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Behavior of the Goldfish As an Early Warning System For the Presence of Pollutants in Water*

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ABSTRACT

After reviewing the literature on the effects of pollutants on fish (particularly goldfish), an experiment is presented showing the effects of two very low concentrations of mercury on the behavior of goldfish. The goldfish were trained to press a lever on various schedules of reinforcement in control clean water and in two concentrations of mercury-polluted water. A reduction in rate of response followed exposure to polluted water (a reduction that exceeded that produced by clean water), thus suggesting the goldfish as a potential monitor of pollution.

Introduction

The purposes of this paper are (1) to present a selective review of the literature on the effects of pollutants on fish, with special attention given to

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goldfish, in terms of lethal dosages and in terms of behavioral changes, and (2) to describe some new data demonstrating that goldfish show marked behavioral changes due to small concentrations of pollutants. We shall present the argument, based on the analysis of the data collected, that goldfish might well constitute rather efficient monitors of our environment.

There is no question about the significance of the water pollution problem. The amount of pollution is huge and in many cases continually increasing, while our knowledge about the effects is not increasing fast enough to make effective inroads into the problem. As Berg [1] indicated, mercury pollution was detected only through the catastrophe of the human deaths in Japan and the death of birds (pheasants, hawks, and eagles) in Sweden. Even though the Swedes acted quickly and decisively to clean up the environment by banning many uses of mercury, some Swedish lakes will not be usable for fishing this century!

The discovery of better methods for the detection of pollutants before a catastrophe occurs is mandatory. In an article aptly titled, "Building a Shorter Life," McCaul [2] describes the effects of cadmium, whose use is twice as great as mercury for various technological processes in the environment, to consist of earlier aging, arterial hypertension, and severe degenerative bone disease. In the United States, McCaul estimated, some 4.6 million pounds of cadmium are emitted into the atmosphere each year. Carroll [3], in a study of cadmium and cardiovascular disease death rate, showed that the two are indeed very much related to one another.

In a recent review of the effects of polychlorinated biphenyls, PCB, Hammond [4] found that most Americans have some residues of PCB, with 33% of more than 600 samples of human adipose tissue having at least 1 ppm. Here, too, the Japanese were the unfortunate dicoverers of the pollutant; some 1000 Japanese who had eaten rice-oil contaminated with PCB suffered from darkened skin, eye discharge, severe acne, and other symptoms of what has come to be called Yusha oil disease; some 3 years later they still suffered from the symptoms. Hammond goes on to say: "Their PCB's presence and persistence there reemphasizes the likelihood that any widely used industrial chemical may become an environmental pollutant . . ." [4]. The number of examples of human catastrophe resulting from the belated detection of a pollutant could easily fill a book if not more.

In 1971 Robert Risebrough reported before the Committee on Commerce of the U.S. Senate that the danger of PCB cannot be underestimated since increases of PCB in fish in the coastal waters might well destroy the use of those fisheries for human consumption. The point is that while one can so far assume that most of the PCB is still on the land, some of it will indeed go into the atmosphere, sewage systems, and rivers.

Many pollution studies using animals have appeared by this time, particularly toxicology studies. Their use is certainly called for, but by relying on death as an index of the effect of the substance involved, they are not sufficiently sensitive to detect subtle or early effects. For that reason this paper presents behavioral indexes of pollution as well.

Changes in our environment are often so precipitous as to require continuous surveillance of the water in order to detect the presence of a pollutant early and before it has caused too much damage. Wolman [5] laments the current state of surveillance when he says "Observational programs appear to be particularly weak with regard to the detection of subtle initial changes from a natural to a polluted condition."

Literature Review

TOXICITY STUDIES

The most widely used method for the study of the effects of pollutants on marine life consists of establishing the dosage that produces lethal toxicity. A lethal dosage (LD) is the level of concentration that causes death in fish after an indefinite exposure time (a relatively long period), or a high concentration that causes death within a brief period of time (1 to 6 hours). One of the indexes of lethal toxicity used generally in drugwork is the LD-50 [6]. It is defined as that dosage which causes death in 50% of the experimental animals. This kind of index provides a gross measure of effect but it is only the first step in the evaluation of any drug or pollutant.

Much of the discussion to follow is based on reviews by Doudoroff and Katz [7]; Doudoroff [8]; and Katz, Sjolseth, Anderson, and Tyner [14]. Doudoroff and Katz [7] state that death by pollution is caused by "coagulation or precipitation of mucus secreted by the gills." The formation of insoluble metal-protein compounds apparently produces death by suffocation.

