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CRITICAL EVALUATION OF MODEL ECOSYSTEMS

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Critical evaluation of model ecosystems

Abstract - This report critically evaluates terrestrial, aquatic and mixed laboratory and outdoor model ecosystems with respect to their use and limitations in studying the fate of pesticides in the environment. Despite the difficulties of comparing the environmental relevance of the different models in use, an attempt has been made to appraise the reported systems in sequence from the point of view of effort involved and their prediction potential. The review concentrates on describing the concepts of the models, typical results which have been obtained and emphasises reliability and prediction potential.

CONTENTS

1. INTRODUCTION

In recent years there have been a number of attempts to develop aquatic, terrestrial and mixed model ecosystems to assess the dispersal and degradation of pesticides. These systems were designed to show how pesticides behave in the environment and to predict ecotoxic effects.

1.1 DEFINITIONS

To fulfill the valid definition of the term 'ecosystem', a model ecosystem needs to be a complex experimental set-up. An ecosystem is in general composed of a variety of fluctuating populations of flora and fauna species, includes their abiotic environment and is self-sustaining. It is generally agreed however, that a model ecosystem need not be self-sustaining. It is partly closed by boundaries and should be composed of more than two compartments, at least two of these compartments being biotic and of different trophic levels. These models can include both completely controlled laboratory systems as well as less controlled outdoor systems. Although soil and sediment are biphasic due to the presence of water as an essential component, they are considered for the purposes of this report as single compartments.

So-called 'microcosms', which generally consist of an undefined variety of microbial and other low-trophic-level organisms in their environment are a special group of model ecosystems.

Consequently, with this interpretation of what constitutes model ecosystems, 'single environmental factors' are all less complex. They consist of at most two compartments, not more than one of which is biotic.

There are also 'single factors' with established high relevance such as some of the physicochemical properties of a pesticide, such as vapour pressure or water solubility. These can be unequivocally determined but their significance in the fate of a pesticide in the environment is not predetermined. The more complex single factor methodology include parameters such as volatility from water which is comparable to model ecosystems with respect to interpretation of resulting data.

1.2 Aims and Scope

Model ecosystems for the investigation of the fate of pesticides in the environment (mobility, transformation, degradation) use a varying number of compartments and well-defined components as well as different boundary conditions. By careful control of variables in the laboratory systems, attempts are made to achieve at least comparative results on the dispersion and degradation of a chemical and qualitative results on metabolic pathways. The extent to which the currently available laboratory and outdoor model ecosystems meet the requirement of quantitative prediction of the fate of xenobiotics in the real environments is uncertain for most of the systems used. Therefore, it is still difficult to compare the different concepts if investigating the fate of pesticides in the environment with each other.

1.3 Concepts of Investigation

The role that model ecosystems can play in the assessment of the environmental fate of pesticides has to take into account their level of complexity. As for other environmental chemicals, there are simple and feasible screening procedures, a large variety of intermediate methods, and at the other end of the spectrum there is environmental real-time monitoring or field experiments which give "absolute" data. Real-time monitoring has the drawback of being very costly and only retrospective in terms of changes of environmental quality. At the other end of the testing scale, screening is frequently considered to be only indicative and of limited value. Therefore, a sequential system of investigations is preferable in order to provide sufficiently reliable data with minimum effort. In such a sequence, model ecosystems fall between the more complicated single factor studies such as (volatility measurements) and field studies. From the conceptual point of view, ecosystems provide data of higher environmental relevance than single factors since they include the interactions of several environmental factors and thus more closely represent actual environmental conditions. However, the more complex and environmentally relevant the models are, the less feasible is their standardization and reproducibility of results due to variation of factors influencing the fate of pesticides. Model systems on the other hand provide more detailed information than field studies since radiolabelled pesticides can be used in laboratory and outdoor models.

According to the above definition, model ecosystems include both laboratory and outdoor methods. Here again there are two principal differences in possible approaches. Laboratory systems can be designed as entirely closed systems with a potential for the establishment of reliable balance sheets including the vapour phase. Additionally, all relevant factors can be conditioned, controlled and standardized as needed. In outdoor studies which aim to utilise the integrity of the environmental factors, an experimentally forced change e.g. of temperature by cooling or heating would lead to the loss of the real outdoor conditions. Thus, laboratory models suffer from the limitation that they are difficult to translate into wider environments whereas outdoor studies have the potential of giving accurate quantitative data but being limited to the place and conditions prevailing of the experiment. With regard to the effort needed to assess the fate of a pesticide by single-factor experiments as compared to the investigations by complex multi-factor systems, the limitations of both approaches have to be considered. In general, strictly single-factor experiments, e.g. the determination of vapour pressure, give data only on the respective behaviour of the parent compound and disregard other competitive factors. For volatilization of any chemical resulting from the use of a pesticide the vapour pressure of each conversion product has to be measured separately. In model systems, on the other hand where in general volatilization of total radioactivity following the application of a pesticide is assessed, the volatilization of conversion and degradation products is included in this single data set. There are, however, also cases of single-factor methods which exclude conversion products.

