# A Method of Predicting the Bioconcentration Potential of Pesticides by Using Fish

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Organic pesticides may enter natural waters through such routes as direct application to control aquatic weeds and insects, leaching and runoff from agricultural lands, drift from aerial and ground applications, discharge of waste waters from manufacturing plants, and discharge of waste waters from cleanup of equipments used for pesticide formulation and application.

In water, pesticides are apt to be hydrolysed chemically and degraded by microorganisms and sun light, and be bound to organic matters in muds and sediments, leaving only a small amount remaining in solution.

Pesticides are also taken up into aquatic organisms through gills and skin during respiration, and by oral feeding. Some are then distributed into various organs of the body, metabolised and finally excreted from the body back to the water. However, certain lipophilic and persistent compounds such as DDT, dieldrin and polychlorobiphenyls are metabolised and excreted by organisms only to a very small extent, with the result that the compounds become concentrated in tissues of the organisms to higher levels than in the surrounding water. This phenomenon is termed biological concentration (bioconcentration). This bioconcentration by aquatic organisms is considered to be the most important problem among the chronic effects of pesticides to non-target organisms, from the viewpoint of protecting the aquatic environment and preventing protein resources for mankind from contamination. It is necessary, therefore, to establish a method of predicting the bioconcentration of candidate chemicals before introducing them as pesticides into the environment.

Fish are the most suitable test organisms to predict the bioconcentration potential of chemicals, because (1) they take up chemicals through respiration and feeding, (2) the concentration of chemicals in the whole body are apt to reach an equilibrium state quite rapidly, and (3) they are easily cultured.

This paper reports bioconcentration factor (BCF) values of 15 important pesticides measured by the use of a freshwater fish, topmouth gudgeon, at the equilibrium state, correlations between BCF values and physicochemical properties such as water solubility (WS), *n*-octanol-water partition coefficient (PC) and molecular weight (MW), and finally the prediction of BCF of pesticides by using their physicochemical parameters.

## Measurement of bioconcentration factors

The BCF has been defined as the ratio of the concentration of a test chemical in an organism to the concentration in the test environment (e.g. water) at a steady state. The continuous-flow water system has been used to keep the concentration of chemicals constant for the testing period. This system is shown in Fig. 1.

Tap water is used after being purified by passing through a commercial water-cleaner containing an active-carbon filter. The constant-flow micro-pump is used to obtain continuous-flow of the test chemical solution. A glass aquarium tank ($45\times24\times30$ cm) containing 20 $l$ of water is used for the system. An aqueous solution containing 2 ppm of the test pesticide is prepared by using a small amount of acetone, and diluted continuously

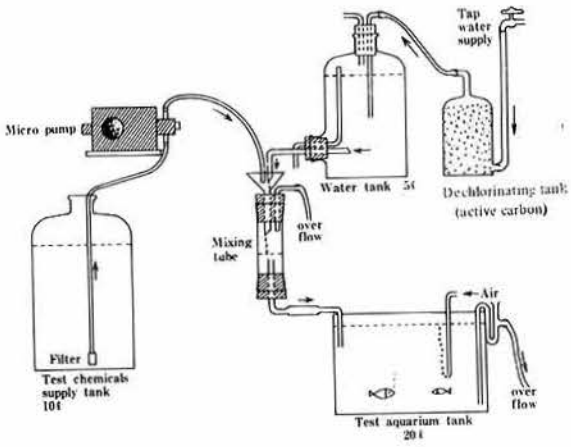


Fig. 1. Continuous flow water system

with 100 volumes of clean water and poured into the test aquarium tank. Therefore, the concentration of the test pesticide in the aquarium water is maintained at 10 to 20 ppb throughout the experiment. The water flow rate is adjusted to 300 ml/min, and the temperature is kept constant, for example, at 20±2°C, with a heater, if necessary. The aquarium water is aerated continuously during the experiment. Used as test species are carp (*Cyprinus carpio*), topmouth gudgeon (*Pseudorasbora parva*), mosquito fish (*Gambusia affinis*), crayfish (*Procambarus clarkii*), red snails (*Indoplanorbis exustus*) and others. Fish of 4 to 8 cm in length and 3 to 6 grams in body weight are desirable. Of these, topmouth gudgeon was selected as the test fish because it had achieved the highest BCF for diazinon among freshwater organisms previously tested by the author.[3] Fifteen to twenty fish were placed in the aquarium tank and reared for 14 days, and then transferred into clean water and reared for further 30 days. Commercial dry food was given once a day throughout the experiment. The concentrations of the test pesticide in the fish and in the water were determined at appropriate intervals of a few days by gas chromatography according to the previously reported procedures.[2,4]

