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Chemical Contamination of Harbor Seal Pups in Puget Sound

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CHEMICAL CONTAMINATION OF HARBOR SEAL PUPS IN PUGET SOUND

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LIST OF ACRONYMS

ABN	acid/base/neutral
CVAA	cold vapor atomic absorption spectrometry
EPA	U.S. Environmental Protection Agency
GC/ECD	gas chromatography/electron capture detection
GC/MS	gas chromatography/mass spectrometry
GFAA	graphite furnace atomic absorption spectrometry
GPC	gel permeation chromatography
ICP	inductively coupled plasma-atomic emission spectrometry
PCB	polychlorinated biphenyl
PSEP	Puget Sound Estuary Program
QA/QC	quality assurance and quality control

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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) Region 10, through the Office of Coastal Waters, has been responsible for providing technical support to the Puget Sound Estuary Program. One component of this program is the evaluation of bioaccumulation of chemical contaminants in the tissues of wildlife species that reside in the sound. Based on discussions with the U.S. Fish and Wildlife Service, the Washington Department of Wildlife, and other government agencies, it was determined that a study of chemical contaminants in the tissues of Puget Sound harbor seal pups (*Phoca vitulina*) would provide an assessment of bioaccumulation at the top level of the Puget Sound food web and thereby help achieve the goals of the estuary program.

Dead newborn harbor seals were collected from Smith Island in the Strait of Juan de Fuca and from Gertrude Island in southern Puget Sound. Seven seals were evaluated from each location. A variety of chemical contaminants were measured in different kinds of tissue from the harbor seal pups. Concentrations of pesticides and polychlorinated biphenyls (PCBs) were measured in blubber tissue and concentrations of metals, bromine, and semivolatile acid/base/neutral (ABN) extractable organic compounds were measured in liver tissue. In addition to measurements of chemical concentrations, various tissues were also evaluated microscopically for histopathological abnormalities.

A key aspect of this study was the comparison of two methods for determining the concentrations of PCBs and DDT (primarily p,p'-DDE) in blubber tissue. One technique (referred to as the "CLP method") was based on a modified version of the EPA Contract Laboratory Program (CLP) procedures (PSEP 1989a). Under this technique, extraction procedures are conducted

according to PSEP guidelines (1989a) and all subsequent analytical procedures are conducted in accordance with EPA CLP procedures. The second analytical technique (referred to as the "Cascadia method") was the one used in past studies of marine mammals in Puget Sound by Cascadia Research Collective. In general, the two methods differed primarily with respect to tissue digestion, cleanup, and quantification techniques. Additional evaluations conducted during this study included comparisons of chemical and histopathological results between the two sampling locations and comparisons with historical data to assess temporal trends of chemical contamination in harbor seal pups.

A summary of the results of this study is as follows (all chemical concentrations are expressed on a wet-weight basis):

- The concentrations of PCBs in blubber tissue of seal pups from Gertrude Island (mean = 17 mg/kg) were significantly higher ($P \leq 0.05$) than the concentrations found in pups from Smith Island (mean = 4.0 mg/kg). The higher concentrations of PCBs at Gertrude Island are likely the result of the closer proximity of that island to some of the chemically contaminated urban bays of central and southern Puget Sound, where concentrations of PCBs are generally elevated in environmental media.

- The concentrations of p,p'-DDE in blubber tissue did not differ significantly ($P > 0.05$) between seal pups from Gertrude Island (mean = 2.2 mg/kg) and Smith Island (mean = 1.5 mg/kg).

- Aside from p,p'-DDE, most of the other 20 pesticides evaluated in this study were not detected in the blubber tissue of seal pups from either Gertrude Island or Smith Island. However, α-hexachlorocyclohexane was detected at concentrations of 33-180 μg/kg in seven seals (four from Smith Island and three from Gertrude Island), ß-hexachlorocyclohexane was detected (38 μg/kg) in a single seal

from Smith Island, and p,p'-DDD was detected at concentrations of 29–74 μg/kg in five seals (one from Smith Island and four from Gertrude Island).

- The concentrations of lead and silver in liver tissue of seal pups from Smith Island (means = 40 and 110 μg/kg, respectively) were significantly higher ($P \leq 0.05$) than the concentrations found in pups from Gertrude Island (means = 30 and 41, respectively). The concentrations of all other metals evaluated (aluminum, arsenic, cadmium, copper, mercury, nickel, selenium, and zinc) did not differ significantly ($P > 0.05$) between the two islands.

- Of the 67 ABN organic compounds evaluated, only 2 were detected in the liver tissue of harbor seal pups. Benzoic acid was detected at concentrations of 140 and 1,200 μg/kg in the single composite tissue samples from Smith Island and Gertrude Island, respectively. 4-Methylphenol was only detected (1,400 μg/kg) in the composite sample from Gertrude Island.

- The concentrations of PCBs and p,p'-DDE in seal pups from both Gertrude and Smith islands have declined significantly ($P \leq 0.05$) since testing began in 1972. The declines have been greatest for seals from Gertrude Island. The concentration ranges of PCBs and p,p'-DDE at Gertrude Island declined from ranges of 16-370 and 2.4-30 mg/kg (respectively) in 1972 to ranges of 7.8-23 and 1.1-2.8 mg/kg (respectively) in 1990.

- The quantification of PCBs using techniques that rely on comparisons with standards (such as those used for the CLP method) are not appropriate for harbor seals, because the composition of PCB congeners in harbor seal tissue does not resemble any single commercial mixture. In future studies, PCBs should be quantified

using methods comparable to those used by Cascadia Research Collective in the present study.

- Although a variety of histopathological conditions were found in seal pups from both sampling locations, none could be conclusively related to chemical contaminants. The prevalence of umbilical infections was higher at Gertrude Island than at Smith Island, which is consistent with the high prevalence of umbilical ulcerations and scarring found in juvenile seals from Gertrude Island in 1984.

- Harbor seal pups provide a useful tool for monitoring trends of tissue chemical concentrations in animals from the upper levels of the Puget Sound food chain. In future studies, seals should be collected within the age range used in the present study to increase the probability that adequate sample sizes can be obtained and to minimize the effects of age on comparisons of tissue chemical concentrations among different studies.

The results of this study demonstrate that tissue contamination of harbor seals by PCBs and p,p'-DDE has declined substantially in Puget Sound since the early 1970s. At that time, PCB concentrations in the blubber tissue of Puget Sound harbor seals were among the highest measured anywhere in the world. Although the concentrations of PCBs measured in the present study remain high compared with many other pinniped species found in other regions, they are no longer among the highest found in the world.

Because harbor seals feed at the top of the marine food chain in Puget Sound, the observed reductions in the concentrations of PCBs and p,p'-DDE in blubber tissue suggest that concentrations of these chemicals have likely declined in environmental media throughout most of the sound. This study demonstrates the value of using harbor seals as indicators of contaminant concentrations throughout Puget Sound and for evaluating trends in the tissue concentrations of stable

chlorinated hydrocarbons. A major disadvantage of previous attempts to use marine mammals as biological indicators has been the high variability of chemical concentrations observed among individual animals. This problem was largely solved in the present study by selecting animals within a narrow age range that met specific acceptance criteria. The variability of concentrations among individual animals was therefore low, increasing the ability to detect differences between the two study sites and among various time periods.

The potential effects of the observed contaminant concentrations on the health of the harbor seal pups are unknown. Acute toxic effects are unlikely because the observed concentrations of chlorinated hydrocarbons are well below the values found to have toxic effects in older animals. However, the potential chronic, sublethal effects of most contaminants (particularly PCBs in marine mammals) are unknown. Of special concern in recent years has been the potential immuno-suppressive effects of PCBs in seals (Brower et al. 1989) and the potential relationship of those compounds to disease-related mass mortalities of seals in other parts of the world (Dietz et al. 1989; Harwood and Grenfell 1990). However, until additional research is conducted, it is not possible to determine whether PCBs may be exerting subtle effects on Puget Sound harbor seals.

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) Region 10, through the Office of Coastal Waters, has been responsible for providing technical support to the Puget Sound Estuary Program (PSEP). One component of this program is the evaluation of bioaccumulation of chemical contaminants in the tissues of wildlife species that reside in the sound. Based on discussions with the U.S. Fish and Wildlife Service, the Washington Department of Wildlife, and other government agencies, it was determined that a study of chemical contaminants in the tissues of Puget Sound harbor seal pups (*Phoca vitulina*) would provide an assessment of bioaccumulation at the top level of the Puget Sound food web and thereby help achieve the goals of the estuary program.

The harbor seal is the most abundant species of marine mammal in Puget Sound. In addition, it is the only species of marine mammal that resides in the sound throughout the year and breeds in the sound (Calambokidis et al. 1979a, 1985; Osborne et al. 1988). Because harbor seals feed at the top of the marine food chain, they are useful for monitoring the trophic transfer of chemical contaminants. Although past studies have found high concentrations of polychlorinated biphenyls (PCBs) and p,p'-DDE in the blubber of harbor seals from several sites in Puget Sound (Arndt 1973; Calambokidis et al. 1978, 1984, 1988), the current concentrations of chemical contaminants in harbor seals from the sound are unknown. The present study serves to fill this data gap.

This study focused on evaluations of dead newborn (stillborn) harbor seal pups because adequate sample sizes could be collected in a predictable manner (i.e., at breeding areas during the pupping season) and because variations in

individual age (which can influence tissue contaminant concentrations) could be minimized. In addition, because the historical database for Puget Sound includes evaluations of chemical contaminants in a relatively large number of harbor seal pups, a detailed evaluation of temporal trends could be conducted. Previous studies (Addison and Brodie 1977; Anas and Wilson 1970; Donkin et al. 1981) have indicated that pups accumulate chlorinated hydrocarbons in their tissues as a result of transplacental transfer and lactation. The use of older seals for evaluations of bioaccumulation is limited because it cannot be predicted when and where seals will die naturally and thereby be available for sampling. Because seals are protected by the Marine Mammal Protection Act, they cannot be killed intentionally without a permit.

