ANALYSIS OF DDT RESIDUES IN ANIMAL TISSUES AND SOILS COLLECTED FROM DIFFERENT REGIONS OF CANADA

By

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File Report No. 3

May 1974

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INTRODUCTION

The chlorinated hydrocarbon insecticide DDT [dichlorodiphenyl-trichloroethane; 2,2-bis-(p- chlorophenyl)-1,1,1-trichloroethane], because of its wide spectrum of insecticidal activity, ease of manufacture, low cost, prolonged stability resulting in good residual activity and low mammalian toxicity, has been used extensively in Canadian forest spray programs prior to 1968 for the control of various lepidopterous defoliators (Fettes and Buckner, 1972). Although it has been phased out of usage because of its acute persistence in terrestrial and aquatic biota, cumulative food-chain concentration and biological magnification, a substantial part of the applied chemical and its degradation products are still present in the environment (Yule, 1970; Yule and Tomlin, 1970; Yule, 1973; Sundaram, 1972, 1974). The extent and significance of their presence in biota over long periods of time, especially at sub acute levels, is still obscure or only partly known primarily due to the lack of an organized environmental monitoring and surveillance system to provide comprehensive and representative data about the locations, amounts and trends of this contamination. Consequently, for an in depth evaluation of the impact produced by the residues of the toxicant, it became necessary to monitor periodically by analyzing quantitatively, their presence in various materials such as plant and animal tissues, soil, air and water samples collected from the forest environment in different regions of Canada. Results obtained earlier on a similar study comprising 362 small mammal samples has already been published (Sundaram, 1972). The present report presents residue data on DDT isomers and the p,p'-DDE metabolite in animal, foliage, water, soil and air samples totalling 460 in number, received from various regions of Canada since mid-1972 along with the analytical methods developed for analysing the insecticide residues. The methods described in the literature and used here are also briefly outlined for the purpose of consolidating the various analytical methods that are available under one cover to serve as a ready source of information for future reference.

MATERIALS AND METHODS

Among the 460 samples analysed and recorded in this report 421 samples had been collected at varying intervals since the early summer of 1972 to the fall of 1973, processed and supplied by Dr. Buckner and his associates of the Invertebrate Biology Section at the Chemical Control Research Institute (CCRI). The samples after identification and processing were preserved in glass jars under methanol (pesticide grade, 20-40 ml) and stored at 10° C in a refrigerator until analysis to prevent any further degradation of the insecticide residues. The 39 samples recorded in Table 11 were collected from Priceville (N.B.) by the personnel at the Analytical Service Section of CCRI with assistance especially from Dr. I.W. Varty of the Maritime Forest Research Centre, to study the persistence and distribution of DDT residues in a localized area which was heavily exposed to DDT spraying.

The breakdown of the 460 samples analysed according to various species, their numbers and sources are as follows:

Species	Numbers	Sources
Mice Fetus	37	(Progeny of 1972 animal samples from N.B., see Sundaram 1972).
Mice brains	332	(Quebec, Manitoba, B.C., Anticosti Is.)
Fish	10	(N.B., B.C.)
Insect larva	1	(B.C.)
Voles	5	(N.B.)
Slugs	5	(B.C.)
Spruce budworm	3	(N.B.)
Soil	50	(Manitoba, N.B., B.C., Anticosti Is.)
Spruce foliage	6	(N.B.)
Air samples	6	(N.B.)
Water	5	(N.B.)
Total	460	

The residue analysis on these samples was started during the latter part of 1972 and continued as time, staff and laboratory facilities permitted until the end of March, 1974. This report is the second of the series, the first published in 1972 (Sundaram 1972) contained the analytical data on 362 samples.

ANALYTICAL METHODS

The analytical methods described here had been tested previously and found to be reliable and practicable; they are also adoptable to analysis of large numbers of samples with high precision. Generally, the methods were developed or modified from the work of others and consisted of seven steps:

1) Sample preparation,
2) Extraction with suitable solvent or solvent mixture,
3) Filtration under suction to separate the solvent and insoluble material,
4) Isolation of the insecticide residues by solvent partition,
5) Cleanup by column chromatography,
6) Concentration by flash evaporation and finally,
7) Detection (identification and quantitation) by gas-liquid chromatography (GLC).

In the analysis of some substrate samples such as air and water, not all the steps were necessary and thus handling and detection were simplified.

Extraction and Cleanup of DDT Residues from Animal Tissues

A number of solvents and procedures were tested (Sundaram, 1972) for efficiency in extracting DDT residues from animal tissues and the selection of acetonitrile was made over dimethylformamide used earlier, as a result of its increased extraction efficiency (> 90%) and ease of handling, with its properties of; boiling point (81.6° C), high polarity, ready miscibility with water, good solubilizing power for insecticides and low toxicity.

Samples (< 2g; fetus and brains of small mammals, insect larva and budworm) were received for analysis already stored in 20 ml methanol*. After blotting on filter paper to remove the solvent, the samples were weighed and homogenized in a Sorvall Omni-Mixer with 25 ml of pesticide grade acetonitrile (Caledon) for 5 min. at speed 6. The macerate was filtered under suction using a fritted glass funnel, the residue was re-extracted with a further 25 ml of solvent and filtered through the same funnel. The residue was washed with 10 ml of acetonitrile, extracts were pooled and flash evaporated to 20 ml. The extract was partitioned twice with 10 ml of hexane (pesticide grade, Caledon), and after clear separation, the nonpolar phase was discarded. The acetonitrile layer was transferred quantitatively to a 250 ml separatory funnel, 100 ml water, 10 ml of 5% Na_2SO_4 and 50 ml of hexane were added. The mixture was equilibrated and the layers separated. The aqueous

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* Gas chromatographic analysis of the methanol used in the sample preservation showed negligible amounts of DDT residues.

layer was re-extracted with 50 ml of hexane and the hexane phase was rinsed with 50 ml of water. The water layers were discarded and the two hexane layers were combined and passed through a column of anhydrous sodium sulphate (ca 50 g) and flash evaporated to 3 ml.

Solid-liquid chromatographic cleanup was accomplished by passing the concentrated extract through a 10 x 300 mm column containing 7 g preconditioned Florisil (60/100 mesh, 0% H_2O) sandwiched between 10 g Na_2SO_4. After rinsing the column with 50 ml hexane, the extract was transferred and eluted with 100 ml of hexane. The eluate was flash evaporated to 0.5 ml for GLC (ECD) analysis. The procedure is schematically illustrated in Fig. 1.

The method outlined above was found to be extremely suitable for analysing the DDT residues found in animal tissues if the sample sizes did not exceed two grams. Almost all the brain, fetus, larva and budworm samples analysed were less than this optimum weight. Fish and slug samples weighing more than 2 g were first cut into small pieces, mixed well and an aliquot was used for analysis. The proportions of acetonitrile, column adsorbent and eluting solvent used were as follows:

1 g tissue: 10 ml CH_3CN

1 g tissue: 4 g Florisil

1 g Florisil: 20 ml eluting solvent

During the course of analysis, it was observed that some of the steps (see Fig. 1) could be eliminated without sacrificing sensitivity and precision. As indicated below the legend in Fig. 1, some of the steps were omitted to save time and simplify the method. The GLC responses before and after the Florisil column cleanup and after eliminating some of the repetitive steps in the procedure are shown in Figs. 3-5.

Extraction and Cleanup of DDT Residues from Soil

The procedure reported here is similar to the one used by Yule and Smith (1971) with minor modifications to increase the sensitivity for soil analysis.

Composited soil sample was screened to remove plant and other debris, sifted through a #8 sieve (Br., opening 2000 µm), 50 g was taken in a Sorvall homogenizer and extracted with 100 ml of 2:1 (v/v) n-hexane: acetone solution for 5 min. at speed 6. The macerate was vacuum filtered through a Buchner funnel using a thin pad of celite, or shark skin (S and S) filter paper rinsed with 25 ml of solvent mixture, then the residue was re-extracted as before. The volume of the combined extracts was made up to 300 ml with hexane and transferred to a 2 liter separatory funnel and mixed with 600 ml of distilled water and 50 ml of 5% sodium chloride solution. The contents were shaken vigorously for 2 min. and allowed to stand overnight for the phases to separate completely. The hexane phase was washed twice with 200 ml of water and the aqueous phase with 100 ml of hexane. The aqueous phase was discarded and the hexane phases were combined and dried by passing through a column of anhydrous sodium sulphate (50 g), the column rinsed with 25 ml hexane and the volume adjusted to 50 ml (1 g/ml) by flash evaporation.

"Shell" design chromatographic column (ID 20 mm, length 400 mm) containing a reservoir (200 ml) at one end and a sealed in coarse porosity fritted disc, and Teflon stopcock to control column flow, at the other, was loaded with 20 g of Florisil (60/100 mesh activated by heating at 160° C for 24 hours in an oven to contain 0% H_2O). Additional anhydrous sodium sulfate (10 g) was placed on top of the Florisil. The column was washed with 100 ml of hexane, then the 50 ml extract was transferred quantitatively to the column and eluted with 200 ml of 15% benzene in hexane. The eluate (ca 250 ml) was collected and concentrated to 10 ml by flash evaporation (1g/0.2 ml) for gas chromatographic analysis. The procedure is schematically illustrated in Fig. 2.

Two 10 g aliquots of the soil were used for moisture* (AOAC, 1955) and pH determinations+ (Atkinson, et al 1958).

Extraction of DDT Residues from Water

An aliquot of water sample (250 ml) was transferred to a one liter separatory funnel and extracted twice by shaking vigorously for 5 min. with 200 ml of hexane. Emulsion formation was minimized by adding 10 ml of 5% sodium sulphate solution. After the phases separated, the aqueous layer was discarded, the hexane phases were combined and passed through a column of anhydrous sodium sulphate (70 g). No column cleanup was necessary for water samples. Prior to GC detection, the extract was concentrated down to 10 ml by flash evaporation followed by

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* Moisture content of soil samples was determined gravimetrically on duplicate samples after drying 16 hours in an air circulated thermostatic oven at 105° C. Percent moisture was calculated as follows:

$$\%\ \text{Moisture} = \left[\frac{\text{Moist weight} - \text{oven dry weight}}{\text{oven dry weight}}\right] \times 100$$

\+ Soil pH was determined in a 1:2 (weight:volume) suspension of soil and distilled water with a IL Porto-matic pH meter (Model 175) employing a glass electrode.

gentle stream of air to 1 ml.

Extraction of DDT Residues from Foliage

Foliage samples were prepared by clipping them from the branches and hand-mixing. The composited sample was finely ground in a Hobart grinder. A 50 g sample was extracted twice with 100 ml of acetonitrile in a Sorvall homogenizer, cleaned-up and analysed as for soil.

Analysis of DDT Residues in Air Samples

The procedure used for analysing the six air samples recorded in Table 11 was similar to the one described recently by Sundaram (1974). The DDT residues present in the Florisil (20 g) samples were extracted twice with 150 ml of benzene and the insecticide residues in dimethylformamide (DMF) (150 ml) bubblers were partitioned twice with aqueous sodium sulphate (500 ml) and hexane (100 ml). The benzene and hexane fractions of each sample were pooled, flash evaporated to 1.0 ml and analysed.

Gas Chromatographic Analysis

Detection (identification and quantitation) of DDT residues (DDE, *o,p*-DDT and *p,p'*-DDT) was by using conventional electron capture gas chromatography.

A Hewlett-Packard 5750 instrument (Avondale, Pa.) equipped with a Ni 63 electron capture detector was used. The operating conditions were as follows:

Column: Glass, 4 ft x 6 mm O.D. packed with 3% DC-200 on Chromosorb W, 80-100 mesh, HP

Temperature:	Injection port = 220° C
	Column oven = 200°C
	Detector = 260° C
Gas flow:	Argon/methane (95/5%) pressure of 40 psi and flow rate of 33.3 ml/min.
Instrument settings:	Attenuation and range, 32 x 10; pulse rate of 150

Standard 4 μl injections of the sample extract were analysed. The extracts were diluted with hexane or air evaporated to the optimum concentrations for GLC analysis after trial injections. The presence of DDT isomers and metabolites in samples was determined by comparison of retention times (R.T.). The relative R.T.'s under the above operating conditions were: DDE, 1.00; o,p- DDT, 1.35; and p,p'-DDT, 1.72. The quantity of DDT isomers and metabolites in samples was determined by comparison of peak heights with standard calibrations for DDE, o,p-DDT, and p,p'-DDT. Quantitative insecticide standards were injected on the same day the samples were analysed to provide for the day-to-day fluctuations in operating conditions.

Reagents

All solvents used were of pesticide grade supplied by Caledon Laboratories, Georgetown, Ontario. Florisil 60/100 mesh (F-100) and reagent grade anhydrous sodium sulphate (S-421) were from Fisher Scientific Co.

During the course of analysis, laboratory sources of contamination, if any, were monitored frequently by conducting blank experiments using the same procedure and analysing for the DDT residues. Contamination was found to be negligible.

The results of the analysis are recorded in Tables 1 to 12 and the abbreviations, symbols and the chemical names of the insecticides mentioned in this report are explained in Appendix I.

