DDT Residues in Cod Livers from the Maritime Provinces of Canada

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Abstract

The residues of DDT and its analogs were estimated in a number of cod liver samples *(Gadus morhua)* collected at six sampling sites off the Atlantic Coast of the Maritime Provinces of Canada during the summer of 1971. The residue levels indicated widespread distribution of DDT over the entire region sampled. Variations in the relative contributions of p,p'-DDE and p,p'-DDT to the total DDT residues (ΣDDT) were noted and the tendency for these residues to preferentially accumulate in lipid rich tissues was demonstrated. The residue levels found in cod livers were compared to the residue levels determined in cod flesh taken from some of the same specimens and also to residue levels in commercially refined cod liver oils. Samples of each of these types were stored at -29°C and analyzed in 1973 for DDT and PCB simultaneously. The ΣDDT residues determined by both methods of analysis were compared.

Introduction

It has long been established that chlorinated insecticides have spread through all segments of the environment, WOODWELL et al. (1967) and JENSEN et al. (1969). It has also been demonstrated that chlorinated insecticides will accumulate in lipid rich tissues, HOLDEN and MARSDEN (1967) and it has furthermore been suggested that the extent of contamination may be in direct relationship to the lipid content of a particular organism, REINERT (1970). Foremost among the persistent pesticides are DDT and its metabolites. The work described in this paper shows the distribution and extent of contamination by DDT residues off the Atlantic Coast of Canada. Similar to the work of STENERSEN and KVALVAG (1972), this investigation employed cod livers as the test material. The initial analyses were carried out analyzing only for p,p-DDT and its principal metabolites, p,p'-DDD, o,p'-DDT and p,p'-DDE.

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The presence of polychlorinated biphenyls (PCBs) was first reported in wildlife samples by JENSEN (1966). Since that time many workers, including RISEBROUGH et al. (1968), JENSEN et al. (1969) and ZITKO (1971) have reported the presence of these industrial chemicals in various biological samples. Several workers have indicated the potential interference of PCBs in the analysis of chlorinated insecticides and have developed techniques to limit the extent of this interference, HOLDEN and MARSDEN (1969), REYNOLDS (1969), ARMOUR and BURKE (1970), ZITKO and CHOI (1971) and BERG et al. (1972). In order to assess the validity of the DDT residue results in this present work, a number of the samples were again analyzed (in 1973) employing the method of REYNOLDS (1969) to reduce any interference by PCBs. A comparison of the two sets of results permitted an estimation of the probable extent of interference in DDT analyses prior to the advent of PCB separation techniques.

Materials and Methods

The samples were collected in the summer of 1971, placed in glass jars with foil-lined lids, and immediately frozen (-29°C). Six cod liver samples were collected at each of six sampling sites off the Maritime Provinces of Canada. These six sampling sites (shown in Fig. 1) were designated as follows:

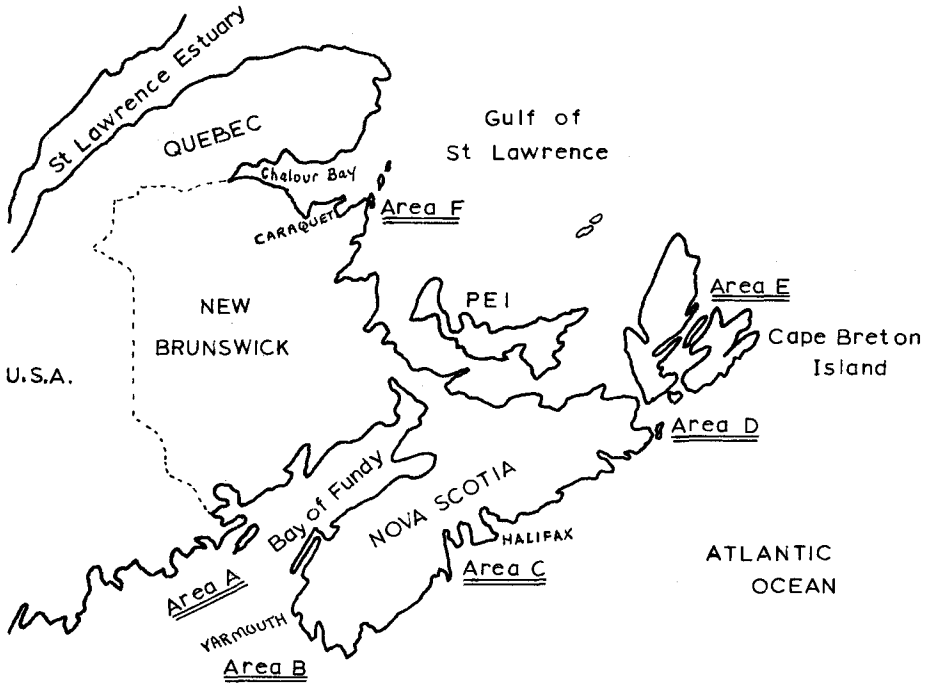
A. Bay of Fundy, off Grand Manan
B. South East Nova Scotia, off Yarmouth
C. Coastal Atlantic Nova Scotia, off Halifax
D. North East Nova Scotia, Chedabucto Bay
E. Northern Nova Scotia, Sydney Bight
F. Northern New Brunswick, off Shippegan

