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BIOACCUMULATION AND METABOLISM OF TRACE ORGANIC COMPOUNDS IN MARINE SEDIMENTS AND ORGANISMS

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ORGANIC:AQUEOUS PARTITION COEFFICIENTS AS A PREDICTOR OF TRACE ORGANIC ACCUMULATION IN MARINE FOOD WEBS

to the

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SECTION 1

ABSTRACT

Of 101 compounds identified in effluent from the Los Angeles County Wastewater Treatment Plant (JWPCP), 27 were selected for analysis in sediments and in eight species of benthic animals collected from adjacent to the discharge zone. These 27 compounds were chosen based on their effluent concentration, their octanol/water partition coefficient and the rat LD50 toxicity values, which were combined into the Toxic Bioaccumulation Factor (TBAF). Results indicated that there was a significant correlation between the concentration of these compounds in sediments and organisms and their octanol/water partition coefficient. Therefore, the octanol/water partition coefficient has proven to be a useful index for predicting the bioaccumulation of organic contaminants.

We attempted to determine the uptake and loss rates for compounds with high partition coefficients by exposing scorpionfish to environmental concentrations of contaminants in cages near the outfall discharge and depurating contaminated scorpionfish in the lab. Our resluts indicated that this approach was not adequate for determining these rates because the natural variation in contaminant concentrations, the natural changes in lipid content and metabolism and the stress of caging had greater effects on the contaminant concentrations than the exposure level.

Finally, metabolism of trace organic contaminants also effects the bioaccumulation and equilibrium of contaminants by the conversion of the parent compounds into oxygenated metabolites. Our results showed that the majority

of contaminants occurred in animals as their oxygenated metabolites and that most analyses of environmemtal samples do not include these metabolites. Also, analysis of the cytosolic pools of liver tissues indicated that the majority of oxy-metabolites occurred conjugated in the low-molecular-weight pool containing glutathione and sequestered away from sensitive cellular sites. However, in some samples these oxy-metabolites were also detectable in the pool containing enzymes, DNA and RNA as well as the pool containing metallothionein, which is a trace metal binding protein. Conjugation of the oxy-metabolites in these high molecular weight pools suggests that spillover has occurred, possibly causing chronic effects.

SECTION 2

INTRODUCTION

2.1 BACKGROUND

Of more than four million chemicals that are registered, it is estimated that approximately 63,000 are in 'common' use (Maugh, 1978). During the manufacture and use of these compounds, it is highly probable that many of these compounds or their waste products will be discharged into the environment and eventually reach the marine ecosystem. Until recently, few laboratories studied more than a few dozen of these constituents and with the establishment of EPA's Priority Pollutant list (US Environmental Protection Agency, 1977), a growing number of wastewaters are now being analyzed for approximately 120 organic compounds. Yet the compounds on this list may not necessarily include the most environmentally hazardous of these chemicals. Therefore, with the increasing number of organic compounds being discharged and the relatively high cost of analysis, it seemed prudent to devise a means of assigning priorities as to which of these compounds should be routinely monitored.

Of ultimate concern with regards to environmental contaminants is the fate and toxicity of the compound. If the contaminant does not bioaccumulate or cause toxicity, then it should be of less concern than a compound that does. Therefore, the ability to predict the fate of an organic compound and its potential to cause toxicity would greatly aid in the decision making process with regards to discharge of organic contaminants into the environment.

Partition coefficients (PCs) have been widely used to predict the fate of organic compounds. The predictive

capability of PCs has been applied mainly in the pharmaceutical field where it was used to predict the partitioning of drugs between water and lipids in the body (Hansch and Leo, 1979). In addition, in the environmental field, Neely et al. (1974) have used PCs to predict the bioconcentration of several organic compounds in trout from their exposure water. Karickhoff et al. (1979) have used PCs to predict the partitioning of trace organic compounds onto sediments, and McCarty (1981) used PCs to predict the desorbtion of trace organic compounds from soils to groundwater. Because of evidence presented in these previous studies, the octanol/water partition coefficient appeared to have a potential use for predicting the bioaccumulation of organic contaminants from a municipal discharge into a marine food web.

2.2 APPROACH

The approach proposed in this study was to use *in situ* measurements to test the predictive capability of the octanol/water partition coefficient. This was to be accomplished by determining which organic compounds were present in the effluent of a large municipal treatment plant. Then measuring the concentrations of several target compounds covering a wide range of partition coefficients in sediments and animals to determine whether or not the tissue concentrations correlated to their partition coefficients. For example, a compound with a high partition coefficient present in the effluent is predicted to accumulate to a higher concentration in an organism than a compound with a

low partition coefficient. Therefore, the tissue concentrations of the animals exposed to the effluent discharge should increase with increasing partition coefficient, and animals should accumulate only those compounds with high PCs to any significant degree.

The main problem with the above approach is that it does not indicate how long an animal needs to reside in the discharge zone to accumulate these compounds. Gossett et al. (1982) have shown that benthic fish feeding near the discharge zone accumulate DDTs and PCBs to significant higher concentrations than pelagic fish that feed for a short time while migrating through the area. Knowing the rate of uptake for organic contaminants would be useful because the residence time needed for an animal to accumulate a significant amount of contaminants could also aid in regulating the rate of discharge of that contaminant. For example, an indicator species from the discharge zone could be chosen and monitored so that the rate of uptake in that organism could be controlled by the rate of discharge so that the assimilative capacity of the environment is not exceeded. Therefore, additional experiments were designed to provide information on the uptake and loss rates for certain contaminants. Originally, intertidal mussels were the organism of choice, however, due to the high variability in the physiology of mussels during the reproductive cycle (Brown et al., 1982a), we decided to try using caged fish instead. Therefore, to measure the uptake and loss rates for organic contaminants, fish that were collected from a

relatively clean area were placed in cages at the discharge zone so the source of contaminants and the period of exposure could be controlled.

