The Accumulation And Loss Of Field-Applied Butoxyethonal Ester Of 2, 4-Dichlorophenoxyacetic Acid In Eastern Oysters, *Crassostrea virginica* And Soft-shelled Clams, *Mya arenaria*[1]

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ABSTRACT

Tidewater Maryland field studies were made in 1966 in the Wicomico River, tributary of the Potomac, to determine the accumulation and loss rate of residues of the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (2,4-D BE) in eastern oysters and softshelled clams. A commercial and a federal laboratory, each using gas electron capture chromatography were involved in the analyses, and reports of residues in similarly exposed, coded animal aliquots varied. Results are graphed or tabulated and were stated to be accurate to ± 0.2 ppm. The lower limit of detection was considered to be 0.01 ppm. Depending on the animal species, the amount of herbicide applied, and the distance the animals were from treated areas, bioassay oysters and clams acquired residues of 2,4-D BE which peaked within 9 days, but were usually lost within detectable limits between the 35th and 72nd days after initial exposure. Reported residue ppm's in divided, washed and unwashed oyster samples differed between the two laboratories, as did data for residues in caged clams compared with residues in natural, bedded populations. Oysters and clams held for two years in freezer storage showed a small, but steadily decreasing residue content.

Earlier residue studies in other Chesapeaks Bay tributaries also are discussed. Suggestions are made for future research, and the need for improved analytical procedures is pointed out. Since no residue tolerance has been established by FDA for 2,4-D BE in finfish or shellfish, proposals are made to assure safety for human consumers utilizing animals harvested from treated areas.

INTRODUCTION

Eurasian watermilfoil, *Myriophyllum spicatum* L., a rooted, perennial, aquatic plant, has been controlled in the Chesapeake Bay and its tributaries on an experimental basis with the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid. A thoroughly researched and carefully tested biological control of milfoil would be preferable to a herbicide control but at present, none is known. Herbicide use in waters from which animal food is harvested for human consumption poses two immediate considerations:
the toxicity to organisms in treated areas, and herbicide residues occurring in edible fauna. Extensive experiments showed that 2,4-D BE at recommended rates in Chesapeake Bay oysters had no primary lethal effects on oysters, softshelled clams, blue crabs, *Callinectes sapidus,* and test fish of various species, mostly pumpkinseed, *Lepomis machrochirus,* (12). No adverse effects were noticed among adult oysters held in treated plots when using new oyster shell growth as a bioindicator in the manner described by Butler (2). Smith and Isom (15) concluded that high application rates of 2,4-D BE in TVA reservoirs did not produce undesirable effects on major forms of aquatic animal life. Studies in Prince Edward Island and in New Brunswick (18) provided like findings when 2,4-D BE was used in controlling eelgrass, *Zostera marina* L. Though 2,4-D BE apparently has little or no direct lethal effects on important aquatic fauna, long term effects of any pesticide should be considered, and when feasible, such possible effects should be carefully researched. Too, any ecological imbalances resulting from habitat manipulation should be closely studied.

Once primary toxicity proved to be a negligible factor in field applications of 2,4-D BE, it then became necessary to determine the accumulation and loss rate, if any, of this herbicide in animals likely to be found in treated locations.

This is important not only from a consideration of potential physiological changes occurring within an exposed animal which accumulates or retains 2,4-D BE residues for many weeks, but it is also important from the standpoint of the human consumer. As pointed out by Rawls (13), little is known of the pharmacological effects of 2,4-D on man, but Dalgaard-Mikkelsen and Poulsen (5) mention that a man in self-experiment consumed 500 mg of 2,4-D daily for 3 weeks with no perceptible effects, and that a young Danish farm worker committed suicide from swallowing not less than 6,500 mg of 2,4-D. Hayes (9) states that the oral dose of 2,4-D required to produce reaction symptons in man is probably 3 to 4 g. Formulations are not identified in these papers.

Because of the paucity of information relating to the effects of 2,4-D BE ingested by people, either directly or indirectly, and because analytical techniques for detection of 2,4-D BE in aquatic animal life are not perfected[1], the Federal Food and Drug Administration conceivably could seize any interstate shipment of animals harvested for human consumption from a treated area. For that matter, FDA has not established a residue tolerance in fish or shellfish for any herbicide (6) and (17).

Other than accumulation and loss rates of 2,4-D BE in oysters and clams, this study was designed to determine changes in the amount of residues in bioassay animals held for two years in deep freezer storage; to determine residue difference between caged clams and clams from a natural

[1]Pers. comm. from J. E. Gilchrist, National Center for Urban and Industrial Health, Cincinnati, Ohio, June 4, 1968.

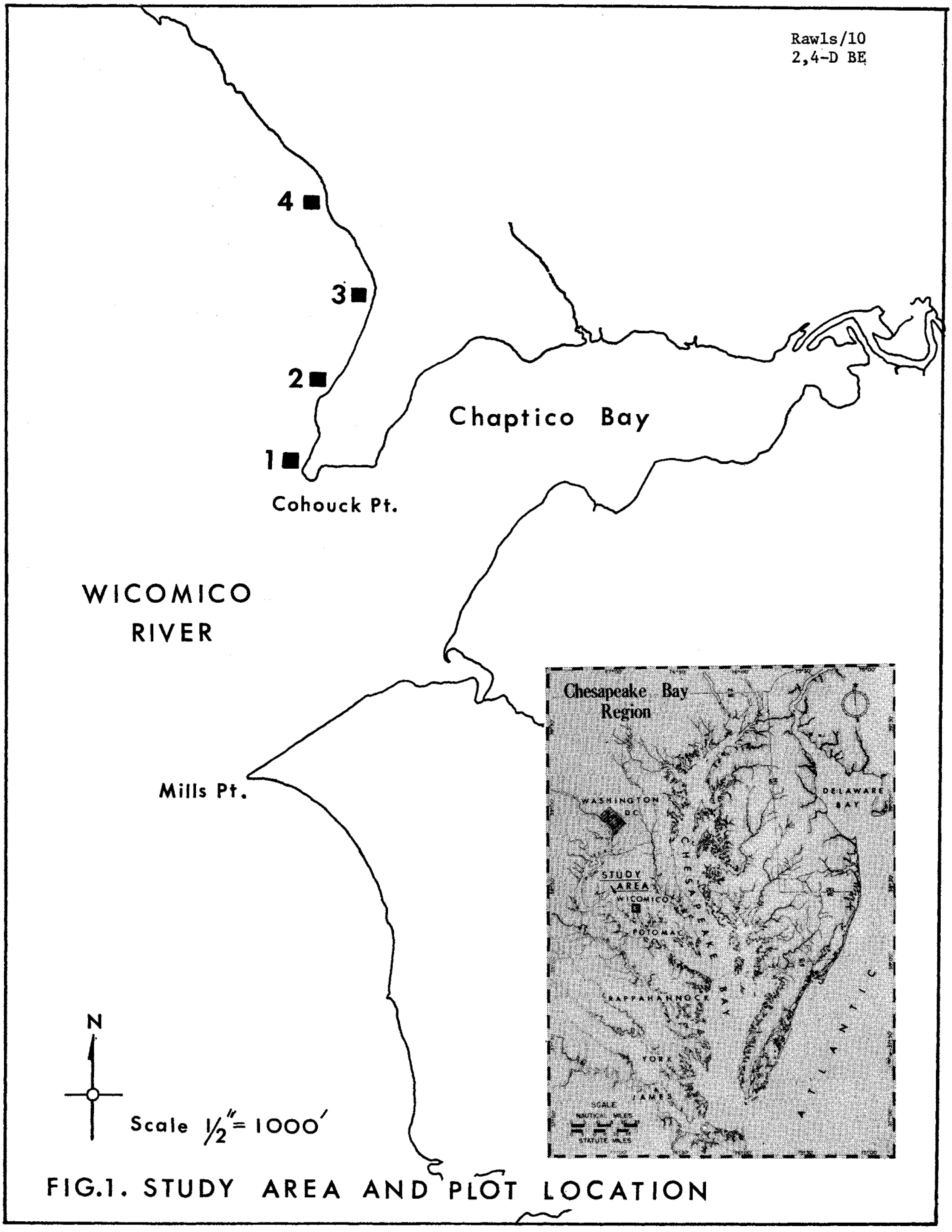

FIG.1. STUDY AREA AND PLOT LOCATION

bedded population in the same location; to determine the effectiveness of analytical procedures; to determine, because oysters are often eaten raw, the residue difference in unwashed ("on the half-shell") oysters and in washed oysters prepared as are those packed for the commercial market; and to determine the length of time a treated shellfish producing area must be closed before animals from it may be harvested free of detectable residues, thereby providing knowledge upon which adequate regulations can be based that will serve to assure the continuance of a local shellfishing industry by precluding possible closure of such harvest areas.

STUDY AREA

In setting up the 1966 field study for 2,4-D BE residues in exposed test animals, a number of site criteria had to be kept in mind: Milfoil had to be present in such sufficiently dense stands that it would be similar to growth conditions requiring 2,4-D BE application; salinity had to be high enough to support oysters and clams; a natural population of clams had to be present; accessibility by outboard boat and clam dredge had to be relatively easy; and a site selected where danger from pilferage hopefully would be at a minimum. Extensive field surveys pointed to one area which appeared to be ideal. Above Chaptico Bay, on the east side of the Wicomico River, a tributary of the Potomac River, vigorous stands of milfoil grew so profusely that they offered adequate vegetative containment for the herbicide to be applied. Empirical observations plus previous hydraulic clam dredge surveys (11) indicated a large population of clams growing beneath the milfoil; a launching ramp for outboard boats was nearby; clam dredges operated within running distance, but were not commercially active in this river section; salinity was ample as shown by existing clam populations, and only pilferage remained an unknown. The section of the Wicomico selected with plot locations noted is shown in Fig. 1, as is its general location with reference to the Chesapeake Bay.

In so far as I know, no previous application of 2,4-D BE had been made there, thus eliminating possible direct contamination of endemic fauna by herbicides. In a field study, no one can be certain what materials may be transported to the test site by air, by water run-off from adjacent land areas, or by tidal currents and net stream flow.

Some environmental characteristics of the test site during the study are shown in Table 1. Air temperatures ranged from 14.5 C to 38 C; surface water from 17.5 C to 31 C; salinities from 9.8°/ _ _ to 15.4 °/_ _; pH from 7.7 to 9.1; oxygen (in ml/l) from 5.05 to 9.3; and Secchi disc readings from 1 1/2′ to 4′ when not obscured by milfoil. Tidal variations averaged 2,.

METHODS AND MATERIALS

Plot establishment

In late June of 1966, 4 one-acre plots, about 209′ to a side, were measured with a floating polypropylene line. Three were to be treated and one would serve as a control. Plot corners and sampling stations were marked with bamboo poles. Weighted buoys made from empty plastic quart oil bottles painted international orange were attached to animal trays and cages to mark their positions. Oysters and caged clams would be placed in the center of each plot and at the 50 and 100 yard stations (Fig. 2). Each plot was separated from the other by a 300 to 500 yard buffer zone of milfoil. Throughout the study location, redhead grass, *Potamogeton perfoliatus* L., and widgeongrass, *Ruppia maritima* L., grew scatteringly among the milfoil while near the shoreline, naiad, *Najas guadalupensis* (Spreng.) Magnus, was common. The average water depth at low tide in the plots was 4′; the bottom was hard-packed, sandy silt with a 2 to 4′ overlay of soft sediments.

