Residue Analyses and Biotests in Rice Fields of East Java Treated with Thiodan®

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In connection with a study concerning the environmental behaviour of endosulfan, which was carried out in East Java during March 1970 in the course of the BIMAS - project*, tests concerning the decomposition of endosulfan, the active ingredient of THIODAN, in water and soil of treated rice fields were performed as well. Under these natural conditions, biotests with native fish could be carried out besides a chemical analysis in order to determine the biological effectiveness of residues in submerged paddy fields. These tests were performed in the vicinity of Pandaan (East Java) during the end of the rainy season in March 1970.

Material and Methods

Two rice fields were rented (height of the rice plants at the beginning of the test was 40-60 cm); one of them was divided into two parts by erecting a dam (A = 64 m^2; B = 173 m^2). These two partial fields remained submerged during the test period and were protected by means of plastic sheets which were fixed alongside the interior wall of the dam against potential heavy losses of water resulting from oozing away. The lower edge of the tape was dug into the mud up to a depth of about 20-30 cm. Part A had no outlet. Losses of water causes by evaporation and porosity of the dam which could not completely be avoided by the plastic sheets were compensated by inflowing water from the dammed up part of the brook, the level of which was kept constant (regulation by a broad overflow). Part B had an in- and outlet. The quantity of water flowing through this field was about 1.5 l/sec (gauged at outlet E). This quantity was kept fairly constant by inflow from basin D, the level of which was controlled. The second field (C = 60 m^2) was used to examine the decomposition of endosulfan in mud which was not covered by water. For protection against rain showers it was partly covered by a plastic roof. (see figure 1).

Puntius javanicus, a tropical freshwater fish and sensitive to endosulfan, was used as a test fish ($LC_{100}24^h$ = 0.001 ppm). This type of fish lives in the rivers and brooks of Java as well as in flooded rice fields and is highly sensitive to oxygen. The eggs normally develop on the sandy or stony ground of small streams or creeks. To hold the fish

*see last page

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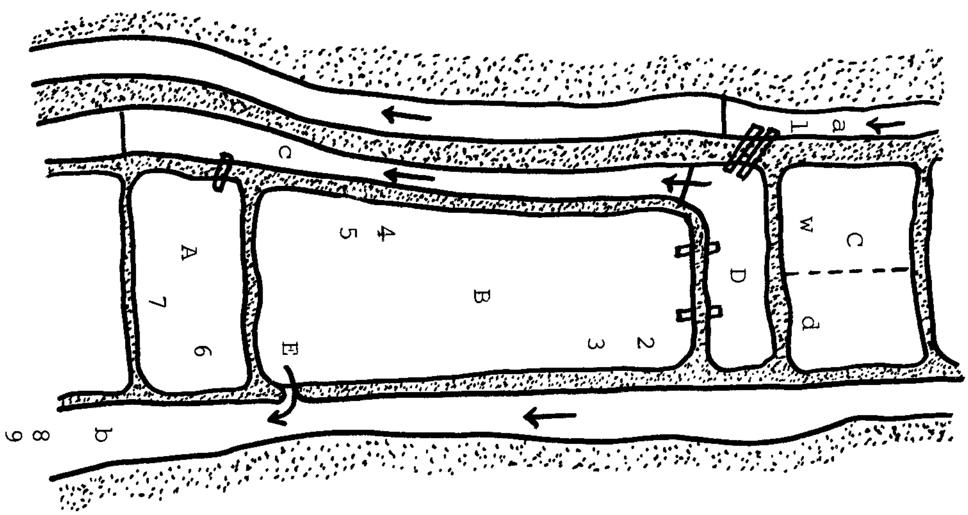

Figure 1. Test rice fields near Pandaan (East Java). 1-9: Position of the weir baskets with caged fish; for other locations see text.

during the test, fully collapsible plastic weir baskets (mesh-width 1, 8mm) with a capacity of about 9-10 liters were used. At the beginning and during the test, each basket was supplied with 5 fish (10-15 cm) brought from a nearby fish breeding station. Then the time required to kill 100% of the fish was determined. For treatment of the fields, a knapsack sprayer (5 atm) was used, applying 1.4 liters THIODAN 35 EC in 750 liters water per hectar. The water temperature of field A varied from 28-38°C; in field B from 28-33°C; pH value being 6.5. The regular precipitation per day during the afternoon was 15-30 mm. The sunshine period was $6\text{-}8^{h}$ per day(estimated).

