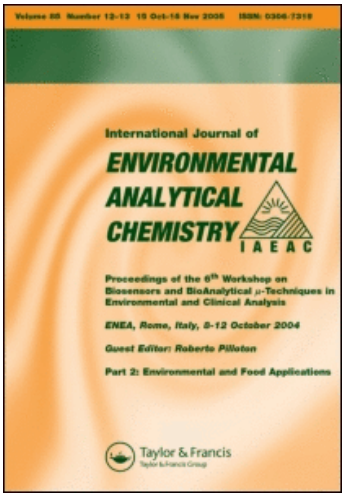

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A Test System for the Determining of the Fate of Pesticides in Surface Water: Protocol and Comparison of the Performance for Parathion of Ecocores and Micro Ecosystems from Two Sources

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A Test System for the Determining of the Fate of Pesticides in Surface Water†

Protocol and Comparison of the Performance for Parathion of Ecocores and Micro Ecosystems from Two Sources

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Protocols are described for constructing laboratory micro-ecosystems (MES), incubating them with radiolabelled pesticides and then using a routine test procedure to ascertain the fate of these pesticides in surface water. The performance of fresh ecocores and acclimated MES from two sources was compared. The influence of the duration of the acclimation to room temperature and a light cycle on the fate of parathion was studied. The variation between replicates of MES was less than that between ecocores. The eutrophic ecocores and MES performed similarly, the oligotrophic ecocores transformed parathion faster than the oligotrophic MES. In eutrophic systems, reduction to aminoparathion was much faster than in oligotrophic systems. The sandy oligotrophic MES needed a longer acclimation to laboratory conditions than the eutrophic MES to produce reproducible results. The results of year-to-year experiments were also more reproducible for the eutrophic MES.

KEY WORDS: Test procedure, fate, parathion, aquatic micro ecosystem.

†Presented at the Workshop on Chemistry and Fate of Organophosphorus Compounds, Amsterdam, Holland, June 18–20, 1986.

INTRODUCTION

Large areas of economically important Dutch agricultural and horticultural land are traversed by ditches and canals of sluggish water. This hydrological system is necessary to cope with the surplus of rainfall and because much of the country is below sealevel. Not surprisingly, therefore, when a pesticide is submitted for registration in the Netherlands, data on its fate in surface water are mandatory. "Fate" is defined as the process of transport, transformation and final disposal by natural means.

For an ecotoxicological evaluation of the environmental loading by pesticides, the conditions for test systems in the laboratory should resemble those of the complex natural systems as closely as possible. This means that at least the naturally present degraders (bacteria, fungi, algae, etc.) should be allowed to develop into a micro-ecosystem in an undisturbed microcosm of sediment and water under aeration of the surface layer and in a day/night light cycle.

J. M. Giddings[1] elaborated a protocol for using aquatic pond microcosms for determining the fate of micropollutants in ecosystems and for studying some of their effects. In his excellent study he concluded that after several weeks of equilibrium under constant laboratory conditions, 70 l microcosms of sediment, water and a complete biotic community can develop into useful test systems that are a realistic simulation of the parent ecosystem. Smaller (7 l) microcosms behaved similarly but were less stable and more easily disturbed.

Routine work requires many replicates of small systems that can be analysed easily and economically. An equilibrated laboratory micro-ecosystem cannot be subsampled without disturbing the complex ecosystem that depends on numerous niches in gradients of light, nutrients, temperature, pH, oxygen and redox potential.

The objective of our study was to develop a routine test system comprising an aquatic micro-ecosystem containing the essential functional components of the parent natural ecosystem, and to develop subsequent analytical procedures to provide data on the fate of pesticides in surface water.

Because of its short half life, the abundance of information on its degradation in aqueous systems,[2] and its easy availability, ^{14}C-parathion was used as a substance to test the model. Using the fate

of parathion and its transformation products as criterion, attention was paid to the following aspects:

1) Constructing replicate laboratory micro-ecosystems (MES);

2) Comparing fresh ecocores with acclimated MES;

3) Ascertaining the influence of the age of the MES on performance;

4) Developing adaptable and economical analytical procedures.

MATERIAL AND METHODS

Constructing the micro-ecosystem

Sediment and water were collected from a ditch in an apple orchard and a small man-made lake near Wageningen. About 25 l of the uppermost 5 cm of the sediment was scooped into a bucket, and the same day was passed through a sieve (2 mm mesh) in the laboratory, with the corresponding surface water. The sieved sediment was allowed to settle overnight in an open plastic box ($60 \times 30 \times 20$ cm). The water was carefully drained off and after mixing, sediment samples were taken so that the physical and chemical properties of the sediment could be determined (Table I).

At the start of our study the sieved sediment was intensely emulsified with an Ultra Turrax homogenizer (Janke & Kunkel, type T 45 S7) to improve the replicability of the weighing of the wet sediment and the sampling of the extracted sediment for combustion afterwards. The experiment on the acclimation of the MES before the incubation was performed with emulsified sediment.

Portions of wet sediment (from 5 to 10 g dry weight) taken with a glass corer were weighed into individually numbered and tared glass centrifuge tubes (70 ml) equipped with a screw thread. The tubes were filled with the drained water, taking care not to disperse the sediment layer, and were then randomly placed in a glass rack in a glass aquarium ($80 \times 40 \times 20$ cm) with a layer (3 cm thick) of the sieved sediment on the bottom and with the corresponding surface water standing 2–3 cm over the open tubes. The aquarium was covered with a glass lid (Figure 1).

