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MONITORING PCB IN WATER AND WILDLIFE

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INTRODUCTION

Although polychlorinated biphenyls (PCB) have been manufactured for various purposes for the past forty years, it was not until 1966 that Jensen identified them in a variety of environmental samples, using the gas chromatographic technique employed since 1962 for the detection and measurement of the persistent organochlorine pesticides such as DDT (and its metabolites) and dieldrin. Confirmation of the identity of the PCB-residues was made by mass spectrometry, which at the same time indicated the presence of a larger number of polychlorinated biphenyls than was suggested by the number of gas chromatographic peaks.

In the analysis of wildlife samples for organochlorine pesticides since 1962, several workers had recorded the presence of a number of unidentifiable GLC peaks. Jensen's discovery of the presence of PCB residues, while assisting in the determination of a further group of detectable contaminants, made it evident that some of the earlier analytical results reported in the literature, particularly for pp'-DDT and pp'-DDE, were probably erroneous. Subsequently techniques have been developed to enable a reasonable degree of separation of PCB from many organochlorine pesticides to be achieved before GLC analysis, but the PCB residues must still be estimated as a group at present.

Polychlorinated biphenyls can be used for a wide variety of purposes, and the mixtures of isomers produced by the various manufacturers may range from those containing about 20% by weight of chlorine, averaging about one chlorine atom per molecule, to some containing about 70% of chlorine, averaging almost nine chlorine atoms per molecule. A total of 209 chlorinated biphenyls, containing from one to ten chlorine atoms, is theoretically possible, and it has been shown (Simmons and Welti, 1971) that one particular 54% chlorinated mixture contains at least fifty different isomers. Few of these have been prepared individually, and consequently for routine analysis of environmental

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samples the commercially-available mixtures must be used as reference standards. Some degradation of the lesser-chlorinated members of the series probably occurs in the environment, and in consequence the pattern of GLC peaks obtained from wildlife samples differs from that of the commercial mixtures. The estimation of total PCB content and toxicity of the wildlife residues are therefore only approximate at the present time.

In view of the persistence of the higher polychlorinated biphenyls, their presence at all environmental levels, their potential interference in the analysis for organochlorine pesticides, and their possible toxicity to various forms of life, considerable attention has been given in recent years to the problem of their world-wide distribution. Most studies have involved fish and birds, but in view of the possibility that global monitoring programmes for pollutants such as organochlorines may be established in the near future some consideration must be given to the types of environmental samples most suitable for the purpose of monitoring.

The Organisation for Economic Co-operation and Development(OECD) has for several years sponsored voluntary international studies of the problem of sampling and analysing wildlife specimens for organochlorine residues, and this paper summarises the results of the first six years of investigation, and indicates the difficulties involved both in the analysis of the samples and in the selection and acquisition of the necessary sample material. It has been possible to compare the efficiency of analysis in different countries, and to assess the importance of analytical errors as compared with the variations between individuals in populations of certain species. The occurrence of PCB concentrations in environmental media as opposed to living organisms is also discussed, media samples sometimes being suggested in preference to wildlife samples for monitoring purposes.

ANALYTICAL METHODS

PCBs are extracted and analysed by the same techniques as are employed for organochlorine pesticides, and appear to be recovered from water and tissue samples with the same efficiency, but must be separated from the majority of pesticides before GLC analysis to avoid interference. Separation is usually made using silica column chromatography,

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the PCB compounds being eluted with p,p'-DDE (Holden and Marsden, 1969), although Armour and Burke (1970) achieved a degree of separation of the PCBs from DDE with a more active type of silica. Several methods have been proposed for the determination of the total PCB content from the gas chromatographic trace, but a recent OECD study (Holden, in preparation) has shown that a considerable measure of agreement can be obtained among analysts using different methods of estimation.

Peak height is usually measured although peak area, measured by electronic integration or mechanical devices, is sometimes used; 4 to 6 GLC peaks are commonly involved. The estimation of PCB concentration is influenced to some extent by the choice of a suitable reference standard, which is usually arbitrary in view of the difference in proportions of the various PCB peaks, as between environmental samples and commercial products. Nevertheless, in the recent OECD study, eight analysts using different methods of estimation but similar 60% chlorinated reference mixtures obtained results on a cormorant sample which showed a coefficient of variation of ±14%, a value no greater than was obtained for some individual pesticides in another exchange sample used in the same OECD study. An alternative method of assessment, not yet widely used, is the complete chlorination of all PCB residues found to the deca-chloro compound, giving a single GLC peak which is compared with the same peak produced from any PCB formulation, or a deca-chloro-biphenyl standard.

