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RESIDUE LEVELS OCCURRING IN ANIMAL PRODUCTS AND TISSUES WHEN ENDRIN IS ADDED TO LIVESTOCK FEED

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INTRODUCTION

The insecticide, endrin, is of potential value in the control of forage crop insects. If used against these pests, it may remain as a residue when the crop is consumed by livestock. The objective of this study has been to determine the extent of endrin deposition in milk, eggs, and certain body tissues when livestock ingest small amounts of this insecticide daily for several weeks.

The animals involved in this study included 14 dairy cows, 13 steers, 11 hogs, 11 lambs, 28 broiler chickens, and 24 laying pullets. Endrin was added to their diets daily in amounts sufficient to obtain total dietary concentrations of 0.10, 0.25, and 0.75 ppm in the case of the hogs, lambs, and chickens and at these concentrations plus an additional level of 2.00 ppm in the case of the cows and steers. The feeding was carried on for 6, 8, or 12 week periods for broilers, layers, and the other livestock, respectively, during which time milk and egg samples were taken and analyzed for endrin content. At the end of the feeding periods, tissue samples were analyzed for endrin.

The project was organized in June and completed in December, 1956.

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SUMMARY OF RESULTS

The results of over 600 analyses of the milk, eggs, and body tissues collected in this study can be summarized as follows:

Milk: After 12 weeks of endrin feeding at 0.10 ppm in the total diet, lactating cows produced milk containing less than 0.01 ppm of endrin. Approximately 0.02 ppm endrin was found in the milk of cows similarly fed 0.25 ppm endrin. Endrin at dietary levels as high as 2.00 ppm resulted in the secretion of milk containing up to 0.10 ppm endrin.

During the experimental period studied, the endrin content of the milk of the endrin fed cows appeared to reach a plateau within a month and to hold this level for at least two months of continued exposure.

Body fat: Less than 0.1 ppm of endrin was deposited in the fat tissues of cows, steers, lambs, and hogs receiving 0.10 ppm endrin daily for 12 weeks. At intake levels of 0.25 ppm endrin the fat tissue of cows and steers reached an endrin content of 0.1 ppm but the fat of hogs and lambs contained less than 0.1 ppm. Endrin fed at 0.75 ppm for 12 weeks resulted in the deposition of from 0.1 to 0.3 ppm endrin in the fat of cows, steers, hogs, and lambs.

Within six weeks after endrin feeding ceased, only the steers at the 2.00 ppm intake level showed appreciable endrin deposits in their body fat. Barely significant amounts remained in the fat of cows, hogs, and lambs after the feed-off period.

Significant amounts of endrin were found in the body fat of the broiler chickens at all levels of intake studied. This apparent difference in tendency to store endrin is thought to be due to the low fat content of these animals and to the relatively higher rate of endrin intake.

Meat cuts: The steaks and roasts of cows, steers, hogs, and lambs receiving endrin at 0.25 ppm in their diet for 12 weeks contained less than 0.1 ppm endrin. The breast and drumstick tissue of broilers ingesting 0.10 ppm endrin daily for 6 weeks contained less than 0.1 ppm endrin. At the 0.25 ppm endrin levels approximately 0.1 ppm was found in these tissues.

Eggs: The eggs of laying pullets on a 0.10 ppm diet of endrin for 8 weeks contained less than 0.1 ppm endrin. Pullets on the 0.25 and 0.75 levels produced eggs with endrin concentrations as high as 0.3 ppm.

Confirmatory analyses: The majority of the data obtained by the analysis of approximately 20% of the samples by an independent biological method confirm the results obtained by the colorimetric method and indicate that endrin is not stored or secreted as a metabolite of greater toxicity than endrin itself.

Effect of endrin feeding: None of the animals were noticeably affected by the endrin feeding. Milk and egg production were normal and growth was in line with that expected under similar conditions of feeding without endrin.

CONCLUSIONS

From a study of the results, the following conclusions can be drawn:

1. At a daily intake of 0.10 ppm endrin for 12 weeks, cows will produce milk containing less than 0.01 ppm endrin and the body fat of cows, steers, hogs, and lambs will contain less than 0.1 ppm endrin.

2. At dietary endrin levels of 0.25 ppm and above for periods up to 12 weeks, the concentration of endrin in milk will exceed 0.01 ppm and the body fat of cows and steers will contain 0.1 ppm or more. Lambs and hogs have less tendency to accumulate endrin in body fat at these levels.

3. At corresponding levels of endrin intake, broiler chickens show a greater tendency to accumulate endrin in fat tissue than cows, steers, hogs, or lambs.

4. Less than 0.1 ppm endrin will be found in eggs produced by laying pullets receiving 0.10 ppm endrin daily for 8 weeks.

5. Less than 0.1 ppm endrin will be deposited in steaks and roasts of cows, steers, hogs, or lambs if the dietary intake of endrin does not exceed 0.25 ppm.

6. When lambs and hogs ingest endrin daily at levels as high as 0.75 ppm for as long as 12 weeks, broilers and layers ingest this amount of endrin for 6 and 8 weeks, respectively, and cows and steers ingest up to 2.00 ppm daily for up to 12 weeks, none of the animals are visibly affected and no interference is noted in their production of milk, eggs, or meat.

EXPERIMENTAL

DAIRY COWS

Source and housing: The dairy cows used in this study consisted of 8 Jersey and 6 Guernsey cows. Six of the Jerseys were purebreds born and raised on the college dairy farm and were a part of the milking herd at the time of purchase. Two grade Jersey and 6 grade Guernsey cows were purchased locally from two herds in an area where little, if any, spraying or dusting of crops is practiced. The 8 grade cows were tested for tuberculosis, brucellosis, leptospirosis, and mastitis and a negative reaction to all tests was reported prior to purchase and transportation to the college farm.

The cows were removed from pasture and stanchioned in a modern dairy barn. Individual feed mangers were constructed so as to prevent food exchange (see figure 4, appendix II). Wood shavings were used for bedding throughout the feeding period. The cows were turned out each day into an unpaved corral for a 2 to 4 hour exercise period. There were no feedstuffs available to the cows while confined to this area. At no time were the cows permitted to come in contact with animals of the college herd.

Feed: The hay fed during the entire period was one lot of first-cutting grass and alfalfa, grown and harvested on the college farm. This crop was not chemically dusted or sprayed. The grain concentrate fed was a simple mixture of locally grown oats and barley. The entire lot of about 6 tons was ground and mixed at the rate of 720 lbs of oats, 250 lbs of barley, 20 lbs of salt and 10 lbs of steamed bone meal per 1000 lbs of concentrate. Enough was prepared to feed the animals throughout the experimental period. The grain mixture was sacked and stored in an area adjacent to the experimental cows. All components of the ration were analyzed for endrin content either separately or in the combined state. Results are shown in tables 19 and 21, appendix I.

To keep feed refusals at a minimum an individual feed consumption level was determined for each cow during the pre-toxicant feeding period. Feed quantities offered daily were thus limited to the amounts the cows willingly consumed. No attempt was made to push the cows to the limit of their appetites. The hay ration was fed immediately after, and the grain ration immediately before, each milking. This feeding sequence resulted in the grain being fed into a clean manger at each feeding.

Fortification of the ration with endrin: Rather than attempt to accurately contaminate the entire ration being fed each day, it was decided to add the toxicant to the grain ration in quantities equivalent to those which would be used if the entire ration was fortified. The amount of endrin solution added to the grain was based on the amount of feed consumed by the animal the day previously. The grain was fortified and fed just prior to the evening milking. The toxicant, in acetone solution, was distributed over the grain in a numbered pail assigned to each cow. A glass syringe, graduated in mls, was used to measure the endrin solution, (see figure 1, appendix II). The endrin solutions were prepared so that the desired level of fortification could be attained by adding one ml of

solution per pound of feed.

Five different levels of endrin were used in the experiments. Two cows were carried as controls, three were fed endrin at 0.1 ppm, four received 0.25 ppm, three received 0.75 ppm, and two received 2.00 ppm.

Records: Throughout the experiment records were kept on feed consumption, milk production, butterfat content, and body weights. This information is shown in tables 30, 31 and 32, appendix II. More detailed records including the amount and concentration of endrin solution added to the rations each day, the amount of feed offered and refused, and daily individual milk weights are on file in the Department of Dairying at Oregon State College.

Sampling, milk: The cows were milked twice each day on 12 hour intervals. Three milking machines were used. One machine was used to milk the two cows receiving toxicant-free rations and the three cows on the 0.1 ppm toxicant level. The other two machines were used to milk the cows on the higher toxicant levels in sequence of the least to the greatest toxicant concentration. The machines were marked so that the same machine and the same sequence of milking was maintained throughout the entire feeding period.

The milk sampling schedule included an initial sample taken three days previous to feeding toxicant rations. The remaining milk samples were taken at intervals of 3/7, 1, 2, 4, 8, 12, 16, and 18 weeks after toxicant feeding began.

On the day milk samples were scheduled the 24-hour milk production of each cow was collected in individual numbered milk cans with lids. Immediately after each cow was milked, the milk was weighed and then thoroughly mixed by pouring back and forth from one container into another three times. A subsample for the determination of the butterfat was withdrawn and placed in a numbered official milk sampling jar. The rest of the milk in the container was then placed in ice water and stored until the second milking. This collection process was repeated for the second milking and the two milkings were combined. On occasion, individual cows failed to produce a sufficient quantity of milk in two milkings to provide the necessary number of 2 quart samples. In this event three consecutive milkings were collected and combined.

The milk samples were taken from the dairy barn laboratory to the dairy where they were processed for freezing and storage. The samples were re-mixed by pouring back and forth between two containers, divided into two quart portions, weighed, poured into pliofilm bags, and sealed. The pliofilm bags were placed in waxed fiber-food tubs, Nestrite No. 5, labeled, and stored at -10° F in a frozen storage room. The time required to completely process the milk samples from barn to frozen storage ranged from 4 to 6 hours.

The butterfat content of the milk samples was determined by the standard Babcock test. All samples were run in duplicate.

Sampling, tissues: At the end of the twelfth week of endrin feeding, eight cows were sacrificed. These included one of the control cows, two of the 0.1 ppm cows, two of the 0.25 ppm cows, two of the 0.75 ppm cows, and one of the 2.00 ppm cows. A college veterinarian was in attendance during slaughtering. The remaining cows were continued on an endrin free diet for six additional weeks and then slaughtered.

The brain, heart, liver, kidney, and renal fat samples were taken immediately after slaughter. Body fat, steak, and roast samples were obtained after the carcasses had chilled sufficiently to facilitate handling and cutting. Brain samples were divided equally into 3 or 4 portions as specified, and the heart, liver, kidney, renal fat, and body fat samples were divided into portions of 1/4 pound or more. Steaks were cut from the round in portions of 1 1/2 pounds or more, and roasts were typical round bone shoulder roasts weighing 2 1/2 to 5 pounds.

An attempt was made to make all tissue samples as uniformly representative as possible. Portions of both kidneys were used and renal fat was taken from both sides of the carcass. Body fat was obtained from the various areas of deposition over the outside of the entire body. The ratio of fat to muscle tissue was also given consideration in the division of sample portions.

Tissue samples were placed in pliofilm bags, weighed, sealed, labeled and placed immediately in frozen storage at a temperature of -10° F. Duplicates of about 1/5 of the tissue and milk samples were shipped frozen to an independent laboratory for confirmatory analysis.

STEERS, HOGS, AND LAMBS

Source and housing: The steers were purchased from two local herds and were brought immediately from pasture to the experimental area. Animals were allotted so that steers from each herd were placed on each endrin level. The lambs were purchased from a local producer. Both the steers and lambs appeared to have been reared under conditions that were at least of average quality with regard to husbandry practices. The hogs were from the Oregon State College swine herd. All hogs had a similar background of breeding, feeding, and management conditions.

The steers were kept stanchioned throughout the experiment, (see figure 6, appendix II). The hogs and lambs were housed in individual pens, (figures 7 and 8, appendix II). All of the animals were kept under shelter.

Feed: The ration for the steers was composed of barley, oats, and grass hay all locally grown. The hog ration consisted of barley, oats, alfalfa meal, tankage, steamed bone meal, oyster shell flour, and iodized salt. The barley and oats were locally grown. The remainder of the ration ingredients were purchased from commercial sources. The lamb ration consisted of barley, oats, and grass hay, all locally grown. Salt and water were available ad libitum. All of the animals were fed twice daily

in individual mangers. Each animal had a separate water trough or shared one with another animal on the same toxicant level.