TABLE I

Physical and chemical properties of the sediments studied.[a]

Site	pH-kCl	Organic matter (%)	$CaCO_3$ (%)	Clay $<2\,\mu m$ (%)	Silt $2–50\,\mu m$ (%)	Sand $>50\,\mu m$ (%)	Total P (%)	Total N (%)	Dry[b] weight (105°C) (%)	Fresh[b] weight of sample (g)
Ditch	7.2	6.4	3.9	23	40	27	0.56	0.37	34.2	19.5
Lake	7.6	0.8	3.9	6	10	79	0.08	0.05	73.3	13.5

[a]Determined by the Laboratory for Soil and Crop Testing, Oosterbeek, The Netherlands.
[b]Determined by the Institute for Pesticide Research, Wageningen, The Netherlands.

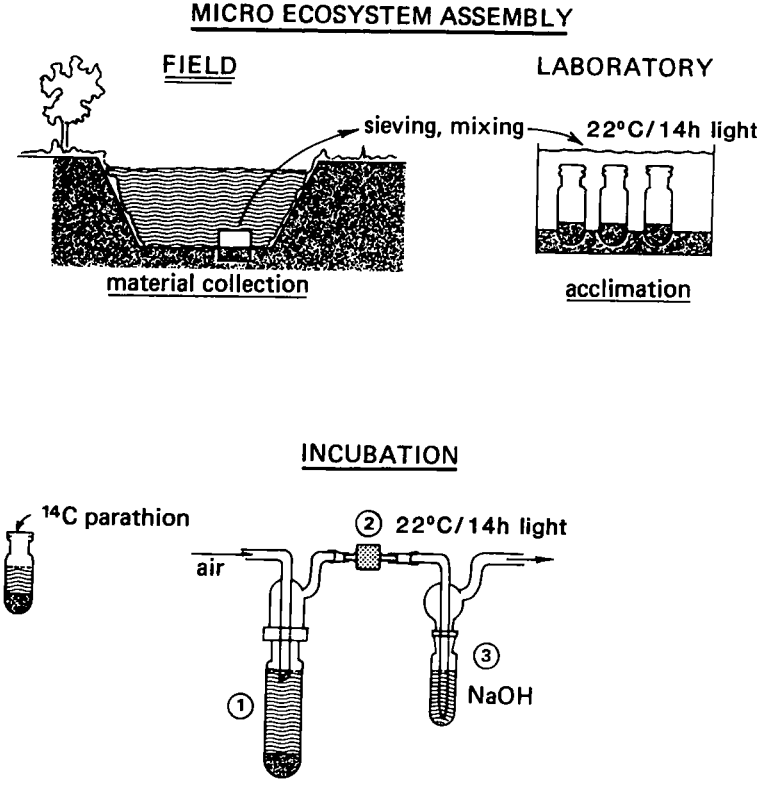

FIGURE 1 Set-up for acclimation and incubation of the micro-ecosystem.

Under identical conditions at room temperature and 14 hrs cool white fluorescent light per day, 108 tubes with a known amount of sediment (variation 0.5 g dry weight) were allowed to develop into replicate MES as part of a larger ecosystem of 3 cm of sediment overlain by 16 cm of water with a total volume of 60 l. Periodically, excess water plants, algae or snails were removed and the evaporated water was topped up.

Ecocores

In the field open glass tubes (20 cm long, 3 cm inner diameter) were pressed 6 cm deep into the sediment that was overlain by shallow surface water (approx. 30 cm deep), next to each other. As the tubes were being pushed into the sediment, the sediment cores in the tubes usually sank about 2 cm and sometimes bubbles of gas escaped. The upper end of each tube was closed with a rubber stopper and the tube was carefully pulled up. While still under water, the lower end of the tube was also plugged with a rubber stopper.

In the laboratory the water was drained off to a level 3 cm above the surface of the sediment. The incubation and further handling were the same as described for the MES. The ecocores were incubated on the day of collection and five replicates were used for each estimation.

Incubation

At appropriate times the MES tubes were taken from the aquariums and their outer surface was cleaned. All handling was done very carefully to minimize the disturbance of the MES. Water was drawn off until the sediment and the water weighed just under 40 g. The radiolabelled test substance was applied in a dose of 10 ml of distilled water so that the final concentration in the MES matched the concentration expected in field situations (in our study, from 1 to 2 mg parathion per litre). The total weight of the MES was made up to 50 g with water.

The dosing solution was made by putting calculated amounts of hexane solutions of unlabelled parathion (0,0-diethyl 0-4-nitrophenyl phosphorothioate, water solubility 24 mg l^{-1}) and [ring-2,6-^{14}C] parathion (Amersham, spec. act. 2.67 M Bq mg^{-1}) in a glass bottle.

After the hexane had evaporated, an appropriate volume of distilled water was added and the glass stoppered bottle was shaken for at least 2 hours or sonicated (Bransonic, Model 52, 50,000 Hz) for 15 minutes. If the radioactivity measurement indicated that the parathion was not yet fully dissolved (in our study ca 10^8 dpm and between 5 and 10 mg l^{-1}) the treatment was continued. Finally, the concentration was determined by HPLC with UV and radiometric detection.