From a scientific point of view, single-factor experiments unequivocally give data on the relative importance of the investigated factor of compound characteristics, e.g. the importance of vapour pressure with respect to volatilization. In multi-factor systems the contribution of each single-factor to the assessed parameter cannot be generally

identified. In single factor experiments, for example, degradation in soil can be assessed comparatively by measuring the degradation in mixed soil populations. The photochemical degradation can be assessed in a strictly abiotic irradiation experiment. In an outdoor model ecosystem where microbes and sunlight act simultaneously on the chemical it is not possible to differentiate between biotic and abiotic degradation. Use of the model results in cumulative data. For a generalization of results in terms of environmental concentrations or kinetics of residues in ecosystems, mathematical models for a respective synthesis using experimental data prefer those from single factor experiments since they are less restricted to undefined conditions, but the variables affecting them are well known.

2. Terrestrial Model Systems

2.1 Introduction

The concept of model ecosystems has been developed mainly for aquatic systems. For experiments with terrestrial flora and fauna species in their abiotic environment, "the model" has usually constituted experimental designs of high complexity or mixed aquatic/terrestrial systems. Terrestrial systems so far developed have been used exclusively for investigating the fate of chemicals, and not their immediate toxic effects on any of the species present. Nevertheless, their aim is to show the effects of interactive processes, which could not be detected from single factor investigations.

Strictly speaking, according to the definition, a small laboratory experiment with a plant growing in soil in a small pot, already includes more than two compartments. Consequently, the range of terrestrial models overlaps at the least complex end with in-vivo experiments in plants growing in soil and extends to closed outdoor field plots.

2.2 Open, plant-soil systems

For a number of years, outdoor plant-soil experiments using boxes maintained in a wire-covered enclosure have been used in many locations. This model(Refs 1,2) comprises wooden boxes of size 60 cm x 60 cm x 60 cm filled with soil (above layers of stones and peat) which are kept in an outdoor enclosure so that radiolabelled compounds can be used in a controlled way. The boxes are in a pit such that the soil surface is level with the soil in the surrounding field. The model size is sufficiently large to avoid the increased uptake rates observed in laboratory pots and is exposed to the same meteorological conditions as the surroundings. Leaching water can be collected in a tray in which the boxes are housed, and in some experiments hinged flaps on one side enable soil cores to be taken horizontally to assess downward movement of pesticides. This approach has been used to study the fate of pesticides applied to crops(Refs 3,4,5) and distinct advantages and limitations of the system have become apparent. Its major advantage is that it permits the fate and persistence of a compound to be observed under outdoor conditions in a radiochemical experiment that is easy to dispose of afterwards. Metabolites formed under the conditions of the experiment are likely to occur under true field conditions. One disadvantage in practice is that, if the labelled pesticide is applied at a concentration close to that which will be employed in commercial use then the amount of metabolites formed will be small, making their identification difficult. This can be overcome by carrying out parallel indoor experiments to obtain larger quantities of metabolites. Another problem with this type of experiment is related to interpretation of the quantitative data obtained. Although the "residue levels" of the pesticide and its metabolites following foliar or soil treatment should be close to those which will occur in the field, the small plot size and careful way in which radiochemical is applied can lead to somewhat higher concentrations in the crop. Nevertheless, reasonable agreement was obtained in foliar treatment experiments with benzoylprop-ethyl(Ref 3). At the same time as the plants growing in boxes were treated at a rate equivalent to 1 kg/ha, wheat in a nearby field was treated at the same application rate with a commercial preparation of benzoylprop-ethyl. There were similar concentrations of benzoylprop-ethyl and its metabolite benzoylprop in both sets of plants. Consequently, the other ^{14}C-containing products detected in the radiochemical samples (for which residue analytical methods were not available at the time) would be expected to occur in field samples at similar concentrations. Such good agreement is not always obtained, however, and there are several possible reasons for this. If the crop density in the boxes is higher than in normal agricultural practice then a greater proportion of a spray application will be deposited on to the plants. This can lead not only to higher residue levels but also to marked differences in the relative concentration of the pesticide and its metabolites. In addition, wire netting can afford some protection from the weather. Despite these limitations, if these outdoor experiments are regarded as falling between glasshouse conditions and field conditions according to recommended use and the data are interpreted accordingly, then they can be very useful.

When crops are grown in treated soil in these boxes, uptake by plants occurs at a somewhat higher rate than that typically observed under field conditions(Refs 2,6,7,8).

Using lysimeters of different size from 0.25 to 1 m^2 surface and a 40 to 110 cm soil layer, including undisturbed soil monoliths, Fuhr et al have investigated the fate of 15 pesticides, especially soil herbicides, in plants and soil (Ref 10). In those cases where data were available from field trials on translocation into plants, plant metabolism, movement leaching in soil, total residues and degradation in soil, these were in rather good agreement with the lysimeter experiments (Ref 11).

