basis, FDA analyzes about 6000 domestic samples, 2100 samples from Mexico, and 2500 other imported samples for pesticide residues. The violation level has been low for domestic samples, 2% annually. Incidence of violations among foreign samples are slightly higher, 3-5%. These violations relate primarily to residues on commodities for which the compound is not registered. High residues are uncommon; violations are very few for the organochlorine insecticides (because of the ban on their use in the 1970's) and for herbicide residues. The cost of the pesticide monitoring program at FDA is roughly $7.5 million. Considering the value of pesticides to U.S. agriculture and the low frequency of violations, this cost seems reasonable.

Residues in red meat and poultry are monitored by the Food Safety and Inspection Service of USDA. Monitoring is done on a random basis from packing plants on paired species samples. In 1981 about 20 000 samples were analyzed and the violation rate was very low, being 0.0% for the organic phosphate insecticides and 0.4% for the organochlorine insecticides in cattle. No comparable costs are available for the USDA monitoring program since the program also includes drugs and other chemicals.

2.5. Specific Compounds and/or their Byproducts

A number of pesticide issues have arisen over the past decade relating to specific compounds, and more particularily impurities related to these compounds. While there are several examples that can be cited here, the dioxins associated with the phenoxy herbicides, ethylenthiourea associated with ethylenebis-(dithiocarbamate) fungicides, and nitrosamines associated with the certain dinitroaniline and acid herbicides will be considered here.

2.5.1. Dioxins

The dioxin issue arose when a feeding study conducted by Courtney et al. [21] showed that high levels of 2,4,5-T (113 mg/kg per day) fed to mice and rats produced cleft palate and fetal mortality in both species and, additionally, cystic kidney in mice. Subsequently, the 2,4,5-T used in the study was found to contain 30 mg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Much intensive environmental and toxicological research has been conducted over the last decade on TCDD.

The early environmental research on TCDD was mainly done with high specific activity [ring-^{14}C]TCDD in model systems due to its high toxicity, which precluded field studies, and due to the lack of a sufficiently sensitive analytical method to detect the low residue levels. TCDD is very insoluble in water (0.2 ppb), which governs many of its properties in soils. Dioxin is relatively persistent ($t_{1/2}$ >0.5 year, depending on initial concentration and climate), does not leach, is not taken up by plants, is rapidly photodecomposed in solution but is not appreciably altered on dry soil surfaces, and is not biosynthesized in soil from 2,4,5-trichlorophenol. In controlled laboratory studies with aquatic ecosystems, TCDD accumulated in amounts ranging from 2-2.6 x 10^4 times the initial water concentration for snails, mosquito, fish, and daphnids and averaging 4.9 x 10^3 for duckweed, algae, and catfish [22]. Most 2,4,5-T samples produced in the 1970's contained TCDD. A 1.1 kg/ha application of 2,4,5-T (typical rates are 0.3-1.1 kg/ha) containing 0.1 ppm TCDD applied to soil would result in a maximum of only 0.1 ppt in

the top 15 cm of soil. Detection of concentrations in the range of 10-50 ppt became feasible using rather elaborate cleanup methods followed by the use of specific ion monitoring on high resolution mass spectrometers interfaced with gas chromatography [23].

From an economic standpoint there have been two major costs associated with the TCDD issue. First, there is the cost associated with loss of the herbicide 2,4,5-T and silvex to the user: this is discussed under Risk/Benefit Analysis. The second major cost has been the research funded by industry and government to learn more about the sources, distribution, and effects of TCDD and related dioxins. This research has cost many millions of dollars, more will be spent in the future. It is currently estimated that residue analyzes for TCDD by use of the specific ion monitoring - high resolution mass spectral method cost ca. $1000 per sample. If all 22 tetra isomers are determined, plus certain higher chlorinated dioxins, the cost rises to ca. $4000 per sample.

The major concerns associated with the dioxins have been nonagricultural: a dioxin-laden oil used to reduce dust in a horse arena; TCDD-containing defoliants used in the Vietnam war; and the chemical plant explosion in Seveso, Italy. Many studies are underway to determine whether veterans of the Vietnam war exposed to Agent Orange suffer any adverse health effects. The dioxin situation has become more complex since several sources of dioxins have been identified. These include chlorophenols, pesticides derived from chlorophenols, production of hexachlorophene and combustion sources. There is evidence that municipal incinerators may serve as emission sources of dioxins, particularly refuse burning incinerators.

2.5.2. Ethylenethiourea

ETU is a manufacturing, processing, and metabolic product of the ethylenebis(dithiocarbamate) (EBDC) fungicides, including nabam, mancozeb, metiram, maneb, and zineb. ETU became an environmental issue when toxicological studies indicated it may be goiterogenic, tumorogenic, and teratogenic to laboratory animals. ETU residues appeared on certain agricultural commodities sprayed with EBDC fungicides. These residues may result from the presence of ETU in the pesticide formulation or from the subsequent transformation, either as a plant metabolite or a byproduct formed during processing of the crop.

In soils, ETU is rapidly oxidized to a number of products, including 2,4-imidazolidinedione (hydantoin), 3-(2-imidazolin-2-yl)-2-imidzaolidinethione (Jaffe's base), and 2-imidazolidone

(ethyleneurea, EU). Soil microorganisms probably play a role in metabolism of ETU and CO_2 is a major product from nonsterile soils. Plants produce a number of unknown products, in addition to EU and 2-imidazoline, from either soil or foliar treatment with the EBDC fungicides. Based primarily on the findings that ETU does not persist or bioaccumulate in plants, soils, or water, much of the initial concern about this product has lessened. Current management practices in agriculture have tended to reduce the usage of the EBDC fungicides for prescribed periods before harvest, thus reducing ETU residues in the crop. For an excellent review on the occurence of ETU as a terminal residue resulting from agricultural use of the EBDC fungicides, the reader is referred to a special IUPAC report [24].

2.5.3. Nitrosamines

Nitrosamines associated with pesticides became an issue when they were detected in certain pesticide formulations, especially dinitroaniline and some acid herbicides, by the use of the thermal energy analyzer (TEA) [25,26]. The combined use of TEA and ^{14}C-labeled nitrosamines has contributed greatly to our understanding of the fate of these contaminates in the environment, and a recent IUPAC report details much of their research [27]. Some, but not all, nitrosamines are carcinogenic. Nitrosamines are subject to photodecomposition, and volatile members of this class of contaminates were partially dissipated by volatilization and subsequent photodecomposition in air. In soils, the nitrosodialkylamines rapidly dissipate, whereas certain nitrosated pesticides were more stable. Due to their rapid disappearance from soils, plant uptake is not an avenue for human exposure to these nitrosamine contaminants. One of the major herbicides that initially showed trace amounts of the nitrosodipropylamine was trifluralin. The economic consequences of loss of this particular herbicide are subsequently discussed under the Risk/Benefit Analysis.

2.6. Risk/Benefit Analysis

There exists very little data on the adverse economic impact of pesticide problems on the agricultural ecosystem. If the user of pesticides is considered a component of that ecosystem, however, then in depth economic benefit data have been developed as part of the RPAR (Rebuttable Presumption Against Registration) process. The RPAR process is a detailed risk/benefit analysis of specific pesticides under review by the U.S. Environmental Protection Agency. The review is initiated after some health or environmental concern has been identified. USDA, in cooperation with the State Land Grant Universities, is responsible for developing biological and economic assessment reports on the

Table V. Economic Short-Term Impact on Users Due to Loss of Selected RPAR Pesticides.[a]

Pesticide	Impact, U.S. $ (millions)
toxaphene	111
silvex	19
2,4,5-T	64
trifluralin	521
EBDC fungicides	60

[a]Data from USDA/State Assessment Activities 1977-1981.

pesticides under review. The information needed for development of these benefit statements is substantial. For example, data inputs include: currently registered uses, where used, how much is used, how frequently used, method of application, geographic range of pests, units infested, formulation used, pests controlled, probability of infestation occurring, units infested at different levels, and annual and seasonal change in infestation. When all of this information is collected and analyzed, a report is prepared. To date, about 40 assessments have been completed. It is estimated that a full RPAR review, including a final position document, costs 4 to 5 million dollars [28]. The documents are extensive and a detailed discussion is beyond the scope of this paper. Some selected results of the short term or first year economic impact on the user or farmer are shown in Table V. Potential loss of the important herbicide trifluralin due to the nitrosamine issue could have cost the user about $521 000 000 on such important crops as soybeans, cotton and other uses. These losses are substantial, although they represent only a fraction of the value of the pesticides shown in Tables I-IV. Unfortunately, farmers in the United States are not in a financial position to sustain any sizeable losses without having a dire effect on their personal income and on agricultural production in general.

3. SUMMARY

How do we balance real or potential costs of economic problems posed by pesticides and the great financial benefits to the user and the national economy of any country?

We suggest there are several steps that can be taken to put these issues into their proper perspective.

(1) There is a need to identify these environmental problems and then through research reduce costs to a reasonable level. We have made significant progress in reducing drift and exposure and in modifying persistence by a better understanding of pesticide behavior on various soil types. The use of labeled pesticides in laboratory and model ecosystem studies has made a major contribution to understanding and reducing some environmental problems. Proper use of the isotopes in well designed studies can play an even greater role in the future.

(2) There is a need to streamline our environmental testing requirements to reduce development costs and time.

(3) There is a need to expand our benefit information gathering process to better evaluate the risks and benefits of pesticides to global agriculture.

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Invited Paper

ECONOMICS OF PEST CONTROL WITH EMPHASIS ON DEVELOPING COUNTRIES*

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Abstract

ECONOMICS OF PEST CONTROL WITH EMPHASIS ON DEVELOPING COUNTRIES.

Insects, weeds and plant diseases cause major losses in potential agricultural production. Their control by chemical methods will play an increasingly important part in raising the productivity of agriculture in the developing world to meet the needs of burgeoning populations. Chemical pest control can offer the farmer very high economic returns to investment, but full realization of these benefits requires good agricultural extension and training and local research to adapt available technologies to local needs. Decisions to employ particular pest control strategies must take account of local economic and social factors, the ecology of the pest and its predators and the consequences of chemical use for human safety and the environment.

1. INTRODUCTION

This conference is concerned, primarily, with one aspect of the complex balance of costs, risks and benefits of the use of biologically active chemicals in agriculture: that is, their fate in food and the environment. The objective of this paper is to set this specific area of concern within the wider context of the economic decisions on pest control facing farmers, governments and chemical suppliers; decisions which have to be taken against a background of rapidly increasing world populations, dominated in absolute number and in percentage growth rate by the developing nations.

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2. WORLD BACKGROUND

Sixty seven per cent of the world's 4.5 bn population currently lives in that group of countries encompassed with the UN category of "developing countries" (footnote [1]). The average rate of population growth within these countries is 2.2% pa compared with 0.7% pa in the developed nations. The high proportion of the population in these countries at or below child bearing age ensures that high growth rates will continue, resulting in 79% of the world's projected 6.2 bn population living in the developing countries by the year 2000. In contrast, these nations produce only 48% of the world's total tonnage of cereal grains and a smaller proportion of its other more varied fruit and vegetable crops and animal products, resulting in their producing only 38% of the world's total agricultural production.

The future demands for food to maintain the living standards of the expanding population, and, in addition, allow improvements in quality of nutrition to reduce the gap between the current average consumption of 2 200 k cal per person per day in the developing nations compared with 3 300 k cal per head per day in the developed countries, and provide a more varied diet, will require large increases in agricultural production. These increases in production must, for political, foreign exchange and other economic and social reasons, occur within the developing nations themselves. Sustained growth rates of at least 2.8% pa are needed; more rapid rates if standards of nutrition are to improve markedly and food deficits be avoided.

A 2.8% pa average rate of production growth has been achieved in the developing world over the twenty years 1960 to 1980, largely due to the introduction of newer high-yielding varieties, increased fertilizer use and other improved management practices. This has been an historically unprecedented rate of growth, yet an even greater achievement is required of the future. FAO suggests a target rate of 3.7% Ref. [1].

Production increases will derive to a limited extent from expansion in area cropped although the potential for this is now very limited in many of the developing countries.

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1 US billion = a thousand millions.

Table 1. PROJECTED CONTRIBUTION OF AREA EXPANSION AND CROP INTENSIFICATION TO AGRICULTURAL PRODUCTION GROWTH IN DEVELOPING COUNTRIES - 1975-2000 (Source: FAO)
(% contribution to output growth)

Region	Arable land growth	Cropping intensity	Yield	Yield + intensity
90 developing countries	26	14	60	74
Africa	27	22	51	73
Far East	10	14	76	90
Latin America	55	14	31	45
Near East	6	25	69	84

The majority of the increase must come from increases in productivity per hectare; productivity improvements resulting from increased use of irrigation, better cultivars, increased fertilizer use, improved husbandry practices and the increased use of crop protection.

In many parts of Asia, for example, expansion in yield per hectare became the main source of rice production growth from the late 1960s (accounting for more than 80% of extra production) and little growth came from area expansion.

FAO [1] project that yield increases and cropping intensity will account for 74% of production growth in developing countries over the period 1975-2000, with even greater dependance on yield performance in Asia (Table 1).

By the year 2000 almost two-thirds of the developing countries population will live in countries with at least 95% of their total potential arable land under the plough.

3. GROWTH OF AGRICULTURAL CHEMICAL USE

Historically the use of agricultural chemicals has been a major part of the package of technical inputs which have brought about the growth of agricultural productivity in

the developed world, and these trends are being repeated as less advanced economies grow. Pest pressures, diversity of insects, and intensity of weed growth are greater in tropical than temperate climates, heightening the need for pest control measures as part of modern production systems.

The overall growth of chemical usage in agriculture seems, therefore, an inexorable part of productivity growth to combat the losses caused by weeds, insects and diseases. These losses are estimated to represent, on average, 30-40% of potential production [2] and in many situations total crop devastation.

It is important that the overall pattern of pesticide use reflects an amalgam of good, rather than poor, decisions on product use by farmers and governments. An important part of that decision-making process is the assessment of costs, risks and benefits as they relate to each particular situation. It is not possible to generalise about the economic merits of chemical use, but it is possible to display a number of principles which should be considered.

Economic considerations embrace financial, social and a range of environmental issues.

4. FINANCIAL COSTS AND BENEFITS FROM PESTICIDE USE

The financial rewards from pesticide use may accrue directly from the reduction of losses caused by pest or disease attack, may be derived from savings in production cost (for example in reducing labour costs for weeding), or may result from a change in crop management system which would not have been possible without chemicals.

Numerous examples of direct financial reward from pesticide use, or losses through their lack of use, have been reported in the literature and a few are quoted here for illustration:

(a) The average losses of grain in storage in Tanzania per year are estimated to be about 100 000 t (30% of the total stored quantity) worth sterling £4.5 m. Cost of treatment to prevent this damage would be about £100 000/y, giving a benefit to cost ratio of 45:1 (ICI Estimates).

(b) In Nigeria in the mid-1970s it was estimated that 50% of stored cowpeas were holed and 25% of their weight lost by Bruchid beetle attack [3].

(c) Fifty per cent of the protein of smoke-dried fish from Lake Chad was destroyed each year by leather beetles (Dermestes maculatus) during the 6 months between drying and sale in Nigerian cities [3].

(d) Fifty experiments over 14 years at IRRI showed that pesticide protected plots yielded 5.7 t/ha compared with 2.9 t/ha on unprotected plots (an additional 2.8 t/ha worth about US $630/ha at 1978 local rice prices to the grower). One hundred and thirty experiments in farmers' fields in the Philippines showed a 25% increase in yield where pesticides were used, and similar results have been generated in other Asian countries [4].

(e) Sugar-cane production in Pakistan was increased by 30% through improved insect control. A $77 000 expenditure on insecticides gave $7.2 m additional sugar; a benefit:cost ratio of c 100:1 [5].

(f) Yields of pyrethroid-treated cotton plots in 28 US University trials between 1976 and 1978 were 2.5 times greater than those on the untreated plots [6]. This corresponded to an increase in crop value of $1 780/ha for an expenditure of $87/ha; a benefit:cost ratio of 20:1.

In plantation crops, where the financial pressures on the business necessitate careful costing of inputs, and where labour management problems demand that available labour is employed on the most rewarding tasks, herbicide use has been demonstrated to result in large savings in cost. For example Yeoh et al. [7] report a reduction in weeding costs in a rubber nursery from M $1 006/ha using manual labour to M $156/ha using herbicides, with additional benefits from reducing injury to the roots and stems of seedlings caused by unskilled labour.
Such large benefits will not always result. Major estates continually review the relative costs of chemical and manual weed control, and the other labour management problems, in evolving their weed control programmes. The optimum system will often integrate chemical and mechanical techniques.

In arable crops the economics of herbicide use will depend upon labour costs, labour availability at the critical stages of crop growth, the physical problems connected with alternative methods of weed control, the levels of use of other inputs such as fertilizer, and the value of the crop per hectare.

In the lowest labour cost areas of South-East Asia or Africa, mechanical or manual weed control will often be the most economic approach. In many areas of transplanted paddy rice the workload for weeding will be contained within manageable proportions by good water management. However it has been estimated that, on average, the tropical farmer spends 60-70% of his working time controlling weeds [8]. As labour costs rise, so there is pressure to substitute labour-saving herbicides. There has been a noticeable transition from manual control through integrated weed control using few sprays of cheap herbicide, eg 2,4-D plus supplementary manual weeding; through wider use of cheap herbicides; finally moving to the more expensive,broader spectrum, herbicides used in, for example, Japan or USA.

The first pressure to use herbicides is likely to come to relieve peak weeding labour inputs. There will be strong inter-farm or even on-farm competition for labour at peak times and loss of yield results from poor timeliness in weed control.

Improved mechanical weed control methods can replace labour in many row crops (eg maize), but in densely planted crops such as rice, effective mechanical techniques are not available. For example, in upland rice, where weed growth is much more intense than in flooded paddies, labour inputs to provide one hand weeding can be as high as 350-600 man-hours/ha. A number of studies reported by De Datta et al. (see Ref. [9]) showed that early competition from weeds reduced upland rice yields by at least 50%, and totally uncontrolled weeds would decimate the crop. Broad-spectrum rice herbicides provide the only practicable solution.

The importance of good weed control increases with adoption of other improved management practices of high yielding (often shorter strawed) varieties and increased levels of fertilizer, which stimulates weed growth as well as crop growth. A number of studies in Philippine and Thai rice [9] show small increases in yield from additional fertilizer under conditions of poor weed

Table 2. INTERACTION BETWEEN THE EFFECT OF FERTILIZER USE AND WEED CONTROL ON YIELD AND NET INCOME (Laguna, Philippines, 1970 wet season and Don Chedi, Supan Buri, Thailand, 1971 wet season) (Source: Ref. [9])

Fertilizer use[a]	Weed control[b]	Yield (t/ha)		Net return[c] (US$/ha)	
		Philippines	Thailand	Philippines	Thailand
Low	Low	3.0	2.4	128	78
Low	High	2.9	2.5	121	76
High	Low	3.4	3.0	137	87
High	High	4.2	3.9	171	127

(a) Fertilizer level : Philippines, low 35 kg nitrogen per hectare, high 75 kg nitrogen per hectare; Thailand, low 5 kg nitrogen per hectare, high 25 kg nitrogen per hectare.

(b) Weed control : Philippines, low $9/ha, high $18/ha; Thailand, low 1 man-day/ha, high 15 man-days/ha.

(c) Return above variable costs.

Table 3. EFFECT OF TILLAGE SYSTEMS ON SOIL TEMPERATURE AND SOIL MOISTURE RETENTION 2 WEEKS AFTER PLANTING VARIOUS CROPS

Tillage system	Max °C at 5 cm depth				% moisture retention at 0-10 cm deph			
	Maize	Pigeon pea	Soya	Cow-pea	Maize	Pigeon pea	Soya	Cow-pea
No tillage	31.6	32.4	32.4	33.4	13.3	12.1	10.6	15.4
Ploughed	41.4	40.0	41.4	41.8	9.7	10.8	7.3	12.3

Source : Rockwood and Lal [10].

Table 4. EFFECT OF TILLAGE SYSTEMS ON RUN-OFF AND SOIL EROSION UNDER MAIZE FOR A RAINFALL OF 44.2 mm

Tillage System	Slope %	Run-off %	Soil loss t/ha
No tillage	1	1.2	0.0007
	5	1.8	0.0007
	10	2.1	0.0047
	15	2.2	0.0015
Ploughed	1	8.3	0.04
	5	8.8	2.16
	10	9.2	0.39
	15	13.3	3.92
Bare fallow	1	18.8	0.2
	5	20.2	3.6
	10	17.5	12.5
	15	21.5	16.0

Source : Rockwood and Lal [10].

control, or, in some situations from added weed control without added fertilizer. The combination of increased fertilizer and better weed control can be very productive (Table 2).

High crop values also stimulate high input use. High levels of fertilizer are used because the marginal quintal of grain is sufficient to justify the marginal kg of fertilizer at the top of a crop's dose response curve. High quality weed and pest control is required to protect the investment in yield.

Rewards may also be attained through beneficial changes in the whole production system. Diverse examples of beneficial changes are available. Rockwood and Lal [10], at the International Institute of Tropical Agriculture, Nigeria, investigated the use of herbicides in place of mechanical weed control as a means of establishing a surface mulch and minimising soil disturbance and erosion. They demonstrated a wide range of benefits from a no-tillage system including reduced soil temperatures

(Table 3) (resulting in better seedling germination, emergence and vigour), better soil moisture conditions (Table 3) (through a dramatic reduction in rainfall run-off and evaporative losses), reduced soil erosion (Table 4) and decreased weed seed germination.

Further studies have led to the establishment of minimal or zero cultivation systems in many parts of Latin America. However, the economics of this herbicide-based change in husbandry systems vary significantly between locations. Initial International Institute of Tropical Agriculture (IITA) work [11] showed that on good soils with high fertility levels maize yields were similar for conventional and zero cultivation. The economic reward was to be gained through long-run benefits of soil conservation and reduced weeding costs rather than immediate yields benefits. Longer run trials at IITA [12], however, showed that, over a run of years, financial benefits began to appear as maize yields on conventionally cultivated plots began to fall in comparison with zero cultivated plots. On poor soils depleted by excessive cropping, there was some yield decrease with zero cultivation compared with conventional cultivation. Correct fertilizer usage is therefore important to the use of the technique and its usage can only be developed on suitable soils, with suitable attendant management practices, and where the economic benefits from erosion control are sufficiently great.

A major area of acceptance has been for large-scale soybean production in Brazil where potential benefits are perceived by government, farmers and chemical and machinery companies and each has been prepared to make the investment in a co-ordinated programme of research to establish a zero-cultivation programme. In southern Brazil the natural vegetation has been cleared to make way for soy bean and coffee. In Parana, the forest cover has been reduced from 84% of the land area to 9% over the last 30 years. The double cropping soybean/wheat rotation, has required two cultivations per year. The major soil types, latosols and podsols,are seriously prone to erosion, exacerbated by rainfall of 1 200-5 000 mm/year and intensities of 30-40 mm/hr. Even with terracing (which reduces soil loss from as much as 400 t/ha per year on a 6% slope to 97 t/ha per year) soil losses are too high to maintain soil fertility and make permanent land use possible [13].

Zero tillage, by leaving a permanent surface mulch, reduces rain impact and erosion enormously (by 95% in

some trials). This is an economic benefit of significant national importance to the Brazilians. Short-term economic benefits accrue to the farmer in savings of diesel fuel, increased area planted per man-hour, improved timeliness of planting and, in the Brazilian rotation, increases in wheat yield (though not soy bean yield) due to improved moisture conservation and soil quality [13], and reductions in the number of terraces and their construction cost.

Analogous benefits from herbicide-induced mulches have been shown in tea in East Africa [14] and in Assam and in young oil-palm in Malaysia [15] where financial savings in weeding costs were augmented by yield benefits through avoidance of root pruning, soil conservation and improved nutrient uptake.

Another example of a change of system is illustrated in the Philippines where herbicides are playing an important part in radical changes of cultural practice. Increased availability of irrigation and more rapidly maturing varieties have made double cropping of rice possible. The growth of non-agricultural employment, however, has resulted in a shortage of labour to meet requirements at the peak transplanting time. One solution to the labour shortage problem has been a switch to direct seeding, a change which was only possible because of the availability of suitable herbicides to control the much more severe weed growth which accompanies the growth of direct-sown rice seedlings. In this situation hand weeding is not a practical alternative to chemical use because of mechanical damage caused by people moving through the crops, the very small size of weed seedlings at the early critical stages of crop growth and the difficulty of telling grass weeds from rice seedlings. While the specific contribution of the herbicide cannot be quantified, the change in system has resulted in dramatic increases in yield per hectare per year.

In addition to benefits accruing to pesticide users through increased value of agricultural output, there is also a wider benefit to the community in the lower food prices which accompany increases in supply. Borlaug [16], for example, estimated that a total ban on pesticide use would not only result in production losses of 50% but could also cause price rises of 4 or 5-fold.

5. VARIABILITY IN THE ECONOMICS OF PESTICIDE USE

While a number of examples has been given to illustrate the rewards which can accrue as a result of pesticide use, the extent to which those benefits are realised will vary considerably between situations. In addition the influence of cost and price factors, such as the cost of the specific pesticide, the cost of labour and the value per tonne of the treated crop, rewards will be affected markedly by the overall level of crop management and the efficiency of pesticide use in relation to levels of pest incidence.

The economic benefits to be derived from protecting a crop against yield losses caused by insect attack or weed competition will be much greater where high-yielding cultivars are grown under good management practices with adequate fertilizer and water than under poorer, lower input management regimes. This has been illustrated above.

In the contrasting agricultural environment of many poor peasant farmers, only very limited amounts of cash will be invested in the production system. Seed will be saved from previous seasons and labour will be provided by the family. For many of them, yields depend more on the luck of good rainfall at critical stages of crop growth than on avoidance of any losses due to pests or weeds. Expenditure on pesticides, rather than being a good insurance policy can, in these circumstances, be a new burden of debt which cannot be financed and may not be economically justified.

Within a particular production system and cost structure, the cost effectiveness of pesticide use depends upon:

(a) selection of the correct chemical for the problem to be solved

(b) correct application timing in relation to crop growth, and pest level

(c) correct application at the chosen time

(d) other associated costs, eg costs of application.

Compound selection must reflect both cost-effectiveness against the target species and spectrum of activity in relation to the target pest complex and the ecology of predators.

Table 5. EFFECT OF TIME OF INSECTICIDE APPLICATION ON FIVE COWPEA CULTIVARS 1973 (IITA Data, Ref. [17])

Cultivar	Resistance rating*	Treatment			
		10 to 70 DAP	45 to 70 DAP	10 to 45 DAP	Control
TVu 1227	MR	844	845	554	311
TVu 2772	MR	611	677	402	236
TVu 1534	MR	637	746	391	226
Pale green	S	1029	858	613	78

* MR = moderate resistance; S = susceptible to leaf hoppers and thrips.

Economics of application can vary considerably with application timing. This is exemplified by studies of insecticide use on cowpeas in northern Nigeria [17] - Table 5.

On varieties moderately resistant to leaf hopper and thrips, insect attacks prior to flowering do not cause levels of injury sufficient to result in economic loss, and plants can recover from moderate to heavy defoliation. Early insecticide applications have no economic merit. Attacks during the critical flowering and pod formation stage, however, cause severe losses and justify chemical use to give yield increase of about 200% [18].

Greater insecticide use can be justified early in the season on the more susceptible variety. As with many high-yielding varieties, genetic potential for yield is often gained at the expense of pest or disease resistance and the variety has to be grown with high levels of other inputs to achieve its potential.

Method of application is important to economics. Many traditional application methods are very crude and the proportion of the applied dose reaching the pest is often small. Unskilled operators often apply wrong rates in the wrong way. This can be improved through good training and also advances in application technology,

such as electrostatic spraying, which can result in more accurate chemical placement, reduced rates of application and reduced application costs (e.g. through reduction in water carrying) [19].

6. NON-FINANCIAL CONSIDERATIONS

The use of biologically active chemicals to achieve particular agricultural benefits carries with it the risk of side effects on humans and non-target organisms in the environment. As the use of crop protection chemicals has become more widespread, so has the awareness of the risks [20], and it is important that hazards to users, food consumers and the environment are fully considered in the economic equation. In the early period of widespread pesticide use, during the late 1940s and 1950s, DDT and other novel compounds were accepted as the salvation from insect pests of a wide range of agricultural crops. Its use gave large increases in yield, and the World Health assembly of 1955 proposed the use of DDT for a global malaria eradication programme. However, by 1962, with the publication of Silent Spring [21], vigorous debates were starting on whether the risk : benefit equation was tilting in favour of, or against, pesticides. During the last two decades there has been increasing concern about the toxicological and environmental consequences of chemical use and increasingly stringent government regulation of the industry, much of which is reflected by the other topics of this conference. The increasingly stringent regulation has increased the cost of new product development by highlighting the need for a very wide range of toxicological and environmental studies as part of the development programme (Table 6).

The average cost of developing a new pesticide is now about $25 m and still rising fast. This means that new pesticides, for which there is a large dossier of data on toxicology, fate in food and in the environment from which to make risk:benefit judgements, are inevitably more expensive than the old "commodity" compounds.

Government authorities have to make a judgement on whether to purchase cheaper, possibly more hazardous compounds, or more expensive, newer products upon which risk:benefit judgements can be more fully made. Decisions have to be taken between financial pressures and the hazards posed by the poor control and misuse of toxic chemicals by peasant farmers.

Table 6. TRANSITION IN MINIMUM REGISTRATION REQUIREMENTS FOR WORLD-WIDE SALES

	1950	1960	1970 Onwards
Toxicology	Acute toxicity 30-90 day rat	Acute toxicity 90 day rat 90 day dog/1 year dog 2 year rat	Acute toxicity 90 day rat 90 day dog/1 year dog 2 year rat 2 year mouse 3 generation rat reproduction Teratogenesis rodent and rabbit Fish, shellfish, etc. Birds
Metabolism		Animal (min)	Rodent and/or dog Plant Animal transfer studies
Analytical	Food crops 1 ppm[1]	Food crops 0.1 ppm[2] Meat 0.1 ppm Milk 0.1 ppm	Food crops 0.01-0.05 ppm Meat 0.1 ppm Milk 0.005-0.03 ppm
Ecology			Environmental impact Stability/degradation in soil Movement (leaching in soil etc.) Accumulation in food chains Effects on micro flora and fauna

1 Pesticides only.

2 Pesticide plus toxic metabolites.

Table 7. NUMBER OF INSECT PESTS RESISTANT TO CERTAIN INSECTICIDES

1948	1951	1954	1957	1960	1963	1968	1976	1980
12	16	25	76	137	159	224	364	>400

The balance of the equation will differ from country to country.

Consider, for example, the question of the use of DDT. DDT use has been restricted in many developed countries, partly because governments have taken the view that society should pay the financial cost of more expensive chemicals in return for a reduction in the environmental concern associated with a compound which is environmentally persistent, accumulates in body fat, tissue and concentrates in the food chain. In the developing world, where DDT still forms an important part of the malaria control programme, shortage of funds and, perforce, other economic priorities tip the balance of judgement in favour of financial rather than environmental considerations. The reduction in environmental risk in moving from DDT may not justify the diversion of scarce foreign exchange from other priorities. Also concern about possible hazards is reduced by the more rapid degradation of the compound under tropical conditions.

Major areas of risk not covered by legislation, of particular concern with insecticides, are those of resistance and resurgence. There is a serious danger that, in maximizing the short-term economic gains which result from liberal use of broad-spectrum insecticides, economic problems could be generated for the future. This is another area well covered by Metcalf [20].

Insect resistance was first observed in 1914 when San Jose scale (Aspidiotus pernicious) became resistant to lime sulphur. Since then the number of carefully documented cases of species/chemical group resistance patterns has grown (Table 7).

Many show multiple or cross-resistance to several groups of insecticide.

Insect resurgence is also an increasing problem. Pests which were not of economic importance can become important because their predators are destroyed, or pest species which are temporarily controlled by pesticides resurge as greater problems because of loss of their predators. A number of important examples exist. Spodoptera exiqua and Trichloplusia ni arose as problems on central American cotton following use of broad-spectrum insecticides during the 1960s. In South-East Asia leaf hoppers and plant hoppers of rice (and virus transmitted by them) became a major problem of the 1970s.

because their predators were decimated by DDT and BHC used for control of stem borers (Chilo suppressalis and Tryporyza incertulus).

Fortunately the international chemical companies have been able to invent new groups of chemicals to replace those which are no longer able to cope. The indiscriminate use of one weapon, and its replacement with a newer more expensive weapon when the first is no longer effective, is unlikely to be the most economically efficient process. It is a treadmill to which the international agricultural community can easily be committed through unskilled product use and short-sighted economics. It is a risk that may be exacerbated by the ambitions of developing countries to have their own pesticide manufacture, locking themselves into certain types of compound.

Long-term economic gain must come from well considered use of the correct chemical at the correct time; taking note of economic thresholds; rotating chemical types; making maximum use of natural enemies; tailoring application rates to the pest pressure and the economic degree of control (which may not be 100%).

The optimum long-term use of pesticides, integrated into the agronomic, ecological and socio-economic system (integrated pest control) will not happen automatically. It must be the result of good adaptive research and extension.

In many cases, improved pest control programmes can be developed with existing pesticides. Development of more sophisticated integrated programmes using novel chemicals with greater selectivity than those currently available, however, requires a much closer partnership between governments and chemical companies to ensure that the potential value of the selective chemicals is fully exploited; farmers will be purchasing a pest management programme rather than cheaper, cruder weapons.

7. INVESTMENT IN RESEARCH AND EXTENSION

The previous sections of this paper have illustrated the need for increased agricultural productivity, and the valuable contribution chemicals can make. They have also highlighted the complexity of the adaptive research needed to exploit pesticides effectively and the need for

local understanding of the balance between risk and reward. It is vital that good technical and economic information is disseminated to pesticide users through a well organised extension system if the full long-run economic benefits are to be gained. Every effort must be made to avoid long-run economic penalties through misguided attempts to obtain short-term gains.

Haskell [2] highlighted the tendency for many developing countries to give low priority and status to research on the optimum use of pesticides and their integration into ecologically sound agricultural systems and to underplay the necessary infra-structure of extension to convert this local adaptive research to practice. Yet investment in good adaptive research and extension is a vital part of the overall investment in pesticides, and an investment which can bring very large rewards for low capital commitment.

It is also probable that the training of farmers and their advisors will have to proceed a long way before alternative more sophisticated and specific pest control techniques, such as pheromones or sterile insect release [22] can make a major impact on the pest control programmes of the developing nations.

UNDP and FAO are taking more initiative to develop international support for plant protection. The closer the integration between the skills of the chemical industry, independent research and extension, the greater will be the economic return to pesticides and the growth of agricultural productivity.

There have also been examples of international chemical companies selling a package of extension, training and pesticide programmes to the governments of developing countries, to the mutual advantage of all concerned. The agricultural chemicals industry has considerable technical expertise, and experience in training technical and administrative staff, and would welcome the opportunity for greater co-operation with international organisations like FAO in the future.

There is also a strong case for aid donors to give more attention to the development of crop protection which can, when correctly applied, generate higher rates of return on investment than many large capital projects.

8. CONCLUSION

The economic decision to use crop protection chemicals has been shown to have many financial and non-financial facets. Used correctly, and with an appreciation of the environmental risks, agricultural chemicals will continue to grow in importance, making a major contribution to the vital growth of agricultural productivity in the developing countries. Investment in the chemicals, and the extension service to promote their correct and timely use will generate excellent economic returns.

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PESTICIDES IN THE AGRICULTURAL ENVIRONMENT
(Session II)

Chairman

F.P.W. WINTERINGHAM
United Kingdom

Invited Paper

FATE OF PERSISTENT PESTICIDES IN THE AGRICULTURAL ENVIRONMENT WITH PARTICULAR EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES

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Abstract

FATE OF PERSISTENT PESTICIDES IN THE AGRICULTURAL ENVIRONMENT WITH PARTICULAR EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES.

A selection has been made from open literature and from studies evaluated by the Joint FAO/WHO Meeting of Experts on Pesticide Residues over the past three or four years to show how isotope techniques have been applied during studies to determine the fate of pesticides in the agricultural environment. While many of these involve investigations to elucidate metabolism in plants, animals and soil, nuclear techniques are also useful in developing and validating analytical techniques and in determining the nature and level of residues transferred to and accumulated in foods of animal origin and the effect of processing and cooking on residues in food commodities of all types.

INTRODUCTION

Pesticides have provided tremendous benefits in the past, through the control of pests that affect man's health, destroy his sources of food, compete with his crops, and damage his belongings. They are toxic chemical compounds that must be treated with the same care and respect as pharmaceutical products and other developments of modern science and technology. There have been problems in the past due to their misuse or over-use but very rarely, if ever, from their proper use. Increasingly precise analytical procedures that can detect minute amounts of residues, the phasing out of undesirable chemicals, more comprehensive regulatory procedures and the less intensive use of pesticides through the development of integrated pest control systems will ensure a minimum of problems and a maximum of benefits from use of pesticides in the future.

An ever-increasing world population demands increased food, fibre and forage production, together with improved public health facilities for man's survival. Improved technology, including the judicious use of pesticides, helps man meet these demands.

Several important factors must be considered in connection with the use of pesticides in today's environment-conscious society. It must be recognised that vast amounts of time, money and effort are being expended to ensure that

these chemicals are capable of being used for the general betterment of mankind through the production of wholesome food and the protection of health. It is also clear that there are some very definite hazards and drawbacks involved when these products are used excessively or unwisely.

One fact that comes through very clearly is that pesticides are now, and will continue to be, an integral and essential part of most pest control systems. The amount of pesticide used in any system or systems will inevitably vary according to the crop to be protected, the pest to be controlled, the nature of the compound itself and of factors such as biological and cultural control practices and climate. By the same token, it is safe to say that society's overall goal should be to use pesticides at the lowest possible levels within the socio-economic constraints of our society and to use them in a manner designed to minimise the risk of side effects.

The term "persistence" cannot be exactly defined. A substance is unduly persistent whenever a measurable quantity thereof continues to exist in some discernable chemical form. Obviously, the most desirable pesticide would be one that has a biological 'life' just sufficient to perform its function as a pesticide, with no residual carry over. Since this is difficult to achieve, the rhythm of use of the pesticide in terms of its function must always be considered. Therefore, "the function, time, rhythm" of a pesticide must take into consideration not only its biological 'life', but also its presence afterwards in terms of any residual terminal chemical.

The desirability of having rapidly bio-degradable pesticides appears attractive as theoretically this would prevent any residues. However, two questions arise; first, are the metabolites from degradation "ecologically safe" and second, how precise is the time of degradation? The first requires extensive study to identify the metabolites and their ecological effect while in the case of the second, conditions determining rate of degradation are important. With short-lived materials, the timing of application for insect control is usually much more critical than with more stable materials. The precision in timing may be well beyond the capabilities of farmers. In the case of insect pests with a long life cycle, a large reproductive capacity or an ability to migrate it would be necessary to apply such short-lived materials repeatedly and frequently with the result that the environmental impact could well be greater.

There are two facts which must be taken into account by the proponents of the use of persistent pesticides. They wander far away from the original area of application and they have been proven to be harmful to some species of fauna. On the other hand, those who favour a complete ban on the use of persistent pesticides, including organochlorine insecticides, should realise that they serve a very valuable function in both public health and agriculture. In spite of claims that less toxic or less persistent materials could serve the same purpose, the expert opinion is that, for many applications, effective substitutes are not yet available.

There are real dangers. There are real advantages. Ways must be sought in which the potential harm can be diminished or eliminated. To accomplish this end, ecologists and physiologists must work together with agriculturists and public health officials.

When the Scientific Secretary responsible for this International Symposium on Agrochemicals invited me to present a review paper on "Persistent Pesticides in the Agricultural Environment" I assumed that I should confine my attention

to the classical "persistent" pesticides. However, a detailed study of the scientific literature revealed that most of this work had already been reviewed at previous FAO/IAEA meetings and I could see no way in which I could present this information in a fresh light. Extensive correspondence with contacts in various tropical countries only served to confirm my views.

I therefore concluded that the recognition that the agricultural environment is finite and that all pesticides are persistent to some degree justified a review of recent studies of the effect of the various components of the agricultural environment on a variety of widely used modern pesticides. I have attempted to make such a review, paying particular attention to the application of nuclear techniques in studying these interactions.

THE APPLICATION OF NUCLEAR TECHNIQUES

It is interesting to consider how the application of isotope techniques has led to a more complete understanding of the properties, effects and fate of pesticides and how radiotracer studies now form a vital part of the multi-disciplinary approach to the study of new pesticides prior, as well as subsequent, to marketing. Before doing so I would like to draw attention to the report of a previous FAO/IAEA Research Co-ordination Meeting held in Vienna, 25-29 October 1971 [1] in which the value of isotopetracer techniques for obtaining complete information on the persistence and fate of foreign substances in living organisms and the environment is summarised in a few, well-chosen words which are as true today as they were eleven years ago:

> "Any foreign chemical added to food or the environment will always be present from that moment onwards either as the original chemical compound or as its equivalent derivatives and breakdown products. Fortunately, most foreign chemicals undergo sufficiently rapid physical dispersion and/or chemical degradation so that the resulting residues are of little or no ecological or toxicological significance. However, in assessing the possible significance of each residue in food or the environment in the first place, it is essential to obtain as complete a picture as possible of the fate and biological activity, not only of the parent compound but of all its likely derivatives. It is not sufficient to study the disappearance of the parent substance only. The derivatives may also be of significance in relation to possible concentration in food chains, chronic toxicity or long-term ecological effects. Isotope-tracer techniques have been found to be especially useful in basic studies of this kind and in the preparation of "balance sheets" to account for the overall fate of the original substance. Another advantage of the tracer technique is that it also provides information on metabolites, derivatives, etc. which may not be recovered or extracted by the usual analytical extraction procedures, especially from aged or stored samples."

If we were to measure the importance of isotope techniques by the number of scientific papers that appear in open literature or in unpublished studies presented to government authorities for evaluation, we would find that studies of metabolism in plants, animals and other environmental niches exceed all of the rest of the studies involving radiotracers. This is not only because of the importance of metabolism studies for evaluation of consumer safety, but because such studies go a long way towards providing a clear picture of the mode of action of the compound, its possible effect on livestock and wild life, the importance of application patterns and techniques, the possibility of phytotoxicity and similar side-effects and the likely influence of environmental factors. There is, in fact, no other convenient method for studying metabolism.

The persistence of a chemical can be studied by conventional residues techniques but these are generally laborious, time-consuming and sometimes imprecise, particularly during the earlier stages of the development of a new pesticide. The use of isotope-labelled compounds permits such persistence studies to be conducted quickly, accurately and economically and with every chance of determining the effects of chemical, physical and meteorological variables on the rate of degradation/disappearance.

Where chemicals have a capacity to be translocated in plants, animals or soil, isotope-labelled compounds can be used to determine the rate, degree and site of translocation without the necessity of developing complex, cumbersome and costly analytical techniques.

Isotope techniques cannot be substituted for conventional residues studies because of the need to determine the maximum levels resulting from approved uses under widely different agricultural, climatic and meteorological conditions. However, such studies are more valuable if the analytical techniques are confirmed against samples obtained from crops or commodities treated with isotope-labelled compounds. The extraction, recovery and clean-up stages can be conveniently checked by such comparisons. The nature of the residues, including bound residues, can be best determined using isotope-tracer studies.

Where there are any doubts concerning the accumulation of residues in fruits, seeds and foods of animal origin these can be dispelled by relatively simple studies involving isotope-labelled compounds. It is possible to similtaneously determine the distribution of residues in edible and non-edible portions of the commodity and the effect of time, temperature or other variables on such distribution.

Many pesticides, including insecticides, nematocides and fungicides, as well as herbicides, have to be applied by way of the soil. Others find their way onto the soil adventitiously. It is important to know the nature and fate of the compounds falling on or formed in the soil and the effects of soil type, moisture and tillage on the soil residues. Such studies are best carried out using isotope techniques which lend themselves to precise, small-scale laboratory studies that are quick, convenient and reproducible.

Many agricultural commodities are stored for varying periods after harvest. Storage may involve controlled atmospheres, refrigeration, moisture control and application of post-harvest treatments with other chemicals. The influence of such storage conditions on the nature, level and distribution of the pesticide residue can be conveniently studied using isotope-tracer studies. Finally, it is important to know the effects of preparation, processing and cooking on the nature and level of the pesticide residue; here too we find many investigators favour the use of labelled compounds in order that results can be obtained from a variety of simulated processes without the complication that would have to be overcome if conventional residues methods had to be developed and validated.

I have been a member of the FAO panel of experts on Pesticide Residues more or less continuously since 1969 and I have been privileged to examine a great variety of studies of the type mentioned above. I have made a selection from studies evaluated by the Joint FAO/WHO Meeting of Experts on Pesticide Residues over the past three or four years to show how isotope techniques have been applied during studies to determine the fate of pesticides in the agricultural environment. These will be presented in alphabetical order by compound.

ALDICARB

Metcalf et al. [2] demonstrated that aldicarb was completely oxidized to aldicarb sulfoxide in cotton foliage within four to nine days. Further hydrolysis yielded the sulfoxide oxime, and oxidation of the aldicarb sulfoxide to aldicarb sulfone occurred. Coppedge et al. [3] confirmed these findings, and identified the sulfoxide nitrile as a definite metabolite in cotton. Once formed, aldicarb sulfone is not reduced to provide a secondary source of aldicarb sulfoxide, nor could evidence of oxidative N-demethylation be found. The total radioactivity in the cotton plant is reduced with time through volatilisation and dilution by plant growth.

Field-grown cotton was treated with radioactive aldicarb in furrow at planting and by side-dressed applications [3]. The residues were identified, quantitated, and the rates of decline determined. The metabolic pattern of aldicarb was in agreement with that described by earlier investigaters. A second field study employed petiole injection and obtained similar results [4]. A complete distribution of radioactivity in the cotton plant was described and the residue in the maturing fruit was characterised. After four weeks, no toxic residues were present in the bolls.

The sulfoxide oxime is further transformed to a mixture of water-soluble products. These consist primarily of sugar conjugates of sulfoxide alcohol, as well as similar quantities of sulfoxide and sulfone acids and sulfone amide [5].

After gaining entrance into the potato plant, aldicarb residues move primarily by xylem transport with higher concentrations appearing in the foliage [6]. Stem injection studies have shown that only limited quantities of aldicarb and its metabolites move downward into the tuber. The toxic carbamate residues appearing in the tuber do not persist, but are actively degraded to non-toxic water-soluble products similar to those formed in the foliage [7, 8].

Systemic movement and concomitant metabolism of aldicarb resulted in a preferential accumulation of the residues in peanut foliage [9]. A small fraction of the observed radioactivity was found in the fruits. Translocation of residues to the forming fruit is facilitated by the polar nature of the metabolic products present in the maturing plants. These water-soluble metabolites were the predominent component of the terminal residues in the foliage and constituted 90 to 95% of the recovered radioactivity in the kernels.

Aldicarb sulfoxide, aldicarb sulfone and the non-toxic water-soluble metabolites constituted the major portion of the residual ^{14}C-materials in sugar beets [10]. Most of the absorbed radioactivity was found in the foliar portion of the plant throughout the growing season. At maturity (140 days after treatment) total ^{14}C-residues were 27.2 mg/kg in the foliage and 2.5 mg/kg in the roots. The corresponding values for total toxic residues was 11 mg/kg in the foliage and 0.6 mg/kg in the roots.

The chemical changes that occur in soil are essentially the same as those in plants, animals and insects. Series of parallel experiments have been performed under the same environmental conditions with single factors varied to assess their roles in controlling the persistence of aldicarb in the soil. These factors include soil types, moisture, pH, and temperature [3, 5, 11].

Under greenhouse and field conditions, aldicarb and its breakdown products leave the soil with unexpected rapidity; a half-life of seven days was

observed [11]. This emission is definitely linked with the degree of soil moisture and consists primarily of an upward movement. This phenomenon has been studied in an elaborate series of percolation experiments with different soil types in assorted sizes of columns [11] and under field conditions [5]. Only in pure sand is downward movement readily achieved through water action.

The dissipation of aldicarb in the soil is sufficiently rapid and complete to ensure that recommended rates will offer no hazard of contamination of subsequent crops in a treated area [12, 13, 14].

Following the administration of a single dose of aldicarb to a lactating cow, approximately 83% of the dosage was eliminated in the urine within 24 hours. A minor quantity of residue was eliminated in faeces and a small residue was observed in the milk (less than 3% of the administered dose was observed in milk over a 5-day interval). Increasing the number of days of treatment from one to fourteen did not change the magnitude or the elimination pattern of aldicarb in milk or excretory products. Approximately 1% of the administered dose was secreted in milk with 95% of the administered dose being eliminated by the other routes. Small levels of residues were observed in tissues with the liver showing the major terminal residues. Continuous exposure of cows to aldicarb in the diet did not significantly alter its absorption and excretion patterns [15, 16].

Aldicarb and/or aldicarb sulfone administered as a single oral dose to laying hens was rapidly excreted in the faeces. Minute quantities of terminal residues were observed in eggs on the first day after treatment, but the residue level declined rapidly. Tissue residues were maximal within six hours of treatment after which a rapid decline was observed. Continuous administration of aldicarb for 21 days did not change the pattern of rapid excretion or of terminal residues in eggs or tissues [17].

CARTAP

Cartap is a recently introduced insecticide which is readily hydrolyzed to nereistoxin, a naturally occurring insecticidal substance isolated from the marine segmented worms, Lumbrineris heteropoda. Extensive information is available on the chemistry and synthesis of nereistoxin and its derivatives, of which cartap hydrochloride is the most potent. One of the most important applications of cartap is for the control of stem borers in rice.

In rice plants grown in hydroponic solutions or under simulated field conditions containing ^{35}S-cartap, absorption and distribution was rapid with cartap accumulating in all parts of the plant. Extensive degradation of cartap was found to occur rapidly with incorporation of ^{35}S into natural components (sulphur-containing amino acids). Prior to complete degradation, cartap was observed to be degraded through a series of similar oxidative pathways as in mammals with the principal exception being that the methylation reaction, as generally occurred in mammals, did not appear to occur in plants. The pathway of metabolism in plants thus results in a series of metabolites that substantially differ from that seen in mammals. Nereistoxin appears to be predominent in plants and undergoes sulphur oxidation to the oxide (sulfinyl), dioxide (sulfonyl) and sulfate, as well as N-demethylation. Thus, the metabolic pathway in plants appears to result in terminal residues that may be substantially different from those observed in mammals. This difference appears to result from the initial equilibrium established with nereistoxin and dihydronereistoxin both of which follow different pathways in plants and animals to their ultimate degradation [18, 19, 20, 21, 22].

The absorption and distribution of ^{35}S-labelled cartap hydrochloride in rice plants grown under various hydroponic conditions were studied by autoradiography. After ^{35}S was absorped from the roots, it was distributed throughout the laminae and high concentrations were observed in the leaf sheaths. Accumulation occurred in the leaf apices. Absorption through the leaf sheaths was also observed, and was found to occur faster in young leaves than in older leaves. Distribution occurred through the vascular tissues. When applied to the leaves, the insecticide diffused from the point of application to the leaf apices and accumulated in the leaf sheaths. ^{35}S was detected in the digestive organs, neural tissues and spiracles of the intoxicated rice stem borers [18].

In 66-day old rice seedlings, the major region of absorption was observed to be the roots. As much as 15% of the applied ^{35}S radioactivity was found to have been taken up by the plant, mainly through the roots. As much as 500 mg/kg accumulated in the rice plant seedlings after six days immersion [19].

When paddy cultivation conditions were simulated, ^{35}S-labelled cartap hydrochloride was rapidly absorped, a maximum level in most tissues being reached after three days. Metabolism to water-soluable components occurred readily. Accumulation of ^{35}S in the panicle was also observed [20]. It was subsequently found [21] that under conventional field practice, the amount of ^{35}S-radioactivity was high in the hull and rice bran but low in the milled rice. Most of the metabolites were water-soluble and many were amphoteric. These metabolites were tentatively identified as methionine sulphoxide, methionine sulphone and S-methyl cysteine sulphoxide [22].

CYCLODIENE INSECTICIDES

Considering the length of time the cyclodiene compounds have been available, the extent and variety of their use and the degree of concern over their persistence, the number and variety of studies that appear to have been conducted using nuclear techniques is relatively small.

Korte [23], after applying ^{14}C-aldrin, dieldrin, endrin, heptachlor, telodin and dihydroheptachlor to microorganisms, mosquito larvae, mammals and, in the case of aldrin, dieldrin and endrin to higher plants, measured remarkable conversion ratios, except for dieldrin, in microorganisms. Some of the break-down products were isolated and identified and had a lower mammalian toxicity than the parent compounds. Long-term feeding experiments with mammals showed that in all cases a sex-dependent saturation level of storage was reached after some time and that after discontinuing the application the radioactivity was eliminated. The investigators observed that when these compounds were applied to plants, a distribution of the insecticide and its metabolites was observed in all plant parts and in the soil as well. More than 50% of the radioactivity disappeared through evaporation and transpiration.

Brooks [24] provided some illustrations of the variety of biotransformations that occur in a small selection of organochlorine compounds, of the relationship of these conversions to those that occur chemically and of the products that might conceivably be expected from what is known about organochlorine chemistry. The author pointed to the important contribution of anaerobic microbial biotransformations to the final disposal of organochlorine compounds and pointed to the observation of a ring cleavage reaction among such sturdy molecules as the cyclodienes as a basis for expecting a considerable degree of biodegradation of such compounds in nature.

Brooks provided two further extensive reviews of cyclodiene metabolism including an extensive discussion of the pathways of enzymatic degradation [25, 26]. A further extensive review was provided by Korte [27]. The bulk of the studies had been conducted with the aid of isotope techniques.

Plimmer in an extensive review of the photochemistry of organochlorine insecticides deals at length with the cyclodiene group and concludes:

> "for the organic chemist, the organochlorine insecticides demonstrate a most interesting series of photochemical transformations. Study of their photo-products, however, presents more than an interesting academic exercise. The majority of the organochlorine insecticides are regarded as 'persistent', i.e., they are metabolised rather slowly by common organisms. Therefore, we might expect photolysis to play ultimately a significant role in their breakdown. Some of these photolysis products have already been recognised in samples of crops and soils. In view of their potential toxicity, it is important that we continue analytical study and identification of new photoproducts, to ensure that we recognise any hazard they might present" [28].

Walker et al. [29], in an extensive radiotracer-aided study of the comparative metabolism of dieldrin analogues by vertebrates, using eight species of animals and birds, showed that two distinct mechanisms are involved in the initial degradation in these species: oxidation and hydration. In vivo experiments produced information on the rates and pathways of metabolic excretion.

Klein [30], reviewing the metabolism of pesticides in higher plants, pointed out that many of this group of pesticides are translocated from soil to the aerial parts of plants and are converted to the corresponding epoxide. The presence of photo-derivatives is noted.

Klein [31] in a review of experimental work carried out between 1969 and 1972 on the fate of cyclodiene residues in soil and crop plants paid special attention to hydrophilic conversion products. The quantitative distribution of residues in various parts of crop plants and different soil depths is tabulated.

Klein et al., reviewing a comprehensive series of isotopic tracer-aided studies of the behaviour of cyclodiene residues in model ecosystems, pointed to the formation of polar metabolites. The degradation of these compounds by green algae was studied as was the abiotic transformation by ultra-violet radiation. The fate of dieldrin in soil/plant/food/animal systems is discussed as a model [32].

FENBUTATIN-OXIDE

Fenbutatin-oxide is used as a specific miticide against a wide range of phytophagous mites. It is recommended especially for the control of the mobile stages on a wide variety of crops, including pome and stone fruits, citrus fruits, grapes, berry fruits, vegetables and ornamentals.

It is interesting to see how isotopic techniques have contributed to the understanding of the effect and fate of such an organo-metallic compound.

Fenbutatin-oxide is slowly lost after application to crops. The degradation to inorganic tin occurs through successive loss of the phenyldimethylethyl

groups. The principal organo tin degradation product found on crops is, 1,1,3,3,tetrakis(B,B-dimethylphenethyl)-1,3-dihydroxydistannoxane, referred to subsequently as SD 31723 [33].

In studies of the fate of fenbutatin-oxide on leaves and fruit of apples [33], small apple trees were treated one to three times with ^{119}Sn-labelled fenbutatin-oxide. Recoveries of the ^{119}Sn 2 to 33 days after the last application were 84-96% of the amount applied. Virtually all the radioactivity in the fruit was found to be on the outer surface of the skin. Substantial amounts of the radioactivity were removed by rinsing with organic solvents, or simply by wiping the fruit-skin with paper tissues. It was shown that less than 5% of the total radioactivity present in the fruit originated from the principal break-down of product (SN 31723) and less than 0.7% from other organo-tin metabolites. Inorganic tin accounted for nearly 10% of the total ^{119}Sn on the apples [33].

Similar experiments were carried out on oranges [34]. Leaves were treated with formulated ^{119}Sn-labelled fenbutatin-oxide at a concentration of 40 mg/L and analysed at intervals up to nine months after treatment. Forty days after the treatment, 65% of the applied radioactivity was still present in the leaves, of which 71% was unchanged fenbutatin-oxide. At the end of the 9-month period the total radioactivity present had declined to about 11% of that applied, and of this amount 60% was still present as fenbutatin-oxide; SD 31723 accounted for about 3% whilst inorganic tin made up the remainder [34].

An experiment was carried out in which three lactating Guernsey cows were fed on a diet containing ^{119}Sn-labelled fenbutatin-oxide [35]. The quantity given was equivalent to 34 ppm fenbutatin-oxide in the whole ration which was given twice daily during a 21-day period. Milk samples were taken from each animal at each milking, and the total radioactivity determined in each sample.

No radioactivity above back-ground level was found, indicating that total residues in milk were equivalent to less than 0.01 mg/kg fenbutatin-oxide. The animals were slaughtered 12 hours after the final feeding and the radioactivity was determined in samples of fat, muscle, brain, kidney and liver. No residues were detected in brain, muscle, fat or liver at a limit of determination equivalent to 0.02 mg/kg fenbutatin-oxide. Similar results were obtained with the kidney sample from two of the animals, but the third was found to contain radioactivity equivalent to 0.03 mg/kg fenbutatin-oxide, i.e. just above the limits of determination in these tissues.

Thin layers of ^{119}Sn fenbutatin-oxide on a glass surface, when exposed to sunlight, slowly decomposed to the derivative SD 31723 and to more polar compounds such as inorganic tin salts. After 230 hours of exposure, 81% of the initial ^{119}Sn deposit was extractable by organic solvents and 6% by aqueous solvents. In this sample 76% of the organo-soluable ^{119}Sn consisted of unchanged fenbutatin-oxide and 21.3% of the degradation product SD 31723 [36].

The degradation of ^{119}Sn-labelled fenbutatin-oxide in soil was studied in a steam-sterilised and unsterilised sandy loam. Ten mg/kg ^{119}Sn-labelled fenbutatin-oxide was mixed into both soils which were stored in the same room at ambient temperature. Almost all the ^{119}Sn was organo-soluble. Fenbutatin-oxide decreased initially faster in sterile than in normal soil. However, the rate of decrease later became less in sterile than in live soils. It was suggested that steam sterilisation activates catalytically

active sites on the soil so that more molecules can be degraded initially. The subsequent decrease in rate as compared with live soils indicates that microbial degradation, which occurred in the latter, became the main factor in degradation. No accumulation of degradation products could be measured [37].

In another experiment in which 10 mg/kg ^{119}Sn-labelled fenbutatin-oxide was mixed into the same sandy loam, the residue degradation was studied under aerobic and anaerobic conditions. One set of soil samples was kept under aerobic conditions at ambient temperatures in a glasshouse; another set was kept under nitrogen after an initial 30 days aerobic period. The amount of solvent-extractable radioactivity, which was nearly all ^{119}Sn-fenbutatin-oxide, decreased slowly during the 180 days of the experiment. No significant differences could be found between the rates of degradation under aerobic and anaerobic conditions [38].

