A PROPOSED BIOCHEMICAL MECHANISM OF THE TOXIC ACTION OF DDT

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ABSTRACT. — DDT, 5.9 x 10^{-4} grams per ml of reaction medium, inhibited oxygen uptake by bluegill liver mitochondria in the presence of succinic acid. DDT increased the hydrolysis of adenosinetriphosphate in the presence of magnesium and manganese ions. A biochemical mechanism of the toxic action of DDT is suggested.

DDT (1,1,1, -trichloro -2,2 bis (P-chlorophenyl) ethane) has been widely used as an insecticide and apparently has spread throughout the world. DDT has been the subject of much research in efforts to explain its mode of action on insects, the primary target organisms. More recent research has attempted to explain its effects on non-target organisms, such as fish and birds. Metcalf (1955) discussed the mode of action of DDT, and later O'Brien (1967) indicated that after 12 years of intensive research the mechanism of the insecticidal properties of DDT has not been elucidated. However, the general agreement was that the primary target of DDT appeared to be the nervous systems of both vertebrates and invertebrates.

This view is untenable, as it does not explain the diverse biological effects, such as the effects on fish and bird reproduction, which have been shown to be related to or caused by DDT. However, some of the observed results, such as increased activity, may be due to the effects of DDT on the nervous system, but there is evidence that DDT affects fundamental biochemical processes in other tissues. The involvement of the production of cellular energy and the effects of DDT on these processes was dismissed by Metcalf (1955) as of little importance in explaining the mode of the toxic action of DDT. The suggested effects of DDT on the nervous systems, extensively reviewed by O'Brien (1967) does not offer a suitable explanation of the effects of DDT on the reproductive processes in birds and fishes, while the effects of DDT on basic biochemical enzymatic processes may explain both phenomena.

We have been investigating the effects of possible pollutants including pesticides on energy production by the mitochondria of the liver of the bluegill sunfish, *Lepomis macrochirus*, (Hiltibran, 1967b) and have found that compounds which were very toxic to fishes, such as rotenone, antimycin A, cyanide, isopropyl ester of 2,4-D altered the oxygen or phosphate uptake by bluegill liver mitochondria (Hiltibran, 1967b). The previous investigations of Sacktor (1950), Johnston (1951), and Anderson *et al* (1954) indicated that DDT altered the oxygen uptake by various tissues. The results of our investigation of the effects of DDT on the oxygen and phosphate metabolism of bluegill liver mitochondria is reported.

Methods

Native, wild bluegills were held in the laboratory aerated aquaria at 25° C and all enzymatic assays were conducted at that temperature. Procedures for the preparation of the mitochondria, for estimating the oxygen and phosphate, for estimating the rate of release of inorganic phosphate from adenosinetriphosphate, ATP, and for estimating the nitrogen content of the mitochondrial preparations have been previously reported (Hiltibran and Johnson, 1965). The data are the average changes observed from three or more experiments and have been corrected for endogenous activity and the effects of the solvent. Reference samples of DDT were used, and redistilled ethyl alcohol or acetone were used as solvents. DDT concentrations are expressed as grams of DDT per ml of reaction medium.

Results

The effects of DDT on oxygen and phosphate uptake by bluegill liver mitochondria in the presence of succinate and alpha-ketoglutarate as substrates are summarized in Table 1. In the presence of succinate, 5.9 x 10^{-4} g of DDT per ml of reaction medium completely inhibited the uptake of oxygen, and at a concentration of 5.9 x 10^{-6} g oxygen uptake was inhibited approximately 40 percent. Lower levels of DDT were not as effective on oxygen uptake. Usually when there is severe inhibition of oxygen uptake there is an increase in the inorganic phosphate content of the reaction medium. However, in the presence of DDT there was not an increase in the inorganic phosphate content of the reaction medium, when oxygen uptake is altered.

DDT inhibited oxygen uptake in the presence of alpha-ketoglutarate

Table 1.—Effects of DDT on Oxygen Uptake by Bluegill Liver Mitochondria.

g/ml of Reaction Medium	Average Change in μ 10_2/hr/mg N	Average Change in μ moles $P0_4$/hr/mg N	Percent Inhibition of 0_2 Uptake
Succinate			
5.9 x 10^{-4}	(—) 118	(±) 16	100
5.9 x 10^{-5}	(—) 89	(±) 18	80
5.9 x 10^{-6}	(—) 39	(±) 9	40
5.9 x 10^{-8}	(±) 30	(±) 4	
Alpha-Ketoglutarate			
5.9 x 10^{-4}	(—) 31	(+) 8	20
5.9 x 10^{-5}	(—) 58	(+) 20	45
5.9 x 10^{-6}	(—) 37	(+) 13	25
5.9 x 10^{-7}	(+) 41	(+) 3	30 (increase)

as substrate, but to a lesser extent. There was no effect of DDT on phosphate uptake in the presence of alpha-ketoglutarate. DDT did not appear to uncouple the phosphate uptake from the oxidation of either substrate, therefore, the primary effect of DDT appeared to be on the utilization of oxygen.