Where low-level concentrations are being determined, as in water or plankton samples, great care is necessary to avoid contamination from laboratory or other sources which may give GLC peaks in positions similar to those from the samples. Such contamination, insignificant in the analysis of samples containing high concentrations of PCBs, can be derived from plastics (particularly PVC), oil used in air compressors, human skin (if not adequately washed beforehand), internal seals of metal containers, and in the solvents or adsorbents used in analysis. All solvents must be purified by distillation, and all adsorbents or drying media (e.g. alumina, silica, sodium sulphate) must be calcined or solvent-washed before use. Blank runs through the complete analytical procedure must be made frequently to check for possible contamination.

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PCB LEVELS IN THE ENVIRONMENT

(a) Air and Rain No reference has been found in the literature to concentrations of PCB in gaseous form in air, and it is quite possible that atmospheric PCB is associated with particulate matter (dust) or water droplets. In the sampling of air, such forms would be removed by filters and analysed as particulate matter. Risebrough et el (1968)[a] found 1 to 64 ppb of organochlorines in dust from air over Barbados, but did not positively identify PCB. Analysis of airborne dust off the California coast did not reveal the presence of PCB, and the deduction was made that PCB do not adsorb to particulate matter.

In the sampling and analyses of atmospheric precipitation for organochlorines, only the common pesticides ∝- and Y-BHC, dieldrin, pp' DDT and its metabolites pp'-TDE and pp'-DDE have been reported in detail. Wheatley and Hardman (1965) and Tarrant and Tatton (1968) found these residues in rain in the United Kingdom, but the latter also noted the presence of other residues believed to be PCB, without quantifying them. Their existence was confirmed in further rain samples collected in London (Annual Report of the Laboratory of the Government Chemist, 1970), with an approximate (TLC) estimate of their concentration as 50-100ng/l.

Rain samples have been collected and analysed from four different sites in Scotland during 1972, and preliminary results indicate that PCB levels are in the range 20-40ng/l, with total DDT levels of 5-20ng/l. These samples are taken in all-glass equipment, protected from birds, the aerial debris (dust, pollen, etc.) being retained separately on pre-washed glass wool filters which are also analysed. Several hundred millilitres of rain must be collected, the sampling periods being irregular according to the prevailing weather conditions. The concentrations of organochlorines found in the samples are nevertheless much higher than in river and stream waters in Scotland. Furthermore, there is so far no evidence that the residues are transported from industrial areas of Scotland by prevailing winds and it seems likely that the contamination is of more distant origin, or perhaps reflects a general level of atmospheric contamination. In this form of sampling, however, it is impossible to exclude the possibility of transient local contamination, whether from

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the excreta of flying birds, from insects, or passing transport, and such events should not be ignored.

(b) Fresh Waters A few references to PCB concentrations in rivers have appeared in the literature, although these generally refer to local contamination. In the Milwaukee River, U.S.A., 20-200ppt of Aroclor 1260 and 80-2070ppb of Aroclor 1242 were detected (Veith and Lee, 1971). In Lake Biwa, Japan, 100-1000ppt of PCB were found (Isono, 1971). Ahling & Jensen (1970) found 0.33 and 0.50ppt in natural waters in Swedon, but particulate material contained 1350ppt. The sediments in Ontario (Canada) rivers have been found to contain up to 0.6p.p.m. (Berg et al, 1972), and in several U.S. rivers up to 3.2ppm (Yates et al, 1972). The latter also found 0.1-4ppb in unfiltered river waters from the United States. The Michigan Water Resources Commission has reported 10-2900ppt in 30 tributary streams to the Great Lakes (Anon, 1972).

Analyses of Scottish streams and rivers for organochlorines in 1971 and 1972 have shown that, even in streams with no evidence of industrial or gross sewage pollution, the PCB concentrations are in the range 2-20ppt in unfiltered samples. The concentrations of organochlorine pesticides found were 0.1-5.1ppt dieldrin, 0.3-5.3ppt DDE, 0.3-6.4ppt TDE and 0.2-5.7ppt DDT, excluding badly-polluted rivers. These residues, with the possible exception of dieldrin (used for about ten years in sheep dips throughout Scotland until 1966), are believed to result from atmospheric, rather than terrestrial contamination. The PCB estimations were made on the basis of fewer peaks than is customary for tissue samples, and further examination is likely to reduce the values quoted above.