IMAZALIL

Imazalil is a systemic fungicide from the group of N-substituted imidazoles. Members of this chemical class affect the cellular permiability barrier of the yeasts. The fungi-toxic action of imazalil on Penicillium may also involve the inhibition of the cell membrane functions. Uptake and membrane effects noted in several studies suggest that imazalil inhibits ergosterol biopynthesis in fungal soils.

It is interesting to note how the use of nuclear techniques have contributed to the understanding of the mode of action, effect and fate of this fungicide which is used as a cereal seed treatment, as a foliar spray and as a post-harvest treatment of citrus, bananas and pome fruit.

Imazalil is rapidly absorped, distributed, metabolised and excreted by rats. Groups of rats were given a single 20 mg/kg oral dose of ^{3}H-labelled imazalil sulphate. Almost 90% of the administered radioactivity was excreted within 96 hours, with approximately equal quantities detected in the urine and faeces. Tissue residues ranged from 5.4 to 6.1% of the administered radioactivity 48 hours after dosing and from 1.8 to 3.5% 96 hours after dosing. The highest levels of radioactivity were found in the liver, lung and kidneys. Analysis of the urine from dosed animals indicated that 4% of the tritium was volatile by lyophilization. Thus, tritium exchange apparently occurred to a minor extent [39].

Studies involving a single 20 mg/kg oral dosing of rats with ^{3}H-labelled imazalil sulphate indicate that extensive metabolism of imazalil occurs in the rats. In one study [39] two major metabolites were identified in the urine. In this study 10% of the radioactivity in the urine and 3% of the radioactivity in the faeces was identified as unchanged in imazalil.

Barley seeds were treated with a dose of ^{3}H-imazalil corresponding to 10 g/100 kg seed. After germination on water agar for nine days, 42% of the radioactivity was found in the agar and 37% in the seed coats. Only 10% of the total radioactivity was present in the roots and 2% in the leaves of the seedlings [40].

Barley seeds treated with ^{3}H-imazalil at 10 g/100 kg were also sown in soil. Plants were harvested after one and three weeks. Soil and plant parts were analysed for radioactivity. Most of the radioactivity (76%) was present in the soil directly around the seed coats and 29% in the seed coats. After

three weeks the green parts of the plants contained only 6% of the radioactivity which had originally adhered to the seeds [40].

The metabolic fate of imazalil on banana plants was studied in a complex series of experiments in which small banana plants was sprayed with a solution of ^{3}H-imazalil sulphate labelled especially on the asymmetric carbon.

The leaves contained 95-100% of the total radioactivity recovered in the plants treated one to nine times. The radioactivity was practically all concentrated on the upper surface of the leaf tested with autoradiography. Transport of the radioactivity to the roots or the rhizome was minimal.

Analysis of various plant extracts on radio-HPLC revealed that the main part of the radio-activity originated from imazalil and its metabolite alpha-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol (R 14821). The results indicated that imazalil was degraded slowly as a function of time and that R 14821 was the main degradation product which was found in alkaline extracts. The remaining part of the radio-activity might be explained by the presence of a large number of minor metabolites [41].

Fourteen weeks after treatment of oranges with a solution containing 1000 mg/kg imazalil labelled with tritium at the 2-ethyl functional group, approximately 50% of the radioactivity remaining was found in the peel in an organo-soluable form: 30% was bound to the insoluable residue of the peel. In the flesh of the fruit 18% of the remaining radio-activity was present as the metabolite R 14821.

The polar, hydrophilic fraction containing nearly 50% of the radioactivity was present in the methanol extract of the peel but did not contain tritiated water or other volatile radio-activity. Since concentrations of radioactivity were identified before and after lyophilisation of this fraction, it seems that no label loss is to be expected during metabolism [42].

The distribution of imazalil in/on oranges was determined at intervals after treatment at 1000 mg/kg [43]. It was shown that the degradation of imazalil on oranges is slow; the half-life is expected to be 12 to 20 weeks; and only small amounts of the imazilil are present in the fruit flesh and part of this may result from contamination during the peeling.

Kraght [44] carried out a study under conditions closer to commercial practice when the distribution of radioactivity in the fruit and the presence of metabolites were studied during and after a storage period of 12 weeks. Most of the radioactivity (89%) remained as unchanged imazalil, in contrast to the previous studies where considerable degradation was reported. This was due to the extreme storage conditions used in the trials reported above where high temperatures and high light intensity prevailed. No tritium exchange was detected, thus confirming the suitability of the tritium labelling for the purpose of the study. Over 99% of the radioactivity in the ^{3}H-imazalil-treated oranges was solvent-extractable. After 12 weeks of storage approximately 10% of the original radioactivity appeared in the form of an unknown water-soluble compound [44].

The half-life of imazalil in a sandy loam or a clay loam was determined to be 4-5 months [45]. After a 36-weeks incubation period 6-7% of the initially applied imazalil was present as the metabolites R 14821. About 13% was found to consist of more polar compounds than imazalil and R 14821 but could not be identified.

LINDANE

It is interesting to note how isotope-tracers studies have helped to illucidate the understanding of the distribution, persistence and metabolism of lindane in plants, animals and processed foods. Saha [46] summarised the results of several biodegradation studies of ^{14}C-lindane in plants and animals. These results, together with those from previous studies, indicate a common biodegradation pattern for lindane in plants, insects and animals, as chlorobenzene and chlorophenolic metabolites are formed in all cases. Various isomers of di-, tri-, and tetrachlorobenzene and chlorophenols; pentachlorobenzene and pentachlorophenol have been found in plants, insects and animals. The gamma isomer of pentachlorocyclohexane has been found in plants and insects but not in mammals.

Studies on weathering, degradation, absorption, translocation and accumulation of ^{14}C-lindane in coffee plants have shown that, 10 days after topical application to the leaf surface, the insecticide can be shown to have been absorbed and translocated to different parts of the plant. It accumulates mainly in the roots and appears in other leaves [47]. In these experiments, when plants are grown in nutrient solution, release of radiocarbon through roots could be detected, indicating exchange of labelled material between plant and surrounding media. When the insecticide is applied to coffee plants through the roots immersed in nutrient solution containing ^{14}C-lindane, the labelled material is absorbed, and after 24 hours, radioactive material can be detected in young leaves of the upper parts of the plant. Loss of ^{14}C-lindane by volatolisation, evaporation and codistillation with water is apparently continuous and represents a significant portion of that applied.

It is not difficult to imagine how many problems would have to be overcome in order to carry out such studies without the aid of isotopes.

Most of us associated with the study, evaluation and regulation of residues in food have long held the desire to know what happens to these residues in processing and cooking.

Mirna et al. Report a study of the type which provides a clear and adequate picture of the influence of processing on the residue content of meat and meat products. Meat from rabbits, which had been fed with ^{14}C-lindane was processed to produce dried sausage and various meat products. It was found that micrococci and lactobacilli from a commercial starter culture metabolised the labelled pesticide to a considerable extent depending upon the temperature and time of incubation. In dried sausage the lindane content decreased in the course of 30 days by an average of about 25%. Curing caused a reduction of about 20% and hot-smoking a reduction of 12% in the lindane content of the meat products. Cooking (1.5 hours at 100°C) was the most effective treatment, more than 50% of the pesticide content being removed. In all of the meat products the residues consisted mainly of lindane together with small amounts of chlorinated benzenes and phenols [48].

OXAMYL

Oxamyl is a new formamidine type of insecticide with pronounced cholinesterase activity which appears to have wide application as a contact insecticide with moderate residual activity against a wide spectrum of insect pests. It has systemic action in many plants and, when applied to the soil, functions as a contact type broad-spectrum nematocide and, bysystemic action, as a miticide/insecticide.

It is interesting to note the extent to which nuclear techniques have featured in the research and development work which led to the clearance and registration of this insecticide in recent years.

Among the studies that were available two years ago was a most extensive study of the metabolism of oxamyl and selected metabolites in the rat [49] and an in vitro study of rat liver microsomal metabolism of oxamyl and selected metabolites [50].

The study of metabolism of oxamyl in plants [51] involved a comprehensive examination of the fate of oxamyl in tobacco, alfalfa, peanuts, potatoes, oranges and tomatoes [51]. These involve not only the identification of the metabolites but also their distribution throughout the plant, plant parts and various components of the fruit. Treatment involves both foliar and soil application and steps were taken to observe the effect of time of application, number of applications and rate of application on the nature and level of the residues.

A ruminant metabolism study involving two lactating goats maintained on diets containing ^{14}C-oxamyl for 10 and 20 days provided information on the degradation, elimination and accumulation in numerous organs, milk, blood and tissues [52]. This was followed by a more recent study in which ^{14}C-labelled oxamyl and selected metabolites were incubated, in vitro, in rumen fluid of a Holstein cow [53].

A livestock-feeding study in which radiolabelled oxamyl was fed to dairy cows in their rations for 30 days [54] clearly showed that no residues were detectable in any samples of milk or milk fractions, liver, kidney, lean muscle or subcutaneous fat at any feeding level. These same samples were later analysed for major metabolites which were also found to be absent [55].

In a poultry-feeding study, adult laying hens were fed diets containing varying amounts of radiolabelled oxamyl for a four-week period [56]. Samples of eggs and tissues were collected and analysed for oxamyl and major metabolites. Residues of oxamyl and major metabolites were found to be at levels below the limit of determination in eggs, liver, muscle, fat and skin.

Numerous studies have been conducted on oxamyl soil metabolism, decomposition, dissipation, absorption, mobility and the effect of water on dissipation and decomposition. In one compilation of studies the decomposition of oxamyl in soil and water was investigated with various soils under aerobic and anaerobic conditions and in distilled water or river water at various levels of pH, with artificial UV light or sunlight, and in the dark. Also included were experiments on soil leaching and mobility in various soils [57].

In a crop rotation study, cabbage, red beets and sorghem seeds were grown in the green house in soils treated 30 and 120 days earlier with ^{14}C-oxamyl [58]. These studies showed that mature crops from soils treated 30 days prior to planting contained low levels of residues but those planted in soils treated with oxamyl 120 days earlier contained no measurable residues of oxamyl or main metabolites.

The availability of these studies did not obviate the need for extensive residues studies but they have certainly provided valuable guidance and a great deal of reassurance about the validity and significance of the residues studies.

PERMETHRIN

The discovery and development of a whole range of synthetic insecticides based on the molecular configuration and mode of action of natural pyrethrum insecticides has stimulated a great deal of scientific research and industrial activity particularly surrounding those pyrethroid compounds which are relatively resistent to photodegradation.

I have chosen permethrin from among the several pyrethroid insecticdes which have recently been evaluated and registered for widespread use but I might equally well have chosen one of four others. There were no less than 47 seperate studies involving isotope-labelled permethrin examined in 1979 and 1980 by the Joint FAO/WHO Meeting of Experts on Pesticide Residues. Many of these were most extensive. I have every reason to believe that considerably more would have been published or available at that time and in the intervening period more have appeared.

A selection of these include a study of the metabolism of trans-permethrin and cis-permethrin and a number of other pyrethroids by microsomal enzynes [59]; a study of metabolism of permethrin in bean plants [60]; a study of permethrin degradation on cotton [61]; and a report on the degradation of trans- and cis-permethrin in cotton and bean plants [62]. There were also studies of the metabolism of permethrin in rats [63] and in rats, cows, bean and cotton plants [64].

Goats which serve as a useful model in animal metabolism and excretion studies featured in two; permethrin metabolism and residues in goats [65]; and distribution and excretion rates of ^{14}C-labelled permethrin isomers administered orally to four lactating goats for 10 days [66].

The fund of information obtained by the aid of isotopic studies included the absorption of permethrin in cows [67]; the distribution and metabolism of trans- and cis-permethrin in lactating Guernsey cows [68] and permethrin residues in cows after dermal application [69]. A similar study on the absorption of permethrin in pigs following dermal treatment was also carried out [70]. The fate in poultry was defined by studies into the absorption of permethrin in chickens after dermal and oral treatments [71], the metabolism of permethrin in hens [72], and the distribution and metabolic fate of trans- and cis- permethrin in laying hens [73].

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Invited Paper

RATIONALE IN THE DESIGN OF PESTICIDE METABOLISM STUDIES USING RADIOISOTOPES

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Abstract

RATIONALE IN THE DESIGN OF PESTICIDE METABOLISM STUDIES USING RADIOISOTOPES.

Numerous physical, chemical and biochemical forces act on pesticides in the environment to regulate their persistence and, to a large extent, determine their environmental and toxicological significance. Radioisotope-aided metabolism studies with pesticides in both target and non-target organisms are important to define the qualitative and quantitative aspects of pesticide degradation involved. Such studies provide valuable information that can be used in a number of ways, including prediction of the nature of potential pesticide interactions with man, estimation of potential residue occurrence in human foods, determination of pesticidal mode of action, and evaluation of potential toxicological effects on non-target species. In designing pesticide metabolism studies that utilize radioisotopes as tracers, the metabolism scientist must exercise careful judgement to ensure that his studies generate data that are scientifically sound and relevant to environmental and human health concerns. Such factors as isotope selection, label position, specific activity, treatment routes, dosage levels, replication, and the utilization of appropriate radiometric and analytical techniques are important considerations to assure the generation of useful data. Because of the very large number of potential pesticide/organism interactions that may be of interest to the metabolism scientist, specific research approaches vary greatly depending on the nature of the study organism and the specific pesticide involved. The rationale often used by metabolism studies in laboratory animals, livestock and poultry, plants, soil and aquatic organisms, arthropods and other pest species, non-target organisms, and for in vitro studies, is considered.

1. INTRODUCTION

The judicious use of pesticidal chemicals contributes in a very positive way to many aspects of human welfare. During the past few decades, pesticides have no doubt spared millions of human lives through their use to control vectors of often fatal

human diseases. Pesticides are also indispensable in the management of a seemingly endless variety of pest organisms, including insects, fungi, bacteria, weeds, mammals, birds, and others that attack or compete with our food and fiber crops, livestock and poultry.

Because of their purpose, pesticides are intentionally toxic. Ideally, these chemicals should be poisonous only to targeted pest organisms; however, such an ideal is almost never fully attained due to the simple fact that, biochemically, all living things have much in common. Thus, we must accept and deal with the unavoidable circumstance that the introduction of pesticides into the environment almost always carries with it some risk of unforeseen toxic consequences to non-target species, including man.

There are numerous physical, chemical and biochemical forces that can act on and chemically transform pesticide residues in the environment. When one considers the multiplicity and complexity of these interactions, it is easily seen that the environmental fate of pesticides must be critically evaluated to provide an adequate basis for estimation of the toxicological significance of these chemicals to man and other organisms. In obtaining such data, the application of radioisotope techniques has, in my opinion, contributed more than any other single factor to the ability of the pesticide scientist to accurately assess the metabolic and environmental behavior of pesticides. By using radioisotope-labeled pesticidal chemicals as tracers, we are in an excellent position to conduct truly definitive fate studies in which, through the application of modern chromatographic, analytical and spectrometric techniques, we can solidly characterize most, if not all, of the metabolites generated in a given system. At the other extreme, radioisotope studies can provide valuable data in research applications where there is essentially no instrumentation backup, either chromatographic or spectrometric. Very useful data can be obtained from isotope studies in which the only instrumentation available to the researcher is that for detection and quantitation of radioactivity. Such applications might include distribution and residue studies in animals or plants where, with straightforward sample preparation and analysis procedures, highly relevant data with respect to pesticide safety can be obtained. Such studies can, for example, reveal the tendency of a pesticide in a given system to yield residues in meat, milk, or eggs of food animals, or in the edible portions of human food plants.

At a somewhat more sophisticated level, low-cost chromatographic techniques, particularly thin-layer chromatography (TLC), can be coupled to isotope studies to provide definitive data on metabolic pathways, or at least on the extent of metabolism of the parent pesticide. Extraction of tissues, excreta, etc. with appropriate solvents and subsequent resolution of radioactive

components by TLC can usually give excellent resolution of the parent compound from any metabolites generated. The metabolites themselves can often be characterized by co-chromatographic studies with analogs of known structure. Metabolite characterizations based on chromatographic techniques certainly must be considered less definitive than those obtained through spectral studies, but such a limitation should in no way discourage the efforts of researchers who do not have access to such costly techniques as nuclear magnetic resonance or mass spectroscopy. Chromatographic-based metabolism studies, if conducted well, generate usually reliable data that are accepted by the scientific community, as evidenced by the fact that most such studies are fully appropriate for publication in major scientific journals.

From the above considerations, it is apparent that radioisotopes can, and often do, represent irreplaceable tools for the researcher whose goal is to define the environmental behavior and fate of pesticides and other agrochemicals. Importantly, it is not necessary for the researcher to have a fully equipped analytical, chemical, or spectroscopic laboratory in order to effectively utilize radioisotope techniques in pesticide metabolism studies. Thus, researchers from a number of scientific backgrounds and laboratory situations are fully capable of conducting such studies to generate toxicologically relevant data on pesticides that have either broad scientific interest and impact or that relate to specific local or regional needs.

In this discussion, my purpose is to convey some thoughts with respect to the rationale that might be appropriate as one considers the design and execution of radioisotope-aided pesticide metabolism studies. This will not be a review of literature on the topic--one would in fact be hard pressed to successfully accomplish a conventional review of such an elusive concept as scientific "rationale", for the literature documents the end product of the process rather than the process itself. It similarly will not be "technique" oriented, for one can through the literature readily gain access to specific and detailed research techniques. Rather, my approach will be more philosophical in nature and is based primarily on my own personal research experience and perspectives developed during the past 16 years as first a graduate student, then a bench scientist, and subsequently as a leader of a small research team. The value of my comments will no doubt be limited by my own bias and shortcomings, particularly in the capacity to view the rather complex discipline of pesticide metabolism chemistry from a very broad perspective.

2. DEFINITION

The word metabolism, from the Greek word "metabole," which means "change", has a rather limited connotation. A pesticide metabolism study is, however, usually considered in a much broader sense to encompass not only the metabolic alterations of the pesticidal chemical in question but also the absorption, transport, storage and elimination of the parent pesticide and its metabolites by the exposed organism. For my purposes, radioisotope-aided "metabolism" studies will be considered to include approaches that, at one extreme, are aimed simply at tracing the labeled chemical in question through an organism or, at the other extreme, are targeted for complete and definitive characterization of all transformation products generated.

3. PURPOSES OF PESTICIDE METABOLISM STUDIES

As with all scientific endeavors, there should be logical reasoning behind the conduct of any pesticide metabolism study. One can quickly think of a number of specific purposes for which metabolism studies might be designed and conducted, and some of these are discussed below.

Studies in mammals as metabolic predictors for man. A primary purpose for evaluating the metabolic behavior of pesticides is to facilitate ultimate assessment of toxicological risk to man that may result from pesticide use. Because ethical and other considerations prevent direct studies of pesticide metabolism in man except in most unusual circumstances, extrapolations to man are usually made on the basis of metabolic data obtained with monogastric laboratory mammals.

Studies in animals and plants to evaluate the potential for residue occurrence in human foods. Dependent upon the proposed use patterns for specific pesticides, it may be crucial to determine the metabolic behavior of these chemicals in animals and plants used as human foods so that predictions can be made relative to the chemical nature and quantity of residues that may enter the human food chain.

Studies to elucidate activation and detoxication phenomena, and mode of action. The metabolic transformation of pesticidal chemicals may result in metabolites of either reduced or enhanced toxicological significance. In either case, it is important to define both the qualitative and quantitative aspects of metabolism that are involved. In addition, metabolic considerations may lead to a more thorough understanding of the mechanisms of pesticidal or toxicological action, be such actions the result of acute or chronic exposures.

Studies to evaluate effects on non-target organisms. In this age of greatly enhanced concern over the environmental impact of pesticides and other man-made chemicals, metabolism studies in non-target organisms provide valuable insight regarding the potential toxicological significance of pesticides to these species.

Studies to define metabolic bases for pesticide selectivity. The development of pesticides that are toxic to target species but have little or no adverse effects on other life forms is a major goal in pesticide development. Selective toxicity can often be attributed primarily, if not totally, to metabolic differences between species, either in the rate of metabolism or the nature of products formed. Thus, metabolism studies in appropriate organisms can be of utmost importance in explaining and, in many cases, predicting selective toxicity. Two well-known examples of pesticidal selectivity attributed to metabolic differences are the highly selective insecticide malathion, which is rapidly detoxified by ester hydrolysis in mammals but not in susceptible insects, and the herbicide linuron, which is detoxified through N-demethylation and N-demethoxylation by tolerant crops but not by certain weeds.

Studies to satisfy regulatory requirements. Many metabolism and other toxicology studies with pesticides are conducted primarily, if not solely, to satisfy regulatory requirements for pesticide registration and use. Although such mandated studies are generally based on solid scientific logic and are justifiable under one or more of the considerations discussed above, it is a simple fact that many such studies would not be conducted in the absence of regulatory needs. To the individual pesticide metabolism scientist, regulatory considerations can be, and often are, major factors to be considered in the design of his studies.

Studies to generate basic research data. This final category is included to cover those studies for which it might be difficult to ascribe a specific need or purpose. However, it can be argued that because of the potential environmental interactions and impact associated with essentially all pesticides, any metabolism study is potentially useful in environmental impact and/or toxicological significance evaluations, irrespective of the pesticidal chemical or the test organism involved.

Considering the seven categories just discussed, and more could probably be added, it is clear that there are a tremendous number of potential pesticide/organism interactions that may be of human health, animal health, or environmental significance. The pesticide metabolism scientist thus finds himself playing a crucial role in the evaluation of the overall impact of pesticides on man and the environment.

Comments will now be directed toward factors that relate to the effective use of radioisotopes to generate pesticide metabolism data that are both scientifically sound and toxicologically relevant.

4. ISOTOPE SELECTION

A number of factors may influence the researcher's selection of a specific radioisotope for use as a tracer in metabolism studies with a given pesticidal chemical. Perhaps the most obvious is the structure of the pesticide itself and the fact that elemental composition naturally limits what isotopes may or may not be considered. Synthesis considerations--the ability to successfully and at acceptable cost incorporate the desired radiotracer into the pesticide molecule--are certainly significant and sometimes limiting considerations. Safety, facility, and instrumentation limitations, and personal preferences are also major factors in isotope selection.

A cursory examination of the literature shows clearly that the vast majority of pesticide metabolism studies conducted during recent years have utilized chemicals tagged either with carbon-14 or with tritium. Radiocarbon has been the isotope of choice for most studies, and the reasons are quite obvious: 1) a variety of ^{14}C precursors are available that can be incorporated into appropriate synthetic schemes for most organic pesticides; 2) radiocarbon is a soft beta emitter that poses no major health, contamination, or disposal problems; 3) this isotope is readily detected and quantitated by standard radiometric techniques; and 4) radiocarbon is a long-lived isotope and thus there are no radioactive decay factors to consider. Tritium is a very acceptable radioisotope for use in metabolism studies for many of the same reasons mentioned above for carbon-14, although the extremely low beta energy of tritium may require somewhat more sophisticated detection and quantitation procedures. Other beta-emitting radioisotopes, particularly sulfur-35 and phosphorus-32, are appropriate for certain pesticidal chemicals but the frequency of their application to pesticide metabolism studies has certainly declined over the years. This is due perhaps to the relatively short half-lives of these isotopes, but probably more than anything else is due to the widespread availability and acceptability of radiocarbon and tritium as alternative isotopes that allow much more flexibility in labeling with respect to label positions. Gamma-emitting isotopes are seldom used in pesticide metabolism studies for a number of reasons, including safety, facility, and instrumentation considerations. Gamma- emitting elements are appropriate for a few inorganic and very few organic pesticides, but the availability of radiocarbon and tritium as more appropriate isotopes usually precludes the use of gamma emitters.

5. LABEL POSITION

The position of incorporation of the radiolabel into the pesticide molecule is of utmost importance to assure the validity of the metabolism data subsequently obtained. In his efforts, the metabolism scientist is seeking to accurately trace the pesticide and its metabolites in the test system, and to define the chemical changes that are inflicted upon the pesticide molecule by the enzymatic systems involved. Although metabolic changes can be grouped into four basic types (oxidation, reduction, hydrolysis, and conjugation), there are many specific metabolic alterations that can occur. Such alterations are dependent upon the chemical nature of individual pesticidal chemicals and upon the enzymatic reactions at work. The importance of label position becomes apparent when one considers that metabolic transformations quite often result in cleavage of chemical bonds; thus, such reactions may split off portions of the tagged chemicals. While the radiolabel is not literally "lost" as a result of such transformations it can, dependent upon its position within the molecule, be separated from the major portion of the pesticide, with the resultant effect that the radiolabel no longer serves its tracer function. In tritium labeled compounds, an additional consideration must be the potential lability or exchangeability of the hydrogen that is under consideration as a label site. Tritium exchange under normal physiological conditions or during metabolic reactions is a relatively common problem that can lead to considerable uncertainties in data interpretation.

Radiotracers incorporated into metabolically labile positions of pesticides can, as a result of metabolic conversions to rather simple labeled derivatives, be assimilated into natural constituents of the test organism. Obviously, such an occurrence can have serious implications with respect to the predictive value of the data obtained. A personal experience illustrates this problem. Early in my career, I was involved in a study in which a lactating cow was fed the carbamate insecticide, carbofuran, labeled with radiocarbon at the carbonyl position of the carbamic acid moiety. Soon after dosing we observed a rather substantial amount of radioactivity in milk, which was unexpected, because residues and metabolites of most carbamate insecticides show little tendency toward secretion into milk. We ultimately showed that the radiocarbon residues in the milk were not associated with carbofuran at all; rather, they arose as a result of ester hydrolysis of the ^{14}C-carbofuran and subsequent degradation of N-methyl carbamic acid-^{14}C to $^{14}CO_2$. The radiolabeled carbon dioxide, which was generated in considerable quantities, had been incorporated by normal biosynthetic pathways into natural milk constituents.

How does one rationally decide on an appropriate label position, particularly when dealing with a specific pesticide or pesticide type for which there are little or no background metabolism data available? The process is usually not that difficult, if the researcher has some understanding of the nature of metabolic reactions and is reasonably familiar with the metabolism literature. Many, if not most, organic pesticides contain aromatic moieties, and because of the relatively high degree of stability of aromatic systems, aromatic rings are generally good label sites for radiocarbon, although less so for tritium because of potential substitution reactions (particularly hydroxylations) that may occur during metabolism. Certain label sites are obviously less than desirable for carbon-14 or tritium labeling; O- and N- substituted moieties are examples of usually poor label positions. Many pesticides are esters (e.g. organic phosphate and carbamate insecticides) that tend to be readily hydrolyzed in living systems. With such chemicals, it is preferable to label that portion of the molecule that is likely to be of the greatest toxicological significance. In the case of the OP and carbamate compounds, the best label position would usually not be the phosphoric acid or carbamic acid moieties.

Some pesticides are of such chemical complexity that a single position of labeling is simply not sufficient to permit accurate tracing of potential metabolic products. Synthetic pyrethroid insecticides, which are esters in which both the acid and alcohol moieties are usually rather complex, are a good example. With the pyrethroids, labeling on both the acid and alcohol moieties is required to permit conduct of definitive fate studies. Most metabolism scientists agree that with chemicals such as the synthetic pyrethroids, more useful and definitive data can be obtained from comparative studies with various preparations labeled at different sites rather than from studies with a single preparation labeled at two or more sites.

Additional factors that must be considered with respect to the position of label incorporation (as well as to the specific isotope to be used) are those of synthetic feasibility and cost. It may be that the most appropriate label position is one that is very difficult or impossible to achieve synthetically or that is attainable only at high cost. In such circumstances, the researcher must consider the obstacles present and the alternatives available, then use his own judgment as to the best approach.

6. RADIOSYNTHESIS AND PURITY CONSIDERATIONS

The pesticide metabolism scientist can obtain radiolabeled pesticides by purchase from radiochemical supply firms, from appropriate pesticide development firms, or by radiosynthesis in

his own or a colleague's laboratory. It is my opinion that in most circumstances, radiosyntheses are not appropriate for individual researchers--such procedures are best left to professionals who are specifically equipped for and trained in radiosynthesis techniques. If the pesticide to be studied is under active development or is one that is in current use, contact with the appropriate pesticide development firm may result in a radiolabeled preparation being provided to the researcher, often at no cost. If a custom synthesis by a commercial firm is required, one can usually anticipate considerable expense. However, putting the cost of the radiochemical in perspective to that of the entire study will more than likely show that the expense of a custom radiosynthesis is not excessive.

Once the radiochemical is in the researcher's hands, one criterion is of utmost importance--the radiochemical purity of the sample. It is quite obvious that the presence of an appreciable percentage of labeled impurities, which may be totally unrelated chemically to the labeled pesticide, could seriously compromise the validity of any studies subsequently conducted with that preparation. On the basis of my own experience with radiochemical supply firms, I do not hesitate to say that one cannot assume adequate radiochemical purity, even if a high percentage of purity is claimed by the manufacturer. Appropriate techniques, usually chromatography, should be used to verify the radiochemical purity of any labeled pesticide, and it is probably safe to say that a radiochemical purity of 98% or greater is acceptable for most studies. Unacceptable preparations should either be returned to the manufacturer or purified by the researcher using appropriate techniques.

The chemical purity of a labeled sample is not as crucial as its radiochemical purity, simply because any non-labeled contaminants present are unlikely to interfere in any appreciable way in most studies. If spectrometric methods of structure confirmation are available (mass spectroscopy, NMR, etc.) one or more of these techniques should be used to verify the chemical identity of the sample. As a minimum, co-chromatographic studies of the radiochemical with a known unlabeled sample of the pesticide should be done to avoid the highly unlikely but always possible tragedy of conducting a study with the wrong compound.

7. SPECIFIC ACTIVITY AND DOSAGE CONSIDERATIONS

The specific activity of a radiolabeled pesticide is an important consideration in many studies, particularly when the researcher wants to quantitate residues at low levels. The use of labeled preparations of very low specific activity can carry with it the inability to detect and quantitate trace residues.

However, labeled preparations having very high specific activity are often similarly inappropriate. The use of high specific activity samples can be wasteful of radiochemical and can result in a level of sensitivity far above that which is reasonably required. The researcher should carefully consider the objectives of the planned study, being fully aware of levels of sensitivity that are required or are appropriate, and not be hesitant to lower the specific activity of his preparation by addition of unlabeled pesticide if this is deemed appropriate.

One must consider dosage levels from two standpoints: 1) the total amount of radioactivity, usually expressed in μCi or mCi, that is applied to the living system; and 2) the total amount of pesticidal chemical itself applied, which may be expressed in a number of ways dependent upon the system under study (e.g. mg/kg for animal dosing studies, μg/cm^2 for plant surface applications, parts per million for aquatic or soil studies, etc.).[1] The total isotope/chemical relationship obviously determines specific activity; thus, in dosage considerations, the researcher cannot consider either independent of the other. The determination of appropriate isotope and chemical dosage levels for a particular study can be a complex and sometimes frustrating exercise. Often, there are constraints on the total amount of radioisotope available to the researcher. Certain studies may require low total dosage and relatively high specific activity preparations to approximate "real-world" exposure conditions and allow detection and quantitation of trace residues. In other studies, the researcher may choose a relatively high chemical dose of low specific activity if his primary goal is to conduct a study in which metabolites are generated in sufficient quantity to permit definitive spectral analysis and structure elucidation. In determining appropriate dosage levels, the researcher should look first at the most critical data needs or the major study objectives, realizing that in a single study it is seldom possible to obtain all the data one might desire. He can then develop specific activity and dosage parameters that are most appropriate for his study, given the limitations and constraints present.

8. RADIOISOTOPE, ANALYTICAL AND SPECTROMETRIC TECHNIQUES

While it is outside the scope of this discussion to deal with detection and analytical techniques in any detail, these will be touched on briefly here, primarily to emphasize the broad range of techniques that are available to the metabolism scientist who uses radioisotopes in his studies.

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[1] 1 Ci = 3.70×10^{10} Bq.

Although ionization detectors are available, liquid scintillation counting is without question the technique of choice for detection and quantitation in most radioisotope applications, particularly those utilizing soft beta emitters such as carbon-14 and tritium. Liquid scintillation counters are not overly expensive, they are highly reliable, and with appropriate sample preparation procedures can be utilized to quantitate radioactivity at high sensitivity in essentially all biological fluids and tissues. In recent years, radioisotope detectors for use with various chromatographic techniques (e.g. TLC plate scanners, HPLC detectors) have become widely available and are finding appropriate applications in a number of research laboratories. However, the simple autoradiographic technique of using X-ray film over TLC plates to detect resolved metabolites that are subsequently quantitated by liquid scintillation counting remains a widely practised, fully acceptable and, in many cases, the preferred technique.

Two chromatographic techniques, TLC and high performance liquid chromatography (HPLC), are widely used by pesticide metabolism scientists to resolve radiolabeled metabolites generated by living systems. TLC offers the advantages of low cost and applicability to a wide range of chemical structures that are likely to be encountered by the metabolism scientist, and TLC is readily adaptable to standard radioisotope detection and quantitation techniques (i.e. autoradiography and liquid scintillation counting). HPLC requires more expensive instrumentation, but can potentially offer much higher resolution power over a complete range of chemical structures and polarity. This is particularly true with more polar pesticide metabolites for which most TLC methods may be only marginally applicable. Gas-liquid chromatography (GLC) is generally not an effective primary chromatographic technique in pesticide metabolism studies because metabolites tend to be rather polar and are often not compatible with the temperature and volatility constraints presented by GLC. However, as a secondary technique or for studies of derivatized metabolites, GLC can be a very powerful tool, particularly when coupled with mass spectrometry for structure elucidation studies. Other chromatographic techniques, such as paper chromatography and conventional column chromatography, have been important in earlier years to the pesticide metabolism scientist, but their usefulness has dramatically diminished as more powerful techniques have become available.

As discussed earlier, the pesticide metabolism scientist need not have access to sophisticated spectroscopic instrumentation in order to conduct toxicologically relevant studies. Various chromatographic techniques, often coupled with relatively simple chemical derivatization or degradation procedures, can result in metabolite characterizations with a

fully acceptable level of confidence. However, the researcher who is fortunate enough to have access to spectral techniques such as mass spectrometry and nuclear magnetic resonance spectrometry can undertake highly definitive pesticide metabolism studies. It is not uncommon today to see reports in the literature in which most, if not all, of the detected metabolites of a pesticide in a given system are fully and unequivocally characterized by spectral means.

One of the major advantages of radioisotope use in metabolism studies is that isotopes provide for a potentially total accountability of dose, irrespective of the metabolic transformations that the parent pesticide may be subjected to. However, the researcher quite often will not routinely design his studies to ensure total accountability. Such an approach would require collection of respired gases from animals and plants, leachate from soils, etc. for isotope determinations. Procedures are of course available for such analyses, but they are often cumbersome and may place an inordinate stress on the study organism. The usual approach, which involves thorough extraction and analysis of excreta, tissues, or other matrices will in many cases be adequate to fully define the pesticide/organism interactions that occur. The extent of volatility losses, metabolism to $^{14}CO_2$, etc. are quite often inferred on the basis of data obtained from the analysis of excreta and tissue samples.

9. SPECIFIC APPLICATIONS

A pesticide metabolism study usually involves a specific radiolabeled pesticide and a single species to be studied. In environments such as soil or water there is of course usually a rather large potential number of species (particularly microorganisms) that may interact with the compound under study. Irrespective of the type of study under consideration, adequate replication in metabolism studies is important where quantitative data are required, but it is probably safe to say that replication is less crucial where one's emphasis is placed more on qualitative aspects of metabolism, i.e. defining the nature of the metabolites generated by a specific organism or system. There are many examples in the literature, particularly with large animals such as cattle, where pesticide metabolism data are reported from a single animal.

Laboratory animals. Metabolism studies with radiolabeled pesticides in laboratory animals, usually rodents, are often done to gather data for extrapolation to man. In most cases, such studies involve oral administration of the radiochemical, which is representative of the major route of human exposure to most

pesticides and which subjects the chemical to potential metabolic and absorption phenomena that may be specific to the gastrointestinal tract. Inhalation and dermal studies with radiolabeled pesticides in laboratory animals are much less common, but can provide very useful absorption data that may have toxicological relevance for humans. Intraperitoneal administration of radiolabeled pesticides, although having no direct environmental relevance, can be useful in comparative studies because it bypasses to a large extent the potential interactions with microflora of the upper gastrointestinal tract. In addition, IP administration can be used to facilitate the generation of rather large amounts of metabolites for isolation and characterization studies.

Livestock and poultry. Metabolism studies in livestock and poultry (cattle, sheep, chickens, etc.) are usually done to evaluate the potential for residue transfer into human foods. Lactating or egg-producing animals are often used in such studies to allow collection of data related to the significance of potential milk or egg residues. The radiolabeled pesticides are usually administered orally, although a few dermal studies in livestock have been reported for pesticides whose use patterns involve direct dermal application to animals for ectoparasite control. In studies designed primarily to elucidate metabolic pathways and gain preliminary insights into patterns of residue retention and elimination, the radiochemical may be administered as a rather large single oral dose, often in the range of 5-100 mg/kg. Where data predictive of environmentally relevant exposures are needed, oral dosages are often multiple (1-2 dosages per day for several days) and are of relatively high specific activity and low total chemical dosage (<1 mg/kg/day). Such studies can provide data that are reasonably predictive of environmental exposures to low levels of a pesticide, particularly with respect to "steady-state" or "plateau" levels of residues that may be expected in tissues, milk, eggs, etc. as a result of continuous environmental exposure to a specific pesticide.

Plants. Metabolism studies in plants may be conducted for a number of reasons, dependent upon the type of pesticide and use pattern involved. As discussed earlier, metabolism studies with herbicidal chemicals in plants may provide valuable insight into selective toxicity phenomena. Studies of pesticide fate in plants can be very important in evaluating the potential for residues appearing in plant products subsequently used either as human foods or as livestock feeds or forage. For obvious reasons, plant metabolism studies generally utilize routes of administration that are consistent with the actual or proposed use patterns of the specific pesticide. Thus, surface application of a radiolabeled pesticide to foliage is usually preferred when studying a

pesticide that is sprayed or dusted on plant foliage, and application of the radiochemical to soil for subsequent uptake by growing plants is preferred for those chemicals normally applied to soils. Quite often, radiolabeled pesticides are administered to plants through stem or petiole injection, or through root uptake from water, in studies designed to evaluate translocation phenomena. In plant metabolism studies, particularly involving foliage application of the radiochemical, it is often difficult if not impossible to distinguish between metabolic reactions that occur in the plant and photochemical or chemical reactions that occur on or within the plant tissues. From a toxicological standpoint, however, the inability to make such distinctions is usually not critical, if the studies are designed to be representative of normal environmental conditions.

Soil and aquatic studies. As with plant metabolism studies, pesticide degradation in soils and water may occur through a number of mechanisms, including metabolism by living organisms, chemical and photochemical reactions. Irrespective of the mechanisms involved, however, it is no doubt true that the environmental persistence of pesticides is governed primarily by their interactions with the soil and water environments. Metabolic studies in soils may be done by surface application or by thorough mixing of the radiochemical with the soil itself. Comparative studies with sterilized versus non-sterilized soils are often done to evaluate more precisely the role of soil flora and fauna in pesticide degradation. Studies utilizing sterilized soils that are subsequently inoculated with specific organisms (usually bacteria or fungi) can provide well-defined data relating to the role of individual organisms in pesticide/soil interactions.

Studies of pesticide metabolism by microorganisms that occur in natural waters are conducted quite often. To obtain environmentally relevant data, care must usually be taken in water studies not to exceed the solubility limits of the pesticide in question. The inclusion of appropriate controls may be needed to properly define the role of non-metabolic conversions that may be related to such factors as pH, photochemical reactions, etc.

Metabolism by pest species. Metabolism studies with pesticides in target pest organisms can be valuable for a number of reasons. Often, a clear understanding of the rate of metabolism or of the nature of metabolites generated will explain why a pesticide is or is not toxic to a particular pest species. Metabolic considerations quite often form the basis for explaining the development of resistance in arthropods and other pest organisms. Certainly, a thorough knowledge of the potential of pest organisms to metabolize specific pesticides or pesticide types is an important consideration in the development of

chemicals that are both efficacious and selective in toxicity toward the target species.

Because of the wide variety of pest species with which it may be appropriate to conduct pesticide metabolism studies, specific research approaches may vary considerably. With insects and other arthropods, exposure to the radiochemical may be topical or oral; studies with aquatic organisms (e.g. mosquito larvae) are usually done by adding the radiochemical to water; soil pests (e.g. nematodes, fungi) may be treated in culture, etc. Where possible, it is certainly preferable to use dosages and exposure routes that most closely approximate those likely to occur under anticipated environmental conditions.

Non-target species. All of the world's living organisms may be potentially exposed to pesticides in the environment, but it is neither possible nor appropriate to attempt to define the nature of these interactions in most instances. However, in circumstances where pesticide/organism interactions with significant toxic consequences have occurred or appear likely to occur, it may be appropriate to conduct both conventional toxicological and metabolic studies. Certainly, the potential of a given pesticide to reduce or adversely affect populations of non-target mammals, birds, fish and many other organisms is of sufficient seriousness to merit rather detailed analysis of the interactions that may occur. Appropriately designed metabolism studies in such organisms can be quite valuable in arriving at a determination of overall risk.

In vitro studies. Although I have emphasized the applicability of radioisotope techniques in evaluating the metabolic and residual behavior of pesticides in intact living organisms, radioisotopes are widely used and can be crucial to the success of in vitro studies as well. There is considerable basis for the belief that the toxic nature of pesticides and other chemicals is quite often attributable to highly reactive metabolites that are generated in trace quantities and that may be short lived. Appropriate in vitro studies with enzyme preparations and radiolabeled chemicals may demonstrate the occurrence of such reactive metabolic intermediates that cannot be detected by conventional in vivo studies. In vitro studies with radioisotopes that utilize subcellular, cellular, tissue, or organ preparations can provide invaluable insight into the metabolic mechanisms involved in pesticide metabolism, and such studies are uniquely suited to identify sites within the organism where major metabolic reactions occur.

10. CONCLUSIONS

In this discussion, I have attempted in a very general way to discuss the rationale that many of us as pesticide metabolism scientists use in the conception, design and execution of our radioisotope-aided metabolism studies. Our discipline is one that is both very broad and quite complex. The data we generate are of far more than academic interest. Our data often have great toxicological significance and direct implications with respect to the impact of pesticides on the environment or on human health and safety.

The nature and number of potential interactions that pesticides may have with living organisms is uncountable! We as individual researchers must therefore carefully apply our good judgment and scientific expertise to ensure that the limited resources available to us are focused in the most appropriate areas. We must carefully establish our research priorities, and design and conduct our studies such that the data we generate are scientifically sound and highly relevant to environmental and human health concerns. Radioisotopes as tracers for our studies have been in the past, and will no doubt continue to be in the future, one of the fundamental tools in our sometimes frustrating but always challenging endeavors.

Invited Paper

FATE OF HERBICIDE CHEMICALS IN THE AGRICULTURAL ENVIRONMENT WITH PARTICULAR EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES

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Abstract

FATE OF HERBICIDE CHEMICALS IN THE AGRICULTURAL ENVIRONMENT WITH PARTICULAR EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES.

The radioisotope tracing technique has been a useful tool in obtaining extensive information on the fate of herbicides in the soil-plant system, including their uptake, transport and metabolism by plants, their photochemical, chemical and microbial degradation, their adsorption-desorption and translocation in soil, and their bioavailability to untreated crops. A balance study under practical field conditions using radiolabelling can examine a number of factors affecting the fate of a compound at the same time and assess the magnitude of the major processes involved. On the basis of these results, more detailed studies are then formulated to be conducted under the precisely defined environment of a growth chamber or a laboratory. Use of tracer techniques in such studies is demonstrated, with results from experiments with different ^{14}C-labelled herbicides.

1. INTRODUCTION

According to data from FAO the annual losses in agriculture due to plant diseases, insect damage and weed competition are estimated to represent a market value of approximately US-$ 130 billion[1)], which is equivalent to the annual crop value of wheat, barley, rye, oat and potatoes[1]. In 1974 alone farmers around the world spent over 5 billion dollars on pesticides to control these losses and the predicted yearly increase of pesticide use is about 10 to 15%. However, even though the highest losses are observed in Asia,

1) 1 US billion = a thousand millions.

Africa and South America, 45% of the produced pesticides are applied in the United States of America, 25% in Western Europe, 8% in Japan and only about 20% in those areas where the use is most needed to produce sufficient food from the limited agricultural lands[2]. Therefore it is expected that especially application of herbicides will be increased to make better use of the area already in agricultural production.

Application of pesticides in agricultural practice must adhere to specific regulations. The pesticide itself has to meet certain standards before it is approved for application. Already during the development of a compound the mode of action, the analytical procedures and the toxicology are investigated, and certain standardized experiments on leaching, degradation and metabolism are conducted by industrial laboratories to provide the required information for the regulating agencies. But it became obvious during the discussion about the relatively stable organo-chlorine compounds that more detailed information is needed concerning the behaviour of pesticides in the soil-plant system. Residue analysis can only trace the original mother compound or certain metabolites of similar structure. However, labelling of a pesticide molecule at a definite position is a unique technique of following the fate of the pesticide and its metabolites formed in the soil-plant system, thus furnishing extensive information on its uptake, transport and metabolism by plants, its photochemical, chemical and microbial degradation, its adsorption-desorption and translocation in soil and its bioavailability to untreated crops (Fig. 1).

Use of the tracer technique is demonstrated in Fig. 2. Depending on the aim of an investigation, the compound is labelled at a definite position of the molecule and applied to the plant or soil, simulating spray application. The treated samples (soil and plant) are then analysed. Different extraction and clean-up procedures are applied, depending on the chemical under investigation. The labelled compounds or metabolites are separated, using thin-layer chromatography, gas chromatography and high performance liquid chromatography in combination with the detection of the radioactivity by liquid scintillation counting. Hypothetical metabolites available as reference substances are cochromatographed to yield information on the possible structure of the unknown metabolites.

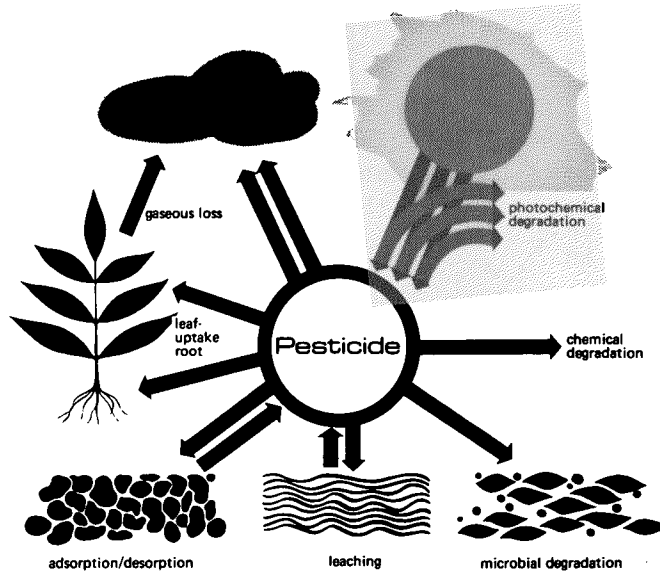


FIG.1. Fate of a pesticide in the soil-plant system.

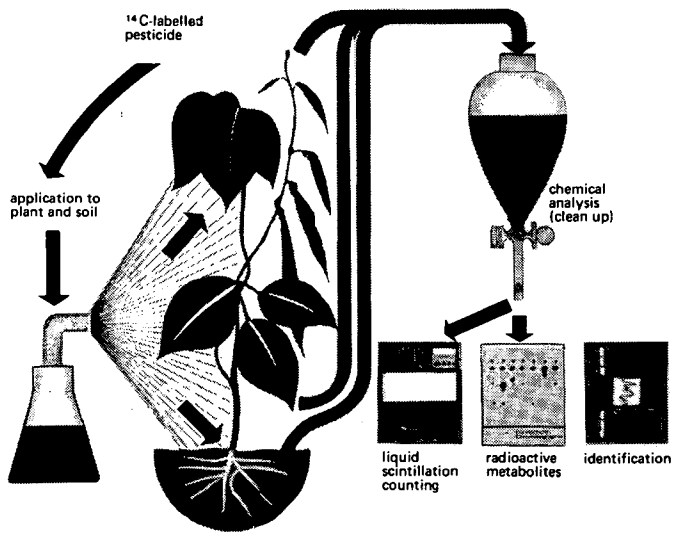
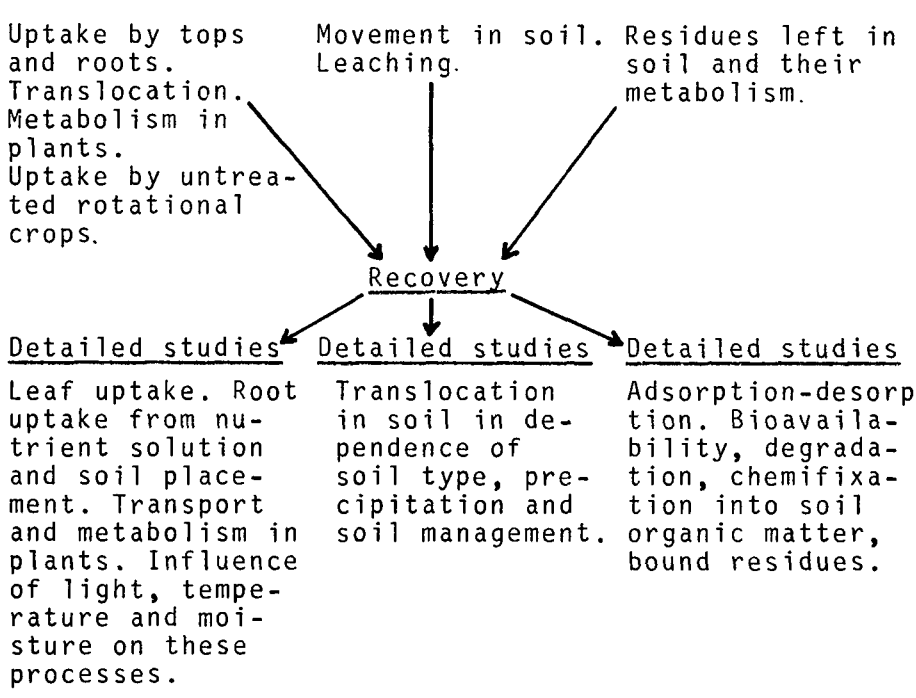


FIG.2. Use of ^{14}C-labelling in herbicide studies.

Table I. General scheme for studies with labelled herbicides



Metabolites representing a substantial percentage of the originally applied amount of radioactivity are specially purified and concentrated for GC/MS identification.

2. LYSIMETER EXPERIMENTS

Many of the tracer studies with herbicides have been conducted under laboratory conditions. The data thus obtained are generally limited to evaluating individual processes. In the Jülich Nuclear Research Centre facilities have been set up to conduct outdoor experiments with ^{14}C-labelled pesticides. This is a chance to make use of a different approach (Table I).

Standardized lysimeters (0.76 or 1 m^2 surface area, 50 cm of packed topsoil) (Fig. 3) and undisturbed soil cylinders (0.25 or 0.50 m^2 surface area, 50 cm of soil profile) have been set up in this area and

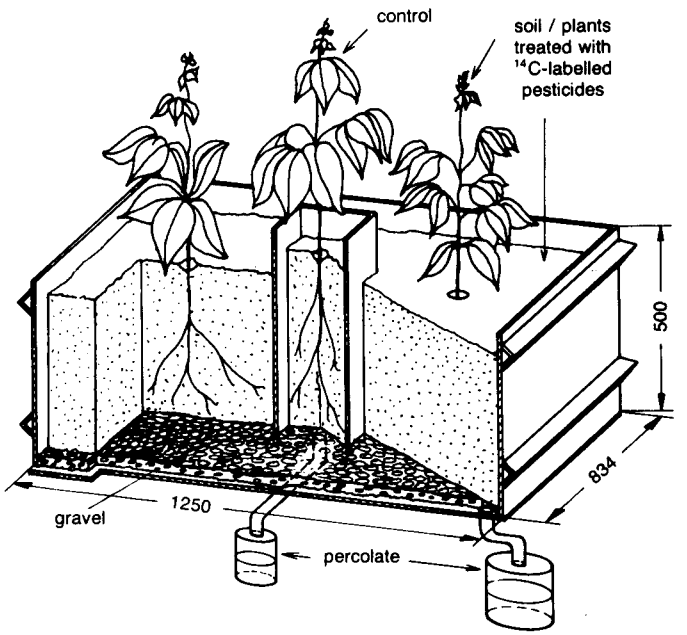


FIG.3. Experimental lysimeter (measurements in mm).

the combined effect of the growth factors, such as the physical, chemical and microbial processes in the soil as well as the climatic factors on the behaviour of a ^{14}C-labelled herbicide in the soil-plant system, are examined throughout the growing season, including uptake by untreated rotational crops. This balance study provides useful information on the magnitude of the uptake, distribution and metabolism of the herbicide in plants, its translocation in the soil and leaching from the root zone as affected by precipitation and root development, as well as its metabolism and mineralization in the soil. More detailed information on these lysimeter experiments and the special problems involved have been described elsewhere [3,4]. In the centre of the lysimeter a separate control vessel is installed (20 x 20 x 50 cm) containing untreated soil and plants.

As an example, data is summarized from a spray application of an urea derivative herbicide (methabenzthiazuron, 1,3-dimethyl-3(2-benzthiazolyl)-urea) to

wheat at the third to fourth leaf stage of plant development [5]. The applied methabenzthiazuron concentration was 140 mg/0.76 m², equivalent to 2.5 kg (R)Tribunil[2]/ha. The specific activity of the [benzenering-U-^{14}C] methabenzthiazuron was 7.85 µCi/mg. The soil was a parabrown earth derived from loess. At harvest time, 111 days after spraying, 83% of the ^{14}C activity was still almost exclusively in the 0-5 cm layer of the loess soil, 1.4% was in the above-ground portion of the treated wheat plants, and only traces were leached from the 40 cm layer of soil (Fig. 4).

In the same lysimeter, immediately after the balance study with spring wheat, an experiment was conducted to investigate the uptake of labelled pesticide residues or metabolites by the subsequent rotational crop rye [5]. The total radioactivity found in the rye grain, chaff and straw amounted to 0.29% of the ^{14}C applied (Fig. 4). Calculated on the specific radioactivity of the sprayed methabenzthiazuron, the following methabenzthiazuron equivalant values were found in the treated spring wheat: straw = 16 mg/kg, chaff = 1.4 mg/kg, and grain = 0.07 mg/kg. In the untreated rotational crop rye these residue values were reduced to about 1/10. Part of the radiocarbon in the experimental plants may result from an assimilation of the $^{14}CO_2$ released via the soil air as a result of mineralization processes. The order of magnitude of this assimilation can range between 1 and 20% of the total ^{14}C absorbed by the experimental plants [6]. Therefore in these experiments radioactivity in the plants does not entirely represent residues in the strict sense.

This lysimeter set up (Fig. 3) represents an agricultural ecosystem which comprises the order of magnitude of uptake and distribution in plant, regardless of leaf or soil application, translocation of active substance and metabolites in the top soil profile, leaching as a function of the precipitation distribution and vegetation as well as the total balance at the end of a vegetation period. All the requirements of good agricultural practice are thus observed: soil cultivation, fertilization and rotation in order to follow the uptake of residues by untreated rotational crops.

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2) Trade name, Bayer AG, Leverkusen, Federal Republic of Germany

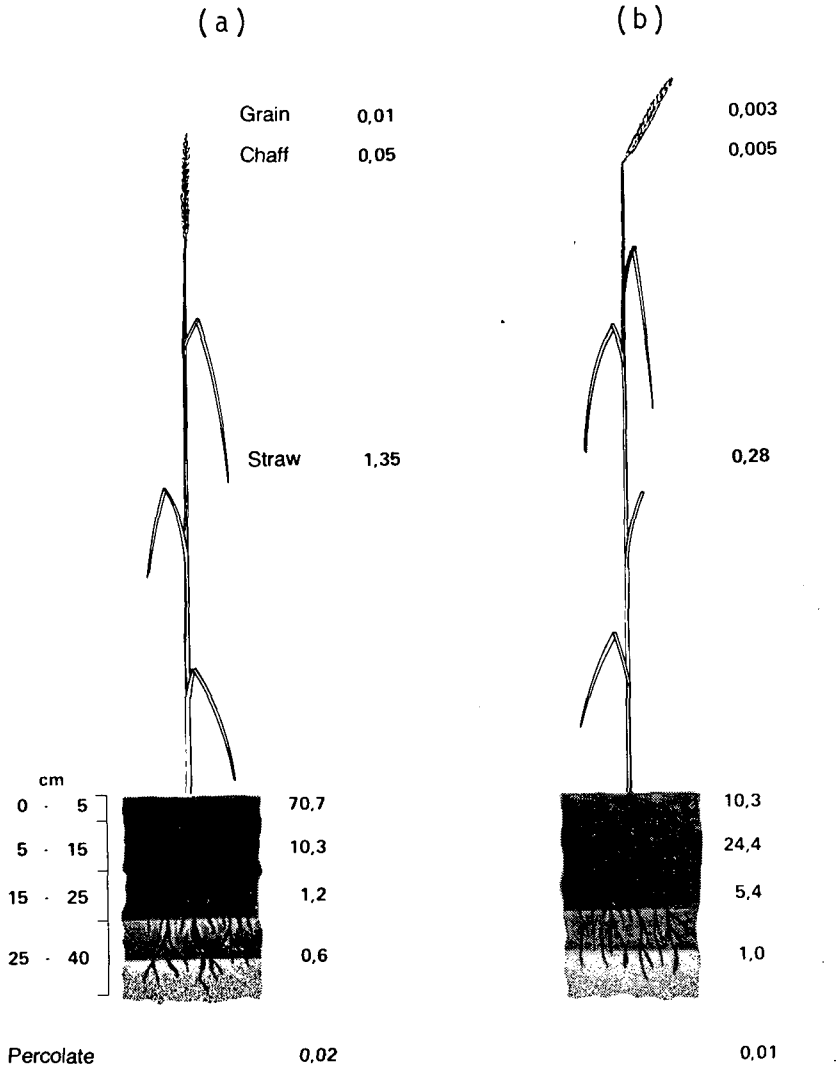

FIG.4. Results of an outdoor lysimeter experiment: ^{14}C distribution in the treated spring wheat, rotational crop rye, and soil after application of [benzenering-^{14}C] metabenzthiazuron. (a) Spring wheat, 111 d after spray application; (b) Rotational crop rye, 503 d after spray application.

3. DETAILED STUDIES

Based on results achieved in the lysimeter experiment, detailed studies are then conducted to investigate the defined steps of plant uptake, transport and metabolism (Table I), and the influence of the different environmental factors on these processes, as well as translocation and behaviour in soil, including all processes which may influence the bio-availability of herbicide residues to untreated rotational crops. Some examples are given for demonstration.

3.1 Small-scale uptake experiments

So far most of the tracer studies with herbicides have been conducted under laboratory conditions using different micro-ecosystems. For metabolism studies these experiments are quite sufficient. However, utmost care must be taken in interpretation e.g. uptake results from small-scale pot experiments. Certain experimental and environmental factors are quite different from field conditions which normally favour the uptake rates:

(1) Distribution of the pesticide in the soil
(2) Ratio of root mass to soil
(3) Maintenance of a relatively optimal soil-water content for plant roots and a steady water supply
(4) Optimal conditions for plant growth and hence high transpiration rates.

Using[ethylene-$^{14}C_2$]isocarbamide, the active ingredient in the selective herbicide (R)Merperlan AZ[3], uniformly mixed into the soil (0.9 kg) of a pot experiment, 64% of the radioactivity was absorbed via the roots and translocated into sugar-beet leaves within 55 days[7]. In a lysimeter experiment, however, the sugar-beet leaves contained only 1.23% of the radioactivity employed 75 days after pre-emergence spray application[8].

3.2 Degradation and metabolism

The temperature-dependent microbial conversion processes in the soil proceeded fairly independently of the other experimental conditions. Therefore, detailed procedures have been described to follow the

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3) Trade name, Bayer AG, Leverkusen, Federal Republic of Germany

degradation in the soil[9]. As an example, degradation studies were conducted in 200 g of soil under standardized condition (Fig. 5) using ^{14}C-labelled methabenzthiazuron at the different positions of the molecule [10] [11].