2.3 Open terrestrial ecosystems

Lichenstein and co-workers(Ref 12) have described a small scale model system consisting of soil, plants and water. Plants were grown in a soil layer treated with radiolabelled compound on top of a layer of untreated soil. Water was percolated through the system so that both movement and metabolism in soil could be studied simultaneously. Uptake and metabolism of soil applied pesticide can also be studied in the same experiment. In a study with [^{14}C]phorate, in which 350g of treated soil and 350g untreated soil were used, information was obtained on the concentration of [^{14}C]phorate, its sulphoxide, sulphone and other metabolites in both soil layers, in corn plants and in leached water. However, no comparative data from earlier work on phorate was given.

From the same laboratory, a more complete, compartmentalised microcosm for studying the fate, metabolism and movement of chemicals in the plant and soil environment was described. Possible bioaccumulation can also be determined under specific conditions. The system consists of terrestrial and aquatic compartments, and soil run-off water (resulting from intermittent watering of the soil-plant part) is run into the aquatic part of the system. The latter contains lake sediment, aquatic plants and fish or insect larvae. The apparatus is described fully elsewhere(Ref 11).

With this system it is possible to use fallow or planted soil and study the metabolism and distribution of pesticide in plants, soil, run-off water (containing soil particles), sediment and aquatic organisms and experiments with [^{14}C]phorate and [^{14}C]fonofos have been described. In the latter case (Ref 13) it was shown that fonofos and its metabolites were less persistent in systems with fallow soil than with a ryegrass cover. Run-off of soil and water (and radiolabelled material) was considerably less from systems with crop cover.

Cheng et al (Ref 14) have used a plant-soil system specifically designed to allow the isolation of the aerial portions of the plants from the roots and soil. Moreover, it was possible to collect any $^{14}CO_2$ released from the soil and root zone. This system was used to study the influence of plants on the degradation of soil applied pesticide. Experiments with methabenzthiazuron(Ref 14) showed that a greater rate of degradation occurred in the presence of peanuts than in soil alone. The authors concluded that the influence of growing plants ought to be taken account of in soil degradation studies, particularly for soil-applied pesticides.

2.4 Closed, aerated systems

The major technical drawback of open terrestrial systems is the difficulty in obtaining a radiochemical (or chemical) balance of the applied compound. In particular, volatile products including carbon dioxide cannot readily be detected and quantified without partially closing in the system to do so. This requires the use of closed, ventilation-controlled plant systems.

Schuphan(Ref 15) and Ebing and Schuphan(Ref 16) have described an apparatus for studying pesticide metabolism under balanced conditions. The system comprises a closed cultivating system for plant growth with a series of traps for absorbing volatilised pesticide and metabolites. Various designs of glass chamber were used, depending on the size of plants to be grown and the apparatus was mounted under a bank of fluorescent lights. Plants could be watered using a side arm (see ref 16 for details).

The system was used for studying the fate of [^{14}C-phenyl]monolinuron applied to soil in which spinach, cress and potatoes were grown in sequence. The overall recovery of radio-label was good (96%) including approximately 5% $^{14}CO_2$ which was collected. In separate experiments with [^{14}C-phenyl] buturon and [^{14}C-carbonyl] diallate recoveries of 89% and 99% respectively were obtained.

Nash and Beall at the Beltsville Agricultural Research Centre have described a "micro-agroecosystem" as an approach for studying the environmental behaviour of compounds, which lies between the laboratory closed system and the field (Refs 17,18,19,20). The "micro agroecosystem" is a large glass chamber (150 cm long, 115 cm high and 50 cm wide) housed

in a greenhouse described in detail in ref. 20. Field soil is placed in the chamber to a depth of 15 cm and the temperature, relative humidity, light intensity and photoperiod are controlled by ambient glasshouse conditions. A large volume of air is pulled through the chamber (2.5 m^3/min or 3.3 chamber volume changes/min) which aids cooling and allows trapping of volatile products. Another feature of the system is that the environmental fate of more than one chemical is usually measured concurrently.

A similar system, a closed plant-metabolism-box, has been developed by Figge (Ref 27) for larger size balance and metabolism studies. It has a controlled climate, uses mainly natural sunlight and is located on the roof of the laboratory building. The system needs much effort but due to its size allows the investigation complex systems (e.g. simultaneously several plant species) and gives reproducible results. As regards potential for quantitative predictions, it has the limitations of closed systems.

Nash(Ref 20) has recently reviewed results from "microagroecosystem" studies and compared them with field and laboratory studies. In particular "half-lives" on foliage and in soil were compared (where data were available) and an attempt was also made to compare volatilisation rates.

Results on dissipation from foliage were in fair agreement with field data and dissipation rates for soil were in fair agreement for several compounds (organochlorine compounds and trifluralin) but not with others. It was found that volatilisation rates were in favourable agreement with those from field measurements.

