PESTICIDE SECTION

# CANADIAN WILDLIFE SERVICE

## MANUSCRIPT REPORTS

No. 1

# Chlorinated Hydrocarbon Residues in Ducklings

William Alexander Charnetski

1965

CANADIAN WILDLIFE SERVICE

PESTICIDE SECTION

MANUSCRIPT REPORTS

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## ABSTRACT



Ducklings of various ages of seven species were collected from an area around Strathmore, Alberta. Methods of analysis and clean-up for the various duckling tissues are described, and discussed as well as the various techniques available for residue determinations, and the chemistry of DDT and dieldrin. The residue analyses were conducted using a Gas Liquid Chromatograph (Wilkens model A-680 Pestilyzer) with an electron capture detector.

Muscle, fat, and preen gland tissue from these birds, have been analysed for DDT, DDD, DDE, and dieldrin, by the author and by the Ontario Research Foundation. Insecticide history of the area shows no significant usage of any DDT or DDT product. The results, however, report DDT (sum of DDT, DDD, DDE) residues in muscle ranging from 0 to 0.97 ppm and in fat from 0 to 36.48 ppm. The occurrence and amount of dieldrin was much less than DDT, with 57.1% of the birds expressing no dieldrin, while 4% no DDT.

It is concluded that the insecticide residue was transferred to ducklings from the hen via the egg. Initial dosages were decreased by excretion and/or growth dilution. The identification of residues of insecticide in the preen gland indicates a possible method of insecticide excretion in birds.

Further research is necessary on methods and analysis techniques, and the importance of environmental contamination and its affect on the biota.

## ACKNOWLEDGEMENTS

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The Hon. J. R. Nicholson, Minister of Forestry, in speaking before the Special Committee on Food and Drugs* in 1963 remarked that:

"There has been comparatively little study of wildlife populations in sprayed forests of Canada; in fact, the real consequences of such treatments on wildlife populations is urgently in need of study. We would like to see much more intensive study carried out by the Department of Fisheries and by the Canadian Wildlife Service on the short-term and long-term impact of insecticides on important fish and wildlife species".

There was other testimony before the committee that was in a similar vein, and as a consequence, one of the recommendations Mr. Harry Harley, the committee's chairman, made to parliament was:

"That pesticide research should be encouraged at all levels and co-ordinated where possible by the Committee on Pesticides. To this end our Committee recommends that the Federal Government give consideration to grants to aid pesticide research".

That recommendation implied a definite responsibility on the part of the Federal Wildlife Service to undertake studies of the effects of insecticide residues on those birds, fish, and mammals we commonly think of as wildlife.

The Alberta Provincial Board of Health Regulations - Division 10 (Regulations respecting water and ice) has taken a stand against chemical pollution of water in Section 14, paragraph 2, which reads:

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*A 24 member Special Committee was appointed to consider and report on (a) the hazards of food contamination from insecticides, pesticides, and other noxious substances; and (b) the safety and cost of drugs.

"No person shall place any chemical in any stream, public lake or public reservoir, or on the shores or banks thereof, to control or kill plant growth, weeds or fish without first obtaining a permit so to do from the Provincial Board of Health. The Provincial Board of Health may refuse to issue such permit or may attach conditions to the issue of such permit where in the opinion of the Provincial Board it is in the interest of the public health so to do (O.C. 187-64)",

It is important to note that this regulation does not include the use of chemicals for insect control in an aquatic environment. That is, control of organisms which constitute part of the food chain of our ducks, birds and fish. By poisoning the insects, we may destroy the food supply of some birds and fish or contaminate the food supply, and thereby poison wildlife.

As for aquatic insect control, people are encouraged to use insecticides to control insects in a terrestrial habitat. Of these insecticides, the use of chlorinated hydrocarbons for the control of grasshoppers was most prominant in the prairie regions of Canada. Unfortunately, chlorinated hydrocarbon insecticides are characterized by long lasting residues and a high toxicity to birds, mammals and fish (Henderson, Pickering and Tarzwell, 1959; Rudd and Genelly, 1956; Tarzwell 1958, 1959). These compounds are fat soluable, and are accumulated in fatty tissue or excreted (to a small extent).

Other biologists (Allison, Kallman, Cope and Van Valin, 1963; Bridges, 1961; Bridges, Kallman and Andrews, 1963; Brown, 1963; Clawson and Baker, 1959; Cope, 1961; Cope, Gjullen and Storm, 1947; Coulson, Huene and Cavanagh, 1960; Coulson and McCarthy, 1963; DeWitt and George, 1960; DeWitt, Menzie, Adomaitis and Reichel, 1960; DeWitt et al. 1963;

to name only a few) have given data regarding the presence of insecticide residues in tissue and in most cases have illustrated the possible harmful effects (reduced fecundity and hatching, mortality under adverse conditions) of insecticides (DDT and dieldrin included) on birds.

Insecticides are applied by hand sprayers, small and large mechanically driven applicators and by airplane. With the increased usage of these insecticides, contamination of the aquatic environment seems inevitable. Contamination of the entire ecosystem is only a matter of time.

With some of the sloughs and marshes being catchment basins for the farm lands of the prairies, questions arose: how much of the persistent insecticides get into these waters and how long do they last; were these residues dissipated evenly throughout the ecosystem, or were they concentrated by food chains; could they be destroyed in the ecosystem through the actions of bacteria, light, temperature, pH or any of the other possible factors which can effect insecticides.

These same sloughs and marshes, however, are the breeding grounds for a large duck population. A shortage of such suitable marsh areas for bird habitats resulted in a reversal of government policy regarding support of wetland drainage. In fact, government policy and support for maintaining the small but productive marshes and ponds was necessary. Officials of the Canadian Wildlife Service questioned the value to wildlife of these bodies of water which were vulnerable to contamination by insecticides particularly chlorinated hydrocarbons.

Government agencies are realizing the possible threat to biota, created by the spread of the long-lasting chlorinated hydrocarbon residues, and are beginning to place severe restrictions on their use.

Insecticides characterized by such a residue are being replaced by the relatively non-residual organophosphorous insecticides. The long-term effects (if any) of large scale usage of these compounds is unknown.

The following study represents a preliminary investigation intended to find out whether dieldrin used in the control of grasshoppers on cereal crops in areas of duck nesting was in fact being accumulated by ducklings. Concurrently, methods of residue analyses were to be examined; the best (for our circumstances) of which were chosen to measure the residues in ducklings taken from a study area around Strathmore, in Southern Alberta. The interpretation of the analytical data and the suggestions for continued research form the basis for long-ranged experiments to trace insecticides through food chains involving wildlife.

The ducklings were analysed for dieldrin, DDT, DDD and DDE residues (for simplicity in this report DDT will represent the summation of DDT, DDD and DDE unless otherwise indicated). Muscle and fat tissues were chosen as representative sampling tissues, but near the completion of the study the preen gland was recognized as a potential excretory organ. Some birds could therefore have become partially independent (with respect to the effects of accumulation of detrimental quantities of insecticide) of their environment because of this excretion.

## 1.1. Site Description

The collection area was within Wheatland No. 16 County and included a series of ponds (selected by Mr. Sugden of the Canadian Wildlife Service) within a nine mile radius of Strathmore, Alberta. Figure 1 shows the geographical location of the sampling sites in

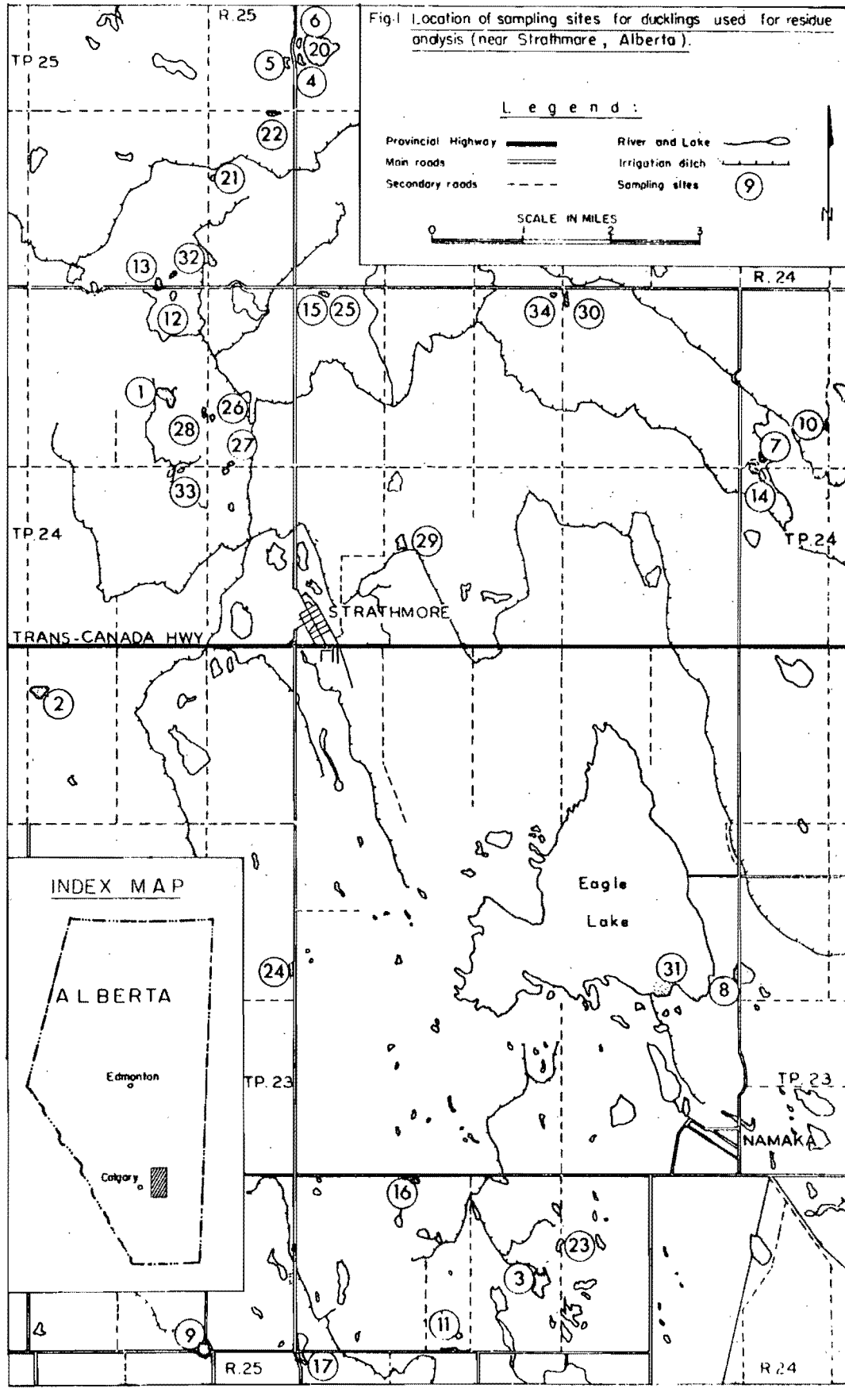


Fig.1 Location of sampling sites for ducklings used for residue analysis (near Strathmore, Alberta).

relation to the town of Strathmore, some of the main side roads, and the network of irrigation canals.

The area is dominated by mixed farming, with a substantial portion north of the townsite classed as unusable scrub pasture land. The nature of the water drainage facilitates movement of insecticides to ponds and sloughs where the persistent insecticide is available to the aquatic biota and the residues of these chemicals may accumulate.

Physical characteristics of the sampling areas differed to such a large extent that no comparison of the average residue content of ducklings sampled on a pond to the geographic location of the ponds could be made. Pond size varied, water depth varied and the marginal vegetation varied both in quantity and type.

Most important, the sites drained water from sprayed and/or unsprayed land. Also, at high water level during the spring and with the use of irrigation canals, some ponds were interconnected and consequently accumulated the drainage from land areas outside the test area.

## 1.2. Insecticide Use in the Study Area

The Strathmore area has a history of heavy grasshopper outbreaks (Province of Alberta 1961, 1962, and 1963), and dieldrin has been used extensively to control these insects.

After searching through the County and Provincial Government records, a summary (Table 1) was prepared of the amount of government - subsidized insecticides sold to farms in the County of Wheatlands No. 16. On the same table, the concentration of the insecticide and its recommended rate of application is shown.

The rather extensive and heavy use of dieldrin (12,585 gallons purchased in the entire Wheatland County No. 16 from 1960 to 1964) is evident.

Small sales in 1964 were attributed to a small grasshopper population due to unfavourable weather conditions for grasshopper development in 1963, rains at hatching time in 1964 and an increase in natural enemies. This was the first time since 1957 that the grasshopper infestation in Southern Alberta showed a decrease; dropping to 80% of the 1963 infestation area (Province of Alberta, 1964). The infestation in the study area (the area from which the ducklings were taken) is indicated by the amount of dieldrin purchased from 1962 to 1964; 261, 295 and 15 gallons respectively.

