Biological Accumulation of Pesticides in an Ecosystem —Evaluation of Biodegradability and Ecological Magnification of Rice Pesticides by a Model Ecosystem—

By CHOJIRO TOMIZAWA

Department of Plant Pathology and Entomology, National Institute of Agricultural Sciences
(Yatabe, Tsukuba, Ibaraki 305 Japan)

Development of agricultural pesticides over the past three decades has contributed enormously to stabilize agricultural production by controlling many pests infesting crops. In particular, organo-chlorine pesticides such as DDT, γ-BHC and cyclodienes suppressed the pest populations below the economic injury level in the early stages of their use. However, the problem of pesticide residues in agricultural commodities arose due to the biological stability of these compounds. Next, the persistent pesticides caused disturbances in the populations of not only the pests themselves but also other habitants in the environment. Finally, the continuous and extensive use of these pesticides built up global contamination by the release of industrial organochlorine chemicals such as polychlorinated biphenyls (PCB's) and other chlorinated solvents into the environment.

Furthermore, heavy metals were components of many classical fungicides, and in particular, a large quantity of organomercury fungicides had been applied to paddy fields in Japan for controlling rice blast disease, and the total amount of mercury applied during the period from 1953 to 1972 was estimated to be somewhat more than 2,300 tons as metalic mercury. Mercury is a non-specific poison. Its methylated product is a powerful nervous poison, and is apt to accumulate in lipoid-rich organs of organisms. At the early stage of organomercury fungicide application, an accident, the "Minamata disease" resulted from human consumption of fishery products containing methyl mercury compound originating in the waste water of chemical factories in the southern part of Japan. Despite the Minamata disease case, mercury residues in rice grains did not attract as much attention due to the absence of any symptom of poisoning among people who consumed rice grains derived from the rice plants exposed to organomercury compounds. Copper and zinc also had been applied as components of classical fungicides on a wide scale, but currently, the greater number of fungicides containing metals have been replaced by organic fungicides.

Effect of pesticides on organisms in the environment

The effects of pesticides on organisms are very complicated. The first effect is the direct toxic action on living organisms. There are considerable differences among different species of organism in their sensitivity to any one pesticide. Even closely related species may differ distinctly in their response to a pesticide. This variation in toxicity to different species means that a pesticide may eliminate one species from an area, but have little

or no effect upon another. The removal of one species from a habitat may enable another species to take its place. In other cases, the natural enemies of the species are more sensitive to a pesticide, and as a result, the species can build up higher numbers than occurred before pesticide application. Conversely, the removal of prey organisms results in the reverse case. It is very important for environmental studies to consider the fact that the populations of organisms are closely correlated with food chains[2].

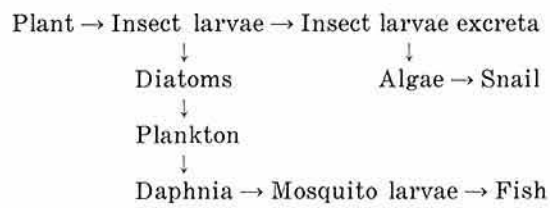
Secondly, the biochemical aspects of pesticidal effects should be considered. As a result of repeated application or continuous contact with a pesticide, an individual organism is apt to induce the enzyme system which catalyzes pesticide metabolism—a mixed-function oxidase (Orrenis et al., 1969)[1]. Persistent organochlorine pesticides are generally non-polar, lipoid soluble, because they must penetrate a lipoid barrier in the insect cuticle and also at the nerve sheath in order to reach the site of action. If a pesticide is persistent and lipoid soluble, it is retained in the fatty tissues of the organism for quite a long period. Such pesticides have a tendency to cause biochemical changes through the induction of a mixed-function oxidase. Not only does the induction of metabolic activity for pesticides in organism develop a resistance to the pesticides, but also the induction of a pesticide-hydroxylating enzyme system is concerned physiologically with the metabolism of steroid hormones and consequently with the fertility of organisms. Thus, persistent and lipoid soluble pesticides have relations with many areas of organism behaviors. These are undesirable characteristics for an ideal pesticide. Therefore, every effort has been made to exclude persistent, lipoid soluble pesticides from our environment. New pesticides which are easily decomposed without any toxic residues are being introduced as alternates. In this connection, if one can predict the persistence of a pesticide in advance of its introduction into our environment, it would undoubtedly be useful for the preservation of the environment.

