DDT AND METHOXYCHLOR POISONING OF LARGEMOUTH BASS

A Thesis

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by

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INTRODUCTION

Much of the early work in the field of pesticide poisoning of fish involved accounts of fish kills resulting from indiscriminant use of various pesticides. When the magnitude of this problem became apparent, researchers began to investigate the degree of toxicity of different pesticides to various species of fish. Some histological studies were completed to determine the effects of certain pesticides on the organs in an attempt to understand the mechanisms of pesticide action (Rudd and Genelly, 1956). Later work involved more sophisticated attempts to explain the acute physiological effects of pesticides. Major concern was centered around chlorinated hydrocarbon pesticides because of their persistence in the environment and their ability to accumulate in biological organisms, especially at higher levels in the food chain. To this date most research in this area has involved the route of entry of pesticides into fish (Bennet and Day, 1970; Predmas and Anderson, 1963), the histological effects of pesticides on various organs (Rudd and Genelly, 1956), and the effect of given pesticides on the nervous system (Anderson, 1968; Aubin and Johansen, 1969). To this author's

knowledge there has been no research conducted on the effects of chlorinated hydrocarbon pesticides on respiratory movements, electrical activity of the heart, or the coordination of heart and respiratory activity of fish. It is this area toward which this study was directed.

The chlorinated hydrocarbon insecticides methoxychlor,[2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane], and DDT,[2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane], were chosen because of their widespread use, high degree of toxicity, and persistence in the environment.

Byington (1966), working with turtles and fish, devised a method for recording electrocardiograms of these animals while they were in water. In this study he noted that the opercular beat and heart beat appeared to be correlated and, in some cases, occurred in definite simple ratios. A mouth jerk or double opercular beat was observed in several fish when the heart beat and opercular beat became unsynchronized. Byington conjectured that the double opercular beat was used by the fish to return the heart beat and opercular beat to synchronization.

In a later study Byington and Rowe (1969) learned that high ammonia concentrations in aquarium

water caused the heart and operculum of largemouth bass to undergo periodic intervals of arrest. They termed this effect cardioventilatory periodicity (CVP) and concluded that the periodic arrest was a response by the fish to minimize the time of exposure of the gills to the harmful ammonia.

In addition to learning the effects of DDT and methoxychlor on the electrocardiogram, heart rate, and opercular rate, it was intended that the present study would extend Byington's work on the correlation of heart beat and opercular beat. It was also intended that the response to ammonia be compared to pesticide poisoning results.

METHODS AND MATERIALS

Recordings were made of the electrical activity of the heart and respiratory movement of largemouth bass, Micropterus salmoides (Lacepede), on an E & M Physiograph "Four" rectilinear recorder. The electrical activity of the heart (EKG), the respiratory movement potentials (RMP), and the time intervals were recorded simultaneously. The EKG and RMP were obtained by placing electrodes near the heart and in the muscle of the isthmus (throat area), respectively.

The electrodes consisted of insect pins that had been soldered to phonograph arm wire, inserted through a polyethylene strip, fortified with epoxy glue, and dipped in Insulex to provide insulation. The positioning of the electrodes was extremely important in obtaining good records of both the EKG and RMP. Byington (1966) described a method of obtaining these records. His method of obtaining the EKG was employed without modification. Byington used a Statham pressure transducer to record RMP. Since his equipment was no longer available, use was made of the fact that voltage changes accompanying respiratory movements are readily recorded from fish (Byington, 1966). These voltage changes are probably a mixture of

movement artifacts and summed muscle action potentials, and are quite reliable indicators of the ventilatory patterns.

Attempts at mounting all four electrodes on a single strip of polyethylene and implanting the electrodes from the ventral side of the isthmus were occasionally successful. Clearer and more consistent recordings were obtained mounting the two EKG electrodes in one polyethylene strip and implanting them through the ventral side of the isthmus. The two RMP electrodes were mounted in smaller pieces of polyethylene and implanted laterally in the isthmus from opposite sides. The placement of electrodes is illustrated in Figure 1. After the electrodes had been implanted a piece of thin wire was used to hold the electrodes securely in position.

Fish rigged in this manner were exposed to test conditions while recordings of EKG and RMP were made. In each case the fish was allowed to acclimate in the aquarium until the cardiac and respiratory rates appeared to stabilize. The time required for the rates to stabilize varied from two to six hours depending on the size of the fish and the depth of anesthesia reached by the fish.

Insecticide was added to the aquarium either in a single dose sufficiently diluted to produce a concentration of one ppm, or in a series of smaller doses at regular intervals until symptoms appeared. The aeration of the water created currents which mixed the pesticide with the aquarium water.

The treatment of fish during the recording of EKG and RMP has been divided into several major groups (Table I) for the sake of clarity and brevity. The three fish in Group I were each acclimated and treated in tap water, thoroughly aerated to drive off chlorine. Two fish were treated with single doses of commercial grade DDT in a 25 L aquarium. The one remaining fish was treated with a single dose of methoxychlor. A 15 L aquarium was used in the tests involving this fish and all subsequent fish. The dosage of all of this group was single. The primary function of this group was to establish an acute dosage level of pesticide and to determine if there were any gross effects on EKG and RMP.

Except for the DDT treated fish in Group I, stock solutions of technical grade (90% pure) methoxychlor and pure DDT were prepared immediately before their use. The fish in Group I were treated with commercial grade DDT which was already in solution. The methoxychlor

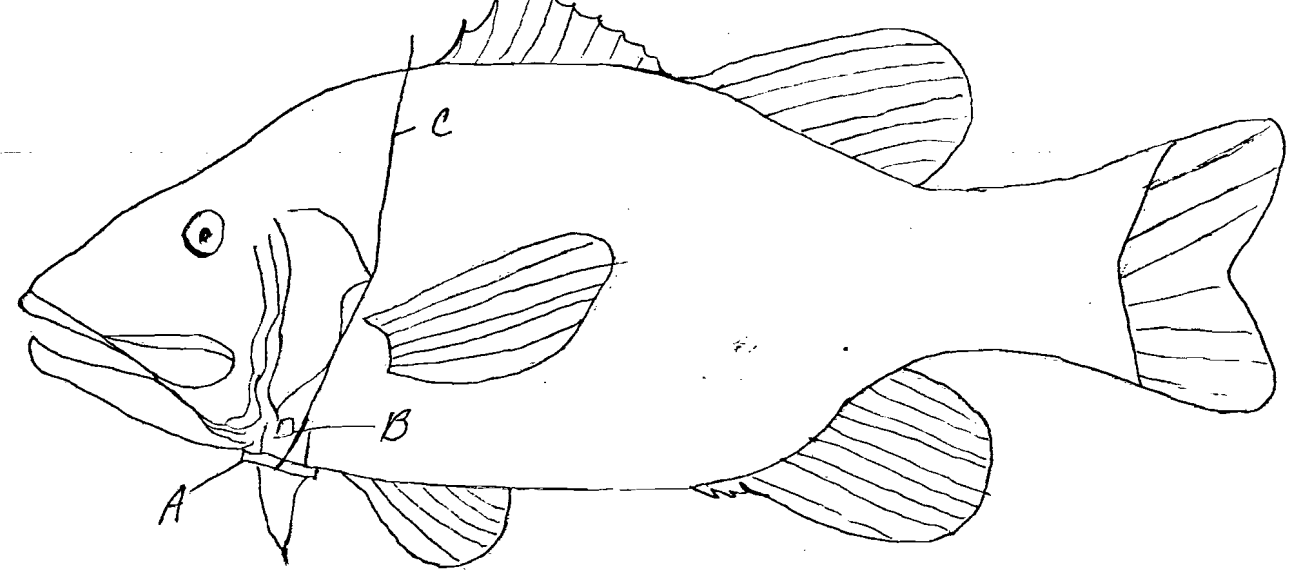

Figure 1. Electrode implantation: A. Electrodes used to record EKG. B. Electrodes used to record RMP. C. Enameled wire used to hold electrodes in place.

stock solutions were prepared by dissolving 16 mg of methoxychlor in 10 ml of ethyl alcohol and diluting to either 500 ml or 1000 ml with distilled water. The DDT stock solutions were prepared by dissolving 15 mg of pure DDT in 10 ml of ethyl alcohol and diluting to either 500 ml or 1000 ml with distilled water. These stock solutions were prepared in this manner to produce concentrations of one ppm or decimal fractions of this concentration upon addition of the entire quantity or 100 ml portions to a 15 L aquarium.

The fish in Group II were acclimated and treated in a soft water solution prepared from ACS grade chemicals and distilled water (Cairns and Sheier, 1964). One fish was exposed to a single dose of 16 mg methoxychlor and three fish were each exposed to a series of increasing doses added at regular time intervals.

The fish in Group III were treated with calcium gluconate prior to and after methoxychlor treatment. Two fish were injected with a calcium gluconate solution, and two fish were treated with calcium by dissolving calcium gluconate in the aquarium water prior to treatment. One of the last pair was transferred upon appearance of symptoms to another aquarium containing calcium gluconate but without any DDT.

Table 1. Grouping of fish according to treatment.