During the incubation each MES with its traps for volatiles and CO_2 was mounted on an aluminium stand. The air perfusion assembly was screwed air-tight on the centrifuge tube with a silicon-rubber ring. Air was bubbled through the 5 mm top layer of the MES water at a rate of 1 ml min^{-1} and subsequently through the volatile trap (SEP PAK®, Waters) and the CO_2 trap (5 ml of 0.5 N NaOH) (Figure 1). At least two replicates were performed for each estimation during incubation at room temperature and 14 hrs light per day.

Analysis

At 2, 7 and 14 days of incubation, replicate systems were analysed. The ^{14}C activity from aliquots of all fractions was measured by liquid scintillation counting in Optifluor (United Technologies Packard) with a Liquid Scintillation Analyser (Phillips Model PW 4540). As no radioactivity from ^{14}C-parathion was detected in the methanol eluate from the SEP PAK® cartridges and the eluted C_{18} adsorbent, these volatile traps were omitted in later experiments with parathion.

The amount of $^{14}CO_2$ that had formed was measured in 0.5 ml of the NaOH trap. The MES tube was centrifuged (10 min, 2,200 rpm, Heraeus Christ Varifuge GL), the weight recorded and the radioactivity in 0.5 ml of the water assessed (Figure 2). The water layer was extracted by suction through a 3 ml Baker SPE C_{18} disposable column.

A special vacuum box was constructed for handling up to 12 extraction columns simultaneously during the prewash, extraction and eluation procedures. This device and the exact procedures will be described elsewhere.

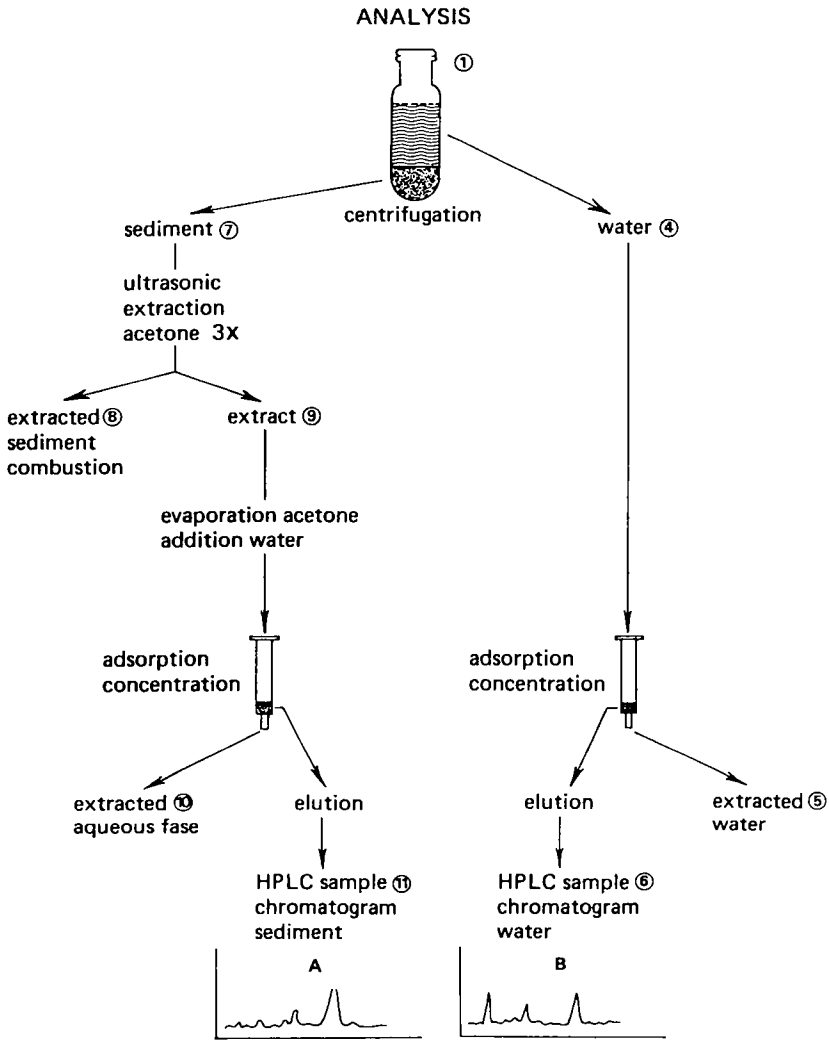

FIGURE 2 Outline of the analytical procedure.

The volume of the eluate was recorded and a 0.5 ml aliquot taken for radioassay. The adsorbent of the column was extracted with 3 ml of methanol directly in the HPLC sample vial. After adding of 1 ml of distilled water and mixing, the radioactivity of a 100 μl aliquot was measured. The weight of the wet sediment was recorded, 25 ml of acetone added, the tube closed with a screw cap and vigorously shaken by hand for a few seconds to disperse the sediment. The sediments were extracted by sonication during 10 min. The extract was collected in a graduated cylinder. This extraction procedure was repeated twice with 25 ml of 10% water in acetone. The volume of the combined extracts was recorded and the radioactivity in an aliquot was measured. The acetone was evaporated in a vacuum rotator until the first drops of water appeared in the condensor. The solids were washed from the wall of the flask with 1 to 2 ml of acetone and 40 ml of distilled water was added. The yellow to dark brown and turbid aqueous phase was extracted by the same extraction column procedure as described for the MES water, and the radioactivity in the eluate and in the HPLC sample was measured.

