THE
ONTARIO WATER RESOURCES
COMMISSION

REPORT ON

THE AUTOPSY OF FISH COLLECTED IN FISH KILLS

801 BAY STREET - TORONTO

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By

Yvonne H. Swabey

Biology Branch

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THE AUTOPSY OF FISH COLLECTED IN FISH KILLS

In the event of a fish kill the usual approach to determining the cause is chemical analysis of the water where the kill occurred. If water samples are taken while the fish are dying, chemical analyses can reveal oxygen lack or the presence of a toxicant. Too often, however, chemical tests are futile. When the kill has been caused by a "slug" of deoxygenating material or toxic industrial waste which moves downstream, late reporting of the incident can render analysis of the water a useless method of detecting the cause.

The question arises, therefore, as to the possibility of determining the lethal agent by examination of the victim: the fish. This possibility was investigated by a search of pertinent literature and consultation with authorities in related fields.

Sources of Information

Publications searched included:

(1) Water Pollution Research, 1960, 1961.

(2) Progressive Fish Culturist, 1934 - 1964.

(3) Fish and River Pollution (Jones, 1964).

(4) Journal of the Water Pollution Control Federation, literature reviews, 1958-1964.

(5) References relating to the effect of pollution on fish and other aquatic organisms. (Ide and Yamazaki, n.d.).

Authorities consulted by correspondence and/or personal interview were:

Dr. A. Dechtiarenko,
Research Branch,
Ontario Department of Lands and Forests,
Maple.

Dr. William Oliver,
Division of Pharmacology and Toxicology,
Ontario Veterinary College,
Guelph.

Dr. Donald I. Mount,
Fisheries Research Biologist,
Basic and Applied Sciences Branch,
Division of Water Supply and Pollution Control,
R. A. Taft Sanitary Engineering Center,
Cincinnati, Ohio.

Causes of Death

Mortality of fishes may result from natural causes (chemical, physical or biological), mechanical injury and polluting substances.

Rarely do diseases and parasites cause the sudden mass mortality which characterizes a fish kill in a stream or lake, and are therefore of minor importance in this investigation. The symptoms of most common diseases have been documented (e.g. Wood and Yasutake 1956a, 1956b, 1957). While some of these may be evident even to a person untrained in pathology, a definitive diagnosis usually requires a trained histologist or parasitologist. The difficulty stems from the overlap in symptoms displayed by fish dying from various causes.

The common causes of fish kills are a lack of oxygen in the water and exposure to a toxic substance. When a fish dies from a lethal dose of a toxicant distinctive changes occur in certain tissues or organs. The tissues most seriously affected depend upon the nature and mode of action of the toxicant.

Mode of Action of Toxicants

Since fresh-water fish swallow very little water, toxicants generally enter via the gill and are carried throughout the body by the blood. Some absorption may occur also by the lining of the mouth and by the skin (Ellis, 1937). Such toxicants may act upon the blood, nerve synapses, respiratory enzymes or internal organs. Detergents, for instance, affect the lipid structure of cells and the enzymatic systems of respiration (Reimann, 1962). Potentially dangerous chemicals can be assimilated also by ingestion of contaminated food organisms.

If the toxicant can be stored in the body it can accumulate to a potentially lethal level. Continued exposure to minute quantities of certain insecticides, for example, can result in high concentrations in the lipoid tissues without acute effects on the fish. When, however, the fat is catabolized during the winter months, the insecticide is released to the bloodstream and death ensues.

Some toxicants need not enter the body of the fish to be lethal. These include heavy metals and corrosives which clog the gills with coagulated mucous and destroy gill tissues. Such injury inhibits respiration, circulation of the blood and excretion from the gills.

SYMPTOMS OF POISONING

Gross Symptoms

Gross symptoms include those changes in the appearance of the fish which are visible to the naked eye.

