Toxicity of Orally and Topically Applied Pesticide Ingredients to Carp, *Cyprinus carpio* Linné. Yasushi HASHIMOTO (Agricultural Chemicals Inspection Station, Kodaira, Tokyo) and Jun-ichi FUKAMI (The Institute of Physical and Chemical Research, Komagome, Bunkyo-ku, Tokyo) Received January 9, 1968. *Botyu-Kagaku* 34, 63, 1969.

7. 経口的および局所的に処理した農薬のコイに対する毒性

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農薬を餌に混入してコイに経口的に摂取させたとき，および，農薬を麻酔したコイのえらにミクロシリンジで局所処理したときの毒性を調べた．この結果を従来の接触法による標準試験法の結果と比較すると，一部の農薬は処理方法により効果が著しく異なることが認められ，農薬の魚に対する毒作用を解明する上での一つの示唆を得ることができた．

The toxicity of pesticides to fish is evaluated by exposing the fish to a candidate toxicant diluted with water in a container. Although this method can give almost enough information for practical pesticide usage, other methods, such as oral or topical application used to test toxicity to mammals and insects, will greatly help to elucidate the mechanisms of toxicity to fish. As an oral application, Allison *et al.* (1964)[1] made pellets of a diet containing DDT to study the chronic effects on fish. No experiments have been carried out to evaluate the acute oral toxicity of pesticides to fish, and no trials of topical application have ever been made on fish.

In the present paper, methods of applying pesticides orally and topically will be explained and the experimental results will be discussed by comparing them with the results obtained from a conventional contact method.

Materials and Methods

Test animal: The test animals were carps, *Cyprinus carpio* Linné, an average of 6.0cm in total length and 2.5g in weight. They were obtained from Kanagawa Prefectural Fresh-water Fishery Station, Sagamihara, Kanagawa Prefecture, and acclimatized to the experimental conditions for more than one week in concrete tanks in the Agricultural Chemicals Inspection Station. They were fed daily on the powdered diet for mice manufactured by Oriental Kobo Co. Ltd. during the acclimatization period, but not fed for a period of about two days before the test.

Pesticide: Technical ingredient or pure compound was used in all the experiments.

Oral application: Pesticide was added to the powdered diet, on which the fishes had been fed during the acclimatization period, so as to give the desired dose in one gram of mixture. After stirring with a spatula, 0.6m*l* of distilled water was pipetted into the mixture, and the resultant paste was formed into a small pellet by hand. Ten fishes, which had been kept in a concrete pot filled with 120*l* of water, were fed on the pellets. The feed containing pesticide was consumed within 15 minutes. The mortality of the fishes was observed 48 hours after feeding. When more than half of the fishes were killed at 48 hours, another ten fishes were added to the pot for 48 hours to confirm whether the fishes were killed by the pesticide in the feed or by other conditions, such as the water.

Topical application: The pesticide was dissolved in distilled water, acetone or tetrahydrofuran to give the desired dose in 5μl of solvent. The fishes to be tested were placed in one litre of 0.1% aqueous solution of MS-222 SANDOZ (methanesulphonate of *meta*-aminobenzoic acid ethylester). At this concentration, most of the fishes were completely anaethetised within three minutes, and they recovered within five minutes after being released into fresh water. No ill effects were observed. Pesticide solutions were applied by a micrometer-syringe onto the gill lamella of the anaesthetised fishes. Each solvent was confirmed to have a non-deleterious effect on the anaesthetised fishes when 20μl was applied to the gill lamella. After treatment, ten fishes which had received each dose were kept in a glass container filled with 10*l* of water for 48 hours

Table 1. Toxicity of pesticide ingredients to *Cyprinus carpio* Linné, expressed as LD-50.

