D-2.3.3 Development of Chemical Analytical Systems

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Abstract

The present paper overviews the ecotoxicological implications of marine mammals by persistent organochlorines. The marine mammals, particularly cetaceans, are one of the animal groups receiving high concentrations of persistent organochlorines arising out of a worldwide contamination. They can amplify much greater amounts of toxic contaminants through feeding and also pass them in large quantities from one generation to the next through lactation. Unfortunately, these animals have smaller capacity for degradation of these contaminants due to the specific mode of cytochrome P-450 enzyme systems. These drug-metabolizing enzyme systems may be related to the possible effects of persistent organochlorines, particularly coplanar PCBs. Furthermore, the residue levels of these contaminants in marine mammals are unlikely to decline in near future. Considering all these facts, it may be concluded that marine mammals are one of the most vulnerable and possible target organisms with regard to long-term toxicity of hazardous man-made chemicals in future.

Key Word organochlorines, PCB, coplanar PCBs, global contamination; marine mammals

Introduction

The demand for many new materials in modern civilization and the concomitant development of the chemical industry has resulted in the production of man-made chemicals in large numbers and quantities, contributing greatly to mankind for a convenient and pleasant life. On the other hand, it should also be noted that many tragic disasters involving environmental deterioration and human health effects have emerged as an unexpected outcome of these developments. The undesirable effects of some of these chemicals are linked to the occurrence of immunologic, reproductive and teratogenic dysfunction in various animals. The abnormalities are generally believed to result from the exposure to persistent organochlorines such as polychlorinated biphenyl (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and their related compounds.

Looking at recent toxic effects by suspected contaminants on wildlife, marine mammals have made a frequent appearance in particular. Stating an anticipant conclusion for those reasons, marine mammals such as pinnipeds and cetaceans may have specific and unique processes for bioaccumulation of toxic contaminants. At the same time, the function of the marine environment as a sink for persistent contaminants should also be considered. These situations lead eventually to very high concentrations of organochlorine residues in marine mammals and partly to the occurrence of toxic effects also. The present paper overviews ecotoxicological implications of marine mammals by the persistent organochlorines.

Ecotoxicological implications in marine mammals

The role of oceans as a sink of toxic contaminants should also be considered in ecotoxicological perspective because these organochlorines are extremely bioaccumulative and impose a toxic threat to resident and migratory marine organisms. Particularly, marine mammals such as whales, dolphins and seals which are at the top of food chain are known to have extremely high rates of contaminant accumulation and thus are a matter of concern on their toxic effects.

In case of the western North Pacific ecosystem, it was reported that the marine food chain has a high capacity to amplify toxic contaminants, despite low residue levels in water (less than part per trillion levels). Striped dolphin, top predator in this food chain, retained very high concentrations of PCBs and DDTs, the values reaching about 10 million times higher than those in water. Such an extraordinary accumulation has also been observed in another case. As shown in Fig. 1, cetaceans inhabiting pristine oceans far from industrial and human activities were found to contain much higher concentrations of PCBs than coastal and terrestrial mammals which live near to pollution sources [1]. This pattern of contamination is against the generally believed notion that the level of contaminants decrease with increasing distance from pollution sources. These observations prompted us to believe that cetaceans might have specific mode of intake and release kinetics for persistent contaminants.