All components of the rations were analyzed for endrin content either separately or combined. The results of these analyses are shown in tables 19 and 21, appendix I.

Fortification of the ration with endrin: The endrin, in acetone solution, was distributed over the entire ration at each feeding using a glass syringe. A separate syringe was used for each level of toxicant and was rinsed with acetone between administrations. The endrin solutions were prepared so that the desired level of fortification could be attained by adding one ml of solution per pound of feed. The feed for each animal was weighed and fed from a separate container. All refused feed was weighed back on an individual animal basis. An attempt was made to keep the amount of feed offered just below the amount consumed.

Two steers, two hogs, and two lambs were given control rations with no endrin added. Three animals of each species received 0.1 ppm endrin in their diets, three received 0.25 ppm, and three received 0.75 ppm. In addition, two steers received 2.00 ppm endrin in their diet.

Records: Weight gain and feed consumption records were kept throughout the experimental period. These are shown in tables 33, 34, and 35, appendix II.

Sampling: One or two of the animals, depending upon the endrin level, were slaughtered at the end of 12 weeks of endrin feeding. The remainder were fed six additional weeks without endrin and then slaughtered. Liver, kidney, body fat, and renal fat samples were collected at the time of slaughter. Kidney and renal fat samples were taken from both kidneys, liver samples were taken from several areas of the liver, and exterior fat was used as the source of the body fat samples. Steak and roast tissue samples were obtained after the carcasses had been chilled 24 to 48 hours. Beef steaks were taken from the short-loin area and roasts from the loin and arm. Pork steaks were taken from the loin and the roasts from the loin and shoulder. Lamb steaks were taken from the loin and leg and the roasts from the leg. Immediately after collection, all samples were labeled, weighed, placed in pliofilm bags, and stored at 0° F.

Duplicates of about one fifth of the samples were sent frozen to an independent laboratory for confirmatory analysis.

POULTRY

Source and housing, broilers: Four lots of 15 Delaware-male X New Hampshire-female crossbred male chicks hatched June 14, 1956, were reared in electric batteries equipped with raised wire floors, floor-type heaters, and constant flow watering systems. The wing-banded chicks were weighed

weekly throughout the test. At three weeks of age the number of broilers per group was reduced to seven by removing the lighter and/or heavier birds within a group. At this time these groups were transferred to finishing batteries (see figure 9, appendix II) with raised wire floors and constant flow watering systems. Feed was supplied ad libitum.

Source and housing, pullets: Four lots of 6 White Leghorn pullets each, hatched February 2, 1956, were placed in individual cages in laying batteries (see figure 10, appendix II) for a period of twenty-three days commencing July 1, 1956, in order to permit the birds to adjust to their environment. The cages were equipped with raised wire floors and constant flow watering systems, and were located in a room ventilated by forced draft. Feed was supplied ad libitum.

Endrin fortification of the ration: Endrin feeding of the broilers began on July 12, and of the pullets on July 23. One group of broilers and one group of the pullets were used as controls with no endrin being added to their diets. The other three groups in each case were given rations fortified at 0.10, 0.25, and 0.75 ppm endrin, respectively. The endrin solutions were added to the rations each week using a separate 10 ml glass syringe for each of the three treatments. The solutions were made so that one ml added to one pound of feed gave the desired endrin concentration. After addition of the toxicant, the rations were mixed in a Hobart model A-200 mixer for approximately 10 minutes commencing with the lowest concentration. All feed remaining in the feed troughs at the end of each week was replaced. To determine if endrin loss occurred during the feeding period, feed samples, both freshly prepared and following storage in the finishing room or laying batteries for one week, were analyzed for endrin content. The basic, unfortified rations were also analyzed. The results of these analyses are shown in table 20, appendix I.

Records: Records kept included egg production, egg weight, mortality, feed consumption, and body weights. These records and the composition of the diets fed throughout the test are shown in tables 24 to 29, inclusive, appendix II.

The broilers were observed during the first and sixth weeks, and the pullets during the first, fifth, and eleventh weeks by Dr. W. E. Babcock, Department of Veterinary Medicine. Any birds that died were autopsied by members of that department.

Sampling, broilers: At the end of the sixth week of endrin feeding, all birds were slaughtered by a commercial poultry processing plant. Samples were composited from each bird. To obtain sufficient drumstick samples, all fourteen drumsticks were utilized. One from each bird was designated to be cooked and the other for analysis without cooking. After removal of the bones, the latter group of drumsticks was further divided into subsamples of 100 grams each. The intact drumsticks were cooked as a group and then subsampled for analysis.

Two breasts were used for each raw and cooked breast sample. Those designated for analysis without cooking were separated from the bones and subsampled as above. Those to be cooked were left intact until after cooking and then subsampled.

Since the amount of fat on the carcasses was very limited, only 50 gram samples were obtained. The fat available from each bird was composited and then subsampled for analysis.

All of the samples were placed in pliofilm bags as soon as taken. They were stored in a deep freezer until analyzed. Duplicates of about one-third of the samples were sent frozen to an independent laboratory for confirmatory analysis.

Sampling, eggs: Endrin feeding of the laying pullets was discontinued after eight weeks. The pullets were continued under the same management conditions for four additional weeks. Egg samples were collected during the 1st, 2nd, 4th, 8th, and 12th week of the experiment. Eggs laid on the sixth, fifth, and fourth days of a given week were composited in that order depending upon the number of samples required. Eggs were collected daily and stored in a 34° F cooler.

The sample eggs were broken into a Waring blender, those from the control pullets being handled first, then proceeding from the low to the high endrin treatments. They were thoroughly homogenized and the required number of 100 gram subsamples taken from the mixture. The samples were poured into pliofilm bags placed in pint waxed paper cups, and stored in deep freezer pending analysis. Duplicates of about one-third of the samples were shipped frozen to an independent laboratory for confirmatory analysis.

ENDRIN ANALYSIS

Sampling: All of the samples designated for analysis were held at freezing temperature until the day of analysis. Milk samples were placed on the steam bath and warmed slightly above body temperature to simplify mixing. The samples were well stirred in a large beaker and the portion for analysis quickly decanted off. The milk remaining was returned to the original container and re-frozen for possible further analysis.

Much attention was given in obtaining representative samples of the various body tissues. The methods of removing the sample from the steak or roast is illustrated in figure 11, appendix II. In all cases the sample was taken so as to get a fair amount of fat and muscle tissue. The sample sizes taken were 618 grams of milk, 100 grams of eggs, and 50 grams of body tissue.

Analytical methods: Most of the analyses were done by a spectrophotometric method specific for endrin. This method involves three main steps; saponification of the sample to allow elimination of fats, chromatography of the fat-free extract to remove additional interferences, and finally,

the development and measurement of the color characteristic of endrin. This method is described in detail in a Shell Development Company publication[1].

As a check on the possibility that endrin is converted to a toxic metabolite not detected by the specific method, about 25% of the samples were analyzed by a non-specific biological method, the mosquito larvae bioassay. The sample preparation steps of saponification and chromatography were utilized in this method also. Graded amounts of the samples were then exposed to mosquito larvae and the resulting mortalities compared with those obtained with known concentrations of endrin. Details of this method are included in appendix I.

To validate the method, samples were occasionally fortified with known amounts of endrin prior to saponification and carried through the procedure in the regular manner. The average recovery obtained by the specific method in over 70 trials was 94%. Fourteen recoveries made by the bioassay averaged 93%. All of the recovery data, with dates of analysis, are given in tables 17 and 18, appendix I.

Verification of endrin content of rations: All components of the rations given to the various test animals were analyzed for endrin prior to the feeding tests. The results of these analyses can be seen in table 19. Apparent endrin levels as high as .09 ppm were found. Confirmatory bioassays indicated that the materials present were not insecticides.

Several times during the feeding experiment samples of rations which had been fortified with endrin were analyzed for endrin content. The results of these analyses, shown in table 21, indicate that the fortification techniques used were quite accurate. In two cases these confirmatory analyses detected errors in the fortification. It was found that during the 8th to 10th weeks the stock solution used to fortify the lamb rations at the .10 ppm level had become contaminated. During this period these lambs probably received five times the intended amount of endrin. Again during the fourth week analyses of the broiler rations indicated that the endrin containing rations were actually fortified at .10 ppm instead of at the three levels, .10, .25, and .75 ppm as intended. Examination of the results from the tissue analyses indicate that neither of these errors produced a noticeable effect on the endrin content.

The fortification of poultry rations presented a special case because it was considered impractical to add endrin each day as was done in the other experiments. Instead, the rations were fortified once a week. The question then arose as to whether the endrin thus added to the rations suffered a significant loss due to evaporation during the week's exposure to feeding room temperatures. To investigate this point, several sets of rations were analyzed before and after this storage period. In comparing

(1) method number SMS 642/56, "Spectrophotometric Determination of Endrin in Animal Tissues, Milk, Butter, and Eggs". Shell Chemical Corporation, 460 Park Avenue, New York, 22, N. Y.

the results of these endrin determinations, shown in table 20, it can be seen that only minor, if any, losses occurred.

Fat determinations: Since any endrin deposited in the body tissues was expected to be most prevalent in the fat, several of the steak, roast, and liver samples from the steers were analyzed for gross fat content. Samples for this determination were prepared by grinding the liver or steak samples and subsampling after thorough mixing of the ground tissues. Roasts were handled similarly except that instead of grinding an entire roast, a lateral section about one inch thick was cut from a roast and then ground.

The determination was done according to the procedure given in the 8th edition of the Methods of the Association of Official Agricultural Chemists, section 23.5, p. 386 and section 22.26, p. 371. The roasts were analyzed in duplicate to establish the reliability of the method. The results of these determinations are listed in table 23, appendix I.

Cooking of meat cuts: Several samples of steaks and roasts which had been found to contain endrin residues were cooked to determine whether the residues were reduced. This was done under the direction of Mrs. Lois Sather, Assistant Food Technologist, Food Technology Department, Oregon State College. Table 22, appendix I, lists the information necessary to describe the cooking methods. With the exception of the lamb and pork chops, thermocouples or meat thermometers were used to follow the progress of the cooking. Samples were weighed before and after cooking to determine weight loss.

Preparation of endrin stock solutions: The endrin used to fortify the animal diets was technical grade, 91% endrin, serial number 20728. Acetone solutions containing 0.0498, 0.1246, 0.3738, and 0.9968 grams per liter were made up in four liter batches and distributed to the feeding areas. These solutions, when added to the animal feed at the rate of 1 ml per pound of feed, gave endrin concentrations of 0.1, 0.25, 0.75, and 2.00 ppm, respectively.

RESULTS AND DISCUSSION

EFFECT OF ENDRIN FEEDING ON THE EXPERIMENTAL ANIMALS

The feed consumption, weight gain, and production records of all animals in the experiment are shown in tables 24 through 35, appendix II.

Dairy cows: All cows receiving endrin gained weight as shown in table 31, appendix II. The physical appearance of the animals during the feeding period, feed-off period, and at slaughter was that of well-fed, thrifty, dairy cattle. Hair coats were not as smooth as might be expected for cattle during the summer season. However, this might be explained as due to barn confinement and a lack of pasture.

Considering the quality and quantity of the rations fed, the season of the year, and the various stages of pregnancy and lactation, it would appear that milk production was normal for all of the cows.

The cows were observed by a veterinarian several times during the feeding period and were examined for general health conditions. At the time of slaughter the animals were observed ante mortem and post mortem. On post-mortem the carcass and the viscera were examined microscopically. The veterinarian reported that none of the animals on the various levels of endrin intake showed noticeable differences from the control animals. He further reported the post-mortem findings on cow number 41, which had experienced a persistent diarrhea for many weeks, as negative for any infective agent. However, the examination did show that the mucous membrane and submucousal area of the small intestine was thickened and congested.

Steers, hogs, and lambs: Visual inspection of the data in tables 33, 34, and 35, appendix II, indicates normal weight gains for steers, hogs, and lambs under the conditions encountered. All animals were normal in appearance throughout the trial and at slaughter.