The effects of DDT on the hydrolysis of ATP by the bluegill liver mitochondria are summarized in Table 2. In the presence of cadmium, DDT inhibited the hydrolysis of ATP at all concentrations of DDT used, and in the presence of zinc, DDT inhibited the hydrolysis of ATP at the highest concentration of DDT. The hydrolysis of ATP was not greatly altered in the presence of either manganese or calcium, but in the presence of magnesium, DDT increased the hydrolysis of ATP from approximately 60 percent at a DDT concentration of 1.5 umoles of DDT per ml of reaction medium to 150 percent at a DDT concentration of 2.5 umoles.

The data suggests that DDT can alter the activities of various enzyme complexes from the bluegill liver mitochondria and that the observed effects appear to be related to the concentration of DDT. The data also suggests a specific effect of the DDT molecule on each liver enzyme complex. When the DDT concentration was increased, complete inhibition of oxygen uptake occurred and this is consistent with the report that as the DDT poisoning of insects increased, the oxygen utilization decreased, until the death of the organisms occurred.

Discussion

Soon after the large scale use of DDT for the control of insect pests was begun, it became evident that bird populations (Robbins *et al*, 1951) declined in the DDT treated areas. Some of the observed changes appeared to be due to the effects of DDT on the viability and hatchability of eggs (Mitchell *et al*, 1951). More recent data have suggested that the wide-spread use of DDT and other organochloro insecticides may be responsible for the decline of populations of the golden eagle, *Aquila chrysaetas* (Lockie and Ratcliffe, 1964), Osprey, *Pandion haliaetus* (Ames, 1966), herring gull, *Larus*

Table 2.—Effects of DDT on Hydrolysis of ATP.

Metal	g of DDT per ml reaction medium		
	5.3×10^{-4}	8.9×10^{-4}	17.7×10^{-4}
	Average Change in μ moles ATP/hr/mg N		
Cadmium	(—) 34	(—) 16	(—) 7
Zinc	(±) 13	(±) 9	(—) 19
Manganese	(+) 40	(+) 45	(±) 19
Magnesium	(+) 16	(+) 36	(+) 23
Calcium	(±) 7	(±) 11	(±) 33

argentatus (Paynter, 1949), and the peregrine falcon, *Falco peregrinus* (Hickey and Anderson, 1968).

Lake trout (*Salvelinus namaycush*) sac fry, hatching from eggs of females from Lake George, New York, did not survive. However, when the sperm from males from Lake George were used to fertilize eggs of females from another watershed, fry survival was normal. The watershed of Lake George had received applications of DDT for the control of gypsy moth. Burdick *et al* (1964) investigated this phenomenon, described the syndrome produced in the lake trout sac fry, and pinpointed the time of its development as late in the yolk-sac utilization period and just before the fry began to feed. This time appeared to be correlated with the period of maximum utilization of the phospholipid content of the yolk-sac. Further, comparison of the affected fry with normal fry did not reveal any histological or pathological differences. They noted that fry hatched from eggs which contained 2.95 ppm of DDT developed the syndrome, whereas fry from eggs with a DDT content of 2.67 ppm did not.

Allison *et al* (1963) reported that mortality among sac fry appeared to be highest in those cutthroat trout (*Salmo clarki lewisi*) which received high concentrations of DDT. Maeck (1968) reported similar observations with brook trout (*Salvelinus fontinalis*) which had received repeated sub-lethal concentrations of DDT.

The recent work reported by Ames (1966) on the Osprey in Connecticut and the work of Paynter (1949) on the herring gull in Lake Michigan indicated that DDT is the causative agent in the decrease in populations of these two species and that the effect was on the development of the embryo. It has been suggested that in the eagle (Lockie and Ratcliffe, 1964) and peregrine falcon (Hickey and Anderson, 1968) DDT caused a reduction in egg shell thickness. However, the effects of DDT on the reproduction of the quail (Dewitt, 1956) and the pheasant (*Phasianus colchicus*) (Genelly and Rudd, 1956) do not fit this pattern, for their chicks die several weeks after hatching.

