1-1-1998

The Occurrence of PCBs and Chlorinated Pesticide Contaminants in Bottlenose Dolphins (Tursiops truncatus) in a Resident Community: Comparison with Age, Gender and Birth Order

Kathleen M. Kuss
Nova Southeastern University, katiekuss@verizon.net

The Occurrence of PCBs and Chlorinated Pesticide Contaminants in Bottlenose Dolphins (*Tursiops truncatus*) in a Resident Community: Comparison with Age, Gender and Birth Order

by

Kathleen M. Küss

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science

in

Ocean Science

With Specialty in:

Marine Biology

Nova Southeastern University
1998

Master of Science

Thesis

of

Kathleen M. Küss

Approved:

Thesis Committee

Major Professor _____

Keith Ronald, Ph.D.

Administrative Advisor _____

Richard E. Dodge, Ph.D.

Professor _____

Curtis Burney, Ph.D.

Research Advisor _____

Randall S. Wells, Ph.D.

Research Advisor _____

Richard H. Pierce, Ph.D.

ABSTRACT

The Occurrence of PCBs and Chlorinated Pesticide Contaminants in Bottlenose Dolphins (*Tursiops truncatus*) in a Resident Community: Comparison with Age, Gender and Birth Order

Kathleen M. Küss

Tissue samples from twenty bottlenose dolphins from a stable, residential community of coastal dolphins in the western Gulf of Mexico were analyzed for toxic PCB congeners and chlorinated pesticides. The tissues analyzed (blubber and melon) were from known individuals in a long-term (27^{+} y) study that stranded and were recovered for necropsy. Substantial demographic data were available on these individuals and utilized in the analysis of maternal transfer of organochlorines to young.

The male dolphins in this study were shown to accumulate organochlorine contaminants with age. In female dolphins the organochlorine levels were found to decline with age. These results are in agreement with previous studies, with gestational and lactational transfer accounting for the decline seen in the females. A lengthening in interreproductive interval by increasing organochlorine levels after approximately age 30 y is noted in the females.

For the first time, this study quantified the organochlorine levels of the first calf of a female, testing the hypothesis that the first-born of a female receives a substantially greater organochlorine load than subsequent calves. The first-born calf (age 5.3 mo) had the highest blubber ΣPCB, total DDT, HCB, and total pesticide levels of all animals in this study. The

organochlorine levels in this calf were 2-5 fold higher than in a similarly aged, fourth-born calf.

All animals in this study had appreciable ΣPCB levels (range 0.07 - 26.9 ug/g wet weight; 2.6 - 203.2 ug/g lipid weight), and ΣDDT (range 0.06 - 10.3 wet weight; 0.9 - 88.1 ug/g lipid weight). These values are in a moderate range compared to other studies, but not far below levels at which western Gulf of Mexico bottlenose dolphins evidenced mortality events in 1990 and 1992. Further monitoring of this population is warranted.

Acknowledgements

I would like to acknowledge first my committee members, with thanks for their interest, support and guidance throughout this study. I am indebted to Dr. Keith Ronald, whose humor and encouragement helped me to get through the rough times.

I would like to acknowledge Mote Marine Laboratory with sincerest gratitude, and Mote Marine's Director of Research, Dr. Richard Pierce, for taking a chance on a biologist who wanted to undertake an analytical chemistry project. Without the guidance and assistance of Rich Pierce and Mike Henry, the Chemical Fates and Effects Laboratory Manager and Staff Chemist, this project would not have been possible. Thanks, Mike. I take responsibility for (a few of) your gray hairs. I would also like to acknowledge the excellent scientific staff at Mote Marine as well as the administrative staff. They are, to a person, a great group of individuals to work with. Many people helped with this project in one manner or another. Wendy Coronis and Lisa Edwards assisted with laboratory work. Charles Manire graciously assisted with statistics. Paul Sherblom helped extensively with equipment loans. Many thanks to all.

I would like to express gratitude to Dr. Randy Wells and the DBRI staff, as well as the MML Marine Mammal Stranding Program for allowing me access to their dolphins and their unique and extensive database. Without Randy's twenty-five years of commitment to the field of dolphin biology and social structure, cetacean research would be far less advanced.

I would like to acknowledge several people for their support over the last few years. Carole McIvor, friend and mentor, was there for me as always with sage advice and commiseration. Debbie McPherson, friend and partner in crime, I wouldn't have made it without you. Finally, I would like to thank my mother, my friend, whose unfailing confidence in me and loving support helped me through this degree.

TABLE OF CONTENTS

List of Tables

List of Figures

1.0 INTRODUCTION

Contamination by chlorinated hydrocarbons has been implicated in the mass mortality of marine mammals in recent years. This study was undertaken to determine the levels of toxic PCBs and organochlorine pesticides in a resident community of bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida, in the eastern Gulf of Mexico. The dolphins in this study have been the subject of research since 1970, and substantial demographic information was available on these individuals. Tissue samples from stranded resident dolphins were analyzed to initiate a database on the organochlorine levels, and to determine if 1) male dolphins showed an increase in organochlorine levels with age, 2) post-reproductive females showed a loss of organochlorines due to gestational and lactational transfer to their young, and 3) any relationship between the order of birth of a calf (a first-born versus a later-born), and organochlorine loading could be established.

In the past 50 years the manufacture and use of synthetic chemicals has increased dramatically. While contributing greatly to human comfort and welfare, many of these chemicals have been demonstrated to have severe and irrevocable toxic implications for the global ecosystem. In particular, attention is focused on synthetic chlorinated hydrocarbons such as polychlorinated biphenyls (PCBs), DDTs (dichloro-diphenyltrichloroethane and its metabolites), hexachlorocyclohexane isomers (HCHs), polychlorinated dibenzofurans and polychlorinated dibenzo-*p*-dioxins (PCDFs and PCDDs), and related compounds due to their persistence and worldwide occurrence in

all compartments of the environment (Risebrough *et al.*, 1968; Hutzinger *et al.*, 1983; Tanabe *et al.*, 1983, 1994; Loganathan and Kannan, 1991).

Technical grade PCBs consist of mixtures of 209 possible chemical congeners with 1 to 10 chlorine atoms attached to a biphenyl group (Mullin *et al.*, 1984). Produced primarily as Aroclor® in the United States by the Monsanto Corporation until their ban in 1979, various mixtures were used extensively in industry as dielectric fluids in sealed capacitors and transformers, as solvent extenders, flame retardants, heat transfer fluids, paint and pesticide additives, plastics, waxes, carbonless "NCR" copy paper, adhesives, and dedusting agents (Erickson, 1986; Safe, 1990). The physical properties that lend such versatility to PCBs - resistance to acids and bases, lipophilicity, compatibility with organic materials, and thermal stability - also contribute to the persistence and high bioaccumulative potential of these compounds, as well as their biomagnification in the food chain (Mullin *et al.*, 1984; Tanabe, 1988).

Organochlorine pesticides such as DDT and its metabolites, dieldrin, mirex, heptachlor, hexachlorocyclohexane (HCH) and its isomers, and chlordane and its metabolites exhibit similar properties to the PCBs in physical and thermal stability, persistence in the environment, worldwide distribution, and demonstration of toxicity. This class of chemicals was introduced into the environment in large quantities beginning in the 1940s, and some are still in wide-spread use. Although many of these pesticides have been restricted or banned in developed countries, some are still manufactured and used throughout the world, including in the United States of America, Europe, Japan and Canada (Iwata *et al.*, 1993).

The primary mode of environmental transport of chlorinated compounds is via the atmosphere, with the open ocean acting as a reservoir and final sink for the major portion of these compounds (Atlas *et al.*, 1986). Although found in low (parts-per-trillion) concentrations in open ocean water, these organochlorines are highly lipophilic, and have been found to be extremely bioaccumulative in the food chain, resulting in high accumulations in top trophic level marine predators (Tanabe and Tatsukawa, 1986).

In recent years, and in response to declines in marine mammal populations and mass strandings of marine mammals in diverse parts of the world, researchers have been investigating both the level of chlorinated hydrocarbon accumulation in marine mammals as well as the toxicological ramifications of this exposure. High organochlorine loading has now been found in marine mammals from even the most remote areas of the globe, the Arctic and Antarctic, as well as all inhabited regions (Tanabe *et al.*, 1983; Bacon *et al.*, 1992; Norstrom and Muir, 1994). Tanabe and Tatsukawa (1991) and Kuehl *et al.* (1991) have found some of the highest organochlorine concentrations ever recorded in any animal in nature in the tissues of striped dolphins (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiops truncatus*). Physiologically, the most toxic of the chlorinated hydrocarbons elicit weight loss, thymic atrophy, hepatic damage, teratogenicity, reproductive toxicity, and immunotoxicity in test animals, with some of these and other symptoms seen in wild populations and in humans exposed accidentally (Safe, 1984; Reggiani and Bruppacher, 1985; Martineau *et al.*, 1994; Norstrom and Muir, 1994).

The implication that organochlorine loading in marine mammals has been a major factor, if not the proximal cause, of recent large scale marine mammal mortalities is now

postulated by many researchers in this field (Kuehl *et al.*, 1991; Kannan *et al.*, 1993; Aguilar and Borrell, 1994*a*; Tanabe *et al.*, 1994). Specifically, they point to the immunosuppressive action of these chemicals, rendering the mammals at high risk when exposed to otherwise defeatable infectious agents. Support for this hypothesis is provided by Lahvis *et al.*, (1995) in their demonstration of decreased immune function relative to organochlorine concentration in bottlenose dolphins.

Three factors appear significant in the accumulation of organochlorine compounds by marine mammals. The first is that the extent of accumulation of chlorinated compounds is dependent to some degree on ambient pollution levels in the animal's habitat and subsequent accumulation in the food chain. The second factor is that marine mammals possess an inefficient metabolic enzyme system for degrading or depurating chlorinated hydrocarbons. This is particularly true in cetaceans and allows for high accumulations of organochlorines in whales and dolphins (Tanabe *et al.*, 1994). The third factor is concerned with mode of reproduction. Female marine mammals are now known to transfer up to 98% of their organochlorine burden to their offspring through gestation and lactation (Aguilar, 1987; Cockcroft *et al.*, 1989; Borrell *et al.*, 1995), not only burdening newborn offspring, but guaranteeing persistence in the species through successive transgenerational loading.

Contamination of marine mammals by chlorinated compounds has been studied extensively since the early 1970s. In that time, many theories and some conclusions have been offered concerning the accumulation of compounds, mode of toxicity, reproductive transfer, and relationship to mass strandings and large scale mortality events.

The majority of these studies have been undertaken with stranded animals that were found dead, animals collected as "by-catch" in fishing operations, and animals collected and sacrificed solely for research purposes. In each of these cases, little is known about the animals other than age and sex. A female's reproductive history may only be assumed by age and knowledge of her species' reproductive habits if comprehensive examination of the uterus, and of the ovaries for corpora lutea and albicantia are not conducted. A beached calf usually renders no information as to his parentage or his birth order. In most cases, little is known about precisely what waters the animal inhabited, and for what length of time.

1.1 Statement of Purpose

This study was conducted in order to integrate substantive demographic data with chlorinated hydrocarbon analysis. Under the direction of Dr. Randall S. Wells, the Dolphin Biology Research Institute (DBRI) has been studying the resident bottlenose dolphin community in Sarasota Bay, Florida (27° 25′ N, 80° 40′ W), as the focus of a long-term study initiated in 1970 (see Wells *et al.*, 1987; Wells, 1991). This stable dolphin community consists of approximately 100 individuals with low (<3%) immigration and emigration, yet substantial opportunity for interaction with neighboring dolphin communities (Wells, 1991). Demographic data on these dolphins as well as on dolphins in adjacent communities have been obtained both by regular observational reconnaissance and by an occasional capture and release sampling program where blood

and other biologic samples are obtained to determine general health and body condition status as well as reproductive status, genetic relationships (Wells, 1991), and immune function (Lahvis *et al.*, 1993; Lahvis *et al.*, 1995; Erickson *et al.*, 1995).

Tissue samples from animals stranded and recovered by the Mote Marine Laboratory Marine Mammal Stranding Program, (part of the Southeast U.S. Marine Mammal Stranding Network), were collected during necropsy and stored for future analysis. Twenty of these animals were identified as DBRI research dolphins, and their blubber (n = 20) and melon tissues (n = 17) were analyzed for toxic PCB congeners and chlorinated pesticides in an effort to evaluate this community as well as determine patterns of accumulation in relation to gender, age, and birth order of these animals. Specifically, the objectives of this study were to ascertain 1) if male dolphins in this community showed significant accumulation of organochlorines with age, 2) if post-parturient females evidenced lower organochlorine levels than pre-parturient females, and 3) if any relationship between the order of the calf's birth in terms of his mother's reproductive history and chlorinated hydrocarbon levels could be established.

1.2 Prior Research / Review of Literature

Chlorinated biphenyls were first developed by Schmidt and Shultz in 1881, and have been widely used since the 1930s (Erickson, 1986). Worldwide, PCBs have been marketed as Clophen® in West Germany, Fenclor® and Apirolio® in Italy, Kanechlor® and Santotherm® in Japan, Pyralene® and Phenochlor® in France, Sovol® in Russia, and

Delor® in Czechoslovakia (Cairnes *et al.*, 1986; Eisler, 1986). In addition to Aroclor®, PCBs have been marketed in the United States as Chloretol®, Dyknol®, Inerteem®, Noflamol® and Pryanol® (Eisler, 1986, Borlakoglu and Haegele, 1991). Although now banned in Japan and Europe as well as the United States, PCBs are still manufactured and used heavily, primarily in developing nations and the Mediterranean basin (Tanabe, 1988; Dachs *et al.*, 1997; Marsili *et al.*, 1997). Coincident with the manufacturing ban, provisions were made for the continued, though restricted use of PCBs in older, "sealed" containers such as transformers and fluorescent light ballasts. Consequently, despite the manufacturing ban, it is estimated that approximately 70% of the world's production of PCBs are still in use today in electrical equipment or deposited in landfills (Bacon *et al.*, 1992; Tanabe, 1988), portending a potential increase in overall contamination in the future as these chemicals make their way into the environment from their present deployments, probably reaching its peak in the 1990's (Tanabe, 1988).

Confirmation of the fact that PCB contamination has not decreased despite bans by industrialized countries come from studies from diverse sectors of the scientific community. In one elegant study of the historical PCB residue record from the Agassiz Ice Cap in Canada, Gregor and his associates found that while mean PCB deposition peaked in 1967-1968 and then reached a minimum in 1980-1981, the deposition increased to nearly the maximum level again in 1989-1990, and remained at fairly high levels as of the study's conclusion in 1993. This group suggests that there has not been a major change in PCB availability in the northern hemisphere over the past thirty years due in part to the volatilization of PCBs from soils and to continued use in Eurasian countries

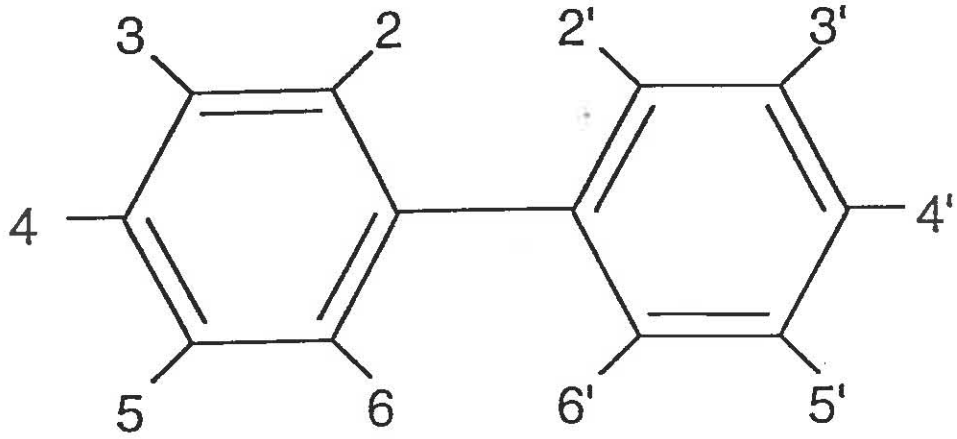
(Gregor *et al.*, 1995).

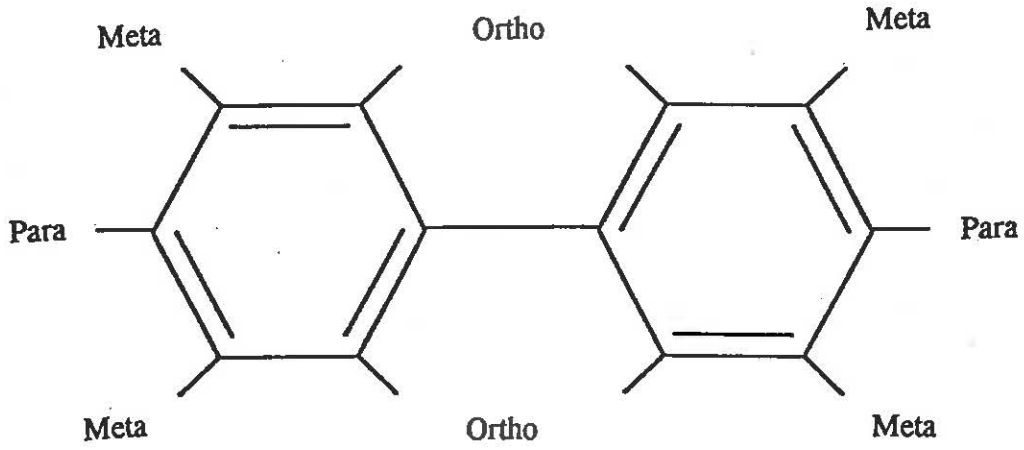
Although banned or restricted for the most part, persistent organochlorine pesticides find continued use as well, in one formulation or another, in almost every country on earth including the United States and other developed countries (Iwata *et al.*, 1993; Voldner and Li, 1995). In some of the developing and third-world countries this use is extensive, such as the heavy DDT and HCH usage in India and China, and DDT use in southeast Asia, Central and South America, and Africa (Gregor, 1991; Iwata *et al.*, 1993). The trend, then, seems to be towards a "southward shift" in chlorinated hydrocarbon use. Tanabe *et al.* in 1983 reported much higher organochlorine levels in the northern than in the southern hemisphere, and this concentrated in the mid-latitudes in both the Atlantic and Pacific oceans, indicating the heavy industrial use by the developed nations such as the United States, Europe and Japan. Presently, increasing use by developing nations in the last decade has shifted the oceanic distribution of organochlorines. Southern hemisphere and tropical northern hemisphere oceans are currently evidencing higher levels of both DDT and HCH (Tanabe *et al.*, 1994). Smaller geographical variations are seen for PCBs and chlordane, with approximately uniform distributions in both hemispheres, but suggest to Tanabe and his colleagues an expansion of their point sources to the tropical developing nations as well.

Transport of chlorinated hydrocarbons is primarily through the atmosphere, where they are in vapor phase (predominantly), and in association with particulate matter. Once airborne, these stable, semi-volatile chemicals are capable of being transported thousands of kilometers and can revolatilize from continental sinks (Atlas *et al.*, 1986). This

transport is driven by both atmospheric conditions and by the physical and chemical characteristics of the compound, in particular its subcooled vapor pressure which is of importance to the global distillation effect, also known as the cold-condenser effect (Van den Brink, 1997). It is thought that these compounds evaporate at warmer places of the globe, travel through the atmosphere and condense at colder places, perhaps helping to explain the relatively high levels of chlorinated compounds found in all environmental compartments of the Arctic, and in the Antarctic (Muir *et al.*, 1988; Van den Brink, 1997). Ultimately, the open ocean and coastal waters act as a reservoir and final sink for the major portion of organochlorine compounds, accounting for 62% of the total PCB load in the environment. Table 1 illustrates the estimated PCB loading in terrestrial and open ocean environments. Incorporation into coastal and terrestrial sediments accounts for another 35%, which is expected to be transported eventually to the oceans (Tatsukawa and Tanabe, 1990). Atmospheric deposition can occur through wet deposition (rain, snow), dry particle deposition, and via vapor exchange across the air-water interface (Gregor, 1991; Erickson, 1986).

Other sources of organochlorine contamination to the oceans include direct industrial discharge, such as the production and subsequent release of dioxins in the paper and pulp mill bleaching process, run-off from dump sites, river transport (Erickson, 1986), incineration disposal methods (Connell and Miller, 1984), and as a result of rupturing of sealed shells which contain generators or other machinery containing PCBs (Tanabe, 1988). Municipal waste incinerators produce dioxins including the extremely toxic 2,3,7,8-tetrachloro-*p*-dibenzodioxin and other semi-volatile organics such as


3
2
2'
3'
4
4'
5
6
6'
5'


Meta
Ortho
Meta
Para
Para
Meta
Ortho
Meta

hexachlorobenzene (HCB) as the result of "incomplete combustion" (Compaan, 1988). In the international North Sea incineration area, incinerator ships at sea combusted organochlorine wastes from 1979 until 1991, with totals of over 100,000 tonnes incinerated per year. Combustion residues including hydrochloric acid gases were released unfiltered into the atmosphere, and are likely to at least partially account for the residues of PCBs, pentachlorobenzene, octachlorostyrene, several HCH isomers, HCB and DDTs found in marine organisms in the vicinity of this incineration area (Dethlefsen *et al.*, 1996).

The pathway from the atmosphere to the food chain is not a lengthy one. Organochlorines are retained in the ocean in particulate (macroparticle), colloidal (nonsettling microparticle) and dissolved forms (Iwata *et al.*, 1993). Due to their physicochemical properties as evidenced by their large octanol/water partition coefficients and lipophilicity, they tend to partition onto lipidic suspended solids, such as plankton (Formica *et al.*, 1988; Tatsukawa and Tanabe, 1990). This allows for the direct uptake of organochlorines into lower order consumers, where they preferentially bind to fatty tissues, fat depots, or free lipids (Gagnon *et al.*, 1990). In this manner, and dependent on an organism's ability to metabolically degrade or depurate these xenobiotics, they progressively accumulate and can biomagnify up the food chain. In a study of the bioaccumulative process, Tatsukawa and Tanabe (1990) demonstrated the amplification of PCBs, DDTs and HCHs in a successive food chain. From ambient seawater up to striped dolphin, they found bioconcentration factors of 10^3 for zooplankton, 10^5 for myctophid fish (*Diaphus suborbitalis*) and squid (*Todarodes pacificus*), and 10^7 for

Table 2. Concentrations and Bioaccumulation Factors of PCBs, ΣDDT, and ΣHCH in Organisms from the Western North Pacific. Tatsukawa and Tanabe, 1990.

	PCBs	ΣDDT	ΣHCH
Concentration			
Surface seawater (ng liter^{-1})	0.04–0.59 (0.28)	0.006–0.48 (0.14)	0.52–8.2 (2.1)
Zooplankton (ng g^{-1}) (mainly copepods)	1.8	1.7	0.26
Myctophid (ng g^{-1}) (*Diaphus suborbitalis*)	48	43	2.2
Squid (ng g^{-1}) (*Todarodes pacificus*)	35–95 (68)	16–28 (22)	0.93–1.5 (1.1)
Striped dolphin (ng g^{-1}) (*Stenella coeruleoalba*)	2800–4100 (3700)	4200–6000 (5200)	48–89 (77)
Bioconcentration factor			
Zooplankton	6.4×10^3	1.2×10^4	1.2×10^2
Myctophid	1.7×10^5	3.1×10^5	1.0×10^3
Squid	2.4×10^5	1.6×10^5	5.2×10^2
Striped dolphin	1.3×10^7	3.7×10^7	3.7×10^4

striped dolphin (see Table 2). In a similar study on PCBs, a bioconcentration factor of nearly 10^8 was found from Lake Ontario water up the food chain to herring gulls (*Larus argentatus*) (Erickson, 1986). The result of the long-range atmospheric transport, physical stability, and bioaccumulative propensity of chlorinated compounds is seen in the present occurrence of these compounds in every compartment of the marine and terrestrial environment investigated (Atlas *et al.*, 1986; Tanabe and Tatsukawa, 1986). Since first discovered to be widely dispersed, PCBs have been measured in air, ice, seawater, and marine mammals in the Antarctic (Tanabe *et al.*, 1983; Iwata *et al.*, 1993), in air, snow, ice, water, birds, fish, marine flora, and marine mammals in the Arctic (Muir *et al.*, 1988*a*; Gregor, 1991; Norstrom and Muir, 1994). Persistent organochlorines are found in every sea (Tatsukawa and Tanabe, 1986), and in virtually every terrestrial and marine organism studied (Connell and Miller, 1984; Atlas *et al.*, 1986; Erickson, 1986; Muir *et al.*, 1988*b;* Zook and Rappe, 1994). (Man, of course, is not immune - all United States residents have measurable PCBs in their adipose tissue (Erickson, 1984)).

Studies of the levels of organochlorine loading in marine mammals have been continuing since the late 1960s. Marine mammals have been found to accumulate organochlorines so efficiently that they now are used as biomonitors to indicate the presence and geographical variation of PCBs and chlorinated pesticides (Tanabe *et al.*, 1983; Tanabe and Tatsukawa, 1986; Muir, 1990). Measurable and perhaps toxicologically significant levels of chlorinated hydrocarbon contaminants have been found, for instance, in white-beaked dolphins (*Lagenorhynchus albirostris*), narwhal (*Monodon monoceros*),

ringed seals (*Phoca hispida*), harp seals (*Phoca groenlandica*), northern fur seals (*Callorhinus ursinus*), walrus (*Odobenus rosmarus*) and polar bears (*Ursus maritimus*) from the Canadian Arctic and Newfoundland (Ronald *et al.*, 1984; Muir *et al.*, 1988*a* and *b;* Bacon *et al.*, 1992; Norstrom and Muir, 1994), beluga whales (*Delphinapterus leucas*) from the St. Lawrence seaway (Massé *et al.*, 1986; Martineau *et al.*, 1994), long-finned pilot whales (*Globicephala melaena*) from the NE Atlantic (Borrell *et al.*, 1995), California sea lions (*Zalophus californianus*) (Bacon *et al.*, 1992), harbour seals (*Phoca vitulina*) in the Wadden Sea and the United Kingdom (Reijnders, 1986; Hall *et al.*, 1992), bottlenose dolphins from the South African east coast, United States east coast and west Wales (Cockcroft *et al.*, 1989; Kuehl *et al.*, 1991; Law *et al.*, 1995), striped dolphins in the Mediterranean (Aguilar and Borrell, 1994*a*), Dall's porpoises (*Phocoenoides dalli*), Pacific white-sided dolphins (*Lagenorhyncus obliquidens*), striped dolphins, finless porpoises (*Neophocoena phocoenoides*), Baird's beaked whales (*Berardius bairdii*), melon-headed whales (*Peponocephala electra*), largha seals (*Phoca larga*), ribbon seals (*Phoca fasciata*) and northern fur seals from the north and west Pacific (Tanabe *et al.*, 1983, 1994; Kannan *et al.*, 1989), bottlenose and common dolphins (*Delphinus delphis*) from the California coast (Muir *et al.*, 1988) and killer whales (*Orcinus orca*) and bottlenose dolphins from Australia (Kemper *et al.*, 1994).

Several reasons exist for the high accumulative tendency in marine mammals. These animals tend to be comparatively long-lived, and therefore have the opportunity for significant accumulation. A large proportion of their bodies is lipidic, with blubber comprising about 43% of the body mass in right whales, 40% in harbour porpoises, 18%

in spinner (*Stenella longirostris*) and common dolphins (Aguilar and Borrell, 1994*a*), 15-20% in fin whales (*Balaenoptera physalus*) (Lockyer, 1976), and 20-30% in bottlenose dolphins (Law *et al.*, 1995). Approximately 70% of total blubber weight is made up of neutral lipids, and as organochlorines are highly apolar, they dissolve in neutral lipids (Aguilar, 1987). The crux of the accumulation problem, however, lies in that marine mammals are quite inefficient when it comes to metabolizing xenobiotics.