TABLE 1. SOME ENVIRONMENTAL CHARACTERISTICS OF TEST AREA, JUNE 28 — OCTOBER 18, 1966.

DATE	TEMPERATURE C Air	TEMPERATURE C Surface H_2O	SALINITY °/oo	pH	O_2 ml/l	SECCHI READING IN FEET	MILFOIL CONDITION
6/28	30	29	9.8	9	5.9	Hidden by milfoil	Original
7/5	35	31	10.5	8.5	5.9	Hidden by milfoil	Original
7/6	26	31	10.3	9.1	5.9	2	Original
7/8	32	31	9.8	9.0	5.9	2	Original
7/10	29	27	11.1	7.7	5.8	2½	Slightly sinking
7/12	38	31	10.6	8.9	5.45	3½	Sinking
7/14	34	31	11.4	8.1	5.05	2	Sinking, some defoliated stems
7/26	33	29	11.2	8.7	6.7	1½	Stems mostly defoliated. Slight regrowth Plot #1
8/9	27	27	14.0	8.1	9.3	4	60 lb. plot virtually bare
9/13	27	22	15.4	7.7	7.6	1½	No milfoil on dredge in any treated plot
10/18	14.5	17.5	13.6	8.1	8.1	2	No milfoil on dredge in any treated plot
11/20	Remaining oysters picked up by dredge boat — no data available.						
Ranges	14.5 / 38	17.5 / 31	9.8 / 15.4	7.7 / 9.1	5.4 / 9.3	1½ / 4	

Average tidal variation = 2 feet.

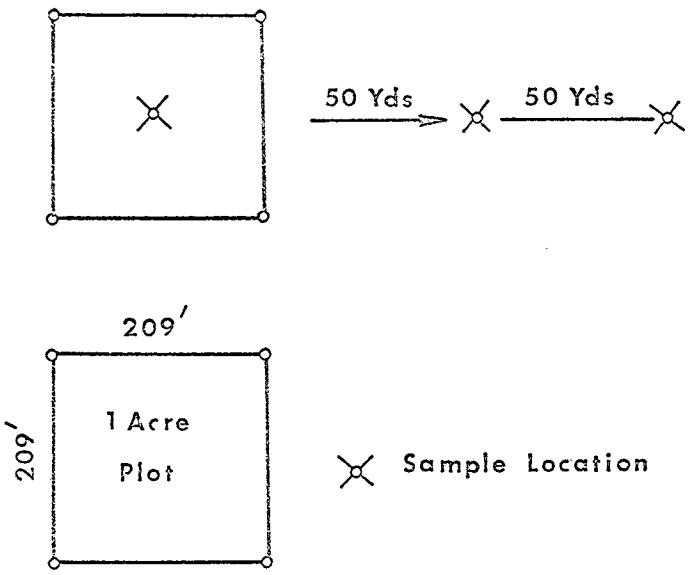

Fig. 2.—Animal sample locations in relation to plot perimeters.

TEST ANIMALS

Clams and oysters were used for test animals since they occupy relatively fixed positions while crabs and fish can leave a treated area if their habitat becomes unsuitable. Too, unconfined motile crabs and fish, if checked for residues, could have acquired them far from test localities, and earlier research had indicated caged fish and crabs would pick up less residues than clams and oysters (4). Adult oysters, taken above highway bridge 301 from Beacon Bar in the Potomac River, were placed on standard oyster trays and stationed as shown in Fig. 2, June 28. The next day, clams dredged from the Potomac near Blakiston Island, were placed in 1/2″ mesh 2′x2′x2′, hardware cloth cages and positioned next to the trayed oysters. Natural clam populations were bedded throughout the area. Ropes, bridled to trays and cages were fastened to the marker poles, and the buoys in turn fastened to the trays. Herbicide application was delayed a week to allow time for bioassay animals to adjust to their new environment.

HERBICIDE TREATMENT

Before herbicide treatment on July 5, each tray and cage of test animals was examined for mortality resulting from natural causes or from mechanical injury incurred during dredging. All oysters were living, but most caged clams were dead either from predation by eels, *Anguilla rostrata,* which had forced themselves through cage seams, or presumably as a result of exposure to extremely hot weather from the time they were dredged until the time they were placed in plot positions. Caged clams were not replaced since weather conditions remained unchanged, and since the planned duration of the overall study permitted little flexibility timewise, no new cages were constructed.

The herbicide used in this experiment was Amchem's "Aqua Kleen", a low volatile 20% acid equivalent (æ) 2,4-D BE impregnated for slow release on size 8/15″ mesh baked attaclay granuales. The herbicide poundage a plot received was determined by random selection. Plot 1 was treated at 30 lb æ/A (30 pounds acid equivalent per acre), plot 2 at 10 lb æ/A, plot 3 at 60 lb æ/A and plot 4, the farthest upstream, served as the control. A single application of 2,4-D BE granules on plots to be treated was made by forced air distribution using a Sol-Fi air blower attached to a flexible 3″ diameter hose connected to a spreader head mounted on the bow of a outboard hull powered by an 18 h.p. Johnson outboard motor. The granular herbicide fed by gravity flow to the air blower. Tidal stage was incoming high.

EXPERIMENTAL DESIGN

Originally it was planned to take oyster and clam samples for bioassay before treatment to establish a baseline for any residues already present in the animals, and to make subsequent collections 1, 3, 7, 21, 35, 70, 105 and 135 days following treatment. These plans were slightly altered. Analyst "B" wanted clam samples from each station but oyster samples only from the center of the plot treated at 30 lb æ/A and at the intervals stated above plus samples taken 5 and 9 days after exposure to the herbicide. Oysters and bedded clams were taken on schedule through the 35th day and processed for "A" and "B". Because of bad weather, the next complete sampling periods were on the 72nd and 104th days. Just prior to the 135th day sample, the hydraulic clam dredge-boat I had contracted for, became mechanically inoperative. Trays of remaining oysters were removed on the 138th day.

At each animal station, a bottom core was obtained with a heavy bamboo pole cut just above a node. Ordinary bottom sampling gear was ineffective because of dense milfoil and the hard-packed, sandy-silt bottom. Milfoil samples were taken by rake at the same place, but water samples were obtained only from the center of the control plot. The water samples were obtained with the idea that they might reveal any movement of 2,4-D BE into the control plot. However, it was realized that in tidal water, a sample would be indicative only of what was present at the instant the sample was taken.

Caged clams were used in addition to naturally bedded clams in test plots to see if there was a difference in residues between completely exposed animals compared to those buried in the sand except for a small hole leading to the clam siphon. Because of predation and natural mortality, clams rarely will live for extended periods when held out of their natural environment, but if caged clams could live successfully for the duration of the study, and if they did not differ significantly from embedded clams in residue accumulation and loss, then future residue studies using clams would not be hindered by the lack of a natural population in test sites. If exposed clams had higher residues, this could suggest, if metabolic differences were ignored, that some portion of residues were acquired by periostracal contact. As previously pointed out, insufficient caged clams survived to offer comparative data.

PREPARATION OF ANIMALS FOR BIOASSAY

Analysts "A" and "B" felt that 25 gm of animal material would be sufficient for each analysis, so 15 to 20 adult oysters and bedded clams were taken at every station on each collection date. A like number of caged clams was sampled until their numbers were exhausted. Individual samples were placed in a gallon bucket permanently marked with the plot number and station from which it had been taken. Buckets were scrubbed with detergent, acetone, and thoroughly rinsed after use. Milfoil, mud and water samples were placed in new polyethylene containers.

All samples were then removed to the Chesapeake Biological Laboratory, Solomons, Maryland, for preparation and labeling. Samples were coded, using numbers from 1 to 100 taken at random from a revolving bingo-ball cage. A master sheet was then set up relating code numbers to complete sample data—animal species, plot number, station location and day of exposure. Oysters and clams were shucked, and as prepared for market, about one-half the clam siphon and all periostracum were discarded. Samples (except for oyster sample duplicates which were unwashed to compare any residue differences washing might cause) were washed separately for 5 minutes under flowing tap water, drained, placed in new 1/2 pint polyethylene containers, covered with just enough water to prevent freezer burn, and after the code number has been marked indelibly on the top and sides of the containers, they were placed in deep freezer to await delivery to "A" and "B". In commercial practice, oysters and clams would have been washed longer and often with forced air agitation, therefore if residues could be washed out, it was felt that our preparation would show more residues than commercially prepared oysters and clams taken from the same site.

Two out of each 10 samples taken to "A" were duplicates; no duplicates were sent to "B" sinve it has been indicated they would be able to analyze only a limited number of samples. Both washed and unwashed oyster samples were prepared for both analysts.

A series of spiked oyster samples were sent to each analyst to determine the effectiveness of their procedures. These oysters came from unexposed stock taken from Beacon Bar. Several hundred oysters were homogenized in a Waring Blendor for 3 minutes. Fifty grams of the homogenate were spiked with a known concentration of 2,4-D BE, again blended for 3 minutes, and 25 gm aliquots of the spiked material were then coded and placed in freezer storage. The Blendor was thoroughly washed with detergent and rinsed in acetone and distilled water after every sample of spiked material was prepared.

Extra oyster and clam samples were collected after 3 days exposure from the center of the 60 lb æ/A plot. These were analyzed by "A" at 6 months intervals over a two year period to determine what effect prolonged freezer storage might have on residues. It was felt that this sample location and time of exposure probably would reflect the highest residue concentration, hence there would be a larger quantity of residue present to analyze and increased accuracy would result. An extra number of oysters and clams were taken from the control plot at the same time for a check against freezer effects on low or no residues.

When a sufficient number of all samples had been prepared, they were removed from freezer storage, placed in insulated styrafoam chests and taken to Washington, D. C., where they were still frozen on arrival. "A" placed his samples in deep freeze at approximately —5 F; the samples for "B" were packed in dry ice and air freighted to the National Center for Urban and Industrial Health in Cincinnati, Ohio. Though it would have been desirable, single animals were not analyzed to establish residue ranges among similarly exposed specimens. One animal did not offer sufficient weight for the analytical methods employed.

RESULTS

"A" and "B" used electron capture gas chromatography for their analyses and both feel their methods are accurate to± 0.20 ppm. J. E. Gilchrist[1] stated the lower limit of detection of "B's" method was 0.01 ppm, so ppm marked 0 are really less than 0.01 ppm. "A's" results are given in ppm's of the methyl ester, while "B's" are given in ppm's of the acid equivalent of 2,4-D. The difference is minor since they have practically the same molecular weights.

OYSTERS IN PLOTS

Figures 3 through 6 show the residues reported as ppm's of 2,4-D by "A". He reported a pre-treatment base level of 0.25 ppm.