Before and during the test, water samples were taken from the rice fields as well as from the part of the brook outlet E, where the baskets 8 and 9 were situated (figure 1). Samples were taken by means of 1 liter glass bottles and then endosulfan was determined after extraction with n-Hexane by means of gas-chromatography. Samples of mud (in a depth of 0-10 cm) were taken from various places in all fields and analyzed according to the following method: 150 g of mud were mixed with about 100 g sodium sulfate and acetonitril and then extracted three times using 50 ml of a mixture of benzene and ethyl alcohol (2:1). The mixed and filtered organic phases were evaporated to a volume of about 50 ml, mixed with 1 liter water, shaken and the water phase was then extracted twice by means of 50 ml benzene. After evaporation (not to dryness) in a glass bowl by a fan and addition of sodium sulfate, the concentrate was transferred into a 10 ml calibrated flask and filled up to the mark with benzene.

TABLE 1

Residue of endosulfan plus metabolite in the water of the test rice field.

No.	Date	α	β	Sulf.	Total	Origin
		(Residues in ppb)				
1	3/8	0.05	0.31	0.46	0.82	a, box 1
2	3/9	0.02	0.08	0.13	0.23	basin D
3	3/9	0.01	0.05	0.11	0.17	field B
4	3/9	0.01	0.06	0.08	0.15	a, box 1
5	3/9	0.04	0.11	0.27	0.42	field B
6	3/10	0.03	0.09	0.30	0.42	a, box 1
7	3/11	110	70	-	180	field A, after treatment
8	3/11	250	300	-	550	field A,a.t.
9	3/11	0.04	0.09	0.11	0.24	E, before t.
10	3/11	14	16	-	30	field B, in time of t.
11	3/11	28	40	-	68	field B, a.t.
12	3/11	4	2.8	-	6.8	E, in time of t.
13	3/11	4.7	1.7	-	6.4	E, in time of t.
14	3/11	12	2.4	-	14.4	E, end of t.
15	3/11	1.6	0.95	0.3	2.9	box 9, in time of t.
16	3/11	0.1	0.14	0.16	0.4	box 9, in time of t.
17	3/11	3.2	2.0	0.2	5.4	box 9, end of treatment
18	3/11	1.4	1.2	0.1	2.7	box 8, in time of t.
19	3/11	0.09	0.09	0.25	0.43	box 8, start of t.
20	3/11	1.9	1.4	-	3.3	field A, 6^h a.t.
21	3/11	0.4	0.4	-	0.8	field B, 6^h a.t.
22	3/12	0.08	0.09	0.3	0.47	box 9
23	3/12	4.7	3.5	-	8.2	field A
24	3/12	0.35	0.45	0.1	0.9	field B
25	3/14	0.05	0.05	0.19	0.29	box 9
26	3/14	0.66	0.68	0.13	1.5	field A
27	3/14	0.14	0.05	0.18	0.37	field B
28	3/16	0.05	0.03	0.26	0.34	box 9
29	3/16	0.37	0.24	0.26	0.87	field A
30	3/16	0.08	0.15	0.20	0.43	field B
31	3/19	0.03	0.14	0.27	0.44	box 9
32	3/19	0.20	0.19	0.18	0.57	field A
33	3/19	0.02	0.05	0.16	0.23	field B
34	3/24	0.03	0.05	0.14	0.22	box 9
35	3/26	0.1	0.12	0.2	0.42	field A
36	3/26	0.05	0.1	0.25	0.4	field B
37	3/26	0.04	0.04	0.14	0.22	box 8

This organic phase was analyzed directly with appropriate dilution by gas-chromatography (Varian-aerograph 1400, 1 m glass column, inner diameter 1.8 mm; 3% OV I on chromosorb W 80-100 mesh; 1 atm N_2; 195°C column temperature).