A white film on the gills, skin and the mouth is evidence of coagulation of mucous which can be caused by acids, heavy metals, and trinitrophenol (Ellis, 1937). Sloughing of the gill epithelium, a sign of extensive gill damage, occurs upon exposure to certain concentrations of copper and zinc (anonymous, 1961), lead (Carpenter 1930), detergents (Schmid and Mann, 1961) ammonia (Kuhn and Koecke, 1956) and quinoline (Shelford, 1917).

Although turbidity is not often the cause of a fish kill, it is readily detectible by the silt-clogged opercular cavities and gill filaments of the victims (Wallen, 1951). Another instance of gill occulsion is that caused by ferric hydroxide precipitated from pickling wastes (Stundle, 1955).

Phenol poisoning results in too much blood in the gills, giving them a dark appearance (Havelka and Effenberger, 1957; Skrapek,

1963). A similar effect is produced by p-cresol, napthalene and a deficiency of oxygen in the water (Alexander et al., 1935). Shelford (1917) noted, however, that the gills of fish poisoned by phenol or cresol may be either red or pale-coloured. Phenol poisoning may also produce a mucous-containing foaming secretion from the skin (Skrapek, 1963).

Fish killed by cyanide exhibit bright red gills. The distinctive colour is due to changes in the arterial blood induced by inactivation of respiratory enzymes (Alexander et al., 1935).

Distended gill covers have been noted in fish killed by phenol and cresols (Shelford, 1917), ammonia (Jones, 1948) and cyanide (Alexander et al., 1935).

Fathead minnows killed by certain chlorinated hydrocarbon insecticides were observed to have swollen abdomens (Henderson et al., 1959). Easterday and Miller (1963) found that fish exposed to molybdenum developed blue stomachs.

Histological Symptoms

Pathological changes in the cells of fish tissues and blood can be detected by a trained histologist.

The sloughing of gill tissue caused by many toxicants as mentioned previously, is a visible manifestation of extensive cellular damage. In poisoning by copper, lead or zinc cellular effects are

generally similar (Anon. 1961): the epithelium separates from the filaments and cells are sloughed off into the spaces between them. Approximately three-quarters of the epithelium has been destroyed by the time the fish dies.

Schmid and Mann (1961) have reported that the respiratory epithelium is destroyed by concentrations of dodecylbenzenesulphonate greater than 5 ppm. Prolonged exposure to 6.5 ppm ABS produced a thickening of the gill lamellae in fish studied by Lemke and Mount (1963).

According to Wood (1960) a thickening of gill lamellae can be caused also by hydrogen sulphide. He stated that this gas produces a layer of edema under the epithelium which swells the lamellae to a non-functional state.

Unlike copper, lead, zinc and mercury, hexavalent chromium does not produce a significant change in the histology of the gills (Fromm and Schiffman, 1958). It does, however, effect widespread destruction of the intestinal epithelium immediately posterior to the pyloric caeca. These authors postulated that the chromium enters the fish by way of the gills, is partially excreted by the liver and reaches the intestine in bile.

In addition to chromium, the alkaline earth metals do not damage the gills (Jones, 1939). These metals, which include sodium,

pottassium, magnesium, calcium, strontium and barium, are much less toxic than the heavy metals.

The histology of several internal organs can be changed by exposure to insecticides. Karper et al. (1962) observed pathological nerve cell changes in the brain of carp after exposure to DDT, lindane, toxaphene and parathion. Cellular lesions in the liver are produced by 3 ppm DDT (Mathur, 1962a,) by 5 ppm lindane and 10 ppm BHC (Mathur, 1962b). Additional effects of DDT poisoning are atrophy of the kidney and degeneration of the mucous membrane of the intestine.

Fujiya (1961) studied the histology of fish exposed in live-boxes to Kraft process pulp mill wastes. He found that necrosis and desquamation of the intestinal epithelium occurred in those exposed to contaminated water of more than 50 mg/l COD. In addition, effects on the circulatory system of the liver suggested that the mill waste contained something harmful to this system.

