Current Progress in the Determination of the Polychlorinated Biphenyls

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The waste products of a global technological society inevitably become components of the environment. Many of these waste materials have the capacity to inflict permanent changes upon the ecosystem. Among the synthetic pollutants which are accumulated by wildlife the chlorinated hydrocarbons of biocide origin appear to be the most abundant. Residue levels in marine fish and birds now frequently surpass those recorded in terrestrial and fresh-water species (1,2,3). In California, the concentrations of chlorinated hydrocarbons in petrels, pelagic birds which do not approach land except to breed on off-shore islands, are many times higher than those recorded in species inhabiting agricultural and urban areas (1). Other classes of environmental pollutants which might be similarly accumulated by organisms and similarly dispersed around the world could be expected to share some or all of the following properties of the biocide derivatives which have contributed to their unexpected distribution in the global ecosystem.

1) High world production. U. S. production of DDT in 1965

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was 140 million pounds (4). World yearly production is therefore in the order of 10^{11} grams, only five orders of magnitude less than the amount of carbon fixed by plants into organic matter (5). Other pollutants which are present in ecologically significant amounts in the global ecosystem must, like p,p'-DDE, therefore derive from primary materials that are produced in comparable amounts.

2) Chemical stability. p,p'-DDE, which may be the most abundant of the synthetic pollutants in the environment, is comparatively resistant to degradation by the usual detoxification mechanisms of vertebrates, to microbial action, and to non-biological breakdown in the environment. Its half-life, therefore, would appear to be greater than ten years.

3) Insolubility in water. Water-soluble waste products will be diluted to a greater extent in the sea and may or may not be subsequently accumulated by organisms. The non-polar nature of p,p'-DDE and the other chlorinated hydrocarbons explains why they are concentrated in fat tissue and why they are present in greatest amounts in the terminal carnivores of food chains.

4) Mobility and aerial dispersal. Even though vapor pressures of potential pollutants might be very low, the amounts entering the atmosphere could become ecologically significant over periods of time, especially when rates of vaporization are greatly increased by codistillation with water (6). Incineration of

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waste materials would also significantly increase the rates of vaporization of some compounds. The global distribution of DDT can be fully explained only by aerial dispersal with subsequent fallout, in conjunction with transport by water (2).

Among industrial products which meet these criteria are the aroclors, which are mixtures of chlorinated polyphenyls. Their usefulness in the manufacture of many plastics, paints and resins derives to a large part from their chemical stability and resistance to degradation.

Unidentified peaks produced by halogen-containing compounds have long been evident in gas chromatograms of extracts of fish and birds. The identification of most of these compounds as polychlorinated biphenyls (PCB) is now based upon the following evidence.

1) Mass spectrometric analysis in Sweden. Mass spectra of compounds present in extracts of fish and sea birds obtained with a combined gas chromatograph-mass spectrometer showed that chlorine-containing compounds which could not be identified as biocides were polychlorinated biphenyls (7).

2) Retention times in gas chromatographic analysis. The majority of peaks in chromatograms of extracts of fish and birds which cannot be identified as biocides that emerge after p,p'-DDE have retention times on both polar and non-polar columns which are identical with those of the principal PCB compounds. The

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major peaks produce a characteristic pattern on each kind of column. The same patterns of peaks are evident in extracts of wildlife from around the world (1,2,8,9).

3) Chlorine content. Microcoulometric analysis shows that the compounds identified as PCB contain halogens.

4) Saponification and nitration. Unlike the peaks of toxaphene (10), p,p'-DDT, p,p'-DDT, and p,p'-DDD, the PCB peaks are not removed or displaced by dehydrohalogenation with alcoholic KOH. Vigorous nitration carried out at room temperature with fuming nitric acid (11) removes the chromatographic peaks of both PCB and the DDT compounds, but does not destroy the toxaphene peaks. At 100° the DDT compounds are tetranitrated by this procedure (12). The nitrated derivatives would not pass through the commonly used GLC columns. A milder nitration process, carried out at 0° with nitric acid rather than fuming nitric acid, destroys the DDT, but not the PCB peaks.