In the course of our studies to examine the above, three factors which cause such a specific contaminant accumulation in cetaceans emerged. First, it can be emphasized that cetaceans have a large pool of persistent toxic contaminants in their bodies. Persistent toxic contaminants like organochlorines are generally lipophilic and hence they have strong affinity to lipid rich tissues and organs. Indeed, cetaceans have a thick subcutaneous fat (blubber) and most of the toxic organochlorines are retained in this tissue. In the case of striped dolphins (*Stenella coeruleoalba*), organochlorines such as PCBs, DDTs and HCHs are mostly accumulated in the blubber, accounting for more than 90% of the whole body burdens of these contaminants [2]. Once these contaminants are retained in such fat rich tissue, they are not easily eliminated and, in consequence, this tissue plays a role as a large stock of persistent contaminants with possible toxic effects. Secondly, a significant factor can be seen in the reproductive process of cetaceans. In general, age-dependent accumulation of persistent organochlorines is rather common in higher animals. This pattern was also found in male animals of cetaceans. However, female animals revealed practically a decreasing pattern in the residue levels of these contaminants after maturity, as represented in Dall's porpoise (Phocoenoides dalli) in Fig. 2[3]. Such a specific pattern in female cetaceans has been explained by the transfer of organochlorines to their offspring in considerable quantities during lactation. It was presumed in the case of striped dolphin that over 60% of PCB residues in the body of the mother animal are transferred to the new-born through milk. Such a high transfer rate is quite unique to cetaceans, never exhibited in other mammals, which can be attributed to the high lipid contents in the milk of this animal and lipophilic nature of organochlorine contaminants. These reproductive processes may impose serious toxic impacts on cetaceans, particularly to new-borns, and also imply the continuous long-term contamination by toxic organochlorines over many generations, even if the status of marine pollution can be improved in near future. Apart from the reproductive point of view, the third factor deals with the metabolic capacity to degrade toxic contaminants. It was found in the course of study on the isomer-specific analysis of PCBs in various species of higher animals including cetaceans [1)] Comparing PCB compositions among various animals, it was noticed that cetaceans retained larger numbers of PCB isomers and congeners than other animals. Additionally, lower chlorinated PCBs that are relatively biodegradable were prominent in cetaceans than in other coastal and terrestrial animals. These residual patterns suggest that cetaceans may have a specific capacity for metabolic PCB degradation. Further findings indicated that cetaceans have a low capacity to metabolize a group of PCB isomers with adjacent non-chlorinated meta- and para-carbons in biphenyl rings [1)]. The comparative approach of PCB compositions also suggests that drug-metabolizing enzyme systems in cetaceans have a smaller functioning of MC (3-methylcholanthrene)-type enzymes but not PB (phenobarbital)-type enzymes (Fig. 3). A study using cetacean liver microsomes demonstrated that these animals have considerably low activity of aldrin epoxidase (PB-type enzymes), whereas the activity of 7-ethoxyresorufin O-deethylase (MC-type enzymes), is comparable to those in non-induced rats, supporting the low capacity to metabolize PCB isomers and congeners [4)]. We believe that the third factor concerning metabolic capacity is the most convincing evidence why the cetaceans retain a wide variety of organochlorines at very high concentrations. All these three factors give an insight into the long-term accumulation of hazardous chemicals in the body of cetaceans and the high risk they are facing in the ecotoxicological context. Regarding the long-term toxicity of environmental contaminants to wildlife and humans, the highly needed studies of great concern are carcinogenicity, teratogenicity, immunologic dysfunction and reproductive abnormalities. Indeed, some of these toxic symptoms are more or less associated with particular enzymes like cytochrome P-450 monooxygenase. These enzyme systems are known to modify some of these contaminants into active toxic intermediates and sometimes they disturb the critical balance of endobiotics like steroid hormones. Some persistent organochlorines are recognized to initiate toxic and biologic effects, since they are potent inducers of P-450 enzyme systems [5)]. Therefore, a research to confirm the possible enzyme induction and disturbance of endobiotics by toxic organochlorines is an urgent need in the natural ecosystem, particularly in marine mammals. A few studies on this line was conducted and some suggestive indication of organochlorine toxicity was observed. For example, Dall's porpoise from the Northwestern North Pacific revealed negative correlations between the residue levels of PCBs and DDE in the blubber and the testosterone levels in blood

[6]. This might well be a causal relationship, since testosterone is metabolized by certain P-450-based monooxygenases which might be susceptible to induction by organochlorine compounds. It is also noteworthy that a significant correlation between 7-ethoxyresorufin O-deethylase (EROD) activities and PCB residue levels was recorded in the liver of short-finned pilot whale from the western North Pacific [7]. Interestingly, similar positive correlation between PCB residues and EROD as well as PROD (pentoxyresorufin O-deethylase) activities was found in the northern fur seal (*Callorhinus ursinus*) collected from the Pacific coast of northern Japan (Fig. 4). These correlations are likely to indicate that the possible enzyme induction by PCBs are taking place largely and commonly in natural ecosystems, at least in marine mammals. A recent study using Mediterranean striped dolphins, which were affected by an epizootic, pointed out that the high concentrations of PCB residues in these animals induced P-450 enzymes and highlighted the possibility of immunosuppression in these animals leading to mass mortality [8]. Considering these observations, it is likely to mention that the present status of contamination by organochlorines in the marine ecosystems has already reached the critical point which might be enough to cause the induction of P-450 enzymes and disturbance of endobiotics in higher organisms such as marine mammals.