Chemical Symptoms

Chemical changes within the body of a fish exposed to a toxicant often involve the accumulation of the toxicant in certain organs. The heavy metals are noteworthy in this respect. Although zinc destroys gill tissues, radioactive zinc tracer studies have shown that it does enter the body of the fish and can possibly act as an internal poison

(Anon. 1958). Joyner and Eisler (1961) reported that zinc accumulates in the vertebral column, head and visceral mass.

In the most pertinent report found in this literature survey, Mount (1964) described an autopsy technique to determine zinc-caused fish kills. The technique is based on analyses which showed extremely high accumulations of zinc in the gill in relation to those in the opercular bone when fish had been exposed to acutely lethal concentrations. It involves chemical analysis of both of these tissues and can be performed on partially decomposed fish.

In view of the similar action of the heavy metals, it would be expected that the gills might provide evidence of kills caused by copper and lead also. Mount (personal communication) has found significantly higher concentrations of copper in the gills of fish killed by this metal than would be expected from accumulation due to sublethal exposure. There is evidence also that during sublethal exposure copper accumlates in the liver. Autopsy techniques for copper and also cadmium are currently being developed by Mount in the U.S. Public Health laboratory at Newtown, Ohio.

Mercury is another metal which can be detected chemically in the fish body. Hamamoto (1960) reported that mercury was deposited in the gills of carp which died within a 48-hour period. When mercury is ingested in food it is accumulated in the gills, kidney and liver (Tsuruga, 1963).

Studies using strontium-85 as a tracer have shown that fish have little ability to store strontium in the body (Schiffman, 1961).

At sublethal concentrations chromium has been found to accumulate in the spleen, posterior gut, pyloric caeca, stomach and kidney (Knoll and Fromm, 1960). Whether significant accumulations occur during lethal exposures is not indicated in the literature. This applied also to arsenic which, at sublethal levels, is stored in the liver (Wiebe et al., 1931).

In regard to anoxia, Pequin and Serfaty (1962) have reported progressive changes in the blood ammonia of carp placed in oxygen-free water.

Burdick (1965) determined the concentrations of cyanide in fish killed by exposure to solutions in the laboratory. Large amounts of cyanide were found in the tissues and gall bladder contents and at high concentrations these varied directly with the exposure concentration. The cyanide content of unexposed fish, however, was highly variable, the higher levels approaching minimal concentrations in exposed fish. Since the toxicity of cyanide is dependent not only on concentration but also on such factors as dissolved oxygen concentration, Burdick considered that the concentrations found in the fish were not sufficiently meaningful and the autopsy method was abandoned.

Pentachlorophenate can be detected chemically in the bodies of fish receiving a lethal dose (Tsuda and Kariya, 1963). Moreover, it is still detectable after the fish have been in running water for 24 hours. Fish absorb phenol at sub-lethal levels in the water, the highest concentrations being found in the liver, gill and kidney (Schulze, 1961).

Studies on the chemical effects of pesticides on fish have dealt largely with DDT and its break-down products. DDT is absorbed rapidly from the water (Holden, 1962). Analysis of tissues and lipid extracts have shown that fish can accumulate hundreds of parts per million of DDT, particularly in the fat (Burdick et al., 1964). In a lake treated with DDD Hunt and Bischoff (1960) found fish containing up to 2500 ppm. Although these instances reflect sublethal exposures, DDT is absorbed so quickly that it can be detected in fish killed in short periods of time. Premdas (1963) subjected young salmon to 1 ppm C-14 labelled DDT and found large amounts of the insecticide throughout the body after only five minutes exposure. The dead fish contained highest concentrations in the gills, liver, spleen, heart kidneys, gonads and swim bladder, and least in the muscles, bone, and integument.

Mount (personal communication) has found that the concentration of pesticides in fish blood is high within one hour of exposure. For blood analysis, samples of about 1 gram are obtained in the field, frozen in dry ice and shipped to the laboratory in a polyfoam container. Mount suggest analyzing the gills and liver of fish suspected of having

been killed by pesticides. Extracts of these tissues are first subjected to thin layer chromatography then analyzed by gas chromatography. Studies utilizing these techniques on fish at the laboratory of the U.S. Public Health Service at Newtown, Ohio, have centered mainly around insecticides such as DDT and endrin.