Only a few per cent of the radioactivity present in the aqueous eluates from the extraction columns proved to be extractable by subsequent extraction with chloroform or ethylacetate. So this rapid extraction, concentration and partial clean-up procedure extracted all non-polar components.

The extracted sediment was dried overnight at 105°C, homogenized in a mechanical mortar (Fritsch, Pulverisette 2) and the $^{14}CO_2$ radioactivity determined after two aliquots (approx. 200 mg each) had been combusted in a Packard Model 306 Sample Oxidizer.

The values found for the radioactivity of the volatile trap, the $^{14}CO_2$ trap, the MES water, the extracted sediment and the sediment extract were used to calculate the total ^{14}C recovery.

The HPLC apparatus was equipped with a Waters Model E 590 solvent delivery system, a Model 710 A WISP sample processor, a column oven, a Schoeffel model 770 spectrophotometric detector (used at 283 nm), a Berthold LB 5026 radioactivity monitor with a HS solid scintillator detection cell and with an Apple IIe computer with an Isomess IM 2013 Tripletrace Radio Chromatographic Program. Automatically 250 μl samples were injected into a 50 × 4.6 mm guard column (Copell ODS, Whatman) and a

150 × 4.6 mm analytical column (Lichrosorb 5 RP_{18}, Chrompack) at 50°C in phosphate buffer/methanol/acetonitrile (30 + 65 + 5) at a flow rate of 1 ml min^{-1}. The phosphate buffer (pH 9) was 0.01 M in the mobile phase, methanol and acetonitrile were "Baker Analyzed" HPLC reagents. Typical retention times under these conditions were parathion 7.8 min, aminoparathion 4.7 min, *p*-aminophenol 3.4 min and *p*-nitrophenol 3.0 min.

RESULTS

Data from a single experiment with fresh ecocores, incubated from the day of their collection, were compared with those from two experiments with MES constituted from material collected from the same sites and on the same days, but acclimated to laboratory conditions for 35 or 41 days and 63 or 69 days (Tables II and III). Small invertebrates, e.g. Cyclops spp. Tubifex spp. and leeches were active in the ecocores during the incubation periods studied. The total ^{14}C recoveries of these experiments were satisfactory and only a slight mineralization was found in all systems. The replicability of the MES was much better than that of the ecocores; this is clearly shown by the larger variation in the measurements (Figure 3) and the standard deviations (Tables II and III).

During the incubation the radioactivity in the water fell and that in the sediment rose. This latter increase was mainly due to the increase in the non-extractable fraction.

The non extractable radioactivity in the lake ecocores was much greater than that in the lake MES. The opposite tended to be found for the ditch systems (Tables III and IV). An almost constant fraction of the radioactivity was extractable from the ditch systems during the incubation. In the older lake MES, however, this fraction was higher than in the younger MES and ecocores.

The persistence of parathion and transformation products in the extracts from water and sediments is given in Tables IV and V and Figure 4.

The polar products had retention times of between 2 and 3.5 minutes in the HPLC chromatograms, i.e. less than aminoparathion. The intermediate products were situated between the amino-parathion and parathion peaks. No identification was undertaken

TABLE II

Recovery of radioactivity in percentage of dose of ^{14}C-parathion, during incubation of ditch systems.[a]

System acclimation time	Incubation time (days)	$^{14}CO_2$	Water	Sediment		Ecotube rinsing	Total
				Extractable	Non-extractable		
Ecocore 0 days	2	nc[b]	65.4± 3.7	23.5±2.5	11.7± 1.7	0.7±0.2	101.3±2.6
	7	nc	33.3±11.7	23.7±3.1	37.6± 5.5	0.5±0.1	95.2±4.5
	14	1.6±1.6	28.2± 7.8	19.1±3.8	40.8±10.5	0.5±0.2	90.2±1.2
MES 41 days	2	<0.05	74.0± 1.1	18.9±0.9	8.3± 2.2		101.2±0.4
	7	0.6±0.1	45.6± 4.5	25.0±1.0	24.6± 3.9		95.7±0.4
	14	1.0±0.6	22.1± 2.7	24.4±1.5	45.9± 2.8		93.4±0.8
MES 69 days	2	<0.05	67.8± 1.1	20.8±1.8	9.9± 0.9		98.5±1.8
	7	0.4±0.1	36.0± 2.0	26.7±2.1	35.4± 4.7		98.6±0.9
	14	0.6±0.3	18.0± 0.4	22.9±0.8	53.9± 1.5		95.3±0.4

[a] $\bar{x} \pm s$, ecocores $n=5$, MES $n=3$.
[b] Not collected.

