

bcc: R. E. Keller - QUEENY.

February 27, 1967

Dr. H. J. Thomas
Research Division
Building No. 33
National Cash Register
Dayton 9, Ohio

Dear Dr. Thomas:

Attached is a photostat of the original paper of Dr. Jensen in Sweden, relating to polychlorinated biphenyls. I will be happy to have your ideas after you read it.

As far as the section on toxicology is concerned, it is true that chloracne and liver trouble can result from large doses. Whether or not this is at all relevant to small quantities existing in human fat is, of course, an entirely different question.

At any rate, I believe before we worry about the toxicological part of the problem, we should settle the analytical part.

Sincerely,

R. Emmet Kelly, M. D.
Medical Director

REK/ln
att.

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$\frac{1}{2} \left(\frac{1}{2} + \frac{1}{2} \right) = \frac{1}{2}$

D. R. Bachman

22. 11. 1956

— 30 —

W. F. O'NEILL

If any of you have any comments or thoughts on something which should be done now, please let me know immediately. I have heard nothing more from our customers or from the field since our meeting in your office. The only G. E. publication that carried the story to date has been 'Chemical Engineering'.

Thank you for your cooperation in helping us follow this so that we can protect our interests properly.

Gene #1100

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Possible Interference by Chlorinated Hydrocarbons—by G. Widmark (Institute of Physical Chemistry, University of Stockholm, Roslagsgatan 90, Stockholm 50, Sweden)

A large number of unknown but chlorinated organic compounds have been detected in food to the ordinary pesticides in the analysis of samples, using both the electron capture and the microcoulometric detector. The spectra of some of these compounds, analysed with a combined gas chromatograph-mass spectrometer (GC-MS) indicate that most of the unknown compounds are polychlorinated biphenyls, potentiated somewhat in nature towards those of higher degree of chlorination. A large number of samples have been examined. Polychlorinated biphenyls are found especially in fish and in plants, but also in conifer needles and in samples of human depot fat. Two hundred samples of soil and water and twenty samples of terrestrial mammals contained no detectable amounts of chlorinated biphenyls. Typical GLC traces are given by Jensen and Schmidt (1). By using a titration method, a lot of the ordinary pesticides can be removed from extracts and the polychlorinated biphenyl will be unaffected. In this way, it has been shown that two of the major chlorinated biphenyl peaks overlap the peaks of *p,p'*-DDT and *o,p'*-DDT, respectively. This overlap is found with SF-95 column, most frequently used for the quantitative residue analyses reported in literature, but not with QF-1 columns.

(i) Confirmation of Identity—

W. K. E. Elgar (Shell Research Ltd., Woodstock Agriculture Research Centre, Sittingbourne, Kent, England)

Multidetector methods would seem to be essential for the analysis of organochlorine compounds because of the number of chlorinated compounds which can be present in any one sample. However, there are problems of interpretation even when techniques are used with the resolution and sensitivity of electron capture GLC. For example, the resolution of the GLC column may not be sharp enough to separate components; or quantities present in the technical pesticide

or other natural products present in the extract may complicate the analysis; or some insecticides may thermally or catalytically decompose during the analysis.

For these reasons, residues of organochlorine compounds should be confirmed by other analytical methods. If enough material can be isolated, infrared spectroscopy or mass spectroscopy can give powerful evidence of identity. However, with other methods in common use the physical basis underlying separation is very similar. Robinson *et al.* (2) reported a highly significant concordance between the "p-values" for pesticides in the various partition systems described by Beroza and Bowman (3). Similarly, retention times on different GLC stationary phases and R_f of pesticides in various TLC or paper chromatographic systems are highly correlated. Furthermore, R_f values in paper chromatography are closely correlated with R_f values in TLC and with p-values. It is possible to do a great deal of work with the intention of establishing the identity of a compound, but very little additional evidence will be gained because the results from the methods used were not independent. GLC retention time, an R_f value from TLC or paper chromatography (or a p-value), a GLC retention time of a derivative formed by chemical reaction, and pesticidal activity against a susceptible organism are considered to be independent parameters giving significant evidence of identity.

5. Organophosphorus Insecticide Residue Analysis

The Commission concluded that while the regulatory methods were adequate for parathion and malathion, clarification of the terminal residue situation would be welcome in some instances. However, more work on the cleanup stage for malathion residues in eggs, milk, and dried fruit was indicated.