5) Distribution in the global environment. Concentrations of PCB in fish and birds are highest in such industrial outfalls as San Francisco Bay and San Diego Bay and concentration gradients apparently exist from these areas to regions more remote (1,2). A consistency in the ratios of p,p'-DDE to PCB among the birds of a given region suggested the existence of regional fallout patterns (1). PCB has been found in all sea birds from the Pacific Ocean so far analysed, but they could not be detected

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in penguin eggs from the Antarctic which did contain DDT compounds (1).

The retention times relative to p,p'-DDE, of peaks in chromatograms of extracts of fish and birds which are identical with those of PCB compounds in the commercial preparations are:

DC-200: 1.25, 1.48, 1.75, 2.05, 2.41, 2.50, 2.90, 3.41, 3.88, 5.53; dieldrin: 1.00; p,p'-DDD: 1.27; p,p'-DDT: 1.68

QF-1: 1.10, 1.33, 1.40, 1.65, 1.72, 2.14, 2.59, 3.23, 3.88, 4.84; dieldrin: 1.49; p,p'-DDD: 1.75; p,p'-DDT: 1.91

The columns employed were 10% DC-200 and 3% QF-1, both on Chromosorb W, 80-100 mesh and were maintained at a temperature of 195°. The retention times of the three principal peaks are underlined. The first of these, with a retention time of 1.25 on DC-200 columns emerges with p,p'-DDD. Another major PCB peak emerges slightly later than p,p'-DDT on DC-200 columns. On QF-1 columns, the compound with retention time 1.33 emerges with o,p'-DDT and the compound with retention time 1.75 interferes with the determination of p,p'-DDD. On this column there is no interference with p,p'-DDT. Dieldrin appears as a trailing shoulder on the peak with retention time 1.40.

As a result of this interference, it appears that many of the values of p,p'-DDD, p,p'-DDT and o,p'-DDT reported in recent literature are erroneous. The DDD values originally reported in Pacific sea birds in a paper from this laboratory

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(13) were too high because of PCB interference and have subsequently been corrected (1). p,p'-DDE is the most abundant of the DDT compounds in the environment and there is no significant PCB interference in the determination of p,p'-DDE. Consequently the total DDT residues reported in the past, before the extent of PCB interference was known, would not be greatly changed after correction for this interference. o,p'-DDT, which has been shown to be an active estrogen (14,15) is present in very low amounts in the global environment, although o,p'-DDE is comparatively more abundant (1,3).

When both DDT and PCB peaks are present in chromatograms, the clue to the relative amount of PCB comes from the height of those PCB peaks which do not interfere with any of the DDT compounds. With the exception noted below, the three major peaks usually have approximately the same height on chromatograms obtained with an electron capture detector. If the peak with retention time 1.48 on DC-200 columns is approximately as high as the "DDD" and "DDT" peaks, no or very small amounts of p,p'-DDD and p,p'-DDT are present. When one or both of these peaks is relatively higher than the PCB peak with retention time 1.48, the amount of p,p'-DDD and p,p'-DDT can be estimated from the changes in peak height with saponification. The saponification procedure is rapid, and since only a relative change in peak heights is determined, it need not be quantitative. An

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aliquot of the extract is refluxed for approximately 5 minutes in ethyl alcohol with 5% KOH, to which hexane and concentrated aqueous NaCl solution are added in turn. Chromatography of the hexane layer shows that the p,p'-DDD and p,p'-DDT peaks have been displaced, but the PCB peaks are unchanged. The contribution of PCB to the peaks of the original chromatograms can thereby be estimated, provided the detector response is linear.

PCB has been shown to be a powerful inducer of steroid hydroxylases in birds (1) and, with p,p'-DDE, may therefore be partially responsible for the aberrant calcium physiology observed in species of raptorial and fish-eating birds which accumulate chlorinated hydrocarbons (16,17). It is probable that the PCB in human food supplies would induce the synthesis of comparable enzymes in man.

Since it is highly unlikely that primary standards of individual PCB compounds will be available in the foreseeable future, the quantification procedures, like those for toxaphene (10), also a mixture of polychlorinated compounds, must for the present be approximate. The quantification procedure we have used (1,2) is based primarily upon the determination with a microcoulometric detector of the total chlorine content of the PCB compounds emerging after p,p'-DDE. The chromatograms of fish and bird extracts most closely resemble those produced by the commercial aroclor which has an average chlorine content

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of 54%. PCB concentrations in fish and birds were therefore derived from the chlorine values by assuming that the chlorine content was also 54%. This procedure would occasionally include other chlorinated pollutants with the PCB. It may, however, be more meaningful to measure total organic chlorine (18) of non-biocide origin in tissues of wildlife than parts per million concentrations of individual compounds. This might be a better index of physiological effects such as enzyme induction.