(c) Sea Water Analysis of a sea-water sample from the Firth of Clyde, West Scotland, an area which receives about one million tons of sewage sludge per annum, showed a concentration of 8ppt. Previous investigations (Holden, 1970) had shown that about one ton of PCBs were probably contained in the annual discharge of sludge but during 1972 the source from which the PCB entered the sewer was removed. Sea-water samples from the Firth of Tay, East Scotland, contained 5-20ppt, the higher values

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being in the vicinity of the city of Dundee. Much of this may have been in particulate matter.

(d) Freshwater Fish The levels of PCB in freshwater fish have been reported from only a few areas, partly due to the fact that pesticide concentrations in rivers and streams in agricultural areas are often greatly in excess of PCB levels, but also, as in Scotland, because terrestrial sources of direct contamination are often non-existent. The PCB problem has so frequently been related to the marine environment that their presence in fresh waters may have been overlooked, yet discharges of industrial wastes or crude sewage to rivers would be expected to result in significant PCB accumulation in the biomass. Possibly the fact that such rivers, if heavily polluted, may contain no fish has produced this situation, as fish are commonly used as the monitoring species for such pollutants.

Yet fish, like many other aquatic species, can concentrate PCB by at least three orders of magnitude, and it would be surprising if PCB residues were not detectable in the fish of the more polluted rivers and lakes. Zitko and Choi (1971) reported a concentration of 14.6ppm in a coho salmon (Oncorhynchus kisutch) from Lake Michigan. Other fish from Lake Michigan analysed by Veith and Lee (1970) contained 15-25ppm. Fish from the Great Miami River (Kroner, 1971) had up to 150ppm, the PCB being discharged by paper mills. Zitko (1971) found 0.36-1.01ppm in American eel (Anguilla rostrata) from New Brunswick (Canada) rivers and, as there was no evidence of industrial or sewage pollution in some of these rivers, Zitko considered atmospheric fallout to be a possible source.

(e) Freshwater Invertebrates Invertebrates are less often studied than fish, but Södergren et al (1972) used the amphipod Gammarus pulex as an indicator organism for both DDT and PCB in streams in southern Sweden. There was evidence of a seasonal variation, with higher residue levels in August than in April, the PCB concentration being 0.002-0.011ppm. A number of other invertebrates were also examined, but PCB concentrations never exceeded 0.020ppm, total DDT being up to 0.046ppm.

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(f) Marine Fish and Shellfish. Zitko and Choi (1971) summarized the analytical data reported for marine fish, the concentrations in the presumably unpolluted areas being less than 0.1ppm. In areas known to be polluted, such as the California coast, the Baltic and the Bay of Fundy, values up to 1.0ppm have been found. In a heavily polluted area such as Escambia Bay, Florida, concentrations from 4.5 to 20ppm were detected. Tissues containing a high proportion of lipids were more heavily contaminated

In a survey of the organochlorine content of commercial marine fish species from waters around the United Kingdom, (Portmann and Holden, unpublished), PCB concentrations in muscle tissue ranged from less than 0.1ppm in fish such as cod (Gadus morhua) up to 1.5ppm in plaice (Pleuronectes platessa) and mackerel (Scomber scombrus) in the polluted waters of the Firth of Clyde. Fish liver reflected contamination of the environment even more markedly, with concentrations in cod liver ranging from 0.4 to 43ppm. Expressed in terms of extractable lipid, PCB levels may range from 1ppm in unpolluted to over 300ppm in polluted areas.

The shellfish most commonly used for pollution studies have been molluscs such as oysters and mussels. However, these species seem to be able to rid themselves of contaminants such as pesticides relatively quickly, and the concentrations found may not always reflect the external conditions at the time of sampling. Nevertheless, persistent pollution in estuaries will give rise to abnormally high residue levels in mussels as found in surveys made in Scotland, using Mytilus edulis. Specimens in the Firth of Clyde contained up to 3.0ppm, while in clean waters the concentrations of 0.1-0.2ppm were more usual. The overall range, however, is not very large.