Under these circumstances, it should be also clarified that what type of organochlorines plays a major role on the ecotoxicological concern. In this context, major concern so far has been focused on the highly toxic contaminants such as polychlorinated dibenzo-p-dioxins and dibenzofurans and many studies have been conducted on their environmental occurrence, biological accumulation and toxic effects. However, several investigators have recently pointed out the ecotoxicological significance of particular PCB isomers which have non- and smaller number of ortho-chlorine substitutions, eliciting similar toxic nature to dioxins and furans [9]. These toxic PCB components, the coplanar PCBs are of growing concern for their association with biological disorders such as mass mortalities of seals in the North Sea and the Lake Baikal, epizootic in Mediterranean striped dolphin and embryonic abnormalities in Great Lakes waterbirds. Results of several recent investigations suggested that the coplanar PCBs were more hazardous because of their greater impact on wild animals than those of dioxins and furans. In wildlife and humans, residue concentrations of coplanar PCBs were generally found to be at much higher levels than those of dioxins and furans [10]. These toxic PCBs were also detected at considerable concentrations in marine mammals, indicating their global distribution similar to common organochlorine insecticides such as DDTs and HCHs [8]. While evaluating the toxic impact of coplanar PCBs using the 2,3,7,8-TCDD equivalent (TEQ) approach [11], marine mammals revealed much higher values of TEQ than terrestrial mammals. In this context, the difference in the TEQ values between coplanar PCB group and dioxin-furan group was apparently larger in marine mammals than terrestrial mammals, where higher values were observed for coplanar PCBs. These results indicated that the toxic significance and ecotoxicological impact of coplanar PCBs were likely to be higher than those of dioxins and furans and this trend is prominent in marine mammals than in terrestrial mammals. Among coplanar PCBs, the mono-ortho congeners were estimated to contribute greater toxic impacts in marine mammals, while non-ortho congeners were prominent in terrestrial mammals (Fig. 5), suggesting different toxic action of PCBs in marine and terrestrial animals. Particularly, three components of PCBs, 2,3,3',4,4'-pentachlorobiphenyl (IUPAC 105), 2,3',4,4',5-pentachlorobiphenyl (IUPAC 118) and 2,3,3'4,4',5-hexachlorobiphenyl (IUPAC 156) seem to be most hazardous with possible long-term toxic potential on marine mammals.

In order to understand the long-term biological effects of hazardous contaminants, the future trend of residue levels should be estimated. The study on the historical trend of contaminant residues is most useful to predict their forthcoming toxic impacts. However, only limited numbers of studies are available regarding the temporal variation of toxic contaminants in marine mammals. To our knowledge, a comparative study using the same age group of adult male striped dolphins from the western North Pacific collected in 1978-79 and in 1986 seems to be one of the reliable examples dealing with the long-term temporal variation of organochlorine residues in cetaceans. In this study, it was elucidated that the concentrations of PCBs and DDTs did not decline during the study period, whereas HCH levels were found to be decreased slightly. Another study was conducted using northern fur seal migrating in a wide range of the northern North Pacific and nearby cold waters[12]. The fur seal samples employed were adult females from 20 to 23 years old, which were preserved in formalin since 1971. As shown in Fig. 6, residue levels of PCBs and DDTs were found to be maximum in 1976 and then decreased. DDT levels continuously declined and the levels at the end of the 1980s reached about one twentieth of the maximum levels in 1976, while PCB levels in 1980s were rather steady reaching about only half of the maximum. On the other hand, HCHs were constant with no apparent decline since 1971. Considering these results, it can be concluded that the recent contamination by organochlorines in marine mammals has recovered from a heavily polluted status in 1970s, but their declining trends are extremely slow in 1980s. Particularly, PCBs are the contaminants of high concern with long-term toxic potential. The slow temporal decrease of PCB residue levels implies no apparent reduction in deleterious toxic impacts leading to disease and mass mortality in cetaceans and pinnipeds.