A variety of chemical contaminants were measured in different kinds of tissue from the harbor seal pups. Blubber tissue was analyzed for pesticides and PCBs because the concentrations of these lipophilic compounds were expected to be highest in that tissue. Analyses for metals were conducted on liver tissue, where concentrations of those chemicals were expected to be high (relative to other kinds of tissue). The concentrations of bromine in liver tissue were also determined because of the reported association between this chemical and selenium and mercury in marine mammals (Martin et al. 1976; Koeman et al. 1973). Martin et al. (1976) suggested that imbalances of these chemicals may have played a role in the premature births of California sea lions. Analyses for semivolatile acid/base/neutral (ABN) extractable organic compounds were performed on a composite liver sample from each sampling area. Although it was not expected that concentrations of ABN organic compounds would be substantially higher than background levels, the data collected during the present study can be used to provide a baseline for future trend analyses. Analyses for volatile organic compounds were not performed because historical studies in Puget Sound have found that those chemicals were rarely elevated in harbor seals. In addition to the chemical analyses, the lipid content of each tissue sample was

determined to evaluate how it may have influenced the observed concentrations of lipophilic contaminants.

The specific objectives of this study were to:

- Measure contaminant concentrations in harbor seal pups from two sites located in different parts of Puget Sound and compare the results between the two sites
- Evaluate histopathological abnormalities in the seals selected for chemical analysis
- Compare the concentrations of PCBs and p,p'-DDE found in the present study with the results of past studies in Puget Sound to evaluate temporal trends of bioaccumulation
- Compare different analytical methods for PCBs and p,p'-DDE to determine how the use of different methods can affect comparisons among different studies
- Develop baseline information on contaminants that have not been measured in past studies of Puget Sound harbor seals
- Evaluate the usefulness of monitoring contaminant concentrations in harbor seal pups.

METHODS

FIELD COLLECTION

Harbor seal pups were collected in the summer of 1990 at two locations in Puget Sound where harbor seals haul out to breed. The two locations included Smith Island in the eastern Strait of Juan de Fuca (48°19'N, 122°50'W) and Gertrude Island in southern Puget Sound (47°13'N, 122°39'W). Smith Island is a wildlife refuge of the U.S. Fish and Wildlife Service that is connected to nearby Minor Island at low tide. Seals from both islands were evaluated in this study and the two islands are collectively referred to as Smith Island in this report. Gertrude Island is a small island in Still Harbor that is connected to McNeil Island (a much larger island) at low tide. It is part of the McNeil Island State Penitentiary and is the largest seal haul-out area in southern Puget Sound.

Beach searches for dead newborn seal pups were conducted at Smith and Gertrude islands using the strategies and methods used in previous studies (Calambokidis et al. 1985; Steiger et al. 1989). Searches were timed to correspond with the pupping season. At Smith Island, searches were conducted on 9 days between 13 June and 14 August 1990. The eastern shore of Smith Island and all of Minor Island were searched on foot. However, portions of the beach could not be searched because seals were hauled out during some searches. At Gertrude Island, searches were conducted on 7 days between 31 July and 13 September 1990. All of Gertrude Island and the eastern shore of Still Harbor (on McNeil Island) were searched on foot. The western shore of Still Harbor (on McNeil Island) was searched from a boat.

Dead pups were selected for analysis and necropsied according to PSEP guidelines (PSEP 1991). Of the 43 dead pups (25 from Smith Island and 18 from Gertrude Island) found during field sampling, 7 were selected for analysis from each island. Each of these 14 pups was selected for analysis based on 1) acceptable postmortem condition of the carcass (i.e., the tissues showed no signs of autolysis based on gross examination), 2) presence of an adequate blubber layer indicating the animal was not emaciated, and 3) evidence that each animal was ≤ 2 weeks old. The carcasses of the 14 pups were either necropsied at the collection site or kept cool on ice and returned unfrozen to the laboratory for necropsy. For each selected pup, the following determinations were made:

- Weight (nearest 0.1 kg wet weight)
- Standard length (nearest cm)
- Axillary girth (nearest cm)
- Sex
- Presence or absence of an umbilical cord
- Tooth development
- Presence of lanugo coat
- Blubber thickness (nearest 0.1 cm, measured over the posterior end of the sternum)
- Signs of blubber deterioration (e.g., gas bubbles or leaching of oil)
- Presence or absence of parasites, injuries, and gross abnormalities
- Signs of lung aeration
- Stomach contents.

Tissue collection procedures followed the PSEP guidelines (PSEP 1989a,b, 1991) and were designed to minimize sample contamination. Tissues were sampled with stainless steel instruments (i.e., knives, scalpels, scissors, and forceps). Prior to use, all instruments were rinsed in sequence with distilled water and dichloromethane and then allowed to dry. A new scalpel blade was used for sampling each kind of tissue to prevent cross-tissue contamination.

Blubber tissue was sampled from the mid-ventral region. These samples included the full thickness of the blubber layer. For liver samples, the posterior portions of the left two lobes were collected. Approximately 50-150 grams of each kind of tissue was removed and immediately transferred to precleaned borosilicate glass jars with Teflon®-lined lids. Samples collected in the field were stored on ice in a cooler and returned to the laboratory. In the laboratory, all samples were frozen at -20°C until analysis.

Several kinds of tissues from the 14 harbor seal pups selected for chemical evaluation were sampled for histopathological analyses. The target tissues included skin, brain (medulla, cerebellum, and cerebral cortex), heart, thymus, liver, kidney, lung, pancreas, gastrointestinal tract, bladder, umbilical region, lymph nodes, thyroid, and adrenal gland. Some of the target tissues could not be collected from all 14 pups because several of these animals had been scavenged.

Following resection, tissues for histopathological analysis were placed in 10-percent buffered formalin. A 1:10 volumetric ratio of tissue to formalin was used to ensure adequate fixation. Samples were returned to the laboratory and stored at room temperature until analysis.

LABORATORY ANALYSIS

Blubber Tissue - PCBs and p,p'-DDE

Split samples of blubber tissue were analyzed for PCBs and p,p'-DDE using two techniques so that the results of the two methods could be compared. One technique (hereafter referred to as the "CLP method") was based on a modified version of EPA Contract Laboratory Program (CLP) procedures (1989a). Under this technique, extraction procedures are conducted according to PSEP guidelines (1989a) and all subsequent analytical procedures are conducted in accordance with EPA CLP procedures. All 14 pups selected for chemical analysis were evaluated using the CLP method. The second analytical technique (hereafter referred to as the "Cascadia method") was the one used in past studies of marine mammals in Puget Sound by Cascadia Research Collective. The Cascadia method was used to evaluate PCB concentrations in 10 of the 14 pups. In general, the two methods differed primarily with respect to tissue digestion, cleanup, and quantification techniques (Calambokidis et al. 1984). Each of the two methods is briefly described below.

CLP Method—Analyses of PCB and p,p'-DDE concentrations in blubber tissue followed modifications to EPA CLP procedures that were consistent with PSEP recommendations for analyses with relatively low detection limits. Modifications recommended by PSEP (1989a) included a larger sample size and a smaller final extract volume for gas chromatography/mass spectrometry (GC/MS) analyses. Tissue samples were homogenized mechanically, as recommended by PSEP (1989a). The analyses were performed using a slightly modified version of the EPA CLP procedures. These analyses included extract cleanup by gel permeation chromatography (EPA Method 3640), alumina column chromatography (EPA Method 3610), and, when necessary, elemental sulfur

cleanup (EPA Method 3660), followed by gas chromatography/electron capture detection (GC/ECD) analysis. The EPA CLP GC/ECD method is analogous to EPA Method 8080; however, quantification and confirmation analyses were performed with megabore capillary columns rather than the packed columns sometimes used in the EPA CLP procedures (U.S. EPA 1988). Calibration procedures for all analyses were consistent with EPA CLP procedures (U.S. EPA 1988).

Cascadia Method—These analyses were performed in the same laboratory (Evergreen State College) using the same equipment and methods that were used for most of the past studies of marine mammals in Puget Sound conducted by Cascadia Research Collective (Calambokidis et al. 1978, 1984, 1988).

A 5- to 10-gram subsample of blubber tissue was excised from the full width of each blubber sample. Approximately 50 mL of BFM solution (glacial acetic acid and perchloric acid) was added to the sample, and the sample was then heated for 2-3 hours on a steam bath until the tissue was completely digested (Stanley and LeFavoure 1965). Following digestion, the sample was extracted three times with 20-mL aliquots of hexane.

Lipid weights were determined by evaporating a portion of the hexane-lipid extract to dryness. A 10-mL portion of the hexane extract was placed in a centrifuge tube and 2 mL of concentrated sulfuric acid was added to destroy the lipids (Murphy 1972). The sample was shaken for 1 minute and then centrifuged for 10 minutes.

A 2- to 8-μL aliquot of each sample was injected on a Hewlett-Packard electron capture gas chromatograph equipped with a glass column (0.25 inches in diameter and 6 feet in length) packed with 10-percent DC-200 on gas chrom Q (80/100 mesh) and a ^{63}Ni electron capture detector. Samples were injected on

a column with a 1-inch alkaline (KOH and NaOH) precolumn (Miller and Wells 1969). Peak areas were determined using integrating computers connected to the gas chromatograph.

The principal DDT compound found in the samples was p,p'-DDE. Therefore, to aid in the analysis of DDT and to minimize its interference with the quantification of PCB peaks, the alkaline precolumn was used to convert all p,p'-DDT to p,p'-DDE. As a result of this conversion, the quantification of p,p'-DDE may have included a small amount of p,p'-DDT. The p,p'-DDE peak was quantified after subtracting the estimated area of the coeluting PCB peak (extrapolated from the preceding adjacent PCB peak).

PCB peaks were quantified by individual homolog analysis using the mean weight-percent figures reported by Webb and McCall (1973) for 21 peaks. Mowrer et al. (1977), Calambokidis et al. (1979b), and Tetra Tech (1986) provide a description of this technique.

Identification and quantification of PCBs and p,p'-DDE were based on comparisons with known standards. DDT standards and PCB standards (mixtures of Aroclors 1242, 1254, and 1260) were injected between every series of three to four samples. If peak areas of the standards varied by more than 20 percent, the corresponding sample runs were rejected and samples were reinjected after an acceptable level of variability was achieved.