Another major factor controlling the bioaccumulation of contaminants is the ability of organisms to metabolize them. In order to accurately predict the bioaccumulation of organic contaminants and also to explain why some contaminants do not accumulate as predicted, the affects of metabolism on the equilibrium between uptake and degradation of contaminants should also be studied. The mechanisms by which this metabolism occurs have been elucidated on studies involving rats and other rodents (Allen et al., 1974, 1975, 1976; Gingell and Wallcave, 1974). The initial response to exposure to hydrocarbons is a proliferation of the endoplasmic reticulum in liver tissue and an increased synthesis of lipids within these cytoplasmic membranes (Allen et al., 1974, 1976). There is a concomitant increase in blood lipids which may serve as a transport mechanism for the transfer of hydrocarbons from the liver to the adipose tissue. Since these compounds are associated with the endoplasmic reticulum, it is postulated that the enzymes which metabolize hydrocarbons are also located there (Hart and Fouts, 1965). These enzymes are known as the mixed function oxidase (MFO) enzymes, and have been found to be inducible by such environmental contaminants as PCBs and benzo-a-pyrene (Spies et al., 1982; Kezic et al., 1983). The metabolism of hydrocarbons usually involves the addition of an oxygen to the hydrocarbon to form a highly reactive and toxic epoxide

intermediate (Brodie et al., 1971; Jerina and Daley, 1974; Shimada, 1976). This highly reactive intermediate can attach to macromolecules including proteins, DNA and RNA with possible toxic effects. Or, these compounds can be deactivated by conjugation onto other cellular molecules such as glutathione or glucuronic acid to form water soluble conjugates that are not toxic to the cell. It has been suggested that whether toxic effects will occur or not depends upon the ratio of the activation of hydrocarbons to their deactivation processes (Brodie et al., 1971; Jerina and Daley, 1974).

While a considerable number of studies have been done on the metabolism of trace organic compounds to oxygenated metabolites by marine organisms in the laboratory (Malins et al., 1979; Varanasi et al., 1979; Krahn et al., 1982), little has been done on the actual concentrations of these oxy-metabolites in the marine environment. Lab studies have indicated that the concentration of oxy-metabolites in organisms could reach up to ten times higher than the concentration of precursor or parent compounds (Varanasi, 1979). Yet, no one is routinely measuring these compounds or considering them as a source of contamination in the marine environment. With the high abundance and diversity of parent trace organic compounds in the marine environment from municipal wastewater discharges (Eganhouse and Kaplan, 1982) and the ability of organisms to accumulate these parent compounds to high concentrations (Gossett et al., 1983; Young et al., 1980), the lack of attention paid to the oxy-

metabolites could mean that regulating agencies are missing a major component of environmental contamination.

2.3 OBJECTIVES

The objectives of this research were to: 1) determine if the octanol/water partition coefficient is useful in predicting the bioaccumulation of trace organic contaminants in marine organisms exposed to wastewater discharges, 2) to measure the uptake and loss rates of trace organic contaminants with high partition coefficients by caging organisms near the outfall discharge, and 3) to measure the concentration and cellular distribution of oxy-metabolites in marine organisms containing low verses high concentrations of trace organic contaminants.

SECTION 3

METHODS

3.1 SAMPLE COLLECTION

From November 1980 to August 1981, quarterly, one week composites of final effluent from the Los Angeles County Wastewater Treatment Plant (JWPCP) were collected for analysis of extractable organics and two grab samples were collected for analysis of volatile organics. Corresponding sediment samples were also collected from station PV3-1 (Figure 1) using a modified Van-Veen grab device. Seven species of animals were collected using a standard otter trawl from station PV3-1 during June 1981. At the same station, a 1mm metal net on a sled was used to collect samples of small invertebrates from just above the bottom. This sample contained 74% (by weight) mysids and 23% decapod shrimp.

For the caged fish experiment, scorpionfish (*Scorpaena guttata*) were collected from station PV7-3 and Dana Pt. (Figure 1) by standard otter trawl. Five cages made of 2.54 square cm galvonized wire mesh that were 1.2 m square and 0.6 m high were placed at each station. The cages were weighted with cement blocks, attached to buoys at the surface and baited to attract food for the fish.

All samples collected were dissected under clean conditions (when appropriate) immediately after collection, frozen at -20 C for chemical analysis or frozen at -80 C for enzyme activities and cytosolic separations.

3.2 SAMPLE EXTRACTION

Extraction of the wastewater was performed according to EPA Priority Pollutant Protocol (US Environmental Protection

Agency, 1977). Extraction of the sediment and animals for volatile organics was similar to the wastewater protocol for volatile organics, except that the samples were placed in a closed container, diluted 3:1 with water and homogenized. Internal standards were added and the vial was swirled again and allowed to equilibrate. The screw cap and septum were removed and a stainless steel cap with an inlet tube and exit port was attached to the sample vial. The vial was immersed in an 80 C water bath then purged with nitrogen gas onto a carbon trap for 12 min. The trap was then heated to 200 C for 5 min and backflushed onto the GC/MS column. For the base-neutral and acid extractable compounds, sediments and animals were sohxlet extracted for 18 hours using pesticide quality hexane/acetone. Then extracted for the base-neutral compounds followed by extraction for the acid compounds. For determination of the DDTs and PCBs, the tissues were homogenized using a high speed blender with pesticide quality acetonitrile, then extracted into hexane.

Samples analyzed for oxygenated metabolites were extracted by homogenization with 2% sodium hydroxide then extracted three times with pesticide quality hexane/acetone (1:1). The top (hexane) layers were combined and analyzed for the base-neutral parent compounds. The bottom (aqueous) layer was heated to 90 C for 30 min, then adjusted to a pH of <2 and extracted three times with pesticide quality methylene chloride. The extracts were combined then dried over sodium sulfate, roto-evaporated dry and 5 ml of methylating agent (5 mg 3-methyl-1-p-tolyltriazene/1 ml diethyl ether) were added.

The sample was then diluted to 50 ml with 30% ether in hexane for analysis for the oxy-metabolites.

Tissues were separated into cytosolic pools by diluting them in 0.1 M TRIS-HCl (pH=7.4) buffer (3 parts buffer to 1 part tissue) then homogenized at high speed. The homogenate was then centrifuged at 100,000 X G and the bottom (pellet) and top (lipid) layers were collected and weighed. Seven ml of the remaining soluble fraction (cytosol) were separated according to molecular weight on a Pharmacia 16/70 column packed with Sephadex G-75 gel, and collected as 3 ml fractions. The fractions were then combined as the high molecular weight (> 20,000 daltons) enzyme-containing (ENZ) pool, the middle molecular weight (3,000 to 20,000 daltons) metallothionein-containing (MT) pool and the low molecular weight (< 3,000 daltons) glutathione-containing (GSH) pool. The identification of these pools was based on the analysis of the individual pools for zinc, which was used to identify the MT pool.