Methods of evaluating biodegradability and ecological magnification of pesticides

To estimate the persistence and bioaccumulation of pesticides in our environment, several methods have been proposed so far.

1) Model ecosystem[2]

This method was proposed by Metcalf et al. (1971) of the University of Illinois, U.S.A. The basic units of the method are glass aquaria $10\times12\times20$ inch with 180 inch^2 of surface. They contain 15 kg of washed white quartz sand molded into a sloping soil/air/water interface. The aquaria contain 7 l of nutrient water for the growth of *Sorghum halpense* plants in the aerial portion of the chamber and *Oedogonium cardiacum* algae in the aquatic portion. The aquaria are also satisfactory for the growth and development of plankton, Physa snail, Cladocera, mosquito larvae and fish. Sorghum is planted along the flattened terrestrial end of the chamber. When the plants are about 4 in. high after 20 days, a radioactive pesticide is applied topically on the surface of the sorghum and fifth instar larvae of *Estigmene acrea*, the salt marsh caterpillar, are fed on the leaves. The chamber is operated at 80°F±1° on a 12 hr diurnal cycle of 5000 ft candles. Mosquito larvae, *Culex pipiens quinquefasciatus* are added to the aquaria on the 26th day following the start of the experiment, and three *Gambusia affinis*, mosquito fish, are added on the 30th day. These fish devour all mosquito larvae and *Daphnia magna*. The experiment is terminated after 33 days, when weighed samples of the organisms are examined for radioactivity. In this experiment, the food chain pathways for radioactive pesticides are as follows:



From the radioactivity and degradation products found in the organisms, it is possible to estimate bioaccumulation and biodegradation of a pesticide. They adopted Ecological Magnification (EM), defined as ppm parent compound in organism/parent compound in water, and Biodegradability Index (BI), defined as ppm polar products in organism/nonpolar products, to provide a quantitative characterization of the environmental behavior of pesticides.

2) Aquatic model ecosystem

This system was developed by Kearney et al. of USDA (1974)[3,4] and is a modification of the model ecosystem of Metcalf et al. This system is composed of soil, water, algae, duckweed, snails, Daphnia, catfish and crayfish. At the start of the experiment, a radioactive pesticide is mixed well with the test soil (11.4 kg soil contains 21.4 mg of pesticide), and the mixed soil is layered on the bottom of 80 *l* of water in 110 *l* all-glass aquarium tank. After one week, aeration is started, and the organisms are added. The system is operated for 23 days, and all organisms are harvested and analyzed. Nine days after the first harvest, new organisms are added, and the second group of organisms is harvested after 20 days exposure. In this method, the adsorption and biodegradation of a pesticide on soil particles may be different with soil types, but it may be considered that the bioaccumulation and biodegradation patterns of a pesticide reflect more accurately the phenomena occurring in a natural environment.

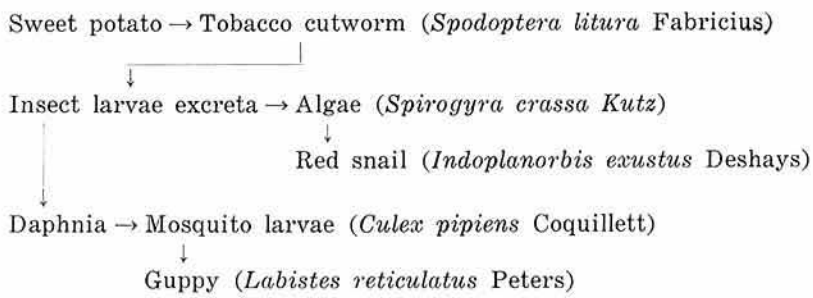
3) Bioaccumulation of pesticides by a single aquatic organism[5]