The reviews discussed here summarize selected parts of the literature on the effects of metal pollutants, and extremes of temperature, pH, salinity, and osmotic pressure as they interact with various pollutants to cause death. Individual metals are listed alphabetically. Attention is given to lowest toxic concentrations, and, where deemed important, interaction effects are mentioned.

Aluminum. Aluminum salts were found to be toxic to fish in quantities ranging from 0.1 ppm to 100 ppm. Aluminum nitrate was found to be fatal in very soft tap water (at pH 6.0 to 6.2) to the stickleback. Goldfish could survive for 7 days in moderately hard tap water containing 100 ppm aluminum sulfate (at pH 5.6) but they died at 0.5 ppm with tap water at pH values of 7.2 to 7.4.

Barium. Barium nitrate was found to be toxic to sticklebacks in soft water at concentrations in the neighborhood of 500 ppm.

Cadmium. Concentration levels ranging from 0.001 ppm to 0.3 ppm have been shown to be fatal to some fish. Goldfish were able to survive only 8.7 to 18 hours in the 0.001 ppm concentration. According to a recent U.S. Geological Survey Report (USGS Water Supply Paper 1879G) cadmium and chromium wastes from a World War II airplane factory in Long Island are polluting groundwater of part of Nassau County, N.Y. Although no visible danger to drinking water exists in the area at this time the pollutants could move. The adverse effect of cadmium given in small doses to rats has been demonstrated to produce hypertension [10]. The significance of cadmium in water supply systems in the United States was pointed out by McCabe et al [11]. In a few cases the water coming out of the consumer's taps were in excess of the U.S. Public Health Service mandatory limit. In a survey of rivers and reservoires [12] 4% exceeded the maximum allowable standard. The various effects of cadmium on human beings are summarized in McCaul [2].

Calcium. Calcium added to distilled or soft waters is toxic to sticklebacks at concentrations varying from 300 to 1000 ppm. Fish have survived for 1 to 3 or more days at concentrations equivalent to 2500 ppm.

Cobalt. Cobalt chloride and cobalt nitrate were found to be toxic to fish in fresh water at concentrations of 7 to 15 ppm. However, there appears to be much variation in the lowest effective levels. Some investigators have found levels in the range of 100 to 1000 ppm. Water hardness was considered an interacting factor. Soft water concentrations of 100 ppm were found to be fatal to goldfish.

Copper. Hard, alkaline water contributes to fish resistance to copper salts in quantities greater than 1 ppm. Usually, however, concentrations between 0.01 and 0.02 ppm are fatal to goldfish in natural water.

Iron. Iron, although frequently investigated, has not yielded consistent toxicity values. The literature indicates an interaction effect between pH values and concentration levels. Goldfish, surviving concentrations of 100 ppm of ferric chloride in hard water, at pH 5.5, and 10 ppm in very soft water, at pH 5.0, for more than 4 days, were killed within 80 minutes by a concentration of 100 ppm in the soft water, at pH 3.4.

Lead. Goldfish appear to be more tolerant to lead than other species, surviving concentrations of 1 ppm indefinitely in very soft water. On the other hand, concentrations of 0.1 to 0.4 ppm were toxic to sticklebacks in distilled and soft waters. Evidence for cumulative poisoning of fish with insoluble lead sulfide was suggested by some long term experiments with goldfish.

Lithium. Higher temperatures were responsible for faster deaths of goldfish (less than 24 hours) when concentrations of lithium ranging from 320 to 620 ppm were used. At moderate temperatures, the range of fatal concentrations ranged from 320 to 400 ppm.

Magnesium. Concentrations from 100 to 400 ppm were found to be toxic to sticklebacks in distilled and tap waters. Some freshwater fish have been found in very saline lake waters containing over 1000 ppm of magnesium, as well as much sodium and calcium.

Manganese. The toxicity concentration range of manganese was found to vary from 50 to 5500 ppm depending upon the water composition and the kind of fish used as subjects. Sticklebacks were susceptible to concentrations of 50 ppm in soft water. At the other extreme, concentrations of 5500 ppm were found to be lethal to eels.

Mercury. Goldfish were killed within 6 days by 1.0 ppm mercury in alkaline water (pH 7.7). Other fish could not survive concentration levels near 0.01 ppm in soft water.

