

## Toxicity of Agricultural Chemicals to Larvivorous Fish in Korean Rice Fields

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(Received for Publication, July 17, 1973)

### Abstract

An important consideration regarding the maintenance or increase of fish populations in rice field areas in relation to Japanese encephalitis is whether the agricultural chemicals applied to rice fields are harmful to fish. This study has shown that the herbicide PCP, which is commonly used in South Korea during the rice-transplanting period, is highly toxic to *Aplocheilus latipes* and *Zacco platypus*. Both species are larvivorous and offer promise in integrated vector control. On the other hand, certain organophosphorus materials applied for rice pest control and the herbicide 2-4 D did not appear to be toxic to fish populations.

### Introduction

The swiftness and agility of the fish *Aplocheilus latipes* make it well adapted for survival in and near rice fields. It moves in trickles of water during draining and sometimes returns to lowland rice fields during flooding. It is seldom seen in upland areas because the lack of rainfall isolates it; moreover the ditches along drained upland rice fields often become dry. The average length of this

species is 2.56 cm (range 1.8 to 3.0) and rural villagers apparently do not bother it. However, stranded specimens are sometimes collected from small pools and mud holes and used to supplement the feed of chickens.

Perhaps the second most common fish found in Korean rice fields is *Zacco platypus*. It is nearly twice as long as *A. latipes* and is more common in northern areas than in

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the south. Other species of fish collected in rice fields by staff of the WHO Japanese Encephalitis Vector Research Unit and identified by the American Museum of Natural History are: *Fluta alba*, *Misgurnus anguillicaudatus*, *Lefua costata*, *Carassius auratus*, *Aphyocypris chinensis* and *Eleotris potamophila*.

*A. latipes* and *Z. platypus* will eat, as shown in this report, mosquito larvae in the laboratory. Both species also overwinter in deep water holes, under ice, in and near rice fields. Marshes south of Chonju city and those close to Pusan are also common year-round habitats of *A. latipes*. In August, a few herons and egrets sometimes are seen searching for fish

in quiet rice field areas, and *Z. platypus* may be the preferred species or easier to catch. After the rice is harvested in October, rural villagers in certain localities are known to eat this latter species, contributing perhaps to its diminishing numbers.

However, a far more important consideration regarding the maintenance or increase of fish populations in rice field areas is whether the agricultural chemicals applied to rice fields, which are essential for increasing local grain production, do so without harming fish. This report dwells on that point and may be of mutual interest to agricultural specialists and to entomologists involved in integrated vector control.

## Methods

### Laboratory feeding tests

During the winter of 1970-1971, about 25 specimens of *A. latipes* and *Z. platypus* were collected from water holes on numerous occasions and acclimated for several days at laboratory temperatures averaging 25°C. The healthy, starved, unsexed specimens were placed individually into 400-ml beakers.

Afterwards, a known number of *Cu. pipiens* larvae or pupae from a mosquito colony were introduced. The average numbers eaten after 24 hours was determined, using from five to 10 fish per test. The numbers exposed in each subsequent test were increased until maximum consumption was obtained. The numbers of immature mosquitos offered (second- to fourth-instar larvae and pupae), ranged from one to 500 per fish.

### Laboratory toxicity tests

Materials tested were those commonly applied against rice pests or potentially use-

full for vector control. Fenitrothion and fenthion covered both categories. Other materials with attractive vector control potential were naled (Dibrom), malathion and Dursban. The herbicides pentachlorophenol (PCP) and 2-4D were included because both are commonly used in Korea. WHO test solutions were obtained or ethanolic stock solutions made from technical material.

Larvae of *Cu. tritaeniorhynchus* and the two common species of rice field fish were exposed. The mosquito larvae were collected from swamps at Banpo Dong village south of Seoul in September 1971, when natural vector populations were high. The fish were obtained from water holes at Paju north of Seoul during February 1970 and 1971. Sufficient numbers of fish and larvae could not be obtained from similar locations and habitats at similar periods. Limited test results from Pusan and from Sintaein in the rice belt, although not reported here, are

similar to the data obtained for the Seoul area.

Using the WHO method, at least 50 fourth-instar larvae were normally exposed at each concentration tested. With the fish, only five specimens were placed into each 400-ml beaker, containing 249 ml of water with toxicant. About 25 to 40 individuals were exposed per concentration. Sometimes, several preliminary tests were made at markedly different concentrations to obtain the range of mortalities required for regression lines. The  $LC_{50}$  values were determined on a computer operated at WHO headquarters in Geneva. Mortalities were determined after 24 hours exposure.