DDT usage, on the other hand, was limited to a 3% powder in home gardens on potatoe plants (no figures available). Local officials could not recall any large-scale DDT use in past years.

The above outline is not completely accurate, but, under the circumstances, it is the best available. The tracing of the insecticide history was complicated by the following factors:

1. Spray operators buy dieldrin and sign the declaration* for their own property and not for the property where it is to be applied, thus the areas treated are not recorded.
2. The possibility of contamination or decontamination by the irrigation canals bringing in and carrying away insecticides. These canals, apparently, at high water connect with several of the ponds which were sampled.
3. The lack of farmer co-operation for spray information, possibly due to fear of legal repercussions.
4. Confusion as to what portions of the property were sprayed

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*A document which must be read, computed and signed by each person purchasing chemicals from the municipalities agents. It is intended to make farmers aware of proper use and prevent food and feed contamination (Gurba, personal communication).

with a particular chemical due to the use of many pesticide mixtures (example, 2,4-D with dieldrin).

Table 1. Quantities of Insecticide Sold (1960 to 1964)[1] to the County of Wheatlands No. 16 by the Government of the Province of Alberta, and the Recommended Application Rate.

| Year | Dieldrin (gal.)[2] | Dimethoate (gal.)[3] | Malathion (gal.)[4] |
|---|---|---|---|
| 1960 | 425 | - | - |
| 1961 | 2670 | - | - |
| 1962 | 4425 | - | 1395 |
| 1963 | 4265 | 1575 | 700 |
| 1964 | 800 | 560 | 100 |

1 Data summarized from the records of insecticide sales for the County of Wheatlands No. 16.

2 2 pounds dieldrin per gallon, applied at 1 to 2 ounces technical per acre.

3 4 pounds dimethoate per gallon, applied at 3 to 4 ounces technical per acre.

4 10 pounds malathion per gallon, applied at 12 to 16 ounces technical per acre.

## 1.3. Collection and Identification of Ducklings

Ducklings used in this study were originally collected for a study of waterfowl food and cover requirements. The birds were shot, weighed, sexed, aged, labelled, and the crops and sometimes gastro-intestinal tract

removed by Mr. Sugden and his assistant. Measurements of sex, age and weight are presented (Tables 6, 7, 8, 9 and 10) together with the results of the residue analyses of the specific bird. Within 24 hours of collection, the ducklings to be used for residue analyses were frozen, a state in which they remained until 24 hours before analysis time.

Ninety ducklings of seven species were used in this study. The species were: the baldpate (American Widgeon), Mareca americana; the gadwall, Anas strepera; the pintail, A. acuta; the blue-winged teal, A. discors; the mallard, A. platyrhynchos; the lesser scaup, Aythya affinis; and the shoveller, Spatula clypeata. Of these, fifty-eight birds (13 baldpates, 14 gadwalls, 16 pintails, 3 blue-winged teals, 8 scaup, 1 mallard and 3 shovellers) were chosen by the author at random for analysis, and the remaining thirty-two (consisting of 6 baldpates, 9 gadwalls, 8 pintails, 7 scaup and 2 blue-winged teal) were sent to the Ontario Research Foundation for analysis.

## 1.4. Duckling Age Classification

The ducklings were aged according to weight and plumage development as described by Gollop and Marshall (1954). Later, when growth rate data were available, more precise ages were designated by Mr. Sugden on the basis of measurements and weights. Table 2 gives the approximate age span (in days) and the midpoint for each age subclass for the pintail, gadwall, baldpate and lesser scaup. These midpoint ages were used to calculate the hatching dates (section 6.6.) of the ducklings analysed in this study.

Table 2. Approximate age span (in days) together with midpoint age, for four species of ducks for each plumage subclass (modified from Gollop and Marshall, 1954).

| Age Class | Age Span (in days) | | | |
|---|---|---|---|---|
| | Pintail | Gadwall | Baldpate | Lesser Scaup |
| Ia | 1 - 5 (3.0) | 1 - 6 (4.0) | 1 - 7 (4.0) | 1 - 6 (3.5) |
| Ib | 6 - 12 (9.0) | 7 - 14 (10.5) | 8 - 12 (10.0) | 7 - 13 (10.0) |
| Ic | 13 - 18 (15.5) | 15 - 18 (16.5) | 13 - 18 (15.5) | 14 - 20 (17.0) |
| IIa | 19 - 23 (21.0) | 19 - 27 (23.0) | 19 - 26 (22.5) | 21 - 28 (24.5) |
| IIb | 24 - 33 (28.5) | 28 - 38 (33.0) | 27 - 35 (33.0) | 29 - 33 (31.0) |
| IIc | 33 - 43 (38.0) | 39 - 44 (41.5) | 36 - 41 (38.5) | 34 - 42 (38.0) |
| III | 44 - 51 (47.5) | 45 - 50 (47.5) | 42 - 50 (46.0) | 43 - 50 (46.5) |
| Flying | 46 - 57 (51.0) | 48 - 52 (50.0) | $47^{+}$ | $47^{+}$ |

## 2. TECHNIQUES AVAILABLE FOR RESIDUE DETERMINATIONS

Since the introduction of DDT in 1942, there has been an increase in concern over public health and pesticide residues. Analytical methods being used before the introduction of DDT incorporated titrimetric, gravimetric, and colour-comparison techniques. With the knowledge that chlorinated hydrocarbon insecticides persisted, penetrated food commodities, and were hazards even with the ingestion of small quantities (Gunther and Blinn, 1955), a refinement of analytical perspectives and techniques was effected. It is these techniques for the detection of chlorinated hydrocarbon insecticides that will be discussed here.

Pesticide analysis by the determination of organically-bound chlorine can be carried out by the Volhard titration procedure (Shell Method Series, 343 and 676); oxygen flask method developed by Schöniger (1955 and 1956) and reviewed by Schöniger (1960) and later modified by Lisk (1960) and St. John and Lisk (1961); combustion - titration method described by Gunther and Blinn (1955); and the neutron activation analysis method described by Guinn and Wagner (1960). It is more satisfactory, however, to analyse for the specific compound as is done by the Schechter-Haller colorimetric test (Schechter et al., 1945) or for the individual compounds of a particular group (example, chlorinated hydrocarbons).

Methods of measurement of specific compounds have been developed by combining some of the following techniques: infrared, ultraviolet fluorescent, phosphorescent, nuclear magnetic resonance and electron spin resonance spectrophotometry, as well as with column, gas, ion-exchange, paper and thin-layer chromatography.

Regardless of the situation, analysis can be broken into three sections (Middlelem, 1963; Zweig, 1963). These sections, processing and

extraction, cleanup, and analysis, are discussed separately in sections 2.1, 2.2. and 2.3.

## 2.1. Processing and Extraction

Processing and extraction is a "stripping" procedure (Mills, 1959) using solvents (benzene, chloroform, hexane, acetonitrile). Zweig (1963) found that this was most easily done by tumbling the sample in one gallon cans fitted with stainless-steel baffles. Bann (1957) used a large metal container and drill press modified to hold the container and stirring assembly. However, "exhaustive" extraction can also be carried out using the soxhlet extractor. The solvent containing the residue can now be condensed to a smaller volume, by Kuderna-Danish apparatus, or by a Rinco (flash) evaporator. This step is not essential and has not been used in the methods outlined in section 4.5.. However, if the time and equipment is available it is strongly recommended, because larger samples can be extracted, increasing the sensitivity of the analyses.

## 2.2. Cleanup Procedures

The samples from the above procedure usually contain fats, waxes or other non-polar substances. Therefore, some type of further cleanup is required.

Solvent partition is a method of physical separation whereby a pesticide has a solubility preference for one of a pair of solvents while the extractive biological interferences have an affinity for the other solvent. The solvents should be immissible. This procedure is used in the separation of fats and waxes from pesticides as shown by Jones and Riddick (1952), Johnson (1962), De Faubert Maunder et al. (1964), and

Moffitt (1963).

Freezing or crystallization of fats and waxes is another physical method of separation. The use of freezing by utilizing low temperature baths has been applied by Gunther and Blinn (1953) in the separation of DDT from avocado oil, by McKinley, Savory and Webster (1961), and also by McCully and McKinley (1964a, 1964b) for cleanup prior to analysis for twelve of the common chlorinated pesticides in a variety of fats and oils. Chemical removal of interference through oxidation (Gunther and Blinn, 1955), saponification (Eidelmann, 1963; Mills, 1959; Prickett, Kunze and Laug, 1950), and hydrolysis (Davidow, 1950; Hoskins and Messenger, 1950; and Hornstein, 1955), without detrimental effect on some compounds are possible. Unfortunately, not all insecticides are stable in these methods, thus, reducing the value of the methods for general cleanup.

Column chromatography is probably the most widely used and readily-adapted cleanup technique. However, the empirical nature of the adsorbant's action with pesticides and interfering substances must be determined by experimentation (Zweig, 1963). The more common adsorbants used are alumina (De Faubert Maunder et al. 1964), charcoal (Rosen and Middleton, 1959), diatomaceous earth (Moats, 1964, Shell Method Series 596/58), Florisil (Moddes, 1961) and ion-exchange resins (Cueto, Barnes and Mattson, 1956; Plapp and Casida, 1958). These materials are also excellent for adsorbing plant pigments, found in extracts of mud and plants.

Paper and gas chromatography have been used for cleanup but with more recent procedures and more sensitive instrumentation, these methods are not as adaptable and have relatively poor recovery.

2.3. Analytical Measurement

If there is a large number of experimental samples to be determined it is desirable to subject samples to a screening technique so as to eliminate non-contaminated samples. Contaminated samples can then be subjected to more specific and sensitive methods. Phillips (1963) reviewed various techniques, the most sensitive being bioassay. Further bioassay techniques are discussed by Sun (1963), Sun et al. (1963), Earle, Pankaskie and Sun (1959), Jensen and Gauffin (1964), Wheatley, Wright and Hardman (1960) and McDonald (1962).

Colorimetry utilizes relatively low cost equipment; however, as commonly applied, it results in the loss of several advantages inherent in other spectrophotometric measurements.

The advantages of spectrophotometry are: specificity of measurements, positive identification of unknown compounds; ability to calculate the concentration of the material in solution since the degree of absorption is proportional to the concentration (Blinn and Gunther, 1963).

The ultimate purpose of spectrophotometry in residue assay is to make a qualitative final identification of a particular chemical. However, it is essential that practically complete isolation of the insecticide from interfering materials in the sample be achieved.

Gunther and Blinn (1955) and Gunther (1959, 1961, 1962) discussed the theoretical and practical aspects of preanalysis cleanup.

For a complete discussion of spectrophotometry consult Ewing (1960), Friedel and Orchin (1951), Harley and Wiberley (1954), Miller (1953), Strouts, Gilfillan and Wilson (1955) and West (1946, 1956). In addition, for infrared spectrophotometry Bellamy (1958), Duncan (1956b), Jones and Sandorfy (1956), Pinder (1961), Newman (1964), Blinn and Gunther (1962 and

1963) should be consulted for reference to pesticide infrared spectra and for metabolic studies of residues employing infrared spectrophotometry. For details of ultraviolet spectrophotometry, one should also consult Duncan (1956a), Matsen (1956) and Pinder (1961).

The gas chromatograph, however, is a most important instrument in a residue laboratory. The functional parts of the instrument are the column (which is often packed with a solid support onto which the liquid may or may not be deposited), the detector, the oven, the electrometer and the recorder.

"Gas chromatography is a process by which a mixture is separated into its constituents by a moving gas phase passing over a sorbent". (Nogare and Juvet, 1962). The sample containing the insecticide to be determined is injected into the column and is carried along the column by a constant flowing inert gas. The components move through the column at rates dependent on the respective volatilities and interaction with the non-volatile liquid phase. Samples are eluted and detected in the inverse order of their solubilities on the liquid phase.

The versatility of gas chromatography is founded upon the fact that there are different detectors, different columns and many different combinations of these. In addition a gas chromatograph may be coupled to a spectrophotometer thus providing the analyst with a check system by qualitative determinations. With these combinations, the analyst can detect insecticides with numerous different functional groups. Of the many detectors available, (thermal conductivity, flame, flame ionization, argon triode, cross section, ultrasonic, sodium thermionic, electron capture (electron affinity) and microcoulometric), the last two are most frequently used for detection of chlorinated hydrocarbons.

Selectivity is improved by better component resolution utilizing different column packings and more sensitive detectors. The electron capture detector has a lower limit of detection of $10^{-13}$ g for lindane (Clark, 1961). Bonelli, Hartman and Dimick (1963) give the sensitivities and minimum detectable quantity (pg) of selected insecticides using a Wilkens model A-680 Pestilyzer with Electrometer setting at 1X attenuation. These are shown in Table 3.