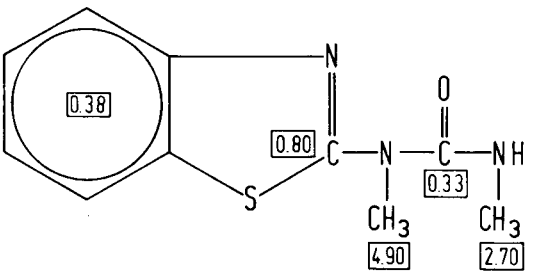
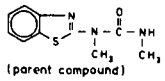
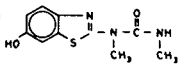
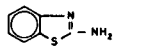
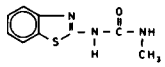
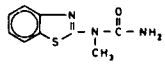


FIG.5. Mineralization rates (%) of the different carbon atoms of methabenzthiazuron under constant conditions in the soil (1.1% C, pH 6.5) at 22°C, 65% WHC, 10 ppm methabenzthiazuron, 11 weeks. Total radioactivity applied = 100%.

Preferentially, the methyl groups are mineralized by microbial action. On the basis of this information, extensive analytical investigations, using soil from the lysimeter experiment [5],were conducted to identify the major metabolite formed in the soil [12]. Thin-layer and high performance liquid chromatography were applied to separate and purify the major metabolite. Cochromatography of hypothetical metabolites [13] as reference substances yielded information on the possible structure of the unknown labelled newly formed metabolite (Fig. 6). Its chromatographic properties were identical to 1-methyl-(2-benzthiazolyl)-urea, which was confirmed by MS fragmentation, yielding a parent ion at m/e 207.

3.3 Formation of bound residues

So far the main interest is focused on the uptake of herbicides by treated crops, but several pesticides are applied to a crop either in combination with other compounds or successively. The question, therefore, remains unanswered as to what is the fate of the residues left in the soil after harvesting, either applied for example as soil herbicides, or leached into the soil from the treated plants by rain, or brought into

No.	Compound Structure	Elution volume [ml] μ-C 18 [1]	μ Porasil [2]
1	 (parent compound)	30,98	4,95
2 [3]		13,65	
3		15,60	
4		21,15	
5		24,83	8,40
Major metabolite		24,75 - 24,83	8,36

[1] Solvent program no. 10, 20 % - 100 % acetonitrile, 20 min; flowrate 1.5 ml/min; chart 10 mm/min

[2] Chloroform, flowrate 1.5 ml/min, chart 10 mm/min

[3] Found as degradation product (Wallnöfer et al., 1976) [13]

FIG.6. Elution volume of methabenzthiazuron and different hypothetical metabolites in comparison with the major metabolite.

the arable soil layer via organic manuring with contaminated plant residues. It has been demonstrated by numerous detailed studies that the fate of these residues depends greatly on the stability of the chemical applied, its adsorption behaviour and the chemical and physical properties of the soil. Therefore two fractions of residues in soil have to be dealt with: extractable pesticides or metabolites and unextractable 'bound' residues.

With time, the second fraction increases and, depending on the herbicide applied, anywhere between a few per cent up to nearly 3/4 will remain in the soil as residue or metabolite in an unextractable form [14]. So far no extraction schemes have been developed to characterize these bound residues [15] and the question still remains whether these 'bound residues' are strongly adsorbed by soil organic matter binding sites or whether they are rather incorporated into or assimilated by soil organic matter [16]. With extraction

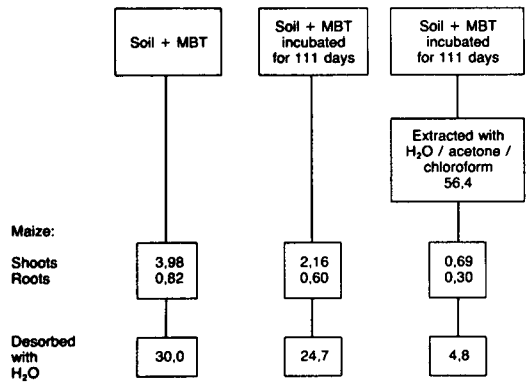


FIG.7. Plant experiment to determine the bioavailability of the non-extractable radioactivity. Test compound: [carbonyl-^{14}C] methabenzthiazuron (MBT); sandy soil, pH 6.4; 1.15% C. Total radioactivity applied = 100%.

procedures commonly used in soil organic matter analysis, normally quite a high percentage can be extracted, and in combination with residue analysis information on the structure and status of binding can be collected. A co-ordinated programme of isotopic tracer-aided studies of unextractable or bound pesticide residues in soils, plants and food was initiated by the Joint FAO/IAEA Division of the IAEA in 1980. In this respect detailed studies will yield information on the degradation rates and, even more important, on the bio-availability of bound residues to plants [17]. As an example, results from experiments with methabenzthiazuron are reported in sub-section 3.4.

3.4 Bio-availability of bound residues

The extractability of methabenzthiazuron residues from soil decreases with time after application [14]. In a special study the bio-availability of this soil bound or adsorbed fraction was investigated using maize plants [17].

A sandy soil and [carbonyl-^{14}C] methabenzthiazuron (10 mg/kg soil) was incubated at 22°C and 65% of the maximum water-holding capacity of the soil for 111 days. After exhaustive extractions with H_2O/acetone/chloroform, unextracted, bound residues remaining in this soil amounted to 41% of that applied. Plant uptake studies were conducted in special experimental pots (~1 L) which allowed an air-tight separation of the

shoots from the root environment[7]. After 29 days the maize shoots contained 0.7% of the radioactivity originally applied to the soil before incubation in comparison with 2.2% from the incubated soil not extracted (Fig. 7). From the carbonyl-^{14}C methabenzthiazuron added to the soil immediately before the plant experiment, about 4% of the radioactivity was found in the shoots. According to these results, after incubation in the soil the plant availability of the unextracted radiocarbon, as compared with the extractable portion, had decreased to about 1/3, and in comparison with the availability after direct methabenzthiazuron application to about 1/6. After extraction with different organic solvents, an equilibrium status is apparently built up again in the soil between bound or adsorbed herbicide residues or metabolites and such compounds in the soil solution. The desorbed radioactivity (Fig. 7) found after the plant experiment supports this. Since the radioactivity in the maize shoots still partially represents the unchanged methabenzthiazuron, it can be concluded that the parent compound is strongly adsorbed by soil-binding sites and is not chemically fixed. Thus, over 90% of the radio activity in the fulvic acid fraction was found to be methabenzthiazuron.

In conclusion, this detailed data has demonstrated the use of radioactivity labelling in investigations on the fate of herbicides in the soil-plant system. Only some of the research topics outlined in Table I could be stressed.

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Invited Paper

ISOTOPE-AIDED STUDIES OF RESIDUE/BIOTA INTERACTIONS

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Abstract

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Current trends in population, demands per capita and land use imply a continuing need and growing use of agrochemicals, especially in developing countries. Studies of the nature, magnitude and persistence of agrochemical residues that find their way into food, the environment and non-target organisms are now an internationally recognized prerequisite for acceptable use. Isotope techniques play a vital role in these studies, as conventional tracers and as monitoring tools for both intended effects and non-intended side effects. They often provide the only effective technique for the detection and characterization of 'bound' residues in soils or plants, which would not be included in conventional chemical analysis based on solvent extraction. Isotope techniques, like all others, have their limitations and certain pitfalls exist. The latter are largely due to an inadequate understanding of the isotope behaviour or biochemistry of the system being studied. It is important to recognize the possibility of effective isotope exchange reactions, whether studying radioisotopically labelled pesticides or ^{15}N-labelled fertilizers. Extensive literature now exists on the fate of agrochemical residues. This indicates that all agrochemical residues tend to undergo chemical modification, biodegradation, dispersion, etc., but some halogenated ring structures appear to be relatively intractable. When considering the significance of known residues it is important to distinguish between acute toxicological effects indicated in the laboratory and the longer term effects on field populations of both 'target' and 'non-target' organisms. The phenomena of pest resistance to pesticides have important implications in this context.

1. INTRODUCTION

The rising use and need of pesticides, fertilizers and other agrochemical aids to agriculture and forestry in recent decades are well documented. Moreover, current trends in land use, population, demands per capita and the effective limits to many agricultural, forestry and fisheries resources imply a continuing rise in agrochemical usage and development during the immediate decades ahead [1, 2].

Existing disparities in pesticide usage [3], in relation to land use and population [4], clearly indicate the particular scope for increased usage in less industrialized regions (Table I). These data imply that if current pesticide usage

TABLE I. AGRICULTURAL LAND, POPULATION AND PESTICIDE USE IN 1978

Region	Total population (10^6)	Total arable and cultivated land (10^6 ha)	Total pesticide use (US \$ X 10^9)
North America	242	231	3.0
Latin America	347	143	0.8
Western Europe	368	96	2.1
Eastern Europe and the USSR	372	279	1.1
Africa and the Middle East	549	262	0.4
Far East	1183	266	1.3
Total			8.7

Note:

Estimated world use if at the N. American rate (ha) would be 17 US \$ X 10^9.
Estimated world use if at the W. European rate (ha) would be 23 US \$ X 10^9.
Estimated world use if at the N. American rate (per capita) would be 38 US \$ X 10^9.
Estimated world use if at the W. European rate (per capita) would be 17 US \$ X 10^9.

Data and estimates based on GIFAP [3] and FAO [4] statistics.

per unit of agricultural land, or per capita, of North America or western Europe were matched in other regions this alone would result in a two- to four-fold increase in world usage.

A large fraction, sometimes almost all, of an applied agricultural chemical appears as residues in food, the environment and non-target animal and plant organisms. For example, it was estimated that when lindane was used for cocoa capsid control in West Africa much less than 0.1% of the applied insecticide was actually taken up by the insect pest population, the remainder appearing as residues in the atmosphere, crop and soil [5]. Experiments in different countries with ^{15}N-labelled fertilizer indicate that usually less than half the applied nitrogen is usefully recovered in the crop [6].

The nature, magnitude and biological significance of the residue are the net result of the interactions between the residue and its abiotic and biotic environment, especially the latter [7, 8], despite one surprising conclusion to the contrary [9]. However, there are exceptions, e.g. chemical decomposition of an atrazine residue in soil was evidently more important than that of microbiological degradation [10].

Isotope techniques now play a well established role in the study of these interactions [11]. Moreover, such studies are an essential prerequisite for

internationally acceptable agrochemical use as implied, for example, by the limits recommended jointly by FAO and WHO for pesticide residues in food.

It is the purpose here to illustrate the wide range of applicable isotope techniques, to mention some limitations and pitfalls, and briefly to consider the significance of the interactions studied.

2. NATURE AND MAGNITUDE OF THE RESIDUE

The nature, magnitude, persistence and distribution of an agrochemical residue depend on many factors [12]. These include the chemical structure of the original compound, formulation, application regime, and transport processes such as spray drift, crop harvest and export. They also include the 'disappearance' factors that remove or transform the original chemical. Abiotic factors include volatilization, photochemical decomposition, weathering, run-off and soil erosion, leaching down the soil profile and inactivations in situ, e.g. the ion-exchange type adsorption of bipyridylium herbicide residues. Biotic factors include plant absorption and metabolism, grazing and metabolism by livestock and wildlife, bioaccumulation and degradation by soil microorganisms, fauna and flora.

The time-concentration curve will depend on the pattern and rate of input and, in its simplest but possibly most useful form, on the effective overall 'disappearance' half-time. It is convenient to recognize the continuous and discontinuous kinds of input. Continuous input would be represented by a person daily ingesting or imbibing a pesticide residue in food and drink; an annual application of fertilizer would be discontinuous. In the former case the significant residue, which may be the original chemical alone or a combination with one or more toxicologically significant metabolites, will tend to rise exponentially with time until a steady-state level is reached; that is, when the input rate is balanced by the disappearance rate due to, for example, the additive effects of metabolism and excretion. In the case of a discontinuous but otherwise equivalent input there will be a series of initially higher peak concentrations, each peak followed by exponential decay according to the disappearance rate constant or related 'half-time'. In both cases steady-state levels or steady-state maxima tend to be achieved over long periods of time. Simple equations for the two kinds of time-concentration curves, and a discussion of their consequences, have been presented elsewhere [12]. Given time, both otherwise equivalent inputs lead to identical time-weighted mean concentrations.

2.1. Isotope techniques

As suggested earlier [11], it is convenient to consider isotope techniques as conventional tracers or as monitoring tools. For tracing, the suitably labelled and

formulated compound is applied, and its physical and chemical fate followed on the basis of subjectively timed sampling. In short, the action of the abiotic or biotic environment on the chemical is studied.

For monitoring the effects of the chemical on the exposed organism isotopes can be used as labelled reagents or substrates, or for labelling a specified biochemical pathway, which in turn can indicate the effects on the organism or ecosystem.

There are many reviews of the conventional use of tracers for pesticides, fertilizers and other agrochemicals (e.g. Refs [11, 13–15]) and, more recently, for studying their fate in model ecosystems [7, 16, 17]. Less attention has been given to the use of isotopes as monitoring tools despite their undoubted potential [18–20]. For examples of both kinds of application attention is drawn to the publications of the Joint FAO/IAEA Division Chemical Residues Programme [21].

There is now a wide range of radioisotopically labelled agricultural chemicals and extensive literature exists on sources, labelled syntheses, sample preparation, extraction, fractionation and assay procedures [22, 23]. Against this background only selected topics are discussed here. The discussion is prompted by some 20 years experience at the research bench level and some 15 years of co-ordinating studies on behalf of WHO, FAO and IAEA.

2.2. Bound residue problem

Once the likely nature of the residue in soil, water or tissue has been established (usually aided by tracer techniques) it is possible to develop analytical chemical procedures for routine monitoring and control purposes, e.g. for pesticide residues in food as studied and reported by the joint FAO/WHO meetings on pesticide residues in food and the environment. However, chemical analysis of organic residues almost invariably involves solvent extraction and clean-up. Part of the total residue may be 'bound', chemically or physically, and will not be recovered by solvent extraction. Although such bound residues may be neither detected nor determined by chemical analysis, they can be of toxicological significance [24]. Alternatively, being undetected they may be wrongly assigned to some other disappearance factor when trying to account for the total residue.

Bound residues formed as a result of exposure to a radiolabelled chemical can be readily detected, either by non-destructive assay of radioactivity of the fully extracted sample or after total oxidative combustion of ^{35}S- or ^{14}C-labelled residues. Use of stable ^{15}N-labelled fertilizer residues similarly provides for the assay of 'bound' nitrogen in the form of the soil immobilized element, this despite the likely great excess of existing native soil nitrogen [6]. However, in this case samples must be chemically prepared for mass or emission spectrometric assay.

By chemical fractionation procedures the nature and magnitude of chemically bound residues in wheat after exposure to ^{14}C-labelled methylbromide as an insect

fumigant were successfully determined [25–27]. Bound residues have similarly been detected and studied in wheat fumigated with ^{32}P-labelled phosphine gas [28–30] and in ^{14}C-phorate- or parathion-exposed soils [31].

2.3. Isotope techniques as monitoring tools

As monitoring tools isotope techniques appear to be relatively unexploited, despite their sophisticated development and use as diagnostic tools in medicine [11]. Use of labelled reagents and enzyme substitutes in the laboratory and determination of natural or environmental isotopic ratios as ecological indicators have been reviewed elsewhere [32].

Use of ^{14}C- or ^{3}H-labelled acetylcholine as the substrate for acetylcholinesterase is probably the most sensitive method available for enzyme assay of carbamate or organophosphorous insecticide residues. It provides for minimal sample dilution, rapid assay and use of very low substrate concentrations. It is, therefore, a very sensitive method for carbamate residues. Higher substrate concentrations, enzyme dilution, etc. dictated by conventional methods tend to reverse the very inhibition it is sought to detect. The techniques have been successfully applied to residues in human blood after occupational exposure, in agricultural products and in aquatic ecosystems [32].

In the labelled pool technique [33] entire metabolic pools are labelled in vitro by feeding or injecting a suitably labelled precursor; for example, ^{14}C-acetate for labelling the ketogenic amino acid and acetylcholine pools of insects, ^{32}P-phosphate for the phosphorylated intermediates and nucleotides of insects, and ^{35}S-sulphate for the sulphur amino acid and protein pools of plants. Extraction, fractionation and comparative radioassay of the labelled metabolites from control and from exposed organisms then provide a quantitative indicator of metabolic disturbances as a result of toxic action or some other undesirable side effect, e.g. formation of undesirable volatile sulphides as a result of fumigating wheat with methylbromide [25].

Related applications include use of labelled DNA-precursors, e.g. ^{3}H-thymidine, for studying DNA repair inhibition by agrochemicals [19, 20], animal hormone disturbances by using radioimmunoassay techniques [34], and the effects of agrochemical residues, e.g. in soil run-off, on the net primary productivity of aquatic ecosystems, or in the microbiological activity of soils [8, 35].

2.4. Some limitations and pitfalls

Isotope techniques, like all others, have their limitations, and certain pitfalls await the investigator [32]. Some points bear reiteration here.

Costs and lack of availability of suitably labelled compounds can be a handicap for workers in less industrialized countries [22]. The possibility of

establishing an international bank or centre for the conservation, storage and provision of labelled agrochemicals and their significant derivatives has been considered, but at the time (1976) it was not felt to be a feasible undertaking for the Joint FAO/IAEA Division [22].

Pitfalls are usually, but not invariably [32], due to data misinterpretation, or to an inadequate understanding of the labelled system. Isotopic exchange can sometimes occur between quite different forms of the same element; for example, between the bromine of an organic alkylbromide and the inorganic bromide naturally present in all plant tissues. Unless recognized this can lead to an overestimate of the bromide residue formed in cereals under the conditions of ^{82}Br-labelled methylbromide fumigation. Effective isotope exchange can also occur when ^{15}N is used to follow the leaching of a labelled nitrogen fertilizer down the soil profile. This is due to the slow but sure mineralization of the unlabelled soil organic nitrogen and the reciprocal microbiological immobilization of the labelled mineral nitrogen. Unless taken into account, the tracer alone would underestimate the loss of mineral nitrogen to the root zone [32, 36].

A pitfall in the context of model ecosystem studies is due to the possible confusion of primary catabolites with secondary anabolites. Many model or simulated ecosystems have been described for studying the fate of an added agrochemical residue [7, 16, 17, 37, 38]. For this purpose the labelled compound, usually labelled with ^{14}C, is added to the model ecosystem. Clearly, if biodegradation in one species leads to a labelled but quite innocuous fragment, such as ^{14}C-acetate, ^{32}P-phosphate, ^{35}S-sulphate or ^{14}C-carbon dioxide, the label will almost certainly reappear in a range of anabolites of other exposed organisms. For example, some ^{14}C-ring or labelled aromatic herbicides can undergo opening of the benzene ring through microbiological degradation, presumably via adipic acid, so that $^{14}CO_2$ could reappear as anabolites in photosynthesizing algae or plankton. Unless recognized this could be wrongly interpreted as undesirable persistence or bioaccumulation in secondary biota.

3. INTERACTIONS

Attention has been drawn to the conventional use of isotopic tracers for studying the fate of an agrochemical and to their use as monitoring tools, in short, to the use of isotope techniques for studying interactions. This is particularly relevant to studies in less industrialized countries where isotope laboratory facilities tend to be concentrated in one 'nuclear' institute and to be used mainly as conventional tracers. However, as already illustrated, these same facilities can be used in the wider and more important context of interactions between chemical and exposed biota. Some observations in this wider context are dealt with in the following sub-sections.

3.1. Physical and chemical fate

Isotopic tracer techniques provide the ideal method for the integrated type of study [39], which is fundamental, for example, to the formulation of realistic pesticide residue 'tolerances' or 'maximum residue limits' (MRLs), as they are now called. For such a study the labelled chemical is applied experimentally to the crop under the simulated conditions of 'good agricultural practice' and its fate followed from the time of application through harvest, post-harvest treatment, processing, cooking, etc., to the 'terminal residue' at the point of human ingestion. Therefore, the study provides especially well for relating the residue at the most convenient point for sampling, analysis and MRL enforcement, and the terminal residue. The MRL is, of course, designed to obviate the presence of a terminal residue likely to exceed that implied by the maximum acceptable daily intake (ADI).

One of the first, if not the first, of such studies was made with a radioactive bromine analogue of DDT more than 30 years ago [40]. The residue was followed and chemically characterized from the point of application to wheat grain through the authentically duplicated conditions of milling, fractionation, baking and feeding to experimental animals, as well as one human volunteer (the present author), and finally to the point of radiochemical analysis for excreted metabolites.

Any review of the now very extensive literature on agrochemical residues is quite beyond the scope of this contribution. The annual series of 'Residue Reviews' and FAO/WHO Monographs on pesticide residues in food, feed and the environment are but two key series of publications. A series of concise summaries of individual or grouped environmental chemicals and radioactive substances was also initiated under the Joint FAO/IAEA Division Chemical Residues Programme as an aid to scientists in developing countries [21].

The literature indicates that agrochemical residues invariably undergo some abiotic chemical modification and/or metabolism, some rapidly with disappearance half-times of the order of minutes, others slowly with half-times measured in years. Halogenated unsaturated rings tend to be especially recalcitrant and persistent. While only minor modification tends to destroy acute toxicity of the parent molecule [41], some residual moieties, e.g. the hexachlorocyclopentadiene moiety of the 'drin' insecticides, can be very persistent and there is as yet little information about their possible long-term ecotoxicological significance [42]. Disappearance rates depend greatly on the chemical structure and climate, and on both abiotic and biotic environment factors. Thus, rates determined under temperate conditions may not be applicable to tropical ones [43].

3.2. Side effects

Given the nature and magnitude of an agrochemical residue as a function of time the most important question remains: what is their significance in terms of effective usage and possible hazards to health and environment?

The most serious side effect of accepted pesticide usage has been the slow but ever widening appearance of 'resistant' strains of pest on the basis of accelerated Mendelian selection [44]. This has involved virtual physiological immunity of entire field populations of hitherto effective acaricides, insecticides, fungicides, herbicides and even rodenticides [45]. These phenomena have implications for unrelated agrochemical effects and emphasize the difference between classical toxicology, which is mainly concerned, directly or indirectly, with human health, and the wider concept of ecotoxicology [46]. In the latter case it is the vast range of microbiological, animal and plant species of the agroecosystem which is of concern. Moreover, it is the longer term effects of the likely exposure of populations over several generations which will be more important than acute effects on individual organisms or initially exposed populations, except for target pest populations. Various compounds are under evaluation to improve the efficiency of nitrogen fertilizer usage by reducing nitrogen volatilization and/or leaching losses. They are based on their ability to inhibit microbiological mineralization of organic nitrogen, e.g. of added urea nitrogen or nitrification of ammonium, since both leaching and denitrification (volatilization) losses imply nitrate formation beyond the immediate needs of the growing crop or retentive capacity of the soil. However, the phenomenon of resistance suggests that while such compounds might appear effective under short-term tests, they would lose effectiveness under field conditions due to the selection of resistant strains of microorganisms [47].

Finally, it is important to consider any demonstrable side effects of agrochemical residues in the context of agrochemical practices as a whole. Thus, the effects of an agrochemical residue on some non-target microorganisms of the soil may be of negligible significance compared with the slow but sure decline of total soil organic matter (and its nitrogen) as a result of clearance and/or intensive agricultural practices generally [1, 48]. Similarly, although DDT at extremely low concentrations can inhibit the growth of some species of marine phytoplankton, more significant interaction may well be the effective removal of DDT by planktonic sedimentation [49].

4. CONCLUSIONS

Isotope techniques have become an indispensable tool for studying the nature, magnitude and persistence of agrochemical residues. The laboratory

facilities for handling radioactive and stable isotopically labelled chemicals also provide for studying the effects of unlabelled chemicals on critical parameters of soil and aquatic ecosystems. They similarly provide for monitoring by using labelled reagents and enzyme substrates.

Methods of labelled synthesis, application and assay are now well advanced. However, as in other instrumental assay techniques the explosive development in applied micro-electronics promises a major impact on the isotope laboratory in terms of programmed and automated analysis and assay, data storage, retrieval and processing [50].

Trends in land use, population and demands per capita imply a continuing rise in pesticide and fertilizer use, particularly in tropical areas. It is, therefore, important that the countries concerned have the full range of isotope techniques available for the study and control of their own agrochemical residue problems under their own local conditions. Wise use of the information and data so acquired will surely facilitate optimal use of agrochemicals, and obviate problems of toxicology and ecotoxicology [5, 11].

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PESTICIDES IN AQUATIC ECOSYSTEMS
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Invited Paper

PESTICIDES IN LIMNIC METABOLISM

A review on the application of nuclear techniques

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Abstract

PESTICIDES IN LIMNIC METABOLISM: A REVIEW ON THE APPLICATION OF NUCLEAR TECHNIQUES.

Pesticides in fresh water ecosystems are discussed with respect to their toxicity, bio-accumulation, degradability and transportation. Water quality problems centre around the balance or imbalance between oxygen supply, primary production and community respiration. Sources of pesticides are runoff, leaching, adverse deposition of pesticides from agriculture and, in certain areas, industrial sources. Several bioassays for toxicity tests are described. For photoautotrophic activity (algae, particularly *Scenedesmus quadricauda*) growth is monitored by: turbidity, cell counting, chlorophyll determination, oxygen production or ^{14}C incorporation; for heterotrophic activity: oxygen consumption, dehydrogenase activity, grazing capacity of protozoa, acidification by *Pseudomonas*, ATP, production of HTO or ^{14}C-dioxide from ^{14}C- or ^{3}H-acetate or glucose; for higher animals: adverse behaviour or death. Radioenzymatic and radioimmunoassays have also been used. Often adverse effects, e.g. on fish (taste, poor health), are observed, but unequivocal causative relationships to particular pesticides or polluters can seldom be established. Metabolic transformations of pesticides in organisms are very involved; they include: oxidation, hydrolysis, reduction, conjugation, dehydrohalogenation, ring scission, hydration and chelation (metals). Some guidelines are established and experiments, particularly with ^{14}C label, are reported. Bioaccumulation occurs mainly in fat and bone. Concentrations take place in the food chain. Research methods are similar to those for degradation. In water, pesticides may be transported as dissolved material or by adhering to suspended sediments. Some studies on isotopic and non-isotopic sediment tagging are reported.

1. INTRODUCTION

Although extended use of pesticides has proved beneficial, as in the production of food, it has also developed into a major threat to the environment. Pesticides will either accumulate in the soil, evaporate, degrade or enter fresh water bodies. These events will be discussed with respect to the application of radionuclides in the context of the following properties:

(1) *Ecotoxicity.* This term is used here to refer to the adverse effects on organisms in the environment but not to the toxic effects on man.
(2) *Degradability.* This means that a pesticide is changed in structure and finally mineralized. Intermediate products need not necessarily be less toxic.
(3) *Bioaccumulation.* Pesticides are often stored somewhere in the organism where they need not necessarily be harmful but where they may be remobilized or eaten by a predator. Accumulation is of major concern with respect to the human food chain.
(4) *Transportation and sources of pesticides.* This is mainly a question of hydrology and sedimentology.

Radiotracers as well as stable isotopic tracers are powerful tools in experiments on these properties.

A short primer on limnic metabolism will be given and the numerous applications of nuclear and some non-nuclear methods for pesticide research will be described.

Limnic metabolism

Some aspects are unique to water ecosystems compared with land systems. Water supports the organisms mechanically and supplies dissolved nutrients. These are the properties that allow development of plankton, a form of life unknown to land systems. Concentration of dissolved oxygen and carbon dioxide or carbonate is not constant, as it is in air. The hypolimneon of deep lakes, or the whole pelagic of shallow lakes at night, may be depleted of oxygen and enriched in carbon dioxide.

Primary production is restricted to a lighted, warm, oxygen-rich surface layer (euphotic zone) where it exceeds community respiration. When dead organic material sinks to the bottom it causes oxygen consumption and carbon dioxide production, which may use up all the oxygen in the depths and cause strongly reducing anoxic conditions. Oxygen is supplied either from photosynthesis (biogenic aeration) or from the atmosphere (atmospheric aeration).

Thermal stratification divides a lake into three layers of different temperature and density: epilimneon (warm), metalimneon (transient zone) and hypolimneon

(cold), if it is deep enough. In moderate climates this stratification is so stable during the summer that oxygen supply to the hypolimneon is prevented completely. Tropical lakes may even show permanent stratification. Detailed discussions of these relations are given in Goltermann's text book [1] and in Ref. [2]. Lake metabolism under oxygen-depleted conditions is described in Refs [3–5].

Almost all water quality problems centre around the balance or imbalance between production and mineralization, and oxygen supply and oxygen consumption. In shallow lakes wind forces are usually strong enough to prevent stratification during the summer. However, lack of biogenic aeration during the night may also cause oxygen depletion. In rivers turbulence prevents stratification. Atmospheric aeration can support a remarkable respiratory turnover, which mineralizes planktic detritus or sewage (self-purification). However, under heavy organic charge large, slow-flowing lowland rivers may be depleted of oxygen. Primary productivity, if it is as warm and bright as in summer, is limited by the availability of plant nutrients. Increased supply (eutrophication) leads to a series of largely undesirable events, such as intense algal growth affecting water-work operation and/or production of toxines [6], overcharge of the self-purification capacity, killing of fish, damage to reed belts and less species diversity. Lake trophic indices have been compiled by Shapiro [7]; for detailed discussions of eutrophication see Refs [1, 8, 9].

In most cases phosphorus is the first limiting factor for primary productivity. Hence, the phosphorus balance of lakes and their watersheds deserves particular attention. Once an algal cell dies most of its phosphorus content leaves the cell at a much faster rate than other cell material (particularly the cellulose walls) is mineralized. Thus, long before the cell detritus has disappeared the phosphorus again helps to build up new organic material (short-circuited phosphorus metabolism [8]).

Biomass is reduced in each step of the food chain, often by a factor of 10 (details are given in Ref. [1]). This means that the biomass of plants is much greater than that of all animals. In spite of this animals play an important role in accumulation problems. They contain more lipids than plants [9] and hence they accumulate hydrophobic xenobiotics, e.g. many insecticides.

2. ECOTOXICITY

Discussion of ecotoxicity often causes a dilemma. Frequently ecosystems are observed that show effects like changes in species composition, adverse behaviour of animals or increased occurrence of sickness, and it is generally known that such systems suffer from pollution by a complex mixture of eutrophicating, toxic and other, probably not dangerous, chemicals. However, it is not always possible to establish a causal relationship between pollution and its effect [10].

On the other hand, there are many ways of determining the toxic levels of selected pesticides or certain mixtures on a well-defined biological property, such as respiratory turnover, photosynthetic activity, etc., some of which are discussed later in this section. Such results establish a stringent relationship between pollution and one of its effects, sometimes called 'hard' methods in contrast to the 'soft' information described above, but the concentrations causing significant effects are usually much higher than those observed in the above-mentioned complex cases (see Refs [11–13]). Radionuclide methods are usually typical examples of 'hard' experiments. Turnover rates of tagged metabolites are accurately measured under well-defined laboratory conditions. Several authors have pointed out that such experiments should be carried out under conditions as near to nature as possible [14, 15]. Armstrong [16] reports an interesting relation between a 'hard' result (the toxicity of waters of Galveston Bay, Texas, was checked in a bioassay measuring the growth depression of blue green algae) and its general ecological relevance (growth depression is correlated to species diversity).

The essential process of self-purification of water bodies is the heterotrophic activity of aquatic bacteria. It is usually limited by oxygen depletion or it may be 'self-limited', i.e. the bacterial activity cannot compensate for the influx of organic material. One of the major concerns of ecotoxicity is the question of whether pesticides reduce self-purification in rivers or whether they affect the proper operation of sewage treatment plants.

Several reviews exist on pesticide interaction on the microflora of soils and water; they report on the negligible influence of pesticides on community gross respiration provided they are applied in reasonably low concentrations [13, 15, 17]. Sewage plants may be affected by intense pulses of poison, although such events seldom occur in urban sewage. A remarkable easing of the organochlorine charge in rivers has been observed since the DDT ban. In the Federal Republic of Germany, for example, lindane and HCB are now of major concern, but these pesticides are controlled more because of their importance to the human food chain than their environmental toxicity. However, there are regionally different situations in particular cases. In the lower Rhine self-purification is assumed to be reduced by one-third owing to a complex pattern of environmental chemicals, including pesticides [18]. Such a reduction may be caused either by damage to the heterotrophic activity which reduces the degradation of organic burden, or by lower primary production which reduces biogenic aeration; this may play an important role in large, slow-flowing lowland rivers. Such a burden is more remarkable, both from the economical and ecological standpoint, than occasional killing of fish, although it is less visible to the public.

A case of damage to primary productivity (measured by the ^{14}C method) by papermill effluents is reported in Ref. [19]. Butler's review [14] compiles the effects of numerous herbicides, insecticides and fungicides on algae. Usually, ^{14}C-dioxide uptake or growth has been used to establish the toxicity levels of

phytoplanktic organisms. Particularly dangerous are the phenylurea derivatives [14, 17, 20], whereas 2,4D is tolerated to much higher levels (approximately 100 ppm) [21].

A study of nonachlor and chlordane effects in the marine fauna off the Canadian east coast has not yet revealed toxic effects [22]. The bulk of the pesticides used are either herbicides acting on phytoplankton or insecticides acting on the nerves of higher animals. An example of the latter is given in Ref. [23], where the insecticide affected fish and zooplankton, whereas it caused a bloom in hydra, which does not have nerves. In experiments with *Chlorella pyrenoidosa* it has been shown [24] that exposure to HCB at levels of 0.1, 1.0 and 5 ppm inhibited photosynthesis but not, or hardly ever, respiration.

In bioassays for toxicity tests one may differentiate according to:

(1) *Time of incubation.* If the time of incubation is short compared with the life span of the test organism the test is called acute. Mortality, metabolic irregularities and adverse behaviour may be observed. In chronic tests the exposure time is comparatively long. Long-term metabolic and reproductive damage is checked.
(2) *Test form.* If the pesticide is administered once and the water is not changed the test is called static. Pesticide concentration will decrease during exposure. If it is to be kept constant automated flow-through methods have to be used.
(3) *Trophic level.* Primary productivity tests require illuminated assays for algal growth. Test parameters may be: oxygen evolution, ^{14}C-dioxide incorporation, chlorophyll measurements or cell counts. The first two parameters are well known in limnology for the measurement of primary productivity; for details see Refs [25–30]. Chlorophyll may be measured photometrically after extraction, if necessary even after chromatographic separation. All these methods require some care concerning incomplete extraction and proper discrimination between the different photosynthetic pigments, particularly as far as the unintentional degradation to phaeopigments is concerned; details are reported in Refs [28] and [31].

These problems are circumvented by in vivo fluorometric methods, which also save much work. However, in living algae the quantum efficiency may vary with physiological conditions. This can be avoided by blocking the electron transport chain with DCMU or CMU [31–34].

Pesticide concentrations preventing growth in phytoplankton are listed in Ref. [17]; they range from 0.02 ppb (urea derivatives) to 1000 ppm (trichlorphon). Fresh water algae that are frequently used for such tests are: *Chlorella* sp., which are distinguished by their storage capacity of lipophilic substances [35], *Scenedesmus quadricaudae, Skeletonema costatum, Microcystis aeruginosa* and *Anabaena flos-aque.*

For high trophic levels Daphnia and Guppy, Golden Orfe which have some central position in β-mesosaprobic waters [35], or Rainbow trout, as well as the mussel *Dreissena polymorpha,* are standard organisms. The relative toxicity of several pesticides to Daphnia are given in Ref. [17].

The heterotrophic activity of microorganisms has frequently been determined by measuring the mineralization of ^{14}C-glucose, ^{14}C-acetate and corresponding ^{3}H-labelled substances. ATP measurement by the luziferine-luziferase method [36] has also been used. Techniques to trap the ^{14}C-dioxide are described in Refs [37–39]. Albright has used heterotrophic activity methods with ^{14}C- or ^{3}H-labelled organic substrates (glucose, acetate or amino acids) to determine the effect of 11 heavy metals; ^{3}H-substrates can be achieved at higher specific activities. Since natural substrate concentrations are very low, such a high specific activity may be necessary not to disturb the natural system by too high a substrate addition. Michaelis-Menthen-kinetics, and deviations from it, have been found [37]. Further details of heterotrophic radiotracer techniques are described in Refs [38–40]. Since most insecticides act via nerve intoxication, anti-cholinesteratic activity is an important property. Horváth [41] and Horváth and Forster [42] have described an extremely sensitive method using carboxyacetylcholine as substrate and the ^{3}H liquid scintillation technique.

3. DEGRADABILITY

Metabolic transformations of the numerous pesticides in numerous organisms modified by numerous different conditions are very involved. It is impossible to establish simple unequivocal guidelines.

The major types of reactions are: oxidation, hydrolysis, reduction, conjugation, dehydrohalogenation, ring scission and, important for heavy metals, chelation. Despite these difficulties it is reasonable to establish a few guidelines which, however, should be applied carefully.

(1) Many pesticides are lipophilic, apolar compounds. They are usually metabolized to more hydrophilic, polar compounds (see Refs [43–45]) or they may be stored 'forever' in the body fat.

(2) Most metabolic transformation leads to less toxic compounds. There are some important exceptions to this principle, e.g. parathion may be transferred to paraoxon, aldrin to dieldrin and metals, particularly mercury, to methyl-metals. Methylation pathways are discussed by Wood and Fanchiang [46] and a radioanalytical method to determine vitamin B-12, the main methylating enzyme, is described in Ref. [47]. There are even pesticides like dichlorbenil, which is formed out of its inactive precursor chlorthiamide in the environment.

(3) Warm-blooded animals show a faster turnover than cold-blooded [48, 49]. In this context it should be mentioned that arctic fauna, with its long-living organisms, shows a particularly slow degradation [50].
(4) Korte [18] gives the following guidelines: alkyl groups are less persistent than branched groups, alkenes less than alkanes, and alkanes less than aromates. The persistence of rings increases with the number of substituted hydrogen atoms.

Degradation of pesticides shows similarities to degradation of natural organic compounds. The most prominent reaction is oxidation. Dagley [51] discusses various biochemical aspects of the biological oxygen activation, the central problem of which is that the ground-state of all organic compounds is a singlet, whereas dioxygen occurs in the triplet state. Thus, a straightforward reaction is forbidden.

In higher animals the mixed function oxydases of the liver are the most important scavengers of the organism [52]. Radiotracer methods are frequently applied in degradation studies. Most of this work is devoted to soil rather than limnic systems. However, the results have relevance for aquatic systems too. The differences are mainly:

(1) Primary producers occupy a much larger volume in the aquatic system (euphotic layer)
(2) Protozoa constitute a larger part of the biomass of the reducers
(3) Transportation, particularly from oxidizing to reducing sites, is much faster owing to turbulence.

Often the complete degradation scheme of a pesticide may be too tedious to be followed in detail. In such cases the ^{14}C-labelled pesticide may be introduced into a bioassay and the ^{14}C-dioxide evolving after complete mineralization is recovered (see Flores-Ruegg et al. [53]). Once the original material is transferred to ^{14}C-dioxide one can be sure that no potentially hazardous remnants are left, whatever the intermediate steps may have been. In such cases care must be taken not to take evaporated pesticides for ^{14}C-dioxide. A comprehensive collection of methods is compiled by Compaan [54]. Experiments on pesticide degradation are much too numerous to be reported on here.

The following papers have been selected to show some typical radiotracer applications:

Metcalf [55] designed the classical approach: an aquarium-like model ecosystem in which radiolabelled pesticides can be administered. Klein et al. [56] report the fate of ^{14}C-labelled aldrin, heptachlor and lindane in an outdoor model ecosystem containing soil and plants. Losses through evaporation and leaching water were checked and degradation products discussed. Sethunathan et al. [57] followed the degradation of ^{14}C-parathion in rice soils and observed that organic

matter enhanced its reduction. Carbon-14-dichlorbenil has been shown to be readily absorbed by fish and weakly hydroxylated; most of it evaporated [58]. The fate of ^{14}C-atrazine and 2,4D in bentic fresh water animals is described in Ref. [59]. A slight increase in 2,4D metabolizing microbes was observed after 2,4D treatment [60]. The fate of ^{14}C-lindane, which is becoming of major concern since the DDT ban, has been studied by Saha [61]. Degradation of pesticides may follow remarkably different pathways, whether or not it occurs in oxidizing or reducing environments. The numerous metabolic pathways of anaerobic organisms can sometimes attack compounds that are persistent in aerobic environments, e.g. DDT is converted to DDD in anaerobic soil [62] or lake water [63]. However, anaerobic conditions are disadvantageous for oxidative degradation reactions. In Refs [64, 65] tracer experiments on thiobencarb and phorate show this effect. Bioassays for such experiments require use of gas-tight containers flushed with helium, carbon dioxide, hydrogen or nitrogen. The ultimate degradation product of ^{14}C-labelled compounds need not be ^{14}C-dioxide [66]. A review on the anaerobic degradation of pesticides is given by Williams [67].

4. BIOACCUMULATION

This term is used here in terms of bioaccumulatory enrichment of environmental substances in organisms. Accumulation in sludge and similar problems are discussed in Section 5. Accumulation of pesticides in the food chain represents a major threat to man. This danger increases with the persistence of the substance and with its affinity to fat tissue. Accumulation and degradation are intimately linked. Often in a radiotracer experiment certain activity is found in some compartment of the bioassay and it is not known whether it is the parent pesticide or some degradation product. This will be dealt with under the term accumulation. This applies, for example, to the 'bound residues' in soil systems that have a certain analogue in limnic systems in the bottom sediments. Examples of bound residues and sediment accumulation of a series of pesticides are given in Ref. [68]. Many of the references discussed in Section 3 also deal with problems of accumulation. The interdependencies of all these questions lead to the concept of ecotoxicological profile analysis [35]. The pesticide that is best investigated with respect to its bioaccumulation is DDT. It has been estimated that the world's biota contain as much DDT as was produced in 4 d in the mid-sixties [69].

Storage in fat tissue is the most important effect in bioaccumulation. Hence, lipophilicity is one of the most important properties of environmental chemicals. For determination of this property one needs a 'standard fat'. In pharmaceutical research n-octanol, although not a fat, has proved that it fits this purpose. The water-n-octanol partition coefficient is currently the universally accepted parameter to predict accumulation in lipids. The experimental procedure comprises shaking

in a water-octanol mixture and determination of the ratio, usually by gas chromatography; details are given by Compaan [70]. If the substance in discussion is identified, the partition ratio can also be determined theoretically on the basis of the chemical structure; for details see Ref. [70]. Data on the fat content of different species are given by Morowitz [71] and particularly by Jorgensen [72].

Bioassays for accumulation tests differ from those for toxicity in so far as the concentration is kept well beyond the toxic level. Animals, with their fat tissues, are most prone to accumulation of lipophilic pesticides and therefore they play a role in the human food chain. Hence, most research has been carried out on crustaceans, clams and fish.

Uptake may be passive diffusion and sorption, active transport, or uptake via food. The different forms are not always easy to discriminate. Gills are usually the most important organ of uptake. The time courses for uptake and elimination have sometimes to be interpreted theoretically, particularly to get reasonable predictions on the times of incubation or exposure. It is usual to apply first-order kinetics similar to the concept of biological half-life applied in radiation biology; for theoretical details see Ref. [73] and for experimental evidence see Ref. [68]. An extended list of half-life values is found in Ref. [72]. Streit and Schwoerbel [59] report hyperbolic kinetics. In the same paper bioaccumulation of 10 pesticides in *Chlorella fusca* is reported. A case study on the accumulation of mercury in a Mexican river system has been carried out by Baez and Nulmann [74].

Interesting background information for pesticide adsorption is given in Ref. [75]. Sorption of 12 ^{14}C-tagged pesticides to humic substances and two synthetic polymers were measured and interpreted in terms of charge-transfer and hydrophobic interactions.

5. TRANSPORTATION AND SOURCES OF PESTICIDES

Sources of pesticides in water are either intentional or unintentional contaminations. The latter comprise surface runoff, leaching, dumping of pesticide remainders, washing of spray facilities and wind drift during spray operations.

Particular problems arise in rivers that flow through heavily industrialized areas. In Ref. [76] the case histories of the Hudson and Rhine rivers and of Galveston Bay, Texas, are given. Intentional contaminations originate from chemical ditch clearing or eradication of aquatic insects. A list of the different sources of pesticides is given in Ref. [17]. The problems of ditch clearing are dealt with in Refs [12, 77]. These authors do not see any serious environmental threat through using these methods provided the herbicide is handled carefully. The poisons in use are not persistent and significant contamination of waters downstream of the spraying area has not been observed. It should, however, be pointed out that no observation on species composition after extended use has been made. Most

of the herbicides in use were strongly bound to a particular material, a fact which stresses the importance of sedimentology in this field. The concentrations in use are given by Koch and Hurle [78], i.e. approximately 1 ppm (assuming 1 m depth). They assume that aeroplane spraying is only a minor source of contamination, whereas Erne [79] claims that negligent dumping of pesticide remainders is a major source.

Particular precaution has to be applied if large water bodies are treated for insect eradication programmes, e.g. problems of blackfly (*Simolinum arcticum* and *S. luggeri*) eradication with methoxychlor in large Canadian rivers have been reported by Haufe [80]. The insecticide was best administered at several points distributed across the river and with careful timing concerning non-target taxa. Absorption to particulate matter is discussed. Another large project is the Onchoceriasis Control Programme in the Volta river. In Ref. [81] the effects of aquatic pesticides are reported; these differ as to whether or not target taxa are emergent, submerged or floating weeds and whether or not they are rooted plants. Water soluble pesticides are usually degraded or evaporated within days or weeks. Vaporization is less important if the material is absorbed by sediments; this was shown with ^{14}C-thiobencarb by Ishikawa [64]. Diquat absorption to the sediments and its incorporation to the bottom fauna are shown in Ref. [82]. Sludge absorption of PCP, PCNB and HCB are described by Klein and Korte [68]. Problems similar to pesticide transport by sediments occur by surface runoff Metribuzin, trifluralin and MSMA have been studied in this concern by Wauchope et al. [83]; Wauchope has also reviewed the problem [84]. It has, for example, also been found that about 0.5% of applied commercial pesticides is lost through runoff. White et al. [85] also found insignificant runoff losses for 2,4D, whereas Vrochinskij [86] maintains that runoff is the commonest cause of surface water contamination.

The properties of sediment, such as grain size and consolidation (squeezing of water out of clay particles), and their relation to erosion and deposition and metal ion absorption are treated by Goltermann [1] and further details are given in Ref. [87]. Vaporization is another important feature of pesticide transportation, e.g. in the ^{14}C-aldrin experiment reported in Ref. [68] about one-half of the pesticide evaporated. In many cases the water-air partition coefficient for sparingly soluble compounds may be calculated theoretically by

$$K = 62.4 * C * T/P$$

where K is the partition coefficient (mol/l), C is the water solubility, T is the temperature (in kelvin), and P is the vapour pressure (mm Hg) [70].
However, one has to be aware that concentration of organic molecules near the surface may not correspond to the bulk concentration. Wu et al. [88] report a three-fold concentration of atrazine in the top 100–150 μm water layer. Similar results are reported by Bidleman and Olney [89] and Moriaty [90].

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Invited Paper

PESTICIDE RESIDUES IN FISH WITH EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES

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Abstract

PESTICIDE RESIDUES IN FISH WITH EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES.

Fish play an important role in the assessment of the fate and effect of pesticides in the aquatic environment, both on intentional and unintentional release. The roles of fish toxicity and human consumption are discussed with respect to uptake, metabolism and excretion, and including residues. In this context a great deal of work has been done with labelled pesticides which has had to be evaluated comparatively with other organisms. Accumulation, as a factor of pesticide mobility and an indicator of toxicity, has been very intensively studied in fish. As the latter are model ecological organisms for accumulation, many parallel investigations have been carried out with labelled and non-labelled material. Owing to the generally low metabolic rate detected, accumulation in fish is largely due to the parent compound in the case of persistent pesticides. The methods used ranged from simple laboratory tests to experiments in the open environment.

INTRODUCTION

Fish belong to the vaste number of non-target organisms exposed to pesticides. Consequently, there is a need for including them in monitoring systems, from the point of view of their use as a food commodity as well as their indicatory function for environmental pollution. Measurements of pesticide residues in fish, however, only provide the results of exposure that has already occurred. In general, they are not prospective and do not provide an understanding of the quantitative exposure, roots of uptake, conversion, degradation and elimination. These factors, which determine the relationship between pesticide residues and the concerned organisms, have to be included in assessment of the hazard potential of a pesticide.

TABLE I. ENVIRONMENTAL HAZARD PROFILE

Concept:	–	Comparative assessment in a system of tests.
Aims:	–	Ranking of organo-chemical substances.
	–	Screening for ecotoxicological potential.
	–	Priority list for further studies

Development of data on the fate and behaviour of pesticides in the aquatic environment are predominantly developed from experiments using labelled material. This method provides the means to assess a pesticide qualitatively and quantitatively at any time of the experiment. Consequently, two types of publications have to be considered in this review: those which only give residue data from monitoring or trace analyses programmes, and those describing experiments with labelled pesticides.

HAZARD-RANKING

Investigating pesticides in fish consists of several approaches and a variety of experimental methods. These also include investigations where fish are but one parameter in a set of investigative factors.

The Environmental Hazard Profile, which has been developed in our institute, is devoted to the hazard-ranking of organic chemicals in general [1]. Table I shows the concept and aims of this system of tests.

The system shown in Fig. 1 comprises simple experiments on bioaccumulation in algae, fish and sewage sludge, storage in rats, as well as biodegradation by sludge and abiotic degradation/mineralization by sunlight. Hazard-ranking is possible by comparative evaluation of the data obtained, which can then be used as a basis for further detailed investigations.

The test apparatus shown in Fig. 2 for the fish accumulation test [2, 3] has no complicating elements.

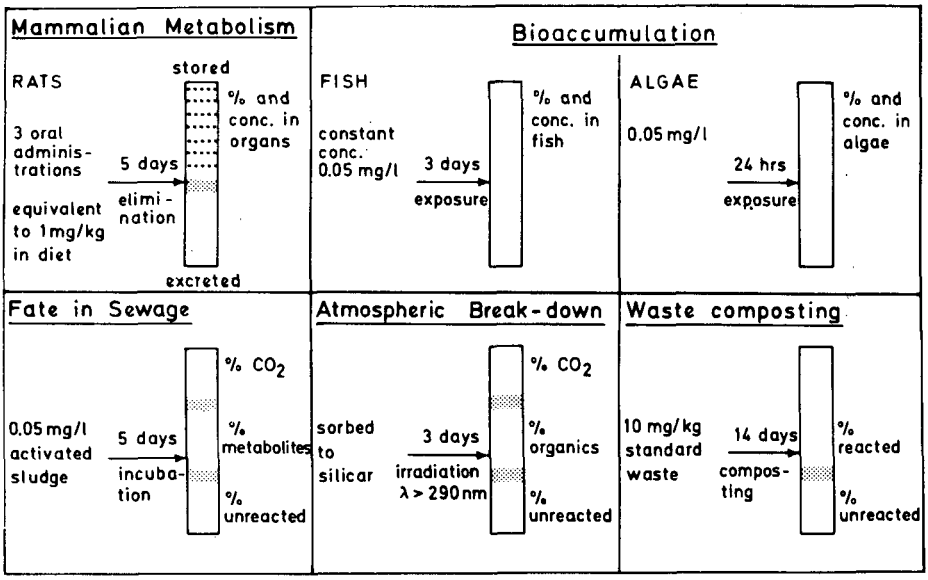
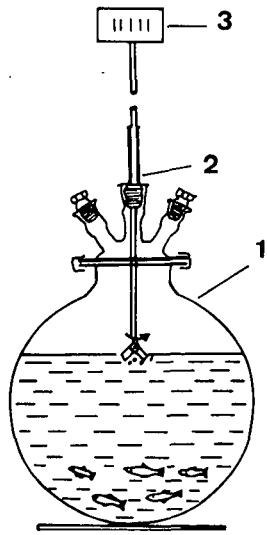


FIG.1. Environmental hazard profile: test system.



1 12.5 l flask
2 stirrer
3 stirrer motor

FIG.2. Fish test apparatus.

TABLE II. BIOACCUMULATION OF 80 ORGANIC CHEMICALS IN FISH (Golden Orfe) AFTER 3 DAYS OF EXPOSURE

Aldrin	3885	Toluene	94	4-Chlorbenzoic acid	*
2,4'-Dichlorobiphenyl	3550	Trichloroethylene	88	Carbontetrachloride	*
2,4,6,2'-Tetrachlorobiphenyl	3150	Benzidine (hydrochloride)	83	Atrazine	*
Pentachlorobenzene	3000	4-Nitrophenol	57	Nitrobenzene	*
Dieldrin	2945	Hexadecanol (Cetylalkohol)	56	Urea	*
DDT	2866	n-Dodecan	52	Belgard (HPMA)	*
2,5,4'-Trichlorobiphenyl	2620	Chlorhexidine	42	Vinylchloride	*
2,2'-Dichlorobiphenyl	2420	Hydrochinone	40	Diethylenglycol	*
2,4,6,2',4'-Pentachlorobiphenyl	2316	2,6-Dichlorobenzinitrile	36	2,4-Dichlorobenzoic acid	*
Phenanthrene	1760	Carbaryl	34	Thiourea	*
Hexachlorocyclopentadiene	1231	Ethylacetate	26	Ethylendiamine-(dihydro-chloride)	*
Hexachlorobenzene	1165	Chlorferon	24		
Pentachloronitrobenzene	1130	Na-Acetate	12	Zineb	*
Pentachlorophenol	1050	Naphthaline	11	Maneb	*
Anthracene	911	Sencor (Metribuzine)	11	Coumarin	*
γ-Hexachlorocyclohexane	754	Cortisoneacetate	10	Ethylenethiourea	*
2,6-Di-tert-butylphenol	687	2,6-Dichlorobenzamide	10	Propylenethiourea	*
3,3'-Dichlorbenzidine	609	Dibenz(a,h)anthracene	10		
Kepone	586	4-Chloroaniline	*		
1,2,4-Trichlorobenzene	491	Perylene	*		
Benzo(a)pyrene	481	Benzene	*		
Di(2-ethylhexyl)-phthalate	456	Monolinuron	*		
ß-Hexachlorocyclohexane	450	Succinic acid	*	Bioaccumulationfactor:	
Benz(a)anthracene	347	4-Bromobenzoic acid	*		
2,4,6-Trichloroaniline	327	Docosane	*	$BF_n = \frac{\mu g \text{ chemical/g fish}}{\mu g \text{ chemical/g water}}$	
2,4,6-Trichlorophenol	310	p-Phenylendiamine-(HCl)	*		
Biphenyl	281	2,4-Dichlorophenoxyacetic acid	*		
n-Dodecylbenzenesulfonate	129	Methanol	*	n = 3d	
4-tert-Butylphenol	118	Benzoic acid	*		
Coumaphos	106	Aniline	*		
2,4-Dichlorophenol	100	Tristearine	*		
Ethylpalmitate	98	Maleic acid anhydide	*		

* = below 10

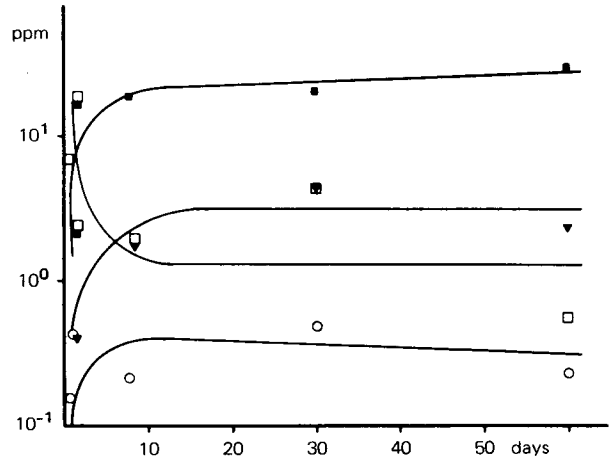


FIG.3. DDT concentrations in various Perch tissues (○ muscle; ▼ gills; □ liver; ■ mesenteric adipose) as a function of time after labelling the pond. From Ref.[4].

The fish are kept for three days under starvation conditions in water containing the ^{14}C-labelled test chemical. Bioaccumulation factors are calculated from the concentration in the fish and in the water at the end of the experiment.

So far 80 chemicals have been screened in the test system. In Table II the accumulation factors are listed with decreasing values. The highly accumulating chemicals include chlorinated benzenes and phenoles, and some of the classical pesticides.

OUTDOOR EXPERIMENTS

Contrary to the above method, experiments in natural ponds are most complex. Salonen and Vaajakorpi [4] applied DDT in a natural pond of 1100 m^2 surface to investigate the distribution of DDT in biota.

In fish, DDT concentrations were determined for muscle, gills and liver; in Perch, concentrations were also measured for mesenteric adipose (Fig. 3) and levels were marked in the gills and liver. The highest level in Perch (Perca fluviatilis L.) (23.8 ppm) was observed in the mesenteric adipose at the end of the test period. As the Crucian carp (Cyprinus earassius L.) has no uniform mesenteric

TABLE III. PER CENT DISTRIBUTION OF ^{14}C-LABELLED PCBs, DDT AND DDE AT END OF EXPERIMENT IN GREEN SUN FISH (*Lepomis cyanellus* Raf.).

	Parent	Polar[a]
Trichlorobiphenyl	18.39	81.61
Tetrachlorobiphenyl	98.84	1.16
Pentachlorobiphenyl	99.41	0.69
DDE	99.00	1.00
DDT	97.23	2.27

[a] RF = 0 Skellysolve B.

adipose, comparisons could not be made. For both species, the lowest levels were found in the muscle tissue. The highest concentration (16.1 ppm) found in the Perch liver was measured in the one-day sample. It declined fairly rapidly thereafter, showing a fast tissue response to the DDT added to the water. The highest concentration in the liver of the Crucian carp (0.39 ppm) was measured after four days. This is below the lowest value of the Perch liver (0.52 ppm), and was measured at the end of the test period.

The differences are probably caused by the physiological differences of the species and their different means of obtaining nutrients. Perch belongs to a group that has a more efficient metabolic system and a higher oxygen demand. Metabolically less efficient species, such as Cyprinids, have been observed to accumulate less toxin in water than species with an efficient metabolism.

Whereas in this work ^{14}C-labelled DDT was used, attempts have also be made to carry out comparable investigations using non-labelled material; for example, work by Gasith and Perry [5], who investigated the fate of parathion in a fish pond ecosystem and the effects of parathion on food-chain organisms. To a limited degree investigations are pos-

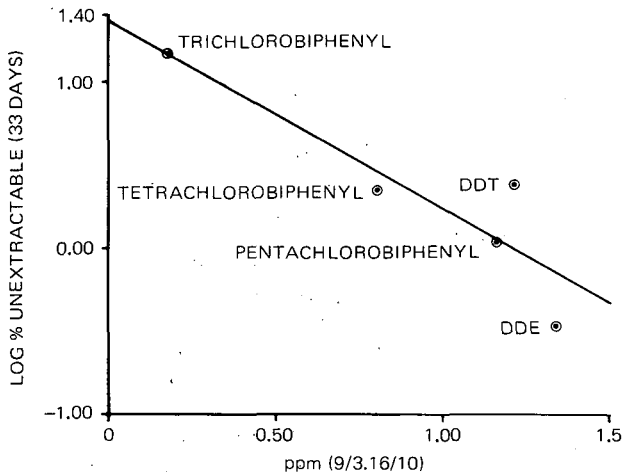


FIG.4. Relationship between the unextractable radioactivity in Mosquito fish (Gambusia affinis) *at the average change in concentration of radioactivity from day 3 to day 9 (ppm 9/3) and day 10 to day 16 (ppm 16/10).*

sible, but they are very time consuming and great effort is needed compared with investigations with labelled material.

OTHER METHODS AND RESULTS

Between the two extremes there are a number of other ways of investigating pesticide/fish chemical interactions.

Sanborn et al. [6] investigated the uptake and degradation of DDT, DDE and three ^{14}C-labelled PCBs in Green Sun fish (Lepomis cyanellus Raf.). As can be seen in Table III, the metabolism rate decreases with increasing chlorination.This well-known correlation has also been confirmed for other methods, e.g. in the model ecosystem by Metcalf [7] using Mosquito fish (Gambusia affinis Baird and Girard).