In the model ecosystem previously mentioned, 5 mg of radioactive pesticide is added to the ecosystem, and therefore, the concentration of pesticide distributed into the aquatic phase is unavoidably high during the early stage of the experiment, and decreases gradually towards the later stage. Such a large fluctuation in concentration would never occur in a natural ecosystem. Actual bioaccumulation is a phenomenon of accumulation of pesticide between the biological system and surrounding phase; that is, it is necessary to maintain a state of pesticide concentration equilibrium between organisms and the aqueous phase. In this model ecosystem, the concept of pesticide concentration equilibrium is not taken into consideration. However, K. Ishizuka, Institute of Physical and Chemical Research, Japan, has developed a new method which works on the basis of the equilibrium concept. He adopted the guppy as the aquatic test organism. Twenty to thirty guppies were placed in 10 *l* of water containing a definite concentration of pesticide, and continuously supplied with water containing the same concentration of pesticide at a rate of 20 *l*/day. The concentration of pesticide in the water and in the guppies was examined at difinite intervals. In this method, the accumulation of a pesticide through a food chain can not be determined, but the operation is extremely simple due to the use of a single aquatic organism. In fish, the accumulation of a pesticide is quite different from that in mammals, and absorption of pesticide takes place not only orally but also via the gills. Although the concentration factor for pesticides administered orally is different with varieties of pesticide, it is generally estimated to be approximately eight times for one step of food chains. The accumulation via gills is remarkably high compared with oral administration, and it was reported that in trout the concentration factor was 7,000 times that of dieldrin in water after about three weeks.

Evaluation of several pesticides by the model ecosystem

Since the proposal of the model ecosystem by Metcalf et al. (1971)[2] many pesticides and industrial chemicals have been evaluated by this method, proving that the model ecosystem approach is useful for evaluating the biodegradability and ecological fate of not only conventional persistent pesticides but also new candidate pesticides. On the other hand, paddy field is a typical terrestrial-aquatic

ecosystem, and therefore, it is reasonable to think that the ecological behavior of any pesticides applied in paddy fields can be evaluated by this method. Therefore, the author conducted a re-evaluation of pesticides using this model ecosystem. Since some of the organisms used in the original method were not available or difficult to obtain in Japan, the organisms used in the model ecosystem were assembled as follows[6]:



To know the feasibility of this system, experiments were first conducted with BHC isomers. Data on the persistence of BHC siomers in the environment are limited as compared with those of lindane, because technical BHC for the pest control has already been replaced with lindane in many countries.

The difference of concentration in the aquarium water was distinct among isomers. A considerable variation in water solubility of BHC isomers has been reported in literatures. The most reliable values show 1.13 ppm for α-isomer, 0.015–0.02 ppm for β-isomer and 5.75 ppm for γ-isomer. This properties might influence the accumulation rate of isomers in the organisms. The translocation of β-isomer to the aquarium water was restricted due to its low water solubility. The results of analyses after 33 days are shown in Fig. 1 and Table 1. In the aquarium water of

Table 1. Concentration of BHC metabolites in the aquarium water and the organisms

		BHC equivalent in ppm			
Isomer	Fraction	Aqueous layer	Toluene layer	Unextractable residues	Percentage of the sum to the initial dosage
α-	Water	0.029	0.010		9.384
	Algae	5.04	14.3	14.8	1.494
	Snail	0.180	0.160	1.03	1.096
	Fish	23.4	17.3	19.2	0.167
β-	Water	0.002	0.010		2.895
	Algae	0.093	0.453	0.038	0.003
	Rice	0.010	0.738	——[1]	
	Cucumber	0.012	0.631	——	
	Snail	0.057	0.603	0.374	0.0002
	Fish	0.056	0.425	0.828	0.002
γ-	Water	0.955	0.009		15.200
	Algae	8.06	93.5	17.3	5.115
	Snail	0.811	0.150	9.79	0.426
	Fish	1.34	2.43	6.18	0.161