6. Dichlorvos Residue Analysis

By R. A. E. Galley (Shell Research Ltd., Woodstock Agriculture Research Centre, Sittingbourne, Kent, England)

Five different approaches have been developed for the determination of traces of dichlorvos: total phosphorus (4, 5), colorimetric

chain is terminated and the completed chain released from the ribosome-mRNA complex to which it is bound by the RNA molecule carrying the last amino-acid incorporated. There must be a mechanism for reading the chain terminating codon (three codons UAA, UAG, and UGA can code for chain termination) and cleaving the last peptidyl-RNA bond. In *Biochem. Biophys. Res. Comm.*, 23, 773 (1967) he describes an experimental system devised to give a rapid assay for chain termination, and in *Proc. US Nat. Acad. Sci.*, 53, 1114 (1967) he reports the discovery of an enzyme required for termination.

The assay for termination employs the RNA from an amber mutant of the coat protein gene of the RNA bacteriophage R17. This mutant has an amber codon, UAG, at the position of the sixth amino-acid, a glutamine residue, in the R17 coat protein molecule. In a cell free system the mutant RNA directs the synthesis of a small N-terminal peptide of the coat protein with the sequence N-formylmet-ala-ser-aspa-phe-thr; the next amino-acid in the coat protein is glutamine, but with the mutant RNA the amber codon causes premature termination of synthesis and the release of the hexapeptide. The great virtue of this system is that the unterminated peptide still attached to the peptidyl-RNA can be distinguished from the terminated peptide released from the RNA and the amounts of each measured. Furthermore, starving the system for any one of the six amino-acids in the peptide arrests the translation machinery before it reaches the UAG codon so termination can be controlled.

Exploiting this procedure, Capēcchi isolated a complex of ribosome, mRNA and pentapeptide

attached by the N-formylmet-RNA simply by starving for threonine. With this he has been able to define the conditions necessary for termination; incubating the complex under suitable conditions with or without threonine or supernatant enzymes showed that to get release it is necessary but not sufficient to complete the peptide by incorporating threonine and that release is not solely dependent on the transfer enzymes required to move the ribosome from the threonyl codon to the UAG terminating codon. Release does, however, depend upon the presence of a supernatant factor. This factor, with a molecular weight of between 40,000 and 50,000, sediments between 3.5S and 4.5S. Because its releasing activity is not affected by RNase, it cannot be an RNA-protein complex. Apparently the substrate of the release enzyme is the ribosome-mRNA-peptidyl-RNA complex so that it must act directly at the ribosome and not cleave the RNA from peptidyl-RNA already released into the supernatant, which was the other possibility.

Capēcchi does not yet know whether the release enzyme works alone or in conjunction with other factors, in particular a hypothetical chain termination RNA which reads the termination codon but does not carry an amino-acid. Many people, including Capēcchi, have searched in vain for such a RNA species, and although negative results can never exclude the possibility that such RNA exists it is reasonable to think about alternative models in which the terminating codons are read either by the ribosome or the release enzyme or in which nonsense codons function simply because they cannot be read at all. Further characterization of the release enzyme could well decide the issue.

Chlorinated Hydrocarbons in British Wildlife

by

D. C. HOLMES
J. H. SIMMONS
J. O'G. TATTON

Laboratory of the Government Chemist,
London

Polychlorobiphenyl compounds have been detected in British wildlife. In birds' livers and eggs they are often in greater quantities than organochlorine pesticide residues. Polychlorobiphenyls are known to be toxic, and their detection in wildlife raises the question of the adverse effect they may have.

When wild birds' eggs or the livers of British wildlife are examined by gas-liquid chromatography (GLC) using electron-capture detection, peaks corresponding to such organochlorine pesticides as DDE, dieldrin and DDT, and their metabolites and breakdown products, often appear on the resultant chromatograms. These peaks sometimes represent amounts which are significant from a toxicological point of view, but the sensitivity of modern instruments also enables completely insignificant amounts to be detected. Analysts concerned with the determination of organochlorine pesticides in wildlife, however, have long been aware that it is not unusual for an additional series of as many as ten peaks, corresponding to unidentified compounds, also to appear on these chromatograms. Little is known of the structure and origin of these compounds, but Hoburn¹ established that they are organo-

chlorine in nature, with a substantial chlorine content, and consequently possess electron-capturing properties.

On silicone or 'Apiezon' GLC columns, the series of peaks for these compounds normally begins at the point at which *pp'*-DDE and *pp'*-DDT are emerging from the column and extends, in terms of retention times, to four or five times that of *pp'*-DDT, which is the last of the commonly occurring pesticides to emerge.