Nash concluded that one of the most important uses of these chambers is for the determination of comparative kinetic data to predict the behaviour of new chemicals.

3. AQUATIC MODEL ECOSYSTEMS

3.1 Introduction

Pesticides are applied intentionally to the environment not only for crop and forest protection but also for public health purposes. Depending on the physico-chemical properties as well as the use patterns, some pesticides may produce adverse effects in the environment. For example, p,p'-DDT and other organochlorine pesticides used widely in the past have been detected in various environmental compartments. Recently, industrial chemicals such as polychlorinated biphenyls and dialkylphthalate plasticizers have also been considered to be significant environmental contaminants.

Among the model ecosystems, several devices have been developed to determine the behaviour of compounds in the aquatic environment. Aquatic model ecosystems are classified roughly into laboratory models and field models, and the former are exemplified by the terrestrial-aquatic model ecosystem developed by Metcalf et al.(Ref 22), the aquatic model ecosystem by Kearney et al.(Ref 23) the recirculating static model ecosystem by Isensee et al.(Ref 24 the river model ecosystem(Ref 25), and the estuarine model ecosystem(Ref 26).

N.B. In this section of the review, bioaccumulation ratio and biodegradility index are designated as BR and BI, respectively. BR can be measured as the ratio of concentration of the parent (radiolabelled) compound (or of any key degradation product) in the organism to that in water at the termination of the test or average concentration during the test. BR indicates how extensively an organism accumulates a compound from its surrounding environment by all processes including absorption, adsorption, ingestion, etc., whereas biomagnification indicates that a compound is concentrated through consumption of organisms at lower food chain with a net increase in tissue concentration, although it is not necessarily clear in the aquatic model ecosystem study to determine the routes of biomagnification. BI is defined as the ratio of the radio-labelled polar compounds to the radiolabelled non-polar compounds in the organism.

3.2 Laboratory model ecosystem

Pesticides and other chemicals may enter the aquatic environment by direct application, spray drift and washing from the atmosphere, by leaching and run-off from agricultural land, and/or by indirect (and unintentional) disposal. In the aquatic environment, these chemicals become bound to organic matter and soil particles in sediment, although small portions remain in the aqueous phase with continuous exchange between sediment and water. The chemicals in the aqueous phase are also taken up and stored in aquatic organisms through direct absorption and indirectly through the food chain. At the same time they are subjected to degradation by metabolic transformation in each component organism and also by various physico-chemical factors.

As the basis of assessing ultimate ecological effects of the compounds, several types of

hexachlorophene (Ref 33), leptophos (Ref 29), lindane (Ref 29,33,35,36), malathion, methoprene (Ref 29), methylchlor (Ref 31,44-46), metrabuzin (Ref 29), "Mirex" (Ref 29,35,36), parathion (Refs 29,33,47), pentachlorophenol (Ref 48), phenmedipham (Ref 29), "Prolan" (Ref 49), propachlor (Ref 29,33), propoxur (Ref 29,34,35), pyrazon (Refs 29,33), temephos, toxaphene (Ref 29),α-trichloromethyl-p-ethoxybenzyl-p-methylaniline (Ref 50) and trifluralin (Ref 29,33) as well as industrial chemicals such as aniline, anisole (Refs 35,48), benzidine, benzo(a)pyrene (Ref 51), chlorobenzene (Ref 35,48), di-2-ethylhexylphthalate (Refs 35,52), di-n-octylphthalate (Refs 33,53), nitrobenzene (Ref 48), PCBs (Refs 33,35,54), phthalic anhydride (Ref 35,48) and vinyl chloride (Ref 51).

The authors observed (a) changes in the chemical constitution of the radiolabelled products in the water and living organisms, (b) bioaccumulation ratio and biodegradability, (c) relation of BR and BI to molecular properties of the compounds and (d) toxic effects on the various organisms. A highly significant correlation between BR and water solubility was also found found; with 12 organochlorine pesticides (Ref 36), log water solubility (ppb) = 5.99-1.176 logBR (fish) r = 0.87, n = 12.

Based on the results of the studies, Metcalf and his collaborators suggested that pesticides with solubility less than 0.5 mg/l are likely to exhibit signficant bioaccumulation, whereas those with solubilities more than 50 mg/l are unlikely to be bioaccumulated. With the pesticides whose solubilities were between these two specified values, the degree of bioaccumulation was variable (Refs 28,29,55,56).

From model ecosystem studies with radiolabelled compounds, useful data about the comparative metabolism of the compounds in a variety of organisms of several different phyla were provided, and Lu and Metcalf (Ref 48) summarized the results for 5 organisms, Oedogonium, Daphnia, Physa, Culex and Gambusia. Hydroxylation occurred most effectively in mosquito larvae for chlorobenzene and anisole. O-Dealkylation of anisole proceeded significantly only in fish. Reduction of nitrobenzene occurred in all organisms. Methylation of aniline was detected only in algae, mosquito larvae and fish. Conjugation of phenol and carboxylic acid was carried out efficiently in all organisms except in fish and in snail, respectively. Epoxidation of aldrin occurred in almost parallel phylogenetic order from daphnia (8% of total radioactivity), mosquito larva (16%), snail (50%) to fish (80%). Dehydrochlorination of DDT to DDE was found in mosquito larva (42% of the total radioactivity), and in daphnia (15%) but to smaller extents in other organisms. Reductive dechlorination of DDT to DDD proceeded slowly throughout all the organisms.