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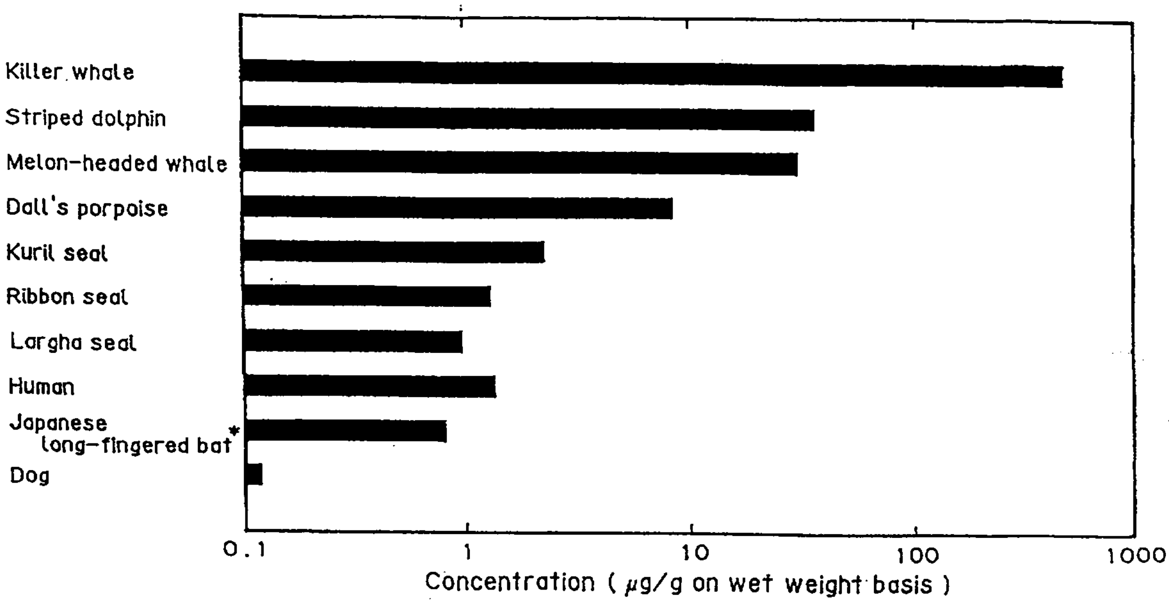

Fig. 1. Comparison of PCB concentrations in fat tissue of cetaceans from the North Pacific, and coastal and terrestrial mammals from several areas in Japan (data from Tanabe et al., 1988 and Kannan et al., 1989).

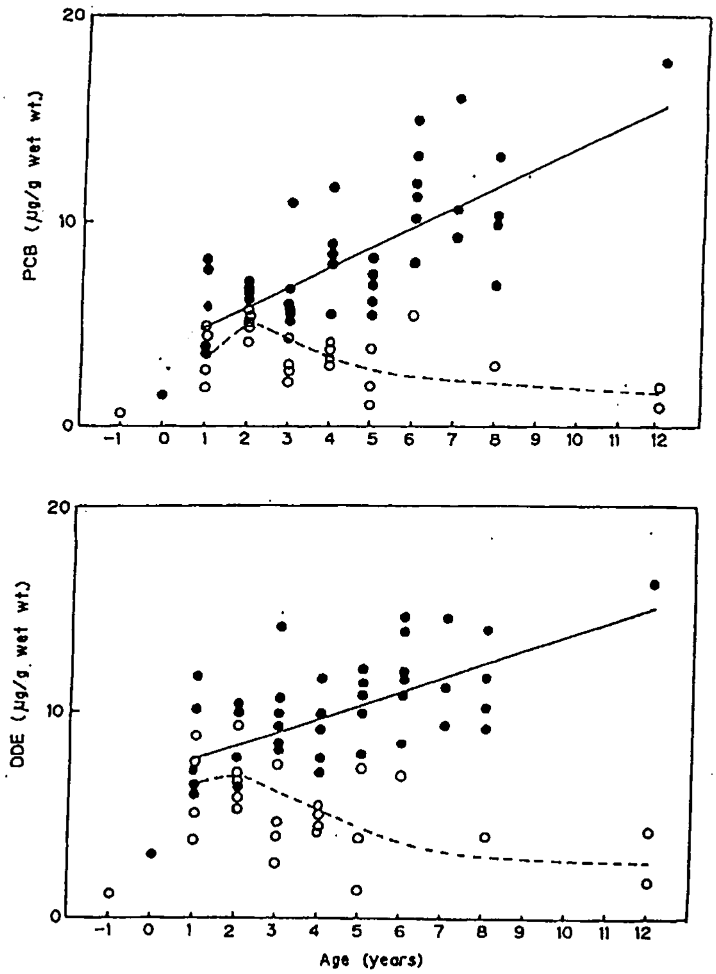

Fig. 2. Age tends of PCBa nd DDE concentrations in male (●) and female (○) Dall's porpoise from the northern North Padific (Subramanian et al., 1988).