In the examination by GLC, samples have normally been subjected to a fairly rigorous clean-up procedure, so that the main group of compounds, which includes the organochlorine pesticides, is purified and separated. These unknown compounds are similar in nature and properties to the organochlorine pesticides, they also pass through this and similar clean-up procedures; consequently, they

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when interfere seriously with determination of *pp*-TDE and *pp*-DDE in wildlife because of overlapping of the chromatographic peaks. Suitable procedures have been devised to overcome this difficulty of interfering peaks. One method is to effect a better resolution of these particular peaks by choice of a suitable stationary phase for GLC⁴. Another method, which also demonstrates the presence of these compounds in the sample, is the use of a preliminary thin-layer chromatographic treatment⁵. The cleaned-up extracts are examined on silica gel chromatographs using a 1 per cent solution of acetone in hexane as mobile phase⁶. Spots corresponding to the compounds can then be found at *R_F* values between 0.8 and 1.0, whereas the commonly occurring organochlorine pesticides and their metabolites produce spots of lower *R_F* values. A similar separation can also be shown with reverse phase paper chromatography⁷ whereby the unknown compounds move only a very short distance from the base line compared with the pesticides and their metabolites.

Gas-liquid chromatography using dual-channel detection systems with separate electron-capture and flame-ionization detectors has also been used to demonstrate the presence of large amounts of weakly electron-capturing material, in addition to known pesticide residues, in the eggs of oyster catchers (*Haematopus ostralegus*)³.

Experience in this laboratory has shown that these compounds occur most frequently and in the largest proportions in the livers, fat and eggs of birds, particularly terrestrial predators such as *Cathartus* (*Accipiter*), *Falco* and *Bubo* and kestrels (*Falco tinnunculus*) and marine feeders such as guillemots (*Uria*), albatrosses (*Diomedea*) and kittiwakes (*Rissa*). They also commonly occur in freshwater fish. We have detected small amounts in human and animal fat samples, but less frequently and in much smaller proportions than in avian samples.

A similar series of chromatographic peaks has been observed by Hildon^{14,15} when examining extracts of seals and fish taken in Scottish waters. He found that the peaks given by the extracts of fresh and sea-water fish represented fairly low concentrations, but those given by extracts of seal blubber corresponded to much larger proportions, of the order found by this laboratory in birds' eggs.

Despite considerable curiosity about the identity of these compounds, little progress has been made in identifying them, but there has long been a general supposition that they were either further breakdown or condensation products of the organochlorine pesticides or possibly loose compounds of these with natural products such as protein matter.

Jönsson, however, succeeded in identifying a series of such peaks as corresponding to polycyclic aromatic compounds, in the order taken in different parts of Sweden, and in the air of Göteborg. We have now been able to show that the long retention time compounds occurring in British wildlife are also polycyclic aromatic compounds.

Polyethyl chloroethyl biphenyl has been in common commercial use for many years as a plasticizer in paints, varnishes and phosphors. It also has electrical insulating properties and can be used for a number of other nonactive purposes. Commercial production of this material are usually produced by addition to the double bond of chlorination, but all of them are usually complex in nature of polycondensation biphenyls made they could be expected to be similar to the other chloroethyl biphenyls in their chemical and physical

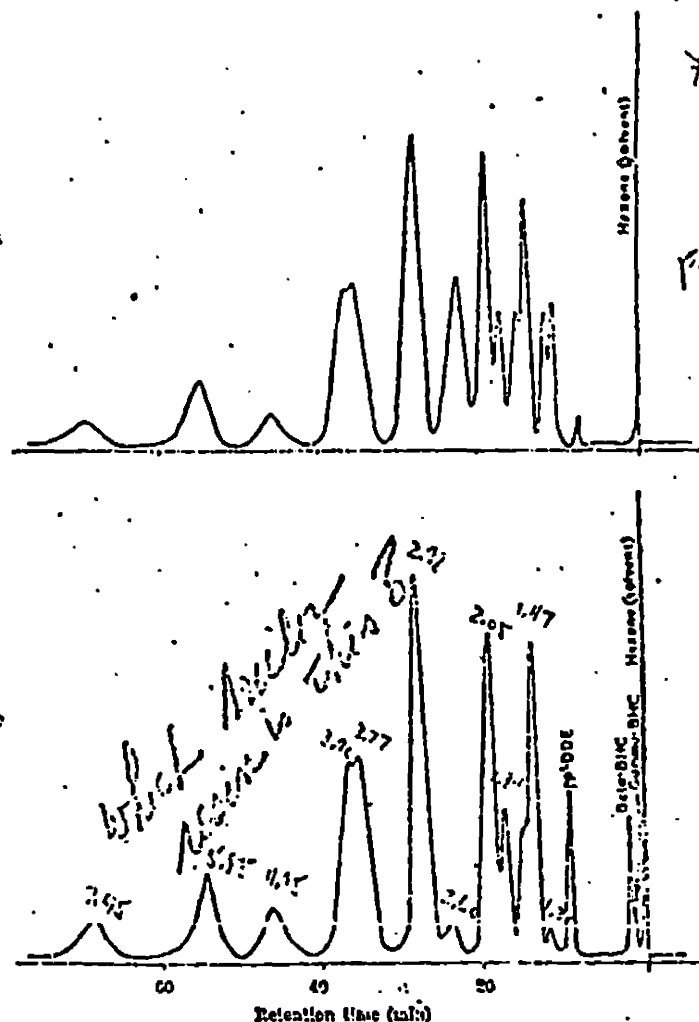


Fig. 1. Gas-liquid chromatogram of (a) extract of kestrel liver and (b) a commercial polychlorobiphenyls.

properties. In the prescribed extraction and GLC conditions, these mixtures produce a series of chromatographic peaks of the type in question.