The flesh (edible portion) from twelve of the fish whose liver was sampled was also collected for analysis. Six commercial perparations of cod liver oil (from different producers) were analyzed. The precise geographic area from which the raw material for the production of these oils originated was not available and can be classed only as "from domestic catches".

The samples were initially analyzed for p,p'-DDT and its principal metabolites, with no attempt at compensation for or separation from any PCB residues which may have been present. The pesticides were extracted from 5 g samples by high speed blending with two 100 ml aliquots of acetonitrile. The acetonitrile extracts were combined, mixed with 500 ml of 2% sodium sulfate in distilled water and partitioned twice with 100 ml aliquots of petroleum ether. The petroleum ether extracts containing the pesticide residues were combined, dried with sodium sulfate, flash evaporated to 2 - 3 ml and then applied to a 2.5 cm I.D. column

containing 20 g of 2% deactivated florisil. The pesticides were eluted with 200 ml of 6% methylene chloride in hexane. This eluate was collected, flash evaporated to a few ml, and the residues were estimated by electron capture gas chromatography. The instruments employed were a Hewlett Packard Model 5750 gas chromatograph equipped with a 200 millicurie tritium detector and a Varian Aerograph Model 1400 gas chromatograph equipped with a 250 millicure tritium detector. The former instrument was fitted with a 6' x 1/4" glass column of 4% SE-30 plus 6% QF-1 on 80-100 mesh Chromosorb W, while the latter was fitted with a 6' x 1/4" glass column of 3% OV-1 on 80-100 mesh Chromosorb W. The DDT and metabolite residues from the cod liver samples were qualitatively confirmed by the thin layer chromatographic method of MOATS (1969). All results were calculated as ppm on a wet weight basis. Recovery factors from the entire procedure were periodically checked by analyzing samples for which the background residues had been established and to which known quantities of the pesticides had been subsequently added. No recovery factors were incorporated into the residue calculations.

Figure 1. Collection sites of cod samples.



For the simultaneous determination of PCBs and DDT, the samples were extracted as above. The PCB fraction was separated by the method of REYNOLDS (1969). The gas chromatographic instruments and columns used were the same as those described above. The PCB residue was estimated by comparison to a PCB standard containing a 4:1 mixture of Aroclors 1254 and 1260. This particular PCB mixture more closely resembled the pattern of peaks observed from the samples than any of the numerous other mixtures tested. The PCB quantitation was effected using the sum of all peak heights for those peaks occurring after peak #7 (inclusive) of Aroclor 1254, using the numbering system of REYNOLDS (1969). Confirmation of the PCB residues was achieved by subjecting the PCB fraction to the KOH-ethanol digestion technique of HOLDEN and MARSDEN (1967) and estimating the PCB content as above.

Results and Discussion

The mean residues found in cod livers taken from the six sampling sites are shown in Table I. Standard deviations were consistently in the range of 30-40% of the mean residue values. Recoveries from spiked samples were 85±6%, 94±8%, 91±8% and 86±4% for p,p'-DDE, o,p'-DDT, p,p'-DDD and p,p'-DDT respectively. The metabolite o,p'-DDT was not confirmed in any of the samples analyzed. The ΣDDT residues found in samples from Area C were appreciably higher than those of the other Areas while the residues in samples from Area E were considerably lower than those of all other Areas. The samples from the remaining four Areas contained ΣDDT residue levels which were not significantly different from each other. Variations were noted in the relative proportions of p,p'-DDT and p,p'-DDE to ΣDDT. The figures in brackets in Table I more clearly show these variations.