In animals exposed to chlorinated hydrocarbons, the metabolic response is dominated by the induction of hepatic and extrahepatic drug metabolizing enzymes (Safe *et al.*, 1985; De Voogt *et al.*, 1990; Tanabe *et al.*, 1994). Lipophilic xenobiotics are eliminated in the liver by cytochrome P-450 mediated mixed-function oxidase (MFO) systems, which convert the xenobiotics to more water-soluble metabolites. Two types of inductive properties are characteristic of the MFO activities of xenobiotics: the phenobarbitol (PB) and the 3-methylcholanthrene (MC) type inducers. Marine mammals, notably cetaceans, have a low activity of 3-methylcholanthrene-type enzymes, and lack phenobarbitol-type enzymes necessary for depurating PCBs and chlorinated pesticides into excretable metabolites. Figure 1 from Tanabe *et al.*, (1988) compares the PB- and MC-type enzyme activities in higher animals, and illustrates the differences between terrestrial and marine animals in metabolic functioning. Pinnipeds fare a little better, demonstrating some limited PB-type activity as well as low MC-type activity, but remain, along with cetaceans, in a vulnerable position in regards to both the toxicity and the accumulative nature of these xenobiotics in comparison with terrestrial mammals (Kannan *et al.*, 1989).

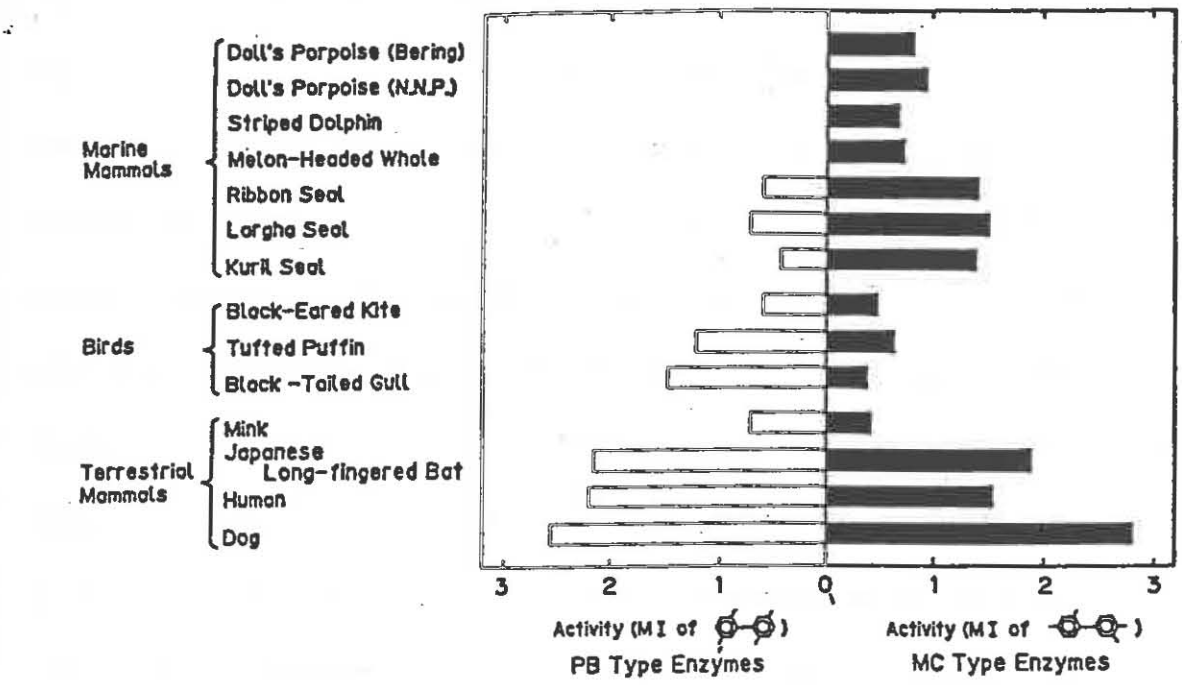


Figure 1. PB (phenobarbitol)- and MC (methylcholanthrene)-type enzyme activities in higher animals estimated by Metabolic Index of 2,2′,5,5′ - and 2,3′,4,4′-tetrachlorobiphenyl isomers. Tanabe et al., 1988.

The toxicity of the chlorinated hydrocarbons, especially of TCDD and structurally-related PCB congeners, lies in the induction of the cytochrome P-4501A1 and A2 hemoproteins and their associated microsomal monooxygenases, which include aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin O-deethylase (EROD); and the binding of the cytosolic arylhydrocarbon (Ah) receptor (Safe *et al.*, 1985; De Voogt *et al.*, 1990; Safe, 1990). This activity elicits a host of biochemical responses including, in brief, the modulation of steroid-metabolizing enzymes, aldehyde dehydrogenase induction, decreased estrogen and progesterone receptor binding, modulation of thyroid hormone levels and function, and decreased glucocorticoid receptor binding (Safe, 1990).

Translated to common toxic responses, this elicits body mass loss, thymic atrophy, gross impairment of immune responses, hepatotoxicity and porphyria, endocrine dysfunction, chloracne and related dermal lesions, carcinogenesis, teratogenicity, and reproductive toxicity (Safe, 1990). Tryphonas (1994) emphasized the mounting evidence that the immune system is one of the most sensitive targets for the toxic effects of PCBs. The affected parameters include bone marrow cellularity and hematologic changes, thymic and splenic atrophy which correlates with humoral or cell-mediated immunosuppression, reduced resistance to microbial infection, and compromised immune surveillance mechanisms against cancer. The enzyme systems in question are also known to modify some of the contaminants into toxic intermediates that can further disturb the critical balance of endobiotics like steroid hormones (Tanabe *et al.*, 1994), in some cases producing metabolites that are more toxic than the parent compound (Erickson, 1986, Norstrom and Muir, 1994).

Accidental poisoning incidents have illustrated the toxicity of the PCBs and the dioxins to humans. In the "Yusho" incident in Japan in 1968, and the "Yu-chen" in Taiwan in 1979, cooking oil contaminated with PCBs, PCDFs and polychlorinated quaterphenyls affected the health of over 4,000 people. In adults, this resulted in severe chloracne, hyperkeratinosis, and abnormalities in hepatic and nervous system function (Seegal, 1996; Tilson and Kodavanti, 1997). In the children exposed *in utero*, multiple developmental abnormalities included low birth weight, motor dysfunction, behavioral and neurological dysfunctions, and lowered intelligence scores (Miller, 1985; Seegal, 1996). Also noted were skull deformities in the form of unusual calcification, wide

separation of the sagittal suture, and large and open fontenelles, thought to be a result of disturbance in calcium metabolism (Miller, 1985).

Research on humans with "ambient" levels of exposure to xenobiotics has demonstrated that humans are being impacted by the loads we currently carry. In adults this includes reduced testosterone levels, increased incidence of diabetes through altered glucose tolerance (DeVito *et al.*, 1995), neurochemical changes such as decreased brain neurotransmitter levels including dopamine, acetylcholine and γ-amniobutyric acid (Seegal, 1996; Tilson and Kodavanti, 1997), and increased risk for non-Hodgkin's lymphoma (Hardell *et al.*, 1996*a*) and leukemia (Hardell *et al.*, 1996*b*). Developmental exposure affects neurological development and impairs cognitive function (Tilson and Kodavanti, 1997). Colborn *et al.* (1993) estimated from current breast milk concentrations nationwide that at least 5% of the babies born in the United States were exposed to quantities of PCBs sufficient to cause neurological effects.

The estrogenic effects of chlorinated compounds are receiving a lot of current attention as evidence of their ability to disrupt endocrine functioning builds. Table 3 from Colborn, *et al.*, (1993) lists wide-spread chemicals reported to have reproductive and endocrine effects. These compounds bind to and interfere with the cellular receptor proteins that mediate the effects of endogenous steroid hormones, and can act as either hormone agonists or antagonists (Colborn et al., 1993; Goldberg, 1995). The major effects seen in endocrine disruption appear to be developmental in nature, occurring during gestation and organogenesis when the development of many tissues is regulated by the endogenous steroid hormones of the mother.

TABLE 3

Chemicals with widespread distribution in the environment reported to have reproductive and endocrine-disrupting effects. From Colborn *et al.*, 1993.

Biocides			
Herbicides	Fungicides	Insecticides	Industrial Chemicals
2,4-D	Benomyl	β-HCH	Cadmium
2,4,5-T	Hexachlorobenzene	Carbaryl	Dioxin (2,3,7,8-TCDD)
Alachlor	Mancozeb	Chlordane	Lead
Amitrole	Maneb	Dicofol	Mercury
Azatrine	Metiram-complex	Dieldrin	PBBs
Metribuzin	Tributyltin	DDT and metabolites	PCBs
Nitrofen	Zineb	Endosulfan	Pentachlorophenol (PCP)
Trifluralin	Ziram	Heptachlor + H-epoxide	Penta- to nonylphenols
		Lindane (γ-HCH)	Phthalates
Nematocides		Methomyl	Styrenes
		Methoxychlor	
Aldicarb		Mirex	
DBCP		Oxychlordane	
		Parathion	
		Synthetic pyrethroids	
		Toxaphene	
		Transnonachlor	

The specific endocrine activity exerted by these compounds can vary widely even within the same family of compounds. One of the earliest compounds to be associated with endocrine disruption, DDT, has several modes of action. One of its forms, o,p′-DDT, has been shown to be an estrogen agonist, while the persistent metabolite p,p′-DDE is a powerful androgen antagonist and is now implicated in the increased incidence of developmental male reproductive system abnormalities in both wildlife and in humans (Colborn *et al.*, 1993; Kelce *et al.*, 1995). A number of the chlorinated chemicals can act as estrogen mimics, and this action is implicated in the etiology of human male prostate hyperplasia, prostate cancer, reduced sperm counts and motility, endometriosis in women, reduced fertility, and cancers of all estrogen-responsive tissues in women (Colborn *et al.*, 1993; Cummings and Metcalf, 1995). Chlorinated compounds have been

associated with abnormal thyroid function in fish and in birds; decreasing fertility in birds, fish and mammals; decreased hatching success in birds, fish and turtles; demasculinization and feminization of male fish, and defeminization and masculinization of female fish, gastropods and birds; precocious sexual maturation in male salmon; greatly reduced birth rate and penis size in alligators; and delayed male puberty (Colborn *et al.*, 1993; Kelce *et al.*, 1995).

Endocrine disruption as evidenced by reproductive dysfunction, along with immunological incompetence are the two main types of xenobiotic impact seen in marine mammals (Reijnders, 1994). These mechanisms are widely thought to be responsible for the large scale mortalities of dolphins, reductions in population size, and epizootics that have occurred in marine mammal populations in the past decade. Chlorinated hydrocarbons, PCBs in particular, have been implicated in premature births seen in California sea lions (DeLong *et al.*, 1973), reduced testosterone levels in Dall's porpoises in the NW Pacific (Subramanian *et al.*, 1987), reproductive failure in harbour seals in the Wadden Sea, with a concomitant population reduction from 3,000 individuals to less than 500 in two decades (Reijnders, 1986), and a strong decline in the population of harbour porpoises (*Phocoena phocoena*) in the coastal North Sea (Duinker *et al.*, 1989). In the heavily polluted St. Lawrence Estuary, the population of beluga whales has declined from an estimated 5000 to about 450 individuals. PCB and DDT metabolite concentrations are high in these animals (Massé *et al.*, 1986), and the incidence of a very high cancer rate in these belugas has been reported, along with a suite of other pathological disorders. Cancers are exceedingly rare in free-ranging odontocete

populations; however, the estimated crude annual incidence of cancer in this beluga population is higher than that for man (Martineau *et al.*, 1994).

Reijnders (1994) reports that certain immunological and reproductive disorders in marine mammals can be linked to tissue concentrations of certain chlorobiphenyls and their metabolites, including lowered immunocompetence (impaired T-cell and natural killer (NK) activity) in harbour seals, uterine stenosis and occlusions in Baltic Sea ringed seals (*Pusa hispida*) and grey seals (*Halichoerus grypus*), adrenocortical hyperplasia, renal glomerulopathy, and lesions in the intestine, kidneys and adrenal glands. The incidence of primary lesions in the endocrine system and suspected disturbances in the developmental processes were reported in 70-90% of the Baltic ringed and grey seals investigated by Zakharov and Yablokov (1990), together with skull-bone lesions (osteoporosis), skull lesions (exotosis) in Baltic harbour seals, and skull lesions in harbour seals from the German Wadden Sea. These authors, in comparing pre-1940 skulls (pre-pollution era), and post-1960 skulls (most significant pollution era), attribute the high degree of developmental instability directly to contaminant levels, principally DDT and PCBs.

The data from recent large scale mortalities of marine mammals is supportive of some degree of immunoincompetence in marine mammal populations. From June 1987 to May 1988, 742 Atlantic bottlenose dolphins washed ashore along the Atlantic coast of the United States. As probably only a fraction of the total dead carcasses washed onto land, it is estimated that over 8,500 deaths occurred, or roughly 60% of the migratory mid-Atlantic dolphin population (Scott *et al.*, 1988). A possible proximate cause of death

for these dolphins had been suggested as brevetoxin, a neurotoxin produced by the Florida red tide phytoplankton *Gymnodinium breve* (Geraci, 1989). However, an EPA study of the stranded animals found very high concentrations of organochlorines which are believed to have been, if not the primary cause of the deaths through immunotoxicity, then an additional stressor (Geraci, 1989; Kuehl *et al.*, 1991). Indications that the dolphins had seriously compromised immune systems came early in the strandings when the dolphins were diagnosed with "dolphin pox", a breakdown and sloughing of the skin allowing infection of vibrio bacteria that the immune system mysteriously was not able to handle (Segars, 1987). Later testing showed that about 50% of the dolphins analyzed had morbillivirus antigens in their tissues (Duignan *et al.*, 1996). The EPA study also found that these dolphins were significantly contaminated with numerous other compounds including polybrominated biphenyls and diphenyl ethers, never previously detected in stranded marine mammals in the United States, as well as yet unidentified polybrominated and polychlorinated compounds (Kuehl *et al.*, 1991).

The actual stranding count in the large scale mortality of 344 bottlenose dolphins washed ashore in the Gulf of Mexico in 1990 was considered low, as reporting and salvage efforts vary considerably along the Gulf coastline (Hansen, 1992). No proximate cause has been determined for this incident, however, Duignan *et al.* (1996) have reported that this event was attributable at least in part to morbillivirus. Chlorinated pesticide and PCB analysis revealed high concentrations similar to those found in the 1987-88 Atlantic coast mortality event (Salata *et al.*, 1995).

During 1988 approximately 20,000 harbour seals in the North Sea died from an

epizootic caused by a previously unrecognized member of the morbillivirus family, phocine distemper virus (Hall *et al.*, 1992; de Swart *et al.*, 1996). Chlorinated hydrocarbon analyses found significantly higher levels of all organochlorine contaminants in the stranded seals than in healthy seals from the same region sampled after the epizootic had run its course. It was concluded that while not the proximal cause of the large scale mortality, immunosuppression had contributed to the scale of the mortality. It was noted as well that these high levels were found in the blubber despite the poor nutritive state and subsequent mobilization of the blubber (and presumably a portion of the organochlorine load) by the affected animals (Hall *et al.*, 1992). This epizootic also reached the Swedish west coast and the Baltic Sea, causing the mortality of approximately 60% of the harbour seals there (Olsson *et al.*, 1994).

In the Mediterranean Sea during 1990 and 1991, an epizootic affected the striped dolphin population causing the deaths of the 700 dolphins who washed ashore in Spain, France and Northern Italy. The actual mortality is thought to be significantly higher, as no stranding information was forthcoming from northern Africa, also affected, and the fact that this species is an offshore dolphin, reaching its highest densities far from the coast. A secondary event occurred in 1991-1992, affecting southern Italy, Sicily, Greece, and Turkey, again producing substantial mortality. The proximal cause of death in both events was the (then) newly identified dolphin morbillivirus. A number of unusual pathological and physiological conditions were seen in conjunction with this epizootic including many individuals with hepatic lesions and ectoparasite loading. The PCB concentrations found in these dolphins were extremely high, and among the highest ever

found in wild mammals, leading researchers to conclude that these pollutants played a role in immune suppression (Kannan *et al.*, 1993; Guitart *et al.*, 1996). Aguilar and Borrell (1994*a*) found that concentrations of PCBs obtained from healthy individuals before and after the epizootic were significantly lower than those stranded during the epizootic, strongly suggestive that mortality due to morbillivirus affected mainly those individuals with higher PCB loads. These results were confirmed by Marsili *et al.*, (1997), who found significantly higher levels of all xenobiotic compounds in tissues from striped dolphins that stranded in 1990 and 1991 than in the tissues from animals stranded prior to 1990. Since this epizootic, morbillivirus has increasingly been seen in large scale mortalities of both seals and dolphins, as well as in individual animals stranded, including both coasts of Florida and the Gulf of Mexico.

The process and mechanisms of the uptake and accumulation of chlorinated compounds by marine mammals is now known to vary not only between orders and species of animals, but between genders and age groups within the same species. In cetaceans as in pinnipeds, it has been established that the concentration of organochlorines increases with age in males while in females it increases until the time of sexual maturity (Martineau *et al.*, 1987; Tanabe, 1988; Muir *et al.*, 1990; Aguilar and Borrell, 1994*a;* Vedder, 1996). The transfer of organochlorines during gestation and lactation results in either stable or declining trends in concentration with age in females until senescence or death (Aguilar, 1987; Tanabe, 1988; Cockroft *et al.*, 1989; Borrell *et al.*, 1995). Whether or not females have a significant post-reproductive stage in their life history appears species-specific. This has been reported, along with a concomitant rise in post-

reproductive organochlorine concentration in Dall's porpoises and in short-finned pilot whales (*Globicephala macrorhyncus*), but has not as yet been reported for other species, and is not believed to be the case for bottlenose dolphin, although interreproductive interval may increase in older females (Cockroft *et al.*, 1989; R.S. Wells, pers. comm.). Parturient female dolphins generally show decreased levels of organochlorines relative to non-parturient (immature) females. The significance, however, of the translocation of contaminants for parturient females will logically be proportional to the number parturitions and subsequent weanings, and may therefore be highly variable even between females of the same age (Aguilar, 1988). An infertile female may continue to accumulate organochlorines in the same manner as males, as seen in reproductively impaired Baltic grey seals (Olsson *et al.*, 1994).

The estimates of gestational and lactational transfer of organochlorines from the mother to the calf range between 72% and 98% of the mother's burden (Aguilar, 1987; Tanabe, 1988). For bottlenose dolphins, it is estimated that about 80% of the maternal body load is transferred by the end of the first complete reproductive cycle (Cockroft *et al.*, 1989). Gestational transfer is not as important as lactational transfer in this process. Estimates with striped dolphins indicate that 4% to 9% of their organochlorine load is transferred during gestation, the remainder (60% to 90%) during lactation (Aguilar, 1988). The lipid content of cetacean milk is considered to as high as 30% in most species (Tanabe, 1988), contributing to this high transfer rate which is rarely seen in terrestrial mammals.

The first-born calf of a female has been estimated to receive a four-fold higher

initial burden of organochlorines than subsequent calves (Fukushima and Kawai, reported in Cockroft *et al.*, 1989). This implies that any potential toxicological effect from the gestational and lactational transfer would be expected to occur, or occur more severely, in the first born calf of a female rather than in subsequent calves. The magnitude of this loading, especially in a first-born calf, may be sufficient to cause significant impairment considering that much of it comes in the late pre-natal and early post-natal stages when important physiological development is occurring as well as the development of the immune system (Aguilar and Borrell, 1994*a*). It is possible that this is being demonstrated within the Sarasota Bay dolphin community as only one first born calf is known to have survived beyond (mother-calf) separation in the past 15 years (R.S. Wells, pers. comm.).

Some variation in the transfer of compounds during gestation and lactation is apparent in the forms and amounts of compounds readily transferred. During pregnancy and lactation, the most easily transferred organochlorines are HCH and HCB (hexachlorobenzene), followed by DDTs and then PCBs. Generally, the higher the lipophilia, the lower the transfer rate. In the case of PCBs, the transfer seems to be inversely proportional to the number of chlorine atoms substituted on the biphenyl ring (Aguilar, 1988). High molecular weight chemicals, and the more highly chlorinated, more lipid-soluble PCB congeners appear to pass less readily through the placental membranes, and are transferred less efficiently from blubber to the circulatory system and from there to milk. Marine mammals tend to be enriched with the more highly chlorinated of the PCBs, suggesting that reproductive transfer of PCBs may be lower

than, for example, that of DDT (Aguilar and Borrell, 1994*a*).

Another potential source of variation in the concentration of chlorinated compounds in cetaceans are the nutritional parameters, specifically, the possible mobilization of fat reserves during periods of low food availability, migration, or a lengthy illness. As fat reserves are mobilized, two processes are possible: either the contaminants leave the blubber in a parallel fashion to the lipids to which they were bound, or the contaminants do not leave, remaining in and concentrating further as the lipids are mobilized. Aguilar (1985) suggests that a combination of these two processes is most probable, with the concentration of contaminants in the blubber rising while some loss is experienced from this compartment to others in the mammal. Some evidence exists, however, supporting the theory of the mobilization of contaminants along with the associated lipids. Comparisons of organochlorine levels in both healthy and non-healthy harbour porpoises, bears out the contaminant mobilization theory, apparently being the more important of the two mechanisms, at least in harbor porpoises (Kuiken *et al.*, 1994). This is supported by nutritional and metabolic studies with a marked increase in the metabolism and excretion of lipophilic chemicals during experimental and seasonal starvation in animals due to the reduction in the mass of fat tissue (Dorea *et al.*, 1997).

The selection of the compartment, or tissue, for analysis can have a great bearing on the resulting concentrations found. In studies of organochlorine concentrations in marine mammals, the compartment most widely studied is the blubber, for several reasons. Ease of collection, from stranded animals as well as from live animals through

dart biopsy, and suitability for long-term storage are certainly important factors (Aguilar and Borrell, 1994*b*). More important, though, is the composition of the tissue. The lipid content in marine mammal blubber is relatively high, accounting for approximately 70% of wet blubber weight in bottlenose dolphins (Aguilar, 1987), and is complexed with connective tissue. The accumulation and bioconcentration of chlorinated compounds, however, correlates not only with the lipid content, but also with the lipid composition of the tissue. Neutral and more non-polar compounds such as PCBs and DDTs have been shown to selectively accumulate more in triglycerides and non-esterified fatty acids (NEFA) than in the more polar lipids such as phospholipids and cholesterol (Kawai *et al.*, 1988). In blubber and in the melon, triglycerides comprise almost 100% of the tissue lipid, whereas other compartments such as the brain and blood are comprised of 90% and 80% phospholipids, respectively (Kawai *et al.*, 1988). Compartments such as the latter are more likely to retain the more polar compounds such as ∝-HCH and the lower chlorinated of the PCBs (Aguilar and Borrell, 1994*b*). Indeed, several researchers have been unable to correlate blood concentrations with blubber or adipose tissue concentrations (Ronald *et al.*, 1984; Archibeque-Engle *et al.*, 1997). The liver, on the other hand, while being comprised primarily of phospholipids and NEFA (approximately 50% and 40% of lipids, respectively), is the main center for degradation of xenobiotics, and has the highest concentration of enzymes involved in metabolism, and of the metabolized forms of the organochlorines, of any of the tissues (Kawai et al., 1988; Aguilar and Borrell, 1994*b*). As has been discussed, blubber concentrations depend to a certain degree on the nutritional state of the animal. The melon, on the other hand,

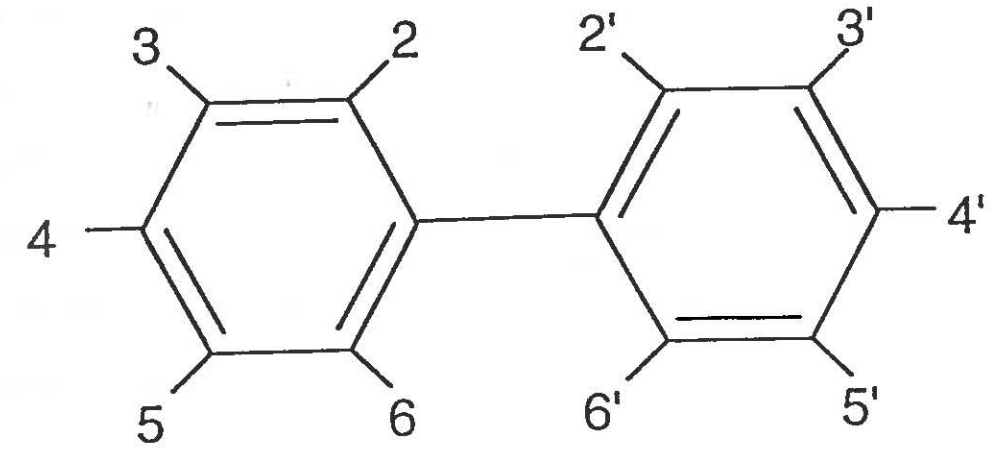
appears to be conserved by the animal, and is not readily mobilized, even in times of starvation. This tissue is thought to be of quite some importance to the animal, aiding in sound transmission, concentration and directionality (Aguilar, 1985), perhaps explaining the conservation of this tissue. As a tissue with little mobilization and very high triglyceride composition, melon tissue, if available for analysis, is an ideal tissue for an accurate comprehension of the organochlorine environment within the animal.

Throughout the 1970s and early 1980s, the principal method of analysis for PCBs in environmental samples involved comparison of the sample to a commercial PCB formulation, for instance, one of the Aroclor® formulations in the United States, or a blend of Aroclors® (Eganhouse and Gossett, 1991). Each PCB congener, however, has slightly different physical and chemical properties. Due to dispersal through the environment, weathering, biological uptake and preferential metabolism, the composition of the environmental samples differed significantly from the compositions of the commercial mixtures, and led to serious under- or overestimation of the concentrations (Duinker *et al.*, 1988*a*; Jones, 1988). The development of high resolution gas chromatography in combination with electron capture detection (GC-ECD) techniques enabled the distinction and quantification of the individual congeners in PCBs (Mullin *et al.*, 1984; Duinker *et al.*, 1988*a*).

The toxic nature of PCB mixtures is associated primarily with certain specific congeners and their individual molecular configurations (Safe *et al.*, 1985). Of the 209 possible isomers and congeners in PCBs, 20 can attain a planar, or coplanar configuration due to a lack of *ortho* substitution in the biphenyl rings (Safe, 1984; Tanabe *et al.*,

1987; Zook and Rappe, 1994). (Fig. 2). The non-*ortho* configuration allows for free rotation around the central phenyl-phenyl bond and the planar configuration (De Voogt *et al.*, 1990). Non-*ortho* PCBs which are substituted in both *para* positions and at least two *meta* positions are approximate isostereomers of the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (T_4CDD), the most toxic synthetic compound ever tested in a laboratory (Eisler, 1986; Safe, 1990). These are the most biochemically active as well as the most toxic of the PCB congeners (De Voogt *et al.*, 1990). The toxicity of these congeners lie in their induction of the drug-metabolizing enzyme systems, the cytochrome P-450 system, their induction of AHH/EROD, and their binding affinity to the cytosolic Ah receptor (Safe, 1984). The introduction of one *ortho*-substituent in the biphenyl ring (mono-*ortho* coplanar) results in decreased coplanarity between the two rings due to steric interactions, but although this diminishes the binding affinities and decreases AHH/EROD, it does not eliminate them. Two *ortho*-substituents (di-*ortho* coplanars) introduce more steric interaction, but some of these congeners are potent inducers as well (Safe *et al.*, 1985; De Voogt *et al.*, 1990).