In Plot 1, Fig. 3, treated at 30 lb æ/A, oysters taken

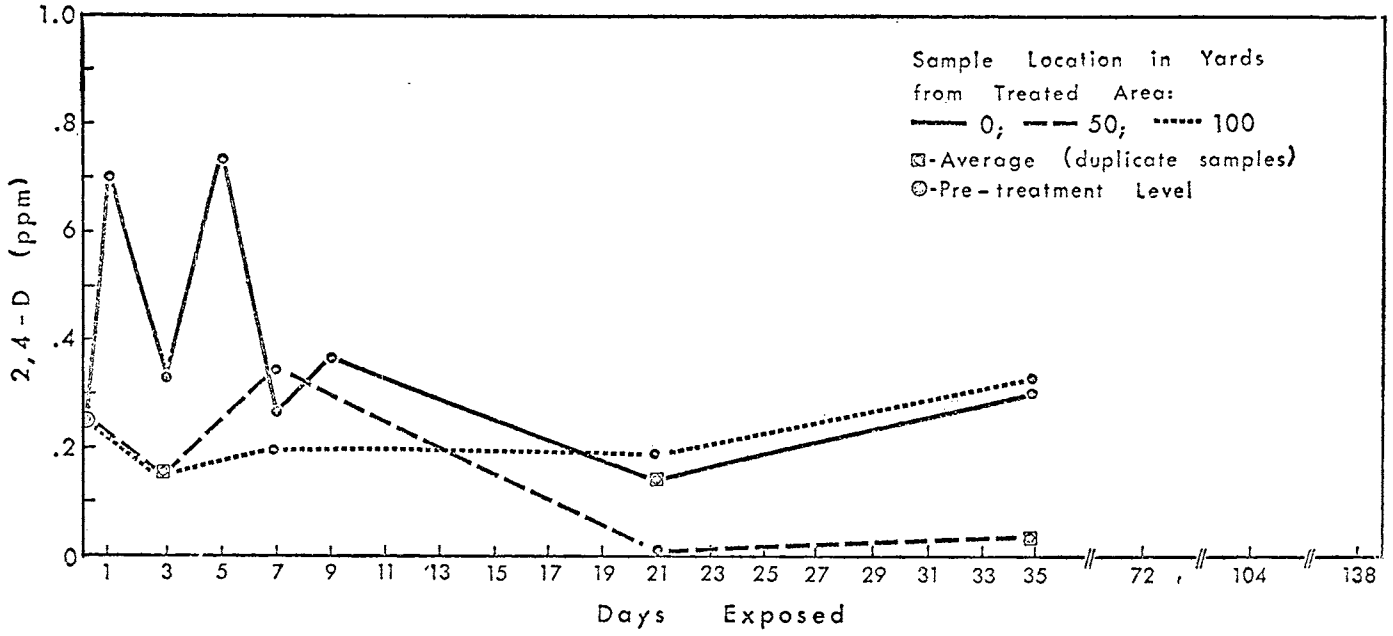

Fig. 3.—Accumulation and loss of 2,4-D in eastern oysters.
Plot 1, 30 lb ae/A, July 5-August 9, 1966.
Analyst A.

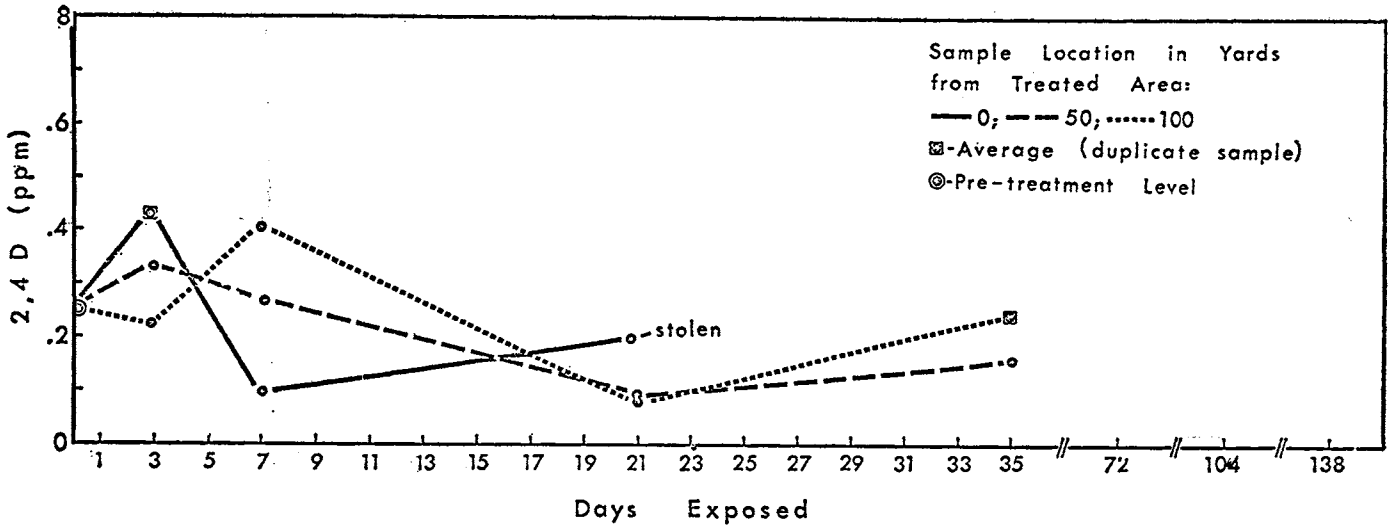

Fig. 4.—Accumulation and loss of 2,4-D in eastern oysters.
Plot 2, 10 lb ae/A, July 5 - August 9, 1966.
Analyst A.

from plot center peaked at 0.73 ppm at 5 days and after 35 days showed 0.30 ppm. Outside the plot, at the 50 and 100 yard stations, residues were 0.14 ppm 3 days after exposure, or less than that reported for the base level. The highest residues in the 50 yard oysters were reported after 7 days exposure, 0.34 ppm. From this peak residues dwindled to an average of duplicate samples at 35 days to 0.03 ppm. The highest residues from the 100 yard station occurred at 35 days when 0.32 ppm were reported.

In Plot 2, Fig. 4, treated at 10 lb æ/A, residues for all samples fluctuated. The highest residues were reported for plot center at the end of 3 days, an average of 0.42 ppm. These oysters were stolen between the 21 and 35 day samplings. At the end of 35 days, oysters at 50 yards showed 0.15 ppm and at 100 yards, residues were reported at 0.24 ppm.

In Plot 3, Fig. 5, treated at 60 lb æ/A, oysters taken at 3 days from plot center had 1.85 ppm, the largest amount of residue reported from any plot for oysters. However, at the end of 35 days, oysters from the same tray showed 0.00

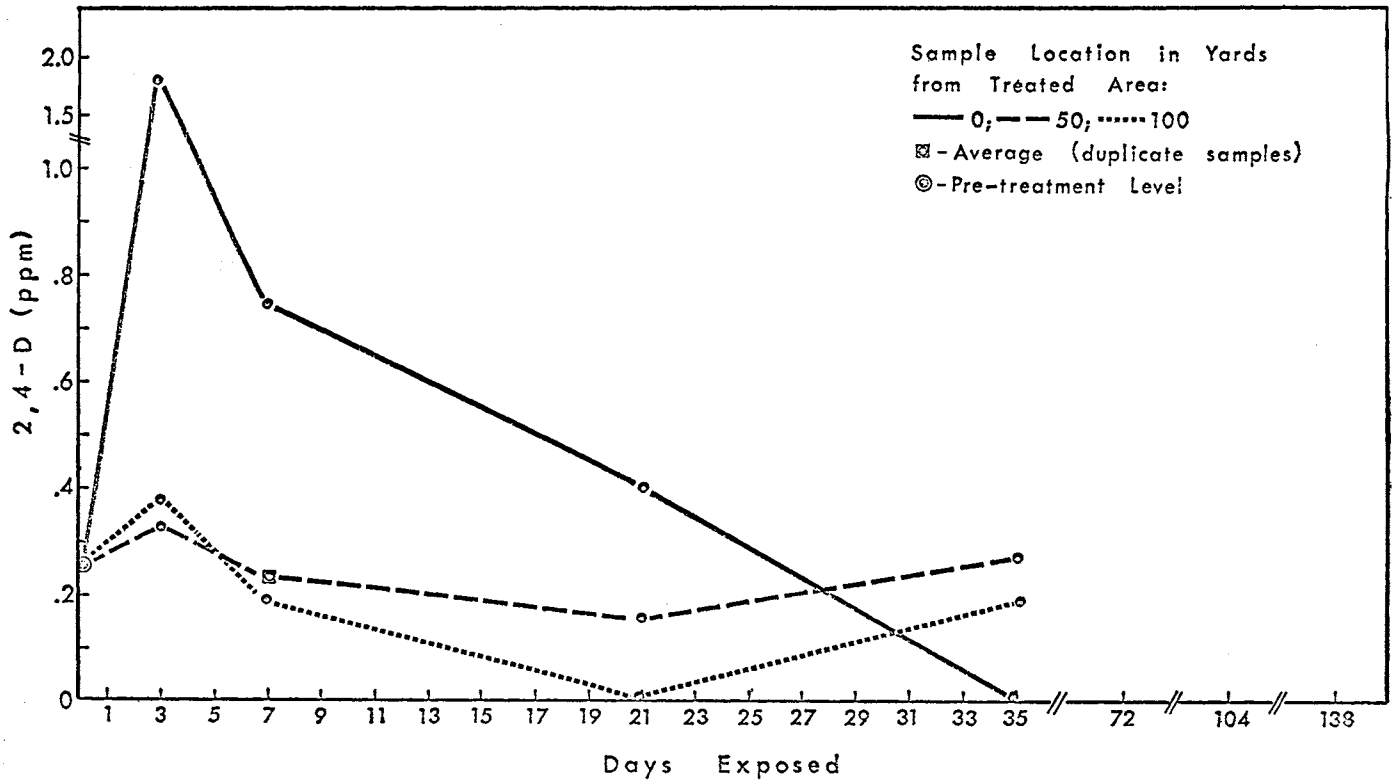

Fig. 5.—Accumulation and loss of 2,4-D in eastern oysters.
Plot 3, 60 lb ae/A, July 5-August 9, 1966.
Analyst A.

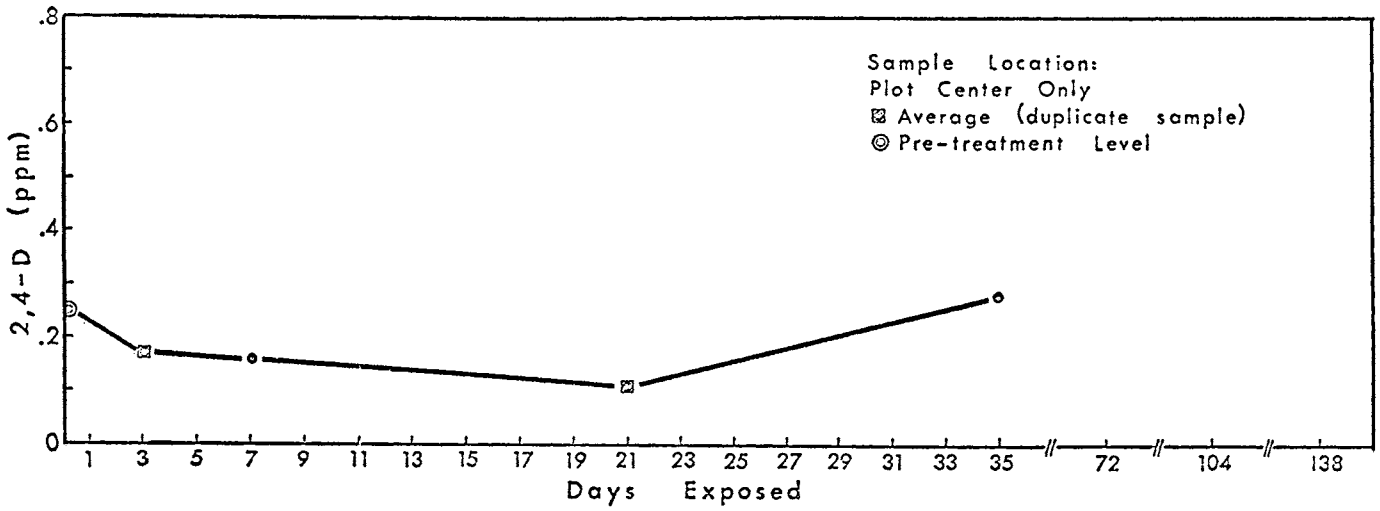

Fig. 6.—Accumulation and loss of 2,4-D in eastern oysters.
Plot 4, Control, July 5-August 9, 1966.
Analyst A.

ppm. In the 100-yard station, residues varied from a peak of 0.38 ppm at 3 days to 0.19 ppm at the 35 day sampling, and at 50 yards, from 0.33 ppm after 3 days to 0.26 ppm at 35 days.