TABLE I

Residues of p,p'-DDE, p,p'-DDD and p,p'-DDT in cod liver samples from the Maritime Provinces

	Mean Residue, ppm (Proportion of ΣDDT)			
Area	p,p'-DDE	p,p'-DDD	p,p'-DDT	ΣDDT
A	2.0(.25)	1.2(.15)	4.9(.60)	8.1
B	1.9(.31)	1.5(.24)	2.8(.45)	6.2
C	5.2(.37)	2.2(.16)	6.5(.47)	13.9
D	2.9(.38)	1.4(.18)	3.3(.44)	7.6
E	1.8(.49)	0.66(.18)	1.2(.33)	3.7
F	4.6(.50)	1.4(.15)	3.2(.35)	9.2
Total	3.1(.38)	1.4(.17)	3.6(.45)	8.1

Table II shows the mean residues found in the livers and flesh (edible portion) of the samples from Areas A and E. These results show that the lipid rich livers (average lipid content of 30%) had accumulated residues of these pesticides in quantities far exceeding the amount present in the flesh (average lipid content of 0.2%). The relative proportions of the metabolites were similar in flesh and liver samples from the same specimens.

TABLE II

Residues of p,p'-DDE, p,p'-DDD, and p,p'-DDT in cod liver and cod muscle samples

Area	Sample	Mean Residues (ppm)			
		p,p'-DDE	p,p'-DDD	p,p'-DDT	ΣDDT
A	Flesh	0.015	0.008	0.026	0.049
A	Liver	2.0	1.2	4.9	8.1
E	Flesh	0.013	0.005	0.012	0.030
E	Liver	1.8	0.66	1.2	3.7

Because of the relatively high levels of DDT which were found in the cod liver samples(mean ΣDDT=8.1 ppm), several commercially refined cod liver oil samples were analyzed. The results in Table III show that the processed liver oils contained much lower residues than the liver samples. This difference would be more striking if the results were expressed on a lipid basis. In that instance the mean ΣDDT residue for the livers would be >20 ppm while the results from the refined cod liver oils would remain at approximately 2 ppm.

TABLE III

Residues of p,p'-DDE, p,p'-DDD, and p,p'-DDT in commercial cod liver oil samples

Sample Number	Residue Concentration(ppm)			
	p,p'-DDE	p,p'-DDD	p,p'-DDT	ΣDDT
1	0.69	0.83	0.57	2.1
2	0.41	0.54	0.50	1.5
3	0.64	0.86	0.51	2.0
4	0.54	0.59	0.67	1.8
5	0.55	0.67	0.45	1.7
6	0.63	0.64	1.0	2.3
Mean	0.58	0.69	0.62	1.9

Four samples each of cod liver, cod flesh, and refined cod liver oil were retained for simultaneous analysis for PCBs and chlorinated pesticides. Table IV shows a comparison of these results to the original re-

sults for which no PCB separation was carried out. The ΣDDT residue results from the later analyses (performed in 1973 employing a PCB separation technique) were an average of 26% lower than the original results (obtained in 1971 using no PCB separation procedures). This figure was obtained without consideration of possible losses or degradation of these residues during their 2 years of storage.

TABLE IV

Comparison of DDT residue results obtained with and without the separation of interferring PCB residues

Sample #	Area	Residue-Without Separation(ppm) ΣDDT	Residue With Separation(ppm) ΣDDT	PCB
Flesh (25)	A	0.040	0.033	0.041
Flesh (26)	A	0.088	0.068	0.091
Flesh (38)	A	0.025	0.018	0.030
Flesh (39)	A	0.016	0.010	0.023
Liver (38)	A	3.3	2.5	4.1
Liver (31)	C	26.4	19.3	23.3
Liver (21)	F	8.0	5.8	3.4
Liver (24)	F	13.2	13.9	7.2
Refined Oil (45)	-	2.1	1.5	2.4
Refined Oil (46)	-	1.5	0.97	1.6
Refined Oil (47)	-	2.0	1.2	2.8
Refined Oil (49)	-	1.7	1.0	2.9

Conclusions

The cod liver samples from Area C showed appreciably higher residue levels than from the other five areas. Area C, off Halifax, represents the collection site near the largest population center in the Maritime Provinces. This is in agreement with the findings of BUTLER (1966) whose work indicated that DDT residues "reflect the proximity of the collection site to human residential centers."