## Uptake and excretion curves of pesticide by fish and determination of BCF

The author determined uptake and excretion curves of the 15 important pesticides by the continuous-flow system. Of these, the uptake and excretion curves of diazinon and IBP by topmouth gudgeon are shown in Fig. 2.

The concentration of diazinon in the whole body of the fish exposed to continuous-flow water containing 10 ppb or 50 ppb of diazinon increased gradually after commencement of the experiment, and reached the equilibrium after 3 days for 10 ppb exposure and after 4 days for 50 ppb exposure, respectively. The concentration of diazinon in the fish was 2.01 ppm for 10 ppb exposure and 11.3 ppm for 50 ppb exposure. Therefore, no significant difference was observed in the BCF values, which was 150 and 210, respectively, although the concentration of diazinon in the test water differed markedly, i.e., 10 and 50 ppb respectively. However, all the fish exposed to 50 ppb diazinon became suffering the vertebral deformation. Accordingly this exposure test was terminated after 7 days and the test fish were transferred into clean water. The excretion of diazinon was relatively rapid, approximately consistent with a linear function in both groups of fish exposed to 10 or 50 ppb diazinon water.

In the case of IBP, the concentration of IBP in the fish rapidly reached the equilibrium within one day. It is already known that BCF of chemicals shown by aquatic organisms is related to physicochemical properties of the chemicals such as water solubility and *n*-octanol-water partition coefficient.[6,7]

## Measurement of water solubility

An amount of test compound in excess of its water solubility, was taken in a 100 ml volumetric flask with about 100 ml of distilled water. The flask was placed in a water-bath at 20–22°C, and treated with ultrasonic