In Plot 4, Fig. 6, the control plot, oysters were stationed at plot center only. Residues varied from an average of 0.16 ppm at the end of 3 days to 0.27 ppm at the end of 35 days.

Fig. 7 shows residues reported by "A" and "B" for oysters taken from the center of Plot 1. These residues peaked after 1 day's exposure at 0.769 ppm, and declined until between the 35 and 72 day of exposure when residues reported by "B" disappeared below detectable levels, while "A" reported increased residue levels at 35 days. Oysters from this station were the only ones analyzed by "B". It should be noted that "B" reported no residues in oysters prior to sampling, while "A" reported a base level of 0.25 ppm. The closest agreement on residues found (disregarding base levels), was from the 1, 7, 9 and 21 day exposure samples.

DUPLICATE OYSTER SAMPLES

Table 2 compares analyses of duplicate oyster samples reported by "A". The difference ranges from 0.00 ppm to 0.24 ppm between aliquots of the same animal sample. These variations can be considered as ±, since it is unknown which figure is more accurate. "A" and I agreed that because of the erratic analytical results, he would analyze oyster and clam samples taken only through the 35th day of exposure. Water, bottom, and milfoil samples were not analyzed for the same reason.

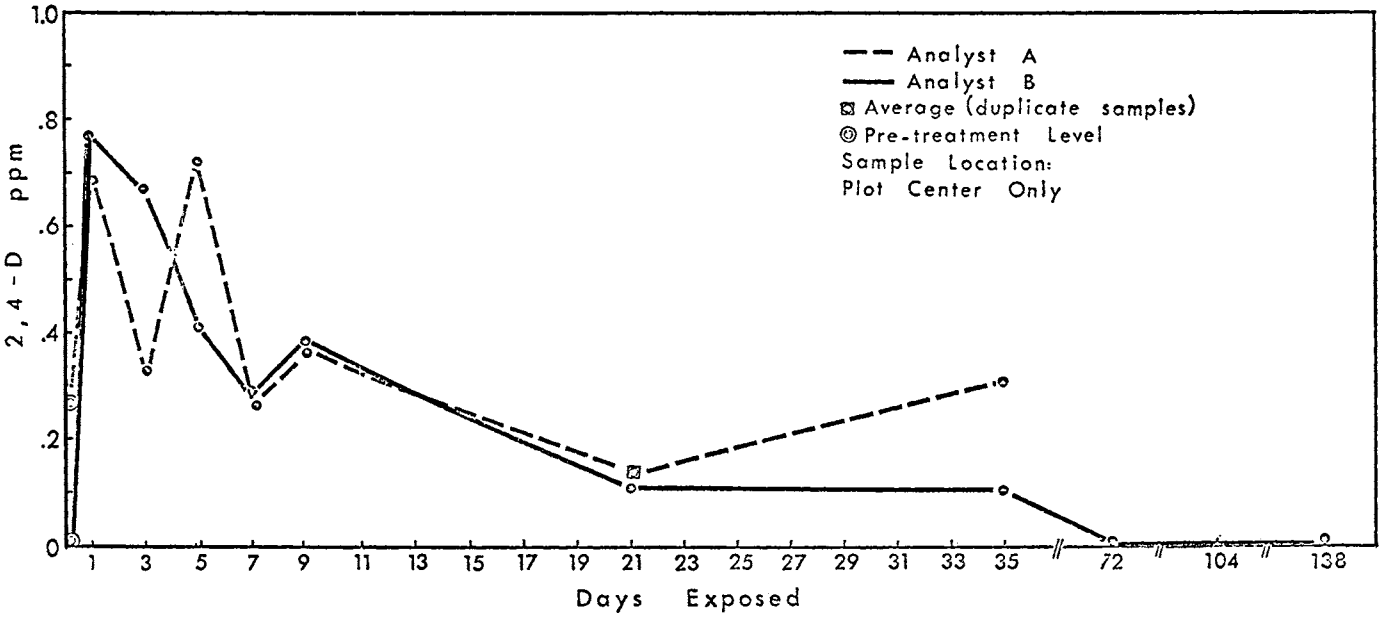

Fig. 7.—Accumulation and loss of 2,4-D in eastern oysters.
Plot 1, 30 lb ae/A, July 5-November 20, 1966.
Analysts A and B.

TABLE 2. COMPARISON OF ANALYSES OF DUPLICATE OYSTER SAMPLES
ANALYST A.

CODE	PLOT	SAMPLE LOCATION[1] (Yards)	DAYS EXPOSED	lb ae/A	ppms 2,4-D BE Sample 1	Sample 2	DIFFERENCE IN ppms
0-82-UNW 0-82-UNWD	3	0	0	60	1.6	1.6	0.00
0-35 0-35-D	4	0	3	Control	0.07	0.24	0.17
0-2 0-2-I	2	0	3	10	0.39	0.45	0.24
0-74 0-74-I	1	100	3	30	0.26	0.02	0.24
0-21-UNW 0-21-UNW-R	1	0	7	30	0.20	0.14	0.06
0-31 0-31-31	3	50	7	60	0.25	0.21	0.04
0-53-W 0-53-WW	1	0	21	30	0.13	0.13	0.00
0-8 0-8-X	4	0	21	Control	0.19	0.00	0.19
0-47 0-47-O	2	100	35	10	0.20	0.28	0.08
0-68-6 0-68	1	50	35	30	0.00	0.06	0.06

DIFFERENCE IN RESIDUE RANGE 0.00 — 0.24

[1] In relation to plot center.

WASHED AND UNWASHED OYSTER SAMPLES

Table 3 compares 2,4-D BE residues in washed and unwashed oysters taken at the same time from the same station. The ppm range difference reported by "A" was 0.06 to 0.48, the washed samples, in most instances, having higher residue amounts than the unwashed. The difference in "B's" reports ranged from 0.167 to 0.384. One sample of washed oysters had greater residues than unwashed. The reports of "A" and "B" agreed in no case where aliquots of the same coded sample were tested as to whether washed or unwashed samples contained the highest residues.

SPIKED OYSTER HOMOGENATE

Eleven 50 gm aliquots of shucked and washed oyster homogenates were spiked with 2,4-D BE in acetone to give residue concentrations ranging from 0.05 to 10.00 ppm. The 12th unspiked sample served for a control. Each 50 gm of spiked material was divided into 2 portions, coded and frozen; one-half of the samples were delivered to "A", the others air-freighted in dry ice to "B." The results are shown in Fig. 8. The stock solution of 2,4-D BE was prepared by "A" and had a purity of 96%.

In general, neither "A" nor "B" recovered all the 2,4-D BE with which the oysters had been spiked. However, "A", in 3 low range samples, reported more residues than had been blended into the homogenate. "B's" recoveries were proportionately more consistent. "A's" recoveries varied from 0.07 ppm in a control blank to 680% of a sample spiked at 0.05 ppm (the other 0.05 ppm spiked sample was reported as such, or 100% recovery), to a low of 28% in a sample spiked with 4.0 ppm. "B's" reports ranged from a reported 0.0 ppm in a 0.05 ppm spiked sample to 51 to 68% recovery in other samples.

CLAMS FROM NATURAL BEDDED POPULATIONS

Figures 9 through 15 graph the residues in clams reported by "A" and "B". "A" reported a pre-treatment level of 0.36 ppm, while "B" reported 0.00. All samples were prepared as previously described, coded and placed in freezer storage to await delivery to "A" and "B".

In Plot 1, Figs. 9 and 10, 30 lb æ/A, "A" and "B" reported the highest residues after 7 days exposure. These occurred in clams dredged from plot center. "A" found less residues at all stations 3 days after exposure than he did in pre-treatment clams, while residues reported by "B" were almost the same for the 3 day period (0.38 ppm) as for the 7 day period (0.41 ppm). At the end of the 35 day period, "A" reported residues from all 3 stations that ranged from 0.18 to 0.24 ppm. The 3-day, 50-yard sample for "B" was lost, but after 7 days, residues were 0.01 ppm. "B's" 21-day sample for clams at 50 and at 100 yards was negative, while plot center clams became negative between the 35th and 72nd day after initial exposure.

In Plot 2, 10 lb æ/A, the findings of "A" can best be understood by looking at Fig. 11. The highest residues, 5.1 ppm, were found in clams taken the 3rd day from plot center, while after 7 days, clams from 100 yards were next highest with 2.1 ppm. Following 35 days exposure, the 100-yard sample was negative, while the 50 yard and plot center clams contained 0.22 and 0.25 ppm, respectively. Plot center clams analyzed by 'B", Fig. 12, also peaked at 3 days with 0.84 ppm. becoming negative after 21 days and showing 0.01 ppm after 35 days, and again becoming negative for the remainder of the test period. 'B's" clams from 50 yards showed negative at 3 days, 0.01 at 21 days and negative through the remainder of the study. At 100 yards, 'B's" clams had the highest residues at 3 days, 0.11

TABLE 3. COMPARISON OF 2,4-D RESIDUES IN WASHED AND UNWASHED OYSTERS.
ANALYST A.

CODE	PLOT	SAMPLE LOCATION	DAYS EXPOSED	lb ae/A	SAMPLE Washed ppms	SAMPLE Unwashed ppms	DIFFERENCE IN ppms
0-99-W	1	0	3	30	0.32	0.73	0.41 (U
0-99-UNW							
0-82	3	0	3	60	1.8	1.6	0.20 (W)
0-82-UNW						1.6	0.20 (W)
0-82-UNWD							
0-21-W	1	0	7	30	0.26	0.20	0.06 (W)
0-21UNW							
0-21-UNWR						0.14	0.12 (W)
0-43-W	3	0	7	60	0.74	0.26	0.48 (W)
0-43-UNW							
0-53-W	1	0	21	30	0.13	0.04	0.09 (W)
0-53-UNW							
0-53-WW					0.13		0.09 (W)
0-27-W	3	0	21	60	0.40	0.24	0.16 (W)
0-27-UW							
		DIFFERENCE IN RESIDUE RANGE			0.06 — 0.48		
				ANALYST B.			
0-99-W	1	0	3	30	0.667	0.500	0.167 (W)
0-99-UNW							
0-21-W	1	0	7	30	0.282	0.474	0.192 (W)
0-21-UNW							
0-53-W	1	0	21	30	0.103		
0-53-UNW						0.487	0.384 (U)
		DIFFERENCE IN RESIDUE RANGE			0.167 — 0.384		