TABLE 1

Analysis of DDT Residues in Fetus Tissues of Mice

Serial No.	Identification No.	Mass (g)	DDE (ppm)	*o,p*-DDT (ppm)	*p,p'*-DDT (ppm)	Total DDT (ppm)
1	1A	1.88	T	N.D.	0.120	0.120
2	1B	1.82	T	0.065	N.D.	0.065
3	1C	1.80	0.005	0.010	0.030	0.045
4	1D	1.82	0.005	0.010	0.010	0.025
5	2A	0.10	0.090	0.030	0.140	0.260
6	2B	0.13	0.015	0.005	0.060	0.080
7	2C	0.12	0.030	0.030	0.290	0.350
8	2D	0.20	0.030	0.020	0.110	0.160
9	2E	0.10	0.020	0.150	0.130	0.300
10	37A	0.60	T	0.005	0.015	0.020
11	37B	0.25	0.005	0.135	0.300	0.440
12	37C	0.50	T	0.010	0.020	0.030
13	37D	0.54	T	0.030	0.035	0.065
14	37E	0.55	T	0.010	0.025	0.035
15	37F	0.59	T	0.005	0.015	0.020
16	72A	0.27	N.D.	N.D.	N.D.	N.D.
17	72B	0.28	0.005	0.010	0.030	0.045

18	72C	0.28	0.005	0.025	0.065	0.095
19	72D	0.23	0.020	N.D.	0.235	0.255
20	72E	0.24	0.010	0.075	0.040	0.125
21	72F	0.27	N.D.	0.035	0.045	0.080
22	106A	0.71	N.D.	T	0.005	0.005
23	106B	0.56	N.D.	0.010	0.225	0.235
24	106C	0.69	N.D.	0.155	N.D.	0.155
25	106D	0.88	N.D.	N.D.	0.010	0.010
26	106E	0.68	N.D.	N.D.	0.100	0.100
27	107A	1.20	N.D.	0.090	N.D.	0.090
28	107B	1.29	N.D.	0.020	N.D.	0.020
29	107C	1.37	N.D.	N.D.	0.250	0.250
30	107D	1.15	N.D.	N.D.	N.D.	N.D.
31	107E	1.18	0.005	0.005	0.100	0.110
32	107F	1.26	N.D.	N.D.	N.D.	N.D.
33	162A	0.92	N.D.	0.010	0.020	0.030
34	162B	0.80	0.005	N.D.	0.010	0.015
35	162C	1.40	N.D.	N.D.	0.020	0.020
36	162D	0.82	N.D.	N.D.	0.075	0.075
37	111 A-E*	0.01	0.120	N.D.	1.600	1.720

* Pooled samples. N.D. = Not detected. T = Traces (<0.005 ppm).

TABLE 2

Analysis of DDT Residues in Mice Brains from Maniwaki and California Lake

Serial No.	Identification No.	Mass of Brain (g)	DDT Residues Concentration (ppm) DDE	o,p-DDT	p,p′-DDT	Total
38	Maniwaki Plot I Cg 10	0.50	0.03	0.59	N.D.	0.62
39	Maniwaki Plot I Cg 11	0.40	N.D.	0.33	0.49	0.82
40	Maniwaki Plot I Pm 26	0.30	N.D.	0.85	N.D.	0.85
41	Maniwaki Plot I Cg 86	0.32	N.D.	2.66	N.D.	2.66
42	Maniwaki Plot I Cg 103	0.40	N.D.	0.43	0.27	0.70
43	Maniwaki Plot I Cg 109	0.45	N.D.	0.54	N.D.	0.54
44	Maniwaki Plot I Pm 82	0.30	N.D.	N.D.	N.D.	N.D.
45	Maniwaki Plot I Ts 48	1.30	0.05	N.D.	N.D.	0.05
46	Maniwaki Plot II Cg 36	0.40	N.D.	0.43	0.11	0.54
47	Maniwaki Plot II Cg 37	0.35	N.D.	0.21	1.36	1.57
48	Maniwaki Plot II Cg 42	0.37	N.D.	1.39	0.65	2.04
49	Maniwaki Plot II Cg 50	0.32	N.D.	3.04	0.66	3.70
50	Maniwaki Plot II Pm 75	0.23	N.D.	N.D.	T	T
51	Maniwaki Plot II Ovenbird 45	0.22	T	N.D.	N.D.	T
52	Maniwaki Plot II Ts 47	1.10	T	T	N.D.	T
53	Maniwaki Plot II Ts 53	1.15	0.09	T	0.04	0.13

54	Maniwaki Plot II Hermit Thrush 107	0.50	0.04	N.D.	N.D.	0.04
55	Maniwaki Plot II Wood Thrush 110	0.39	N.D.	N.D.	N.D.	N.D.
56	Maniwaki Plot III Cg 9	0.30	0.04	0.89	1.84	2.77
57	Maniwaki Plot III Cg 35	0.29	N.D.	2.05	N.D.	2.05
58	Maniwaki Plot III Cg 39	0.38	N.D.	1.69	0.21	1.90
59	Maniwaki Plot III Cg 52	0.25	N.D.	1.88	N.D.	1.88
60	Maniwaki Plot III Cg 62	0.43	N.D.	0.68	N.D.	0.68
61	Maniwaki Plot III Cg 67	0.37	N.D.	4.17	N.D.	4.17
62	Maniwaki Plot III Cg 72	0.30	N.D.	11.69	0.23	11.92
63	Maniwaki Plot III Cg 73	0.35	N.D.	0.86	N.D.	0.86
64	Maniwaki Plot III Cg 111	0.38	N.D.	1.19	0.40	1.59
65	Maniwaki Plot III Cg 121	0.28	N.D.	2.33	N.D.	2.33
66	Maniwaki Plot III Ni 19	0.28	0.15	N.D.	N.D.	0.15
67	Maniwaki Plot III Cg 44	0.30	N.D.	0.16	N.D.	0.16
68	Maniwaki Plot III Cg 51	0.28	N.D.	T	N.D.	T
69	Maniwaki Plot III Cg 66	0.20	N.D.	N.D.	N.D.	N.D.
70	Maniwaki Plot III Cg 68	0.32	N.D.	T	N.D.	T
71	Maniwaki Plot III Pm 70	0.40	N.D.	T	T	T
72	Maniwaki Plot III Cg 71	0.33	N.D.	T	T	T
73	Maniwaki Plot III Pm 74	0.35	N.D.	T	N.D.	T
74	Maniwaki Plot III Ni 76	0.32	N.D.	T	T	T

75	Maniwaki Plot III Pm 78	0.30	N.D.	N.D.	N.D.	N.D.
76	Maniwaki Plot III Cg 84	0.30	N.D.	0.17	N.D.	0.17
77	Maniwaki Plot III Cg 88	0.38	N.D.	0.95	N.D.	0.95
78	Maniwaki Plot III Cg 89	0.39	N.D.	0.34	N.D.	0.34
79	Maniwaki Plot III Cg 90	0.25	N.D.	1.71	N.D.	1.71
80	Maniwaki Plot III Cg 92	0.30	N.D.	0.57	N.D.	0.57
81	Maniwaki Plot III Cg 93	0.27	N.D.	N.D.	2.15	2.15
82	Maniwaki Plot III Pm 107	0.40	N.D.	T	T	T
83	Maniwaki Plot III Pm 113	0.48	N.D.	0.06	0.12	0.18
84	Maniwaki Plot III Pm 116	0.40	N.D.	0.23	N.D.	0.23
85	Maniwaki Plot III Cg 123	0.40	N.D.	0.32	N.D.	0.32
86	Maniwaki Plot III Cg 126	0.35	N.D.	0.28	N.D.	0.28
87	Maniwaki Plot III Cg 128	0.62	N.D.	0.17	0.62	0.79
88	Maniwaki Plot III Cg 132	0.43	N.D.	0.72	N.D.	0.72
89	Maniwaki Plot III Pm 140	0.50	0.08	0.09	0.11	0.28
90	Maniwaki Plot III Cg 144	0.53	N.D.	0.15	N.D.	0.15
91	Maniwaki Plot III Cg 145	0.45	0.05	0.16	N.D.	0.21
92	Maniwaki Plot III Cg 146	0.60	N.D.	0.14	N.D.	0.14
93	Maniwaki Plot IV Cg 18	0.40	N.D.	2.45	0.18	2.63
94	Maniwaki Plot IV Pm 25	0.30	N.D.	1.67	N.D.	1.67
95	Maniwaki Plot IV Cg 29	0.30	N.D.	0.99	0.19	1.18
96	Maniwaki Plot IV Cg 59	0.50	N.D.	0.60	0.10	0.70
97	Maniwaki Plot IV Cg 60	0.46	0.14	1.09	N.D.	1.23

98	Maniwaki Plot IV Cg 80	0.50	N.D.	0.76	N.D.	0.76
99	Maniwaki Plot IV Cg 102	0.50	0.01	4.50	N.D.	4.51
100	Maniwaki Plot IV Cg 142	0.53	N.D.	0.74	N.D.	0.74
101	Maniwaki Plot IV Cg 162	0.70	N.D.	12.50	N.D.	12.50
102	Maniwaki Plot IV Pm 12	0.25	N.D.	0.46	N.D.	0.46
103	Maniwaki Plot IV Cg 15	0.45	N.D.	0.17	N.D.	0.17
104	Maniwaki Plot IV Cg 17	0.58	N.D.	0.49	0.16	0.65
105	Maniwaki Plot IV Pm 22	0.40	N.D.	0.99	0.09	1.08
106	Maniwaki Plot IV Pm 24	0.30	N.D.	0.36	N.D.	0.36
107	Maniwaki Plot IV Pm 27	0.30	T	0.53	N.D.	0.53
108	Maniwaki Plot IV Pm 28	0.28	N.D.	0.26	T	0.26
109	Maniwaki Plot IV Pm 31	0.25	0.12	T	N.D.	0.12
110	Maniwaki Plot IV Pm 32	0.23	N.D.	0.76	N.D.	0.76
111	Maniwaki Plot IV Pm 33	0.28	N.D.	N.D.	T	T
112	Maniwaki Plot IV Cg 61	0.46	N.D.	0.19	N.D.	0.19
113	Maniwaki Plot IV Cg 85	0.32	0.14	0.48	2.22	2.84
114	Maniwaki Plot IV Cg 94	0.30	N.D.	1.97	N.D.	1.97
115	Maniwaki Plot IV Cg 95	0.30	N.D.	0.53	N.D.	0.53
116	Maniwaki Plot IV Cg 96	0.40	N.D.	0.86	0.52	1.38
117	Maniwaki Plot IV Cg 97	0.38	N.D.	1.36	N.D.	1.36
118	Maniwaki Plot IV Ni 98	0.29	0.13	0.53	N.D.	0.66
119	Maniwaki Plot IV Cg 106	0.32	N.D.	2.37	N.D.	2.37

120	Maniwaki Plot IV Pm 108	0.30	0.51	T	N.D.	0.51
121	Maniwaki Plot IV Ni 115	0.32	N.D.	0.08	T	0.08
122	Maniwaki Plot IV Cg 133	0.33	N.D.	0.31	N.D.	0.31
123	Maniwaki Plot IV Pm 134	0.30	N.D.	0.36	N.D.	0.36
124	Maniwaki Plot IV Pm 135	0.31	N.D.	0.16	0.07	0.23
125	Maniwaki Plot IV Pm 136	0.35	N.D.	T	N.D.	T
126	Maniwaki Plot IV Cg 139	0.37	N.D.	N.D.	N.D.	N.D.
127	Maniwaki Plot IV Bb 143	0.20	N.D.	0.33	N.D.	0.33
128	Maniwaki Plot IV Cg 147	0.38	N.D.	2.03	0.19	2.22
129	Maniwaki Plot IV Cg 148	0.32	N.D.	1.25	N.D.	1.25
130	Maniwaki Plot IV Pm 152	0.30	N.D.	2.09	N.D.	2.09
131	Maniwaki Plot IV Pm 153	0.31	N.D.	9.67	N.D.	9.67
132	Maniwaki Plot IV Cg 155	0.35	N.D.	6.95	N.D.	6.95
133	Maniwaki Plot IV Pm 156	0.28	0.08	T	N.D.	0.08
134	Maniwaki Plot IV Pm 157	0.37	N.D.	0.10	N.D.	0.10
135	Maniwaki Plot IV Pm 160	0.36	N.D.	0.50	T	0.50
136	Maniwaki Plot IV Cg 164	0.27	N.D.	5.10	N.D.	5.10
137	Maniwaki Plot IV Cg 165	0.35	N.D.	1.32	N.D.	1.32
138	Maniwaki Plot IV Pm 166	0.28	N.D.	22.82	N.D.	22.82
139	Maniwaki Plot IV Squirrel 4	3.40	0.05	N.D.	N.D.	0.05
140	Maniwaki Plot IV Water Thrush 5	0.32	0.09	N.D.	N.D.	0.09