The following steps were used for the quantification of samples. PCB peaks were identified using retention times. One sample peak did not match the standards as well as the other PCB peaks. This peak was accepted, however, because it was identical to the peak occurring in previous analyses of harbor seal tissue, and it has been included in the results of previous studies conducted by Cascadia Research Collective. Because the peak is a merged peak (even in the

PCB standard), the observed time difference was probably the result of different proportions of the PCB congeners coeluting under this peak.

Quantification of PCBs and p,p'-DDE was conducted using the mean of the standards injected before and after each sample. The five primary PCB peaks were quantified in all samples. Four additional PCB peaks were present, but were not integrated in the four samples with the lowest concentrations. For those four samples, estimated values were added for the four additional peaks based on a proportional relationship to the primary PCB peaks in the six samples for which most or all peaks were quantified. One additional PCB peak was obscured by the p,p'-DDE peak. This interference was accounted for by assuming that the area of the PCB peak was equivalent to the preceding eluting PCB peak (as is the case in an Aroclor 1254 standard).

Blubber Tissue - Pesticides Other Than p,p'-DDE

The concentrations of pesticides other than p,p'-DDE were evaluated in blubber tissue according to the CLP method described previously for the evaluation of p,p'-DDE in blubber tissue.

Liver Tissue - Metals and Bromine

For all metals except mercury, tissue samples were freeze-dried and digested with concentrated nitric acid in a closed pressure vessel in a microwave oven. This digestion procedure is a modification of the strong-acid digestion for tissue described in PSEP (1989b), which was shown by the analytical laboratory to be equivalent to total digestion methods (PSEP 1990b) in terms of accuracy and precision. Metals concentrations (except mercury) in tissue digestates were determined by graphite furnace atomic absorption spectrometry (GFAA) or

inductively coupled plasma-atomic emission spectrometry (ICP). Cold vapor atomic absorption spectrometry (CVAA) was used for determination of mercury.

For analysis of total bromine concentrations, samples were freeze-dried, placed in a Teflon® digestion vessel with sulfuric acid, and heated at 105°C for 1 hour. After cooling, the samples were filtered and analyzed by ion-exchange chromatography.

Liver Tissue - ABN Organic Compounds

The analyses of ABN organic compounds in liver tissue followed modified EPA CLP procedures that were consistent with PSEP recommendations for analyses with relatively low detection limits. Modifications to the protocols included a larger sample size (i.e., 30-64 grams) and a smaller final extract volume for GC/MS analyses. An additional modification was the use of isotope dilution to monitor and correct for recovery of the ABN compounds. Tissue samples were homogenized mechanically, as recommended by PSEP (1989a). Gel permeation chromatography (GPC), an optional step under EPA CLP procedures that is analogous to EPA Method 3640, was performed on all sample extracts to reduce matrix interferences. Care was taken by the laboratory to minimize mechanical losses during GPC cleanup. GC/MS was used to measure ABN compounds using EPA Method 1625C (U.S. EPA 1989).

Histopathological Analysis

In the laboratory, each formalin-fixed tissue sample was dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. The embedded tissue was sectioned at 4-5 μm using a rotary microtome and then stained. Prepared slides were examined using conventional light microscopy.

DATA ANALYSIS

All data collected during the study were entered into a single database and analyzed used SYSTAT (Wilkinson 1990). Results of historical analyses of chemical contamination in harbor seal pups from Puget Sound were also coded in the same format using information from Cascadia databases and information from past reports (Arndt 1973; Calambokidis et al. 1978, 1984). In many cases, biological information was limited for the animals collected in historical studies.

All chemical concentrations were log-transformed prior to statistical analysis. Pairwise comparisons of variables were made using paired and unpaired *t*-tests and evaluations of relationships among variables were made using Pearson's product-moment correlation coefficient (*r*) or bivariate and multiple regression techniques.

RESULTS AND DISCUSSION

Prior to analysis, all laboratory data were subjected to a quality assurance and quality control (QA/QC) review. The results of that review are described in PTI (1991). With the exception of five values, all data generated during this study passed the QA/QC review and were considered acceptable for characterizing chemical contamination in harbor seals from Puget Sound, including those data that were qualified as estimates during QA/QC review. The five values that did not pass the QA/QC review included the liver concentrations of fluoranthene and 3,3'-dichlorobenzidene from Smith Island and Gertrude Island and the liver concentration of butyl benzyl phthalate from Gertrude Island. Each of those values failed the QA/QC review because the criterion for acceptable recovery of the isotopically labeled internal standard for each compound was not met.

The remainder of this section presents the results of the study and a discussion of the implications of those results with respect to chemical contamination of harbor seals in Puget Sound. All of the chemical concentrations presented in this section are expressed on a wet-weight basis. The characteristics of those harbor seal pups selected for chemical evaluations are presented in Table 1.

BLUBBER TISSUE - PCBs AND p,p'-DDE

In this section, comparisons are first made between the results obtained using the CLP and Cascadia methods. Comparisons of tissue chemical concentrations between seals from Smith Island and Gertrude Island and among different time

TABLE 1. CHARACTERISTICS OF THOSE HARBOR SEAL PUPS SELECTED FOR CHEMICAL ANALYSIS FROM SMITH AND GERTRUDE ISLANDS

Seal No.[a]	Sampling Date[b]	Tissues Evaluated for Contaminants: Blubber	Tissues Evaluated for Contaminants: Liver	Sex[c]	Age (weeks)	Standard Length (cm)	Axillary Girth (cm)	Weight (kg)	Blubber Thickness (cm)
S1	4 July	X[d]	X	M	< 1	85	48	11.5	1.5
S2	6 July	X	X	F	< 1	80	49	9.3	1.0
S3	10 July	X	--[e]	F	--	87	44	--	1.2
S4	10 July	X	X	M	1-2	85	41	7.1	0.6
S5	10 July	X	--	M	1	98	56	--	1.4
S6	11 July	X	X	M	1	83	45	8.2	0.9
S7	11 July	X	X	M	1	85	56	13.5	1.8
G1	31 August	X	X	F	2	86	46	--	1.4
G2	4 September	X	X	M	2	86	42	9.6	0.9
G3	4 September	X	X	M	2	86	40	9.2	1.0
G4	9 September	X	X	F	1	85	54	11.1	1.4
G5	12 September	X	X	F	2	85	44	8.5	1.2
G6	13 September	X	X	M	1	87	51	12.6	1.2
G7	13 September	X	X	F	1	84	45	9.5	0.8

[a] The relationships of seal numbers to the sample numbers given in the field and presented in PTI (1991) are as follows: S1 = CRC-344, S2 = CRC-347, S3 = CRC-348, S4 = CRC-355, S5 = CRC-356, S6 = CRC-349, S7 = CRC-350, G1 = CRC-379, G2 = CRC-385, G3 = CRC-387, G4 = CRC-388, G5 = CRC-389, G6 = CRC-392, G7 = CRC-393.

[b] All dates are 1990.

[c] M = male; F = female.

[d] X indicates tissue was evaluated.

[e] -- indicates data not available because seal was partially scavenged, but acceptable for evaluation of other characteristics.

periods are then made using the data considered most appropriate based on the results of the comparison of analytical methods.

Comparison of Analytical Methods

The concentrations of p,p'-DDE determined using the CLP and Cascadia methods were similar (Table 2; Figure 1) and did not differ significantly ($P > 0.05$) between the two methods. By contrast, concentrations of PCBs differed significantly ($P \leq 0.05$) between the two methods. The concentrations that were determined using the Cascadia method were 2-7 times higher than the concentrations determined using the CLP method (Table 2, Figure 2). Although there were large differences in the PCB concentrations based on the two methods, the two sets of concentrations were correlated significantly ($r=0.92$, $P \leq 0.001$). These results indicate that although the relative concentrations among different samples were similar between the two sets of results, one or more factors appeared to be acting in a systematic manner to account for the large differences observed between the two data sets.

Most of the differences in the PCB concentrations obtained using the two methods appeared to be related to differences in the quantification procedures. The total PCB concentrations that were obtained using the Cascadia method were determined by summing the concentrations represented by each separate PCB peak identified on the gas chromatograph. By contrast, the concentrations of PCBs obtained using the CLP method were determined by matching chromatograms to a particular PCB commercial mixture, and then comparing the areas of several "representative" peaks present in the sample and the standard. The quantification of PCBs using the CLP method only included the quantification of Aroclor 1260 because that was the only aroclor identified by the analytical laboratory as being present in the samples.

TABLE 2. CONCENTRATIONS OF PCBs AND p,p´-DDE IN BLUBBER TISSUE OF HARBOR SEAL PUPS FROM SMITH AND GERTRUDE ISLANDS

	Cascadia Method[a]			CLP Method[a]			Adjusted CLP Values for PCB Aroclors[a,b]		
Seal No.[a]	PCBs	p,p´-DDE	Lipids	PCBs	p,p´-DDE	Lipids	1254	1260	1254/1260
Smith Island									
S1	1.4	0.66	78	0.40	0.36	76	0.98	0.98	1.1
S2	1.8	1.0	83	0.56	0.66	73	1.7	1.6	1.9
S3	--[c]	--	--	1.2	0.70	74	2.1	2.5	2.7
S4	19	6.5	50	4.5	8.2	44	13	11	13
S5	--	--	--	0.40 *U*[d]	0.37	75	0.88	0.60	0.92
S6	1.2	0.74	75	0.58	0.44	72	1.5	1.4	1.6
S7	1.1	0.40	84	0.42	0.46	75	1.9	1.4	1.9
Gertrude Island									
G1	18	1.9	--	4.9	2.3	80	13	11	14
G2	--	--	--	5.2	2.7	73	16	14	16
G3	--	--	--	2.9	1.1	70	7.7	6.3	7.8
G4	12	1.5	39	2.5	1.6	75	7.5	6.0	7.5
G5	23	2.7	84	5.3	2.5	80	20	17	21
G6	22	2.6	92	5.6	2.4	82	14	12	15
G7	21	2.8	73	3.1	1.4	69	9.0	8.2	9.5

[a] Concentrations of PCBs and p,p´-DDE are mg/kg wet weight; lipid content is expressed as percent wet weight.

[b] Values are presented for Aroclors 1254 and 1260 both separately and combined. Values were adjusted as described in the text.