Table 3. Sensitivity and Minimum detectable quantities (pg) for chlorinated hydrocarbon insecticides.[1]

| Compound | Sensitivity[2] | Minimum Detectable Quantity (pg)[3] |
|---|---|---|
| Aldrin | 2060 | 0.4 |
| DDD (TDE) | 540 | 4 |
| p, p' DDE | 1030 | 1 |
| p, p' DDT | 770 | 13 |
| o, p' DDT | 800 | 7 |
| Dieldrin | 1600 | 1 |
| Endrin | 125 | 70 |
| Heptachlor | 1010 | 0.9 |
| Heptachlor Epoxide | 1600 | 0.9 |
| Lindane | 1400 | 0.1 |

1. Rearranged into alphabetical order from Bonelli, Hartmann and Dimick, 1963.

2. D.U./µgm x 100,000 using Wilkens Model A-680 Pestilyzer, with the Electrometer at 1-X attenuation. Disc Unit (D.U.) = 1/100 of a full Disc Integrator pen stroke using a 60 RPM motor.

3. 1 picogram (pg) = $1 \times 10^{-12}$ grams.

## 3. CHEMISTRY OF DDT AND DIELDRIN

There are different forms (stereoisomers and metabolites) of DDT which could be involved in this residue study, each having different chemical (reaction times, etc.), physical (melting points), and biological (toxicity, etc.) properties. It is only necessary to present a general picture of those forms most pertinent to the duckling analyses.

DDT, one of the most widely known and used insecticides, is described and depicted in Table 4. It has only three commonly identified isomers, called p-p' DDT, o-p' DDT and o-o' DDT. We are concerned mostly with the p-p' DDT isomer which makes up 70% of the commercial compound.

DDT is not attacked by acids or alkaline permanganate, or by aqueous acids or alkalies. However, it is readily dehydrochlorinated when in solution by alkali or organic bases (Martin, 1963). Its degradation in mud was reported by Jones and Moyle (1963); in fish by Bridges, Kallman and Andrews (1963).

The degradation of DDT results in the formation of two common fat soluble products, DDD and DDE (shown on Table 4 below) as well as water soluble DDA, (diphenyl dichloroacetic acid, reported by White and Sweeny, 1945). In addition to these, Peterson and Robinson (1963) found four other DDT metabolites. These were isolated and identified as: 1 - chloro - 2, 2 - bis (p - chlorophenyl) ethylene (DDMU), 1 - chloro - 2, 2 - bis (p - chlorophenyl) ethane (DDMS), unsymmetrical bis (p - chlorophenyl) ethylene (DDNU) and 2, 2 - bis (p - chlorophenyl) ethanol (DDOH).

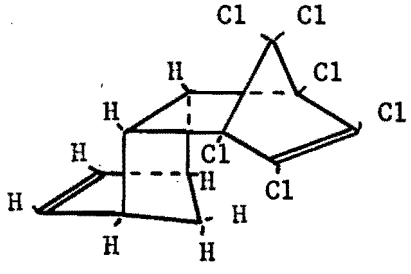
In Canada, aldrin is the approved name of the pure compound listed in Table 4. In the United States and Great Britain, aldrin is the approved name for a material containing not less than 95% of the pure form listed; this pure compound is also known as HHDN (hexachloro-hexahydro-dimethano-naphthalene).

Dieldrin is the approved name in Canada for the pure compound

given in Table 4. In the United States and Great Britain, this refers to a material containing not less than 85% of the pure form listed on Table 4. As with aldrin, the pure form of dieldrin can also be abbreviated as HEOD (hexachloro - epoxy - octahydro - dimethanonaphthalene).

For this study, the Canadian terminology will be used.

The planar form shown as aldrin in Table 4 has in fact four stereoisomers. Aldrin is the endo - exo structure represented below (Martin, 1963):



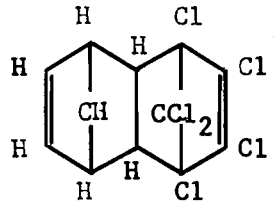
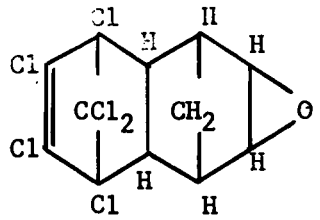
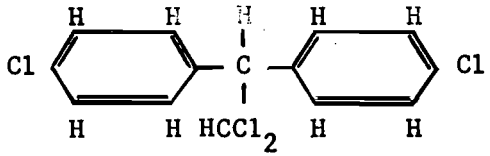
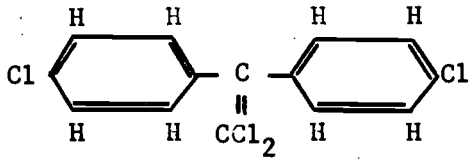
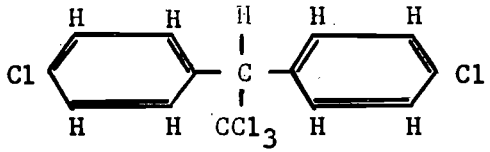

ALDRIN

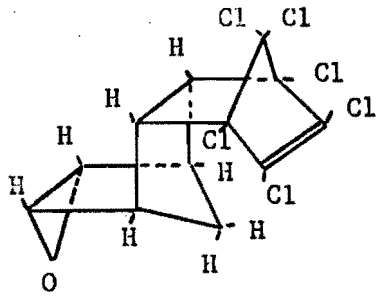
Although stable to alkali and to mild acids, oxidising agents and strong acids attack the unchlorinated ring to form the epoxide form, dieldrin.

The conversion of aldrin to dieldrin in animal tissues was shown by Bann, DeCino, Earle and Sun, 1956; and has been demonstrated to occur in soils (Edwards, Beck and Lichtenstein, 1957; Gannon and Bigger, 1958; Bollen, Roberts and Morrison, 1958; Lichtenstein and Schlulz, 1959a), as well as in plant tissue after aldrin adsorption through the root (Lichtenstein and Schlulz, 1960). The oxidation process is less in muck soils than wet-nonautoclaved Carrington loam; less in soils containing a lower number of micro-organisms; and less in dry soils. (Lichtenstein and Schulz, 1960).

The planar form given in Table 4 as dieldrin has eight stereoisomers. The insecticide dieldrin is the exo-epoxide of the endo - exo - isomer (Aldrin).

Table 4. Common and Accepted Names; Structural and General Formulae of Aldrin, Dieldrin, DDT, DDD and DDE

| Common Name | General Formula | Structural Formula | Accepted Chemical Name |
|---|---|---|---|
| Aldrin | $C_{12}H_8Cl_6$ |  | 1, 2, 3, 4, 10, 10 - Hexachloro -1, 4, 4a, - 5, 8, 8a -hexahydro - endo - 1, 4 - exo - 5, 8 - dimethano - naphthalene. |
| Dieldrin | $C_{12}H_8Cl_6O$ |  | 1, 2, 3, 4, 10, 10 - hexachloro - exo - 6, 7, - epoxy - 1, 4, 4a, 5, 6, 7, 8, 8a - octahydro - 1, 4 - endo, exo - 5, 8 dimethano - naphthalene. |
| DDD | $C_{14}H_{10}Cl_4$ |  | 2, 2 - bis (p - chlorophenyl) - 1, 1 - dichloroethane. |
| DDE | $C_{14}H_8Cl_4$ |  | 2, 2 - bis (p - chlorophenyl) - 1, 1 - dichloroethene. |
| DDT | $C_{14}H_9Cl_5$ |  | 2, 2 - bis (p - chlorophenyl) - 1, 1, 1 - trichloroethane. |




DIELDRIN

This compound is stable to alkali, mild acids and to ultra-violet light; the epoxide group is unusually stable. Lichtenstein and Schulz (1959b) suggest that residue decay is approximately exponential, over a number of years, and that the half-life of dieldrin in soils is "more than four years" in the United Kingdom.

# 4. METHOD OF RESIDUE ANALYSIS

The methods and equipment discussed in this section are the result of many discussions and much searching and letter writing. Their selection has been made on the basis of ease of operation, sensitivity, availability of equipment and financial restrictions.

## 4.1. Materials

### 4.1.1. Reagents

It was necessary that all the liquid reagents (except those purchased as spectranalyzed) be redistilled in a glass still.

The following reagents were used:

Column packing for gas chromatograph. 6% Q.F. - 1 and 4% SE - 30 mixed silicones on 60/80 mesh-acid-washed Chromosorb W. (Wilkens Instrument and Research Incorporated).

Dimethylformamide (Fisher, D-19)

Dimethylformamide saturated with hexane (DMF reagent)

Florisil ® (Fisher F-100)

n-hexane

1. reagent grade (Fisher, H-291)
2. spectranalyzed (Fisher H-334)

Hexane saturated with dimethylformamide

Methanol - spectranalyzed (Fisher A-408)

Methylene chloride (Fisher, D-37)

Methylene chloride: Petroleum ether mixture (1:5 v/v)

Pesticides - E.S.A. standards in n-hexane

Petroleum ether (Fisher, E-139)

4.1.2. Apparatus

The following items were used in this study:

Analytical Columns - for gas chromatograph. Pyrex glass columns 5 feet long, 1/8 inch diameter in a 2 1/2 inch helix (Wilkens Instrument and Research Inc., part number 11-005)

Balances

Column chromatograph equipment

Gas Chromatograph - Wilkens Model 680-A Pestilyzer with built-in 3" strip chart recorder.

Hamilton 10 ul syringes (Chromatographic Specialities Ltd., model 701 NCH)

Purging oven - made by author (refer to section 4.4)

Recorder - Westronics dual pen

Ultrasonic cleaner (Wilkens Instrument and Research Inc., model 9650)

Variac Transformer - for gas chromatograph and purging oven

Voltage regulator for gas chromatograph

## 4.2. Modifications Made to the Gas Chromatograph

A stable base line could not be achieved and only after much experimentation was it found that the thermostatic control was not sensitive enough, resulting in oven temperature fluctuations. The heat sensitive detector, mounted in the cast aluminum oven, responded by a fluctuation in standing current and therefore caused the oscillating base line. The problem was corrected by disconnecting the thermostat and controlling the heat with a constant voltage regulator and a variac transformer.

In addition, the recorder supplied with the pestilyzer did not produce accurate tracings, and therefore the chromatograph was attached to

a Westronics recorder (with a 12 inch span), as described in Figure 2.

## 4.3. Operation of Gas Chromatograph

The Wilkins Pestilyzer used in this study is an excellent routine gas chromatograph (after modifications) because of the small number of variable factors. There are controls for oven temperature and electrometer attenuation only. The oven temperature was maintained at approximately 180°C. Because of the location of the heating cartridges, however, the detector and injection port temperature was actually 5°C above the oven temperature. At this temperature the investigator is assured of complete vaporization of insecticide in the injection port and no condensation on the detector. In addition, the injection port was lined with a pyrex insert tube, which prevented decomposition of insecticides as reported by Cassil (1961).

The oven, the electrometer, and the nitrogen carrier were never turned off. Once in operation, the nitrogen carrier flow was maintained at approximately 40 ml/min., while the electrometer attenuation was set at 8. Only when a very low concentration was suspected, was the attenuation changed; to x 4, a high sensitivity setting. If a sample contained a large amount of residue, instead of increasing sensitivity by attenuating up, the sample was diluted until the peak height did not exceed three quarters of the recorder scale (i.e. 30% standing current).