Group No.	No. in Group	Type of Pesticide	Type of Dosage	Aquarium Water Preparation	Preventive or Remedial Treatment
I	3	2 DDT	Single	Aerated tap water	None
		1 Methoxychlor	Single	Aerated tap water	None
II	4	1 Methoxychlor	Single	Aerated soft water preparation	None
		3 Methoxychlor	Incremental	Aerated soft water preparation	None
III	4	1 Methoxychlor	Single	Aerated soft water	Calcium injection, preventive
		1 Methoxychlor	Single	Aerated soft water	Calcium injection, preventive and remedial
		1 DDT	Incremental	Calcium treated water	Transfer to pesticide free Calcium treated water, preventive and remedial
		1 DDT	Incremental	Calcium treated water	Calcium in aquarium water, preventive
IV	3	1 DDT	Incremental	Distilled water	None
		1 DDT	Incremental	Distilled water	Transfer to pesticide free water, remedial
		1 DDT	Single	Distilled water	None

The three fish in Group IV were acclimated in distilled water prior to DDT treatment. The doses of two were incremental while the third received a single dose. One of those treated with an incremental dose was transferred to distilled water upon appearance of symptoms.

RESULTS AND DISCUSSION

The visible symptoms of DDT and methoxychlor poisoning followed a general pattern in all fish observed. The first sign to appear was a slight twitch of the mouth. The occurrence of this symptom was irregular and occurred more frequently in methoxychlor poisoning than in DDT poisoning. It became more pronounced and more frequent as the poisoning progressed. Soon after the appearance of the mouth twitch, a jerking motion of the tail appeared. These actions coincided and the severity of both increased until the entire body lurched forward with the occurrence of each tremor. The fish soon began to swim about the aquarium in short bursts. Shortly thereafter it began to show signs of difficulty in maintaining equilibrium. By this time the recordings of respiratory movement usually had to be discontinued due to the severely erratic movement of the recording pen. With the loss of equilibrium the fish assumed a position on its side on the floor of the aquarium and only occasionally attempted to swim. The final externally visible sign of life was an extremely erratic eye movement which gradually became slower and ceased. The EKG showed that the heart continued to function for a short while longer. Although the duration of each stage of the observed symptoms varied,

the overall sequence of symptoms progressed uniformly once the first sign of poisoning had occurred.

Several serial additions of pesticides were made in an attempt to extend the length of the different stages of symptoms and reduce interference of other symptoms with the recordings. It was found that the length of the different stages could be extended by serial addition of pesticide, but this method of administering the pesticide did not consistently reduce the interference with recording.

Figure 2 shows the EKG and RMP of Fish No. 1 prior to methoxychlor treatment and represents normal patterns. Figures 3 and 4 show the EKG and RMP after treatment. Figure 3 was recorded at the mouth jerk stage of symptoms and shows the mouth jerks on the record. Figure 4 was recorded after the fish had lost equilibrium and showed no externally visible signs of life. Before this figure was recorded the RMP had been discontinued due to the extremely high amplitude and erratic movement of the recording pen.

The abnormal potential changes that occurred on the RMP at the same time as the mouth jerks were of two types (Figure 3). One type appeared as a pulse of high frequency changes. The other appeared as a spike or rapid single high amplitude change.

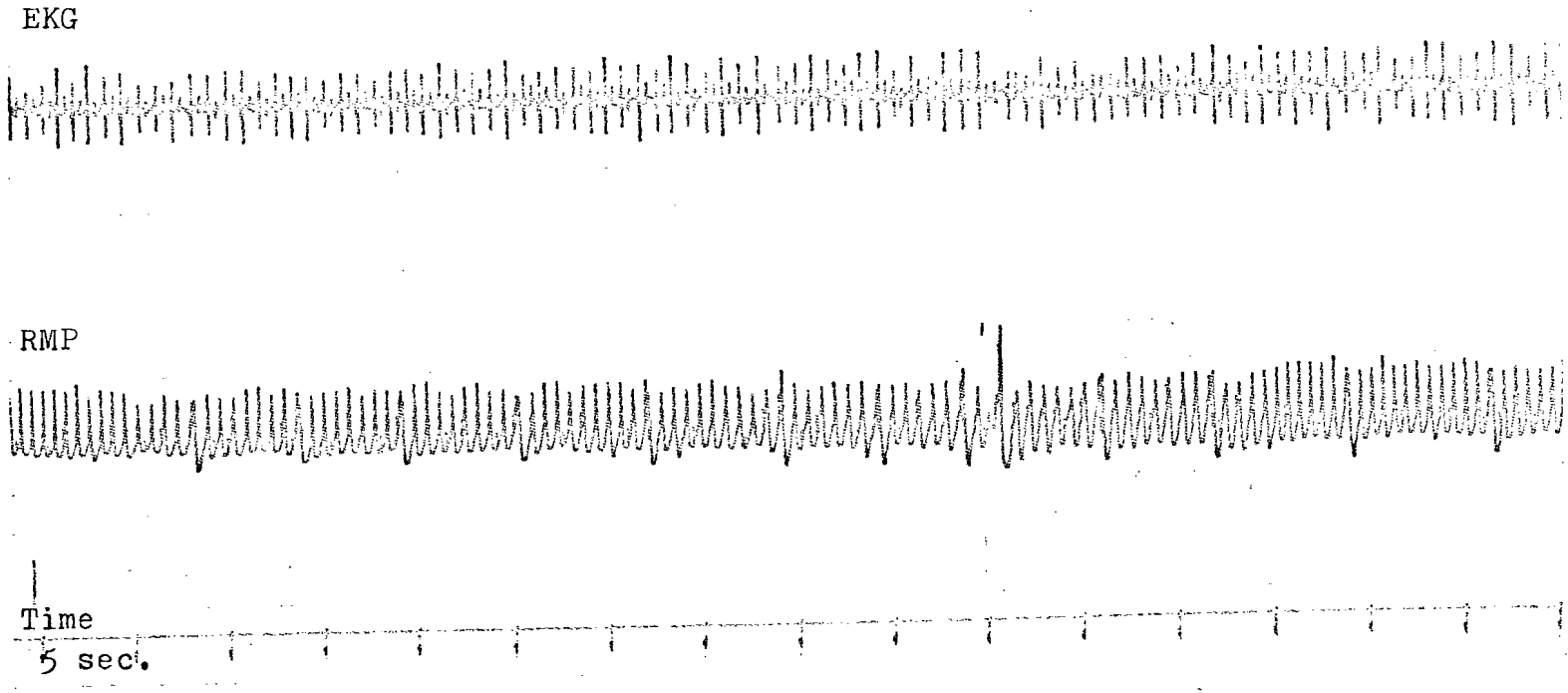

Figure 2. EKG and RMP of Fish No. 1 prior to treatment with methoxychlor, represented normal patterns.

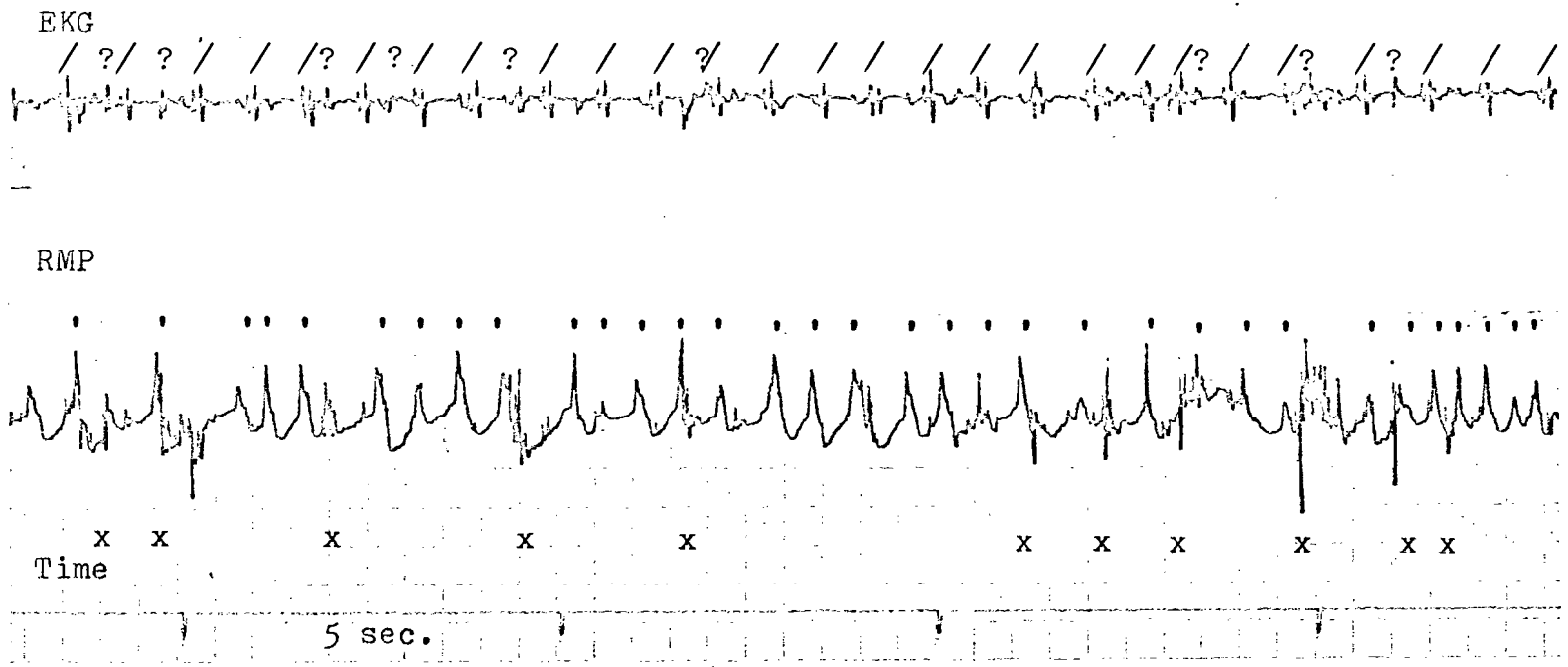

Figure 3. EKG and RMP of Fish No. 1 one hour after the single addition of methoxychlor to a level of one ppm. Hash marks above EKG indicate those potentials interpreted to be QRS waves, hence indicating heart beats. Apostrophes above RMP record indicate voltage deflections counted as respiratory movements. Mouth jerks occurred at the points marked x. Question marks indicate potentials of unknown origin.