The report of the veterinarian in attendance at slaughter states: "The steers, swine, and sheep on the endrin feeding trials were all observed within 48 hours before slaughter. Slaughter was observed and carcasses and viscera were carefully examined. No observable abnormal conditions either ante mortem or post mortem were observed that may be attributable to the endrin feeding."

The numbers of steers, hogs, and lambs used in these trials are inadequate for statistical analysis of the weight gains and feed efficiency data. No unusual behavior patterns or feedlot characteristics were noted during the course of the trial.

Poultry: Average egg production, feed consumption, and body weight data for the experimental periods are shown in table 28, appendix II. On the

basis of the limited number of pullets and the short test periods involved, apparent differences in egg production, feed consumption, and body weights cannot be attributed to the toxicant with any degree of certainty.

Average egg weights are shown in table 29. The data indicate some depression in egg weight at the 0.75 ppm level. This effect appears to be overcome following withdrawal of the toxicant. Interpretation of the data is complicated, however, by the fact that this depression was evident during the first week of toxicant administration. That this effect may be an artifact, therefore, should not be discounted.

One pullet in the control group died during the fourth week of the trial. Upon autopsy a diagnosis of neural leucosis was given.

Average weights, gains, and mortality data for the broilers are shown in table 26, appendix II. Due to the limited numbers of birds used in the tests, no conclusions can be drawn in regard to weight gains. The apparent differences noted for the group receiving 0.25 ppm endrin can be traced to two broilers that failed to gain in a satisfactory manner. No abnormalities were encountered during the experimental period. When the feed conversion values in table 27 are examined, no differences due to endrin administration are observed.

ANALYTICAL RESULTS

The results of the analyses of the samples obtained from the endrin fed animals are shown in appendix I, tables 5 through 16. In all of the analytical work, results which seemed in question were checked by reruns. The rerun values are included in table 5, but have been averaged in the other tables.

Tables 1 through 4, pages 20, 21, and 22, summarize the results of the milk, body fat, and steak and roast analyses.

Reliability of the analytical methods: A statistical study of the spectrophotometric method based on the differences between duplicate endrin determinations on various 50 gram tissue samples indicated that the endrin content of a single sample could be determined with a precision of $\mp$ .026 ppm at the 1% level of significance. From this it might be estimated that the sensitivity of the method is of the order of 0.05 ppm with a 50 gm sample. Calculation of the standard deviation of a group of analytical results obtained in the analysis of endrin fortified milk samples gave a value of $\mp$ .005 ppm. This value could be assumed to be a fair estimate of the sensitivity of the method for 600 gram milk samples.

Before arriving at a final estimate of the reliability of the spectrophotometric method, however, other factors require consideration. The limit of detection by the analytical method appears to be about 5 micrograms of endrin. That is, the spectrophotometric reading obtained with this amount of endrin corresponds to the upper limit of variability of the reagent blank.

With a 50 gram sample, this would place the amount of endrin which could be measured with confidence at 0.1 ppm and with a 600 gram sample it would be approximately 0.01 ppm. A reproduction of the standard curve obtained by the colorimetric method is shown in figure 15, appendix II.

The sensitivity of the bioassay method is limited only by the toxicity of the control samples. With the cleanup methods employed in this study all tissue samples except the brain could be assayed with a sensitivity of 0.02 ppm. The latter analyses were sensitive to 0.04 ppm. Milk samples allowed a sensitivity of 0.002 ppm.

In comparing the analytical results of samples analyzed by both methods, it is found that 62% of the milk results agree within 0.01 ppm, while 81% of the egg and body tissue sample results agree within 0.1 ppm. This seems to confirm the estimate of the reliability of the two methods.

A final estimate of the confidence which can be placed in the analyses performed in this study is given by the recovery studies. As shown in tables 17 and 18, appendix I, the average recovery in 70 determinations by the specrophotometric method was 94%, while that of 14 bioassays was 93%. The samples were fortified at several different levels, all of the same order of magnitude as those experienced in the analysis of the unknown samples.

Correcting the analytical data for experimental and sample errors: The analytical data given in appendix I is uncorrected except for averaging occasional duplicate analyses. To facilitate the study and discussion of the results, it seemed necessary to correct the data as completely as possible for the experimental errors which were encountered. This was done in tables 1 through 4. The critical reader has the privilege of making his own interpretations of the original, uncorrected data which will be found in the appendices.

Two sources of error were encountered in the study. One was due to the presence of a background level of apparent endrin as indicated by the results of the analyses of the control samples. The second error was the analytical error due to variations in the techniques and reagents employed. This error was discussed in the previous section.

In tables 1 through 4, the data have been corrected for the sample error by averaging the apparent endrin values of the control samples and subtracting this average amount from all values. The corrected results can be considered to be measures of the true endrin content of the tissues. These values still include the analytical error, however, which makes all values less than 0.1 and 0.01 ppm in the tissue and milk analyses, respectively, of doubtful significance. The data are further corrected, therefore, by referring to all values below these two limits of sensitivity as less than 0.1 and 0.01 ppm, respectively. Values above these limits were rounded to the nearest 0.1 and 0.01 ppm in keeping with the analytical standards thus set.

An examination of the bioassay results found throughout the tables in appendix I seems to justify the corrections discussed above.

Endrin residues in milk: From a study of tables 1 and 5 it appears that definite evidence of endrin accumulation in milk is found at the 0.25 ppm intake level, although limited bioassay data indicate that endrin is present to the extent of 0.003 ppm at the 0.10 ppm intake level. The deposition of endrin began to be evident within one week after intake started and, except for the 2.00 ppm intake level, had disappeared from the milk within one month after intake ceased. It is apparent that an increase in the dietary level of endrin did not result in a corresponding equal increase in endrin secretion. Furthermore, it appears that, within the limits imposed by this study, the endrin content of milk reaches a plateau within a month after intake begins and remains relatively close to this level for the remainder of the exposure.

In table 4, the endrin concentration of cow body fat is compared with that of milk from the corresponding dietary endrin level after adjusting the milk to a 4% fat content. Also included in this table is the expected endrin content of a 4% milk calculated from the corresponding endrin content of the body fat. In arriving at these figures the assumption was made that the endrin content of milkfat and body fat from an animal would be the same. A study of the values in table 4 indicates that such a correlation does exist.

Good agreement between results obtained by the bioassay and colorimetric methods indicate that no metabolites more toxic than endrin are secreted in milk.

Endrin in fat tissues: The results of the endrin analyses of body and renal fat tissues are given in table 2, page 21, and in tables 8, 10, 12, 13, and 15, appendix I. Except for broilers, there was less than 0.1 ppm endrin in any of the fat samples from the animals on the 0.10 ppm dietary endrin level. There are two possible explanations for the high concentrations of endrin found in the broiler fat samples. The amount of body fat present in the broiler carcasses at slaughter was probably less than 2% of the total body weight, judging from the amount available for the samples, thus greatly concentrating the fat soluble insecticide. In addition, if one compares the relationship between feed consumption and body weight of the various animals in the test, it will be seen that during the six week period of endrin feeding the broilers consumed a weekly average of approximately 50% of their body weight. With the other animals this figure varies from 15 to 30%. Therefore, the broilers not only ingested nearly twice as much endrin according to their size, but the endrin was concentrated in a much smaller amount of fat. It should also be pointed out that in practice the possibility of poultry rations being contaminated with such levels of endrin as were fed in these studies is probably remote.

The hogs showed little tendency to accumulate endrin in their fat tissue. This may be due to the higher ratio of fat to protein tissue with the correspondingly greater chance for dilution.

During the feed-off period, all but a trace of the endrin deposited in the fat tissues of the dairy cows was eliminated. In contrast to this, approximately 50% of the endrin originally present in the steer fat at the climax of the endrin feeding period, was still present after six weeks without endrin intake. This difference in rate of decline may be due to the greater mobility of fat tissue in the lactating cow. In both hogs and lambs, the feed-off period was sufficient to allow removal of any endrin present to levels below those detectable by the spectrophotometric method. Limited bioassay data indicate that some endrin was still present at the top level of dietary intake.

Endrin residues in steaks, roasts, and broiler tissues: Analyses of steaks and roasts, tables 7, 9, 11, and 14, appendix I, and as summarized in table 3, page 22, indicate that only the higher levels of endrin intake resulted in deposition in this type of tissue. The cow steaks appeared to contain less than 0.1 ppm endrin at all levels of feeding while the roasts gave a definite test for endrin at the 0.75 and 2.00 ppm levels. It can be seen that higher endrin concentrations were found in the steer steaks than in the roasts. This may be due to the greater fat content of the steaks as shown in table 23 which lists fat analyses for steaks, roasts, and liver tissue from steers. In a supplementary experiment with a steak and a roast from steer number 41, the samples were divided into gross fat and protein fractions and analyzed separately. It was found that practically all of the endrin, nearly 1.0 ppm, was located in the fat.

Broiler breast and drumstick tissue, table 15, exhibited some tendency to accumulate endrin at the 0.25 ppm level of intake. The explanation for this may be the same as that given previously for broiler fat.

After the six weeks feed-off, all of the meat cuts examined were found to contain less endrin than could be detected by the method. No feed-off period was included with the broilers.

All of the meat cuts which appeared to contain significant amounts of endrin at the end of the twelve weeks feeding period were analyzed again after cooking. The cooking data is shown in table 22, appendix I, where it can be seen that the weight loss of the tissues during cooking was approximately 40%. A survey of the endrin concentrations of the various cooked steaks, roasts, and broiler cuts, shows that little or no decrease in endrin content was detected. In fact, there are several examples where the endrin concentration was increased. This increase, in most instances, is approximately equal to the concentrating effect produced during the cooking. The experiment indicates that cooking cannot be expected to have any diminishing effect on the endrin content of such meat cuts.

Endrin content of eggs: A detectable level of endrin was found in the eggs of the laying pullets at the 0.25 ppm intake level after 8 weeks of intake. This is shown in table 16. At the 0.75 level of intake, the positive endrin tests began to occur after 4 weeks exposure. After 4 additional weeks without endrin, a positive test was still obtained at both the 0.25 and 0.75 ppm intake levels.

Endrin content of body organs: Of all body organ tissues examined, only the liver tissue of the cows and steers showed positive endrin at the end of the twelve week feeding period.

Statistical analysis of the analytical data: A study of the milk analyses indicates that the difference between the controls and the 0.10 ppm endrin intake level is not statistically significant. However, the endrin content of the milk at the 0.25 ppm and higher levels is significantly above that of the controls. The least significant difference at the 5% probability level, table 5, is 0.018 ppm and at the 1% probability level it is 0.024 ppm.

A statistical study of the results of analyses of the body fat samples from the cows, steers, hogs, and lambs on the control, 0.10, 0.25, and 0.75 ppm endrin intake levels, indicates that there is no significant difference in endrin content between controls and the 0.10 ppm intake levels. Between the controls and the 0.25 ppm endrin intake levels, the difference in endrin content approaches significance. The least significant difference at the 5% probability level, fat analyses in tables 8, 10, 12, and 13, is 0.10 ppm and at the 1% probability level it is 0.14 ppm.

The study also indicates that although the level of endrin storage in body fat differs from species to species, the rate of storage as endrin intake increases, is similar. 1/

1/ Statistical analyses made by Dr. Roger Peterson, Station Statistician, Oregon Agricultural Experiment Station.

Confirmatory analyses by bioassay: The analyses by the biological method were included in the study as a means of detecting any toxic metabolites produced as a result of the endrin feeding. Except for the poultry tissue, it seems safe to conclude that no such toxic materials were produced. The bioassays, being more sensitive than the colorimetric method, allowed an extension of the lower limit of detection of endrin and indicate that small levels of endrin occurred in most of the milk and fat tissues even at the lower levels of endrin intake.

Relationship between endrin intake and deposition: An examination of the data in tables 6 through 16 leads to the conclusion that the ratio of dietary endrin concentration to fat tissue endrin is less than 1. That is, the highest concentration of endrin found in fat tissue was approximately 1 ppm while the upper level of endrin intake in the diet was 2.00 ppm. This is contradicted by the poultry experiment where the ratio is about 2 using the spectrophotometric method results. A possible explanation for this species difference has been given.

Another peculiarity of the broiler body fat analyses is the higher endrin concentration indicated by some of the bioassays than by the specific method. The results suggest the possibility that in this species a metabolite more toxic than endrin itself is being formed although such an interpretation must be very tentative due to the limited number of analyses in which this relationship was found.