#### *Field toxicity tests*

Materials were applied to experimental plots (.0025 ha) at Paju during April and May 1971. Many specimens could be routinely collected before transplanting time and farmer pesticide treatments. The organo-phosphorus materials evaluated were normally applied at recommended rates for first brood (0.35 kg/ha) and second brood (0.70

kg/ha) stem borers. It has been previously shown (WHO/VBC/71.270) that these dosages kill mosquito larvae, being about five to 10 times higher than required for larval control.

In each plot, which had negligible vegetation, about 20 specimens were placed into each of two fine mesh wire cages. They had diameters of 45 cm and were open at the top. Several organo-phosphorus materials were applied by knapsack sprayers. With lindane and the herbicides, the desired quantities of granules or dusts were weighed in beakers and equally distributed over the plots. Most of the applied dosages reached the water, whereas a good portion of the toxicants applied by farmers in July and August undoubtedly lands on rice foliage.

Mortality counts, normally based on about 75 specimens, were made at 24, 48 and 72 hours after treatment. Cages were sometimes disturbed, and the tests repeated once or twice. The mortality in controls usually ranged from 5 to 15%.

## Results

#### *Laboratory feeding tests*

Table 1 shows that *A. latipes* and *Z. platypus* are larvivores. This is consistent with Sandosham's (1965) comment that fish are useful as mosquito destroyers when they are small, say up to 10 cm. Larger fish may also eat mosquitos in the laboratory but will require larger food in the field. Conversely, the small larvivores will feed on many kinds of small plant and animal life. An examination of the stomach contents of these two Korean species under varied ecological conditions is very much desired.

*A. latipes* consumes few fourth-instar larvae but hardly any pupae. The small openings of its mouth parts however, do not prevent it from chasing and wounding some big larvae. Its average consumption of 40 third-instars within 24 hours is formidable, nonetheless. It is only about three-quarters as long as the famous mosquito fish *Gambusia affinis*.

*Z. platypus* consumes, on the average, a strikingly high number of fourth-instar larvae (188). Here, we consider the possibility that an overwintered fish, in its "excess"

**Table 1.** Laboratory tests showing numbers of *Cu. pipiens* larvae and pupae eaten in 24 hours by rice field fish.

Immature stages offered	Total No. of immatures offered	Total No. of fish used	Total immatures eaten	Average No. eaten per fish	Maximum No. eaten by one fish
<i>Aplocheilus latipes</i>					
Second-instar	5 749	113	5 635	50	120
Third-instar	2 687	56	2 231	40	88
Fourth-instar	426	8	136	17	40
Pupae	45	15	3	1<	1
<i>Zacco platypus</i>					
Fourth-instar	4 820	20	3 760	188	280
Pupae	1 620	48	1 468	31	90

Fish were individually placed in 400-ml beakers with known number of immature mosquitos.

starved state, may be hungrier than a fish captured in the summer, starved, and soon released. Although this has not been studied in Korea, a clear understanding on this point may be of some interest regarding any mass rearing and timely release of aggressive larvivores into unstable habitats.

In these tests, *Z. platypus* eats more larvae than reported for *G. affinis* in California by Rees(1958). There, one fish 4.5 cm in length devoured 163 third- and fourth-instar larvae in an eight-hour period. The average length of *Z. platypus* is 4.6 cm (range 3.3 to 5.0) and, like *G. affinis*, it readily devours pupae. Feeding usually begins immediately after fish and mosquito immatures are confined together.

In further tests, seven *Z. platypus* specimens were each offered 25 fourth-instars for 10 consecutive days. Only 2% of the larvae were not eaten.

Because the fish were not sexed in these experiments, the average numbers consumed by females may be higher than the figures shown in Table 1. The data showing maxi-

mum numbers eaten probably refers to females.

#### Laboratory toxicity tests

Table 2 compares the LC<sub>50</sub> values for *Cu. tritaeniorhynchus* larvae to those for both species of fish. The safety factors for fish, based on the ratios of the respective LC<sub>50</sub>s, provide some indication of whether the dosages required for larval control also kill fish. However, a more meaningful safety criterion is whether the dosages applied by rice farmers kill fish.

The compounds showing the greatest differences between the dosages required to kill mosquito larvae and the small paddy fish are trichlorfon (Dipterex), methyl parathion, Dursban, fenthion, fenitrothion, naled (Dibrom), phenthoate (Cidial), EPN and malathion. With *Z. platypus*, a somewhat similar order is obtained, although this species is considerably less susceptible, particularly to malathion.