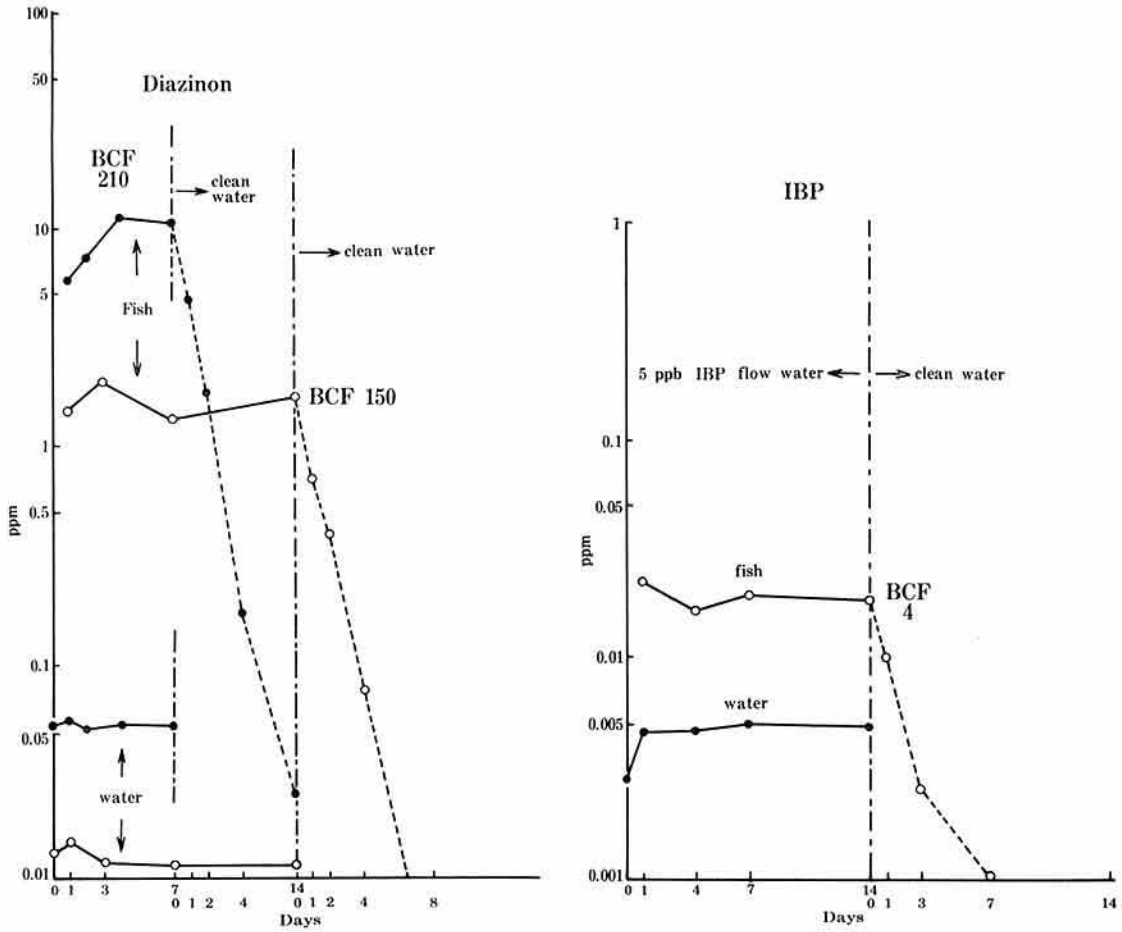


Fig. 2. Uptake and excretion curves of diazinon and IBP by topmouth gudgeon

vibration for 10 min. It was stood overnight and again subjected to ultrasonic treatment for 5 min. This saturated solution was then filtered with Toyo filter No. 5C. An appropriate amount of the filtrate was taken and extracted with dichloromethane ($2\times10$ m*l*). The combined dichloromethane solution was evaporated and the residue was dissolved in a determinate amount of acetone for gas chromatographic estimation. Measurement of the water solubility of leptophos is difficult by this method, because it is very hydrophobic. The measurement was therefore made by the Haque and Schmedding method[1]. In this method, the restoration of the equilibration between the chemical and the water takes a long time. The chemical under investigation is first deposited as a thin film inside the wall of a large vessel, e.g. a round bottom flask, water is then added, and the system is slowly stirred, and the concentration of the chemical in water is determined at various intervals of time until it becomes constant. The water solubilities quoted in the literature[9] were adopted, for phenthoate, IBP, thiobencarb and molinate.

## Measurement of partition coefficient

A stock solution containing approximately one mg of the test compound per m*l* of *n*-octanol was prepared. Five m*l* of this solution was shaken with distilled water (15 m*l*)

for 2 hr at 20±1°C in a closed 25 m*l* centrifuge tube. The solution was then allowed to stand overnight. After equilibration and centrifugation (3,500 rpm for 10 min), the two phases were separated and extracted with the appropriate solvent, or diluted as necessary, for analysis by gas chromatography. The partition coefficient (PC) was determined by dividing the concentration of the pesticide in the octanol by that in the water.

## Relation between bioconcentration factors and physico-chemical properties

BCF values of the 15 pesticides determined with topmouth gudgeon, together with their values of WS, PC and MW are shown in Table 1.

A marked difference was found among the BCFs of the pesticides tested. Compared with dieldrin, which was selected as a reference compound, the BCF of leptophos was the highest, followed by that of trifluralin, EPN, gamma-HCH (lindane), whereas, the BCFs of carbaryl, IBP and BPMC showed very low values. A logarithmic (log) plotting of the BCF against the log of the WS is shown in Fig. 3.