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Table 1 - Endrin Content of Milk, Summary [1/]

animal number	endrin in diet, ppm	endrin content of milk, ppm: before endrin fed	during endrin feeding: 3/7 wk	1 wk	2 wks	4 wks	8 wks	12 wks	after endrin feeding ceased: 4 wks	6 wks
1,2	.00	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
11,12,13	.10	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
21,22,23,24	.25	<.01	<.01	.01	<.01	.01	.02	.02	<.01	<.01
31,32,33	.75	<.01	<.01	.01	.01	.02	.04	.02	<.01	<.01
41,42	2.00	<.01	.01	.07	.08	.10	.10	.08	<.01	.03

1/ This table is a summary of the analytical data given in table 5, appendix I, after correcting for apparent endrin content (0.006 ppm) of control samples, averaging values within the dietary intake groups, and rounding averages over 0.01 ppm to the nearest 0.01 ppm. Since the sensitivity of the analytical method is 0.01 ppm, values below this level are indicated.

Table 2 - Average Endrin Content of Fat Tissue of Cows, Steers, Lambs, and Hogs After Twelve Weeks of Endrin Feeding 1/

endrin in diet, ppm	endrin content of fat, ppm			
	cows	steers	lambs	hogs
.00	<.1	<.1	<.1	<.1
.10	<.1	<.1	<.1	<.1
.25	.1	.1	<.1	<.1
.75	.3	.3	.2	.1
2.00	.9	.9		

1/ This table is a summary of renal and body fat data given in tables 8, 10, 12, and 13, appendix I, after correcting for apparent endrin content of controls, indicating as less than 0.1 ppm the averages obtained which were less than the sensitivity of the method, (0.1 ppm), and rounding the values above this figure to the nearest 0.1 ppm.

Table 3 - Endrin Content of Steaks and Roasts from Cows, Steers, Lambs, and Hogs after 12 Weeks of Endrin Feeding

endrin in diet, ppm	endrin content of steaks and roasts, ppm 1/							
	cows		steers		lambs		hogs	
	steaks	roasts	steaks	roasts	steaks	roasts	steaks	roasts
.00	<.1	<.1	<.1	<.1	<.1	<.1	<.1	<.1
.10	<.1	<.1	<.1	<.1	<.1	<.1	<.1	<.1
.25	<.1	<.1	<.1	<.1	<.1	<.1	<.1	<.1
.75	<.1	.1	<.1	<.1	<.1	<.1	<.1	<.1
2.00	<.1	.1	.3	.2				

1/ This table is a summary of data found in tables 7, 9, 11, and 14, appendix I after correcting for apparent endrin content of controls, indicating as less than 0.1 ppm the values obtained which were less than the sensitivity of the method (0.1 ppm), and rounding the values above this figure to the nearest 0.1 ppm.

Table 4 - A Comparison of the Endrin Content of Milk with That Calculated from the Corresponding Body Fat Content, Expressed on a 4% fat Basis.

endrin in diet, ppm	endrin content, ppm		
	cow body fat tissue	milk, 4% fat, based on body fat analyses 1/	milk, actual, on a 4% fat corrected basis 2/
.00	<.1	<.01	<.01
.10	<.1	<.01	<.01
.25	.1	<.01	.01
.75	.3	.01	.02
2.00	.9	.04	.06

1/ These calculations assume that the endrin content of body fat and milk fat are the same.

2/ Corrected values, using endrin content data from table 5, and average daily milk and butterfat production records for September, table 30, appendix II, and assuming that all of the endrin present in the milk is contained in the milk fat.

APPENDIX I - ANALYTICAL DATA

Explanation of Tables:

In the following tables the abbreviation "sp." refers to analyses performed by the spectrophotometric method specific for endrin. The abbreviation "bio." refers to analyses performed by the mosquito larvae bioassay method.

Some of the samples from animals on the same endrin levels were composited for analysis. These are indicated by brackets.

Because of their special importance, the data shown in Table 5, "Analysis of Milk", are as obtained originally. No averaging of duplicates nor rounding of values was done.

In the remaining tables duplicates, where encountered were averaged and only the average value given.

Table 5 - Endrin Content of Milk

animal number	endrin in diet ppm	endrin content of milk, ppm: before endrin feeding sp.	before endrin feeding bio.	during endrin feeding: 3/7 wk. sp.	3/7 wk. bio.	1 week sp.	1 week bio.	2 week sp.	2 week bio.	4 weeks sp.	4 weeks bio.	8 weeks sp.	8 weeks bio.	12 weeks sp.	12 weeks bio.	after endrin feeding ceased: 4 weeks sp.	4 weeks bio.	6 weeks sp.	6 weeks bio.
1	.00	.000 .000		.007		.000		.000		.007		.023 .005	<.002	.000	<.002	--		--	
2	.00	.000 .004	.002	.003		.000	<.002	.000		.000 .003		.016 .007		.005		.005	<.002	.000	<.002
11	.10	.022 .000		.000		.003		.000		.017 .005		.019	.004	.002	.003	--		--	
12	.10	.022 .013		.002	.004	.005	.002	.010		.012		.008	.002	.015	.003	--		--	
13	.10	.000 .000		.016 .000		.016		.005		.01		.013	.003	.007		.003 .021		.007	<.002
21	.25	.000 .013		.018 .024		.026 .020		.028 .031		.047 .008		.027 .026	.007	.017	.009	--		--	
22	.25	.003 .000		.01		.024 .008		.000		.005		.029 .005		.05 .039	.02	--		--	
23	.25	.000 .008		.013 .011		.006	.003	.005	.006	.016		.023 .043		.023	.03	.006 .023	.003	.015	.002
24	.25	.013 .002		.023 .011		.034 .019		.016		.016		.037 .027		.016	.02	.01		.01	

Table 5 - Endrin Content of Milk (continued)

animal number	endrin in diet ppm	endrin content of milk, ppm: before endrin feeding sp.	before endrin feeding bio.	during endrin feeding: 3/7 wk. sp.	3/7 wk. bio.	1 week sp.	1 week bio.	2 week sp.	2 week bio.	4 weeks sp.	4 weeks bio.	8 weeks sp.	8 weeks bio.	12 weeks sp.	12 weeks bio.	after endrin feeding ceased: 4 weeks sp.	4 weeks bio.	6 weeks sp.	6 weeks bio.
31	.75	.01 .000		.002 .032		.008		.028 .026		.018		.05 .046		.013 .026		--		--	
32	.75	.016		.016		.023 .023	.009	.008		.028 .039		.035	.03	.009 .035	.04	--		--	
33	.75	.000 .005		.006		.028 .028		.013		.016 .034	.01	.05		.04	.02	.004		.011	.002
41	2.00	.016		.014		.075		.097 .140		.13		.136		.065		--		--	
42	2.00	.008		.022 .023		.075 .071	.03	.062		.083		.074	.05	.113	.06	.014 .01	.022	.035	.018

Table 6 - Endrin Content of Cow Brain and Heart

animal number	endrin in diet ppm	endrin content, ppm							
		brain				heart			
		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	<.04				.00			
2	.00			--				--	
11	.10								
12	.10	<.05				.00	<.02		
13	.10			--				--	
21	.25								
22	.25	<.04				.03			
23	.25			--				--	
24	.25			--				--	
31	.75								
32	.75	<.04				.04			
33	.75			--				--	
41	2.00	.04				.04			
42	2.00			--				--	

Table 7 - Endrin Content of Cow Steaks and Roasts, Raw and Cooked

animal number	endrin in diet ppm	endrin content, ppm													
		steak, raw				steak, cooked				roast, raw				roast, cooked			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.00	<.02			.04				.03				.01			
2	.00			.01				--				.00				--	
11	.10	(.01				--				(.06				--			
12	.10					--								--			
13	.10			--				--				--				--	
21	.25	(.02	<.02			(.07	<.02			(.04				(.04			
22	.25																
23	.25			--				--				--				--	
24	.25			--				--				--				--	
31	.75	(.06				(.07				(.16	.07			(.06			
32	.75																
33	.75			.00				--				.00				--	
41	2.00	.10				.06				.16	.17			.04			
42	2.00			.00	.02			--				.06	.04			--	

Table 8 - Endrin Content of Cow liver, Kidney, Renal Fat and Body Fat

animal number	endrin in diet ppm	endrin content, ppm															
		liver				kidney				renal fat				body fat			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.06				.00				.00				.04			
2	.00			.03				--				.05				.03	<.02
11	.10	(.03				(.02				(.04	.08			.08			
12	.10													.02			
13	.10			--				--				.04				.04	.02
21	.25	(.02	<.02			(.03				(.22				.11	.09		
22	.25													.14	.11		
23	.25			--				--				(.03				.06	
24	.25			--				--								.02	.03
31	.75	(.18	.11			(.03				(.32				.42			
32	.75													.36			
33	.75			.08				--				.04	.05			.00	
41	2.00	.21	.15			.03				.80				1.05	.68		
42	2.00			.07				--				.14	.11			.12	

Table 9 - Endrin Content of Steer Steaks and Roasts, Raw and Cooked

animal number	endrin in diet ppm	endrin content, ppm											
		steak, raw				steak, cooked				roast, raw				roast, cooked			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.00	<.02			.05		--		.00	<.02			.10			
2	.00			.00								.04	.02			--	
11	.10	(				--				(				--			
12	.10	(.03				--				(.04	.03			--			
13	.10			--				--				--				--	
21	.25	(				(				(				(			
22	.25	(.07				(.00				(.00				(.03	<.02		
23	.25			--				--				--				--	
31	.75	(				(				(				(			
32	.75	(.04				(.14				(.06	.04			(.12			
33	.75			--				--				--				--	
41	2.00	.29	.18			.29	.15			.18				.35			
42	2.00			.03				--				.04				--	

Table 10 - Endrin Content of Steer Liver, Kidney, Renal Fat, and Body Fat

animal number	endrin in diet ppm	endrin content, ppm													
		liver				kidney				renal fat				body fat			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.00	<.02			.00				.10				.10	<.02		
2	.00			.02				--				.00				.06	
11	.10	(				(				(				.09			
12	.10	(.00	<.02			(.07				(.15				.14	.17		
13	.10			--				--				.22	.10			.03	.16
21	.25	(				(				(				.20			
22	.25	(.00				(.10				(.15				.30	.20		
23	.25			--				--				.08	.14			.26	
31	.75	(				(				(				.40			
32	.75	(.21	.02			(.04				(.34				.56	.34		
33	.75							--				.12				.20	
41	2.00	.20	.12			.03				.98				.95			
42	2.00			.11	.02			--				.32				.34	.47

Table 11 - Endrin Content of Lamb Steaks and Roasts, Raw and Cooked

animal number	endrin in diet ppm	endrin content, ppm											
		steak, raw				steak, cooked				roast, raw				roast, cooked			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.03				.05				.07				.06			
2	.00			.06				--				.03 <.02				--	
11	.10	(.03				--				(.06				--			
12	.10	(.03				--				(.06				--			
13	.10			--				--				--				--	
21	.25	(.06				--				(.10				--			
22	.25	(.06				--				(.10				--			
23	.25			--				--				--				--	
31	.75	(.12	.05			(.13				(.07	.06			(.12	.09		
32	.75	(.12	.05			(.13				(.07	.06			(.12	.09		
33	.75			.06				--				.05				--	

Table 12 - Endrin Content of Lamb Liver, Kidney, Renal Fat, and Body Fat

animal number	endrin in diet ppm	endrin content, ppm													
		liver				kidney				renal fat				body fat			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.03	<.02			.01				.02				.00	<.02		
2	.00			.03				.01				.03				.07	
11	.10	(				(				(							
12	.10	(.04	<.02			(.05				(.00				.00	.04		
13	.10			--				--				--		.01		--	
21	.25	(				(				(							
22	.25	(.03				(.00				(.12				.08	.07		
23	.25			--				--				.06		.13	.08	.14	
31	.75	(				(				(							
32	.75	(.07				(.09				(.26				.24			
33	.75			.06				.12				.14	.05	.29		.10	.07