[c] -- indicates not analyzed.

[d] *U* - Undetected at detection limit shown.

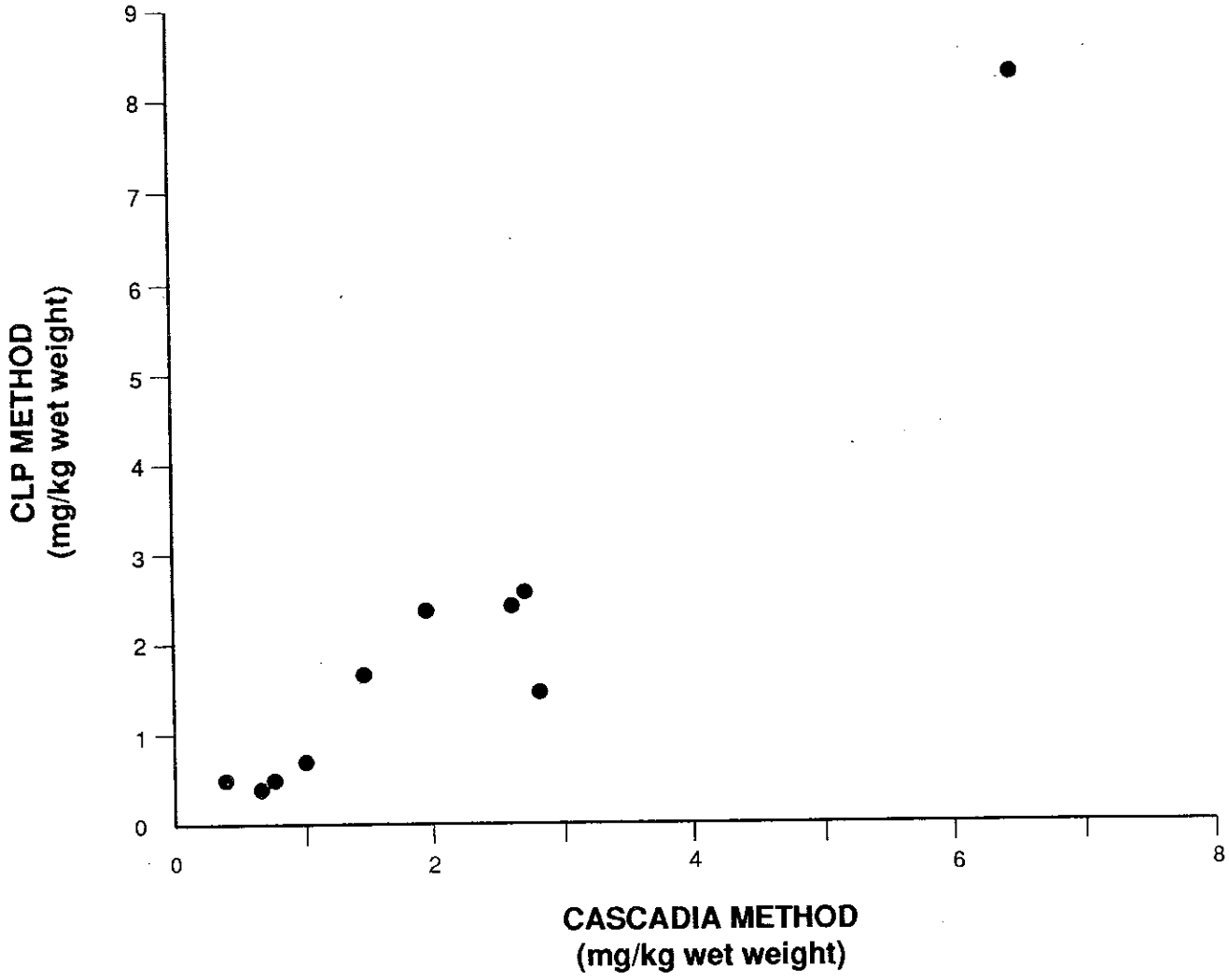

Figure 1. Comparison of p, p'-DDE concentrations in split samples of blubber tissue determined using the CLP and Cascadia methods.

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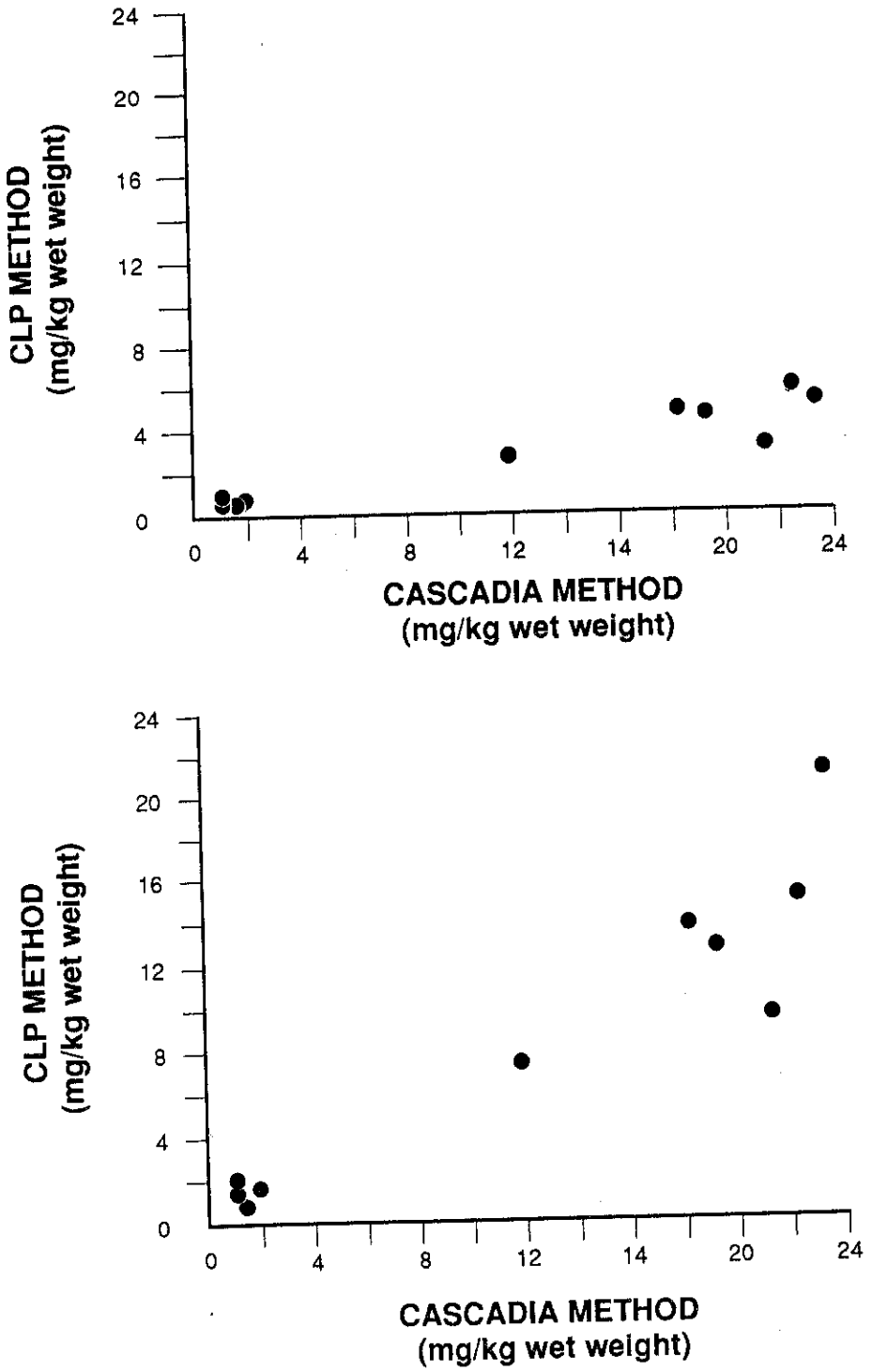

Note: CLP values in upper and lower graphs are unadjusted and adjusted, respectively, as described in text.

Figure 2. Comparison of PCB concentrations in split samples of blubber tissue determined using the CLP and Casadia methods.

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The quantification of PCBs using the CLP method is appropriate for the quantification of samples that contain mixtures of PCBs that resemble commercial PCB mixtures. However, when the sample mixtures do not resemble the commercial mixture, it is difficult to match the sample to a particular standard, and large differences in results can occur depending on which standard or mix of standards is selected for comparison and which peaks are used for quantification of the sample in relation to the standard (see additional discussion in Tetra Tech 1986). This problem is particularly apparent for tissue samples from seals. Because seals feed high on the food chain, the proportion of PCB components can be altered because of selective uptake, retention, and metabolism by different organisms in the food web (Calambokidis et al. 1979b). In addition, seals can selectively metabolize some PCB congeners (Calambokidis et al. 1984; Boon et al. 1987; Jansson et al. 1975; Jensen and Jansson 1976).

Two factors appeared to be responsible for the fact that PCB concentrations obtained using the CLP method were lower than the concentrations obtained using the Cascadia method. The first factor was that the quantification of PCBs by the CLP method was performed using only the standard Aroclor 1260, which did not include PCB congeners outside the range of congeners found in that standard. Based on the results of the Cascadia method, more than one-half of the total PCB concentrations were attributable to peaks that were more typical of Aroclor 1254. The second factor was that only the last three peaks that eluted were selected for quantification of Aroclor 1260 using the CLP method. These peaks were selected because their ratios to each other closely matched the standard. However, because the largest PCB peaks in the Aroclor 1260 range in the sample were actually the earlier eluting peaks, selecting only the three latest peaks underestimated the amount of Aroclor 1260 in the samples.

Given the above considerations, the PCB results obtained using the CLP method were requantified using the standard matching technique for both Aroclor 1254 and 1260, as well as for a mixture of Aroclor 1254 and 1260 (calculated

after analysis using the separate Aroclor 1254 and 1260 standards). Five of the largest peaks from each standard were used for the quantification. Three of these peaks were shared by both Aroclor 1254 and 1260, resulting in the use of seven peaks in the Aroclor 1254/1260 mixture. Because the calculated values of Aroclor 1254 and 1260 in the samples were similar, the Aroclor 1254/1260 mixture was considered the most appropriate standard to use for quantification.