(g) Marine Plankton Marine zooplankton, with a significant lipid content, might be expected to reflect PCB levels in the surrounding aqueous medium, but two major difficulties arise in their use for monitoring. Zooplankton samples usually vary in species composition, and this may result in a between-sample variation in PCB content by virtue of the different lipid contents of the various species. Analyses based on lipid content might, however, reduce the influence of species composition. A more serious problem arises from the possibility of collecting in the plankton net

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such extraneous material as/from ships, and tar or oil, which are found almost everywhere on the ocean surface. Nets used for plankton should be closed when entering or leaving the water surface. As oil is an excellent solvent for PCB, even a small amount may produce a significant enhancement of PCB concentrations in plankton samples. Paint, hydraulic fluid or lubricating oil from ships could also be sources of PCB.

Despite these potential errors, analyses of mixed zooplankton samples in the eastern Atlantic and Firth of Clyde have shown a consistent pattern, the latter containing higher concentrations, as expected from the local PCB contamination. The samples from the north-east Atlantic contained 0.01-0.11ppm wet weight (0.1 - 4.2ppm in lipid), while samples from the Firth of Clyde, including some unpolluted areas, contained 0.04 - 2.20ppm wet weight (1.1 - 20.0ppm in lipid). Extremely high PCB concentrations, expressed in terms of extractable lipid, were obtained from plankton (more correctly seston) samples in the Bristol Channel, S.W. England, where the sediment is continually disturbed by tidal movements. The area receives a considerable amount of industrial waste, and PCB values of up to 7000ppm in lipid were calculated for the seston. Such a situation would be unlikely in the open ocean. Recent analyses of zooplankton from the Clyde suggest that species composition may have little influence on the PCB levels in zooplankton samples.

(h) Birds. Both terrestrial and aquatic species of birds may accumulate high PCB concentrations, but the piscivorous species, particularly in the marine environment, generally contain most. Risebrough et al (1968) recorded the results of analyses of both DDT group and PCB residues from a wide range of avian species in North America. Some species contained less than 0.01ppm in muscle tissue, but values between 0.1 and 1.0ppm were common, and in species feeding on fish in polluted areas concentrations between 10 and 100ppm were common. Zitko and Choi (1971) summarised the analyses of birds from a number of literature references, and in a few instances, such as in the Stockholm Archipelago, values between 100 and 10,000ppm PCB were found. Such concentrations would seem to be potentially, if not actually, toxic to the birds. The eggs of several species in contaminated areas have also been found to contain high levels of PCB, sometimes 10 - 100ppm.

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The range of PCB residues in birds from various locations is thus extremely wide, from 0.01 to 10,000ppm, and much larger than is found in fish, for example. In this respect, birds would appear to be most appropriate organisms for monitoring the distribution of PCB in the environment, although other considerations must qualify this judgement, as discussed later.

(i) Marine Mammals Holden, (1972, and 1973 in press) has described the analysis of seal and porpoise fat (blubber) for organochlorine residues, and the variation in PCB concentrations found in seals from different parts of the world. Concentrations in extractable lipid range from 1ppm in the Arctic to 250ppm on the west coast of the United Kingdom, but in starving specimens, which have metabolised most of their fat reserves, up to 2800ppm has been found. The increase in concentrations in lipid, as the result of starvation stress and reduction of body fat reserves, is a complicating factor in the use of residue concentrations in organisms for assessing the extent of local environmental contamination.

MONITORING PCB IN THE ENVIRONMENT

The above summary has indicated the range of concentrations of PCB to be found in the environment, but before any selection of species appropriate for monitoring is made several aspects of the problem of environmental contamination must be considered. The contaminant in question may enter an organism from the external medium, such as air or water, or from sediments in the case of an aquatic filter-feeder, and thus the media might be appropriate for sampling and analysis. However, contamination of water or air might fluctuate appreciably with time, and organisms, in which the fluctuations would be reduced, could reflect the general situation more effectively, while at the same time providing higher concentrations for easier determination. As uptake from food is an alternative, and often the only, source of contamination in most species, the concentration in the external medium may not be directly relevant.