Table 13 - Endrin Content of Hog Liver, Kidney, Renal Fat, and Body Fat

animal number	endrin in diet ppm	endrin content, ppm											
		liver				kidney				renal fat				body fat			
		12 wks		18 wks		12 wks		18 wks		12 wks		13 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.00				.03	<.02			.01				.04			
2	.00			--				--				.09				.00	<.02
11	.10	(				(				(				.04			
12	.10	(.00	<.02			(.01				(.04				.04	.02		
13	.10			--				--				--				--	
21	.25	(				(				(				.16	.09		
22	.25	(.03				(.08				(.02				.20			
23	.25			--				--				--				--	
31	.75	(				(				(				.20	.22		
32	.75	(.01	<.02			(.00				(.10				.16			
33	.75			--				--				.09	.03			.03	

Table 14 - Endrin Content of Hog Steaks and Roasts, Raw and Cooked

animal number	endrin in diet ppm	endrin content, ppm													
		steak, raw				steak, cooked				roast, raw				roast, cooked			
		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks		12 wks		18 wks	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1	.00	.01				.08	<.02			.00	<.02			.00			
2	.00			.04				--				.06				--	
11	.10																
12	.10	.03				--				.04	.03			--			
13	.10			--				--				--				--	
21	.25																
22	.25	.02	.03			--				.02				--			
23	.25			--				--				--				--	
31	.75																
32	.75	.12	.08			.04	.04			.10				.03	.04		
33	.75			.02				--				.11				--	

Table 15 - Endrin Content of Various Tissues from Endrin Fed Broilers

animal number	endrin in diet ppm	endrin content, ppm, after 6 weeks of endrin intake									
		fat		breast raw		breast cooked		drumstick raw		drumstick cooked	
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1-6	.00	.00	<.02	.00		.02		.00		.00	
11-16	.10	.54	.64	.00	.04	.05		.00		.07	
21-26	.25	.40	1.1	.13		.11		.03		.35	.16
31-36	.75	1.45	2.90	.20	.24	.30		.25		.53	1.0

Table 16 - Endrin Content of Eggs From Endrin Fed Pullets

animal number	endrin in diet ppm	endrin content of eggs, ppm									
		during endrin feeding								4 weeks after end of endrin feeding	
		1 week		2 weeks		4 weeks		8 weeks			
		sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.	sp.	bio.
1-5	.00	.06	<.01	.08		.06		.02		.02	
11-15	.10	.02		.02		.08		.06		.07	
21-25	.25	.07		.04		.03	.05	.22	.31	.08	.13
31-35	.75	.05		.03		.19		.38	.36	.21	.17

Table 17 - Recovery of Endrin from Fortified Samples, Specific Method

sample	date analyzed	ppm endrin added	ppm endrin found	% recovery	
milk	7/16	.032	.018	56	
"	7/25	.032	.023	72	
"	7/26	.032	.019	59	
"	7/27	.032	.018	56	
"	7/30	.032	.025	78	
"	7/31	.032	.014	44	
"	8/1	.016	.013	81	
"	8/1	.032	.020	59	
"	8/1	.049	.040	82	
"	8/3	.016	.020	125	
"	8/9	.032	.011	34	
"	8/13	.016	.01	62	
"	8/14	.032	.024	75	
"	8/15	.048	.042	88	
"	8/16	.032	.018	56	
"	8/20	.016	.011	69	
"	8/21	.032	.033	103	
"	8/22	.049	.026	54	
"	8/23	.030	.036	120	
"	9/7	.032	.029	91	
"	9/13	.016	.015	94	
"	9/24	.016	.017	106	
"	10/12	.016	.008	50	
"	10/15	.032	.027	84	
"	11/8	.032	.036	112	
"	11/13	.016	.021	130	ave. 79%
eggs	9/5	.20	.19	95	
"	9/10	.09	.10	112	
"	9/11	.30	.29	96	
"	9/24	.10	.09	90	ave. 98%
ration	7/25	.20	.08	40	
"	9/6	.57	.50	88	
"	9/12	.40	.32	80	
"	9/12	.32	.27	84	
"	9/12	.30	.31	103	
"	9/17	.30	.27	90	
"	9/19	.30	.29	97	
"	9/26	.10	.10	100	ave. 85%

Table 17 - Recovery of Endrin from Fortified Samples, Specific Method (continued)

sample	date analyzed	ppm endrin added	ppm endrin found	% recovery	
fat	9/13	.10	.06	60	
"	9/13	.30	.34	113	
"	9/19	.30	.32	107	
"	10/2	.30	.24	80	
"	10/4	.10	.13	130	
"	10/4	.30	.26	87	
"	10/5	.10	.12	120	
"	10/9	.10	.08	80	
"	10/10	.30	.27	90	
"	10/18	.30	.28	93	
"	11/12	.30	.30	100	
"	11/14	.10	.07	70	ave. 94%
meat cuts	10/3	.10	.11	110	
"	10/3	.30	.30	100	
"	10/16	.10	.08	80	
"	10/19	.30	.32	107	
"	10/30	.10	.13	130	
"	10/31	.30	.29	97	
"	11/1	.10	.11	110	
"	11/2	.30	.26	87	
"	11/6	.10	.13	130	
"	11/7	.30	.33	109	
"	11/7	.30	.29	98	
"	11/9	.10	.09	90	
"	11/9	.30	.36	120	ave. 105%
liver, kidney, and heart	9/25	.15	.10	67	
"	9/25	.30	.20	67	
"	9/26	.10	.15	150	
"	9/26	.30	.36	120	
"	9/28	.30	.31	103	
"	10/10	.30	.30	100	
"	10/17	.30	.31	103	ave. 101%
				overall average	94%

Table 18 - Recovery of Endrin from Fortified Samples, Bioassay

sample	date bioassayed	ppm endrin added	ppm endrin found	% recovery
milk	8/9	.02	.016	82
lamb ration	8/16	.30	.20	67
eggs	9/12	.10	.11	110
milk	9/18	.033	.023	70
fat	10/8	.30	.32	107
brain	10/17	.20	.13	65
liver	10/18	.10	.09	90
eggs	10/19	.05	.07	140
brain	10/23	.25	.18	72
fat	10/25	.10	.11	110
meat cut	10/26	.30	.32	106
fat	10/29	.10	.09	90
milk	11/12	.016	.014	87
meat cut	11/13	.30	.30	100
			average	93

Table 19 - Analysis of Ration Components

sample	stage of feeding	endrin, content ppm expected	ppm found	analyzed by
hay...for steers, lambs, and dairy cows	initial	.00	.01	Shell
barley...for steers and lambs	initial	.00	.04	Shell
oats...for steers and lambs	initial	.00	.04	Shell
wheat...for dairy cows	initial	.00	.05	Shell
oats...for steers and hogs	12th wk.	.00	.09	Shell
barley...for steers, lambs and hogs	12th wk.	.00	.05	Shell
barley...for steers, lambs, and hogs	12th wk.	.00	.07	OSC
oats...for steers and hogs	11th wk.	.00	.09	OSC

Table 20 - Analysis of Endrin Fortified Poultry Rations

Ration	endrin level ppm	stage of feeding	endrin content, ppm expected	endrin content, ppm found*	analyzed by
broiler	.25	1st wk.	.25	.29	OSC
"	.25, stored 1 wk.**	"	.25	.31	OSC
layer	.25	1st wk.	.25	.22	OSC
"	.25, stored 1 wk.**	"	.25	.18	OSC
layer	control	1st wk.	.00	.05	OSC
"	control	1st wk.	.00	.08	Shell
"	.75	5th wk.	.75	.81	OSC
"	.75	5th wk.	.75	.75	Shell
"	.75, stored 1 wk.**	5th wk.	.75	.70	Shell
"	.10	8th wk.	.10	.15	OSC
broiler	control	1st wk.	.00	.06	Shell
"	"	3rd wk.	.00	.04	OSC

* corrected for blank and recovery
** ration stored in feeding room

Table 21 - Analysis of Endrin Fortified Rations

ration	endrin level ppm	stage of feeding	endrin content, ppm expected	endrin content, ppm found*	analyzed by
dairy	control	2nd wk.	.00	.05	Shell
"	"	7th wk.	.00	.09	OSC
"	"	12th wk.	.00	.00	OSC
"	.25	7th wk.	.25	.26	OSC
"	.10	12th wk.	.10	.11	OSC
"	.25	12th wk.	.25	.21	OSC
steer	control	12th wk.	.00	.10	OSC
"	.10	12th wk.	.10	.21	OSC
"	.25	10th wk.	.25	.33	OSC
"	.75	12th wk.	.75	.79	OSC
"	.25	6th wk.	.25	.25	OSC
"	.75	6th wk.	.75	.90	OSC
swine	control	initial	.00	.13	Shell
"	"	6th wk.	.00	.13	OSC
"	.25	6th wk.	.25	.28	OSC
"	.75	6th wk.	.75	.78	OSC
"	.10	12th wk.	.10	.05	OSC
"	.25	12th wk.	.25	.22	OSC
"	.75	12th wk.	.75	.74	OSC
lamb	control	6th wk.	.00	.04	OSC
"	"	10th wk.	.00	.15	OSC
"	.25	6th wk.	.25	.16	OSC
"	.75	6th wk.	.75	.98	OSC
"	.25	10th wk.	.25	.23	OSC
"	.75	12th wk.	.75	.72	OSC

* corrected for blank and recovery

Table 22 - Cooking Data, Meat Cuts

Sample Description and endrin level in diet	cooking method	cooking temp.	cooking time	% wt. loss cooking
pork chop, control	fried, covered pan	medium	15 min.	30
pork chop, .75 ppm	" "	"	"	35
lamb chop, control	broiled, well done	medium	25 min.	35
lamb chop, .75 ppm	" "	"	"	29
beef steak, control	broiled, med. well dn.	medium	30-45 min.	31
beef steak, .25 ppm	" " "	"	"	29
beef steak, .75 ppm	" " "	"	"	36
beef steak,2.00 ppm	" " "	"	"	43
cow steak, control	covered roaster,oven	325°	60 min.	49
cow steak, .25 ppm	" " "	"	"	47
cow steak, .75 ppm	" " "	"	"	46
cow steak,2.00 ppm	" " "	"	"	52
broiler, breast, control	baked, open pan, oven	350°	65 min.	35
broiler, breast, .10 ppm	" " "	"	"	41
broiler, breast, .25 ppm	" " "	"	"	43
broiler, breast, .75 ppm	" " "	"	"	37
broiler, drumstk., control	baked, open pan, oven	350°	55 min.	39
broiler, drumstk., .10 ppm	" " "	"	"	35
broiler, drumstk., .25 ppm	" " "	"	"	40
broiler, drumstk., .75 ppm	" " "	"	"	35
pork roast, control	roasted, open pan	325°	2 3/4 hr.	30
pork roast, .75 ppm	" "	"	3 1/4 hr.	28
lamb roast, control	roasted, open pan	325°	1 1/2 hr.	28
lamb roast, .75 ppm	" "	"	2 hr.	23
beef roast, control	roasted, covered pan	325°	3 1/2 hr.	43
beef roast, .25 ppm	" "	"	2 3/4 hr.	40
beef roast, .75 ppm	" "	"	4 hr.	38
beef roast,2.00 ppm	" "	"	2 3/4 hr.	36
cow roast, control	roasted, covered pan	325°	2 3/4 hr.	38
cow roast, .25 ppm	" "	"	3 1/4 hr.	38
cow roast, .75 ppm	" "	"	3 3/4 hr.	38
cow roast,2.00 ppm	" "	"	2 3/4 hr.	39

Table 23 - Total Fat Content of Beef Steaks, Roasts, and Liver

animal number	fat content, %		
	steak	roast*	liver
1	56.5	26.2 26.1	7.0
11	45.9	28.7 28.3	8.1
21	50.0	31.2 28.2	6.5
31	57.1	32.9 25.1	12.7
41	48.7	25.0 23.6	7.4

*duplicate determinations made

THE MOSQUITO LARVAE BIOASSAY METHOD

Rearing techniques: The test insects are reared in a constant temperature room. Adult mosquitoes are held in a wire screen cage containing a pan of water in which the eggs are deposited. The female mosquitoes are given a blood meal by introducing a small rooster into the cage overnight. Approximately four days after receiving blood, the female mosquitoes deposit rafts of eggs on the surface of the water in the pan. These rafts, made up of from 100 to 200 eggs, are transferred with a medicine dropper to another pan of water. Larvae emerge from the floating rafts in a day or two and are separated from the unhatched eggs at 24 hour intervals so that their age is uniform. One gallon wide mouth mayonnaise jars are used as rearing containers. About 600 larvae in 3 quarts of water make up a population for each jar. The larvae are fed 100 mgs of powdered dog food each day by dusting it lightly on the surface of the rearing jars. When the larvae are five days old they are ready to be used in bioassay.