The requantified PCB concentrations (which were obtained using the CLP method) were more similar to the results obtained using the Cascadia method than were the original values (Table 2, Figure 2). However, the two sets of values were still significantly different ($P \leq 0.05$), with concentrations obtained using the Cascadia method generally being higher.

Because the quantification techniques used for the Cascadia method were similar to those used in the past and are generally more appropriate for samples that do not closely match commercial mixtures, they were used as the basis for the evaluations conducted in the present study. However, because the Cascadia method was only used for 10 of the 14 blubber samples, the requantified results (from the CLP method) were used as the best available estimates for the remaining 4 samples.

Comparisons Between Study Areas

PCBs and p,p'-DDE were detected in all 14 harbor seal pups selected for evaluation. Concentrations of PCBs ranged from 0.92 to 23 mg/kg and concentrations of p,p'-DDE ranged from 0.37 to 6.5 mg/kg. As noted in the previous section, the PCB values used for the evaluations conducted in this study included the values determined using the Cascadia method for 10 samples and the requantified values (from the CLP method) for the remaining 4 samples. Concentrations of PCBs were significantly higher ($P \leq 0.01$) in the blubber tissue

of seals from Gertrude Island (mean = 17.1 mg/kg) compared with the concentrations in seals from Smith Island (mean = 4.02 mg/kg). Concentrations of p,p'-DDE in blubber tissue did not differ significantly ($P > 0.05$) between the two study areas (means = 2.19 and 1.48 mg/kg, respectively).

In general, concentrations of both PCBs and p,p'-DDE were similar among the seals sampled within each study area. This consistency was probably related to the restricted sampling period and similar condition of the sampled pups. Unusual concentrations of both kinds of chemicals were found only in a single seal from Smith Island (Seal S4), in which concentrations of both kinds of chemicals were more than 5 times higher than the concentrations found in any other seal from that area. Because Seal S4 had the smallest blubber thickness and lowest lipid concentration of the seals collected at Smith Island, fat may have been mobilized from the blubber reserves, resulting in the unusually high concentrations of contaminants in the remaining blubber tissue.

The higher concentrations of PCBs observed in seals from Gertrude Island, compared with Smith Island, were consistent with the relative concentrations found in different age classes of seals from the two areas in past studies (Calambokidis et al. 1978, 1984). This consistent difference between the two areas is likely the result of the closer proximity of Gertrude Island to some of the chemically contaminated urban bays of central and southern Puget Sound where the concentrations of PCBs are generally elevated in environmental media.

Comparisons with Historical Data

The historical studies used for comparison with the results of the present study included evaluations of the concentrations of PCBs and p,p'-DDE in blubber tissue of seal pups collected from Smith Island and from southern Puget Sound within 20 km from (and including) Gertrude Island. Seals collected from

locations in southern Puget Sound other than Gertrude Island were included in the evaluations to provide a larger sample size. Calambokidis et al. (1984) found that tissue concentrations of PCBs and p,p'-DDE were similar among seals collected throughout southern Puget Sound.

Most of the historical studies used the Cascadia method for determining concentrations of PCBs and p,p'-DDE in the tissue of seal pups. Although some of those studies used different analytical methods, the results were considered appropriate for comparison with the results of the present study. Analyses were conducted in 1972 for three pups from Gertrude Island and one pup from Smith Island (Arndt 1973), using a method in which PCB quantification involved a comparison only to Aroclor 1254. Those results may therefore be underestimates of the concentrations of total PCBs. However, the magnitude of the underestimates should be small, because most of the PCBs in the blubber tissue of harbor seals fall within the range of congeners included in Aroclor 1254. Additional chemical analyses were conducted for seals collected in the late 1970s and early 1980s by the Bodega Bay Institute (Calambokidis et al. 1984). A comparison of duplicate samples run by the Bodega Bay Institute and Cascadia Research Collective showed that the mean concentrations of PCBs were similar between the sets of analyses (Calambokidis et al. 1984).

The historical studies of chemical contamination in harbor seal pups included a wider age range of pups (< 1 week to 3-4 months) than the range used in the present study (< 1-2 weeks). In addition, blubber thicknesses of some of the pups sampled in past studies differed from the thicknesses found in the present study. Because both age and blubber thickness can influence tissue contaminant concentrations, the potential confounding effects of these variables on temporal trends were evaluated using multiple regression analysis.

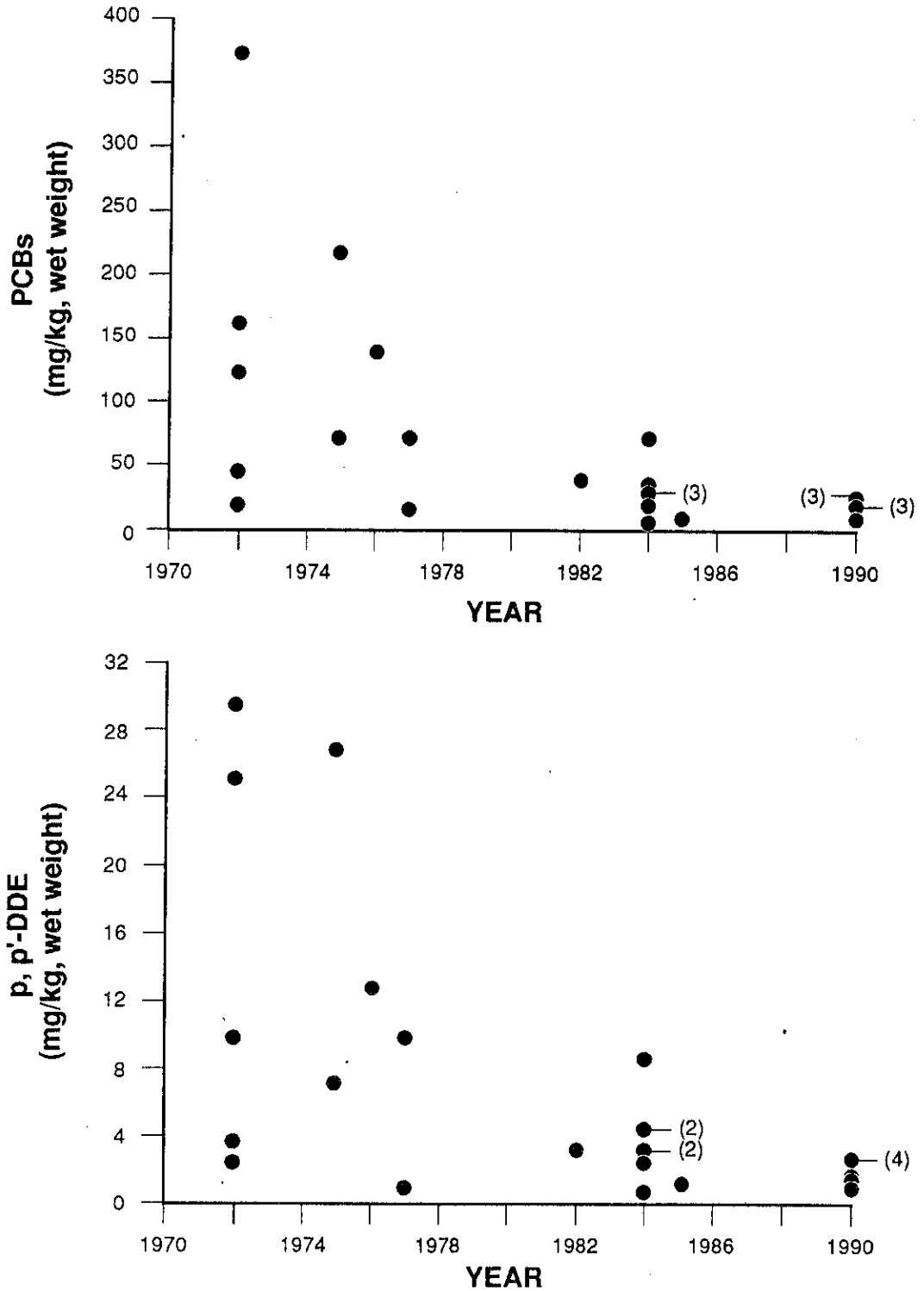
Concentrations of PCBs and p,p'-DDE in the blubber tissue of harbor seal pups from Puget Sound appear to have declined substantially over the past 18

years (Figures 3 and 4). Based on bivariate regression analysis, the concentrations in pups examined from 1972 to 1990 showed a significant decline for both the area near Gertrude Island ($n=26$, $r^2=0.46$, $P \leq 0.001$) and Smith Island ($n=15$, $r^2=0.41$, $P \leq 0.01$). Declines in p,p'-DDE concentrations were also significant for Gertrude Island ($n=26$, $r^2=0.41$, $P \leq 0.001$), but not for Smith Island ($n=15$, $r^2=0.14$, $P > 0.05$).

The results of the multiple regression analysis indicated that PCB concentrations were positively related ($P \leq 0.01$) to pup age at Gertrude Island and negatively related ($P \leq 0.01$) to blubber thickness at Smith Island. However, the temporal decline of PCB concentrations remained significant ($P \leq 0.01$) even after accounting for the effects of age and blubber thickness. The multiple regression analysis also indicated that concentrations of p,p'-DDE were positively related ($P \leq 0.01$) to pup age at Smith Island, and that concentrations declined significantly ($P \leq 0.05$) with time when the effects related to pup age were accounted for.

Factors other than age and blubber thickness were also evaluated for potential relationships with the tissue concentrations of PCBs and p,p'-DDE in harbor seal pups collected between 1972 and 1990. Those factors included pup weight, length, girth, lipid content, and causes of death. None of those factors exhibited a significant relationship ($P > 0.05$) with tissue chemical concentrations. However, these comparisons could only be conducted with limited data sets because information on all of these factors was not available for all historical samples.

To provide additional support for the observed temporal declines in the concentrations of PCBs and p,p'-DDE in blubber tissue, pups from previous years that fell outside the neonatal age range sampled in the present study (< 1-2 weeks) and those from other southern Puget Sound sites besides Gertrude Island were excluded. Significant declines in concentrations were found for both PCBs ($n=18$, $r^2=0.59$, $P \leq 0.001$) and p,p'-DDE ($n=18$, $r^2=0.41$, $P \leq 0.01$) in Gertrude Island pups, which agrees with the results of the analyses conducted for



Note: If points represent multiple samples, the number of samples is given in parentheses

Figure 3. Temporal trends of concentrations of PCBs (above) and p, p'-DDE (below) in blubber tissue of harbor seal pups from southern Puget Sound.