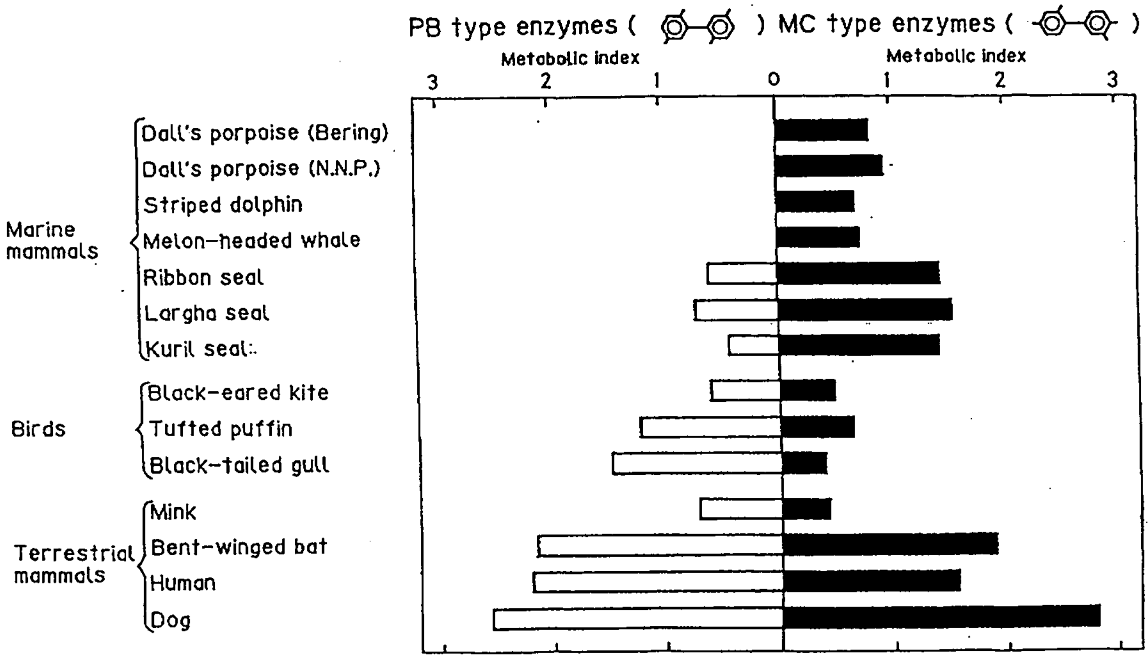

Fig. 3. PB (phenobarbital)- and MC (3-methylcholanthrene)-type enzyme activities in higher animals as estimated by Metabolic Index (MI) of 2,2',5,5'- and 2,3',4,4'-tetrachlorobiphenyl isomers which indicate the capacity of PCB metabolism. For more details of MI see Tanabe et al. (1988).

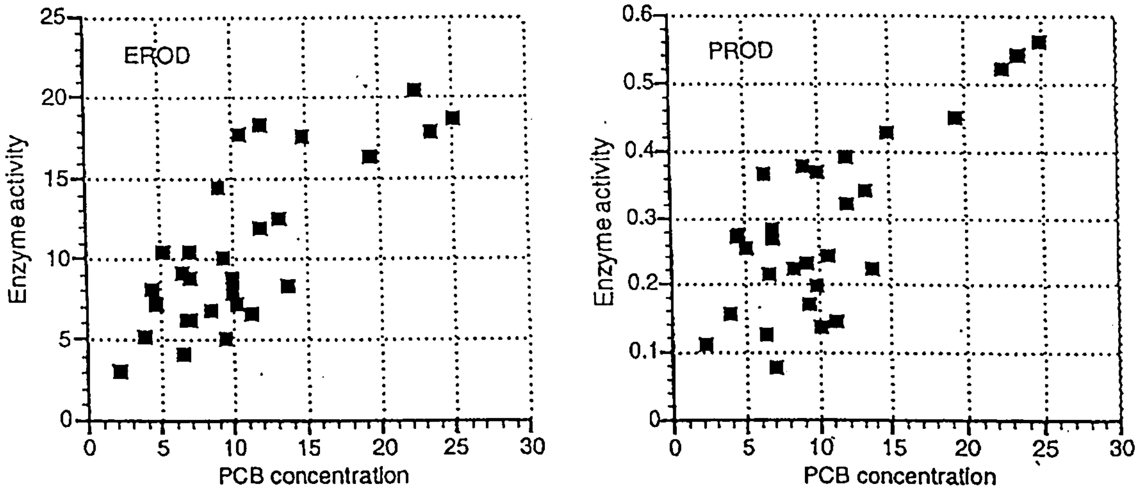

Fig. 4. Relationship between PCB residue levels and EROD (7-ethoxyresorufin O-deethylase) / PROD (pentoxyresorufin O-deethylase) activities in northern fur seals collected from the Pacific coast of northern Japan (Tanabe et al., unpublished).