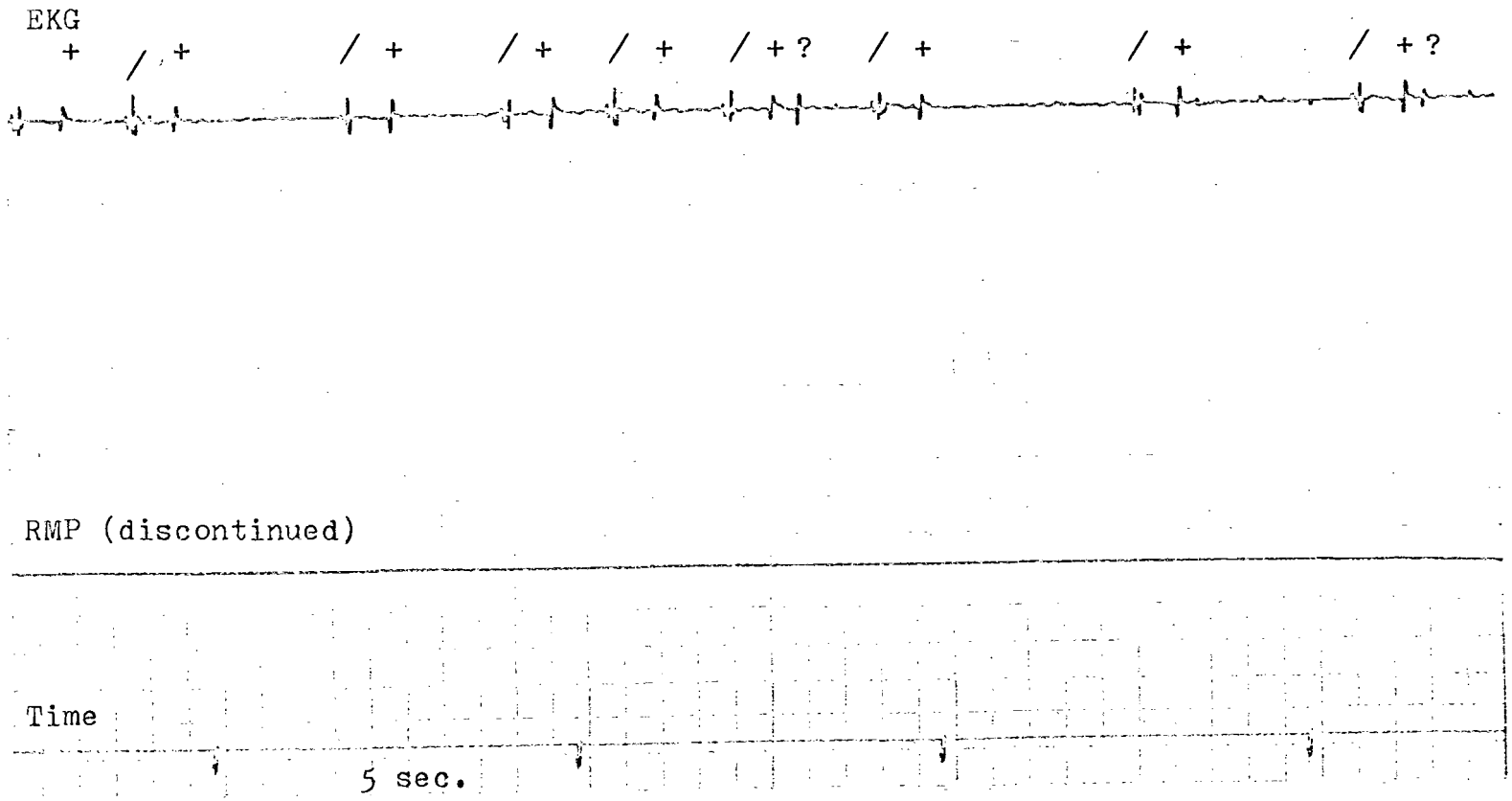

Figure 4. EKG and RMP of Fish No. 1 one and one half hours after the single addition of methoxychlor to a level of one ppm. Hash marks above EKG indicate those potentials interpreted to be QRS waves. Plus signs indicate those potentials believed to be T waves. Question marks indicate potentials of unknown origin.

These changes also appeared on the EKG but their amplitude was not as great there. These changes are marked by an x on the RMP record and an ? above the EKG. Since the externally visible symptoms had subsided before Figure 4 was recorded, it could not be determined if the points marked ? were of the same origin as those in Figure 3, but this was believed to be the case. Figure 3 was recorded before the potential changes had reached such a high frequency and large amplitude that cessation of RMP recording had become necessary. That it was possible to continue recording EKG even after the potential changes had necessitated discontinuance of RMP recording indicates that the respiratory mechanism was affected to a much greater degree than was the heart. Whether the appearance of abnormal potential changes on the EKG is an indication of abnormal heart electrical activity or a result of an imperfect recording technique could not be determined because the heart could not be observed during recording.

It was believed, however, that the abnormal potential changes on the EKG were primarily a recording of electrical activity of tissue near the heart. At least the heart was not as greatly affected by the methoxychlor poisoning as the EKG as a whole would

indicate. The simultaneous occurrence of the mouth jerks, spikes, and high frequency pulses and the increasing amplitude and frequency of these symptoms is strong evidence that methoxychlor's effects are produced in the central nervous system (CNS). The symptoms observed here may be merely recordings of the centrally induced abnormal potential changes. The respiratory mechanism is under much more strict control of the CNS and therefore reflects the disorder created in the CNS more clearly than does the heart.

Figures 5-7 are also presented to show the effect of methoxychlor on EKG and RMP. These records represent the effect on the RMP more clearly because the recording of this channel was possible throughout the entire range of symptoms. As seen in Figure 6 the respiratory movement remains apparent but is more disordered than prior to treatment as in Figure 5. At the time Figure 6 was recorded respiratory movement still exhibited a rhythm, while at the time Figure 7 was recorded no rhythm could be observed. The operculi would occasionally move, but the movement was not coordinated and therefore could not be interpreted as respiratory movement. The electrical activity apparent on the RMP channel in Figure 7 appears as continuous

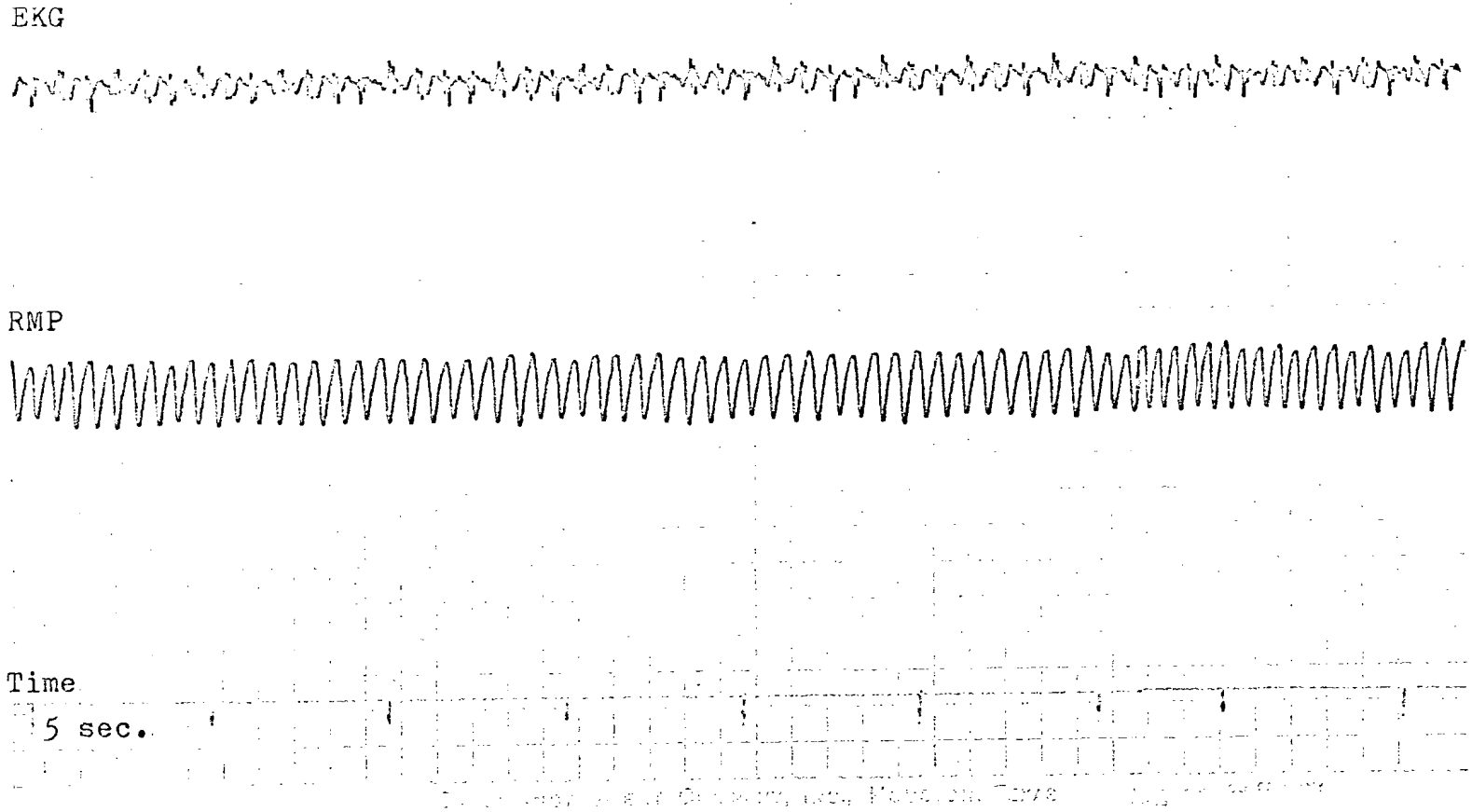

Figure 5. EKG and RMP of Fish No. 2 prior to methoxychlor treatment. EKG shows electrical activity from respiratory movements superimposed on baseline. RMP record shows smooth and even respiratory movements.