The compounds occurring in British wildlife correspond to the more highly chlorinated biphenyl compounds rather than the more lightly chlorinated types. These highly chlorinated biphenyls also occur to some extent in the analogous polychloroterephenyl preparations which have commercial uses similar to those of polychlorobiphenyl. It is possible that some of the compounds with very long retention times occurring in British wildlife are lightly chlorinated polychloroterephenyl compounds.

Fig. 1a shows the gas-liquid chromatogram of an extract from a control liver. The peaks corresponding to *pp'*-DDE (0.43 ng), beta-BHC (0.33 ng) and gamma-BHC (0.03 ng) are labelled. The other peaks form the series referred to above and until now would have been regarded simply as "unknown organochlorine compounds" not corresponding to any known pesticide or its metabolites. Fig. 1b shows the analogous chromatogram of a commercial polychlorobiphenyl resin. So far as retention times are concerned and apart from the pesticide peaks, there is exact matching for all but a few peaks in the two chromatograms.

The oligomers shown were obtained by use of a silicone gum (Gidex 55) column, but this precise correspondence between the retention times of the peaks for the unknown compounds and those from the polydichlorobutadiene has also been demonstrated on Avidon L.

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Table 1. RELATIVE RETENTION TIMES OF POLYCHLOROBIPHENYL ESTERS ON DIFFERENT TYPES OF COLUMN

Silicone column		(D. Glaxo, 100)		Cyanosilicone column	
KEL	PCB	KEL	PCB	KEL	PCB
1.42	1.42	2.51	2.19	1.21	1.21
1.73	1.73	2.67	2.67	1.24	1.24
1.91	1.90	3.01	2.93	2.24	2.19
2.81	2.81	4.02	3.95	2.62	2.60
3.69	3.68	5.98	5.85	2.67	2.66
3.71	3.70	6.37	6.26	3.69	3.69
4.68	4.68			4.62	4.62
5.62	5.62			5.71	5.71
7.10	7.11				

KEL, Kestrel liver extract; PCB, polychlorobiphenyl.

and cyanosilicone columns. The actual retention times recorded for the peaks on these three types of column are given in Table 1.

Further confirmation as to the identity of these compounds has been obtained by a study of their behaviour on thin-layer chromatoplates, alumina and silica gel absorption columns and reverse phase paper chromatograms. In addition, these compounds have been shown to resemble the polychlorobiphenyl compounds in their chemical inertness. Thus they are not easily modified by simple chemical reactions to produce readily identifiable shifts in their retention times on GLC as can be done, for instance, to *pp'*-DNT by hydrolysing it to *pp'*-DDT or to aldrin by oxidizing it to dieldrin.

A number of livers and eggs taken from birds in the British Isles or their coastal waters have been examined in this laboratory with results similar to those obtained for the kestrel liver referred to earlier. While the identification of these compounds in British wildlife may solve the mystery of these peaks on GLC chromatograms, it raises, however, many other questions. Why, for example, do these compounds seem to be accumulated, most frequently and in the largest proportions, in certain types of wildlife such as birds, seals and fish, and only at the most in very small proportions in man or his domestic animals such as cows or sheep? In this respect they seem to differ markedly from the organochlorine pesticides which seem to have invaded nearly all aspects of our environment and

to which we bear a strong resemblance chemically and physically.

Some of the samples of bird examined in this laboratory have seemed to contain higher proportions of polychlorobiphenyl compounds than organochlorine pesticides. For example, the peaks on the chromatogram for the kestrel liver shown in Fig. 1 were estimated to be equivalent to about 12 ng of polychlorobiphenyl compounds. This must be contrasted against the organochlorine pesticide residues listed above. These total only about 0.8 ng of which 0.33 ng is beta-BHC, an isomer of BHC which is generally regarded as non-toxic to wildlife.

Great concern has often been expressed by conservationists about the effects of pesticides on wildlife, particularly birds. The identification of these other organochlorine compounds in wildlife prompts enquiries as to the possible toxic effects they may be having. That these compounds are toxic is generally agreed^{1,2,3}; the polychlorobiphenyls are in fact regarded as an industrial hazard and threshold limits for them in air have been advised^{4,5}.