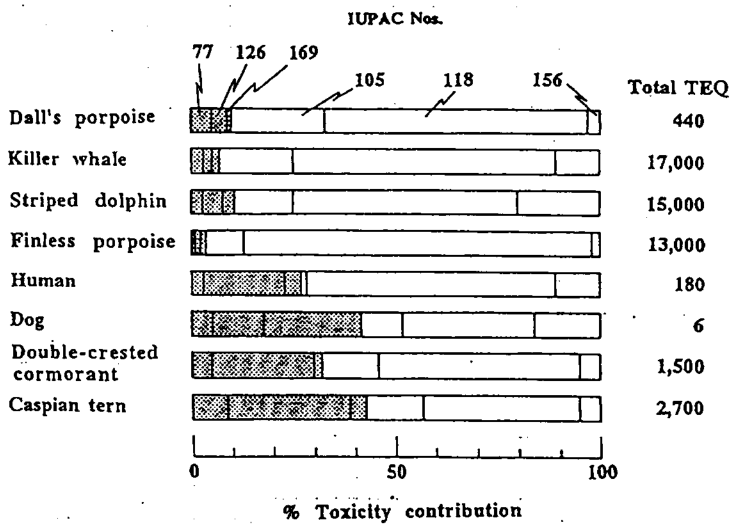

Fig. 5. Comparison of relative contributions of 2,3,7,8-TCDD toxic equivalents (TEQ) by non- and mono-ortho polychlorinated biphenyls (PCBs) in different species of marine and terrestrial mammals. Total TEQ (in pg/g wet wt) is the sum of toxicities estimated for non- and mono-ortho PCB congeners only. Concentrations of non-ortho PCBs in Dall's porpoise, killer whale, finless porpoise, human and dog were obtained and caluculated from Kannan et al. (1989), those of striped dolphin from Kannan et al. (1993), and those of double-crested cormorant and Caspian tern from Yamashita et al. (1993).

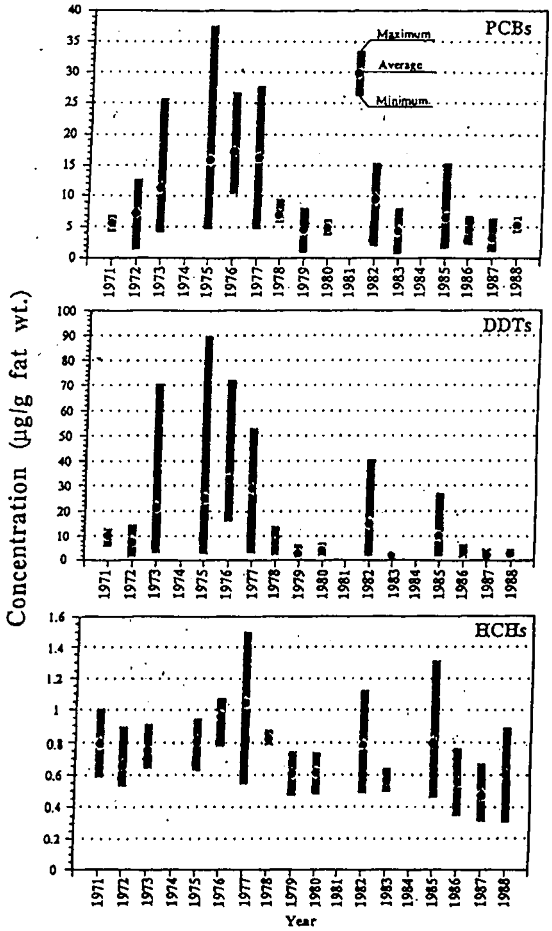

Fig. 6. Temporal trend of organochlorine residue levels in female northern fur seal collected from the Pacifc coast of Japan during 1971 to 1988 (Tanabe et al., 1994).