DDT can cause mortality among fishes and birds. However, repeated sub-lethal doses of DDT did not cause mortality. Further, sub-lethal doses of DDT to fishes and birds did not cause any reduction in the number of viable eggs produced. In fishes, normal sac-fry were produced from females from the Lake George watershed which apparently were not susceptible to the effects of DDT until late in the sac-fry stage. The fish affected during sac-fry stages did not show any histological or pathological damage. Bird embryos, however, appeared to die in embryonic life. Thus, it would appear that DDT must be altering some fundamental biochemical or physiological processes common to all the organisms and to the various situations discussed. Energy production would be such a common denominator.

The increased oxygen consumption and the associated random, awkward body movements observed in insects treated with DDT suggest a neuromuscular involvement. It is generally agreed that the increased oxygen consumption is the result of increased activity of the organisms (Metcalf, 1955, O'Brien, 1967). However, Riker (1946), Jandorf *et al* (1946), and Laug and Fitzhugh (1946) found that the oxygen consumption of liver slices of animals in sub-acute and chronic DDT poisoning had an increased oxygen consumption, and

that the oxygen consumption from animals in advance stages of DDT poisoning was decreased. The changes in body coordination could be considered as a partial impairment of functions due to the decreased ability of the nervous system to function normally, a possible result of reduced energy output.

Part of the reaction of the organisms would appear to be a response to stress. It was observed that as the poison symptoms became more severe, oxygen uptake declined (O'Brien, 1967), which is consistent with the observed effects of DDT on oxygen uptake as described above. The first system to be involved might be the nervous system because of its high fat content. This point will be discussed later.

Rotenone, which has been used as an insecticide but more recently has been widely used as a fish toxicant, has been shown to decrease oxygen uptake by insects (Tishler, 1935) and was thought to cause damage to the tissues (Danneel 1933). Fukami and Tomizawa (1956) reported that rotenone inhibited the oxidation of glutamate, and later it was shown that this compound did not cause tissue damage (Oberg, 1955) but that its primary effect was the inhibition of the transfer of electrons from the substrate to the cytochrome chain (Lindahl and Oberg, 1961). This has been the only biochemical effect shown for rotenone. It should be pointed out that in rotenone poisoning, excessive, random movements of fishes are observed, but rotenone has not been considered a nerve poison (O'Brien, 1967). The effects of rotenone on the oxygen uptake by bluegill liver mitochondria have been reported (Hiltibran and Johnson, 1965).

Cyanide and antimycin A have been used as fish toxicants (Bennett, 1962), (Walker *et al*, 1964) and appear to block the uptake of oxygen by bluegill liver mitochondrial systems (Hiltibran, 1965, 1967a). They interrupt the electron flow from the substrate to the electron acceptor, oxygen (Chance, 1956). The data strongly suggest that when the oxidative pathways are blocked, energy cannot be produced, various cells cannot function, and if some regulatory cells or organ would be affected, for example the respiratory center, the organism cannot maintain its integrity and dies. This has been suggested previously (Hiltibran, 1971), (Skidmore, 1965).

It is well known that some metals are extremely toxic to bluegills (McKee and Wolf, 1963). Recently we observed that cadmium and zinc at relatively low levels (Hiltibran, 1965; 1967a) severely inhibited the uptake of oxygen by bluegill liver mitochondria. We found also that calcium and manganese can interrupt energy production, but the effect was primarily on phosphate metabolism, whereas the primary effect of cadmium and zinc was on the uptake of oxygen (Hiltibran, 1971).

Previously we had been intrigued by the fact that certain derivatives of 2,4-D were more toxic to small bluegills than were others (Hiltibran, 1967c). Therefore, we investigated the effects of nine derivatives of 2,4-D and found that the most toxic 2,4-D derivatives used in the study, the butyl and isopropyl esters, altered the uptake of oxygen and phosphate by the bluegill liver mitochondria. Thus it would appear that the primary effect of these derivatives was on oxygen uptake and that their toxic action could be produced via their effects on energy production (Hiltibran, 1969a; 1969b). It appears, then, that the

effects of DDT on the oxygen uptake cited above would be of paramount importance in explaining the mode of action of DDT, since we have shown the similarity of the effects of DDT, rotenone, cadmium, zinc, the butyl and isopropyl esters of 2,4-D, and other electron flow inhibitors and oxidative phosphorylation uncoupling agents.