(W) — Washed
(U) — Unwashed

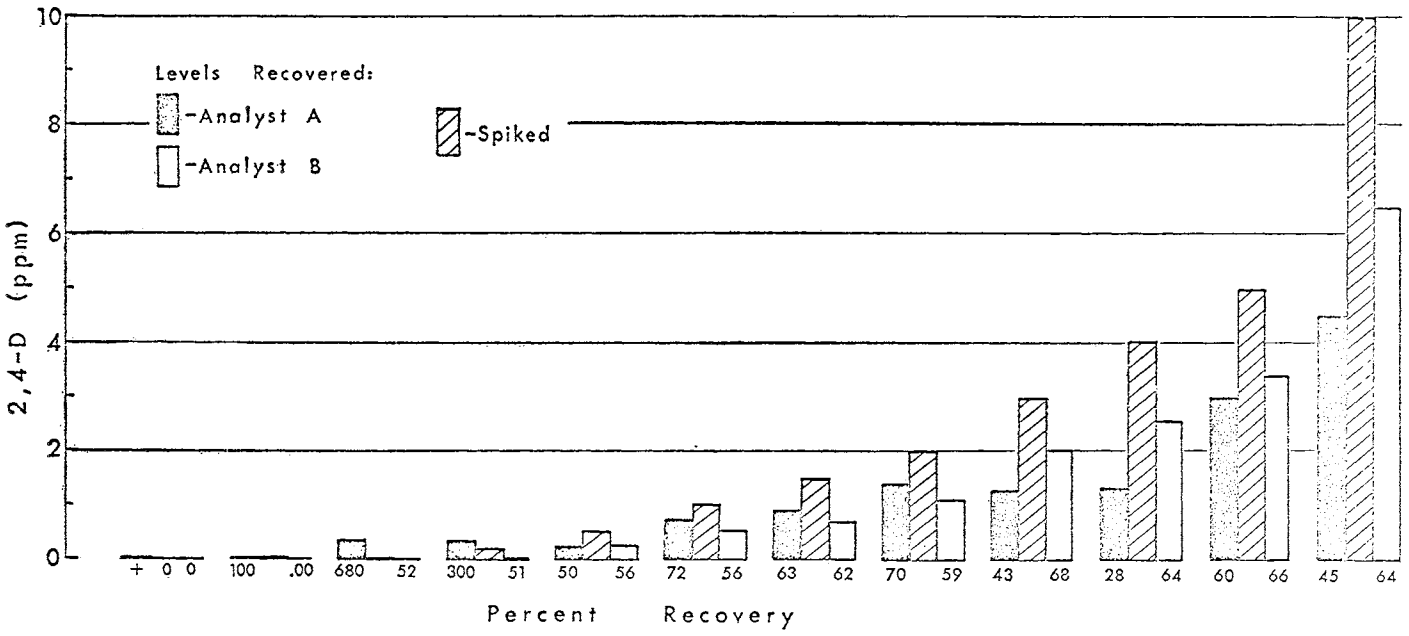

Fig. 8.—Recovery of 2,4-D from spiked oysters homogenates.
Analysts A and B.

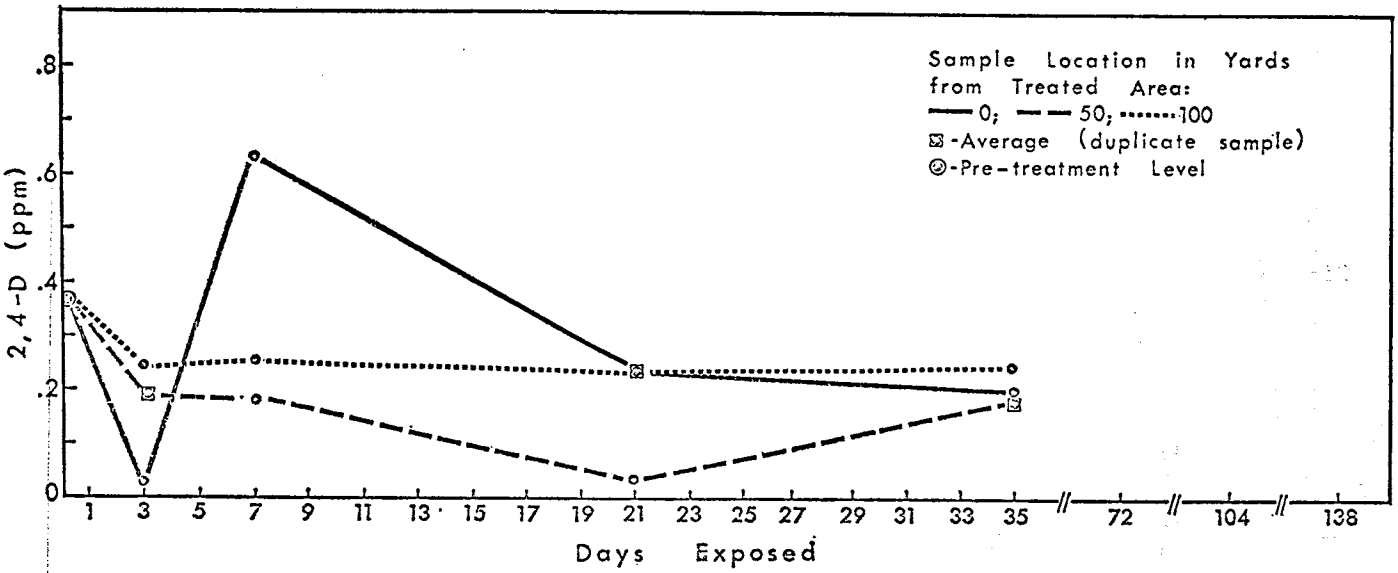

Fig. 9.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 1, 30 lb ae/A, July 5-August 9, 1966.
Analyst A.

ppm. becoming negative at the 21, 35 and 72 day exposure period and at the 104 day sampling, showing 0.05 ppm.

In Plot 3, 60 lb æ/A, Fig. 13, "A's" reported residues varied widely, from a peak at 3 days of 1.5 ppm for plot center clams, to 0.04 ppm at 35 days. Fifty and 100-yard samples were up and down, with the highest residue, 0.28 ppm, at 50 yards on the 7th day, dropping to 0.06 ppm on the 21st day and climbing to an average of 0.15 ppm at 35 days. The 100-yard sample reached 0.25 ppm on the 21st day, and dropped to 0.13 ppm on the 35th day. "B" reported, Fig. 14, the most residues, 0.66 ppm, for plot center clams on the 3rd day, as did "A". By the 35th day, residues were 0.02 ppm. No residues were reported for the 72nd and 104th day exposures. At the 50-yard station, clams varied from 0.01 to 0.02 ppm, and at the 100-yard station, no residues were found throughout the study period.

In Plot 4, Fig. 15, the control plot, "A" reported ressidues ranging from a high of 0.26 ppm at 21 days to a low of 0.12 ppm after 35 days. "B" found no residues in any clams collected from the control plot.

DUPLICATE CLAM SAMPLES

Table 4 compares analyses of duplicate bedded clam samples as reported by "A". No duplicates were sent to "B" because "B's" time to work on all residue samples was limited. The residue differences varied from 0.00 to 0.44 ppm.

CAGED CLAMS

The caged clams died so rapidly, mostly from eel predation, mechanical injury from dredging, and hot weather—several weeks of surface water temperatures up to 31 C—that only a partial collection was made after 3 days exposure. There was insufficient data to graph, hence the limited results are presented in Table 5. When compared to "A's" findings in bedded clams, all caged clams in Plot 1 had higher residues; Plots 2, 3 and 4, at all stations, had lower amounts fo 2,4-D BE.

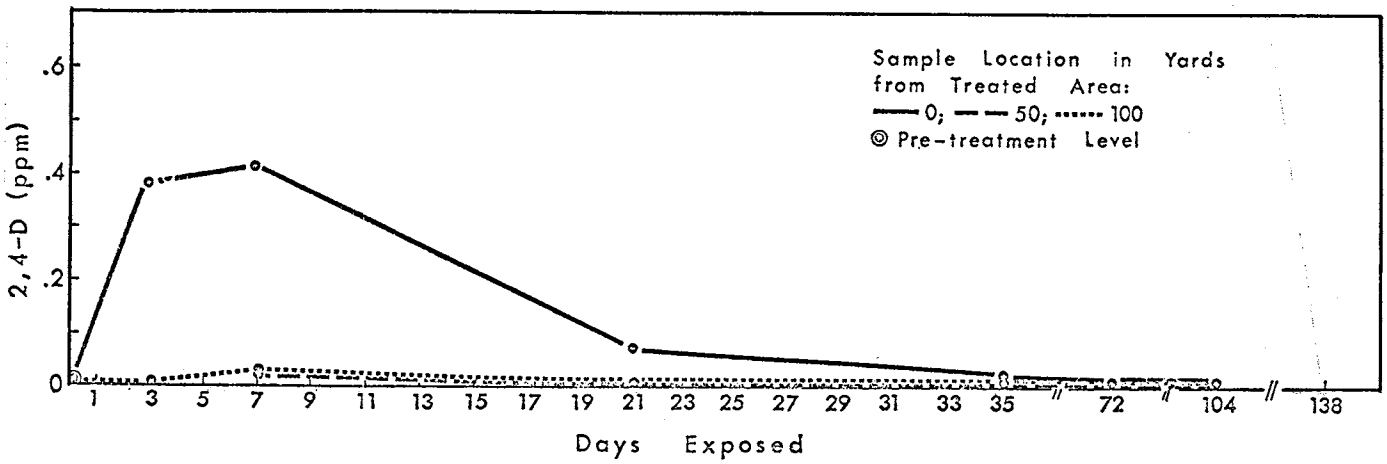

Fig. 10.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 1, 30 lb ae/A, July 5-October 17, 1966.
Analyst B.

