THE USE OF FISH AS TEST ANIMALS FOR THE STUDY OF INSECTICIDES

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SOME of the most noteworthy of modern insecticides of plant origin were originally used as fish-poisons by primitive tribes in various parts of the world. Consequently, the use of fish as test animals for insecticides is quite obvious. However, the method does not seem to have been so popular. Pittenger and Vanderkleed[1] were the first to employ this method and they found that gold-fish was a suitable test-animal for the assay digitalis preparations. Powers[2] and subsequently Gersdorff[3] used these fish for toxicological studies of other substances. The former observed in his experiments the death-point of the fish at which complete cessation of movements occurred, and he noted as the survival time, the time interval between addition of the fish-poison to the fish-tank and the death point of the fish. Plotting the survival times as ordinates against the concentrations of the test solutions as abscissæ, he obtained a curve which was logarithmic in function and whose middle portion approached an equilateral hyperbola. Further details of the calculation of toxicity are given in the paper of Gersdorff. According to them phenol, potassium cyanide and rotenone have respectively the toxicities 0·0008, 0·16, and 4. Surprisingly these values indicate that rotenone is 25 times as toxic as potassium cyanide and the latter is 200 times as toxic as phenol to fish.

More recently, in connection with their investigation on natural coumarins, Spaeth and his collaborators examined the effect of a number of synthetic and natural coumarins using tropical fresh-water fish, known as *Lebistes reticulatus* (Spaeth[4]). For purposes of comparison they noted the concentration of substances necessary for causing death within a time of 8 hours. This is obviously meant as a very rough comparison.

In the course of our work on insecticides, there was need for a rapid and reliable method for comparing the toxicities of natural and synthetic compounds. The procedure adopted by Spaeth and collaborators is not reliable, and details are lacking. The common time interval employed by them seems to be too long. In the procedure of Powers used subsequently also by

Gersdorff, the death-point was employed. We noticed that this is rather indefinite, however carefully the mouth and gill movements be followed. These movements persisted for a long time after the fish sank to the bottom. In some cases fish that were given up for dead came back to life in about an hour when removed to fresh water. Consequently some other criterion which is more well defined had to be sought for. As noted by Gersdorff, with toxic doses the activity of the fish gradually diminished and there was apparent difficulty to preserve equilibrium. Next they overturned and started swimming upside down and after a little while they sank to the bottom and lay on their backs. The stage at which they get overturned seems to be fairly definite and could be taken as a sign of toxicity. By using this point, there are several advantages; (1) there is definiteness in the record, (2) the period of the experiment becomes shorter and (3) the fish could be revived by transfer into fresh water and consequently could be used again for the experiments after the lapse of a few days.

When the above 'turning-point' is employed as a criterion of toxic effect and the time interval plotted on a graph against concentrations of the solutions, a curve very similar to that of Powers is obtained, thus indicating that the new procedure yields results as valid as those of previous authors.

With a view to effect simplification in the technique, we have determined the concentration for each substance corresponding to a 'turning-time' of 10 minutes. This interval has been chosen since with most substances the point falls within the 'hyperbolic' portion of the curves and hence in the region where the time interval is inversely proportional to the concentration. Outside this portion of the curve the relation obviously does not hold good due to various factors that affect this biological property. Consequently the reciprocal of the concentration employed for each substance within this range gives a measure of its toxicity.

In order to facilitate comparison there is need for a standard and a system of expressing toxicity in terms of units. As the most easily available and the most well-known of insecticides, rotenone is chosen, and the standard preparation containing CCl_4 of crystallisation (B.D.H.) is employed. The toxicity is expressed as fish units and that of rotenone is arbitrarily fixed as 1000 'fish-units'. This is a convenient number since otherwise the toxicities of other substances which are much weaker have to be expressed in terms of inconveniently small fractions.

The fish employed for these experiments have to be of the fresh-water type and they should be adapted to still water conditions. Gold fish come under this category. A few varieties of fresh-water fish obtainable in the neighbourhood of Waltair were examined and among them one variety was found to be very suitable. These fish are very handsome and when fully grown measure about 1½ inches in length and weigh about 700–800 milligrams. They have been identified as *Haplochilus panchax*, a common larvicidal fish belonging to the family 'Cyprinodontidæ'. On an average six fish were employed for each experiment.

