Monsanto

FEB 23 1967

FROM (NAME & LOCATION): D.V.N. Hardy, London

DATE: 21st February, 1967

SUBJECT: Sweden, Aroclor

REFERENCE: DVNH/ARW

TO: Dr. R. Emmet Kelly, St. Louis

cc D. Wood, Brussels
G.R. Buchanan, St. Louis
J. Filer, Brussels
E. Wilde, St. Louis.

Re your memo of February 10th 1967 addressed to D. Wood, I am following up your request for information concerning the work at Monks Wood Experimental Station and at the Laboratory of the Government Chemist. We think that all this work is comparatively recent, and I cite below two recent communications from the latter.

1. "Organochlorine Pesticides in the Atmospheric Environment" by T.C. Abbot, R.B. Harrison, J.O'G. Tatton and J. Thompson, Nature 208, 25.12.1965 No. 5017 p. 1317-8.

2. "Organochlorine Pesticides in the Atmosphere" by D.C. Abbot, R.B. Harrison, J.O'G. Tatton and J. Thompson, Nature 211, 16.7.1966 No. 5046, p.259-261.

Photostat copies of these are attached for your convenience.

I have also been in touch with Mr. Richardson of Shell, to whom I supplied the samples of pure Aroclor components, and he reports that the characteristics of the unknown material in the organochlorine residues are quite different from those of any of the chlorinated biphenyls I submitted to him. He also said that he is carrying out metabolic experiments on fowls and that the chlorinated biphenyls appear substantially unchanged in the egg. I told him that Jensen has been asking for pure samples of Aroclor constituents, and we came to the conclusion that Jensen must therefore have jumped the gun in blaming chlorinated biphenyls. Richardson is going to see Jensen around March 2nd and they will discuss the position and Richardson's latest results. The situation will be reported to me on Richardson's return and I will pass it on directly to you.

D.V.N. HARDY

EXHIBIT
WBP M-10

MONS 096494

LEXOLDMON000028
WATER_PCB-00035495

ORGANOCHLORINE PESTICIDES IN THE ATMOSPHERE

By Dr. D. C. ABBOTT, R. B. HARRISON, J. O'G. TATTON and Dr. J. THOMSON

Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1

THE problems introduced by the pollution of our atmospheric environment have been concerned largely with those pollutants the effects and presence of which are readily demonstrated, for example, smog, sulphur dioxide, sooty materials and dusts. More recently, attention has been directed to the polynuclear hydrocarbons[1-3], although their presence in air is by no means solely of modern origin.

Recent publications have given evidence for the presence of some organochlorine pesticides in rainwater[4,5]. It has been suggested[5] that the minute traces found arose from the 'scrubbing' action of the falling rain on the air through which it passed, in a manner analogous to the collection of the inorganic constituents of rainwater[6]. The present work describes the experiments which have been carried out to ascertain the extent to which these organochlorine compounds are present in the atmosphere.

Pesticidal compounds may enter the atmospheric environment in several ways and in various physical states. Direct drift from spraying operations will contribute particulate or globular matter at concentrations which are likely to vary inversely as the distance from the site of application, such effects being mainly local but possibly appreciable in extent. It has been shown[7,8] that several organochlorine insecticides volatilize from treated soils, thus adding a slow, long-term contribution. Effluents and vapours from industrial processes, such as pesticide manufacture or moth-proofing of garments, may also contribute, while further quantities may accrue from the use of domestic aerosol insecticides and thermal vaporizers, and the dust from treated clothing and carpets.

Wheatley and Hardman[4] examined rainwater for traces of pesticides in order to try to account for residues found in soil from untreated plots of land. The amounts which they found were insufficient to account for more than a very small proportion of the pesticides present in such soil. Although the concentration of these compounds in air is lower by a factor in the range of 10–100 than that in rainwater, the vastly greater volumes of air may render this contribution of greater significance. As well as the normal, constantly changing contact between air and soil, the mass of air in surface soil may alter by up to 5 per cent owing to diurnal variations in temperature. Pesticides may thus be absorbed by the soil acting as a gas-solid chromatographic 'column' as well as by direct surface adsorption mechanisms. Assuming a tendency to equilibrium, treated soils will lose pesticides while untreated plots will gain them.