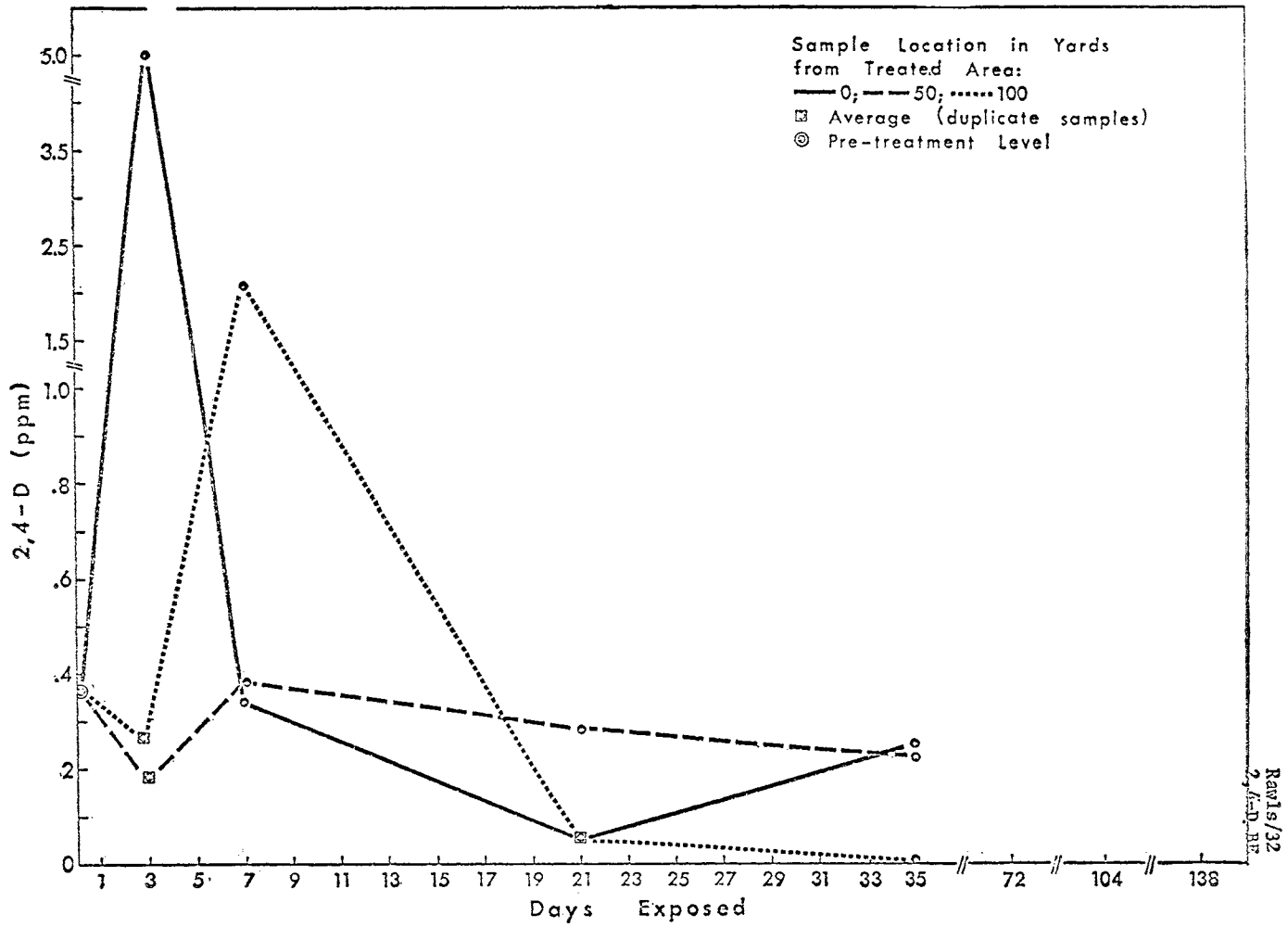

Fig. 11.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 2, 10 lb ae/A, July 5 - August 9, 1966.
Analyst A.

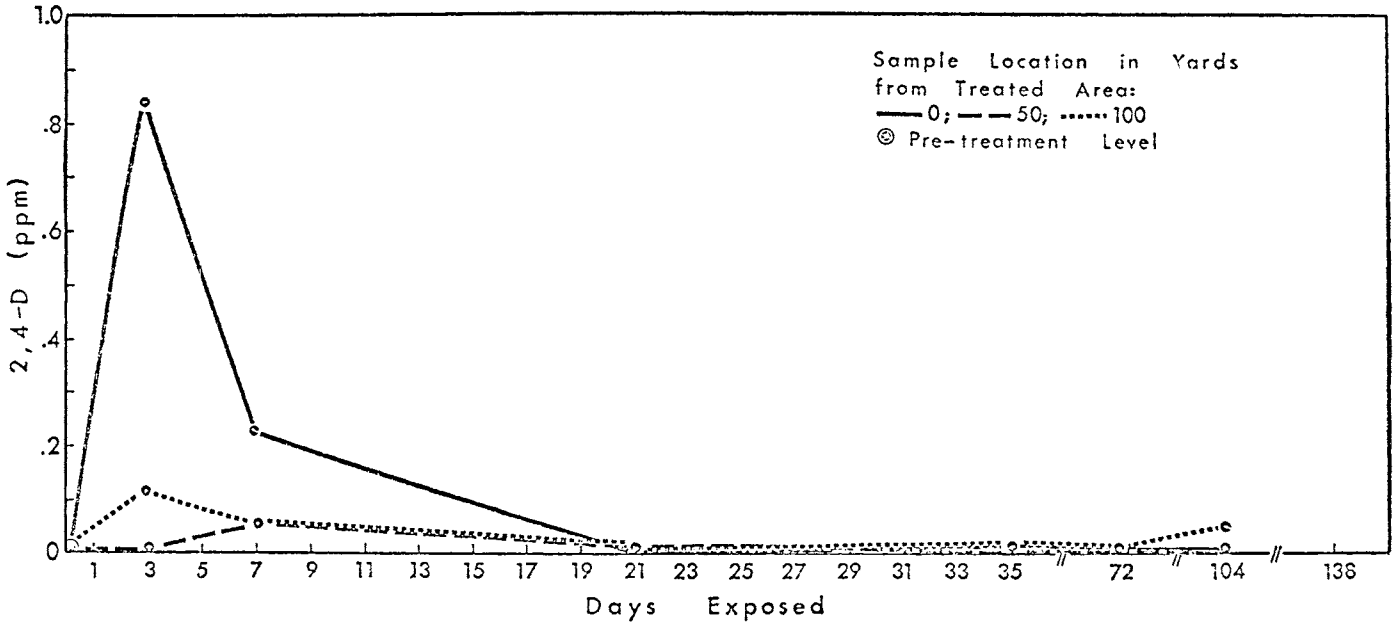

Fig. 12.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 2, 10 lb ae/A, July 5-October 17, 1966.
Analyst B.

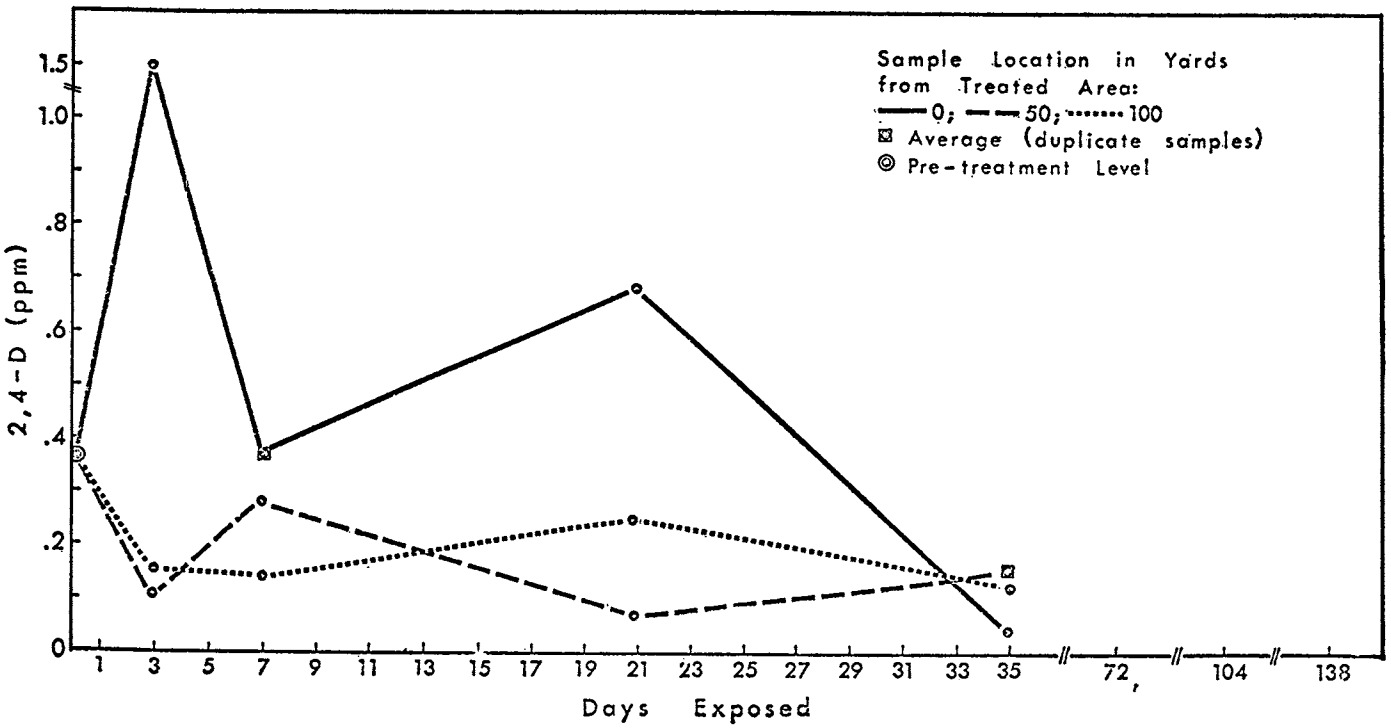

Fig. 13.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 3, 60 lb ae/A, July 5-August 9, 1966.
Analyst A.

PROLONGED FREEZER STORAGE OF OYSTERS AND CLAMS

To determine what effects, if any, prolonged freezer storage might have on the residue in oysters and clams, extra samples of each were obtained after 3 days exposure in Plot 3, treated at 60 lb æ/A, and in Plot 4, the control plot. Analyses were made by "A". The results are presented in Table 6. From the 3 day exposure to analyses of animals held for 24 months, there is a general but inconsistent loss. The net difference between the 3 day sample and the residues in the same sample 24 months later was minus 0.60 ppm for oysters in the treated plot, and a gain of 0.10 ppm for control plot oysters. The net residue difference for clams from the treated plot was minus 0.30 and in the control plot, it was minus 0.06.

DISCUSSION

1966 STUDIES

Any apparent incongruities mentioned here stem from a biological viewpoint, not that of a bio-chemist. Variation between laboratories in analyses for residues of another herbicide, diquat, has been reported by Gilderhus (8).

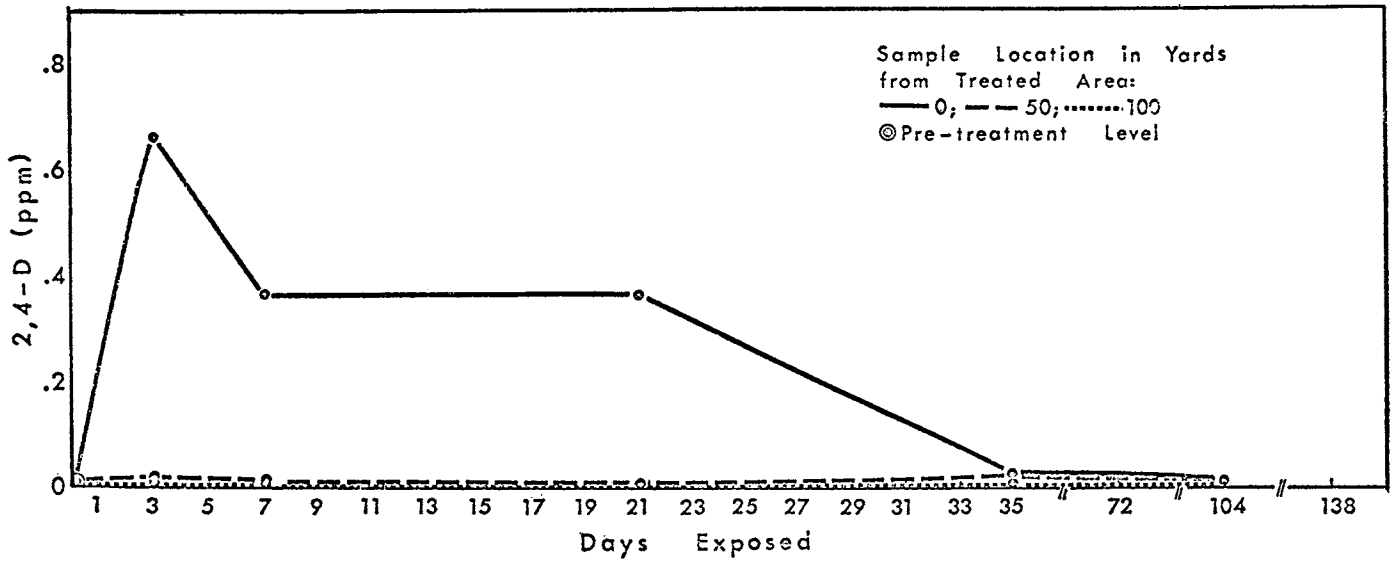

Fig. 14.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 3, 60 lb ae/A, July 5-October 17, 1966.
Analyst B.