In the 33-day model ecosystem experiment (Fig. 4) an interesting relationship is shown between the dynamics of uptake, that is the change in concentration of the biphenyls in the fish after labelled compounds are added to the water, and the per cent unextractable radioactivity in Mosquito fish.

This is as logical a relationship as could be expected. The greater the metabolic susceptibility the larger the amount of incorporation of ^{14}C radioactivity into highly polar compounds.

In an investigation by Herbst et al. [8] only 3% metabolism were found for 2,2-dichlorobiphenyl in Gold fish (Carassius auratus). It should be mentioned, however, that the radioactivity in the open experimental facility was reduced by 87% with fish and by 98% without fish (control system) during the duration of the experiment. This is due to the extremely high volatility of this chemical and demonstrates that positive balances are urgently needed for an appropriate interpretation of experimental data. Guiney et al. [9] investigated the distribution and elimination of 2,2',5,5'-tetrachlorobiphenyl during the early life stages of Rainbow trout (Salmo giardueri).

Figure 5 shows that there was a rapid loss of radioactivity during the first day after transferring exposed eggs to uncontaminated water. This was seen by the percentage ^{14}C-residue in the whole egg on day 0 (100%), which dropped to 83% on day 1. After this initial, rapid loss elimination of the chemical from the egg was slow, namely with an elimination rate of t 1/2 = 231 days. This continued until near the end of the sac-fry stage of development. At this time (day 49), the oil globules in the yolk fluid were beginning to be absorbed by the larvae and the PCB residue level in the whole sac fry began to decrease at a more rapid rate. Thus, in days 42 to 49 of the sac-fry stage the percentage of initial PCB residue in sac-fry dropped from 74 to 62%. This more rapid rate of elimination continued through the fry stage of development, until only 5.5% of the initial PCB-derived radioactivity remained in the fry at the end of 105 days. The t 1/2 for elimination from day 49 of the sac-fry stage to day 105 of the fry stage was 14.6 days.

This rapid elimination is in sharp contrast to excretion by adult Rainbow trout, where very slow rates were found, namely an elimination of t 1/2 = 1.76 years in females and 1.43 years in males. There are two explanations for this. One is the low fat content of the fry compared with the adult, the other is the higher metabolic activity of the fast-growing fry.

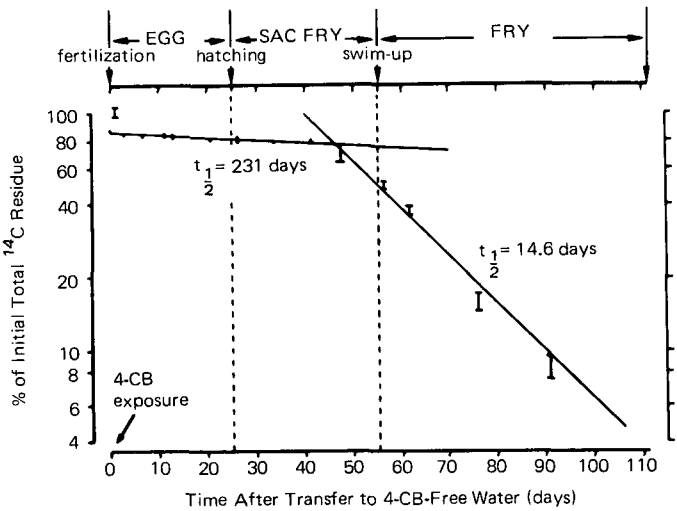


FIG.5. Elimination of ^{14}C-4-CB residues during early development of Rainbow trout. Each point and associated vertical line represents the mean ± SE (n = 15). The t1/2 of 231 days was calculated from means for days 1 – 42; t1/2of14.6 days was calculated from means for days 49 – 105. The time course of the early development in this study is given at the top of the figure and the duration of 4-CB exposure at the lower left. From Ref.[9].

Work by Melancon and Lech [10] demonstrated the formation in Rainbow trout bile of ^{14}C-labelled conjugated metabolites following exposure to ^{14}C-labelled TCB. The experimental demonstration was done by glucuronidase hydrolysis and derivatization followed by gas chromatography.

Although the extent of TCB metabolism by Rainbow trout and also by Sun fish appears to be low, even this level could be important in view of the high concentration of PCBs found in various fish species. Frequently, significant amounts of hydroxylated PCBs being released into the environment from the large fish biomass have been discussed as an additional hazard.

Pritchard et al. [11] described the limited ability of Flounder to metabolize and eliminate injected ^{14}C-DDT to DDE and more polar metabolites. Only 2% of the total contained in fractions other than urine were metabolites, whereas more than 75% of the ^{14}C-urine (2% of injected dose) appeared as metabolites.

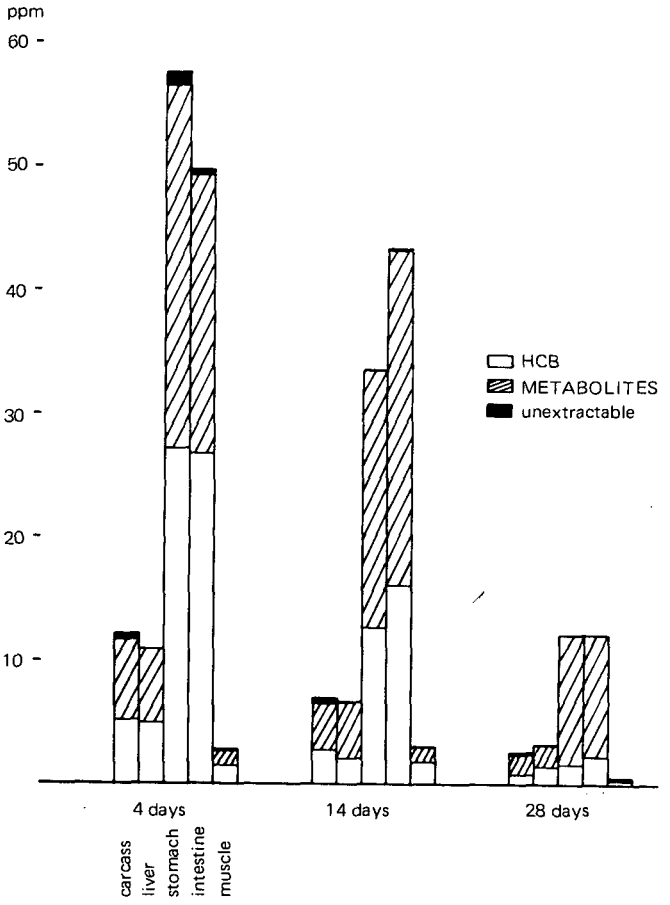


*FIG.6. Distribution of ^{14}C-hexachlorobenzene and its degradation products in Green Sun fish (*Lepomis cyanellus *Raf.). From Ref.*[6].

The majority of experiments used exposure of fish via the aqueous medium. Fewer experiments have been done with exposure by food. Sanborn et al. [12] investigated the fate of ^{14}C-hexachlorobenzene in Green Sun fish after feeding on contaminated food.

Figure 6 shows that HCB, which is claimed to be highly persistent, is fairly well metabolized. This is contradictory to a vast number of investigations where HCB has been demonstrated to be persistent, so the work mentioned here should be carefully interpreted.

Feeding ^{14}C-hexachlorobenzene-contaminated food to Green Sun fish at 1, 10, and 100 ppm resulted in residues as expected, i.e. related to the concentration in the food. As regards distribution in organs and tissues, the highest levels were found in the alimentary tract of the fish and the lowest levels in the skeletal muscle. PCP was a major metabolite.

The apparent half-life for elimination of HCB, PCP and the other derivatives ranged from 8.0 days for elimination of HCB from the stomach-pyloric caeca to 16.6 days for elimination of PCP from the same organ.

During the experiment the ratio of HCB to PCP changes in the various organs and tissues of fish. This is in agreement with the behaviour of chemicals in other organisms. For all tissues, with the exception of the muscle, the ratio of HCB to PCP decreases from day 4 to day 28 as HCB is metabolized to PCP. Values range from near unity for the liver to approximately 0.20 for the stomach-pyloric caeca. However, the skeletal muscle has an increasing HCB:PCP ratio. A nearly 22 times greater HCB content is reached in muscle than in the stomach, the tissue with the lowest HCB:PCP ratio. This can easily be explained by continuous redistribution in organs and tissues, parallel to metabolism.

Pentachlorophenol was intensively studied by Kobayashi [13] and other groups. It was rapidly absorbed by Gold fish from the nutrient media and it accumulated in various organs, especially the gall bladder, where the concentration increased rapidly with exposure time and even displayed an increase after termination of exposure, whereas a decrease was observed in all other organs (Fig. 7). Excretion into surrounding water was mostly in a conjugated form, it being identified as pentachlorophenylsulphate.

Most of the PCP found in the gall bladder was also a conjugate, it being identified as pentachlorophenyl-ß-glucuronide. It was revealed that fish dispose of PCP by both the sulphate and glucuronide conjugations, contrary to the conclusion of earlier investigations.

The sulphate conjugation could also be found in in vitro experiments for different chlorophenols.

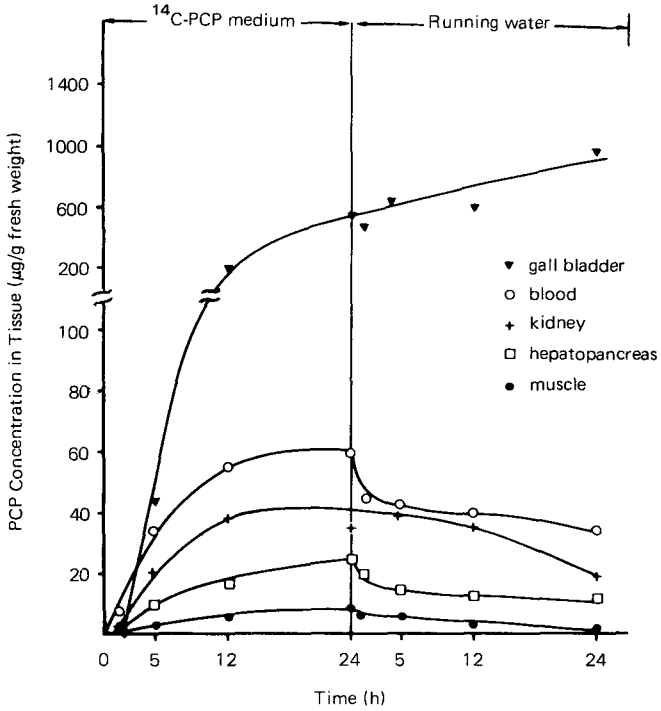


FIG. 7. Change in concentration of ^{14}C-PCP in various tissues of Gold fish during exposure to PCP medium and running water after 24-h exposure.

With the exception of 4-chlorophenol, low concentrations of activity (0.07-0.13 mM in incubation media) were found for each of the investigated chlorophenols. There was an abrupt decrease of activity by increasing the substrate concentrations, as shown in Fig. 8 [13] . An increase of the Cl-atom number in the chlorophenols caused a decrease of the sulphate conjugation activity of the soluble fish liver fractions. The decrease in conjugation capacity goes parallel with an increase in fish toxicity [13].

The major elimination pathways for the two conjugates of PCP in fish are bronchial excretion of the sulphates and biliary excretion of the glucuronides.

Differences between fresh and sea water have been demonstrated by Tulp et al. [14].

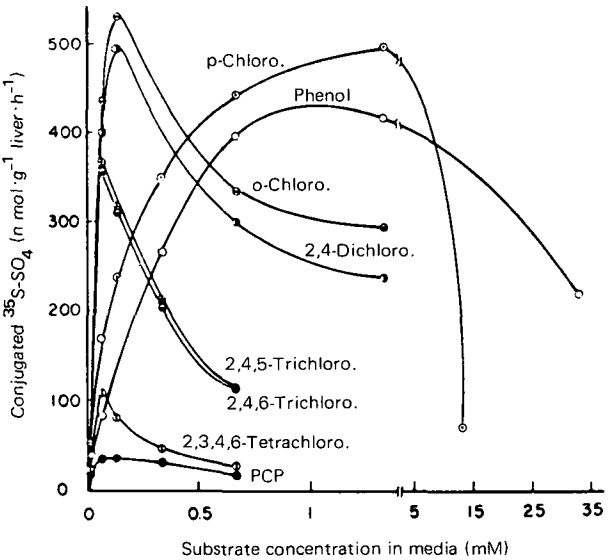


FIG.8. Sulphate conjugation of various chlorophenols by the soluble liver fraction of Gold fish. (From Ref.[13].

Uptake of ^{14}C-2,2',4,5,5'-pentachlorobiphenyl by appropriately adapted juvenile Atlantic salmon (Salmo salar) is more efficient from fresh water than from sea water (Fig. 9). Consequently, the concentrations of ^{14}C-PCP in the whole body as well as in a number of organs and tissues were correspondingly higher in salmon exposed in fresh water.

These differences in one organism exposed to a chemical either in fresh or in sea water can be explained by different osmotic regulation, as shown in Fig. 10.

In fresh-water fish, water enters the bloodstream mainly through the gill epithelium; in salt-water fish, it is transported into the bloodstream across the intestinal epithelium, assuming that compounds dissolved in water enter the body via the same route. It can be concluded that absorption of ^{14}C-PCB is more efficient through the gill epithelium than through the intestinal epithelium.

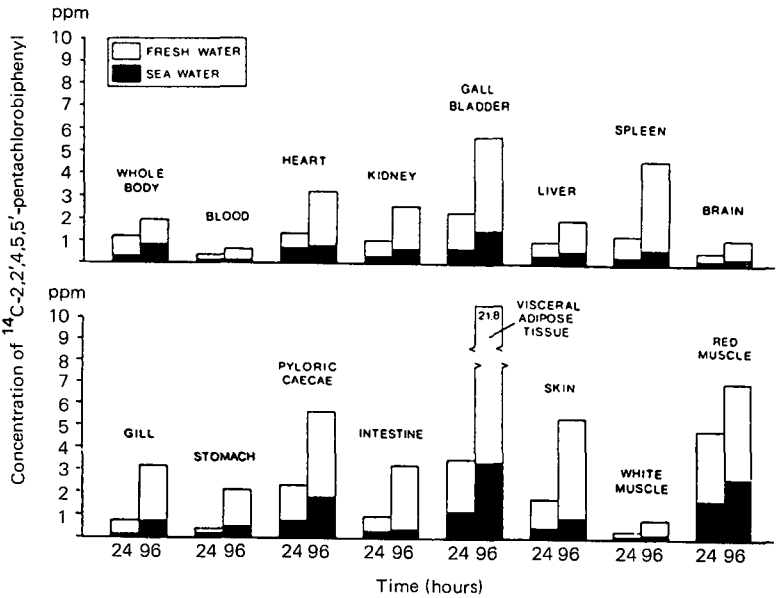


FIG. 9. Tissue distribution of ^{14}C-PCB in juvenile Atlantic salmon after exposure to contaminated fresh water and sea water. From Ref.[14].

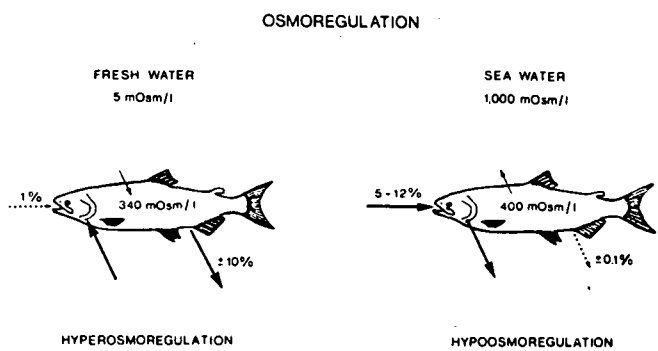


FIG. 10. Hyper- and hypoosmoregulation: differences in water balance. Large arrows indicate major routes of water intake and excretion; dotted arrows are minor routes. The direction of the flow of water through the skin is indicated by a small arrow. Percentages are per body weight per day. From Ref.[14].

Lee at al. [15] studied uptake, metabolism and elimination of ^{14}C-naphthalene and ^{3}H-3,4-benzopyrene by marine fish. These chemicals are mentioned although they are not pesticides but occur globally.

Three species of marine fish (Mudsucker or Sand goby, Gillichtys mirabilis; Sculpin, Oligocottus maculosus; Sand dap, Citharichthys stigmaeus) were exposed to the two chemicals for various periods of time. Within minutes all three fish species rapidly absorbed the hydrocarbon through the gills. Radioactivity build-up was mainly in the liver where metabolism took place. Elimination was via the bile. Naphthalene resorption was more efficient than uptake of benzpyrene. Based on the radioactivity data in the liver, there was already a steady-state of concentration after a few hours of exposure. Major metabolites were identified as dihydroxynaphthalene and dihydrodihydroxybenzpyrene. The gall bladder was the major organ for storing chemicals and metabolites, consequently urine was the most important excretion route. After termination of exposure the radioactivity in various tissues was reduced tenfold within 24 hours. Elimination of naphthalene was in total greater than that of benzpyrene. Again, in this case there are the same detoxification mechanisms prevailing as already mentioned, namely conjugation with sulphate and glucosiduronic acid.

The oncogenetic effects of the so-called carcinogen benzo(a)pyrene (BAP) on three species of larval Flat fish were investigated by Hose et al. [16], using concentrations from 0.10 to 4.2 ppb, which were comparable to levels found in polluted harbours. It is not usual that specific toxicity investigations are done using the labelled material, but in this case the ^{14}C-labelled BAP was used to facilitate analyses, e.g. for taking autoradiographs. BAP-treated sand sole (Psettichthys melanostichus) eggs showed a decline in hatching success and a higher incidence of anomalies than did control eggs. Flathead sole (Hippoglossoides elassodon) eggs exposed to a single dose of a water-soluble BAP-bovine serum albumin complex demonstrated evidence of toxic injury with pycnotic nuclei present in the integument and, more commonly, in ocular and neural tissues. An increased incidence of morphological anomalies in English sole (Parophyrs vetulus) eggs and larvae exposed to BAP was not detected [16].

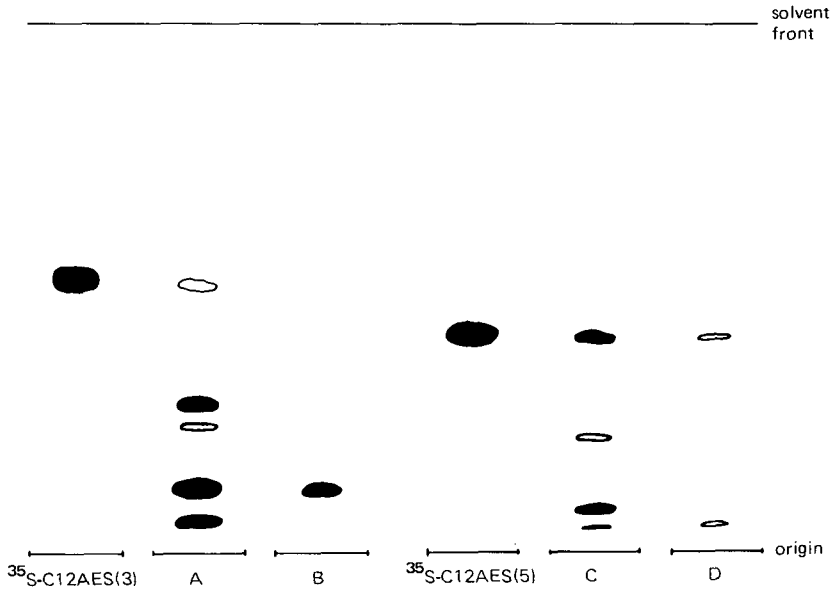


FIG.11. Radio-thin-layer chromatograms of ^{35}S-C12-AES(3), ^{35}S-C12-AES(5) and their metabolites on silica gel developed in 1-butanol: acetic acid: water (4:1:1) in Carp exposed to 0.56 – 0.63 mg/L solutions A-bile extract of fish exposed to ^{35}S-C12-AES(3); B-hepatopancreas extract of fish exposed to ^{35}S-C12-AES(3); C-bile extract of fish exposed to ^{35}S-C12-AES(5); D-hepatopancreas extract of fish exposed to ^{35}S-C12-AES(5). From Ref.[17].

Detergents in Carp (Cyprinus carpio) were investigated among others by Kikushi et al. [17]. They studied ^{35}S-labelled material, namely sodium dodecyltri(oxyethylene) sulphate (^{35}S-C_{12}-AES(3)) and sodium dodecylpenta (oxyethylene)sulphate (^{35}S-C_{12}-AES(5)) (Fig. 11). The number of oxyethylene units affects the distribution patterns in organs and tissues. The ^{35}S-radioactivity derived from the test chemical with three oxyethylene groups was higher in the gills and lower in the gall bladder than that derived from the chemical with five oxyethylene groups. This is in agreement with the increased rate of metabolism in the detergent with three oxyethylene groups.

A further example that small variation in the molecule leads to a significant difference in the behaviour is the compound pair pentachlorophenol and pentachloroanisole (Fig. 12), which were investigated by Lech et al. [18].

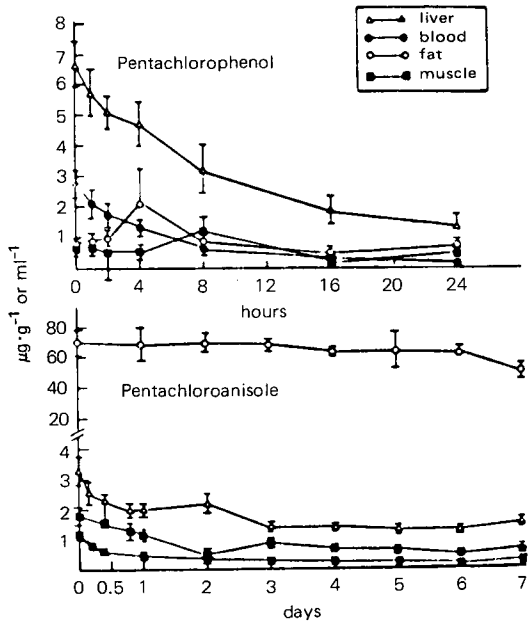


FIG.12. Elimination of ^{14}C from PCP and PCA exposed Rainbow trout. From Ref.[18].

Pentachlorophenol and pentachloroanisole were rapidly taken up by Rainbow trout at the concentrations of these compounds in water of 0.025 µg/ml. Following a 24-h exposure of trout to ^{14}C-PCP the liver, blood, fat and muscle contained 16, 6.5, 6.0 and 1.0 µg/g PCP, respectively. Exposures of trout to ^{14}C-PCA resulted in tissue concentrations of a similar magnitude, with the exception of fat where PCA concentrations reached levels of up to 80 µg/g. Elimination studies indicated that PCA was retained in most tissues considerably longer than PCP. The half-lives for PCP residues in blood, liver, fat and muscle were about 6, 10, 23 and 7 h, respectively, while the half-lives for PCA in the same tissues were 6, 7, 23 and 6 d. Thin-layer chromatography and GC-MS analyses of the PCP-exposed trout failed to demonstrate methylated PCP (PCA) in any of the tissues studied. Bile from trout exposed to PCP showed high concentrations (250 µg/g) of PCP-glucuronide. The bile of trout exposed to PCA contained both PCA and PCP glucuronide. The presence of PCP glucuronide in bile indicated some demethylation of PCA in vivo by Rainbow trout.

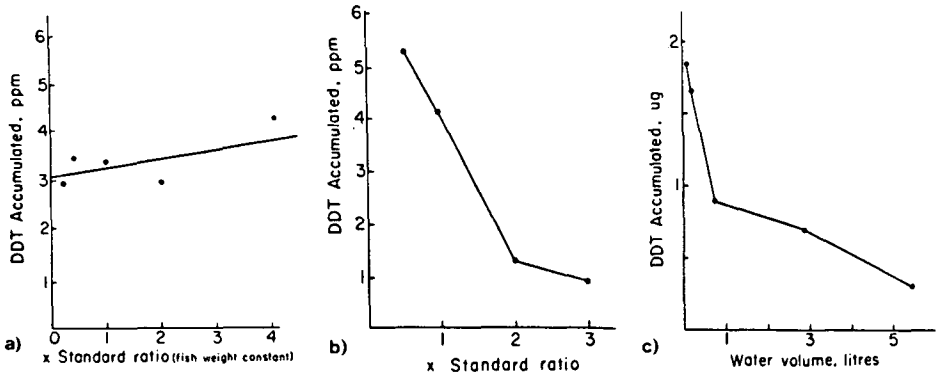


*FIG. 13(a). Effect of varying the scale of the system on the level of bioaccumulation in fish Common minnow (*Pimephales promelas*). The volume of water, amounts of DDT and sand were proportionally varied, except that the size of fish was kept constant. (b) Effect of varying the scale of the system including the size of fish on the level of DDT accumulation. (c) Variation of the water volumes affecting the level of bioaccumulation. In this experiment, the amount of DDT and sand and the size of fish were not changed proportionally.*

The experiments cited demonstrate that there is a vast number of factors influencing the behaviour of pesticides in fish, even if some examples were not pesticides.

Absorption, accumulation and elimination of several labelled pesticides in different aquatic organisms were investigated by Matsumura [19] using a model system. Since many pesticides have a low water solubility, sufficient exposure was ascertained by mixing the pesticide with sand or sediment, respectively, thus simulating the naturally occurring sedimentation of pesticides.

In this system DDT accumulation was studied in the Common minnow (Pimephales promelas Rafinesque). The size of the system has no influence on accumulation provided the weight of the fish is constant (Fig. 13(a)). The increase of the fish weight shows a proportional decrease of the accumulated amount (Fig. 13(b)). An increase in water volume leads to a decrease of the accumulated amount (Fig. 13(c)).

Keeping all other variables constant and only increasing the amount of exposed fish leads to a decrease in accumulation (Fig. 14(a)). Decreasing

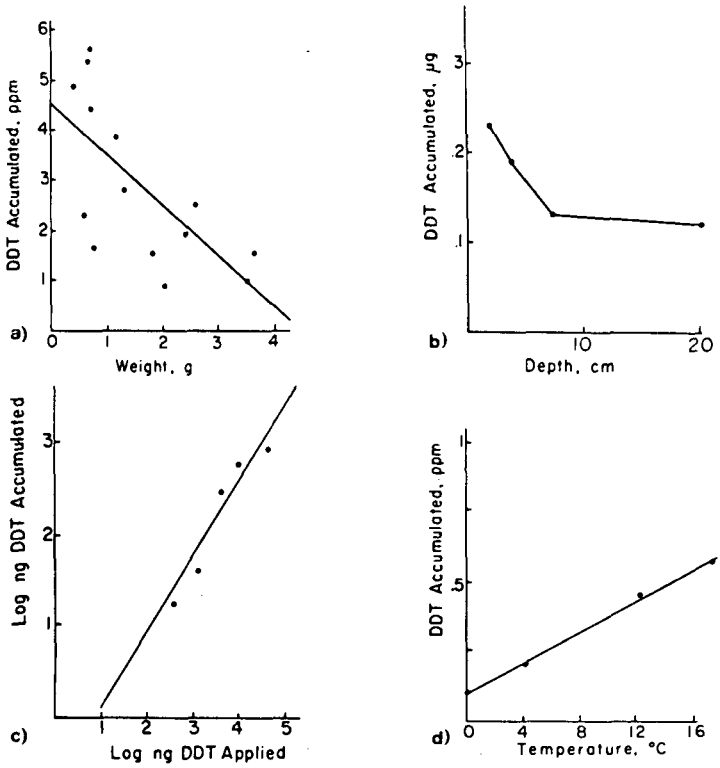


*FIG. 14(a). Effect of variation of fish sizes on the level of bioaccumulation of DDT in Common minnow (*Pimephales promelas*) under standard model conditions. (b) Effect of varying the depth of the system, at a constant volume of water, on the level of bioaccumulation of DDT in fish. (c) Effect of changes in the amount of applied DDT on the level of bioaccumulation of DDT (the amount of sand was also proportionally changed). (d) Effect of changes in ambient temperature on the level of DDT accumulation in fish.*

the depth of water (Fig. 14(b)) but keeping the water volume constant brings the fish nearer to the dosed sand surface; by direct contact the uptake is increased. An increase of DDT concentration (Fig. 14(c)) results in an increased accumulated amount of the chemical, whereas within a certain range the accumulation factor remains constant. An increase of temperature results in a proportional enhancement of accumulation (Fig. 14(d)).

These results were also shown in experiments using carbon-14-labelled pentachlorophenol and a

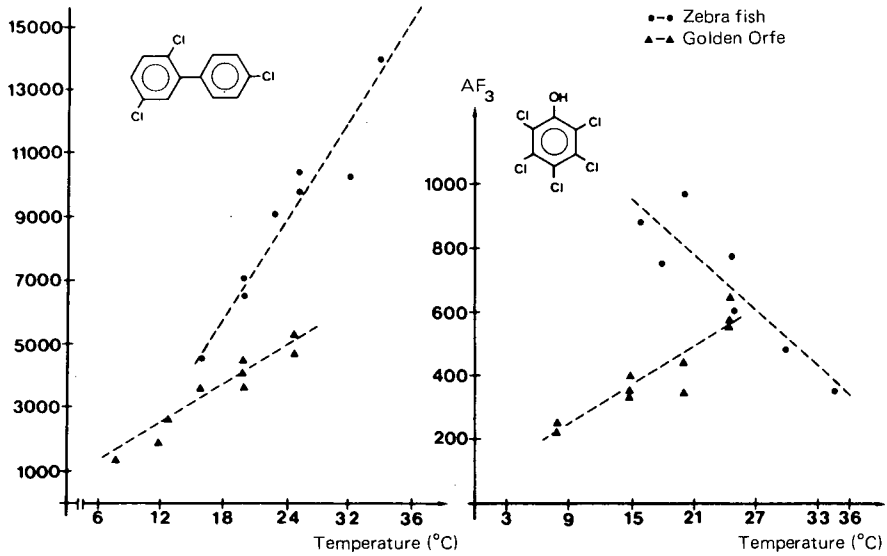


*FIG. 15. Accumulation factor of TCB and PCP in Golden Orfe (*Leucisens idus melanolus*) and Zebra fish (*Brachydanio rerio*) (temperature).*

trichlorobiphenyl. In these experiments by Bude [20] with Golden Orfe and Zebra fish (Brachydanio rerio) in the test apparatus shown earlier, these factors were investigated systematically.

The opposite effect of temperature using pentachlorophenol in Zebra fish can be explained by increased detoxification and elimination at elevated temperature (Fig. 15).

The pH-value has no influence on the accumulation of TCB. For PCP, however, dissociation leads to increased water solubility and consequently a decrease of the accumulation factors (Fig. 16). Thus, accumulation decreases with increasing pH-value.

The situation is similar when assessing the influence of water hardness on accumulation (Fig. 17).

The distribution of radioactivity was measured in organs and tissues of Golden Orfe for both TCB and PCP, and the highest values were found in the viscera. Compared with carcass PCP storage in the viscera it was 20-fold higher and TCP storage 3-fold

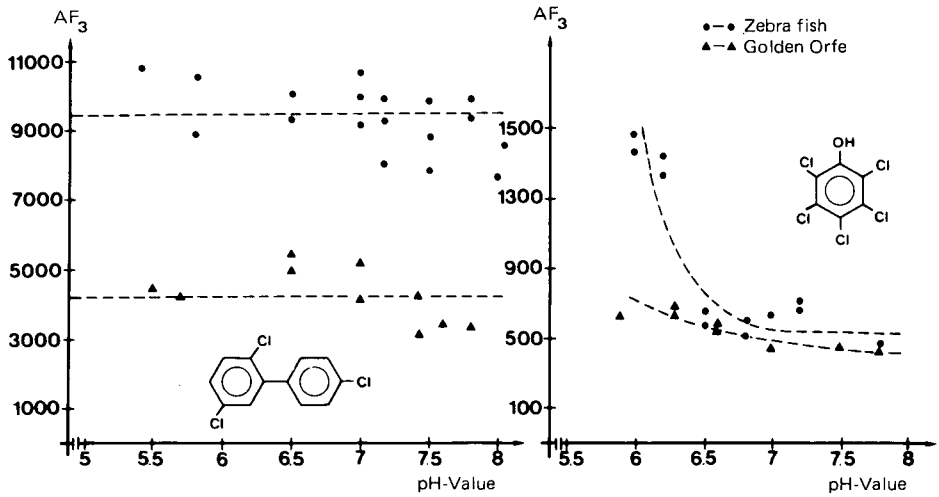


*FIG.16. Accumulation factor of TCB and PCP in Golden Orfe (*Leucisens idus melanolus*) and Zebra fish (*Brachydanio rerio*) by different pH-values of the water.*

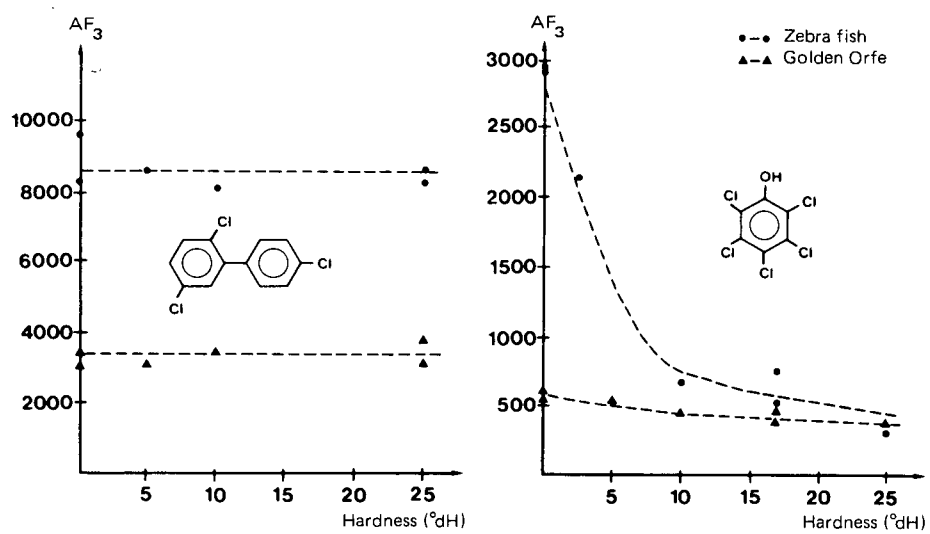


*FIG.17. Accumulation factor of TCB and PCP in Golden Orfe (*Leucisens idus melanolus*) and Zebra fish (*Brachydanio rerio*) (water hardness).*

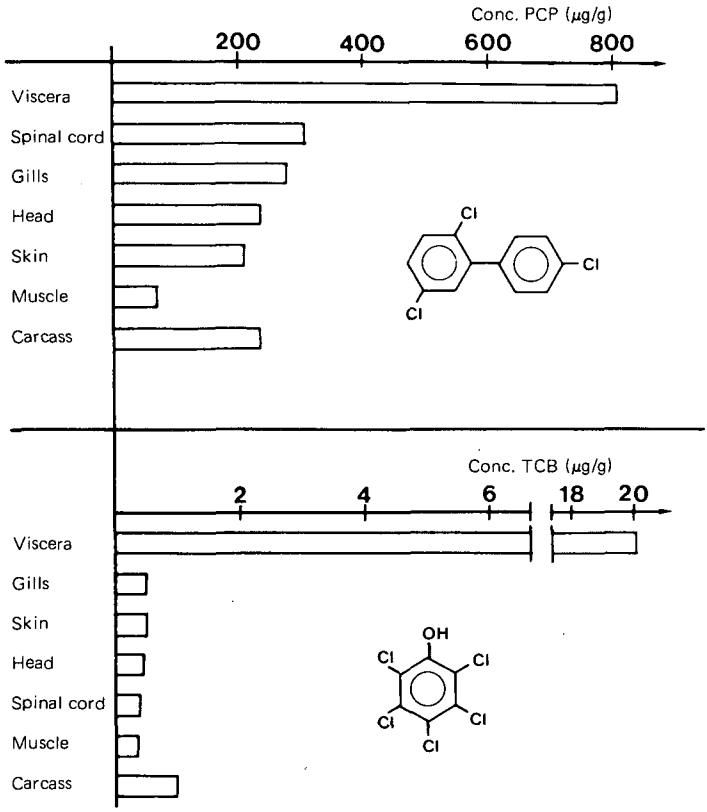


*FIG.18. Distribution of TCB and PCP in organs of Golden Orfe (*Leucisens idus melanolus*).*

higher. The gall bladder of PCP-exposed Golden Orfe had a 50- to 60-fold concentration compared with the whole fish (Fig. 18).

These results are comparable to data from other investigations and they show that using a more feasible experimental method appropriate indications on the fate of a pesticide can be obtained. If these indications show a certain potential for persistence or increased accumulation, a step-wise assessment with more sophisticated methods is indicated.

CONCLUSIONS

In general, there are two major questions to be considered in the frame work of pesticides in fish.

One is their accumulation, the other is their metabolism or degradation in the organism. Most work has been done on accumulation of chemicals in fish and many correlations have been elaborated, such as water solubility and accumulation. Is accumulation in fish indicative for other aquatic organisms, or even in organisms in general? Another question that is still being debated is whether determination of the toxicity of a chemical to fish is indicative for other aquatic organisms. This leads to the problem of the low metabolic activity of fish compared with terrestrial animals.

For future research it will be essential to study the fate of a new pesticide in fish, or to study the fate of a pesticide in fish with a new use or in a new area. However, one major topic should be a comparative investigation of aquatic organisms as well as aquatic and terrestrial organisms in order to establish reliable correlations and thus reduce the impact on ecosystems by repeated testing in too many species.

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APPLICATION OF A RADIOMETRIC ENZYMIC METHOD FOR MONITORING ORGANOPHOSPHOROUS AND CARBAMATE INSECTICIDE RESIDUES IN WATER OF THE RIVER DANUBE*

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Abstract

APPLICATION OF A RADIOMETRIC ENZYMIC METHOD FOR MONITORING ORGANOPHOSPHOROUS AND CARBAMATE INSECTICIDE RESIDUES IN WATER OF THE RIVER DANUBE.

The necessity of a monitoring method that is sensitive to the anti-cholinesteratic quality of pesticides is obvious. For this purpose we applied a radiometric version of the enzymatic determination technique. Acetylcholine labelled with tritium in the acetyl moiety was used as substrate. The method is suitable for detection of the ppm level of organophosphorous or carbamate insecticide residues in water. From 1978 onwards water of the river Danube was regularly monitored by means of the radiometric enzymic method. In the minority of cases significant levels of residues were detected. Samples of extreme values were extracted and analysed by gas chromatography. During the monitoring programme it was recognized that water samples possess an acetylcholine hydrolysing ability. This parameter was also detected and plotted. The method was also used in laboratory model experiments for investigating degradation of pesticides in various water samples.

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INFLUENCE OF THE ORGANOPHOSPHOROUS INSECTICIDES ACEPHATE AND PARATHION ON THE HETEROTROPHIC BACTERIA OF TWO FRESHWATER ECOSYSTEMS*

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Abstract

INFLUENCE OF THE ORGANOPHOSPHOROUS INSECTICIDES ACEPHATE AND PARATHION ON THE HETEROTROPHIC BACTERIA OF TWO FRESHWATER ECOSYSTEMS.

Acephate, a relatively new organophosphorous insecticide, is increasingly used in North America for control of forest insects, whereas parathion, an older organophosphorous insecticide, is currently employed in Israel for crop protection with emphasis on cotton. In both instances, since application is usually by aerial spraying, these pesticides often enter adjacent and commercially important water bodies. In western Canada/United States of America these are salmonid spawning rivers, whereas in Israel they are fish (*Tilapia*/carp) ponds. Concern has often been expressed about the effects of these pesticide additions on these aquatic ecosystems, which support a fishery. This project was therefore initiated to investigate the effects of acephate and parathion on one biotic component of aquatic food webs, the bacteria of the plankton and benthos. Limnocorrals of 2 m in diameter by 5 m in height were placed in Shirley Lake, a Canadian west coast temperate/dystrophic lake (49° 21′ N, 122° 33′ W), whereas limnocorrals of 1.2 m in width by 1.2 m in length by 1 m in height were placed in a fish pond located at the Fish and Aquaculture Research Station, Dor, Israel (32° 37′ N, 34° 4′ E). The former limnocorrals were treated with 1, 10 or 25 ppm acephate, whereas the latter were treated with 30–40 ppb parathion. Both acephate and parathion additions at these concentrations had little effect on the bacterial activities as determined by the glucose heterotrophic potential technique. In addition, parathion treatment at 30–40 ppb had little apparent effect on the bacterial numbers and productivities within the fish pond limnocorrals. Acephate,

* L.J. Albright and G.H. Geen were associated with the acephate portion and L.J. Albright, A. Gasith, Y. Mozel and A.S. Perry with the parathion portion of the experiments described. The abstract only is published here, since it is intended that the full paper will appear in the IAEA-TECDOC Series (unpriced publication).

however, when added at concentrations of 1, 10 or 25 ppm, stimulated benthic and planktonic bacterial productivities. It was therefore concluded that there is no appreciable adverse influence of these two insecticides on the heterotrophic bacterial component of freshwater food webs at concentrations likely to be encountered in water bodies within and adjacent to these sprayed areas.

Poster Presentation

IAEA-SM-263/53

A TEST FOR ENVIRONMENTAL CHEMICALS AFFECTING PHOTOSYNTHESIS

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A chlorophyll fluorescence test has been designed to measure the effect of environmental chemicals in aqueous solution. It was checked by comparison with radiotracer experiments.

Fluorescence of chlorophyll occurs from the lowest singlet to the ground state. It may be quenched by triplet transitions and radiationless transitions. Of the latter, natural quenching by the photosynthetic process is the most important. Its intensity depends on the photosynthetic turnover of the plants, but it is additionally influenced by factors such as previous illumination, temperature and nutrient supply, or metabolic inhibitors such as DCMU, or environmental chemicals.

The basic concept of this method is to add the substance under investigation to a healthy alga culture and to observe the increase in fluorescence. The fluorescence of any plant after a certain dark interval (some minutes) is not constant, but follows the curve described by Kautsky, which displays a maximum peak some seconds after the start of excitation and then tapers out to an asymptotic value. With increasing damage by the herbicide the difference between the maximum and the asymptotic value decreases.

These effects have been used to test a series of chemicals, mainly pesticides (Fig.1). They occurred within 5 min of addition of the substance, whereas heavy metals (mercury, cadmium and lead) needed 24 h to show an effect. Synergisms between cadmium, lead and monuron could not be detected.

This fluorescence test has the advantage of being very quick and owing to the portability of the instrument it can easily be carried out in the field.

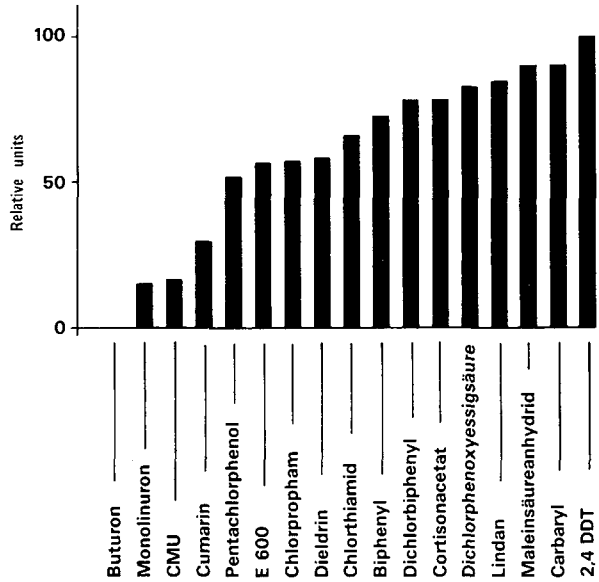

FIG.1. Kautsky effect of 17 environmental chemicals.

The fluorometer designed for these measurements incorporates the following features:

(1) Fixed wavelengths. Since the fluorometer is only for chlorophylla measurements, the excitation and emission wavelengths are fixed; hence, filters were used instead of costly monochromators.
(2) Sedimentation of algae out of the light path during measurement. A vertical light path avoids this error.
(3) Weight. The apparatus is designed as a light weight field instrument; a flash lamp minimizes battery weight and a photodiode avoids the expense of a multiplier.
(4) High efficiency and low background noise require a special design. Excitation light from the lamp is successively filtered three times; light that is not absorbed in the cuvette is reflected back to the sample.

The reliability of this fluorometric measurement of the turnover of the electron transport chain has been checked by incorporating ^{14}C-labelled CO_2 into algae.

Environmental chemicals may be bound to particulate matter. To follow such substances in the water body, methods are being planned for tagging aqueous sediments with tracers that can be activated by neutrons (e.g. dysprosium).

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EFFECTS OF PARATHION ON THE ECOLOGY OF A EUTROPHIC AQUATIC ECOSYSTEM

*Limnocorral experiment**

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Abstract

EFFECTS OF PARATHION ON THE ECOLOGY OF A EUTROPHIC AQUATIC ECOSYSTEM: LIMNOCORRAL EXPERIMENT.

Repetitive exposure of a eutrophic fish pond ecosystem, enclosed in limnocorrals to 30 ppb parathion, resulted in elimination of the potentially dominant zooplankton species, *Moina micrura*. Consequently, populations of the rotifers *Brachionus* and *Asplanchna* increased markedly. Changes in the zooplankton composition and abundance were followed by increased fluctuations in phytoplankton biomass, phytoplankton photosynthesis, plankton respiration and community metabolism. Similar responses to parathion treatment were observed in previous studies under fish pond conditions. In the presence of fish the effect of parathion on the ecosystem was generally less pronounced and not uniform. Collapse of the zooplankton community and rapid changes in limnological conditions in the control (untreated) limnocorrals severely limited the duration of the experiment. The results suggest that under eutrophic conditions small enclosures may be useful for evaluation of the effect of toxicants on the ecosystem only in short-term experiments with short-lived chemicals.

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PESTICIDES IN SOIL
(Session IV)

Chairman

E.P. LICHTENSTEIN
United States of America

Invited Paper

BOUND PESTICIDE RESIDUES IN SOIL, PLANTS AND FOOD WITH PARTICULAR EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES

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Abstract

BOUND PESTICIDE RESIDUES IN SOIL, PLANTS AND FOOD WITH PARTICULAR EMPHASIS ON THE APPLICATION OF NUCLEAR TECHNIQUES.

Although so-called bound residues have been detected for all classes of chemicals investigated so far, their quantitative levels have wide-ranging differences, depending on the chemical structure of the pesticide; phenols and nitrogen-containing pesticides exhibit the highest binding rates. The portion of bound residues in soil and plants increases with time and varies with environmental conditions (soil and plant type, climatic conditions, etc.). So far the chemical identity of bound residues has only been elucidated for a limited number of model substances by using various liberation techniques. Most information is available on anilines; models have been developed demonstrating their copolymerization into natural macromolecules (humic acids, lignin). Misinterpretation of natural products assimilated from totally degraded pesticides, as bound xenobiotic residues can only be excluded by the sophisticated separation and identification procedures of all residues. Studies on the persistence of bound residues showed differing mineralization rates, depending on the chemical structures of the pesticides; research on the bioavailability revealed that for all pesticides low amounts of soil-bound residues are taken up by plants, and that plant-bound residues are eliminated rather quickly by mammals in the faeces.

INTRODUCTION

Although non-extractable or non-recoverable pesticide residues have been known for decades, their intensive discussion started only when it became obvious that all chemicals lead to some strongly adsorbed or covalently bound portions in the biosphere. This was primarily the consequence of increasing use of nuclear techniques which, after application of radiolabelled pesticides, led to the

detection of residues which were undetectable by any analytical method, presupposing the extraction of the residue. In contrast to traditional techniques, nuclear techniques - the most commonly used being labelling of the pesticide with ^{14}C - enable detection and exact quantitation of residues by combustion and $^{14}CO_2$ determination without preceding extraction steps. However, the chemical nature of bound residues as well as mechanisms and consequences of binding cannot be established in this way and require more sophisticated research which, up to now, is only at its beginning for a few chemical classes. Thus, the topic of non-extractable pesticide residues remains controversial despite a vast amount of effort spent in recent years. The Pesticide Commission of the International Union of Pure and Applied Chemistry initiated a project on non-extractable pesticide residues. For convenience the definition and some statements adopted by the IUPAC Commission are cited.

"IUPAC Pesticide Commission: NON-EXTRACTABLE PESTICIDE RESIDUES IN PLANTS AND SOILS

Definition

Non-extractable residues (sometimes referred to as "bound" or "non-extracted" residues) in plants and soils are defined as chemical species originating from pesticides, used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues. These non-extractable residues are considered to exclude fragments recycled through metabolic pathways leading to natural products.

Chemical species in this context refers either to the parent material or to derivatives or fragments of it.

Methods in this context refer to any procedures, such as solvent extraction and distillation, used to exhaustively remove chemical species from a soil or plant matrix. In each reference to a non-extractable residue, the extraction procedure must be given.

Properties and relevance of non-extractable pesticide residues

When significant concentrations of the non-extractable pesticide residues (structurally related to the parent pesticide) occur in soil or plants, the properties of these residues should be investigated, the most relevant being:

In soil

- The bio-availability to plants and to soil organisms
- The persistence in soil
- The mobility in soil

In plants

- The bio-availability to man and animals
- The distribution within the plant.

Following these investigations, if a non-extractable pesticide residue in soil is
a) not bio-available to plants or soil animals
b) not persistent or
c) not mobile
or if a non-extractable residue in plants is
a) not bio-available to man or animals or
b) not located within the edible parts of the plant,
then such residues can be considered insignificant. If, however, based on the above criteria, a non-extractable pesticide residue is considered relevant, further work on a case-by-case basis, depending on the chemical nature of the residue, may be required. The following paper critically reviews experimental approaches to the study of non-extractable pesticide residues."

The major question still under discussion is the significance of non-extractable residues even when the magnitude formed is known. This is even valid for binding in animal tissues, especially to proteins. In the following sections, a survey is given on the results obtained in the last years on the correlation of binding with the chemical structure of the parent compounds, time course of binding, environmental factors influencing binding rates, binding sites and mechanisms, laboratory techniques for the liberation of bound residues, their chemical identity as well as their persistence and bioavailability.

TABLE I. PESTICIDE RESIDUES BOUND IN SOIL
(in % of applied amount)

Chemical class	No. of representatives evaluated	Time of exposure	% residue bound (range)	References
Free phenols	2	1 vegetation period	50–58	[1, 2]
Anilines without N-substitution	2	1 vegetation period	31–58	[3, 4]
	4	6 weeks	56–65	[5]
Triazines	2	4–12 months	49–57	[6, 7]
Urea herbicides	3	1 vegetation period	28–41	[3, 8, 9]
Carbamates	2	30–32 days	17–57	[10, 11]
Organophosphates	6	7–84 days	18–80	[12–17]
Anilines with N-substitution	5	7 months	7–21	[18]
	3	12 months	20–56	[19]
Dodecachloropentacyclodecane insecticides	2	1 vegetation period	1–9	[20, 21]
Cyclodiene insecticides	2	1 vegetation period	1–8	[22, 23]

PESTICIDE RESIDUES BOUND IN SOIL

Table I does not give a complete review of all bound residues reported thus far in soil, but gives only a few examples to demonstrate their occurrence in all the chemical classes investigated [1-23].

The table shows that chlorinated hydrocarbons, like dodecachloropentacyclodecane insecticides (e.g. kepone and kelevan) or cyclodiene insecticides (e.g. aldrin and dieldrin), as shown in the last lines, form only small portions of bound residues in soil, whereas phenols and anilines and their derivatives

TABLE II. INFLUENCE OF ANILINE FORMATION TENDENCY OF ^{14}C-LABELLED CHEMICALS ON THE FORMATION OF BOUND RESIDUES IN SOIL
(outdoor conditions)

Chemical applied	Aniline metabolites identified	%[a] unextractable in soil
p-chloroaniline		95
3, 4-dichloroaniline		84
Monolinuron	p-chloroaniline derivatives	74
Buturon	p-chloroaniline	53
Imugan	3, 4-dichloroaniline	32
Pentachloronitrobenzene	Pentachloroaniline	11

[a] % of total radioactivity recovered in soil at harvest.

TABLE III. INFLUENCE OF PHENOL FORMATION TENDENCY OF ^{14}C-LABELLED CHEMICALS ON THE FORMATION OF BOUND RESIDUES IN SOIL
(outdoor conditions)

Chemical applied	Phenolic metabolites identified	%[a] unextractable in soil
2, 4, 6-trichlorophenol		91
Pentachlorophenol		87
2, 2′-dichlorobiphenyl	Dichlorobiphenylols	42
Chloroalkylene-9	Dichlorobiphenylols	40
2, 5, 4′-trichlorobiphenyl	Trichlorobiphenylols	19
Lindane	Tri-, tetra- and pentachlorophenols	–
Pentachloronitrobenzene	Pentachlorophenol	11
2, 4, 6, 2′, 4′-pentachloro-biphenyl	Not identified	7
Hexachlorobenzene	Not identified	1

[a] % of total radioactivity recovered in soil at harvest.

have a high binding tendency. Carbamates, triazines and organophosphates also form considerable amounts of bound residues. It should be mentioned that some of the organophosphates evaluated in this list (parathion, methyl-parathion, fonofos, fenitrothion, phorate and phosalone) contain amino groups in their molecules or form amino groups by metabolic reactions, which might contribute to their high soil binding rates.

Tables II-IV give further examples on the relation of soil-binding tendency of pesticides to their chemical structure. The data have been obtained in our Institute by long-term experiments with plant-soil systems under outdoor conditions.

Table II shows the percentages of unextractable residues, based on total radioactivity recovered in soil, for two free anilines and four pesticides which have been shown to form anilines by metabolic reactions. In the case of free anilines, the portion of bound residues is lower for the representatives with higher chlorine content. For the pesticides, also, the percentage of unextractable residues decreases with increasing number of chlorines present in the metabolic aniline molecules.

Table III gives a similar relationship for two phenols and seven chemicals which form phenols in soil. Here also, the amounts of unextractable residues decrease with the increasing number of chlorine atoms in the phenolic metabolites. Although polychlorinated biphenyls are not used as pesticides, they have been included into this table to demonstrate the clear relationship between the chlorination degree of metabolites and the formation of bound residues. For 2,4,6,2',4'-pentachlorobiphenyl and hexachlorobenzene, the formation of phenolic metabolites is suspected but could not be confirmed unequivocally due to the very low level of soluble metabolites present. The very low amount of bound residues corresponds to these observations.

Table IV summarizes the reverse relationship of chlorination degree and formation rate of unextractable residues for three chemical classes investigated in our laboratory (polychlorinated biphenyls, phenols and anilines).

TABLE IV. INFLUENCE OF CHLORINE CONTENT OF ^{14}C-LABELLED CHEMICALS ON THE FORMATION OF BOUND RESIDUES IN SOIL (outdoor conditions)

Substance class	Chemical applied	%[a] unextractable in soil
Polychlorinated biphenyls	2, 4, 6, 2′, 4′-penta-chlorobiphenyl	7
	2, 5, 4′-trichlorobiphenyl	19
	Chloroalkylene-9 (2 chlorines)	40
	2, 2′-dichlorobiphenyl	42
Phenols	Pentachlorophenol	87
	2, 4, 6-trichlorophenol	91
Anilines	3, 4-dichloroaniline	84
	p-chloroaniline	95

[a] % of radioactivity recovered in soil (0–10 cm depth) at harvest.

Laboratory experiments carried out by several groups show an increase of bound residues in soil with time, as shown in Fig. 1 for two organophosphates, methyl-parathion and dyfonate, and two chlorinated hydrocarbon insecticides, dieldrin and DDT [13].

Within 28 days all substances show a decrease in total residues, a decrease in extractable residues, and an increase in bound residues. However, as discussed below, bound residues are susceptible to microbiological degradation. Thus, if the experimental time is extended to several years, the proceeding formation of bound residues from the parent compound or from soluble metabolites may compensate for a slow mineralization, resulting in a nearly constant level of bound residues in soil for many years, in spite of a considerable decrease of total residues, as shown in Table V for aldrin under outdoor conditions.

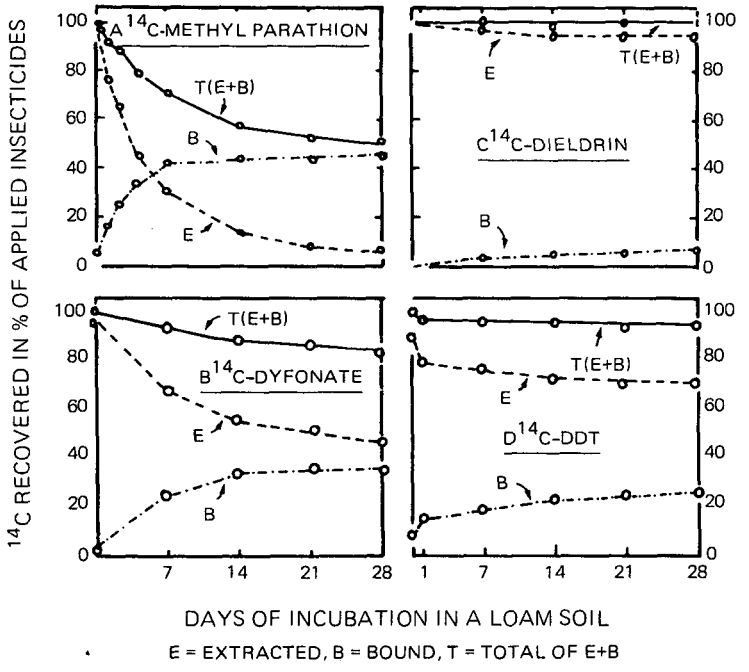

FIG. 1. Time course of binding of ^{14}C-labelled insecticides to soil.

TABLE V. BOUND AND TOTAL RESIDUES OF ^{14}C-ALDRIN IN SOIL DURING 10 YEARS AFTER APPLICATION
(0–10 cm depth, outdoor conditions)

Year	Bound residues (μg/g dry soil)[a]	Total residues (μg/g dry soil)[a]
1969, after application	0	2.72
1969, after harvest	0.17	1.87
1970	0.11	1.18
1971	0.06	0.57
1972	0.13	0.64
1973	0.11	0.50
1974	0.09	0.44
1975	0.11	0.43
1979	0.11	0.35

[a] μg equivalent to aldrin.

TABLE VI. DISTRIBUTION OF ^{14}C-RESIDUES OF ^{14}C-HYDROXYMONOLINURON-β-GLUCOSIDE IN TWO STERILIZED AND NON-STERILIZED SOILS AFTER 34 DAYS
(laboratory conditions; % of applied radioactivity)

% ^{14}C recovered as	Non-sterilized soils		Sterilized soils	
	Soil 1	Soil 2	Soil 1	Soil 2
$^{14}CO_2$	23.0	22.6	0.20	0.20
Extractable	21.1	23.0	74.1	92.2
Unextractable	55.7	52.7	27.0	7.1
Sum recovered	99.8	98.3	101.3	99.5

From Ref. [24].

TABLE VII. INFLUENCE OF SOIL COMPOSITION ON THE FORMATION OF BOUND CARBARYL RESIDUES IN SOIL

Soil number	Clay (%)	Silt (%)	Organic matter (%)	Bound residues (% of applied amount)
D	19	29	1.5	17.4
F	11	6	3.3	32.1
E	28	32	4.1	17.2
A	40	29	5.8	34.7
C	21	35	12.8	49.0

From Ref. [11].

Table VI [24] demonstrates the contribution of biotic reactions to the overall formation rate of bound residues in soil for a metabolite of the herbicide monolinuron under laboratory conditions.