3.3 SAMPLE ANALYSIS

Analysis of the wastewater for volatile organics was performed using a Finnigan high resolution gas chromatograph/mass spectrometer (GC/MS) equipped with an Incos data system and a 60 m SE-54 fused silica capillary column for the base-neutral and acid extractable compounds or a 0.2% Carbowax packed column for the volatile compounds. Analysis of tissues and sediments for the base-neutral and acid extractable compounds was performed on a Varian GC equipped with a 30 m SE-54 fused silica capillary column and

either an electron capture detector (ECD) or a flame ionization detector (FID). DDTs and PCBs were determined on a Tracor GC/ECD equipped with a column packed with 1.5% OV-17 and 1.95% QF-1 held at 200 C. Analysis for naphthalene and related compounds was by C-18 reverse phase high pressure liquid chromatography (HPLC) using UV detection at 254 nm.

GC/MS quantification of EPA Priority Pollutants was accomplished by comparison with individual standards. All other GC/MS quantifications were based on ion intensity relative to an internal standard and are accurate to within a factor of ten. Results obtained by GC/ECD, GC/FID and HPLC were based on individual standards. DDT results were corrected for PCB interferences using previous reported methods (Liu-HU et al., 1980). Procedural blanks and standards were analyzed with every set of sample extractions and results were corrected for blanks and extraction efficiencies.

Analysis for the oxy-metabolites was performed using a 1.5% OV-17 plus 1.95% QF-1 packed column on a Tracor GC/ECD and using a 30m SE-54 fused silica capillary column on a Varian GC/ECD. The oxy-metabolites were also confirmed by a Dupont GC/MS equipped with an identical 1.5% OV-17/1.95% QF-1 packed column using selected ion monitoring and a Finnigan high resolution GC/MS equipped with a SE-30 fused silica capillary column using the total ion chromatogram.

SECTION 4

RESULTS

4.1 PREDICTING BIOACCUMULATION

Approximately 101 compounds were identified in effluent from the Los Angeles County Wastewater Treatment Plant (JWPCP) and are presented in Table 1. The concentrations of identified compounds ranged from 0.003 ug/l for 2,4'-DDT to 2500 ug/l for hexadecanoic acid. Eleven of the compounds were present at concentrations greater than 100 ug/l, while 65 were 10 ug/l or greater. Of the 101 compounds identified, 46 were base-neutral extractable, 39 were acid extractable and 16 were volatile organics. Thirty-six of the 101 compounds were on the EPA's Priority Pollutant list (US Environmental Protection Agency, 1977). Twenty-three were base-neutral extractables, 3 were acid extractables and 10 were volatile organics. Only 11 of the 36 Priority Pollutants were above the quantification limits set by the EPA.

The 101 compounds were ranked by a factor divised by us called the toxic bioaccumulation factor (TBAF) which is calculated by multiplying the effluent concentration by the partition coefficient (PC) and dividing by the rat LD50 (NIOSH, 1978). This factor ranks the compounds by their potential to bioaccumulate and cause toxicity. From the list of 101 compounds, 27 were selected for subsequent analysis in sediments and in eight species of marine benthic animals collected from the discharge zone at station PV3-1 (Figure 1). Ten of these compounds were base-neutral extractable, 4 were acid extractable and 13 were volatile organics. These covered a range of partition coefficients from 1.48 to 6.19 (on a log basis) and effluent concentrations from 0.013 to

980 ug/l (Table 2).

Results from the analysis of sediment and animals for the 27 selected compounds are presented in Table 2. DDE (2,4'-DDE + 4,4'-DDE) was present at the highest concentration in sediments and animal tissues. Pacific sanddab liver contained the highest concentration of DDE at 122 mg/wet kg. With the exception of phenol in tissues, all the base-neutral and acid extractable compounds were detectable while only 6 of the 13 volatile organics were detectable at low concentrations in animal tissues and none were dectectable in sediments.

Results of Spearman's (nonparametric) rank correlation test (Zar, 1974) indicated: 1) that sediment and tissue concentrations of the 27 selected compounds were positively correlated with their partition coefficients (Log Kow), 2) that sediment and tissue concentrations of these compounds were negatively correlated with their effluent concentrations, and 3) that tissue concentrations were positively correlated with sediment concentrations (Table 3).

4.2 CAGED FISH EXPOSURE RESULTS

California spotted scorpionfish (*Scorpaena guttata*) were chosen for exposure to contaminated and clean conditions to determine the uptake and loss rates of trace organic contaminants with high partition coefficients. Scorpionfish were chosen because they occur at both clean and contaminated areas, have large livers and provide sufficient sample for analysis and accumulate trace organic compounds to significant concentrations (Table 2). Also, they do not have swim bladders and can withstand the changes in pressure during handling and are benthic and can survive being caged on the bottom by feeding on the natural fauna.

Scorpionfish were caught from several areas to determine the total DDT (ortho and para isomers of DDE, DDD and DDT) and total PCB (Aroclor 1242 and Aroclor 1254) concentrations and their coefficient of variation. Results of these analyses indicated that the natural variation in fish with elevated concentrations is very high (Tables 4 and 5). In liver tissue of scorpionfish taken from the Channel Islands (Catalina and Anacapa), the mean concentration ($\pm$ 1SD) of total DDT was 1.6 $\pm$ 0.4 mg/wet kg and total PCB was 0.30 $\pm$ 0.21 mg/wet kg. If these fish were separated by island, the variation in PCB concentrations would compare to that for DDTs. This would indicate that these fish were exposed to the same concentration of DDTs but not PCBs. In fish caught at Dana Pt. (Figure 1) in March, the mean concentration for total DDT was 6.1 $\pm$ 6.6 mg/wet kg and for fish caught in October the mean total DDT was 6.7 $\pm$ 3.3 mg/wet kg. Total PCB in the

March fish was 0.39 ± 0.28 mg/wet kg and in the October fish, total PCB was 1.2 ± 0.4 mg/wet kg. Fish caught near the outfall discharge at Palos Verdes (PV7-3; Figure 1) in March had a mean total DDT concentration of 35 ± 21 mg/wet kg and for total PCB, the mean was 2.1 ± 1.3 mg/wet kg. Several scorpionfish caught at PV7-3 were placed in cages at PV7-3 for 72 days to determine if the coefficient of variation could be lowered by insuring that the fish had resided in the area for a significant period of time. This resulted in a slight increase in the concentration of total DDT from 35 ± 21 mg/wet kg to 37 ± 25 mg/wet kg. The total PCB also increased from 2.1 ± 1.3 mg/wet kg to 2.2 ± 1.5 mg/wet kg.