## 4.4. Sample Injection

Samples were injected with a Hamilton Syringe equipped with a Chaney Adaptor, which provides a simple means of making repetitive injections of constant size and prevents twisting and bending of the

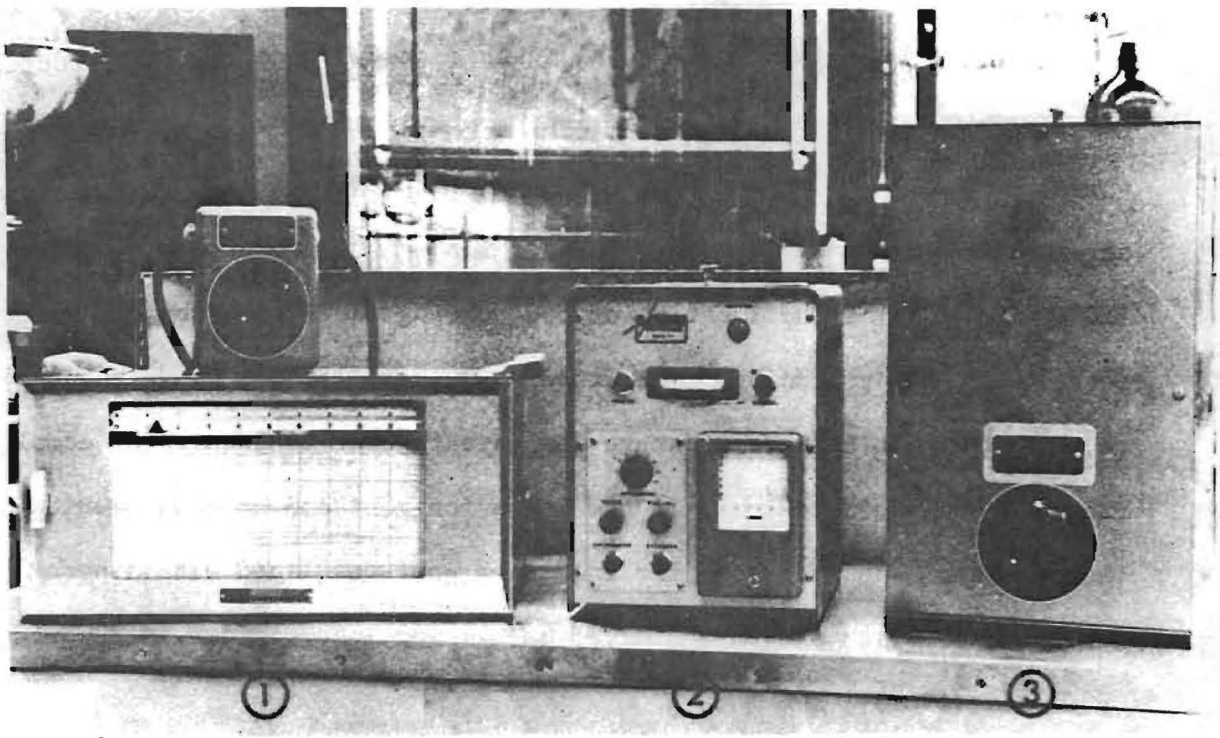



Figure 2. Gas chromatograph, purging oven and recorder used for insecticide analysis of ducklings.

1. Recorder
2. Gas Chromatograph
3. Purging Oven

delicate plunger. Usually 10 µl were injected, although at times it was found more convenient to use 1, 2 or 5 µl.

Reproducible results could be achieved only by leaving the injection needle in the injection port for 10 seconds (a count of ten). This gave sufficient time for the high injector temperature to completely evaporate the solvent and the sample in it. Because this needle volume contributes materially to the analysis it was necessary to calculate the volume of sample in the needle. The calculation involved drawing a sample up the syringe and expelling it in the normal fashion. When the plunger was drawn back, the liquid in the needle was carried with it, and could be measured using the calibrations on the syringe barrel.

The actual volumes given in Table 5 were used in the calculation (section 5.2.) of the amount of insecticide in tissue samples.

Table 5. Theoretical and actual volumes of Chromatograph injection sample, using hamilton Syringe with a Chaney Adaptor.

| Theoretical Volume ul. | Actual Volume ul. |
|---|---|
| 1 | 1.91 |
| 2 | 2.91 |
| 5 | 5.91 |
| 10 | 11.77 |

4.5. Purging Oven

Newly packed columns for the gas chromatograph require purging for at least 48 hours and preferably for 96 hours at 225°C. This treatment removes volatiles which otherwise might contaminate the detector.

During this time the gas chromatograph cannot be used for insecticide detection.

In view of this, I designed and helped build a "purging oven" (Figure 2) which has a cast aluminum oven with a controlled (variac autotransformer) heat source, and a glass thermometer. To avoid duplication of nitrogen gas tanks and valves, the purging oven was connected to the main gas chromatograph system (Figure 3) and a small nitrogen control valve mounted on its side.

## 4.6. Methods of Sample Clean-up and Analysis

Much time was spent investigating various procedures for sample clean-up, preparatory for gas chromatography. The Langlois procedure (discussed in 4.6.1.) was chosen because of its simplicity and good recovery. This method, however, could be applied only to the duckling muscle and fat. The preen gland appeared to either overload the column or contain waxes or other materials which would not adsorb onto the Florisil column. Therefore, other procedures and formulations were attempted. The recently developed procedure by De Faubert Maunder et al. (1964) was found most reliable and reproducible (see 4.6.2.).

The Ontario Research Foundation staff used an entirely different procedure (Section 4.6.3.) for their analyses. However, if their freezing procedure (essentially that of McCully and McKinley, 1964a and 1964b) did not clean up the sample they reverted to a florisil column for final clean-up similar in principle to that in 4.6.1.

### 4.6.1. Fat and Muscle Clean-up and Analysis

After reviewing methods of insecticide analysis, a method

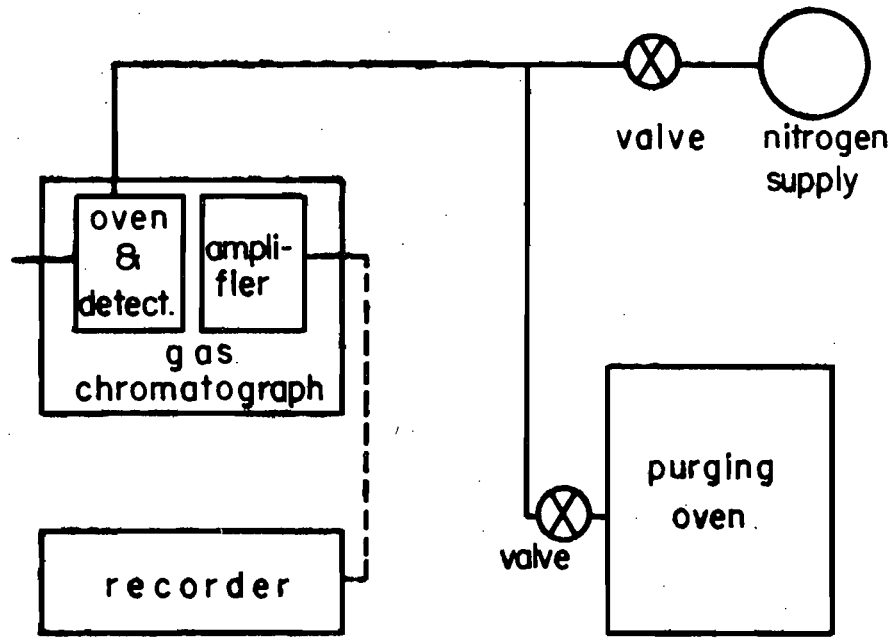



Fig. 3. Schematic diagram illustrating connections for the gas chromatograph and the purging oven.

originally reported by Moats (1963) and adapted by Langlois, Stemp and Liska (1963a) and later extended by Langlois, Stemp and Liska (1964), and Stemp, Langlois and Liska (1964), was selected, since it was found efficient and requiring a minimum of equipment. These investigators used an analytical column packing (either a 2.5 or 5% Dow 11 Silicone on 60/80 mesh hexamethyl-disilizane (HMDS) treated Chromosorb W) which could not distinguish between p, p' - DDE and dieldrin. They, therefore, found it necessary and practical to split the eluate from the column cleanup; collecting the first 300 ml which contained the DDE separate from the last 300 ml which contained the dieldrin. In this study, it was found that this method was not effective, and therefore required modification.

A new analytical column packing, a mixture of 6% QF-1 and 4% SE-30 mixed silicone on 60/80 mesh acid-washed Chromosorb W, allowed the separation of the DDE and dieldrin peaks (McCully and McKinley, 1964b). With this column packing the following method was adopted: 25 grams of deactivated florisil (5% water was mixed with the activated florisil and the mixture held in an air tight container for 48 hours) was added to a 20 mm I.D. x 600 mm pyrex glass chromatographic column with a glass wool plug at the base. This florisil was washed with a 50:50 mixture of methylene chloride and petroleum ether. The sample (1 gm muscle or fat tissue) was ground with 30 gm deactivated florisil until a free flowing powder was obtained. This was added to the prewashed column and eluted with 700 ml of a 20% methylene chloride in petroleum ether. The eluate was collected in a 1000 ml pyrex beaker and evaporated to dryness at 55°C.

Ten milliliters of spectroanalyzed n-hexane was added to the

final extract and a 10 µl aliquot of the resulting solution was injected into the gas chromatograph.

A control extraction (using DDT, DDD, DDE, and dieldrin standards) was performed along with each set of sample extractions.

4.6.2. Preen Gland Clean-up and Analysis

Only one of the bilobed portions of the gland was analyzed at a time - the other was used as a replicate. These lobes were treated in a manner similar to the muscle and were cleaned up using the florisil chromatographic column technique. Evaporation to dryness revealed large amounts of materials which would not permit injection into the gas chromatograph.

The sample was then dissolved in n-hexane and transferred to a separatory funnel. (From this point a procedure outlined by De Faubert Maunder et al. (1964) was followed). The sample solution was extracted with 10 ml of dimethylformamide saturated with hexane (DMF reagent). The mixture was set aside for 2 to 3 minutes after which the clear DMF phase was run into a 125 ml separatory funnel, retaining any interfacial emulsion in the first separatory funnel. The extraction of the sample solution was repeated with two further 10 ml of DMF reagent. The DMF extracts were combined and washed with 10 ml of n-hexane saturated with DMF, to remove any traces of fat. This 10 ml of hexane was separated and was washed with a further 10 ml of DMF which was added to the previous 3 combined DMF extracts in a 250 ml separatory funnel. These extracts were shaken, briskly, with 200 ml of 2 percent aqueous sodium sulphate solution for 2 minutes. After a 20 minute settling period, the hexane previously held in the DMF reagent separated. The aqueous layer (DMF, sodium sulphate and water) was run out to waste, and the stem of the separatory funnel was dried. The remaining hexane

layer and subsequent rinsings were run into a 10 ml graduated cylinder.

This hexane extract was then treated using the Langlois procedure (see section 4.6.1.), to insure that no contaminants were present which would reduce the sensitivity of the analytical column.

An alternate procedure was to grind up the preen gland sample with 50 ml of hexane for 2 minutes, let it stand for 10 minutes, decant, and repeat 3 times. Evaporate the combined extracts to a 25 ml volume and continue with the De Faubert Maunder procedure. At the end of the partition chromatography, treatment with an activated-alumina column (De Faubert Maunder, 1964) may be better than the florisil chromatographic column.

4.6.3. Ontario Method of Clean-up and Analysis

The Ontario Research Foundation analysed the fat, muscle and preen glands of thirty ducklings using the following procedure:

The sample was macerated in a Waring blender and then extracted with 100 ml of acetonitrile in the presence of anhydrous sodium sulphate in a Waring blender for 5 minutes. The resulting suspension was centrifuged at 2000 rpm for 15 minutes and the supernatant liquid decanted into a separatory funnel. Water (5 ml), saturated sodium chloride solution (15 ml) and hexane were added and the total shaken for 2 minutes. The hexane fraction was separated and evaporated just to dryness in a rotary flash evaporator at 50 - 60°C. The residue was then taken up in 100 ml of benzene acetone (1:19). This solution was then cooled, with stirring, to -70°C, and then filtered through a carbon Solkafloc pad (2 gms; 10 gms) which had also previously been cooled to -70°C. The filtrate was then

dried by addition of anhydrous sodium sulphate and concentrated to 4 ml. One to four microlitres are injected into the chromatograph for analysis. The column used consists of 2.0% (or 10%) QF-1 on 60 - 80 mesh Chromosorb W.

All solvents were checked for interfering impurities and purchased, when possible, as spectroanalyzed grades. A standard sample of Dieldrin (and DDT) was run after every two samples.

If there was appreciable background in the chromatograph, it was necessary to submit the samples to further clean-up. In this event, the concentrate was passed through a Florisil column (5 gms anhydrous sodium sulphate, 10 gms florisil) previously washed with 100 ml hexane. The sample was eluted with 100 ml hexane and the eluate (100 ml) concentrated to 4 ml. One to four microlitres were chromatographed for final analysis.

# 5. RESULTS

## 5.1. Reference Standards

Figure 4 shows a tracing of the mixed standard (a 1 µl injection) used in this study. The insecticides, lindane, aldrin, heptachlor, heptachlor epoxide, dieldrin, DDD, DDE and DDT, were chosen as representative chlorinated hydrocarbon and as most likely to be found in the ducklings. Each insecticidal peak represents $1 \times 10^{-10}$ g/ml except for DDD ($2 \times 10^{-10}$ g/ml) and DDT ($5 \times 10^{-10}$ g/ml). This standard was injected approximately every two hours during continuous chromatograph operation, to enable the investigator to determine variation in relative retention times of insecticides injected as a single quantitative standard (usually 10 µl injected at a time) or as the mixed standard.