The unextractable portion, as shown in the third line, for both soils is considerably lower after

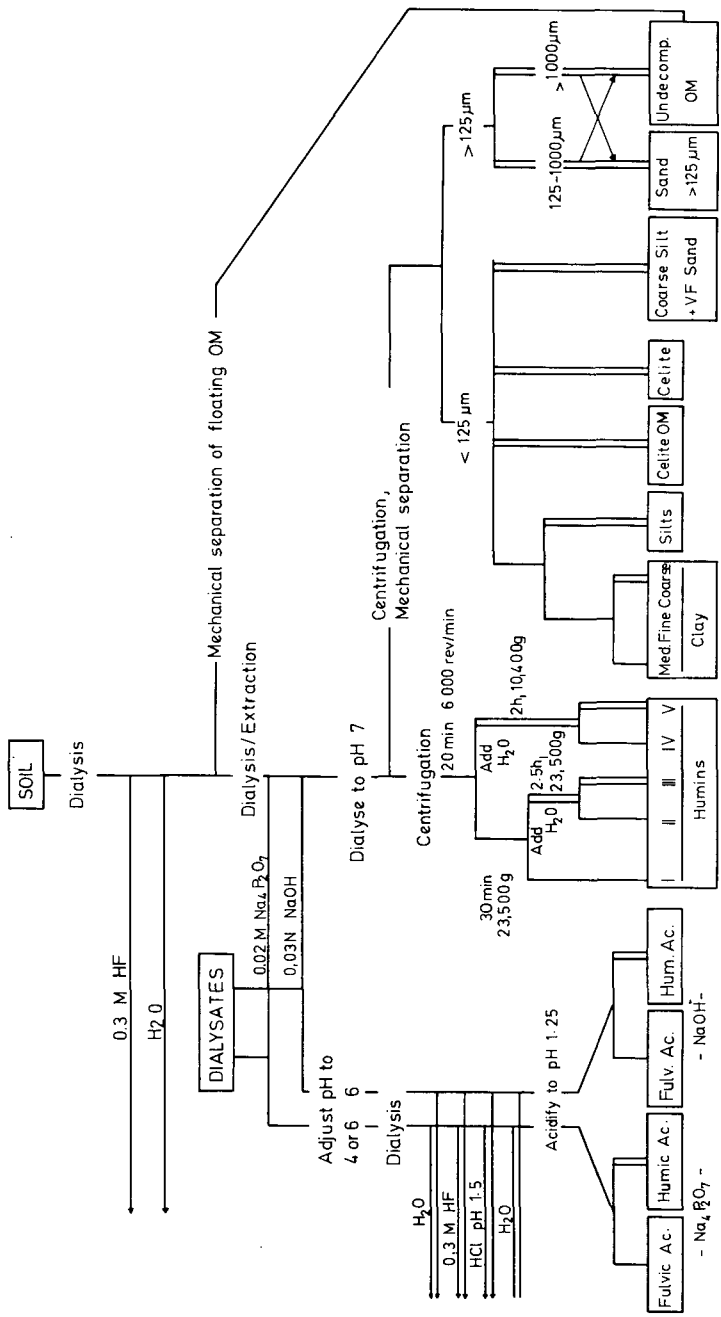

FIG. 2. Separation scheme of soil into various organic and inorganic fractions.

sterilization; however, binding is not fully suppressed. Indeed, for anilines it has been demonstrated that abiotic chemical reactions contribute significantly to the formation of unsoluble complexes with inorganic as well as organic soil constituents, as will be discussed below.

In Table VII [11] the formation of bound residues from carbaryl is presented for five different soils. The binding rate is roughly correlated to the organic matter content; no correlation seems to exist with the particle size distribution (content of clay or silt).

However, in most cases the formation of unextractable residues in soil cannot be correlated to a unique soil fraction. Therefore, the question of localizing the binding sites in soil has been a focus of research interest. The classical fractionation scheme separating soil organic matter into humin, humic acids and fulvic acids by alkaline treatment followed by acid precipitation has been used by several authors [7, 18, 25, 26]. Bound pesticide residues were detected in all of these fractions. A more sophisticated fractionation scheme is presented in Fig. 2.

This scheme [18, 27] permits the complete separation of soil into a number of organic and inorganic fractions. Bound residues of the herbicide butralin were detected in all of these fractions, but humic acid and silt were the major binding sites. It may be concluded that organic as well as mineral soil constituents must be considered in the research for binding mechanisms of pesticides.

Figure 3 is a scheme of the structure of a so-called expanding clay mineral. The adsorption sites between the tetraedral and octaedral silicate sheets normally are discussed in the context of adsorption-desorption studies, i.e. the adsorption to these sites is assumed to be reversible. However, the occurrence of bound residues in mineral soil fractions suggests that also at these sites an unextractable fixation of pesticide molecules is possible. Hysteresis (= non-coincidence of adsorption and desorption isotherms), a phenomenon which has been observed in many pesticide adsorption studies including

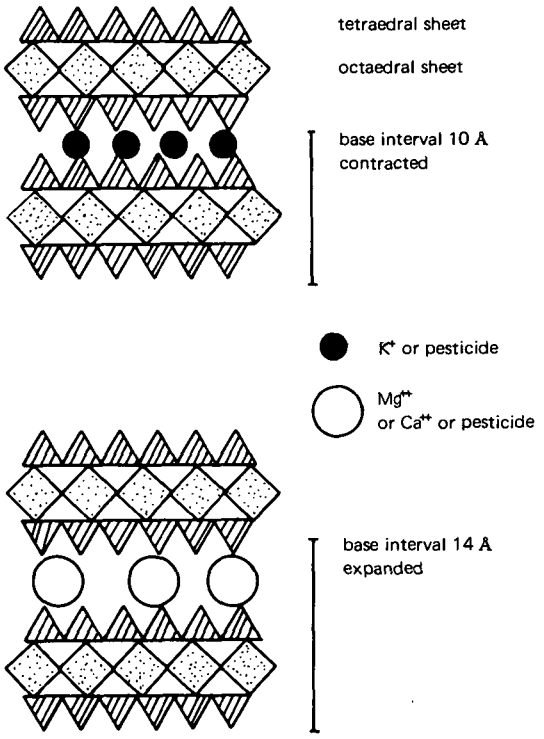

FIG.3. Interlamellar binding of pesticides into expanding clay minerals.

those with sterilized soils [28], is a further indication supporting this assumption. Chemical structures suitable for such a kind of binding would be ionic or basic substances such as phenols or anilines.

Normally, however, the organic soil matter is regarded as the main site of irreversible fixation of chemical residues. As an example for soil organic matter, Fig. 4, shows a section of a humic acid macromolecule.

It is evident that, in the genesis of humic acids, the aniline units (marked by the spotted squares) could be replaced easily by chlorinated anilines present from the use of herbicides. Similarly, chlorinated phenols may be incorporated.

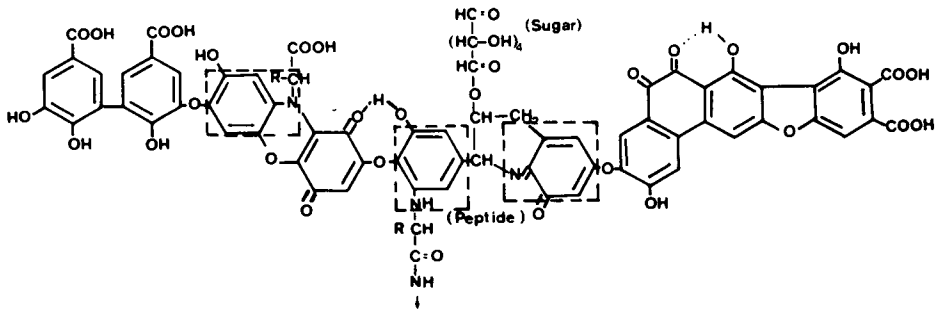

FIG.4. Type structure for humid acid.

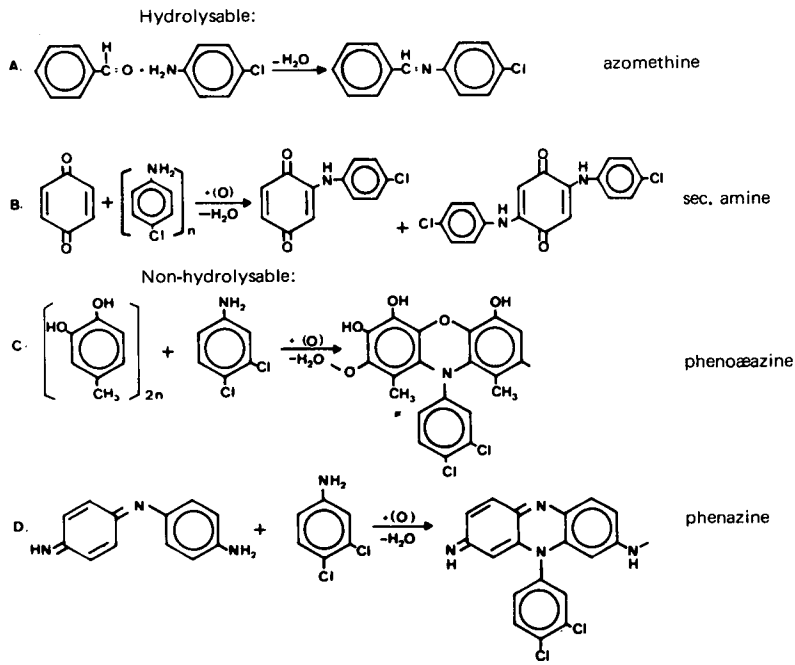

FIG.5. Binding of chloroanilines to humic acid monomers.

Hsu and Bartha [29] have carried out model experiments by incubating humic acid monomers with chlorinated anilines at room temperature and obtained polymeric precipitations with benzaldehyde (reaction A), p-benzoquinone (reaction B), 4-methylcatechol (reaction C), and indamine (reaction D). The reactions A and B in Fig. 5 are models for mechanisms resulting in hydrolysable, azomethine - or

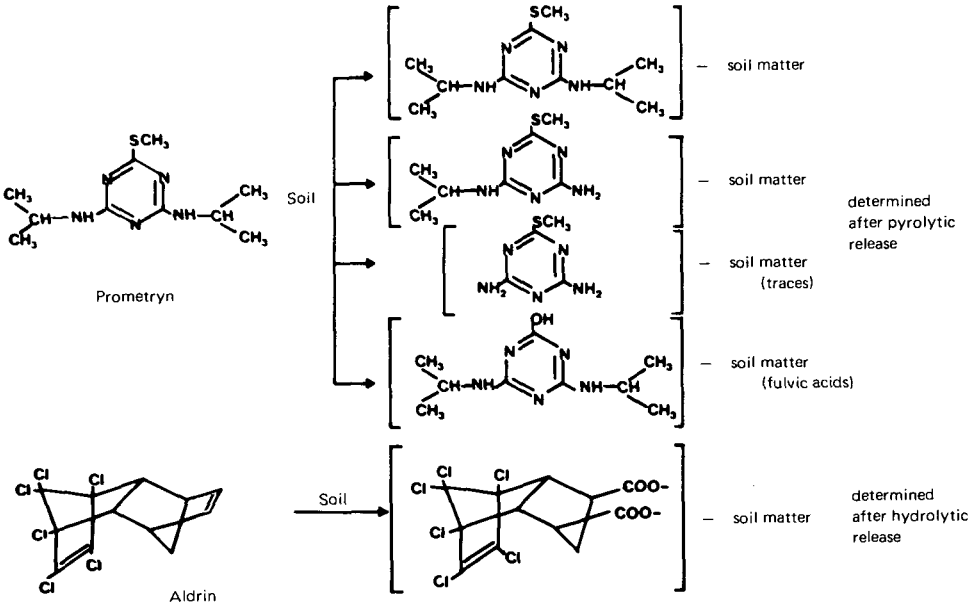

FIG. 6. Chemical identity of pesticide residues bound in soil.

amine-like bonds. The reactions C and D, however, result in the incorporation of the anilines into heterocyclic ring systems of the phenoxazine or phenazine type, which cannot be hydrolysed. Since neither inoculum nor any other biotic reagent was added, the reactions may be regarded as mainly abiotic. The same authors also determined the ratio of hydrolysable and non-hydrolysable-bound residues of 3,4-dichloroaniline and found that the hydrolysable portion decreased with time.

Methods to elucidate the chemical identity of bound pesticide residues should cleave the bonds fixing the xenobiotic to the natural macromolecule as completely as possible but, on the other hand, should not cause chemical changes at the xenobiotic molecule itself. Hydrolytic 3,[29-31] and pyrolytic [7, 32] methods have been proposed. For anilines, both methods are applicable and release the bound xenobiotic molecule unchanged for identification. However, for chemicals sensitive to hydrolytic or thermal attack the problem of identifying soil-bound residues remains unsolved.

The xenobiotics bound to soil constituents may represent the parent compound applied to soil or a conversion product formed biotically or abiotically in soil. Figure 6 gives some examples of identified bound pesticide residues in soil.

Whereas anilines are bound in soil mostly in an unchanged form [3, 29, 30, 32], the herbicide prometryn is bound as parent compound, as well as in the form of its mono- and didealkylated and its hydroxylated derivatives [7]. The insecticide aldrin, however, is bound only in the form of its polar ring cleavage product. This product was identified after alcaline hydrolysis of bound residues in soil treated either with the insecticide itself [33] or with the aldrin metabolite trans-4,5-dihydroxy-4,5-dihydroaldrin [31].

All bound residues discussed so far may be regarded as xenobiotic residues in soil. However, in studies with ^{14}C-labelled non-persistent pesticides, natural organic soil constituents assimilated by soil microorganisms from $^{14}CO_2$ resulting from total degradation are included in the determination of bound pesticide residues. This assumption is supported by the finding that, for 12 chemicals tested in a laboratory soil-plant system, a linear correlation exists between the mineralization rate and the formation of bound residues in soil [34], as shown in Fig. 7.

It is noteworthy that two substances, namely aniline and phenol, do not fit into this correlation. This is in line with the previously discussed mechanisms of incorporation of anilines and phenols into humic acids, which occurs without any preceding biotic degradation. An analytical separation and quantitation of bound residues into xenobiotic and natural compounds is difficult and would imply the degradation of all macromolecules involved to low-molecular, identifiable compounds.

The persistence of bound residues, i.e. their susceptibility to mineralization mechanisms, is, as mentioned in the introduction, a central point of interest for the evaluation of their significance. Figure 8 shows, as an example, the $^{14}CO_2$ evolution from cultures of Aspergillus versicolor utilizing 3,4-dichloroaniline - humic acid complexes [29].

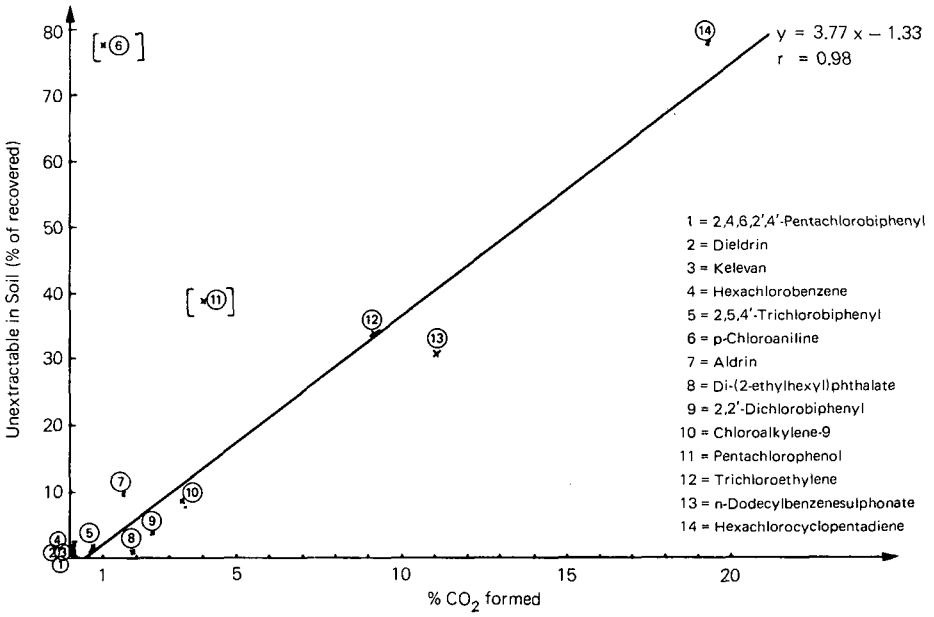

FIG. 7. Correlation of formation of soil-bound residues with soil mineralization rates.

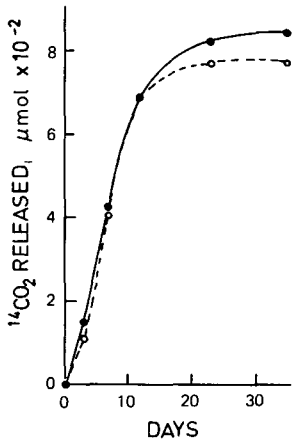

FIG. 8. Mineralization of hydrolysable and non-hydrolysable 3, 4-dichloroaniline-humic acid complexes by Aspergillus versicolor.

TABLE VIII. BIOAVAILABILITY OF SOIL-BOUND PESTICIDE RESIDUES TO PLANTS

Pesticide	Plant species	Time	% of soil-bound residue taken up	References
Butralin	Soy bean	4 weeks	0.27	[36]
		10 weeks	0.74	
Chlornidine	Soy bean	4 weeks	0.16	
		10 weeks	0.46	
Dinitramine	Soy bean	4 weeks	1.07	
		10 weeks	1.07	
Fluchloralin	Soy bean	4 weeks	0.45	
		10 weeks	0.90	
Profluralin	Soy bean	4 weeks	0.58	
		10 weeks	0.70	
Trifluralin	Soy bean	4 weeks	0.56	
		10 weeks	0.59	
Prometryn	Oats	3 weeks	0.53	[35]
Methabenz-thiazuron	Maize shoots	4 weeks	1.68	[9]
	Maize roots	4 weeks	0.73	
2, 4, 6-trichloro-phenol	Wheat	10 days	<0.1	[1]

The solid circles represent the intact complexes (hydrolysable plus non-hydrolysable complexes), the open circles only the non-hydrolysable portion, corrected by a factor resulting from change in specific radioactivity of the complex due to removal of the hydrolysable dichloroaniline. The curves reveal that Aspergillus versicolor makes little, if any, distinction between hydrolysable and non-hydrolysable complexes and oxidizes both at comparable rates. Similar biodegradation studies with bound residues of other pesticides show, depending on the chemical nature of pesticides, large variations. However, the fact that microorganisms are able to break heterocyclic bonds that resist acid as well as alkaline hydrolysis, indicates that xenobiotic residues bound in any form may be bioavailable to plants.

TABLE IX. PESTICIDE RESIDUES BOUND IN PLANTS
(in % of total residue in plants)

Chemical class	No. of representatives evaluated	Time of exposure	% residue bound (range)	References
Free phenols	2	1 vegetation period	29–38	[1, 2]
Anilines	2	1 vegetation period	87–90	[3, 4]
Triazines	2	20–100 days	20–63	[6, 37]
Urea herbicides	3	48–105 days	46–72	[3, 38, 39]
Dodecachloro-pentacyclodecane insecticides	2	1 vegetation period	3–5	[20, 21]
Cyclodiene insecticides	2	1 vegetation period	1–2	[22, 23]

TABLE X. INFLUENCE OF ANILINE FORMATION TENDENCY OF ^{14}C-LABELLED CHEMICALS ON THE FORMATION OF BOUND RESIDUES IN PLANTS
(outdoor conditions)

Chemical applied	Aniline metabolites identified	%[a] unextractable in plants
p-chloroaniline		90
3, 4-dichloroaniline		87
Monolinuron	p-chloroaniline derivatives	72
Buturon	p-chloroaniline	58
Imugan	3, 4-dichloroaniline	50
Pentachloronitrobenzene	Pentachloroaniline	31

[a] % of total radioactivity recovered in plants at harvest.

Table VIII gives some examples for the bioavailability of bound pesticide residues to plants [1, 9, 35, 36]. This table confirms the assumption that, based on the findings on biodegradation of soil complexes, in principle every bound residue can be available to plants. However, the table shows that the uptake is mostly below 1% of the amount bound in soil. Uptake of soil-bound residues by earthworms has also been reported [15].

PESTICIDE RESIDUES BOUND IN PLANTS AND FOOD

Table IX gives some examples on the occurrence of bound residues in plants, derived from different classes of chemicals [1-4, 6, 20-23, 37-39]. As in the case of residues bound in soil, the chlorinated hydrocarbons in the two last lines form the lowest portion of unextractable residues, and higher portions are formed by phenols and nitrogen-containing compounds. One difference between soil and plant binding seems remarkable; in soil the free phenols and anilines form bound residues of comparable high levels, whereas in plants the percentage is considerably lower for phenols than for anilines. It may be concluded that the incorporation rates of phenols into plant macromolecules (lignin, as discussed below) is low as compared to anilines, but the incorporation of anilines into plant material occurs as easily as that into soil material. Table X reveals that the percentage of unextractable pesticide residues in plants is correlated to the metabolic formation of anilines and decreases with increasing chlorine content of the aniline metabolites.

Table XI shows that plant growth conditions are a very important factor affecting the formation rate of residues bound in plants. The three upper lines show good growth conditions for lettuce plants (i.e. relatively high temperatures), high crop yields, and high portions of unextractable lindane residues. In the two last lines, poor growth conditions (low temperatures) result in poor crop yields and low lindane binding rates in plants [40].

To identify the binding sites of pesticide residues in plant material, various schemes for the fractionation may be used, one of which is shown in Fig. 9 for rice and wheat straw [41]. It primarily

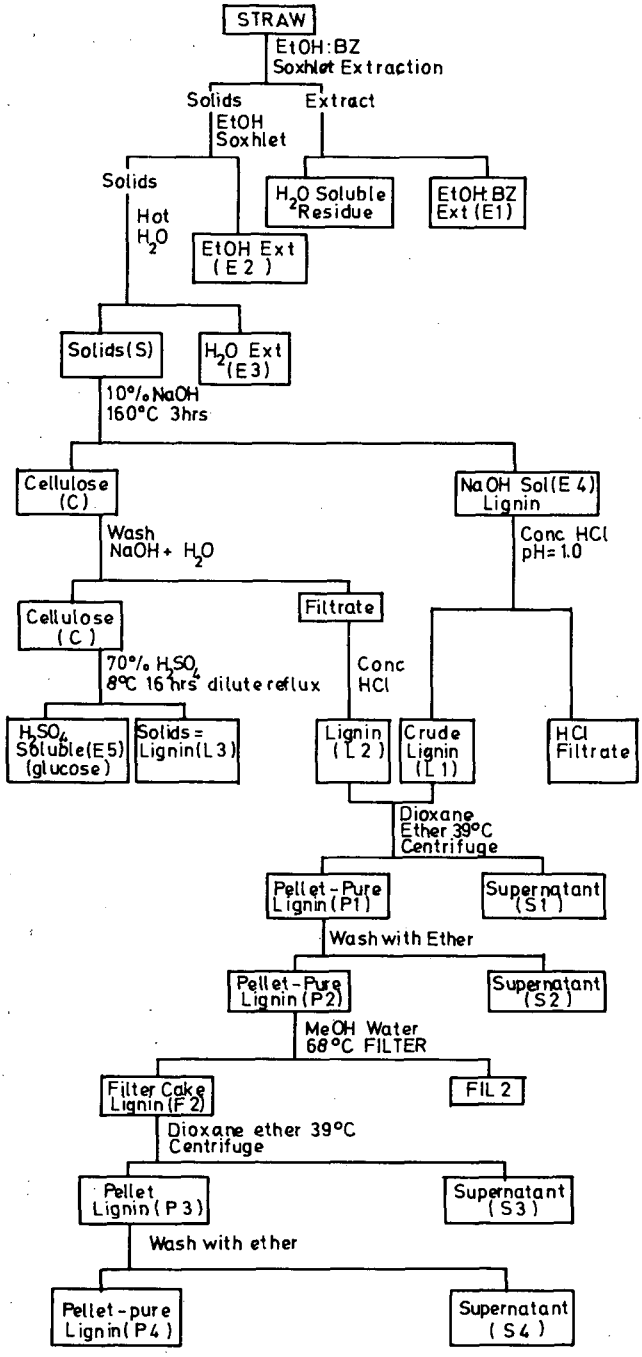

FIG. 9. Straw fractionation scheme.

TABLE XI. INFLUENCE OF PLANT GROWTH CONDITIONS ON THE FORMATION OF BOUND RESIDUES OF ^{14}C-LINDANE IN LETTUCE AFTER FOLIAR APPLICATION (outdoor conditions)

Mean daily max. and min. temperature (°C)	Growth conditions	Crop yield (g)	%[a] unextractable in plants
28/15	good	442	31
29/13	good	1146	25
30/16	good	577	33
14/5	poor	12.5	4
13/7	poor	26.5	2

[a] % of total radioactivity recovered in plants at harvest.

aims at isolating lignin and cellulose fractions. Other separation methods also include the isolation or specific enzymatic degradation of pectin [42], protein [39, 43] and starch [44]. Bound residues were detected in all of these fractions.

Since most of plant-bound pesticide residues were shown to be localized in lignin, model experiments were carried out to copolymerize chlorinated anilines with a lignin monomer, coniferylalcohol. 3-Chloroaniline, 3,4-dichloroaniline [45, 46] and 4-chloroaniline [47] were used as representative anilines. In all cases, copolymers were found. The postulated mechanism as shown in Fig. 10 is an addition of the aniline to a quinone methide intermediate.

In order to identify the chemical nature of plant-bound residues, hydrolytic [48] or pyrolytic [45, 49] degradation methods have been recommended, which are similar to those recommended for soils. Simple dissolution of lignin by hot dimethylsulfoxide has been successful for the isolation and identification of lignin-bound carboxin residues [50]. In this case, the residue probably is not copolymerized into the lignin but fixed only physically. Another

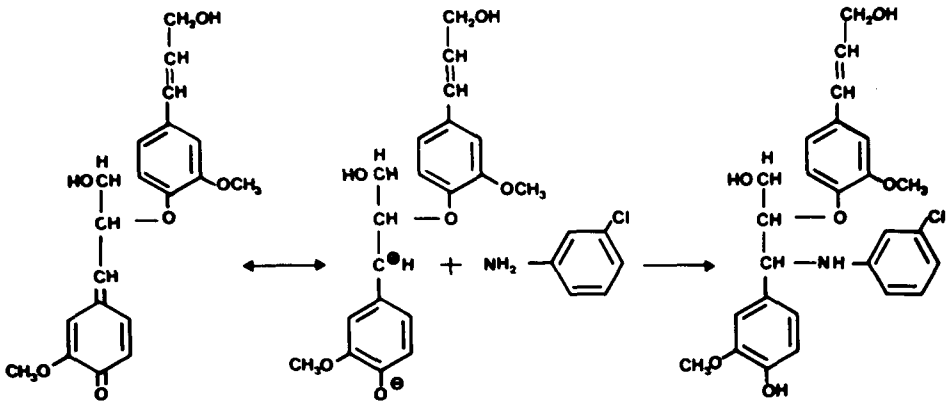

FIG.10. Postulated mechanism of arylamine incorporation into lignin.

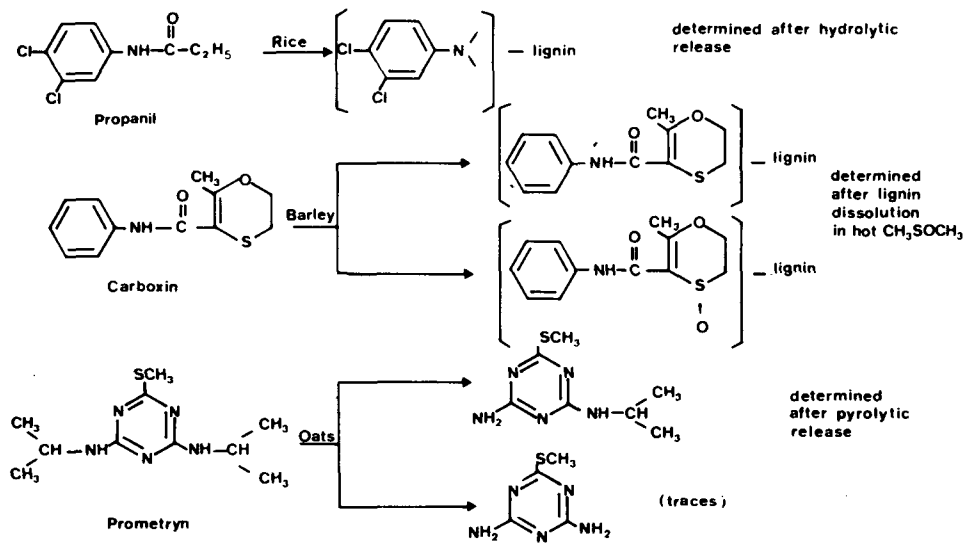

FIG.11. Chemical identity of pesticide residues bound in plant material.

method for the liberation of bound 3,4-dichloroaniline from plant material - nitric acid digestion - resulted in chemical alteration of the liberated residue [51].

Figure 11 gives some examples for the chemical identity of plant-bound pesticide residues. The anilide propanil is bound to lignin in form of its me-

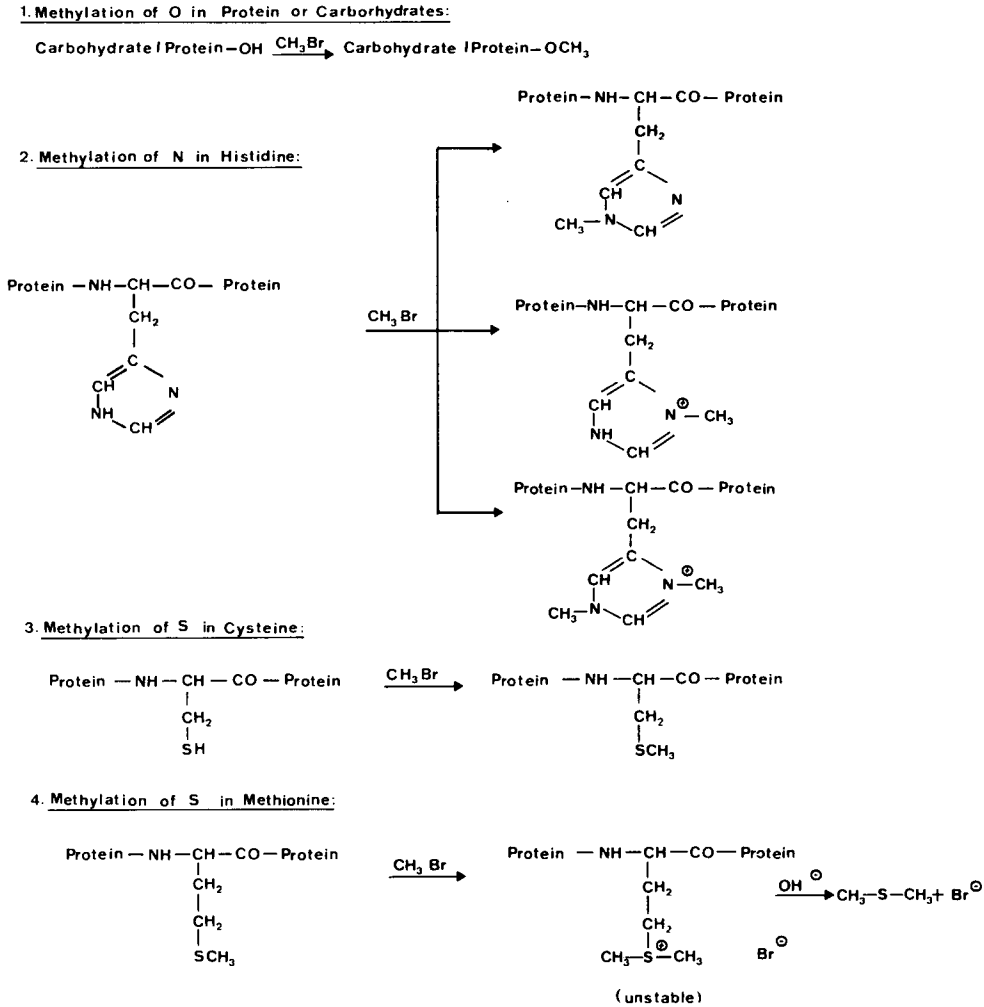

FIG.12. Methylation of cereal food constituents by methyl bromide fumigant.

TABLE XII. BIOAVAILABILITY OF PLANT-BOUND PESTICIDE RESIDUES TO MAMMALS

Pesticide bound	Animal species	Plant feed	Excretion in faeces within 24 h (% of applied)	Excretion in urine within 24 h (% of applied)	References
Atrazine	Sheep	Sorghum	32.3	3.1	[37]
Atrazine	Rat	Sorghum	83.5	2.1	[37]
Propham	Rat	Alfalfa	84.2	0.7	[53]

tabolite 3,4-dichloroaniline [48], the anilide carboxin in form of unchanged parent compound and of its sulfoxide metabolite [50]. The triazine prometryn is bound in form of its mono- and didealkylated metabolites [49].

As in the case of soils, for plant-bound residues also, the potential occurrence of natural plant constituents must be considered, which have been assimilated from the ^{14}C resulting from total pesticide degradation, and which, thus, may be misinterpreted for xenobiotic residues. To demonstrate that ^{14}C is really part of such a natural polymer, e.g. a polysaccharide, sophisticated procedures are needed including purification, degradation and derivatization, in order to exclude the physical adsorbence of a radioactive xenobiotic to the polysaccharide. The utilization of ^{14}C derived from ^{14}C-labelled pesticides for the biosynthesis of cellulose can be demonstrated by derivatization of cellulose hydrolysate to a glucosazone [41]. Identification of radioactive starch is carried out similarly [44].

A further, very important aspect of pesticide residues in food plants is the alkylation of food constituents by alkylating pesticides. As an example, the reactions of the fumigant methylbromide with food constituents are presented in Fig. 12. Cereal foods and feed and relatively small areas of agricultural soils have been intermittently exposed to this alkyl halide under conditions of fumigation for many years. Methylbromide is a powerful methylating agent. Its reaction products in cereals have been characterized as indicated in Fig. 12 [52]. According to this scheme, the oxygen in proteins or carbohydrates (reaction 1), the nitrogen in histidine (reaction 2), the sulphur in cysteine (reaction 3) or methionine (reaction 4) may be methylated. These methylation products may be regarded as bound methylbromide and, thus, as unwanted residues in food.

From the toxicological point of view, bioavailability of pesticide residues bound in plants and food to mammals is in the focus of interest and concern. Therefore, extracted plant material containing bound pesticides was fed to mammals, and excretion was monitored. The excretion in urine is regarded as a measure for absorption of ^{14}C from the gastroin-

testinal tract by the blood and for potential metabolism, whereas ^{14}C excreted in feces represents the portion which passes the gastrointestinal tract without being involved into metabolism. As examples, in Table XII the results are given for two substances, the triazine herbicide atrazin [37] and the carbamate herbicide propham [53]. In the case of these two pesticides, the plant-bound residues are excreted nearly quantitatively in feces, by rats within one day and by sheep within two days. Thus, in both these cases bound residues in food might be regarded as harmless to mammals.

CONCLUSIONS

The chemical identity of bound pesticides covers a large scale of products, from physically adsorbed xenobiotics via xenobiotics copolymerized with natural macromolecules and natural products altered by alkylation, up to normal plant constituents assimilated from pesticide-derived CO_2 formed by total degradation. The latter are not included in the definition of bound pesticide residues; in practice, however, it is very difficult to recognize them as such and to determine them separately from real xenobiotical residues. The soil-bound residues investigated thus far seem to be susceptible to microbial attack and, thus, are in principle bioavailable to plants; however, the amounts taken up by plants are small in all cases reported. The few studies available today on the uptake of plant-bound residues by animals indicate that they enter the blood and participate in natural metabolism only to a small extent. All information available today on bound residues is related to a few model compounds. Further information on other substance classes is urgently needed.

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EFFECTS OF FERTILIZERS, FUNGICIDES AND HERBICIDES ON THE FATE OF ^{14}C-PARATHION AND ^{14}C-FONOFOS IN SOILS AND CROPS*

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Abstract

EFFECTS OF FERTILIZERS, FUNGICIDES AND HERBICIDES ON THE FATE OF ^{14}C-PARATHION AND ^{14}C-FONOFOS IN SOILS AND CROPS.

Metabolism and translocation of ^{14}C-parathion and ^{14}C-fonofos deposits in and from an agricultural loam soil into oat or corn plants growing in an open agro-ecosystem were significantly affected by the presence of organic and inorganic fertilizers as well as by the fungicide captafol (Difolatan) and the herbicide atrazine. Utilizing another soil type in a closed system, the interaction of selected fungicides, herbicides and N-fertilizers with microorganisms in cranberry soils and their effects on the degradation of ^{14}C-phenyl-parathion were investigated. Soil microorganisms were responsible for the oxidative as well as reductive degradation of the insecticide. Incubation of soils with parathion or p-nitrophenol for 4 d, followed by the addition of ^{14}C-parathion resulted after 24 h in an enhanced degradation of the insecticide to $^{14}CO_2$ (34–39% of the applied radiocarbon as opposed to 2% in controls), and also in an increased binding of ^{14}C to the soil. The fungicide captafol inhibited the degradation of soil-applied ^{14}C-parathion as evidenced by a reduction of both $^{14}CO_2$ evolution and ^{14}C-bound residues. Maneb and benomyl suppressed the degradation of ^{14}C-parathion to $^{14}CO_2$ but not the formation of bound residues. PCNB had no effect. Addition of 2, 4D to ^{14}C-parathion-treated soil also resulted in an increased persistence of the insecticide. Studies conducted with the insecticide and $(NH_4)_2SO_4$, NH_4NO_3, KNO_3 or urea showed that under all experimental conditions the total amounts of ^{14}C recovered were similar, yet the distribution of ^{14}C-compounds into benzene-soluble, water-soluble and bound residues was not. This possibly indicated a change in the pathway of ^{14}C-parathion degradation. The insecticide was most persistent in soils containing $(NH_4)_2SO_4$, as demonstrated by a recovery of 29% of the applied radiocarbon in benzene-soluble form. Analyses by TLC of this benzene extraction phase revealed the presence of ^{14}C-parathion, ^{14}C-p-aminophenol and ^{14}C-aminoparathion. It appears that the form of the N-soil amendment and not the N amendment as such affected the degradation of ^{14}C-parathion. Results reported here stress the importance of investigating the environmental fate of a particular pesticide in relation to the presence of other agricultural chemicals.

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* The abstract only is published, since it is intended that the full paper will appear in the IAEA-TECDOC Series (unpriced publication).

FATE OF 2, 4D HERBICIDE IN SOIL-PLANT ECOSYSTEMS*

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Abstract

FATE OF 2, 4D HERBICIDE IN SOIL/PLANT ECOSYSTEMS.

The behaviour of 2, 4D herbicide was investigated in soil/plant ecosystems under laboratory, greenhouse and field conditions, using isotope tracer techniques. Laboratory studies included: (a) Degradation of ^{14}C-2, 4D in three different soils by incubating them in closed systems and collecting the evolved $^{14}CO_2$ in sodium hydroxide. Four weeks after incubation 18.7, 19.7 and 24.6% of the applied ^{14}C activity were recovered from the three soils in the alkaline solution. (b) Degradation of 2, 4D at room temperature with three different moisture contents (10, 15 and 20%) was studied in open systems. Within 4 weeks 94.0–96.0% of the applied herbicide was degraded and the degradation rate was higher at the 20% moisture content. Greenhouse investigations included: Distribution of 2, 4D in soil/plant ecosystems in two soil types with barley, wheat and oats. After extracting the herbicide from the soil samples, the bound residues in the soil were determined by using the wet combustion method. Uptake of 2, 4D in the two soils by barley, wheat and oats during 4 weeks under these conditions was very low and most of the herbicide remained in the soil. In field studies aimed at studying the degradation and leaching of 2, 4D, polyethylene tubes were placed in the field and labelled herbicide was applied to the top of the tubes. After 4 weeks the soil found in each joint of the tube was analysed for the extractable and bound residues. The balance figure of this experiment was 62.9% and the significant loss of herbicide (37.1%) was attributed to degradation. In 4 weeks the chemical was leached to a depth of 25 cm in the soil. In a field experiment aimed at studying the effect on crops and fertilizer of 2, 4D, barley, wheat and oats were grown in small boxes in the field and diammonium phosphate fertilizer was mixed with the soil in some of the boxes. After 4 weeks uptake of the herbicide by the plants was very low, in the range of 0.2–0.9% of the applied herbicide. Uptake of the chemical by the plants grown with fertilizer was much lower and was of similar magnitude (0.2–0.7%).

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BEHAVIOUR OF CARBARYL IN SOILS UNDER THE INFLUENCE OF DIFFERENT CARBON SOURCES*

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Abstract

BEHAVIOUR OF CARBARYL IN SOILS UNDER THE INFLUENCE OF DIFFERENT CARBON SOURCES.

The effect of adding various carbon sources (microcristalline cellulose, filter paper, sucrose, milk, soy bean leaf and soy bean oil) on the persistence of carbaryl was investigated in samples of Humic Gley soil (43% organic matter; 57% clay; 12% silt; pH 5.7) and Yellow Red Latosol soil (0.36% organic matter; 77% clay; 9% silt, pH 6.4) using radiometric techniques. For these studies an aqueous solution of ^{14}C-carbaryl labelled on the carbonyl group was added to 10 g of soil after treatment with the carbon sources. Samples were extracted with 20 ml of dichloromethane and analysed by liquid scintillation counting. Extracted carbaryl was identified by thin-layer chromatography by spotting on silica-gel plates with 1 ml extract and using hexane-acetone (4:1) as the solvent system. After extraction, radiocarbon remaining in the soil was determined by wet combustion to CO_2. Recovery of carbaryl from soils as a function of incubation time and differing carbon sources showed that addition of milk, sucrose and soy bean oil induced in the Humic Gley soil a moderate increase in the rate of degradation of carbaryl, while incubation with the other organic nutrient sources, such as cellulose, filter paper and soy bean leaves, had practically no influence on degradation. In contrast, addition of nutrient sources on the Yellow Red Latosol greatly increased degradation of carbaryl, very little of this compound being left after 6-weeks treatment. Carbaryl was very poorly degraded on the Humic Gley soil, probably due to its higher content of organic matter which causes its higher absorption to the soil particles, thus reducing availability of the insecticide for metabolic processes. Nutrient sources that contribute towards increasing degradation of carbaryl in soils are certainly those readily available for microorganisms. In the studies performed here pure cellulose and filter paper are slowly metabolized and milk and soy bean oil, which are deprived of cellulose, are more efficiently attached by microorganisms than cellulose itself.

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PESTICIDE METABOLISM IN PLANTS
(Session V)

Chairman

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Invited Paper

METABOLISM OF PESTICIDES IN PLANTS

Some applications of nuclear techniques

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Abstract

METABOLISM OF PESTICIDES IN PLANTS: SOME APPLICATIONS OF NUCLEAR TECHNIQUES.

Metabolism of pesticides in plants is reviewed, using selected references to illustrate the development and application of nuclear techniques. The significance of metabolic processes is discussed and some possible developments in the application of nuclear technology.

INTRODUCTION

Radiotracers have been used for investigating the metabolism of pesticides in plants for over 30 years, and at least one publication exists which dates from 1951 [1]. There has been a continuous expansion of the effort to learn the fate of pesticides in crops and the environment and, while it is possible that the main driving force remains fear of the unknown, in this case pesticide residues, there are other good reasons for these studies and some may become apparent later. However, the main objective here is to consider the application of nuclear techniques to studies of the metabolism of pesticides in plants, but similar methods are also used to study enzyme activities and normal metabolic pathways. Since living organisms are so closely interdependent, having similar basic metabolites and pathways in common and because it is sometimes difficult to distinguish chemical from biological degradation, it is perhaps not surprising that the nuclear techniques used to study metabolism in plants are much the same as those used to study degradation of chemicals on surfaces or in soil, water or organic solvents or in the vapour phase, and by other organisms from microorganisms to mammals. Techniques must obviously be adapted to the morphology and chemistry of the system being investigated and to the ultimate objectives of the studies.

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Pesticides are here defined as chemicals used to control pests, and in broad terms pests may be defined as any organism (insects, fungi, plants, micro-organisms) that causes crop losses or deterioration of stored products. To do this safely the pesticides must remain active long enough to control pests but without leaving harmful residues.

Simply to measure persistence is not enough. If the chemical can no longer be detected we need to know why not; has it moved on or has it been modified? We need to know the fate of the modified chemical to ensure efficient and safe use. Thus, we are led to study pathways of degradation and metabolism.

Isotopic labelling is not essential for all studies, especially if products are formed that can be recognized and assayed by other methods. However, this is not always the case and isotopes have obvious advantages in that they facilitate detection and identification of degradation products by providing a unique nuclear property to distinguish them from normal materials.

A detailed and comprehensive review is neither appropriate nor possible here and would merely supplement existing compilations [2–6]. The aim, therefore, is to present an overall view of the subject illustrated by reference to selected publications.

HISTORICAL

Historically, the introduction and rapid spread of the use of organophosphorous insecticides caused much anxiety because most of these compounds were toxic to mammals; anxiety was not allayed when some, which are taken up into and move in plants, began to be used. At about the same time radioisotopes became freely available and labelled pesticides were synthesized and used to study the behaviour of these pesticides.

In 1951 Hartley and Heath [1] published work using ^{32}P-labelled octamethylpyrophosphamide and they concluded that this systemic insecticide was metabolized by plants. The decomposition products formed in plants differed from those formed by chemical hydrolysis, which was also much slower. The parent compound was separated from decomposition products by partition between water and chloroform. Differential solubility in the presence of sodium salts and solvent partition was used to confirm that products of chemical decomposition differed from those in the plant.

This early demonstration of the metabolism of pesticides in plants emphasizes both the importance of establishing the identity, if not the structure, of radioactive materials and the need for methods to do this. The radioactive isotope ^{32}P served not only as a label for the pesticide but also as a ready means of measuring small amounts of chemicals, since at that time no suitable chemical methods had been developed.

Subsequent developments based on principles already well established have transformed the techniques available for identifying and measuring small concentrations of pesticides or other chemicals. Simultaneously, nuclear techniques were developed and, as with many other processes, equipment has improved with developments in electronics and application of microprocessors to control equipment and to compute.

ORGANOPHOSPHOROUS COMPOUNDS

Results were soon published from other studies of the fate and behaviour in plants of an increasing range of organophosphorous insecticides. These show the importance and value of technique and a progression of improvements. Studies of systox (now demeton) and related compounds employed paper chromatography to separate isomers of the insecticide from metabolites [7], to relate these to oxidation products [8–10] and to their anti-cholinesterase activity [11].

Although the compounds were synthesized with ^{32}P and ^{35}S, most of the work was undertaken with ^{32}P since ^{35}S label was found to be generally less useful than ^{32}P with the techniques available at the time, possibly because samples at first were combusted for counting so that recovery of the sulphur was uncertain and also because some sulphur was lost by metabolism. Techniques developed during the work and combusting before counting, using a Geiger-Muller tube and planchets, was abandoned, activity being counted on sections of dried chromatograms. Autoradiography was also introduced to locate activity on paper chromatograms.

In later studies of the structure of metabolites a system of solvent partition and column chromatography was used to remove plant constituents from isolated metabolites, which were then examined by infra-red spectroscopy to confirm structures deduced by chemical reaction and co-chromatography of labelled metabolites with authentic materials.

Metabolism of these compounds in insects and mammals was examined in parallel work, using the same basic techniques and with generally similar results, although some metabolic differences were observed.

More recent investigations of the metabolism of organophosphorous insecticides show a general similarity of approach to studies of the fate, although technology has changed. One example is investigation of the metabolism of Etrimfos [12] in plants, which was made to evaluate the metabolic residues and their fate before the chemical was used in extensive field trials of insect control. Instead of the short-lived isotope ^{32}P the isotopic marker used was ^{14}C, which has the advantage of a longer half-life and greater flexibility, not being limited to P-containing molecules. Radioassay was by liquid scintillation counting, but plant pigments in

extracts cause quenching and have to be removed by some means; in this instance column chromatography was used. Many alternative methods have been devised to avoid quenching, each tailored to suit particular problems; methods include solvent extraction [13] separation by thin-layer chromatography [14] and even by total combustion to destroy the interfering compound, the $^{14}CO_2$ being absorbed in an alkaline medium for counting. Combustion is also valuable to assay labelled metabolites associated with other insoluble material from which it cannot be separated, as was found with some metabolites of Etrimfos [12].

The increased range of nuclear techniques now available is complemented by the development of analytical techniques, and in the study of Etrimfos leaves were rinsed with water then macerated and extracted with chloroform, followed by acetone and then water. Solvent extracts and leaf residues were examined separately, each fraction providing a partial separation of materials. Solutions were chromatographed on thin layers of silica, using seven different solvents in all, to separate or purify materials that were located by radioautography. Separated materials were assayed by scintillation counting or subjected to gas chromatography and mass spectrometry for identification. Oxidation and hydrolysis products were identified, as well as conjugated metabolic products, although neither recovery of ^{14}C nor identification of metabolic products was complete.

ALDICARB

Metabolism of the oxide carbamate aldicarb in plants [15, 16] was examined using similar methods: extraction, separation by solvents, thin-layer and ion-exchange chromatography for isolation and identification of metabolites. Liquid scintillation counting was used for quantitive measurements of activity. Radioautography was used to locate material separated on thin-layer plates and authentic samples of unlabelled materials were used for tentative identifications, which were confirmed by mass spectrometry and NMR. It was shown that the major pathway of degradation was by oxidation of the thioether group to the sulphoxide and by hydrolysis to liberate the corresponding oximes. Recovery of radioactivity declined with time, and only 50% or less was accounted for after one month. No attempt was made to determine if the loss resulted from incorporation into insoluble materials, root or leaf exudation, or by complete metabolism to CO_2.

Similar methods were used by Belgian workers [17, 18] to investigate the metabolism of ^{14}C-aldicarb in sugar-beet plants, but the work was extended to investigate conjugated products of metabolism that were not soluble in the solvent (50% aqueous ethanol) used for extraction.

Results from localized applications of aldicarb to sugar-beet plants growing in the field showed that at harvest only 13% of the radioactivity in the leaves was

present as aldicarb or its sulphoxide or sulphone; about 15% appeared to be incorporated into plant polymers. Much of the remaining ^{14}C was probably conjugated to plant materials, as shown by liberation by hydrolysis with strong acids or an enzyme (-glucuronidase). When roots were examined for compounds labelled with ^{14}C the proportions of aldicarb metabolites in the form of conjugates differed from those in leaves and as much as a fifth of the ^{14}C found in roots occurred as sugar, indicating incorporation of label into the normal metabolic pools of sugar-beet. On reflection it is not surprising that no ^{14}C-sugar was found in leaves, since the root is the normal sink for sugar in sugar-beet.

FURTHER EXAMPLES OF PESTICIDE METABOLITES ENTERING METABOLIC POOLS AFTER BREAKDOWN OF PESTICIDES

Incorporation of ^{14}C from aldicarb into sugar is not a unique example of metabolic products from pesticides entering normal metabolic pathways. Such incorporation is to be expected when small molecules containing carbon result from degradation, as in the case of the organophosphorous systemic insecticide mephospholan [19]. Applied to rice, about half the residual ^{14}C from mephospholan could not be extracted with methanol for a period of up to 18 weeks after treatment, and ^{14}C was shown to be incorporated into the glucose or starch, cellulose and lignin of the plants. Labelled material extracted by methanol was almost all the unchanged parent compound, indicating rapid breakdown of hydrolysis products containing ^{14}C.

Cellulose, lignin and many other normal metabolites were shown to be derived from the ^{14}C-labelled fungicide cymoxanil [20]. After foliar application to grapes, tomatoes and potatoes the fungicide was rapidly metabolized and much (30–55%) of the ^{14}C was incorporated into glycine and other amino acids, while another considerable proportion (7–15%) of the ^{14}C was reported as sugar or starch, the proportions depending on the crop and its physiology or biochemistry. Among other compounds identified as containing ^{14}C were polycarboxylic acids, such as citric acid and fatty acids.

Residues of the fungicide and its hydrolytic degradation products were sought using thin layer chromatography, the radioactive bands being located using a TLC radio scanner (not radioautography), and assay was by scintillation counting of bands scraped from TLC plates. Various analytical methods were used to separate and identify other radioactive products, including derivatization, assay by radio gas chromatography or by gas chromatography trapping column affluents for assay of radioactivity by scintillation counting. Chemical structures were confirmed by GC/MS.

MORE PERSISTENT STRUCTURES AND CONJUGATES

Fragments of pesticides once incorporated into normal metabolic pools behave indistinguishably from material derived from natural sources and so have no toxicological interest and cannot be recognized as pesticide residues except in so far as their origin may be determined by the presence of unusual isotopes. However, many substances are less completely metabolized and parts, if not all, of the pesticide structure may remain chemically recognizable, if not intact, so that the residues may have significant biological activities.

Recent investigations of such pesticides provide examples of the use of all types of techniques and instrumentation already mentioned for identification, location and assay both of radioisotopes and chemicals.

Development of this methodology makes possible identification and measurement of the multiplicity of metabolites that may be derived from compounds such as pentachloronitrobenzene [21–23], and some of the products may represent less than 1% of the original pesticide [20, 22].

Metabolism of pentachloronitrobenzene in onions and peanuts provided evidence of a whole range of mechanisms, including reduction of elimination of the NO_2 group, formation of phenols, their methylation and acetylation, introduction, methylation and oxidation of a thiol group into the aromatic ring, and dechlorination. Such reactions occur separately or in sequence to produce a variety of products which may in turn form conjugates. A variety of conjugates containing cysteine or glutathione were identified, but some insoluble radiolabelled products remain unidentified [22–24].

Complex patterns of metabolism occur with many pesticides that are susceptible to degradation at several sites. Typically, pathways of metabolism of pesticides with aromatic groups are related and a range of pesticides may have some metabolites in common so that subsequent pathways of metabolism are also the same. Phenols and carboxylic acids, whether pesticides or degradation products, are likely to be conjugated with a variety of substances, such as amino acids and sugars. Examples include herbicides, such as phenoxyacetic acids [5, 25] and synthetic pyrethroid insecticides, reported to be degraded in plants by hydrolysis at the ester group, followed by oxidation and conjugation with sugars of both the cyclopropyl and aromatic moieties of the molecule [6,26,27].

SIGNIFICANCE OF PESTICIDE METABOLISM IN PLANTS

Many types of metabolism have been demonstrated in plants using nuclear techniques, but the significance of pesticide metabolism cannot readily be

generalized. Obviously the time scale and extent of metabolism have immense practical importance, but experimentally these are probably best examined separately and after major metabolic products (pathways) are established. However, once metabolites are known it is necessary to establish their significance in terms of toxicology and pest control and for purely scientific purposes. Frequently, the amounts of metabolites may be too small to be of practical interest, but much remains to be learned about the toxicology of chemicals formed in organisms, which may differ greatly from chemicals applied externally or injected, since toxicity is often much influenced by presentation of chemicals. Metabolism changes both the chemical and physical properties of pesticides modifying reactivity and behaviour, which together determine biological activity, and a metabolite could be generated at sites it would never reach if applied to an organism. Assessment of the significance of metabolism is relatively simple in the extreme case of little or no metabolism, for example, diflubenzuron [28–30], which is scarcely taken up and degraded by plants, although once injected into a plant it appears to be readily degraded. Similarly, the biological significance of the primary metabolite, which remains largely unchanged like the imide formed from Techlofthalan [31] in plants, is relatively easy to assess.

Component atoms or groups of pesticides that are incorporated into normal metabolic pools will obviously have no toxic effects so that only the period of survival of the pesticide intact will be of paramount importance. However, this only applies when all parts of the pesticide molecule are metabolized and part of the molecule remains unchanged. Interpretation is more difficult when metabolism gives highly toxic products such as result from the oxidation aldicarb to the toxic sulphoxide and sulphone. These metabolites are not expected to distribute in the same way as the plant compound inside an organism (plant). Work by colleagues at Rothamsted has shown that the polarity of molecules influences their behaviour (uptake and movement) in plants and worms, and suggests that cell walls and cuticles are more freely permeable to aldicarb than its oxidation products. Formation of conjugates, like other forms of metabolism, modifies biological activity. Usually the conjugate is more stable and less biologically active than the parent compound, but if the process can be reversed then the conjugate may provide a reservoir of inactive material which may be reactivated to release a chemical with useful or harmful biological activity. The presence of conjugates provides a challenge to determine their biological significance. This may conceivably range from a stabilized reserve of active compound, which may be released by degradation of the conjugate, to a detoxication mechanism. Both extremes provide interesting possibilities for using isotopes, even if only to develop analytical techniques for residue determination which will distinguish between free and conjugated forms of biologically active compounds.

METABOLISM, ISOTOPES AND THE FUTURE

There seems little doubt that while chemicals continue to be developed and applied to crops there will remain a need for continued studies of metabolic pathways and metabolites. Doubtless, studies of metabolism in plants and other organisms will continue to be made in parallel, using radioisotopes to label either all or parts of the pesticide molecule. There is evidence of the continuing development of techniques and instrumentation for both isotopic and chemical assay, which will further increase power to gather information.

Resolution of the problem of unidentified residues has begun with demonstration of the incorporation of ^{14}C from pesticides into normal plant constituents. Increasing information on conjugates of pesticides and their metabolites suggests that understanding of their toxicological importance may emerge.

Work on the identification of a conjugate of a pyrethroid metabolite [27] presents an interesting application of ^{14}C glucose, which may find wider applications of labelled normal metabolites to investigate conjugates.

Radioimmunoassay techniques have been applied to determination of pesticides (benzimidazoles [32] and parathion [33]). It is an extremely sensitive technique and if it can be adapted to the assay of metabolites closely related to pesticides it may advance metabolic studies, but the method has yet to be fully assessed for specificity.

More studies are needed to assess the biological significance, if any, of metabolites and also to develop a more systematic approach to the metabolism of groups of related compounds, such as substituted phenols, which may be derived from pesticides containing similarly substituted aromatic groups but with widely differing biological activities. Such systematic investigations would provide information to allay anxieties about effects on non-target organisms more economically than detailed investigations of individual compounds.

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Invited Paper

CONTROLLED RELEASE FORMULATIONS OF AGRICULTURAL CHEMICALS

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Abstract

CONTROLLED RELEASE FORMULATIONS OF AGRICULTURAL CHEMICALS.

Chemical agents of agricultural importance can be formulated in a polymeric matrix and their slow continuous release effected through internal transport mechanisms or triggered by external environmental factors. The five basic release systems are all amenable to agricultural usage. The major advantages include reduction of the total quantity of agent necessary, economy in labour through increasing the between-application intervals, greater safety in handling and dissemination of hazardous materials, and reduced environmental impact arising from usage of non-persistent pesticides. It is noted that continuous target exposure to ultra-low pesticide concentration provides much lower insect or weed control at agent level than conventional technology. An increasing number of commercial controlled release products indicates not only a growth in technology but also acceptance on the market. Controlled release systems lend themselves to the scientific study of agricultural agent movement through the soil and plant tissue in that the dispensing unit serves as a relatively stationary retrievable focal source for agent emission. Measurement of agent emission into the ambient environment is thus facilitated through analysis of dispenser residue.

1. INTRODUCTION

1.1. Background

During the early 1960s dichlorvos and other insecticides were monolithically incorporated in plasticized polyvinylchloride matrices and emission in air was noted for a few months [1]. The concept was applied to confined volume vapour, insecticide usage, endoparasitic action [2] and pet collars [3].

Release was based on the so-called 'blooming' phenomenon common to plastic materials overloaded with various fillers [4]. The agent used was soluble in the plasticizer and the plasticizer migrated to the dispenser surface, carrying with it the toxic agent. Blooming arises from segmental stress of the macromolecules caused by filler incorporation beyond the interstitial free volume holding capacity.

Agent emission ceases when the carrier content drops to a level equivalent to the available free volume. This release mechanism has been observed with other agents, organophosphates and carbamates in particular, and in various polymers [5–9]. More recently three-phase systems have been reported [10] involving agent A in solution in agent B, with both solutes in a carrier.

A variation of the carrier concept has been used in the membrane/reservoir-type systems in wide commercial application as anti-bacterial fabrics, contact insecticidal tapes, etc. [11–13].

In 1964 one of the authors formulated specific organotin anti-fouling agents in elastomers and developed the diffusion-dissolution mechanism of release [14]. The resulting product has exhibited over 10 years biologically effective life in commercial usage [15]. The technique was extended to include molluscicides [16], membrane/reservoir systems for even longer agent emission [17] and three-phase systems based on a monolithic absorbant reservoir mechanically dispersed within a rubber matrix [18]. The diffusion-dissolution release mode was found applicable to aquatic insect larvicides [19], aquatic herbicides [20, 21], schistosome larvicides [22] and bactericides [23].