Next, scorpionfish were reciprocally transplanted between PV7-3, a highly contaminated zone, and Dana Pt., a relatively uncontaminated zone (See Section 3 for Methods). Five cages with 20 fish in each cage were placed at both sites. Four of the cages contained fish from the opposite collection site while the fifth cage contained fish from the original collection area, which were used as experimental controls. Approximately 10 fish from the transplanted cages and 3 fish from the control cage were sampled on a periodic basis and analyzed for total parent compounds (total DDT plus total PCB). Unfortunately, the survival rate of the caged fish was lower than we expected. All of the fish caged at Dana Pt. were missing at the first sample period at four weeks. This was probably due to small isopods that stormed the cages and ate the fish within hours after placement (Stepien, 1983). Since these isopods are absent at

contaminated station PV7-3 (Word, 1977), we were able to obtain samples from these cages for up to 45 days and used these fish to determine the uptake rate of the total parent compounds.

The results of the analyses for total DDT and total PCB are presented in Tables 4 and 5. The scorpionfish livers ranged in mean (± 1SD) total parent compound concentrations from 1.9 ± 0.5 mg/wet kg in the Channel Island fish to 39 ± 26 mg/wet kg in the PV7-3 fish caged at PV7-3 for 72 days. Analysis of variance (ANOVA) techniques revealed a significant difference in liver concentrations of total parent compounds ($F= 8.79$, $p<.001$). Scheffe's multiple comparison test indicated that the data could be divided into two groups that were significantly different. One group contained the PV7-3 non-treated fish and the PV7-3 fish caged at PV7-3 for 72 days. The other group contained the Channel Island fish, the Dana Pt. non-treated fish and the Dana Pt. fish caged at PV7-3 for 12 and 45 days. The PV7-3 fish depurated in the lab for 28, 56 and 126 days and the Dana Pt. fish caged at PV7-3 for 27 days were not significantly different from either group.

When compared on a mass basis, the mean (± 1SD) mass of total parent compounds in the livers of the Channel Island fish was 0.027 ± 0.015 mg. The mean mass of total parent compounds in the Dana Pt. non-treated scorpionfish livers was 0.16 ± 0.19 mg in the fish collected in March and 0.072 ± 0.063 mg in fish collected in October. The Dana Pt. fish caged at PV7-3 contained 0.10 ± 0.08 mg total parent

compounds in the 12 day sample, 0.12 ± 0.15 mg in the 27 day samples and 0.057 ± 0.074 mg in the 45 day samples. The PV7-3 non-treated fish contained 0.22 ± 0.15 mg total parent compounds and the PV7-3 fish caged at PV7-3 for 72 days contained 0.55 ± 0.25 mg. The PV7-3 fish depurated in the lab resulted in 0.23 ± 0.15 mg for the 28 day samples, 0.28 ± 0.14 mg from the 56 day samples and 0.19 ± 0.10 mg from the 126 day samples. ANOVA indicated a significant difference in the mean mass of total parent compounds ($F= 9.08$, $p<.001$), but Scheffe's multiple comparisons test indicated that only the 0.55 ± 0.25 mg measured in the PV7-3 fish caged at PV7-3 for 72 days was significantly different from the other group. The PV7-3 fish depurated in the lab for 28 and 56 days were not different from either the PV7-3 fish caged at PV7-3 for 72 days or the other group containing the remaining samples.

The whole body weight (WBW) of a normal fish is related to its total length (TL). To determine if fish used in the different treatments suffered a weight loss due to lack of food in the cages or lab aquaria, the log of the whole body weight was regressed against the log of the total length (Log WBW= Log a + b Log TL). The slopes (b) of the regression lines were then tested for a significant difference, which would indicate a change in body weight. The results of this test indicated that there was no significant difference in the data ($F=.172$, $p>.2$). Therefore, the fish suffered no change in body weight due to the treatments.

4.3 METABOLISM AND EFFECTS OF TRACE ORGANIC COMPOUNDS

The result of the metabolism of trace contaminants within an organism is that the oxy-metabolites are conjugated, which excludes them from a normal scan for environmental contaminants. This is because the conjugated metabolites are water soluble and would be difficult to extract with an organic solvent even at a low pH. Even if they were extracted, they would not chromatograph at the same position as the other environmental contaminants. Therefore, we adapted an extraction procedure from mammalian literature (Gold et al., 1980) which would allow us to analyze for the oxy-metabolites in tissues of marine organisms and sediments.

There were two major changes added to our extraction technique which were used in the mammalian technique for the analysis of cellular compounds. First, we used centrifugation instead of filtration to separate the organic solvent from the remaining part of the sample, which included water and the components of the tissue which were not soluble in the organic solvent at a base-neutral pH. This change made it possible to carry the entire remaining sample on to the acid extraction instead of filtering the solid part of the sample out. Second, we added a heat-catalyzed base-hydrolysis step between the base-neutral extraction and the acid extraction. This step deconjugated the oxy-metabolites from their cellular cofactors and made the oxy-metabolites available for extraction and analysis by conventional techniques.

Samples of sediments, shrimp (*Sicyonia ingentis*) and scorpionfish (*Scorpaena guttata*) were collected from two

stations off southern California (SMB2-3 and PV7-3; Figure 1). The results comparing these samples extracted with and without the heat-catalyzed base-hydrolysis step are presented in Tables 6 and 7. The oxy-metabolites of both total DDT and PCB were not detectable without the heat-catalyzed base-hydrolysis step included in the technique. When this step was included in the extraction, results indicated that the concentration of the oxy-metabolites of DDT (DDTols) and PCB (PCBols) comprised 85 to 99% of the total contaminants (parent compounds + oxy-metabolites) in shrimp, 36 to 90% in scorpionfish and 80 to 99% in sediments. Therefore, without the analysis of environmental samples for oxy-metabolites, approximately only 10% of the total contaminants present are measured.

Results from analyzing the cytosolic pools (See Section 4 for Methods) in mussels (*Mytilus californianus*) from 3 sites along the southern California coast collected at 2 different time periods are presented in Figure 2. The majority of oxy-metabolites in the cytosol of mussel soft parts were present in the GSH pool. However, there were trace amounts of oxy-metabolites present in both the MT and ENZ pools. There were higher concentrations of oxy-metabolites present in the December samples compared to those collected in September (Table 8). The ratio of oxy-metabolites to parent compounds was similar to that in the sediment, shrimp and scorpionfish livers presented earlier. This ratio (Table 8) indicated that the oxy-metabolites of both total DDT and total PCB were the majority of contamination present (greater

than 96%). Histopathological examination resulted in no discernable pathological condition related to contamination (Brown et al., 1982a).