1) Not determined

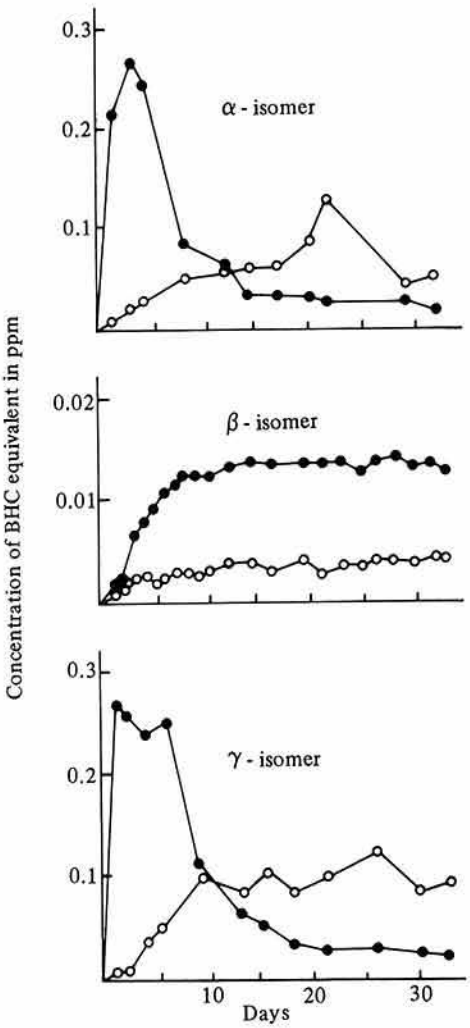


Fig. 1. Translocation of radioactive materials derived from BHC-^{14}C to the aquarium water.
○ Toluene layer
● Aqueous layer

α-and γ-isomers, the concentration in the aqueous layer was higher than that in the toluene layer, but in the organisms, partition rates between both layers were variable. In the snail, the concentration in the aqueous layer was higher than that in the toluene layer for both of α-and γ-isomers. With β-isomer, the concentration in the organisms was extremely low, and the concentration in the toluene layer was always higher than that in the aqueous layer for all of the organisms.

As shown in Table 2, the results of TLC with the toluene layers revealed that β-isomer was highly persistent, while α-and γ-isomers were moderately persistent though the residue patterns of three isomers were somewhat different from those occurred in the natural environment.

The pesticides examined by the model ecosystem showed a diversity of environmental fate in the system. Values of EM and BI were calculated for the pesticides which had been or are used for controlling rice pests. As shown in Table 3, EM values in fish were much higher than those in snail. It appeared that the red snail was the most active member participated in the degradation of the pesticides in the present experiments. On the other hand, it was suggested from the experiment with BHC β-isomer that the translocation rate of radioactive materials to the aquarium water was a limiting factor for bioconcentration in the organisms. Usually, organo-soluble materials in the aquarium water was observed at the early stage of the experiment except β-isomer. In such cases, the organisms in the system may be exposed to a pesticides at relatively high concentration at the early stage of the experiment, and at low concentration at the later stage. Mixing the pesticide with carriers such as quartz sand or soil from the start of the experiment is possible to avoid such sharp decline of concentration. Kearney et al.[4] adopted such a procedure in their aquatic microecosystem. Total recovery of radioactivity in the organism was relatively low at the end of the experiment; usually less than 30% except disulfoton, cartap and edifenphos. This fact appeared to suggest the importance of labelling position or element of pesticide molecules. CNP appeared to be less persistent than BHC β-isomer, but it was apt to accumulate in the tissues of the organisms and showed remarkably high EM values for both the fish and snail. In CNP, ratio of water soluble metabolites was much higher in the aquarium water than the terrestrial portion. As far as the present experiment is concerned, it appears that EM values did not reflect the cases of

environmental contamination occurred in the past, and BI values were more realistic than EM values as seen in the order of persistence of BHC isomers.

In conclusion, the persistence and distribution of the rice pesticides in the model ecosystem depend on their chemical structures. In spite of the limitation of short experimental period, the model ecosystem may be applicable and useful for predicting the environmental fate of rice pesticides.