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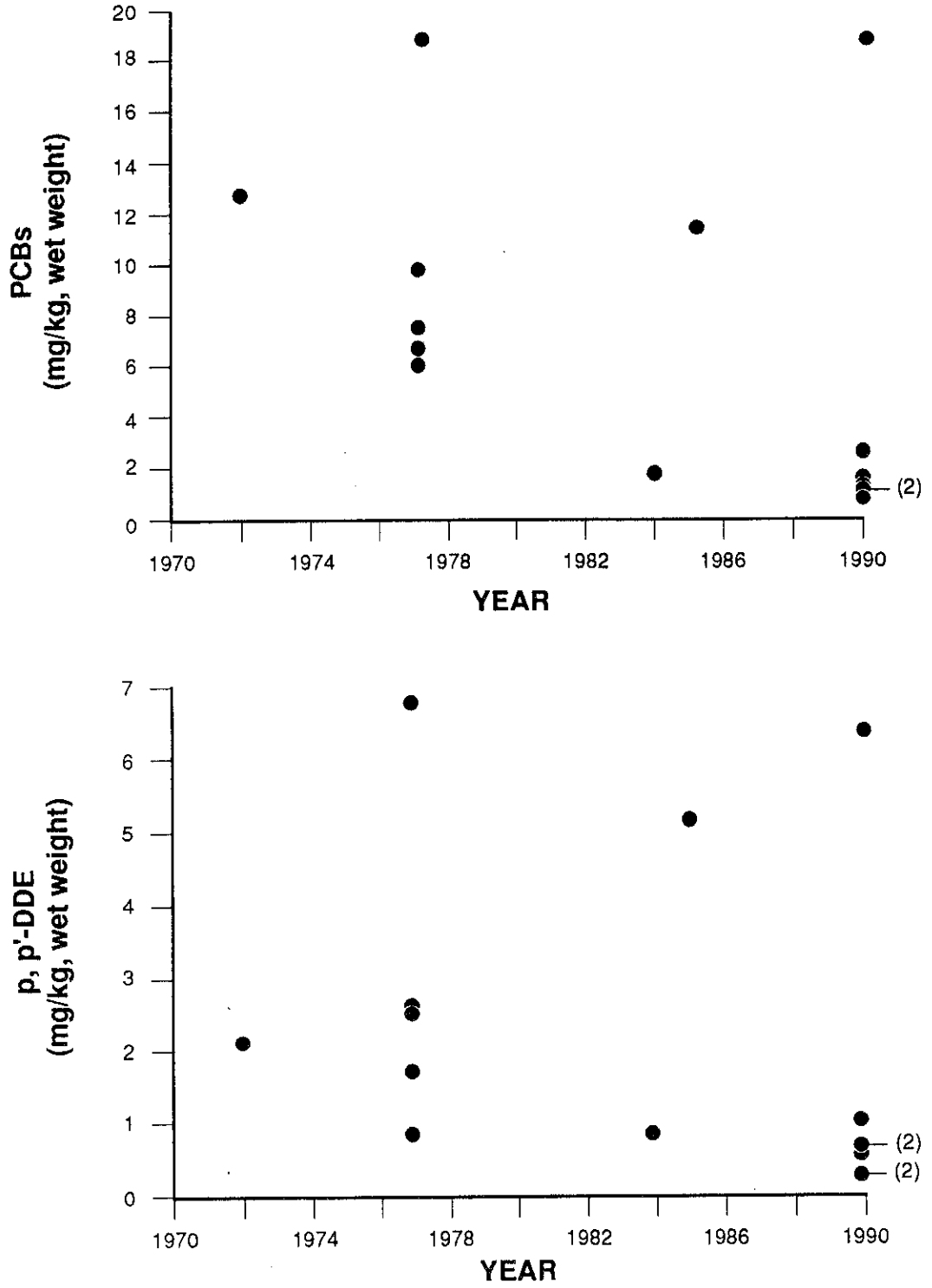

Note: If points represent multiple samples, the number of samples is given in parentheses

Figure 4. Temporal trends of concentrations of PCBs (above) and p, p'-DDE (below) in blubber tissue of harbor seal pups from Smith Island.

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all seals from southern Puget Sound. A similar evaluation could not be conducted for Smith Island because only three of the pups evaluated during historical studies satisfied the age constraint.

Concentrations of PCBs and DDT compounds found in the blubber of harbor seals during this study can be compared with the limited amount of information available for pinniped pups outside Puget Sound. However, these comparisons are complicated by the use of different species, pups in different degrees of emaciation, and different analytical procedures. Nevertheless, several general conclusions can be made based on comparisons with other areas.

The concentrations of both PCBs and DDT compounds measured in the harbor seal pups evaluated during the present study were generally lower than the concentrations reported in pups of several pinniped species from European waters in the 1970s (Holden 1972) and in harbor seal pups collected along the California coast in 1975 and 1976 (Risebrough 1978). In addition, the concentrations of PCBs and DDT compounds measured in harbor seal pups from Gertrude Island in the present study were similar to the concentrations of those chemicals measured in harbor seal pups from England and gray seal pups (*Halichoerus grypus*) from the Farne Islands in 1988 (Law et al. 1989). The concentrations of both PCBs and DDT compounds in harp seal pups (*Phoca groenlandica*) from the northwest Atlantic Ocean and the Arctic Ocean (Ronald et al. 1984) generally were lower than the concentrations of those chemicals measured in the present study.

BLUBBER TISSUE - PESTICIDES OTHER THAN p,p'-DDE

A limited number of pesticides other than p,p'-DDE were detected at low concentrations in the blubber tissue of selected harbor seal pups (Table 3). In 7 of the 14 seals (4 from Smith Island and 3 from Gertrude Island), α-hexachloro-

TABLE 3. CONCENTRATIONS OF PESTICIDES IN BLUBBER TISSUE OF HARBOR SEAL PUPS FROM SMITH AND GERTRUDE ISLANDS

	Seal Number													
	Smith Island[c]							Gertrude Island[c]						
Pesticide[a,b]	S1	S2	S3	S4	S5	S6	S7	G1	G2	G3	G4	G5	G6	G7
Aldrin	-16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	25 *U*	22 *U*	20 *U*	25 *U*	16 *U*	16 *U*
α-Chlordane	24 *U*	24 *U*	24 *U*	120 *U*	24 *U*	24 *U*	24 *U*	24 *U*	24 *U*	24 *U*	24 *U*	80 *U*	24 *U*	24 *U*
γ-Chlordane	24 *U*	24 *U*	24 *U*	80 *U*	24 *U*	24 *U*	24 *U*	130 *U*	24 *U*	60 *U*	60 *U*	24 *U*	110 *U*	50 *U*
Chlorpyrifos	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*
Dieldrin	32 *U*	32 *U*	32 *U*	45 *U*	32 *U*	32 *U*	32 *U*	110 *U*	40 *U*	32 *U*	32 *U*	130 *U*	32 *U*	32 *U*
Endosulfan sulfate	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*	64 *U*
α-Endosulfan	16 *U*	16 *U*	16 *U*	80 *U*	35 *U*	16 *U*	16 *U*	16 *U*	25 *U*	24 *U*	32 *U*	55 *U*	40 *U*	20 *U*
ß-Endosulfan	32 *U*	32 *U*	32 *U*	45 *U*	32 *U*	32 *U*	35 *U*	170 *U*	32 *U*	32 *U*	80 *U*	85 *U*	95 *U*	32 *U*
Endrin	32 *U*	32 *U*	32 *U*	55 *U*	32 *U*	32 *U*	32 *U*	60 *U*	61 *U*	32 *U*	32 *U*	80 *U*	32 *U*	32 *U*
Endrin ketone	48 *U*	48 *U*	48 *U*	55 *U*	48 *U*	48 *U*	48 *U*	80 *U*	70 *U*	48 *U*	48 *U*	95 *U*	48 *U*	48 *U*
Heptachlor	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	21 *U*	80 *U*	20 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*
Heptachlor epoxide	16 *U*	16 *U*	16 *U*	20 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*
α-Hexachlorocyclohexane	48	72	33	180	16 *U*	45 *U*	16 *U*	130 *U*	78	16 *U*	36 *U*	72	110	20 *U*
ß-Hexachlorocyclohexane	16 *U*	16 *U*	16 *U*	38	45 *U*	16 *U*	16 *U*	560 *U*	50 *U*	16 *U*	16 *U*	25 *U*	16 *U*	16 *U*
γ-Hexachlorocyclohexane	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	45 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*	16 *U*
δ-Hexachlorocyclohexane	24 *U*	24 *U*	24 *U*	24 *U*	24 *U*	24 *U*	35 *U*	340 *U*	24 *U*	120 *U*	24 *U*	24 *U*	24 *U*	24 *U*
Methoxychlor	64 *U*	64 *U*	64 *U*	120 *U*	64 *U*	64 *U*	64 *U*	220 *U*	200 *U*	64 *U*	64 *U*	300 *U*	64 *U*	64 *U*
p,p'-DDD	32 *U*	32 *U*	32 *U*	50	32 *U*	32 *U*	32 *U*	45 *U*	57	32 *U*	29 *E*	74	51	32 *U*
p,p'-DDT	32 *U*	60 *U*	60 *U*	370 *U*	32 *U*	60 *U*	32 *U*	240 *U*	280 *U*	210 *U*	200 *U*	320 *U*	400 *U*	270 *U*
Toxaphene	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*	2,400 *U*

[a] Concentrations are µg/kg wet weight.

[b] Concentrations of p,p'-DDE are presented in Table 2.

[c] All of the chemical concentrations presented in this table pass PSEP (1989b) guidelines and are considered acceptable for characterizing tissue contamination in Puget Sound harbor seals. The following qualifiers provide additional information for specific values:

E - Estimated value. These values have a greater degree of uncertainty than unqualified data. Data are generally assigned *E* qualifiers when one quality assurance and quality control result (i.e., matrix spike or matrix duplicate) falls outside of the control limits.