As a result of the large expenditure of time and monetary resources necessary to analyze all congeners in environmental samples, many researchers and some national and international agencies (*e.g.* the International Council for the Exploration of the Sea (ICES), and World Health Organization (WHO)) now advocate the selection of a set of congeners for analysis (Tanabe *et al.*, 1987; Duinker *et al.*, 1988*a* and *b*, 1989; Jones, 1988). Depending upon the environmental compartment to be analyzed, criteria for the selection may include the distribution of congeners between various matrices, contribution



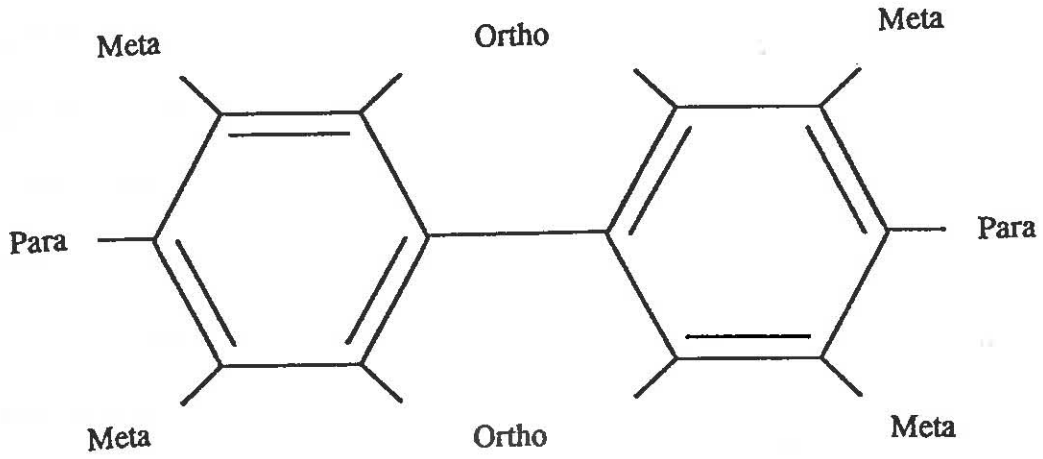


Figure 2. Polychlorinated biphenyl ring structure and chlorine substitution positions.

to environmental samples, volatility, persistence in one compartment, or toxicity, among others. Ecologically, it has been suggested that toxicity may be the more significant criteria in environmental samples and has been advocated as the criterion to be adopted in the future (Duinker *et al.*, 1988*b*). Recently, the WHO-European Center for Environment and Health and the International Programme on Chemical Safety selected 14 of the toxic coplanars as a subset in establishing internationally agreed upon toxic equivalency factors for PCBs (Hühnerfuss *et al.*, 1995). This recommendation has been followed in the present study. The congener set examined is composed of the toxic non-*ortho*, mono-*ortho*, and di-*ortho* inducers (#19 congeners), and 1 congener (IUPAC #153) included for its prevalence in all environmental compartments. The toxic coplanars studied are as follows in IUPAC #'s: non-*ortho*: #77, #81, #126, and #169; mono-*ortho:* #105, #114, #118, #123, #156, #157, #167, #189; di-*ortho* inducers: #101, #128, #138, #158, #166, #170, #180. This congener set is the same as adopted by WHO, with the addition of one mono-*ortho* congener, #189, and three di-*ortho* congeners, #128, #158, and #166. Congener #189 is an AHH inducer, and the three di-*orthos* have demonstrated mixed-induction activity (De Voogt *et al.*, 1990).

The manner of the reporting, or expressing, of organochlorine concentrations is still being debated in the literature. The use of fresh weight, or "wet weight" basis, dry weight, or a lipid weight basis have all been criticized on many occasions as these all have both advantages and disadvantages (Aguilar, 1987; Guitart *et al.*, 1996). Most commonly, the concentrations of chlorinated hydrocarbons in animal tissue have been reported in terms of the concentration in the fresh weight of the piece of tissue analyzed

(wet weight basis). Aguilar (1985) considers this method of expression to be inadequate in the analysis of marine mammal tissues for establishing comparisons between different organs in the same individual, different individuals in a population, or different species, and advocates the reporting be done on lipid basis - pollutant level/unit/weight of lipids. The rationale for this approach is that the most widely sampled tissue in marine mammals, the blubber, is neither homogeneous nor constant, and subject to physiological and environmental factors exist that can affect the lipid composition, and so the organochlorine content in this tissue. This lack of homogeneity in the tissue is, however, more marked in the larger cetaceans, while smaller cetaceans such as dolphins show much greater homogeneity (Granby and Kinze, 1991; Davis, 1993). When expressing concentrations on the basis of lipid weight, the great magnitude in difference between the concentrations in blubber and in other tissues disappears, and in fact come close to a proportion of 1:1, useful if comparisons are to be made between many tissues of an animal (Aguilar, 1985). Another cause for criticism, however, is the fact that quantity of lipid extracted is dependent upon the type of solvent(s) used, which moreover can vary from study to study (Guitart *et al.*, 1996). Recognizing the validity of Aguilar's argument while bearing in mind the advantage of comparison with studies that have expressed concentration as wet weight, the concentrations in this study have been reported on both a wet weight and a lipid weight basis.

2.0 METHODS AND MATERIALS

2.1 Sample Acquisition and Storage

The samples used in this study were collected during necropsies performed on dolphins that stranded and were recovered by the Mote Marine Laboratory Marine Mammal Stranding Program in conjunction with the Southeast U.S. Marine Mammal Stranding Network. The blubber and melon tissues sampled for organic analysis were stored frozen in aluminum foil at -20° C until thawing prior to weighing and extraction. Tissue samples of blubber from 20 dolphins and melon samples from 17 of these dolphins were selected for analysis based on their identification as locally resident dolphins of known histories, as provided by the Dolphin Biology Research Institute (DBRI). A stranding code of 1, 2 or 3 was required to ensure reliable samples (1=Live animals, 2=Carcass in good condition-fresh/edible, 3=Fair-decomposed but organs basically intact, 4=Poor-advanced decomposition, 5=Mummified or skeletal remains) (Geraci and Lounsbury, 1993). Table 4 (pg.43) contains descriptions of the dolphins analyzed in this study, along with stranding codes and necropsy findings.

2.2 Extraction Procedures

Approximately 1 g of tissue was cut from the centers of the thawed samples with a pre-cleaned and solvent-rinsed stainless steel scalpel, and weighed. When possible, blubber samples included the entire blubber layer from skin to muscle to avoid possible bias in blubber composition (Aguilar and Borrell, 1991). The exact sample weight was

recorded, and the sample placed in a solvent-rinsed 250 ml beaker. A recovery surrogate, DBOB (4,4'- dibromooctafluorobiphenyl) was added to each sample as an internal standard, along with matrix spikes in 10% of the samples. Approximately 25 g of solvent-cleaned anhydrous sodium sulfate was added to remove water and facilitate grinding. Dichloromethane was then added (approximately 100 ml), and the sample macerated for 3 min using a Tekmar Tissuemiser® (Tekmar Co., Cincinnati, OH) with a stainless steel probe. The extract was filtered via vacuum filtration into a solvent rinsed 500 ml flask. The sample residue was macerated 2 more times with an addition of dichloromethane each time, and the solvent extract again collected into the receiving flask. The extract volume was concentrated on a Büchi Rotovapor® (Brinkman Instruments Inc., Westbury, NY), transferred with several dichloromethane rinses to a glass vial, and concentrated again to a 5 ml volume by gentle nitrogen stream evaporation.

2.3 Lipid Weight Determination

Gravimetric analysis was used to determine the lipid weight of each sample. A 1 ml aliquot of the sample extract was applied to a tared, solvent-rinsed 70 mm aluminum weigh pan on a Mettler® AE 163 analytical balance (Mettler Instrument Corp., Hightstown, NJ). The dichloromethane was allowed to evaporate and the weight of the lipids recorded (Sherblom and Eganhouse, 1991). Every tenth sample was analyzed in triplicate to assess the analytical precision. The percent lipid weight was calculated using the original volume of the extract, the sample wet weight, and the measured lipid weight.

2.4 Clean-up of Lipids - Gel Permeation Chromatography

Gel permeation chromatography was used to effect the separation of lipids from organochlorines through size-exclusion (Shan, *et al.*, 1994; Salata, *et al.*, 1995). An HPLC was modified to work in a low-pressure mode to deliver solvent through a column packed with Bio-Beads® S-X3 (BioRad Laboratories, Hercules, CA), neutral, porous styrene divinylbenzene copolymer beads that are crosslinked to facilitate separations of low molecular weight organic polymers and other hydrophobic substances. The Bio-Beads® S-X3 were saturated in 50:50 dichloromethane:hexane overnight to swell the beads. Before the column was packed, the slurry was sonicated for approximately 20 min to degas the solvent. The slurry was packed under low pressure into a 10 x 500 mm Omni® glass chromatographic column. The solvent was graduated to 75:25 dichloromethane:hexane, and then to 100% dichloromethane.

The 1 ml sample was diluted with 1 ml of dichloromethane and added to the column via a 2.5 ml sample loop. The solvent (100% dichloromethane) was passed through the column at 0.75 ml/min. An ISCO® V^4 Absorbance Detector (ISCO Inc., Lincoln, NE) was used to ascertain the elution time of the lipids and the organochlorines. The organochlorine fraction was collected in a solvent-rinsed 25 ml pear-shaped flask. This fraction was then concentrated on a Büchi Rotovapor® at <38 C to remove most of the dichloromethane, and transferred to a glass vial. This was evaporated to dryness under a gentle nitrogen stream and brought up in hexane to a final volume of 1 ml.

2.5 Separation of Compounds - Silica Gel Fractionation

A glass chromatography column, 6 mm x 400 mm, was fitted with a hexane-rinsed glass wool plug. Five g of silica (Merck® silica gel, Aldrich Chemical Co., Milwaukee, WI, prepared as in "Solvents and Reagents") was weighed in an aluminum weigh boat and poured into the dry column while gently tapping the column. One centimeter of cleaned anhydrous sodium sulfate was added to the top of the column. The column was prewetted with 20 ml of hexane, and this eluent was discarded. The sample was then added to the column with a pipet, eluted with 40 ml of hexane, and collected as fraction 1. This fraction generally contained the PCBs, HCB, heptachlor, aldrin, DDE, and some DDT. The next fraction was eluted with 40 ml of 25:75 dichloromethane:hexane, and optimally contained the HCH compounds, heptachlor epoxide, chlordane, some DDE, DDT and DDD. Fraction 3 was eluted with 40 ml of 40:60 dichloromethane:hexane, and contained some endrin, dieldrin, and endrin aldehyde, as well as endosulfan sulfate. The above methodology followed Metcalfe, 1994. Fractions 1, 2 and 3 were individually concentrated on a Rotovap® and brought up in 1 ml of hexane to which the quantitation standard had been added. PCB #207 was chosen as the quantitation standard for this study as it is a "theoretical" PCB congener, not found in commercial mixtures or in the environment, and because it was found not to co-elute with other compounds in these samples. The fractions were then ready for analysis by injection into the gas chromatograph interfaced with an electron capture detector.

2.6 Apparatus and Materials

2.6.1 Glassware: Glassware was cleaned by hot water wash with Micro® cleaning solution (International Products Corp., Trenton, NJ), and then rinsed with tap water followed by deionized water. Glassware was then sequentially rinsed with wash acetone, followed by the solvent(s) used in the specific procedure.

2.6.2 Solvents and Reagents: All solvents used in this study were Burdick and Jackson® High Purity grade (AlliedSignal Specialty Chemicals, Muskegon, MI), certified for use in pesticide analysis. Silica gel (Merck® 70-230 mesh, grade 7754), and sodium sulfate (Mallinckrodt® AR anhydrous, Mallinckrodt Inc., Paris, KY) underwent cleaning before use in these procedures. Each were washed sequentially, sonicated, and decanted with methanol for 10 min, dichloromethane for 5 min, dichloromethane for 10 min, hexane for 5 min, and hexane for 10 min, then placed on top of the drying oven for 2-3 d loosely covered with foil, and stirred each day until the solvent had evaporated. The silica was then placed in an oven at 130° C for 24 h before use. The sodium sulfate was placed in the muffle furnace at 250° C for a minimum of 6 h. Both materials were then placed in the drying oven for storage. Before use, the silica and the sodium sulfate were placed in a desiccator to cool.

2.6.3 Standards: All individual analyte standards were purchased from Accustandard Inc., (New Haven, CT), ULTRA Scientific (Kingstown, RI), and Supelco Inc. (Bellefonte, PA).

2.7 Instrumental Analysis

Organochlorine analyses were performed using a Varian® 6000 gas chromatograph (Varian Associates, Inc., Walnut Creek, CA) equipped with ^{63}Ni electron capture detector and a 30 meter, 0.25 mm i.d., 0.25 μm (film thickness) J&W DB5® fused silica capillary column (J&W Scientific, Fulsom, CA). The inlet was operated in a splitless mode with a purge delay of 0.75 min. 1.0 μl of sample was injected via syringe. Helium was used as the carrier gas. The injector temperature was set at 275° C and the detector temperature at 325°C. The GC oven temperature program used was: initial temperature 50°C for 1 min, 12° C/min to 175°C with a hold of 0.5 min, then 1.4°C/min to 226°C, and 10°C/min to 285°C with a 6 min hold, with a total run time of 60.23 min.

2.7.1 Data acquisition and integration: Data acquisition, integration and quantification were performed using P.E. Nelson Model 2600 Multiple Instrument Chromatographic Software Rev. 5.2.0 operating on an IBM compatible PC.

2.7.2 Compound quantification of PCBs and Chlorinated Pesticides: PCB congeners and pesticide compounds were identified and quantified using external congener standard mixtures. A full analyte list appears on page 42. Relative response factors were determined from serial analyses of each of 4 calibration standard concentrations to compensate for the nonlinear response of the electron-capture detector. Recoveries of the compounds were determined by comparison to the quantitation standard (PCB #207). A Varian® Saturn II capillary gas chromatograph coupled with

ion trap mass spectrophotometer (GC-MS) was used to confirm identification of congeners. Limits of detection (LOD) were as listed: 0.001 μg/g for PCB congeners 101, 118, 114, 126, 105, 138, 128, 156, 180, 169, 170, and pesticides $\propto$-HCH, lindane, d-HCH, aldrin, γ-chlordane, $\propto$-chlordane, dieldrin, endrin, endosulfan II, endosulfan sulfate, o,p-DDE, and o,p-DDT. LOD of 0.002 μg/g for PCB congeners 28, 52, 81, 77, 123, 158, 166, 167, 157, 189, and pesticides b-HCH, heptachlor, heptachlor epoxide, endosulfan I, endrin aldehyde, p,p-DDE, and p,p-DDD. LOD of 0.004 μg/g for PCB congener 153, and pesticides HCB, o,p-DDD, and p,p-DDT.

2.7.3 Quality Assurance/Quality Control: A method blank, a sample triplicate, a reference standard and a matrix spike were each run for a minimum of 10% of samples. An instrument blank was run on the GC-ECD a minimum of once a day, and generally after every third run to verify non-contamination of the column. A minimum of 10% of samples were confirmed by gas chromatograph-mass spectrophotometer (GC-MS). Qualitative results obtained by ECD were in agreement with those obtained by MS detection. Data for sample sets were considered acceptable if the recovery of the internal standard (recovery surrogate) was within 50-115%, and the coefficient of variation of the concentration of each analyte in the triplicated samples was less than 30%. Mean standard deviations in the triplicates were less than 10% (range 0.05-9.31%).

2.7.4 Data Analysis: All data analyses were performed with an IBM® compatible personal computer. The programs utilized for data management were Excel® v. 4.0 and

Paradox® v. 5.0. Statistics were performed using SigmaStat® v. 1.0 and graphing using SigmaPlot® v. 1.02. The data were not adjusted for recoveries. All statistical tests were performed at the 95% confidence interval. Data were tested initially for normality and equal variance. When these tests were passed, Student's t-Test was used to test for differences between two groups, one-way ANOVA was used to test the differences between three or more groups, and linear regression was used to determine the significance of values of dependent variables to independent variables. Correlational analysis utilizing the Pearson Product Moment Test was conducted to test the strength of association between the values of two variables. As parametric tests are not reliable when used on non-normal populations, in the few cases where the data did not pass either the test for normality or equal variance, the Mann-Whitney Rank Sum Test was used to test for differences between two groups, the Kruskal-Wallis ANOVA on Ranks was used to test the differences between three or more groups. Correlational analysis was tested non-parametrically by the Spearman Rank Order test. Data analyses were performed using both wet weight and lipid weight values. The statistical results presented are based on the wet weight values.

2.8 ANALYTES

PCB Congeners: IUPAC numbers

Non-*ortho* (planar):	**# 77**		3,3′,4,4′ - tetrachlorobiphenyl
	81		3,4,4′,5 - tetrachlorobiphenyl
	126		3,3′,4,4′,5 - pentachlorobiphenyl
	169		3,3′,4,4′,5,5′ - hexachlorobiphenyl
Mono-*ortho:*	28	◂▸	2,4,4′ - trichlorobiphenyl
	105		2,3,3′,4,4′ - pentachlorobiphenyl
	114		2,3,4,4′,5 - pentachlorobiphenyl
	118	◂▸	2,3′,4,4′,5 - pentachlorobiphenyl
	123		2′,3,4,4′,5 - pentachlorobiphenyl
	156		2,3,3′,4,4′,5 - hexachlorobiphenyl
	157		2,3,3′,4,4′,5′- hexachlorobiphenyl
	167		2,3′,4,4′,5,5′- hexachlorobiphenyl
	189		2,3,3′,4,4′,5,5′ - heptachlorobiphenyl
Di-*ortho*:	52	◂▸	2,2′,5,5′ - tetrachlorobiphenyl
	101	◂▸	2,2′,4,5,5′ - pentachlorobiphenyl
	128		2,2′,3,3′,4,4′ - hexachlorobiphenyl
	138	◂▸	2,2′,3,4,4′,5′ - hexachlorobiphenyl
	153	◂▸	2,2′,4,4′,5,5′ - hexachlorobiphenyl
	158		2,3,3′,4,4′,6 - hexachlorobiphenyl
	166		2,3,4,4′,5,6 - hexachlorobiphenyl
	170		2,2′,3,3′,4,4′,5 - heptachlorobiphenyl
	180	◂▸	2,2′,3,4,4′,5,5′ - heptachlorobiphenyl

◂▸ = The subset of 7 congeners used for study by the ICES (International Council for the Exploration of the Seas)

Organochlorine Pesticides:

Aldrin
Dieldrin
Endosulfan I, II and -sulfate
Chlordane and metabolites
- heptachlor
- heptachlor epoxide
- alpha-chlordane
- gamma-chlordane

Endrin
Endrin aldehyde

DDT compounds
- 2,4′ & 4,4′ DDE
- 2,4′ & 4,4′ DDD
- 2,4′ & 4,4′ DDT

Hexachlorobenzene (HCB)
Hexachlorocyclohexanes (HCH):
- alpha HCH
- beta HCH
- gamma HCH (lindane)
- delta HCH

Table 4. Descriptions of Sarasota Bay Dolphins Used for Analysis: Sex, Age, Stranding Code and Necropsy Findings

MML#	DBRI#	Sex	Age	Strand. Code	Cause of death/comments
9309	C07-2	F	3 wk	sc 2/3	2nd calf, no diagnosis, stomach empty
9224	C65-1	F	5.3 mo	sc 2	Discharge L. mammary; net entanglement possible
9417	C75-4	F	6 mo	sc 2/3	Monofilament entanglement, shark bite, ematiated, respiratory failure, cardiac arrythmia
9314	C33-3	F	1.25	sc 2	Monofilament entanglement, septicemic shock. stomach empty
9118	FB21	F	3	sc 3	Small hemorrhages in ventral blubber - net entanglement suspected
9221	FB103	F	4	sc 2	Emaciated, lung abcesses + bronchopneum. Brain: evid. of toxic metabolic disorders
9225	FB37	F	9.5	sc 2	Paralysis secondary to stingray barb
9115	FB31	F	11	sc 2	Stabbed in lung, heart, pregnant with yearling calf at side, lactating
9212	FB67	F	24	sc 3	Died during parturition - breech-type birth, ruptured uterus. Anthracosis found.
9108	FB45	F	35	sc 3	Severe fatty liver, chronic mastitis, kidney involvement., ematiated, Hg toxicity suspected
9514	FB41	F	36	sc 2	Monofilament strangulation
9625	FB57	F	44	sc 2	Shark foraged/anthracosis. Had 6 week old calf at side
9401	FB19	F	50	sc 2	Old age / septic shock suspected, anthracosis found
9308	C17-3	M	3 mo.	sc 2	Pneumonia, was nursing
9621	C71-7	M	3.5 mo	sc 1/2	Enlarged liver, ematiated, +milk in intestine, 2nd stomach ulcerated
9104	FB22	M	4	sc 2	Net entanglement suspected, rope marks found
9215	FB50	M	4	sc 2	No acute diagnosis / Stress due to stranding secondary to sinus parasites + disorientation
9226	FB52	M	9	sc 3	No acute diagnosis on necropsy
9012	FB74	M	39	sc 3	Ruptured aorta
9509	FB98"G"	M	42	sc 2	Old age assumed. No acute necropsy diagnosis
	"Young animals" for analysis of birth order include calves less than 2 years old and are designated by a DBRI # of C**-*.				
			The last digit indicates the birth order.		
	"Dependent calves" for analysis of post-weaning decline in OC levels includes all calves less than 5 years old.				

3.0 RESULTS

Seven male dolphins were included in this study and range in age from 3 months to 42 years old. The thirteen female dolphins in this study range in age from 3 weeks to 50 years old. Tables 5 and 6 at the end of this section summarize the data into major organochlorine groups in wet weight and lipid-normalized weight values, respectively, along with mean values and standard deviations. Raw data are presented in appendices A-D, again in wet weight and lipid-normalized weight concentrations.

3.1 Data from Male Dolphins:

A comparison of organochlorine content in male dolphin blubber as a function of age is given in Figure 3. These results show a significant correlation with PCB content with age (linear regression analysis $p<0.04$; $r=0.75$, $n=7$), and total DDTs (ΣDDT) ($p=0.05$; $r=0.75$). Total chlorinated pesticides (ΣPests) also appeared to increase with age, however the difference was not significant at the 95% confidence level ($p=0.18$; $r=0.57$). The concentrations of the major organochlorine groups in melon samples ($n=6$) also exhibited an increase with age, with a significant increase seen in ΣPCB ($p<0.04$; $r= 0.83$). Although the mean value increased with age, the increases in melon ΣDDT and ΣPests were not statistically significant. Figure 4 illustrates the levels of melon organochlorines with age in the male dolphins. Individual PCB congeners and pesticides that either increased or decreased significantly with age are as follows:

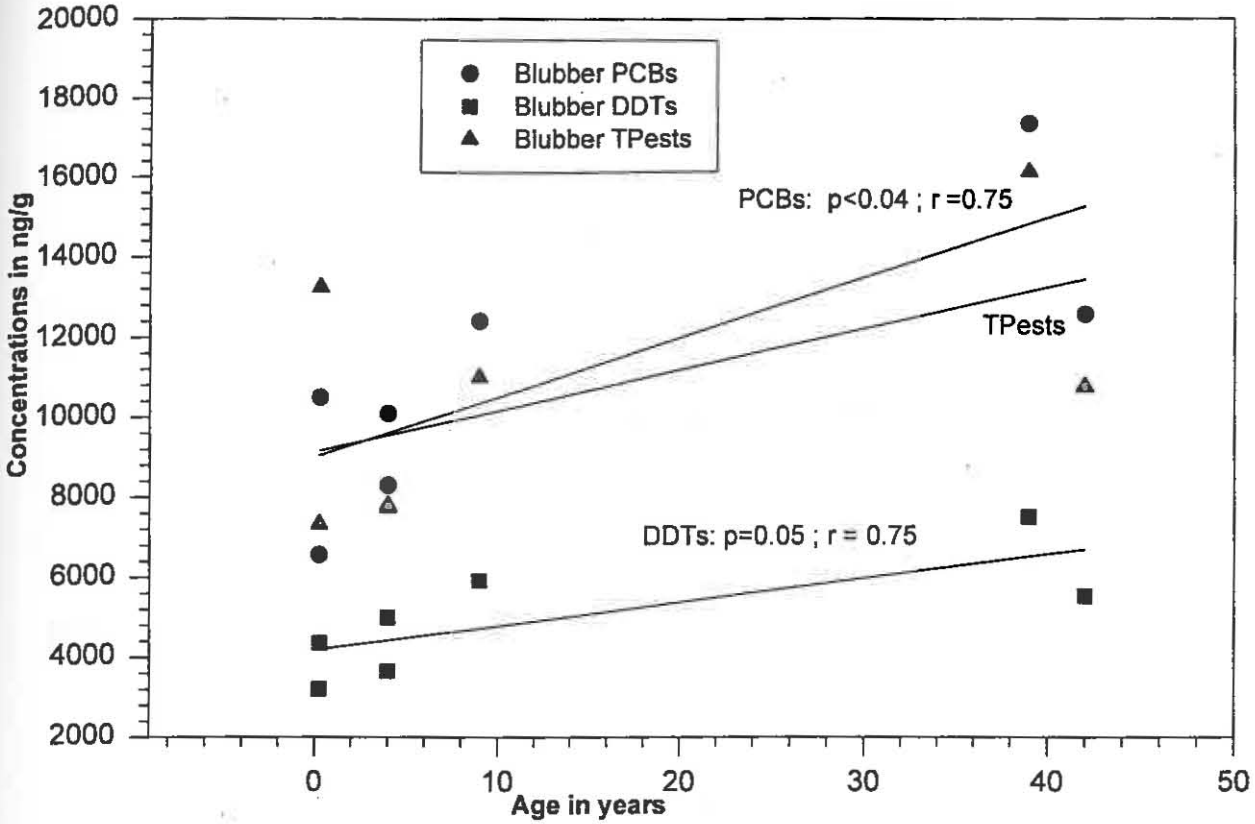


Figure 3: Concentrations of Organochlorines in Blubber as a Function of Age in Male Dolphins

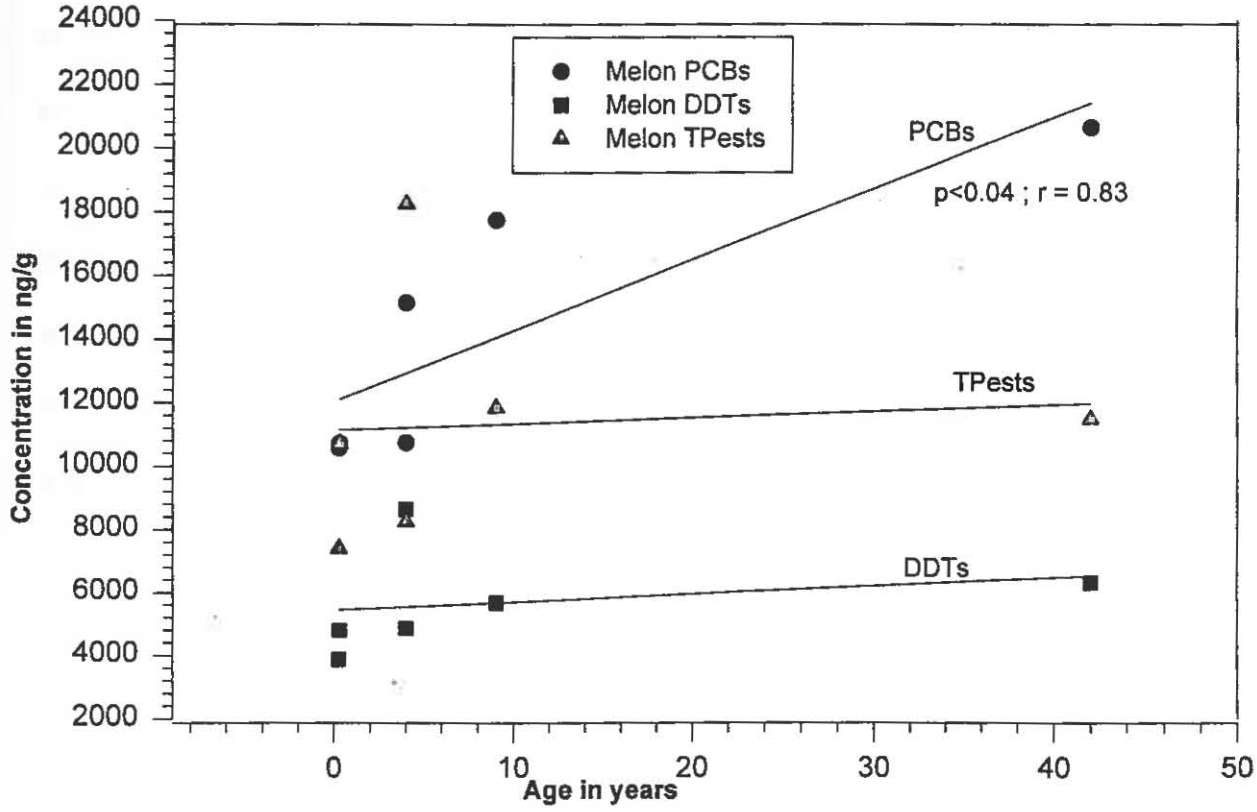


Figure 4: Concentrations of Organochlorines in Melon as a Function of Age in Male Dolphins

Blubber increases:	PCBs:	#126	($p<0.02$; $r=0.89$)	#166	($p<0.002$; $r=0.94$)
		#105	($p<0.05$; $r=0.76$)	#128	($p<0.005$; $r=0.91$)
		#138	($p<0.05$; $r=0.76$)	#157	($p<0.001$; $r=0.96$)
		#158	($p<0.05$; $r=0.75$)	#189	($p<0.04$; $r=0.79$)
		#153	($p<0.002$; $r=0.93$)		
	Pesticides:	endrin	($p<0.02$; $r=0.83$)		
		endrin aldehyde	($p<0.02$; $r=0.84$)		
Melon increases:	PCBs:	#153	($p<0.02$; $r=0.87$)	#170	($p<0.02$; $r=0.89$)
		#167	($p<0.01$; $r=0.92$)	#189	($p<0.05$; $r=0.82$)
Melon decreases:	PCBs:	#101	($p<0.02$; $r=0.89$)		

In male blubber samples, the congeners that significantly increased with age are as shown above. No significant decreases with age in PCB congener or pesticide concentration were found in male blubber. The melon PCB congeners that showed either an increase or decrease with age are shown above. No significant increase or decrease with age in any of the pesticides was evident in the melon. Tests to determine any relationship between the blubber levels and the melon levels in male dolphins of the major organochlorine groups were run using the Pearson Product Moment Correlation. No significant correlations were found between the blubber and the melon in concentrations of the ΣPCB, the ΣDDT or the ΣPests.