Potassium. Potassium concentrations ranging from 50 to 200 ppm were toxic to sticklebacks in soft tap water. Toxicity was closely tied to exposure duration and lower levels may prove fatal with longer exposure than those used.

Silver. Silver nitrate was toxic to sticklebacks in soft water at concentrations in the neighborhood of 0.004 ppm.

Sodium. Concentrations between 500 and 1000 ppm were toxic to sticklebacks in distilled and very soft water. Fresh water fish were less tolerant than salt water species. Increased water hardness increased resistance to sodium salts. Concentration levels between 1500 and 2000 ppm were found to be fatal to fresh water fish in harder, alkaline waters.

Strontium. Goldfish survived for 4 days at concentration levels of 3200 ppm. Lower toxicity levels around 1500 ppm were fatal to other species.

Tin. Young eels withstood concentrations of 1.2 ppm for approximately 50 hours. Concentrations as high as 6 ppm proved fatal in 2.8 hours.

Zinc. Experimenters have reported toxicity levels ranging from 0.13 to 100 ppm for various zinc salts. Species, types of water, and water makeup determine the lethal concentration. Concentration as high as 1000 ppm were necessary to kill goldfish in hard water (pH 7.6). A more recent study [13] showed soluble zinc to be much more dangerous than insoluble zinc.

Doudoroff [8] and Katz et al. [14] also reviewed the effects of pesticides on marine life. It was found that 1 ppm DDT was fatal to 50% of the goldfish exposed to the toxic concentration for 2.5 hours. Chlorinated camphene, known as toxaphene, which is an organic pesticide, was fatal to hardy goldfish in concentrations as low as 0.005 ppm.

BEHAVIORAL STUDIES

This section will review studies dealing with the effect of sublethal dosages of pollutants on behavior. The first category of such behavioral measurement consists of the monitoring of instinctive behaviors under relatively "natural" conditions, i.e., where a minimum of experimental intervention is employed.

Examples of such behaviors are movement patterns and rates shown by Cairns [15], Shirer, Cairns, and Waller [16], Waller and Cairns [17], and Cairns, Sparks, and Waller [18] to be sensitive to pollution by zinc, feeding rates shown by Cairns and Loos [19] to be affected by zinc and other pollutants, and breeding behavior shown by Foster and Cairns [20] to be influenced by alkyl benzene sulfonate. Finally, Scheier and Cairns [21] used the optokinetic response of following moving stripes as an index of degree of parathion pollution.

Other approaches to the behavioral study of pollutants have consisted of three basic experimental paradigms: the preference paradigm (swimming away from areas containing the pollutants), conditioned reflex (classical conditioning) paradigm, and the operant conditioning paradigm.

Preference Studies. In the typical preference study the concentration of the pollutant is maintained at one end of the tank; clear water is at the other end. The fish is introduced into the tank and his behavior is observed. Frequency of visit and length of time spent in the polluted end of the tank serve as the measure of avoidance.

Sprague [22] used the same apparatus to investigate the avoidance of copper or zinc solutions by young salmon. Fish were acclimated to the trough for 30 minutes before the pollutant was introduced. The fish's behavior was monitored throughout the testing. The salmon actively avoided both substances even at very low levels of concentration.

In a similar experiment Sprague [23] investigated the effects of zinc sulphate on rainbow trout. The apparatus and procedure were the same as in the earlier experiment. The rainbow trout, like the salmon, showed increasingly stronger avoidance behavior to increasing concentrations of zinc sulphate.

Conditioned Reflex Studies. In the conditioned reflex paradigm, shock was the unconditioned stimulus (US), swimming from one end of the tank to the other was the unconditioned response (UR), and presentation of an unusual hydrogen ion concentration, salinity, temperature, or pressure served as the conditioned stimulus (CS).

Medved et al. [24] reviewed the literature on effects of insecticides on the conditioned reflex in vertebrates, particularly cats and rats. Organophosphate pesticides and other such poisons produce changes in the higher nervous system activities of the organism, as shown by inhibition of the conditioned reflex. Of particular interest is the fact that changes in the conditioned reflex were found at doses too small to affect the liver function or carbohydrate metabolism. A later review on the effect of insecticides on fish behavior was written by Katz et al. [14]. It also showed that the site of action was in the central nervous system of the fishes. To take but one example, goldfish exposed to 1 ppm DDT for 2.5 hours showed a change in spontaneous electrical activity of the cerebellum.