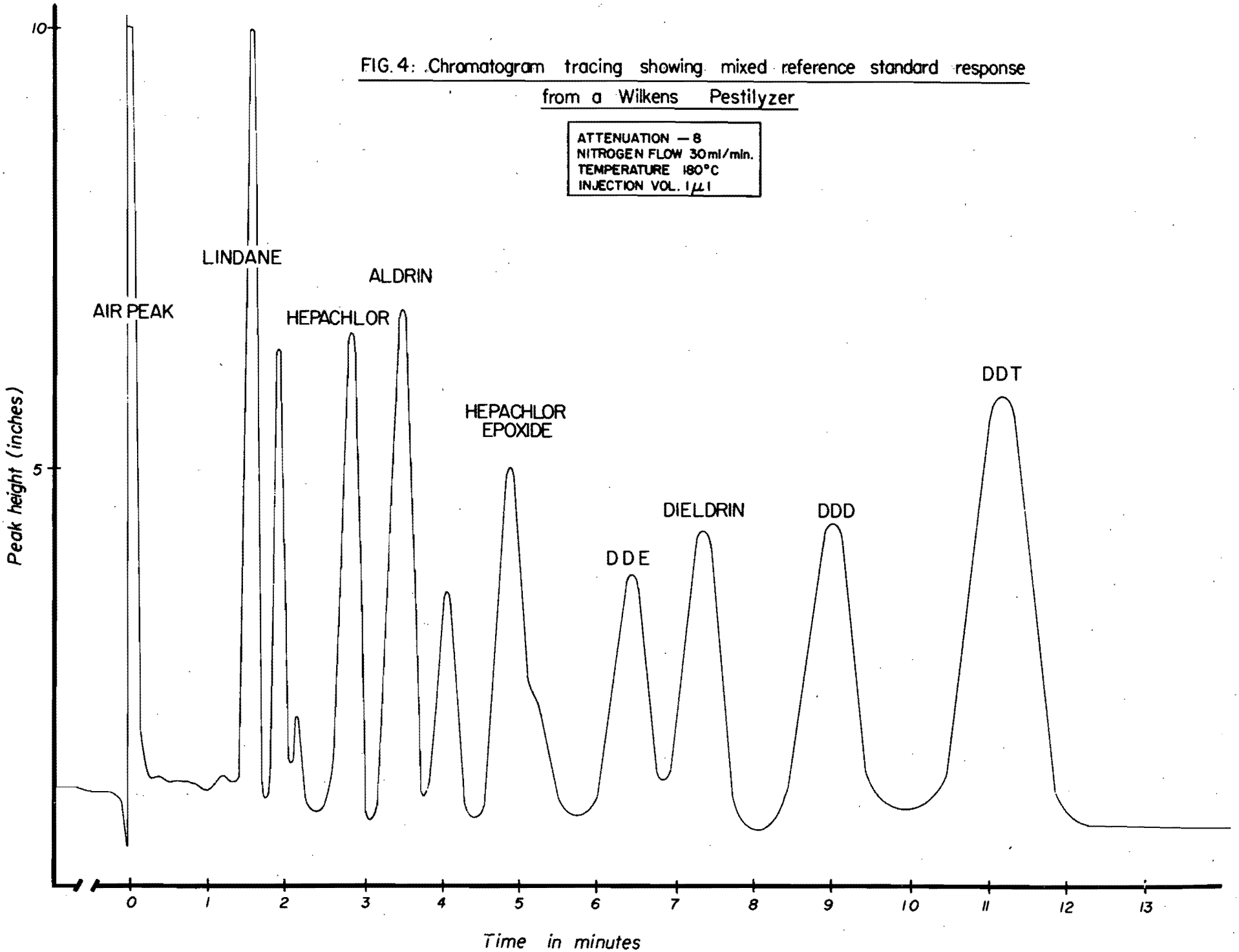
Single insecticide quantitative standards were necessary, even though the mixed standard was quantitative as well as qualitative. The single standards were kept frozen, and only removed from the freezer when it was necessary to fill the syringe for a positive determination whereas the mixed standard remained at room temperature. To maintain the quantitative nature of the mixed standard, a new one was formulated every four weeks. The single standards, on the other hand, were formulated every three months.

Relative retention times were calculated (using aldrin at 1.0) and are presented in Table 12 (section 6.1) where they are compared to those published by Wilkens Instrument and Research, Incorporated (1964). The results of the Wilkens researchers were apparently obtained using a pestilyzer with a pyrex column 5 feet long, 1/8 inch diameter, similar to those used here for analysis.

By comparing the relative retention times of an unknown peak with those

FIG. 4: Chromatogram tracing showing mixed reference standard response from a Wilkens Pestilyzer

ATTENUATION — 8
NITROGEN FLOW 30 ml/min.
TEMPERATURE 180°C
INJECTION VOL. 1 μl



of the standards, one can (see section 6.11) determine which peaks represented insecticides.

## 5.2. Interpretation of Chromatograms

The amount of insecticide is calculated by comparing the area of the unknown peak to a reference standard (where the concentration is known). The area is best calculated in this situation (Scott and Grant 1964) by taking the product of peak height times the width of the peak at half the peak height. Using these areas and the following formula (Jonasson, personal communication), it was possible to find the concentration of the insecticide in ppm:

$$\text{PPM} = \frac{\text{area of unknown peak}}{\text{area of standard peak}} \times \frac{\text{Vol. standard inj. x conc of st'd}}{\text{wt. of sample}} \times \frac{1}{\text{Dilution factor}} \times \frac{1}{\text{vol. of unknown injected}} \times \frac{\text{attenuation of unknown}}{\text{attenuation of standard}}$$

## 5.3. Duckling Analyses

Originally only dieldrin was to be identified. However, by using a mixed reference standard, quantitative determinations for lindane, aldrin, heptachlor, heptachlor epoxide, endrin, dieldrin, DDE, DDD, DDT were carried out. Of these, only DDT and its metabolites DDD and DDE showed any significant values and therefore they are reported here with the dieldrin. Of particular importance was DDE, a dehydrochlorinated metabolite of DDT, which occurred in all but two fat analyses and all but 18 muscle analyses.

Tables 6, 7, 8, 9, and 10 give a complete list of data for each bird investigated. The DDT (total of DDD, DDE and DDT) and dieldrin residues are summarized in Figure 5 according to the levels in fat and muscle, and later in section 6 according to insecticide, tissue, age, weight, and species difference.

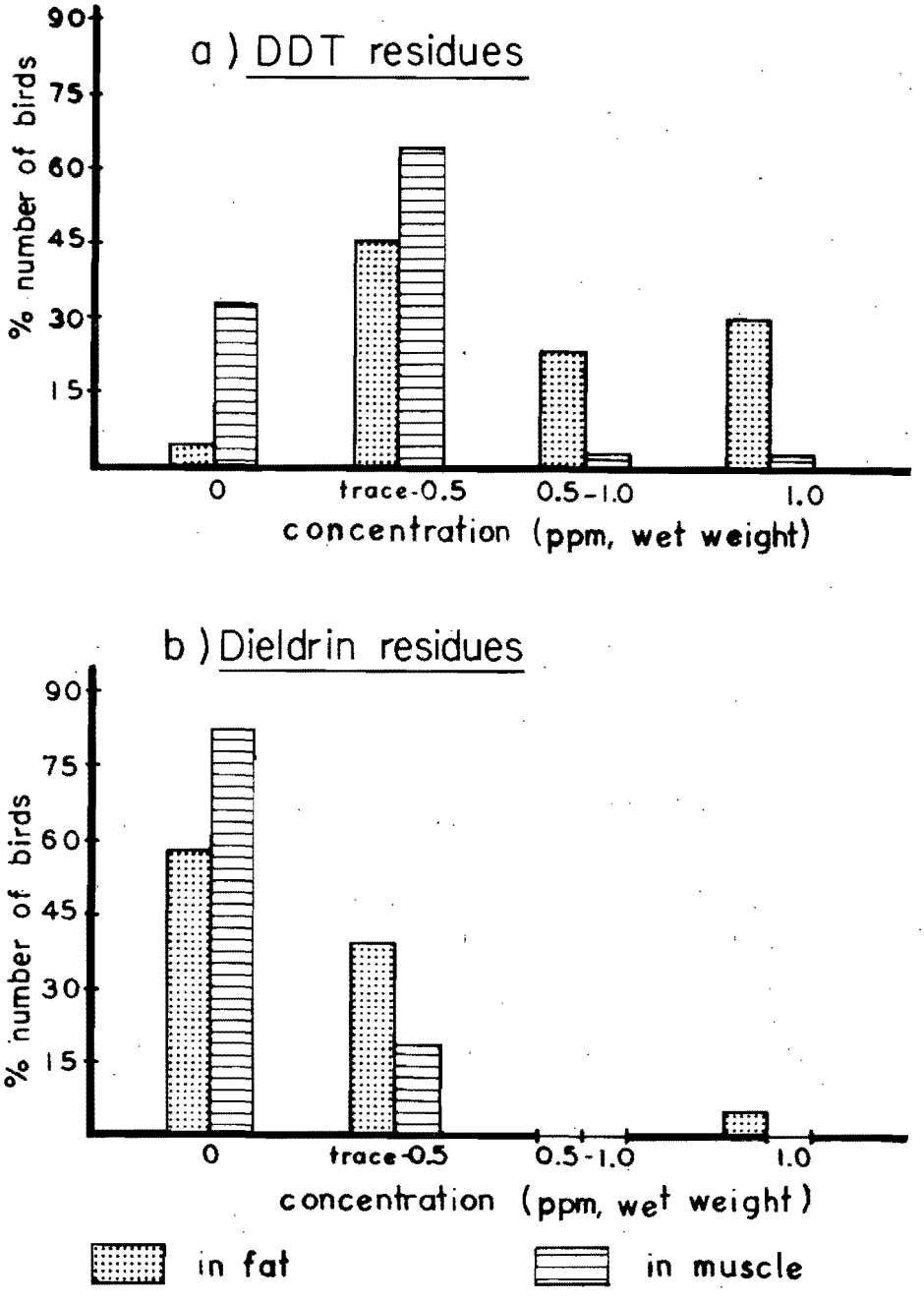



Fig.5. Frequency of DDT and Dieldrin residues in duckling fat and muscle tissue.

The levels of DDT ranged from 0 to 36.48 ppm in fat with only 4% of the birds free of DDT; 44% had a trace to 0.5 ppm; 22% had from 0.5 to 1.0 ppm and 30% had greater than 10 ppm. In the muscle samples, the range was 0 to 0.97 ppm with 32.7% of the birds with no DDT, 63.7% with a trace to 0.5 ppm, 1.8% with 0.5 to 1.0 ppm and 1.8% with greater than 1.0 ppm.

The dieldrin levels, ranging from 0 to 2.62 ppm in the fat and from 0 to 0.11 ppm in the muscle, were much lower than those of DDT. Figure 6 shows that 57.1% of the birds analyzed showed no dieldrin in fat, 38.8% with trace to 0.5 ppm and 4.8% with greater than 1.0 ppm. Dieldrin-free muscle samples occurred in 81.4% of the ducklings while a trace to 0.5 ppm levels occurred in 18.6%.

Table 6. Sampling data and results of analyses for insecticides and their degradation products in Baldpate ducklings from Strathmore, Alberta

| Bird No. | Age | Wt (gm) | Sex | Site | Tissue Sampled | Sample Size (g) | Concentration (ppm, wet weight) DDD | DDE | DDT | Dieldrin |
|---|---|---|---|---|---|---|---|---|---|---|
| 22 | Ib | 82.6 | M | 6 | Muscle | 1.0 | 0.00 | 0.04 | 0.00 | 0.00 |
| 24 | Ib | 79.1 | M | 6 | Fat | 0.1 | 0.00 | 0.04 | 0.00 | 0.02 |
| | | | | | Muscle | 1.0 | 0.00 | trace | 0.00 | 0.00 |
| 27 | Ib | 51.2 | M | 8 | Fat | 0.13 | 0.00 | 1.53 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.07 | trace | trace |
| 28 | Ib | 48.0 | M | 7 | Fat | 0.031 | 0.00 | 0.36 | 0.00 | 0.01 |
| | | | | | Leg | 1.0 | 0.00 | 0.04 | 0.00 | 0.00 |
| 29 | Ib | 52.8 | F | 7 | Fat | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | Leg | 0.68 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21 | Ic | 166.0 | M | 6 | Fat | 0.06 | 0.00 | 0.31 | 0.00 | trace |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 23 | Ic | 179.4 | M | 6 | Fat | 0.19 | 0.00 | 0.01 | 0.00 | trace |
| | | | | | Muscle | 1.0 | 0.00 | trace | 0.00 | trace |
| 30 | IIa | 242.1 | F | 17 | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.01 |
| 31 | IIa | 274.6 | M | 17 | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 32 | IIa | 267.3 | M | 17 | Fat | 0.02 | 0.00 | 0.29 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 33 | IIa | 271.7 | F | 17 | Fat | 0.09 | 0.00 | 0.31 | 0.00 | 0.00 |
| | | | | | Breast | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 37 | IIc | 496.6 | F | 1 | Fat | 0.86 | 0.00 | 0.04 | 0.09 | 0.00 |
| | | | | | Breast | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 35 | III | 590.0 | F | 29 | Fat | 0.71 | 0.00 | 0.04 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.08 | 0.00 |