U - Undetected at detection limit shown.

cyclohexane was detected at concentrations of 33-180 μg/kg. ß-Hexachlorocyclohexane was detected in a single seal from Smith Island at a concentration of 38 μg/kg. In 5 of the 14 seals (1 from Smith Island and 4 from Gertrude Island), p,p'-DDD was detected at concentrations of 29–74 μg/kg.

LIVER TISSUE - METALS AND BROMINE

Bromine and all metals except aluminum were detected in the livers of at least some seals (Table 4). The concentrations of two metals, lead (means = 39.7 and 29.5 μg/kg, respectively) and silver (means = 112 and 40.8 μg/kg, respectively), were significantly higher ($P \leq 0.05$) in seals from Smith Island compared to seals from Gertrude Island. These results were unexpected, given the closer proximity of Gertrude Island to known areas of chemical contamination and the higher concentrations of PCBs found in the seals from that location.

Bromine was correlated ($P \leq 0.05$) negatively with selenium, but was not correlated significantly ($P > 0.05$) with mercury. Selenium also was not correlated significantly ($P > 0.05$) with mercury. The reason for the lack of positive associations among these three chemicals was not clear. As noted in the introduction of this report, positive relationships among these chemicals have been found for California sea lions (Martin et al. 1976; Koeman et al. 1973).

The concentrations of metals in liver tissue found in the present study were compared to the concentrations found in seven harbor seal pups collected from Puget Sound between 1972 and 1982 (Calambokidis et al. 1984). The cadmium concentrations found previously ranged from below unspecified detection limits to a value of 480 μg/kg. Some of those values were much higher than the concentrations found in the present study (2.2 *U* - 6.1 μg/kg). The concentrations of lead and mercury found in the historical samples were approximately twice as high as the concentrations found in the present study. The concentrations of

TABLE 4. CONCENTRATIONS OF METALS AND BROMINE IN LIVER TISSUE OF HARBOR SEAL PUPS FROM SMITH AND GERTRUDE ISLANDS

	Seal Number											
	Smith Island[b]					Gertrude Island[b]						
Metal[a]	S1	S2	S4	S6	S7	G1	G2	G3	G4	G5	G6	G7
Aluminum	567 *UE*	487 *UE*	449 *UE*	463 *UE*	505 *UE*	406 *UE*	392 *UE*	645 *UE*	329 *UE*	502 *UM*	501 *UE*	537 *UE*
Arsenic	53.8 *WET*	329 *WET*	29.2 *WET*	32.4 *WET*	27.8 *WET*	102 *UWE*	19.6 *UWE*	161 *UWE*	90.4 *WET*	132 *M*	25 *UWE*	161 *WET*
Cadmium	3.7 *T*	2.4 *U*	2.2 *U*	2.3 *U*	2.5 *U*	2.4 *T*	6.1 *T*	3.2 *U*	3.8 *T*	2.5 *UM*	2.5 *U*	2.7 *U*
Copper	12,700	26,800	5,010	6,630	24,800	12,400	6,110	7,850	4,140	6,630 *M*	4,470	4,910
Lead	48.2 *T*	36.5 *T*	29.2 *T*	41.6 *T*	42.9 *T*	28.4 *T*	35.3 *T*	41.9 *T*	21.4 *T*	27.6 *LM*	25 *UWE*	26.8 *U*
Mercury	610	910	650	2,200	400 *M*	480	340	640	550	500 *M*	520	850
Nickel	28.3 *U*	24.3 *U*	22.4 *U*	23.1 *U*	25.2 *U*	20.3 *U*	78.4 *T*	32.2 *T*	16.4 *U*	26.3 *LM*	25 *U*	26.8 *U*
Selenium	975	1,050	664	1,040	798	536	400	561	615	1,050 *M*	776	714
Silver	83.6	163	27.6 *T*	203	80.8	40.0 *T*	44.3 *T*	49.6 *T*	47.8	31.8 *LM*	23.8 *T*	48.1 *T*
Zinc	65,100 *E*	126,000 *E*	54,000 *E*	64,500 *E*	65,900 *E*	79,400 *E*	39,500 *E*	115,000 *E*	34,300 *E*	84,700 *EM*	52,600 *E*	117,000 *E*
Bromine	14,900	16,600	19,600	15,600	22,600	26,500	50,100	17,500	20,500	18,200	28,700 M	19,600 M
% Moisture	71.1	73.7	76.9	75.4	74.6	79.0	78.5	65.0	79.2	73.9	73.2	72.6

[a] Concentrations are µg/kg wet weight.

[b] All of the chemical concentrations presented in this table pass PSEP (1989a) guidelines and are considered acceptable for characterizing tissue contamination in Puget Sound harbor seals. The following qualifiers provide additional information for specific values:

E - Estimated value. These values have a greater degree of uncertainty than unqualified data. Data are generally assigned *E* qualifiers when one quality assurance and quality control result (i.e., matrix spike or matrix duplicate) falls outside of the control limits.

L - Value is less than the maximum shown.

M - Value is mean.

T - Detected between the limit of detection and the quantification limit; these values are acceptable as estimates.

U - Undetected at detection limit shown.

W - Graphite furnace atomic absorption analytical spike recovery > 115 percent.

copper and zinc found in the present study fell within the same ranges as those found in the historical samples. Because five of the seven pups evaluated from 1972 to 1982 were older (weaned) than the pups evaluated in the present study, some of the observed differences in metals concentrations observed between the two data sets may have been related to age.

Mercury was the only metal for which a number of values for pinniped pups in areas outside Puget Sound were available for comparison with the results of the present study. Mercury concentrations in liver tissue of gray seal pups from Nova Scotia in 1972 (Freeman and Horne 1973) and from harbor seal pups from the Netherlands in 1974 (van de Ven et al. 1979) were higher than the values observed in the present study. By contrast, mercury concentrations in fur seal pups (*Callorhinus ursinus*) from the Pribilof Islands in 1970 (Anas 1973, 1974) and in harp seal pups from the Gulf of St. Lawrence in 1973 (Jones et al. 1976) were lower than the values observed in the present study. Mercury concentrations in harbor seal pups and gray seal pups from the British Isles in 1988 and 1989 (Law et al. 1991) were similar to the values observed in the present study.

LIVER TISSUE - ABN ORGANIC COMPOUNDS

Only two ABN organic compounds were detected in the composited samples of liver tissue collected from harbor seal pups from Smith and Gertrude islands (Table 5). Benzoic acid was detected in the composited samples at estimated concentrations of 140 and 1,200 μg/kg, respectively. 4-Methyl phenol was detected in the composite sample from Gertrude Island at an estimated concentration of 1,400 μg/kg.

TABLE 5. CONCENTRATIONS OF SEMIVOLATILE ORGANIC COMPOUNDS IN COMPOSITE SAMPLES OF LIVER TISSUE FROM HARBOR SEAL PUPS FROM SMITH AND GERTRUDE ISLANDS

Compound	Smith Island[a,b]	Gertrude Island[a,b]
Low Molecular Weight PAH[c]		
Naphthalene	62 *U*	330 *U*
2-Methylnaphthalene	62 *U*	330 *U*
Acenaphthylene	62 *U*	330 *U*
Acenaphthene	62 *U*	330 *U*
Fluorene	62 *U*	330 *U*
Phenanthrene	62 *U*	330 *U*
Anthracene	62 *U*	330 *U*
High Molecular Weight PAH		
Fluoranthene	--[d]	--[d]
Pyrene	62 *U*	330 *U*
Benz(a)anthracene	62 *U*	330 *U*
Chrysene	62 *U*	330 *U*
Total benzofluoranthenes (B + K)	62 *U*	330 *U*
Benzo(a)pyrene	62 *U*	330 *U*
Indeno(1,2,3-c,d)pyrene	62 *U*	330 *U*
Dibenz(a,h)anthracene	62 *U*	330 *U*
Benzo(g,h,i)perylene	62 *U*	330 *U*
Phenols and Substituted Phenols		
Phenol	300 *ZU*	2,600 *ZU*
2-Methylphenol	62 *U*	330 *U*
4-Methylphenol	120 *U*	1,400 *E*
2,4-Dimethylphenol	150 *U*	730 *U*
2-Chlorophenol	62 *U*	330 *U*
2,4-Dichlorophenol	62 *U*	330 *U*
4-Chloro-3-methylphenol	150 *U*	730 *U*
2,4,5-Trichlorophenol	62 *U*	330 *U*
2,4,6-Trichlorophenol	62 *U*	330 *U*
2-Nitrophenol	120 *U*	670 *U*
2,4-Dinitrophenol	120 *U*	670 *U*
4-Nitrophenol	120 *U*	670 *U*
2-Methyl-4,6-dinitrophenol	120 *U*	670 *U*
Pentachlorophenol	120 *U*	670 *U*
Phthalate Esters		
Dimethyl phthalate	62 *U*	330 *U*
Diethyl phthalate	62 *U*	330 *U*
Di-*n*-butyl phthalate	62 *U*	330 *U*
Butyl benzyl phthalate	62 *U*	--[d]

TABLE 5. (Continued)

Compound	Smith Island[a,b]	Gertrude Island[a,b]
Phthalate Esters, Continued		
Bis(2-ethylhexyl)phthalate	64 *ZU*	350 *ZU*
Di-*n*-octyl phthalate	62 *U*	330 *U*
Chlorinated Hydrocarbons		
1,2-Dichlorobenzene	62 *U*	330 *U*
1,3-Dichlorobenzene	62 *U*	330 *U*
1,4-Dichlorobenzene	62 *U*	330 *U*
1,2,4-Trichlorobenzene	62 *U*	330 *U*
2-Chloronaphthalene	62 *U*	330 *U*
Hexachlorobenzene	62 *U*	330 *U*
Hexachlorobutadiene	62 *U*	330 *U*
Hexachloroethane	200 *U*	950 *U*
Hexachlorocyclopentadiene	62 *U*	330 *U*
Halogenated Ethers		
Bis(2-chloroethyl)ether	62 *U*	330 *U*
Bis(2-chloroisopropyl)ether	62 *U*	330 *U*
Bis(2-chloroethoxy)methane	62 *U*	330 *U*
4-Chlorophenyl phenyl ether	62 *U*	330 *U*
4-Bromophenyl phenyl ether	62 *U*	330 *U*
Miscellaneous Oxygenated Compounds		
Benzyl alcohol	310 *U*	1,700 *U*
Benzoic acid	140 *E*	1,200 *E*
Dibenzofuran	62 *U*	330 *U*
Isophorone	62 *U*	330 *U*
Organonitrogen Compounds		
Aniline	120 *U*	670 *U*
Nitrobenzene	62 *U*	330 *U*
N-nitroso-di-*n*-propylamine	62 *U*	330 *U*
N-nitroso-dimethylamine	62 *U*	330 *U*
1,2-Diphenylhydrazine	62 *U*	330 *U*
Carbazole	62 *U*	330 *U*
4-Chloroaniline	190 *U*	1,000 *U*
2-Nitroaniline	310 *U*	1,700 *U*
3-Nitroaniline	310 *U*	1,700 *U*
4-Nitroaniline	120 *U*	670 *U*
2,6-Dinitrotoluene	62 *U*	330 *U*
2,4-Dinitrotoluene	62 *U*	330 *U*
N-nitrosodiphenylamine	62 *U*	330 *U*
Benzidine	3,100 *U*	17,000 *U*
3,3'-Dichlorobenzidine	--[d]	--[d]

TABLE 5. (Continued)

[a] Concentrations are μg/kg wet weight.