Preliminary experiments to illustrate the presence of pesticides in air were carried out by using a small portable constant-rate air-sampler which was used to suck air through a hexane trap. Although this method gave a total sample of only about 180 l. of air per power-canister, gas-liquid chromatography showed the presence of traces of BHC, DDT, DDE, TDE and dieldrin in samples taken in central London and its suburbs. Dieldrin alone was observed in a sample taken in Norfolk, while Aberystwyth air gave no detectable amounts of pesticide at this sample size. It was clear from these preliminary observations that an analytical problem existed and that larger samples would be required before reliable data could be obtained. Accordingly, an electrically operated vacuum pump capable of drawing 15 l. of air per min for long periods of time was brought into use. At this rate of flow hexane was too volatile, but dimethylformamide proved to be a suitable, non-volatile extractant. Examination of the separate contents of a sequential train of four solvent traps showed that nearly all the pesticide content was retained in the first vessel. Attempts have also been made to ascertain whether the pesticides present were in the vapour state or in association with the particulate matter of the air. Interposing bacteriological membrane filters (pore size 0·22 μ) between the air inlet and the solvent trap showed that variable proportions of the pesticides were retained on the filter. The amount retained, however, tended to reach an equilibrium value, indicating that the compounds were volatilizing again from the filter into the air stream. Similar effects have been observed with some polynuclear hydrocarbons[3]. Thus, although the use of membrane filters offers a simple qualitative test for pesticides in air, quantitative results can only be obtained by solvent scrubbing procedures.

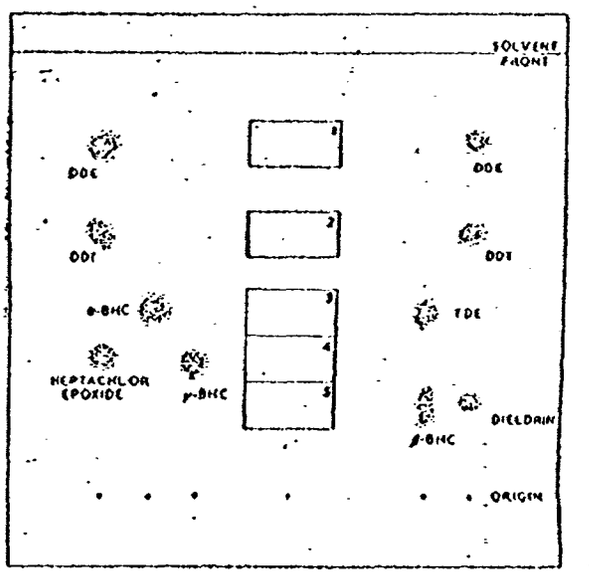

Fig. 1. Thin-layer chromatoplate

Metropolitan air was sampled by suction at about 15 l./min through 100 ml. of dimethylformamide contained in a Drechsel bottle fitted with a coarse sinter at the inlet to the solvent to ensure adequate diffusion. Dispersion of the solvent in 500 ml. of 5 per cent sodium sulphate solution followed by hexane extraction yielded a clean solution which, after evaporation to small bulk, was applied to a 250 μ silica-gel chromatoplate. This was developed with hexane : acetone, 90 + 1, for 35 min and appropriate fractions eluted with hexane[10] for examination by gas-liquid chromatography on both silicone and 'Apiezon' columns with electron-capture detection[11].

Fig. 1 shows the positions on the thin-layer plate of the more important organochlorine pesticides and indicates the areas containing the residues which were re-

MON-MT-008554

EXHIBIT WBP M-10.1

LEXOLDMON000029
WATER_PCB-00035496

moved for examination. Figs. 2–4 illustrate gas-chromatograph traces obtained from some of those areas; peaks corresponding to known pesticidal materials are marked—the other peaks have not been positively identified as yet. The appearance of a peak at the appropriate retention time on two chromatographic columns of differing polarity on injecting the eluate from the chromatoplate area of correct R_F value for the compound concerned has been taken as evidence of the identity of the compounds. Table 1 lists the quantities of pesticides found on examination of 8,000 l. of air taken on the roof of Cornwall House in August, 1965. The presence of alpha-BHC, gamma-BHC, dieldrin (HEOD), *pp'*-DDE, *pp'*-TDE and *pp'*-DDT was confirmed as already described; substantial agreement between quantitative results on both columns confirmed the absence of any appreciable amount of interfering non-pesticidal material of similar retention time.