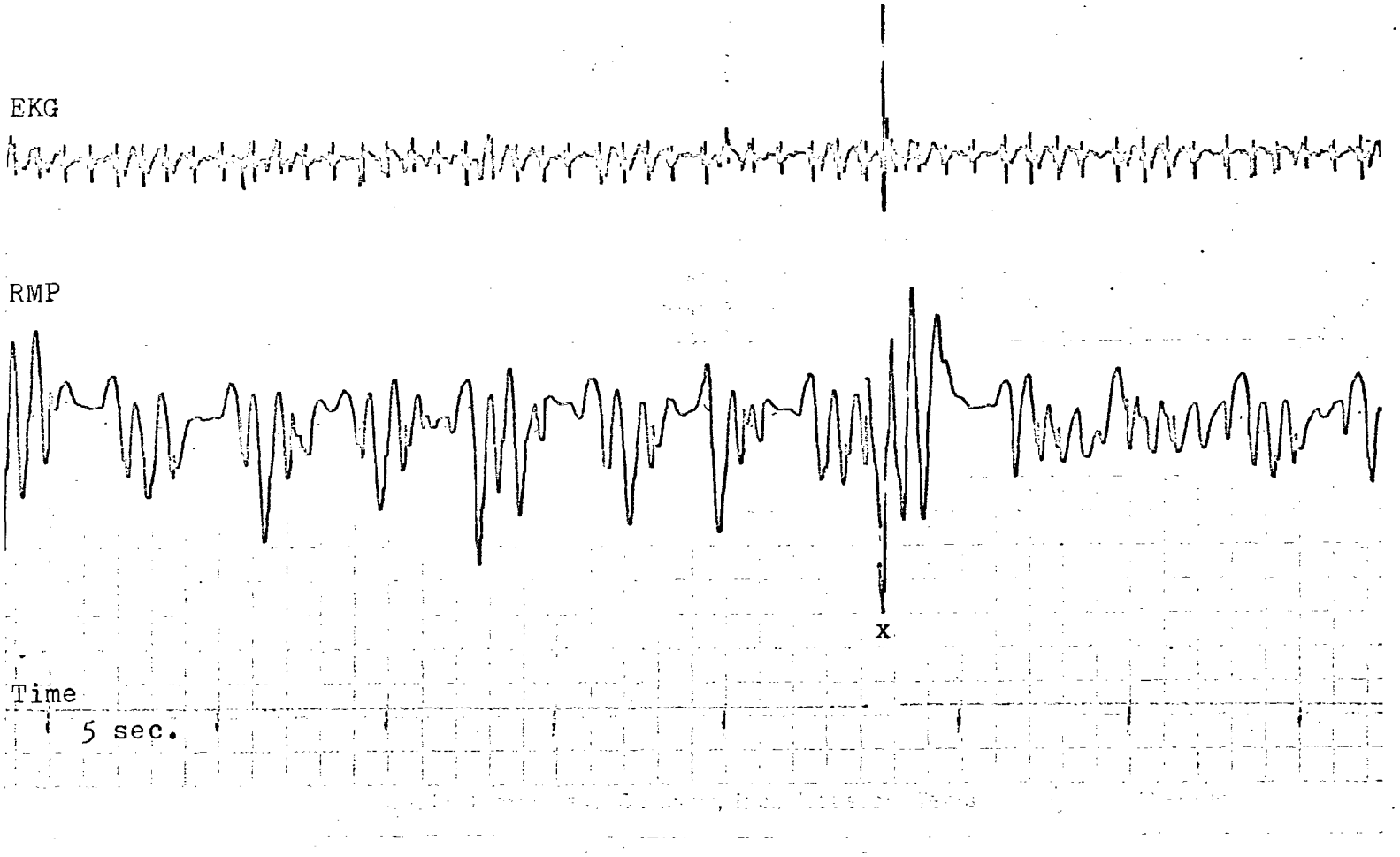

Figure 6. EKG and RMP of Fish No. 2 three hours after beginning of serial additions of methoxychlor. The concentration at this point was approximately 0.2 ppm. Note uneven respiratory movement shown by RMP. Also note the mouth jerk (x) apparent on both records.

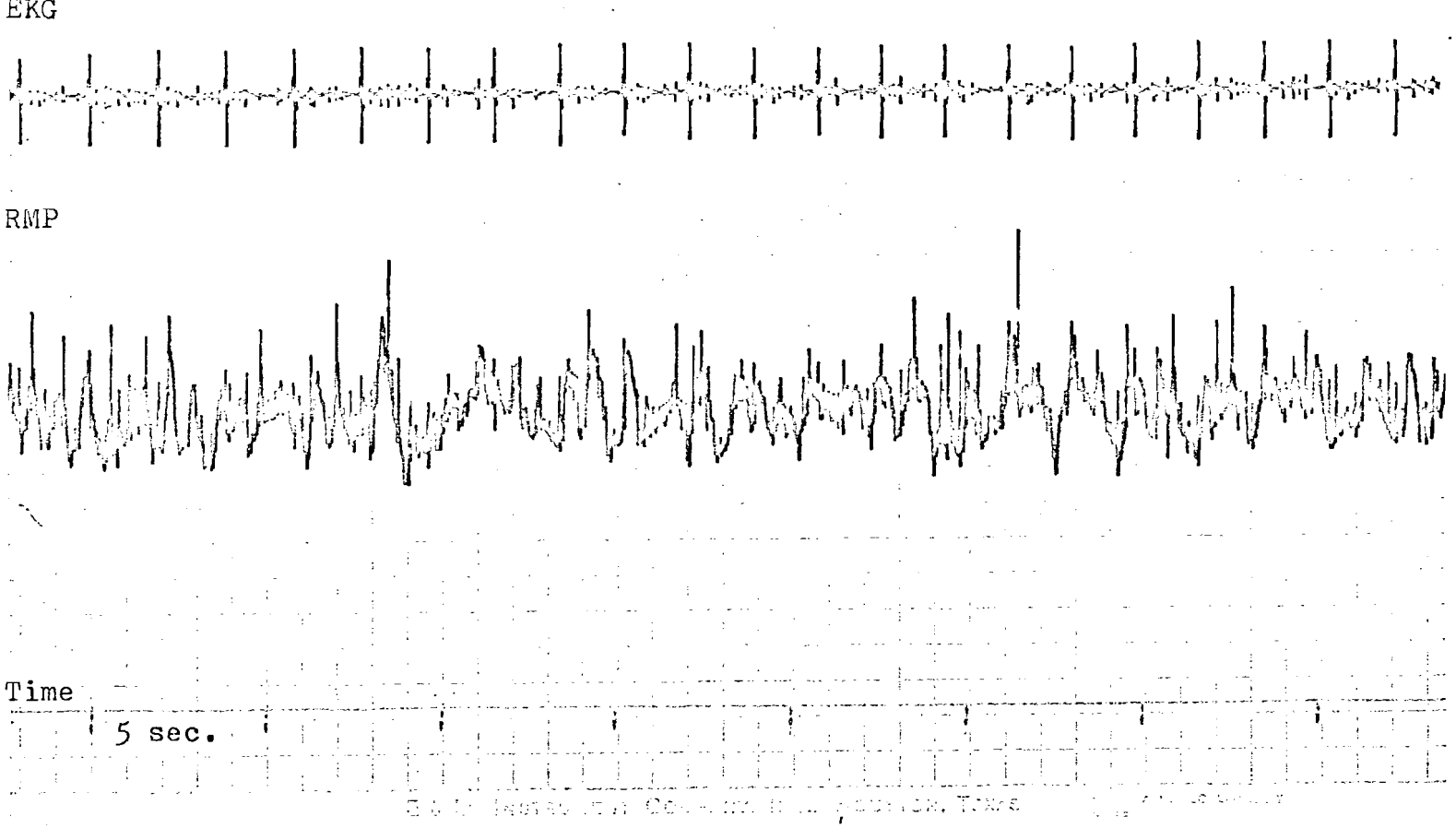

Figure 7. EKG and RMP of Fish No. 2 five hours after beginning of serial additions of methoxychlor, approximate concentration 0.4 ppm. The fish had lost its ability to maintain equilibrium and was no longer attempting to swim. Better placement of RMP recording electrodes allowed more extended recording of respiratory movement than with fish No. 1.

high frequency potential changes punctuated with larger single changes or spikes. These two types of activity were also present on the records in Figure 3, although they were less pronounced and less frequent there. They would probably have appeared in Figure 4 much like they appear in Figure 7 had it not been necessary to discontinue recording on the RMP channel prior to the recording of that figure. Thus although at first glance the recordings of these two fish may appear to show different effects of methoxychlor, closer examination of the records shows that the effects were very similar. The difference in methods of dosage is very likely responsible for the difference in the appearance of the records. It is reasonable to assume that the more acutely toxic concentration of the single dose of methoxychlor would produce more rapid appearance of symptoms and thus possibly obscure some of the symptoms more readily observable under more extended addition of the same amount of pesticide. Therefore, it does not seem unusual that the symptoms of the two different types of dosage were not identical.