Both fish seem resistant to trichlorfon, a compound once commonly used against rice pests. Also, the chlorinated hydrocarbons

**Table 2.** LC<sub>50</sub> values for the larvae of *Cu. tritaeniorhynchus* and two rice field fish after 24 hours exposure.

Material tested	LC <sub>50</sub> mosquito larvae ppm	LC <sub>50</sub> <i>Aplocheilus</i> <i>latipes</i> ppm	LC <sub>50</sub> <i>Zacco</i> <i>platypus</i> ppm	Safety factor fish/larvae	
				<i>Aplocheilus</i>	<i>Zacco</i>
Trichlorfon	0.019	259	299	13 632	15 737
Methyl parathion	0.00354	2.9	4.6	5 371	8 519
Dursban	0.00032	0.31	1.7	969	5 312
Fenthion	0.0056	2.6	7.2	464	1 286
Fenitrothion	0.019	4.5	7.4	450	740
Naled	0.0094	3.0	5.2	319	553
Phenthoate	0.0013	0.38	4.1	292	3 153
EPN	0.0011	0.29	2.2	264	2 000
Malathion	0.021	2.8	9.7	133	462
Lindane	0.17	0.73	2.5	4	15
DDT	0.16	0.21	0.35	1.3	2.2
2-4D	91.8	110	140	1.2	1.5
Dieldrin	0.21	0.14	0.75	0.67	3.6
PCP	20.9	0.14	0.23	0.0067	0.011

allow much lower margins of safety than the organophosphorus materials. Regarding the effect of dieldrin against *A. latipes* and PCP against *A. latipes* and *Z. platypus*, the dosages required to kill larvae also kill fish.

#### Field toxicity tests

Table 3 shows that fenitrothion and fenthion cause negligible mortality to fish even at rates twice those recommended for second brood stem borers. Moreover, the theoretical target dosages in ppm, calculated on water depths ranging from 8 to 12 cm, are lower than the laboratory LC<sub>50</sub> values.

Malathion is clearly more toxic than fenitrothion and fenthion at very high application rates. However, this rate (1.4 kg/ha) is not used for vector or pest control, the latter being limited to seedbeds in the spring.

Therefore, malathion seems to pose little toxic hazard to fish in Korea; indeed an ULV aerial application at 5 fl/oz per acre for vector control did not kill *A. latipes* in marshes and open ditches at Pusan (Unpublished document WHO/VBC/71.305).

EPN and phenthoate are very toxic to caged *A. latipes* when applied at the rates recommended for control of second-brood rice-stem borers. These materials appear to be more hazardous to the fish present in rice fields where the water is shallow at the time of treatment. However, on-site checks of treatment made by farmers in July and August 1970 did not confirm the toxicity of these materials in the field. Moreover, many rice fields in September do not contain fish because they become dry before harvest. With the organo-phosphorus insecticides, *A. latipes*

**Table 3.** Toxicity of insecticides and herbicides to caged rice field fish.

Formulation applied	Kg/ha active ingredient	Theoretical dosage ppm	% mortality to <i>A. latipes</i> and ( <i>Z. platypus</i> )		
			24 hours	48 hours	72 hours
Fenthion 50% EC	0.1	0.13	0 (0)	0 (0)	0 (0)
	0.35	0.46	10 (0)	10 (0)	10 (0)
	0.70	0.92	10 (0)	15 (0)	20 (10)
	1.4	1.1	0 (5)	0 (10)	15 (10)
Fenitrothion 50% EC	0.70	0.92	5 (0)	5 (0)	5 (0)
	1.4	1.2	15 (0)	15 (0)	15 (0)
Malathion 50% EC	0.35	0.38	10 (0)	10 (0)	10 (0)
	0.70	0.58	30 (0)	35 (0)	35 (0)
	1.4	1.6	90 (10)	90 (25)	95 (25)
EPN 50% EC	0.35	0.39	0 (5)	0 (5)	0 (5)
	0.70	0.92	90 (45)	90 (45)	95 (50)
	1.4	1.2	60 (50)	90 (70)	95 (-)
Phenthoate 47.5% EC	0.35	0.31	80 (5)	85 (10)	85 (10)
	0.70	0.6	75 (10)	80 (15)	80 (15)
	1.4	1.1	100 (35)	- (35)	- (35)
Lindane 3% dust	1.2	1.2	5 (0)	45 (5)	45 (10)
Lindane 6% granule	1.2	1.3	5 (5)	10 (5)	15 (5)
	4.8	4.8	5 (0)	75 (60)	85 (65)
2-4D 7% granule	14.0	15.5	5 (0)	5 (0)	5 (0)
	28.0	35.0	5 (20)	20 (50)	35 (55)
PCP 86% dust	0.17	0.19	40 (65)	40 (65)	40 (65)
	0.34	0.34	100 (100)	-	-
	0.85	0.85	100 (100)	-	-

Each treatment made to .0025/ha plots, with water depth from 8 to 12 cm, using about 75 specimens of each species of fish.

proved to be more susceptible than *Z. platypus*, its mortality rate in the field being similar to that found in the laboratory.