Table 1. Organochlorine Pesticides found in London Air

Compound	R_F range of chromatoplate area	Concentration found (parts per 10^{10} w/w) by gas-liquid chromatography on (a) Silicone *E*.301	(b) 'Apiezon *L*'
Alpha-BHC	0·37–0·43	1	1
Gamma-BHC	0·27–0·36	6	11
Dieldrin (HEOD)	0·17–0·26	16	21
pp'-DDE	0·76–0·84	4	7
pp'-DDT	0·51–0·64	3	3
pp'-TDE	0·37–0·48	3	3

Silica-gel chromatoplate developed with hexane : acetone, 99 + 1.
Silicone column: 1·2 per cent silicone *E*.301 plus 0·12 per cent 'Epikote 1001' on 'Silanized Chromosorb G' (70–80 mesh) at 165° C; nitrogen carrier gas, 100 ml./min.
'Apiezon' column: 3 per cent 'Apiezon *L*' plus 0·3 per cent 'Epikote 1001' on 'Celite 545' (100–120 mesh) at 186° C; nitrogen carrier gas, 100 ml./min.

Great care must be taken in the interpretation of the gas-liquid chromatograms obtained from air samples. Even after the thin-layer separation process used, each fraction from the plate contained several peaks of unidentified materials showing electron-capturing properties. Some of the short-retention time peaks (Fig. 3) corresponding to section 2 of the chromatoplate (R_F 0·54–0·66) have been tentatively identified as the polynuclear hydrocarbons acenaphthylene (R_F 0·57), anthracene (0·67), fluoranthene (0·57) and fluorene (0·70). Retention times on both columns confirmed those identities; these substances are known to be among the *polynuclear* hydrocarbon ingredients of atmospheric dusts[9,11].

The three peaks of *longest retention times* obtained from section 1 of the chromatoplate (Fig. 2) would seem to result from compounds the behaviour of which on the plate and on each column is identical with that shown by the compounds giving the first three of a series of unidentified peaks often found in chromatograms of extracts from