The EKG in Figure 7 shows some smaller spikes that cannot be interpreted as normal heart electrical activity. But the heart beat appears regular and is

actually clearer than in the previous two figures. In view of the more pronounced symptoms at this stage of poisoning and the greater amplitude of the spikes on the RMP channel, it seems logical to conclude that the small spikes on the EKG are not indicative of heart electrical activity at all, but that they are the recording of electrical activity of tissue surrounding the heart.

Figures 8-10 are presented to show the effect of DDT on EKG and RMP. Figure 8 was recorded prior to incremental dosage with DDT. Figure 9 was recorded at the onset of the visible mouth jerk stage, and Figure 10 was recorded at the body tremor stage of symptoms. It would appear from these figures that the DDT had a less disruptive effect on the records than did methoxychlor, and this did appear to be the case in recordings of other DDT treated fish also but the externally visible symptoms of DDT and methoxychlor were very similar. The most notable difference in visible symptoms was the less frequent appearance of the mouth jerk and subsequent whole body tremor in DDT poisoning.

The EKG's in Figures 8-10 show no detrimental effect due to DDT treatment. The EKG in Figure 9 shows less interference. The only abnormality is the one mouth jerk. In Figure 10 the EKG exhibits no abnormality. The

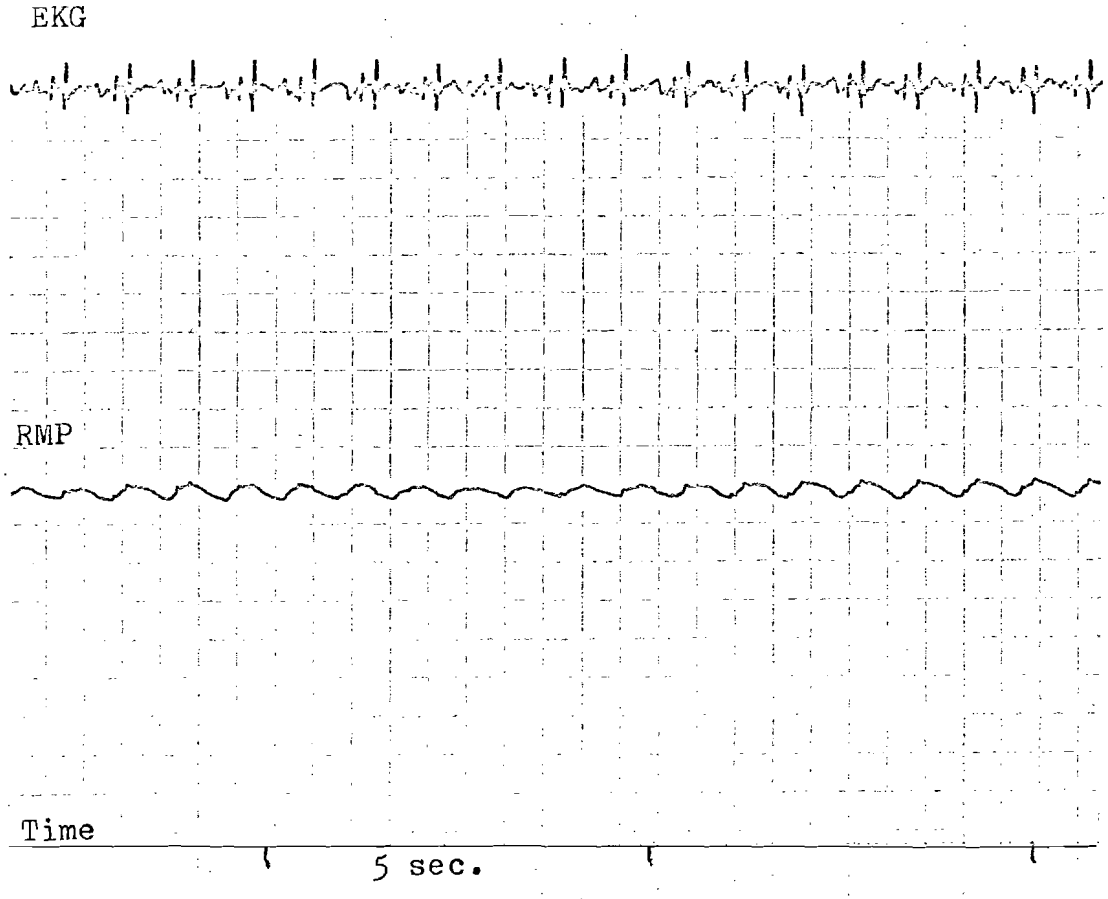

Figure 8. EKG and RMP of Fish No. 3 prior to DDT treatment.

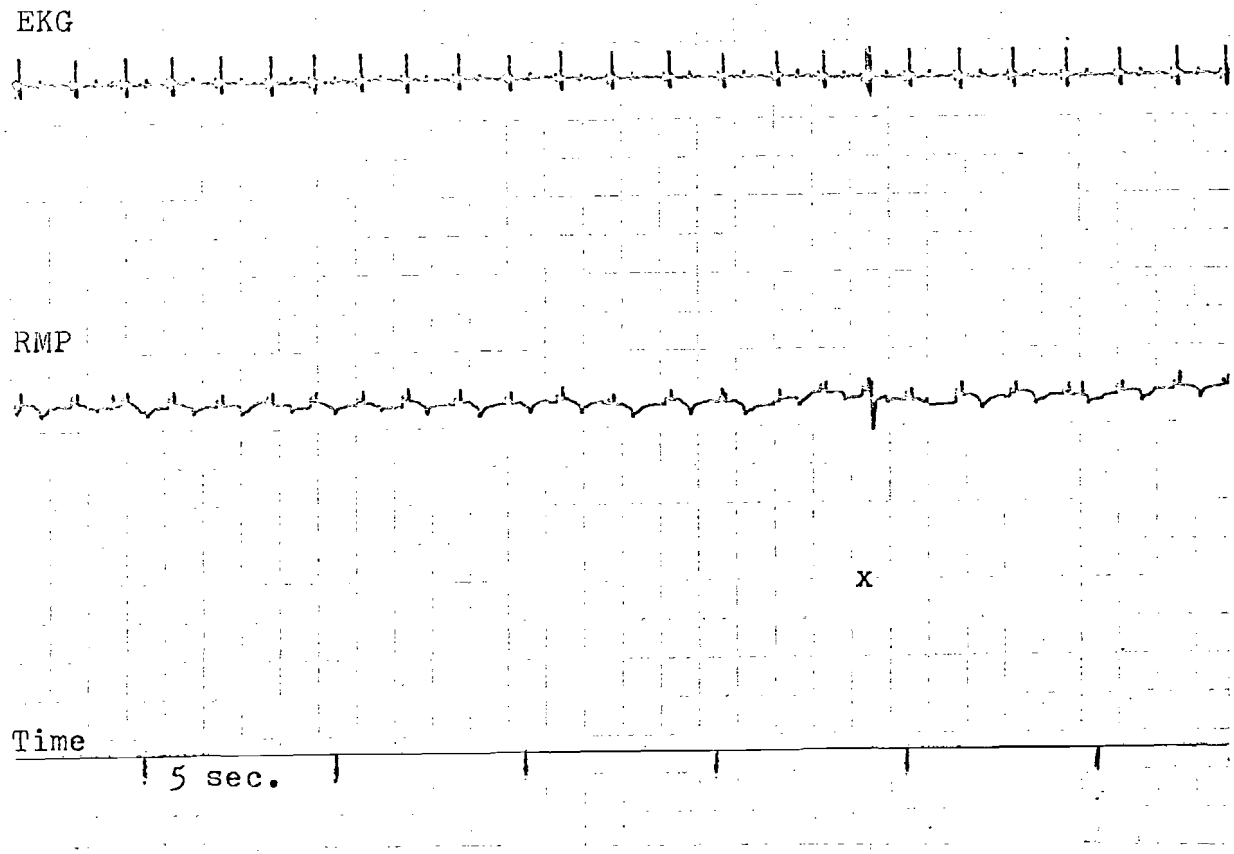

Figure 9. EKG and RMP of Fish No. 3 three hours after beginning incremental dosage of DDT. Concentration of DDT at this point was approximately 1 ppm. One mouth jerk (x) is apparent. RMP begins to show electrical activity of heart.