Table 7. Sampling data and results of analyses for insecticides and their degradation products in Gadwall ducklings from Strathmore, Alberta

| Bird No. | Age | Wt (gm) | Sex | Site | Tissue Sampled | Sample Size (g) | Concentration (ppm, wet weight) DDD | DDE | DDT | Dieldrin |
|---|---|---|---|---|---|---|---|---|---|---|
| 96 | Ic | 132.4 | M | 12 | Fat | 0.30 | 0.00 | 4.31 | 0.35 | 0.28 |
| | | | | | Muscle | 1.0 | 0.00 | 0.12 | 0.00 | 0.00 |
| 97 | Ic | 123.1 | M | 12 | Fat | 0.49 | 0.00 | 18.62 | 0.42 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | 0.19 | trace | 0.00 |
| 98 | Ic | 113.4 | F | 12 | Fat | 0.52 | 0.00 | 36.48 | trace | trace |
| | | | | | Breast | 1.0 | 0.00 | 0.35 | 0.00 | 0.00 |
| 99 | IIa | 141.2 | M | 10 | Fat | 0.51 | 0.00 | 0.48 | 0.10 | 0.05 |
| | | | | | Leg | 1.0 | 0.00 | trace | trace | 0.00 |
| 104 | Ic | 87.0 | M | 2 | Fat | 0.25 | 0.17 | 10.99 | 0.05 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.06 | 0.00 | 0.00 |
| 107 | Ic | 95.3 | M | 2 | Fat | 0.16 | 0.00 | 2.45 | 0.00 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | 0.01 | 0.00 | trace |
| 123 | Ic | 204.0 | M | 31 | Fat | 0.27 | trace | 0.11 | 0.10 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | trace | 0.03 | 0.00 |
| 109 | IIa | 142.8 | M | 12 | Fat | 0.20 | 0.00 | 0.93 | 1.94 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.04 | 0.00 | 0.00 |
| 110 | IIa | 132.0 | M | 12 | Fat | 0.07 | 0.00 | 1.68 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.01 | 0.00 | 0.00 |
| 111 | IIa | 123.1 | M | 12 | Fat | 0.12 | 0.00 | 0.05 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 112 | IIa | 122.9 | F | 12 | Fat | 0.12 | 0.00 | 0.01 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | trace | 0.04 | 0.00 |
| 116 | IIa | 251.3 | F | 12 | Fat | 0.24 | 0.00 | 0.29 | 0.00 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | trace | 0.00 | 0.00 |
| 130 | IIc | 601.0 | F | 32 | Fat | 0.48 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 128 | III | 784.1 | F | 17 | Fat | 0.879 | 0.00 | 0.02 | 0.00 | 0.02 |
| | | | | | Breast | 1.0 | trace | 0.02 | trace | 0.03 |

Table 8. Sampling data and results of analyses for insecticides and their degradation products in Pintail ducklings from Strathmore, Alberta

| Bird No. | Age | Wt (gm) | Sex | Site | Tissue Sampled | Sample Size (g) | Concentration (ppm, wet weight) DDD | DDE | DDT | Dieldrin |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Ia | 46.7 | M | 21 | Fat | 0.07 | 0.00 | 0.25 | 0.51 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.16 | 0.21 | trace |
| 6 | Ic | 70.8 | F | 22 | Fat | 0.15 | 0.00 | 3.64 | 5.34 | 1.42 |
| | | | | | Leg | 1.0 | 0.00 | 0.17 | 0.05 | 0.05 |
| 8 | Ic | 65.3 | M | 22 | Fat | 0.06 | 4.83 | 6.25 | 5.10 | 2.61 |
| | | | | | Leg | 1.0 | 0.06 | 0.24 | 0.12 | 0.11 |
| 14 | Ic | 113.2 | F | 6 | Fat | 0.07 | 0.00 | 19.04 | 8.60 | 0.00 |
| | | | | | Breast | 1.0 | 0.06 | 0.58 | 0.33 | 0.00 |
| 15 | IIa | 244.6 | F | 1 | Fat | 0.54 | 0.10 | 1.66 | 1.75 | 0.03 |
| | | | | | Breast | 1.0 | trace | 0.07 | trace | 0.00 |
| 16 | IIb | 490.6 | M | 8 | Fat | 1.0 | 0.00 | 0.24 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | trace | 0.00 | 0.00 |
| 20 | IIb | 451.6 | F | 8 | Fat | 0.50 | 0.00 | 0.58 | 0.12 | 0.04 |
| 21 | IIb | 422.8 | M | 8 | Fat | 0.67 | 0.00 | 0.05 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | trace |
| 22 | IIb | 429.5 | M | 8 | Fat | 0.65 | 0.04 | 0.68 | 0.11 | 0.08 |
| | | | | | Muscle | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 24 | IIb | 319.3 | F | 6 | Fat | 0.05 | 0.00 | 0.53 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 28 | IIb | 377.6 | M | 9 | Leg | 1.0 | 0.00 | 0.01 | 0.02 | 0.00 |
| 38 | III | 603.7 | F | 25 | Fat* | 0.3* | 0.00 | 0.86 | 0.00 | 0.46* |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 51 | III | 537.0 | M | 2 | Fat | 0.3 | 0.00 | 0.29 | 0.24 | 0.06 |
| | | | | | Breast | 1.0 | 0.03 | 0.01 | 0.06 | trace |
| 53 | III | 608.2 | F | 9A | Fat | 0.24 | 0.00 | 0.07 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | trace | 0.00 | 0.00 |

* estimated amount

Table 9. Sampling data and results of analyses for insecticides and their degradation products in Scaup ducklings from Strathmore, Alberta.

| Bird No. | Age | Wt (gm) | Sex | Site | Tissue Sampled | Sample Size (g) | Concentration (ppm, wet weight) DDD | DDE | DDT | Dieldrin |
|---|---|---|---|---|---|---|---|---|---|---|
| 45 | Ib | 106.4 | F | 28 | Fat | 0.21 | 0.00 | 0.08 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 47 | Ib | 119.3 | F | 28 | Fat | 0.32 | 0.00 | 0.80 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.01 | 0.00 | 0.00 |
| 29 | IIa | 307.0 | F | 31 | Fat | 0.57 | 0.00 | 0.06 | 0.04 | 0.01 |
| | | | | | Leg | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 36 | IIa | 251.7 | F | 29 | Fat | 0.41 | 0.00 | 0.35 | 0.00 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | 0.02 | 0.00 | 0.00 |
| 39 | IIa | 242.0 | M | 29 | Leg | 1.0 | 0.00 | 0.30 | 0.00 | 0.00 |
| 40 | IIa | 252.2 | F | 28 | Fat | 0.5 | 0.00 | 0.69 | trace | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 48 | IIa | 248.9 | M | 28 | Fat | 0.2766 | 0.00 | 3.68 | 0.00 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.02 | 0.00 | 0.00 |
| 1 wk old | Ia | 0 | 0 | 0 | Breast | 0.85 | 0.17 | 0.33 | 0.60 | 0.00 |

Table 10. Sampling data and results of analyses for insecticides and their degradation products in Blue-winged Teal, Mallard and Shoveller ducklings from Strathmore, Alberta.

| Bird No. | Age | Wt (gm) | Sex | Site | Tissue Sampled | Sample Size (g) | Concentration (ppm, wet weight) DDD | DDE | DDT | Dieldrin |
|---|---|---|---|---|---|---|---|---|---|---|
| (a) Blue-winged teal | | | | | | | | | | |
| 25 | Ia | 32.8 | F | 12 | Fat | 0.05 | 0.00 | 1.04 | 0.85 | 0.00 |
| | | | | | Leg | 1.0 | 0.00 | 0.05 | trace | 0.00 |
| 31 | III | 283.0 | F | 28 | Fat | 0.34 | 0.00 | 0.26 | 0.05 | 0.00 |
| | | | | | Breast | 1.0 | 0.00 | 0.01 | 0.02 | 0.00 |
| 30 | Adult | 417.6 | F | 17 | Fat | 0.56 | 0.00 | 0.18 | 0.08 | 0.02 |
| | | | | | Breast | 1.0 | 0.00 | 0.02 | 0.05 | 0.00 |
| (b) Mallard | | | | | | | | | | |
| 5 | IIa | 238.3 | F | 6 | Fat | 0.56 | 0.04 | 0.38 | 0.38 | 0.03 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| (c) Shoveller | | | | | | | | | | |
| 20 | Ic | 96.2 | M | 6 | Fat | 0.0225 | 0.00 | 1.56 | 0.00 | 0.00 |
| 19 | IIa | 237.0 | M | 7 | Fat | 0.4 | 0.00 | 0.47 | 0.07 | 0.06 |
| | | | | | Breast | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 23 | IIb | 274.7 | M | 17 | Fat | 0.42 | 0.00 | 0.02 | 0.10 | 0.07 |
| | | | | | Breast | 1.0 | 0.00 | 0.01 | 0.03 | 0.00 |

# 6. DISCUSSION

## 6.1. Sampling and Sample Size

Fat samples varying in weight from 0.02 grams to 0.88 grams were taken from the subcutaneous region along the thigh; the only easily removed storage fat available. The variation in sample size resulted in a reduction in sensitivity of the experimental procedure for fat analysis.

The muscle samples, were taken from the breast; however, if sufficient breast muscle was not available, leg muscle was taken. For these samples a constant sample size of 1.00 gram was maintained except in one analysis where only 0.85 grams was used. The inexactness of these latter analyses was not realized until the fat content* of the muscle was investigated. The results of such a fat analysis of the muscle of four ducklings (three scaup of age class IIa and one baldpate of age class III) are given in Table 11. The variation of from 3.69 to 17.18% (mean of 8.85%) shown here is further proof of the necessity of expressing insecticide residues in terms of pure fat.

Table 11 implies the need to sample a specific muscle or muscle set. It is conceivable that there would be a variation in the fat composition and amount of fat between different muscle layers.

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* Add an amount of tissue greater than 2 grams to a tared beaker, weigh and place in an oven (approximately 40°C) for 24 hours. Remove, cool in a desicator and re-weigh. Remove the dry tissue from the beaker, grind and add to a soxhlet extractor (using a tared petroleum ether flask) and reflux with 200 ml petroleum ether for 8 hours. Evaporate the petroleum ether to dryness. Re-weigh the flask and calculate the percentage fat content.

Table 11. Percentage fat in breast muscle of four ducklings collected from Strathmore, Alberta.

| Duckling Sampled | | Percentage Fat |
|---|---|---|
| Scaup | 29 | 17.18 |
| Scaup | 36 | 12.98 |
| Scaup | 39 | 5.42 |
| Baldpate | 35 | 3.69 |

Similarly, because the insecticide concentrations are calculated on a wet weight basis, a moisture loss during storage or transit of the sample from the field to storage will give misleading results. Therefore, it would be more satisfactory if residue levels were expressed as parts per million of pure fat. Analyses carried out with this in mind would require very little extra time and equipment. The investigator would be required to extract the fat, calculate the percentage of fat in the muscle, organ or glandular (preen gland) tissue and then analyze the extracted fat for insecticide residues.

## 6.2. Possible Misidentification of Insecticides

Elution time of a compound from an analytical column is constant for a particular set of instrumental conditions. A comparison of elution times (relative retention times), of eight insecticides for two different column packings is shown in Table 12. The packing used by Wilkens Instrument and Research Incorporated (5% Dow 11 Silicone on 60/80 mesh HMDS treated Chromosorb W) is the same packing as was used initially

(see section 4.6.1.),

Table 12. Comparison of insecticide retention times as established in the Alberta laboratory[1], with those published by the Wilkens Instrument and Research, Inc.[2]

| | Wilkens Retention Times | Alberta Retention Times |
|---|---|---|
| Aldrin | 1.00 | 1.00 |
| DDD (TDE) | 2.64 | 2.59 |
| DDE | 1.98 | 1.86 |
| DDT | 3.44 | 3.21 |
| Dieldrin | 2.00 | 2.10 |
| Heptachlor | 0.84 | 0.81 |
| Heptachlor epoxide | 1.29 | 1.40 |
| Lindane | 0.45 | 0.44 |

[1]using 6% QF - 1 and 4% SE - 30 on 60/80 mesh acid-washed chromosorb W.

[2]using 5% Dow 11 Silicone on 60/80 mesh HMDS

in this study, while the second packing (6% QF - 1 and 4% SE - 30 60/80 mesh acid-washed chromosorb W), was used for the major portion of the analyses. The difference in relative retention times of DDE and dieldrin between the two column packings is illustrated.

A similar relative retention time for an insecticide reference standard and unknown peak is not unequivocal proof of identification. Many pesticides have similar relative retention times (Watts and Klein, 1964) under normal operating conditions (for example, o-p' DDT, endrin, perthane, esters of herbicides). Contaminants may also produce responses at relative retention times similar to several chlorinated

hydrocarbon insecticides (for example, a contaminant from Florisil has the retention time of aldrin). Careless interpretation could produce misleading results. Some of the contaminants are removed by the clean-up, while others are recognized from control chromatographic tracings and are ignored. However, the possibility of not recognizing all the contaminants, or even interpreting actual insecticide peaks as contaminants does exist. In this study the most frequent contaminants had relative retention times of 3.47, 4.57, 4.96 and 5.44. Other contaminants occurred too infrequently to report.