Thus I believe that the interruption of the production of energy is of primary importance in explaining the toxic action of DDT. I suspect that similar cases can be developed for some of the other organochloro insecticides on the basis of available data (Hiltibran unpublished data; Colvin and Phillips, 1968).

DDT is very soluable in or has a high affinity for the tissue fat. Further, it is assumed that the incorporation of DDT into the tissue fat is a passive process and is not an active "detoxification" process. It does not appear that definitive data are available to determine whether this incorporation is a biological active or a biological passive process.

Exposure of target or non-target organisms to lethal levels of DDT would result in a DDT uptake which would exceed the rate at which DDT could be incorporated into the tissue fat and/or exceed the DDT storage capacity of the total body fat content. This amount of DDT would be in addition to that which would be metabolized. The remaining "unbound DDT" or "circulating DDT" would be able to exert its toxic action by blocking the oxygen uptake in the various tissues. Repeated light doses of DDT to fishes and birds would give time for the storage of the DDT in the tissue fat, and this could account for the large buildup of DDT without apparent damage.

Apparently the DDT is transferred to the eggs of fishes or birds which would account for the high levels of DDT in these eggs. In fishes, most of the DDT would remain bound throughout the development of the fish embryo, and DDT could not exert its toxic action until the late stage of the development when the yolk fat was mobilized, and the DDT again became "circulating" or "unbound". If only small amounts of DDT were involved, the effects on fry would not be lethal. This is consistent with the data in Table 1 and with the observations of Burdick *et al* (1954). The DDT content of bird eggs which did not develop normal embryos has not been as well documented as has the DDT content of fish eggs; the effects of DDT on bird reproduction is not as clearly defined as with the fishes. However, the developing bird embryos apparently were severely affected by high levels of "circulating DDT".

There are intraspecies differences in the quail and pheasant that complicate the picture, and these will remain a mystery until the comparative biochemistry of the organisms involved is known. These differences might help to explain why quail and pheasant chicks are the susceptible unit to the lethal effects of DDT. It is suspected that the "circulating DDT" is the causative agent, but apparently the stress develops later which may coincide with the production of feathers or using the remaining portion of the yolk, which may contain large quantities of DDT.

Data to support this hypothesis comes from the work of Johnston (1951), Anderson (1954), Riker (1946), Jandorf *et al* (1946), and Laug and Fitzhugh (1946). Judah (1949) could not demonstrate any effect of DDT on the succinic oxidase,

but Johnston could. Judah had administered the DDT in his experiments in an oil emulsion, whereas Johnston had administered the DDT in acetone. Further, when Johnston administered DDT in an oil emulsion, he could not demonstrate any effect of DDT on the succinic oxidase. This demonstrates the protective effect of the oil or fat on the DDT. It is not surprising, therefore, that with the high fat and phospholipid content of the nervous tissue that nervous systems might be one of the first tissues affected.

It has been suggested that DDT altered the production of the egg shell by the peregrine falcon (Hickey and Anderson, 1968) and the golden eagle (Lockie and Ratcliff, 1964), and this effect of DDT was confirmed in experiments with the American sparrow hawk, *Falco sparverius* (Porter and Wiemeyer, 1969). The synthetic formation of the egg shell, primarily calcium carbonate, would suggest that its synthesis is an energy-requiring process for the mobilization of the required calcium and for the production of the carbonate. Torda and Wolff (1959) demonstrated that DDT inhibited the carbonic anhydrase enzyme complex, which has been suggested as one enzyme complex involved in the formation of the egg shell (Sturkie, 1954). Further, the effects of organochloro pesticides on the metabolism of endogenous steroids is not known (Kupfer, 1967), but recently Welch *et al* (1969) reported that a DDT isomer was converted to an estrogentic hormone metabolite which would indicate that organochoro insecticides, particularly DDT, may have some effect on the steroid hormones involved in calcium metabolism.