Preparation of samples for bioassay: The samples are saponified and chromatographed in the same manner as the samples being analyzed by the chemical method. These steps are described in the Shell Development Company method number SMS 642/56, "Spectrophotometric Determination of Endrin in Animal Tissues, Milk, Butter, and Eggs".

After the samples have been treated for the removal of interfering materials they are concentrated to a small volume on a steam bath and final traces of solvent removed under an air jet at room temperature. The dry residue is redissolved in a small volume of acetone, not over 1% of the final volume, and is suspended by vigorous shaking in a volume of water sufficient to allow proper sensitivity. Usually the ration of water suspension to original sample weight is 1:1. The sensitivity of the method under the conditions encountered in the analysis of eggs, meat, fat, and other animal tissues is 0.02 ppm of endrin. The sensitivity in assaying milk is 0.002 ppm.

The bioassay: Each bioassay includes a check consisting of an untreated sample, a reference standard consisting of a water suspension of the insecticide being measured, and one or more unknown or treated samples. Recovery studies are made frequently by adding known amounts of the standard insecticide to untreated samples and comparing the mortalities with those obtained with the reference standard.

The assays are performed in 40 ml glass vials. The sample and sufficient water to make a volume of 10 mls are added first followed by ten larvae in 5 mls of water. The amount of the sample suspension required to give an optimum mortality range must be determined by preliminary experiment. The assay levels are run in duplicate or triplicate.

The mortalities of the test insects are determined after 24 hours. Total lack of movement upon probing with a wire probe is taken as the criterion of death.

Evaluating the results: Two methods of interpreting the results can be used. If it is desired to know the actual level of insecticide residue present in the sample, the dosage-mortality curves of the standards and unknowns can be compared. The use of log-probit paper, expressing concentration of the standard in micrograms and that of the unknown in grams simplifies this comparison. In case there is no kill in the unknown sample, an estimate of the upper limit of residue present can be made by comparing the lowest level which gives a significant kill in the standard with the highest level of unknown.

APPENDIX II - ANIMAL RECORDS AND PHOTOGRAPHS

Table 24 - Composition of Experimental Ration
Broilers

Ingredient	%
Ground yellow corn	57.55
Prime tallow[1/]	3.0
Soybean meal, sol., 44% protein	22.7
Fish meal, herring, 70%	5.0
Corn gluten meal	3.0
Whey, dried	2.5
Alfalfa Meal, sun cured, 15% protein	2.0
DL-Methionine (98%)	0.05
Bone meal, st., sp.	2.25
Limestone flour	1.25
Salt, iodized	0.3
Choline chloride (25%)	0.2
Vitamin A, dry (10,000 U.S.P.U./gm.)	0.05
Vitamin D, dry (1,500 I.C.U./gm.)	0.05
Riboflavin concentrate (8 mg./gm.)	0.07
Antibiotic-B_{12} supplement (2 gm. procaine penicillin & 3 mg. vit. B_{12}/lb.)	0.05
	gm./100 lbs.
Manganese sulfate (70%)	18.1
Ca-pantothenate concentrate (70.5 mg./gm.)	3.6
Sulfaquinoxaline	7.1
Niacin	1.0

1/ Stabilized with Tenox R.

Table 25 - Composition of Experimental Ration Layers

Ingredients	Pounds
Corn, grd., yellow	1464
Soybean meal, sol. 44% protein	275
Fish meal, 70% protein	60
Alfalfa meal, s.c., 18% protein	60
Bone meal, sp. st.	50
Limestone flour	75
Salt, iodized	10
Manganese sulfate (70%)	0.3
A & D feeding oil (2250 U.S.P.U. A & 300 I.C.U.D./gm.)	3
Vitamin D_3, dry (1500 I.C.U./gm.)	0.5
Choline Chloride (25%)	2
Riboflavin Conc. (3632 mg./lb.)	0.5
	grams
Niacin	10
Total.....	2000.3

Table 26 - Average Body Weights, Gains and Mortality Data
Broilers

	Endrin ppm			
	0.0	0.1	0.25	0.75
		Preliminary Period		
Body weights (gm.)				
Initial Weight	41 (15)[1/]	41 (15)	43 (15)	41 (15)
1st Week	84 (14)	80 (15)	85 (15)	75 (15)
2nd Week	162 (14)	156 (15)	162 (15)	144 (15)
3rd Week)	258 (14)	264 (15)	263 (15)	233 (15)
) [2/]	253 (7)	272 (7)	255 (7)	252 (7)
4th Week	378 (7)	399 (7)	389 (7)	386 (7)
		Experimental Period		
Initial Weight	378 (7)	399 (7)	389 (7)	386 (7)
1st Week	537 (7)	577 (7)	545 (7)	560 (7)
2nd Week	714 (7)	758 (7)	717 (7)	729 (7)
3rd Week	938 (7)	981 (7)	930 (7)	943 (7)
4th Week	1149 (7)	1176 (7)	1104 (7)	1157 (7)
5th Week	1353 (7)	1389 (7)	1323 (7)	1373 (7)
6th Week	1579 (7)	1606 (7)	1501 (7)	1596 (7)
Av. Gain (gm.)	1201	1207	1112	1210

1/ Figures in parentheses represent survivors

2/ Divided into experimental lots of 7 broilers each

Table 27 - Average Feed Consumption and Conversion - Broilers

	Endrin ppm											
	0.0			0.10			0.25			0.75		
	By Week	cumulative by periods	cumulative	by week	cumulative by periods	cumulative	by week	cumulative by periods	cumulative	by week	cumulative by periods	cumulative
Feed Consumed (lbs)					Preliminary Period							
1st week	0.07	---	---	0.06	---	---	0.07	---	---	0.06	---	---
2nd week	0.35	0.42	0.42	0.30	0.36	0.36	0.33	0.40	0.40	0.29	0.35	0.35
3rd week	0.51	0.93	0.93	0.45	0.81	0.81	0.47	0.87	0.87	0.41	0.76	0.76
4th week	0.59	1.52	1.52	0.65	1.46	1.46	0.61	1.48	1.48	0.54	1.30	1.30
					Experimental Period							
1st week	0.73	---	2.25	0.79	---	2.25	0.77	---	2.25	0.81	---	2.11
2nd week	0.99	1.72	3.24	1.04	1.83	3.29	0.98	1.75	3.23	1.04	1.85	3.15
3rd week	1.06	2.78	4.30	1.04	2.87	4.33	1.11	2.86	4.34	1.14	2.99	4.29
4th week	1.23	4.01	5.53	1.21	4.08	5.54	1.04	3.90	5.38	1.17	4.16	5.46
5th week	1.38	5.39	6.91	1.39	5.47	6.93	1.35	5.25	6.73	1.38	5.54	6.84
6th week	1.54	6.93	8.45	1.57	7.04	8.50	1.31	6.50	8.04	1.47	7.01	8.31
Feed conversion		2.62[1]	2.43[2]		2.65	2.40		2.65	2.43		2.63	2.35

1/ F.C. = $6.93 \div \frac{1201}{454}$ = (See Table 2) = 2.62

2/ F. C. = $8.45 \div \frac{1579}{454}$ (See Table 2) = 2.43

Table 28 - Average Egg Production, Feed Consumption and Body Weight Data
Layers

	Endrin (ppm)			
	0.0	0.1	0.25	0.75
No pullets surviving	5	6	6	6
Hen Days				
Experimental Period	305	336	336	336
Post Experimental Period	140	168	168	168
Total	445	504	504	504
Eggs Laid				
Experimental Period	175	230	145	193
Post Experimental Period	90	92	78	104
Total	265	322	223	297
Egg Production (%)				
Experimental Period	57.4	68.6	43.2	57.4
Post Experimental Period	64.3	54.8	46.4	61.9
Total	59.6	63.9	44.2	58.7
Feed Consumed/Hen Day (lbs)				
Experimental Period	.179	.191	.192	.184
Post Experimental Period	.208	.186	.202	.213
Total	.188	.189	.196	.194
Body Weight (lbs)				
Initial	3.9	3.8	3.6	3.6
Experimental Period	4.1	3.7	3.7	3.8
Post Experimental Period	4.1	3.8	3.8	4.1

Table 29 - Average Egg Weight Data
Layers

	Endrin (ppm)			
	0.0	0.1	0.25	0.75
	(gm)			
1st week	49 (12)[1/]	47 (23)	53 (7)	43 (8)
2nd week	49 (22)	51 (30)	55 (18)	46 (25)
3rd week	48 (29)	51 (30)	51 (17)	46 (21)
4th week	49 (27)	49 (30)	50 (21)	48 (27)
5th week	50 (19)	50 (31)	50 (20)	49 (26)
6th week	50 (21)	52 (27)	50 (19)	48 (26)
7th week	54 (17)	54 (24)	51 (18)	48 (23)
8th week	52 (15)	52 (25)	50 (13)	49 (26)
Average	50	51	51	47
9th week	52 (19)	53 (20)	51 (19)	49 (26)
10th week	52 (22)	53 (22)	50 (18)	51 (28)
11th week	51 (22)	54 (25)	51 (26)	50 (22)
12th week	53 (27)	55 (25)	51 (22)	52 (23)
Average	52	54	51	51

1/ Figures in parentheses represent number of eggs per week

Table 30 - Milk Production, Butterfat Test, Total Fat, and Average Daily Milk and Fat for Cows on Endrin Feeding Studies

Cow No.	June, 1956 Total Milk lbs	B.F. Test %	Total Fat lbs.	Days in Milk	Ave. Daily lbs. Milk	Ave. Daily lbs. Fat	July, 1956 Total Milk lbs.	B.F. Test %	Total Fat lbs.	Days in Milk	Ave. Daily lbs. Milk	Ave. Daily lbs. Fat
1	713.8	4.36	31.1	29	24.6	1.07	745.8	4.00	29.8	31	24.1	0.96
2	925.4	4.35	40.3	28	33.1	1.44	960.0	4.20	40.3	31	31.0	1.30
11	543.5	4.58	24.9	27.5	19.8	0.91	574.4	4.80	27.6	31	18.5	0.89
12	615.3	4.78	29.4	28	22.0	1.05	667.6	4.60	30.7	31	21.5	0.99
13	621.2	4.16	25.8	28	22.2	0.92	663.0	4.15	27.5	31	21.4	0.89
21	458.4	6.18	28.3	29	15.8	0.97	464.4	6.17	28.7	31	15.0	0.93
22	375.8	5.26	19.8	27.5	13.7	0.72	358.5	5.57	20.0	31	11.6	0.65
23	533.8	4.73	25.2	28	19.1	0.90	583.4	5.00	29.2	31	18.8	0.94
24	742.6	4.61	34.2	28	26.5	1.22	782.3	5.17	40.4	31	25.2	1.30
31	390.8	5.63	22.0	29	13.5	0.76	405.7	5.60	22.7	31	13.1	0.73
32	453.5	5.16	23.4	28	16.2	0.84	923.2	4.60	42.5	31	29.8	1.37
33	627.8	4.85	30.4	26.5	23.7	1.15	785.3	4.55	35.7	31	25.3	1.15
41	386.6	4.93	19.1	28	13.8	0.68	528.0	4.80	25.3	31	17.0	0.82
42	473.7	5.47	25.9	29	16.3	0.89	474.9	5.70	27.1	31	15.3	0.87