TABLE III

Recovery of radioactivity in percentage of dose of ^{14}C parathion, during incubation of lake systems.[a]

System acclimation time	Incubation time (days)	$^{14}CO_2$	Water	Sediment		Ecotube rinsing	Total
				Extractable	Non-extractable		
Ecocore 0 days	2	nc[b]	76.9±7.0	12.4±3.9	8.9±2.8	0.5±0.0	98.7±0.7
	7	nc	44.9±2.2	14.7±0.9	31.6±2.0	0.7±0.1	91.9±0.7
	14	1.0±1.0	23.5±6.5	14.2±2.7	49.1±4.3	0.5±0.2	88.2±3.6
MES 35 days	2	<0.05	76.7±2.5	16.3±0.3	3.4±0.4		86.0±2.0
	7	0.4±0.3	57.8±1.1	19.7±0.6	15.2±0.2		93.1±1.7
	14	3.3±0.5	42.1±0.8	15.6±0.4	31.9±1.1		93.0±1.4
MES 63 days	2	<0.05	70.9±2.3	22.2±1.4	3.0±0.2		96.1±0.8
	7	0.5±0.2	55.0±0.7	25.7±0.7	13.7±0.6		94.9±1.3
	14	3.5±0.1	42.6±3.6	21.4±1.9	26.4±0.9		93.9±0.9

[a] $x \pm s$, ecocores $n = 5$, MES $n = 3$.
[b] Not collected.

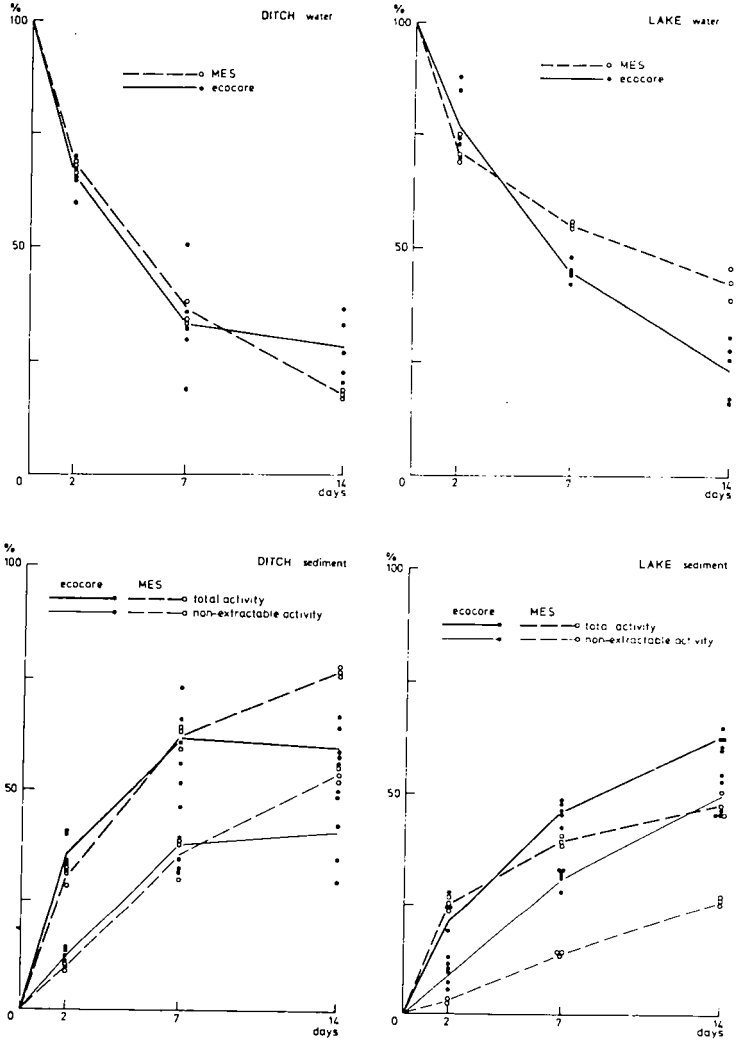

FIGURE 3 Performance of ecocores and MES from ditch and lake. Remaining total radioactivity in water and sediments and non-extractable radioactivity in sediments after 2, 7 and 14 days of incubation.

TABLE IV

Parathion and transformation products in ditch systems in percentage of dose.[a]

System acclimation time	Incubation time (days)	Water extract			Sediment extract		
		Polar products	Amino-parathion	Parathion	Amino-parathion	Intermediate products	Parathion
Ecocore 0 days	2	3.7±0.8	13.2±3.3	29.0± 6.5	7.3±1.4	2.3±0.7	9.2±2.9
	7	3.4±0.8	18.0±5.1	5.7± 2.7	8.9±1.8	2.8±1.5	3.5±0.9
	14	4.3±0.6	17.3±6.0	—[b]	11.5±2.4	-	1.4±0.5
MES 41 days	2	2.5±0.6	31.7±8.3	30.9±11.4	5.8±0.8		3.1±0.8
	7	6.4±0.7	29.7±2.6	2.2± 0.4	8.7±0.6	—	1.8[c]
	14	4.9±0.6	12.8±1.8	—	12.5±0.9	—	—
MES 69 days	2	4.6±0.3	30.9±2.9	26.6± 4.3	6.9±1.2	—	5.6±0.4
	7	4.3±0.1	29.7±1.3	—	14.4±0.7	2.3±0.9	0.9±0.5
	14	4.9±0.2	10.6±0.7	—	11.9±0.7	—	-

[a] $\bar{x} \pm s$, ecocores $n = 5$, MES $n = 3$.
[b] Not detected.
[c] Single measurement.