The diffusion-dissolution mechanism required agent solubility in the selected elastomer. Non-soluble materials will not release from polymers by this method and some sort of leaching mechanism had to be developed, as with most anti-fouling paints.

It was found possible to incorporate copper sulphate at a 30% or higher loading in an ethylene-propylene-diene terpolymer and achieve 6 to 8 months continuous Cu++ emission, provided a secondary co-leachant was used to adjust interfacial pH [24]. It was discovered that two materials could be simultaneously leached from an elastomer.

One basic problem retarding commercial acceptance of controlled release agent/elastomer systems was not efficacy, which has been well demonstrated under field conditions, but the high cost of processing. Use of thermoplastics would be ameliorative and studies began in 1975 with this view in mind.

1.2. Controlled release mechanisms

Other than the 'carrier type' mechanism there are four distinct methods through which a chemical agent can be slowly released from a polymeric matrix. Each method will be summarized in the following sub-sections.

1.2.1. Micro-encapsulation

In this method a polymeric envelope is created that surrounds, or partially surrounds, a small mass of the agent through the co-acervation process. Once the capsule is formed two distinct phases exist, namely a solid or highly viscous

liquid enveloping film encompassing a solid, liquid or gaseous core. This technology arose in 1939 and underlines hundreds of commercial products. There are over 180 US patents covering formulations, processes and uses. In the late 1960s it was applied to pesticides. Efficacy depends on the agent molecule leaving the core area and moving to the target. This can occur through vapour diffusion of the agent permeating the envelope or destruction of the enveloping and protecting film by chemical or physical natural processes. Micro-encapsulation of pesticides provides continuous agent emission for several weeks, efficacy and improved safety to humans involved in dispersal operations.

1.2.2. Pendent substitution

This technique consists of chemically bonding a pest control agent to a polymeric backbone. The bond in question is cleaved through natural processes such as hydrolysis and the agent molecule emitted into the surrounding media, usually water. The technology is new and, except for anti-fouling agents, has yet to be evaluated under rigorous field conditions.

1.2.3. Monolithic agent incorporation in polymers

In this technology a pesticidal agent is incorporated in a polymeric material under unique conditions. Polymer selection is limited to elastomers wherein the agent is soluble (elastomers are highly viscous liquids obeying the laws of the liquid state). Useable agents are liquids or solids soluble to an appropriate degree in the elastomeric matrix. On addition of the agent to the elastomer a condition of true solubility exists. Agent release occurs as follows: solute molecules on or near the elastomer/medium interface pass into the medium through volatilization or dissolution processes. This occurrence creates a localized solution disequilibrium and molecules internal to the elastomer migrate under solution pressure to the depleted elastomer surface where the loss process is continuous. Molecules move as a liquid or solid by diffusion processes.

The mechanism is termed diffusion-dissolution in that essential agent migration occurs through diffusion and loss into the ambient media occurs through the passing of the agent from solution in the elastomer to the external environment. Elastomers may be cross-linked or not, although in the practical sense the necessary physical properties necessitate cross-linking (vulcanization). Through proper use of compounding additives, such as carbon black, and proper vulcanization as regards heat history (i.e. degree of cross-linking) both the solubility and diffusion rate of the agent in the elastomer matrix is controllable.

This technology arose with the need for long-term anti-fouling coverings but was quickly extended into the public health area through development of molluscicidal [16, 22, 25] and insecticidal [19] compounds.

1.2.4. Monolithic agent incorporation in plastics

Plastic and elastomers vary greatly in thermodynamic and kinetic properties, although both categories consist of high molecular weight polymers. Thermosetting plastics are usually solids at room temperature, and the cross-linking process, once it has occurred, is not reversible. Thermoplastic materials are usually solids but can be liquids at ambient temperatures. Cross-linking is reversible. Also, materials called 'plastomers' exist which combine some elastomeric properties with some plastic properties in the same molecule. Pesticidal agent solubility in plastics is very low or non-existent. Consequently, the diffusion-dissolution release mechanism is not attainable. Release must arise from some other modus operandi.

It was discovered that a leaching-type mechanism would allow the continuous release of an agent into the water or soil provided a proper porosity was induced in the dispenser. Thus, a formulation additive was necessary that would enhance porosity. It was known from the earlier art that a secondary co-leachant could be added to a plastomer in order to effect agent release [24].

It was recently discovered that pesticides and fertilizers could be incorporated in a thermoplastic matrix, and through addition of a porosity-inducing agent, or 'porosigen', caused to emit in water or moist soil [25–27].

The porosity-inducing agent, on emission in water, creates a pore structure through which ingressing water contacts, solubilizes and removes the agent. The mechanism involved is leaching in that, unlike diffusion-dissolution, the agent does not physically move within the matrix but depends on water contact via a growing pore structure. The nature and amount of the porosigen additives is determinant to the rate of agent release. The polymers used are not plasticized. Technology was extended to include methods for adjusting free volume (i.e. voids between polymer molecules) to increase or decrease the size of the developing porosity and the various geometries that the agent dispenser can take [28–30].

2. THERMOPLASTIC-BASED INSECTICIDE DISPENSERS

Initial studies were addressed to the development of thermoplastic-based controlled release molluscicides, algicides and insect larvicides where agent emission is in water. Since the diffusion-dissolution method is inoperable and carrier-type systems are inadequate (as well as expensive) for emission beyond a few months, the remaining economic choice was to develop a long-term leaching system.

It was discovered that water soluble co-leachants in thermoplastics gradually solvate into an external water medium, leaving a porosity network for further water egress and subsequent contact and partitioning of the agent. A secondary key discovery was also needed before very efficient leaching systems could be developed. Porosigens and many chemical agents are generally, although not

TABLE I. TEMEPHOS RECIPE [28]

Ingredient	Amount (%)	Use	Manufacturer
Vistalon 707	34.0	Thermoplastic binder	Exxon Chemical Co.
Microthene 718	34.0	Thermoplastic binder	U.S.I. Chemical Co.
Zinc stearate	0.2	Dispersant	–
Calcium carbonate	16.5	Porosigen	–
Ammonium sulphate	1.0	Porosigen initiator	–
Silicon dioxide	6.0	Secondary porosigen	–
Temephos	8.2	Agent	90% technical grade, American Cyanamid Co.

always, solids and thus matrix incorporation is not of dispersed molecules, but of molecular aggregates. Such aggregates are relatively large and cannot traverse intramolecular voids normal to polymers at rates conducive to the desired dispenser surface emission. It was found that free volume is dramatically increased by using polymer blends wherein the several polymers have a disparity in melt index.

Consequently, through proper adjustment of free volume and use of one or more porosigens a new class of long-term controlled release dispensing systems was developed. The concept has been extended from emission in water to emission in soil, air and living organisms.

3. AQUATIC LARVICIDES

Initial larvicide work was aimed at the mosquito. The agents used were temephos, which has been cleared for use in potable water [31], and tributyltin fluoride, a potent non-persistent molluscicide and highly selective insect larvicide [32, 33]. Early formulations have been disclosed elsewhere and the test results described [25, 27, 33]. Proper selection of the porosigen in terms of processing compatibility and water solubility, porosigen/porosigen and porosigen/agent ratios leads to desired release rates.

Controlled release temephos is commercially available as ECOPRO™ 1707 (Environmental Chemicals Inc., Barrington, Illinois, USA). It has been produced in various dispenser geometries: sinking pellet, suspending strand, suspending chip, bimodal (mud floating) pellet, etc. tailored to the environmental conditions [28, 34, 35]. The formulation shown in Table I has efficaciously released temephos for over 3 years (Table II) in repeat challenge laboratory tests.

TABLE II. LONG-TERM IMMERSION REPEAT CHALLENGE BIOASSAY OF CONTROLLED RELEASE TEMEPHOS AGAINST *C. quinquefasciatus* LARVA: 1st AND 2nd INSTAR

Pre-test water immersion time (d)	LT_{100} (d)[a] Active agent pellet concentration (ppm)										
	0.035 ppm	0.06 ppm	0.14 ppm	0.23 ppm	0.46 ppm	0.54 ppm	0.84 ppm	1.3 ppm	1.5 ppm	2.2 ppm	3.4 ppm
30	6	5	5	2	2	2	3	2	3	2	2
70	11	9	6	4	4	3	3	3	3	3	2
110	10	13	7	8	8	3	3	5	4	3	2
160	11	9	11	4	–	6	6	3	1	2	1
225	14	9	5	4	4	4	5	7	3	2	1
310	–	–	–	6	5	5	5	3	6	3	3
416	–	–	–	8	7	5	5	5	5	1	2
532	–	–	–	10	9	5	2	3	3	2	2
710	–	–	–	–	12	5	3	3	3	3	2
820	–	–	–	–	–	5	5	5	5	1	1
950	–	–	–	–	–	2	3	3	3	2	1
1108	–	–	–	–	–	7	8	6	5	1	2

[a] LT_{100} = lethal time to 100% population mortality.

TABLE III. LONG-LASTING CONTROLLED RELEASE CONTACT INSECTICIDES [30]

Ingredient	Recipe (%)		
	A	*B*	*C*
Vistalon 707	84.0	69.0	–
Microthene MU 763[a]	1.0	–	68.8
Zinc stearate	1.0	1.0	1.0
Diazinon SOW/P[b]	10.0	20.0	–
Lecithin	5.0	–	–
Soy oil	–	10.0	10.0
Baygon (70%)[c]	–	–	15.7

[a] Ethylene vinyl acetate co-polymer; manufactured by U.S.I. Chemicals, Inc.
[b] 0,0-diethyl-o-(isopropyl-6-methyl-5-pyrinidinyl) phosphorothioate, used as 50% powder; from Ciba-Geigy Corp.
[c] 2-(1-methylethoxy)phenol methylcarbamate, used as 70% active powder; from Mobay Chemical Corp.

Field evaluations have been extensive [35] and are generally favourable. Several dispenser types are on the commercial market, e.g. an anchored floating strand for use against catch-basin mosquitoes and an anchored floating chip used by the World Health Organization for mosquito control in potable water reservoirs.

4. INSECT ADULTICIDES

Air release systems have been developed that rely basically on insect contact with the pesticide. In one system the agent, an organophosphate or carbamate insecticide, is coupled with an additive, soy oil or lecithin, that serves both as a volatile porosigen and an attractant in a polymer alloy to ensure adequate free volume [30]. Over 18 months effective control of cockroaches and ants has been demonstrated, as well as considerable promise for use against Silver fish.

Several recipes are shown in Table III. Effective and rapid control of *Blatella germanica* (cockroach) and *Formica fusca* (black ant) for at least 18 months has been noted.

This technique, using either a pheromone or other attractant, should be readily extendible to agricultural usages in crawling or flying insect control.

5. CONTROLLED RELEASE HERBICIDES

5.1. Aquatic herbicides

Aquatic herbicides keyed to the control of unwanted plant life in navigable waterways and irrigation systems were first developed in 1969 [4]. It was discovered that the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (2,4-D BEE) was soluble in natural rubber and other elastomers. Field and laboratory evaluations showed long-term (in excess of three years) efficacy [36]. However, processing, and thus manufacturing costs, are high and this material has never achieved commercialization. In follow-up studies it was noted that other esters and amines of 2,4-D could be used, as well as a number of other herbicides.

Two important facets of this effort lie in demonstration of the versatility of use of polymeric materials, i.e. the wide variation possible in dispenser design, and discovery of the so-termed 'chronicity phenomenon'.

5.2. Chronicity phenomenon

Conventional pesticide treatment methodology consists of application of a relatively large amount of the chemical agent to the target habitat. The quantity used depends on the agent of choice, the target species and the nature of the environment to be intoxicated. In use, conventional herbicide dosages vary from less than 1 ppm to over 30 ppm. Normal dosages are 'massive' in the sense that the amount applied is always far in excess of the amount required if the lethal dose per individual target is multiplied by the number of target organisms to be destroyed.

Of necessity large quantities of the given pest control agent are used in order to overcome dispersal through water flow, resulting in a rapid drop in concentration, and detoxication processes arising from the chemical reaction with dissolved or suspended matter, hydrolysis, solar radiation, etc. The effective half-life of a given pesticide molecule in a natural water body may be but a few hours. During this restricted exposure time the target species must absorb a lethal dose. If it were necessary to maintain a high toxicant concentration in the water course treated, say 5 ppm or greater, the volume of controlled release material necessary would be prohibitive. Consequently, one of the problems addressed early in the development of controlled release pesticides was to determine the application dosages wherein the target species population is exposed continuously to the agent present rather than periodic exposure as with conventional technology.

It has long been presumed that the Concentration X Time (CT) relationship is a valid method of determining the pesticide application dosage. That is, if target exposure at 2 ppm for 6 h provides a lethal dose for some segments of the

population, then 1 ppm for 12 h or 4 ppm for 3 h are equally effective [37]. Universal acceptance of this doctrine is convincing evidence as to at least an approximate reliability. Obviously there are upper limits based on respective kill mechanisms. Snails exposed to 1 ppm copper ion concentration under laboratory conditions succumb within 6 h to acute intoxication. At 100 ppm copper ion concentration the same species, *Biomphalaria glabrata*, does not die in 0.06 h (3.6 min). Sufficient time is necessary for the movement of copper ion into the molluscan target, transport through internal tissue and accumulation of a lethal dosage at sites of cidal activity.

The critical question as regards controlled release is whether the CT relationship holds at dosage levels practical to the emission of an agent from a polymeric matrix. If the necessary dosage is 2 ppm for a given plant species to succumb to a given herbicide, and the practical emission rate will provide only 0.01 ppm/d, then 200 d would be required for control. Obviously such a formulation would have severely limited value, if any.

Fortunately the CT relationship is not valid at ultra-low dosage levels.

A number of laboratory experiments were performed to determine dosage levels necessary to destroy various aquatic weeds through herbicide emission from a polymeric matrix. During the course of this activity it was noted that the CT presumption was not valid when a given target was exposed to continuous herbicide stress over a long duration of time at very low concentrations [38].

Continuous water plant exposure to aquatic herbicide dosages in the 0.1 to 0.001 ppm concentration range, as continuously maintained by release from a dispensing polymer granule, will kill a wide variety of plants within several weeks [39–41]. This phenomenon has been also observed with snail and mosquito species exposed to ultra-low, but continuous, molluscicide and larvicide dosages [4, 25].

The chronicity phenomenon has been observed with the herbicides noted in Table IV and evaluated against the various major aquatic weeds listed in Table V. Most of the herbicides evaluated against aquatic weeds are in agricultural use. The phenomenon is aptly described by the data given in Table VI. This refers to one of numerous 56-d laboratory evaluations published in full [42].

Assessment of the chronic intoxication syndrome has been initiated. The working hypothesis is that the target organism's biochemical response to xenobiotic entry is not triggered in that the ultra-low agent concentrations involved are sub-threshold. That is, normal penetration barriers are not erected as the target's defence mechanism fails to detect toxicant ingress. This level varies for different species, but is generally below 0.1 ppm water concentration.

5.3. Controlled release terrestrial herbicides

In the summer of 1980 several controlled release terrestrial herbicides based on a thermoplastic dispensing system were prepared and evaluated [36].

TABLE IV. HERBICIDES EXHIBITING THE CHRONICITY PHENOMENON

Trade or laboratory designation	Chemical name	Type
Diquat™ (Chevron Chemical Co.)	6,7-dihydrodipyrido (1,2-a:2′, 1′-c) pyrazidiinium dibromide	Technical 97%
2,4-D BEE (Dow Chemical Co.)	Butoxyethanol ester of 2,4-dichlorophenoxyacetic acid	Technical 97%
Fenac™ (Amchem Products Inc.)	2, 3, 6-trichlorophenylacetic acid	Technical 100%
Silvex™ (Dow Chemical Co.)	2-(2, 4, 5-trichlorophenoxy) propionic acid	Technical 100%
2,4-D acid (Dow Chemical Co.)	2,4-dichlorophenoxyacetic acid	Technical 96%
2,4-D butylester (Dow Chemical Co.)	Butylester of 2,4-dichloro-phenoxyacetic acid	Technical 96%
Endothall (Pennwalt Corp.)	7-oxabicyclo (2,2,1) heptane-2,3-dicarboxylic acid	Technical 96%
Fenuron (E.I. DuPont de Nemours & Co.)	1,1-dimethyl-3-phenylurea	Technical 98%
2,4-D oleylamine (Dow Chemical Co.)	Oleylamine salt of 2,4-dichloro-phenoxyacetic acid	Technical 94%
2,4-D amine (Dow Chemical Co.)	Dimethylamine salt of 2,4-dichloro-phenoxyacetic acid	Technical 97%
Acrolein (Shell Chemical Co.)	2-propenal	Chemically Pure 100%
Hydrothol (Pennwalt Corp.)	Sodium salt of endothall	Technical 96%
Dichlobenil (Thompson-Hayward Chemical Co.)	2,6-dichlorobenzonitrile	Technical 99%

TABLE V. PLANT SPECIES EVALUATED

Common name	Biological name
Water hyacinth	*Eichornia crassipes*
Alligator weed	*Alternanthera philoxeroides*
Elodea	*Elodea canadensis*
Eurasian watermilfoil	*Myriophyllum spicatum*
Vallisneria	*Vallisneria americana*
Cabomba	*Cabomba caroliniana*
Water lettuce	*Pistia stratiotes*
Southern naiad	*Najas guadalupensis*
Coontail	*Ceratophyllum demersum*
Smart weed	*Polygonum*
Duck weed	*Lemna minor*

A small 40′ X 200′ weed-grown field was plowed and disced to a depth of approximately 6 in.[1] All existing weeds and grass were raked off before the field was seeded with common dandelion. Sections of 30′ X 10′ were marked off. Each section contained three 10′ X 10′ plots. One plot was treated at a high dosage, another received a low dosage and the third plot served as the control. Growth of the weeds was checked weekly or when weather permitted. During each examination period the number of all weeds (except dandelions) were counted in five 1 ft^2 segments selected randomly.[2] Average weed heights were also noted. The formulations evaluated are shown in Table VII [43, 44] and the results in Tables VIII–X.

Formulations of bromocil and 2,4-D acid were also evaluated in these preliminary studies. Results indicate the feasibility of the controlled release methodology against terrestrial weeds, although optimum compounds have yet to be developed. It was observed that the dichlobenil formulations remained active not only during the summer of 1980 but carried over with similar efficacy throughout the 1981 growing season.

[1] 1 ft = 3.048×10^{-1} m; 1 in = 2.54×10^{1} mm.
[2] 1 ft^2 = 9.290×10^{-2} m^2.

TABLE VI. LT VALUES FOR *Myriophyllum spicatum* EXPOSED TO VARIOUS HERBICIDES AT A CONSTANT DOSAGE
(five replicates X three plants per replicate)

Herbicide	Concentrations (ppm/d)	Days to a given mortality			
		LT_{25}	LT_{50}	LT_{90}	LT_{100}
Diquat	1.0	6	9	11	11
	0.1	6	9	13	19
	0.01	6	9	13	16
	0.001	15	19	25	32
2,4-D BEE	1.0	7	8	11	14
	0.1	7	9	14	22
	0.01	7	11	22	27
	0.001	12	20	32	56+[a]
Fenac	1.0	12	19	30	36
	0.1	24	28	37	39
	0.01	20	56+	–	–
	0.001	22	56+	–	–
Silvex	1.0	6	8	12	15
	0.1	13	14	18	21
	0.01	18	21	23	28
	0.001	22	24	27	32
2,4-D acid	1.0	9	12	18	20
	0.1	14	17	21	29
	0.01	26	34	35	39
	0.001	29	40	46	56+
2,4-D butyl ester	1.0	4	5	7	7
	0.1	5	6	8	10
	0.01	6	7	9	10
	0.001	15	34	60+[a]	–
2,4-D oleylamine ester	1.0	4	5	6	7
	0.1	7	9	11	12
	0.01	9	12	16	17
	0.001	19	28	47	60+

[a] 56+ and 60+ = over 56 and 60 d.

TABLE VII. CONTROLLED RELEASE TERRESTRIAL HERBICIDES [43, 44]

Formulation code[a]	Active agent	% active agent in formulation
2508F	Dichlobenil	20
2508H	Dichlobenil	20
2508J	Dichlobenil	20
2501F	Diuron	31
2501H	Diuron	31
2501J	Diuron	31

[a] Variation is in the matrix/porosigen system used.

6. TRACE NUTRIENTS

Water-soluble salts of trace metals have been successfully incorporated in thermoplastic matrices and are currently undergoing extensive field evaluation in Australia. Salts and oxides of zinc, iron, copper, molybdenum, boron, cobalt, manganese, magnesium and selenium have been evaluated [45]. Typical formulations are shown in Table XI. A laboratory evaluation of several of the controlled release zinc compounds with soy bean plants is presented in Tables XII and XIII. The soil content in each growth pot was 1300 g and soil containing less than 0.01 ppm soluble zinc came from the state of Arizona.

7. IN-FLIGHT MICRO-ENCAPSULATION

A major advance in the micro-encapsulation of pesticides and pheromones was first announced in 1978 [46]. In previous technology micro-capsules were, and still are, processed within a manufacturing facility and commercially distributed at a given mesh size and agent concentration. It was discovered that co-acervation was possible from an agent/thermoplastic/solvent system during flight [47]. That is, a liquid solution is sprayed by conventional air or ground spray equipment and during flight solvent evaporation initiates co-acervation with a dry micro-capsule impinging on the foliage or soil. By a proper mix of low and high molecular weight carboxylated polyacrylates it is possible to formulate envelope surface tack sufficient for adhesion to foliage even under adverse climatic conditions.

TABLE VIII. CONTROLLED RELEASE TERRESTRIAL HERBICIDE DATA (weeds/ft^2)[a]

Number	Code	Active dosage (lb/acre)[b]	Days 16	26	34	44	54	64
1	2508F	4	30	11	21	13	15	22
2	2508F	12	4	3	4	5	3	6
3	Control	–	24	12	42	50+[c]	50+	50+
4	2508H	4	8	9	9	9	6	15
5	2508H	12	5	2	3	2	3	4
6	Control	–	8	9	17	50+	50+	50+
7	2508J	4	10	9	5	5	6	10
8	2508J	12	2	2	1	2	1	6
9	Control	–	15	17	43	50+	50+	50+
10	2501F	5	19	8	7	13	8	16
11	2501F	20	12	3	3	2	3	8
12	Control	–	39	30	34	50+	50+	50+
13	2501H	5	10	9	9	10	7	9
14	2501H	20	10	2	1	3	2	3
15	Control	–	46	24	52	50+	50+	50+
16	2501J	5	12	9	8	11	8	14
17	2501J	20	10	6	8	14	7	7
18	Control	–	29	20	21	50+	50+	50+

[a] 1 ft^2 = 9.290 X 10^{-2} m^2.
[b] 1 lb = 0.4536 kg; 1 acre = 4.047 X 10^3 m^2.
[c] 50 + throughout = over 50 d.

TABLE IX. TERRESTRIAL WEED DATA (DANDELIONS)
(count per plot; initial date 28 May 1980)

Number	Code	Active dosage (lb/acre)	Days 16	26	34	44	54
1	2508F	4	0	0	0	2	0
2	2508F	12	0	1	1	0	0
3	Control	–	0	11	8	*[a]	*
4	2508H	4	0	1	1	12	2
5	2508H	12	0	1	1	2	0
6	Control	–	0	15	14	*	*
7	2508J	4	0	1	1	0	3
8	2508J	12	0	3	3	0	2
9	Control	–	0	30	28	*	*
10	2501F	5	0	2	2	2	3
11	2501F	20	0	5	5	9	16
12	Control	–	0	15	14	*	*
13	2501H	5	0	0	1	16	4
14	2501H	20	0	0	0	2	2
15	Control	–	0	12	20	*	*
16	2501J	5	0	6	6	60	50
17	2501J	20	0	6	9	43	40
18	Control	–	0	14	13	*	*

[a] Asterisk throughout = other weeds obscured dandelion count.

TABLE X. TERRESTRIAL HERBICIDE WEED HEIGHT DATA
(average)

Number	Code	Active dosage (lb/acre)	Height 64 d post-application (in.)[a]
1	2508F	4	4.6
2	2508F	12	9.4
3	Control	–	23.0
4	2508H	4	11.7
5	2508H	12	9.2
6	Control	–	25.0
7	2508J	4	10.0
8	2508J	12	7.7
9	Control	–	30.0
10	2501F	5	6.3
11	2501F	20	5.0
12	Control	–	29.0

[a] 1 in = 2.54×10^1 mm.

Agents usable in this application consist of a number of trace nutrients, especially zinc compounds, insecticides, acaricides, several nematicides, fungicides such as benomyl pheromones, and various terrestrial herbicides. Several recipes are given in Table XIV.

Cross-linking occurs with volatilization of ammonia and the solvent system. Once cross-linked the formed micro-capsule is insoluble in water at pH values below 9.5. During flight micro-spheres are formed of remarkably narrow size range, about 20 to 40 μm dependent on nozzle parameters, and no fines are observed, thus dramatically reducing the spray drift problem.

Test results with the first commercialized product, a horse barn spray, have shown an increase in effectiveness from $\frac{1}{2}$ d with conventional repellent/insecticides to 19 to 28 d using in-flight encapsulation.

8. DISCUSSION

Controlled release pesticides used as anti-foulants, molluscicides and larval insecticides in the public health area, and bait/contact toxicant systems have shown considerable efficacy, as evidenced by the increasing number of commercial-

TABLE XI. CONTROLLED RELEASE TRACE NUTRIENT FORMULATIONS

Ingredient	Code (recipe by parts weight)						
	1-D	3-A	4-D	5-I	9-D	11-D	12-K
MU-763[a]	50	100	100	–	–	100	–
LDPE 718[b]	40	–	–	50	50	–	50
Zinc stearate[c]	2	2	2	2	2	1	2
Ammonium sulphate[d]	5	5	–	–	5	–	–
Zinc sulphate[e]	60	–	–	–	–	–	–
Zinc chloride[e]	–	80	–	–	–	–	–
Zinc carbonate[e]	–	–	80	–	–	–	–
Vistalon 702[f]	–	–	–	50	50	–	50
Copper sulphate[e]	–	–	–	100	–	–	–
Ferric oxide[e]	–	–	–	–	80	–	–
Boric acid[e]	–	–	–	–	–	50	–
Sodium bicarbonate[d]	–	–	–	–	–	2	3
Sodium molybdate[e]	–	–	–	–	–	–	75
Emission rate (% agent loss in water per day)	0.38	2.1	0.029	0.31	0.0017	0.72	1.63

[a] Ethylene vinylacetate co-polymer.
[b] Low density polyethylene (U.S.I. Chemicals Co. code MN 718).
[c] Dispersant.
[d] Porosigen.
[e] Nutrient agent.
[f] Ethylene propylene co-polymer.

ized products. Application of this technology to agricultural usage has been slow, although discovery of the porosigen-type system using thermoplastic matrices should see increasing technology transfer from the public health domain to agronomy and agriculture.

The special advantages, well discussed in a rapidly growing volume of scientific literature, lie basically in extension of the between-application interval, thus providing significant economy of labour. In agriculture this may be of considerable value as regards trace nutrients and soil insecticides. Controlled release herbicides lasting beyond one growing season may be undesirable, especially where crop rotation is commonly practised. In general, less chemical agent is required than that necessary in the conventional art. Reductions to 10% or less of the agent quantity will give equivalent results, at least with the materials currently commercialized. The luxurious consumption seen early in the herbicide and fertilizer treatment cycle is avoided. Environmental contamination is significantly decreased.

TABLE XII. GROWTH RATE OF SOY BEANS IN ZINC-POOR SOIL TREATED WITH CONTROLLED RELEASE ZINC FORMULATIONS

Compound	Pot dosage (g)	Average post-germination stem growth (cm/d)	Zinc content in leachate (ppm/d)
1-D	1	2.75	0.0015
	0.5	1.94	0.0011
	0.7	1.40	0.0007
	0.1	1.14	0.0002
Control	0.0	1.09	0.00008
4-E	1	3.13	0.003
	0.5	2.20	0.004
	0.2	2.14	0.002
	0.1	1.95	0.002
Control	0.0	1.07	0.00008

TABLE XIII. ZINC ANALYSIS OF PLANT TISSUE AT 56 d POST-GERMINATION OF SOY BEANS

Compound	Post dosage (g)	Zinc ion content		
		Soil (ppm)	Leaf (ppm)	Root (ppm)
1-D	1.0	0.075	0.03	0.10
	0.5	0.060	0.05	0.09
	0.2	0.050	0.03	0.08
	0.1	0.050	0.04	0.07
Control	0.0	0.00	0.05	0.04
4-E	1.0	0.01	0.08	0.05
	0.5	0.01	0.05	0.03
	0.2	0.04	0.08	0.02
	0.1	0.02	0.08	0.01
Control	0.0	0.005	0.05	0.01

TABLE XIV. IN-FLIGHT MICRO-ENCAPSULATION RECIPES

Ingredient	Recipe (%)				
	1	2	3	4	5
Carboset 525[a]	16	6	6	–	12
Carboset 526[b]	–	7	–	–	–
Carboset XL11[c]	–	–	6	6.25	12
Carboset 514[d]	–	–	6	–	–
Ethyl acetate[e]	–	–	1	–	–
Calcium chloride[f]	0.1	0.2	0.2	–	0.1
Ammonium hydroxide[g]	0.4	0.1	0.1	–	0.05
Ethanol[h]	50	69	66	–	37.85
Water[h]	–	–	–	87.5	–
Agent	33.5	17.7	20.7	6.25	30
Calcium benzoate[i]	–	–	–	–	1

[a] Moderate molecular weight carboxylated polyacrylate, manufactured by the B.F. Goodrich Co.
[b] High molecular weight carboxylated polyacrylate.
[c] Water soluble carboxylated polyacrylate.
[d] Low molecular weight carboxylated polyacrylate, which provides tackiness to the developed envelope.
[e] Adhesion promoter.
[f] Source of divalent calcium ion for cross-linking.
[g] Maintains alkaline pH to prevent premature cross-linking.
[h] Solvent.
[i] Preservative.

Once the agent is bound within a thermoplastic dispenser, such as a granule or pellet, it is not available in quantity to man or other non-target organisms. The health hazard exposure to agricultural workers has been dramatically reduced. For instance, controlled release tributyltin fluoride, a molluscicide, shows no acute rodent toxicity compared with around 200 mg/kg rat LD_{50} for the non-formulated powder.

Thermoplastics are inert and non-toxic. Once in the environment chemical degradation, generally to water, carbon dioxide and lower alkyls, proceeds extremely slowly and should have a reasonably negligible impact.

Thermoplastics are produced from petroleum or natural gas stocks and thus shortages can be predicted. However, current work indicates that controlled

release agricultural chemical formulations can be made, using cellulosics and other products from renewable natural resources. The binding matrix element is relatively unimportant as long as free volume constraints to agent movement are overcome through proper compounding. The vital element is selection of the appropriate porosigen or porosigen mixes.

Controlled release granules, pellets or other geometrical dispenser forms lend themselves readily to the scientific study of agent movement. Once inserted into the soil, for instance, the dispenser is relatively stationary, being water insoluble and too large for movement within the soil. Thus, the site of agent injection is focal and agent transport through the surrounding soil is readily measurable. Through using radiolabelled herbicides, insecticides, etc. it is possible to analyse reliably soil, or plant, aliquots at a known distance from the dispenser and thus compute transport parameters and a time profile of agent movement. Conventional agricultural chemical application does not readily allow such studies in that no stable and stationary injection foci exist. Obviously the total amount released can be calculated by retrieval and analysis of dispenser residues.

The authors have not studied radiolabelled agricultural agents in this regard but have, to date, confined their studies to ^{14}C-labelled and ^{113}Sn-labelled molluscicidal tin compounds. However, the above method of determining agent movement is workable in both soil and water organotin transport studies.

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Poster Presentations

IAEA-SM-263/16

ESTUDIO COMPARATIVO DE PERSISTENCIA DE TRES INSECTICIDAS CLORINADOS EN LECHUGA POR CROMATOGRAFIA GASEOSA

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INTRODUCCION

Se analizaron residuos de BHC, Heptacloro y Aldrín en lechuga mediante cromatografía de gases.

MATERIALES Y METODOS

Aplicación de los insecticidas

Los insecticidas fueron aplicados por aspersión foliar a plantas de lechuga de dos meses de edad, en las dosis indicadas en las recomendaciones técnicas de cada uno de los plaguicidas usados. Se efectuaron dos aplicaciones con una semana de intervalo.

Muestreo

De las hojas se llevaron a cabo los siguientes muestreos:

1) Antes de la primera aplicación (A-P1);
2) Después de la primera aplicación (D-P1);
3) Antes de la segunda aplicación (A-P2);
4) Después de la segunda aplicación (D-P2);
5) Una semana después de la última aplicación (1-S);
6) Dos semanas después de la última aplicación (2-S).

Análisis por cromatografía de gases

Se extraen 30 gramos de muestra con una mezcla de hexano-acetona al 3:1, por licuado durante tres minutos. Dos particiones líquido-líquido con acetonitrilo preceden al "clean-up" por columna de Florisil, de la cual una mezcla de hexano-éter dietílico al 6,15 y 50% como eluatos y una concentración previa a la inyección en cromatógrafo de gases con detector de captura de electrones de ^{63}Ni permiten cuantificar las concentraciones de plaguicidas en las muestras [1].

RESULTADOS Y CONCLUSIONES

Los resultados encontrados indican de una manera evidente lo que sucede con cada uno de los plaguicidas al ser aplicados en lechuga.

El BHC, dos semanas después de la última aplicación está a un nivel de 0,67 ppm, lo que está por bajo del límite máximo (2) [2].

El Heptacloro, dos semanas después de la última aplicación está a un nivel de 0,09 ppm, cifra que es superior al límite máximo (0,05 ppm).

El Aldrín, dos semanas después de la última aplicación está a un nivel de 0,14 ppm, frente al límite máximo que es 0,1 ppm.

Así, se puede concluir que si se siguen las recomendaciones de cada producto, y si se tiene muy en cuenta el límite de la última aplicación que se puede dar a un cultivo antes de la cosecha, se da tiempo a que el producto, si bien no desaparece completamente, por lo menos los residuos de plaguicidas estarán muy por debajo de los límites máximos y, por tanto, se podrán ofrecer alimentos libres de residuos tóxicos.

Tenemos proyectado continuar este trabajo comparando los resultados con los que se obtengan por ensayo radioinmunológico (RIA).

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IAEA-SM-263/5

ETUDE SUR LES RESIDUS DE PRODUITS PHYTOSANITAIRES DANS LES CULTURES DE TOMATES ET DE PETITS POIS

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L'étude sur tomates a été effectuée sur une trentaine de matières actives (fongicides, insecticides et herbicides) choisies parmi les plus employées sur cette culture. La même étude sur petits pois porte sur trois insecticides.

Méthode

Les traitements ont été effectués aux conditions normales (dose recommandée par les firmes phytosanitaires et par l'ACTA) et à dose double. Le délai d'application avant la récolte est normal ou réduit de moitié.

Afin de juger de l'influence des traitements technologiques sur les teneurs finales en résidus, les dosages ont été faits aux différents stades de fabrication (parage, lavage, stérilisation) et sur les déchets de fabrication (peaux de tomates et gousses de petits pois).

Résultats et conclusion

Si les conditions d'emploi sont respectées, les teneurs résiduelles obtenues sur tomates et petits pois frais ou transformés sont en général inférieures aux tolérances FAO/OMS.

Les teneurs trop élevées en produits phytosanitaires sur et dans les tomates ou petits pois ne peuvent provenir, dans nos conditions d'essais, que d'une application du produit en quantité trop importante ou bien du non-respect du délai de traitement.

IAEA-SM-263/2

CONTRIBUTION DE L'ACTIVATION NEUTRONIQUE A POSTERIORI A L'AMELIORATION DES TECHNIQUES DE TRAITEMENTS PHYTOSANITAIRES

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L'amélioration des techniques de traitements phytosanitaires par l'étude des facteurs de constitution des dépôts initiaux suppose que l'on dispose de techniques efficaces permettant de caractériser localement ces dépôts.

Etant donné les inconvénients inhérents à l'utilisation de la radioactivité dans un processus expérimental ayant pour cadre un site naturel, la technique mise en œuvre est celle du traceur activable a posteriori [1–4].

Le choix du traceur inactif a été dicté par un certain nombre d'impératifs d'ordre nucléaire et caractéristiques de l'expérimentation proprement dite.

La technique consiste à pulvériser une solution de chlorure de scandium ($ScCl_3$) dans l'eau ou une solution d'un complexe de scandium (Na(Sc, EDTA)) en mélange avec des produits phytosanitaires dans l'eau.

Des languettes en polyéthylène de 4 X 10 cm disposées en fonction des objectifs de l'expérimentation, ou la matière végétale elle-même, servent de substrats de récolte. Après séchage à l'air libre, chaque languette traitée est introduite dans un conteneur en polyéthylène et le scandium ainsi recueilli est soumis à un flux intégré de neutrons thermiques de l'ordre de $6 \cdot 10^{16}$ neutrons par cm^2 (réacteur BR 1, CEN, Mol, Belgique). Des étalons sont soumis à la même irradiation. La réaction nucléaire produite est $^{45}Sc(n,\gamma)^{46}Sc$. Après refroidissement approprié, la radioactivité gamma des échantillons est mesurée à l'analyseur d'amplitude multicanaux équipé d'un détecteur NaI(Tl) et relié à un petit ordinateur.

Cette installation permet de doser le scandium par analyse de son pic à 1120 keV et donc de quantifier le dépôt par unité de surface.

Dans une publication récente [5], nous avons montré que l'utilisation du traceur radioactivable a posteriori constituait une méthode d'approche simple et très satisfaisante pour le contrôle de la régularité des répartitions spatiales des

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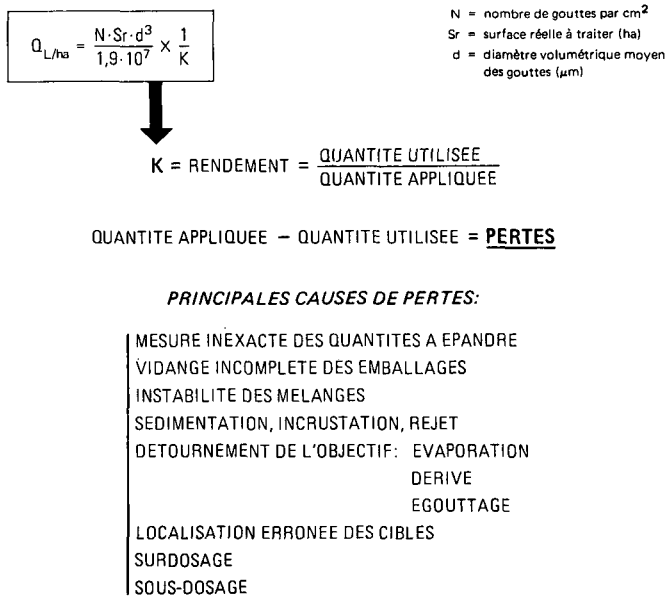

FIG.1. Les composantes du volume épandu (Q).

liquides pulvérisés. Depuis, l'intérêt de cette recherche n'a cessé de croître. La nécessité d'optimiser les conditions d'emploi des produits chimiques en agriculture en vue d'alléger la charge globale en matières en est le principal motif. Les utilisateurs, les consommateurs et l'environnement sont les bénéficiaires directs de cette démarche. L'essentiel des préoccupations et des contraintes relatives à ce problème est repris dans la figure 1. Celle-ci fait apparaître les composantes entrant en ligne de compte pour l'obtention d'une bonne pratique phytosanitaire.

Il est apparu que la valeur de K, paramètre défini dans cette figure, pouvait dans certains cas ne pas excéder 0,3. Or, une augmentation de ce rendement permettrait de réduire les volumes/hectare appliqués avec, pour conséquence, des économies substantielles en eau et en énergie à toutes les étapes de l'utilisation (approvisionnement, transport, distribution, etc.) [6].

Au stade actuel des recherches, l'analyse par activation a posteriori comme méthode de contrôle des répartitions a permis de mettre en évidence quelques unes des principales causes de pertes qui affectent le rendement K.

Le phénomène de *dérive* a pu être quantifié en champ d'essai constitué d'un damier de petites parcelles et sur différents types de cultures. Dans le même esprit, ont été examinés les *surdosages* et *sous-dosages* localisés aux différents niveaux de la masse végétale traitée.

D'autre part cette technique a également contribué à la sélection de différents moyens de correction: c'est ainsi, à titre d'exemple, qu'elle a été utilisée comme méthode de référence pour la mise au point d'un dispositif original de pulvérisation pour l'application tardive sur cultures de céréales.

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IAEA-SM-263/17

DETERMINATION DU MERCURE, DU MANGANESE, DU CUIVRE ET D'AUTRES ELEMENTS DANS LE SOL ET LES PLANTES A L'AIDE DE L'ANALYSE D'ACTIVATION DE NEUTRONS APRES UTILISATION DE PESTICIDES

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Les méthodes de radioactivation et leurs variantes radiochimiques et instrumentales permettent de déterminer un grand nombre d'éléments dans un échantillon, la sensibilité de la détermination atteignant souvent 10^{-11} g de l'élément. A la Section de radiobiologie et d'analyse par activation de l'Institut de protection des plantes, elles ont été appliquées pour déterminer la teneur en

métaux (mercure, cuivre, manganèse) de pesticides et d'autres éléments introduits dans le sol par l'intervention directe et indirecte de l'homme.

Une méthode radiochimique élaborée par nous a été utilisée pour déterminer la pollution en mercure du sol et des plantes par le fait de grains traités avec des fungicides contenant du mercure. La pointe de ^{197}Hg a été mesurée après activation des échantillons et des étalons dans un réacteur. Les résultats obtenus ont démontré que les mesures agro-techniques prises dans notre pays n'entraînent pratiquement pas de pollution du sol par le mercure. La teneur en mercure de la récolte de blé obtenue de grains traités n'est pas supérieure à 0,2 μg/g.

La détermination de la quantité de cuivre et de manganèse dans le sol et les plantes a été faite également à l'aide d'une méthode radiochimique. Les données démontrent que le cuivre est concentré dans la couche labourée (15–25 cm) du sol. Une pollution importante du sol – plus de 2200 μg/g – n'a été établie qu'autour des réservoirs contenant des pesticides à base de cuivre. L'analyse de concombres cultivés en serre a prouvé que la teneur en cuivre et en manganèse dans leur peau n'excède pas sensiblement la quantité trouvée dans leur chair.

La détermination du brome dans des fruits traités avec du bromure de méthyle contre les parasites est aussi d'un grand intérêt. Une variante instrumentale de l'analyse par activation a été appliquée. Les résultats ont démontré que la peau des pommes contient beaucoup plus de brome que leur chair. La méthode a été appliquée aussi pour déterminer la teneur en brome du sol de serres où le bromure de méthyle a été utilisé comme désinfectant contre les nématodes.

Une variante instrumentale a été appliquée également pour déterminer simultanément les éléments Ce, Cr, Cs, Mn, Rb, Fe, Zn, Co, Sb dans les sols et les plantes de la partie industrielle de Sofia. Les résultats de la spectrométrie ont été traités au moyen d'un calculateur électronique relié directement à un analyseur multicaux et ils figurent dans un tableau.

La méthode peut être utilisée avec succès pour étudier l'action des pesticides sur les plantes. A l'Institut, nous avons utilisé avec succès un générateur de neutrons rapides de 14 MeV pour déterminer la teneur en macroéléments principaux (N, P, K) après avoir pris des mesures de protection des plantes. La méthode élaborée permet en même temps de déterminer les éléments Si, Al, Mg et Cl. C'est une des méthodes permettant d'étudier la teneur des plantes en macroéléments principaux et, par là, de déterminer la teneur en protéines générales des plantes et des produits végétaux. Elle permet aussi de juger des changements ayant eu lieu dans les plantes après l'emploi de pesticides différents.

La méthode d'analyse par activation combinée avec les autres méthodes radioanalytiques permet de faire une étude complète de la pollution de l'environnement par des pesticides et d'autres polluants. La présente publication a pour but d'illustrer ses possibilités et son application dans l'agriculture et la protection de l'environnement dans la République populaire de Bulgarie.

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PESTICIDES IN FOOD
(Session VI)

Chairman

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Invited Paper

PHARMACOKINETIC STUDIES ON ^{14}C-LABELLED PHENANTHRIDINE AND AROMATIC DIAMIDINE DRUGS USED TO CONTROL AFRICAN TRYPANOSOMIASIS IN DOMESTIC ANIMALS

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Abstract

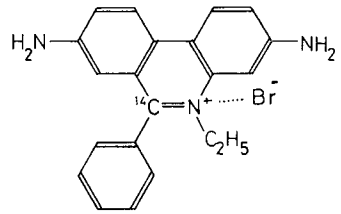
PHARMACOKINETIC STUDIES ON ^{14}C-LABELLED PHENANTHRIDINE AND AROMATIC DIAMIDINE DRUGS USED TO CONTROL AFRICAN TRYPANOSOMIASIS IN DOMESTIC ANIMALS.

For many years ethidium (homidium) bromide (3, 8-diamino-6-phenyl-5-ethyl phenanthridinium bromide) has been used curatively and for limited prophylaxis against *Trypanosoma congolense* and *Trypanosoma vivax* infections in ruminants in Africa and aromatic diamidine berenil (diminazeneaceturate, 4, 4′-(diazoamino) benzamidine) has been used as a curative drug against these parasites, but there is little published information about the pharmacokinetics of either drug. Reviewed here is the present knowledge and report results of recent experiments using ^{14}C-labelled drugs to measure blood and tissue fluid levels and tissue residues of ethidium in uninfected and *T. congolense*-infected laboratory animals and bovines and berenil in laboratory animals. In the case of ^{14}C-ethidium levels of radioactivity in blood and tissue fluids reached a maximum within 1 h of intramuscular injection (1 mg/kg) and then fell rapidly; after 96 h 80–90% of the radioactivity injected had been excreted, approximately one-third in urine and two-thirds in faeces. It is estimated that ca 3–4% of the radioactivity injected is present in tissues of animals sacrificed 9–10 d after administration of drug, the highest residues/unit wet weight of tissue being present in liver and kidney. In similar experiments with ^{14}C-berenil (3.5 mg/kg) radioactivity in blood reached a peak within 30 min, fell rapidly over the next 5 h, but remained at a significant level for 4–6 d. Radioactivity in tissue fluids did not rise as rapidly or to such a high peak level as in blood, but after 2 h remained at approximately twice the level detected in blood for up to 7 d. At 7 d, in marked contrast to ethidium, only 65% of the administered radioactivity had been excreted (44% in urine and 21% in faeces), and in animals sacrificed at that time it was found that the majority (95%) of the residual drug was present in the liver.

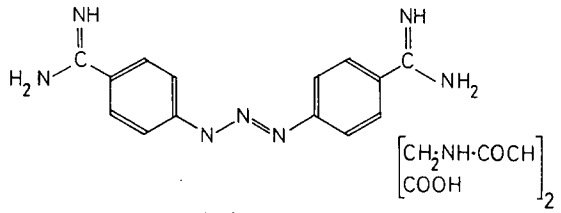
1. INTRODUCTION

In 1976 the Joint WHO Expert Committee and FAO Expert Consultation on African Trypanosomiasis [1] stressed the need for more detailed information about the uptake, distribution and excretion of currently used anti-trypanosomal agents and particularly, in the case of drugs used to treat the disease in cattle, the need for information about the location and level of drug residues in carcasses. It was recognized at that meeting that the lack of sufficiently sensitive methods of drug assay has been a major factor delaying pharmacokinetic studies on existing anti-trypanosomal compounds; radiotracer techniques are clearly applicable and it was recommended that synthesis of radioactively labelled drugs should be given high priority. Today, in the pharmaceutical industry, synthesis of radioactive 'lead compounds' is an accepted step in drug development, but many of the drugs used for the treatment of African trypanosomiasis in man and animals were marketed before such procedures were commonplace, consequently most of the published data on their pharmacokinetics is based on chemical, physical or biological assays. While all these methods have yielded valuable information each has its limitations: small amounts of drug distributed in tissues are difficult to determine by chemical methods; cell constituents not easily separated from the drug may interfere with fluorometric and spectroscopic measurements; a biological assay may have the advantage of measuring an activated or modified form of the drug but may yield spurious results if other biologically active substances (e.g. antibodies) are present. Use of radioactive drugs does not overcome all these problems but the obvious advantage of high sensitivity makes this the method of choice, particularly for investigation of transport across membranes.

Many early studies on the pharmacokinetics of anti-trypanosomal drugs were concerned with the ability of compounds to penetrate the 'blood-brain barrier' in order to assess their value for the treatment of advanced cases of sleeping sickness in man, but it is now recognized that the African trypanosomes do not just confine themselves to the bloodstream and cerebrospinal fluid. Losos [2] has emphasized that *T. congolense* and *T. rhodesiense* differ considerably in their distribution in the body of their hosts; *T. congolense* and *T. vivax* are generally parasites of the bloodstream, whereas *T. rhodesiense* and other members of the *brucei* group become more widely distributed in the intercellular fluids of the connective tissue stroma of various organs. These variations in distribution may account for some of the observed differences in drug sensitivity between these species. Clearly a better understanding of the distribution, and the factors affecting the distribution, of existing anti-trypanosomal drugs is highly desirable; recent progress in this field is reviewed here, with particular reference to the use of radiotracer techniques in the study of two important representatives (Fig. 1) of the phenanthridine and aromatic diamidine trypanocides used in the control of African trypanosomiasis in animals.



(A)



(B)

FIG.1. Structure of: (A) ethidium (homidium) bromide (3, 8-diamino-5-ethyl 1.6 phenyl phenanthridinium bromide); (B) berenil (diminazene) aceturate (4, 4'-(diazoamino) benzamidine).

The trypanocidal activity of phenanthridines was first reported by Browning and co-workers [3] in 1938 and ethidium (homidium) bromide (3, 8-diamino-6-phenyl-5-ethyl phenanthridinium bromide) has been used to treat *T. congolense* and *T. vivax* infections in ruminants in Africa since 1948. Field trials [4] showed that ethidium has limited prophylactic activity and it has been recommended to provide short-term protection to trade cattle en route to market through tsetse-infested areas [1]. Mass treatment with ethidium resulted in the 1960s in the appearance of resistant strains of *T. congolense* in both East and West Africa, but Whiteside [5] reported that ethidium-resistant strains were not resistant to the aromatic diamidine berenil (diminazeneaceturate), which had been introduced by Jensch [6] in 1955, and he proposed that either these two drugs, or samorin (isometamidium) and berenil, should be used as 'sanative' pairs, i.e. used alternately in the field when resistance to either drug appears.

During the last 25 years much has been learnt about the mechanisms of action of phenanthridine and diamidine drugs; ethidium is known to be a selective inhibitor of DNA synthesis [7] combining with the DNA molecule by intercalation into the helix [8] so causing it to unwind [9]. Ethidium has been widely used as a biochemical probe [10] and there is now a very detailed knowledge of the molecular basis of its interaction with DNA [11]. Similar investigations of the mode of action of aromatic diamidines have revealed that these drugs also combine with

DNA but in a different manner to phenanthridines [12–14]; diamidines do not intercalate into the DNA helix but are probably situated in the small groove, astride the two complementary strands, slightly distorting the helical structure [15].

In contrast to this detailed knowledge at a molecular level of drug/DNA interactions little is known about the pharmacokinetics of either ethidium or berenil. Results of the earliest pharmacokinetic studies on ethidium, carried out by the manufacturers (The Boots Co. Ltd), were not published; ^{14}C-ethidium of very low specific activity was used (ca 0.12 μCi/mg) and the results indicated that, in cats, the drug is rapidly excreted (Watkins, personal communication).[1] Kandaswamy and Henderson [16] came to a similar conclusion from experiments with mice in which intraperitoneal injection of ^{14}C-ethidium (15 mg/kg) resulted in 51% being excreted unchanged in the urine in 24 h. Similarly MacGregor and Clarkson [17], using a chemical assay, found 50–55% of an intravenous dose (15 mg/kg) to rats was recovered in the bile in 16–18 h, about a quarter of the recovered drug being in an unchanged form and the remainder as a monoacetyl-amino conjugate.

As mentioned above, field studies [4] have indicated that ethidium may have some prophylactic activity and protection for periods of up to 20 weeks has been reported [5]. Such protection is surprising if the drug is excreted as rapidly in cattle as it has been reported to be by laboratory animals. There are similarly anomalous results in the literature for berenil; it has frequently been stated that this drug is noteworthy among trypanocides in being rapidly excreted, all of a parenterally administered dose being cleared through the kidneys within 24 h [18]. However, Van Hoeve and Cunningham [19], using a biological assay, detected trypanocidal activity in bovine blood 3 weeks after intramuscular injection of berenil and Cunningham et al. [20] have reported protection of cattle exposed to high infection by fortnightly treatment with this drug. Clearly there is a need for more detailed investigation of the pharmacokinetics of both ethidium and berenil, preferably in bovines, if we are to explain these anomalies. We have recently used ^{14}C-ethidium (3,8-diamino-5-ethyl 1.6 ^{14}C-phenyl phenanthridinium bromide, specific actvitiy 45 mCi/g, obtained from Modichem Ltd., UK, courtesy of The Boots Co., UK) and berenil (bis-phenyl-U-^{14}C, specific activity 18.4 mCi/g, the generous gift of F. Bauer, Hoechst Farbwerke, AG, Frankfurt-am-Main) for such studies. With ethidium initial experiments were carried out in rabbits to establish suitable techniques and the work was then extended to uninfected and *T. congolense*-infected calves; preliminary reports of this work have been published [21–23] and a full account is in press [24]. The experiments with berenil have only been performed in rabbits and, due to the limited amount of labelled drug available, are only at a preliminary stage.

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[1] Ci = 3.70 X 10^{10} Bq.

2. MEASUREMENT OF THE DISTRIBUTION OF TRYPANOCIDES IN BODY FLUIDS

A technique developed by Calnan et al. [25] involving subcutaneous implantation of small plastic cages permits serial samples of blood-free tissue fluid to be collected. Calnan and his collaborators used this method to study the concentrations attained by antibiotics in tissue fluid [26] and Goodwin and Tierney [27] used it in conjunction with a microbiological assay to compare the trypanocidal activity of tissue fluids and blood from drug-treated uninfected and *T. brucei*-infected rabbits. Two of the drugs studies were the cattle trypanocides berenil and samorin (isometamidium) and it was found that trypanocidal activity persisted longer in both plasma and tissue fluids of drug-treated trypanosome-infected animals than in uninfected animals. This difference was attributed to the immune response of the infected animals and the experiment serves to illustrate the sort of problem in interpretation that can rise when a biological assay is used as a basis for pharmacokinetic studies. Nevertheless, these experiments provide the first detailed comparison of the blood and tissue fluid levels of anti-trypanosomal drugs and clearly illustrate the value of this elegant technique devised by Calnan and co-workers [25]. It should be more widely used in pharmacokinetic studies.

3. DISTRIBUTION AND EXCRETION OF ^{14}C-ETHIDIUM IN RABBITS

After intramuscular injection of 1 mg ^{14}C-ethidium(specific activity 22.5 mCi/g)/kg, radioactivity was measured in blood and tissue fluid. Peak levels occurred less than 1 h after injection and, assuming that all the radioactivity was present as ethidium, corresponded to 180 ng ethidium/ml in blood and 50 ng/ml in tissue fluids. These concentrations fell rapidly to less than 10 ng/ml after 96 h and over this period one-third of the drug administered was excreted in urine and two-thirds in faeces. Cannulation of the bile duct showed that biliary excretion of radioactivity corresponded closely to the amount excreted in the faeces of uncannulated animals over a similar time period. These results confirm earlier reports of biliary excretion of phenanthridines [17, 27, 28]. A ten-fold increase in the amount of drug injected resulted in only a two- to three-fold increase in blood and tissue fluid levels and did not affect the proportion of drug excreted in urine and faeces. Rabbits dosed with 1 mg/kg were sacrificed after 9 d and the distribution of radioactivity in tissues was studied; only 2–3% of the radio-activity injected was present at this time, the highest residues per unit weight of tissue being present in the liver and kidney.

4. DISTRIBUTION AND EXCRETION OF ^{14}C-ETHIDIUM IN CALVES

Similar results were obtained in uninfected and *T. congolense*-infected calves. Radioactivity in blood and tissue fluids (Fig.2) reached a maximum (equivalent to ca. 120–170 ng/ml) within 1 h of injection of 1 mg ^{14}C-ethidium/kg. The levels of radioactivity fell rapidly during the next 24 h and then more slowly during the next 8 d. Within 2 d of injection 20% of the administered drug was excreted in urine and 50% in faeces (Fig.3) and after 8 d radioactivity in blood and tissue fluids was equivalent to 15 ng ethidium/ml. Tissue residues (Table I) were determined in animals sacrificed after 10 d; it is estimated that about 4% of the initial dose was retained at this time and, as in the rabbit experiments, the highest levels were observed in the liver and kidney. These findings are in broad agreement with other studies on the distribution of phenanthridines [16, 28, 29] in other experimental animals.

Chromatography of serum collected from animals after intramuscular injection of ^{14}C-ethidium has demonstrated that all the radioactivity is in a single substance, which chromatographs in the same position as a ^{14}C-ethidium marker in the gel and thin-layer systems used. Chromatography of urine and bile from ethidium-treated animals has shown that up to 46% of the radioactivity separates from an ethidium marker. Two excretion products have been detected, which are acid labile and are not mono- or diacetyl derivatives of the drug; they have not yet been identified.

These findings seem to be incompatible with field studies, which indicate that ethidium can provide protection for up to 20 weeks [5]. The very low levels of ethidium (ca. 10–20 ng/ml) detected in blood and tissue fluids are about two orders of magnitude less than the trypanocidal level measured in vitro, but this may not be relevant; we have no idea what the in vivo trypanocidal level is. It has been suggested by a number of workers that host defence mechanisms play an important role in potentiating the action of anti-trypanosomal drugs and, in the case of ethidium, Leach et al. [4] and Goodwin and Tierney [27] hint at this possibility. Our own experiments [23] with *T. brucei* and *T. congolense*-infected rabbits indicate that there is a short 'apparent' prophylactic period when infected animals are treated with ethidium and subsequently re-infected; this has been correlated with the host immune response. In view of these results it is interesting to speculate whether the success of ethidium as a prophylactic for slaughter cattle is related to the time they become infected after administration of the drug, or to whether they have a sub-patent infection at the time the drug is given. Our findings also bring into question the suggestion that failure of ethidium to protect cattle in the field is always due to drug resistance. Clearly the efficacy of this drug in field cattle should be re-examined and the possibility of slow release formulations explored.

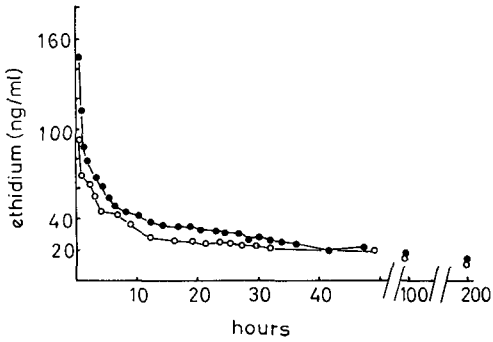

FIG.2. Levels of radioactivity in blood (● ——— ●) and tissue fluid (○ ——— ○) of calf after intramuscular injection of 1 mg ^{14}C-ethidium bromide (specific activity 22.5 mCi/g)/kg. Results expressed as ng ethidium/ml, assuming all radioactivity is present as ethidium; each point represents the mean of three determinations. Standard deviations were in the range 3–9% of the values given. (1 Ci = 3.70 × 10^{10} Bq.)

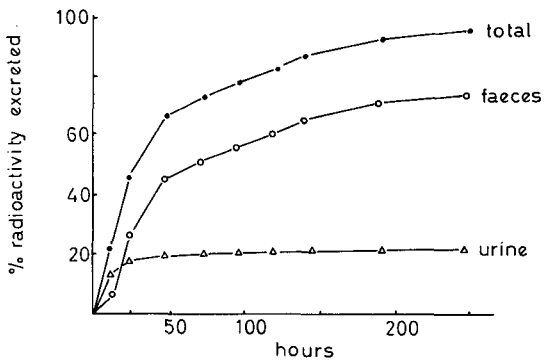

FIG.3. Excretion of ^{14}C-ethidium in urine and faeces by calf after intramuscular injection of 1 mg ^{14}C-ethidium bromide/kg.