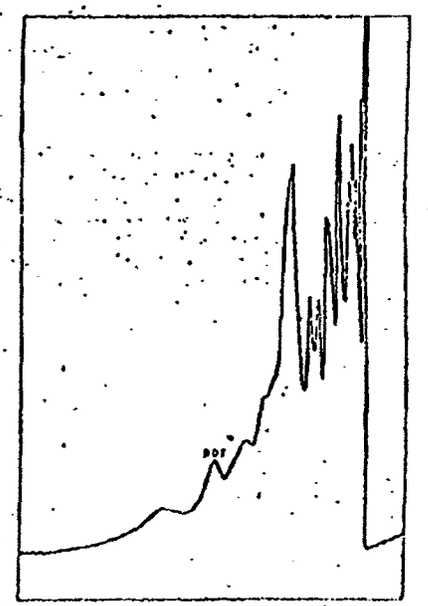

Fig. 3. Gas-liquid chromatogram from section 2 of the thin-layer chromatoplate

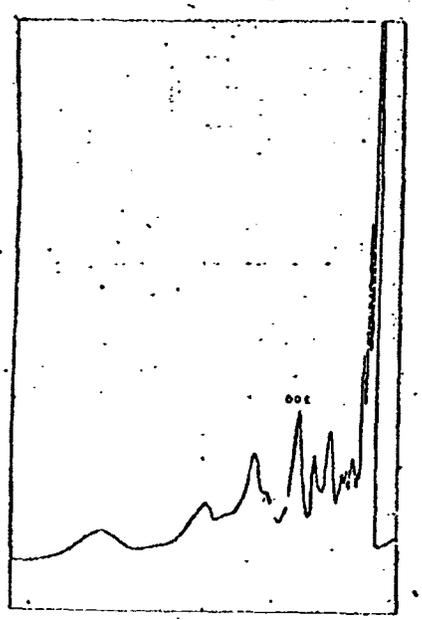

Fig. 2. Gas-liquid chromatogram from section 1 of the thin-layer chromatoplate

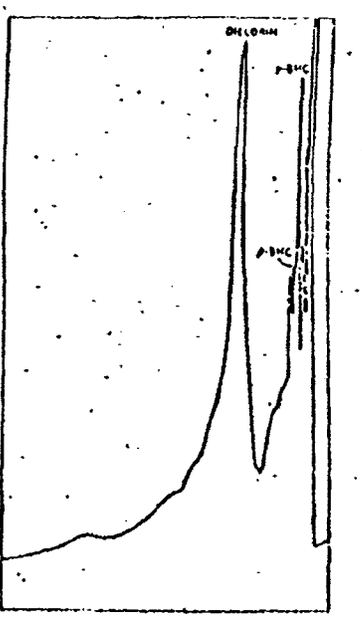

Fig. 4. Gas-liquid chromatogram from section 5 of the thin-layer chromatoplate

MON-MT-008555

LEXOLDMON000030
WATER_PCB-00035497

the tissue and eggs of some predatory birds. Their concentrations relative to each other in these two sample sources are also closely similar. Total chlorine determinations on avian tissue samples have indicated that these compounds contain chlorine, and because they are only found in samples containing appreciable residues they are probably breakdown products of organochlorine pesticides.

The evidence presented here and from rainwater examination[4,5] indicates the general occurrence in the atmosphere of minute traces of some of the persistent organochlorine pesticides. Such compounds have also been found in samples of air taken during the spraying season in American peach-orchard districts[10]. The concentrations of these residues are so low that it is necessary to work almost at the limit of sensitivity of the detection systems at present in use. For this reason, scrupulous care is necessary in order to avoid contamination at each manipulative stage, from the initial apparatus preparation to the final injection on to the gas chromatograph. Blank experiments must be performed at each stage and on all materials used. Particular care is required to exclude laboratory dust, for it is found that it is frequently a carrier of interfering electron-capturing materials.

Although the levels concerned are extremely low, these indications of the presence in air of organochlorine pesticides call for further and more detailed investigation. Confirmation of the identity of these residues by other methods such as infra-red spectroscopy or mass spectrometry would also be desirable, although far larger samples would be required in order to obtain sufficient material in a suitably pure state. It would also be of interest to compare the levels found in London with those found in other parts of the country; seasonal variation and correlation with other indices of atmospheric pollution would also be worth investigation. In these ways it may be possible to obtain some indication as to whether the pesticides derive from within the shores of the British Isles or from further afield.

[4] Sawicki, E., Stanley, T. W., Elbert, W. C., and Pfaff, J. D., *Anal. Chem.*, 36, 497 (1964).
[5] Liberti, A., Cartoni, G. P., and Cantuti, V., *J. Chromatog.*, 18, 141 (1965).
[6] Sawicki, E., Necker, J. E., and Morgan, M. J., *Intern. J. Air Water Poll.*, 9, 291 (1965).
[7] Wheatley, G. A., and Hardman, J. A., *Nature*, 207, 486 (1965).
[8] Abbott, D. C., Harrison, R. B., Tatton, J. O'G., and Thomson, J., *Nature*, 208, 1317 (1965).
[9] Egan, H., Brodin, O., and Johansson, O., *Annal. Agric. Coll. Sweden*, 22, 309 (1956).
[10] Decker, G. C., Weinmann, C. J., and Dunn, J. M., *J. Econ. Entomol.*, 43, 910 (1950).
[11] Harris, C. R., and Lichtenstein, E. P., *J. Econ. Entomol.*, 54, 1038 (1961).
[12] Rondia, D., *Intern. J. Air Water Poll.*, 8, 135 (1964).
[13] Harrison, R. B., *J. Sci. Food Agric.*, 17, 10 (1966).
[14] de Faubert Maunder, M. J., Egan, H., and Roburn, J., *Analyst*, 89, 357 (1964).
[15] Cantuti, V., Cartoni, G. P., Liberti, A., and Torri, A. G., *J. Chromatog.*, 17, 60 (1965).
[16] Breidenbach, A. W., *Arch. Envir. Health*, 10, 827 (1965).

HORMONES OF THE VERTEBRATE HOST CONTROLLING OVARIAN REGRESSION AND COPULATION OF THE RABBIT FLEA

By the Hon. MIRIAM ROTHSCHILD and BOB FORD

Ashton, Peterborough

In Nature, the rabbit flea (*Spilopsyllus cuniculi* Dale) only undergoes maturation on the healthy host during the last 10 days of the doe's pregnancy, or on nestlings 1–7 days old. Soon after the young rabbits are born, the fleas transfer to the nestlings, on which they feed avidly and pair: egg-laying on the body of the young and in the nest follows[1,2]. Between 8 and 22 (average 11) days later, the fleas abandon the young rabbits and return to the lactating doe. Any gravid fleas which remain on the doe after parturition, or are still gravid when they return to the lactating doe, regress rapidly and the developing oocytes are resorbed[1]. If, at a later date, the doe has a second pregnancy, these fleas are capable of undergoing maturation for a second time[3].