Bull [25], making extensive use of the conditioned reflex paradigm as outlined above, investigated the effects of hydrogen ion concentration, salinity, temperature, and pressure changes on the conditioned reflex in fish. Bull's findings demonstrate the extreme sensitivity of the fish to its environment. To take but two examples, Bull reported that it took *Spinachia vulgaris* Flem. only 41 associations to produce a conditioned response to an increase in temperature of .05°C; *Gobius flavescens* Fab. required 14 associations to respond to a minimal change of .05°C.

Initial conditioning was to very large temperature changes; the final temperature changes used in the discrimination procedures were of course considerably smaller. In some cases, fish are sensitive to temperature changes as small as .03°C. With respect to pH, Bull found that a reduction of 0.04 to 0.10% could be detected by 20 different species of fish. Again, it must be noted that this is the end point of generalization tests (after differential reinforcement) and that original changes were much larger. Using the same sample of fish as above, Bull noted that initial changes of 34 to 30% salinity were discriminated and that after discrimination training, conditioned responses occurred to changes as small as 0.5% in the salinity.

Anderson and Prins [9] conditioned brook trout to exhibit the propeller-tail reflex. Electric shock served as the US and light as the CS. Prior to conditioning, one-half of the fish tested were exposed for 24 hours to sublethal DDT (20 ppb). Ten of the 16 DDT-treated fish failed to become conditioned after 100 trials and the remainder took 60 or more trials with an average of 76 trials. Of the 16 fish in the control group, 14 took no more than 50 trials.

Operant conditioning studies. Operant responses consist of the emission of behavior that acts on the environment. Most often, in behavioral experiments, such behavior activates a food magazine that dispenses a small amount of food. In this situation, the food is called a positive reinforcement since its occurrence immediately after the animal's response strengthens its behavior, as manifested in its increased frequency of making such responses. The other large class of operant conditioning experiments makes use of negative reinforcement. Here the animal's behavior results in the cessation or prevention of the occurrence of electric shock or other negative reinforcement. The reinforcing effect stems from the fact that the animal escapes from or avoids an aversive event. The reinforcing effect is demonstrated by the increased frequency of occurrence of responses removing the aversive events.

Rozin [26] conditioned goldfish to respond on a fixed interval (FI) 1 minute schedule. Fish were free to respond any time but were only reinforced (by food) for the first response at the end of each 1 minute interval. After stable behavior was reached, the ambient temperature in the fish's tank was reduced by 10°C, then increased once more to the original temperature while the conditioning continued. The overall response rate increased with an increase in temperature but the relative rate of response over the course of each minute remained unchanged. The conclusion reached by Rozin was that the patterning of responses in a temporal discrimination is independent of temperature even though overall rate varies.

Weir and Hine [27] trained goldfish to make an avoidance response to a warning light which was followed by an electric shock, unless that avoidance response occurred. After consistent avoidance responses were established, the fish were exposed to various pollutants for periods of 24 and 48 hours. Concentrations at which statistically significant behavioral impairment was obtained were as follows: arsenic 0.1 ppm; lead 0.07 ppm; mercury 0.003 ppm; selenium 0.25 ppm. Weir and Hine point out that the concentration of lead is at a level which approximates potable water standards and that no traces of lead were found in the tissue of the affected fish.

An Experiment

The literature reviewed has shown that behavior is sensitive to pollutants of smaller concentrations than is the usual physiological index, and particularly the lethal dosage index. Furthermore, the area of behavioral pharmacology has shown the superiority of behavioral studies over pharmacological ones with respect to many drugs [6, 28]. The behavioral experiment [27] most relevant to this one found a change in behavior after 24 and 48 hours. Unfortunately, electric shock administered in such avoidance conditioning experiments produces somewhat variable results (making them less useful than they might otherwise be), since magnitude of shock received depends in part upon the orientation of the fish with respect to the electrodes [29, 30]. The resultant greater variability requires the use of groups of fish thus making monitoring less economical.

The object of this experiment was to investigate the effectiveness of appetitive operant conditioning paradigms (where the animals' behavior is controlled by food) in making manifest the effect of small concentrations of mercury in water inhabited by goldfish for very brief periods of time.