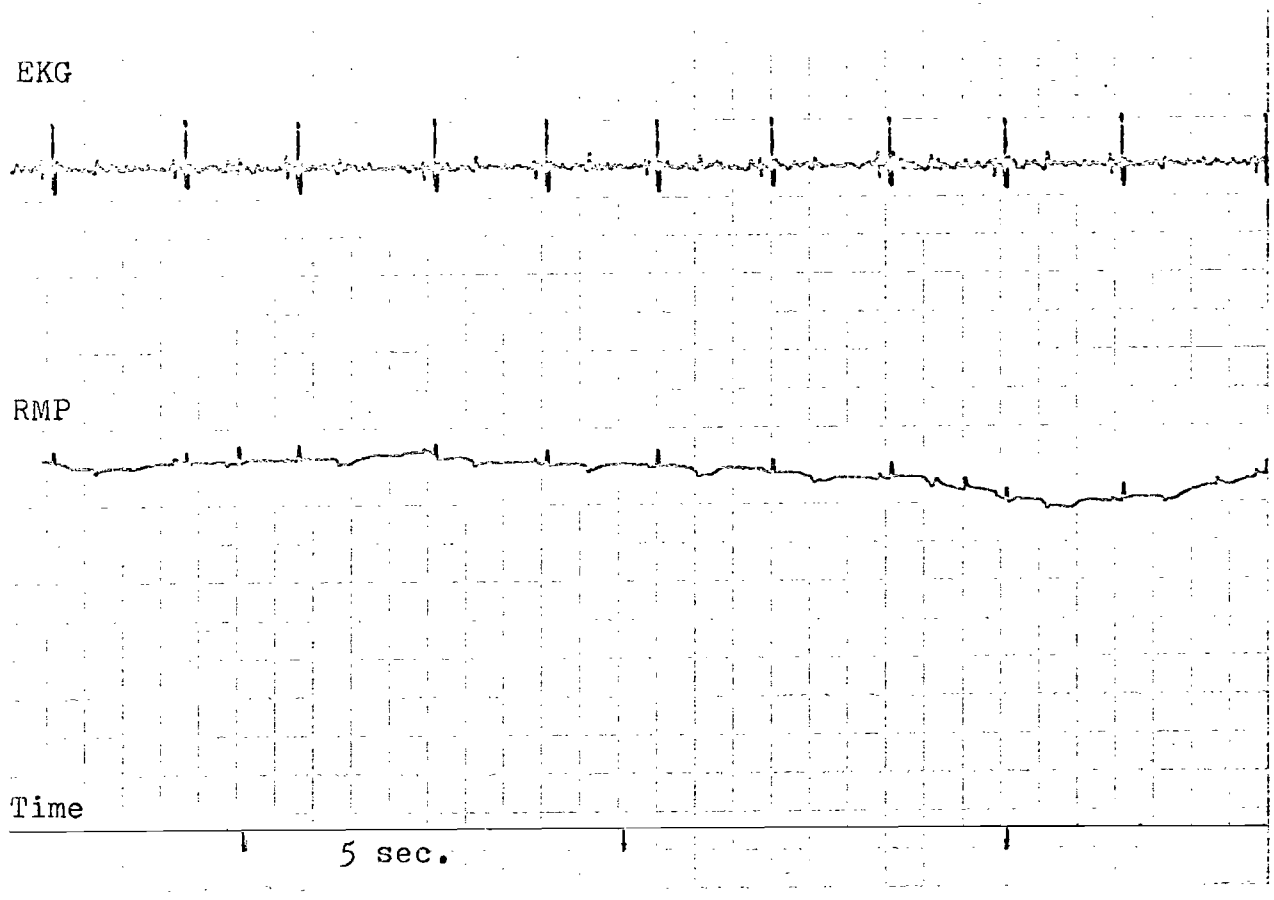

Figure 10. EKG and RMP of Fish No. 3 four hours after beginning incremental dosage of DDT. Concentration of DDT at this point was approximately 1.5 ppm. RMP shows reduced amplitude of respiratory movements.

RMP in Figures 8-10 shows a gradual decrease in the amplitude of respiratory movements. The electrical activity of the heart appears on the RMP in Figure 8 and becomes increasingly evident in Figure 10.

By allowing the cardiac and respiratory rates of the fish to stabilize prior to addition of the pesticide, and by holding environmental conditions constant, except for the addition of DDT or methoxychlor, it was assumed that any rate change observed would be a result of pesticide treatment. Data from such treatment is presented in Figures 11, 12, and 13. As can be seen in Figure 11, the respiratory rate increased and the cardiac rate decreased after the addition of methoxychlor. The cardiac rate later increased to pretreatment level before decreasing again as death approached.

In Figure 12 the respiratory rate appears to be very unstable after the addition of the first amount of methoxychlor. It first increases greatly, drops to below pretreatment level, then increases again before stabilizing somewhat in a downward trend. The cardiac rate appears to drop after the initial addition of methoxychlor, then stabilizes with only small variation through the remaining doses.

In Figure 13 the cardiac and respiratory rates appear more stable through the entire dosage with DDT.

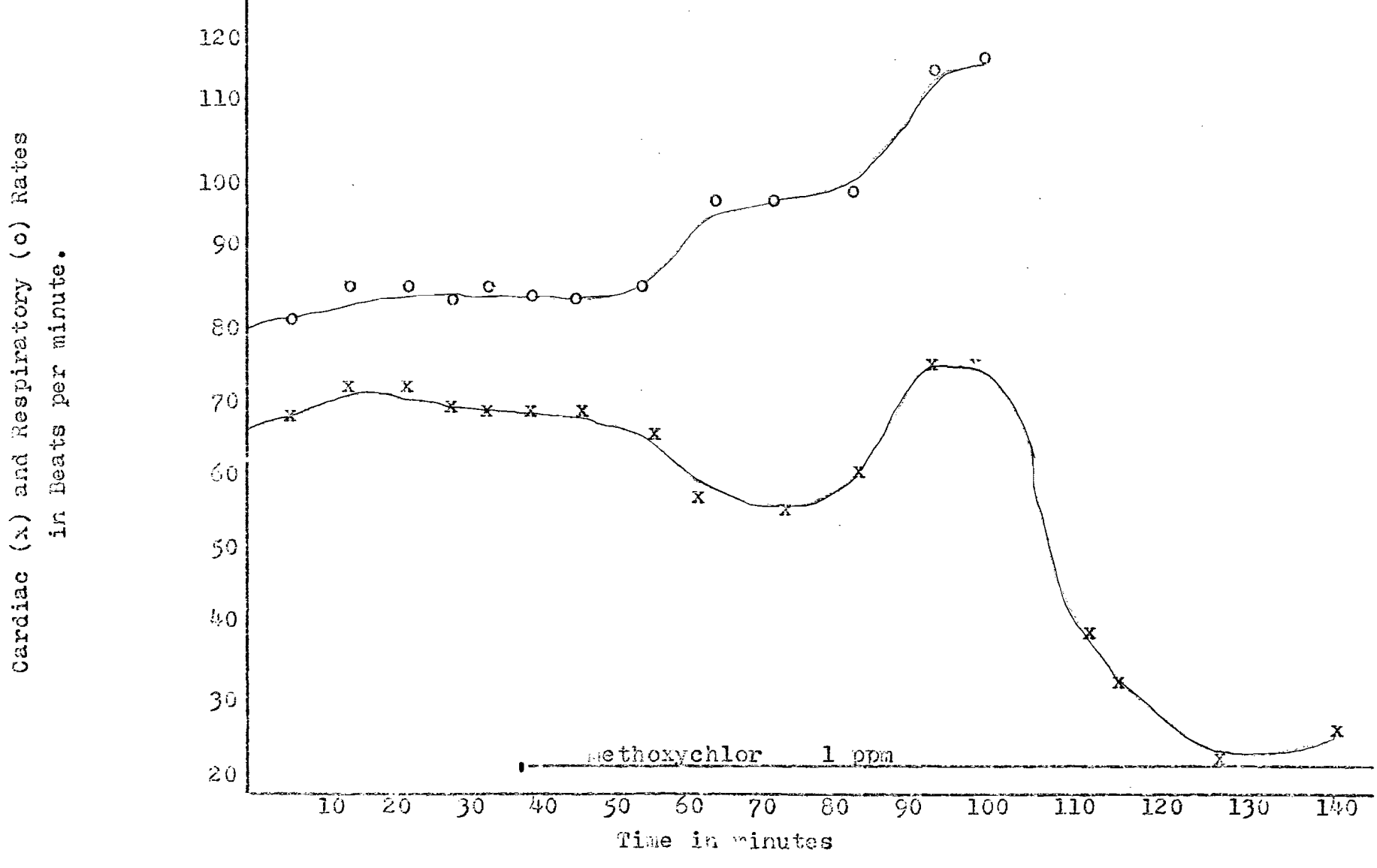

Figure 11. Cardiac and respiratory rates of Fish No. 1. Single dosage of methoxychlor.