Thus, it was demonstrated that a variety of aquatic organisms can metabolize various xenobiotics, whose metabolic alteration varies with the species.

Several modifications of the terrestrial-aquatic model ecosystem have been made. For example, Tsuge et al. reconstituted the model containing sweet potato plant, rice seedling and tobacco cutworm larvae in the terrestrial compartment and algae, red snail, daphnia, mosquito larvae and guppy in the aquatic compartment. Whenα-naphthaleneacetic acid was examined in this model, the BR value for snail, rice plant, fish and alga was 170, 260, 490 and 540, respectively(Ref 57). Comparative studies withα-,β-,δ-BHC showed that theβ-isomer was least biodegradable(Ref 58,59).

Kazana et al reported that when radiolabelled 3,5-xylyl N-methylcarbamate (XMC) was applied to the above model, the recovery of radioactivity was 2.9% for carbonyl-labelled XMC, 3.9% for N-methyl-^{14}C labelled and 21.0% for 3,5dimethyl-^{14}C labelled (Ref 60).

To improve recovery of the applied radioactivity, they designed a closed type of model ecosystem which allowed trapping of the volatile and gaseous metabolites. By using the closed system, 44.6% of the applied radioactivity was recovered as $^{14}CO_2$ from N-methyl-^{14}C-labelled XMC(Ref 61) and the recovery of the radioactivity associated with naphthyl-1-^{14}C labelled carbaryl was 51.4% of which 25.1% was $^{14}CO_2$, whereas the ^{14}C recovery of ^{14}C-ring-DDT was 62.1% with 0.54% of $^{14}CO_2$(Ref 62).

Since white quartz sand has been used in Metcalf's model ecosystem to eliminate the complicated effects caused by soil, the results are generally reproducible, and the tested compounds can be compared with one another with respect to their bioaccumulation and biodegradability. However, because of the artificial nature of white quartz sand, the results will often be inevitably artificial, simulating the natural environment to only a limited extent.

3.3 Aquatic model ecosystems

3.3.1 Static model ecosystems

The aquatic environment is generally a reservoir of pesticides and xenobiotics, and the compounds tend to adsorb to suspended solids, and become bound on to bottom sediment. Consequently, the biota are continually exposed to the

compounds. To evaluate interaction between compounds and aquatic organisms under these circumstances, Kearney et al. designed an aquatic model ecosystem, especially to clarify the fate of chemicals with regards to (a) total balance, (b) biodegradability, (c) bioavailability of bound residues and (d) bioaccumulation in aquatic organisms (Refs 23,63,64).

In the system(Refs 2,43), 11.4 kg of soil mixed with a radiolabelled compound was layered on the bottom of the 110 l glass aquarium, flooded with 80 l of distilled water and allowed to equilibrate for 1 week. A screen was used to vertically bisect the aquarium to protect the catfish from the predaceous crayfish. After 1 week, catfish, crayfish, daphnids, snails, algae and duckweed plants were added. After maintenance for 1 month under glasshouse conditions, the ecosystem was terminated and aquatic organisms as well as soil and water were analysed for radioactivity.

By using this system, the behaviour of cacodylic acid(Refs 23,65), carbaryl (Ref 66), dimethylarsine (Ref 65), 6-dinitroaniline herbicides (R67), monosodium methanearsonic acid, sodium arsenate(Ref 64), 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD)(Ref 68) and 3,5-xylyl N-methylcarbamate (XMC)(Ref 66) were investigated.

When TCDD was added to sandy loam and silty loam soil, the concentration of TCDD in water was higher with sandy loam than with silty loam soil and increased with the amount applied to soils(Ref 68). TCDD accumulation in all organisms was directly related to the water concentration; log TCDD content in tissue (ppb) = $\alpha+\beta$ log TCDD content in water (ppt), r_ 0.94, and averaged 2.0-2.6 x 10^4 (snail, fish and daphnid) and 4-9 x 10^3 (duckweed, algae and catfish) times the water concentration. Most (85-99%) of the ^{14}CTCDD originally added to the ecosystem remained in the soil at the end of the experiment, indicating that soil serves as a reservoir for the compound.

Kearney et al. investigated the distribution, accumulation and degradation of 6 dinitroaniline herbicides in the aquatic model ecosystem with emphasis on effects of sunlight(Ref 67). These studies revealed that BR values for fish were greatly dependent on exposure to sunlight: the aquarium afforded higher BR values for fish when held under dark conditions afforded higher BR values for fish (240-760) than in sunlight (30-80). It seems likely that under natural conditions these herbicides would be easily photodegraded to more polar products.