Four different behavior patterns were investigated in order to find which pattern is most sensitive. Twelve goldfish (Carrasius auratus), deprived of food for periods of 48 hours, were conditioned according to the following schedules:

1. *Fixed ratio.* Three goldfish were trained until they struck a movable target 10 times for each reinforcement consisting of a tubifex worm.
2. *Fixed interval.* Three goldfish were trained to strike the target, receiving a reinforcement for the first response emitted after each successive period of 30 seconds.

3. *Extinction.* Three goldfish were trained to strike the target on a fixed ratio of 10:1 as in 1 above; after reaching stable behavior, the fish were extinguished, that is, the fish received no further worms. Instead, every tenth strike of the target was following only by the solenoid noise of the worm dispenser and the dropping of water earlier associated with the delivery of the worm. In other words, these fish were extinguished with the use of conditioned reinforcers [31].
4. *Discrimination.* Three goldfish were trained to strike the target in the presence of one stimulus (a red or a green light), called the discriminative stimulus, S^D, and not to strike the target in the presence of the other stimulus (a green or a red light, respectively), that is, the S^Δ. Every eighth response emitted during S^D condition yielded a worm but there was no consequence for responding during the S^Δ condition.

Each of the goldfish (ranging in weight from 29 to 52 grams) was housed individually in a 10-liter tank with a filter. Before the experiments began, all goldfish were fed the tubifex worms individually so that they learned to swim up to get the worms as they floated down in the tank. With the exception of those that struck the target spontaneously within a short period of time, they were trained in target-striking behavior by the method of approximation conditioning (shaping).

When the goldfish started to respond, they were reinforced on a continuous schedule of reinforcement until their response rate was high enough to warrant increasing either the time between reinforcements or the number of nonreinforced responses. This process was continued until they reached the various levels of schedule described above.

After the behavior of each goldfish was stabilized on its respective schedule of reinforcement, three goldfish in each condition were randomly selected. Two were placed in polluted water, one in a high concentration of $HgCl_2$ (.01 parts per million), and one in a low concentration of $HgCl_2$ (.006 parts per million). The third goldfish in each of the conditions was placed in a new tank of unpolluted water. Each fish remained in the new tank for one hour and then was tested in that tank (clear water, high pollution, or low pollution) for a half hour. The placement of some of the goldfish into a new solution of unpolluted water served as a control for the effect of transferring the organism into a new environment.

The results were as follows: All four goldfish placed in the highly polluted water showed the largest percentage drop in response rate. Furthermore, all the goldfish but one showed the second largest decrease in the low pollution water. Figure 1 presents the data in terms of percentage change, i.e., rate during the experimental condition, R_e, ($R_e = 0$, .006, or .01 ppm mercury) minus the control rate, R_c, divided by the control rate $(R_e - R_c)/R_c \times 100$. The fish on the fixed ratio schedule of reinforcement showed the greatest amount of differentiation between polluted and clean water. Like the fish on he FI schedule, the FR-fish showed little change in response when in a new unpolluted environment, indicated by the "O" point. On the other hand, the fish trained on the discrimination procedure showed a drop greater than 30% simply as a consequence of being in different water, a drop almost as great as that found for the fish in extinction. Although this will need to be checked with additional animals, it suggests that an animal in a discrimination task may be more attuned to the environment and, therefore, its behavior more easily disturbed, than an animal on a simple response schedule. As for the fish tested under the extinction condition, both values of pollution show a drop in rate beyond that produced by extinction alone. In general, the fish on FR10:1 appear to provide the greatest sensitivity to the polluted water, at the same time being uninfluenced by new clean water.

Figure 2 presents the cumulative number of responses over a half hour on

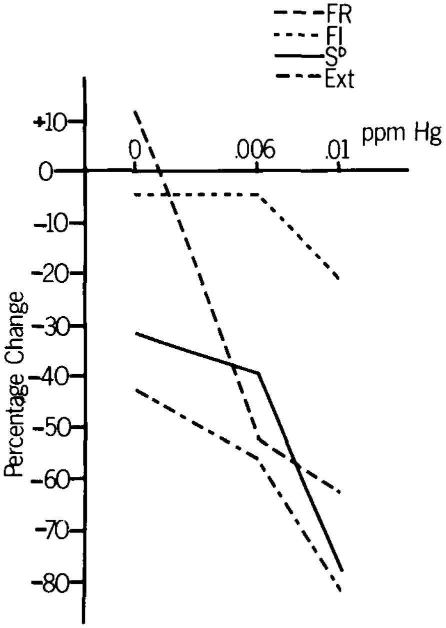

Figure 1. Percentage change in response rate between the control (before) and the experimental (after) water condition for 3 fish on an FR 10:1 reinforcement schedule, for 3 fish on an FI 30 seconds reinforcement schedule, for 3 fish on an FR 8:1 reinforcement schedule during the S^D condition of a discrimination procedure (responses during S^Δ were negligible), and for 3 fish tested during extinction after having been trained on an FR 10: 1 reinforcement schedule.