Results

The fish placed into the weir baskets two days before application showed no signs of mortality until that time. The biocoenosis, which could be determined in both submerged rice fields, showed species of Oligochaeta (Tubificidae), larvae of Coleoptera, Tipulidae and Hydrocoridae, Cylopidae, Decapoda (Brachyura) and Gastropoda (Ctenobranchia). A slight growth of blue-green algae could be observed too. The water analyses showed a relatively constant level of endosulfan ($\alpha + \beta$) and endosulfan-sulfate in an amount of 0.0005-0.0008 ppm (see table 1). This may be explained by the fact that the test fields were located in the BIMAS-project area. During the period (March) considerable quantities of THIODAN were applied in this area.

After treatment, the initial residue level of field A was 0.068 ppm and field B 0.2-0.5 ppm (sum of endosulfan α and β) (see table 1). The degradation in both fields was different. At the third day after treatment, the survival time of *Puntius javanicus* reached 24 hours in field A and the residue analyses showed the concentration of the $LC_{100}24^h$. (see table 2,3). Within 5 days, this concentration dropped down to the original value(0.0005-0.0008ppm).

TABLE 2

Mortality of the caged fish in field A

Day	No.*	100% mortality after minutes		New Fish
-2	1,2,3, 4,5,6		no m.	+
0	7,8	box 6	60'	-
		box 7	43'	
1	23	box 6	3 h 6'	+
		box 7	3 h	
3	26	box 6	22 h	+
		box 7	23 h	
5	29	box 6	no m.	-
		box 7	no m.	
8	32	box 6	no m.	-
		box 7	no m.	
15	35	box 6	no m.	-
		box 7	no m.	

(*samples see table 1)

TABLE 3

Mortality of the caged fish in field B

Day	No.*	100% Mortality After Minutes		New Fish
-2	1,2,3, 4,5,6		no m.	+
0	11	box 2	97'	-
		box 3	102'	
		box 4	72'	
		box 5	82'	
1	24	box 2-5	no m.	+
3	27	box 2-5	no m.	-
5	30	box 2,3,5	no m.	-
		box 4	one fish dead	
8	33	box 2-5	no m.	-
15	36	box 2-5	no m.	-

(* samples - see table 1)

In field B, only a mortality of the fish at the day of application was observed. New fish, caged the first day after treatment showed no significant mortality up to the end of the experiment. Around the second day the residue level was down at the normal range of 0.0008-0.0005 ppm.

TABLE 4

Residues of endosulfan plus metabolite in the mud of the test rice fields

No.	Date	α (Residues in ppm)	β	Sulfate	Total	Origin
1	3/11	0.03	0.02	<0.003	0.053	field A
2	3/12	0.004	0.003	<0.002	0.009	field A
3	3/16	0.008	0.007	0.002	0.017	field A
4	3/11	1.0	0.55	-	1.55	field C,w
5	3/12	0.17	0.14	0.04	0.35	field C,w
6	3/16	0.6	0.68	0.63	1.61	field C,w
7	3/19	0.26	0.38	0.3	0.94	field C,w
8	3/26	0.09	0.15	0.07	0.31	field C,w
9	3/11	0.27	0.14	-	0.41	field C,d
10	3/12	0.36	0.28	0.04	0.68	field C,d
11	3/16	0.1	0.15	0.16	0.41	field C,d
12	3/19	0.06	0.12	0.15	0.33	field C,d
13	3/26	0.03	0.08	0.12	0.23	field C,d
14	3/16	0.002	0.002	0.004	0.008	field B
15	3/10	<0.001	<0.002	<0.004	<0.007	field C, before treatment

The mud analysis in field A and B showed the very low residue values of initially 0.05 ppm total directly after treatment. (see table 4). The residues declined to about 0.01-0.02 ppm (endosulfan plus metabolite) in the mud of field A on the fifth day after treatment. At the same time, the ratio endosulfan (α + β): endosulfan-sulfate was clearly shifting in favor of endosulfan-sulfate, a sign of degradation. The residues in the mud of field B were below the detection limit on the fifth day after treatment (0.008 ppm). Mud samples from field C (the field not covered with water) had a residue content of 1.9 ppm maximum (total endosulfan) in the part of the field covered with a plastic roof; and 0.68 ppm (total endosulfan) in the non-covered part. The residue values clearly decreased within 14 days after application. In this case, the ratio of endosulfan (α + β): endosulfan-sulfate was also shifting in favor of the sulfate.