3.2 Data from Female Dolphins:

Female dolphins showed a significant decrease with age in one major organochlorine group, the concentration of ΣDDTs in melon ($n=11$) ($p=0.02$; $r=0.68$),

utilizing linear regression. Although not statistically significant, declines in all organochlorine groups with age are noted and are illustrated in Figure 5 (organochlorine content in female blubber as a function of age), and Figure 6 (organochlorine content in female melon as a function of age). Specific PCB congener and pesticide concentrations that either increased or decreased significantly with age are as follows:

Blubber decreases: Pesticides: endosulfan I ($p<0.05$; $r=0.56$)

Melon decreases: PCBs: #126 ($p<0.02$; $r=0.70$) #170 ($p<0.05$; $r=0.60$)
#138 ($p=0.04$; $r=0.62$) #189 ($p<0.04$; $r=0.64$)
#157 ($p<0.01$; $r=0.76$)

Pesticides: γ-chlordane ($p<0.05$; $r=0.60$)
o,p -DDE ($p<0.02$; $r=0.70$)
p,p -DDE ($p=0.01$; $r=0.73$)
endrin ($p=0.04$; $r=0.62$)
endosulfan sulfate ($p<0.04$; $r=0.63$)

There was one pesticide that showed significant decrease with age in the blubber of the female dolphins, as shown above. No PCB congeners were found to significantly decrease with age in the blubber of the female dolphins. In the melon samples, the PCB congeners and the pesticides that showed significant decreases with age are as shown above. There were no PCB congeners or pesticides found to increase significantly with age in either the blubber or the melon. Tests to determine any relationship between the blubber levels and the melon levels in female dolphins of the major organochlorine groups were run using the Pearson Product Moment Correlation. No significant correlations were found between the blubber and the melon in concentrations of the

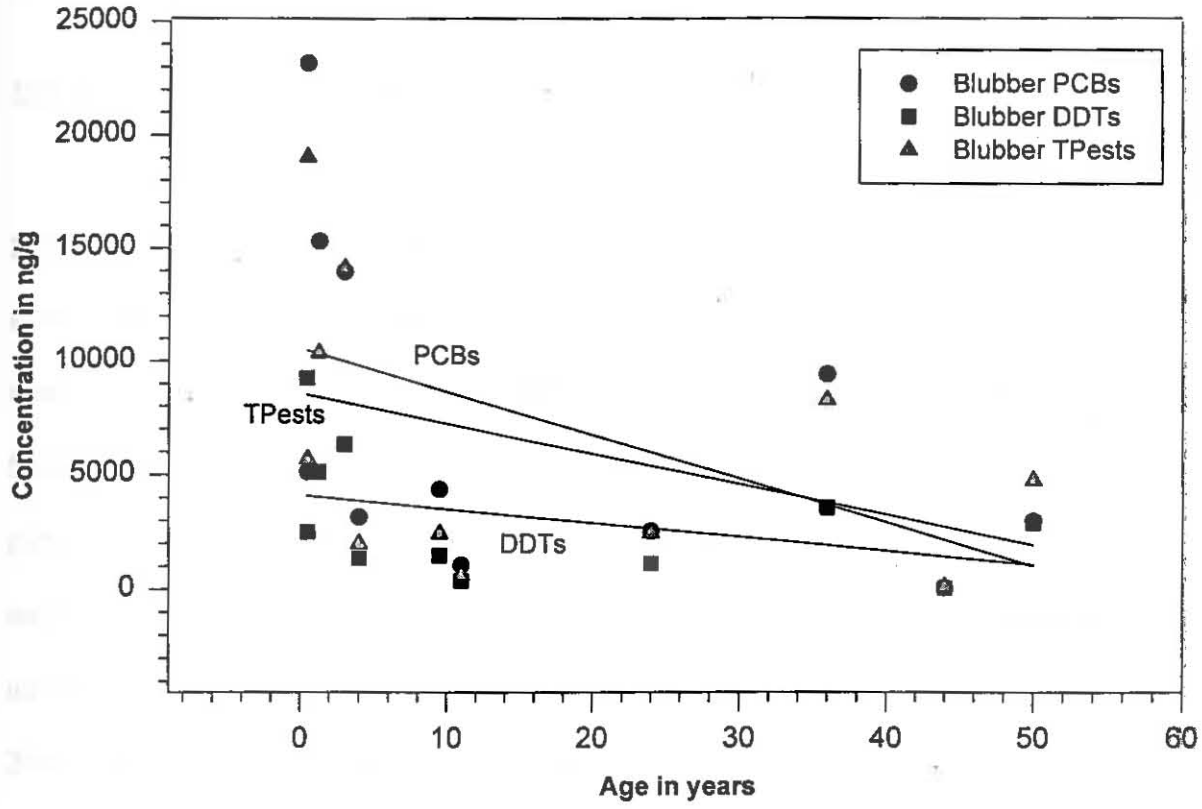


Figure 5: Concentration of Organochlorines in Blubber as a Function of Age in Female Dolphins

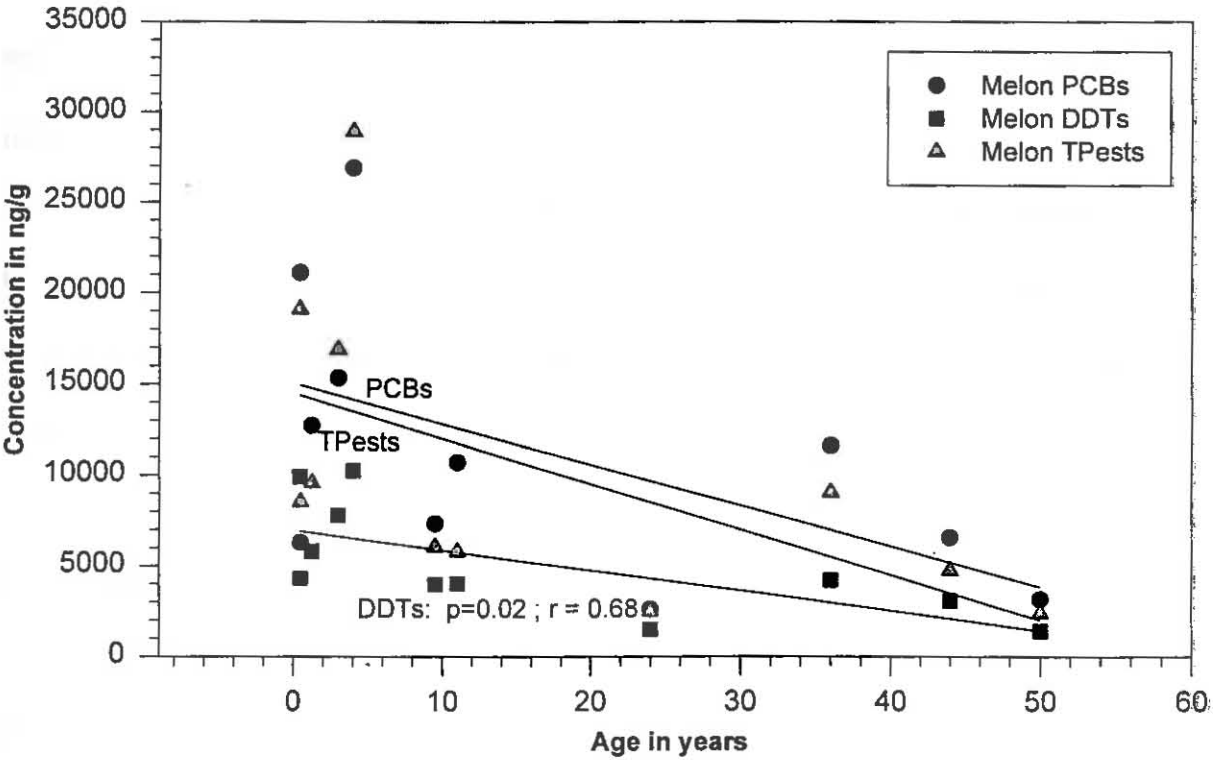


Figure 6: Concentration of Organochlorines in Melon as a Function of Age in Female Dolphins

ΣPCB, the ΣDDT or the ΣPests.

To test for significant differences between the levels of organochlorines in pre-parturient and post-parturient females, Student's t-Test (S-T) was applied. When the assumption of equal variance was not met, the Mann-Whitney Rank Sum Test (M-W) was used. The reproductive state of all females was known definitively from DBRI records. Significant differences in all organochlorine groups were found between the pre- and post-parturient females, in both the blubber and the melon. For the blubber analyses, the number of individuals was: pre-parturient $n=6$ and post-parturient $n=7$. Blubber ΣPCB differences by M-W were significant at $p=0.02$, medians 9593.7 ng/g pre-parturient, and 2969.7 ng/g post-parturient. Blubber ΣDDT differences by S-T were significant at $p<0.05$, means 4432.5 ng/g pre-parturient and 1666.4 ng/g post-parturient. Blubber ΣPests differences by S-T were significant at $p=0.04$, means 9415.6 ng/g pre-parturient and 3316.8 ng/g post-parturient. For the melon analysis pre-parturient $n=5$ and post-parturient $n=6$. Melon ΣPCB differences were significant by M-W at $p<0.05$, medians 15325.1 ng/g pre-parturient and 6933.9 ng/g post-parturient. Melon ΣDDT differences by M-W were significant at $p=0.004$, medians 7770.9 ng/g pre-parturient and 3526.8 ng/g post-parturient. Melon ΣPests differences were significant by M-W at $p<0.009$, medians 16878.5 ng/g pre-parturient and 5275.4 ng/g post-parturient.

3.3 Data by Age and Sex Class

The two age classes of "immature" and "mature" animals were determined through the DBRI demographic database. For females, the immature age class was from 3 weeks

- 4 years old (n=6), and the mature age class from 9.5 years - 50 years old (n=7). For males, the immature age class was from 3 months - 9 years old (n=5) and mature age class 39 - 42 years old (n=2).

Significant differences in organochlorine levels between age classes and between different genders in the same age classes were tested for by Student's t-Test (S-T). If the assumptions of normality or equal variance were not met, the Mann-Whitney Rank Sum Test (M-W) was employed. No significant differences in blubber organochlorine levels were found between immature (n=11) and mature (n=9) animals. In analyses of the melon data, significant differences were found between immature (n=10) and mature animals (n=7) in melon ΣDDT by S-T, ($p<0.01$), means 6588.7 ng/g immature and 3495.2 ng/g mature. A significant difference was also found by S-T in melon ΣPests at $p<0.02$, means 13940.9 ng/g immature and 5998.7 ng/g mature. The result for melon ΣPCB by S-T was not a significant one at $p=0.07$.

In the immature age class, no significant differences were found between males and females in either the blubber or the melon organochlorine groups. In the mature age class, significant differences were found in all blubber organochlorine groups between females (n=7) and males (n=2). For blubber ΣPCBs by S-T, $p=0.002$, means were 3406.0 ng/g for females and 14947.0 ng/g for males. Blubber ΣDDT was significant by S-T at $p=0.002$, means 1666.4 ng/g for females and 6518.5 ng/g for males. The blubber ΣPests were significant by S-T at $p<0.004$, means 3316.8 ng/g for females and 13434.9 ng/g for males. Melon organochlorines in the mature age class could not be tested statistically as male n=1.

3.4 Data by Birth Order

No significant relationship was found by linear regression between birth order and either blubber or melon organochlorine levels.

Table 5: Data Summaries of PCBs and Chlorinated Pesticides in Sarasota Bay Dolphins
Concentrations are in ng/g (ppb) on a wet wt. basis

MML#	DBRI#	Sex	Age	Lp Wt%	B PCBs	B DDTs	B HCB	B HCHs	B TPests	Lp Wt%	M PCBs	M DDTs	M HCB	M HCHs	M TPests
9309	C07-2	F	3 wk	64.65	5249	2057	219	121	5302						
9224	C65-1	F	5.3 m	76.95	23171	9243	954	318	19034	83.55	21088	9894	1378	494	19083
9417	C75-4	F	6 m	65.00	5112	2500	445	264	5690	84.10	6285	4307	546	383	8513
9314	C33-3	F	1-1.5	75.00	15288	5108	273	149	10352	87.40	12693	5795	108	78	9558
9118	FB21	F	3	65.50	13939	6321	705	247	14127	74.60	15325	7771	611	414	16879
9221	FB103	F	4	1.55	3149	1366	45	19	1989	82.55	26888	10250	1952	569	28900
9225	FB37	F	9.5	55.95	4356	1475	14	35	2439	86.55	7323	3992	43	69	6055
9115	FB31	F	11	41.60	1071	371	43	6	634	89.55	10694	4025	20	45	5801
9212	FB67	F	24	75.40	2534	1128	102	96	2447	70.25	2615	1481	109	78	2497
9108	FB45	F	35	42.70	3430	2174	204	114	4521						
9514	FB41	F	36	53.25	9408	3554	169	40	8276	89.85	11604	4203	140	67	9039
9625	FB57	F	44	0.75	74	55	38	8	131	82.65	6545	3062	234	130	4749
9401	FB19	F	50	53.20	2970	2907	114	111	4771	76.00	3146	1413	76	107	2380
		Mean		51.65	6903.93	2943.08	255.77	117.54	6131.67	82.46	11291.30	5108.38	474.27	221.27	10313.91
		St. Dev.		25.16	6736.34	2592.06	285.24	102.83	5543.18	6.34	7502.57	3029.48	633.42	199.77	8151.05
		%St Dev		48.70	97.57	88.07	111.52	87.49	90.40	7.69	66.45	59.30	133.56	90.28	79.03
9308	C17-3	M	3 m	78.70	6555	3207	664	305	7328	83.50	10564	3874	775	328	7413
9621	C71-7	M	3.5 m	64.25	10489	4356	941	369	13239	84.60	10706	4803	1111	604	10713
9104	FB22	M	4	57.60	8292	3659	502	214	7749	86.80	15128	8642	908	331	18249
9215	FB50	M	4	69.40	10081	4998	152	76	7802	79.20	10739	4873	345	152	8261
9226	FB52	M	9	46.15	12403	5913	273	108	11002	81.20	17742	5679	378	272	11840
9012	FB74	M	39	49.65	17332	7504	254	94	16115						
9509	FB98"G"	M	42	62.20	12562	5533	176	37	10755	79.15	20624	6291	150	66	11470
		Mean		61.14	11456.77	5186.55	458.37	181.25	11014.76	81.30	14403.89	5990.27	611.14	309.57	12117.86
		St. Dev.		11.23	3373.43	1428.37	289.75	120.01	3279.51	4.10	3938.26	1709.09	342.22	175.10	4077.17
		%St Dev		18.37	29.44	27.54	63.21	66.21	29.77	5.04	27.34	28.53	56.00	56.56	33.65

B = blubber values; M = melon values
PCBs = sum of 22 congeners (see analyte list)
DDTs = total o,p-DDT, p,p-DDT, o,p-DDE, p,p-DDE, o,p-DDD and p,p-DDD
HCHs = sum of a-HCH, b-HCH, d-HCH and lindane (g-HCH)
TPests = sum of all pesticide analytes (see analyte list)

Table 6: Data Summaries of PCBs and Chlorinated Pesticides in Sarasota Bay Dolphins
Concentrations are in ng/g (ppb) on a lipid wt. basis

MML#	DBRI#	Sex	Age	Lp Wt%	B PCBs	B DDTs	B HCB	B HCHs	B TPests	Lp Wt%	M PCBs	M DDTs	M HCB	M HCHs	M TPests
9309	C07-2	F	3 wk	64.65	8118	3181	339	187	8201						
9224	C65-1	F	5.3 m	76.95	30112	12012	1240	413	24735	83.55	25240	11842	1650	591	22840
9417	C75-4	F	6 m	65.00	5582	3847	685	407	8754	84.10	7473	5121	650	455	10122
9314	C33-3	F	1-1.5	75.00	20384	6811	364	199	13803	87.40	14523	6630	123	89	10936
9118	FB21	F	3	65.50	21281	9650	1076	377	21567	74.60	20543	10417	818	555	22625
9221	FB103	F	4	1.55	203186	88139	2932	1201	128325	82.55	32572	12417	2365	689	35009
9225	FB37	F	9.5	55.95	7785	2637	25	63	4359	86.55	8461	4612	49	80	6996
9115	FB31	F	11	41.60	2575	892	104	13	1525	89.55	11942	4495	22	51	6478
9212	FB67	F	24	75.40	3360	1496	135	127	3245	70.25	3722	2108	155	111	3555
9108	FB45	F	35	42.70	7949	5092	479	267	10589						
9514	FB41	F	36	53.25	17668	3116	318	76	11983	89.85	12914	4677	156	75	10060
9625	FB57	F	44	0.75	9836	7321	5089	1091	17410	82.65	7919	3704	283	157	5746
9401	FB19	F	50	53.20	5582	5465	214	208	8967	76.00	4139	1860	99	140	3131
		Mean		51.65	26416.77	11512.23	1000.00	356.08	20266.38	82.46	13586.18	6171.18	579.09	272.09	12499.82
		St. Dev.		25.16	53748.27	23243.96	1452.37	373.93	33179.21	6.34	9115.97	3730.48	766.47	245.72	10050.41
		%St Dev		48.70	203.46	201.91	145.24	105.01	163.72	7.69	67.10	60.45	132.36	90.31	80.40
9308	C17-3	M	3 m	78.70	8330	4075	844	388	9312	83.50	12651	4639	928	393	8878
9621	C71-7	M	3.5 m	64.25	16326	6780	1464	574	20606	84.60	12655	5677	1313	714	12664
9104	FB22	M	4	57.60	14396	6353	872	371	11513	86.80	17428	9956	1046	381	21024
9215	FB50	M	4	69.40	14527	7202	220	109	11242	79.20	13599	6153	436	192	10430
9226	FB52	M	9	46.15	26875	12814	592	235	23840	81.20	21850	6994	477	335	14581
9012	FB74	M	39	49.65	34908	15144	511	189	29570						
9509	FB98"G"	M	42	62.20	20196	8895	283	59	17291	79.15	26057	7948	189	84	14492
		Mean		60.96	19365.43	8751.86	683.71	275.00	17624.86	82.41	17373.33	6894.50	731.50	349.83	13678.17
		St. Dev.		12.29	8923.56	3900.83	425.02	180.09	7500.14	3.09	5554.43	1876.80	429.43	215.06	4244.04
		%St Dev		20.16	46.08	44.57	62.16	65.49	42.55	3.75	31.97	27.22	58.71	61.47	31.03

B = blubber values; M= melon values
PCBs = sum of 22 congeners (see analyte list)
DDTs = total o,p-DDT, p,p-DDT, o,p-DDE, p,p-DDE, o,p-DDD and p,p-DDD
HCHs = sum of a-HCH, b-HCH, d-HCH and lindane (g-HCH)
TPests = sum of all pesticide analytes (see analyte list)

4.0 DISCUSSION

The concentrations of the chlorinated pesticides and those of the PCBs within individual dolphins in this study showed consistent patterns, with the sum of the PCB congeners studied (ΣPCB) being 2-3 times higher than total DDTs (ΣDDT) and approximately equivalent to or slightly higher than the sum of all chlorinated pesticides studied (ΣPests). The differences in concentrations in the animals are gender and age related. Due to the relatively few dolphins available at this time from the Sarasota Bay community, and consequently small sample sizes involved in the analyses, the results from this study are best viewed as trends rather than incontrovertible conclusions.

Data analyses were performed using both wet weight and lipid weight values. Concentrations normalized for lipid weight may have the advantage of compensating for inhomogeneity in blubber composition, and for some correction of differences in nutritive state. In very extreme cases, such as severely low lipid content, this normalization may result in overestimation of the analyte concentration in the tissue, producing anomalously high values. This appeared to be the case in this study with one animal, and was verified by comparison with the values derived from the melon concentrations of the animal in question. As inhomogeneity of blubber composition is not found in small cetaceans, and has not been found in bottlenose dolphin, compensation for this was not required. The question of nutritive state has yet to be resolved, but Kannan *et al.*, (1993) offer results of their research with striped dolphins, stating that they found no prominent variation in the PCB and DDT concentrations between well and poorly nourished animals. The

statistical results presented are based on the wet weight values.

The male dolphins in this study showed a significant increase with age in both the blubber and the melon ΣPCB concentrations, and in the blubber ΣDDT. Male dolphins, without benefit of any means by which to transfer or off-load chlorinated compounds, are expected to show this accumulation with age. The results from this study indicate that this process is occurring in these dolphins. A larger sample size might illustrate this more clearly in the future.

The female dolphins as a group show a great deal more variability than the males in body condition, lipid content of the blubber, and contaminant levels. The female dolphins showed a 49% relative standard deviation (RSD) in their blubber lipid weight, compared to 18% in the males. This variation is seen again in the RSDs of the blubber organochlorine concentrations, with females ranging from 88-112% while male RSDs ranged from 28-66%. The melon showed much less variability in lipid weight, as expected, with a 7.7% RSD in the females and 5% RSD in the males. The blubber lipid weight is a good indicator of the animal's general condition and nutritive state, therefore this variability appears to reflect the rigors of the female dolphins' circumstance in reproduction and subsequent lactation.

The organochlorine concentrations in these females show a decrease with age, although this decrease was only significant in one organochlorine group, the melon ΣDDT. Post-parturient females showed significantly lower levels of organochlorines than pre-parturient females in all organochlorine groups in both the blubber and the melon. These results agree with previous studies (Aguilar, 1987; Borrell *et al.*, 1985;

Cockroft *et al.*, 1989; Vedder, 1996), and indicate that the females in Sarasota Bay are off-loading a portion of their organochlorine loads to their young, and this appears to continue throughout their years of parturition. Little is currently known about senescence in female cetaceans, and bottlenose dolphins have not been shown to go through a significant post-reproductive period. For descriptive purposes, a second-degree regression was run on the female organochlorine data, and suggested an increase of organochlorine levels in the latter part of the female age group, after age 30-35 (Figure 7). This apparent rise in the chlorinated hydrocarbon concentrations of older females supports the reported increase in interreproductive interval as noted by Cockroft *et al.*, (1989), and in the Sarasota Bay dolphins, R.S. Wells, (pers. comm.). Interestingly, the rise suggested by the melon data lags behind the rise in blubber concentrations by at least 5 years, and the rise is not as large in the melon. This may be expected in a denser, more static organ which is not readily mobilized and may take longer for the xenobiotics to partition into and out of it. In the females' HCH levels, again by second degree regression, the rise in levels in older females appears to begin earlier in both blubber and melon. That the HCHs appear to accumulate to even a greater degree in the melon is supportive of Aguilar's data (1985), which show that the melon, although composed totally of triglycerides and non-esterified fatty acids (NEFA) like the blubber, nevertheless has physicochemical properties that favor the attachment of compounds of greater polarity.