141	Maniwaki Plot IV Mh Juv 6	0.15	0.45	T	N.D.	0.45
142	Maniwaki Plot IV Wood Thrush 7	0.75	0.13	0.12	N.D.	0.25
143	Maniwaki Plot V Cg 16	0.22	N.D.	1.02	1.07	2.09
144	Maniwaki Plot V Cg 40	0.30	N.D.	N.D.	N.D.	N.D.
145	Maniwaki Plot V Cg 43	0.31	N.D.	0.66	0.56	1.22
146	Maniwaki Plot V Cg 58	0.33	N.D.	3.01	0.65	3.66
147	Maniwaki Plot V Cg 87	0.34	N.D.	7.94	N.D.	7.94
148	Maniwaki Plot V Cg 99	0.40	N.D.	0.39	0.05	0.44
149	Maniwaki Plot V Cg 101	0.40	N.D.	N.D.	N.D.	N.D.
150	Maniwaki Plot V Cg 130	0.40	N.D.	T	N.D.	T
151	Maniwaki Plot V Cg 137	0.30	N.D.	T	T	T
152	Maniwaki Plot V Cg 141	0.30	N.D.	T	T	T
153	Maniwaki Plot V Pm 46	0.22	N.D.	N.D.	N.D.	N.D.
154	Maniwaki Plot V Cg 63	0.40	N.D.	0.17	0.94	1.11
155	Maniwaki Plot V Cg 69	0.20	N.D.	1.28	N.D.	1.28
156	Maniwaki Plot V Pm 114	0.40	N.D.	T	N.D.	T
157	Maniwaki Plot V Pm 117	0.41	N.D.	0.28	N.D.	0.28
158	Maniwaki Plot V Pm 118	0.24	N.D.	N.D.	0.12	0.12
159	Maniwaki Plot V Pm 119	0.32	N.D.	T	N.D.	T
160	Maniwaki Plot V Pm 120	0.20	N.D.	0.44	N.D.	0.44
161	Maniwaki Plot V Pm 124	0.30	N.D.	T	0.29	0.29

162	Maniwaki Plot V P_m 125	0.31	N.D.	T	T	T
163	Maniwaki Plot V Pm 129	0.30	N.D.	N.D.	N.D.	N.D.
164	Maniwaki Plot V Pm 131	0.22	T	T	T	T
165	Maniwaki Plot V Pm 138	0.27	N.D.	T	0.09	0.09
166	Maniwaki Plot V Ni 154	0.29	N.D.	0.10	N.D.	0.10
167	Maniwaki Plot V Cg 161	0.30	0.13	N.D.	0.89	1.02
168	Maniwaki Plot V Cg 100	0.38	N.D.	T	T	T
169	Maniwaki Plot VI Cg 38	0.35	N.D.	T	T	T
170	Maniwaki Plot VI Cg 49	0.40	0.06	0.49	N.D.	0.55
171	Maniwaki Plot VI Cg 56	0.26	N.D.	0.29	N.D.	0.29
172	Maniwaki Plot VI Cg 57	0.35	0.03	0.23	N.D.	0.26
173	Maniwaki Plot VI Cg 65	0.35	N.D.	0.78	N.D.	0.78
174	Maniwaki Plot VI Cg 77	0.16	N.D.	1.09	N.D.	1.09
175	Maniwaki Plot VI Cg 91	0.41	N.D.	0.27	N.D.	0.27
176	Maniwaki Plot VI Cg 105	0.17	N.D.	T	N.D.	T
177	Maniwaki Plot VI Cg 122	0.30	N.D.	T	T	T
178	Maniwaki Plot VI Cg 150	0.38	N.D.	T	N.D.	T
179	Maniwaki Plot VI Pm 1	0.40	N.D.	0.27	N.D.	0.27
180	Maniwaki Plot VI Cg 2	0.30	T	0.46	T	0.46
181	Maniwaki Plot VI Pm 3	0.30	0.03	N.D.	1.02	1.05
182	Maniwaki Plot VI Zh 8	0.29	0.09	T	N.D.	0.09

183	Maniwaki Plot VI Pm 13	0.40	N.D.	T	N.D.	T
184	Maniwaki Plot VI Cg 14	0.37	N.D.	T	T	T
185	Maniwaki Plot VI Cg 18	0.24	N.D.	T	T	T
186	Maniwaki Plot VI Cg 20	0.22	0.06	N.D.	T	0.06
187	Maniwaki Plot VI Pm 21	0.27	N.D.	N.D.	T	T
188	Maniwaki Plot VI Pm 23	0.30	N.D.	N.D.	N.D.	N.D.
189	Maniwaki Plot VI Cg 30	0.30	N.D.	N.D.	N.D.	N.D.
190	Maniwaki Plot VI Pm 34	0.26	N.D.	N.D.	N.D.	N.D.
191	Maniwaki Plot VI Pm 41	0.31	N.D.	N.D.	0.22	0.22
192	Maniwaki Plot VI Cg 55	0.23	0.04	8.99	N.D.	9.03
193	Maniwaki Plot VI Cg 64	0.42	N.D.	0.42	N.D.	0.42
194	Maniwaki Plot VI Cg 79	0.23	N.D.	T	T	T
195	Maniwaki Plot VI Cg 83	0.30	N.D.	N.D.	T	T
196	Maniwaki Plot VI Pm 112	0.31	N.D.	N.D.	N.D.	N.D.
197	Maniwaki Plot VI Pm 127	0.29	N.D.	0.30	0.13	0.43
198	Maniwaki Plot VI Pm 149	0.30	N.D.	T	N.D.	T
199	Maniwaki Plot VI Pm 151	0.30	N.D.	N.D.	N.D.	N.D.
200	Maniwaki Plot VI Pm 158	0.31	N.D.	0.15	N.D.	0.15
201	Maniwaki Plot VI Pm 159	0.33	N.D.	0.24	N.D.	0.24
202	Maniwaki Plot VI Ts 54	0.96	0.05	N.D.	N.D.	0.05
203	Maniwaki Plot VI Ts 163	1.30	0.32	N.D.	0.09	0.41

204	California Lake	20	0.39	N.D.	N.D.	T	T
205	California Lake	21	0.23	N.D.	T	0.13	0.13
206	California Lake	22	0.21	N.D.	0.02	N.D.	0.02
207	California Lake	6	0.33	T	T	N.D.	T
208	California Lake	7	0.31	N.D.	T	N.D.	T
209	California Lake	8	0.29	T	N.D.	N.D.	T
210	California Lake	9	0.24	N.D.	N.D.	T	T
211	California Lake	10	0.30	N.D.	0.01	T	0.01
212	California Lake	11	0.29	N.D.	T	N.D.	T
213	California Lake	12	0.19	T	N.D.	N.D.	T
214	California Lake	13	0.31	N.D.	T	N.D.	T
215	California Lake	14	0.28	T	0.03	0.01	0.04
216	California Lake	15	0.27	N.D.	T	N.D.	T
217	California Lake	16	0.31	N.D.	0.01	N.D.	0.01
218	California Lake	17	0.40	N.D.	0.02	T	0.02
219	California Lake	18	0.36	N.D.	0.03	N.D.	0.03
220	California Lake	19	0.32	N.D.	N.D.	T	T
221	Progeny	♀ Cg 1	5.40	T	N.D.	T	T
222	Progeny	♂ Cg 2	4.90	T	N.D.	T	T
223	Progeny	♂ Cg 3	5.20	T	N.D.	T	T

224	Progeny	♀ Cg 4	3.75	0.01	N.D.	T	0.01
225	Progeny	♀ Cg 5	4.70	T	N.D.	0.01	0.01

N.D. = Not detected

T = Traces (<0.005 ppm)

TABLE 3

Analysis of DDT Residues in Mice Brains from Manitoba

Serial Number	Identification No.	Species	Mass (gm)	DDT Residues (ppm) DDE	o,p-DDT	p,p'-DDT	Total DDT (ppm)
226	Sprucewoods Aug. 6, 1973 Fenitro-2 #2	Pm	0.50	T**	T**	0.012	0.012
227	Sprucewoods Aug. 4, 1973 Fenitro-2 #1A	Cg embryos	0.11	T**	0.030	0.050	0.080
228	Sprucewoods Aug. 4, 1973 Fenitro-2 #1	Cg	0.60	T**	0.009	0.016	0.025
229	Belair Aug. 3, 1973 West - 2 #2	Pm	0.52	T**	T**	0.014	0.014
230	Belair Aug. 3, 1973 West - 2 #1	Cg	0.42	T**	N.D.	0.015	0.015
231	Sprucewoods Aug. 4, 1973 Sevin - 1 #1	Pm	0.50	T**	0.009	0.010	0.019
232	Sprucewoods Aug. 6, 1973 Sevin - 1 (N) #7	Pm	0.41	0.008	0.006	0.008	0.022
233	Sprucewoods Aug. 5, 1973 Sevin - 1 #3	Sc	0.11	0.031	0.090	0.105	0.226
234	Sprucewoods Aug. 5, 1973 Sevin - 1 #4	Cg	0.51	T**	T**	0.008	0.008
235	Sprucewoods Aug. 5, 1973 Sevin - 1 #6	Cg	0.42	T**	T**	T**	T**
236	Sprucewoods Sevin-1 #1E	Pm embryo	0.92	T**	0.005	0.005	0.010
237	Sprucewoods Sevin-1 #1C	Pm embryo	0.94	T**	N.D.	0.005	0.005

238	Sprucewoods Aug. 5, 1973 Sevin - 1 #5	Cg	0.42	0.009	N.D.	0.015	0.024
239	Sprucewoods Aug. 5, 1973 Sevin - 1 #2	Pm	0.31	0.010	0.016	0.018	0.044
240	Sprucewoods Sevin-1 #1B	Pm embryo	1.41	T**	0.004	0.006	0.010
241	Sprucewoods Sevin-1 #1A	Pm embryo	1.11	0.003	T**	0.013	0.016
242	Sprucewoods Sevin-1 #1D	Pm embryo	1.10	T**	0.004	0.007	0.011
243	Belair Aug. 3, 1973 West-1 #2	Cg	0.40	0.011	0.014	0.026	0.051
244	Belair Aug. 1973 West-1 #1	Least Chipmunk	1.44	T**	T**	T**	T**
245	Sprucewoods Aug. 6, 1973 Sevin - 2 #4	Sc	0.11	0.039	N.D.	0.071	0.110
246	Sprucewoods Aug. 6, 1973 Sevin - 2 (S) #5	Sc	0.12	T**	N.D.	N.D.	T**
247	Sprucewoods Aug. 4, 1973 Sevin - 2 #1	Cg	0.21	0.014	0.021	0.070	0.105
248	Sprucewoods Aug. 5, 1973 Sevin - 2 (S) #3	Cg	0.31	0.012	0.075	0.015	0.102
249	Sprucewoods Aug. 5, 1973 Sevin - 2 (S) #2	Cg	0.41	T**	N.D.	N.D.	T**
250	Belair Aug. 1, 1973 Control -2 #4	Cg	0.51	T**	N.D.	N.D.	T**
251	Belair Aug. 3, 1973 Control -2 #9	Least Chipmunk	1.31	T**	N.D.	N.D.	T**
252	Belair Aug. 1, 1973 Control -2 #2	Least Chipmunk	0.31	0.010	0.015	0.043	0.068
253	Belair Aug. 1, 1973 Control -2 #1	Least Chipmunk	0.32	T**	0.032	0.028	0.060

254	Belair Aug. 1, 1973 Control -2 #5	Cg	0.51	0.007	0.011	0.018	0.036
255	Belair Aug. 2, 1973 Control -2 #7	Least Chipmunk	1.42	T**	N.D.	0.003	0.003
256	Belair Aug. 1, 1973 Control -2 #3	Pm	0.11	0.025	0.060	0.080	0.165
257	Belair Aug. 2, 1973 Control -2 #8	Pm	0.41	T**	N.D.	0.009	0.009
258	Belair Aug. 2, 1973 Control -2 #6	Least Chipmunk	1.41	0.003	0.009	0.006	0.018
259	Sprucewoods Aug. 5, 1973 BT-2 #1	Least Chipmunk	1.72	0.003	T**	0.004	0.007
260	Sprucewoods Aug. 6, 1973 BT-2 #2	Pm	0.43	T**	N.D.	0.006	0.006
261	Belair Control - 1 #1E	Cg embryo	0.11	0.039	0.106	0.059	0.204
262	Belair Aug. 3, 1973 Control -1 #2	Least Chipmunk	1.42	T**	0.003	0.007	0.010
263	Belair Control -1 #1F	Cg embryo	0.11	T**	0.114	0.071	0.185
264	Belair Control -1 #1B	Cg embryo	0.11	0.034	0.095	0.053	0.182
265	Belair Aug. 2, 1973 Control -1 #1	Cg	0.31	T**	T**	0.045	0.045
266	Belair Control -1 #1D	Cg embryo	0.11	T**	0.023	0.034	0.057
267	Belair Control -1 #1A	Cg embryo	0.11	T**	N.D.	0.026	0.026
268	Belair Control -1 #1C	Cg embryo	0.11	T**	N.D.	0.026	0.026
269	Sprucewoods Aug. 6, 1973 Fenitro-1 #1	Zh	0.41	0.005	0.006	0.018	0.029