Analysis of the cytosolic pools of white croaker (*Genyonemus lineatus*) livers from Dana Pt. also indicated that the main portion of oxy-metabolites present were sequestered in the GSH pool and were 95% of the total contaminants present (Table 9, Figure 3). White croaker livers from Palos Verdes resulted in the majority of oxy-metabloites being detected in the MT pool and they were also detectable in the ENZ pool. In these fish, oxy-metabolites were only 41 % of the total cytosolic contaminants. Along with the higher concentration of contaminants in the PV fish, there was a decrease in enzyme activity as well as detectable liver pathology (Perkins et al., 1982).

The scorpionfish used for determination of uptake and loss rates of DDTs and PCBs (Section 4.2 of this report) were also examined for cytosolic contaminants, enzymes and histopathology. Results from the analysis for the oxy-metabolites of DDT in cytosolic pools (Figure 4) indicated no difference in the concentrations of oxy-metabolites in the GSH pools during the exposure period. There were low concentrations of oxy-metabolites in the MT pools and no detectable oxy-metabolites in the ENZ pools.

Analysis for the activity of the enzyme cytochrome P-450, which is the enzyme associated with the metabolism of organic contaminants, in scorpionfish liver tissues showed increased P-450 activity in the Dana Pt. fish caged at PV7-3

for 45 days (Figure 5). This activity was higher than the non-treated fish taken from the contaminated PV7-3 station. Free glutathione, which is responsible for binding with the epoxide intermediates to detoxify them (Jerina and Daley, 1974), also increased in the Dana Pt. fish caged at PV7-3 for 45 days over the Dana Pt. non-treated fish (Figure 4). The activity of the enzyme catalase, which catalyzes the reduction of hydrogen peroxide and conjugation of the epoxide intermediates onto glutathione (Lehninger, 1982), was also measured and showed a slight increase in activity for the caged fish. However, the results for cytochrome P-450, oxy-metabolites in cytosolic pools, glutathione and catalase were on single analyses of composites of 10 individuals and could not be analyzed statistically. These results were only preliminary and should only be used to suggest possible trends and recommendations for further research.

Seventeen histopathological conditions were rated in the scorpionfish livers from the PV7-3 fish, the Dana Pt. non-treated fish and the Dana Pt. fish caged at PV7-3 for 12, 27 and 45 days. Each fish was given a rating of 0,1,2 or 3 (0 being best) for each of the 17 conditions without knowledge of the origin of the sample. Then each condition factor was totalled for the fish from each treatment (n=10). These results are presented in Table 10 along with results of the statistical analyses. ANOVA results indicated significant differences in hepatic sclerotic foci (HSF) and cytoplasmic inclusions (CI) between livers of the different treatments. No significant differences for the other histopathological

conditions were detected. Student-Neuman-Keuls (SNK) tests of the HSF results indicated that: 1) the Dana Pt. non-treated fish, the Dana Pt. fish caged at PV7-3 for 12 days and the PV7-3 non-treated fish were not significantly different from each other, 2) the Dana Pt. fish caged at PV7-3 for 27 days grouped by itself, and 3) the Dana Pt. fish caged at PV7-3 for 45 days were not significantly different from either group. For the CI condition, the Dana Pt. non-treated fish formed one group and the Dana Pt. fish caged at PV7-3 for 27 days formed the other group. The Dana Pt. fish caged at PV7-3 for 12 and 45 days and the PV7-3 non-treated fish were not significantly different from either group.

SECTION 5

DISCUSSION

Results of this study indicate that the concentrations of organic compounds in organisms from the open coastal ocean are inversely correlated to their effluent concentrations. Also, there was a high positive correlation between the PCs, the sediment concentrations and the concentrations of the target compounds in the organisms (Table 3). This bioaccumulation is directly related to the potential of the compound to accumulate in sediments, and this potential can be predicted by the octanol/water partition coefficient. For example, Aroclor 1254, which has a low effluent concentration (0.052 *ug*/l) and a high PC (log Kow=6.11) was present in fish liver at high concentrations (615-4920 *ug*/wet kg), while benzene, which has a relatively high effluent concentration (220 *ug*/l) but low PC (2.15), was present in fish liver at low concentrations (1-52 *ug*/wet kg). These data indicate that the EPA priority pollutant criteria would have eliminated from consideration those substances which are accumulated to the highest concentrations in organisms and which are the most toxic.

The PC may give a good indication of an organism's ability to bioaccumulate organic contaminants because octanol has the same hydrophobic properties as lipids in cells upon which dermal absorption is dependent (Freed and Chiou, 1981). However, absorption from the gastrointestinal tract is probably independent of the PC since organisms are designed to absorb both lipophilic and hydrophilic substances (Marshall and Hughes, 1967). Since the main route of organic contaminants in marine organisms appears to be via their food

supply (Young et al., 1980; Schafer et al., 1982), uptake may depend largely upon availability of contaminants to the base of the food web. The base of marine food webs consists largely of particulates in both the pelagic and benthic environments. A large portion of those organic compounds with high partition coefficients, which are present in effluent, would most likely be attached onto paticulates in the effluent since these compounds would be highly insoluble in water (Karickhoff et al., 1979). A large portion of these particulates are known to settle in the region of the outfalls (Hendricks, 1977) and therefore could provide a source of organic contaminants with high PCs to foodwebs in these areas. Contaminants with low PCs would be less likely to attach to particulates, would dilute out in the ocean as they are discharged and therefore would be less available to foodwebs. It is concluded that partition coefficients in addition to wastewater concentrations of contaminants, are important tools for making management decisions concerning safe or allowable inputs to the environment.

Following the determination of the predictive capabilities of the octanol/water partition coefficient, we proposed exposing caged mussels to the sediments near the outfall zone to determine rates of uptake as well as depuration for several of the target compounds. However, since mussels undergo severe physiological changes during their reproductive cycle causing up to a 30% variation in contaminant levels (Farrington et al., 1983; Brown et al., 1982a), we decided to choose another organism that is

resident in the outfall zone. California scorpionfish were chosen for determining bioaccumulation and depuration rates by caging near the outfall zone because of their large livers, lack of a swim bladder and overall hardiness to capture and handling.