To evaluate bioavailability of the non-extractable (bound) residues, the soil was treated for 7 months with 10 mg/kg of (^{14}C)-butralin or profluralin and then the obtained non-extractable bound residues were added to the ecosystem. The radioactivity was released into the water phase rapidly and reached a relatively higher concentration. However, BR values for fish were considerably lower (76, 120, for butralin and profluralin, respectively). The results suggest that the polar products released from the bound state possess less potential for biomagnification.

The results from Kearney's aquatic model ecosystem studies revealed that

(a) the major portions of the applied pesticides remain in the bottom sediment,
(b) the amount of pesticide in the water phase depends on the soil characteristics, concentration of chemicals in the soil and water solubility of the compound,
(c) the compound in solution is taken up by aquatic organisms depending on water solubility, as pointed out by Metcalf(Ref 28),
(d) the non-extractable (bound) residues in soil are readily released into the water phase due to their polar nature,
(e) the bound residues are biologically inactive to a certain extent and are less readily accumulated, and
(f) the behaviour of compounds may be affected by exposure to sunlight.

3.3.2 Recirculating static model ecosystem

Isensee et al.(Ref 24) designed a recirculating static model ecosystem with a major advantage over the previous static system that fish as well as daphnids and other organisms are exposed to chemicals for the entire 30 day period. The system was designed so that some daphnids passed through the screen to provide a part of the food for fish and thereby establish the food chain from daphnids to fish. In the larger compartment of the aquarium, soil treated with a pesticide was placed prior to flooding with water. After one day, daphnids, snails and algae were added to the larger compartment, and mosquito fish to the other smaller compartment. The percolating water pump ensured uniform mixing of water between the 2 compartments.

Using this system, the distribution and bioaccumulation of atrazine (Ref 69), hexachlorobenzene (Ref 74), N-nitrosoatrazine (Ref 69), oxadiazione, phosalone (Ref 70), 2,4,5-T, TCDD (Ref 71), toxaphen (Ref 72) and trifluraline (Ref 73) were investigated.

In the case of TCDD, for example, any toxicity to fish by TCDD was not observed in the previous aquatic model ecosystem where fish were exposed to the compound for only 3 days (Ref 68), although TCDD showed high toxicity to fish and other aquatic organisms in the acute test. On the other hand, according to Yockim et al.'s study with the recirculating ecosystem (Ref 71), the radioactivity in the water from the soil treated with 0.1 mg/kg TCDD reached equilibrium by day 1, ranging from 2 to 4 ppt , and all of the fish died between days 14-15. BR values of 2-6 x 10^3 were obtained for the organisms and the values agreed with those found in the aquatic model ecosystem (1-6 x 10^4) (Ref 68).

Isensee et al. determined the distribution and degradation of unfractionated toxaphene and three of its fractions in the recirculating model ecosystem(Ref 72). The concentrations found in water were about one order of magnitude higher with the initial soil concentrati of 1.0 mg/kg, as compared with the experiment using initial 0.1 mg/kg. The tissue concentrations were directly related to the amounts available in water, namely, organisms in the 1.0 mg/kg experiment contained about 10 times more compounds. For most organisms, the increase in tissue content over the first 7 to 15 days was observed and then the tissue concentrations generally decreased. BR values for fish were least for the most polar compound (fraction 1, BR = 600-800) and those of unfractionated toxaphene and 2 other fractioned compounds were 4000-10000. Bioaccumulation in fish was much higher than in snails (average of 6000 vs. 480).

Distribution and bioaccumulation of fenvalerate were investigated in the recirculating static mode ecosystem, in comparison with DDT(Ref 74). After the radiocarbon level in water had reached equilibrium at day 7 and day 5 after introduction of fenvalerate- and DDT-treated soil, respectively, aquatic orgnaisms were added. The soil contained 90-93% of the radiocarbon added to the soil during the running period of both 7 and 30 days, suggesting that the soil acted as a reservoir. The BR value of fenvalerate for fish and other organisms (algae, snails and daphnids) was 120 and 400-600, respectively, after 7 days exposure, and this value was about 10 times less than that of DDT in fish. After 30 days exposure, the BR for fenvalerate was 160-300 for fish and 630-1180 for other organisms.

When radiolabelled fenitrothion was introduced into the water phase of a similar ecosystem(Ref 75,76), the radiocarbon was rapidly taken up by the organisms. However, it also decreased rapidly with a half-life of 2, 3, 3 and 4 days in fish, daphnids, snails and algae, resectively. The maximum BR value was 181 for fish and algae, 33 for snails and 69 for daphnids. Although the radioactivity applied to water remained mainly in water (4659%), the radiocarbon in soil increased with time and reached 25% of the applied ^{14}C at day 21. In the organisms several metabolites were found which were never produced in a simple water-organism system, and these included demethylaminofenitrothion, N-acetylaminofenitrothion and 3-methyl-4-acetyl-aminophenol. These metabolites were actually produced in soil. When DDT was introduced into the same system, the BR value was 53400 for fish, 6110 for snails and 3440 for daphnids and algae, indicating higher bioaccumulation in the organisms at a higher trophic level in the food chain.