Our results have indicated that p,p'-DDE and p,p'-DDT comprise different proportions of the ΣDDT residues of samples taken from different sampling areas. This variation is most pronounced when comparing the results from Areas A and B to those from Areas E and F. Several possible factors which may be involved are:

i) the proximity of the sampling site to the source of the parent compound,

ii) the time elapsed since the most recent application of the parent compound,

iii) the identity of the parent compound. Some areas may have been exposed to Rothane (p,p'-DDD) as well as to DDT (largely p,p'-DDT),

iv) the aquatic biota, which in different areas may vary in such a way as to affect the rates of degradation of these compounds.

A combination of these circumstances has probably resulted in this situation which indicates that different areas may reflect very different ratios of these metabolites, even within the same species.

This work has again demonstrated the ability of chlorinated pesticides to accumulate in lipid rich tissues. In this instance, lipid rich organs within the specimens investigated were found to preferentially accumulate these residues. Twelve cod livers had a mean ΣDDT level of 5.9 ppm while the corresponding flesh samples showed a mean ΣDDT level of only 0.040 ppm.

The mean ΣDDT residue content of the 34 cod livers expressed on a lipid basis would be more than 20 ppm. However, the results of refined cod liver oil analyses suggested that the ΣDDT residues were severely reduced through processing. The mean value of 1.9 ppm ΣDDT found in commercial cod liver oils represented only about 10% of the quantity implied from the cod liver results. Reductions in DDT as well as PCB residues during processing of oils for edible use have been previously reported, ADDISON and ACKMAN (1974).

In almost all samples analyzed for PCBs, the PCB residues were higher than the ΣDDT residues. Similar tendencies were reported by ZITKO (1971) for samples of freshwater and marine fishes of the Maritime Provinces. The ΣDDT analyses which had included compensation for the presence of PCBs gave results which were an average of 26% lower than the analyses of the same samples without consideration for the presence of PCBs. However, if recovery factors were incorporated into the calculations for the later analyses, then the resultant values would be nearer the true residue levels and would be within an average of 15% of the original results (where PCB interference was not removed and recovery factors were not included). Thus, it is suggested that these original results were probably not in serious error due to the influence of PCBs.

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References

ADDISON, R.F., and ACKMAN, R.G., J. Amer. Oil Chem. Soc., 51, 192 (1974).

ARMOUR, J.A., and BURKE, J.A., J.A.O.A.C.,53, 761 (1970).

BERG, O.W., DIOSADY, P.L., and REES, G.A.V., Bull. Environ. Contam. Toxicol., 7, 338 (1972).

BUTLER, P.A., Transactions of the Thirty-First North American Wildlife and Natural Resources Conference, Pittsburg, U.S.A. (1966).

HOLDEN, A.V., and MARSDEN, K., Nature, 216, 1274 (1967).

HOLDEN, A.V., and MARSDEN, K., J. Chromatog., 44, 481 (1969).

JENSEN, S., New Scientist, 32, 612 (1966).

JENSEN, S., JOHNELS, A.G., OLSSON, M., and OTTERLIND,G., Nature, 224, 247 (1969).

MOATS, W.A., J.A.O.A.C., 52, 871 (1969).

REINERT, R.E., Pest. Monit. J., 3, 233 (1970).

REYNOLDS, L.M., Bull. Environ. Contam. Toxicol., 4, 128 (1969).

RISEBROUGH, R., RIECHE, P., PEAKALL, D.B., HERMAN, S.C., and KIRVEN, M.N., Nature, 220, 1098 (1968).

STENERSEN, J., and KVALVAG, J., Bull. Environ. Contam. Toxicol., 8, 120 (1972).

WOODWELL, G.M., WURSTER, C.F., and ISAACSON, P.A., Science, 156, 821 (1967).

ZITKO, V., Bull. Environ. Contam. Toxicol., 6, 464 (1971).

ZITKO, V. and CHOI, P.M.K., Fisheries Research Board of Canada, Technical Report No. 272 (1971).