Gas liquid chromatography with electron capture detection is extremely sensitive for compounds having high electron affinity, thus a number of functional groups produce a response. Therefore, all insecticide analyses using electron capture detection should be confirmed by another technique.

The Ontario Research Foundation has partially confirmed our results by using a different cleanup procedure and model of gas chromatograph. These results are discussed further in the following section (6.3.).

## 6.3. Collaboration Study - Muscle and Fat

Analyses by the Ontario Research Foundation were most fruitful. Some of the value of the data, however, has been lost because some of the samples were grouped with the idea that certain tissues were too small to analyze conveniently (Canadian Wildlife Service Report, ORF 65-2). However, in the results given in this reference, one can find that individual samples weighing as little as 0.25 grams were analyzed. The indiscriminate grouping therefore does not allow age and weight and in

some cases species comparison.

Nevertheless, it is possible to make a comparison of the total DDT and dieldrin concentration in all the fat and muscle analyses (Table 13).

The Alberta and Ontario analyses of the DDT and dieldrin in the fat, unlike those in muscle, compare very favourably. The Ontario values for muscle analyses are approximately four times (3.5 for DDT and 4.3 for dieldrin) larger than the Alberta values for both insecticides. An explanation for the difference in the values cannot be given without further collaboration using control samples.

## 6.4. Total Amount of Insecticides per Bird

The ideal situation in a program of insecticide analysis in wildlife would be to calculate the actual amount of insecticide consumed or absorbed by the specimen under study.

Table 13. Comparison of DDT and dieldrin analyses carried out at the University of Alberta and at the Ontario Research Foundation on Fat and Muscle of Ducklings.

| | Average concentration (ppm, wet weight) | |
|---|---|---|
| | Alberta Method | Ontario Method |
| Fat | | |
| DDT | 0.71 (48) | 0.915 (5) |
| Dieldrin | 0.11 (52) | 0.113 (5) |
| Muscle | | |
| DDT | 0.05 (52) | 0.1690 (28) |
| Dieldrin | 0.01 (59) | 0.0232 (26) |

For larger animals, the only practical method would be to calculate what proportion of the animal each organ occupies, then sample each organ and analyze for insecticides. For small animals and insects, this is impractical and in most cases impossible.

For small animals it is quite sufficient to homogenize the entire animal, analyze an aliquot for insecticide* and divide the result by the fraction of the homogenate analyzed, to obtain the number of milligrams of an insecticide in the animal.

By just analyzing the fat and muscle, one cannot calculate the total milligrams of insecticide per bird without creating misleading results. Therefore, a comparison of the residues found in this study and the total weight of the birds has not been attempted here.

## 6.5. Insecticide-Site Relationship

The small sample number characteristic of most sites, plus the fact that this number may represent only birds from the same family ( brood ) could result in a misleading comparison of sampling site (bird collection site) and average insecticide concentration.

Tables 6, 7, 8, 9, 10 and 14 are given to enable individual interpretation of any comparison of insecticide levels to geographical

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*When analyzing the aliquot for insecticide, extract the fat by the method outlined in 6.1., and separate the insecticide from this fat by using the procedure outlined in 4.6.1. A preliminary step, however, of calculation of the percent moisture content must be carried out. With this, the investigator can compare his results with those of any other study, regardless of the amount of moisture loss.

Table 14. Average concentration of DDT and dieldrin in fat and muscle tissue of ducklings computed according to collection site.

| Site No. | DDT | | Dieldrin | |
|---|---|---|---|---|
| | Fat | Muscle | Fat | Muscle |
| 1 | 1.83 (2) | 0.04 (2) | 0.01 (2) | 0.00 (2) |
| 2 | 4.76 (3) | 0.59 (3) | 0.03 (3) | trace (3) |
| 5 | 0.05 (1) | 0.72 (1) | 0.49 (1) | 0.01 (1) |
| 6 | 4.57 (7) | 0.14 (7) | 0.03 (7) | 0.00 (7) |
| 7 | 0.02 (2) | 0.02 (3) | 0.01 (2) | 0.00 (3) |
| 8 | 0.46 (4) | trace (3) | 0.03 (4) | trace (3) |
| 9 | 0.07 (1) | 0.01 (2) | 0.00 (1) | 0.00 (2) |
| 10 | 0.58 (1) | trace (1) | 0.05 (1) | 0.00 (1) |
| 12 | 7.49 (9) | 0.09 (9) | 0.03 (9) | 0.00 (9) |
| 17 | 0.23 (5) | 0.02 (7) | 0.02 (5) | trace (7) |
| 21 | 0.76 (1) | 0.37 (1) | 0.00 (1) | trace (1) |
| 22 | 10.17 (2) | 0.29 (2) | 2.02 (2) | 0.08 (2) |
| 25 | 0.86 (1) | 0.00 (1) | 0.47 (1) | 0.00 (1) |
| 28 | 1.63 (5) | 0.02 (5) | 0.00 (5) | 0.00 (5) |
| 29 | 0.25 (2) | 0.13 (3) | 0.00 (2) | 0.00 (3) |
| 31 | 0.16 (2) | 0.03 (2) | 0.01 (2) | 0.00 (2) |
| 32 | 0.00 (1) | 0.00 (1) | 0.00 (1) | 0.00 (1) |

location of the sampling site. The occurrence of the highest levels of DDT in duckling fat tissue occurs in birds from site number 1, 2, 6, 12, 22 and 28. It should be noted that these high levels do not always correspond to those in the muscle tissue. Likewise for dieldrin, the highest levels were recorded in birds from site number 5, 10, 22 and 25. With the exception of site number 22, high levels of DDT and dieldrin do not correspond.

## 6.6. Insecticide - Weight - Species Relationship

Table 15 compares the average DDT and dieldrin concentrations for baldpate, gadwall and pintail ducklings by weight groupings. The gadwalls show a greater difference in residue level in fat between the weight groupings than pintails. However, the baldpates have residue levels much lower than either the gadwalls or pintails.

Weight and age comparisons in a small sample population such as this could be considered parallel. That is, ducklings are increasing in weight up until maturity at a very fast rate. Therefore, since this study deals only with immature birds, the similarity of insecticide residue comparisons by age and weight is not surprising; it is considered in greater detail in the following section (6.7.).

## 6.7. Insecticide - Age - Species Relationship

By grouping age classes, the total analyses have been summarized (Figure 6) to show the number of birds (percentage) which had residues occurring in each of four insecticide levels in fat and muscle. No strong trends or correlations are evident except in Figure 6a which

Table 15. The average concentrations of DDT° and dieldrin found in duckling fat and muscle compared to duckling weights by species.

| Species | Wt.* group | AVERAGE CONCENTRATION ppm, wet weight | | | |
|---|---|---|---|---|---|
| | | DDT° | | Dieldrin | |
| | | Fat | Muscle | Fat | Muscle |
| Baldpate | I | 0.37 (6)+ | 0.02 (7) | 0.01 (6) | 0.00 (7) |
| | II | 0.30 (2) | 0.00 (4) | 0.00 (2) | 0.00 (4) |
| | III | 0.09 (2) | 0.04 (2) | 0.00 (2) | 0.00 (2) |
| Gadwall | I | 7.90 (10) | 0.08 (10) | 0.03 (10) | 0.00 (10) |
| | II | 0.25 (2) | 0.01 (2) | 0.00 (2) | 0.00 (2) |
| | III | 0.01 (2) | 0.01 (2) | 0.01 (2) | 0.01 (2) |
| Pintail | I | 12.18 (4) | 0.48 (4) | 1.01 (4) | 0.04 (4) |
| | II | 2.02 (2) | 0.03 (3) | 0.01 (2) | 0.00 (3) |
| | III | 0.40 (6) | 0.02 (6) | 0.03 (6) | 0.00 (6) |

° The term DDT includes a sum of DDD, DDE and DDT levels

* Weight group I = 0 to 200 grams

Weight group II = 201 to 400 grams

Weight group III = 401 grams and over

+ Number of analyses used to determine average

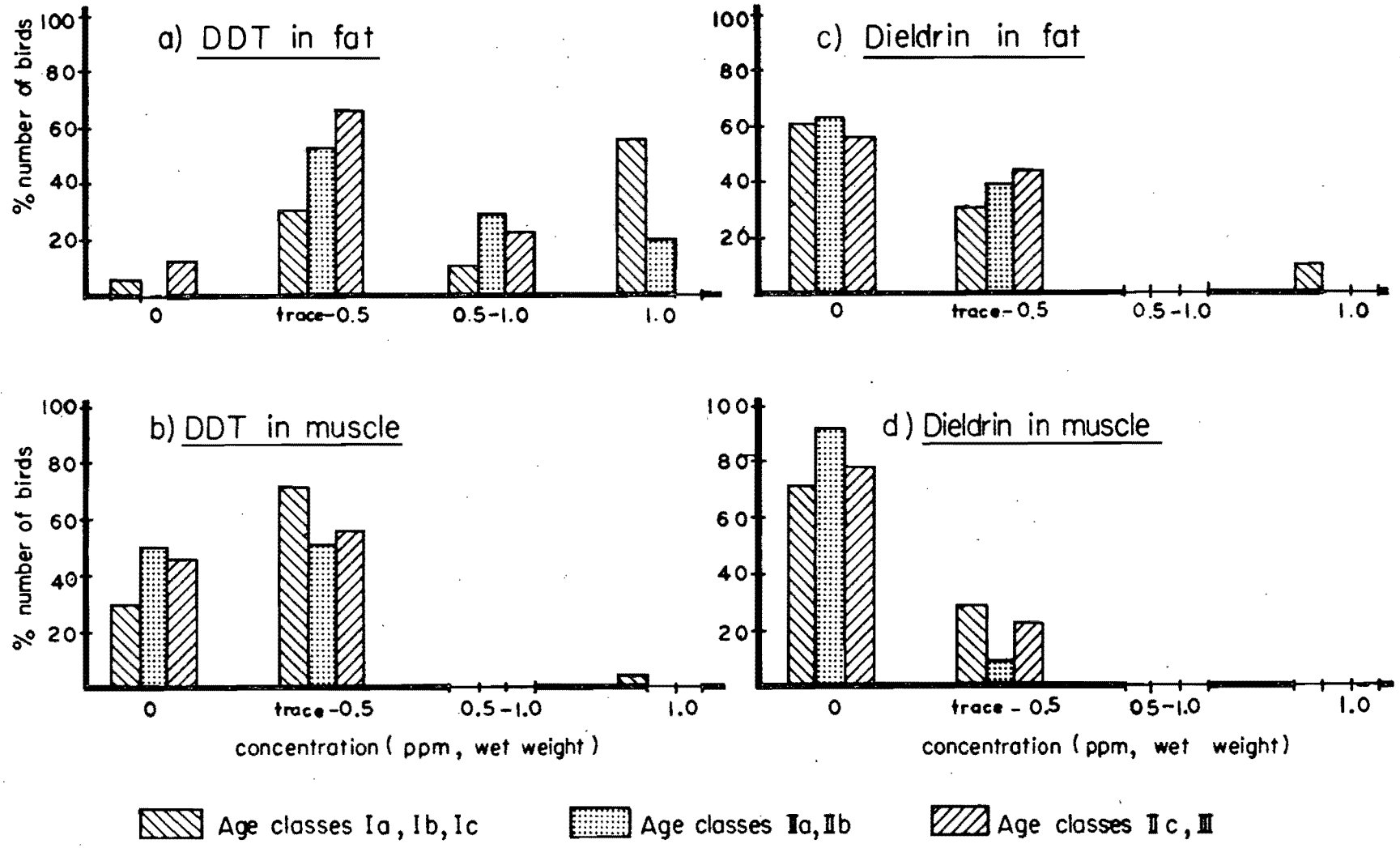


Fig. 6. Frequency of DDT and Dieldrin in ducklings as compared by age classes.

suggests the possible dilution of DDT by growth.

This relationship is more pronounced in Table 16 which summarizes the average residue levels of DDT and dieldrin for 3 species of ducklings grouped into 3 age divisions. Both DDT and dieldrin in fat and muscle samples show a general decrease in concentration with an increase in bird age. The trend is accentuated to a greater degree in the DDT comparison than that of dieldrin because of the higher DDT levels.

Here the value of expressing concentrations as ppm of pure fat again becomes evident.

The fat tissue results of the baldpate analyses indicate a much lower concentration than the pintails and gadwalls. There is no difference in residue level between the first two age groups in baldpates, but there is between the second and third. The pintails and gadwalls, on the other hand, express a large difference between the three age groups. This would indicate a species difference and a physiological quality (excretion) which enables the baldpate to avoid excessive insecticide residue accumulation.