Species of which the individuals move over considerable distances, either for feeding or on migration, may give residue information which is difficult to interpret, as the residues may not have been ingested in the area in which the specimens were

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obtained. Thus adult migratory birds are not appropriate monitoring organisms. For the same reason, marine fish, which may move from estuarine to oceanic waters, could also be unsuitable. Freshwater fish which remain in a lake over a complete life-span, or invertebrates, which move over relatively short distances, can on the other hand provide information relevant only to their local environment.

A further important consideration in the choice of species for monitoring is that sufficient individuals must be available for sampling at the required time, without having a significant influence on the total population. As residue levels often increase with age, and may also differ between the sexes, the specimens may have to be selected both for age and sex, this increasing the problem of sampling. Furthermore, there is always a natural and sometimes considerable variation among individuals within a population, even when of one sex and age. In consequence, to obtain a reasonably accurate estimate of the mean level of contamination in any one population, a large number of individuals may be required, creating problems both in sampling and analysis.

At this point, it is worth considering the purpose for which a monitoring programme may be designed. Initially it is intended that the level of contamination in several areas will be determined, so that the more heavily contaminated areas can be identified, and the sources of pollution controlled. Yet, unless these are already known or suspected, and sampling areas chosen accordingly, a general monitoring programme is unlikely to reveal such "hot-spots". On the other hand, contamination over large areas, or due to aerial fallout from distant sources, may be detected in a general survey.

The information obtained must, however, be sufficiently accurate to establish statistically that levels of contamination appearing to be high are in fact high, by comparison with those in other areas. Similarly, it should be possible for changes in the contamination level from year to year to be detected with appropriate statistical significance. Such information is of value in assessing the consequences of government or industrial action which has been taken to reduce pollution. The accuracy of the estimates of contamination levels depends in part on the precision of the analyses, which in turn is dependent on the experience and efficiency of the analysts,

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In large-scale monitoring programmes many analysts will be involved, but an adequate assessment of their agreement can be obtained by the use of exchange samples.

Finally, the acquisition of analytical data is not the ultimate purpose of monitoring. The underlying fear is that contamination levels may be sufficient to have adverse biological effects at one or more points in an eco-system, but as yet adequate evidence of such effects has been established in very few instances. It is essential that the concentrations in various species which are capable of causing adverse effects should be determined, whether the effects are for example, reduced photosynthetic activity in algae, the failure to reproduce in invertebrates, abnormal behaviour in vertebrates, or mortality in any species.

OECD STUDIES OF MONITORING

The Organisation for Economic Co-operation and Development (OECD), in a series of international collaborative studies on the monitoring of organochlorine residues in the environment, has attempted to examine some of the problems referred to in the preceding paragraphs.

In the second programme (Holden, 1970), the ability of analysts in several countries to obtain the same results, both qualitatively and quantitatively, in the analysis of exchange samples was measured. At that time (1968-69), the variation between analysts increased with the difficulty of processing the samples, and for the only residues in which there was general agreement on identity (dieldrin, DDE, TDE and DDT), the coefficient of variation was ±25 to ±60%. In the third programme (Holden, in preparation) a substantial improvement was noted for the period 1969-71, the coefficient of variation for six pesticide residues and PCBs in samples requiring full processing being ±10 to ±18% among 17 laboratories.

The question of the number of individuals of a population required to provide a mean value of sufficient precision for detecting differences between populations, or changes from year to year, was also examined in the second programme. In both sterling and pike populations (with no selection for age or sex), fifty individuals would have been required to detect a 25% difference between means, but ten would have been

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sufficient to detect a 75% difference. The main reason for such low precision was that the residues in the individuals were usually skewly distributed, but this may have been due to the variation in age within samples.

The third study programme included the analysis of PCB residues in addition to organochlorine pesticide residues in selected wildlife species, and samples were taken from uncontaminated and contaminated areas. The results of this programme have provided information on the extent of PCB contamination, and also on the difficulty of identifying the contaminated areas.

It must be borne in mind that, in common with all other PCB analyses at the present time, the values obtained are estimates of the total PCB concentration expressed in terms of a commercial mixture of specified chlorine content. The proportions of the various isomers and homologues present in the samples usually differ from those in the commercial mixtures, and no information on the concentrations of individual compounds is afforded by the analysis. The assessment of the potential toxicity of the residues detected is thus rendered difficult, and any such estimate must be regarded with caution.