It appeared that the scorpionfish from the contaminated station at PV7-3 (Figure 1) depurated in the lab for 126 days decreased in mean liver concentrations of parent compounds (DDTs + PCBs) from 37 mg/wet kg to 13 mg/wet kg. However, when the mass of parent compounds in the liver was compared, no significant change was detected (0.22 mg parent compounds in the PV7-3 non-treated fish and 0.19 mg in the fish depurated for 126 days). Actually, the concentration changed because the weight of the liver changed due to an increase in lipid content, not because of a loss in contaminants. The mean liver weight of the PV7-3 non-treated fish was 7.3 g and the mean liver weight of the 126 day depuratd fish was 15 g, while there was no significant difference in whole body weight.

Since changes in the lipid content of organisms will also affect the contaminant concentration, factors controlling lipid content should also be considered with contaminant concentrations. These results indicate that there are two processes controlling the lipid/contaminant cycle. First, you have the uptake of contaminants causing the liver to increase its lipid production (Allen et at., 1974, 1975). This ultimately results in pathological changes caused by increased vacuolation of the liver tissue. Zeh (1982)

reported a significantly higher frequency of idiopathic specific degeneration/necrosis, neoplasia, preneoplasia or storage disorders among English sole with total chlorinated hydrocarbons in liver tissue exceeding 0.2 mg/wet kg. Also, Sherwood (1978) reported an increase in lipid content of Dover sole livers collected from Palos Verdes over those collected from Dana Pt., along with an increase in liver pathology. These results were also seen by us. The livers of white croaker from Palos Verdes had significant liver pathology (Perkins et al., 1982) and were 1.5 times larger than white croaker livers from Dana Pt. This size increase was due to an increase of lipid content from 6.0% to 15%, respectively. Also, our results from scorpionfish livers showed no change in pathology related to changes in contaminant concentrations, but all the scorpionfish used contained more than 1 mg/wet kg chlorinated hydrocarbons. It is possible that pathological changes had already occurred. However, we have been unable to obtain adequate control fish to substantiate this.

Second, the lipid content of an organism is also controlled by its metabolism. During reproduction or periods of starvation, lipids are mobilized from the liver and adipose tissue for use by the organism as an energy source. The lipophilic contaminants will also be associated with this process and therefore their concentration will also be affected. For example, the reproductive cycle has a significant effect on lipid content in the liver. The mean concentration of total parent compounds in the liver of

scorpionfish caught at Dana Pt. was 6.5 mg/wet kg for fish caught in March and 7.9 mg/wet kg for fish caught in October. The liver weight of the March fish was 23 g and the liver weight of the October fish was 8.2 g, while the whole body weight was not different. Even though the concentration was similar, the actual mass of parent compounds in the livers was different (0.16 mg for March and 0.072 mg for October). The affect caused by seasonality in the reproductive cycle was also seen in the histology of mussels collected from 3 sites in southern California (Brown et al., 1982a). The changes in histology caused by seasonality were so great that no correlation with contaminant concentrations could be detected, even though the concentrations were elevated in the mussels from White Point (Palos Verdes).

Another conclusion that can be drawn from our data is that fish from a zone thought to be relatively clean based on distance from the discharge zone and the structure of the invertebrate infauna, had quite high contaminant concentrations. Scorpionfish taken from as far away as the Channel Islands still contained more than 1 mg/wet kg parent compounds in their livers. This evidence indicates that as contaminant concentrations are decreasing near the outfall zone, the area affected by this contamination is growing larger.

Finally, there is a significant fraction of polar oxygenated metabolites present at concentrations up to ten times higher than their non-polar parent compounds. These results also indicated that the oxy-metabolites of DDTs and

PCBs represent from 35 to 99% of the total contaminants within the sediment, shrimp and scorpionfish, which agrees with results reported by Varanasi (1979) on English sole.These oxygenated metabolites can not be detected in any samples without inclusion of the heat-catalyzed base-hydrolysis step (Tables 6 and 7). This suggests that the oxy-metabolites present were conjugated onto cellular molecules, possibly including important molecules like DNA or RNA with resultant chronic effects. Most of these compounds are as of yet not reported to any extent in literature and could possibly cause as much environmental damage as their precursors. Since these oxy-metabolites are not integrated into the regulations governing the discharge of contaminants into the marine environment, a major component of contamination is not being regulated.

The source of these oxy-metabolites in animal tissues is uncertain. The mixed-function oxidase (MFO) system is known to be inducible in marine fish in response to exposure to PCBs, but not DDTs (Franklin et al., 1980). However, it is possible that once induced, the MFO system can metabolize the DDTs as well. A comparison of DDTols verses PCBols in scorpionfish livers shows that their concentrations are similar even though the parent compound concentrations are quite different (Tables 6 and 7). This was not true for the shrimp muscle or the sediments. Also, DDE, the main form of total DDT in southern California coastal waters, can be metabolized by microorganisms in marine sediments (Lee and Ryan, 1978). The oxy-metabolites of DDT have octanol/water

partition coefficients which suggest that these could be bioaccumulated from sediments. Although the concentrations of parent compounds and oxy-metabolites were correlated in sediments ($r= 0.86$, $p< 0.1$), they were not correlated in shrimp muscle ($r= -.50$, $p>0.1$) or scorpionfish liver ($r= 0.17$, $p>0.2$). Since the ratio of parent compounds to metabolites in tissues do not reflect those in sediments, it is more likely that the animal controls their metabolite concentrations more than the concentrations being a reflection of the sediments.

The energy cost to the organism to metabolize organic contaminants is insignificant compared to all the other biological functions. Therefore, chronic effects caused by this metabolism must be from a source other than the direct depletion of energy reserves. The oxy-metabolites were detectable in cytosolic pools other than the GSH pool where they are detoxified. This would suggest that an excess of the highly reactive epoxide intermediates were being produced, over and above the ability of the detoxifying mechanisms to remove them. Once the capacity of the detoxification mechanism is overloaded, the reactive intermediates are available to attack important cellular molecules such as DNA, RNA, enzymes and metallothionein. Brown et al. (1983) reported that spillover of oxy-metabolites into the MT pool could displace essential trace metals causing their depletion, explaining the apparent depression of trace metal concentrations in animals from Palos Verdes where there is an excess of trace metals in the sediments. The presence of

epoxide intermediates has also been associated with being the mechanism by which organic pollutants cause cancer (Thakker et al., 1979). From our results, it is entirely possible that the oxy-metabolites detected in the MT and ENZ pools may have caused chronic effects. However, these effects were not significant enough to be detected by histopathological or enzymatic examinations.