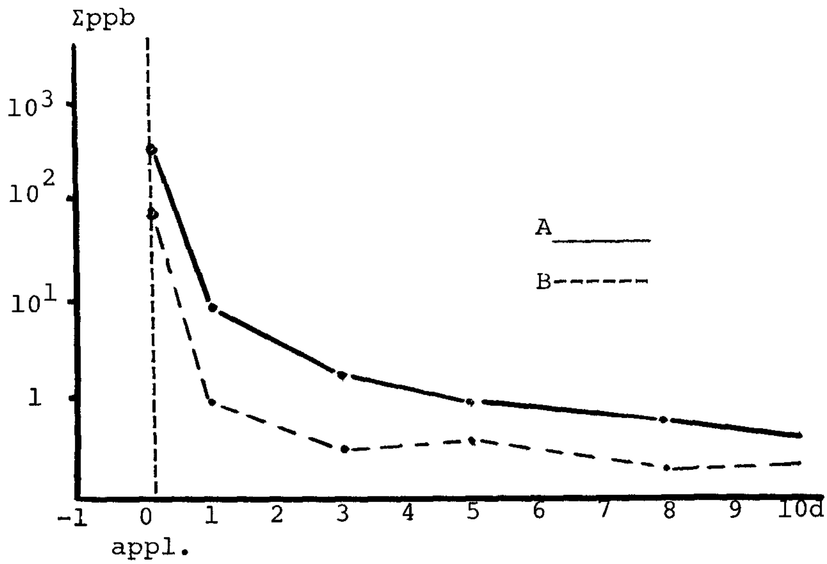

FIGURE 2

Degradation of residues in the test rice field A and B.

On the first day after application all Brachyura as well as the major part of the Coleoptera and larvae of Tipulidae had been killed in fields A and B. Tubificidae, Hydrocorisidae, Cyclopidae and Gastropoda showed no signs of mortality. About 5 days after treatment, the biocoenos: formerly prevailing began to reappear due to immigration.

In the weir baskets, located about 50 m (8) and 1,000 m (9) beyond the outlet of field B, all fish put in two days before application survived and showed no reaction. In basket 8, the residue value after initial increase to 0.0027 ppm decreased to 0.0003-0.0004 ppm within a few hours; the same applied to the residues at basket 9 (reduction from 0.0054 ppm to 0.0003-0.0004 ppm; see table 1). It is quite interesting that for a short time, residue concentrations prevailed 4 times the LC_{100} of *Puntius javanicus*. It may be assumed, therefore, that fish are able to tolerate without harm residues of a multiple of the LC_{100}, if exposed to them for a short time.

Summary

Endosulfan residues declined rapidly - within three to five days - in the water of THIODAN treated test rice fields near Pandaan (East Java). Terminal residues in the water amounted to 0.0005-0.0008 ppm. These residues were due to the constant large scale application in this area. It was noted, that fish are able to tolerate short time exposure of endosulfan concentrations 4 times the LC_{100}. In the mud of submerged as well as dried rice fields, only very low residues (1.9 ppm maximum) were found. The increasing sulfate equivalent in the total residue points to decomposition of the chemical.

The biocoenosis, which was impaired at the beginning of the test, reappeared after completion of the test.

Acknowledgement

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Reference

1. GORBACH,S; HAARING,R; KNAUF,W; WERNER,H-J: Residue Analysis in the Water Systems of East Java (River Brantas, Ponds, Sea Water) after continued large scale Application of THIODAN in Rice. This Journal December (1970).