Cow No.	August, 1956 Total Milk lbs	B.F. Test %	Total Fat lbs.	Days in Milk	Ave. Daily lbs. Milk	Ave. Daily lbs. Fat	September, 1956 Total Milk lbs.	B.F. Test %	Total Fat lbs.	Days in Milk	Ave. Daily lbs. Milk	Ave. Daily lbs. Fat
1	691.8	4.55	31.5	31	22.3	1.02	120.8	4.88	5.9	6	20.1	0.98
2	852.1	4.80	40.9	31	27.5	1.32	652.6	6.05	39.5	30	21.8	1.32
11	569.3	4.55	25.9	31	18.4	0.84	98.0	5.65	5.5	6	16.3	0.92
12	579.9	5.25	30.4	31	18.7	0.98	89.9	6.05	5.4	6	15.0	0.90
13	659.4	4.50	29.7	31	21.3	0.96	571.9	4.90	28.0	30	19.1	0.93
21	425.8	6.65	28.3	31	13.7	0.91	67.9	7.25	4.9	6	11.3	0.82
22	254.4	6.65	16.9	31	8.2	0.55	42.2	6.68	2.8	6	7.0	0.47
23	576.7	4.48	25.8	31	18.6	0.83	500.5	5.50	27.5	30	16.7	0.92
24	737.6	4.35	32.1	31	23.8	1.03	613.5	6.65	40.8	30	20.5	1.36
31	323.5	6.35	20.5	31	10.4	0.66	38.8	6.50	2.5	6	6.5	0.42
32	495.6	4.30	21.3	31	16.0	0.69	71.1	5.50	3.9	6	11.9	0.65
33	740.6	5.20	38.5	31	23.9	1.24	592.3	4.75	28.1	30	19.7	0.94
41	411.3	4.82	19.8	31	13.3	0.64	66.8	5.05	3.4	6	11.1	0.57
42	452.5	6.60	29.9	31	14.6	0.96	378.3	5.52	20.9	30	12.6	0.70

Cow No.	October, 1956 Total Milk lbs	B.F. Test %	Total Fat lbs.	Days in Milk	Ave. Daily lbs. Milk	Ave. Daily lbs. Fat
2	306.4	5.25	16.1	17.5	17.5	0.92
13	277.4	5.60	15.5	17.5	15.9	0.89
23	254.2	6.25	15.9	17.5	14.5	0.91
24	320.6	6.90	22.1	17.5	18.3	1.26
33	303.0	4.00	12.1	17.5	17.1	0.69
42	181.5	6.70	12.2	17.5	10.4	0.70

Table 31 - Weights of Dairy Cows

cow no.	body weight, lbs.										
	6/8 [1]	6/14 [2]	6/28	7/12	7/26	8/9	8/23	9/5 [3]	9/22	10/10	10/17
1	862	855	868	864	890	932	906	953	---	---	---
2	863	875	865	887	884	900	872	907	893	914	893
11	892	862	891	824	889	907	885	939	---	---	---
12	887	901	906	975	966	975	967	997	---	---	---
13	885	887	873	921	930	983	943	992	990	1001	996
21	1028	1036	1035	1063	1028	1059	1038	1056	---	---	---
22	1061	1075	1088	1104	1100	1145	1131	1153	---	---	---
23	718	735	744	748	768	785	797	839	836	847	874
24	697	753	731	732	767	783	747	781	797	792	807
31	890	893	894	881	899	932	932	932	---	---	---
32	814	840	821	838	862	876	877	876	---	---	---
33	824	818	866	877	883	922	921	944	955	991	1004
41	812	813	830	839	861	908	897	915	---	---	---
42	864	850	864	860	875	902	900	929	937	960	955

1/ Average of 3 consecutive day's weighings before endrin feeding began

2/ Endrin feeding started June 14

3/ Endrin feeding stopped September 7

Table 32 - Average Daily Feed Consumption, Dairy Cows

Animal number	average daily feed consumption, lbs. before endrin feeding	during endrin feeding June	July	August	Sept.	October	Overall Average
1	32	33	32	32	32	--	32
2	36	38	38	37	37	37	37
11	28	29	28	29	29	--	28
12	30	33	37	38	38	--	36
13	31	33	38	38	38	38	37
21	28	29	31	32	31	--	31
22	28	29	30	29	29	--	29
23	27	29	30	32	32	32	31
24	31	34	33	33	33	33	33
31	27	29	30	29	29	--	29
32	27	29	34	35	35	--	33
33	29	30	33	36	36	36	34
41	30	33	33	33	33	--	33
42	26	29	29	29	29	29	29

Table 33 - Weight Gain and Feed Consumption Records for Hogs

animal number	weight gain and feed consumption during endrin feeding: weight, lbs. 6/19	7/3	7/17	7/31	8/14	8/29	9/11	daily gain ave.	daily feed ave.	after endrin feeding ceased: weight, lbs. 9/25	10/9	10/24	daily gain ave.	daily feed ave.
1	55	65	77	94	115	131	143	1.05	4.41	slaughtered 9/13				
2	40	47	57	70	89	108	131	1.08	3.99	148	174	197	2.47	6.38
11	44	52	64	79	104	124	142	1.17	4.36	slaughtered 9/13				
12	47	58	70	85	109	129	154	1.27	4.37	slaughtered 9/13				
13	29	37	45	56	73	83	102	.87	2.63	121	152	184	2.58	5.93
21	52	63	78	94	121	139	167	1.37	5.23	slaughtered 9/13				
22	44	58	65	83	104	116	141	1.15	4.11	slaughtered 9/13				
23	27	32	41	51	68	75	91	.76	2.36	111	140	162	2.28	5.73
31	51	64	81	102	124	141	168	1.39	5.23	slaughtered 9/13				
32	41	50	63	79	98	115	135	1.12	4.14	slaughtered 9/13				
33	28	36	45	57	79	93	115	1.04	3.34	134	161	184	2.26	6.35

Table 34 - Weight Gain and Feed Consumption Records for Lambs

animal number	weight gain and feed consumption: during endrin feeding: weight, lbs. 6/19	7/3	7/17	7/31	8/14	8/29	9/11	daily gain ave.	daily feed average grain	forage	after endrin feeding ceased: weight, lbs. 9/25	10/9	10/24	daily gain ave.	daily feed average grain	forage
1	67	73	81	82	85	91	100	.39	1.12	2.66	slaughtered 9/13					
2	70	75	86	89	93	98	105	.42	1.12	2.98	101	103	109	.21	1.52	3.45
11	72	80	89	91	96	97	104	.38	1.12	2.80	slaughtered 9/13					
12	73	79	89	93	96	99	101	.33	1.12	2.92	slaughtered 9/13					
13	67	72	81	84	90	91	98	.37	1.12	2.82	98	99	103	.12	1.52	3.30
21	67	72	82	85	92	99	106	.46	1.12	2.91	slaughtered 9/13					
22	74	76	87	87	93	97	107	.39	1.12	2.90	slaughtered 9/13					
23	73	80	89	93	97	102	106	.39	1.12	3.01	108	108	115	.21	1.52	3.45
31	76	78	86	87	92	94	99	.27	1.12	2.99	slaughtered 9/13					
32	69	74	85	87	92	96	104	.42	1.12	2.94	slaughtered 9/13					
33	68	73	87	84	87	89	94	.31	1.12	2.89	97	101	104	.23	1.52	3.52

Table 35 - Weight Gain and Feed Consumption Records for Steers

animal number	weight gain and feed consumption: during endrin feeding: weight lbs. 6/19	7/3	7/17	7/31	8/14	8/30	9/11	daily gain ave.	daily feed average grain	daily feed average forage	after endrin feeding ceased: 9/25	10/9	10/23	daily gain ave.	daily feed average grain	daily feed average forage
1	665	668	718	725	739	759	752	1.04	8.95	8.32	slaughtered 9/12					
2	620	639	700	687	720	724	735	1.37	7.95	8.47	761	765	773	.90	6.98	7.06
11	475	516	560	573	627	651	657	2.17	8.49	8.32	slaughtered 9/12					
12	643	667	684	715	763	766	780	1.63	8.01	8.99	slaughtered 9/12					
13	568	609	614	642	657	681	691	1.46	7.58	8.53	734	768	759	1.62	9.62	8.93
21	503	516	575	556	597	597	601	1.17	6.92	8.36	slaughtered 9/12					
22	667	685	779	700	738	766	783	1.38	8.05	8.33	slaughtered 9/12					
23	541	563	685	630	676	693	704	1.94	8.58	9.51	723	755	756	1.24	9.62	8.19
31	594	629	705	655	663	701	716	1.45	7.40	8.26	slaughtered 9/12					
32	584	615	720	660	700	722	731	1.75	8.69	9.41	slaughtered 9/12					
33	659	650	690	660	748	769	766	1.27	8.26	8.05	839	855	840	1.76	9.17	8.31
41	590	614	683	717	778	797	819	2.73	8.98	10.22	slaughtered 9/12					
42	586	595	601	650	671	701	698	1.33	7.24	7.48	719	718	720	.52	7.24	6.81

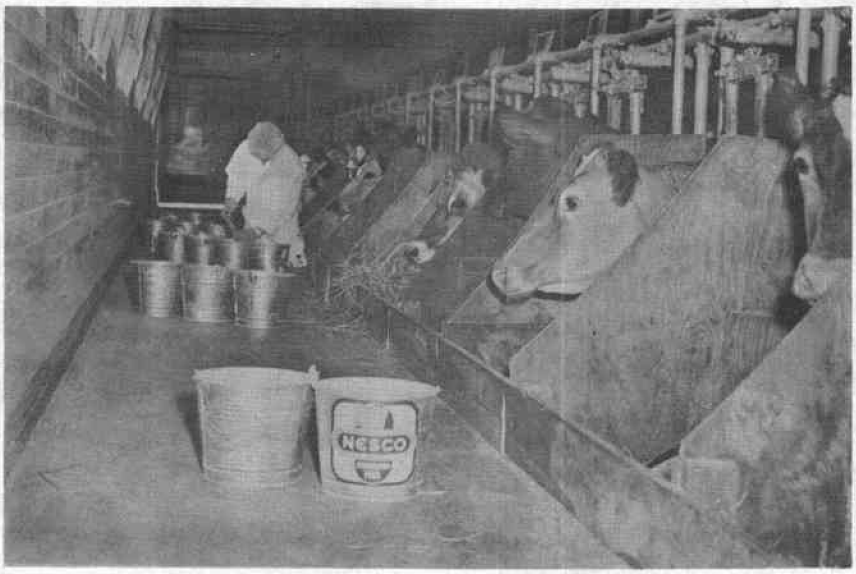

Figure 1. Dairy ration being fortified with endrin.

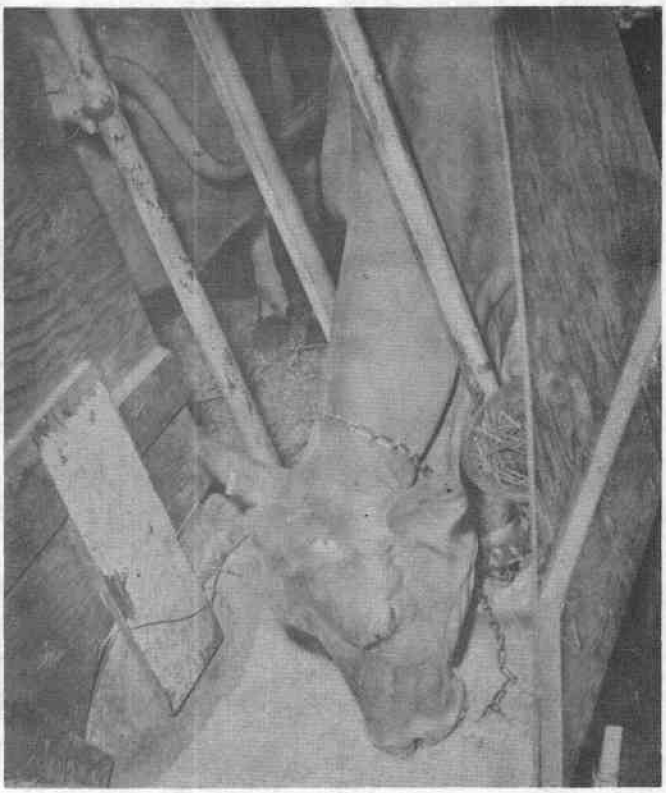

Figure 2. Cow consuming freshly fortified grain ration.