SECTION 6

RECOMMENDATIONS

The n-octanol/water partition coefficient can be used to predict the bioaccumulation of trace organic compounds in marine organisms. This would make a very useful tool for regulating agencies when making decisions relating to the discharge of chemicals into the sea. It is recommended that this index be used for this purpose, possibly by incorporating it into an index such as the Toxic Bioaccumulation Factor used by us. This approach toward the regulation of the discharge of compounds into the environment is much more practical than the existing method based on effluent concentration. For example, based on effluent concentration, pentachlorophenol would not surpass any of the existing guidelines for discharge by the treatment plant researched by us. As a matter of fact, its not even required in the state monitoring regulations. However, when the TBAF was calculated, it resulted in the highest TBAF value of all the identified compounds, based on its effluent concentration, octanol/water partition coefficient and its acute toxicity.

Clearly, from our results a major fraction of environmental contamination is not being regulated. The ability of organisms to metabolize organic compounds to oxygenated metabolites may be the most important component of environmental contamination in terms of toxic chemicals. This metabolism may be the main source of long term chronic effects that cause the community changes in the marine ecosystem, whereas, the parent compounds may only be responsible for acute problems. The research presented here

indicates that research should be funded to determine the biochemical mechanism and its regulation for the metabolism of organic compounds by the mixed function oxidase system. Also, the toxicity of the oxygenated metabolites and their availability to marine organisms should be determined. It is possible that once these compounds are conjugated to detoxifying agents within the organisms, they no longer have a potential for toxicity.

With a combination of the octanol/water partition coefficient and the point at which trace organic compounds can be detoxified by their metabolism and conjugation without harm to the organism, it may be possible to control the rate at which contaminants can be discharged into the marine environment so that no adverse effect will be seen.

SECTION 7

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SECTION 8

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APPENDIX A
TABLES AND FIGURES

TABLE 1. Concentrations (ug/l) and n-octanol/water partition coefficients (Log Kow) for compounds identified in Los Angeles County (JWPCP) effluent collected quarterly from November 1980 until August 1981.

BASE-NEUTRAL EXTRACTABLES

	Effluent Conc.	Log Kow
trimethyl-3-cyclohexene-1-methanol	90	3.38
2-butoxy ethanol	50	0.83
1,2-dimethyl benzene	40	2.77
2,3-dimethyl phenol	40	2.33
caffeine	40	0.01
2-methylpropyles propanoic acid	35	*
1,4-dimethyl benzene	30	3.15
3,4-dimethyl phenol	20	2.23
4-hydroxy-4-methyl-2-pentanone	20	0.29
1,2,3-trimethyl benzene	20	3.42
naphthalene	15	3.45
1,2,4-trimethyl benzene	10	3.42
1-ethyl-2-methyl benzene	10	*
2,5-dimethyl phenol	10	2.33
2-methyl naphthalene	10	3.86
1-methyl naphthalene	10	3.87
methyl heptanol	10	*
1-(2-ethoxypropoxy)-2-propanol	10	*
1-(2-butoxyethoxy)-ethanol	10	-1.26
9-octadecanoic acid	10	5.36
3-(1,1-dimethylethyl)-phenol	8	*
1-ethyenyl-1,2-methyl benzene	5	*
1,2-dimethyl naphthalene	5	4.31
2,4-bis(1,1-dimethylethyl) phenol	5	*
tetramethyl hexane	5	*
trimethyl dodecatrien-1-ol	5	*
di-n-butyl phthalate	4.2	2.88
bis(2-ethylhexyl) phthalate	3	3.62
1,2-dichloro benzene	2.2	3.38
diethyl phthalate	2	3.02
1,4-dichloro benzene	1.8	3.38
Aroclor 1242	0.94	5.58
1,2,4-trichloro benzene	0.77	4.08
4,4'-DDD	0.60	5.69
Heptachlor	0.43	3.05
4,4'-DDE	0.16	5.69
2,4'-DDD	0.10	5.69
4,4'-DDT	0.084	6.19
Heptachlor Epoxide	0.071	2.65
Aroclor 1254	0.052	6.11
Dieldrin	0.038	2.60
1,3,5-trichloro benzene	0.035	4.08
2,4'-DDE	0.031	5.69
hexachloro benzene	0.013	6.18
Aldrin	0.008	3.01
2,4'-DDT	0.003	6.19

Table 1.(continued)

ACID EXTRACTABLES

	Effluent Conc	Log Kow
hexadecanoic acid	2500	6.20
tetradecanoic acid	1200	5.20
octadecanoic acid	1000	*
dodecanoic	1000	4.20
phenol	980	1.48
benzene methanol	500	1.10
octanoic acid	400	2.92
benzoic acid	400	2.03
pentadecanoic acid	200	5.70
hexanoic acid	55	1.92
pentanoic acid	50	1.21
2-methyl phenol	40	1.97
3-methyl phenol	40	1.96
propanoic acid	20	0.33
1-methyl-4(1-methyl) cyclohexane	20	3.94
3-ethyl phenol	15	2.40
2-propyl phenol	15	2.93
butyl cyclohexane	10	*
decahydro-2-methyl naphthalene	10	*
pentyl cyclohexane	10	*
tri-ethyl silane	10	*
undecane	10	*
3-methyl octane	10	*
4-fluoro biphenyl	10	4.23
trimethyl-1-hexene	10	*
tetramethyl pentane	10	*
o-decyl hydroxylamine	10	*
hexadecane	10	*
hexatricocane	10	*
nonadecanol	10	*
butyl octanol	10	*
trimethyl-1-nonene	10	*
(1-methyldecyl) benzene	5	*
(1-butylheptyl) benzene	5	*
(1-propylnonyl) benzene	5	*
2,4,6-trichloro phenol	2.3	3.69
pentachloro phenol	2.3	5.12
tetrachloro phenol	0.15	4.42
2,4,5-trichloro phenol	0.12	3.72

Table 1. (continued)

VOLATILE ORGANICS

benzene	220	2.15
toluene	210	2.73
dimethyl disulfide	45	1.77
4-methyl-2-pentanol	45	1.77
1,2-dichloro ethane	41	1.48
2,2'-oxybis propane	40	*
1,1,1-trichloro ethane	31	2.49
tetrachloro ethylene	29	2.60
2-methyl-1-pentene	25	3.56
methyl cyclohexane	20	3.94
trichloro ethylene	17	2.29
ethyl benzene	14	3.15
chloroform	8.0	1.97
vinyl chloride	6.2	*
1,2-dichloro ethene	5.2	1.98
1,1-dichloro ethane	3.5	1.48

TABLE 2

Partition coefficients (Log K_{ow}) and concentration of 27 compounds selected from Table 1, in effluent ($\mu g\ l^{-1}$), sediments (μg dry kg^{-1}) and tissues (μg wet kg^{-1}).