Table 2. Concentration of BHC metabolites in the toluene layer

		BHC equivalent in ppm			
Isomer	Rf value[1)]	Water	Algae	Snail	Fish
	0.89		6.36	0.05	4.10
	0.79	0.00054	5.16	0.0447	1.99
	0.71	0.00092			
	0.65 (α)	0.00058			
	0.43	0.00013			
α-	0.36	0.00006			
	0.29	0.00011			
	0.20	0.00455	1.13		10.2
	0.14	0.00045			
	0.09	0.00166	0.988	0.038	0.624
	0	0.00080	0.688	0.0302	0.433
	0.90	0.00010	0.00226	0.0103	0.283
	0.67		0.0172		
	0.57 (β)	0.0100	0.431	0.577	9.359
β-	0.36	0.00003			
	0.23	0.00003			
	0.09	0.00003			
	0	0.00001	0.00272	0.0157	0.0377
	0.99		5.52	0.0100	0.170
	0.87	0.00013		0.0306	
	0.75 (γ-PCCH)	0.00241	53.9	0.0566	1.53
	0.66 (γ)	0.00132	10.2	0.0175	0.430
	0.53	0.00008			
	0.43	0.00009		0.00479	
γ-	0.37	0.00002			
	0.31	0.00009	7.29		
	0.25	0.00244		0.0109	
	0.19	0.00075	7.01		0.0680
	0.13	0.00075	2.99	0.0133	0.143
	0.08	0.00037			
	0	0.00037	6.64	0.00599	0.238

1) Developing solvent: hexane: acetone=10:2 for α-isomer and 10:1 for β-isomer, and hexane: ethyl acetate=8:2 for γ-isomer.

Table 3. EM values and BI values for rice pesticides[8)]

Pesticide	Snail		Fish	
	EM	BI	EM	BI
DDT[1)]	6, 241	0. 076	13, 047	0. 069
BHC α-isomer	——[2)]	1. 125	——	1. 350
BHC β-isomer	5	0. 094	936	0. 131
BHC γ-isomer	13	5. 416	326	0. 550
Disulfoton	9	4. 100	2, 487	0. 010
Pyridaphenthion	——	3. 037	——	0. 465
Cartap	——	248. 546	——	67. 612
Kitazin P	4	11. 526	15	6. 609
Edifenphos	——	133. 872	——	1. 043
PCP	——	1. 476	——	1. 165
CNP	5, 316	0. 085	31, 791	0. 099

EM, defined as ppm parent compound in organism/parent compound in water
BI, defined as ppm in aqueous layer/solvent layer
1) Data from Tsuge et al. (1976)[8)]
2) No parent compound was detected or could be estimated.

Refernces

1) Orrenius, S., Das, M. & Gnosspelius, Y.: Overall biochemical effects of drug induction on liver microsomes. Microsomes and drug oxidation Ed. Gillette, J. R. et al. Academic Press, p. 251 (1969).
2) Metcalf, R. L., Sangha, G. K. & Kapoor, I. P.: Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. *Environ. Sci. Technol.*, **5**, 709 (1971).
3) Kanazawa, J., Isensee, A. R. & Kearney, P. C.: Distribution of carbaryl and 3,5-xylyl methylcarbamate in an aquatic model ecosystem. *J. Agr. Fd. Chem.*, **23**, 760 (1975).
4) Schutch, G. K.: Distribution of ^{14}C and arsenic derived from ^{14}C cacodylic acid in an aquatic ecosystem. *J. Agr. Fd. Chem.*, **22**, 999 (1974).
5) Ishizuka, K.: 1973 Report on the analysis of biological concentration of pesticides. Environmental Agency, Japan (1974) [In Japanese].
6) Kazano, H., Asakawa, M. & Tomizawa, C.: Fate of 3,5-xylyl methlycarbamate insecticide (XMC) in a model ecosystem. *Appl. Ent. Zool.*, **10**, 108 (1975).
7) Tomizawa, C. & Kazano, H.: Environmental fate of rice paddy pesticides in a model ecosystem. *Environ. Sci. Health.* **B14**, 121 (1979).
8) Tsuge, S., Kazano, H & Tomizawa, C.: Some devices in a model ecosystem for volatile compounds and its application to carbaryl and p,p-DDT. *J. Pest. Sci.*, **1**, 307 (1976).

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