Johnston (1951) found that DDT did not alter the succinic dehydrogenase but that the succinic oxidase complex was affected. The data in Table 2 indicate that DDT did not appreciably alter the oxidation of alpha-ketoglutarate. These observations indicate that DDT inhibited the flow of electrons at the flavoprotein transferring site between the succinic dehydrogenase and the cytochrome chain. Matsumura and O'Brien (1966) have isolated complexes of DDT and tissue components and suggested that the complexes involved were of the charge-transfer type. These data would further support the biochemical hypothesis suggested, since DDT could form a complex with the flavoprotein of the electron transferring site between succinic acid and the cytochrome chain. Such action could block the flow of electrons, which would block the production of energy via the oxidative pathways. DDT has been found to have an effect similar to rotenone, cyanide, and antimycin A, and it is suggested that this is the primary effect of DDT. DDT also has been shown to have an effect on phosphate metabolism, as indicated by its effect on the hydrolysis of ATP, which rotenone, cyanide, and antimycin A did not alter. The data suggest that the primary effect is the inhibition of electron flow from succinic acid to the cytochrome chain.

Acknowledgments

The author acknowledges the technical assistance of Messrs. Michael Johnson and Richard Schumacher and Mrs. Charlene Mathis during this investigation.

Literature Cited

Allison, D., B. J. Kallman, O. B. Cope, and C. C. Van Valin. 1963. Insecticides: Effects on Cutthroat Trout of Repeated Exposure to DDT. Science 142: 958-961.

ANDERSON, A., R. MARCH, and R. L. METCALF. 1954. Inhibition of the succinoxidase system of susceptible and resistant house flies by DDT and related compounds. Ann. Entomol. Soc. 47: 595-601.

AMES, P. S. 1966. DDT Residues in the eggs of the Osprey in the North-Eastern United States and their Relation to Nesting Success. J. Applied Ecol. Supplement 3, 87-97.

BENNETT, G. W. 1962. Management of Artificial Lakes and Ponds, Reinhold Publishing Co. N.Y. p. 142-149.

BURDICK, G. E., E. J. HARRIS, H. J. DEAN, T. M. WALKER, J. SHEA, and D. COLBY. 1964. The Accumulation of DDT in Lake Trout and the Effect on Reproduction. Trans. Amer. Fish. Soc. 93: 127-136.

CHANCE, B. 1956. "Enzymes: Units of Biological Structure and Function." Academic Press, Inc. New York. p. 450.

COLVIN, H. J., and A. T. PHILLIPS. 1968. Inhibition of Electron Transport Enzymes and Cholinesterases by Endrin Bull. Environmental Contamination and Toxicology 3: 106-115.

DANNEEL, R. 1933. Die Giftwirkung des rotenons and seinen Derivativ auf fische. III Der Angriffspunkt der Gifte. Zeit. vergl. Physiol. 18: 524-535.

DEWITT, J. B. 1956. Chronic toxicity to Quail and Pheasants of some Chlorinated Insecticides. J. Agr. and Food Chem. 10: 863-866.

FUKAMI, J., and C. TOMIZAWA. 1956. Effects of rotenone on the l-glutamic oxidase systems in insects. Japanese Contributions to the Study of the Insectcide-Resistance Problem. p. 212-216. Originally published by Boytu Kagaku 21: 129-133.

GENELLY, R. E., and R. L. RUDD. 1956. Effects of DDT, Toxaphene and Dieldrin on Pheasant Reproduction. The Auk 73: 529-539.

HICKEY, J. J., and D. W. ANDERSON. 1968. Chlorinated Hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162: 271-273.

HILTIBRAN, R. C. 1965. Oxidation of succinate by bluegill liver mitochondria. Trans. Ill. Acad. Sci. 58: 176-182.

HILTIBRAN, R. C. 1966. Hydrolysis of adenosinetriphosphate by bluegill liver mitochondria. Trans. Ill. Acad. Sci. 59: 249-253.

HILTIBRAN, R. C. 1967a. Oxidation of alpha-ketoglutarate by the bluegill liver mitochondria. Trans. Ill. Acad. Sci. 60: 244-249.

HILTIBRAN, R. C. 1967b. Abstracts. 7th International Congress of Biochemistry. Tokyo, Japan. p. 899.

HILTIBRAN, R. C. 1967c. Effects of some herbicides on fertilized eggs and fry. Trans. Am. Fish. Soc. 96: 414-416.

HILTIBRAN, R. C. 1969a. The hydrolysis of adenosinetriphosphate by bluegill liver mitochondria in the presence of 2,4-dichlorophenoxyacetic acid derivatives. Trans. Ill. Acad. Sci. 62: 36-43.

HILTIBRAN, R. C. 1969b. Oxygen and phosphate metabolism of bluegill liver mitochondria in the presence of 2,4-dichlorophenoxyacetic acid derivatives. Trans. Ill. Acad. Sci. 62: 175-180.