Practical Applications

The information obtained in this investigation indicates that gross examination alone of fish collected from fish kills usually cannot provide the answer to the cause of the kill. While sloughing of the gill epithelium, for instance, is visible evidence of a toxicity problem, it is non-specific for the toxicant. Such a condition, however, in conjunction with knowledge of pollutants entering the stream could indicate which chemical analyses should be made on the fish.

The histological approach can provide valuable insight into the cause of fish mortality but there are obstacles to its routine use. Histological examinations require fresh tissue which must be obtained from dying or recently dead specimens; any decomposition of the flesh renders an examination useless. In addition, the examination must be made by a trained histologist capable of recognizing normal and abnormal cell structures of fish.

The most practical approach to fish autopsy in the Commission laboratories appears to be chemical analysis of the victims of kills.

According to available information, copper, zinc, mercury, chromium, arsenic, cadmium, pentachlorophenate, cyanide and chlorinated hydrocarbon insecticides can be determined in fish tissues. Undoubtedly there are others. Merely finding a certain concentration of an ion in the fish does not, however, prove that that ion was responsible for its death. There must be some scale of values with which to compare the concentrations. In some cases it may be possible to show a significantly higher content of toxic ion in victims of a kill than is present in fish living upstream of a source of pollution. But, as two investigators have found, concentrations in killed fish may be similar to those living under natural conditions. Then there is the problem of a relatively low concentration of toxicant causing death in conjunction with other stresses such as low dissolved oxygen. Here again the amount of toxicant found in the fish would be smaller than might be expected, making interpretation difficult.

Burdick's problems in developing an autopsy method for fish killed by cyanide lead him to make the following statement: "With the great number of possible contaminants to be found in water that can enter into interaction to produce death, the use of any post-mortem procedure of fishes seems theoretically impossible." It is obvious that identifying the causal agent of fish mortality by autopsy is a complex and difficult task. Mount's work with zinc, however,

shows that it is not impossible. His technique of using a ratio of the concentrations found in two tissues could have a fairly wide application.

Urgently needed in the Commission laboratories are autopsy procedures and equipment for analyzing pesticides in fish. Analytical methods are available which can detect these complex chemicals but they utilize the techniques of thin layer and gas chromatography for which we are not equipped at the present time.

Recommendations

When a fish kill occurs dying or freshly dead fish specimens should be examined for gross abnormalities, frozen and transported to the Commission laboratories. When a lay person collects the fish this initial examination would not be done, so that an additional examination should be made on receipt by personnel of the Biology Branch.

If abnormalities in the fish and/or available information on the kill indicate that a toxicant or group of toxicants might be determinable by a chemical analysis for which the laboratory is equipped, arrangements for this should be made with the Supervisor of the Chemistry Branch. It would be advantageous for all concerned if a file of chemical methods applicable to fish autopsy were maintained by personnel of the Chemistry Laboratory.

Laboratory facilities should be expanded to include equipment and staff for analysis of pesticides, and staff should be trained in analytical procedures for fish.

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Prepared by:

Yvonne H. Swabey, B. A. (Hon.)
Biology Branch.

Supervised by:

Carl F. Schenk,
(Acting) Supervisor,
Biology Branch.

Tsuda, T. and T. Kariya, 1963. Studies on the post mortem identification of the pollutant in the fish killed by water pollution. III Confirmation method of pentachlorophenate in the fish. Bull. Japan Soc. Sci. Fish 29: 828.

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Prepared by:

Yvonne H. Swabey

Yvonne H. Swabey, B. A. (Hon.)
Biology Branch.

Supervised by:

C F Schenk

Carl F. Schenk,
(Acting) Supervisor,
Biology Branch.

Date Due