As the substances to be tested were almost insoluble in water, they were as a rule dissolved in alcohol and a measured volume of the solution poured into a measured amount of tap water. The amount of alcohol thus added was such that it usually did not exceed 1%, which according to blank experiments was not found harmful. A number of fish, usually six were placed in the solution and the turning point for each was recorded. The average for all the fish was taken as the 'turning-time'.

The actual procedure for the determination of the toxicity may be described as below. Using a number of concentrations the corresponding turning-times are observed and the results are plotted on a graph. From this graph, the concentration corresponding to 10 minutes turning-time is obtained and this is taken as the 'toxic concentration'. For rotenone it is found to be 0·2 mgm. per litre. Toxicity of a substance is proportional to the reciprocal of the toxic concentration. Consequently the following equation is obtained:

$$\frac{\text{toxicity of substance}}{\text{toxicity of rotenone}} = \frac{0 \cdot 2}{x}.$$

The toxicity (T) as defined above is then obtained from the following formula $T = \frac{0 \cdot 2}{x} \times 1000$ fish-uits (toxicity of rotenone) where x is the toxic-concentration of the substance. When plotting the results it could be seen if the 10 minutes interval falls in the region of sensitiveness. The results will not be quite reliable, if the point be far outside this limit.

The following table gives the results obtained in a few typical cases of organic compounds:

TABLE I

Name of Compound	Toxic Concentration in mgm. per litre	Toxicity in fish-units
(i) Rotenone ..	0·2	1000
(ii) Karanjin ..	6·6	30
(iii) Psoralen ..	38·5	5
(iv) Angelicin (Isopsoralen) ..	6·8	29

The number of compounds examined do not warrant any generalisation regarding the influence of chemical constitution on toxicity. But a probable inference is that pyronofuran derivatives of the angular type (i, ii and iv) are definitely more powerful as fish poisons than the linear type (iii). It is expected that by the adoption of the above procedure the toxicity determinations made by different workers and made with the use of other fish may not vary much. The values may be expected to be independent of these variations.

Experimental

Rotenone.—A standard solution of rotenone (10 mgm. in 50 c.c. of rectified spirits) using a sample supplied by B.D.H. was prepared. Measured volumes of this solution were added to different troughs, containing a measured amount of tap water (1 litre). The turning-points were determined in the case of six fish at each of these concentrations and the intervals of time elapsing between the commencement of the experiment and of the turning upside down of the fish were noted. At each concentration the mean of the time-intervals of the fish was calculated and the figure used in plotting the graph. The toxicity-curve thus obtained was a rectangular hyperbola (Table II).

TABLE II

Temperature of experiment : 31·5° C.

Concentration in mgm. per litre	Mean turning-time in minutes
0·065	75
0·130	26
*0·260	9·0
0·660	7·0
1·300	6·0

TABLE III

Strength of standard solution: 20 mgm. in 25 c.c. rectified spirits

Temperature of experiment : 32·6° C.

Concentration in mgm. per litre	Mean turning-time in minutes
2	125
4	17
6	11
*8	9·0
12	5·5

* With concentrations higher than the toxic concentration it is possible to measure turning-time correct to half a minute.

Toxic concentration from the graph: 0·2 mgm. rotenone per litre. This was confirmed experimentally as follows. To a trough containing 1 litre of tapwater, 1 c.c. of the above rotenone solution (1c.c. = 0·2 mg.) was added. Six fish were put into the trough and the mean turning-time determined; it was found to be in agreement with expectations (*i.e.*) 10 minutes.

In Table III are given the results obtained with karanjin, as another illustration of the method employed.

Toxic concentration from Graph is 6·6 mgm.

$$\text{Toxicity} = \frac{0\cdot2}{6\cdot6} \times 1000 = 30 \text{ fish units.}$$

Summary

A simple procedure employing fish is described for determining the toxicity of chemical compounds. The 'turning-point' is taken as the criterion of toxic effect and the toxicity is expressed in terms of fish units that of rotenone being arbitrarily fixed as 1000. A few organic compounds belonging to the group pyronofurans have been studied.

REFERENCES

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