Since we do not yet have in our laboratory the permanent use of a microcoulometric detector, we have had to depend upon electron-capture for routine quantification of PCB. Each PCB compound was initially assumed to produce the same peak height, with the electron capture detector, as an equivalent amount of p,p'-DDE. Amounts of sample injected into the gas chromatograph were adjusted in order to fall within the linear response range of the electron capture detector. The amount of total PCB considered as p,p'-DDE was then multiplied by a factor which would yield a value equal to that obtained with a microcoulometric detector. This procedure was periodically checked by electron capture determination of standard PCB mixtures.

Although this method yields approximate results, it does permit accurate determinations of relative concentrations. It has shown that some species of birds, notably certain raptorial

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species, shearwaters and petrels, accumulate high concentrations of PCB (1). Terminal carnivores of food chains may accumulate and concentrate the PCB present in prey species (1). It has also shown that regional fallout patterns exist (1), with highest concentrations in industrial regions.

The PCB compound with retention time 1.25 on DC-200 columns and 1.33 on QF-1 columns produces one of the prominent peaks in chromatograms of extracts of fish and birds from San Francisco Bay and in the commercial mixtures, yet it is not present, or is present in relatively low amounts, in extracts of birds from the Gulf of California. PCB in this area must derive from aerial fallout, since there are few industrial areas in Baja California or along the coast of western Mexico. We therefore tested the possibility that this compound might have been selectively degraded by ultraviolet light during its transport in the atmosphere. Standard PCB solutions in hexane were evaporated to dryness in Petri dishes, or were applied to Petri dishes containing either water of 3% NaCl solution. They were then irradiated at a distance of 4 inches with a 15-watt germicidal lamp emitting at 2537 Å. In each case the pattern of peaks on the chromatograms of the irradiated PCB was essentially unchanged, except for a relative reduction in the height of this peak. The effect was more pronounced when PCB was irradiated on the aqueous surfaces.

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References

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

2. R. W. RISEBROUGH, Chemical Fallout, First Rochester Conference on Toxicity, in the press.

3. R. W. RISEBROUGH, D. B. MENZEL, D. J. MARTIN and H. S. OLCOTT, in preparation.

4. The Pesticide Review, United States Department of Agriculture (1966).

5. E. S. NIELSON, Ann. Rev. Plant Physiol. 11, 341 (1960).

6. F. ACREE, Jr., M. BEROZA, and M. C. BOWMAN, Agric. Food Chem. 11, 278 (1963).

7. G. WIDMARK, J. Aas. Off. Anal. Chem. 50, 1069 (1967).

8. D. C. HOLMES, J. H. SIMMONS, and J.O'G. TATTON, Nature 216, 227 (1967).

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

10. T. E. ARCHER and D. G. CROSBY. Bull. Environ. Cont. Toxicol. 1, 70 (1966).

11. F. ERRO, A. REVENUE, and H. BECKMAN, Bull. Environ. Cont. Toxicol. 2, 372 (1967).

12. M. S. SCHECHTER and H. L. HALLER, J. Am. Chem. Soc. 66, [illegible] (1944).

13. R. W. RISEBROUGH, D. B. MENZEL, D. J. MARTIN, and H. S. OLCOTT, Nature 216, 589 (1967).

14. R. M. WELCH, W. LEVIN, and A. H. CONNEY, Toxicol. Appl. Pharmacol., in the press.

15. J. BITMAN, H. C. CECIL, S. J. HARRIS, and G. F. FRIES, Science 162, 371 (1968).

16. D. A. RATCLIFFE, Nature 215, 208 (1967).

17. J. J. HICKEY and D. W. ANDERSON, Science 162, 271 (1968).

18. F. A. GUNTHER and J. H. BARKLEY, Bull. Environ. Cont. Toxicol. 1, 39 (1966).

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