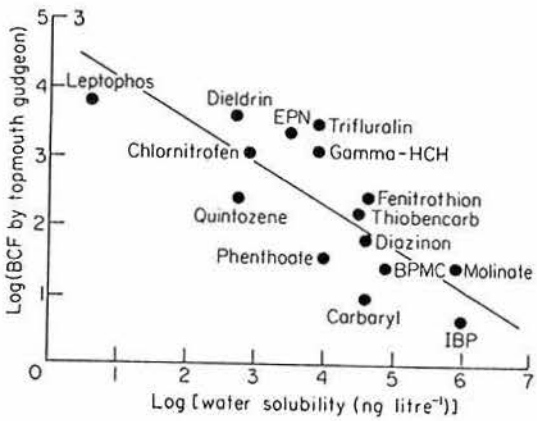


Fig. 3. Relationship between the water solubilities for 15 pesticides and their bioconcentration factors (BCF) for topmouth gudgeon; $r=-0.784^{**}$

A satisfactory linear relationship was observed between the log of the BCF and the

**Table 1. Bioconcentration factors and physicochemical properties of pesticides tested**

| Pesticide | MW | WS at 20–25°C ppb | PC octanol/water | BCF by topmouth gudgeon |
|---|---|---|---|---|
| Gamma-HCH[a] | 290.9 | 7,880 | 4,611 | 1,246 |
| Dieldrin | 380.9 | 468 | 20,785 | 4,430 |
| Diazinon | 304.4 | 40,500 | 1,386 | 152 |
| EPN | 323.3 | 3,113 | 7,027 | 2,346 |
| Fenitrothion | 277.2 | 38,700 | 2,767 | 246 |
| Leptophos | 411.8 | 5 | 20,833 | 6,058 |
| Phenthoate | 320.3 | 11,000 | 781 | 36 |
| Carbaryl | 201.2 | 34,000 | 197 | 9 |
| BPMC | 207.3 | 89,000 | 1,500 | 26 |
| IBP[b] | 288.3 | 400,000 | 1,630 | 4 |
| Quintozene | 259.4 | 550 | 16,552 | 238 |
| Thiobencarb[c] | 257.8 | 30,000 | 2,650 | 170 |
| Chlornitrofen[d] | 318.6 | 764 | 4,709 | 1,109 |
| Molinate | 187.3 | 880,000 | 1,628 | 26 |
| Trifluralin | 335.3 | 8,110 | 9,328 | 3,142 |

a: γ-hexachlorocyclohexane, lindane
b: S-benzyl O,O-diisopropyl phosphorothioate
c: S-4-chlorobenzyl diethylthiocarbamate
d: 2,4,6-trichlorophenyl 4′-nitrophenyl ether

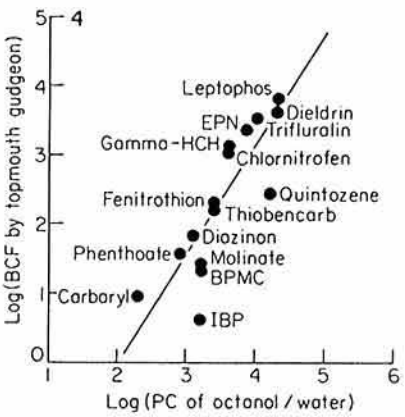


Fig. 4. Relationship between the partition coefficient (PC) for 15 pesticides and their bioconcentration factors (BCF) for topmouth gudgeon; $r=0.843^{**}$

log of the WS, which extends to more than six orders of magnitude for the WS (10 to $10^6$) and four orders of magnitude for the BCF (10 to $10^4$). The regression equation is as follows:

$$\log BCF = 4.68 - 0.59 \log WS \quad \cdots\cdots(1)$$

The correlation coefficient was $-0.784$, which is significant at the 1% probability level.

The log of the BCF is shown in Fig. 4 as a function of the log of the PC.

A satisfactory linear relationship was also observed between the log of the BCF and the log of the PC, extending over more than three orders of magnitude for the PC ($10^2$ to $10^5$) and four orders of magnitude for the BCF (10 to $10^4$). The regression equation is as follows:

$$\log BCF = 1.53 \log PC - 3.03 \quad \cdots\cdots(2)$$

The correlation coefficient was 0.843, which is significant at the 1% probability level. Therefore, it is possible to predict the bioconcentration potential of pesticides by means of these two indices.