It was shown previously[2] that one of the principal groups of hormones governing maturation was the corticosteroids under the control of adrenocorticotrophic hormone (*ACTH*), while the oestrogens (and, to a lesser degree, thyroxine) played an important contributory part in development and egg-laying. It is probable that prolactin is a subsidiary factor in maintaining and promoting maturation on the nestling rabbits. The experiments described here indicate that lutoinizing hormone (*LH*) and the progestins are two of the principal factors governing ovarian regression and that somatotropin (growth hormone), also secreted by the anterior lobe of the pituitary gland, constitutes one of the major "copulation factors".

Earlier, it was claimed[1-3] that not only fleas feeding on buck rabbits injected with cortisone, corticosterone or hydrocortisone, but also fleas sprayed externally with these hormones, matured their eggs and displayed the various characteristics associated with maturation. It was thus shown that the hormones acted directly on the flea and not indirectly by way of the host. During the early stages of the investigation, it was thought that the rise and fall of the corticosteroid-levels in the blood of the host, and, to a lesser degree, in the levels of oestrogens and thyroxine during pregnancy, were sufficient to account for both maturation and regression. Thus the increased defaecation rate, which indicated an increased flow of blood through the body of the flea in response to the rise in hormone-level, automatically ensured that larger amounts of both hormone and food were available to the parasite. Conversely, a fall in the hormone levels cut down the defaecation rate (Table 2) and the amount of blood passing through the body of the flea, thus further reducing the amount of available hormones and nutrient material. Reduction in food intake alone does not account for the rapid regression of the ovaries of fleas feeding on the postpartum or oestrous doe; gravid fleas removed from the host and completely starved do not

Table 1. Movement of Fleas between Doe and Nestlings

Total No. of litters observed	66
Successful litters in which at least some young survived 10 days or longer	51
Unsuccessful litters: (a) all dead	15
(b) some dead	18
(16 of these litters born during winter months)	
No. of litters in which some or all fleas transferred from postpartum doe to nest or nestlings (9 litters in which fleas were removed by hand are not included)	33
No. of litters in which no fleas transferred (14 were winter litters)	19
No. of litters in which all fleas transferred to nestlings or nest	
Mean No. of days in nest before returning to doe (range 8–22)	11
Average time (approx.) for transfer from doe to young after parturition (range 10 min–20 h)	7–8 h
Day on which fleas of F^1 generation first emerged from pupa:	
Range 21–56 days	
Mean 30 days	

MON-MT-008556

LEXOLDMON000031
WATER_PCB-00035498

Table 2. Constants of the given equation for H_2O and CO_2. The calculations were made with eight numbers in order to obtain suitable results also for lower pressures

Const.	H_2O	CO_2
k	$1{\cdot}3805561 \times 10^{-16}$	$1{\cdot}3805730 \times 10^{-16}$
f_0	$1{\cdot}4864141 \times 10^{-16}$	$7{\cdot}744915 \times 10^{-17}$
f_0	$5{\cdot}2289137 \times 10^{-16}$	$7{\cdot}682670 \times 10^{-17}$
σ	$7{\cdot}8147519 \times 10^{-8}$	$6{\cdot}015895 \times 10^{-8}$
n	6·9	1·4229
v_{calc}	$2{\cdot}5057914 \times 10^{-5}$	$2{\cdot}5295745 \times 10^{-5}$

The values of the constants of Table 2 were calculated by the given form of the equation which, of course, is only an approximate one on several grounds. We will always be forced to use approximate expressions for such complicated processes of short-range molecular forces, the values of the constants being dependent therefore not only on the conceptual approximation of the problem, and even on the selected points of the liquid state used for the calculation, but also on the selected form of simplification.

The position of the points on the experimental curves used for the calculation of the constants (Fig. 1) leaves no doubt that the equation gives good to excellent results for these broad ranges of liquid states. This surprising fact means that the given relatively simple three-term equation, in comparison with the two-constant—two-term and even the more complicated formulae mentioned here, seems to contain some appropriate new step as to equations of state. Also the calculated points of the saturated vapour state, obtained, of course, with the same constants, are favourable or acceptable ones; this is especially so when it is taken into account that this is only the first stage in a new direction. The greatest divergence of the calculated pressure values is about 10 per cent for water and about 20 per cent for carbon dioxide near the critical point, generally rapidly decreasing at greater vapour volumes, and finally scarcely differing from the experimental data. The same equation, evidently, can represent the corresponding volume values of liquids with a considerably increased precision.