HILTIBRAN, R. C. 1971. Effects of cadmium, zinc, manganese and calcium on the oxygen and phosphate metabolism of bluegill liver mitochondria. J. Water Poll. Contr. Fed. In press.

HILTIBRAN, R. C., and M. G. JOHNSON. 1965. The effect of rotenone on oxygen uptake by liver mitochondria of the bluegill, *Lepomis machochirus*. Trans. Ill. Acad. Sci. 58: 140-143.

JANDORF, B. J., H. B. SARETT, and O. BODANSKY. 1946. Effect of oral administration of DDT on the metabolism of glucose and pyruvic acid in rat tissues. J. Pharmacol. and Exp. Therap. 88: 333-337.

JOHNSTON, C. 1951. The *in vitro* effect of DDT and related compounds on the succinic oxidase system of rat heart. Arch. Biochem. 31: 375-382.

JUDAH, J. D. 1940. Studies on the metabolism and mode of action of DDT. Brit. J. Pharmacol. 4: 120-131.

KUPFER, D. 1967. Effects of some pesticides and related compounds on steroid function and metabolism. Residue Rev. 19: 11-30.

LAUG, E. P. and O. G. FITZHUGH. 1946. 2, 2-Bis(p-chlorophenyl) 1,1,1-trichloroethane (DDT) in the tissues of the rat following oral ingestion for periods of six months to two years. J. Pharmacol. and Exp. Therap. 87: 18-23.

LINDAHL, P. E., and K. E. OBERG. 1961. The effect of rotenone on respiration and its point of attack. Exp. Cell. Res., 23: 228-237.

LOCKIE, J. C. and D. A. RATCLIFFE. Insecticides and Scottish golden eagles. British Birds. 57: 89-101.

MACEK, K. J. 1968. Reproduction in Brook Trout (*Salvelinus pontinalis*) fed sublethal concentrations of DDT. J. Fish. Res. Bd. of Canada. 25: 1787-1796.

MATSUMURA, F., and R. C. O'BRIEN. 1966.

Absorption and Binding of DDT by the Central Nervous System of the American Cockroach. J. Agr. Food Chem. 14: 36-45.

McKee, J. E., and H. W. Wolf. 1963. Water Quality Criteria. Pub. 3A California State Water Quality Control Board.

Metcalf, R. L. 1955. Organic insecticides, their chemistry and mode of action. Interscience Publishers, Inc. New York, p. 127-189.

Oberg, K. E. 1955. The structure of gill ephithelium and the circulation in gills of fishes poisoned with rotenone. Arkiv. Zool. 12: 383-384.

O'Brien, R. C. 1967. Insecticides: Action and Metabolism. Academic Press New York, p. 108-132.

Paynter, R. A. 1949. Clutch size and the eggs and chick mortality of Kent Island herring bulls. *Ecology*. 30: 146-166.

Porter, R. C. and S. N. Wiemeyer. 1969. Dieldrin and DDT: Effects on Sparrow Hawk eggshells and reproduction. Science. 165: 199-200.

Riker, W. F., V. R. Huebner, S. B. Raska, and M. Cattell. 1946. Studies on DDT 2,2-Bis(parachlorophenyl) 1,1,1-trichloroethane: Effects on oxidative metabolism. J. Pharmacol. and Exp. Therap. 88: 327-332.

Robbins, C. S., P. F. Springer, and C. J. Webster. 1951. Effects of five-year DDT application on breeding bird populations. J. Wildl. Mgmt. 15: 213-216.

Sacktor, B. 1958. A comparison of the cytochrome oxidase activity of two strains of house flies. J. Econ. Entomol. 43: 832-837.

Skidmore, J. F. 1965. Toxicity of zinc compounds to aquatic animals, with special reference to fish. Quart. Rev. Biol. 39: 227-248.

Sturkie, P. D. 1954. Avian Physiology. Compstock Publishing Associates, Ithaca, New York.

Tishler, N. 1935. Studies of how derris kills insects. J. Econ. Entomol. 28: 215-220.

Torida, C. and H. G. Wolff. 1959. Effect of convulsant and anticonvulsant agents on the activity of carbonic anhydrase. J. Pharmacol. Exp. Therap. 95: 444-447.

Walker, C. R., R. E. Lennon, and B. L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. Cir. 186. Bur. of Sport Fish. and Wildl.

Welch, R. M., L. Levin, and A. H. Conney. 1969. Estrogenic action of DDT and its analogs. Toxic. Appl. Pharmacol. 14: 358-367.

Manuscript received May 4, 1970