270	Belair Aug. 1, 1973 East - 2 #4	Least Chipmunk	1.41	T**	0.003	0.014	0.017
271	Belair Aug. 1, 1973 East - 2 #3	Least Chipmunk	1.51	T**	T**	0.003	0.003
272	Belair Aug. 2, 1973 East - 2 #1	Pm	0.51	T**	N.D.	0.010	0.010
273	Belair Aug. 3, 1973 East - 2 #6	Cg	0.21	0.013	T**	0.025	0.038
274	Belair Aug. 2, 1973 East - 2 #2	Least Chipmunk	1.51	0.003	T**	0.005	0.008
275	Sprucewoods Aug. 4, 1973 BT-4 #1	Pm	0.41	T**	0.013	0.018	0.031
276	Sprucewoods Aug. 5, 1973 BT-4 #3	Pm	0.42	T**	T**	0.013	0.013
277	Sprucewoods Aug. 5, 1973 BT-4 #2	Pm	0.31	T**	0.007	0.027	0.034
278	Sprucewoods BT-4 #3A	Pm embryos	0.11	T**	T**	T**	T**
279	Belair Aug. 1973 East -1 #1	Em	1.51	T**	0.009	0.009	0.018
280	Belair Aug. 2, 1973 East -1 #7	Em	1.41	0.003	0.003	0.034	0.040
281	Belair Aug. 3, 1973 East -1 #8	Ct	2.01	T**	T**	0.004	0.004
282	Belair Aug. 1, 1973 East -1 #2	Em	1.41	T**	T**	0.005	0.005
283	Belair Aug. 3, 1973 East -1 #9	Em	1.11	0.004	0.008	0.007	0.019
284	Belair Aug. 3, 1973 East -1 #3	Em	1.00	0.003	0.011	0.010	0.024

285	Belair Aug. 2, 1973 East -1 #5	Em	1.31	T**	T**	0.003	0.003
286	Belair Aug. 3, 1973 East -1 #4	Em	1.22	T**	T**	0.006	0.006
287	Belair Aug. 3, 1973 East -1 #10	Em	1.11	T**	T**	0.004	0.004
288	Belair Aug. 2, 1973 East -1 #6	Em	1.51	0.003	0.004	0.004	0.011

T** = Trace (<0.003 ppm)

N.D. = Not Detected

TABLE 4

Analysis of DDT Residues in Mice Brains - North Vancouver Island

Serial Number	Sample Description	Mass (g)	DDE (ppm)	o,p-DDT (ppm)	p,p'-DDT (ppm)	Total DDT (ppm)
289	Keogh R.B.C. Aug 21, 1973 Plot 12 #7 Pm	0.60	0.012	0.020	T	0.032
290	Keogh L.B.C. Aug 21, 1973 Plot 7A #7 Pm ♂	0.30	T	T	N.D.	T
291	Keogh L.B.C. Aug 19, 1973 Plot 7 #5 Pm ♀	0.10	T	T	N.D.	T
292	Keogh R.B.C. Aug 19, 1973 Plot 12A #2 Pm ♂	0.10	T	T	N.D.	T
293	Keogh R.B.C. Aug 19, 1973 Plot 12A #3 Pm ♂	0.10	0.017	T	0.046	0.063
294	Keogh R.B.C. Aug 21, 1973 Plot 12A #17 Pm ♂	0.60	T	T	0.014	0.014
295	Keogh L.B.C. Aug 19, 1973 Plot 7A #2 Pm	0.50	T	T	T	T
296	Keogh R.B.C. Aug 19, 1973 Plot 12A #5 Pm ♀	0.50	T	T	T	T
297	Keogh L.B.C. Aug 19, 1973 Plot 7 #4 Pm ♂	0.10	T	0.015	T	0.015
298	3 Island L.B.C. Aug 19, 1973 # 6 Pm ♀	0.10	0.016	0.032	0.066	0.114
299	3 Island L.B.C. Aug 21, 1973 #12 Pm ♂	0.60	T	T	T	T
300	Keogh R.B.C. Aug 19, 1973 Plot 12A #4 Pm ♂	0.60	T	T	T	T

301	3 Island L.B.C. Aug 21, 1973 # 14 Pm ♂	0.40	0.010	T	T	0.010
302	Maynard L.B.C. Aug 21, 1973 Plot 9 #6 Pm ♂	0.60	T	T	0.010	0.010
303	Keogh L.B.C. Aug 19, 1973 Plot 7 #2 Pm ♂	0.50	T	T	T	T
304	Keogh L.B.C. Aug 19, 1973 Plot 7B #2 Pm ♂	0.10	T	N.D.	0.035	0.035
305	Keogh R.B.C. Aug 19, 1973 Plot 12 #1 Pm ♀	0.50	T	N.D.	T	T
306	Keogh L.B.C. Aug 19, 1973 Plot 7A #4 Pm ♂	0.10	T	T	T	T
307	Keogh L.B.C. Aug 19, 1973 Plot 7B #1 Pm ♀	0.10	T	T	0.048	0.048
308	3 Island L.B.C. Aug 19, 1973 # 8 Pm ♂	0.10	T	T	T	T
309	Keogh R.B.C. Aug 21, 1973 Plot 12A #15 Pm	0.40	0.004	T	0.020	0.024
310	Keogh L.B.C. Aug 19, 1973 Plot 7 #1 Pm ♂	0.10	T	N.D.	0.051	0.051
311	3 Island L.B.C. Aug 19, 1973 #2 Pm ♂	0.10	T	T	0.048	0.048
312	Keogh L.B.C. Aug 19, 1973 Plot 7 #3 Pm ♂	0.20	T	T	0.019	0.019
313	Keogh R.B.C. Aug 20, 1973 Plot 12 #2 Pm	0.50	0.003	T	0.010	0.013
314	Keogh R.B.C. Aug 20, 1973 Plot 7 #12 Pm	0.50	T	T	0.012	0.012

315	Keogh L.B.C. Aug 19, 1973 Plot 7 #7 Pm ♂	0.20	T	T	0.018	0.018
316	3 Island L.B.C. Aug 13, 1973 #7 Pm ♀	0.10	T	T	0.045	0.045
317	Maynard L.B.C. Aug 20, 1973 #3 Pm ♂	0.05	0.004	0.006	0.045	0.055
318	Keogh R.B.C. Aug 20, 1973 Plot 7 #12 Pm	0.10	T	T	0.042	0.042
319	Keogh L.B.C. Aug 21, 1973 Plot 7 #13 Pm ♀	0.40	0.005	0.008	0.025	0.038
320	3 Island L.B.C. Aug 21, 1973 # 13 Pm ♂	0.30	T	N.D.	T	T
321	Keogh R.B.C. Aug 20, 1973 Plot 7 #11 Pm ♂	0.50	T	T	T	T
322	Keogh R.B.C. Aug 21, 1973 Plot 12A #16 Pm ♂	0.50	T	T	0.006	0.006
323	3 Island L.B.C. Aug 19, 1973 #3 Pm ♀	0.30	T	T	0.012	0.012
324	Maynard L.B.C. Aug 21, 1973 Plot 9 #7 Pm ♂	0.60	T	T	T	T
325	Keogh R.B.C. Aug 20, 1973 Plot 12A #12 Pm ♂	0.50	T	T	0.008	0.008
326	Maynard L.B.C. Aug 20, 1973 #4 Pm ♂	0.50	T	0.006	T	0.006
327	Keogh R.B.C. Aug 20, 1973 Plot 12 #3 Pm	0.60	T	0.004	T	0.004
328	Keogh R.B.C. Aug 20, 1973 Plot 12 #6 Pm	0.50	T	T	0.009	0.009

329	3 Island L.B.C. Aug 19, 1973 # 9 Pm ♀	0.40	T	T	0.015	0.015
330	Keogh L.B.C. Aug 21 1973 Plot 7A #6 Sc	0.20	T	T	0.021	0.021
331	Maynard L.B.C. Aug 21, 1973 Plot 9 #9 ♂	0.60	T	T	T	T
332	3 Island L.B.C. Aug 20, 1973 # 11 Pm	0.50	T	T	T	T
333	Keogh R.B.C. Aug 20, 1973 Plot 12A #8 Pm ♂	0.10	T	T	0.024	0.024
334	3 Island L.B.C. Aug 20, 1973 # 10 Pm ♀	0.40	0.006	T	0.007	0.013
335	Keogh R.B.C. Aug 20, 1973 Plot 7B #7 Pm ♂	0.50	T	T	0.011	0.011
336	Maynard L.B.C. Aug 19, 1973 # 1 Pm ♀	0.10	T	T	0.011	0.011
337	Keogh R.B.C. Aug 20, 1973 Plot 12A #9 Pm	0.10	T	T	T	T
338	Keogh R.B.C. Aug 20, 1973 Plot 7B #6 Pm ♂	0.40	T	T	T	T
339	Keogh L.B.C. Aug 20, 1973 Plot 7A #5 Pm ♂	0.70	T	T	T	T
340	3 Island L.B.C. Aug 19, 1973 # 1 Pm ♂	0.60	0.003	T	T	0.003
341	Keogh L.B.C. Aug 21, 1973 Plot 7B #8 Pm ♂	0.30	T	T	0.013	0.013
342	Keogh R.B.C. Aug 20, 1973 Plot 7B #5 Pm ♀	0.60	T	T	0.005	0.005

343	Keogh R.B.C. Aug 20, 1973 Plot 12A #14 Pm	0.10	T	T	T	T
344	Keogh R.B.C. Aug 20, 1973 Plot 7 #9 Pm ♀	0.20	T	T	0.013	0.013
345	Keogh R.B.C. Aug 20, 1973 Plot 7 #10 Pm ♀	0.70	T	T	0.006	0.006
346	Keogh R.B.C. Aug 20, 1973 Plot 12 #13 Pm ♀	0.10	T	T	0.035	0.035
347	Keogh R.B.C. Aug 20, 1973 Plot 12 #5 Pm	0.60	T	T	0.007	0.007
348	Keogh R.B.C. Aug 20, 1973 Plot 12A #7 Pm	0.20	T	T	T	T
349	3 Island L.B.C. Aug 19, 1973 #5 Pm ♂	0.10	0.015	T	0.108	0.123
350	Keogh R.B.C. Aug 20, 1973 Plot 12 #4 Pm	0.10	T	T	0.060	0.060
351	Keogh R.B.C. Aug 20, 1973 Plot 12A #11 Pm ♂	0.10	0.017	T	0.470	0.487
352	Keogh R.B.C. Aug 20, 1973 Plot 12A #10 Pm	0.50	0.003	T	0.016	0.019
353	Maynard L.B.C. Aug 21, 1973 Plot 9 #8 Pm ♂	0.70	T	T	T	T
354	Keogh R.B.C. Aug 20, 1973 Plot 7B #4 Pm ♂	0.50	T	T	T	T
355	Keogh R.B.C. Aug 20, 1973 Plot 7B #3 Pm	0.30	T	N.D.	T	T
356	3 Island L.B.C. Aug 19, 1973 # 4 Pm ♀	0.50	T	N.D.	T	T

357	Keogh R.B.C. Aug 20, 1973 Plot 12A #6 Pm	0.50	T	T	0.021	0.021
358	Keogh L.B.C. Aug 19, 1973 Plot 7 #6 Pm ♂	0.10	T	T	0.105	0.105
359	Keogh L.B.C. Aug 19, 1973 Plot 7A #3 Pm	0.10	0.014	T	0.074	0.088
360	Keogh R.B.C. Aug 20, 1973 Plot 7 #8 Pm ♀	0.50	T	T	0.012	0.012
361	Maynard L.B.C. Aug 20, 1973 #5 Pm ♂	0.60	T	T	0.018	0.018
362	Maynard L.B.C. Aug 19, 1973 #2 Pm ♂	0.10	T	T	0.032	0.032

T = Trace (< 0.002 ppm)

N.D. = Not detected.

TABLE 5

Analysis of DDT Residues in Mice Brains - Anticosti Island

Serial Number	Sample Description	Mass (g)	DDE (ppm)	o,p-DDT (ppm)	p,p'-DDT (ppm)	Total DDT (ppm)
363	Anticosti Is. Sept. 21, 1973 Plot A #4 Pm ♀	0.40	T	N.D.	0.362	0.362
364	Anticosti Is. Sept. 20, 1973 Plot A, Line 1 #1 Pm ♂	0.50	T	T	0.148	0.148
365	Anticosti Is. Sept. 21, 1973 Plot A #3 Pm ♂	0.80	T	T	0.040	0.040
366	Anticosti Is. Sept. 21, 1973 Plot D #1 Pm. ♀	0.80	T	T	0.008	0.008
367	Anticosti Is. Sept 19, 1973 Plot A #1 Pm ♀	0.60	T	T	0.012	0.012
368	Anticosti Is. Sept. 20, 1973 Plot A #2 Pm ♀	0.50	T	T	T	T
369	Anticosti Is. Sept. 20, 1973 Plot B, Line 2 #1 Pm. ♀	0.40	0.004	T	0.008	0.012

T = Trace (0.002 ppm)

N.D. = Not Detected.