The essentially increased applicability of the formula pointed here gives the impression that the modifications incorporated could also be of theoretical significance. Improvements and applications of the solution given and the possible or probable theoretical background will be reported separately.

I thank Prof. P. H. Emmett of the Johns Hopkins University, Baltimore, Maryland, for discussions. I also thank the Petroleum Research Fund of the American Chemical Society and the Cabot Co., Boston, for financial support.

István Kürti*

Department of Chemistry,
The Johns Hopkins University,
Baltimore, Maryland.

* On leave from the Department of Physics of the Engineering Faculty of the Buenos Aires University, Buenos Aires, Argentina.

1 Shah, K. K., and Thodos, G., *Indust. Eng. Chem.*, 57 (3), 30 (1965).
2 Redlich, O., and Kwong, J. N. S., *Chem. Rev.*, 44, 233 (1949).
3 Ree, F. H., *J. Chem. Phys.*, 36, 3373 (1962).
4 Berthelot, D. J., *J. Phys.*, 8, 263 (1889).
5 Wohl, A., *Z. physik. Chem.* (Leipzig), 87, 1 (1914).

Organochlorine Pesticides in the Atmospheric Environment

Pollution of the environment has long been a subject of public concern and much attention has been paid recently to the new ecological factors introduced by the wide agricultural usage of pesticidal chemicals. Contamination of soil and crops has often featured in environmental studies of these pesticides, but so far little attention has been given to atmospheric pollution, although, as long ago as 1961, Harris and Lichtenstein[1] showed that the loss by volatilization of aldrin, dieldrin, heptachlor and gamma-benzene hexachloride was a major factor in their disappearance from soils treated with these compounds. Later, in the United States, the President's Science Advisory Committee drew attention to the fact that inhalation of air contaminated with pesticides might present a hazard to man[2] and recommended that air should be continuously monitored for pesticide residue levels. In 1965, an Advisory Committee of the United States Food and Drug Administration referred to studies which showed the presence of up to 0·075 p.p.m. of dieldrin in total diets[3] and considered the possibility that half as much again could be absorbed from "air or other exposures".

Little practical work seems to have been undertaken to determine pesticide residues in the atmospheric environment, but in the United Kingdom, Wheatley and Hardman[4] recently reported indications of the presence of organochlorine insecticides in rain-water collected in an agricultural area at Wollaston in Warwickshire. Steps were therefore taken at this Laboratory to examine rain-water collected in the Metropolitan area.

Two collecting stations were set up in Central London, one being on the roof of the Laboratory of the Government Chemist in Lambeth, S.E.1, and approximately 20 m above ground-level. The other was set up, with the co-operation of the Meteorological Office, at the London Weather Centre's station on the roof of State House, High Holborn, W.C.1, some 53 m above ground-level and about 1·45 km distant from the other station, across the River Thames. At these two stations, rain was collected in all-glass apparatus, with amber-coloured receiving vessels to reduce the incidence of photochemical degradation[5]. Samples were removed monthly for examination.

The organochlorine pesticides in the water were determined by a gas-liquid chromatographic method using electron-capture detection and silicone and 'Apiezon' columns as described by de Faubert Maunder *et al.*[6]. The limits of detection by this method were 5 parts per million-million for the isomers of benzene hexachloride (BHC) and 10 parts per million-million for the other organochlorine pesticides in general use in the United Kingdom.

The results for the months of February to July 1965, expressed in parts per million-million, are given in Table 1. Because of the small amounts of rain collected and the low levels of the pesticides found, it was not possible to confirm the results obtained for the individual samples by an alternative technique. However, at the end of the 6 months, the combined residues from each station were examined by thin-layer chromatography on silica-gel plates with hexane : acetone, 99 : 1, as mobile phase.

The appropriate areas of the chromatoplates were eluted and the separate eluates individually re-injected on to the gas-liquid chromatograph in columns. In this way, the presence of dieldrin, *pp'*-DDT and the α- and γ-isomers of BHC was confirmed for both stations. The identity of the *pp'*-DDT from both stations was further confirmed by conversion to *pp'*-DDE by treatment with ethanolic potassium hydroxide[7]. The *pp'*-DDE formed by this reaction was identified and determined by gas-liquid chromatography.