In comparing the data from the males with the data from the females by Student's t-test, no statistically significant difference was found in either the blubber or the melon

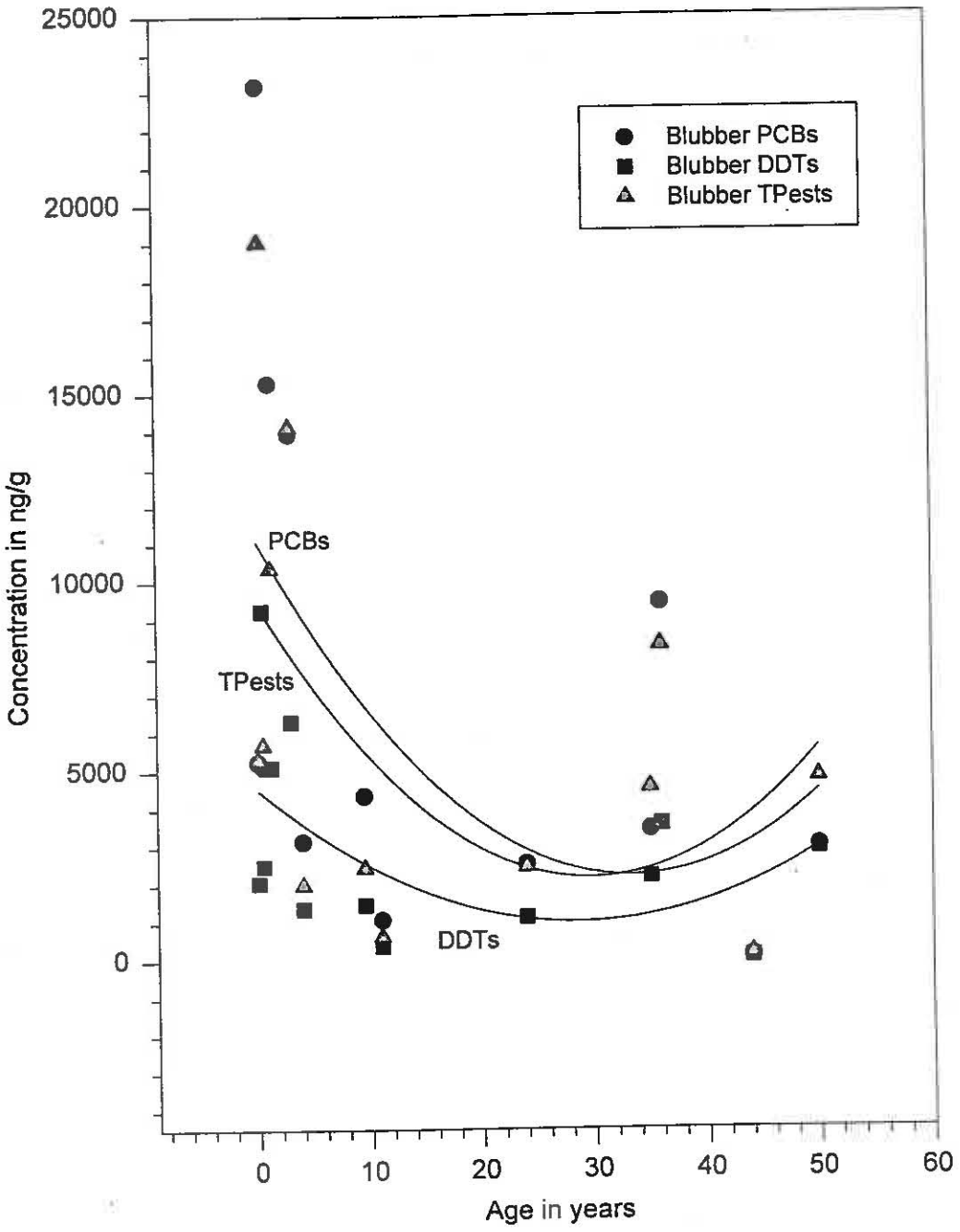


Figure 7: Second Degree Regression of Blubber Organochlorines with Age in Female Dolphins

concentrations of the major organochlorine groups. When the data are separated into two major age classes of mature dolphins and immature dolphins, the results are somewhat different. Looking at the immature age group, no significant difference is found between young males and young females in either the blubber or the melon organochlorines, suggesting similar maternal loading and subsequent food sources. Mature males and females, on the other hand, show significant differences using Student's t-test in the blubber in all organochlorine groups. This reflects the basic trends for males to increase their contaminant loading with age, while in females, metabolism and excretion, and offloading to young exceeds their dietary uptake (Figures 8 and 9).

It is apparent that the young dolphins in this study were subject to high amounts of gestational and lactational contaminant loading. It is generally assumed that the older males in a population will have the highest amounts of organochlorines. The first and third highest blubber PCB levels recorded in this study, however, are seen in a 5.3 month old (23.2 μg/g, wet wt.) and a 15 month old (15.3 μg/g, wet wt.). A 39 year old male had the second highest, with 17.3 μg/g (wet wt.). In the melon, the first and second highest PCB concentrations were in young dolphins, with the highest concentration in a 4-year old (26.9 μg/g wet wt.), and the second highest in the aforementioned 5.3 month old (21.1 μg/g wet wt.).

An interesting trend is seen when examining the organochlorine concentrations of immature dolphins 0 - 4 years old (the age group prior to mother/calf separation). In this group, blubber organochlorines appear to decrease at approximately 2 years old, which is at about the time of weaning. This is illustrated again by second-degree regression in

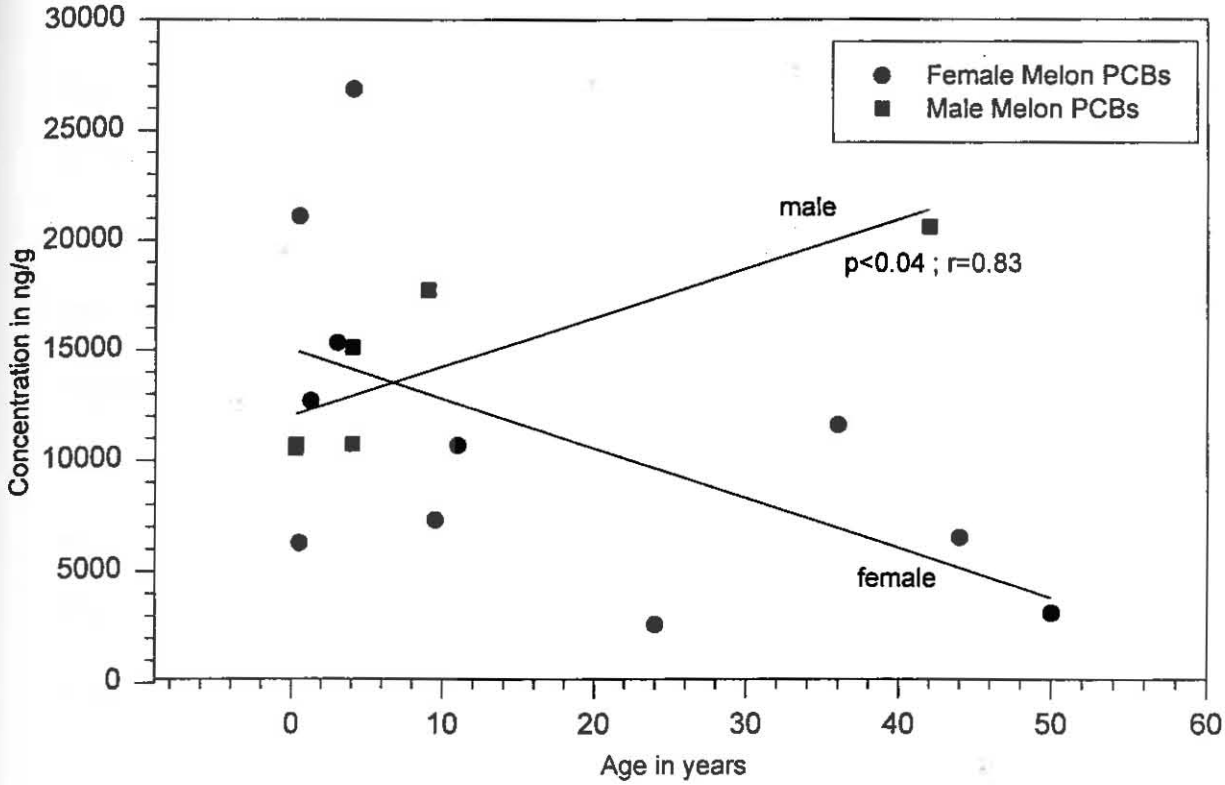


Figure 8: Concentrations of PCBs in Melon as a Function of Age in Male and Female Dolphins

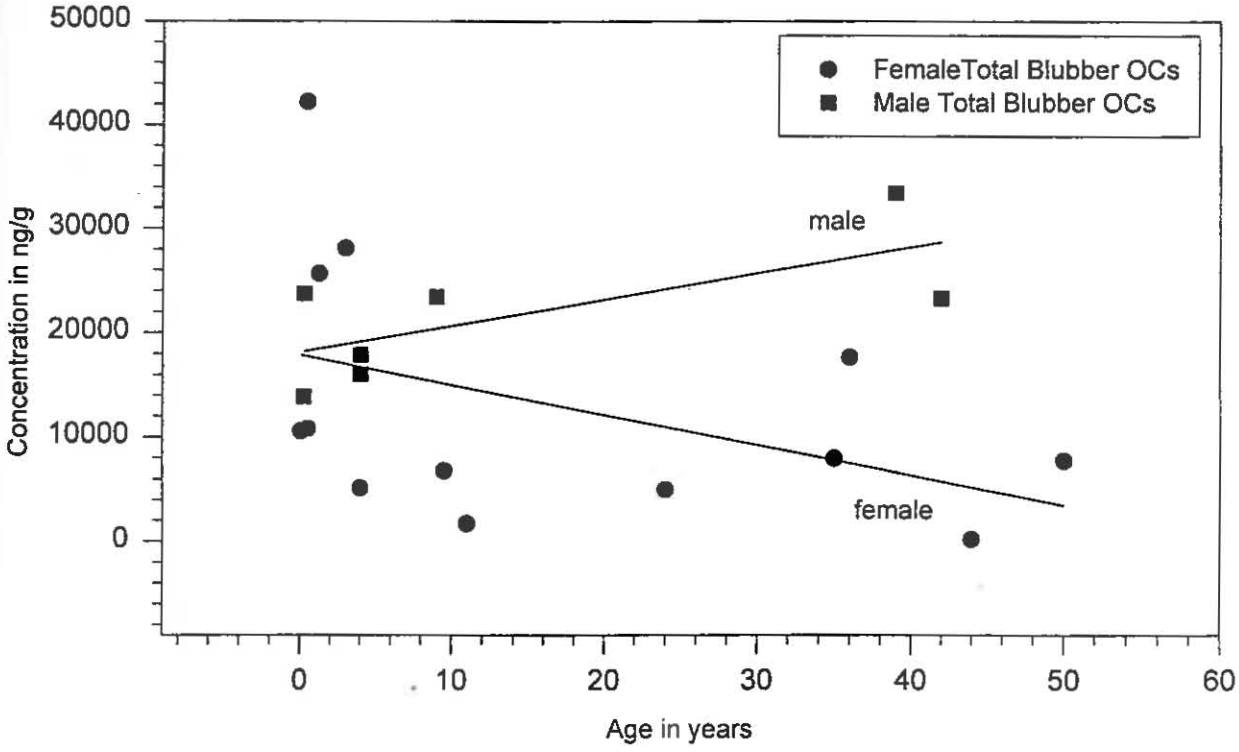


Figure 9: Concentration of Total Organochlorines in Blubber as a Function of Age in Male and Female Dolphins

Figure 10. This post-weaning decrease in blubber organochlorines, though not mentioned in the literature, makes a good deal of intuitive sense. As a calf begins feeding on "solid" food and decreasing intake of the mother's lipid-rich milk, metabolism and excretion may well begin to exceed organochlorine intake, especially if the calf is beginning to mobilize some of its own lipid stores through the energetic demands involved with increased time away from mother in play and in foraging. Even more interesting is the fact that this same decrease is not seen in the melon organochlorines. This is consistent with the concept of the melon as a more static tissue which is not readily mobilized.

It has been speculated that birth order could have a great bearing on the xenobiotic loading of calves, with the first-born calf of a female receiving a much higher burden than subsequent calves (Cockroft *et al.*, 1989). In this study, birth order was conclusively known for 6 animals, all calves, aged 3 weeks - 15 months. (Melons were only available for 5 of these animals). Although not statistically significant, when birth order is graphically plotted against organochlorine levels, a trend may be seen in organochlorines appearing to decrease with increasing birth order (Figure 11). While of interest, this requires caution as a larger number of animals within a closer age association is necessary to draw any conclusion from this analysis.

The first-born calf in this study, however, had 2-5 times the contaminant levels of any of the other birth orders, validating to some degree Fukushima and Kawai's estimation of the first-born receiving up to a 4-fold higher organochlorine burden than the subsequent calves of a female (see Cockroft *et al.*, 1989). In fact, this animal, at 5.3

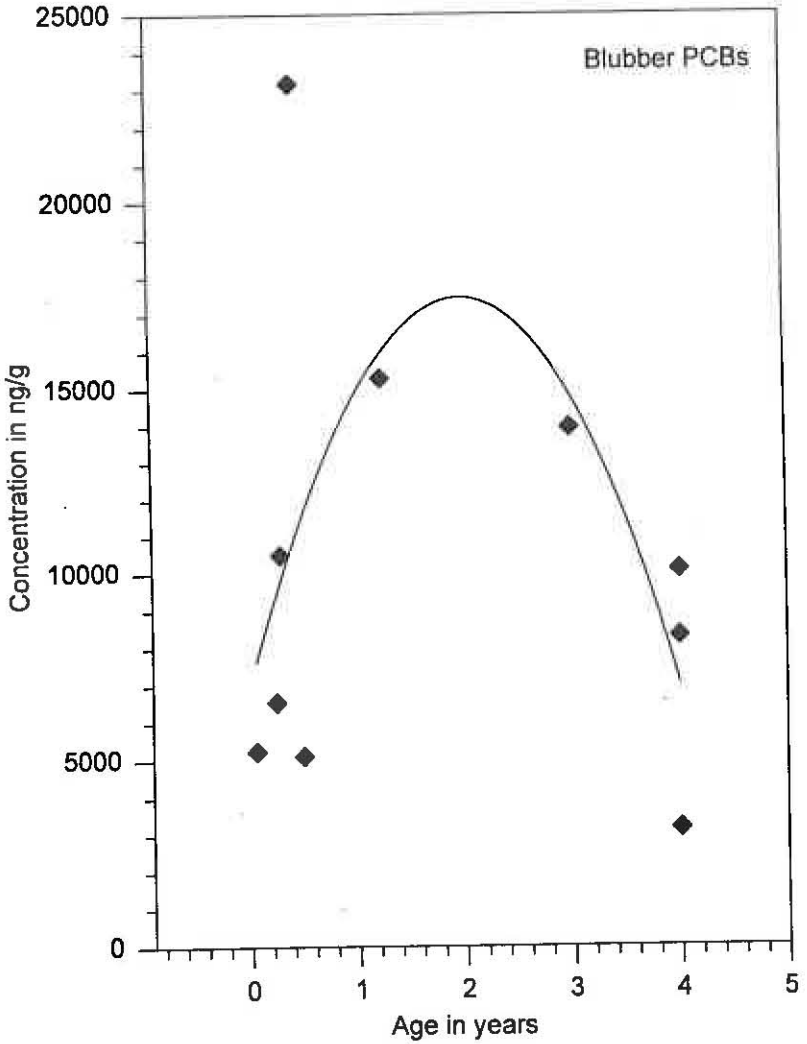


Figure 10: Second Degree Regression of PCB Concentrations in Blubber as a Function of Age in Young (0-4 year old) Dolphins

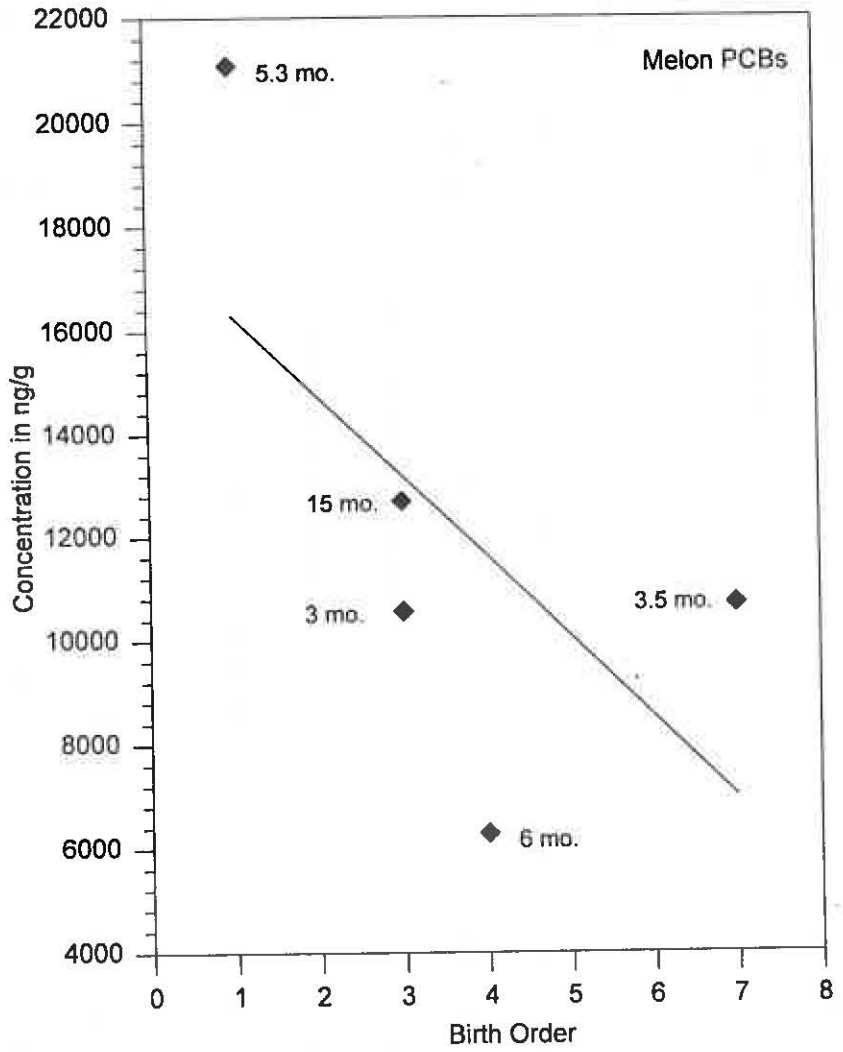


Figure 11: Concentrations of PCBs in Melon as a Function of Birth Order Order of Young (3 month - 15 month) Dolphins

months old, had the highest blubber ΣPCB, ΣDDT, HCB, and ΣPests of all animals in this study, and the second highest melon ΣPCB, ΣDDT, and ΣPests. Compared to the next closest calf in age, a fourth-born 6 month old female, the first-born had 5 times the blubber ΣPCB, 4 times the blubber ΣDDT, and between 2 - 3.5 times all of the other major organochlorine groups. Whether these concentrations are toxic or even harmful is not fully known as yet. Cockroft *et al.*, (1989), however, warn that the initial high dose and rapid, heavy transfer of these chlorinated (and immunosuppressive) compounds to dolphin neonates may constitute a greater risk than the actual concentrations suggest. Aguilar and Borrell (1994) agree, and state that the most critical period in the translocation process will be right around birth, as the flow rate of the umbilical circulation is greater, allowing more chemicals to cross the placenta. As well, they state that neutral lipids in foetal tissues tend to increase near term, facilitating the retention of organic compounds. This late prenatal and early postnatal exposure to chemicals is of pronounced significance as the development of the immune system is particularly susceptible to chemical interruption and damage at this time (Aguilar and Borrell, 1994*a*; Cogliano *et al.*, 1996; Miller, 1985).

A population of dolphins or other small cetaceans, therefore, could be impacted by this mechanism even in light of contaminant levels that are not comparatively high. This could also help to explain the extremely high mortality of first-born calves in the Sarasota Bay community. Another first-born calf was recovered by the Stranding Program and the DBRI in 1988, and although tissue samples were not available for analysis, the skull has been archived. Ruth DeLynn, an adjunct researcher at MML

studying early skull development in dolphins, found the skull from this two and a half month old to be microcephalic - abnormally small, with severe elongation and distortion, and premature fusion. This is reminiscent of the findings in the Baltic which correlated skull exotosis, skull bone lesions and other skull abnormalities in several seal species with high PCB and DDT levels (Olsson *et al.*, 1994; Reijnders, 1994). In time MML and the DBRI may be able add to their database on first-born calves, and substantiate this phenomenon.

The contaminant levels at which a population may be at risk for adverse health effects were postulated in 1984 by Wagemann and Muir, and remain the guidelines currently accepted by researchers. For cetaceans, the contaminant levels in blubber for PCBs and for ΣDDT are suggested as 50-200 μg/g wet weight (Wagemann and Muir, 1984). The ΣPCB levels found in the Sarasota community ranged from 0.07 μg/g to 23.2 μg/g wet weight in the blubber, and 2.62 μg/g to 26.9 μg/g in the melon. The ΣDDT ranged from 0.06 μg/g to 9.2 μg/g in blubber, and 1.48 μg/g to 10.3 μg/g in the melon. These concentrations, while below the suggested level of toxicological concern, should not be discounted. Subramanian *et al.* (1987) found testosterone levels significantly reduced in male Dall's porpoises with an increase in DDE and PCB levels at 11.0 μg/g and 9.02 μg/g (wet weight), respectively. Serious effects in beluga whales from the St. Lawrence River are proposed at the population's mean PCB levels of 76 μg/g in males and 37 μg/g in females (Muir *et al.*, 1990; Norstrom and Muir, 1994).

A comparison of organochlorine levels between the Sarasota Bay community and other bottlenose dolphins and small cetaceans is presented in Table 7. The numbers in

Table 7: Comparison of Organochlorine Levels in Sarasota Bay Dolphins with Other Small Cetaceans

Species	Location	Mortality Event Yr	Sex	PCBs	DDTs	HCHs	HCB	References
Wet Weight in ug/g (ppm)								
Bottlenose Dolphin	**E. Gulf of Mexico**		**M**	**11.5**	**5.2**	**0.18**	**0.46**	**Present study**
(Tursiops truncatus)			**F**	**6.3**	**2.7**	**0.11**	**0.22**	
	Gulf of Mexico	1990	M&F	31	20			Varanasi et al., 1992a
	S.E. Indian Ocean		M	20	14			Cockcroft et al., 1989
			F	9	5.9			
	Mediterranean	1992	M	562	187.6			Corsolini et al., 1995
			F	230	54			
Striped Dolphin	W. Mediterranean	1990	M	430	150			Kannan et al., 1993
(Stenella coeruleoalba)			F	94	22			
Harbor Porpoise	N. Atlantic		M&F	14.8	7.3	0.49	0.52	Becker et al., 1997
(Phocoena phocoena)								
	Black Sea		M	16	70	10	0.4	Tanabe et al., 1997
			F	12	50	7.2	0.4	
White-beaked Dolphin	Newfoundland		M	0.034	0.043	0.0008	0.001	Muir et al., 1988
(Lagenorhynchus albirostris)			F	0.022	0.028	0.0008	0.0009	
Pilot Whale	Newfoundland		M	0.009	0.012	0.0002	0.0003	Muir et al., 1988
(Globicephala melaena)			F	0.003	0.005	0.00008	0.0001	
	N. Atlantic		M&F	7.9	7.7	0.04	0.2	Becker et al., 1997
Lipid Weight in ug/g (ppm)								
Bottlenose Dolphin	**E. Gulf of Mexico**		**M**	**19.6**	**8.9**	**0.29**	**0.73**	**Present study**
(Tursiops truncatus)			**F**	**26.8**	**11.7**	**0.35**	**0.99**	
	W. Gulf of Mexico		M&F	36.1	15.3	0.1	0.51	Salata et al., 1995
	U.S. Atlantic Coast	1987/88	M	138.4	38.6		0.042	Kuehl et al., 1991
			F	62.4	7.5		0.035	
Common Dolphin	W. North Atlantic		M	36.5	14.4		0.015	Kuehl et al., 1991
(Delphinus delphis)								
Striped Dolphin	W. Mediterranean	1990	M	1300	480			Kannan et al., 1993
(Stenella coeruleoalba)			F	290	69			

this table are mean concentrations, and reflect various methods of reporting PCBs, necessitating caution in making direct comparisons. In some cases, the analyses were conducted in response to a large scale mortality event, either an epizootic or a mass stranding, and in these cases the date of the event is given. Animals involved in large scale mortality events exhibit quite high chlorinated hydrocarbon levels. In comparison with these values, the Sarasota community has a lower organochlorine load, and a higher load than whales from more pristine waters (*i.e.* Newfoundland). The values for the Sarasota Bay community are probably representative of northern hemisphere coastal marine mammals.

An accurate assessment of risk to the health of individuals or populations from xenobiotics should take into account not only the concentrations of these contaminants, but also the effects of the metabolites and reactive intermediaries of the compounds. Some chlordane, DDT and PCB compounds are metabolized to non-degradable compounds that are more toxic and persistent than the parent compounds, yet little research has been done on this factor. The toxicity of one hydroxylated PCB, (2′,4′,6′-trichloro-4-biphenylol), is greater not only than its precursor, but also more toxic than other PCBs tested (Norstrom and Muir, 1994; Matta *et al.*, 1997). The methylsulphone ($MeSO_2$) metabolites of PCBs and DDE are of particular concern as there are hundreds of possible congeners, and have been found to have a similar persistence to unmetabolized PCBs. Further, $MeSO_2$ - DDE has been found to be a potent adrenocortical toxin, and DDE yields a fairly high percentage of $MeSO_2$ metabolites (Norstrom and Muir, 1994).

Synergistic effects between toxic xenobiotics have been widely noted as well.

Borlakoglu and Haegele (1991) report on one study of the toxic effects of two individual PCB congeners that did not cause toxicity alone, but acted synergistically to produce chromosomal damage to lymphocytes when used in combination. Relatively non-toxic mixed-inducing PCBs have been shown to have a modulating action on the dioxin TCDD, enhancing its toxicity by 10-fold (McKinney *et al.*, 1985). Beyond the myriad of possible and troubling synergistic effects, some of the PCBs that were thought to be non-toxic are now proving to have serious detrimental effects, as in the ortho-substituted, non-dioxin-like PCBs that have now been shown to mediate the neurotoxicity of the PCBs (Maier *et al.*, 1994; Kodavanti *et al.*, 1996; Seegal, 1996). And, numerous non-organohalogenated compounds may be acting along with, or even synergistically with chlorinated compounds to suppress immune systems and cause reproductive dysfunction. Such is thought to be the case with the tributyltin compounds (TBTs) used widely as anti-fouling agents in marine paints. The TBTs are known immunosuppressors, exert acute toxic effects, and have been found in marine mammals in high concentrations in mass mortality events (Kannan *et al.*, 1997). The occurrence of these compounds, which are relatively biodegradable, in top-level predators has been suggested as the result of a reduced capacity of drug-metabolizing enzyme systems due to the co-occurrence of PCBs and DDTs (Iwata *et al.*, 1994).

A great number of synthetic compounds and by-products of industrial process have been demonstrated to have toxic effects on living organisms, and must be acknowledged when assessing a very small subset of compounds for toxic implications on a population. The bottlenose dolphins in the Tampa Bay to Charlotte Harbor corridor, which includes

Sarasota Bay, have evidenced other forms of anthropogenic effects. Routine necropsies of stranded dolphins have revealed a high incidence of anthracosis, better known as brown lung disease in humans, as well as liver abnormalities from high mercury levels (Rawson *et al.*, 1991, Rawson *et al.*, 1995). The Sarasota Bay population of bottlenose dolphin have concentrations of contaminants in their tissues that have been correlated with decreased lymphocyte response in this community (Lahvis *et al.*, 1995). While not apparently exerting acute affects, (with the possible exception of the first-born calves) these contaminants are nearing levels that may have caused chronic long-term effects in other small cetaceans, and could possibly exert immunosuppressive effects if an infectious agent is introduced into this community.

Immunological studies of the dolphins in this community reveal previous exposure to morbillivirus, though this exposure appears in the older animals and not in the younger animals. The discreteness of this population generally lessens opportunity for contact with more offshore species such as pilot whales, false killer whales (*Pseudorca crassidens*), and Fraser's dolphins (*Lagenodelphis hosei*), which associate with and sometimes strand with offshore bottlenose dolphins, facilitating viral transmission (Duignan *et al.*, 1996). The immune systems of the Sarasota community, therefore, do not appear to have been challenged in more recent years. The levels of the subset of organochlorines analyzed in this study, although in the "moderate" range, remain of concern and should continue to be monitored along with the other health evaluations routinely conducted on this population.