TABLE 6

Analysis of DDT Residues in Forest Slugs - North Vancouver Island

Serial Number	Sample Description	Mass (g)	DDE (ppb)	o,p-DDT (ppb)	p,p'-DDT (ppb)	Total DDT (ppb)
370	Keogh R.B.C. Aug., 1973 Plot 12-12A DDT/57 Forest Slugs Slug #1	20.0	0.6	0.6	1.2	2.4
371	Keogh R.B.C. Aug., 1973 Plot 12-12A DDT/57 Forest Slugs Slug #2	20.0	0.3	0.7	T	1.0
372	Keogh L.B.C. Aug. 20, 1973 Plot 7B Forest Slugs Slug #1	20.0	0.4	1.0	0.9	2.3
373	Keogh L.B.C. Aug. 20, 1973 Plot 7B Forest Slugs Slug #2	20.0	T	T	T	T
374	Keogh, L.B.C. Aug. 20, 1973 Plot 7B Forest Slugs Slug #3	20.0	0.3	0.5	T	0.8

T = Trace (<0.2 ppb)

TABLE 7

Analysis of DDT Residues* in Five Fish and Larva Samples

Keough River - Vancouver Island

Serial No.	Identification No.	Mass of the Sample (g)	Concentration (ppm) of DDT Residues **				
			DDE	o,p-DDT	DDD	p,p'-DDT	Total DDT
375	Pacific Salmon Parr	5.10	0.002	0.001	0.001	0.002	0.006
376	Rainbow Trout Parr	5.10	0.002	0.002	0.003	0.007	0.015
377	Rainbow Trout	5.00	0.002	0.001	0.001	0.003	0.007
378	Freshwater Sculpins	3.50	0.001	0.001	0.001	0.001	0.004
379	Caddisfly Larva +	0.50	0.007	0.011	0.010	0.024	0.052

* Average of two determinations

** Residue concentrations are expressed on wet weight basis.

\+ Results uncertain due to insufficient sample.

TABLE 8

Analysis of DDT Residues* in Manitoba Soil: Spruce-wood Area, 1973

Serial No.	Identification No.	Wt of Soil (g)	Moisture Content (percent)	Soil pH	Concentration (ppm) of DDT Residues**				
					DDE	o,p-DDT	DDD	p,p'-DDT	Total DDT
380	Belair No. 1 Control	50	11.0	6.54	0.002	0.001	N.D.	0.004	0.007
381	Belair No. 1 East	50	9.0	5.90	0.001	0.001	N.D.	0.002	0.004
382	Belair No. 1 West	50	8.5	5.68	0.001	0.001	N.D.	0.004	0.006
383	Line 1 Scots Pine	50	17.0	6.37	0.004	0.008	0.001	0.036	0.048
384	Belair No. 2 Control	50	7.0	6.56	0.001	0.001	N.D.	0.001	0.003
385	Belair No. 2 East	50	6.0	5.99	0.001	N.D.	N.D.	0.003	0.004
386	Belair No. 2 West	50	9.0	5.46	0.002	0.001	N.D.	0.009	0.012
387	BT 2	50	21.5	6.79	0.001	0.001	T	0.004	0.006
388	BT 4	50	19.5	6.77	0.003	0.001	T	0.003	0.007
389	Sevin	50	29.0	7.22	0.003	0.001	T	0.009	0.013

* Average of two determinations.

** DDT concentration expressed in wet weight basis

T Traces (< 0.001 ppm)

N.D. Not Detectable.

TABLE 9

Analysis of DDT Residues in Soil - North Vancouver Island

Serial No.	Sample Description	pH	Moisture %	DDE (ppb)	o,p-DDT (ppb)	p,p' DDT (ppb)	Total DDT (ppb)
390	3 Island L.B.C. Aug. 21 1973 Plot 8	4.90	53.5	4.7	1.2	28.8	34.7
391	Keogh L.B.C. Aug. 21, 1973 Plot 7B	4.70	20.5	T	T	1.0	1.0
392	Keogh L.B.C. Aug. 21, 1973 Plot 7A	4.40	20.0	0.4	T	0.3	0.7
393	Maynard L.B.C. Aug. 21, 1973 Plot 9	4.50	43.0	3.2	0.8	12.6	16.6
394	Keogh R.B.C. Aug. 21, 1973 Plot 12A	5.10	50.5	2.9	2.3	4.8	10.0
395	Keogh L.B.C. Aug. 21, 1973 Plot 7	4.50	21.5	1.4	0.6	5.4	7.4
396	Keogh R.B.C. Aug. 21, 1973 Control Plot 12	4.85	14.0	T	T	5.5	5.5

T = Trace (< 0.3 ppb)

Sample size = 50 g.

TABLE 10

Analysis of DDT Residues in Soil - Anticosti Island

Serial No.	Sample Description	pH	Moisture %	DDE (ppb)	o,p-DDT (ppb)	p,p'-DDT (ppb)	Total DDT (ppb)
397	Anticosti Is. Plot A	4.70	57.0	0.7	0.5	5.3	6.5
398	Anticosti Is. Plot B	5.05	43.0	0.3	T	4.6	4.9
399	Anticosti Is. Plot C	4.10	74.5	0.7	0.8	8.9	10.4
400	Unmarked Sample	4.40	73.0	1.8	T	3.2	5.0
401	Anticosti Is. Plot D	5.00	62.5	0.4	T	1.2	1.6

T = Trace (< 0.3 ppb)

Sample size = 50 g.

TABLE 11

Analysis of DDT Residues in Soil, Sediment, Water, Foliage, Fish, Mammal, Insect and Air

Samples Collected from Priceville Area, New Brunswick - May 1972

Serial No.	Sample Description	sample size (g)	moisture (%)	pH	Temp. (°C)	DDT (ppm) DDE	o,p-DDT	p,p'-DDT	Total DDT (ppm)
402	Soil * Plot I	50	46	6.7		0.074	0.098	0.552	0.724
403	Soil * Plot II	50	44	6.5		0.096	0.117	0.818	1.031
404	Soil * Plot III	50	41	6.4		0.058	0.076	0.543	0.677
405	Sediment 1 Crooked Bridge Brook	25	> 55	6.8		0.170	0.300	0.046	0.516
406	Sediment 2 Crooked Bridge Brook	25	"	6.6		0.163	0.419	0.098	0.680
407	Sediment 3 Crooked Bridge Brook	25	"	6.7		0.241	0.613	0.925	1.779
408	Sediment 4 Crooked Bridge Brook	25	"	6.2		0.175	0.235	0.110	0.520
409	Sediment 5 Crooked Bridge Brook	25	"	6.3		0.056	0.134	0.110	0.300
410	Water - Crooked Bridge Brook	250		6.0	12.5	T	T	0.001	0.001
411	Water - Crooked Bridge Brook	250		6.3	12.1	T	T	T	T
412	Water - Pond	250		6.7	8.3	T	T	0.008	0.008
413	Water - Creek	250		6.4	10.7	T	T	0.001	0.001
414	Water - Spring	250		6.5	11.2	N.D.	N.D.	0.002	0.002
415	Spruce* Foliage	20	37			N.D.	N.D.	0.182	0.182
416	Spruce* Foliage	20	41			N.D.	N.D.	0.193	0.193
417	Spruce* Foliage	20	39			N.D.	N.D.	0.196	0.196
418	Spruce* Foliage	20	43			0.010	0.020	0.150	0.180
419	Spruce* Foliage	20	46			0.045	0.068	0.492	0.605
420	Spruce* Foliage	20	42			0.034	0.095	0.654	0.783

421	Spruce budworm - Sample 1	4	0.178	0.024	0.108	0.310
422	Spruce budworm - Sample 2	3	0.450	0.032	0.270	0.752
423	Spruce budworm - Sample 3	5	0.033	0.023	0.120	0.176
424	Fish - Trout # 1	5	4.570	0.322	0.674	5.566
425	Fish - Trout # 2	5	3.800	0.522	1.120	5.442
426	Fish - Trout # 3	5	6.680	0.641	0.662	7.983
427	Fish - Trout # 4	5	5.390	0.747	1.301	7.438
428	Fish - Trout # 5	5	6.270	0.589	0.627	7.486
429	Fish - Trout # 6	5	8.250	0.813	0.650	9.713
430	Vole # 1 (Whole body)	2.5	0.018	0.022	0.353	0.393
431	Vole # 2 (Whole body)	1.8	0.070	0.052	0.323	0.445
432	Vole # 3 (Whole body)	2.1	0.061	0.041	0.470	0.572
433	Vole # 4 (Whole body)	2.4	0.055	0.050	0.430	0.535
434	Vole # 5 (Whole body)	1.3	0.026	0.099	0.232	0.357
435	Air samples* (in DMF bubbler) Ground level A-I		0.016	0.045	0.075	0.136
436	Air samples* 6' high A-I		0.018	0.060	0.030	0.108
437	Air samples* Ground level B-II		0.031	0.061	0.081	0.173
438	Air samples* 6' high B-II		0.031	0.085	0.037	0.153
439	Air samples* Ground level C-III		0.020	0.042	0.045	0.107
440	Air samples* 6' high C-III		0.022	0.051	0.029	0.102

T = Traces (< 0.001 ppm)

N.D. = Not detectable

* Average of two determinations.

TABLE 12

Moisture Control, pH and DDT Residues in Soil Samples Collected from Maniwaki - 1972

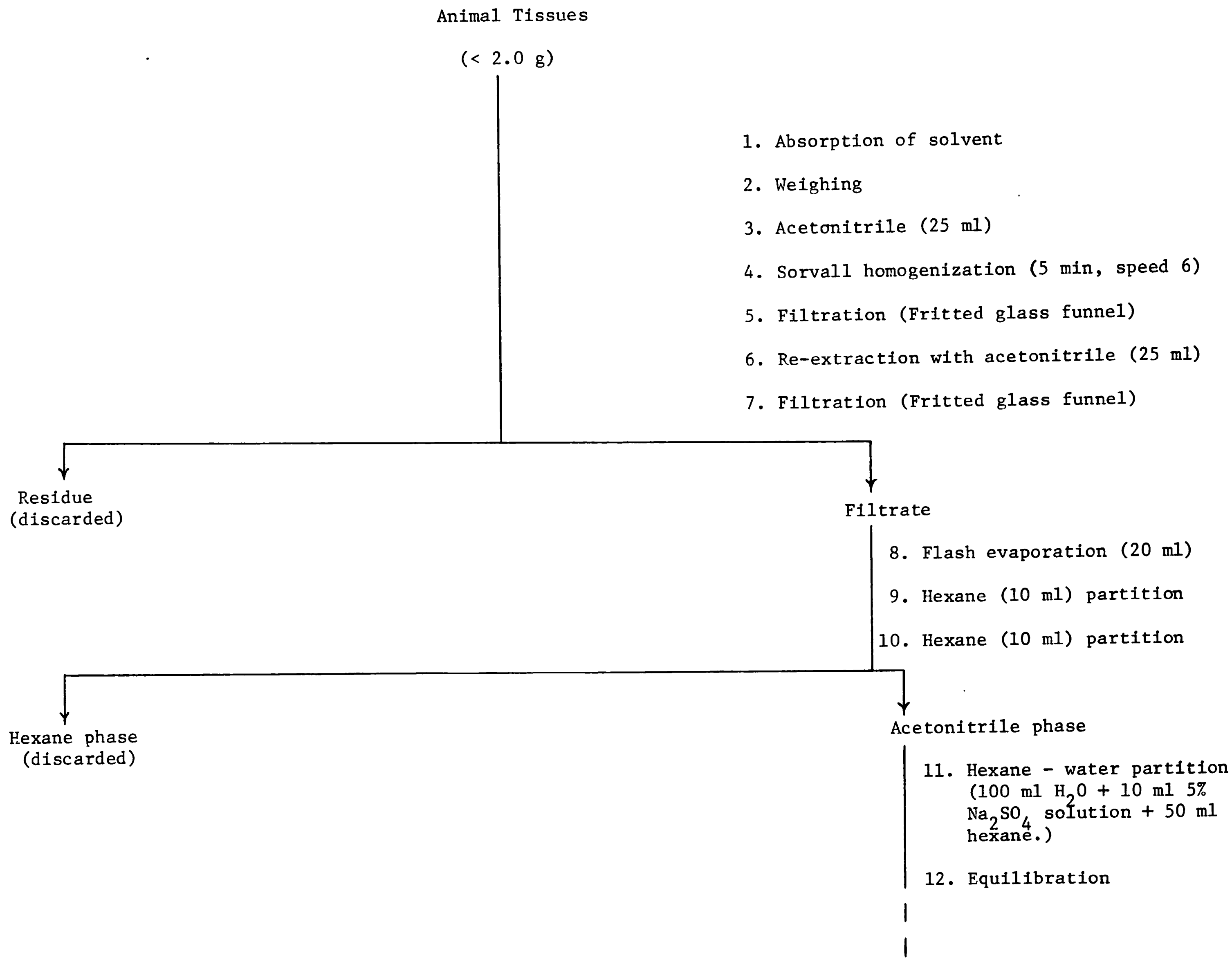
Serial No.	Plot No.	Soil Moisture (Percent)	pH	DDT Residues (ppm-wet mass)			Total DDT (ppm)
				DDE	o,p-DDT	p,p'-DDT	
441	1A	42	5.9	0.023	N.D.	0.056	0.079
442	1B	34	5.7	0.029	N.D.	0.041	0.070
443	1C	27	5.7	0.017	N.D.	0.034	0.051
444	2A	27	5.5	0.016	N.D.	0.049	0.065
445	2B	30	5.4	0.023	N.D.	0.041	0.064
446	2C	33	5.9	0.023	N.D.	0.037	0.060
447	3A	27	6.0	0.019	N.D.	0.034	0.053
448	3B	78	5.6	0.272	N.D.	0.052	0.324
449	3C	56	4.7	0.014	N.D.	N.D.	0.014
450	4A	37	5.8	0.022	N.D.	0.049	0.071
451	4B	42	5.8	N.D.	N.D.	0.086	0.086
452	4C	42	5.5	0.013	N.D.	0.047	0.060
453	5A	29	4.9	N.D.	N.D.	N.D.	N.D.
454	5B	27	5.2	0.007	N.D.	0.016	0.023
455	5C	37	4.9	0.013	N.D.	0.024	0.037
456	6A†	83	5.5	0.010	N.D.	0.037	0.047

457	6B	64	4.7	N.D.	N.D.	N.D.	N.D.
458	6C	69	4.9	N.D.	N.D.	0.072	0.072
459*		32	7.3	1.720			
460*		34	7.3	1.200			

N.D. = Not detected

† = Very wet sample, hard to sieve.