TABLE I. RESIDUES OF RADIOACTIVITY IN THE TISSUES OF NORMAL AND *T. congolense*-infected CALVES TREATED WITH 1 mg/kg ^{14}C-ETHIDIUM BROMIDE (SPECIFIC ACTIVITY 22.5 mCi/g) [a] (Residues are expressed as μg drug/g wet weight tissue, assuming all the radioactivity to be present as ethidium. Figures in parentheses represent an estimate of the drug residue in each tissue (as a percentage of the total drug injected). Standard deviations are in the range 1–10% of the value shown.)

Tissue	Radioactivity residual 10 d after dosage	
	Normal calf	*T. congolense*-infected calf
Liver	1.54 (2.8%)	1.47 (2.8%)
Kidney	1.02 (0.56%)	0.48 (0.3%)
Eye (cornea)	0.31 (0.1%)	0.19 (0.1%)
Skeletal muscle (neck)	0.35	0.37
Injection site	0.29	0.41
Adrenal	0.32 (0.1%)	0.35 (0.1%)
Ovary	0.30 (0.1%)	0.21 (0.1%)
Lung	0.22 (0.29%)	0.36 (0.6%)
Spleen	0.16 (0.1%)	0.14 (0.1%)
Ileum	0.08	0.20
Skin	0.08	0.05
Heart	0.09 (0.1%)	0.16 (0.1%)
Bladder	0.11 (0.1%)	0.05 (0.1%)
Brain	0.03	0.09

[a] 1 Ci = 3.70 X 10^{10} Bq.

5. DISTRIBUTION AND EXCRETION OF ^{14}C-BERENIL IN RABBITS

Using the techniques already described for the ethidium experiments we have shown (Fig.4) that intramuscular injection of ^{14}C-berenil (3.5 mg/kg) results in high blood levels (ca. 1000 ng/ml) within 30 min, which then fall rapidly over the next 5 h but remain at a significant level (ca. 10–20 ng/ml) for 4–6 d. Similar very rapid rises and falls in bloodstream levels have been described after subcutaneous or intramuscular injection of berenil in rats and monkeys [30,31].

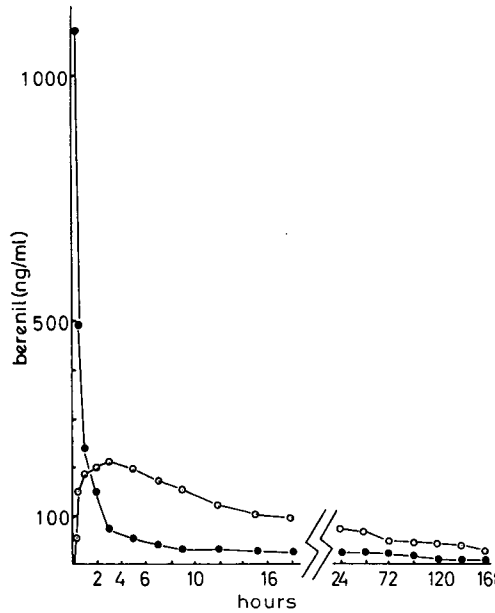

FIG.4. Levels of radioactivity in blood (● ——— ●) and tissue fluid (○ ——— ○) of rabbit after intramuscular injection of 3.5 mg ^{14}C-berenil (specific activity 18.4 mCi/g)/kg. Results expressed as ng berenil/ml, assuming all radioactivity is present as berenil. (1 Ci = 3.70 × 10^{10} Bq.)

Tissue fluid levels did not rise as rapidly or to such high levels (ca. 200 ng max.) but remained at approximately twice the level detected in blood for up to 7 d. At 7 d, in marked contrast to ethidium, only 65% of the administered drug had been excreted (44% in urine and 21% in faeces) and in animals sacrificed at that time it was found that the majority (95%) of the residual drug was present in the liver (Table II). These findings suggest that the pharmacokinetics of berenil in bovines should be investigated as a matter of urgency; such studies could well provide a rational explanation for conflicting data in the literature on the protective action of this drug [19, 20].

6. CONCLUSIONS

Advances in our knowledge of the pharmacokinetics of anti-trypanosomal drugs that have resulted in recent years from the use of radiolabelled drugs of high specific activity justify the WHO/FAO recommendation [1] and stress the need for radiolabelling other currently used trypanocides to probe all aspects of

TABLE II. EXCRETION OF ^{14}C-BERENIL AND RESIDUAL RADIOACTIVITY IN RABBIT LIVER AND KIDNEY 7 d AFTER INTRAMUSCULAR INJECTION OF 1 mg ^{14}C-BERENIL (SPECIFIC ACTIVITY 18.4 mCi/g)/kg [a] (results expressed as percentage of the total drug injected)

Rabbit	%
Urine	44
Faeces	20
Liver	35
Kidney	1

[a] 1 Ci = 3.70 X 10^{10} Bq.

their pharmacokinetics and the molecular basis of their action. Such knowledge may suggest ways of improving existing drugs or designing new ones and throw light on the problem of drug resistance.

ACKNOWLEDGEMENTS

The authors wish to record their thanks to The Boots Co. Ltd., UK and F. Bauer, Hoechst Farbwerke, AG, Federal Republic of Germany, for generous gifts of ^{14}C-ethidium and ^{14}C-berenil, respectively. This work was funded in part by a grant from the Food and Agriculture Organization of the United Nations.

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UPTAKE AND DISTRIBUTION OF SOIL-APPLIED LABELLED HEAVY METALS IN CEREAL PLANTS AND PRODUCTS*

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Abstract

UPTAKE AND DISTRIBUTION OF SOIL-APPLIED LABELLED HEAVY METALS IN CEREAL PLANTS AND PRODUCTS.

Uptake of isotopically labelled mercury, cadmium and zinc from a calcareous chernozem and a podzolized brown earth by spring and winter varieties of wheat, rye and barley was investigated in pot experiments carried out until maturity of the plants. The labelled heavy metals, applied at concentrations innocuous to plant growth (0.5 ppm Hg or Cd, 50 ppm Zn), were determined radiometrically in the straw and in the grains of the harvested plants, as well as in the milling products (bran, semolina and flour) obtained by standard procedures of grain processing. Uptake of mercury was several hundred times smaller than the uptake of cadmium, if both metals were applied to the soil in equal amounts. Whereas the uptake of mercury from the acid soil was insignificant or not detectable, cadmium was taken up from this soil at a much higher rate than from the alkaline soil. Thus, not mercury, but cadmium imposes the greatest hazard on the food chain. Winter varieties of cereals took up more mercury and cadmium than did spring varieties. The heavy metal content in the plants decreased considerably when plants approached maturity. During translocation through the plants the metals were gradually retained when passing from the stalks ('straw') into the grains, and from the seed cover ('bran') into the endosperm ('flour'). The heavy metal content of the grain fractions decreased in the order: bran > semolina > flour. Concentrations of heavy metals in flour were 3–8 times smaller than in straw, showing that flour is least affected by heavy metal pollutions of cereals via the soil. The metal content of the various flour types was correlated with their percentage of bran and with their ash content. By adding an ion exchanger to the soil the pattern of relative distribution of heavy metals in mature plants was not changed, but the cadmium content of all cereal products was considerably lowered.

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* The abstract only is published, since it is intended that the full paper will appear in the IAEA-TECDOC Series (unpriced publication).

Poster Presentations

IAEA-SM-263/21

UPTAKE AND CLEARANCE OF MERCURY ($^{203}Hg(NO_3)_2$) IN THE GUPPIE *(Lebistes reticulatus)*

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Fish weighing between 20.60 and 536.90 mg were exposed to 25.50 and 100 ppb of $^{203}Hg(NO_3)_2$ for 20 d in plastic aquaria containing 4 L of soft aerated water (hardness = 36 mg $CaCO_3$; pH = 7.2; OD = 7.0 mg/L; T = 23°C). At each sampling interval six fish were collected and immediately sacrificed by placing them in a deep freeze [1]. They were then wrapped in aluminium foil, placed in a glass tube and the dose determined by gamma counting, using a single-channel spectrometer and a 3 X 3 in NaI (TL) well crystal [2].[1] The average content of mercury, in nanograms per gram fresh fish against time, is plotted in Fig 1. It was found that the highest mercury content in fish was achieved after 100 h, that is, nearly the time indicated for a fish toxicity bioassay test [3]. A concentration factor (CF) of 168.4 of $^{203}Hg(NO_3)_2$ by Guppies after 72 h incubation was also determined [4]. The rate at which mercury moves in and out of fish depends on its concentration in water, as proposed by Neely [5]. Standard deviations of experimental data decreased when the sampled fish had approximately the same weight. Whole body clearance was carried out by transferring fish from the aquaria containing 35 and 70 ppb of $^{203}Hg(NO_3)_2$ to mercury-free water. The doses were measured as already described. The data shown in Figs 2 and 3 give a similar rate of clearance for both concentrations tested (λ_{35} = 0.03868 and

[1] 1 in = 2.54 X 10^1 mm.

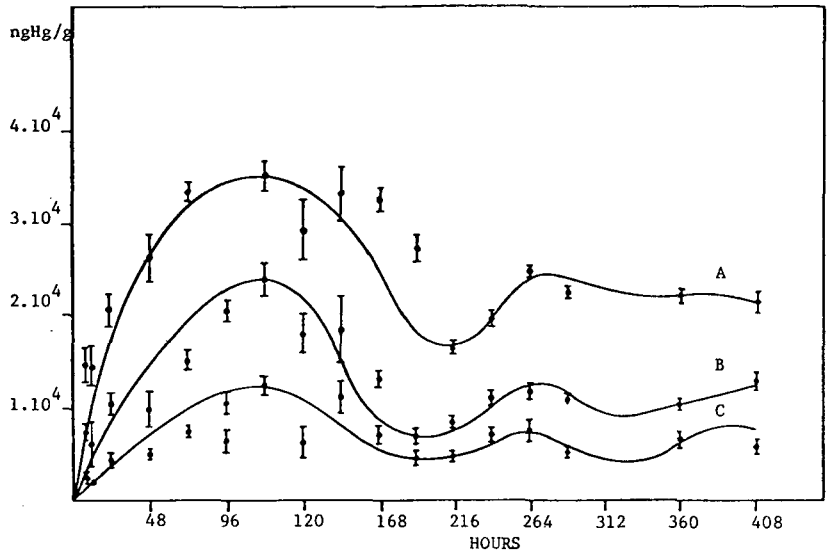

FIG.1. Uptake and clearance of $^{203}Hg(NO_3)_2$, in nanograms of mercury per gram fresh fish, by Lebistes reticulatus *with three treatments: A = 100 ppb; B = 50 ppb and C = 25 ppb. Points indicate average content of six fish, bars indicate one standard deviation.*

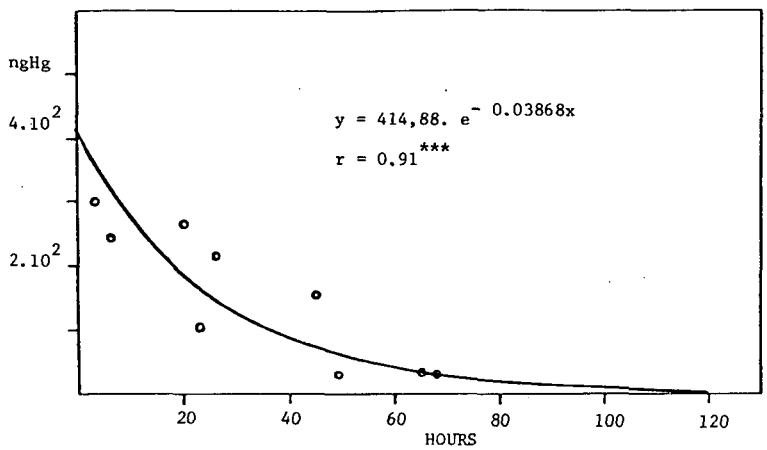

FIG.2. Elimination of mercury by Lebistes reticulatus *in uncontaminated water. Fish were transferred from a water solution of 35 ppb of mercury as $^{203}Hg(NO_3)_2$ after a period of exposure.*

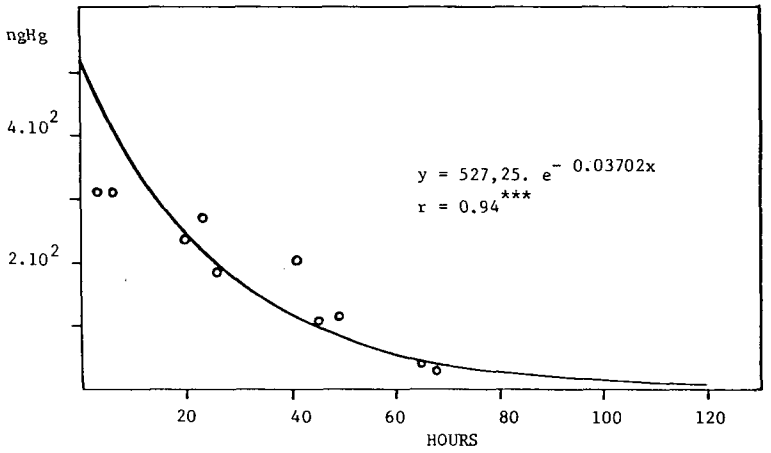

FIG.3. Elimination of mercury by Lebistes reticulatus *in uncontaminated water. Fish were transferred from a water solution of 70 ppb of mercury as* $^{203}Hg(NO_3)_2$ *after a period of exposure.*

λ_{70} = 0.03702), and after 120 h of exposure to uncontaminated water the fish eliminated the mercury previously absorbed. This did not occur when the fish were in mercury-contaminated water as elimination of mercury was reduced to a low value in about 5 d, but was retained in whole-body doses in equilibrium with the water. The equations achieved for mercury clearance were $y_{35} = 414.88\ e^{-0.03868x}$ and $y_{70} = 527.25\ e^{-0.03702x}$. The regression coefficients (r) were 0.91 and 0.94 for 35 and 70 ppb, respectively. The equations differ from those obtained for whole-body retention of mercury nitrate in Gold fish [1].

The conclusions were as follows:

(1) Uptake rate of mercury by Guppie is related to the mercury content in the water, which increases with the metal concentration in water
(2) The highest bioconcentration of mercury occurred at the same time in all treatments, i.e. about 100 h
(3) Guppies eliminated their whole-body mercury content in about 5 d when transferred to a mercury-free environment
(4) The rates of mercury clearance obtained in mercury-free water were quite similar (Tb_{35} = 17.9 h and Tb_{70} = 18.7 h)
(5) Standard deviations of the retained dose were lower when the weight of the sampled fish was uniform.

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IAEA-SM-263/4

A STUDY ON THE FATE OF ^{14}C-RADIOLABELLED CHLORPYRIFOS-METHYL ON WHEAT GRAIN

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Chlorpyrifos-methyl is an organophosphorous insecticide that has been approved in certain countries for admixture to stored cereals. It is very effective against insects that develop within the kernel of wheat grains. To determine why the compound should be so effective it was necessary to treat individual grains with chlorpyrifos-methyl. However, a preliminary study had suggested that after treating grains in this manner there was an unexpectedly rapid loss of insecticide. The aims of the current work were to treat individual grains topically with ^{14}C-chlorpyrifos-methyl to measure the rate of loss of insecticide and to identify the nature of the materials lost.

The chlorpyrifos-methyl used was radiolabelled because it was necessary to measure accurately small changes in the minute doses of insecticide (300 ng or 5 ppm) applied to single grains, with the further advantage that liquid scintillation counting (lsc) would provide information additional to that obtainable by gas liquid chromatography (glc). The treated grains were placed in glass scintillation counting vials and either left open to the atmosphere or sealed. Small filter papers were similarly treated to provide a non-biological system for comparison. All vials were kept at 27°C, 70% RH for up to 7 d. Each grain or filter paper and the

inner surfaces of each vial were analysed for both chlorpyrifos-methyl alone (by glc) and for the ^{14}C-insecticide, together with any radioactive degradation products (by lsc).

The results confirmed that grains stored in unsealed vials lose their ^{14}C-chlorpyrifos-methyl rapidly, possibly by volatilization directly to the atmosphere. The loss was virtually complete after 7 d. By storing some of the grains in sealed vials it was possible to prove that a portion of the insecticide is lost as intact chlorpyrifos-methyl by volatilization to the inner vial surface where it collects together with 3,5,6-trichloro-2-pyridinol. Some of the latter arises by degradation of the chlorpyrifos-methyl on the glass; indeed this discovery was exploited as a convenient method of preparing the ^{14}C-pyridinol. Work is in progress to see whether any of the pyridinol could arise from degradation by the grains and subsequent volatilization to the vial surface.

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METABOLISM OF THE ORGANOPHOSPHOROUS INSECTICIDE BUTONATE IN WATER, PLANTS AND ANIMALS

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The metabolic fate of butonate, an organophosphorous insecticide of reduced mammalian toxicity, was investigated in chemical and biological media using ^{32}P-labelled compounds synthesized in our laboratory. Analytical methods were two-phase partition, TLC scanning and autoradiography.

Butonate can be degraded by the following four routes (Fig.1):

(a) By deacylation of the butyryl group, yielding trichlorphone with possible non-enzymatic formation of dichlorvos according to the range of pH: increase of biological effectivity

(b) By dehydrochlorination of the trichlorohydroxyethyl group, yielding vinylbutonate: about the same biological effectivity

(c+d) By dealkylation of the methylester bond to give demethyl butonate and by cleavage of the P-C phosphonester bond to give dimethyl phosphate: decrease of biological effectivity.

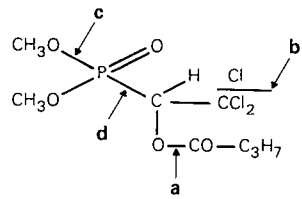

FIG. 1. Butonate.

In aqueous non-biological media butonate is degraded at pH < 5.5 to trichlorphone, demethyl trichlorphone and demethyl butonate, at pH > 5.5 preferably to vinylbutonate, demethyl butonate and demethyl vinylbutonate with traces of trichlorphone. Formation of dichlorvos is possible in traces only according to the range of pH.

In plants butonate is metabolized by deacylation via trichlorphone and demethyl trichlorphone with dichlorvos as a minor metabolite and by demethylation via demethyl butonate. Evaporation from plant surfaces is increasing in the order trichlorphone < butonate < dichlorvos, which is a minor part of evaporating insecticidal compounds following spraying of butonate.

Photodegradation following 5 h of ultraviolet irradiation on glass plates is more expressed than 20 h of sunlight irradiation and is preferred for dichlorvos (100 and 75%) in relation to butonate (30 and 2%) and trichlorphone (7 and 6%).

In mammals, according to the preferred pH > 7 and the increased activity of certain enzymes, the formation of vinylbutonate and trichlorphone with preferred rapid detoxication by demethylation to give the non-toxic demethylated metabolites is observed. The blood level as well as residues in milk are given by the graduation: trichlorphone > vinylbutonate > butonate > dichlorvos.

The half-life of butonate and metabolites in water, plants and animals in vitro and in vivo and the complete scheme of suggested chemical degradation and metabolic pathways are given.

FERTILIZERS AND PESTICIDES
(Session VII)

Chairman

K. REICHARDT
Brazil

Invited Paper

FATE OF FERTILIZER NITROGEN IN SOIL-PLANT SYSTEMS WITH EMPHASIS ON THE TROPICS*

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Abstract

FATE OF FERTILIZER NITROGEN IN SOIL-PLANT SYSTEMS WITH EMPHASIS ON THE TROPICS.

The fate of fertilizer nitrogen in soil-plant systems is considered by analysing the different components of nitrogen balance. Special emphasis is given to research conducted in the tropics, using isotope techniques. Nitrogen fertilization, biological fixation, rainfall additions, nitrogen movement in the soil profile, nitrogen leaching, run-off and gaseous losses, and crop extraction/export are considered. Data indicate that the fertilizer application rates cover a wide range; in developed areas they are approximately 6.5 times higher than in developing areas. Nitrogen fixation is 17 to 40% of plant nitrogen and rainfall inputs are very low. A more extended analysis is made of nitrogen leaching losses, and it is concluded that they are not a problem if fertilizer is applied at normal levels, being of the order of 90 kg N/ha. Run-off losses do not seem to be a major nitrogen loss mechanism; gaseous losses are extremely variable and can rise to 30% of fertilizer nitrogen, and crop export is the most variable component of the balance.

1. INTRODUCTION

In most developing countries food production has practically stabilized, whereas the human population continues to grow at rates of over 2% per year. The need to increase agricultural production and productivity is becoming more and more crucial, and this has lead to more intensive fertilizer use. Its high cost

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demands rational use, something that has still not been established for most agrosystems. The need for basic information on the dynamics of nitrogen under different soil-plant conditions is essential to achieve this goal.

Nitrogen is a nutrient element of vital importance to all organisms. Although it is the most abundant element in the atmosphere, it is the one that most frequently limits agricultural production in both temperate and tropical regions. While in most natural ecosystems the mineral nutrient balance is found to be in equilibrium, in agrosystems the highly mobile nutrients are lost, often on a catastrophic scale, as has been observed after deforestation of rain forests. Soil management practices can cause enormous losses, especially of nitrate, due to rapid oxidation of organic matter, which is accelerated by these practices.

Owing to constant losses in agrosystems, use of fertilizers has increased. This in turn might lead to progressive losses, mainly to the atmosphere (in the case of nitrogen) and by leaching. Many studies have been developed to evaluate nitrogen movement in soil profiles and also leaching losses, but it is first necessary to obtain both information and an understanding of the interaction of the different factors affecting the nitrogen balance in a system, as well as to optimize productivity, diminish losses and reduce the risk of pollution. Isotope techniques are in this context of extreme importance and in many instances they are the only means of achieving an answer.

Nitrogen balance in a soil-plant system is the summation of all inputs (N_{in}) and outputs (N_{out}) of this element in a known soil volume (which should include the entire root system) in a given period of time ($t_2 - t_1$), so that

$$\Delta N = (\Sigma_{N\,in} - \Sigma_{N\,out}) = N_2 - N_1 \qquad (1)$$

where ΔN is the change in nitrogen storage (kg/ha) in a soil layer L (cm) and N_1 and N_2 are the nitrogen storages (kg/ha) at times t_1 and t_2 (d) in a soil layer L.

Nitrogen inputs can come from mineral fertilizers and/or organic matter additions (N_f), biological nitrogen fixation (N_b) and rainfall (N_r). Outputs can occur by leaching (N_ℓ), run-off (N_{of}), gaseous losses (N_g) and crop export (N_{ce}), so that

$$\Delta N = \underbrace{N_f + N_b + N_r}_{N_{in}} - \underbrace{(N_\ell + N_{of} + N_g + N_{ce})}_{N_{out}} \qquad (2)$$

A partial review of all these components is presented here, but special emphasis is given to the leaching component under tropical Latin American conditions.

2. NITROGEN INPUTS

2.1. Nitrogen fertilization

The amount of nitrogen applied to crops depends on the mineral nitrogen content that the soil can supply during crop growth, and the crop need. Malavolta [1] suggests that nitrogen fertilization should be equal to the difference between crop need and soil supply, multiplied by a coefficient K (equal to or greater than 1), which is a function of fertilizer type, soil conditions, climate and plant, it being a measure of the fertilizer use efficiency of the plant. Regarding soil supply, it is generally assumed that organic matter is the main source of nitrogen. According to Bremner [2] and Cheng [3], the nitrogen content of organic matter is about 5%. Total soil nitrogen, normally more than 85% [4] or 98% [2], is in the organic form and its content is extremely variable.

Organic nitrogen has to be mineralized to the ammonia form ($N\text{-}NH_4$) and the nitric form ($N\text{-}NO_3$) before it can be used by plants. Mineralization is a complex biological process affected by a great number of factors, but these are not discussed here. The extreme variability of these factors leads to variable mineralization rates, but these are generally very low. Only 1 to 2% of the total organic nitrogen is mineralized annually in temperate climate areas. Under humid tropical conditions information is very limited, but there is evidence that its contribution to the plant is low due to the high rainfall rates [5]. In these areas good correlation between organic nitrogen content and crop response is not found, probably due to the intense leaching of mineral nitrogen by rainfall. In many of these areas it can be observed that during periods of low rainfall, in which high soil temperatures promote partial sterilization, there is an accumulation of products that can be nitrified. These, at the beginning of the rainy season, are highly subject to leaching at a time when crop roots are not sufficiently developed to use all the nitrates produced [4, 6–10]. In Brazil, in order to estimate the soil nitrogen availability, an average mineralization rate of organic matter of 2% is assumed for the period of 1 year. In the past, the total soil nitrogen content used to be considered [5].

Crop needs are related to crop growth rates which in turn depend on species, variety and yield. They also depend on the climatic factors and the technology level. Malavolta [1] and Sanchez [8] present tables of the nitrogen needs (extraction and export) of the main tropical crops in which the influence of the above factors can be seen, mainly the effect on yield. In Brazil [5], because of lack of an efficient criterium to discriminate crop response to nitrogen fertilization, average crop response curves for nitrogen are used with which it is possible to define economic application rates. On a world-wide basis the level of agricultural development is influencing significantly the quantities of fertilizer applied per hectare. Cooke [11] shows that greater use of fertilizer is restricted to developed areas, in which rates are 6.5 times higher than in developing areas.

2.2. Biological nitrogen fixation

Biological nitrogen fixation constitutes one of the main forms by which nitrogen is added to soil-plant systems, in temperate as well as in tropical regions. Epstein [12] and Postgate and Hill [13] present a list of the different biological nitrogen fixing systems that occur in nature. Among these, the legume-*Rhizobium* associations are considered the most efficient [8]. Henzel and Norris [14] report that these associations contribute inputs of 16 to 500 kg N/ha per year. The wide range depends to a large extent on the methodology and conditions under which the evaluation was made. Sanchez [8] affirms that in most cases the fixed quantities of nitrogen are below those expected, mainly in tropical areas. This might be due to low concentrations of phosphorus and calcium and high concentrations of toxic elements, as Al in the majority of soils.

In the last decade much evidence of non-symbiotic associative N_2-fixation, especially with C-4 tropical grasses such as *Digitaria decumbens, Panicum maximum* and *Cynodon dactylon,* has accumulated. *Azospirillum* spp. appears to be ubiquitous in many of these systems for C-3 barley, rice, rye and wheat, while *A. lipoferum* has the greatest affinity for maize, sorghum and C-4 grasses [15]. Other diazotrophic biocoenoses have been found in spring wheat (*Bacillus polymixa*), rice (*Achromobacter*) *Spartina (Campylobacter)* and sugar-cane (*Enterobacteriaceae* and *Bacillaceae*), and recent information is presented in Vose and Ruschel [16].

Estimating amounts of N_2 fixed in associative systems is very difficult. Rennie [17] presented a consensus that it was at least 30 kg N/ha per year. Ruschel [16] noted that for sugar-cane there was reasonable evidence that 17 to 30% of plant nitrogen was due to fixation. Boddey and Döbereiner [15] noted that for *Azospirillum* inoculation experiments in Israel and Brazil as much as 40% of the plant nitrogen might come from biological fixation.

An especially important symbiotic association is *Azolla-Anabaena,* which is frequently used in south Asia, mainly in flooded rice paddies. Under field conditions fixation values of 62 to 125 kg N/ha per year have been reported [18].

In a general way, according to Sanchez [8], additions of atmospheric N_2 to the soil-plant system in the tropics can be as low as 4 and not greater than 50 kg N/ha per year in annual crops; however, in tropical forest systems it can vary from 46 to 147 kg N/ha per year.

2.3. Rainfall inputs

In humid tropical areas, far from industries, some nutrients are incorporated into the soil-plant systems by rainfall. They are transported by wind, as fine ash of forest combustion products in shifting cultivation, or as dust mainly from desert areas [8, 19]. Cooke [11] shows that in industrial inland areas, such as

those in England and the United States of America rainfall contains appreciable quantities of nutrients, some of which come from the sea as an aerosol and others from fossil fuel combustion, which also contributes nitrogen and sulphur to the atmosphere. Owing to the great variability of all these processes, composition of rainfall varies a great deal from place to place and from time to time. Cooke [11] states that in industrial areas nitrogen incorporated into soil by rainfall is of the order of 17 kg/ha per year and in undisturbed areas of the order of 1.5 kg N/ha per year. Sanchez [8] noted that for five different ecosystems, including agricultural and forest systems, the contribution of rainfall nitrogen was in the range of 4 to 8 kg N/ha per year. Libardi and Reichardt [20] report that for tropical conditions (during the rainy season) 4.6 kg N/ha were received by the crop in a total of 661.4 mm of rain, which fell in 120 d. So it seems that an average contribution of 8 kg N/ha per year is reasonable; slightly higher values could be expected in areas of intense atmospheric lightning, where ammonia inputs are significantly higher.

3. NITROGEN LOSSES

3.1. Nitrogen movement in the soil profile and leaching

Mineral soil nitrogen in the $N\text{-}NO_3$ form (and sometimes $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$) is normally not strongly retained by soil colloids, it remaining free in the soil solution and being subject to water movement in the soil profile. In this way it can be lost by drainage (leaching), which decreases soil fertility, thereby increasing the cost of production through the need for higher fertilization rates and opening the possibility of groundwater contamination. The quantities of $N\text{-}NO_3$ in the soil profile that are susceptible to leaching are extremely variable in space and time, depending on the amount of nitrogen applied to the soil, mineralization rates, crop removal, soil management practices, type of crop and volume of water drained. All these factors are significantly affected by soil properties and climate.

Sampling and analysis of the soil below the root zone in areas of free drainage is a procedure used by many scientists for estimation of leaching losses [20–26]. Other more sophisticated methods involve measurement of mass-flow and hydrodynamic dispersion [27–32] which, coupled with mathematical models, give the actual rates of movement in soil profiles.

Wild [33], studying $N\text{-}NO_3$ movement in a well aggregated Alfisol in Nigeria, found that this ion moved downward at a rate of 0.5 mm per millimetre of rainfall, which is considered low when compared with the rates found by Terry and McCants [34] of 1 to 5 mm per millimetre of rainfall for sandy soils in North Carolina. Pratt et al. [35], working with soils of different textures, found

that the volume of drained water, the concentration of $N\text{-}NO_3$ and the leaching losses of these ions were significantly related to irrigation treatments and soil characteristics, mainly saturated hydraulic conductivity. The whole picture becomes more complicated when it is realized that these soil characteristics can present a high spatial variability over large field areas.

Over four years Sotiriou and Korte [36] evaluated the balance and fate of urea ($CO(^{15}NH_2)_2$) in sandy and loamy soils, with and without addition of organic matter, and in loamy soil with different crops. They found that the applied nitrogen decreased linearly with the soil depth for sandy soil and exponentially for loamy soil. Organic matter additions did not affect losses. Youngdahl et al. [37], in a study to evaluate the potential losses of urea of different granule size in soils of different textures, showed that for moderate and high drainage rates (say 10–20 mm), especially with soils of low CEC, the leaching losses of super-granulated urea can be total. In sandy loam ^{15}N-analysis showed that the crop (rice) recovered 63% of the supergranulated fertilizer when the drainage rate was 4.4 mm/d but recovered only 5% for a 18.3 mm/d rate. The leaching losses of supergranulated urea were always lower than the losses for other nitrogen fertilizers. In Sri Lanka Golden [38] reports that normally 200 kg N/ha are applied to tea crops and that the efficiency of utilization is in the range of 30 to 50%, the remainder being potentially available for leaching.

Libardi and Reichardt [20], studying the fate of urea-^{15}N (120 kg N/ha) applied to a bean (*Phaseolus vulgaris* L.) crop on an Oxic Paleudalf in Brazil, found that during the cropping season (120 d) 6.7 kg N/ha were lost by leaching, this including both fertilizer and soil nitrogen. The contribution of fertilizer nitrogen to leaching could not be estimated owing to the small size of the samples obtained from porous cup extractors (Reichardt et al. [39]). In these studies they were faced with the problem of spatial variability of soil properties, which was found to be very significant. Reichardt et al. [40, 41] found that for this Oxic Paleudalf the spatial variability of soil bulk density, soil water retention curves and soil hydraulic conductivities could not be neglected. Nascimento et al. [42] and Reichardt et al. [43], using a pulse of ^{36}Cl during steady infiltration of water into the same soil, found saturated hydraulic conductivities varying from 1.26 to 6.86 cm/h inside a 10 × 10 m plot. Using Gaussian statistics they obtained an average value of 1.513 ± 0.687 cm/h. The time for ^{36}Cl to reach to a depth of 120 cm varied from 38.5 to 95 h.

Meirelles et al. [44] and Cervellini et al. [45], in the same soil [20], applied 100 kg N/ha as ammonium sulphate-^{15}N to a bean crop (*Phaseolus vulgaris* L.) and found that a total of 15 kg N/ha were leached with only 1.35 kg of this derived from fertilizer. These are average values for which spatial variability was not taken into account.

Reichardt et al. [26], also in Brazil, studied the dynamics of nitrogen (80 kg N/ha, $(^{15}NH_4)_2SO_4$) applied to a maize crop (*Zea mays* L.) on sandy

Oxisol, for which spatial variability was a minor problem [46]. They found that 9.2 kg N/ha were lost below the 127.5 cm depth by leaching, with only 4.8% derived from fertilizer, which gives a total of 0.4 kg N/ha. The study covered a period of 150 d during the rainy season, but rainfall was very well distributed and as a result the crop recovered 89.1% of the applied fertilizer nitrogen.

Libardi et al. [23], in order to increase understanding of the fate of applied nitrogen in a *Phaseolus vulgaris* crop grown under tropical conditions, applied ^{15}N-labelled urea to bean crops grown on Oxic Paleudalf [20] and followed with a pulse of enriched ^{15}N-fertilizer for three consecutive cropping periods. Results showed that 31.2% of the nitrogen in the first crop was derived from the applied urea (100 kg N/ha), which represents a nitrogen utilization efficiency of 38.5%. The second crop had 6.2% of the fertilizer nitrogen applied to the first crop, and only 1.4% of the nitrogen in the third crop was derived from fertilizer. In total, the three crops recovered 44.3% of the nitrogen applied to the first crop, and the remainder was either still in the soil profile or had been lost by leaching, volatilization or denitrification. Nitrogen-15-enrichment of mineral nitrogen ($NO_3 + NH_4$) suggests that at the end of the second crop the pulse of fertilizer applied to the first crop had probably passed the 120 cm depth. Nitrogen-15-enrichment of organic nitrogen suggests that the root activity of beans and weeds transported nitrogen to the 90–120 cm depth (or deeper). They accounted for 109 kg fertilizer nitrogen per hectare in harvested biomass, crop residue and soil at the end of the first cropping period. This indicates an experimental error of about 10%; on nitrogen it was lost by volatilization, denitrification or leaching below 120 cm. ^{15}N-data at the end of the first cropping period support the assumption of leaching. At the end of the second and third cropping periods 76 and 80 kg N/ha, respectively, could be accounted for, suggesting that 20 to 25% of the applied nitrogen was lost over a two-crop period.

Calvache [47] and Araújo Silva [48] studied in detail the fate of fertilizer nitrogen in a corn (*Zea mays* L.) crop on Oxic Paleudalf [20] in Brazil. During the cropping period 30% (195 mm) of the water balance corresponded to drainage below the 120 cm depth. Assuming that nitrates are leached only by mass flow, they accounted for a loss of 32.4 kg N/ha, with a contribution of 34% from fertilizer (11.0 kg N/ha). The application rate was 100 kg urea-N/ha.

In the latest study in Brazil, Urquiaga [49] used a highly enriched (56% ^{15}N atom excess) ammonium sulphate (42 kg N/ha) on a bean crop grown on Oxic Paleudalf [20]. The crop had an extremely high fertilizer efficiency use of 76% (32.0 kg N/ha). At the end of the cropping period 9.2 kg N/ha derived from fertilizer were found in the soil profile. Leaching losses were not calculated, but even assuming no other losses, leaching would amount to only 0.8 kg N/ha.

Table I summarizes the nitrogen leaching data found by seven authors. Using average values, which certainly has many limitations, it can be seen that for nitrogen application rates of about 90 kg N/ha, under tropical conditions, only

TABLE I. TYPICAL LEACHING LOSSES UNDER TROPICAL CONDITIONS (Piracicaba, Brazil)

Authors	Soil	Crop	Period (d)	Fertilizer rate (kg N/ha)	Total nitrogen leached (kg N/ha)	Leached nitrogen from fertilizer (kg N/ha)	Rainfall (mm)
Libardi and Reichardt [20]	Alfisol	Beans	120	120	6.7	–	661
Meirelles et al. [44]	Alfisol	Beans	365	100	15.0	1.4	1382
Reichardt et al. [26]	Oxisol	Corn	130	80	9.2	0.4	717
Araújo Silva [48]	Alfisol	Corn	150	100	32.4	11.0	620
Urquiaga [49]	Alfisol	Beans	86	42	–	0.8	403
Average				88.4	15.8	3.4	757

4.5 g of fertilizer nitrogen are lost by leaching, per hectare and per millimetre of rainfall. It is clear that a minor proportion of the leached nitrogen derives from fertilizer, and it can be concluded that losses by leaching for fertilizer applied at normal levels are not a problem.

3.2. Run-Off Losses

The soil surface layer is generally the richest in nutrients and therefore its loss can, in many cases, bring decreases in fertility and productivity levels. In tropical areas water erosion is the most important, and is related to conditions of climate, soil, relief, crop cover and management practices. So, due to the different factors that affect run-off and erosion, information about losses is also very variable. On the other hand, most reports deal with soil losses as a whole, generally expressed as tonnes of soil per hectare per year, and little attention is given to fertilizer losses.

Lal et al. [50, 51] report that in Nigeria large amounts of soil and nutrients are lost by run-off and erosion when Alfisols with a sandy surface are left without crop protection. Under these conditions soil losses can amount to 115 t/ha per year, productivity is reduced to less than 50% and additional fertilization does not

correct the fertility state of the soil. This, however, is an extremely high loss. In Brazil Bertoni et al. [52] report losses ranging from 0.9 to 27 t/ha per year, and most of the values are less than 15 t/ha per year. A loss of 15 t/ha per year represents a loss of soil layer of 1.2 mm thickness, considering a soil bulk density of 1.3 g/cm^3.

Therefore, it seems that erosion is not the main cause of the low fertility of tropical soils. Nye and Greenland [19] report that on a cultivated forest soil the greatest nutrient concentration is in the first 7.5 cm, even though erosion losses are minimal when the slopes are not greater than 10%. Owing to the mobility of ions like NO_3^-, $SO_4^=$, Cl^-, they are generally leached downwards before being lost by run-off.

3.3. Gaseous losses

Volatilization of ammonia occurs with high probability when fertilizer is applied to the soil surface, but it depends on the nature of the fertilizer, soil CEC, pH, organic matter content, and other properties like temperature and water content. In the case of urea, hydrolysis forms ammonium carbonate in the presence of urease, an enzyme of high activity in soils of high organic matter content and high CEC. The carbonate, owing to its low stability, breaks down into ammonia and carbon dioxide. Gasser [53] reports that in slightly acid or neutral soils, hydrolysis of urea can increase soil pH and stimulate NO_2^- accumulation, and thereby increase the probability of formation of volatile compounds (NO_2, NO), which are subject to losses. This mechanism seems to operate more efficiently with urea than with ammonium salts. On the other hand, Gasser stresses that the pH increase due to hydrolysis in acid soils favours the nitrification of ammonium, it being faster for ammonium derived from urea than from other salts.

NH_3 losses are common in soils with a high calcium content or in those that have received heavy liming, but quantities vary mainly with fertilizer type. According to Malavolta [1] and Terman [54], in ammoniacal fertilizers the complementary anion has a great influence on NH_3 losses; the following sequence is considered to be valid for the losses: urea > ammonium sulphate > ammonium nitrate > monoammonium phosphate.

In Brazil Anjos and Tedesco [55] studied the volatilization of ammonium derived from urea and ammonium sulphate. For urea losses ranged from 12 to 30% and for ammonium sulphate only from 0.5 to 1.1%. Libardi and Reichardt [20], in their study of the fate of fertilizer nitrogen, did not find volatilization losses for urea applied in the furrow of an Alfisol, probably due to the soil pH which was 5.5.

In summary, according to Terman [54], N-NH_3 losses are practically eliminated in acid soils when nitrogen fertilizers are applied at depths greater than

5 cm. Surface applications have a high risk of loss, especially in soils of low CEC, high pH and low water content.

Losses in the form of N_2O and N_2, that is by denitrification, are more significant under reducing conditions, which occur in soil profiles with deficient drainage. Terman [54] reports that the low recovery of applied nitrogen in flooded rice crops is usually due to N_2O and N_2 losses to the atmosphere. Also, Thenabadu [56] states that gaseous losses of nitrogen are of special significance in flooded rice growing in soils where ammonium salts could be nitrified on the soil surface and which, on reaching the lower reduced horizons, get denitrified.

Many reports are available and in many cases much of the nitrogen that could not be accounted for in attempts to prepare balance sheets is considered lost to the atmosphere in gaseous forms.

Another important aspect is nitrogen losses from the tops of plants, which are discussed in detail by Wetselaar and Farquhar [57]. They report that for annual field crops the losses reach 40–50 kg N/ha, at mean rates of 1.2 kg N/ha per day, between maturity and harvesting.

3.4. Crop extraction and export

It is generally assumed that crop extraction is the total quantity of a given element accumulated in the whole plant, and that crop export is the fraction of the total within the harvested product. In most cases the non-exported material remains on the field as harvest residue.

As was mentioned in Section 2.1 crop needs are strongly related to plant species and are affected by factors such as climate and the technology level, which certainly affect yield (or crop export). The available literature on the subject is enormous and a complete survey is not appropriate here. An analysis of data presented by Malavolta [1] and Sanchez [8] gives a good idea of the order of magnitude of crop exports. They report that under tropical conditions export of nutrients by the main crops (corn, rice, wheat and sorghum) range from 20 to 25 kg N/ha per tonne of product, corn and sorghum being the best 'exporters' of nitrogen, due to their high productivity. In tubers and roots, nitrogen exports range between 3.8 and 5.4 kg/t per hectare. Grain legumes show much higher values (32 to 40 kg N/t per hectare) due to their high protein content. Sugar-cane crops export practically all the extracted nitrogen, accounting for 0.8 to 1.3 kg N/t per hectare.

4. CONCLUSIONS

An attempt has been made to establish the nitrogen balance of agriculturally productive soils in tropical Latin America. Data indicate that fertilizer applica-

tion rates cover a wide range, being dependent on crop needs and growth rates. Application rates are approximately 6.5 times higher in developed areas of the world than in developing countries. The amount of N_2 fixed in associative systems depends on many factors, therefore estimation is very difficult and data range from 17 to 40% of the plant nitrogen when fixation occurs. Inputs of nitrogen through rainfall seem to be very low under most conditions. Nitrogen leaching losses also depend on many factors, as indicated by their wide ranges; approximately 10 to 20 kg N/ha are leached at fertilizer rates of about 100 kg N/ha, but only about 20% of these amounts are derived from the fertilizer. In Brazil only 4.5 g of fertilizer nitrogen are lost by leaching per millimeter of rainfall at application rates of 90 kg N/ha. It can therefore be concluded that losses by leaching are not a problem at normal fertilizer rates. Run-off losses do not seem to be a major nitrogen loss mechanism; gaseous losses are extremely variable and can constitute up to 30% of fertilizer nitrogen. Finally, crop export of nitrogen is the most variable component of the nitrogen balance.

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Poster Presentation

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A TRACER METHOD TO MEASURE NUTRIENT UPTAKE FROM THE PLOUGH LAYER AND SUB–SOIL

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Tracer methods used for root studies employ either the plant injection or soil injection approach [1]. The first involves injection of the radiotracer at the stem base of the plant and subsequent analyses for its presence in the soil profile [2, 3]; the second involves injection of the radiotracer in the soil profile at various locations and subsequent analysis for its presence in above-ground parts of the plant [4]. A special version of the latter approach, developed and applied to estimate the nutrient uptake from the plough layer and sub-soil [5, 6], is described here.

The method consists of homogenously contaminating the plough layer with a tracer, radioactive or stable, in two treatments: in a reference treatment without connection with the sub-soil, and in an experimental treatment where the sub-soil is freely available for root penetration and root uptake of water and nutrients; uptake of the latter causes increased dilution in the shoot. Based on the specific activities or isotope ratios in the shoot of both treatments, and according to inverse isotope dilution and the A-value concept [7] as extended for determination of biological dinitrogen fixation [8, 9], nutrient uptake from the plough layer and sub-soil can be quantified as percentages (yield independent criteria) as well as in absolute units per hectare (yield dependent criteria).

The tracers used to label the nutrient source in the plough layer of both treatments were ^{32}P for phosphorus, ^{86}Rb or stable rubidium for potassium and ^{85}Sr or stable strontium for calcium. Regarding the nutrients studied, phosphorus and potassium are highly mobile in plants and are adsorbed by young as well as older roots, while calcium is slightly mobile and is mainly transferred from young roots towards the shoot. Owing to this different behaviour uptake of calcium was considered to correlate with water uptake in the soil profile, whereas this was not the case for phosphorus and potassium.

Sampling of the spring-sown cereals grown at different times during the growing season showed that uptake of nutrients from the sub-soil varied with many factors but usually increased with the growth stage and especially during stem elongation [5, 6]. At tillering or early jointing uptake from sub-soil ranged from 0–25% for phosphorus and potassium and 10–40% for calcium; at heading or early maturing it ranged from 10–50% for phosphorus and potassium and 40–80% for calcium. It was usually lower for phosphorus than for potassium at all growth stages, and lower for potassium than for calcium. Clay sub-soils were usually superior to sandy sub-soils as the nutrient sources of all three elements investigated. A high uptake of sub-soil calcium, indicating a high utilization of the sub-soil as a water source, usually led to a high uptake of sub-soil potassium. As shown by cultivating soils from long-term phosphate fertilizer experiments, a good phosphorous state of the plough layer was a pre-requisite for a high dry matter yield and for a high utilization of the sub-soil as a nutrient and water source.

Experiments carried out on many sites in Sweden showed that the nutrient uptake from the plough layer and sub-soil varied with the soil type and location, the year and sowing date, and the time and cereal grown. Uptake of sub-soil potassium was usually higher in eastern Sweden than elsewhere, while uptake of sub-soil phosphorus usually increased from the north to south of the country. Early sowing in spring induced early penetration of the sub-soil. Barley, and early rather than late varieties, was usually more aggressive in establishing contact with the sub-soil early in the season than oats and wheat. Later in the season oats was comparable with barley, while wheat was still inferior. A wheat cultivar with a normal straw length showed a higher nutrient uptake from the sub-soil than a dwarf cultivar of the same variety.

The results obtained show the value of optimizing cereal root development in the soil profile, and the value of certain cultivation measures to increase the nutrient, and especially the water uptake, from the sub-soil. Early root penetration of the plough layer provides a later extensive root activity in the sub-soil, which enhances shoot development. The main conclusion is that increased shoot development by itself is a driving force to use distant sources in the soil profile such as water and nutrients from the sub-soil. The method, based on inverse isotope dilution and the A-value concept, is useful to estimate the nutrient uptake from the plough layer and sub-soil, with the plough layer as a reference or standard. Applying the method under field conditions gives quantitative information on percentage and absolute uptake of calcium, phosphorus and potassium from the two compartments, integrated over the time elapsed from the start of the experiment to sampling the crop.

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EFFECTS OF ATRAZINE ON THE RATES AND GASEOUS PRODUCTS OF DENITRIFICATION IN SOIL*

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Abstract

EFFECTS OF ATRAZINE ON THE RATES AND GASEOUS PRODUCTS OF DENITRIFICATION IN SOIL.

Experiments were conducted to evaluate the effects of the herbicide atrazine on denitrification in two soils, Yolo loam from California, United States of America, and S. Cataldo silt loam from Italy. The rates of denitrification and amounts of N_2 and N_2O gases produced during denitrification were measured from incubation experiments and from miscible displacement experiments on the transport and transformation of $^{15}NO_3$ in soil with and without incorporated atrazine. The amount of $^{15}NO_3$ appearing in the effluent of the soil columns was measured as a function of time and fitted to analytical solutions of the transport and transformation equation in order to determine the constants for first order kinetics. The soil columns were also equipped with a means of flowing He and N_2 perpendicular to the water and NO_3 flow, in order to slowly purge the evolved N_2O and $^{15}N_2$ gases from the soil columns. At 25 ppm atrazine, denitrification was substantially inhibited during the first 8 days of incubation. However, at 5 ppm atrazine, denitrification remained the same as with no atrazine or was slightly stimulated. The presence of atrazine in the soil during NO_3 transport greatly inhibited the reduction of N_2O to N_2, resulting in small N_2/N_2O ratios. Thus, atrazine, at concentrations reasonable for field application, resulted in a slight increase in the nitrate reduction rate over that without herbicide. The results also show that atrazine can have a profound effect on the enzyme systems that are responsible for reduction of N_2O to N_2 during denitrification. Thus, the presence of atrazine in the soil may result in increased evolution of N_2O from fertilizer-applied N during time periods when field soils become anoxic.

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* The abstract only is published here, since it is intended that the full paper will appear in the IAEA-TECDOC Series (unpriced publication).

INFLUENCE OF A HERBICIDE ON CERTAIN METABOLIC AND SYMBIOTIC ACTIVITIES OF A *Rhizobium**

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Abstract

INFLUENCE OF A HERBICIDE ON CERTAIN METABOLIC AND SYMBIOTIC ACTIVITIES OF A *Rhizobium*.

The influence of Basalin (a pre-emergence herbicide) on the metabolism and symbiosis of a *Rhizobium* sp. (strain P 47) with cowpea (*Vigna unguiculata* var. C 152) was studied. The herbicide was applied at 2, 5 and 10 ppm levels to simulate the recommended and higher accumulated levels, respectively. Basalin reduced the in vitro growth of the *Rhizobium* at 5 and 10 ppm, while oxidation of ^{14}C-glucose by the bacterium was significantly affected at all three levels. At 2 and 5 ppm Basalin did not affect the oxidation of acetate, pyruvate, succinate and fumarate, while citrate oxidation alone was enhanced at the 2 ppm level. However, the 10 ppm level of the herbicide significantly reduced oxidation of the above TCA-cycle intermediates. Soil application of Basalin significantly suppressed plant growth as well as nodulation (nodule number and weight per plant) at 5 and 10 ppm, but not at the 2 ppm level. Reduction in the dry matter production and total nitrogen content of plants was also observed in plants raised in soil treated with 5 and 10 ppm of the herbicide. Both nodular respiration and leghaemoglobin content of nodules were adversely affected by all three levels of the herbicide, whereas the nitrogenase activity was reduced significantly at 5 and 10 ppm. Translocation of foliar applied ^{14}C-glucose to roots and nodules was suppressed in cowpea plants raised in Basalin-incorporated soil. In general, the results indicated that, while the recommended level (2 ppm) of the herbicide Basalin was less harmful, the higher accumulated levels (5 and 10 ppm) were highly toxic to the metabolic and symbiotic activity of the cowpea *Rhizobium*.

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Poster Presentation

IAEA-SM-263/10

ACCUMULATION OF ^{15}N-LABELLED (2–CHLOROETHYL)TRIMETHYL–AMMONIUMCHLORIDE (CCC) IN THE NODES OF WHEAT

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Techniques using the stable isotope ^{15}N have been widely used in agricultural research and are also going to gain wide application in medical research and diagnostics. Therefore, our Central Institute of Isotope and Radiation Research has made many efforts to develop simple and highly automated methods for analysing this isotope. These have resulted in two commercial emission spectrometrical devices: the ^{15}N analysers NOI-5 and Isonitromat 5201, which have been developed in co-operation with the VEB Statron, Fürstenwalde, German Democratic Republic. During the last few years we have designed and constructed a third, not yet commercial, device for this purpose, which we have named NOI-6. It is the aim here to describe the technical parameters of these devices and to discuss the results of our investigations on the accumulation of (2-chloroethyl) trimethylammoniumchloride (CCC) in the nodes of wheat plants, using an emission spectrometrical ^{15}N analyser.

All our ^{15}N analysers are based on the same principle:

(1) Chemical reaction between nitrogen compounds (ammonium compounds, amines, amides) and ammonium hypobromite to form gaseous nitrogen
(2) Purification of nitrogen
(3) Introduction of purified nitrogen into a discharge tube
(4) High frequency excitation to effect light emission ($C^3\pi - B^3\pi$)
(5) Separation of the bands emitted by the isotopic molecules by a monochromator
(6) Recording the intensity of the isotopic bands by a secondary electron multiplier
(7) Calculation of the relative abundance of ^{15}N.

TABLE I. NITROGEN-15 EXCESS ABUNDANCE IN DIFFERENT PARTS OF THE STEM (in at.%)

Level of insertion (numbered from the base)	Region		
	Internode	Subnode	Node
5	0.20	0.27	0.90
4	0.06	0.12	0.33
3	0.04	0.07	0.13
2	0.07	-	-

^{15}N excess abundance in the ear: 0.20 at.%.

The ^{15}N analyser NOI-5 only performs steps 4, 5 and 6; our ^{15}N analysers, NOI-6 and Isonitromat 5201, perform steps 1 to 6. The additional advantage of Isonitromat 5201 is the possibility to hold up to 380 test samples that are sampled and introduced into the analyser automatically. Both NOI-6 and Isonitromat 5201 calculate the relative ^{15}N abundances by an analogous computer and then print them. Our new ^{15}N analyser NOI-6 combines most of the advantages of NOI-5 and Isonitromat 5201, particularly the high accuracy and small amounts of nitrogen needed which are characteristic of NOI-5 and part of the high degree of automation of Isonitromat 5201.

As is known synthetic CCC is a growth-retarding substance used for stem shortening of wheat, particularly when applying large amounts of nitrogen fertilizers. Former investigations at our institute and other research institutions on the uptake and transport of CCC in wheat have proved that it is rather effectively accumulated in the stem. The stem-shortening effect might be associated with differences in the CCC concentration in different parts of the plants showing different responses to the biologically active substance. Therefore, we studied the distribution and persistence of CCC in the stems of wheat plants, using ^{15}N-labelled CCC and the analytical procedures already described.

Spring wheat was grown in a water culture using Zinsadse I nutrient solution. When the plants had reached a height of 25 cm their roots were dipped into an aqueous 7×10^{-4} M solution of ^{15}N-labelled CCC for 6 h (the ^{15}N abundance used was 50.7 at.%). After a further period of 7 weeks, when the ears had emerged, the plants were harvested. The main shoots of the harvested plants were divided into different parts to determine the CCC content by the inverse isotope dilution technique.

Fresh samples were treated with alcohol, resulting in a 'soluble N' fraction and a precipitate 'protein fraction'. Nitrogen-15 was determined by our emission spectrometric method. The results of these experimental investigations are shown in Table I.

No ^{15}N excess abundance was found in the protein fraction, indicating that the CCC molecule was virtually not metabolized. Therefore, we can conclude that the ^{15}N excess abundance is representative of the CCC content.

Usually the upper region of the wheat plant exhibits the highest ^{15}N abundance, values decreasing in the basipetal direction. Moreover, the nodes show considerably higher ^{15}N abundances and higher CCC contents, respectively, than the neighbouring internodes and subnodes.

POSTER PRESENTATIONS

Poster Presentations

IAEA-SM-263/8

ACCUMULATION AND MIGRATION OF HEAVY METALS IN SOILS AND CONTAMINATION TO GROUNDWATER AS A RESULT OF LONG-TERM APPLICATION OF URBAN WASTES

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Standards for waste utilization in soil improvement and crop production should provide reasonably high levels of public health protection. By utilization of waste water, sewage sludge and municipal waste compost on farmland, heavy metals and other constituents accumulate in the soil to a different degree.

To investigate their behaviour in the soil over long periods different sites were selected for investigation and sampling of undisturbed soil columns:

(a) Sewage farm in operation for 85 years
(b) Field experiments with sewage sludge
(c) Field experiments with municipal waste compost.

Isotope studies and neutron activation analysis were carried out on the accumulation and migration of antimony, arsenic, cadmium, chromium, mercury, lead, selenium and zinc.

A significant accumulation of most of the 27 metals investigated was detected in the upper layers of the sewage farm soils:

(a) The concentration of Cr, Hg and Zn already approached the permissible limit of elements in soils
(b) Relatively high accumulations of Sb, Ba, Cd, Hg, Pb, Sn and U were also found.

In the event of adequate water flux, a specific differentiation in the heavy metal migration rate in soils could be identified. The soil and concentration factors were then determined. The order of mobility observed was
Se > As > Zn > Cd > Pb > Cr, Hg > Sb.

TABLE I. TOLERABLE CONCENTRATION OF VARIOUS ELEMENTS IN SOILS (PROPOSAL) AND POSSIBLE REMOVAL BY PLANTS

Elements	Tolerable limits in soils (ppm) (proposal)	Removal by plants ($g \cdot ha^{-1} \cdot a^{-1}$)
Arsenic	20	1–50
Boron	25	200–800
Beryllium	10	0.5–1
Bromine	10	50–150
Cadmium	3	0.3–8
Cobalt	50	1–6
Chromium	100	1–10
Copper	100	30–150
Fluorine	200	20–200
Mercury	2	0.2–1.5
Nickel	50	10–30
Lead	100	1–80
Antimony	5	1–5
Selenium	10	1–15
Tin	50	5–50
Zinc	300	100–500

An absolutely stationary state was not noticed so that under certain conditions a sudden enhancement of the mobility could take place. Leaching, as well as contamination of the surface and groundwater, should be expected.

The length of time during which a given organic waste can be applied to a soil and the total load without resulting in a metal concentration in excess of the permissible levels listed in Table I can be calculated according to the following formula

$$n = \frac{(M_{sp} - M_{si}) \times 4.2 \times 10^6}{M_w \times w}$$

where n is the number of years till the permissible limits have been reached, M_{sp} is the permissible metal concentration in soils (see Table I), M_{si} is the initial metal concentration in soils, M_w is the metal concentration in waste, and w is the weight of wastes in kilogram dry matter per hectare per year.

IAEA-SM-263/7

IS NITRATE ABLE TO SUBSTITUTE FOR OXYGEN AS THE ELECTRON ACCEPTOR IN ANAEROBIC ENVIRONMENTS TO SUPPORT DEGRADATION OF AROMATIC NUCLEI?

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Many pesticides are halogenated aromatic compounds that are markedly more refractile to microbial attack than non-halogenated aromatics [1]. It was observed that microbial degradation of such compounds, especially dehalogenation, occurs more rapidly in flooded anaerobic soils than in aerobic upland soils [2]. Complete mineralization with methane or carbon dioxide as metabolic end-products was minimal under these conditions. On the contrary, anaerobic conditions support the accumulation of partially mineralized pesticides such as TDE [3].

Therefore, we examined the possibility of mineralizing aromatic nuclei in absolutely anaerobic environments only at the expense of nitrate. We used ring-U-^{14}C-benzoate as the carbon and energy source for the utilization by actively denitrifying *Pseudomonas aeruginosa, Acinetobacter* sp., *Moraxella* sp., and a consortium of various bacteria [4]. Experiments were done with the aromatic compound as the only carbon source in the culture medium and with glycerol as an additional carbon source. At the end of the tests the fate of the labelled carbon was examined as well as the conversion of nitrate.

Under absolutely anaerobic conditions, with nitrate as the only terminal electron acceptor, rupture of the benzene nucleus was not possible, neither with pure strains nor with mixed denitrifying populations. Also, when a second carbon source was present in the culture medium no $^{14}CO_2$ production was detected, indicating that no ring fission occurred. However, if traces of oxygen were left in the culture vessel, both mineralization of the aromatic moiety and denitrification could be observed. With increasing amounts of oxygen, mineralization as well as denitrification was intensified. In the presence of 10 or

20% oxygen (vol./vol.) in the culture vessel the relationship between produced CO_2 and denitrification gases was constant. Only when the oxygen concentration increased to 40% (vol./vol.) did the amount of released denitrification gases drop relatively to the produced carbon dioxide. These data lead to the conclusion that small quantities of molecular oxygen are sufficient to activate oxygenases and to rupture the benzene nucleus, and that the aliphatic products are then metabolized immediately by nitrate respiration. With a limited oxygen supply the molecular oxygen is used primarily for the ring fission.