We thank the Nature Conservancy for samples of wildlife and Monsanto Chemicals Ltd. for samples of polychlorobiphenyl resins.

Received October 5, 1967.

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Quasi-stellar Sources, Supernovae and Novae

by

P. F. BROVINE

University of Manchester Institute of Science and Technology

By considering the opacity of a surface layer of a star, the author concludes that quasi-stellar sources, supernovae and novae may be different manifestations of the same mechanism.

It is usually considered that the outbursts of novae and supernovae are fundamentally different processes, but I shall here propose a mechanism which can account for both phenomena, and which at the same time can account for other problems concerned with quasi-stellar sources (quasars). In particular, I suggest that these outbursts are likely to be anisotropic (as indeed they are observed to be for novae). Because the energy released during a supernova outburst is comparable with the stellar rest energy, an anisotropic outburst could mean that the star is accelerated to relativistic velocity in order to conserve momentum, thus affording a Doppler explanation of the large red-shifts of the quasi-stellar sources.

Consider the opacity of a layer, L , at the surface of a star. Let ΔR be the thickness and R the radius of L , and let L contain plasma at temperature, T , with electron and positive ion densities N_e and N_i respectively. L will be opaque to radiation of wavelength λ provided that τ is about 1, where τ is the optical depth of L . A major contribution to τ is τ_{λ} , the optical thickness due to free-free

absorptions. It is known that $\tau_{\lambda} \propto \lambda^2 N_e N_i \Delta R$ (ref. 1) where induced emissions are included. Suppose, now, that L is compressed or dilated without changing the total number of ions; that is, ΔR is varied under conditions which maintain constant $4\pi R^2 \Delta R N_i$, so that $\Delta R N_i = \text{constant}$. Evidently $\tau_{\lambda} \propto N_e N_i \Delta R \propto N_e \propto 1/\Delta R$. Thus compression of L increases its opacity, while dilation reduces the opacity.

Of course, the total number of ions will not remain constant in practice, because the recombination rate is proportional to $N_e N_i$. If the percentage of atoms ionized is large, the density of un-ionized atoms, N_u , will therefore be proportional to $N_e N_i$. This means that the contribution to the opacity made by bound-bound and bound-free transitions within un-ionized atoms is also proportional to $N_e N_i \Delta R$. This effect, recognized to be important for stellar pulsation², further increases the opacity of L on compression.

Some consequences of this variable surface opacity will now be examined. Under equilibrium conditions the

XXXX/CS

1st November, 1967

Mr. A. Richardson,
Well Chemists,
Marshall Laboratory,
Littingbourne, Kent.

Dear Mr. Richardson,

A week or two back you phoned me concerning the stable chlorine-containing residues found in wild life, and you indicated that you had established identity (or substantial identity) with one or other of the two color samples which I had made available to you. You undertook to let me have the details, but so far these have not come to hand.

I have now seen the communication from the Government Laboratory (London, reference No. 107-229) which claims that the stable components are substantially identical with known residues (probably 345 and/or 346).

I would be glad to know how far your observations confirm the work of the Government Laboratory and to what extent, if any, you disagree.

Yours sincerely,

D. V. M. HAYES

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TELEPHONE
SITTINGBOURNE 4-1441
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TUNSTALL LABORATORY,
BROAD OAK ROAD,
SITTINGBOURNE,
KENT.

2nd November, 1967.

AR/SB

Dr. V.N. Hardy,
Monsanto Chemicals Ltd.,
Monsanto House,
10-18 Victoria Street,
London, S.W.1.

Dear Dr. Hardy,

Thank you for your letter of 1st November, 1967. I apologise for not having answered your letter sooner.

As you know, we have been for some time examining extracts of marine birds and their eggs in connection with the possible occurrence of Polychlorinated Biphenyls.

In one particular experiment we have extracted approximately 100 eggs of the Shag (*Phalacrocorax aristotelis*) and examined the resulting extract following liquid-solid chromatography on Silica Gel, by gas-liquid chromatography-mass analysis and by mass spectroscopy. Mass spectroscopy of a fraction from the Silica Gel column showed the presence of a compound of molecular weight 426 which contained 8 chlorine atoms, this is consistent with an octachloro biphenyl; gas-liquid chromatography-mass analysis indicated the presence of compounds of mass 324, 358, 392 and 426 respectively. These masses are consistent with pentachloro, hexachloro, heptachloro and octachloro biphenyls by being present.