Insufficient quantities of the residues were available to confirm the presence of β-BHC, *pp'*-TDE and *pp'*-DDE

Table 1. Organochlorine Pesticide Residue Levels in London Rain-water

(Parts per million-million)

Station—Cornwall House, London, S.E.1

Month 1965	α-BHC	β-BHC	γ-BHC	HEOD (dieldrin)	*pp'*-DDE	*pp'*-TDE	*pp'*-DDT
Feb.	40	90	90	60	70	—	400
Mar.	15	—	60	60	20	—	115
Apr.	30	—	80	50	—	—	300
May	20	65	70	95	—	—	190
June	30	—	65	25	—	15	70
July	25	—	40	10	15	85	85

Station—State House, High Holborn, London, W.C.1

Month 1965	Rain (mm)	α-BHC	β-BHC	γ-BHC	HEOD (dieldrin)	*pp'*-DDE	*pp'*-TDE	*pp'*-DDT
Mar.	33·1	10	—	20	20	—	—	—
Apr.	37·3	20	—	70	70	—	100	140
May	20·0	20	25	65	80	—	—	220
June	36·2	20	—	165	20	—	60	125
July	76·0	30	—	70	15	10	20	80

MON-MT-008557

LEXOLDMON000032
WATER_PCB-00035499

by alternative techniques or to make an infra-red study of any of the residues".

Some individual rain-water samples which had been taken between September 1964 and January 1965 at a number of other places in the British Isles were also examined. The collecting sites ranged from Stornaway in the Outer Hebrides to Cunnborne in Cornwall, and the samples showed residues of organochlorine pesticides in amounts similar to those found in London rain-water. Samples of snow collected in different suburban areas within 25 km of London gave similar results. The size of these other samples, and the low levels found, again precluded the application of any confirmatory technique to the individual samples, but by appropriate fulfilling of the residues it was possible to confirm, by thin-layer chromatography, the presence, in the rain-water and in the snow, of dieldrin, *pp'*-DDT and the α- and γ-isomers of BHC.

The levels at which these pesticides have been detected are extremely low, near to the limits of sensitivity of the delicate electron-capture detection system used. Strict precautions were taken throughout to ensure that no fortuitous contamination of samples or extracts occurred within the Laboratory. Two samples taken from remote areas of Scotland during periods of heavy rainfall showed practically no residue content and these results suggest that adventitious contamination of samples in the Laboratory was negligible.

Present results suggest that the atmosphere carries, either as vapour or by occlusion on dust particles, small amounts of the organochlorine pesticides in common use in the United Kingdom and that they are 'scrubbed out' by rain and snow.

Work is now proceeding to determine the residue levels in air samples, and preliminary results show the presence of organochlorine pesticides in the range of 10–20 parts per million-million. Most of these residues appear to be associated with particulate matter. These experiments may eventually make it possible to determine the respiratory intake by man and to compare the result with his intake from certain foods of known pesticide-levels[6].

We thank the Director-General of the Meteorological Office for permission to quote from the London Weather Centre's records of the rainfall on the roof of State House.

D. C. Abbott
R. B. Harrison
J. O'G. Tatton
J. Thomson

Laboratory of the Government Chemist,
Cornwall House, London, S.E.1.

[1] Harris, C. R., and Lichtenstein, E. P., *J. Econ. Entomol.*, **54**, 1038 (1961).
[2] *Use of Pesticides, Rep. President's Sci. Advis. Comm.* (U.S. Government Printing Office, Washington, 1963).
[3] *Rep. Panel and Their Advis. Advis. Comm. appointed to Review Established Procedures for Practice of Advice and Decision on Uses Agricultural Commodities* (U.S. Government Printing Office, Washington, 1963).
[4] Wheatley, G. A., and Hardman, J. A., *Nature*, **207**, 460 (1965).
[5] Roburn, J., *Chem. and Ind.*, 1555 (1963).
[6] de Faubert Maunder, M. J., Egan, H., and Roburn, J., *Analyst*, **89**, 167 (1964).
[7] Hamence, J. H., Hall, F. S., and Caverly, D. J., *Analyst*, **90**, 649 (1965).
[8] Abbott, D. C., Crosby, N. T., and Thomson, J., *Proc. Soc. Anal. Chem. Conf.* (In the press).
[9] *Residues of the Persistent Organochlorine Pesticides*, Appendix F (H.M.S.O., London, 1964).