5.0 SUMMARY AND CONCLUSIONS

Analyses of xenobiotic compounds in marine mammals have been conducted since the discovery of their occurrence in this compartment of the environment in the late 1960s. As top trophic predators with high lipid mass and inefficient xenobiotic metabolizing systems, marine mammals are unique in their capacity to accumulate contaminants, most notably the halogenated compounds. Many of these polychlorinated and brominated substances are known to exert toxic effects on individuals and on populations. These effects include endocrine system disruption, neurotoxicity, reproductive failure and immunoincompetence, and have been implicated in the mortalities of marine mammals since the 1970s.

The majority of the studies conducted on small cetaceans have been without the benefit of demographic or reproductive history data on the individuals tested. This investigation is the first to be conducted on known individuals in a community that has been the subject of study for more than 27 years. As a result, this study has been able to demonstrate what has previously only been hypothesized concerning maternal transfer of chlorinated hydrocarbons to their young.

Tissue samples (blubber and melon) of seven male bottlenose dolphins and thirteen female bottlenose dolphins were analyzed for toxic PCB congeners, DDT and its metabolites, and a suite of other chlorinated pesticides. In agreement with many previous studies, the male dolphins were found to increase their organochlorine levels with age. The female dolphins were found to decrease their organochlorine levels through

gestational and/or lactational transfer to their calves. Although not statistically significant, these data are consistent with the fact that female bottlenose dolphin demonstrate a lengthening of interreproductive interval in their later lives as seen by an increase in organochlorine levels after the age of approximately 30 yrs.

The dependent calves (ages 0-4 yrs., previous to mother/calf separation) were found to have their highest levels of organochlorine loading initially, and until approximately 2 yrs., when the levels begin to show a decrease. Weaning in bottlenose dolphin calves occurs at around the age of two yrs., and is suggested as the reason for the decrease in the blubber organochlorine levels, as the calf terminates its lipid-rich food source. This decrease is not, however, seen in the melon tissue, supporting the premise of this as a tissue that is conserved and not utilized for energetic requirements. The first-born calf in this study had 2-5 times the levels of the organochlorines analyzed as the closest calf in age to it, which was a fourth-born calf. This is the first study to demonstrate this conclusively. These data also suggest that there may be a gradient in organochlorine loading with birth order, with the first calf receiving the largest loading, and a seventh-born the least initial loading, but many more similarly-aged calves are required to determine this conclusively.

The chlorinated hydrocarbon levels in the Sarasota Bay community of dolphins appears to be a moderate level of loading as compared to other small cetaceans. This level is not as high as is evidenced in animals involved in large scale mortality events, yet is higher than levels of organochlorines from animals in more pristine areas, and is ominously close to levels seen in recent mortality events in the western Gulf of Mexico.

While this community does not appear to be impaired by their current organochlorine loads, the extremely high mortality of first-born calves, and the skull deformities of the 1988 first-born calf may be an indication of impact from xenobiotics. To state that this community is unequivocally healthy and without effects from xenobiotics would be premature and incautious at this time. Further monitoring of this population is warranted.

6.0 LITERATURE CITED

Aguilar, A. (1985). Compartmentation and reliability of sampling procedures in organochlorine pollution surveys of cetaceans. *Residue Rev.*, 95:91-114.

Aguilar, A. (1987). Using organochlorine pollutants to discriminate marine mammal populations: A review and critique of the methods. *Mar. Mammal Sci.*, 3:242-262.

Aguilar, A. and A. Borrell. (1994*a*). Abnormally high polychlorinated levels in striped dolphins (*Stenella coeruleoalba)* affected by the 1990-1992 Mediterranean epizootic. *Sci. Tot. Environ.*, 154:237-247.

Aguilar, A. and A. Borrell. (1994*b*). Assessment of organochlorine pollutants in cetaceans by means of skin and hypodermic biopsies. In: *Non-Destructive Biomarkers in Vertebrates*, M.C. Fossi and C. Leonzio (Eds.). Lewis Publishers, Boca Raton, FL. pp. 245-267.

Archibeque-Engle, S.L., Tessari, J.D., Winn, D.T., Keefe, T.J., Nett, T.M. and T. Zheng. (1997). Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. *J. Toxicol. Environ. Health*, 52:285-293.

Atlas, E., Bidleman, T. and C.S Giam. (1986). Atmospheric transport of PCBs to the ocean. In: *PCBs and the Environment, vol. I*, J.S. Waid (Ed.). CRC Press Inc, Boca Raton, FL. pp. 79-97.

Bacon, C., Jarman, W. and D. Costa. (1992). Organochlorine and polychlorinated biphenyl levels in pinniped milk from the Arctic, the Antarctic, California and Australia. *Chemosphere*, 24:779-791.

Becker, P.R., Mackey, E.A., Demiralp, R., Schantz, M.M., Koster, B.J. and S.A. Wise. (1997). Concentrations of chlorinated hydrocarbons and trace elements in marine mammal tissues archived in the U.S. National Biomonitoring Specimen Bank. *Chemosphere*, 34:2067-2098.

Borlakoglu, J.T. and K.D. Haegele. (1991). Comparative aspects on the bioaccumulation, metabolism and toxicity with PCBs. *Comp. Biochem. Physiol.*, 100C:327-338.

Borrell, A., Bloch, D. and G. Desportes. (1995). Age trends and reproductive transfer of organochlorine compounds in long-finned pilot whales from the Faroe Islands. *Environ. Pollut.*, 88:283-292.

Cairnes, T., Doose, G., Froberg, J., Jacobson, R. and E. Siegmund. (1986). In: *PCBs and the Environment, vol. I*, J.S. Waid (Ed.). CRC Press Inc, Boca Raton, FL. pp 1-46.

Coburn, T., vom Saal, F. and A. Soto. (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect.*, 101:378-384.

Cockcroft, V.G., De Kock, A.C., Lord, D.A. and G.J.B. Ross. (1989). Organochlorines in bottlenose dolphins *Tursiops truncatus* from the east coast of South Africa. *S. Afr. J. Mar. Sci.*, 8:207-217.

Cogliano, V.J., Hiatt, G. and A. Den. (1996). Quantitative cancer assessment for vinyl chloride: Indications of early life sensitivity. *Toxicology*, 111:21-28.

Compaan, H. (1988). Environmental acceptability on the North Sea of incineration ship emissions: Needed future research. *Mar. Pollut. Bull.*, 19:11B:594-598.

Connell, D.W. and G.J. Miller. (1984). *Chemistry and Ecotoxicology of Pollution.* John Wiley and Sons, New York. pp. 251-275.

Corsolini, S., Focardi, S., Kannan, K., Tanabe, S., Borrell, A. and R. Tatsukawa. (1995). Congener profile and Toxicity Assessment of polychlorinated biphenyls in dolphins, sharks and tuna collected from Italian coastal waters. *Mar. Environ. Res.*, 40:33-53.

Cummings, A.M. and J.L. Metcalf. (1995). Effects of estrogen, progesterone, and methoxychlor on surgically induced endometriosis in rats. *Fundam. Appl. Toxicol.*, 27:287-290.

Dachs, J., Bayona, J.M. and J. Albaigés. (1997). Spatial distribution, vertical profiles and budget of organochlorine compounds in Western Mediterranean seawater. *Mar. Chem.*, 57:313-324.

Davis, J.W. (1993). An analysis of tissues for total PCB and planar PCB concentrations in marine mammals stranded along the Gulf of Mexico. [MSc thesis]. Galveston, (TX): Texas A&M University. 134 pp.

Dethlefsen, V., Söffker, K., Büther, H. and U. Damm. (1996). Organochlorine compounds in marine organisms from the international North Sea incineration area. *Arch. Fish. Mar. Res.*, 43(3):215-242.

DeLong, R.L., Gilmartin, W.G. and J.G. Simpson. (1973). Premature births in California sea lions: association with high organochlorine pollutant residue levels. *Science*, 181:1168-1170.

DeVito, M.J., Birnbaum, L.S., Farland, W.H. and T.A. Gasiewwicz. (1995). Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ. Health Perspect.*, 103(9):820-831.

De Swart, R.L., Ross, P.S., Vos, J.G. and A.D.M.E. Osterhaus. (1996). Impaired immunity in harbour seals (*Phoca vitulina*) fed environmentally contaminated herring. *The Veterin. Quarter.*, 18(3):s127-s128.

De Voogt, P., Wells, D.E., Reuthergårdh, L. and U.A.T. Brinkman. (1990). Biological activity, determination, and occurrence of planar, mono- and di-ortho PCBs. *Intern. J. Environ. Anal. Chem.*, 40:1-46.

Dorea, J.G., Granja, A.C.C. and M.L.L. Romera. (1997). Pregnancy-related changes in fat mass and total DDT in breast milk and maternal adipose tissue. *Ann. Nutr. Metab.*, 41:250-254.

Duignan, P.J., House, C., Odell, D.K., Wells, R.S., Hansen, L.J., Walsh, M.T., St. Aubin, D.J., Rima, B.K. and J. R. Geraci. (1996). Morbillivirus infection in bottlenose dolphins: Evidence for recurrent epizootics in the western Atlantic and Gulf of Mexico. *Mar. Mammal Sci.*, 12:499-515.

Duinker, J.C., Hillebrand, M.T.J., Zeinstra, T. and J.P. Boon. (1989). Individual chlorinated biphenyls and pesticides in tissues of some cetacean species from the North Sea and the Atlantic Ocean; tissue distribution and biotransformation. *Aquat. Mammals*, 15:95-124.

Duinker, J.C., Knap, A.H., Binkley, K.C., Van Dam, G.H., Darrel-Rew, A. and M.T.J. Hillebrand. (1988*a*). Method to represent the qualitative and quantitative characteristics of PCB mixtures. *Mar. Pollut. Bull.*, 19:74-79.

Duinker, J.C., Schultz, D.E. and G. Petrick. (1988*b*). Selection of chlorinated biphenyl congeners for analysis in environmental samples. *Mar. Pollut. Bull.*, 19:19-25.

Eganhouse, R.P. and R.W. Gossett. (1991). Sources and magnitude of bias associated with determination of polychlorinated biphenyls in environmental samples. *Anal. Chem.*, 63:2130-2137.

Eisler, R. (1986). Dioxin hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. 85(1.8). 37 pp.

Eisler, R. (1986). Polychlorinated biphenyl hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. 85(1.7). 72 pp.

Erickson, K.L., DiMolfetto-Landon, L., Wells, R.S., Reidarson, T., Stott, J.L. and D.A. Ferrick. (1995). Development of an interleukin-2 receptor assay and its use in evaluation of cellular immune responses in the bottlenose dolphin, *Tursiops truncatus. J. Wildl. Diseases,* 31: 142-149.

Erickson, M.D. (1986). *Analytical Chemistry of PCBs*. Butterworth Publishers, Stoneham, Mass. 499 pp.

Formica, S., Baron, J., Thibodeaux, L. and K. Balsaraj. (1988). PCB transport into lake sediments. Conceptual model and laboratory simulation. *Environ. Sci. Tech.*, 22:1435-1440.

Gagnon, M., Dodson, J., Comba, M. and K. Kaiser. (1990). Congener-specific analysis of the accumulation of polychlorinated biphenyls (PCBs) by aquatic organisms in the maximum turbidity zone of the St. Lawrence estuary, Quebec, Canada. *Sci. Tot. Environ.*, 98:739-759.

Geraci, J.R. (1989). Clinical investigations of the 1987-1988 mass mortality of bottlenose dolphins along the U.S. central and south Atlantic coast. Final report to NMFS and U.S. Navy, Office of Naval Research, and Marine Mammal Commission, Guelph, Ontario, Canada. 63 pp.

Geraci, J.R. and V.J. Lounsbury. (1993). *Marine Mammals Ashore: A Field Guide for Strandings*. Texas A&M Sea Grant Publication, Galveston, Texas. 305 pp.

Granby, K. and C.C. Kinze. (1991). Organochlorines in Danish and west Greenland harbour porpoises. *Mar. Pollut. Bull.*, 22:458-462.

Gregor, D.J. (1991). Organic micropollutants in seasonal snowcover and firn. In: *Seasonal Snowpacks: NATO ASI series, vol. G28*, T.D. Davies (Ed.). Springer-Verlag Publ., Berlin. pp. 324-358.

Gregor, D.J., Peters, A.J., Teixeira, C., Jones, N. and C. Spencer. (1995). The historical residue trend of PCBs in the Agassiz Ice Cap, Ellesmere Island, Canada. *Sci. Tot. Environ.*, 161:117-126.

Goldberg, E.D. (1995). Emerging problems in the coastal zone for the twenty-first century. *Mar. Pollut. Bull.*, 31:152-158.

Guitart, R., Guerrero, X., Silvestre, A.M., Gutiérrez, J.M. and R. Mateo. (1996). Organochlorine residues in tissues of striped dolphins affected by the 1990 Mediterranean epizootic: Relationships with fatty acid composition. *Arch. Environ. Contam. Toxicol.*, 30:79-83.

Hall, A.J., Law, R.J., Wells, D.E., Harwood, J., Ross, H.M., Kennedy, S., Allchin, C.R., Campbell, L.A. and P.P. Pomeroy. (1992). Organochlorine levels in common seals (*Phoca vitulina*) which were victims and survivors of the 1988 phocine distemper epizootic. *Sci. Tot. Environ.*, 115:145-162.

Hansen, L.J. (1992). Report on investigation of 1990 Gulf of Mexico bottlenose dolphin strandings. NMFS/SEFC Miami, Contribution MIA-92/93-21. Miami, FL. 219 pp.

Hardell, L., Van Bavel, B., Lindström, G., Fredrikson, M., Hagberg, H., Liljegren, G., Nordström, M. and B. Johansson. (1996*a*). Higher concentrations of specific polychlorinated biphenyl congeners in adipose tissue from non-Hodgkin's lymphoma patients compared with controls without a malignant disease. *Int. J. Oncol.*, 9:603-608.

Hardell, L., Liljegren, G., Lindström, G., Van Bavel, B., Broman, K., Fredrikson, M., Hagberg, H., Nordström, M. and B. Johansson. (1996*b*). Increased concentrations of chlordane in adipose tissue from non-Hodgkin's lymphoma patients compared with controls without a malignant disease. *Int. J. Oncol.*, 9:1139-1142.

Hühnerfuss, H., Pfaffenberger, B., Gehrcke, B., Karbe, L., König, W.A. and O. Landgraff. (1995). Stereochemical effects of PCBs in the marine environment: Seasonal variation of coplanar and atropisomeric PCBs in blue mussels (*Mytilus edulis* L.) of the German Bight. *Mar. Pollut. Bull.*, 30:332-340.

Hutzinger, O., Safe, S. and V. Zitko. (1983). *The Chemistry of PCB's*, Krieger Publishing Co., Malabar, FL. 269 pp.

Iwata, H., Tanabe, S., Miyazaki, N. and R. Tatsukawa. (1994). Detection of butyltin compound residues in the blubber of marine mammals. *Mar. Pollut. Bull.*, 28:607-612.

Iwata, H., Tanabe, S., Sakai, N. and R. Tatsukawa. (1993). Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean in their global transport and fate. *Environ. Sci. Technol.*, 27:1080-1098.

Jarman, W.M., Simon, M., Norstrom, R.J., Burns, S.A., Bacon, C.A., Simonelt, R.T. and R.W. Risebrough. (1992). Global distribution of tris(4-chlorophenyl) methanol in high trophic level birds and mammals. *Environ. Sci. Technol.*, 26:1770-1774.

Jones, K.C. (1988). Determination of polychlorinated biphenyls in human foodstuffs and tissues: Suggestions for a selective congener analytical approach. *Sci. Tot. Environ.*, 68:141-159.

Kannan, K., Senthilkumar, K., Loganathan, B.G., Takahashi, S., Odell, D.K. and S. Tanabe. (1997). Elevated accumulation of tributyltin and its breakdown products in bottlenose dolphin (*Tursiops truncatus*) found stranded along the U.S. Atlantic and Gulf coasts. *Environ. Sci. Technol.*, 13:296-301.

Kannan, K., Tanabe, S., Borrell, A., Aguilar, A., Focardi, S. and R. Tatsukawa. (1993). Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the western Mediterranean Sea. *Arch. Environ. Contam. Toxicol.*, 25:227-233.

Kannan, K., Tanabe, S., Ono, M. and R. Tatsukawa. (1989). Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: Increasing impact of non-*ortho* and mono-*ortho* coplanar polychlorinated biphenyls from land to ocean. *Arch. Environ. Contam. Toxicol.*, 18:850-857.

Kawai, S., Fukushima, M., Miyazaki, N. and R. Tatsukawa. (1988). Relationship between lipid composition and organochlorine levels in the tissues of striped dolphin. *Mar. Pollut. Bull.*, 19:129-133.

Kelce, W.R., Stone, C.R., Laws, S.C., Gray, L.E., Kemppainen, J.A. and E.M. Wilson. (1995). Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature*, 375:581-585.

Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S. and C. Lenghaus. (1994). A review of heavy metal and organochlorine levels in marine mammals in Australia. *Sci. Tot. Environ.*, 154:129-139.

Kodavanti, P.R.S., Ward, T.R., McKinney, J.D., Waller, C.L. and H. Tilson. (1996). Increased [^{3}H]Phorbol ester binding in rat cerebellar granule cells and inhibition of $^{45}Ca^{2+}$ sequestration in rat cerebellum by polychlorinated diphenyl ether congeners and analogs: Structure-activity realtionships. *Toxicol. Appl. Pharmacol.*, 138:251-261.

Kuehl, D.W. and R. Haebler. (1995). Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphins (*Tursiops truncatus*) collected during an unusual mortality event in the Gulf of Mexico, 1990. *Arch. Environ. Contam. Toxicol.*, 28:494-499.

Kuehl, D. W., Haebler, R. and C. Potter. (1991). Chemical residues in dolphins from the U.S. Atlantic coast including Atlantic bottlenose obtained during the 1987/88 mass mortality. *Chemosphere*, 22:1071-1084.

Kuiken, T., Bennett, P., Allchin, C., Kirkwood, J., Baker, J., Lockyer, C., Walton, M. and M. Shelrick. (1994). PCBs, cause of death and body condition in harbour porpoises (*Phocoena phocoena*) from British waters. *Aquat. Toxicol.*, 28:13-28.

Lahvis, G.P., Wells, R.S., Casper, D. and C.S. Via. (1993). In-vitro lymphocyte response of bottlenose dolphins (*Tursiops truncatus*): Mitogen-induced proliferation. *Mar. Environ. Res.*, 35:115-119.

Lahvis, G.P., Wells, R.S., Kuehl, D.W., Stewart, J.L., Rhinehart, H.L. and C.S. Via. (1995). Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ. Health Perspect.*, 103:67-72.

Law, R.J., Allchin, C.R. and R. Morris. (1995). Uptake of organochlorines (chlorobiphenyls, dieldrin; Total PCB & DDT) in bottlenose dolphins (*Tursiops truncatus*) from Cardigan Bay, West Wales. *Chemosphere*, 30:547-560.

Lockyer, C. and T. Waters. (1986). Weights and anatomical measurements of northeastern Atlantic fin (*Balaenoptera physalus*, Linnaeus) and sei (*B. borelais*, Lesson) whales. *Mar. Mamm. Sci.*, 2:169-185.

Loganathan, B. and K. Kannan. (1991). Time perspectives of organochlorine contamination in the global environment. *Mar. Pollut. Bull.*, 22:582-584.

Maier, W.E., Kodavanti, P.R.S., Harry, G.J. and H. Tilson. (1994). Sensitivity of adenosine triphosphatases in different brain regions to polychlorinated biphenyl congeners. *J. Appl. Toxicol.*, 14:225-229.

Marsili, L., Casini, C., Marini, L., Regoli, A. and S. Focardi. (1997). Age, growth and organochlorines (HCBs, DDTs and PCBs) in Mediterranean striped dolphins Stenella coeruleoalba stranded in 1988-1994 on the coasts of Italy. *Mar. Ecol. Prog. Ser.*, 151:273-282.

Martineau, D., De Guise, S., Fournier, M., Shugart, L., Girard, C., Lagacé, A. and P. Béland. (1994). Pathology and toxicology of beluga whales from the St. Lawrence Estuary, Quebec, Canada. Past, present and future. *Sci. Tot. Environ.*, 154:201-215.

Massé, R., Martineau, D., Tremblay, L. and P. Béland. (1986). Concentrations and chromatographic profile of DDT metabolites and polychlorobiphenyl (PCB) residues in stranded beluga whales (*Delphinapterus leucas*) from the St. Lawrence estuary, Canada. *Arch. Environ. Contam. Toxicol.*, 15:567-579.

Matta, M.B., Cairncross, C. and R.M. Kocan. (1997). Effect of a polychlorinated biphenyl metabolite on early life stage survival of two species of trout. *Bull. Environ. Contam. Toxicol.*, 59:146-151.

McKinney, J.D., Chae, K., McConnell, E.E. and L.S. Birnbaum. (1985). Structure-induction versus structure-toxicity relationships for polychlorinated biphenyls and related aromatic hydrocarbons. *Environ. Health Perspect.*, 60:57-68.

Metcalfe, C.D. (1994). Polychlorinated biphenyls. In: *Analysis of Contaminants in Edible Aquatic Resources*, J.W. Kiceniuk and S. Ray (Eds.). VCH Press, New York. pp. 305-333.

Miller, R.W. (1985). Congenital PCB poisoning: A reevaluation. *Environ. Health Perspect.*, 60:211-214.

Muir, D.C.G., Ford, C.A., Stewart, R.E.A., Smith, T.G., Addison, R.F., Zinck, M.E. and P. Béland. (1990). Organochlorine contaminants in belugas, *Delphinapterus leucas*, from Canadian waters. *Can. Bull. Fish. Aquat. Sci.*, 224:165-190.

Muir, D.C.G., Norstrom, R.J. and M. Simon. (1988*a*). Organochlorine contaminants in Arctic marine food chains: Accumulation of specific polychlorinated biphenyls and chlordane related compounds. *Environ. Sci. Technol.*, 22:1071-1079.

Muir, D.C.G., Wagemann, R., Grift, N.P., Norstrom, R.J., Simon, M. and J. Lien. (1988*b*). Organochlorine chemical and heavy metal contaminants in white-beaked dolphins (*Lagenorhynchus albirostris*) and pilot whales (*Globicephala melaena*) from the coast of Newfoundland, Canada. *Arch. Environ. Contam. Toxicol.*, 17:613-629.

Mullin, M., Pochini, C., McCrindle, S., Romkes, M., Safe, S. and L. Safe. (1984). High-resolution PCB analysis: Synthesis and chromatographic properties of all 209 PCB congeners. *Environ. Sci. Technol.*, 18:468-476.

Norstrom, R.J. and D.C.G. Muir. (1994). Chlorinated hydrocarbon contaminants in Arctic marine mammals. *Sci. Tot. Environ.*, 154:107-128.

Olsson, M., Karlsson, B. and E. Ahnland. (1994). Diseases and environmental contaminants in seals from the Baltic and Swedish west coast. *Sci. Tot. Environ.*, 154:217-227.

Reggiani, G. and R. Bruppacher. (1985). Symptoms, signs and findings in humans exposed to PCBs and their derivatives. *Environ. Health Perspect.*, 60:225-232.

Reijnders, P.J.H. (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature*, 324:456-457.

Reijnders, P.J.H. (1994). Toxicokinetics of chlorobiphenyls and associated physiological responses in marine mammals, with particular reference to their potential for ecotoxicological risk assessment. *Sci. Tot. Environ.*, 154:229-236.

Risebrough, R.W., Reich, P., Herman, S., Peakall, D. and M. Kirven. (1968). Polychlorinated biphenyls in the global ecosystem. *Nature*, 220:1098-1099.

Ronald, K., Frank, R.J., Dougan, J.L., Frank, R. and H.E. Braun. (1984). Pollutants in harp seals (*Phoca groenlandica*). I. Organochlorines. *Sci. Tot. Environ.*, 38:133-152.

Safe, S. (1984). Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): Biochemistry, toxicology, mechanism of action. *CRC Crit. Rev. Toxicol.*, 13:319-395.

Safe, S. (1990). Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Toxicology*, 21:51-88.

Safe, S., Bandiera, S., Sawyer, T., Robertson, L., Safe, L., Parkinson, A., Thomas, P.E., Ryan, D.E., Reik, L.M., Levin, W., Denomme, M.A. and T. Fujita. (1985). PCBs: Structure-function relationships and mechanism of action. *Environ. Health Perspect.*, 60:47-56.

Salata, G. G., Wade, T.L., Sericano, J.L., Davis, J.W. and J.M. Brooks. (1995). Analysis of Gulf of Mexico bottlenose dolphins for organochlorine pesticides and PCBs. *Environ. Pollut.*, 88:167-175.

Seegal, R.F. (1996). Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *CRC Crit. Rev. Toxicol.*, 26(6):709-737.

Segars, H. (1987). Dolphin pox: ongoing investigation reveals east coast dolphins dying of viral infection. *Underwater U.S.A.*, 4:5.

Sericano, J., El-Husseini, A. and T. Wade. (1991). Isolation of planar polychlorinated biphenyls by carbon column chromotography. *Chemosphere*, 23:915-924.

Scott, G.P., Hansen, L.J. and D.M. Burn. (1988). Preliminary report on: status of bottlenose dolphin stocks in the US Gulf of Mexico and US Atlantic Ocean. NOAA/NMFS/SEFC/Miami Laboratory Coastal Fishery Resources Division Contribution CRD-87/88-23. 11 pp.

Shan, T.-H., Hopple, J.A. and G.D. Foster. (1994). Alternative tissue analysis method developed for organochlorine contaminants in aquatic organisms. *Bull. Environ. Contam. Toxicol.*, 53:382-389.

Sherblom, P.M. and R.P. Eganhouse. (1991). Bioaccumulation of molecular markers from municipal waste by *Mytilus edulis*. In: *Organic Substances and Sediments in Water, vol. 3: Biological*, R.A. Baker (Ed.). Lewis Publishers, Boca Raton, FL. pp.139-158.

Subramanian, A.N., Tanabe, S., Tatsukawa, R., Saito, S. and N. Miyazaki. (1987). Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of the northwestern North Pacific. *Mar. Pollut. Bull.*, 18:643-646.

Tanabe, S. (1988). PCB problems in the future: Foresight from current knowledge. *Environ. Pollut.*, 50:5-28.

Tanabe, S., Iwata, H. and R. Tatsukawa. (1994). Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Sci. Tot. Environ.*, 154:163-177.

Tanabe, S., Madhusree, B., Öztürk, A.A., Tatsukawa, R., Miyazaki, N., Özdamar, E., Aral, O., Samsun, O. and B. Öztürk. (1997). Persistent organochlorine residues in harbour porpoise (*Phocoena phocoena*) from the Black Sea. *Mar. Pollut. Bull.*, 34:338-347.

Tanabe, S., Mori, T., Tatsukawa, R. and N. Miyazaki. (1983). Global pollution of marine mammals by PCBs, DDTs, and HCHs (BHCs). *Chemosphere*, 12:1269-1275.

Tanabe, S., Watanabe, S., Kan, H. and R. Tatsukawa. (1988). Capacity and mode of PCB metabolism in small cetaceans. *Mar. Mamm. Sci.*, 4:103-124.

Tanabe, S. and R. Tatsukawa. (1991). Persistent organochlorines in marine mammals. In: *Organic Contaminants in the Environment: Environmental Pathways and Effects*, K.C. Jones (Ed.). Elsevier Applied Sciences, New York. pp. 275-289.