In addition we have fed chickens for two months on a diet containing 100 ppm Arochlor 1254 after which we placed them on normal diet and collected the eggs for a further three months. Extracts of these eggs have been examined by gas-liquid chromatography and indicate that a substantial amount of Arochlor 1254 is excreted into the egg, the maximum concentration being 89 ppm. Examination of an egg taken three months after feeding was discontinued shows that these materials are very persistent and that the less chlorinated constituents are more readily metabolised.

I would say that our results are in broad agreement with that of the Government Laboratory and that we have no disagreement with them; however, I believe that they may have over-stressed the likely toxic effects of these materials but as you know there is only very limited evidence for us to go on.

We are continuing our studies on the chlorinated biphenyl and will let you have further information as soon as it is available.

Yours sincerely,

A. Richardson

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Fractions and Multiples		
fraction	prefix	symbol
10 ⁻¹	deci	d
10 ⁻²	centi	c
10 ⁻³	milli	m
10 ⁻⁶	micro	μ
10 ⁻⁹	nano	n
10 ⁻¹²	pico	p
10 ⁻¹⁵	femto	f
10 ⁻¹⁸	atto	a
multiple	prefix	symbol
10	deka	da
10 ²	hecto	h
10 ³	kilo	k
10 ⁶	mega	M
10 ⁹	giga	G
10 ¹²	tera	T

*To be restricted as much as possible.

Compound prefixes should not be used. Thus 10⁻⁹ metre is represented by 1 nm, not 1 mμm. The attaching of a prefix to a unit in effect constitutes a new unit, so that 1 km² = 1 (km)² = 10⁶ m² not 1 k(m²) = 10³ m².

Where possible any numerical prefix should appear in the numerator of an expression.

Examples of Units Contrary to SI, with their Equivalents

unit	equivalent
length	
angstrom	10 ⁻¹⁰ m
inch	0.0254 m
foot	0.3048 m
yard	0.9144 m
mile	1.609 34 km
nautical mile	1.852 km
square inch	6.4516 mm ²
square foot	0.092 903 m ²
square yard	0.836 127 m ²
square mile	2.580 10 km ²
cubic inch	1.638 71 × 10 ⁻⁴ m ³
cubic foot	0.028 316 8 m ³
U.K. gallon	0.004 546 092 m ³
pound	0.453 592 37 kg
slug	14.593 9 kg
pound/cubic inch	2.767 99 × 10 ⁴ kg m ⁻³
pound/cubic foot	16.0185 kg m ⁻³
dyno	10 ⁻⁵ N
poundal	0.138 255 N
pound-force	4.448 22 N
kilogramme-force	9.806 65 N
atmosphere	101 325 N m ⁻²
torr	133 322 N m ⁻²
pound (f)/sq.in.	6894.76 N m ⁻²

Organochlorine Pesticides in Seals and Porpoises

by

A. V. HOLDEN
K. MARSDEN

Freshwater Fisheries Laboratory,
Bullockley, Leithshire

Seals and porpoises in Scotland and Canada, far from the sites of application of pesticides, can accumulate high concentrations of residues in their blubber. These chemicals are spreading through the long food chain which ends with seals and porpoises and obviously cannot be confined to their place of discharge.

The presence of organochlorine residues in marine life in many areas of the world, including the Antarctic, has been reported recently^{1,2}. In British waters the accumulation of residues in the eggs of sea birds³ and the process of concentration of pesticides through certain food chains in the marine environment⁴ have been studied. Analyses of various species of marine fish and mammals from Scottish waters during the past 5 years^{5,6} have shown that organochlorine residues are now a normal occurrence, the highest concentrations being found in organs or tissues with the highest lipid content. Thus the muscle tissue of salmonids, the livers of cod and the blubber of seals act as storage sites for these residues.

The aquatic mammals, such as whales, seals and porpoises, have a relatively high proportion of the body weight in the form of subcutaneous fat (blubber). Seals feed chiefly on piddoids and salmonids, and porpoises chiefly on small piddoids and clupeids. The process of concentration of pesticides through their food chain might be expected to result in high pesticide levels in the fat of these mammals, and samples analyzed at this laboratory confirm this expectation. In recent years, specimens of the grey seal (*Halichoerus grypus*), common seal (*Phoca vitulina*) and porpoise (*Stenonema phocaena*) have been sampled from the coasts of Scotland. Samples of blubber were available for all the specimens examined, and in a few cases other organs were also available. As well as recognized pesticide residues, other electron-capturing substances have been detected by gas-liquid chromatography and, in order to detect any other pesticides, samples were widely distributed to the providers, but for samples from grey seals and

harp seals (*Phoca groenlandica*) on the Canadian Atlantic coast have also been examined and the results of these analyses are reported here. Examination of samples of seal blubber from other areas is continuing in order to assess the variation between different regions of the marine environment.