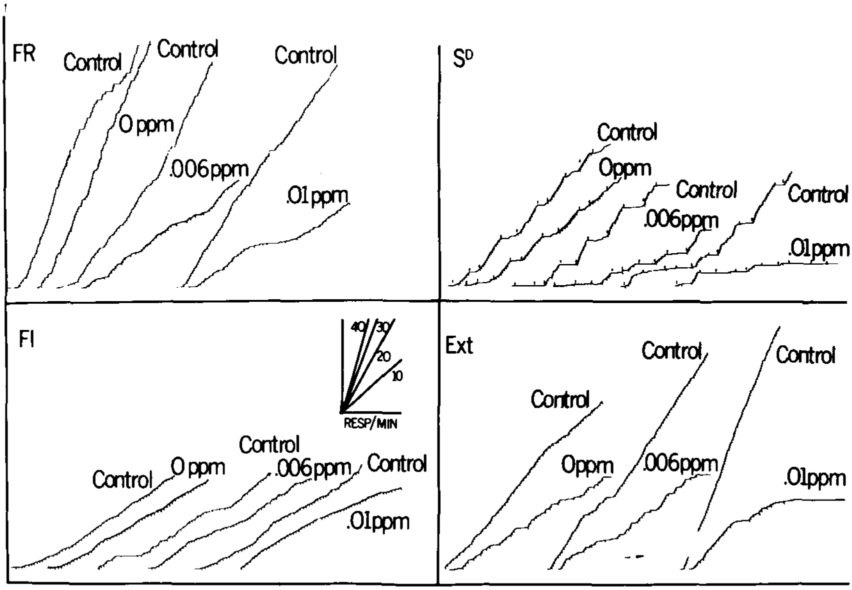

Figure 2. Cumulative number of responses for fish tested under various reinforcement contingencies (FR 10:1, S^D condition-FR 8:1 and S^Δ-no reinforcement, FI 30 seconds. Extinction following FR 10:1) under control (clean water) conditions, in different clean water, and under two concentrations of mercury pollution (.006 ppm and .01 ppm of mercury). The three sets of curves corresponding to the discrimination condition contain curved lines ‘ ’ to signify S^D periods and ’ ‘ to signify S^Δ periods.

the different schedules of reinforcement for individual fish under various conditions of pollution. Note that, as in Figure 1, the changes taking place are greatest for the fixed ratio schedule of reinforcement and least marked (but still clear) for the fixed interval reinforcement schedules.

Future research will have to make clear why the fixed interval schedule of reinforcement is least sensitive to the effects of pollution. One possible interpretation is that it requires less work than the fixed ratio schedules used for the other fish. Inspection of Figure 2 shows that the control rate of response is considerably less for the fixed interval than for the fixed ratio fish.

The results are very interesting since the behavior of these goldfish appears to be influenced even after as short a period of time as one hour of pollution exposure (plus a half hour during which the data are collected) and, therefore, the goldfish appears to be a rather good animal for such monitoring. Although this experiment dealt with the behavioral effect of mercury only, the findings suggest the possibility of using goldfish to alert authorities early enough (i.e., before the human population is harmed) concerning a pollutant not ordinarily present or expected in a water supply, river, or lake. Since chemical tests must be for known specific substances, they would fail to detect the presence of unexpected material. The behavioral tests, on the other hand, if subsequent experimentation shows them to be generally sensitive to other pollutants as well, would have the advantage of alerting the inspector to the fact that a new substance is in the water. Following a general alert concerning a foreign substance, chemical analyses could take place to learn precisely what the foreign substance is. Although the foreign substance need not be toxic on an immediate basis, that is, have an immediate deleterious effect, it would be important to know that a new substance is in the water supply since it may have long range or cumulative effects. Like the canary in the coal mines, the goldfish may well turn out to be a worthwhile companion to man as he tries to protect himself against a hostile environment.

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