Tanabe, S. and R. Tatsukawa. (1986). Distribution, behavior, and load of PCBs in the oceans. In: *PCBs and the Environment, vol. I*, J.S. Waid (Ed.). CRC Press Inc., Boca Raton, FL. pp. 143-161.

Tatsukawa, R. and S. Tanabe. (1990). Fate and bioaccumulation of persistent organochlorine compounds in the marine environment. In: *Oceanic Processes in Marine Pollution*, D. Baumgartner and I. Duedall (Eds.). Kreiger Publishing Co., Malabar, FL. pp. 39-52.

Teichman, J., Bevenue, A. and J.W. Hylin. (1978). Separation of polychlorinated biphenyls from chlorinated pesticides in sediment and oyster samples for analysis by gas chromatography. *J. Chromatogr.*, 151:155-161.

Tilson, H.A. and P.R. Kodavanti. (1997). Neurochemical effects of polychlorinated biphenyls: An overview and identification of research needs. *Neurotoxicology*, 18(3):727-744.

Tryphonas, H. (1994). Immunotoxicity of polychlorinated biphenyls: Present status and future considerations. *Exp. Clin. Immunogenet.*, 11:149-162.

Varanasi, U., Stein, J.E., Reichert, W.L., Tilbury, K.L., Krahn, M.M. and S-L Chan. (1992*a*). Chlorinated and aromatic hydrocarbons in bottom sediments, fish and marine mammals in U.S. coastal waters: Laboratory and field studies of metabolism and accumulation. In: *Persistent Pollutants in Marine Ecosystems*, C.H. Walker and D.R. Livingstone (Eds.). Pergamon Press, Oxford, England. pp. 83-115.

Varanasi, U., Tilbury, K., Brown, D., Krahn, M., Wigren, C., Clark, R. and S-L Chan. (1992*b*). Chemical contaminants in bottlenose dolphins stranded along the Gulf of Mexico during 1990. In: Report on investigation of 1990 Gulf of Mexico bottlenose dolphin strandings. NMFS/SEFC Miami, Contribution MIA-92/93-21. Miami, FL. pp. 57-86.

Van den Brink, N.W. (1997). Directed transport of volatile organochlorine pollutants to polar regions: The effect on the contamination pattern of Antarctic seabirds. *Sci. Tot. Environ.*, 198:43-50.

Vedder, J.M. (1996). Levels of organochlorine contaminants in milk relative to the health of bottlenose dolphins (*Tursiops truncatus*) from Sarasota, Florida. [MSc thesis]. Santa Cruz, (CA): University of California, Santa Cruz. 35 pp.

Voldner, E.C. and Y. Li. (1995). Global usage of selected persistent organochlorines. *Sci. Tot. Environ.*, 161:201-210.

Wagemann, R. and D.C.G. Muir (1984). Concentrations of heavy metals and organochlorines in marine mammals of northern waters: Overview and evaluation. *Can. Tech. Rep. Fish. Aquat. Sci.*, 1279: v+97 pp.

Wells, Randall. Dolphin Biology Research Institute, Chicago Zoological Society. Mote Marine Laboratory, Sarasota, Florida.

Wells, R.S. (1991). The role of long-term study in understanding the social structure of a bottlenose dolphin community. In: *Dolphin Societies: Discoveries and Puzzles*, K. Pryor and K.S. Norris (Eds.). Univ. of Calif. Press, Berkeley, CA. pp. 198-225.

Wells, R.S., Scott, M.D. and A.B. Irvine. (1987). The social structure of free-ranging bottlenose dolphins. In: *Current Mammology, Vol. 1*, H.H. Genoways (Ed.). Plenum Press, New York and London. pp. 247-305.

Zakharov, V.M. and A.V. Yablokov. (1990). Skull asymmetry in the Baltic grey seal: Effects of environmental pollution. *Ambio*, 19:266-269.

Zook, D.R. and C. Rappe. (1994). Environmental sources, distribution, and fate of polychlorinated dibenzodioxins, dibenzofurans, and related organochlorines. In: *Dioxins and Health*, A. Schecter (Ed.). Plenum Press, New York. pp. 79-113.

Appendix A

Blubber Data in ng/g Wet Wt.

Animal ID #	9012	9104	9108	9115	9118	9212	9215
Lipid Wt%	49.65	57.6	42.7	41.6	65.5	75.4	69.4
PCBs:							
pcb-28	19.91	104.01	194.31	30.06	91.46	89.35	48.45
pcb-52	917.82	1332.55	500.24	50.37	1778.43	156.41	593.78
pcb-81	686.42	456.96	236.91	37.80	870.57	106.22	516.74
pcb-101	272.91	282.98	344.84	51.58	577.40	207.66	324.77
pcb-77	143.55	20.57	50.63	1.45	16.61	31.72	76.62
pcb-123	432.71	46.79	35.45	nd	132.66	40.16	551.26
pcb-118	287.03	530.07	168.55	20.75	909.83	49.84	683.19
pcb-114	3367.23	1402.28	480.00	131.98	2200.36	475.97	1322.31
pcb-126	4073.43	1235.53	519.99	155.22	2342.03	547.08	1938.46
pcb-105	256.43	188.97	67.36	0.21	329.21	45.12	262.90
pcb-138	2184.29	1098.50	328.75	151.82	1755.05	278.92	1370.61
pcb-158	267	110.19	31.84	17.51	297.87	12.72	196.69
pcb-153	393.27	34.09	24.03	nd	102.61	49.24	214.76
pcb-166	40.44	5.68	3.17	0.82	21.83	3.43	16.24
pcb-128	513.26	201.01	36.38	8.98	465.58	46.15	208.17
pcb-167	104.27	104.67	2.00	75.76	131.51	25.87	99.70
pcb-156	36.86	90.82	18.77	8.72	178.53	25.90	98.73
pcb-157	106.04	33.47	8.88	2.99	57.10	6.15	37.15
pcb-180	2132.94	515.26	254.88	196.27	1194.33	261.06	818.90
pcb-169	85.82	300.58	0.81	44.11	4.80	nd	3.89
pcb-170	981.41	188.24	84.44	84.77	454.72	71.13	681.23
pcb-189	28.83	9.06	2.12	nd	26.49	3.54	16.94
Sum PCBs	17331.87	8292.28	3429.85	1071.17	13938.99	2533.65	10081.49
Pesticides:							
a-bhc	26.62	69.67	39.05	2.58	61.06	38.07	18.68
hcb	253.66	502.45	204.40	43.09	705.06	101.87	152.40
b-bhc	19.61	29.86	10.35	2.97	37.00	8.54	8.96
lind	47.53	109.55	63.55	nd	145.90	45.88	48.29
d-bhc	nd	4.58	0.85	nd	2.86	3.55	0.00
hept	38.83	45.64	38.95	nd	62.82	23.50	22.71
aldr	20.99	27.73	11.18	nd	19.69	16.60	16.95
hept e	873.3	498.72	191.35	11.14	686.86	66.95	218.00
y-chlor	66.47	50.03	22.92	71.87	87.14	27.68	37.05
op dde	776.55	328.11	171.90	14.18	666.60	78.74	291.06
endo I	146.81	16.92	9.71	nd	29.27	5.06	5.70
a-chlor	701.18	647.94	266.78	49.07	1626.67	150.71	697.88
dield	3465.91	1121.19	1036.56	9.82	2821.75	594.13	851.84
pp dde	3680.55	1889.97	1546.81	290.45	2864.80	615.97	2175.50
op ddd	532.94	115.49	84.77	4.03	207.89	25.08	135.79
endr	719.7	374.17	96.27	30.37	361.23	63.68	63.52
endo II	925.24	370.67	255.82	33.38	684.47	104.16	448.47
pp ddd	1310.1	765.67	281.22	37.44	1266.61	148.16	378.91
op ddt	898.31	396.12	3.77	1.66	520.74	200.07	1883.23
endr ald	568.6	28.03	3.54	nd	57.38	7.14	49.69
endo ss	736.35	192.73	95.89	8.64	416.40	61.19	163.93
pp ddt	305.77	164.10	85.82	23.18	794.36	60.09	133.73
T/ DDTs	7504.21	3659.45	2174.28	370.94	6321.01	1128.10	4998.21
T/ Cyclos	4775.20	1551.12	1147.56	40.58	3260.05	681.55	982.00
T/ HCHs	93.76	213.66	113.80	5.56	246.83	96.04	75.93
T/ Chlors	1679.78	1242.32	519.99	132.08	2463.50	268.83	975.64
T/ Endos	1808.40	580.32	361.42	42.02	1130.13	170.41	618.10
T/ Pests	16115.01	7749.33	4521.44	634.25	14126.59	2446.79	7802.28

Blubber Data in ng/g Wet Wt.

Animal ID #	9221	9224	9225	9226	9308	9309	9314
Lipid Wt%	1.55	76.95	55.95	46.15	78.7	64.65	75
PCBs:							
pcb-28	7.70	30.74	49.69	90.72	147.99	381.89	88.91
pcb-52	356.07	3985.10	106.83	1781.67	852.73	593.83	1182.41
pcb-81	57.23	1903.57	36.52	351.14	308.24	280.28	882.75
pcb-101	118.88	1259.70	97.21	470.76	534.69	380.29	772.31
pcb-77	21.16	51.80	5.49	30.38	35.88	63.82	243.90
pcb-123	59.58	216.49	89.15	490.52	18.51	195.08	526.13
pcb-118	70.36	721.79	52.36	373.86	389.16	236.76	946.43
pcb-114	508.63	2686.72	389.68	1857.65	1614.66	460.01	2245.94
pcb-126	505.06	4060.84	2040.82	1350.16	669.27	1503.04	2290.84
pcb-105	79.78	478.82	48.41	136.19	146.55	71.83	263.01
pcb-138	468.35	3248.30	352.65	2423.79	805.26	595.87	2196.25
pcb-158	46.23	487.63	35.88	203.80	52.13	44.69	161.33
pcb-153	12.84	339.06	nd	204.74	18.26	30.42	374.35
pcb-166	3.22	36.94	8.13	12.99	2.37	5.14	18.24
pcb-128	99.80	700.80	60.37	268.71	112.56	103.53	324.63
pcb-167	42.96	177.53	82.04	191.07	86.88	22.50	173.72
pcb-156	25.20	253.95	29.13	65.11	50.67	10.31	146.77
pcb-157	7.77	83.86	13.34	44.93	23.52	7.37	69.86
pcb-180	518.47	1809.58	486.86	1461.93	438.59	179.35	1780.70
pcb-169	nd	8.05	19.80	nd	1.28	nd	nd
pcb-170	133.84	586.80	337.00	574.82	240.31	82.48	557.32
pcb-189	6.25	43.29	14.56	17.99	5.89	nd	42.06
Sum PCBs	3149.39	23171.36	4355.90	12402.91	6555.40	5248.51	15287.67
Pesticides:							
a-bhc	5.82	58.23	10.92	26.70	87.80	41.90	26.33
hcb	45.45	954.39	14.23	273.37	664.00	218.89	272.91
b-bhc	4.56	73.76	2.04	16.05	20.69	10.37	17.68
lind	6.77	184.16	19.10	61.68	193.72	64.91	105.16
d-bhc	1.46	1.89	3.12	4.03	3.07	3.96	0.00
hept	1.62	199.10	19.26	91.80	93.44	26.25	23.88
aldr	5.44	84.28	7.50	21.34	34.87	20.48	30.80
hept e	52.29	906.50	25.90	405.88	342.91	160.35	401.32
y-chlor	11.23	98.42	31.68	54.80	113.04	20.51	158.44
op dde	120.88	862.32	58.50	359.69	336.60	15.93	1141.09
endo I	0.57	45.19	1.51	15.63	23.60	13.66	36.99
a-chlor	109.71	993.65	104.71	501.55	1028.44	315.23	1265.20
dield	104.45	3560.86	581.15	2178.15	900.14	2004.46	1717.24
pp dde	945.75	4939.87	939.35	4221.01	1509.25	1236.37	2426.74
op ddd	23.46	332.02	27.27	114.61	87.69	87.68	263.64
endr	85.74	757.51	39.71	487.31	194.93	120.16	444.94
endo II	121.15	923.82	58.18	393.21	242.98	121.17	358.36
pp ddd	180.34	2626.17	131.97	884.30	598.68	247.76	1059.19
op ddt	1.55	70.71	196.74	26.83	482.16	464.23	34.74
endr ald	22.41	412.93	12.66	77.53	24.16	55.08	155.55
endo ss	44.22	535.93	31.81	479.74	153.21	48.06	229.44
pp ddt	94.16	411.87	121.37	307.04	192.94	4.76	182.51
T/ DDTs	1366.15	9242.95	1475.20	5913.48	3207.32	2056.74	5107.92
T/ Cyclos	218.03	4815.58	641.02	2764.34	1154.09	2200.18	2348.53
T/ HCHs	18.62	318.05	35.17	108.46	305.28	121.14	149.17
T/ Chlors	174.84	2197.68	181.55	1054.05	1577.83	522.33	1848.84
T/ Endos	165.94	1504.94	91.51	888.58	419.78	182.89	624.79
T/ Pests	1989.04	19033.59	2438.68	11002.29	7328.30	5302.17	10352.15

Blubber Data in ng/g Wet Wt.

Animal ID #	9401	9417	9509	9514	9621	9625
Lipid Wt%	53.2	65	62.2	53.25	64.25	0.75
PCBs:						
pcb-28	122.75	253.43	53.97	71.86	261.66	3.73
pcb-52	138.24	665.42	957.91	822.38	1990.64	13.06
pcb-81	134.71	212.20	573.09	328.76	713.44	2.23
pcb-101	261.48	583.44	259.83	376.65	861.85	1.66
pcb-77	14.70	111.14	127.17	80.41	182.43	nd
pcb-123	21.24	40.77	490.14	80.33	238.88	0.76
pcb-118	248.34	244.56	340.35	373.51	701.50	nd
pcb-114	681.99	722.85	1580.28	1364.18	1318.86	10.05
pcb-126	388.95	772.46	2377.78	1684.07	1712.58	19.72
pcb-105	28.45	111.18	400.85	217.08	198.21	nd
pcb-138	504.13	554.78	2325.96	1453.02	814.55	5.23
pcb-158	13.13	38.18	209.96	119.54	83.60	nd
pcb-153	9.02	47.01	479.20	141.87	nd	nd
pcb-166	2.48	10.13	61.99	13.51	14.44	nd
pcb-128	29.28	79.70	370.46	311.82	199.70	nd
pcb-167	23.77	40.06	63.90	118.98	72.58	nd
pcb-156	19.69	47.57	23.35	63.51	63.05	nd
pcb-157	7.13	15.62	83.49	25.93	30.71	nd
pcb-180	258.11	444.86	1072.37	1461.97	738.56	6.92
pcb-169	13.11	nd	45.47	11.85	nd	10.07
pcb-170	45.10	113.04	645.70	273.36	281.65	0.35
pcb-189	3.87	4.06	18.83	13.49	10.26	nd
Sum PCBs	2969.66	5112.49	12562.06	9408.09	10489.14	73.77
Pesticides:						
a-bhc	35.37	66.14	4.66	6.41	94.25	2.78
hcb	114.03	445.26	176.23	169.45	940.60	38.17
b-bhc	4.16	8.71	5.36	4.99	31.81	2.12
lind	65.25	188.43	26.93	28.03	241.12	nd
d-bhc	5.96	1.04	nd	0.81	1.43	3.28
hept	18.21	1.56	nd	3.41	12.22	nd
aldr	10.13	21.96	24.82	8.48	59.84	1.41
hept e	107.51	180.69	229.41	170.92	594.78	nd
y-chlor	40.54	83.14	67.60	33.47	114.77	7.56
op dde	196.58	615.31	587.71	501.71	660.11	1.43
endo I	nd	10.13	16.48	nd	8.08	nd
a-chlor	305.16	416.27	725.58	520.68	1264.98	3.43
dield	867.93	1338.12	2399.73	3044.89	4061.48	10.58
pp dde	1099.24	1119.12	3501.73	6.00	2254.56	46.45
op ddd	46.39	106.71	214.57	124.12	214.05	nd
endr	64.10	57.26	571.29	72.72	260.14	nd
endo II	140.31	257.24	419.65	323.52	948.83	6.33
pp ddd	328.72	490.50	773.67	713.48	964.83	1.92
op ddt	1172.67	nd	274.58	30.97	42.45	nd
endr ald	nd	23.95	221.87	180.26	34.20	nd
endo ss	84.53	89.66	332.39	153.71	215.05	nd
pp ddt	63.70	168.77	180.56	282.73	219.92	5.10
T/ DDTs	2907.30	2500.42	5532.82	3553.90	4355.92	54.91
T/ Cyclos	942.16	1441.29	3217.72	3306.35	4415.66	11.98
T/ HCHs	110.74	264.32	36.94	40.23	368.61	8.18
T/ Chlors	471.43	681.65	1022.59	728.48	1986.75	11.00
T/ Endos	224.85	357.03	768.52	477.24	1171.96	6.33
T/ Pests	4770.50	5689.97	10754.81	8275.66	13239.50	130.58

Appendix B

Melon Data in ng/g Wet Wt.

Animal ID #	9012	9104	9108	9115	9118	9212	9215
Lipid Wt%		86.80		89.55	74.6	70.25	79.2
PCBs:							
pcb-28		186.77		36.81	65.63	110.80	54.22
pcb-52		2272.10		92.70	2758.83	195.92	723.30
pcb-81		1099.23		41.12	1256.90	90.34	263.24
pcb-101		1132.93		138.05	1060.26	231.00	719.21
pcb-77		170.75		51.74	65.64	148.41	nd
pcb-123		331.57		201.02	34.77	45.66	978.44
pcb-118		1068.06		174.18	588.85	94.23	393.75
pcb-114		1913.28		987.14	2710.21	448.07	1572.39
pcb-126		2077.49		3382.11	2662.70	351.56	1928.26
pcb-105		332.41		55.50	192.54	30.90	276.27
pcb-138		1709.22		2266.16	1716.14	389.29	1480.03
pcb-158		182.38		92.58	194.96	24.89	104.44
pcb-153		189.16		107.71	7.23	39.95	nd
pcb-166		27.23		131.62	32.04	3.16	6.81
pcb-128		447.32		217.75	302.94	34.27	382.67
pcb-167		194.66		93.58	201.42	105.13	125.38
pcb-156		183.49		58.63	91.91	29.99	122.18
pcb-157		64.00		30.04	46.08	8.36	25.47
pcb-180		1197.62		1794.74	969.67	163.93	1095.61
pcb-169		nd		2.96	1.53	nd	nd
pcb-170		328.77		714.51	352.64	65.85	470.39
pcb-189		19.41		23.26	12.15	3.22	16.67
Sum PCBs		15127.87		10693.92	15325.05	2614.94	10738.71
Pesticides:							
a-bhc		93.18		13.67	94.62	18.16	33.04
hcb		908.16		19.99	610.54	109.10	345.42
b-bhc		68.87		3.69	86.38	7.46	45.46
lind		168.17		21.17	231.88	50.09	73.30
d-bhc		0.36		6.86	0.89	2.03	nd
hept		64.99		nd	82.32	27.73	24.77
aldr		46.42		13.11	42.20	6.10	20.81
hept e		611.00		31.40	719.85	105.18	330.13
y-chlor		117.89		44.34	146.47	11.50	59.68
op dde		1468.71		190.47	1334.98	60.89	660.90
endo I		12.82		nd	70.66	1.66	68.78
a-chlor		1771.06		386.89	2325.31	141.98	885.10
dield		3089.08		641.48	2472.67	316.82	133.89
pp dde		4007.91		3381.29	3747.85	896.14	1923.64
op ddd		420.68		42.59	315.09	30.34	162.58
endr		353.56		123.09	392.18	102.46	332.61
endo II		1738.39		297.93	921.54	81.37	808.72
pp ddd		1386.94		175.32	1487.63	126.49	496.29
op ddt		880.29		20.91	73.85	320.22	1286.93
endr ald		130.47		29.71	147.08	11.81	58.60
endo ss		433.15		143.09	763.01	22.43	167.06
pp ddt		477.09		214.48	811.51	47.10	342.98
T/ DDTs		8641.60		4025.06	7770.92	1481.17	4873.33
T/ Cyclos		3619.52		807.39	3054.13	437.19	545.90
T/ HCHs		330.58		45.38	413.76	77.74	151.79
T/ Chlors		2564.95		462.63	3273.95	286.39	1299.68
T/ Endos		2184.35		441.03	1755.21	105.46	1044.57
T/ Pests		18249.16		5801.48	16878.51	2497.06	8260.68

Melon Data in ng/g Wet Wt.

Animal ID #	9221	9224	9225	9226	9308	9309	9314
Lipid Wt%	82.55	83.55	86.55	81.2	83.5		87.4
PCBs:							
pcb-28	182.02	119.39	80.46	140.22	150.41		68.65
pcb-52	4758.98	4517.58	229.55	2786.81	1363.44		1234.19
pcb-81	2118.00	686.57	87.40	1068.60	405.42		594.35
pcb-101	1267.22	639.39	215.31	904.78	892.32		795.97
pcb-77	129.26	98.83	55.31	85.28	102.73		41.06
pcb-123	330.96	413.02	37.89	147.63	650.03		89.71
pcb-118	1506.16	497.15	194.67	630.41	596.31		538.93
pcb-114	3541.05	2154.73	1039.06	2810.19	1823.28		1451.92
pcb-126	3252.43	4588.51	2425.89	3236.39	2131.44		2573.31
pcb-105	1175.02	647.86	155.51	219.28	226.97		326.80
pcb-138	3611.73	3190.13	1017.60	1883.74	1217.76		1974.25
pcb-158	477.70	364.21	113.29	486.03	102.42		130.62
pcb-153	74.72	77.52	nd	174.80	42.26		159.41
pcb-166	82.62	26.12	8.65	44.36	8.55		46.71
pcb-128	1139.14	745.92	194.07	486.60	116.39		324.12
pcb-167	247.20	187.57	116.92	195.17	91.18		123.09
pcb-156	199.14	173.82	85.57	100.81	56.77		81.25
pcb-157	70.09	56.13	40.42	63.42	12.95		62.48
pcb-180	2086.91	1162.22	775.73	1941.13	396.17		1632.92
pcb-169	nd	7.26	0.47	nd	3.91		65.85
pcb-170	604.82	704.37	433.60	307.44	168.34		358.86
pcb-189	32.71	29.36	15.30	29.05	4.63		18.77
Sum PCBs	26887.88	21087.67	7322.66	17742.14	10563.69		12693.22
Pesticides:							
a-bhc	94.64	82.85	27.12	40.61	93.87		5.98
hcb	1952.31	1378.29	42.64	387.13	774.73		107.77
b-bhc	164.28	134.21	5.43	117.56	33.74		22.72
lind	307.74	273.09	36.74	112.08	198.33		48.82
d-bhc	2.28	3.48	nd	1.54	2.43		nd
hept	202.37	249.64	11.33	78.45	nd		23.81
aldr	48.53	103.67	10.21	76.94	28.40		34.12
hept e	1208.97	645.22	44.58	807.57	260.62		367.92
y-chlor	226.01	214.69	62.38	105.35	76.76		92.48
op dde	1624.78	2188.95	409.55	456.83	813.68		583.03
endo I	88.66	104.60	5.17	56.31	32.65		31.01
a-chlor	2120.93	1342.15	523.37	1057.67	391.94		908.22
dield	7890.91	16.77	316.66	884.57	549.28		909.41
pp dde	3404.48	3775.08	2827.02	2787.80	1758.92		3842.33
op ddd	478.19	523.25	70.23	350.19	118.17		82.80
endr	719.64	896.01	316.59	708.06	423.41		386.07
endo II	2519.69	2175.28	335.95	801.48	375.86		366.05
pp ddd	2667.47	1991.29	454.94	1565.05	862.71		841.44
op ddt	736.90	533.67	nd	39.33	115.92		205.42
endr ald	273.58	695.30	60.28	407.33	69.68		94.65
endo ss	829.58	873.82	264.30	518.38	227.72		364.41
pp ddt	1338.12	881.45	230.23	479.93	204.10		239.94
T/ DDTs	10249.94	9893.69	3991.97	5679.12	3873.52		5794.98
T/ Cyclos	8932.66	1711.76	703.74	2076.89	1070.76		1424.24
T/ HCHs	568.94	493.64	69.29	271.79	328.37		77.52
T/ Chlors	3758.28	2451.70	641.66	2049.05	729.31		1392.43
T/ Endos	3437.93	3153.70	605.42	1376.17	636.23		761.47
T/ Pests	28900.07	19082.78	6054.72	11840.16	7412.92		9558.40

Melon Data in ng/g Wet Wt.

Animal ID #	9401	9417	9509	9514	9621	9625
Lipid Wt%	76	84.1	79.15	89.85	84.6	82.65
PCBs:						
pcb-28	110.41	321.14	41.55	92.57	312.95	75.92
pcb-52	205.58	781.48	1011.03	867.80	2356.74	447.99
pcb-81	97.56	365.16	489.15	554.87	409.83	329.00
pcb-101	168.92	482.91	261.84	801.24	996.59	551.32
pcb-77	15.64	95.93	255.02	73.41	151.52	18.64
pcb-123	30.64	138.31	372.22	165.55	nd	5.93
pcb-118	96.14	388.40	318.97	563.80	777.02	248.94
pcb-114	585.94	1229.40	1677.54	2731.87	1649.42	1508.33
pcb-126	305.70	883.51	3228.74	1065.78	1786.37	925.24
pcb-105	201.12	56.68	145.14	230.79	216.38	33.27
pcb-138	224.22	723.28	2020.94	1333.22	804.94	606.03
pcb-158	11.05	38.68	233.85	126.71	100.82	49.79
pcb-153	3.09	46.30	358.10	109.17	44.00	25.78
pcb-166	120.81	5.38	50.99	31.77	11.53	16.69
pcb-128	98.62	107.65	496.06	329.95	228.33	55.51
pcb-167	504.12	36.48	324.17	736.78	85.68	37.79
pcb-156	12.94	38.84	99.12	112.34	62.89	nd
pcb-157	2.43	17.12	49.63	22.54	18.08	9.47
pcb-180	155.42	391.88	1530.78	961.95	421.69	359.07
pcb-169	149.31	1.03	6906.05	355.60	51.46	1127.38
pcb-170	45.90	129.94	716.62	325.03	213.00	106.95
pcb-189	0.00	5.28	36.29	10.84	6.74	6.19
Sum PCBs	3145.57	6284.78	20623.80	11603.57	10705.96	6545.20
Pesticides:						
a-bhc	29.79	77.85	6.40	9.71	142.95	32.40
hcb	75.54	546.34	149.50	140.18	1110.78	233.90
b-bhc	3.23	45.17	15.02	13.66	73.95	22.03
lind	73.53	252.60	44.94	43.47	384.43	75.13
d-bhc	nd	7.27	nd	0.35	2.46	nd
hept	nd	45.87	9.36	17.72	90.34	18.86
aldr	8.43	24.26	17.78	12.95	55.92	13.88
hept e	132.05	209.08	289.71	188.90	507.21	321.52
y-chlor	68.22	145.86	44.58	85.89	147.76	26.44
op dde	37.28	1117.86	453.10	357.25	637.42	14.19
endo I	33.31	16.92	42.98	137.64	34.49	2.55
a-chlor	183.27	263.68	501.20	803.24	1023.67	436.64
dield	175.25	1076.64	2458.72	2008.99	617.40	171.34
pp dde	855.60	1837.12	3047.79	2470.82	2092.34	1699.36
op ddd	13.16	116.34	206.47	112.86	195.35	29.75
endr	40.79	183.07	311.55	209.69	247.04	142.89
endo II	47.84	1026.33	640.74	805.87	947.06	143.39
pp ddd	226.34	941.03	583.42	853.20	1538.31	221.25
op ddt	213.93	nd	1666.18	19.47	32.49	1017.35
endr ald	nd	47.66	294.55	74.80	200.50	nd
endo ss	95.10	237.26	352.39	282.89	324.84	46.76
pp ddt	66.97	294.53	333.76	389.02	306.74	79.79
T/ DDTs	1413.27	4306.89	6290.72	4202.62	4802.65	3061.68
T/ Cyclos	224.46	1331.63	3082.60	2306.43	1120.87	328.10
T/ HCHs	106.55	382.90	66.36	67.19	603.78	129.56
T/ Chlors	383.54	664.48	844.86	1095.75	1768.98	803.46
T/ Endos	176.25	1280.51	1036.11	1226.40	1306.40	192.70
T/ Pests	2379.61	8512.76	11470.14	9038.56	10713.46	4749.42

Appendix C

Blubber Data in ng/g Lipid Wt.