All tissue samples from Scottish waters were frozen at -18°C until required for analysis. The Canadian samples were preserved in 10 per cent formalin, which showed no evidence of any influence on the analytical procedure, and in this and other cases involving fish it has not affected the efficiency of recovery of pesticide residues. Aliquots of the samples (5 g) were ground to a powder with anhydrous crystalline sodium sulphate (AR grade) and extracted in a Soxhlet apparatus with redistilled AR grade n-hexane (previously tested and found to be free of electron-capturing materials by gas chromatography). The hexane extracts were made up to 100 ml and 25 ml aliquots were subjected to clean-up by the hexane-dimethylformamide partition method of de Lambert Mauder *et al.*⁷, followed by column clean-up using alumina containing 5 per cent water. The loss of pesticide residues from fat extracts so treated is up to 20 per cent, but, as is customary in pesticide analyses, no correction has been made for such losses in the results presented here.

The cleaned-up hexane extracts were analysed by gas-liquid chromatography on a Varian Aerograph 205-2B instrument employing two glass columns 5 ft. long × 1/8 in. outer diameter. The column was packed with Chromosorb W AW/DMCS 80-100 mesh coated with 10 per cent

DC-200) and coated with fluorosilicon nitrogen for detection. While for residues in interferences with p,p'-DDT, analytical were over and the alcoholic water-but with mix sulphate then ana technique DDE, an tained, a the origi and p,p' from the in these where I affected been us interfe The exami in muc lower e Scottis tions of the DC by a 25 per Canad DDT about 30 po intere meter value ferent lyed notice comb

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nitrogen and flow of nitrogen may be controlled by a valve for diethyl in equivalent to 1.0 on theoretical plates. There was no significant detection of DDT on the column. While complete separation of the common pesticide residues is possible on one or either of these columns, interference by polychlorinated biphenyl (PCB) residues with *p,p'*-TDE and *p,p'*-DDE peaks necessitated a further analytical stage. Aliquots (5 ml.) of some of the extracts were evaporated to dryness in a stream of cold filtered air, and the residues refluxed with 2 ml. of 2.5 per cent alcoholic potassium hydroxide for 15 min. on a warm water-bath. After cooling, 5 ml. of *n*-hexane was added, with mixing, followed by 50 ml. of 2 per cent sodium sulphate solution. The supernatant hexane layer was then analysed by gas-liquid chromatography. By this technique, quantitative conversion of *p,p'*-DDT to *p,p'*-DDE, and of *p,p'*-TDE to *p,p'*-MDE (=DDEMU), is obtained, and the conversion products confirm the identity of the original residue. The residual peaks in the *p,p'*-DDT and *p,p'*-TDE positions, where present, can be subtracted from the values of the pesticides originally determined in these positions. Hydrolysis also destroys α and γ -BHC, where present, but polychlorinated biphenyls are unaffected. A cyanosilicone (XE-60) column¹¹ has also been used to separate *p,p'*-DDT and *p,p'*-TDE from PCB interference, confirming the latter.

The principal pesticide residues found in all samples examined were those of the DDT group, with dieldrin in much smaller quantities. Canadian seals contained lower concentrations of dieldrin than Scottish seals, while Scottish porpoises contained remarkably high concentrations of all pesticide residues (see Table 1). Interference on the DC-200 silicone column in the *p,p'*-TDE position caused by a non-hydrolysable residue was responsible for about 25 per cent of the total estimated *p,p'*-TDE in the Canadian and Scottish samples examined. In the *p,p'*-DDT position, the non-hydrolysable residue constituted about 10 per cent in the Canadian samples and about 30 per cent in the Scottish samples examined for this interference, the total amount of non-hydrolysable material being smaller in the Canadian samples. The values in Table 1 have not been corrected for this interference, for only a proportion of the samples were hydrolysed. Results from the various species of seals were not noticeably different in a given area, and have been combined in Table 1.

Table 1. CONCENTRATION RANGES OF ORGANOCHLORINE RESIDUES IN FAT OF SEALS AND PORPOISES (p.p.m.) (Means in parentheses)