* Soil samples from the C.C.R.I. premises for comparison; chromatograms showed numerous interference peaks around the o,p and p,p'DDT peaks; so no estimatdon was made. Presence of these isomers in appreciable quantities was apparent.


Animal Tissues
(< 2.0 g)
1. Absorption of solvent
2. Weighing
3. Acetonitrile (25 ml)
4. Sorvall homogenization (5 min, speed 6)
5. Filtration (Fritted glass funnel)
6. Re-extraction with acetonitrile (25 ml)
7. Filtration (Fritted glass funnel)
Residue (discarded)
Filtrate
8. Flash evaporation (20 ml)
9. Hexane (10 ml) partition
10. Hexane (10 ml) partition
Hexane phase (discarded)
Acetonitrile phase
11. Hexane - water partition (100 ml H_2O + 10 ml 5% Na_2SO_4 solution + 50 ml hexane.)
12. Equilibration

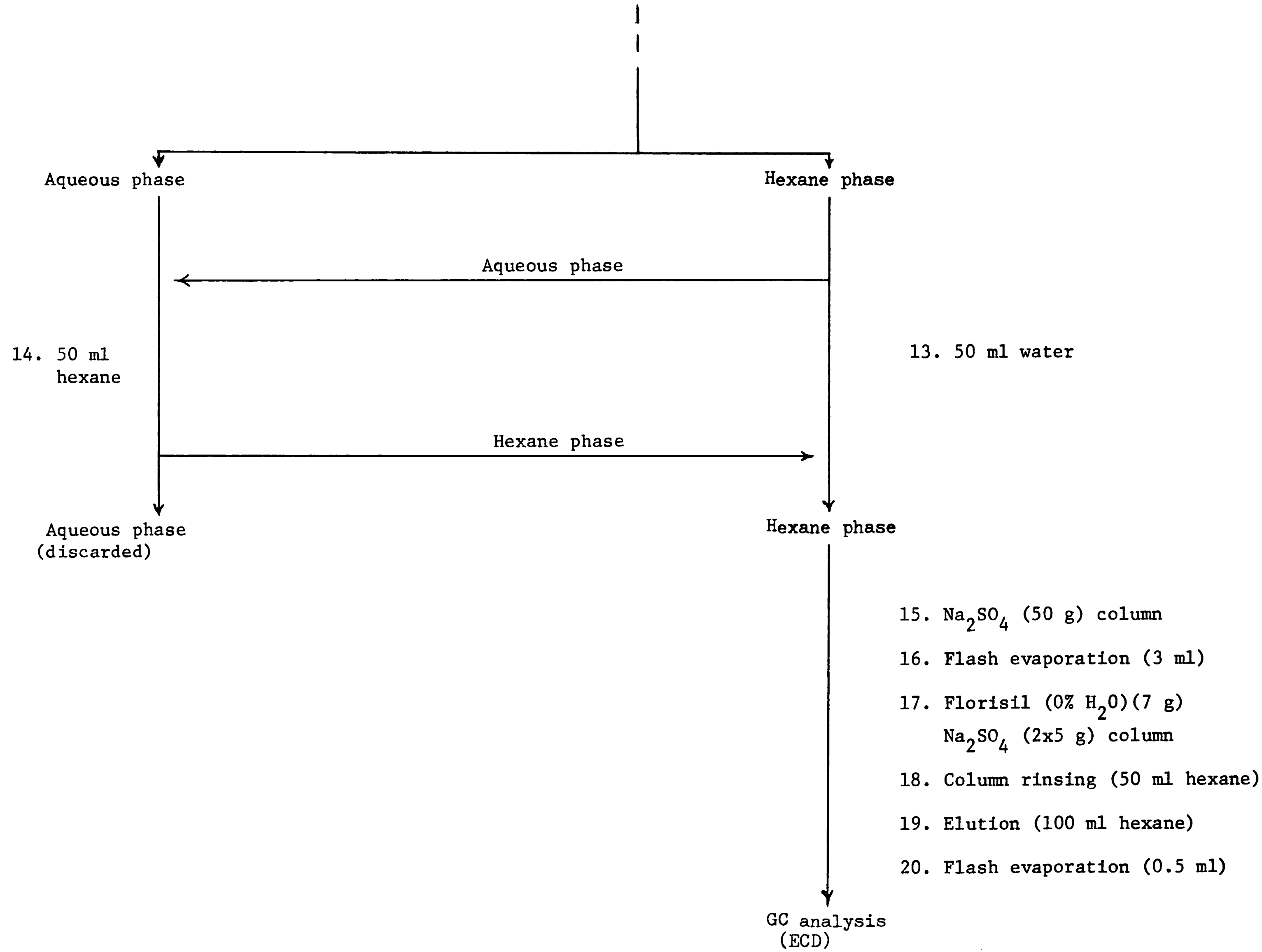
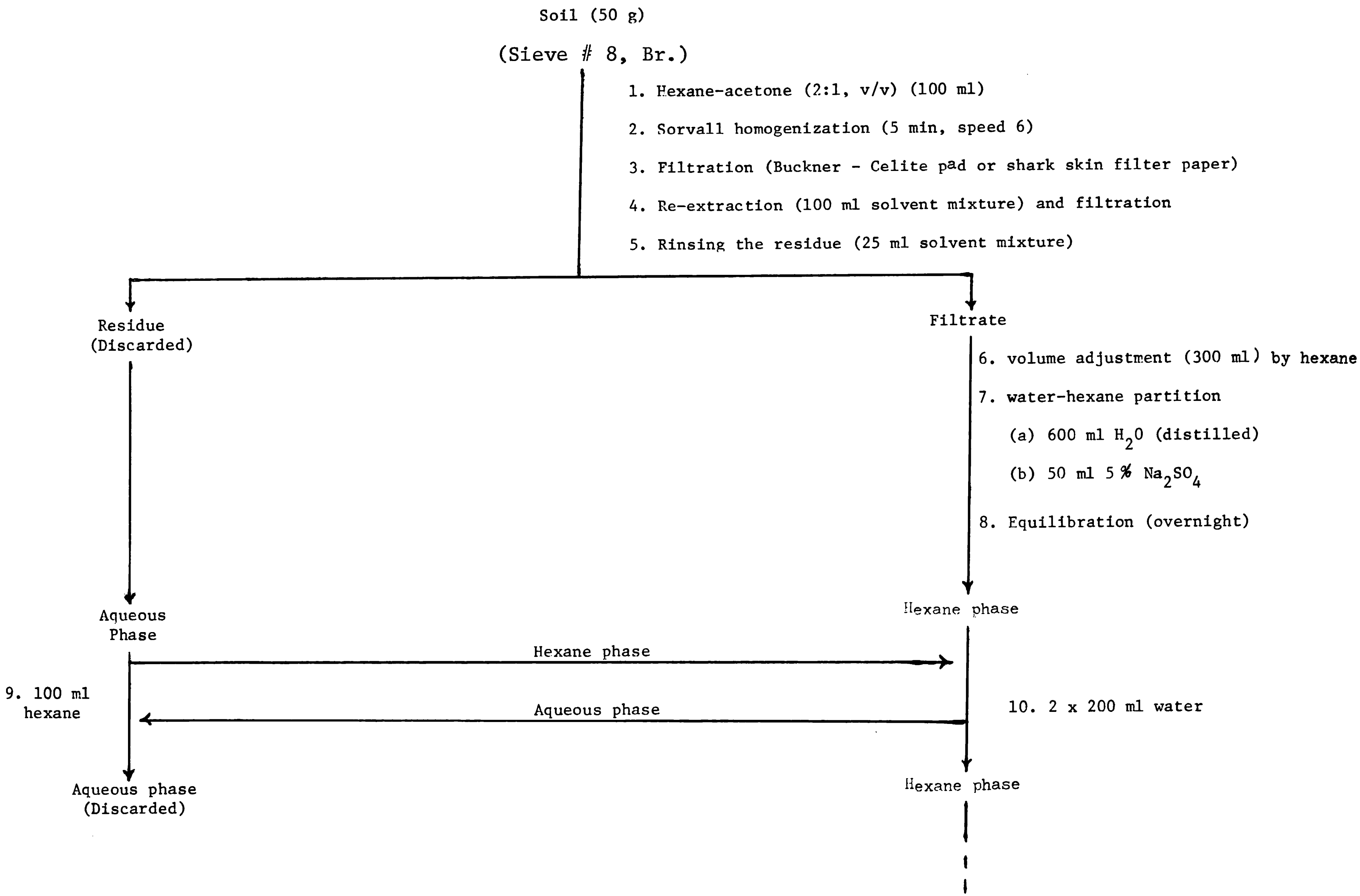


Fig. 1. Schematic representation of extraction and cleanup procedure for DDT residues in animal tissues. [The procedure was simplified for samples recorded in tables 3 to 5 by omitting steps 6,7,8,10,13 and 14].


Soil (50 g)
(Sieve # 8, Br.)
1. Hexane-acetone (2:1, v/v) (100 ml)
2. Sorvall homogenization (5 min, speed 6)
3. Filtration (Buckner - Celite pad or shark skin filter paper)
4. Re-extraction (100 ml solvent mixture) and filtration
5. Rinsing the residue (25 ml solvent mixture)
Residue
(Discarded)
Filtrate
6. volume adjustment (300 ml) by hexane
7. water-hexane partition
(a) 600 ml H2O (distilled)
(b) 50 ml 5% Na2SO4
8. Equilibration (overnight)
Aqueous
Phase
Hexane phase
Hexane phase
9. 100 ml
hexane
Aqueous phase
10. 2 x 200 ml water
Aqueous phase
(Discarded)
Hexane phase

11. Na_2SO_4 column (50 g)

12. Column rinsing (25 ml solvent mixture)

13. Flash evaporation (50 ml)

14. Florisil (0% H_2O, 20 g) column. Na_2SO_4 (10 g) sandwich

15. Column rinsing (100 ml hexane)

16. Extract (50 ml) transference

17. Elution (200 ml of 15% benzene in hexane)

↓

Eluate

18. Flash evaporation (10 ml)

↓

GC analysis (ECD)

Fig. 2 Schematic representation of extraction and cleanup procedure for DDT residues present in soil samples.