Pesticide	LD-50 (mg/fish)		
	Oral application	Topical application	Contact method
DDT	0.34	0.065	0.11
γ-BHC	3.6	0.060	0.17
Endrin	0.065	0.002	0.00084
Dieldrin	0.18	0.058	0.018
Aldrin	6.4	>0.1	0.12
Parathion	>10	>0.1	4.5
Methylparathion	>10	>0.1	1.7
Diazinon	>10	>0.1	3.2
Sumithion	>10	>0.1	4.4
EPN[1]	3.3	>0.1	0.35
NAC (Sevin)	>10	>0.1	>10
Salithion[2]	>10	>0.1	>10
Lannate[3]	4.2	0.036	2.0
Cartap[4]	9.0	>0.1	0.78
Rotenone	6.5	0.014	0.032
Allethrin	>10	>0.1	1.5
FABB[5]	>10	>0.1	>10
EDDP[6]	>10	0.1	2.5
PCBA[7]	7.8	>0.1	1.8
Dyrene (Triazine)	>10	>0.1	0.095
Ferbam	>10	>0.1	0.090
Ziram	>10	>0.1	0.075
Dichlone	>10	0.018	0.07
ETM[8]	>10	>0.1	0.48
Blasticydin	>10	>0.1	>40
NBT[9]	>10	>0.1	0.0075
Dithane	>10	>0.1	>10
NIP[10]	>10	>0.1	2.1
Triethazine	10	>0.1	0.85
PCP-Na[11]	7.2	0.062	0.12
PCP-OH[12]	10	0.016	0.11

1) *O*-ethyl *O*-*p*-nitrophnyl phenylphosphorothioate
2) 2-methoxy-4H-1, 3, 2-benzoxaphosphorine-2-sulfide
3) *S*-methyl-*N*-〔(methylcarbamoyl)-oxy〕-thioacetamidate
4) 1, 3-*bis* (carbamoylthio)-2-(*N*, *N*-dimethylamino) propane
5) monofluoroacetic acid *p*-bromobenzylamide
6) *O*-ethyl-*S*, *S*-diphenyldithiophosphate
7) pentachlor benzylalcohol
8) ethylene thiuram monosulfide
9) 2, 4-dinitrophenyl thiocyanate
10) 2, 4-dichlorphenyl 4-nitrophenyl ether
11) sodium pentachlor phenolate
12) pentachlorphenol hydroxide

to observe mortality.

Contact method: The standard method for the evaluation of acute toxicity of agricultural chemicals to fish[2], was used essentially as recommended by Ministry of Agriculture and Forestry. The fishes were introduced into a glass container filled with 10*l* of each test solution. The water temperature was maintained between 20° and 22°C throughout the experiments.

Calculation of TLm and LD-50 values: TLm and LD-50 values were derived by the straight-line graphical interpolation method of P. Doudoroff *et al.* (1951)[3].

Results and Discussion

The experimental results expressed in LD-50 are shown in the first and second columns of Table 1. TLm values in the contact method in the previous papers[4,5] were converted to LD-50 values assuming that the total amount of pesticide ingredient in a 10*l* solution in a glass container was completely accepted by the test fishes. The converted LD-50 values are shown in the third column of the table for comparison.

Generally speaking, all the pesticides tested showed lower toxicity when they were applied orally than by the contact method. The difference in LD-50 values between the two methods is greater than the figures in Table 1 suggests because a considerable amount of active ingredient still remained in the test solution in the container when it was applied by the contact method. In the case of insecticides, it seems that there is a positive correlation between both LD-50 values, while for fungicides and herbicides, oral toxicity is generally of such a low level that it cannot be evaluated, most of them show high contact toxicity.

The low oral toxicity implies that the pesticides either do not act as stomach poisons or that they do not reach their site of action. It seems that they are either detoxified in the alimentary canal or are prevented from reaching the target site by some physico-chemical barriers. This hypothesis fits well in the case of fungicides, herbicides and most of the insecticides since there is a great difference between both the LD-50 values. In the case of DDT and Lannate there are small differences between LD-50 values, so they may act as stomach poisons, but further study is required to elucidate this.

A toxic substance is presumed to kill fishes, when it reaches a site of action such as the brain or nervous system through the gills. Holden (1962)[6] and Lloyd (1960)[7] studied the distribution of a toxic substance in the body of a fish exposed to a solution of the toxicant. They found that some of the substance accumulated in the gill parts. As the pesticides were administered exclusively onto the gill parts by the topical application method in the present experiment, it was anticipated that the true lethal dose and the site of action could be determined. As the maximum volume of solution which can be applied by the micrometer syringe, without ill effect to the fishes did not enable more than 0.1 mg of active ingredient to each individual fish, LD-50 values could not be calculated for less than half of the pesticides tested.