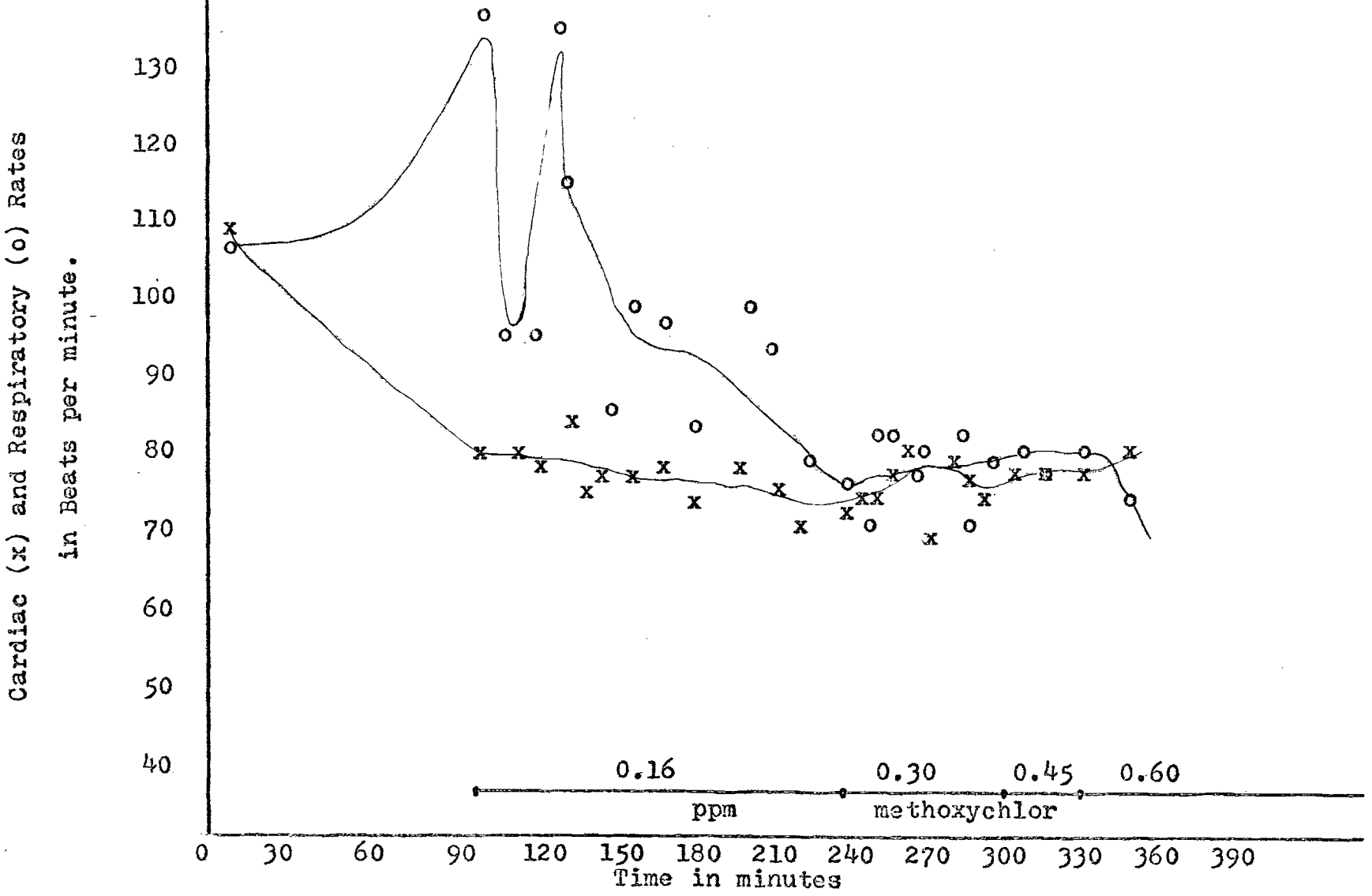

Figure 12. Cardiac and respiratory rates of Fish No. 2. Incremental dosage of methoxychlor as indicated.

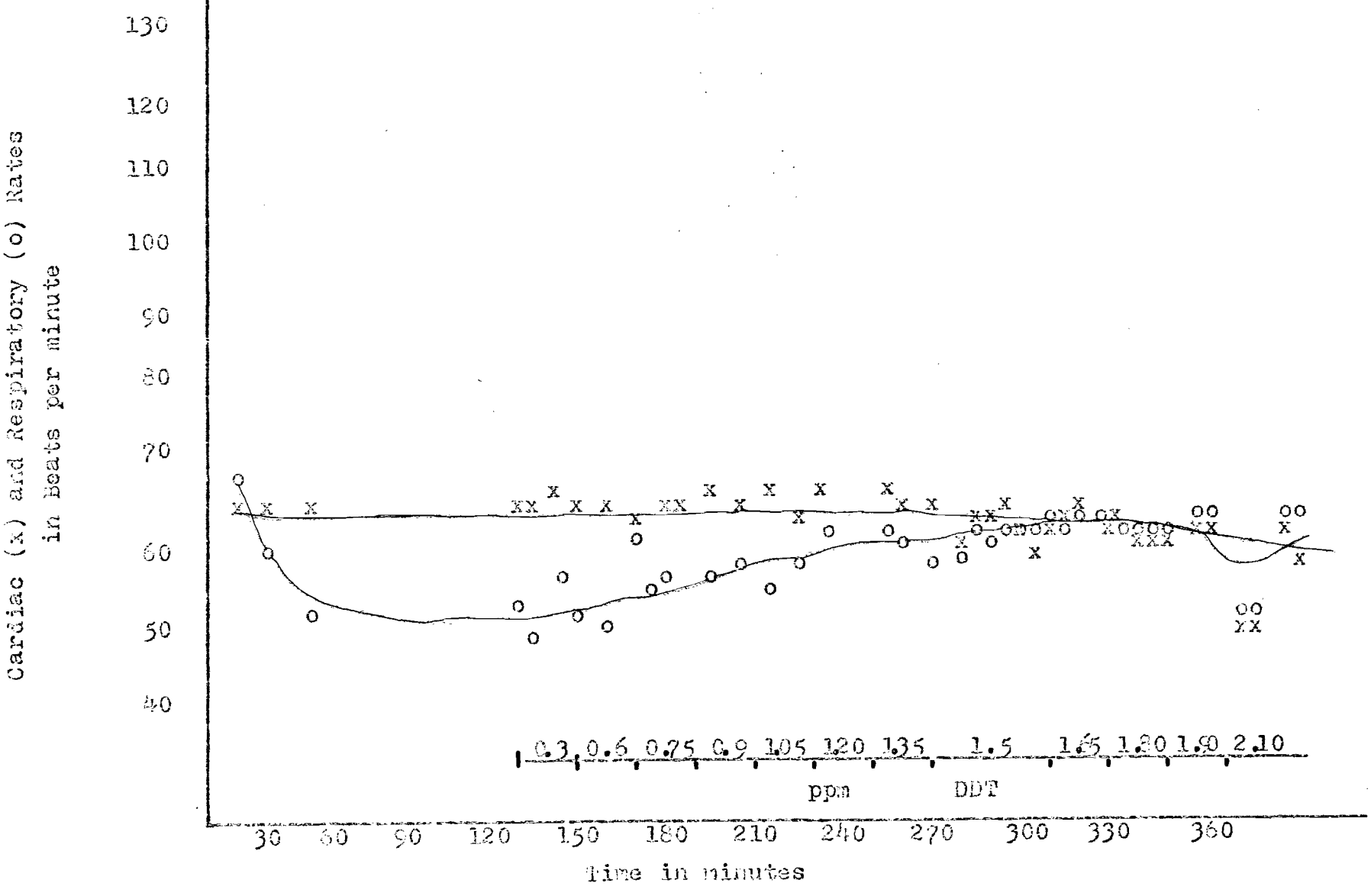

Figure 13. Cardiac and respiratory rates of Fish No. 3. Incremental dosage of DDT as indicated.

The greater change in rates observed with methoxychlor treatment and methoxychlor's more disruptive effect on the pattern of respiratory movement and record of heart electrical activity suggest slightly different types of action of these pesticides in fish.

It would appear from Figures 2-10, presented to show the patterns of RMP and EKG that the pesticides both caused a rather drastic reduction in the cardiac and respiratory rates. But it must be understood that the figures here presented were chosen to depict the pattern of activity during and prior to poisoning and therefore do not provide a true picture of the effect of the pesticides on these rates. The range of rates is indeed large when the first and last figures representing a given fish are compared. However, the first patterns of each fish (Figures 2, 5, and 8) were recorded prior to the stabilization of rates and the last patterns (Figures 4, 7, and 10) were recorded after the fish had lost equilibrium and were approaching death. There is not sufficient evidence to conclude that the decreased rates were a result of the pesticide acting directly on the cardiac and respiratory control systems. The primary site of action may have been elsewhere and the lower rates may have been simply a reflection of the generally deteriorated condition of the fish.

It was necessary to discontinue recordings of both EKG and RMP at different stages of poisoning of different fish due to the appearance of violent movement of the recording pens. It was believed that these movements were due to muscle spasms in the tissue near the electrodes as a result of pesticide poisoning. Recordings of respiratory movement were more sensitive to interference than recordings of heart activity, and recordings of both showed more interference from methoxychlor poisoning than from DDT poisoning. Better placement of the electrodes was probably the reason some recordings were obtained at more advanced stages of poisoning than in other instances. In several cases the EKG was recorded through the entire range of symptoms. In only one case of methoxychlor poisoning was the respiratory movement recorded through the entire range of symptoms. After handling several of the fish to change aquariums as remedial treatment for pesticide poisoning, large increases of rates were noted. Calcium treatment resulted in reduced heart rate.