Lindane as applied in the field was less toxic than EPN and phenthoate. Since the laboratory data indicated that it should have caused higher fish mortality than these two OP compounds, the solid formulations of lindane used in the field may have been safer than spray formulations, although this point requires more study. Farmers normally apply

lindane granules at 1.8 to 2.4 kg/ha shortly after transplanting.

The granular herbicide 2-4D is not toxic to fish. The recommended rate is 2.1 kg/ha, and negligible fish mortality was found to occur at 14 kg/ha.

PCP is highly toxic. The recommended rate of 6 kg/ha, when applied to water 30 cm deep, gives a theoretical dosage of 2 ppm, which is about 10 times higher than the LC<sub>50</sub> values found in the laboratory. The field

dosages would be even higher when the PCP was applied to shallow water. The PCP dust floats temporarily on the water surface and is usually applied to vast areas of flooded rice fields shortly before and after transplanting in late May and early June. Most of the dosage reaches the water, unlike the foliar pesticide sprays made later. A PCP dosage of 0.34 kg/ha, being 18 times lower than the rate recommended for weed control, killed every fish exposed in our tests.

It is certain that the agricultural applica-

tions of PCP are devastating to rice field fish populations of all species and sizes. Observations at Sintaein in the rural rice belt in 1970 revealed that application of PCP at 6, 8 or 26 kg/ha were equally effective in eliminating seven species of fish in rice fields during June. In addition to dead fish which float on the water surface soon after treatment, frogs, snails, earthworms and leeches were also found to have been killed. Yet these application rates normally do not kill the mosquito larvae.

### Discussion

*A. latipes* and *Z. platypus* are two native fish of Korea which are found in and near rice fields. Laboratory tests show that both species are larvivorous, and the results with *Z. platypus* appear particularly promising. Thus the limitations and advantages of larvivorous fish in integrated vector control programmes can be initially explored in Korea without the introduction of a foreign species.

In Korea, the vector of Japanese encephalitis, *Cu. tritaeniorhynchus*, and the vector of malaria, *An. sinensis*, occur in rice fields, marshes, swamps and ditches, and here the larvivorous fish are also usually found. However, fish are absent from the stable swampy areas constituting abandoned land near large cities, and for that reason they are attractive sites for fish release studies. The marsh areas populated with fish normally do not contain many mosquito larvae, provided that the outer edges are not used as garbage-dump sites, which permit isolated breeding pockets to

develop.

Safety factors for fish for the common insecticides were established in laboratory tests by comparing the  $LC_{50}$  value for *Cu. tritaeniorhynchus* to those for *A. latipes* and *Z. platypus*. Such data are particularly useful for vector control operations planned for areas not treated by agricultural chemicals. Before attempting integrated vector control with fish in Korean rice fields, a thorough understanding of rice culture practices, including the effects of repeated applications of agricultural chemicals, seems desirable.

The only agricultural chemical clearly found to be exceedingly toxic to fish under natural field conditions in this study is the herbicide PCP. This compound is imported into Korea in much larger quantities than any other herbicide currently being used. Its substitution by more selective materials may help increase fish populations in rice field areas.

### Acknowledgements

We thank Dr. A. W. A. Brown and Mr. J. W. Wright of the Vector Biology and Control division of the World Health Organization for supporting this study and for permission

to publish this paper. We are also grateful to Dr. Y. Wada for reviewing the paper and for his kind assistance.

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韓国の水田の蚊幼虫捕食性魚類に対する農薬の毒性

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### 摘 要

水田地域における魚類個体群の維持と増加にとって、水田に撒布される農薬が魚に有害かどうか重要である。韓国南部で田植時期に普通に使用される除草剤 PCP は、*Aplocheilus latipes* メダカ及び *Zacco platypus* オイカワに対して強い毒性を示す。両種は蚊の幼虫を捕食するので、蚊の総合防除に当り考慮されるべきものである。一方、稲作害虫に対して撒布される有機燐剤や除草剤 2-4D は魚類個体群に有毒ではないようであった。