A similar model ecosystem composed of soil sediment, plants, animals, and water which were collected from a lake was constructed to trace the movement of radiolabelled diquat applied to water(Ref 77). When the radiocarbon had fallen close to the detectable background levels in water after 9 days, the concentration of diquat was found to be highest in sediment followed by snailshell and fish viscera. The ^{14}C levels of the latter organisms were about 2 times higher than the applied level in the water.

The results obtained from studies in the recirculating model ecosystem provide data on
(a) uptake and elimination of the compounds in aquatic organisms as a function of time by periodically analyzing the compound in water and organisms,
(b) bioaccumulation of the compounds in a state of equilibrium, and
(c) toxicological effects of the test compounds to aquatic organisms during a long period.

3.3.3 River model ecosystem

In the river environment where pesticide residues are present, the aquatic organisms are continuously exposed to the effluent containing pesticides.

Nambu et al. constituted a river model ecosystem to investigate the fate of methylmercury (Ref 25). Gravel was placed at the bottom of the water-course (25 cm W x 30 cm H) and algae (mainly diatoms) were inoculated with addition of growth media to water. Thereafter the solution containing 5 ppb of methylmercury was allowed to flow for 4 times during a 6 day period. Then fresh water was introduced, and aquatic insect larvae (dayfly) were put into the system to live on algae. Finally, fish (dace) were added 4 days after introduction of the insect larvae. Thus, in the ecosystem the test compound can be supplied or removed

at specified intervals.

The total mercury content (organic and inorganic) in algae became 43 mg/kg, with a BR of 10700 at the maximum after the initial 6 days. Methylmercury content relative to total mercury was 68% on the next day, and decreased to 42% 5 days thereafter, suggesting mineralization of mercury in algae. The concentration of methylmercury in the insect larvae and fish was above 7 mg/kg and 3 mg/kg (in edible tissues), respectively, and the BR was higher than that encountered when each organism was separately exposed to 5 mg/kg of methylmercury, namely, 1.70 mg/kg for insect larvae and 0.34 mg/kg for fish. These findings suggest that the compound is biomagnified through both the food chain and direct absorption.

3.3.4 Estuarine model ecosystem

Since estuaries receive the outflow of rivers, much of the suspended solid in water carrying pesticides is deposited in this area. So, the estuarine model ecosystem was designed to investigate the distribution and accumulation of test compounds in the components in the ecosystem.

Schoor(Ref 78) constructed a system containing beach sand, artificial seawater, turtle grass plants and grass shrimp, and Mirex was introduced into the aquarium by air-lift columns containing Mirex bait. The Mirex concentration in the sand increased linearly with time, and reached about 85% of the applied Mirex, suggesting that sand was a reservoir. The Mirex residue in shrimp exposed for 13 days was 130 mg/kg and the BR value was about 7000. To the above system, pinfish were introduced to investigate distribution and biological effects of Mirex on predator-prey interaction(Ref 26). The maximum BR value in turtle grass leaves and shrimp after 13 days exposure and in pinfish after 3 days was 1300, 8000 and 3800, respectively.

3.3.5 Other aquatic model systems

Lichtenstein et al. described a microcosm apparatus consisting of terrestrial and aquatic components to study the movement and contamination of pesticides into aquatic systems by soil run-off by irrigation or rainfall(Ref 79). Silt loam treated with (^{14}C)-phorate at 4 ppm was placed into terrestrial components and corn seedling were then planted. Each 1875 ml of rain was applied on days 42 and 49, and this resulted in run-off of about 650 ml by slope of the soil (5°) and dense leaves of corn plants. Five hundred and fifty ml of run-off water and soil were then poured into an aquarium, into which a layer of lake bottom mud had been previously deposited, and 2 days later fish and water ferns were added. The system was analyzed on day 70. Each of 2 rainfalls yielded soil-water run-off containing 1.5% of the totally applied radiocarbon. Two-thirds of the radio-carbon recovered from the aquaria was associated with the soil-lake mud mixture, and ^{14}C in water, fish and water fern was 0.8, 0.02 and 0.2% of the applied radiocarbon, respectively.

This study revealed that with soil slope of 5° and rainfall applied twice, the transported radioactivity to the components of the aquatic system was mainly adsorbed on the sediments.

3.4 Field model ecosystems

Unlike the above mentioned laboratory model ecosystems, a field model (actually a part of the natural environment) is relatively large in size, and the system can be an accurate simulation of the natural environment. With field models, observations can be continued for a long period of the fate and effects of the test chemicals on organisms.

On the other hand, it is difficult to grasp the behaviour of the degradation products and the total balance of the applied compound, even when labelled compounds are used.