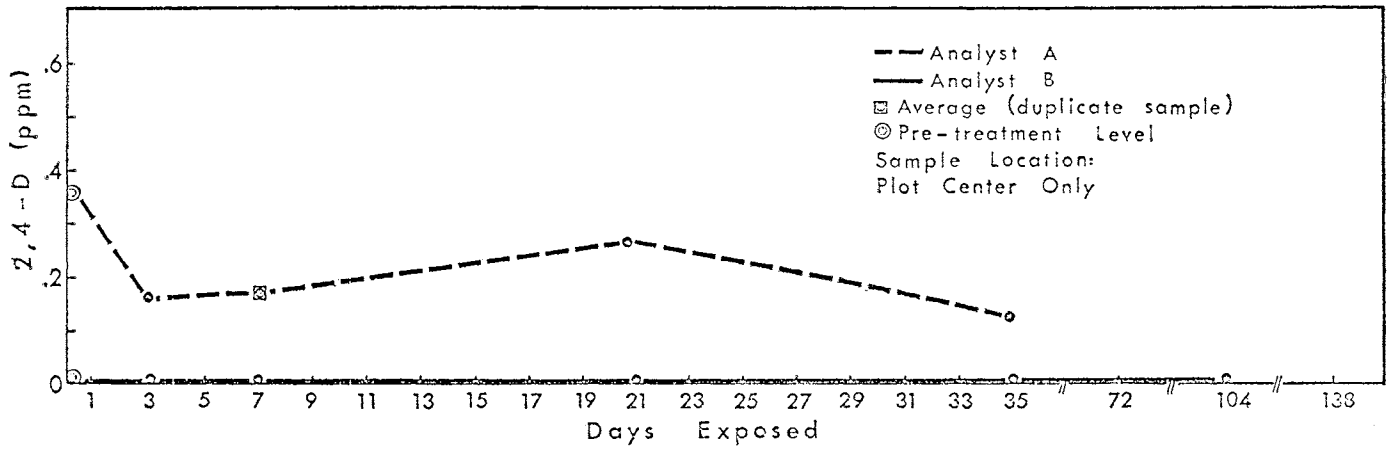

Fig. 15.—Accumulation and loss of 2,4-D in soft-shelled clams.
Plot 4, Control, July 5-October 17, 1966.
Analysts A and B.

Burchfield, Johnson and Storrs (1), point out that reproducibility of results obtained between different laboratories making pesticide residue analyses is almost always poorer than that which is obtained in a single laboratory. They also state that most variability is incurred during extraction and clean-up, and to some extent in the determinative steps. Further, they feel that precision obtainable in residue analysis is usually no better than ± 10 percent, and that individual results may depart far from the average. Schechter (14) describes as "appalling" the results obtained both qualitatively and quantitatively when on several occasions replicate samples spiked with known amounts of pesticides have been sent to a number of laboratories for analysis.

There are some anomalies which should be pointed out in the 1966 study. "A" reported pre-treatment 2,4-D residues of 0.25 ppm for oysters and of 0.36 ppm for bedded clams. It seems highly unlikely that these animals had been

TABLE 4. COMPARISON OF ANALYSES OF DUPLICATE BEDDED CLAM SAMPLES
(NALYST A.

CODE	PLOT	SAMPLE LOCATION	DAYS EXPOSED	lb ae/A	ppms 2,4-D BE Sample 1	Sample 2	DIFFERENCE IN ppms
CC-29 CC-29-A	Blakiston Pretreat			Pretreat	0.00	No reply	
C-82 C-82-F	3	0	3	60	1.5	1.5	0.00
C-71 C-71-D	1	50	3	30	0.04	0.34	0.30
C-59 C-59-Z	2	100	3	10	0.18	0.33	0.15
C-62 C-62-P	2	50	3	10	0.06	0.30	0.24
C-74 C-74-2	1	100	3	30	0.23	0.25	0.02
C-5 C-5-C	4	0	7	Control	0.02	0.13	0.07
C-43 C-43-C	3	0	7	60	0.15	0.59	0.44
C-58 C-58-N	2	100	21	10	0.09	0.01	0.08
C-53 C-53-K	1	0	21	30	0.27	0.18	0.09
C-70 C-70-B	3	50	35	60	0.12	0.18	0.06
C-68 C-68-8	1	50	35	30	0.32	0.03	0.29

DIFFERENCE IN RESIDUE RANGE 0.00 — 0.44

TABLE 5. RESIDUES OF 2,4-D BE (AS METHYL ESTER) PPM IN CAGED CLAMS[1] COMPARED WITH BEDDED CLAMS. ANALYST A.

LOCATION	3 DAY EXPOSURE Caged	Bedded	DIFFERENCE
Plot #1			
30 lb ae/A			
0	0.86	0.02	0.84 (C)
50	0.22	0.19*	0.03 (C)
100	0.42	0.24*	0.18 (C)
Plot #2			
10 lb ae/A			
0	—	5.1	—
50	0.14	0.18*	0.04 (B)
100	0.00	0.26*	0.26 (B)
Plot #3			
60 lb ae/A			
0	0.25	1.5	1.25 (B)
50	—	0.10	—
100	0.04	0.15	0.11 (B)
Plot #4			
Control			
0	0.15	0.16	0.01 (B)

PRE-TREATMENT LEVELS = 0.00

1 From Blakiston Island area.
— Insufficient clams for analysis.
* Average.
(C) Caged.
(B) Bedded.

exposed to enough 2,4-D BE to contain these residues, particularly so since in several instances, (Figs. 3, 4, 6, 7, 9, 11 and 13) animals at some stations showed less residues 3 days after treatment than at the pre-treatment level. Too, it would not appear logical that some animals would possess more residue at the end of 35 days than they did at the end of 21 days (Figs. 3, 4, 5, 6, 7, 9, 11 and 13) when exposed to a single application of granular material. This material releases most of the active herbicide within 96 hours.[1]. "B" reported a generally decreasing amount of residues in oysters from the 30 lb æ/A, and in clams after the 3rd to 7th day. "B" reported no residues from any control plot clams, while "A" reported residues of 0.36 ppm before treatment of 0.12 ppm at the end of 35 days. Pretreatment clams were obtained from the control plot location.

Milfoil is extremely sensitive to minute quantities of 2,4-D BE and in sub-lethal amounts reveals this sensitivity by apical aberrations. At no time were such physical distortions as abnormal leaf growth, curling, chlorosis, or defoliation noted in milfoil thriving in the control plot. Normally, milfoil is most vulnerable to 2,4-D BE in these waters after it reaches the surface, before it flowers, and when the surface water temperature is at least 18 C. In the Chesapeake, these conditions usually exist from the last of May until mid-June. Herbicide application was made to the study plots on July 5 since we were primarily interested in residues, not in killing milfoil. However, by July 10, some milfoil was sinking, and by August 9, 35 days after treatment, only a few strands of milfoil came up on the clam dredge from 30 and 60 lb ae/A plots; the 10 lb ae/A plot was considerably thinned; and the control plot and all 50 and 100-yard stations continued to support such heavy milfoil growth that it was necessary to use a discarded clam cage as a strainer over the water intake pipe of the dredge before it could function properly. Previously, this had been true for all plots. Therefore vegetative containment of the herbicide was apparently excellent.

Since the analytical procedures seemed to be imperfect, "A" and I decided not to subject any mud, water, or milfoil samples to analysis. One important fact that could very well account for part of the variance between similar sample residues reported by "A" and "B", is that "A" did not homogenize his entire sample and take aliquots for analysis as did "B". The metabolic activity of individual animals certainly varies and as pointed out by Butler (3), in discussing organochlorides, the sensitivity and efficiency in storing residues varies with the species. Yet another difference in analytical procedures was brought out in personal discussions with "A" and "B"; "A" stated that he had more difficulty in analyzing oysters because of two pigments which would not come out on the Florisil column,

1 J. E. Gallagher, pers. comm.

TABLE 6. EFFECTS OF PROLONGED FREEZING ON 2,4-D RESIDUES (PPM) IN OYSTERS AND CLAMS. ANALYST A.

SAMPLE	3 DAYS 7/8/67	6 MOS. Jan. '67	12 MOS. Jul. '67	18 MOS. Jan. '68	24 MOS. Jul. '68	NET DIFFERENCE
			OYSTERS			
0-82 60 lb ae/A Center	1.8	1.6	1.4	1.2	1.2	−0.60
0-35 Control	0.07	0.24	0.26	0.18	0.17	+0.10
			CLAMS			
C-82 60 lb ae/A Center	1.5	1.6	1.3	1.3	1.2	−0.30
C-35 Control	0.16	0.15	0.12	0.11	0.10	−0.06

while "B" encountered more difficulty with clams but did not state the reason. Why clams in the center of the 10 lb ae/A plot contained higher residues at the end of 3 days than did the clams at plot center of the 60 lb æ/A plot (as reported by both "A" and "B") is conjecture. Human error could be involved regardless of the fact that all samples were triple checked before and after coding for proper identification, or it could be that maximum vegetative containment of released herbicides occurred in this plot, or even that the light treatment irritated or stimulated clams into increased metabolic activity.

During the course of "A's" analyses, it was suggested that the addition of a protein digestion agent (Papain), might be helpful in 2,4-D recovery. This was done on 2 oyster and 1 clam samples, but yielded only a slightly higher recovery. It also was thought that if the residues were 2,4-D acid, the Federal Food and Drug Administration might be more willing to set a residue tolerance limit for oysters and clams. Fifteen oyster and clam samples were analyzed for the free acid of 2,4-D. The chromatograms of these samples indicated that some "X" substances was present, and had a molecular weight similar to that of the free acid, but definitely was not 2,4-D.

Analyses made by "B" seem to be more biologically plausible than those reported by "A", and if this is true, then oysters and clams probably accumulate most residues of 2,4-D within 3 to 9 days and lose them to the extent that current detection procedures indicate little, if any, residues after the 35th day of exposure. Again, if "B's" results are to be taken as criteria, where vegetative containment exists there appears to be little residue pickup 50 and 100 yards from plot perimeters and at 300 to 500 yards, as shown by control plot animals, none at all.

Lowe (10) in a laboratory study of 2,4-D BE uptake by large oysters, found that when exposed continuously in flowing sea water containing 0.1 ppm of 2,4-D BE for 7 days, they accumulated residues averaging 18.0 ppm of 2,4-D acid. This was determined by thin layer paper chromatography. After remaining oysters were placed in uncontaminated water and allowed to "flush" for 7 days, no residues were found. This bears out the proclivity for oysters to accumulate and then lose residues rapidly.

Earlier Cooperative Studies

Studies in 1961 using oysters, clams, blue crabs and fish (mostly pumpkinseed) for bioassay have been reported upon by Coakley, Campbell and McFarren (4) and by Rawls (13). The animals were held in the center of a one-acre plot treated with 2,4-D BE at 30 lb æ/A in St. Patrick Creek, a small, relatively confined tributary to the Potomac River. Pre-treatment residue levels were 0.00. At the end of 3 days, oyster samples were reported to have between 3.5 and 3.8 ppm residues, clams between 3.5 and 3.6 ppm, fish 0.3 ppm and crabs 0.8 ppm. Oysters removed after 3 days and held at the Chesapeake Biological Laboratory piers showed no residues after 300 days.