Animal ID #	9012	9104	9108	9115	9118	9212	9215
Lipid Wt%	49.65	57.6	42.7	41.6	65.5	75.4	69.4
PCBs:							
pcb-28	40.10	180.58	455.06	72.26	139.63	118.50	69.81
pcb-52	1848.58	2313.45	1171.53	121.09	2715.16	207.44	855.59
pcb-81	1382.52	793.33	554.82	90.86	1329.12	140.87	744.58
pcb-101	549.67	491.29	807.59	123.98	881.53	275.41	467.96
pcb-77	289.12	35.71	118.58	3.49	25.36	42.07	110.41
pcb-123	871.52	81.24	83.03	nd	202.54	53.26	794.32
pcb-118	578.11	920.27	394.73	49.87	1389.06	66.11	984.43
pcb-114	6781.93	2434.51	1124.12	317.27	3359.33	631.26	1905.34
pcb-126	8204.29	2145.01	1217.78	373.12	3575.63	725.57	2793.16
pcb-105	516.48	328.07	157.76	0.50	502.61	59.84	378.82
pcb-138	4399.38	1907.12	769.91	364.94	2679.47	369.92	1974.95
pcb-158	537.76	191.31	74.56	42.08	454.76	16.87	283.42
pcb-153	792.08	59.18	56.29	nd	156.66	65.31	309.45
pcb-166	81.45	9.86	7.43	1.97	33.32	4.55	23.40
pcb-128	1033.76	348.97	85.20	21.58	710.82	61.21	299.95
pcb-167	210.01	181.73	4.68	182.12	200.78	34.30	143.66
pcb-156	74.24	157.68	43.95	20.96	272.56	34.36	142.26
pcb-157	213.58	58.10	20.81	7.19	87.17	8.16	53.53
pcb-180	4295.95	894.56	596.90	471.81	1823.40	346.23	1179.98
pcb-169	172.85	521.83	1.90	106.04	7.32	0.00	5.61
pcb-170	1976.66	326.80	197.76	203.79	694.23	94.34	981.60
pcb-189	58.07	15.73	4.96	nd	40.45	4.70	24.41
Sum PCBs	34908.10	14396.32	7949.34	2574.93	21280.90	3360.28	14526.64
Pesticides:							
a-bhc	53.62	120.96	91.44	6.21	93.23	50.50	26.92
hcb	510.90	872.32	478.68	103.58	1076.43	135.10	219.60
b-bhc	39.50	51.84	24.24	7.14	56.50	11.32	12.91
lind	95.73	190.19	148.82	nd	222.75	60.85	69.58
d-bhc	nd	7.96	2.00	nd	4.37	4.71	nd
hept	78.21	79.24	91.21	nd	95.91	31.17	32.72
aldr	42.28	48.15	26.19	0.92	30.07	22.02	24.42
hept e	1758.91	865.83	448.12	26.78	1048.65	88.79	314.11
y-chlor	133.88	86.85	53.67	172.75	133.04	36.71	53.39
op dde	1564.05	569.63	402.57	34.10	1017.71	104.43	419.39
endo I	295.69	29.37	22.74	nd	44.68	6.71	8.21
a-chlor	4.00	1124.89	624.77	117.96	2483.47	199.88	1005.59
dield	6980.68	6.00	2427.53	23.61	4308.02	787.97	1227.43
pp dde	7412.99	3281.19	3622.50	698.20	4373.75	816.94	3134.72
op ddd	1073.39	200.51	198.53	9.70	317.39	33.27	195.67
endr	1449.55	649.60	225.46	73.00	551.49	84.45	91.53
endo II	1863.52	643.52	599.10	80.23	1044.99	138.14	646.20
pp ddd	2638.67	1329.28	658.59	90.00	1933.76	196.50	545.97
op ddt	1809.28	687.71	8.82	3.98	795.03	265.34	2713.58
endr ald	1145.22	48.66	8.30	nd	87.60	9.47	71.61
endo ss	4.03	334.60	224.58	20.77	635.72	81.16	236.21
pp ddt	615.85	284.90	200.97	55.71	1212.76	79.69	192.69
T/ DDTs	15114.24	6353.219	5091.984	891.6901	9650.393	1496.155	7202.025
T/ Cyclos	9617.72	752.41	2687.49	97.54	4977.18	903.91	1414.98
T/ HCHs	188.84	370.94	266.50	13.36	376.84	127.38	109.42
T/ Chlors	1975.00	2156.81	1217.77	317.49	3761.07	356.54	1405.82
T/ Endos	2163.24	1007.50	846.42	101.00	1725.39	226.00	890.63
T/ Pests	29569.94	11513.19	10588.85	1524.65	21567.31	3245.09	11242.48

Blubber Data in ng/g Lipid Wt.

Animal ID #	9221	9224	9225	9226	9308	9309	9314
Lipid Wt%	1.55	76.95	55.95	46.15	78.7	64.65	75
PCBs:							
pcb-28	497.01	39.95	88.82	196.57	188.04	590.70	118.54
pcb-52	22972.01	5178.81	190.94	3860.60	1083.52	918.53	1576.55
pcb-81	3692.51	2473.78	65.27	760.88	391.66	433.53	1177.00
pcb-101	7669.49	1637.04	173.74	1020.06	679.40	588.23	1029.75
pcb-77	1365.31	67.32	9.81	65.82	45.59	98.72	325.20
pcb-123	3843.59	281.34	159.33	1062.88	23.52	301.75	6.00
pcb-118	4539.23	938.00	93.58	810.09	494.48	366.22	1261.91
pcb-114	32815.08	3491.51	696.48	4025.24	2051.67	711.54	2994.59
pcb-126	32584.30	5277.25	3647.58	2925.59	850.41	2324.89	3054.46
pcb-105	5147.08	622.25	86.52	295.10	186.22	111.10	350.68
pcb-138	30216.06	4221.31	630.30	5251.98	1023.20	921.69	2928.33
pcb-158	2982.75	633.70	64.12	441.60	66.24	69.13	215.10
pcb-153	828.18	440.62	0.00	443.64	23.20	47.05	499.14
pcb-166	207.81	48.00	14.53	28.15	3.01	7.96	24.32
pcb-128	6438.86	910.73	107.90	582.24	143.02	160.15	432.84
pcb-167	2771.80	230.71	146.63	414.03	110.40	34.81	231.62
pcb-156	1625.85	330.02	52.06	141.07	64.38	15.95	195.69
pcb-157	501.59	108.98	23.84	97.36	29.89	11.40	92.91
pcb-180	33449.91	2351.63	870.17	3167.79	557.30	277.42	2374.27
pcb-169	0.00	10.46	35.39	0.00	1.63	0.00	0.00
pcb-170	8634.92	762.57	602.32	1245.55	305.35	127.59	743.09
pcb-189	402.93	56.26	26.02	38.97	7.48	0.00	56.08
Sum PCBs	203186.26	30112.23	7785.34	26875.22	8329.60	8118.34	20383.56
Pesticides:							
a-bhc	375.20	75.68	19.51	57.85	111.56	64.81	35.11
hcb	2932.18	1240.28	25.43	592.36	843.71	338.58	363.88
b-bhc	294.44	95.85	3.64	34.77	26.29	16.04	23.57
lind	437.01	239.33	34.13	133.66	246.15	100.40	140.22
d-bhc	94.49	2.46	5.58	8.74	3.91	6.13	nd
hept	104.77	258.75	34.43	198.92	118.73	40.60	31.84
aldr	351.26	109.53	13.40	46.25	44.30	31.68	41.06
hept e	3373.35	1178.04	46.29	879.49	435.71	248.02	535.09
y-chlor	724.29	127.90	56.63	118.75	143.64	31.72	211.25
op dde	7798.93	1120.62	104.55	779.39	427.70	24.64	1521.45
endo I	36.62	58.72	2.70	33.86	29.98	21.13	49.32
a-chlor	7077.88	1291.30	187.14	1086.79	1306.79	487.60	1686.93
dield	6738.40	4627.49	1038.70	4719.72	1143.76	3100.47	2289.65
pp dde	61016.25	6419.59	1678.91	9146.28	1917.72	1912.41	3235.65
op ddd	1513.44	431.47	48.74	248.34	111.42	135.63	351.52
endr	5531.44	984.42	70.97	1055.93	247.68	185.86	593.26
endo II	7816.37	1200.55	103.99	852.03	308.74	187.42	477.81
pp ddd	11634.99	3412.82	235.88	1916.15	760.72	383.23	1412.26
op ddt	100.09	91.89	351.64	58.14	612.66	718.07	46.32
endr ald	1445.66	536.62	22.63	168.01	30.70	85.20	207.40
endo ss	2852.98	696.46	56.86	1039.53	194.67	74.34	305.92
pp ddt	6074.99	535.24	216.93	665.31	245.15	7.37	243.35
T/ DDTs	88138.69	12011.63	2636.647	12813.62	4075.375	3181.343	6810.56
T/ Cyclos	14066.77	6258.06	1145.70	5989.90	1466.44	3403.21	3131.37
T/ HCHs	1201.14	413.32	62.87	235.02	387.90	187.37	198.90
T/ Chlors	11280.28	2855.98	324.49	2283.96	2004.87	807.94	2465.12
T/ Endos	10705.97	1955.73	163.55	1925.42	533.39	282.89	833.05
T/ Pests	128325.03	24735.01	4358.68	23840.27	9311.69	8201.34	13802.87

Blubber Data in ng/g Lipid Wt.

Animal ID #	9401	9417	9509	9514	9621	9625
Lipid Wt%	53.2	65	62.2	53.25	64.25	0.75
PCBs:						
pcb-28	230.74	389.89	86.77	134.94	407.25	497.66
pcb-52	259.85	1023.73	1540.05	1544.38	3098.28	1741.28
pcb-81	253.21	326.46	921.36	617.39	1110.41	296.73
pcb-101	491.51	897.60	417.73	707.33	1341.39	221.90
pcb-77	27.63	170.98	204.45	151.01	283.94	nd
pcb-123	39.93	62.72	788.01	150.85	371.79	100.91
pcb-118	466.81	376.24	547.19	701.43	1091.82	nd
pcb-114	1281.93	1112.08	2540.64	2561.85	2052.70	1339.97
pcb-126	731.11	1188.40	3822.80	3162.56	2665.50	2629.07
pcb-105	53.48	171.05	644.46	407.66	308.49	nd
pcb-138	947.61	853.51	3739.49	2728.67	1267.78	696.90
pcb-158	24.68	58.73	337.56	224.49	130.12	nd
pcb-153	16.96	72.33	770.42	266.41	nd	nd
pcb-166	4.67	15.59	99.66	25.37	22.47	nd
pcb-128	55.04	122.62	595.59	585.58	310.82	nd
pcb-167	44.68	61.63	102.73	223.43	112.96	nd
pcb-156	37.01	73.19	37.53	119.26	98.14	nd
pcb-157	13.40	24.03	134.23	48.69	47.79	nd
pcb-180	485.18	684.41	1724.07	2745.49	1149.51	923.15
pcb-169	24.64	nd	73.10	22.26	nd	1342.57
pcb-170	84.77	173.91	1038.11	513.35	438.37	46.13
pcb-189	7.27	6.25	30.27	25.34	15.98	nd
Sum PCBs	5582.07	7865.37	20196.23	17667.77	16325.51	9836.26
Pesticides:						
a-bhc	66.48	101.75	7.49	12.03	146.70	371.17
hcb	214.34	685.02	283.32	318.22	1463.96	5089.40
b-bhc	7.82	13.41	8.61	9.37	49.51	282.47
lind	122.64	289.89	43.29	52.63	375.29	0.00
d-bhc	11.21	1.60	0.00	1.52	2.22	437.20
hept	34.23	2.40	0.00	6.41	19.03	0.00
aldr	19.05	33.79	39.91	15.92	93.13	187.35
hept e	202.09	277.99	368.83	320.98	925.72	0.00
y-chlor	76.21	127.90	108.68	62.85	178.63	1008.66
op dde	369.51	946.64	944.87	942.17	1027.41	191.14
endo I	0.00	15.58	26.49	0.00	12.58	0.00
a-chlor	573.61	640.41	1166.53	977.81	1968.84	457.80
dield	1631.44	2058.65	3858.09	5718.10	6321.38	1410.33
pp dde	2066.24	1721.72	5629.79	11.27	3509.04	6193.73
op ddd	87.21	164.18	344.97	233.08	333.15	0.00
endr	120.49	88.09	918.47	136.56	404.88	0.00
endo II	263.74	395.76	674.68	607.56	1476.78	844.56
pp ddd	617.89	754.62	1243.84	1339.86	1501.68	256.00
op ddt	2204.26	0.00	441.45	58.17	66.07	0.00
endr ald	0.00	36.84	356.71	338.52	53.23	0.00
endo ss	158.90	137.93	534.39	288.67	334.72	0.00
pp ddt	119.74	259.64	290.28	530.95	342.28	680.40
T/ DDTs	5464.842	3846.794	8895.207	3115.505	6779.638	7321.27
T/ Cyclos	1770.98	2217.37	5173.18	6209.10	6872.62	1597.68
T/ HCHs	208.16	406.65	59.39	75.55	573.71	1090.85
T/ Chlors	886.14	1048.70	1644.04	1368.04	3092.22	1466.46
T/ Endos	422.64	549.28	1235.56	896.22	1824.07	844.56
T/ Pests	8967.11	8753.81	17290.69	11982.65	20606.22	17410.22

Appendix D

Melon Data in ng/g Lipid Wt.

Animal ID #	9012	9104	9108	9115	9118	9212	9215
Lipid Wt%		86.80		89.55	74.6	70.25	79.2
PCBs:							
pcb-28		215.18		41.11	87.98	157.73	68.45
pcb-52		2617.63		103.52	3698.16	278.90	913.25
pcb-81		1266.39		45.92	1684.86	128.60	332.37
pcb-101		1305.22		154.16	1421.27	328.82	908.10
pcb-77		196.72		57.78	87.99	211.26	nd
pcb-123		382.00		224.48	46.61	65.00	1235.41
pcb-118		1230.48		194.51	789.34	134.14	497.16
pcb-114		2204.24		1102.34	3632.99	637.82	1985.34
pcb-126		2393.43		3776.79	3569.31	500.44	2434.68
pcb-105		382.96		61.98	258.10	43.99	348.82
pcb-138		1969.15		2530.61	2300.45	554.14	1868.72
pcb-158		210.12		103.39	261.34	35.43	131.87
pcb-153		217.92		120.28	9.69	56.87	nd
pcb-166		31.37		146.98	42.96	4.50	8.60
pcb-128		515.34		243.16	406.09	48.78	483.16
pcb-167		224.27		104.50	270.00	149.65	158.31
pcb-156		211.40		65.48	123.20	42.69	154.27
pcb-157		73.74		33.55	61.77	11.90	32.15
pcb-180		1379.74		2004.17	1299.82	233.35	1383.34
pcb-169		nd		3.30	2.05	nd	nd
pcb-170		378.77		797.89	472.70	93.74	593.92
pcb-189		22.36		25.97	16.29	4.59	21.04
Sum PCBs		17428.42		11941.85	20542.96	3722.34	13558.98
Pesticides:							
a-bhc		107.35		15.26	126.83	25.85	41.71
hcb		1046.27		22.32	818.42	155.30	436.13
b-bhc		79.34		4.12	115.79	10.62	57.40
lind		193.75		23.64	310.83	71.30	92.55
d-bhc		0.41		7.66	1.19	2.89	nd
hept		74.88		nd	110.35	39.47	31.27
aldr		53.48		14.64	56.57	8.68	26.27
hept e		703.92		35.06	964.95	149.73	416.83
y-chlor		135.82		49.51	196.34	16.37	75.35
op dde		1692.06		212.70	1789.52	86.67	834.47
endo I		14.77		nd	94.72	2.36	86.84
a-chlor		2040.39		432.04	3117.03	202.11	1117.55
dield		3558.85		716.34	3314.58	450.99	169.05
pp dde		4617.41		3775.87	5023.93	1275.64	2428.84
op ddd		484.65		47.56	422.38	43.18	205.27
endr		407.32		137.45	525.71	145.86	419.97
endo II		2002.75		332.70	1235.31	115.83	1021.12
pp ddd		1597.85		195.77	1994.14	180.05	626.63
op ddt		1014.15		23.35	99.00	455.83	1624.91
endr ald		150.31		33.17	197.15	16.82	73.99
endo ss		499.02		159.79	1022.80	31.93	210.94
pp ddt		549.64		239.51	1087.81	67.05	433.06
T/ DDTs		9955.76		4494.77	10416.78	2108.43	6153.19
T/ Cyclos		4169.96		901.61	4094.00	622.34	689.27
T/ HCHs		380.85		50.68	554.64	110.67	191.65
T/ Chlors		2955.01		516.62	4388.67	407.68	1641.01
T/ Endos		2516.54		492.49	2352.83	150.12	1318.90
T/ Pests		21024.38		6478.49	22625.35	3554.54	10430.16

Melon Data in ng/g Lipid Wt.

Animal ID #	9221	9224	9225	9226	9308	9309	9314
Lipid Wt%	82.55	83.55	86.55	81.2	83.5		87.4
PCBs:							
pcb-28	220.50	142.89	92.96	172.68	180.14		78.54
pcb-52	5764.97	5407.03	265.22	3432.04	1632.86		1412.12
pcb-81	2565.71	821.75	100.98	1316.00	485.54		680.03
pcb-101	1535.09	765.28	248.77	1114.27	1068.65		910.72
pcb-77	156.58	118.29	63.90	105.03	123.03		46.98
pcb-123	400.93	494.34	43.78	181.81	778.48		102.64
pcb-118	1824.54	595.03	224.92	776.36	714.15		616.63
pcb-114	4289.58	2578.97	1200.54	3460.82	2183.57		1661.23
pcb-126	3939.95	5491.94	2802.87	3985.70	2552.62		2944.29
pcb-105	1423.41	775.42	179.67	270.05	271.82		373.91
pcb-138	4375.20	3818.23	1175.74	2319.88	1458.39		2258.87
pcb-158	578.68	435.92	130.90	598.56	122.66		149.45
pcb-153	90.52	92.79	nd	215.27	50.61		182.39
pcb-166	100.08	31.26	9.99	54.63	10.24		53.44
pcb-128	1379.94	892.78	224.23	599.27	139.39		370.85
pcb-167	299.45	224.49	135.09	240.36	109.20		140.83
pcb-156	241.24	208.04	98.87	124.15	67.99		92.96
pcb-157	84.91	67.18	46.71	78.10	15.51		71.49
pcb-180	2528.06	1391.05	896.28	2390.55	474.45		1868.33
pcb-169	nd	8.69	0.54	nd	4.68		75.34
pcb-170	732.67	843.05	500.98	378.62	201.61		410.60
pcb-189	39.62	35.15	17.68	35.77	5.54		21.47
Sum PCBs	32571.63	25239.58	8460.62	21849.92	12651.12		14523.13
Pesticides:							
a-bhc	114.65	99.17	31.33	50.02	112.42		6.84
hcb	2365.01	1649.66	49.26	476.76	927.82		123.31
b-bhc	199.01	160.64	6.28	144.78	40.41		25.99
lind	372.80	326.86	42.45	138.03	237.52		55.85
d-bhc	2.76	4.16	nd	1.89	2.91		nd
hept	245.15	298.79	13.09	96.62	nd		27.24
aldr	58.78	124.08	11.80	94.75	34.01		39.04
hept e	1464.53	772.26	51.51	994.55	312.11		420.96
y-chlor	273.79	256.95	72.08	129.74	91.92		105.82
op dde	1968.24	2619.93	473.19	562.60	974.47		667.08
endo I	107.40	125.19	5.97	69.35	39.10		35.49
a-chlor	2569.26	1606.40	604.70	1302.55	469.39		1039.15
dield	9558.95	20.08	365.87	1089.37	657.83		1040.51
pp dde	4124.14	4518.35	3266.34	3433.25	2106.49		4396.26
op ddd	579.28	626.27	81.15	431.27	141.53		94.74
endr	871.77	1072.43	365.79	871.99	507.07		441.72
endo II	3052.32	2603.57	388.16	987.04	450.13		418.82
pp ddd	3231.34	2383.36	525.63	1927.40	1033.19		962.75
op ddt	892.67	638.75	nd	48.44	138.83		235.04
endr ald	331.41	832.20	69.65	501.64	83.44		108.29
endo ss	1004.94	1045.86	305.38	638.40	272.71		416.94
pp ddt	1620.98	1055.00	266.01	591.04	244.43		274.54
T/ DDTs	12416.64	11841.64	4612.33	6993.99	4638.94		6630.41
T/ Cyclos	10820.91	2048.79	813.11	2557.75	1282.35		1629.57
T/ HCHs	689.21	590.83	80.06	334.72	393.26		88.69
T/ Chlors	4552.73	2934.41	741.37	2523.46	873.43		1593.17
T/ Endos	4164.67	3774.62	699.50	1694.79	761.95		871.25
T/ Pests	35009.17	22839.95	6995.63	14581.48	8877.75		10936.39

Melon Data in ng/g Lipid Wt.

Animal ID #	9401	9417	9509	9514	9621	9625
Lipid Wt%	76	84.1	79.15	89.85	84.6	82.65
PCBs:						
pcb-28	145.28	381.86	52.50	103.02	369.92	91.85
pcb-52	270.50	929.22	1277.36	965.83	2785.74	542.03
pcb-81	128.37	434.20	618.00	617.55	484.43	398.07
pcb-101	222.26	574.21	330.81	891.76	1178.00	667.06
pcb-77	20.58	114.06	322.20	81.70	179.10	22.55
pcb-123	40.32	164.46	470.27	184.25	nd	7.17
pcb-118	126.51	461.84	403.00	627.49	918.46	301.19
pcb-114	770.97	1461.83	2119.44	3040.48	1949.67	1824.96
pcb-126	402.23	1050.54	4079.27	1186.18	2111.55	1119.47
pcb-105	264.64	67.39	183.38	256.86	255.77	40.25
pcb-138	295.03	860.03	2553.30	1483.83	951.46	733.25
pcb-158	14.54	45.99	295.46	141.02	119.17	60.24
pcb-153	4.07	55.05	452.44	121.50	52.01	31.19
pcb-166	158.97	6.40	64.42	35.36	13.62	20.20
pcb-128	129.76	128.00	626.73	367.22	269.89	67.17
pcb-167	663.31	43.37	409.57	820.02	101.28	45.72
pcb-156	17.03	46.18	125.23	125.03	74.34	0.00
pcb-157	3.20	20.36	62.71	25.08	21.37	11.46
pcb-180	204.50	465.96	1934.03	1070.62	498.45	434.44
pcb-169	196.46	1.22	8725.27	395.77	60.83	1364.04
pcb-170	60.39	154.51	905.39	361.75	251.78	129.40
pcb-189	nd	6.28	45.84	12.07	7.97	7.48
Sum PCBs	4138.90	7472.98	26056.61	12914.38	12654.80	7919.18
Pesticides:						
a-bhc	39.20	92.57	8.09	10.81	168.97	39.21
hcb	99.39	649.64	188.88	156.01	1312.98	283.01
b-bhc	4.25	53.72	18.97	15.20	87.41	26.65
lind	96.75	300.36	56.77	48.38	454.40	90.91
d-bhc	nd	8.65	nd	0.39	2.91	nd
hept	nd	54.54	11.83	19.72	106.78	22.82
aldr	11.09	28.85	22.47	14.41	66.10	16.79
hept e	173.75	248.61	366.03	210.24	599.53	389.01
y-chlor	89.76	173.43	56.32	95.59	174.65	31.99
op dde	49.05	1329.21	572.45	397.61	753.45	17.17
endo I	43.83	20.11	54.30	153.19	40.77	3.09
a-chlor	241.15	313.53	633.23	893.98	1210.02	528.30
dield	230.59	1280.20	3106.40	2235.93	729.79	207.31
pp dde	1125.79	2184.45	3850.65	2749.94	2473.22	2056.09
op ddd	17.31	138.34	260.86	125.61	230.91	35.99
endr	53.67	217.68	393.62	233.38	292.01	172.88
endo II	62.95	1220.37	809.53	896.91	1119.46	173.50
pp ddd	297.81	1118.95	737.10	949.59	1818.34	267.70
op ddt	281.49	nd	2105.09	21.66	38.40	1230.91
endr ald	nd	56.66	372.14	83.26	237.00	nd
endo ss	125.14	282.12	445.22	314.85	383.97	56.58
pp ddt	88.12	350.22	421.69	432.97	362.58	96.53
T/ DDTs	1859.57	5121.16	7947.85	4677.37	5676.89	3704.40
T/ Cyclos	295.35	1583.39	3894.63	2566.98	1324.90	396.98
T/ HCHs	140.19	455.29	83.84	74.78	713.69	156.76
T/ Chlors	504.66	790.11	1067.41	1219.54	2090.99	972.12
T/ Endos	231.91	1522.60	1309.04	1364.94	1544.20	233.16
T/ Pests	3131.07	10122.19	14491.64	10059.61	12663.66	5746.42

Appendix E

EHIME UNIVERSITY
COLLEGE OF AGRICULTURE

3 TARUMI,
MATSUYAMA 790, JAPAN
TELEPHONE:

Feb. 16, 1998

To:
Ms. Kathleen M. Küss
451 Avenida De Mayo
Sarasota
FL 34242
U.S.A.

From:
Dr. Shinsuke Tanabe
Professor of Environmental Chemistry and Ecotoxicology
Department of Environment Conservation
Ehime University
Tarumi 3-5-7, Matsuyama 790
JAPAN
TEL/FAX: +81-899-46-9904 E-mail: shinsuke@agr.ehime-u.ac.jp

Dear Ms. Kathleen;

Thank you for your letter dated 2 Feb., 1998. I am pleased to inform you that you may reproduce of our any figure and table for use in your thesis and defence presentation. Thank you for your interests to our research.

Sincerely yours,

(S. Tanabe)

WWF

January 23, 1998

Kathleen M. Kuss
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236

Dear Kathleen Kuss:

Thank you very much for your letter of January 19, 1998. I write to inform you that Dr. Colborn has given her consent for the reproduction of material you requested from her article, "Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife & Humans" published in the Environmental & Health Perspectives Journal vol. 101, no. 5,1993.

We hope that this information will add great credibility to your research. Good Luck in your studies.

Sincerely.

C. Percival-Deigh
Administrative Assistant.

World Wildlife Fund
1250 Twenty-Fourth St., NW Washington, DC 20037-1175 USA
Tel: (202) 293-4800 Fax: (202) 293-9211
Affiliated with World Wide Fund for Nature