TABLE V

Parathion and transformation products in lake systems in percentage of dose.[a]

System acclimation time	Incubation time (days)	Water extract			Sediment extract		
		Polar products	Amino-parathion	Parathion	Amino-parathion	Intermediate products	Parathion
Ecocore 0 days	2	8.8±0.8	7.5±2.0	55.9±8.3	2.5±0.9	[b]	6.0±2.6
	7	7.6±1.6	20.8±1.5	11.6±1.6	5.9±1.3		3.4±1.0
	14	5.9±1.5	13.8±6.0	—	8.2±1.7		
MES 35 days	2	—		67.5±1.8	—	—	15.5±0.3
	7	2.4±1.1	9.0±0.5	42.3±2.7	5.2±0.4	—	12.7±0.5
	14	2.4±0.6	13.2±1.8	21.6±1.7	3.6±0.9	—	9.2±0.3
MES 63 days	2	2.3±0.1	2.5[c]	65.4±4.0	—	—	20.7±1.6
	7	2.9±0.7	8.0±1.8	41.0±4.2	2.7±0.3	—	15.7±3.6
	14	3.0±0.7	11.1±0.2	23.8±2.9	3.8±0.7	—	12.5±1.5

[a] $\bar{x}+s$, ecocores $n=5$, MES $n=3$.
[b] Not detected.
[c] Single measurement.

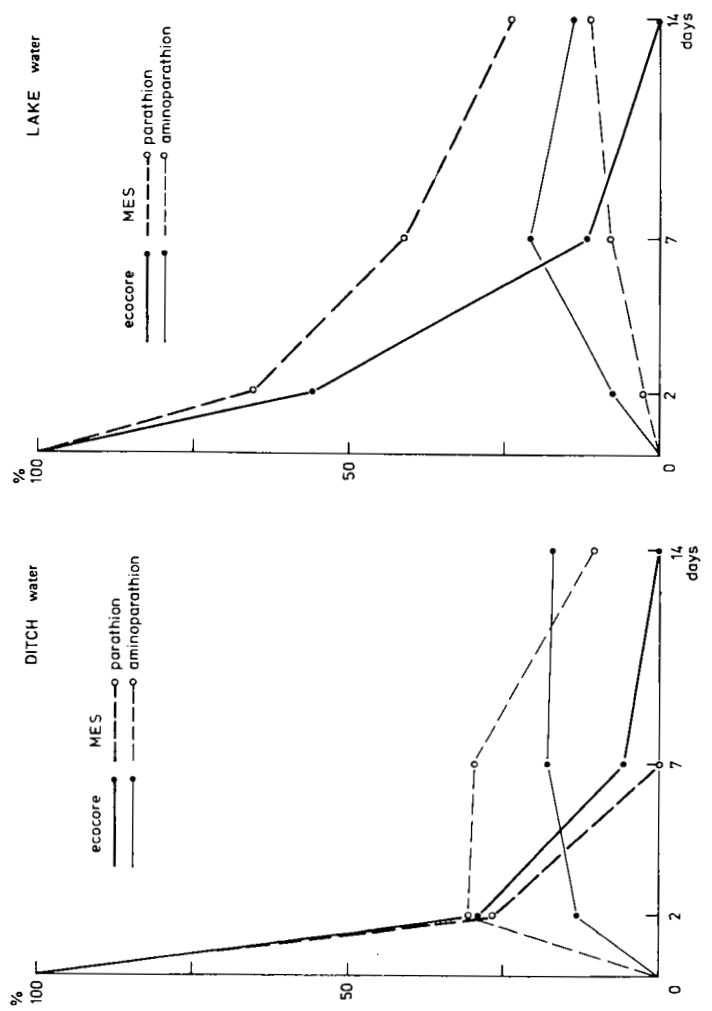

FIGURE 4 Performance of ecocores and MES from ditch and lake. Persistence of parathion and aminoparathion in water after 2, 7 and 14 days of incubation.

and even the aminoparathion peak was not homogeneous and contained other radiolabelled products.

Between 3.7% and 4.7% of the radioactivity remained in the extracted water from the ditch ecocores and between 1.4% and 2.9% in that from the ditch MES. The corresponding fractions of the lake systems contained about 1% more radioactivity. Thus, only a minor fraction of the transformation products consisted of very polar components.

It should be mentioned here that for unknown reasons about 20% of the radioactivity was lost during the extraction of the first ecocore water sample processed, notably the ditch ecocores after 2 days of incubation.

Parathion disappeared rapidly from the water in the ditch systems concomitantly with its transformation into aminoparathion. This latter component decreased gradually in the water of the MES.

Almost twice the amount of parathion was present in the ditch ecocore sediment than in the MES sediment (Table IV). This may have been caused by the turbulent activity of Tubifex worms that were present only in the loose organic upper layer of the ditch ecocore sediments. The percentage of aminoparathion increased gradually in the sediments of the younger ditch MES and ecocores, but reached a maximum of 14.4% at 7 days in the older MES.

A totally different pattern was found in the lake systems (Table V, Figure 4). After 2 days, about 60% of the radioactivity in the water was still present as parathion, and more than 20% remained in the MES water after 14 days. The aminoparathion fraction had peaked in the lake ecocore water, but was in an early stage of build up in the lake MES water. The polar products formed a remarkably high fraction in the lake ecocore water. In the lake MES sediment the percentage of the parathion was the highest of all systems, whereas that of aminoparathion was the lowest.