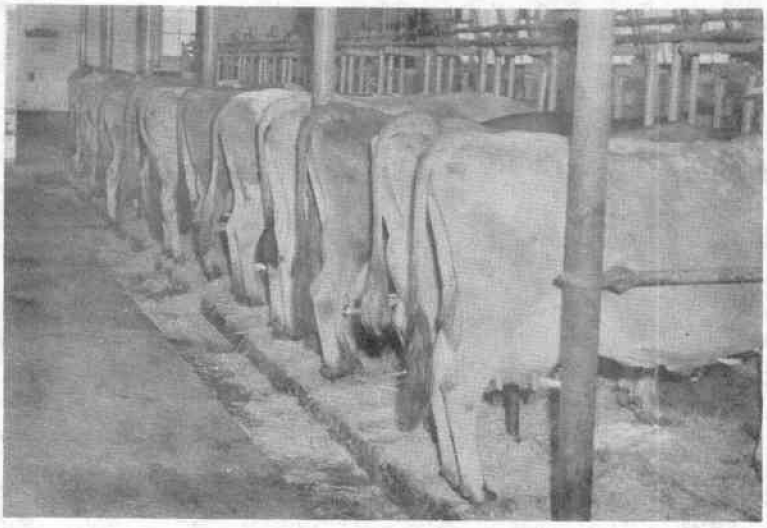

Figure 3. View of cows used in the endrin-feeding experiment.

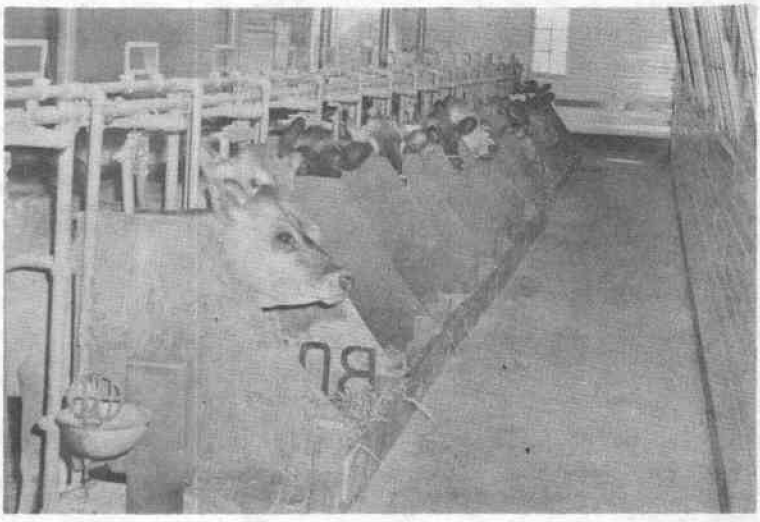

Figure 4. Mangers designed to prevent mixing of rations.

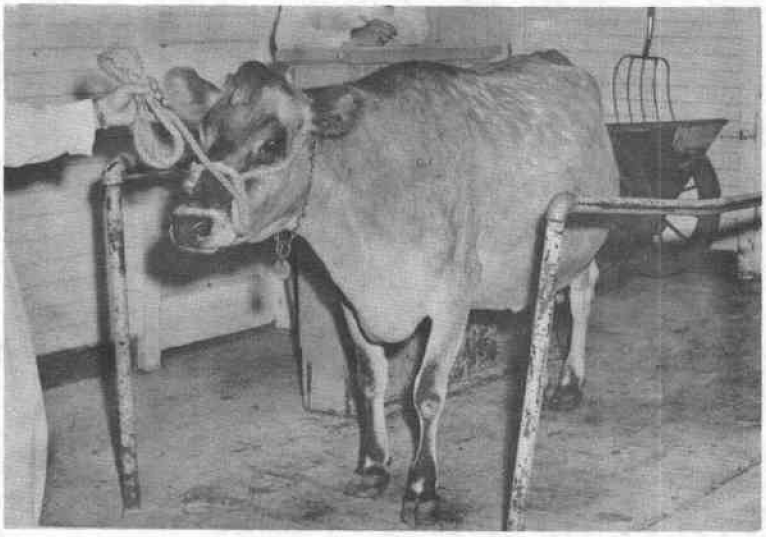

Figure 5. Weighing a cow that received an endrin-fortified ration.

Figure 6. Steers with individual mangers and drinking fountains.

Figure 7. Lambs in individual pens. Note individual feeding racks.

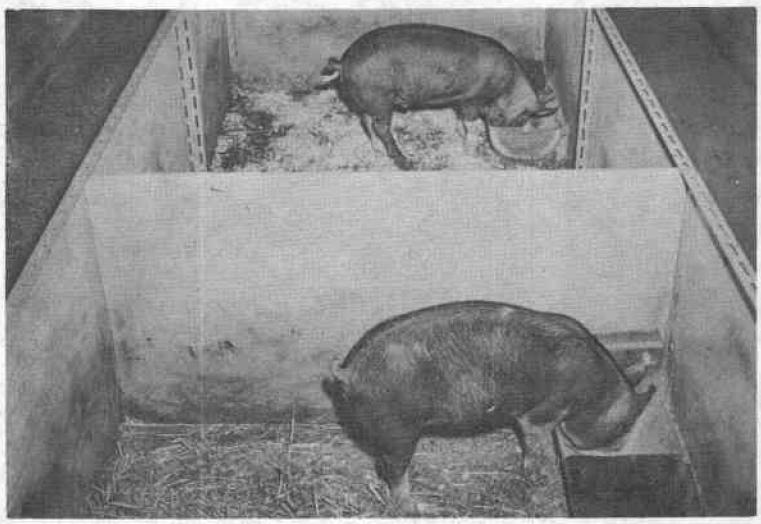

Figure 8. Hogs in individual pens showing separate feeding and watering facilities.

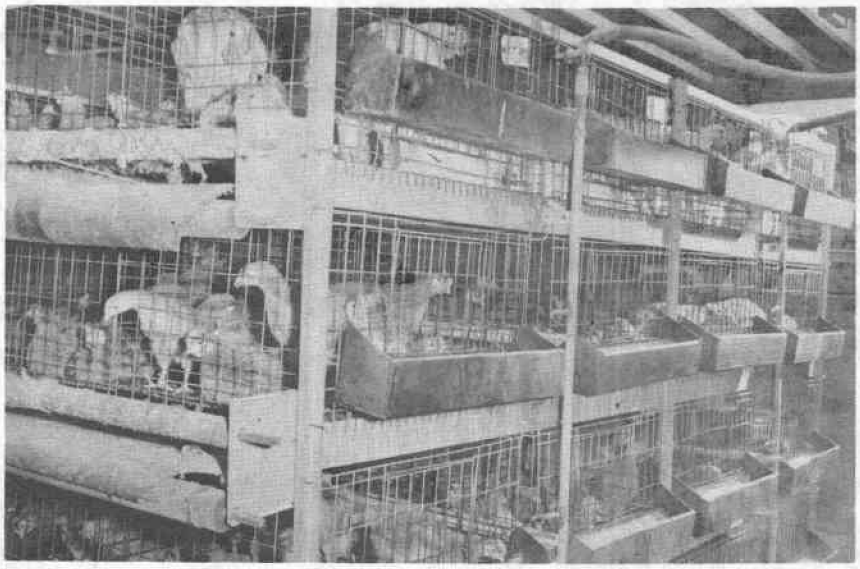

Figure 9. Finishing batteries.

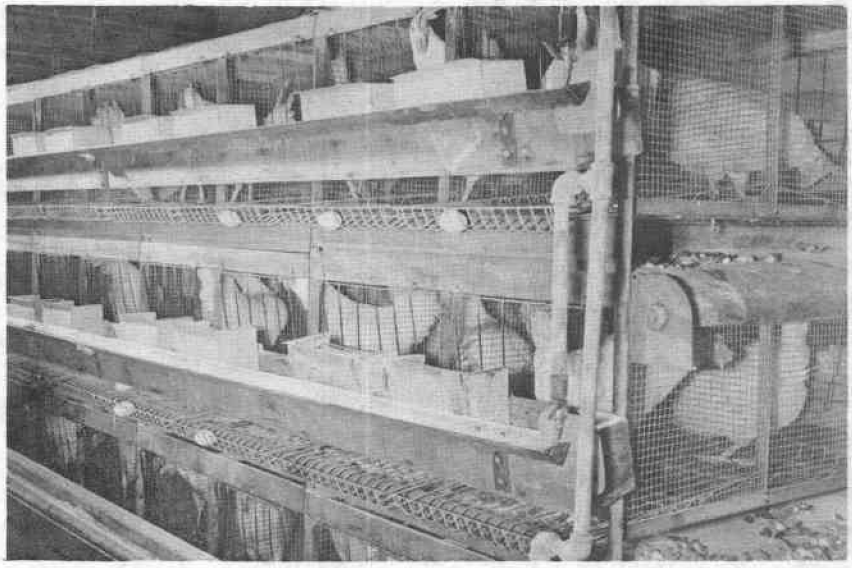

Figure 10. Laying batteries.

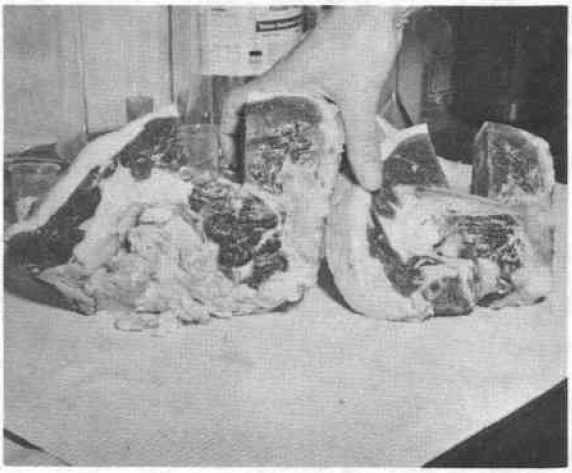

Figure 11. Beef steak and roast showing method of sampling for analysis.

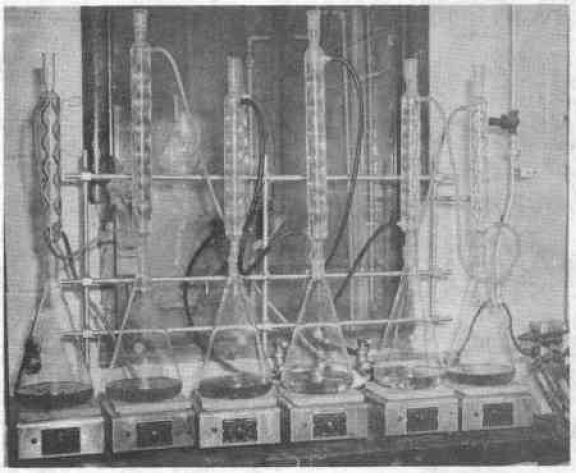

Figure 12. Saponification setup.

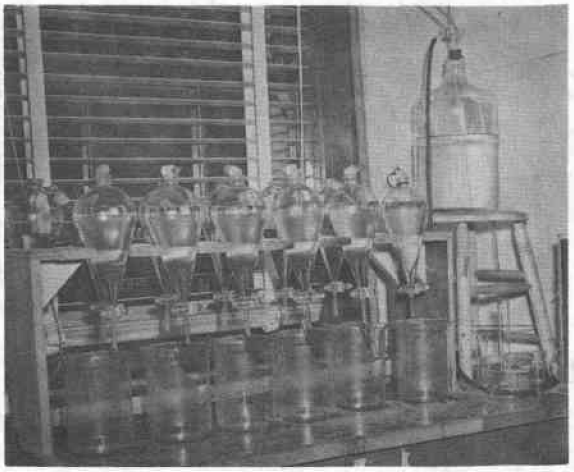

Figure 13. Solvent extraction after saponification.

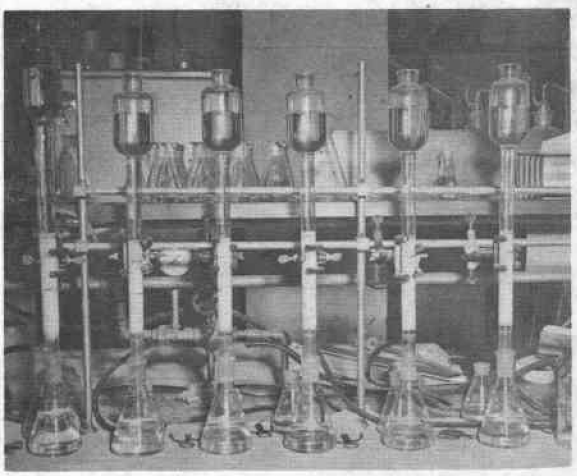

Figure 14. Chromatography of tissue extracts.

Figure 15 - Standard Curve for Endrin Determination by Spectrophotometric Method.