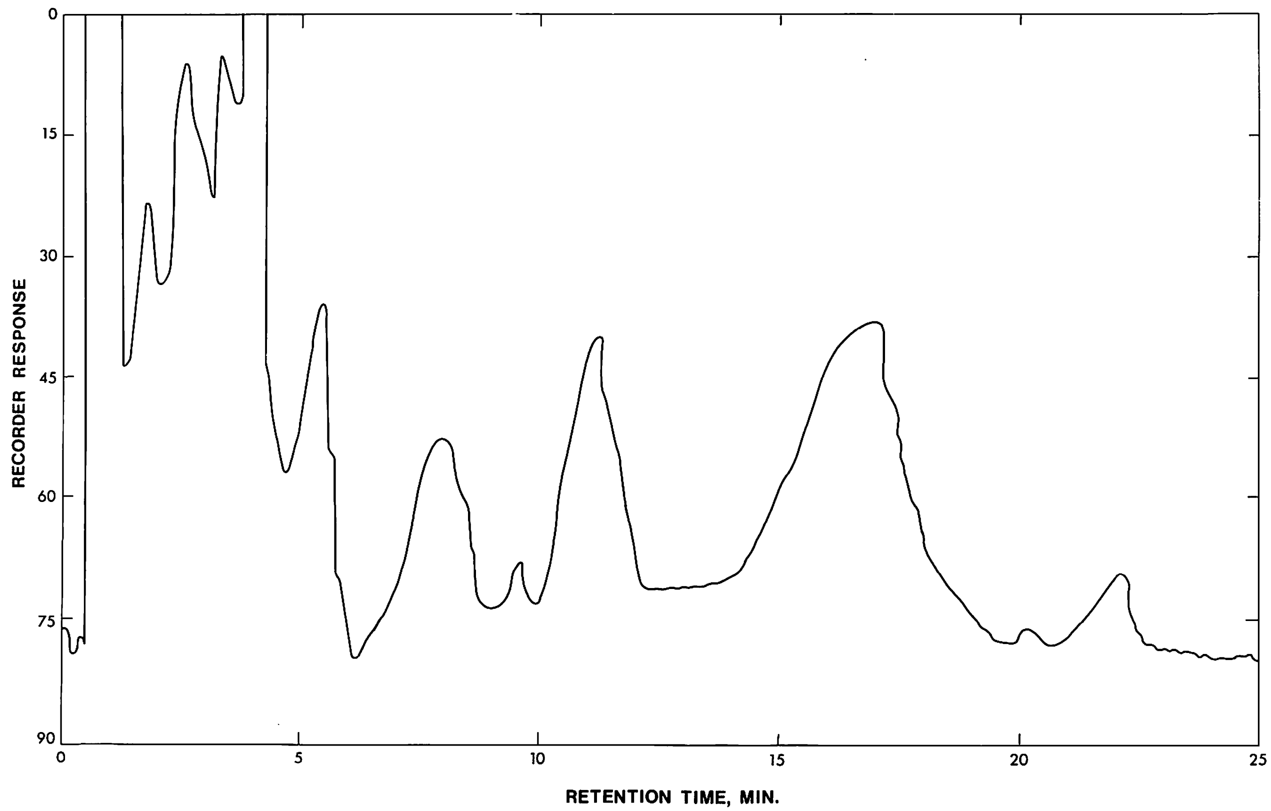


Fig. 3. Chromatogram of mouse brain extract before cleanup.

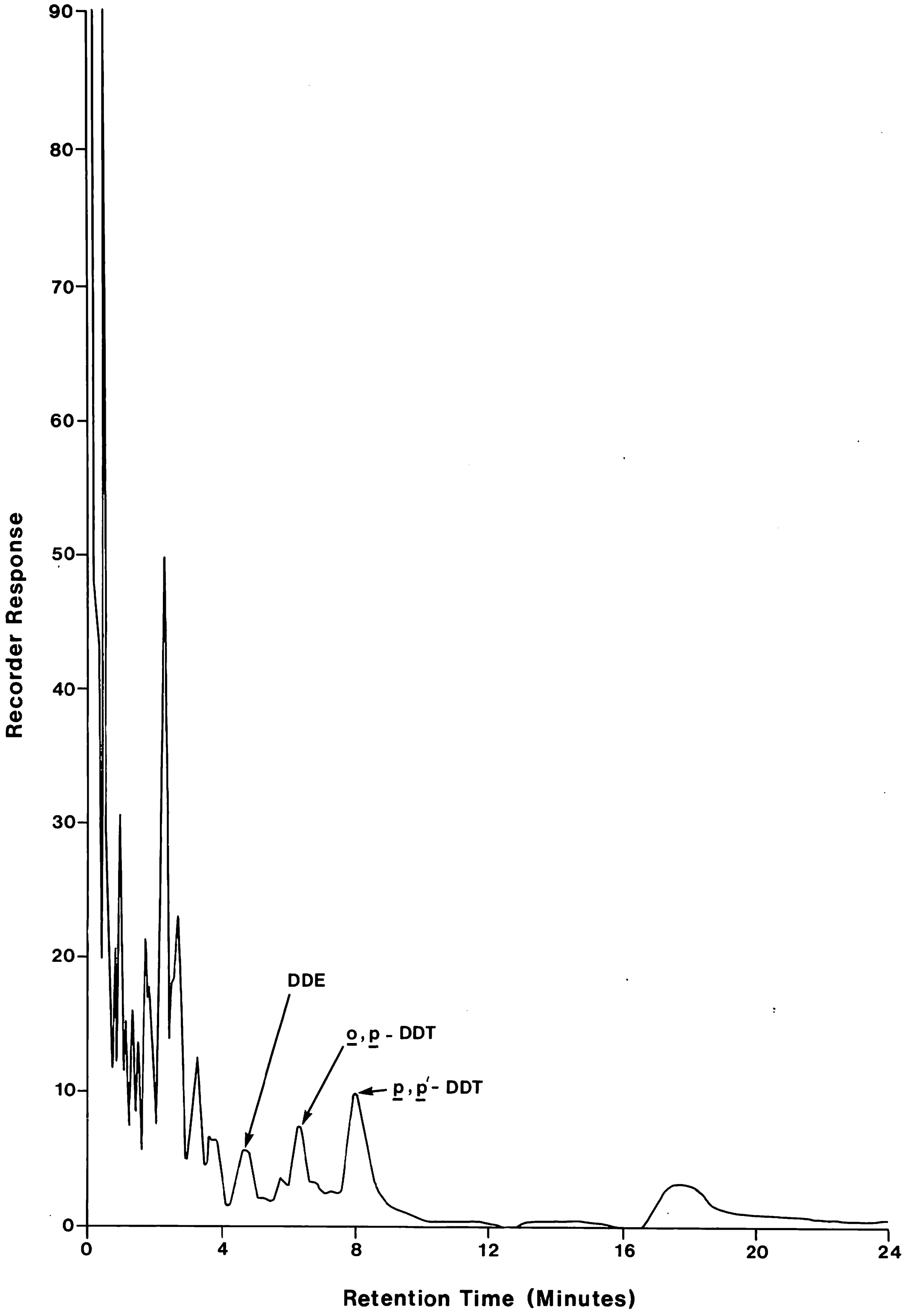


Fig. 4. Chromatogram of mouse brain extract after multistage cleanup.

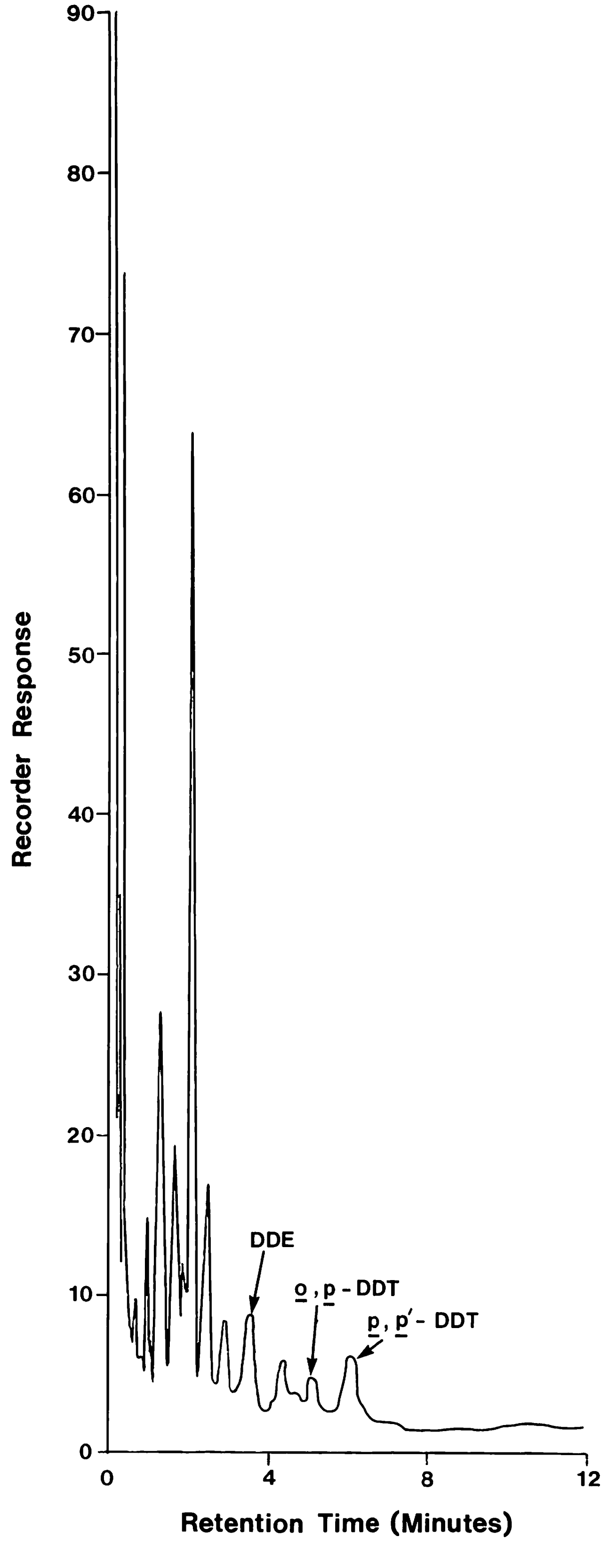


Fig. 5. Chromatogram of mouse brain extract after cleanup (Simplified).

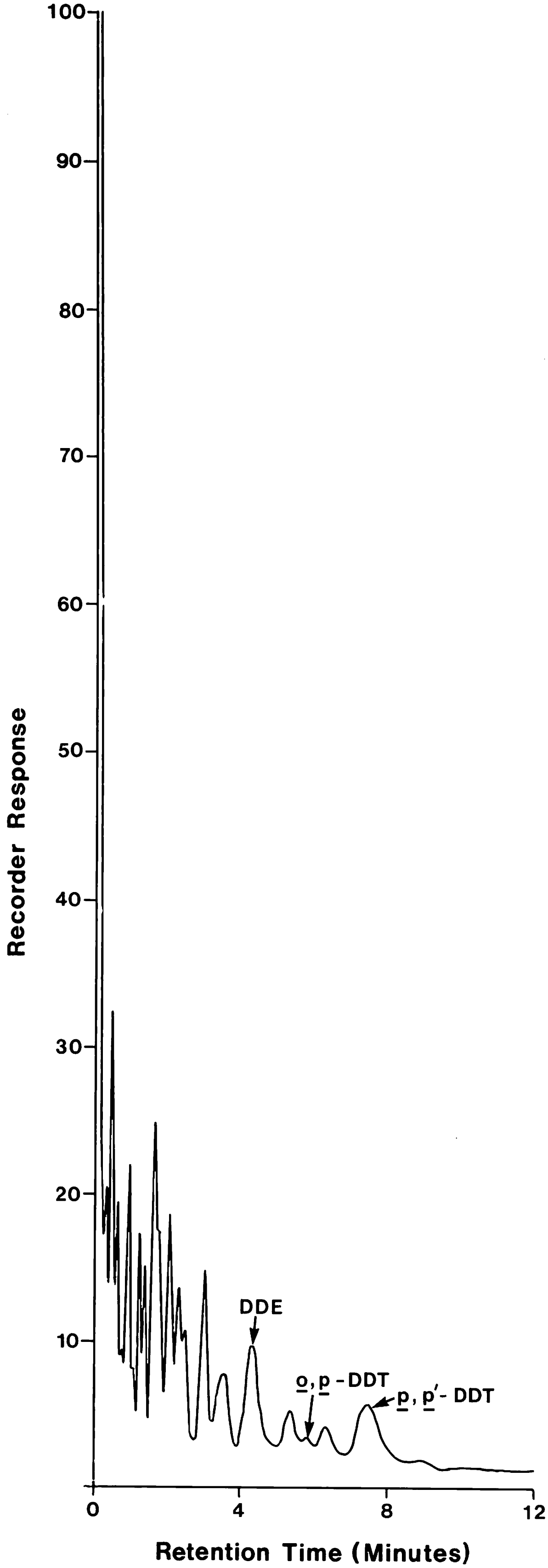


Fig. 6. Chromatogram of fish tissues after cleanup.