Earlier experiments had indicated that the MES performance changed gradually during the acclimation period. In our study trends could be seen by comparing ditch MES acclimated for 41 and 69 days (Tables II and IV), the clearest being the percentage of ^{14}C in water, the non-extractable fraction of sediment and the amount of aminoparathion in the sediment. In the present study we therefore investigated the influence of ageing of the MES on its performance with the fate of parathion as criterion. Sieved and emulsified

sediments (10 ± 0.3 g dry weight) and surface water (40 g) collected from both sites were acclimated to laboratory conditions. Incubations were performed at 1, 3, 5, 8, 16, 26 and 71 weeks. In Figure 5 the percentages of total ^{14}C, parathion and aminoparathion show trends until about 26 weeks of acclimation in the case of the lake MES and 16 weeks in the case of the ditch MES water.

The aminoparathion curves during the ageing of the lake and ditch MES are compared in Figure 6. The lake MES mainly showed a build up of the aminoparathion content, with only a small decline between 7 and 14 days. The ditch MES showed an increasing amount of aminoparathion at 2 days of incubation and an increasing rate of decline from 2 days onwards.

DISCUSSION

The rapid reduction of parathion to aminoparathion in the water and the sediment and the rapid increase of the non-extractable fraction in the sediment agree with the well documented transformation of parathion by algae[3] and anaerobic micro-organisms[2,4,5] and the strong adsorption of aminoparathion in soils.[6] Acclimation and incubation in a light regime are necessary to provide the natural elements of ecosystems (the production of oxygen and organic nutrients in the water columns, the degrading capacity of algae and their associated biomass, and the diurnal changes in pH). In our ditch aquariums the largest fluctuation in pH (from 7 to 9) was measured after several weeks of acclimation. Within 4 to 7 days an E_h below -50 mV was measured in the sediments. To support the autotrophic organisms, perfusion with compressed air containing a normal CO_2 content was preferred above the use of CO_2-free air during incubation.

In our search for an economical test procedure we attempted to miniaturize MES by acclimating replicates of 70 ml microcosms in a 70 l MES. The use of centrifuge tubes for the determination of the persistence of pesticides in an estuarine environment has recently been described.[7] However, that research was not aimed at an ecosystem test, and unacclimated sediment and seawater were agitated continuously at 100 rpm on a rotator table. The individual acclimation of MES-tubes to laboratory conditions has also been

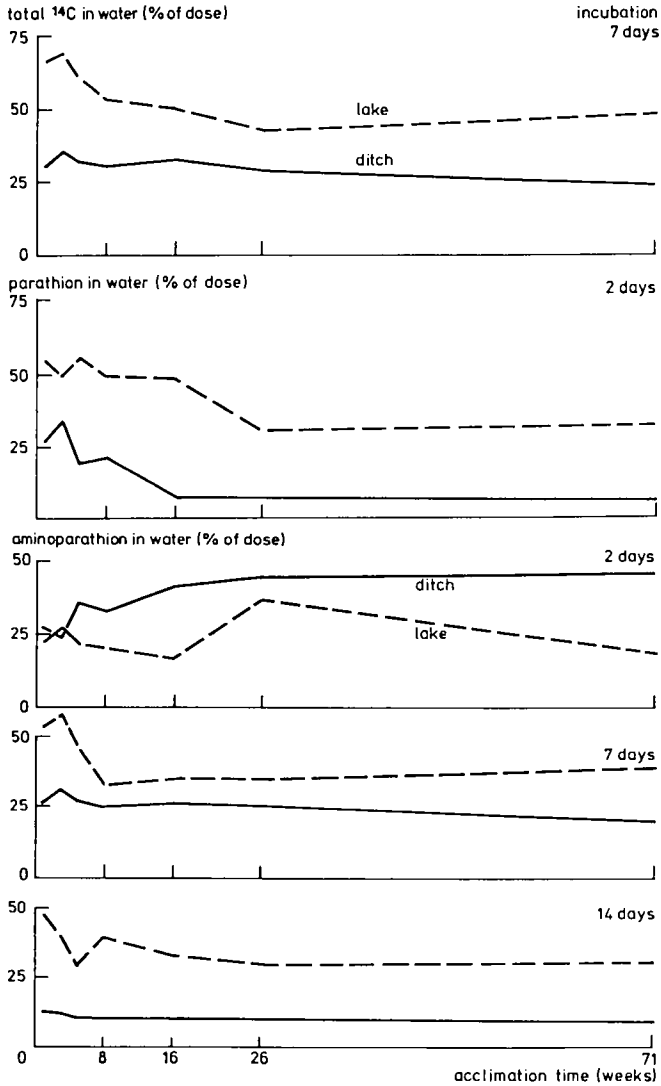

FIGURE 5 Effect of acclimation on performance of homogenized lake and ditch MES. Incubation after 1, 3 5, 8, 16, 26 and 71 weeks of acclimation to laboratory conditions. Contents in water of total ^{14}C after 7 days, parathion after 2 days and aminoparathion after 2, 7 and 14 days of incubation.