[b] All of the chemical concentrations presented in this table pass PSEP (1989b) guidelines and are considered acceptable for characterizing tissue contamination in Puget Sound harbor seals. The following qualifiers provide additional information for specific values:

E - Estimated value. These values have a greater degree of uncertainty than unqualified data. Data are generally assigned *E* qualifiers when one quality assurance and quality control result (i.e., matrix spike or matrix duplicate) falls outside of the control limits.

U - Undetected at detection limit shown.

ZU - Value is less than the related detection limit because blank contamination was present.

[c] PAH - Polycyclic aromatic hydrocarbon.

[d] The results for this compound were rejected during QA/QC review because the criterion for acceptable recovery of the isotopically labeled internal standard for this compound was not met (PTI 1991).

HISTOPATHOLOGICAL ANALYSIS

Results of the histopathological analysis of tissue samples collected from the 14 harbor seal pups selected for chemical evaluations are summarized in Table 6. The prevalence of various histopathological conditions at Smith and Gertrude islands are presented in Table 7. Statistical comparisons of the prevalence of histopathological abnormalities and tissue chemical concentrations were not made because seals were collected from only two sites. Differences in the prevalence of abnormalities between the sites could have been influenced by any variables that differed between the two sites and therefore could not be attributed exclusively to chemical contaminants. In addition, postmortem autolysis of some of the liver samples made it difficult to evaluate the subtle cellular changes that may have been related to chemical contaminants.

Conditions related to infection were found in many of the pups. Umbilical infection (or omphalitis) was found in 7 of the 10 pups for which this tissue was examined. Several of the other infectious conditions were probably related to omphalitis, including septicemia, interstitial pneumonia, cellulitis, peritonitis, and suppurative hepatitis. Multifocal acute hemorrhage was found in a second pup with omphalitis. This condition can be caused by viruses, bacteria, or bacterial toxins.

Most of the pups from Gertrude Island showed signs of infection. In the pups for which 15 or more tissues were examined, all 4 pups from Gertrude Island and 2 of 5 pups from Smith Island had infectious conditions.

Thymic lymphoid atrophy, a sign of stress in young animals, was found in 4 of the 11 pups in which the thymus was examined. All of these animals had omphalitis and therefore would be expected to be stressed. Of these 11 pups (including the 4 with thymic lymphoid atrophy), 10 showed signs indicative of

TABLE 6. SUMMARY OF HISTOPATHOLOGICAL ABNORMALITIES OBSERVED IN INDIVIDUAL HARBOR SEAL PUPS FROM SMITH AND GERTRUDE ISLANDS

	Seal Number													
	Smith Island[a]							Gertrude Island[a]						
Tissue/Abnormality[b,c]	S1	S2	S3	S4	S5	S6	S7	G1	G2	G3	G4	G5	G6	G7
Lungs														
Congestion	3	2	2	2	2	2	3	2	--	0	0	2	2	3
Pulmonary edema	2	0	2	2	2	0	2	0	--	1	0	0	2	2
Hemorrhage	2	0	2	0	0	0	0	0	--	0	0	0	0	2
Presence of amniotic squames	0	0	3	0	2	3	0	0	--	0	0	0	0	0
Presence of other debris	0	0	0	0	2	0	0	0	--	0	2	2	0	0
Interstitial pneumonia	0	0	0	0	0	0	0	2	--	0	0	0	1	0
Atelectasis	0	0	0	0	2	0	0	2	--	0	0	2	2	3
Little or no aeration	0	0	0	0	0	0	0	0	--	0	2	0	0	2
Liver														
Congestion	0	0	--	3	--	3	2	0	0	2	0	0	0	3
Hepatitis	0	0	--	0	--	0	0	3	0	0	0	0	0	0
Vacuolar hepatopathy	0	0	--	0	--	0	0	2	0	2	2	2	0	1
Hemorrhage	0	0	--	0	--	2	0	0	0	0	0	0	0	0
Hemosiderosis	2	3	--	1	--	3	0	1	1	1	0	0	1	0
Bile stasis	0	0	--	0	--	0	0	0	3	0	1	2	1	1
Adrenal Gland														
Multifocal acute necrosis	--	0	--	0	--	0	0	2	--	--	--	2	0	0
Hemorrhage	--	0	--	0	--	0	0	0	--	--	--	0	2	0

TABLE 6. (Continued)

Tissue/Abnormality[b,c]	Seal Number													
	Smith Island[a]							Gertrude Island[a]						
	S1	S2	S3	S4	S5	S6	S7	G1	G2	G3	G4	G5	G6	G7
Thymus														
Thymic lymphoid atrophy	0	0	0	2	0	0	0	0	--	--	--	3	2	2
Degeneration of Hassall's corpuscles	1	1	1	2	2	0	1	2	--	--	--	3	2	2
Other														
Omphalitis	0	0	--	2	--	2	0	3	--	--	2	2	3	2
Peritonitis	0	0	--	0	--	0	0	2	--	0	0	0	2	2
Kidney lesions	0	0	--	0	--	0	0	0	--	0	--	0	2	0

[a] Conditions:

-- not examined
0 not present
1 mild or equivocal
2 present
3 severe.

[b] Autolysis of tissues was absent in Seals S2, S7, and G7; mild to moderate in Seals S1, S3, S6, G3, G5, and G6; and moderate to severe in Seals S4, S5, G1, G2, and G4.

[c] The number of tissues examined for each seal was: S1 = 28, S2 = 28, S3 = 9, S4 = 30, S5 = 7, S6 = 29, S7 = 30, G1 = 32, G2 = 1, G3 = 7, G4 = 9, G5 = 29, G6 = 30, G7 = 35. The number of tissues varied among seals because the organs of some seals were scavenged (and therefore could not be evaluated) and because multiple slides were evaluated (particularly for lymph nodes) for some seals.

TABLE 7. SUMMARY OF PREVALANCES OF HISTOPATHOLOGICAL ABNORMALITIES IN HARBOR SEAL PUPS FROM SMITH AND GERTRUDE ISLANDS

Tissue/Abnormality	Prevalence (percent)	
	Smith Island	Gertrude Island
Lungs		
Congestion	100	83
Pulmonary edema	71	50
Hemorrhage	29	17
Presence of amniotic squames	43	0
Presence of other debris	14	33
Interstitial pneumonia	0	33
Atelectasis	14	67
Little or no aeration	0	33
Liver		
Congestion	60	29
Hepatitis	0	14
Vacuolar hepatopathy	0	71
Multifocal acute hemorrhage	20	0
Hemosiderosis	80	57
Bile stasis	0	71
Adrenal Gland		
Multifocal acute necrosis	0	50
Hemorrhage	0	25
Thymus		
Lymphoid atrophy	14	75
Degeneration of Hassall's corpuscles	86	100
Other Tissue		
Omphalitis	40	100
Peritonitis	0	50
Kidney lesions	0	20

premature involution of thymus cells (i.e., degeneration of Hassall's corpuscles), which is also an indication of stress.

Vacuolar hepatopathy, a sign of accumulation of fat in the liver, was present in 5 of the 12 pups for which the liver was examined. All five of the pups with this condition were from Gertrude Island. In four of those pups, the alteration was compatible with fatty change in the liver. This condition would be expected for animals suffering from starvation. However, blubber thickness in the five pups with vacuolar hepatopathy did not differ significantly ($P>0.05$) from blubber thickness in the pups without that condition. Other possible causes of vacuolar hepatopathy could be interference with protein metabolism or abnormalities resulting from exposure to toxic chemicals.

The high prevalence of umbilical infections found for Gertrude Island seals was consistent with the high prevalence of umbilical ulcerations and scarring found in juvenile harbor seals at Gertrude Island in 1984 (Calambokidis et al. 1985). In that study, the prevalence of omphalitis in seals from Gertrude Island was found to be substantially higher than the prevalence found in seals from other parts of Puget Sound. The umbilical infections noted as prevalent in the present study were probably related to the externally visible conditions found in juvenile seals in 1984.

Several factors may account for the higher prevalence of the umbilical infections at Gertrude Island compared with Smith Island. These factors include:

- The sand substrate at Gertrude Island (compared with the predominantly rock and cobble substrate at Smith Island) could increase the probability of infection through the umbilical opening because of increased abrasion to the umbilical area.

- The lack of substantial wave action on the protected haul-out area at Gertrude Island could result in elevated concentrations of bacteria from seal feces. Exceptionally high concentrations of fecal coliform bacteria from seal feces were found in shellfish collected from this haul-out area in a past study (Calambokidis et al. 1989).

- The higher concentrations of PCBs or other contaminants in southern Puget Sound may result in a reduction of immunological responses in the seals.

Without additional information, the factors responsible for the high prevalence of umbilical infections at Gertrude Island cannot be determined conclusively.

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