McFarren (16), reported at an interagency workshop meeting on milfoil, that 1962 studies involving only oysters and clams exposed to the 2-ethyl hexyl ester of 2,4-D at 30 lb æ/A in Chaptico Bay (Fig. 1) revealed residues in oysters after 3 days exposure of 0.3 ppm and in clams of 0.18 ppm. After 35 days oyster residues had dropped slightly to 0.26 ppm, while residues in clams had increased to 0.44 ppm. The determinative procedure (Coakley, Campbell and McFarren, unpublished) was similar to that used for 2,4-D BE (4), except that the hydrolyzing time was extended from 1½ to 3 hours. Wimsatt, Gilchrist and Campbell (unpublished) reported in a personal communication from Campbell, June 22, 1966, on 2,4-D residues from bioassay animals used in 1963 field tests in Chaptico Bay, Coatigun Run, and Chaptico Wharf, downstream from Chaptico Bay. Because of limited clam and oyster populations in these locations, caged clams and trayed oysters were utilized. Rawls (13), describes these field studies more fully, but gives no residue report since it was not available until after the paper had been written. The residues in oysters and clams in Chaptico Bay peaked at the 3 day exposure collection period; oysters at 4.7 ppm in plot center of the 60 lb æ/A plot, and at 4.1 ppm for plot center of the 30 lb æ/A plot; clams in the 60 lb ae/A plot at 3.5 ppm and in the 30 lb ae/A plot at 2.1 ppm. By the 35th day, all plot center clams and oysters showed 0.2 ppm residue or less. At the 50-yard station, clams from the 60 lb ae/A plot had residues of 0.50 ppm at 3 days, declined slightly and then reached 0.50 ppm again at 35 days. Residues in clams in the 30 lb ae/A plot peaked at 7 days with 0.80 ppm and fell to 0.10 ppm at 35 days. Oysters in both the 60 and 30 lb ae/A plots had residues which closely paralleled each other (0.10 ppm at 3 and 7 days, less than 0.10 thereafter). An irregularity appeared in clams held 100 yards from the 60 lb ae/A plot. They contained 1.7 ppm at 3 days, 0.2 ppm at 7 days, rose to 0.40 ppm at 21 days and dropped to 0.10 ppm at 35 days. Oysters from the 60 lb ae/A plot and clams from the 30 lb æ/A plot had the most residues at 21 days, 0.20 and 0.40 ppm, respectively. Oysters in the 30 lb test plot accumulated and lost residues similarly to those held at 50 yards outside the plot perimeter.

In Coatigan Run, a very small (2.5 acres), shallow, virtually cut off embayment draining into the Patuxent River, it seemed likely that the bioassay animals would reveal higher residues when periodically sampled because of the physiographic features of the test area, even though the Run was treated with just 20 lb ae/A 2,4-D BE. The animals were placed in the center of the area to be treated. Control animals were placed outside the Run 500 sparsely vegetated yards upstream in the Patuxent River. Oysters and clams in the Run and at the control station contained the highest residues after 3 days exposure; the oysters with 5.1 ppm, clams with 2.8 ppm, and control oysters with 0.8 ppm and clams with 0.6 ppm. Controls were probably contaminated by strong tidal flushing in and out of the Run and a lack of blocking vegetation between controls and the mouth of the Run. After 7 days, controls showed about 0.1 ppm, the clams rising to 0.2 ppm at 21 and 35 days. Extremely low tides were responsible for killing all clams within the test area after the 7 day exposure collection. At this time they showed 0.6 ppm while the oysters had 0.7 ppm of 2,4-D BE. Control oysters became negative between the 21 and 35 day samplings, while test plot oysters had 0.1 ppm at 129 days.

In 1963, test plots were established near Chaptico Wharf and treated with 30 and 60 lb ae/A 2,4-D BE. One other plot served as a control. Only naural bedded clams were used in this portion of the study, and it was designed to determine any residue differences between embedded clams here and caged clams in Chaptico Bay. In none of these plots did residues exceed 0.5 ppm after 35 days and the average for both plots was less than 0.2 ppm. Controls averaged about 0.08 ppm. Vegetation was sparse compared to that in Chaptico Bay, and in the 1966 Wicomico study. Data for caged vs. uncaged animals is inconclusive for 1963,

but if 1961 and 1963 caged clam residue data are compared with 1966 naturally embedded clam residue data, it appears that, other considerations being equal, caged clams will pick up greater amounts than natural bioassay populations (periostracal absorption, metabolic differential?).

The analytical procedures used by "B" in 1961 were continuously refined and applied to recovery techniques used in analyzing 1962, 1963 and 1966 test animals. The 1961 and 1963 residues reported in clams and oysters were much greater than those announced for animals tested in 1962 and 1966. Aside from possible discrepancies in analytical and/or field procedures, I can explain this only by pointing out that St. Patrick Creek and Coatigan Run are small areas with limited water circulation (other than tidal action), and hence less dilution when compared to the open Wicomico River. Too. in 1962, low residues may have been influenced by the different (2 ethyl hexyl ester) formulation of 2,4-D. Another factor in considering low 1962 and high 1963 Chaptico Bay results is that in 1962, only one plot was treated at 30 lb ae/A in the Bay, whereas in 1963, plots were treated at 30 lb ae/A and at 60 lb ae/A, hence residues may be compounded when heavy applications are made in embayments or like bodies of water. Winds can also affect residue concentrations by slowing or increasing normal tidal dispersion and heavy rains may contribute a diluting effect. Vegetative barriers also seem to depress residue concentrations.

Though studies by Smith and Isom (15) in TVA reservoirs and lakes, and Thomas and Duffy (18) at Prince Edward Island, Canada, cannot be compared precisely with those which I made because of differences in environment, bioassay animals, and analytical procedures, as well as intrinsic differences between any human investigators, their studies and mine do emphasize the possibility and even probability of wide variations in results that can be expected from residue field studies using current detection techniques.

RESIDUE STUDIES

General Considerations of 2,4-D BE

DeVaney (6) states that any phenoxy compound will cause a taste in water that may persist for several months and will impart an unpleasant flavor to fish. This may be true for fish in relatively still water, but several of us at the Chesapeake Biological Laboratory have eaten raw oysters which were exposed for as long as 28 days in the center of a plot treated with 2,4-D BE at 120 lb ae/A, and could detect no phenolic taste. When these exposed oysters were intermixed with unexposed oysters, no one was able to organoleptically differentiate them.

When analytical techniques for detection of 2,4-D BE are perfected, it would be worthwhile to determine the difference between raw bioassay animals, and bioassay animals cooked by various methods. Duggan (7), mentions that generally, the same kinds of pesticides are found in foods after preparation for consumption, but that there are substantial reductions in the levels. He mentions, for example, DDT levels in ready-to-eat food, as being about one-tenth that found in the raw product.

Another important study should be designed to determine residue variation among similarly exposed individual animals. Results would point not only to the highest residue a human might be likely to consume, but would also furnish a basis for determining the number of animals necessary — when their residues were averaged — which would reflect a reasonably accurate figure of overall residue gain and loss rates. Such a study would also indicate variations which might be expected if individual animals are analyzed as compared to aliquots of homogenized samples.

Yet another study should be made by feeding known amounts of 2,4-D BE to animals containing predator species, such as crabs and fish, taken from untreated waters to learn if these residues tend to build up in certain organs and tissues as does DDT and its metabolites.

Before contracts are awarded to laboratories offering to perform residue analyses, it seems wise to submit coded, spiked samples of the bioassay animals to be used in the study to determine the effciency of the analytical technique employed by these laboratories.

But even with the variance in residue determinations discussed in this paper, it seems reasonable to expect that oysters and clams will pick up 2,4-D BE residues which generally peak at 3 to 9 days, and are lost rapidly after 21 to 35 days. Following 2,4-D BE applications to shellfish areas, it appears that a 2 to 3 month closure to harvest would assure adequate safety to humans consuming oysters and clams taken from treated locations. Too, in open tidal waters, a 500-yard safety zone from any treated locality should be sufficient if such a place is surrounded by heavy vegetative stands.

Studies such as those reported in this paper, should be instigated any time a pesticide is considered for use in aquatic situations which maintain important floral and faunal populations or in which potability is a consideration.

SUMMARY AND CONCLUSIONS

Crabs, fish, clams and oysters will accumulate and lose 2,4-D BE residues in varying amounts dependent on the species of bioassay animal, the distance animals are held from treated areas, the dosage of herbicides applied, water movement through study plots, and the degree of vegetative containment within and around sites of herbicide application. Clams and oysters acquire greater residues than do fish and crabs. In most instances, when using analytical techniques presently known, it appears that residues become undetectable between the 35th and 72nd day of exposure. Residue peaks are reached in from 1 to 9 days after treatment and decline rapidly thereafter; they may be amplified in semi-enclosed areas.

Methods of detecting residues of 2,4-D BE in fish and shellfish need to be improved. Wide variation in reported residues occurs between laboratories analyzing aliquots of similarly exposed test animals. When analytical techniques are perfected, further residue studies should be made to determine variation in residue accumulation and loss among individual animals, the fate of residues in food prepared for human consumption, and the number of exposed animale necessary in a sample to reflect a typical average of residues acquired or lost at the time of collection.

If shellfish are to be harvested immediately near an area of 2,4-D BE application, there should be a minimum 500-yard buffer zone of vegetation separating the harvest area from the treated area; if shellfish are to be harvested from the treated area, a waiting period of 2 to 3 months should be enforced. These minimums should assure that little or no 2,4-D BE will be present in animals taken for human consumption.

A study should be made to determine whether or not 2,4-D residues are magnified as they pass through a food chain.

ACKNOWLEDGEMENTS

Field residue research using pumpkinseed, blue crabs, oysters and clams for bioassay were begun in 1961. Biologists of the Chesapeake Biological Laboratory performed the field work and personnel of the U. S. Public Health Service, Department of Health, Education and Welfare, Robert A. Taft Sanitary Engineering Center, Cincinnati; Ohio, made the analyses. Prominent in these studies from 1961 to 1964 were Mr. Earl McFarren, Dr. J. E. Campbell, Mr. J. E. Coakley, and Dr. L. A. Richarson (13). Since 1964, Dr. Campbell, Mr. John Wimsatt, and Mr. James E. Gilchrist of the same organization, now the National Center for Urban and Industrial Health, have carried out the examinations. Their cooperation, interest and aid is deeply appreciated.

Improvements in analytical techniques and the desirability of obtaining services of a commercial laboratory unhindered by higher priority research justified some repetition of earlier field studies. This 1966 research was made possible largely by a contract with the Bureau of Commercial Fisheries, U. S. Fish and Wildlife Service of the Department of the Interior. I am grateful to Dr. Philip A. Butler for his encouragement and aid in securing the appropriation. With funds provided, the University of Maryland contracted with the C. W. England Laboratories of Washington, D. C., to determine residues in exposed oysters and clams. Dr. Harold Windlan, who performed the analyses has been most cooperative, and his personal enthusiasm in the project has far exceeded that generated by commercial motives. For the sake of brevity and because of the number of analysts at the National Center for Urban and Industrial Health involved in determining residues from 1966 and earlier field tests, I shall refer to the work of Dr. Windlan as being that of Analyst "A", and to findings reported from N.C.U.I.H. as being that of Analyst "B".

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