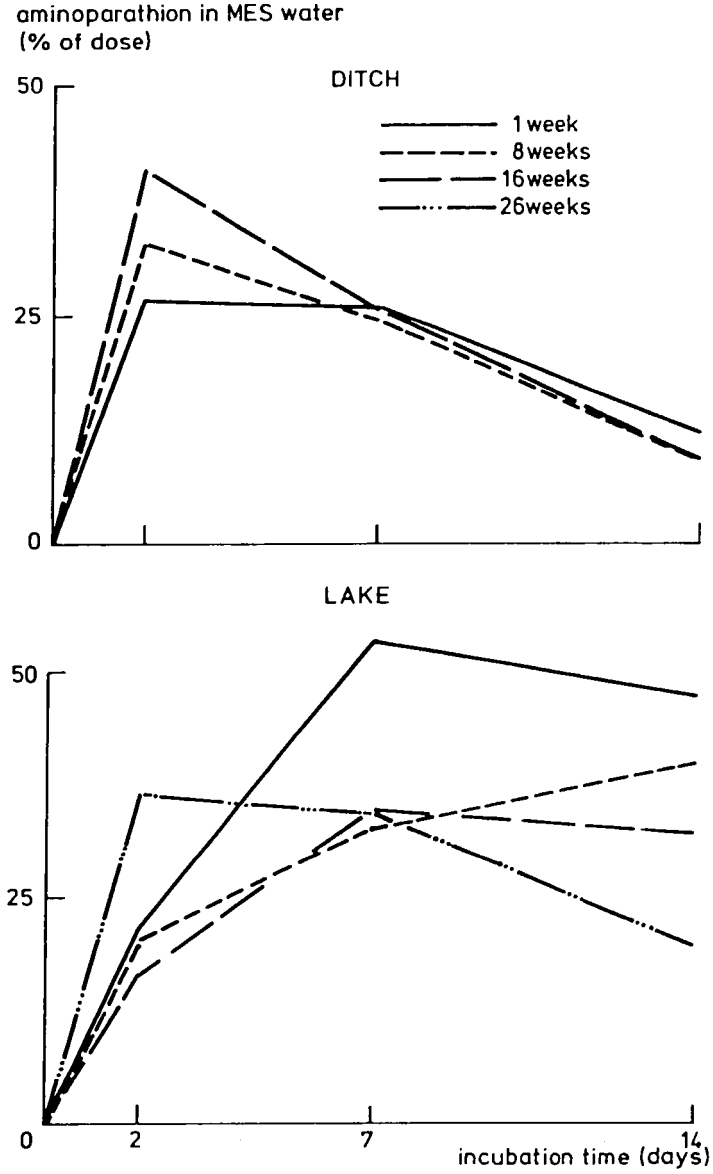

FIGURE 6 Persistence patterns of aminoparathion in water of lake and ditch MES after acclimation to laboratory conditions for 1, 8, 16 and 26 weeks.

described.[8] However, in that study, glass ecocore tubes, closed at both ends with rubber stoppers and thus not part of a larger ecosystem, were used. No advantage for the subsequent chemical analysis was obtained and the sediment had to be transferred for extraction.

In our experiments the replicability of all acclimated MES of the same batch was very good and better than that of the fresh ecocores. In our set-up a longer acclimation to laboratory conditions (16 to 26 weeks) is indicated than in one non-compartmentalized 70 l aquarium, where at least 6 weeks of acclimation was necessary.[2] Our data conform with the longer equilibrium time found for aquariums of 7 l.[2] Unlike the lake MES, our ditch MES performed similarly or even better than the fresh ecocores. The ecocores were collected early September 1985 when outdoor temperature was approximately 18°C and there was 13 hours daylight.

The reproducibility of the system's performance was examined by comparing the aminoparathion content in the water of homogenized MES prepared in October 1984 (outdoor temperature approx. 14°C, 11 hrs daylight) and acclimated for 8 weeks, with that in the water of 63 and 69 days acclimated 1985 MES. An excellent reproducibility was obtained for the ditch MES: the aminoparathion contents for 2, 7 and 14 days of incubation were 33, 25 and 9% in 1984 and 33, 27 and 12% in 1985. The corresponding data for the lake systems were 20, 33 and 40% in 1985 versus 1, 19 and 18% in 1985.

Our data agree with the conclusion reached by Giddings[2] that a microcosm with sand as sediment does not develope into an MES as readily as one with a richer pond sediment, and that sandy MES are less stable. It is therefore doubtful if sandy sediments from oligotrophic waters can be used for this test. Even if these poor sandy MES simulate the performance in an oligotrophic surface water, the erratic data are less useful for evaluating risk.

In the hands of untrained students our test system has proved to be reliable and easy. Some modifications have been introduced during the last three years. Instead of intensely homogenizing the sediment prior to composing of the MES and to improve the recovery from the combustion of the sediment for radioanalysis, the extracted sediment is now homogenized with a mechanical mortar, without loss of recovery or replicability.

The use of centrifuge tubes with ground glass joints was found to

be impractical because of the leakage during incubation caused by the ground part being fouled by "aufwuchs".

If compounds that are less soluble in water have to be tested, the solubility might be increased several fold by using MES water. The use of organic solvents for dosing, or of organic nutrients during incubation, is not recommended because of the unpredictable effect on the degradation of xenobiotics.[9–12] The use of relevant formulation ingredients that simulate the application of pesticides in field situation seems more realistic. The influence of the formulation on the behaviour and effects of pesticides in aquatic systems may be very large.[13]

Tenax®, XAD-resins, activated charcoal or other modified silicas can be substituted for the SEP PAK® C_{18} cartridge or the Baker SPE disposable columns. If these adsorbents are packed in small interchangeable columns suitable for HPLC, most of the analytical procedure can easily be automated.

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