Elimination of pesticides in soils is favoured by alternating changes between anaerobic and aerobic conditions. An anaerobic environment enables dechlorination, whereas even traces of molecular oxygen permit fission of the aromatic ring [5]. For fast and complete mineralization of the aliphatic components of the pesticide a sufficient nitrate supply is needed.

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IAEA-SM-263/22

METABOLISM OF ^{14}C-CHLORFENVINPHOS IN RAPE PLANTS

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Rape plants were treated twice at 1-week intervals with 0.3 mg (0.8 μCi) of ^{14}C-chlorfenvinphos.[1] Application was made in field conditions at the full flowering stage, radioactive material being spread on the leaves and stems. After sampling, separate parts of the plants were extracted into organic and water phases and the extracts were studied by liquid scintillation counting. Most radioactivity was found on the leaves and/or stems where the total radioactive residue ranged from 4.4 ppm one hour after application to 0.33 ppm at harvest. Radiolabelled material was translocated to the siliques and roots and was extracted mainly into the water phase where at harvest 0.01 ppm and 0.02 ppm, respectively, were found. In the stem solids that remained after extraction of the samples taken at harvest time 0.13–0.18 ppm were found. At harvest 9.1–11.7% of the total radioactivity found in stems were in the hexane phase, 36.4–50% in the water phase and 38.3–54.5% in the remaining solids. The solids were further extracted with absolute ethanol and then with water, and after the extractions 0.1 ppm of unextracted radioactive residue was still present. The unextracted residue fraction was treated as follows:

(1) Hydrolysis with 0.7% KOH and precipitation with ethanol, which released 0.04 ppm in saccharide solution and 0.01 ppm in starch precipitate
(2) Hydrolysis with 17.5% NaOH and precipitation by acidification, which released 0.01 ppm in hemicellulose precipitate
(3) Digestion with concentrated HCl, which released no radioactivity, either in the cellulose precipitate obtained by neutralization or in the neutralized solution. The remaining solids, believed to be lignins, contained 0.04 ppm, which constituted 12.1% of the total radioactive residue found in the stems at harvest.

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[1] 1 Ci = 3.70×10^{10} Bq.

IAEA-SM-263/15

MICROBIAL DEGRADATION OF THE INSECTICIDE AZINPHOS-METHYL

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Azinphos-methyl (0,0-dimethyl-S-(4-oxo-1,2,3-benzotriazine-3[4H]-yl-methyl)-phosphorodithioate; trademark ®Guthion or ®Gusathion) is a widely used non-systemic insecticide for the control of plant-sucking insects. Since it is used in large crop areas significant amounts will directly contaminate the soil during the application.

During a search for soil microorganisms capable of degrading azinphos-methyl, *Pseudomonas fluorescens* DSM 1976 was selected because of its ability to form different metabolites from azinphos-methyl [1]. The results presented here clearly show that *P. fluorescens* DSM 1976 is able to degrade the organophosphorous insecticide azinphos-methyl. Formation of bis-(benzazimidyl-methyl)-disulphide is most probably the result of a simple hydrolysis of the sulphur-phosphorus bond of the starting compound leading to thiomethyl-benzazimide, a metabolite which is readily oxidized enzymatically to bis-(benzazimidyl-methyl)-disulphide (Figs 1,2).

Azinphos-methyl obviously is degraded to benzazimide via enzymatic oxidation. However, the most significant reaction type of azinphos-methyl degradation by *P. fluorescens* DSM 1976 is the cleavage of the heterocyclic ring, thus forming anthranilic acid. Anthranilic acid is not degraded further by *P. fluorescens* DSM 1976. It is, however, easily metabolized via catechol by several other *Pseudomonas* strains or via gentisic acid by *Nocardia* [2]. As no anthranilic acid was detected in cultures of *P. fluorescens* DSM 1976 grown in the presence of benzazimide, it is suggested that anthranilic acid is formed from an intermediate other than benzazimide, presumably from bis-(benzazimidyl-methyl)-disulphide or thiomethyl-benzazimide.

Benzazimide is considered to be the main metabolite of azinphos-methyl during the degradation in soil [3]. Degradation studies with this metabolite have so far failed although some indications exist for the transformation of this compound by selected soil microorganisms.

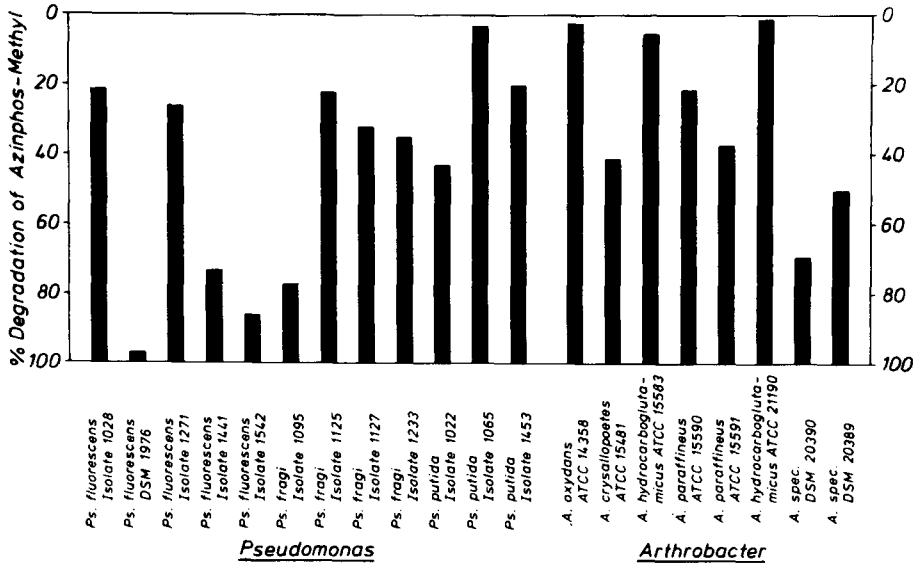

FIG. 1. Degradation of azinphos-methyl by different Pseudomonas *and* Arthrobacter *species (incubation period 10 d).*

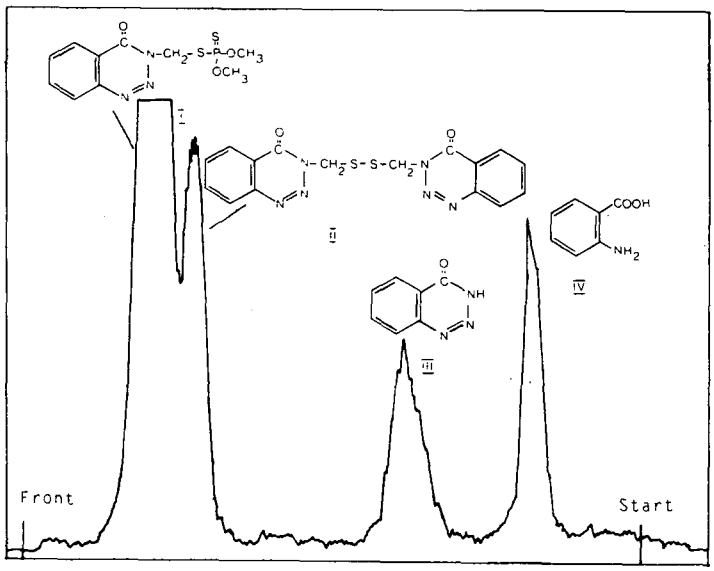

FIG. 2. TLC-scanning of metabolites formed during degradation of ^{14}*C-azinphos-methyl (I) by* Pseudomonas fluorescens *DSM 1976: bis-(benzazimidyl-methyl)-disulphide (II), benzazimide (III), and anthranilic acid (IV); solvent system: toluene-acetone-triethylamine, 8:2:1 (vol./vol./vol.).*

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IAEA-SM-263/14

PERSISTENCE OF ATRAZINE METABOLITES IN SOIL AFTER A SINGLE APPLICATION

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The fate of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) in soil was investigated under field conditions. In spring 1973 culture boxes (150 × 30 × 48 cm) filled with naturally grown soil were buried in soil and the surface area of the soil was treated with 25.6 mg ^{14}C-ring-labelled atrazine. Between 1973 and 1981 different plants were cultivated in these culture boxes. In summer 1981 soil samples were collected and analysed. Eighty-three per cent of the initial ^{14}C activity persisted in soil. The metabolites identified in the soil samples were: diethylatrazine (2-chloro-4-amino-6-isopropylamino-s-triazine) in less than phytotoxic amounts, 2-chloro-4,6-diamino-s-triazine, hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine), diethylhydroxyatrazine (2-hydroxy-4-amino-6-isopropylamino-s-triazine) and diisopropylhydroxyatrazine (2-hydroxy-4-ethylamino-6-amino-s-triazine).

Data show that a single application of atrazine results in the persistence of some of its degradation products, mainly hydroxylated analogues, in soil even 8 years after herbicide application. N-dealkylation and hydrolysis reactions were involved in the breakdown of atrazine in soil. Only 50% of the ^{14}C activity present in the soil samples could be extracted. This suggests that the atrazine metabolites are strongly bound to the soil complex and probably slowly set free. The metabolism of atrazine leading to harmless products, such as carbon dioxide, ammonia or water, is surely a slow process. It is conceivable that long-term annual applications of atrazine for weed control result in accumulation of metabolites in soil, which could possibly affect the soil biochemistry.

IAEA-SM-263/18

EFFECT AND PERSISTENCE OF CARBARYL IN SOILS

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The effects of carbaryl and 1-naphthol residues in soils on plant growth and microbial activities were investigated. Carbaryl had no phytotoxic effect at normal field concentrations. There was a gradual decrease in the seedling height of barley with higher concentrations of carbaryl in soil. Stimulation in plant height was observed with 1-naphthol. The nodule number in peanut plants was increased at 2.5 and 25 mg/kg levels of carbaryl and 1-naphthol. Carbaryl reduced the nodule number at higher application rates. There was stimulation in soil microbial numbers with carbaryl. The increase in fungal and bacterial numbers was seen up to the first 7-d incubation period only. Soil respiration was enhanced in carbaryl-treated moist (aerobic) soil; however, in flooded (anaerobic) soil there was less CO_2 production. 1-naphthol in aerobic soil increased CO_2 production and decreased it in anaerobic soil. Soil dehydrogenase activity was not affected by carbaryl and 1-naphthol in aerobic soil.

The persistence of ^{14}C-carbaryl was studied in black clay loam soil under aerobic and anaerobic conditions for 56 d. The extractable radioactivity was less in aerobic than in anaerobic soil; by the end of the 56-d incubation period 1.9 and 41.1% remained in aerobic and anaerobic soils, respectively. Carbaryl was degraded both in aerobic and anaerobic soils. 1-naphthol was identified as the degradation product in anaerobic soil by TLC. More $^{14}CO_2$ was produced in aerobic than in anaerobic soil. Carbaryl was less stable in alkaline than in acid soil.

IAEA-SM-263/55

MEASUREMENTS ON ^{14}C PRIMARY PRODUCTIVITY AND ALGAL ASSAY IN THE STUDY OF WATER POLLUTION EFFECTS IN THE CITARUM RIVER AND JATILUHUR RESERVOIR

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Various methods are used for evaluation of water pollution, i.e. physical, chemical and biological, and productivity. The relation of physical and chemical parameters to biological and productivity parameters is not yet clearly known. In this study measurements were taken on the various physical and chemical parameters and primary productivity (by the ^{14}C method) [1] and the algal growth potential [2].

Investigations were carried out in the Citarum river, which receives a considerable amount of pollutants originating from human and industrial wastes and from silt due to erosion. This river is dammed downstream to form the Jatiluhur reservoir. The site of investigation covers the area upstream of the river, down to the reservoir and beyond. These investigations were carried out during the wet and dry seasons in the years 1979-1982.

Based on statistical multivariate analysis, it has been concluded that a close relationship exists between all physical and chemical parameters to primary productivity (PP) and algal growth potential (AGP); either it is a relationship of nutrients or of depression due to toxic characteristics. Results of the correlation formula prove that the primary productivity and algal growth potential form parameters which can be considered as water pollution indicators.

The results of studies on water quality, primary productivity and algal growth potential indicate that the Citarum river has a high silt content, especially during the wet season, which depresses primary productivity. Starting upstream, water pollution originating from human and industrial waste from Bandung and its vicinity causes a high content of nutrients, consequently increasing primary productivity, but the toxic content is quite high, even resulting in a reduction of algal growth potential. However, after the river has flowed 100 km downstream and approaches the Jatiluhur reservoir a process of self-purification takes place, which improves the water quality, as indicated by an increase of PP and AGP.

The water quality becomes even better in the Jatiluhur reservoir due to the sedimentation of silt, resulting in a significant increase in PP. After passing

through the reservoir the pollution process starts again, owing to silt and industrial waste, resulting in a reduction of PP.

From the aspect of productivity, water of the Citarum river is able to self-purify itself against the influence of pollution and toxic contents, but the additional nutrient content has created another problem in the reservoir because it generates a process of eutrophication.

ACKNOWLEDGEMENTS

This work was carried out with the support and co-operation of the IAEA, Research Contract No. 2393/RI/SD, and the Ministry of Public Works, Indonesia. The author would also like to thank the Research Centre for Nuclear Techniques in Bandung for the ^{14}C counting.

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IAEA-SM-263/20

EFFECT OF COMMON INSECTICIDES ON NITROGEN-FIXING ORGANISMS IN NORTH IRAQI SOILS

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North Iraq is by and large different from the rest of the country regarding the factors that control plant growth, such as soil, temperature and its variations, water regime, pests and diseases. Quite a large part of the region is fairly rich in horticultural crops; thus, the potentiality of higher production from land

resources has a bright future here. This is mainly due to good soil fertility with respect to soil organic matter and native soil nitrogen. Preliminary investigations have suggested that such a condition is mainly due to the abundance of effective nitrogen-fixing organisms in most north Iraqi soils [1]. Close examination of individual organisms has shown that various species of the free-living bacteria *Azotobacter* are particularly active in Sulaimaniyah Governorate soils [2]. *Vicia faba* has also been reported to significantly enrich the soil in nitrogen when inoculated with a local strain of *Rhizobium leguminosarum* [3]. Similarly, numerous blue-green algae have been found to fix large amounts of atmospheric nitrogen [4].

With the present stress on higher agricultural production it is now necessary to use higher levels of fertilizers and also diverse pesticides to control various insect pests, disease fungi and weeds. It is considered probable that these chemical compounds may affect the various nitrogen-fixing organisms in these potentially fertile soils. Earlier unpublished studies have indicated that these agrochemicals had a deleterious effect on some of the nitrogen-fixing blue-green algae. In 1981 Khider [3] carried out a trial using four insecticides, nogos, kelthane, melathion and diptrex, and three fungicides, robigan, benlate and sulphur powder, on a local strain of *R. leguminosarum*. He found that higher doses of almost all the pesticides, when applied to the nutrient culture medium containing a known count of the rhizobia, lowered the count to a significant level.

A series of experiments have been carried out by the authors on the following soil nitrogen-fixing organisms isolated from different locations in north Iraq: *Azotobacter chroococcum, A. vinelandii, Nostoc* spp. and *R. leguminosarum* 307 (Obtained from Nitragan Co., Milwaukee, USA). The various pesticides used were diptrex, melathion, kelthane and parathion. These agrochemicals were used at four doses, namely 0.1, 1.0, 4.0 and 8.0 ml per litre of medium, on the basis of active ingredients of the respective pesticides.

The results of the experiments can be summarized as follows:

(1) Both *A. chroococcum* and *A. vinelandii* were found to be suppressed by the insecticides at all the levels used, but fungicides caused injury at high doses only. When medium doses were applied the organisms regained their usual growth pattern after 20 d incubation. It is thought that the organisms probably metabolized these agrochemicals.

(2) Growth of *Nostoc* spp. was very significantly affected by all the pesticides. However, even with high doses of different pesticides, it was noted that organisms regained their usual growth pattern when removed from the media containing the agrochemicals.

(3) *R. leguminosarum* 307 was found to be quite tolerant to all the pesticides used, but at high doses the growth of the organisms was very markedly influenced and in most cases irreversibly. Respiration studies using

^{14}C-glucose [5] indicated that glucose oxidation was inhibited in consonance with the doses of the insecticides. The behaviour of fungicides was not clear.

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IAEA-SM-263/19

EFFECT OF GAMMA IRRADIATION ON PESTICIDE RESIDUES IN FOOD PRODUCTS

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Use of chemicals in agriculture results in many cases in the appearance of terminal residues in food products. This study was undertaken to determine the effect of ionizing irradiation on the magnitude and nature of pesticide residues in food products and model systems. For this purpose gamma irradiation from ^{60}Co was used at doses normally applied for food preservation, as well as doses higher than 50 kGy. Lindane, methoxychlor and carbaryl were irradiated in a solid-state or in hexane solutions at concentrations of 4–5 ppm for the chlorinated hydrocarbons and 1 ppm for carbaryl. Food included luncheon meat and onions.

TABLE I. RECOVERY OF PESTICIDES IN GAMMA-IRRADIATED HEXANE SOLUTIONS

Dose (kGy)	Lindane (%)	DMDT (%)	Carbaryl (%)
0	100	100	100
4	96	99	95
6	91	89	93
10	85	81	90
30	76	78	62
48	73	73	55
80	64	68	29

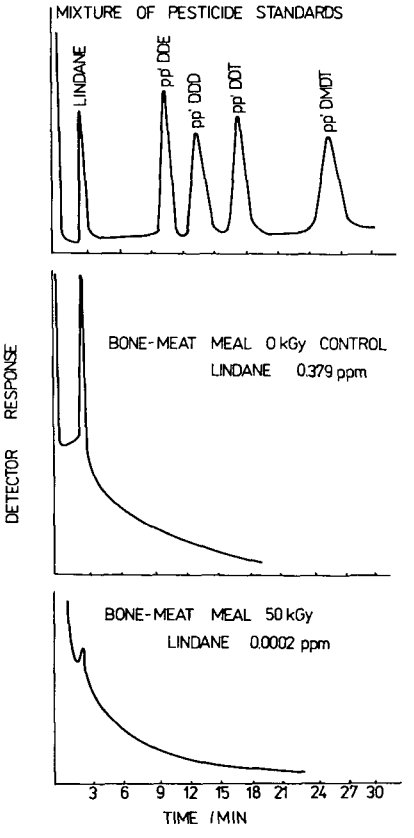

FIG. 1. Influence of irradiation on the content of lindane in commercial bone-meat meal.

The chemicals were mixed with the meat by dissolving in purified lard to provide pesticide levels at the concentrations indicated above. Animal feed included bone meat, blood and fish meals. At 30 kGy chemical degradation in model systems amounted to 24% for the organochlorine pesticides and 40% for carbaryl (Table I). Pesticide residues in luncheon meat did not suffer any apparent degradation when exposed to a dose range of 25–48 kGy. Normally occurring residues of p,p'-DDT (0.05 ppm) in onions were not affected at doses of 40–80 Gy. On the other hand, sterilizing doses of 25–50 kGy resulted in a reduction of lindane residues of 0.038 ppm in bone-meat meal to 0.0002–0.016 ppm (Fig.1). In this case, sterilization by gamma irradiation may provide the additional advantages of degrading and possibly detoxifying chemical residues.

IAEA-SM-263/56

A COMPARATIVE STUDY OF THE NITROGEN METABOLISM OF PHYTOPLANKTON AND PERIPHYTON IN A SUB-ALPINE LAKE*

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Current limnological research in meso-oligotrophic Castle Lake, California, USA, has emphasized the effects of nutrient enrichment and deficiency on primary producers. Nitrogen availability, in particular, has been shown to regulate rates of nitrogen uptake and primary production during a major portion of the growing season [1–5]. Beginning in 1980, the programme was expanded to include studies of benthic communities (eulittoral, sublittoral, and epipelic periphyton) and whole-epilimnion enrichments with NH_4NO_3 (+75 $\mu g\ N \cdot l^{-1}$) to more fully delineate the effects of increased N-loading on different algal communities co-existing within the same lake.

* This work was supported by a US National Science Foundation grant (DEB-8019918) to C.R. Goldman.

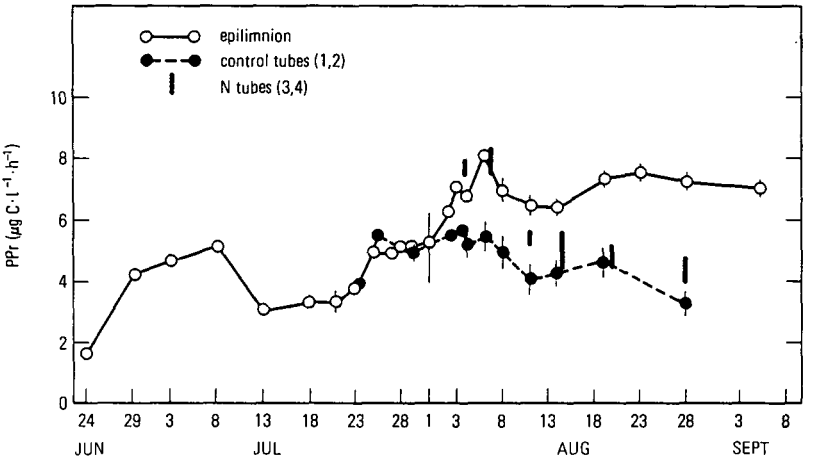

FIG. 1. Seasonal plot of ^{14}C primary productivity (PPr). The lake was spiked on the evening of 1 August 1981.

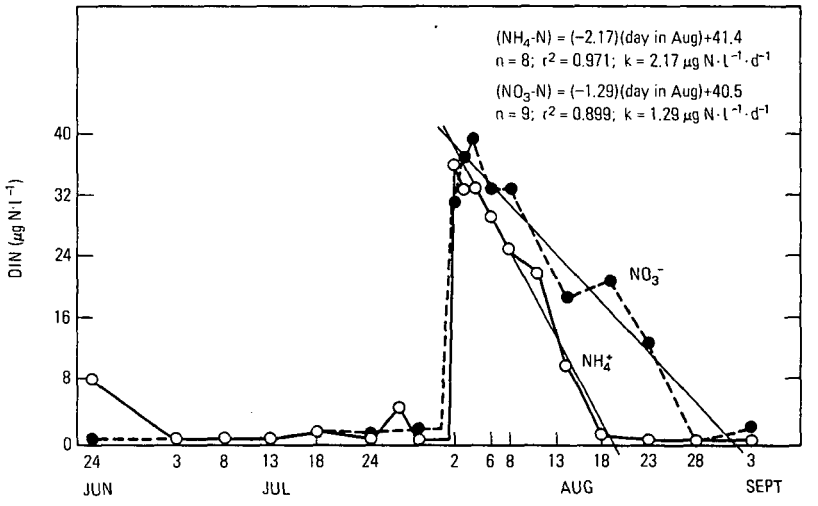

FIG. 2. Depletion of added DIN from the Castle Lake epilimnion in 1981.

Our experimental approach was to:

(1) Intensively monitor in situ rates of primary productivity, using $^{14}CO_2$ in both planktonic and benthic algae

(2) Compare and contrast strategies of N-utilization by planktonic and benthic algae, using $^{15}NO_3$, $^{15}NH_4$ and $^{15}N_2$ (used to calibrate the acetylene reduction method for assaying nitrogenase activity)

(3) Utilize $^{14}CO_2$, $^{15}NH_4$, $^{15}NO_3$ and ^{14}C- and ^{3}H-labelled organic compounds in a variety of physiological assays, the results of which could then be compared with actual in-lake responses.

At the time of writing some of the data sets remain incompletely analysed; however, we are able to provide preliminary interpretations of our findings. Phytoplankton rates of primary production in 1980 exhibited essentially no effect to epilimnetic enrichment for a period of about 12 d, after which rates rapidly increased by more than 300%. However, in 1981, a year in which mid-summer phytoplankton productivity was 400-500% higher than in 1980, rates of ^{14}C primary productivity (PPr) increased rapidly to a level approximately 60% above the pre-spike rate after one week (Fig. 1). Large (>5000 l) polyethylene tube enclosures containing pre-spike water remained near pre-spike values during this period, and paralleled open-lake rates of primary production for the duration of the experiment, although at lower values. Replicate 'N'- tubes tracked the open-lake with regard to DIN concentrations for >20 d (depletion), but did so for only 7–10 d with regard to ^{14}C-PPr. An obvious conclusion is that large volume enclosures do not necessarily simulate open-lake production processes for extended periods of time.

Chemical analyses following the enrichment showed that ammonium and nitrate depletion proceeded linearly with time (Fig. 2), in accordance with Michaelis-Menten kinetics and a low ($K_t \sim 5\ \mu gN \cdot l^{-1}$) half-saturation constant. Furthermore, NO_3 decreased at a rate of only about 60% of the NH_4 removal rate. Although this preferential NH_4 uptake is in general agreement with numerous studies [6], previous shorter-term ^{15}N and ^{13}N experiments using Castle Lake water had indicated that NO_3 uptake occurred at rates of about 1–5% of that for NH_4 [4,7]. This suggests that nitrate reductase activity was induced over a period of days following whole-lake enrichment.

Our studies of nitrogen cycling in Castle Lake have now delineated five distinct communities of algae, which differ with regard to their sources of available nitrogen. Briefly, these are: (1) upper euphotic zone phytoplankton, which have high affinity for low levels of DIN (half-saturation constants for N-uptake, $K_t < 10\ \mu gN \cdot l^{-1}$); (2) lower euphotic zone (~1% surface light) phytoplankton, which are located immediately above aphotic bottom waters (higher DIN) and have K_ts in the range of 20-80 $\mu gN \cdot l^{-1}$; (3) eulittoral (splash zone) epilithic periphyton, which lack the ability to fix atmospheric nitrogen and have a K_t for NH_4-N of 175 $\mu gN \cdot l^{-1}$ (and 162 $\mu g[NO_3\text{-}N] \cdot l^{-1}$ in ultra-oligotrophic

Lake Tahoe, California); (4) sublittoral epilithic periphyton, which have relatively higher K_ts (lower affinity) in the range of 300–2000 $\mu gN \cdot l^{-1}$ and which depend primarily upon N_2 fixation for their N metabolism; (5) epipelic periphyton living on the surface of the bottom mud immediately adjacent to the large pool of DIN in interstitial pore waters, which do not exhibit saturation uptake kinetics for DIN levels below 1000 $\mu gN \cdot l^{-1}$ ($K_t > 1000$ $\mu gN \cdot l^{-1}$).

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IAEA-SM-263/52

ИЗУЧЕНИЕ ДЕЙСТВИЯ РАЗЛИЧНЫХ ЗАГРЯЗНИТЕЛЕЙ ВОДНОЙ СРЕДЫ НА ГИДРОФИТЫ

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Роль различных химических веществ в загрязнении водоемов в первую очередь оценивается по их воздействию на важнейшие функции организмов, в частности, на их рост, развитие, размножение, ионный и энергетический обмены со средой, а также по генетическому действию этих веществ. Состояние ионного и энергетического обменов со средой в растительных клетках отражают мембранные потенциалы, проницаемость клеточных мембран и скорость движения протоплазмы. Эти параметры могут быть использованы как экспресс-показатели функционального состояния клеток при загрязнении водной среды. Одним из основных биологических процессов растительной

клетки является фотосинтез, который обеспечивает ее рост и развитие. Генетический эффект, вызываемый в популяциях одноклеточных водорослей различными загрязнителями, в том числе и радионуклидами, можно оценить по числу мутантных и летально поврежденных клеток.

Целью настоящей работы явилось изучить: действие различных концентраций Pb, сточных вод целлюлозно-бумажного производства (ЦБП) и ДДТ на физиологическое состояние водорослей, которое оценивали по мембранным потенциалам (МП), скорости движения протоплазмы и выживаемости их клеток, действие термального загрязнения на физиологическое состояние водорослей, которое оценивали по подвижности протоплазмы и выживаемости клеток, а также на уровни накопления радионуклидов в водных растениях, и действие радионуклидов на популяции хлореллы, состояние которой оценивали по плотности клеток, интенсивности фотосинтеза и числу мутантных и летально поврежденных клеток.

Pb при концентрациях 0,02 и 0,2 мг/л оказывает незначительное отрицательное влияние на клетки водорослей Nitellopsis obtusa, вызывая небольшую деполяризацию мембран, а при концентрации 2,0 мг/л уменьшает МП на 30-50 мВ или вызывает генерацию "потенциалов действия" и увеличивает чувствительность МП к повышению концентрации KCl в среде. Следовательно, Pb при концентрации 2,0 мг/л уже индуцирует переход клеток из энергизированного состояния с активированными H-насосами в деэнергизированное состояние с увеличенной проницаемостью для ионов K^+ и Cl^-. При длительном действии Pb с концентрацией 0,5 мг/л в клетках Nitellopsis obtusa снижается уровень АТФ, доступной для энергообеспечения движения протоплазмы, и на десятые сутки опыта погибает 50% клеток. Пороговой концентрацией Pb для клеток Nitellopsis obtusa следует считать 0,02 мг/л, а критической – 0,5 мг/л.

Сточные воды ЦБП при концентрации 0,4% вызывают у клеток Nitellopsis obtusa значительное, но у отдельных клеток обратимое снижение МП (до 70 мВ). В 6%-ом растворе этого загрязнителя МП снижается до величин K – Na-диффузионных потенциалов (– 150 мВ), а в 12%-ом растворе – до величин близких к потенциалу оболочки (около – 70 мВ). Таким образом, сточные воды при концентрации от 0,4% до 12% индуцируют снижение или полное подавление той части МП клеток Nitellopsis obtusa, которая определяется работой электрогенных насосов. В 12%-ом растворе этого загрязнителя, по-видимому, начинается деструкция клеточных мембран, обуславливающая снижение МП до уровня потенциала оболочки. При длительном действии 3%-го раствора сточных вод ЦБП в водорослях уже снижается уровень АТФ, доступного для энергообеспечения движения протоплазмы. В 12%-ом растворе сточных вод на 16-е сутки опыта погибало 50% клеток. Пороговой концентрацией сточных вод ЦБП для клеток Nitellopsis obtusa следует считать 0,4%, а критической – 12%.

ДДТ в концентрациях 0,03 мг/л, 0,3 мг/л и 3,0 мг/л вызывают у клеток Nitellopsis obtusa различные биоэлектрические реакции: в одних клетках возникают колебания МП, в других же – волнообразная деполяризация или гиперполяризация на 10-20 мВ, а некоторые клетки не реагируют на присутствие ДДТ, что может свидетельствовать о замедленном токсическом действии этого пестицида на клеточные мембраны и био-

энергетическую активность водорослей. При длительном действии ДДТ на клетки Nitellopsis obtusa достоверное снижение подвижности протоплазмы происходит при концентрации 0,3 мг/л, гибель отдельных клеток – при концентрации 3,0 мг/л, а гибель 50% клеток, которая отмечена в первые семь суток опытов, – при концентрации 30 мг/л. Пороговой концентрацией ДДТ для клеток Nitellopsis obtusa следует считать 0,03 мг/л, а критической – 3,0 мг/л.

При повышении температуры водной среды (до 30°С) происходит ухудшение физиологического состояния водорослей Nitellopsis obtusa , что выражается в снижении скорости движения протоплазмы и гибели отдельных клеток растений, а также подавлении образования и роста верхушечных клеток. Повышенные температуры водной среды (до 25-29°С) в основном не оказывают существенного влияния на уровни накопления ^{90}Sr и ^{137}Cs в водных растениях и несколько увеличивают уровни накопления ^{210}Pb и ^{144}Ce в них, а также поступление этих радионуклидов, особенно ^{210}Pb, в растительные клетки. Разница в накоплении радионуклидов в водных растениях при действии термального загрязнения, по-видимому, связана, главным образом, с различным физико-химическим состоянием этих радионуклидов как в водной среде, так и в самих растительных клетках. Повышение температуры водной среды увеличивает токсическое действие Pb на водоросли.

^{90}Sr, ^{137}Cs и ^{144}Ce (в диапазоне концентраций $37 \cdot 10^{-6}$ - $37 \cdot 10^{-4}$ Бк/л) на популяции хлореллы действуют подавляюще, при этом повышается число летально поврежденных и мутантных клеток. Однако, эти радионуклиды по их физиологическому и генетическому действию на популяции хлореллы весьма различаются. Если ^{144}Ce, КН которого в хлорелле достигает 273000 ед. и который в растительных клетках в основном аккумулируется на оболочке, несколько сильнее действует на интенсивность фотосинтеза и на число летально поврежденных клеток, то ^{90}Sr, КН которого в хлорелле равняется лишь 60 ед. и который в растительных клетках интенсивно накапливается и во внутриклеточных компартментах, сильнее действует на плотность клеток и изменения числа мутантных клеток, чем ^{137}Cs (КН 350 ед.) и ^{144}Ce. Различия в действии ^{90}Sr и ^{137}Cs, который, как и ^{90}Sr, в растительных клетках интенсивно накапливается во внутриклеточных компартментах, по-видимому, можно объяснить неодинаковым физико-химическим состоянием этих радионуклидов, как аналогов Ca и K, в протоплазме растительных клеток. Следовательно, степень действия радионуклидов (в пределах концентраций $37 \cdot 10^{-6}$ - $37 \cdot 10^{-4}$ Бк/л) на популяции хлореллы, особенно на плотность клеток и число мутантных клеток, главным образом, зависит от места локализации радионуклидов в ее клетках, которое в свою очередь обусловлено физико-химическим состоянием радионуклидов в водной среде и клетке, и в значительно меньшей степени – от величин КН радионуклидов в них.

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MICROORGANISMS FROM AN AQUATIC ECOSYSTEM
Dichlobenil interaction

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Granular dichlobenil was applied to an isolated portion of two irrigation channels at a rate to give a final concentration of 1 ppm for the control of macrophytes which impede water flow. The dominant plants *Ceratophyllum demersum, Myriophyllum spicatum, Ranunculus circinans* and *Potamogeton pectinatus* were suppressed. Despite a significant reduction in the fresh weight of submersed plants, i.e. 86.6 and 75.2% in the two channels, respectively, no significant drop in dissolved oxygen was noticed, but the pH of the water increased to 9. A slight increase in the number of amonifiers occurred between the 5th and 14th day after treatment, and an increase in fungi number was also noticed.

Disappearance of dichlobenil from the water was rapid, with the rate dependent on conditions in the channel. The concentration in water was below the detectable level on the 14th and 28th day, respectively, in the two channels. The highest concentration was found on the 2nd and 3rd day after application. Accumulation in water plants was observed from the 3rd day and in bottom sediment from the 7th day onwards after treatment.

To investigate the influence of dichlobenil on consumer activity, fresh channel sediment was used and BOD was measured, using the conventional manometric technique with Warburg apparatus. The low concentration of 1 ppm showed a stimulatory effect after 50 h incubation. For a concentration of 10 ppm 100 h were needed for adaptation; for 100 ppm no increase in oxygen consumption was recorded, remaining below the values of the control.

An accepted method for testing the toxicity of industrial contaminants on microorganisms has been tried to assess the toxicity of herbicides [1]. This method showed slight toxicity at the 1 ppm concentration of dichlobenil and was higher at 10 ppm. The toxicity increased after 40 h, which could indicate that more toxic substances were generated.

Two selected organisms were used, namely the fungi P. *lilacinum* Thom and *Aspergillus* sp. 15, which were isolated by the enrichment technique [2] with labelled dichlobenil and checked by autoradiography as it was hoped that they

would be capable of accumulating the herbicide. They showed a positive reaction in the amidase test. Respiration measurements with Warburg apparatus showed the ability of the tested species to use benzamide, 2,6-dichlorobenzamide and 2,6-dichlorobenzamide as a carbon source. The 2,6-dichlorobenzamide was shown to be the least prominent. In the presence of glucose, as an alternative substrate, the first two chemicals increased oxygen consumption, but at a lower level than pure glucose; in the case of dichlobenil the consumption was higher.

By autoradiography several species were isolated which belonged to the genera *Penicillium, Aspergillus, Fusarium, Trichoderma, Phoma* and *Coniothyrium.*

To obtain information on possible metabolism a study in pure culture with labelled dichlobenil was conducted. The autoradiography of silica gel ^{254}F TLC plates indicated microorganisms capable of degrading dichlobenil.

The main metabolites in pure culture were 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid. Another five labelled metabolites were also detected in co-metabolism studies but their identification has not yet been carried out.

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IAEA-SM-263/3

ANALYSIS OF RADIOLABELLED XENOBIOTICS AND THEIR METABOLITES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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High performance liquid chromatography (HPLC) has been extensively used in the last decade for drug and pesticide analysis. Classical TLC, by virtue of its ease of operation and ability to separate compounds of widely differing polarities, has come to occupy a central role in studying the metabolism of xenobiotics in animals and plants. HPLC has similar operating parameters but in many cases has the additional advantages of more rapid analysis, greater sample

capacity and superior resolution, and is thus well suited for studying the metabolic alterations in drugs and pesticides.

Examples are given of the application of radio-HPLC to the analysis of radio-labelled novel chemicals and their metabolites in selected animal species. These HPLC analyses utilized straight phase and reversed phase systems often with the addition of anionic or cationic surfactants.

Detection of radiolabelled pesticide xenobiotics and their metabolites in HPLC eluate can be by continuous (e.g. continuous flow radioactivity detected) or discontinuous methods (e.g. eluate fraction collection and quantitation by an independent liquid scintillation counter).

HPLC analysis with radio-detection is suited for use in the following applications:

(1) Qualitative and quantitative comparison of xenobiotic metabolic profiles in various animal species and following different routes of administration
(2) Isolation and purification of xenobiotic metabolites before identification by GC-MS
(3) Stability/degradation studies
(4) Pharmacokinetics of unchanged test compounds.

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STUDY OF THE EXCRETION, SECRETION AND RETENTION OF AN ANTI-LIVERFLUKE COMPOUND IN A LACTATING COW

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The metabolic fate of a drug, 4,4′,6,6′-tetrabromo-2,2′biphenyldiol-mono (dihydrogen phosphate), has been investigated in a lactating dairy cow at the experimental farm of the Belgian Nuclear Centre in Mol.

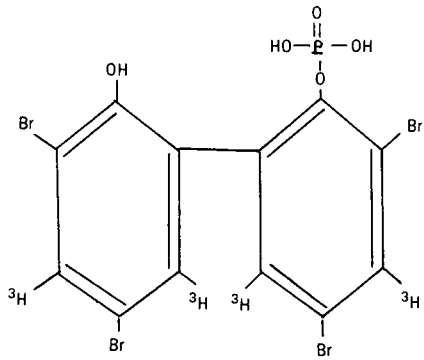

FIG.1. ALAC-II.

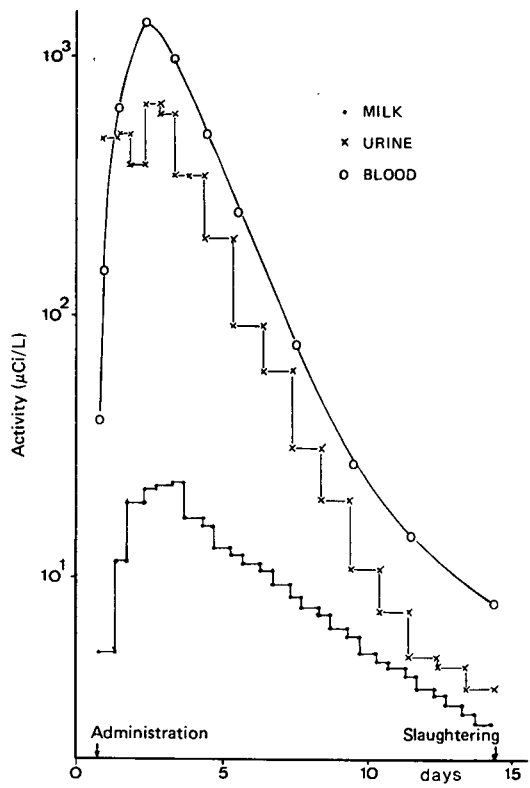

FIG.2. Urinary excretion, secretion into milk and concentration in blood for 14 d after administration of ALAC-II (1 Ci = 3.70 X 10^{10} Bq).

TABLE I. CONCENTRATIONS OF ALAC-II IN DIFFERENT TISSUES OF A COW AT SACRIFICE 14 DAYS AFTER ADMINISTRATION
(expressed as μCi/kg and as mg/kg fresh weight)

	μCi/kg[a]	mg/kg
Liver	377 ± 4.2	5.42 ± 0.06
Kidneys	12.6	0.18
Muscle	1.91	0.027
Bile fluid	13.4	0.19
Spleen	6.42	0.09
Fat	1.02 ± 0.16	0.015 ± 0.0023
Brain	0.17	0.0024
Bone marrow	1.09	0.016

[a] 1 Ci = 3.70×10^{10} Bq.

TABLE II. RECOVERY AND DISTRIBUTION OF ALAC-II IN A COW AT SACRIFICE 14 DAYS AFTER ADMINISTRATION

		mCi[a]	% of dose
Excretion-secretion	Faeces	350	92.7
	Urine	23.3	6.17
	Milk	0.91	0.24
Tissues-organs	Blood	0.32	0.008
	Meat	0.54	0.14
	Liver	2.31	0.62
	Kidneys	0.02	0.005
	Spleen	0.005	0.001
	Fat	0.069	0.018
	Brain	6.9×10^{-5}	1.8×10^{-5}

[a] 1 Ci = 3.70×10^{10} Bq.

In collaboration with the inventors, synthesis of the tritium ^{3}H-labelled compound was done; as indicated in the formula (Fig.1), tritium was incorporated in the 3,3′ and 5,5′ positions.

The specific radioactivity was 69.5 mCi/g and the prepared quantity of compound was 5.375 g, to which were added 2.687 g of sodium bicarbonate.[1] The trial compound was coded ALAC-II. A 4-year old 'pie noire' cow of 450 kg received a single dose in 3 gelatine capsules, each containing one-third of the ALAC-II-^{3}H bicarbonate mixture. The amount administered corresponded to the usual therapeutic dose for cows (1.2 g ALAC-II/100 kg body weight).

Faecal and urinary excretion, secretion into milk and concentration in blood were monitored for 14 d after administration. Fourteen days after administration the cow was slaughtered and distribution in different organs and tissues (liver, meat, kidney, spleen, fat, bone marrow, brain, bile) was determined.

Figure 2 shows that maximum concentrations are reached in blood and urine approximately 48 h after administration and in milk 72 h after administration and that these concentrations decrease by half-life times of 1 d (urine, blood) and 4 d (milk) between days 3 and 9.

Concentrations of the compound in different tissues and organs at sacrifice are given in Table I. Recovery and distribution are given in Table II.

Fourteen days after administration recovery was quite good and various organs and tissues contained less than 1% of the administered dose. The highest concentration was observed in the liver (5.42 ± 0.06 mg/kg); bile fluid, kidneys, spleen and muscle showed lower concentrations.

[1] 1 Ci = 3.70×10^{10} Bq.

PANEL DISCUSSION ON RESEARCH NEEDS FOR EFFECTIVE AND SAFE PESTICIDES IN DEVELOPING COUNTRIES
(Session VIII)

Chairman

F.P.W. WINTERINGHAM
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SUMMARY REPORT

On the last day of the Symposium a Panel Discussion was held on the Research Needs for Effective and Safe Pesticides in Developing Countries. Preliminary remarks were made by scientists from Brazil, the Federal Republic of Germany, India, the Philippines and the United Kingdom, followed by a lively discussion on the topic which continued for one and one-half hours. The following is a brief résumé of the Panel Discussion, as interpreted by the Scientific Secretary of the Symposium.

The need for appropriate use of pesticides to improve food production was pointed out repeatedly. A number of scientists also noted that the most effective way to increase food production is to intensify agricultural production, which implies increased investment in all aspects of production and protection. This increase in investment must be protected by the appropriate use of pesticides. Thus, use of pesticides will increase, particularly in developing countries where it is currently rather limited.

The most consistent comment throughout the discussion was that great differences exist between nations which influence research priorities in relation to pesticides. These differences are not limited only to those between developed and developing countries, even though this was the one most frequently mentioned. Differences in climate (e.g. between tropical and temperate zone agriculture) affect the fate of pesticides, so that in at least some and perhaps many cases the residues in soil, water and foodstuffs will be very different after the same usage patterns. Certain data indicate that some chlorinated hydrocarbon pesticides do not appear to be as environment damaging in tropical climates as in temperate ones. However, these differences have not been investigated sufficiently to allow definitive statements about the fate of some of the more long-residual pesticides, such as chlorinated hydrocarbons.

Another dissimilarity mentioned was that in some cases priorities were established by governments of developing countries regarding pesticide usage and pesticide residues which did not coincide with the priorities set by governments in developed countries. These priorities should be recognized as having been set by government authorities who have taken into account such things as levels of education and training of their farmers, economic conditions of the country and of the farmers, and availability of trained research scientists and equipment and facilities with which to carry out the research. Economics was repeatedly pointed out as one of the major deterrents to substituting some of the newer, more expensive pesticides for some of the older and cheaper ones, many of which have been severely restricted or banned in developed countries.

It was mentioned that the pesticide formulations available in many developing countries are the same ones used in developed countries. This has resulted in occasional failures of the pesticide to effectively control the pest because the

formulation available was not designed for the tropics, for high rainfall, etc. In turn more pesticides are used and, thus, a potential for higher pesticide residues ensues.

Several speakers mentioned the possibility of establishing regional centres for pesticide research, using similar climatic zones as regions. These centres would conduct research on the primary pesticides used in the region, including the fate of pesticides and residues. This information would then be available to interested governments and organizations.

The ever expanding need for trained people was repeatedly emphasized and pleas were made to increase the amount of training available for scientists from developing countries. Requirements include not only technical training but also training for technicians, electronics equipment maintenance personnel, and others. The need for better library facilities and reprint exchanges was also stressed.

The specific types of research required for the development of effective and safe pesticides in developing countries, which require or will greatly benefit from the use of radiolabelled pesticides, include the following:

(1) Adaptive research to seek ways of using existing pesticides effectively, efficiently and safely under local conditions
(2) Special studies aimed at evaluating the actual risk of using the older, more established pesticides, such as DDT, under tropical conditions
(3) Studies to improve pesticide formulations
(4) Studies to improve methods of application
(5) Studies aimed at determining the potential problems associated with pesticides moving into aquatic systems in which fish and other food are being produced
(6) Specific pesticide research aimed at solving pest problems and pesticide residue problems for intensive agricultural production, such as multi-cropping and no-till agriculture.

There was no question that more pesticide research is needed in developing countries. Governmental authorities should establish national priorities for the types of pesticide research required to solve local problems. Experiments using isotopically labelled pesticides can be a great assistance in solving many specific pesticide problems in developing countries.

HIGHLIGHTS OF THE CONFERENCE

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REVIEW

A wealth of information has been produced to illuminate many aspects of the use of agrochemicals and their effects on the environment.

Perhaps the major highlight of the Symposium was the conception of a programme that brought together so many differing aspects of the fate and behaviour of agrochemicals in the environment. The very breadth of the programme was bold, including the importance of raising agricultural output to feed the growing world population, with other topics ranging from the cost of safety in terms of finance or lost potential for producing food, to the development of techniques to study problems. All play an important and interacting part.

One might possibly draw an analogy between the various facets of the use of agrochemicals and some of the aqueous ecosystems described during the Symposium. For example, in some conditions distinct communities of algae, differing in their sources of nitrogen, may exist in zones in lakes, but use of a barrier (container) to isolate part of the water for experimental purposes significantly changes the conditions. Movement of nitrogen and other chemicals is prevented, and the results of measurements, while important, have a different significance from any made in the absence of restraints. Lakes and other bodies of water themselves receive water from land bearing material which affects the aquatic environment. Some substances are naturally occurring, others may be agrochemicals that disturb the ecosystem, either by providing additional nutrients or by toxic effects.

There was much emphasis on the so-called bound residues. These are not easily defined with precision but represent potentially biologically active materials not readily assayed because they are difficult to separate from the surrounding matrix.

Evidence was presented of the degradation of pesticides in soil being affected by inorganic and organic fertilizers and materials providing ready carbon sources for microbial growth. Both increased and decreased degradation were observed and there was evidence of different metabolic end-points, indicating changes in metabolic pathways and possibly soil microbial populations.

Despite emphasis on the results of investigations and their significance, development of new applications and possible pitfalls in the use of nuclides were not neglected, although nuclear techniques are established and accepted as making important and unique contributions to the study of agrochemicals in the environment. There were elegant illustrations of combining techniques to show both movement and degradation by using the results from specific gas chromatographic determination of the parent compound and tracer techniques to measure the total material. At the same time the work illustrated the ability of glass vessels to adsorb and degrade materials, a property which may cause misleading results. There was a forceful reminder of the value of the short-lived ^{32}P isotope for

investigations of organophosphorous compounds, which illustrated that differing metabolic pathways predominated in various organisms, resulting in different active materials predominating.

Use of neutron activation of elements was an interesting development that could prove of use in improving pesticide efficiency. Improved application of pesticides obviously eliminates the risk of unwanted biological effects by diminishing the environmental burden of xenobiotic compounds, and examples of achieving control with controlled release formulations were described, using much diminished amounts of pesticides.

Studies of the environmental behaviour of elements using isotopes were not overlooked. The example of nitrogen using stable isotopes clearly showed the interrelation between soil, crop, air and water and recirculation among the components and organisms.

Heavy metals are used as fungicides and tend to accumulate in land and, even more, may accumulate from fertilizing land with sewage sludge contaminated with industrial wastes. As such, there is concern about the transfer of heavy metals to crops for human consumption. Here, studies with radioisotopes and nuclear activation provide some reassuring evidence about uptake from contaminated soils and distribution in cereals.

CONSULTANTS' MEETING ON PERSISTENT PESTICIDES AND HERBICIDES IN THE TROPICS USING ISOTOPE TECHNIQUES

The following pages are a Summary Report of the Consultants' Meeting that was held concurrently with the Symposium

SUMMARY REPORT

INTRODUCTION

A group of consultants met in Rome during the Symposium to assist the Joint FAO/IAEA Division in determining whether or not programmes involving persistent pesticides and herbicides in the tropics should be developed by the Joint Division and, if so, what specific types of programmes should be considered. Since there was a natural division of subject matter, the consultants' group considered each separately. There was little difficulty at arriving at consensus regarding the advisability of initiating programmes on the use of herbicides in the tropics. However, in the case of persistent pesticides, such as DDT, dieldrin, lindane, BHC, there was considerable discussion regarding the advisability of such a programme. Scientists and administrators familiar with pesticide regulations in developed countries are well aware that persistent pesticides, such as DDT and dieldrin, have either been banned or their use patterns greatly reduced because of environmental or human health concerns. The primary point of discussion was whether or not there was sufficient evidence available to indicate that the fate, including metabolism, degradation, residues, etc., was sufficiently different under tropical conditions than that under temperate conditions to warrant initiation of a study of persistent pesticides under tropical conditions. It is known, for example, that the more intensive UV light in the tropics degrades pesticides more rapidly and that the increased rainfall and higher ambient temperatures also influence degradation of pesticides.

The following is a brief summary of the consultants' report.

PERSISTENT PESTICIDES IN THE TROPICS

Information in scientific literature suggests that organochlorines and other persistent pesticides are degraded much more rapidly under tropical or sub-tropical than under temperate climatic conditions, but more data are needed from experiments deliberately designed to explore this phenomenon. A reappraisal of the properties of persistent pesticides should be conducted under conditions closely proximating their use in tropical and sub-tropical climates; in other words, using open-air techniques that can provide data comparable as far as is possible with data obtained by similar techniques in temperate climates.

The specific sequence of events recommended by the consultants is as follows:

(1) A literature search should be conducted for publications on organochlorines, etc., relevant to their fate and behaviour in temperate and tropical/sub-tropical climates.

(2) Experiments should be planned between the IAEA and its collaborators in tropical climates to determine the half-lives of, initially, DDT, technical BHC, and possibly lindane. These experiments should be designed to include degradation in soils, on and in plants, and aquatic environments in order to compare differences between temperate and tropical conditions.
(3) The effectiveness of microbial degradation must be considered in the soil studies.
(4) Volatilization of unchanged pesticides must be taken into account in the experimental designs.

HERBICIDES IN THE TROPICS

Owing to the very rapid increase in the use of herbicides in developing countries, primarily under tropical conditions, the consultants' group recommended consideration of two research programmes: for effective chemical control of *Imperata cylindrica* (L.) *beauv.* and related weeds, and for improving weed control in aquatic ecosystems.

Imperata cylindrica and related weeds are among the ten most damaging pest weeds in the world. This weed infests enormous land areas in the tropics and effective chemical control has not yet been developed. Little is known about the absorption and metabolism of herbicides by this weed. Studies with radiolabelled herbicides would readily answer these questions and, as a continuation, it may be possible to develop special formulations of herbicides that would control this weed effectively.

Regarding weed control in aquatic systems, the primary technical difficulties at present involve herbicide application technologies that effectively control water weeds while not causing environmental problems involving fish, use of the water for human consumption, etc. Some work has been done on herbicide formulations, specifically for aquatic situations, and the consultants urged that this be used as a building block for the Joint FAO/IAEA Division programme. Radiolabelled herbicides are ideal for these studies because of precise answers that can be obtained on movement and distribution of the herbicide in water, uptake by the plant, inactivation in sediments, and metabolism of the herbicide in the water, and in the plant.

In both programmes, the consultants listed possible research contractors and research agreement holders.

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THIRD FAO/IAEA RESEARCH CO-ORDINATION MEETING ON AGRICULTURAL CHEMICAL RESIDUE-BIOTA INTERACTIONS IN SOIL AND AQUATIC ECOSYSTEMS

The following pages are a Summary Report of the Third Research Co-ordination Meeting that was held concurrently with the Symposium

SUMMARY REPORT

1. INTRODUCTION

This report documents the recommendations of the Third Research Co-ordination Meeting on Agricultural Chemical Residue-Biota Interactions in Soil and Aquatic Ecosystems, which was held concurrently with the Symposium. The accomplishments of the programmes are reviewed and proposals for the future activities and direction of the programmes are presented.

Use of agrochemicals will continue to be indispensable in the desperate battle of mankind against starvation. Agrochemicals are essential ingredients in agricultural production. Environmental contamination represents unwanted side-effects of agrochemical use. Frequent monitoring of the fate and effects of pesticides in the environment is necessary because these chemicals are subject to continuous change. Nuclear techniques are an essential tool in this context. Both isotope tracer techniques and labelled substrate techniques are used and are found to be of great importance in this field. With the aim of promoting extension of this research and monitoring to developing countries, the Joint FAO/IAEA Division initiated two co-ordinated research programmes:

(a) Isotopic tracer-aided research and monitoring programme on Agricultural Residue-Biota Interactions in Aquatic Ecosystems (water programme) in 1975, which had the objectives:

(i) To develop, standardize and apply isotopically labelled substrate techniques for comparative assays of primary autotrophic and micro-heterotrophic production and decay
(ii) To develop, standardize and apply complementary isotopic tracer techniques to determine fate, persistence and bioconcentration of trace contaminants
(iii) To use these techniques to obtain comparable data on the current status of water bodies and the changes to be expected.

(b) Isotopic tracer-aided studies of Agrochemical Residue-Soil Biota Interactions (soil programme) in 1977, which had the objectives:

(i) To develop and evaluate labelled substrate techniques for measuring the soil capacity to decompose undesirable contaminants and residues and to promote desirable transformations
(ii) To perform comparative studies of the fate and biological effects of selected agrochemical residues and additives, and of soil biological activity
(iii) To apply the techniques as a diagnostic tool, with priority to be given to rice ecosystems.

2. OBJECTIVES

The following objectives were outlined at this meeting:

(a) To review the progress of the programme
(b) To discuss the problems related to methodology and labelled substrates
(c) To discuss application of a common methodology developed since the previous Research Co-ordination Meeting
(d) To prepare for publication the scientific data and information obtained since the last report (IAEA-TECDOC-247, 1981)
(e) To identify the problems and priorities of related subject areas and to make appropriate recommendations.

3. ACHIEVEMENTS

The contributions of the programme participants were presented at the Symposium. The results were discussed by a larger group of scientists and they attracted generally favourable comments. In a separate meeting, the programme participants reviewed the results and highlighted the achievements. They commented that the system of research programmes is quite satisfactory. Major importance lies in development of an international understanding of the subject matter. The programme was successful in the sense of having established over the years an excellent co-operation and exchange of experience among the participants. Through scientific visits, close collaboration was established among scientists from developing and developed countries.

Some highlights are presented in the following sub-sections.

3.1. Water programme

Studies in Hungary showed that the labelled substrate-anti-cholinesterase method is a simple, sensitive and reliable tool to estimate specific pesticide residues in rivers and lakes. Application of the labelled substrate-heterotrophic activity technique in fresh water fish ponds has been demonstrated in Canada and Israel. With the help of this technique it was concluded that the tolerance of parathion in this system is 30 ppb for a single treatment and that repeated treatments with 15 ppb, if a recovery time of 3 to 7 days is allowed, are also tolerated.

Algal growth measurements using $^{14}CO_2$ correlated well with other primary production measurement methods. It was shown to be a good monitoring method for water quality, both of an Indonesian river system and of a lake in the Federal Republic of Germany. Use of labelled substrate techniques for estimating the nitrogen and the carbon cycles in fresh water lakes has been demonstrated in the United States of America.

The fate of the herbicide dichlobenil in Yugoslav irrigation channels was investigated, using a radiolabelled chemical. A half-life was estimated and some metabolites could be identified. Accumulation and effects of radionuclides, heavy metals (^{210}Pb) and DDT were studied in connection with paper mill effluents in Baltic estuaries. Under certain conditions aquatic plants and algae were found to accumulate considerable amounts of the contaminants, to the disadvantage of some algal species.

3.2. Soil programme

Despite some promising publications and laboratory attempts, use of labelled substrates to investigate agrochemical effects in the soil environment is not as well developed as in the aquatic system. Labelled substrates are, however, used to demonstrate effects on the metabolism of soil organisms in vitro. In India it was found that nitrogen fixation of cowpea plants was inhibited by herbicides at a concentration of 5 to 10 ppm. This was correlated with a reduced metabolism of labelled substrates by the *Rhizobia* strains. In the US it was found that at application levels of 25 ppm atrazine began to exert an inhibiting effect on the appearance of gaseous denitrification products. In Iraqi soils nitrogen fixation and ^{14}C-glucose respiration were only inhibited at very high concentrations of benlate, sevin and dipterex.

On the other hand, in the US, Turkey and Brazil labelled pesticides are widely used in the soil ecosystem as a unique tool to investigate pesticide residues and metabolites. In Austria it has been shown that, under certain conditions, elements such as zinc, mercury and cadmium accumulate to undesirable concentrations in some crop plants, although the edible parts usually have lower concentrations compared with other plant parts.

4. RECOMMENDATIONS

The following recommendations were outlined to ensure essential continuity and the most effective contribution of related research programmes to the growing problems of environmental contaminants:

(a) To initiate, as funds become available, a co-ordinated research programme with the possible title of Fate of Pesticides and their Effects in Soil and Water Environments of Tropical and Non-tropical Regions. The programme should include the flooded soils of rice fields. A condition for participants in the programme is that they would undertake common experiments.
(b) To allocate extra funds for procurement of agrochemicals and their metabolites.

(c) To continue publication of scientific documents, since they were found to be of great value to institutes, especially in developing countries, where literature sources are often limited.
(d) To hold future Research Co-ordination Meetings at locations where the work is being done, preferably in developing countries, as previously recommended in IAEA-TECDOC-247, 1981.

5. CONCLUSIONS

In the light of the original objectives and earlier recommendations, the meeting noted with satisfaction that the programme had achieved most of its goals. Labelled substrates and other isotopic tracer techniques have been improved and their use has been extended to applications in developing countries. Comparative assays have been devised that are ready to be applied whenever required. Results obtained with the techniques in various countries generally confirmed that pesticides, when used at the recommended levels, do not reach alarming concentrations in food and the environment. It should be stressed that the results are preliminary and some aspects, such as the possible differences between pesticide behaviour in tropical and temperate zones, need further investigation. A topic that requires specific attention is temporarily and permanently flooded soil. There is insufficient information on the transport of agrochemicals from agricultural land into water bodies. Rice field water, running from one field into the other, is likely to increase residues on its way. It is concluded that there is a greater need to use tracer methodologies to help solve these problems.

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