More recently, the author found a significant positive correlation between molecular weight of pesticides and their BCF in fish. In

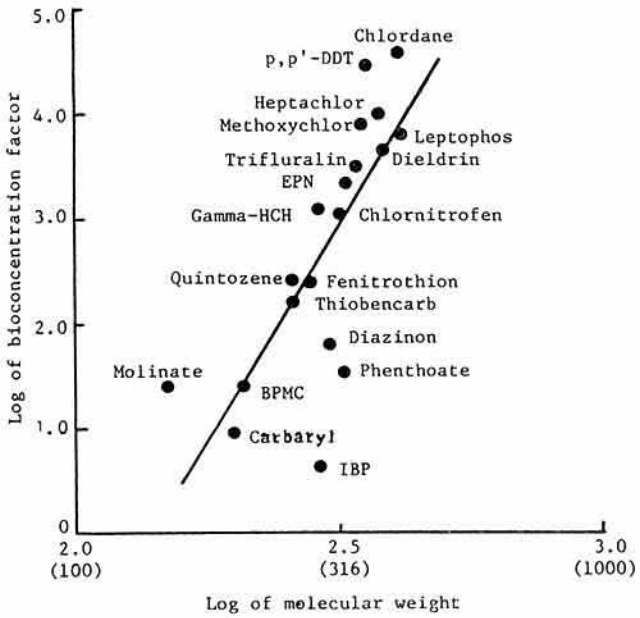


Fig. 5. Relationship between the molecular weights of some pesticides and their bioconcentration factors by topmouth gudgeon and fathead minnows
Parenthesis shows an integral number.

addition to 15 pesticides shown in Table 1, the BCF of four reference pesticides such as p,p'-DDT, methoxychlor, heptachlor and chlordane, in fathead minnows (*Pimephales promelas*) reported by Veith et al.[8] were used to examine relationship between BCF and MW.[5]

A plot of log of the BCF vs log of the MW is shown in Fig. 5. With a total of 19 pesticides showing a wide range of structures, it was found that the BCF increased as the MW increased from 187 to 412. A satisfactory linear relationship is observed between the log of BCF and log of MW. The regression equation is:

$$\log \mathrm{BCF} = 10.9 \log \mathrm{MW} - 24.2 \quad \cdots\cdots\cdots\cdots (3)$$

The correlation coefficient was 0.846, which was significant at 1% probability level. Thus, it was shown that the bioconcentration potential of a pesticide can be predicted by its MW.

The result of this study suggests that the lipid solubility of chemicals related to the passing of the chemicals across the gills of fish is more influential to bioconcentration than the structure of molecule itself. However, as pointed out by Zitko and Hutzinaer,[10] the uptake of chlorinated paraffins by Atlantic salmon from water takes place only up to a molecular weight limit of 600. Therefore, the correlation obtained by this study may exist only within the range of the pesticides tested.

**Table 2. Comparison between the observed and calculated BCF of various pesticides**

| Pesticide | Observed BCF | Calculated BCF |
|---|---|---|
| Gamma-HCH | 1,246 | 376 |
| Dieldrin | 4,430 | 3,768 |
| Diazinon | 152 | 60 |
| EPN | 2,346 | 717 |
| Fenitrothion | 246 | 172 |
| Leptophos | 6,058 | 3,782 |
| Phenthoate | 36 | 25 |
| Carbaryl | 9 | 3 |
| BPMC | 26 | 68 |
| IBP | 4 | 77 |
| Quintozene | 238 | 2,660 |
| Thiobencarb | 170 | 161 |
| Chlornitrofen | 1,109 | 389 |
| Molinate | 26 | 77 |
| Trifluralin | 3,142 | 717 |

## Prediction of BCF from PC

BCF of 15 pesticides actually measured with topmouth gudgeon and those calculated from PC by using the equation (2) are shown in Table 2.

The variation of calculated BCF values from observed BCF values was as much as one or two orders of magnitude. Nevertheless, such a degree of variation does not invalidate calculated values for this parameter, PC, as a useful tool for preliminary potential bioconcentration assessment. Moreover, BCF in fish can also be estimated by using other parameters such as WS and MW in a similar manner as PC.

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