The difference in effect of DDT and methoxychlor on the RMP is interesting because the general effect of the pesticides on respiratory movement were the same. The mouth-tail jerk appeared to over-ride the normal respiratory movements. When the jerk occurred the

operculum moved with the mouth. In the early stage of poisoning the respiratory rhythm appeared nearly normal with only the occasional jerk. As the frequency and severity of the symptomatic jerks progressed the respiratory rhythm became more and more disordered until the fish lost equilibrium and the ability to coordinate muscular movement. At this time the operculi would occasionally move, but did not exhibit a rhythm. From attempts to reverse the symptoms of poisoning it appeared that once the rhythmic movements had disappeared, death of the fish was inevitable.

Henderson and Woolley (1970), in a study of mechanisms of neurotoxic action of DDT in rats, noted an increase in respiratory rate prior to the appearance of visible symptoms of poisoning in adult rats. The respiratory rate remained above normal with only a slight decrease until shortly before death, when a precipitous drop occurred. A gradual increase in heart rate occurred during DDT poisoning, but no apparent abnormalities were observed in the EKG. In all instances the EKG continued after the cessation of respiration and EEG. Body temperature remained unaffected until the onset of tremoring and hyperexcitability, then rose as the intensity of the tremors increased. They believed that a marked sympathetic stimulation is important in the symptomatology of DDT poisoning, and cite the hyperthermia and early increase in respiratory

rate as evidence of sympathetic stimulation.

The increased respiratory rate and heart rate observed in DDT poisoned rats were not observed in the DDT poisoned fish in this study. There is an appealingly simple explanation for the lack of an initial increase in heart rate. Randall (1968) has stated that there appear to be no sympathetic fibers innervating the fish heart and this lack of sympathetic innervation may account for the absence of an increase in heart rate in DDT poisoned fish. The situation in respiratory control is more complex and requires more detailed discussion.

It is generally accepted that the respiratory rhythm is generated in the medulla in both mammals and fish. The rate of the rhythm may be modified by the higher centers of the brain as well as the sympathetic and parasympathetic centers of the hypothalamus. It is also generally accepted that the control these centers exert over the rhythm of the medulla is dependent on sensory inputs to these centers. The way in which sensory inputs are integrated to modify the rate of respiratory rhythm is not understood in either fish or mammals.

Fish differ from mammals in the arrangement of respiratory motor pathways. In mammals efferent pathways from the respiratory center run down the cord to spinal motors neurons. Fish show a more compact arrangement with the motor connections being established from the anterior part of the medulla.

This difference in arrangement of motor pathways may be important in understanding the different effect of DDT observed in fish and rats, but the observed alterations in respiratory rate suggest the medulla (or centers modifying the medulla) is more sensitive in the rat than in the fish.

It is interesting to note that Henderson and Woolley (1970) observed no hyperthermia in immature rats treated with DDT. The hypothalamus is responsible for the regulation of body temperature and is not considered functional until after it is myelinated at an age more advanced than in the immature rats tested. If the role of the hypothalamus in regulating body temperature is not profoundly affected by DDT prior to its myelination, then its role in regulating respiratory rate might not be profoundly affected either. However, since the heart and respiratory rates of the immature rats were not recorded this last suggestion remains speculation.

It is interesting to note that the effect of methoxychlor poisoning on cardiac and respiratory rates in fish agrees more closely with the effect of DDT poisoning on these rates of rats than does the effect of DDT poisoning in fish.

Bahr and Ball (1971) have concluded that the central nervous system is the primary target for DDT in fish. They found that the frequency of spontaneous neural discharge of the lateral line nerve was not affected by DDT treatment. This finding and the obvious tremoring of DDT treated fish in the absence of lateral line involvement led these researchers to the conclusion that afferent activity of the lateral line nerve does not play an important role in the neurotoxic action of DDT.

This conclusion may lend some support to the concept that the medulla or motor neurons controlling respiratory movement may be the site or sites where DDT produces its lethal effect in fish. If the sensory nerves can be eliminated as sites of DDT disruption, then the central nervous system and/or motor neurons must be the sites of action.

The purpose of calcium treatment of DDT and methoxychlor poisoned fish was to determine if the symptoms of poisoning could be alleviated by calcium. Relief of symptoms of DDT poisoning by calcium in dogs has been reported (Vaz et al., 1945). The theoretical explanation for the ability of calcium treatment to decrease sensitivity to and remedy symptoms of DDT

poisoning was advanced by O'Brien (1967). The DDT molecule is believed to produce a destabilizing effect on the nerve axon by competing with calcium for binding sites on the nerve membrane. The DDT membrane complex alters membrane permeability to potassium and sodium ions causing spontaneous conduction of nerve impulses and producing tremors. The increased resistance of calcium treated dogs to DDT and the similarity of DDT poisoning symptoms with calcium deficiency symptoms were the bases of this theory.

The lack of success with calcium treatment in this study may be a result of the difficulty encountered in introducing the calcium gluconate into the fish. The first attempts at intramuscular injection were unsuccessful because the solubility of the compound dictated the use of too large an injection. Attempts of introducing calcium gluconate into the fish via the environmental water did not produce consistent results. Due to the difficulty encountered in this portion of this study the results obtained here were inconclusive.

There is some disagreement on the degree of branchiocardiac coordination in fish. Satchell (1960) states that,

> "The hearts of both elasmobranch and teleost fish are known at times to beat at a particular phase of the respiratory cycle and thus to exhibit a regular rhythm locked to that of respiration".

> "In the resting teleost there is a tendency for the heart beat and breathing to become synchronized. The synchrony is by no means perfect and, though the heart beats more frequently in the "mouth-closing" phase, there is usually a large number of occasions when the beat occurs in other phases. A more marked synchrony is seen in anesthetized fish and, in some, a very strict relationship has been seen over long periods of time. The fact that the unanesthetized animal must be at rest and undisturbed for the described relationship to appear, suggests that a similar mechanism is at work in the anesthetized animal. The synchrony in teleosts is easily lost and so is clearly subordinate to other factors in the coordination of breathing and heart output," (Shelton and Randall, 1962).

Apparently the teleost fish, the largemouth bass included, exhibit the synchrony described by Byington (1966) normally only at rest or under other special conditions - hypoxia, anesthesia, etc., (Shelton and Randall, 1962). At these times the simple ratios of heart beat and respiratory rate may occur as long as the conditions are maintained. Upon exposure to new conditions one or both of the rates may be changed and the coordination of both may appear as a complex dynamic situation such that the respiratory and circulatory requirements of the fish are met. The detection of any synchronization after the shift from the state of simple ratio of rates thus becomes extremely difficult.

The synchrony of heart beat and opercular beat referred to by Shelton and Randall (1962) and Byington (1966) was only occasionally seen in the bass observed in

this study. The fact that on several occasions steady rates of undisturbed and resting fish were sampled over periods of several hours without showing any apparent synchrony suggests that the conditions required to establish synchrony in largemouth bass may be even more stringent than those of rest and lack of disturbance described by Shelton and Randall (1962).

The test conditions of Byington's study in which he observed the marked synchrony between heart beat and opercular beat involved the use of a more concentrated anesthetic than was employed in the present study. The use of smaller aquaria by Byington provides further reason to believe that the more static conditions in his study would promote the continuance of branchio-cardiac synchrony after recovery of the fish from the anesthetic. The fish observed in this study were prone to swim around the aquarium after being transferred from the anesthetic. The exercise produced a more complex situation confronting the animals circulatory and respiratory systems than would be expected under the more static conditions of deeper anesthesia.

It is difficult to compare the symptoms of DDT and methoxychlor poisoning to the cardiovascular periodicity resulting from ammonia poisoning reported by Byington and Rowe (1969) as was originally intended because the responses were so different. An elevated free ammonia level in rat

brain as a result of chlorinated hydrocarbon poisoning had been reported by Hathaway (1965) and St. Omer (1969). This has led Ecobichon (1970) to suggest that ammonia may be the source of the centrally induced excitation in chlorinated hydrocarbon poisoning. Whether the symptoms of cardiac and respiratory arrest reported in fish by Byington and Rowe (1969) are a result of ammonia poisoning or a response to prevent such poisoning may have some bearing on this issue. The concept of elevated free ammonia in the brain producing centrally induced excitation lends credibility to the suggestion of Byington and Rowe (1969) that the condition noted in fish is a response which prevents ammonia poisoning. Injection of ammonia into fish might eliminate the cardioventilatory periodicity and produce symptoms similar to those observed in this study. However, as was learned in the calcium treatment section of this study, the anatomy of the fish does not lend itself to injection of any material. Devising a method of intravenous injection for fish would prove fruitful in further study of this problem.

SUMMARY

The electrical activity of the heart and respiratory movements of largemouth bass were recorded before and after treatment with DDT and methoxychlor to determine the effects of these pesticides on these parameters as well as their effects on the cardiac rate and respiratory rate. Correlation of heart beat and respiratory beat was also investigated.

Results of this study showed no direct effect of either pesticide on the electrical activity of the heart. Methoxychlor appeared to produce its lethal effect by causing a disruptive action on the respiratory mechanism of the fish. Although the visible symptoms of DDT poisoning appeared to be very similar to methoxychlor poisoning, the recording of respiratory movements showed a gradual reduction in the amplitude of respiratory movement with less of a disruptive effect. Calcium treatment of fish to prevent or remedy symptoms of poisoning was inconclusive. The cardiac and respiratory rates appeared to be unaffected by DDT treatment, but methoxychlor appeared to destabilize both rates with the greatest effect being shown on the respiratory rate.

Synchrony of heartbeat and opercular beat was rarely

observed. It was concluded that synchrony occurs only under special circumstances and persists only under very static conditions.

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