Ten countries sampled and analysed the mussel (Mytilus edulis) in coastal waters, both in areas considered to be uncontaminated and in other areas known to be polluted. The assessment of pollution varies considerably, some polluted areas giving PCB concentration in mussels as low as 0.02mg/kg, and the unpolluted area giving 0.67mg/kg. Areas with varying degrees of pollution gave values of 0.02 - 1.7mg/kg. A similar situation arose with the samples of herring (Clupea harengus) analysed by eight countries. Those from areas classed as unpolluted had PCB concentrations in muscle tissue ranging from 0.10 to 0.54mg/kg, but the range in fish/from polluted areas was 0.19 to 3.2mg/kg.

Freshwater fish show less evidence of PCB contamination. The pike (Esox lucius) sampled in six countries usually contained little PCB even in polluted waters, the values generally being less than 0.1mg/kg. Yet where serious pollution by PCB exists the concentrations can be much higher, as in the samples analysed by the Netherlands, with PCB levels of 0.47 - 0.95mg/kg.

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The eggs of fish-eating birds can demonstrate the accumulation of organochlorine residues from their food very effectively. Eggs of the heron Ardea cinerea were analysed by the United Kingdom and the Netherlands. In samples from remote areas of the United Kingdom concentrations below 0.5mg/kg were found in the eggs, with up to 7.3mg/kg in a very polluted area. In the Netherlands the concentrations in polluted areas were up to ten times greater, 33 - 74mg/kg. This ability to concentrate residues in eggs was also shown by the samples from two common tern (Sterna hirundo) colonies in Canada. The relatively uncontaminated colony gave 2 - 4mg/kg PCB but that in a polluted area contained a mean of 149mg/kg in eggs.

DISCUSSION

The third OECD programme has indicated the difficulties of identifying areas polluted by organochlorines, and in particular PCB, by evidence other than chemical analysis. Consequently the identification of local sources of PCB pollution by the analysis of indicator or monitoring species would entail a very large and detailed sampling and analytical programme involving numerous sites. On the other hand, the detection of more general pollution over large areas, if such pollution exists, should be disclosed by a pattern of fewer sampling sites at which an appropriate species could be used for monitoring on a regular basis. Ideally, this species should accumulate the contaminants to such an extent that there is a considerable difference between PCB content of uncontaminated and polluted specimens. The species should therefore be relatively resistant to the effects of the pollutant.

Referring to the information provided by the third OECD study, concentrations of PCB in mussels showed an overall range of about 1:100, with only a 1:30 range for herring. The eggs of herons showed at least a range of 1:150, and an even greater range might be revealed by further samples. A heron in the Stockholm Archipelago was found by Jensen et al (1969) to contain 9,400mg/kg in muscle, which suggests that eggs from such birds could also contain exceptionally high levels.

While eggs may be particularly useful in revealing areas of pollution, the numbers available for analysis could be limited. On the other hand species such as mussels or

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herring can usually be collected in quantity. The greater precision of the estimation of the mean level of contamination of a population of mussels, herring or similarly abundant species could therefore be as important in the choice of a monitoring species as the wider range of contamination levels likely to occur in the eggs of piscivorous birds.

A further restriction is placed on sampling by the habitat of the species. Mussels can only be collected from coastal waters, for example, and seals, in which extremes in contamination level have also been demonstrated (Holden, 1972) are limited in their distribution. As many seal colonies are protected, it may be difficult to acquire more than a few specimens for analysis. The major problem which arises in the selection of an appropriate monitoring species for global surveillance, however, is that very few occur in all areas of the world, although an ecologically equivalent species can often extend the range. Species which seem ideal from many points of view (adequate numbers, ease of sex and age determination, ease of sampling) may be limited to the northern hemisphere, or to one continent. Marine species are sometimes more widespread, but clearly cannot reflect contamination in terrestrial or freshwater environments, where pollution may originate.

The OECD studies have enabled some assessment to be made of analytical accuracy, of the ability to obtain samples of chosen species in the required numbers (or of the attendant difficulties), and of the ranges of various residues which exist in polluted and unpolluted areas. They have also shown the difficulty of positively identifying such areas prior to performing the analyses. In particular, the choice of species which would be appropriate for global monitoring has been under constant discussion, and no species has yet been proposed which meets all the requirements for such a purpose. It is likely that a wide variety of species must eventually be proposed, the assessment of the extent of pollution being based on the results from several species in each area, rather than on any particular species.

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