Area and type of sample	No. in sample	Dieldrin	<i>p,p'</i> -DDE	<i>p,p'</i> -TDE	<i>p,p'</i> -DDT
E. Scotland, adult seals (1964-65)	18	0.13-2.1 (0.79)	1.0-11.1 (5.2)	0.27-3.0 (1.2)	1.5-23.3 (7.6)
N. and W. Scotland, adult seals (1965-66)	6	0.05-0.80 (0.20)	1.2-7.0 (3.4)	0.17-0.83 (0.41)	1.7-5.7 (3.2)
N. and W. Scotland, seal pups (1966)	9	0.09-0.24 (0.18)	1.0-4.0 (2.6)	0.15-0.50 (0.32)	1.4-3.7 (2.3)
Canada, Magdalen Is., juv. and adult seals (1967)	2	0.62-0.64 (0.63)	0.67-1.8 (1.2)	0.08-0.16 (0.12)	0.08-0.43 (0.26)
Canada, Cabot Straits, juv., imm. and adult seals (1967)	6	0.03-0.10 (0.07)	1.2-17.3 (5.9)	0.35-2.1 (0.78)	2.1-15.4 (5.8)
Scotland, Orkney, adult porpoise (1967)	1	0.19	1.1	0.33	2.2
E. Scotland, adult porpoises (1965, 1967)	3	4.0-14.0 (9.0)	0.6-33.3 (12.6)	1.2-14.3 (5.0)	13.1-25.7 (21.1)

Contamination by pesticides is greater in the seals on the east coast of Scotland (taken in an area roughly from Aberdeen to the Tay estuary) than in specimens from the west and north coasts, including the Orkney Islands. Seal pups from the Orkney and Harris (west coast) breeding grounds contained only slightly lower residues than adults from the same areas. By comparison, the Canadian samples from the Magdalen Islands (Gulf of St. Lawrence)

low porpoises examined, that from Orkney was the least contaminated, but the three taken on the east coast of Scotland had markedly higher contents of all the residues measured. One specimen contained a total residues concentration (dieldrin and DDT group) of 73.3 p.p.m. Analyses of a few tissues other than the blubber have been made, but concentrations in the blubber have always been found to be the highest (Table 2). Concentrations in other tissues seem likely to be consistent with their lower contents.

Table 2. TOTAL PEST CONCENTRATIONS (p.p.m.) IN VARIOUS TISSUES

Sample	Blubber	Liver	Brain	Kidney	Spleen	Muscle
Grey seal	1.8	0.77	0.26	—	—	—
Grey seal	0.2	0.36	0.16	—	—	—
Grey seal	14.8	0.97	0.21	—	0.53	—
Grey seal	8.4	0.10	0.17	—	0.13	—
Grey seal	2.1	0.11	0.07	—	0.07	—
Porpoise	3.6	0.55	0.02	0.01	0.10	0.20

The proportion of blubber extractable by hexane was determined for some samples. Values ranged from 72 to 86 per cent (mean 80 per cent) for the Canadian seals, and from 88 to 98 per cent (mean 93 per cent) for Scottish seals samples on the breeding grounds off the west coast. (Values for other seal samples were not estimated.) Concentrations of pesticides in terms of extractable fat for these samples would thus be up to 40 per cent greater than the values in Table 1, but the general comparison is not significantly affected. The lower extractable fat values for the Canadian samples may possibly be a result of being preserved in formalin.

Concentrations of pesticide residues in the extractable fat of various organs and tissues of one porpoise show an important relationship (Table 3). While the concentrations of the DDT group in the original tissues vary widely, those expressed in terms of extractable fat are in much closer agreement, with the sole exception of the brain. A similar anomaly for tissues of trout was described by Halden¹¹. It seems likely that a large proportion of the lipid fraction of the brain may not contain pesticides, and the brain lipid composition is known to differ considerably from that of deposit fat.

Table 3. CONCENTRATIONS OF TOTAL DDT (p.p.m.) IN TISSUES OF A PORPOISE

Tissue or organ	Blubber	Muscle	Liver	Spleen	Kidney	Brain
Concentration in tissue	2.6	0.30	0.55	0.12	0.01	0.03
Percentage extractable fat	87.0	8.1	13.2	8.1	1.4	8.3
Concentration in extractable fat	2.6	0.2	4.8	2.4	2.9	0.27

The unidentified peaks on the chromatograms seem to be similar to those produced by polychlorinated biphenyls and, to enable comparison to be made, the ratios (*R_x*) of the retention times of all regularly occurring peaks (relative to dieldrin = 100) have been calculated for the operating column temperature of 200°C, these values being temperature-dependent. The *R_x* values of PCB compounds from commercial PCB formulations were also determined for both types of column (Table 4). At least seven PCB-type peaks were found, including those which interfere with *p,p'*-TDE and *p,p'*-DDT. Heptachlor epoxide and *p,p'*-MDE could not be confirmed on both columns and are not therefore recorded.

The most significant aspect of the results is that, although seals and porpoises inhabit an environment far removed from the site of application or discharge of pesticides, they can contain much greater concentrations of dieldrin and DDT group residues than those recorded for most other species, including man. Robinson and Hunter¹² found a mean concentration of dieldrin of 6.22 p.p.m. (range 0.10-0.73 p.p.m.) and a mean total con-