BIOCHEMISTRY

Separation of High-molecular Deoxyribonucleic Acid and Ribonucleic Acid

So far there has been no really satisfactory method for the quantitative separation in high-molecular form of a mixture of DNA and RNA isolated from cells. The procedure based on the different solubility of DNA and RNA in salt solutions of varying concentration[1–3] is quantitatively unsatisfactory. Another method uses chromatographic separation on methylated albumin[4–7], but involves a series of steps which entail the danger of degradation in the recovery of the macromolecule. Specifically, single-stranded DNA cannot be quantitatively recovered without loss of its high-molecular character, because complete elution occurs only at strongly alkaline *p*H. Nygaard and Hall[8] have recently described a micro-method for the detection of DNA/RNA hybrids based on their adsorption on membrane filters of nitrocellulose containing material.

On the basis of the selective adsorption of single-stranded DNA on nitrocellulose fibres of a certain nitration degree (the most satisfactory preparation proved to be 'Nitrocell', supplied by the Serva Laboratories, Heidelberg), we have developed a simple method for the quantitative separation, in high-molecular form, of the two components from a nucleic acid mixture. While RNA and double-stranded DNA (dissolved in 2 × standard saline citrate (SSC), *p*H 6·7) pass through such a column (Fig. 1), single-stranded DNA is quantitatively retained. Elution occurs only with very diluted solutions at *p*H > 9 (Fig. 2). The fact that the sedimentation constant (S_{20} = 23·6) of a single-stranded DNA from bacteriophage *FD* (courtesy of Prof. H. Hoffmann-Berling, Max-Planck Institut für Medizinische Forschung, Heidelberg) remained unchanged (S_{20} = 22·1) proves that the molecule size is not altered during the adsorption and elution steps.

For the isolation of the nucleic acids, approximately 6 × 10^8 HeLa cells, cultivated in Hanks's solution with the addition of lactalbumin hydrolysate and calf serum, were washed and gently disintegrated by repeated freezing and thawing; during the last thawing, dodecylsulphate *tris*-buffered to *p*H 7 was added (final concentration 0·5 per cent). The resulting viscous solution was diluted and deproteinized in the usual way by treating it twice with phenol[9]. After two-fold precipitation by 67 per cent alcohol, the nucleic acids were dissolved in 0·1 × SSC, dialysed against SSC, and centrifuged for 10 min at

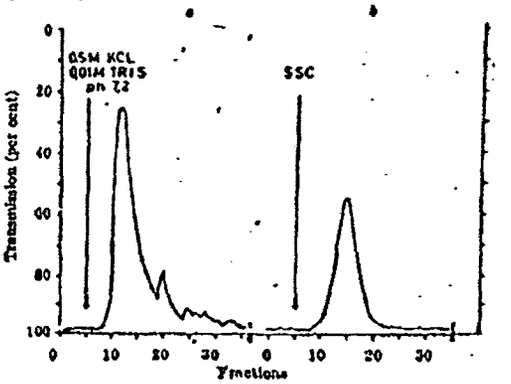

Fig. 1. a, Elution diagram of high-molecular RNA from yeast; b, that of a double-stranded DNA isolated from thymus. Optical densities were measured with the Uvicord (LKB, Stockholm)

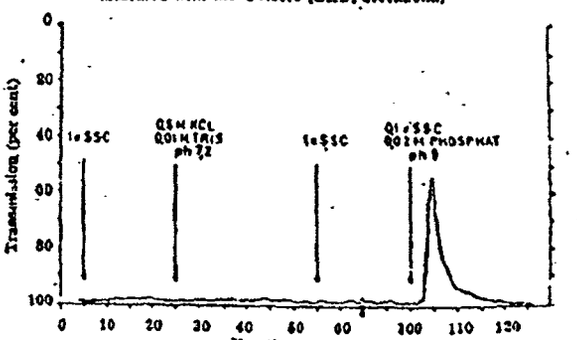

Fig. 2. Elution of a circular form of the single-stranded DNA from phage, dependent on the eluting agent

MON-MT-008558

LEXOLDMON000033
WATER_PCB-00035500