If the pesticides reach the site of action selectively through the gill parts, LD-50 values in the topical application method are expected to be smaller than, or at least of the same level as those in the contact method. DDT, γ-BHC, Lannate, rotenone, dichlone, PCP-Na and PCP-OH fall into this category.

In addition, since a considerable amount of active ingredients is supposed to be accepted on or in the fishes body other than the gills, or to remain in the test solution, the actual amount remaining in the gill parts or reaching the site of action through the gill parts must be very small in the contact method.

Therefore, if the gill parts are the only the site of action of pesticides or the sole route introducing them to the target site, LD-50 values in the topical application method would be much smaller than those obtained in the present experiment with the exception of Lannate. However, LD-50 values in the topical application method were significantly higher than those in the contact method in the case of the organosulfur compounds, such as triazine, ferbam and ziram. Although these fungicides are of rather low oral toxicity to mammals and fish, as found in the present experiment, they often cause dermatitis when

they remain on the human skin.

Therefore, it seems probable that the site of action of these fungicides is not in the brain or other internal parts readily accessible to the fungicides through the gill parts, but on the surface of the fish body or a site accessible through the epidermis.

In conclusion, though the gill parts may be one of important site of action or route through which pesticides can reach the target sites, other sites or route probably exist in the case of the organochlorine compounds. It was shown here that the gill parts provide neither site nor route in the case of the organosulfur compounds. Lannate is the only pesticide showing a distinct possibility of acting on the actual gill parts selectively, or another site of action readily reached through them. To confirm the above hypothesis, the distribution of pesticides in the fish body after topical application to the gill part or to other sites which might predictably be the sites of action should be studied. As the first step, the distribution of rotenone, diazinon, methyl parathion and their metabolites in the fish body are being studied.

Summary

Oral and topical applications of pesticides to carp were made by newly devised techniques. When the experimental results were compared with those of the contact method, the effect of pesticides on carp was found to differ with the application methods. This evidence suggests further studies to elucidate the mode of action of pesticides on fish.

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Syntheses and Insecticidal Activities of 2-β-Substituted Ethoxy-4H-1, 3, 2-benzodioxaphosphorin-2-oxides and -sulfides. Ken KOBAYASHI, Tadayoshi HIRANO, Shigeki WAKAMORI, Morifusa ETO* and Yasuyoshi OSHIMA** (Kumiai Chemical Co. Ltd., Odawara, Institute, Odawara, *Department of Agricultural Chemistry, Kyushu University, Fukuoka, and **Faculty of Agriculture, Meiji University, Kawasaki). Received March 28, 1969. *Botyu-Kagaku* 34, 66, 1969. (with English Summary 69)

8. 2-β-置換 ethoxy-4H-1, 3, 2-benzodioxaphosphorin-2-oxides および sulfides の合成と殺虫性.

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サリゲニン環状リン酸エチルエステルの β-置換誘導体10種を合成し，イエバエとニカメイガ幼虫に対する殺虫性について検討した．置換基としてハロゲン，アルコキシルおよびフェノキシル基を導入した．これら置換基の導入によって殺虫性は減少した．さらに，種類によって程度は異なるが，一般に置換基が大きくなるほど殺虫力は低下した．従って母体のエチルエステルあるいはサリチオン，サリオキソンなどメチルエステルより殺虫力のすぐれたものは見出されなかった．

江藤，大島およびその協力者たちはすでに実用化に成功したサリチオン (2-methoxy-4 H-1, 3, 2-benzodioxaphosphorin-2-sulfide) を含む一連のサリゲニン環状リン酸エステル類の合成法と殺虫性について報告して来ているが[1~7]，本報ではアルキル側鎖の置換基が殺虫力に及ぼす影響を調べる目的で，環状リン酸エチルエステルのβ位にハロゲン，アルコキシルおよびフェノキシル基等を導入することを試み，得られた10種の誘導体の殺虫力を検討した．

実 験 の 部

1. 合　　成