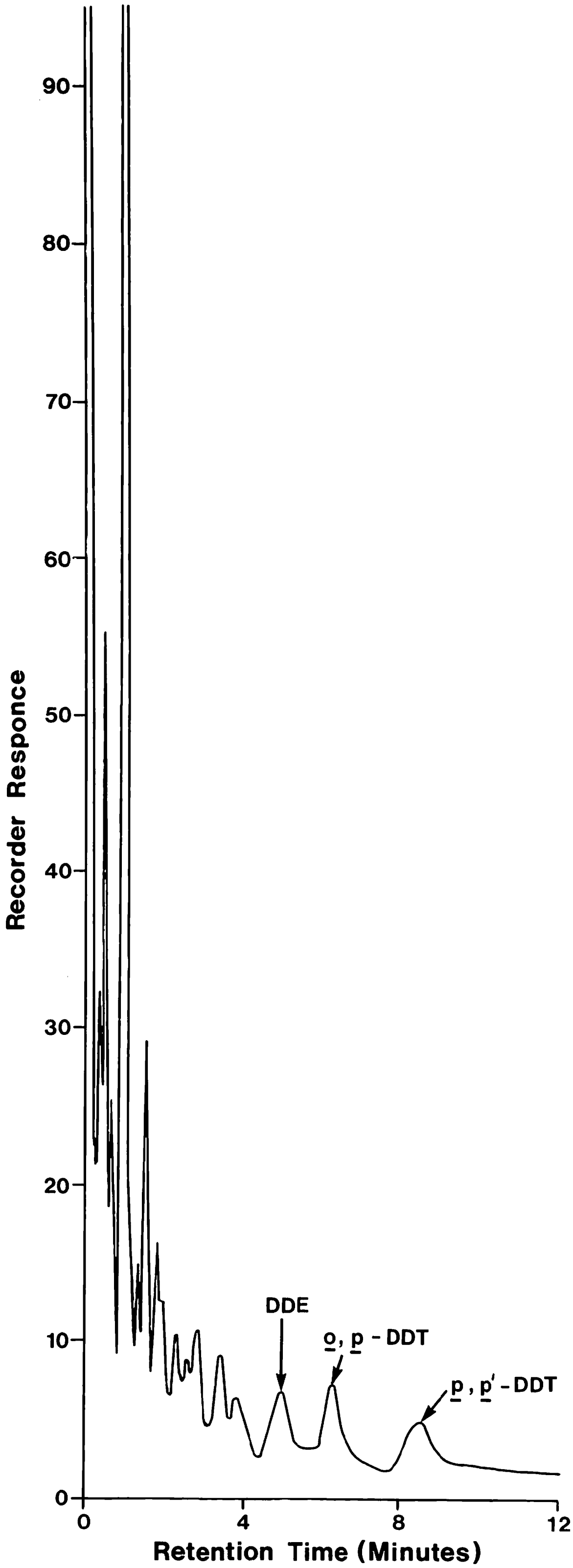


Fig. 7. Chromatogram of forest slug after cleanup.

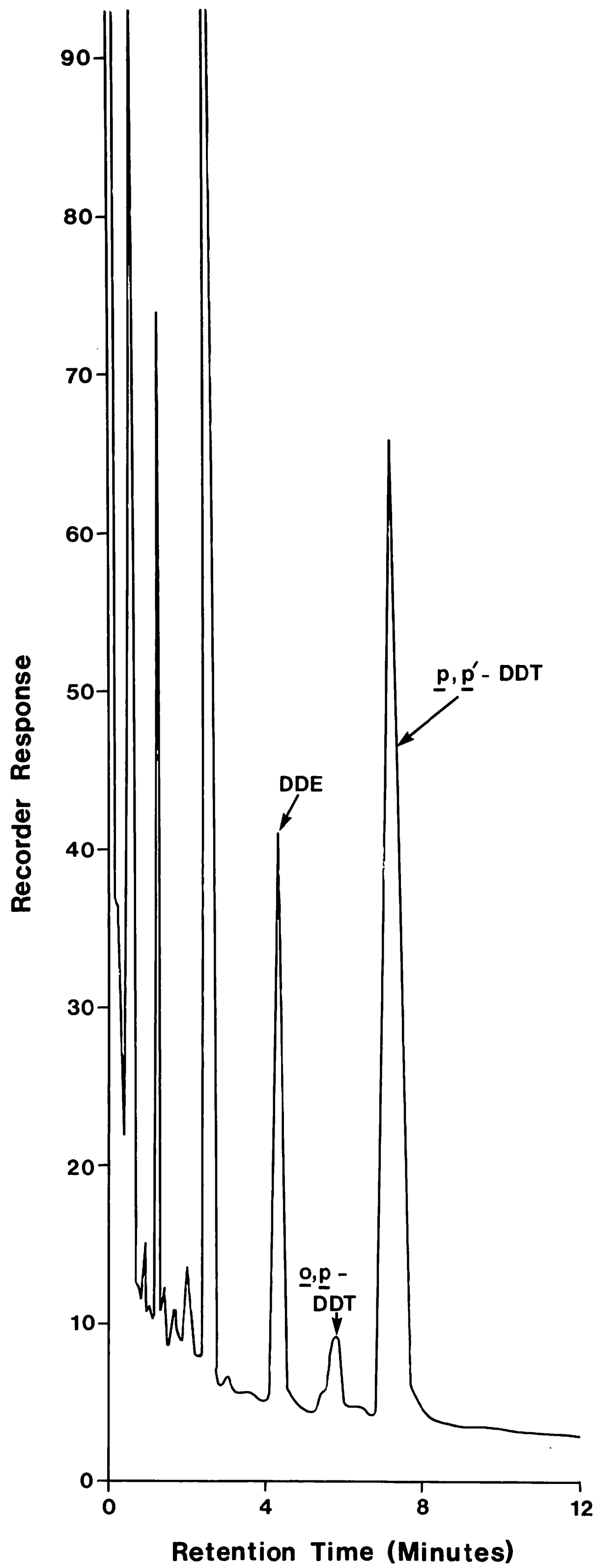


Fig. 8. Chromatogram of soil samples after cleanup.

The analytical methods used were sensitive, reliable and practicable giving minimum interference in the terminal quantitation using EC gas chromatography (see Figs. 3 to 8). The procedures might also be applied with slight modifications to other environmental samples not listed in this report. The minimum detectable limit of the DDT residues varied from 0.2 to 5 ppb depending on the co-extractive impurities present in the substrate samples. The simplified procedure used later on, in analysing some of the animal tissues (Tables 3 to 5) was found to be good demonstrating the practicality of the method developed.

Among the 460 samples of various types (animal tissues, soil, foliage water and air) collected from different parts of Canada and analysed, 445 (97%) contained the insecticide residues showing that DDT is distributed and persistent in all the components of forest and its cycling in the environment involves complex processes. So far little is known about its environmental reactions, partitioning and interactions among the various components in forest, transport in air, soil, water and living organisms. It is likely that much of the sprayed parent insecticide would have disappeared by photodegradation, microbial attack, chemical decomposition, volatilization and leaching (Gould 1966).

The DDT residues in fetus tissues of mice are recorded in Table 1. The fetuses are obtained from the mice whose brain samples were analysed for DDT residues during the early part of 1972 (Sundaram 1972). The average DDT residues (ppm) present in brain and fetus samples were as follows:

	DDE	o,p-DDT	p,p'-DDT	Total DDT
Brain	0.004	T	0.027	0.031
Fetus	0.010	0.026	0.112	0.147

The total DDT in the fetus samples was nearly five times higher than that of the brains indicating that DDT residues accumulate in the fetus tissues more than in the adipose tissues of brain.

No spray histories for the 188 mice samples (Table 2) collected from Maniwaki and California Lake areas in Quebec and 7 similar samples (Table 5) obtained from Anticosti Island were available, consequently no meaningful interpretations could be made. The average residue level in brain samples from mainland Quebec was found to be rather high, 0.988 ppm, compared with that in samples from Anticosti Island (0.082 ppm). The soil samples from these areas (Tables 10 and 12) contained only 0.065 (soil:brain, 1:15) and 0.006 (soil:brain, 1:11) ppm respectively.

The mice brain and soil samples (Tables 3 and 8) collected in 1973, 6 years after DDT application at 0.75 lbs A.I./acre, (DeBoo and Hildahl 1967) from Sprucewood area in Manitoba contained 0.037 and 0.011 ppm (soil: brain, 1:3) respectively. The DDE concentration in tissues was 35% of the total whereas in soil the amount was only 18%. Similar studies on samples received from North Vancouver Island (Keough River Basin), sprayed with DDT at the rate of 1 lb A.I./acre in 1957, showed (Tables 4 and 9) on average, the presence of 0.026 ppm of DDT residues in brains and 0.011 ppm in soils. The percent DDE concentration in the samples were 8 and 17 respectively. The concentration levels observed in these samples were lower than the earlier ones, probably due to the lapse of 16 years since the spray operation and sampling; during the long interval much of the insecticide residues were lost by various physicochemical processes outlined earlier. Forest slug (Table 6), fish and larvae (Table 7) samples collected from this area contained 0.001, 0.008 and 0.052 ppm respectively which are comparatively lower than the values obtained from Priceville (N.B.) samples (see Table 11).

Table 11 gives the DDT residue data of various types of samples (air, water, soil, sediment, foliage, budworm, fish and vole) collected from Priceville (N.B.) during 1972. This area received the heaviest dosage of DDT totalling 70 oz. A.I. applied per acre since 1956 to 1967 (Yule 1973). It is reflected in the high amounts of DDT residues observed in the samples. The "oven-dry" soil, sediment and spruce foliage samples contained on average 0.810, 0.759 and 0.358 ppm of DDT residues respectively. Water (0.002 ppm) and air samples (0.156 ppm) (see Sundaram 1974 for more information) showed measurable amounts of DDT. The five voles trapped in the area had a mean DDT content of 0.460 ppm. The six fish (trout) samples collected from the stream contained an unusually high amount (8.726 ppm) of DDT residues; 80% of it was DDE (6.992 ppm), 8% was _o,p_-DDT (0.727 ppm) and only 12% was _p,p'_-DDT (1.007 ppm). The budworm samples also contained significant amounts of DDT residues (0.413 ppm), the source for the toxicant in the insects was the spruce foliage serving as food which contained the translocated insecticide _via_ root penetration from soil.

Apart from the loss of the applied DDT by physicochemical processes for the past seven years, _i.e._, from 1967, the Priceville area still contains appreciable quantities of DDT in all the four components of the forest and, as seen from the data is undergoing dynamic cycling (Woodwell et al 1971). An intensive and long-term study would be necessary to understand the complex pathways by which DDT cycles and accumulates from one component to another, its potential interaction within the components, and its long term consequences on the biological and ecological systems of the area. The total effect of the chemical on these systems is proportional to its persistence and its toxicity.

In addition to the DDT residues found in nearly all the samples analysed, the presence of PCBs called aroclors* which are widely distributed in the environment like DDT, are indicated in the chromatograms of animal (Figs. 3-7) and soil (Fig. 8) samples studied. Figures 3-8 contain multiple peaks of the three PCBs (aroclor 1242, 1254 and 1260) most commonly used. An aroclor has many different chlorinated biphenyls present. It can easily be seen by observing these chromatograms (Figs. 3-8), how this can present a problem as interfering contaminants in the analysis of DDT residues. No attempt has been made to estimate them and their presence in these samples is a puzzling one which requires an intensive study.

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* The polychlorobiphenyls (PCBs) are mixtures of compounds (> 200) derived from biphenyl containing chlorine on any of the ten positions. These PCBs, called aroclors, are designated by a number, e.g., aroclor 1254. The first two numbers represent the fact that it is a biphenyl and the second two numbers represent the weight percent of chlorine. Many of these compounds are more stable and have longer half-life than DDT. They have the necessary physical and chemical characteristics for persistence and biological magnification.

SUMMARY

Animal, foliage, water, soil and air samples amounting to 460 were collected from various forest regions of Canada sprayed with DDT. Quantitative estimation of the DDT isomers and DDE was carried out using gas chromatography, after suitable analytical methodologies have been developed. The residue data confirmed the protracted persistence and dynamic cycling of DDT through nontarget species inhabiting the forest ecosystem even after several years of cessation of spraying. The various inter related factors which influence the insecticide accumulation in non-target species were obscure. The presence of PCBs in the samples analysed was evident but no attempt was made to quantify them.

ACKNOWLEDGMENTS

The authors are indebted to Dr. C.H. Buckner and Mr. Bruce B. McLeod for collecting and supplying nearly all the samples recorded in this report. Thanks are extended to Dr. I.W. Varty (MFRC, Fredericton) for his assistance in sampling some of the samples recorded in Table 11.

The technical assistance of Messrs. W. O'Brien and G.G. Smith is greatly appreciated.

The authors acknowledge with thanks the suggestions and criticisms of Messrs W.W. Hopewell and W. Haliburton while preparing this report, and finally, it is a pleasure to acknowledge the support of Dr. J.J. Fettes in this work.

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APPENDIX I

ABBREVIATIONS AND SYMBOLS

Bb	*Blarina brevicauda* (Say), Short-tailed shrew
Cg	*Clethrionomys gapperi* (Vigors), Red-backed shrew
Ct	*Citellus tridecemlineatus* (Mitchill), Striped ground squirrel
Em	*Eutamias minimus* (Bachman), Least Chipmunk
Mh	*Microsorex hogi* (Baird), Pigmy shrew
Ni	*Napaeozapus insignis* (Miller), Woodland jumping mouse
Pm	*Peromyscus maniculatus* (Wagner), Deer mouse
Sc	*Sorex cinereus* (Kerr), Common or Masked shrew
Ts	*Tamias striatus* (Linn.), Eastern chipmunk
Zh	*Zapus hudsonius* (Zimmermann), Meadow jumping mouse
♀	Female
♂	Male
R.T.	Retention time (min.)
o,*p*-DDT	2,2-*Bis*(*o*,*p*-chlorophenyl)1,1,1-trichloroethane
p,*p*'-DDT	2,2-*Bis*(*p*-chlorophenyl)1,1,1-trichloroethane
DDE	2,2-*Bis*(*p*-chlorophenyl)1,1,-dichloroethylene
DDD	2,2-*Bis*(*p*-chlorophenyl)1,1-dichloroethane