Simazine was applied to 0.1 ha ponds to give estimated concentrations of 0.1, 1.0 or 3.0 mg/kg, and the residues in mud, water, benthic invertebrates and fish were determined over 1 year by gas chromatography(Ref 80).

The concentration of simazine in the pond mud was directly related to application rates and declined with time, but the residues were still present after 346 days (0.002-0.32 mg/kg). In the next year's trials with the same application rates, the concentrations in water were 2 to 8 times higher than with the previous treatment, and the residues were detected in all the treated ponds even 456 days after the application (0.001-0.16 mg/kg). The concentration of simazine residues in water were somewhat lower than in mud. The simazine residues in benthic invertebrates and fish were also directly proportional to the amounts applied, but usually far exceeded those in water for at least 86 days (BR = 2-100). Thereafter the residues declined, and after 367 days were still detected (0.10

mg/kg and 0.04 mg/kg after treatment with 0.3 mg/kg and 0.1 mg/kg, respectively). The simazine residues in benthic invertebrates sampled at the second trial were comparable to or less than the residues at first treatment, and gave little evidence of bioaccumulation. Simazine or its degradation products appeared to have no adverse effects on survival of aquatic organisms.

The distribution and retention of atrazine and carbofuran in farm ponds were examined for 2 years (Ref 81). Soon after atrazine was applied, it was found in all non-biological and biological components, and no biological magnification was observed. Carbofuran was detected only in the water and mud immediately after application and thereafter it was absent from all components. No adverse effects due to the pesticides were observed in the biological components.

Korte et al use small, experimental but essentially natural, and matured ponds to study the long-term fate and effects of pesticides and other chemicals (Ref 82,83). For studying the long-term fate, the labelled material was dosed for a period of about 4 weeks at 50 ppb level to 4 m^2 surface and 7 m^3 volume ponds, and the concentrations in flora, fauna and abiotic compartments were measured over several years. The pesticides investigated so far are hexachlorobenzene, pentachlorinitrobenzene, pentachlorophenol and 4-chloroaniline as a pesticide metabolite.

The general conclusion of these investigations is the observation of seasonal fluctuations of total residues in biota with an overall sharp decline between the first and second season following the application, whereas in the sediment, after the build-up of residues, there is a rather steady decline. Compartments of 200 l volume in these ponds as well as 1 m^3 compartments in a nearby larger natural pond have been used to study effects on species' diversity and abundance and on physico-chemical limnological parameters.

Using toxic doses based on daphnia acute toxicity tests, the major finding was a decrease in zooplankton and microphytoplankton diversity with an increase of abundance of surviving species (Ref 84).

Crossland and co-workers have used outdoor ponds to study the fate and effects of chemicals. Twelve outdoor experimental ponds were constructed for studying fate and biological effects of chemicals under semi-natural conditions. Each pond is 5m wide, 10m long and 1m deep. The longer, dividing walls are vertical and made from concrete. The shorter sides of the ponds slope at an angle of 45° and consist, as do the bottom of the ponds, of a mixture of clay, alluvial silt and organic debris. When not in use the ponds are interconnected by pipes and the water is occasionally mixed by circulation between ponds, thus providing uniform distribution of organisms between ponds. Management of aquatic vegetation is carried out to minimise the variability of habitats for invertebrate communities.

The fate and biological effects of various reference chemicals have been evaluated. Replicated field experiments have been carried out with methyl parathion (Ref 85,86), pentachlorophenol (Ref 87) 3,4-dichloroaniline (Ref 88,89) and 2,5,4'-trichlorobiphenyl Process analysis and mathematical models were used to predict the fate of these chemicals in the ponds and the observed fate was compared with predictions. Biological effects in the ponds were compared with predictions based on single-species toxicity tests.

Conclusions

Laboratory model ecosystems using radiolabelled compounds are useful screening tools for assessing the environmental fate of chemicals. Results are generally reproducible and permit comparisons of pesticide behaviour under identical conditions to be made.

Laboratory model ecosystems have several limitations:

- the number of organisms and environmental parameters is limited,
- food chain organisms are not truly representative of the natural environment,
- lower trophic organisms are frequently consumed in too short a period,
- component organisms are not kept under their physiologically optimum conditions.

To overcome limitations of the laboratory models, larger open and outdoor model ecosystems have been developed. They enable:

- the biological components and biomass balance to be brought much closer to the natural environment,

- the fate of chemicals to be observed for longer periods, since the ecological system is stable by self-control.

However, open air systems also have several limitations. It is difficult to obtain the total balance of the applied chemicals and to obtain reproducible results. Above all, the costs are very high.

In certain critical situations, confidence in the results from model systems can be increased by specific field tests, although these situations need to be considered on a case-by-case basis.

When using model ecosystems a compromise must be reached between using a simple system with limited biotic and abiotic interactions, which is easy to analyse and is highly reproducible, and using a more complex, more expensive system, closer to the natural environment, from which it is more difficult to obtain reproducible analytical data.

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