It is impossible to conclude that the concentration differences by species are due to different geographical wintering areas, since the ducks from the Strathmore area use a common migratory path along the Central and Pacific Flyways (Sugden, personal communication). In addition, the random manner of collection would not be selective for ducklings whose parents had not been subjected to insecticides.

Table 16. The average concentrations of DDT° and dieldrin found in duckling fat and muscle compared to duckling ages by species.

| Species | Age* Group | MEAN CONCENTRATION ppm. wet weight | | | |
|---|---|---|---|---|---|
| | | DDT° | | Dieldrin | |
| | | Fat | Muscle | Fat | Muscle |
| Baldpate | A | 0.37 (6)+ | 0.02 (7) | 0.01 (6) | trace (7) |
| | B | 0.30 (2) | 0.00 (4) | 0.00 (2) | trace (4) |
| | C | 0.09 (2) | 0.04 (2) | 0.00 (2) | 0.00 (2) |
| Gadwall | A | 12.34 (6) | 0.13 (6) | 0.05 (6) | trace (6) |
| | B | 0.91 (6) | 0.02 (6) | 0.01 (6) | 0.00 (6) |
| | C | 0.01 (2) | 0.01 (2) | 0.01 (2) | 0.01 (2) |
| Pintail | A | 12.18 (4) | 0.48 (4) | 1.01 (4) | 0.04 (4) |
| | B | 0.98 (6) | 0.05 (6) | 0.03 (6) | trace (6) |
| | C | 0.30 (2) | 0.04 (3) | 0.02 (2) | trace (3) |

° The term DDT includes a sum of DDD, DDE and DDT levels

* Age group A includes age classes Ia, Ib and Ic

Age group B includes age classes IIa and IIb

Age group C includes age classes IIc and III (not flying)

+ Number of analyses used to determine average

## 6.8. Insecticide - Approximate Hatching Date Relationship

If the average age (using the mid point of the age classes given in Table 2) of a duck is extrapolated from the birds' collection date, the approximate hatching date is attained. By comparing* the amount of DDT and dieldrin residue found in the entire bird to the calculated hatching date, it would be possible to see if there is any one point before which there is a zero or a very low concentration of insecticide. Such a point would be expected if the study area had been treated with DDT or dieldrin and any exposed birds had accumulated large amounts of the insecticide. That is, before the date of such a treatment, the insecticide residue levels would be very low; after the date the residue levels would be high.

Such a comparison could not be carried out, because of insufficient data for the calculation of the total amount of insecticide in the whole bird (as discussed in section 6.4.). Once again it is evident that calculation of the percentage of total fat and then the insecticide content in the birds would be profitable.

## 6.9. Preen Gland - Insecticide Relationship

Ducks have a relatively large bilobed sebacious preen gland or uropygial gland, located at the base of the tail. The secretion of this gland is partially ether soluble, with the lipoidal fraction containing both saponifiable and non saponifiable fractions as well as lecithin (Weitzel, Fretzdorff and Wajahn, 1952a and 1952b). This oily secretion is collected

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* This is best done by plotting insecticide concentration of the whole bird in ppm or milligrams (the ordinate) against the approximate date of hatching in days (the abscissa) on a graph.

in the central cavity of this gland and secreted through a nipple-like process at the skin surface. Because of the high fat and oil content, one would assume that this gland could accumulate DDT and dieldrin.

No specific indispensible function has yet been assigned to the preen gland. Thomson (1923) suggests that it aids in feather cleaning. Hou's view (1928, 1930) is that the secretion is a source of vitamin D taken in via the mouth when preening feathers.

However, there is secretion and with the results obtained for the insecticide levels in the preen gland, the author would like to believe that this gland can be used effectively for the excretion of these chemicals.

The Ontario Research Foundation Analyses of preen glands of ducklings (Table 17) show an average DDT (and metabolite) level of 0.63 ppm (with a range from 0.17 to 4.65 ppm) which is about one-third less than their result for DDT in fat, and similar to the result obtained for DDT in muscle. Their average result for dieldrin was 0.23 ppm (a range from 0.03 to 2.29 ppm per bird).

These results differ significantly from those obtained in Alberta (only one duckling from this study has been sampled). Most significant is the lack of DDT or dieldrin in this analysis. The high value of 1.68 for DDE could be attributable to chance; that is, it was chance that the bird chosen had the very high level.

The amount of fat surrounding the gland which is included in the preen gland analysis could result in an inaccurate picture. These analyses included the whole gland, not just the secretion. It is possible that the glandular tissue in the gland wall may restrict the insecticide passage through to the secretion. If this is possible, a conclusion of

Table 17. Analyses* of Preen Gland of Ducklings collected at Strathmore, Alberta.

| Species | Bird No. | Sample Weight | CONCENTRATION, ppm DDE | DDT | Dieldrin |
|---|---|---|---|---|---|
| Gadwall | 103<br>95<br>102<br>96 | 1.9 | 0.785 | 0.437 | 0.135 |
| Baldpate | 44<br>26<br>20 | 2.7 | 0.270 | 0.252 | 0.090 |
| Gadwall | 105<br>125 | 2.35 | 0.437 | 0.362 | 0.106 |
| Scaup | 14 | 0.25 | 0.877 | 3.770 | 2.290 |
| Pintail | 26 | 1.15 | 1.160 | 1.070 | 0.452 |
| Pintail | 36<br>9<br>7<br>13<br>11 | 2.95 | 0.446 | 0.458 | 0.374 |
| Gadwall | 119 | 0.8 | 0.454 | 0.782 | 0.437 |
| Pintail | 48<br>23 | 2.85 | 0.350 | 0.261 | 0.175 |
| Blue-winged teal | 28<br>27 | 1.1 | 0.134 | 0.850 | 0.113 |

*Conducted by the Ontario Research Foundation

insecticide excretion would be misconceived. Therefore, it would be necessary to analyse only that material which is collected in the central cavity of the gland.

6.10. Excretion - Growth Dilution

Insecticides in the preen gland could be excreted. Other forms of insecticide excretion have been shown elsewhere. Bernard (1963) showed that house sparrows maintained on a DDT-free diet following an initial 6-day exposure, reduced the amount of DDD in their tissues. Gannon, Link and Decker (1959a, 1959c) and Gannon and Decker (1960) have shown excretion of chlorinated hydrocarbons in the milk of dairy cattle; while Egan et al. (1965) have reviewed and reported residues in human milk.

Although excretion could play an important part, along with volatization and degradation of insecticides, the results as outlined in this study best indicate growth dilution of residues in tissues. Such a growth dilution is indicated in Table 16 in this study. This is also shown in the Canadian egg and duck samples, which Sheldon and his associates (1962) collected. The eggs contained more DDT and metabolites than any mature duck he collected, and the immatures averaged more DDT and related compounds than did the adults.

Hopkins, Norton and Gyrisco (1952) and Miles et al. (1963) have investigated growth dilution as a factor in the decline of insecticide residues in forage crops. Hopkins and his associates concluded that residue loss from growth alone would be from 60 to 80% of the original deposit in a period of four to six weeks.

Further research into all types of excretion and growth dilution would be most valuable.

## 6.11. Origin of Contamination

The information outlined in this thesis indicates that the most probable source of contamination of the ducklings was the hen. That is, there was transfer of the insecticide through the egg. DeWitt et al. (1963) found DDT residues of from 11 to 37 ppm in eagle eggs. Bernard (1963) has found DDT in reproductive organs and in unhatched eggs of robins. He suggests that DDT may be passed on directly from the female to the eggs and young.

Sheldon et al. (1962) found that 61% of the collected waterfowl and all clutches of eggs (duck range was 0.0 to 1.0 ppm DDT and metabolites while eggs over 2.2 ppm with range 1.3 to 4.0 ppm) collected near Yellowknife, N.W.T. contained insecticide residues. Sheldon et al. (1963) again identified insecticides in waterfowl eggs from North America in 1963. Stickel, Reichel and Addy (1963) found DDT or its metabolites in black duck eggs and osprey eggs from the Atlantic coast.

## 6.12. Physiological and Feeding Habit Differences

In this study we have noticed that the pintail and gadwall species have higher residues than the baldpate and yet, Sugden (1964), in a progress report, has shown that the feeding habits of the gadwall and baldpates are more similar to each other than to the pintail. The latter being more of an omnivore in later stages of development, than the former.

These differences in residue concentrations could be due to differential contamination of food and/or to physiological dissimilarities (mentioned in section 6.7.), as shown by Gannon, Link and Decker (1959b) with lambs and hens fed 0.4 ppm dieldrin over an 84 day period. The lambs built up residues of 0.5 ppm while the hens 10.0 ppm.

However, the Nature Conservancy in England has found marked differences in residue content between raptorial and fish eating birds, and herbivores. Similarly, in eggs analysed by the Government Chemist and Department of Agriculture for Scotland, higher residues were found in peregrine falcons, great crested grebes and herons than in the herbivorous Canada Goose and the omnivorous pheasant and carrion crow, while the golden eagle and terns occupied an intermediate position (Moore and Walker, 1964). Taylor and Brady (1964) collected eggs of eight species of wild birds in England and Wales and found that bullfinch eggs taken from the same locality as the song thrush and blackbird eggs contained very much lower residues. This result was presumed related to their habit of feeding off the ground.

## 6.13. Effects of Insecticides

The effects of the DDT and dieldrin residues on ducklings are virtually unknown. From the results of controlled feeding experiments on other birds some effects can be speculated. However, the literature reports conflicting results. Also, it is well known that results of laboratory tests do not conform with results of field tests.

DeWitt (1956a and 1956b) established that sub-lethal amounts of aldrin and dieldrin at levels as low as 1.0 ppm in feed resulted in decreased fertilization of eggs and viability in chicks of quail. Rubin et al. (1941) found that DDT in the diet of laying hens resulted in reduced egg production and hatchability. These investigators, as well as Cross, King and Haynes (1962) did not determine the levels of insecticide in the eggs. Ash and Taylor (1964) found that residues of from 0.4 to 22.1 ppm BHC did not impair hatchability of eggs from

treated birds.

Difference in pheasant reproductive performance between an area treated with insecticides according to "good" agricultural practice and another, untreated area, have been shown in California. Differences were small in fertility of the eggs, hatchability of the eggs and capacity of the young birds to survive. However, differences between the areas were significant when the total differences were compared (Buckley, 1963).

The above evidence indicates that there is great need for experimentation to measure the effects of various insecticide residues on various species of ducks. Such an experiment should be conducted in as natural an environment as possible, and continued through several generations. Without the information from such a study one cannot conclude what effects residue levels in tissue have on an animal or ecosystem.

## 6.14. Spread of Insecticides

The Wheatland County No. 16 had no large scale DDT spraying or use in past years (section 1.2). The finding of DDT in the ducklings indicates the spread of insecticides from another source.

Certainly this is not an original finding but it adds another point to the wide spreading of insecticides through our wildlife.

A number of the residue figures for predators (e.g. 4.20 ppm of dieldrin in the liver of 8 species found by Moore and Walker (1964) are comparable with values obtained for poisoned non-predators reported by DeWitt and George (1960). Moore and Walker (1964) report residues found in birds and eggs of 37 countries from 20 out of 21 taxonomic

families from terrestrial, freshwater and marine habitats. Similar widespread distributions of organic chlorine insecticide residues have been reported from North America (George, 1962). George (1965) also reported DDT and metabolite residues in penguins of the Antarctic.

Examples such as these above, confirm the fact that food chains can accumulate and spread these insecticides. Migrating wildlife, fish or insects can move insecticides from a contaminated area to non-contaminated areas. Only a small percentage of the ducklings hatched in the spring will migrate south in the fall. Those which die or are killed during development, deposit insecticides into an ecosystem possibly not previously contaminated.

## 7. CONCLUSIONS

The preceding discussion and results indicate an insecticide residue transfer to the ducklings from the hen via the egg. However, since the Strathmore area has no record of significant DDT usage, and since the levels in the ducklings were relatively high, it is concluded that the adult birds had accumulated this insecticide in an area other than the breeding area.

The initial dosages have been decreased by excretion and/or growth dilution. Evidence of insecticide excretion by the preen gland has been presented and is to be seriously considered. Growth dilution on the other hand, has been proven and is probably the best way of explaining a decrease in residue levels with an increase in age and weight.

It is suggested that some environmental contamination by chlorinated hydrocarbon insecticides is directly related to the spreading of insecticides by migratory animals. Therefore, investigations should be made to find out to what degree general environment contamination is important and to what degree the biota are affected.

A standardization of techniques and methods is to be desired. The concentration of insecticides would be better expressed on a ppm of pure fat basis, than a ppm wet weight basis. In addition, further research on methods and analysis techniques with collaboration studies involving control samples is necessary. Analysts must be accurate and have complete confidence in their interpretation of chromatograph tracings.

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