Compound	Class	Log K_{ow}	Effluent Conc	(n)	Sediment Conc	(n)	P. Sanddab liver Conc	(n)	Halibut liver Conc	(n)	Scorpionfish liver Conc	(n)	Dover sole liver Conc	(n)	Croaker liver Conc	(n)	Crab digestive gland Conc	(n)	Shrimp muscle Conc	(n)	Invertebrates whole Conc	(n)
1 DDT (2)*	B†	6.19	0.087	(2)	191	(1)	1600	(5)	1870	(3)	422	(5)	168	(5)	20	(5)	344	(5)	9	(5)	5	(3)
2 Hexachlorobenzene	B	6.18	0.013	(2)	5	(1)	29	(5)	2	(3)	5	(5)	6	(5)	4	(5)	5	(5)	<1	(5)	<1	(3)
3 Aroclor 1254	B	6.11	0.052	(2)	678	(1)	4920	(5)	2080	(3)	1140	(5)	615	(5)	1100	(5)	1200	(5)	18	(5)	19	(3)
4 DDE (2)	B	5.69	0.19	(2)	11 700	(1)	122 000	(5)	19 200	(3)	20 100	(5)	19 000	(5)	21 200	(5)	51 900	(5)	294	(5)	383	(3)
5 DDD (2)	B	5.69	0.70	(2)	558	(1)	2280	(5)	36	(3)	384	(5)	549	(5)	305	(5)	122	(5)	22	(5)	8	(3)
6 Aroclor 1242	B	5.58	0.94	(2)	256	(1)	772	(5)	139	(3)	143	(5)	166	(5)	224	(5)	379	(5)	<2	(5)	13	(3)
7 pentachlorophenol	A‡	5.12¶	2.3	(3)	15	(4)	5	(5)	5	(2)	74	(4)	70	(5)	8	(5)	19	(5)	5	(3)	24	(2)
8 tetrachlorophenol (3)	A	4.42	0.15	(2)	4	(2)	19	(5)	<1	(2)	<1	(4)	17	(5)	30	(5)	<1	(5)	<1	(3)	9	(2)
9 trichlorobenzene (2)	B	4.08	0.81	(2)	9	(1)	28	(5)	<1	(3)	15	(5)	7	(5)	<1	(5)	8	(5)	<1	(5)	<1	(3)
10 methyl cyclohexane	V§	3.94	20	(1)	<0.5	(2)	<0.3	(1)	NA‖		<0.3	(1)	<0.3	(1)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)
11 trichlorophenol (2)	A	3.69	2.4	(3)	14	(2)	47	(5)	29	(2)	164	(4)	85	(5)	629	(5)	55	(5)	10	(3)	28	(2)
12 naphthalene	B	3.45	15	(2)	15	(1)	15	(2)	<20	(3)	<20	(2)	20	(5)	42	(5)	<20	(3)	<20	(2)	<20	(3)
13 dichlorobenzene (2)	B	3.38	4.0	(2)	33	(1)	10	(5)	180	(3)	851	(5)	27	(5)	<1	(5)	575	(5)	403	(5)	59	(3)
14 ethylbenzene	V	3.15	14	(5)	0.5	(2)	<0.3	(1)	NA		<0.3	(1)	0.3	(1)	4	(1)	NA		<0.3	(1)	<0.3	(1)
15 Heptachlor	B	3.05	0.43	(2)	7	(1)	40	(5)	8	(3)	5	(5)	3	(5)	1	(5)	5	(5)	<1	(5)	2	(3)
16 toluene	V	2.73	210	(5)	<1	(2)	<1	(1)	NA		<1	(1)	1	(1)	25	(1)	NA		<1	(1)	<2	(1)
17 tetrachloroethylene	V	2.60	29	(5)	<0.5	(2)	23	(1)	NA		29	(1)	19	(1)	11	(1)	NA		<0.3	(1)	8	(1)
18 1,1,1-trichloroethane	V	2.49	31	(5)	<0.5	(2)	7	(1)	NA		2	(1)	1.5	(1)	<0.3	(1)	NA		<0.3	(1)	4	(1)
19 trichloroethylene	V	2.29	17	(5)	<0.5	(2)	2	(1)	NA		6	(1)	4	(1)	2	(1)	NA		0.3	(1)	7	(1)
20 benzene	V	2.15	220	(5)	<1	(2)	<1	(1)	NA		16	(1)	52	(1)	15	(1)	NA		<1	(1)	8	(1)
21 1,2-dichloroethene	V	1.98	5.2	(5)	<0.5	(2)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)
22 chloroform	V	1.98	8.0	(5)	<0.5	(2)	<10	(1)	NA		<10	(1)	<10	(1)	<10	(1)	NA		<10	(1)	<10	(1)
23 4-methyl-2-pentanol	V	1.90	45	(1)	<0.5	(2)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)
24 dimethyldisulfide	V	1.77	45	(1)	<0.5	(2)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)
25 vinyl chloride	V	1.52	6.2	(5)	<0.5	(2)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)
26 phenol	A	1.48	980	(1)	10	(1)	<10	(1)	<10	(1)	<10	(1)	<10	(1)	<10	(1)	NA		<10	(1)	<10	(1)
27 dichloroethane (2)	V	1.48	44	(5)	<0.5	(2)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)	<0.3	(1)	NA		<0.3	(1)	<0.3	(1)
Lipid – % wet weight			0.0022	(4)	0.34	(1)	31.5	(3)	10.4	(3)	21.8	(3)	9.51	(3)	6.10	(3)	7.09	(3)	1.05	(3)	1.17	(3)
Dry – % by weight			0.016	(4)	36.1	(2)	46.0	(2)	23.3	(3)	42.9	(3)	24.5	(3)	27.7	(3)	23.3	(3)	24.1	(3)	11.3	(3)

*Number of isomers of compound combined from Table 1.

†Base-neutral extractable organic.

‡Acid extractable organic.

§Volatile organic.

‖Not analysed.

¶Log K_{ow} may approach 2.4 at physiological pH (Leo, personal communication).

Table 3. Results of Spearman's rank correlation test (r_s) indicating the relationship between the n-octanol/water partition coefficient (Log Kow), effluent concentration, sediment concentration and tissue concentrations of the 27 selected compounds.

	Log Kow	Effluent	Sediment
Sediment	.737	-.553	*
Sanddab Liver	.754	-.691	.839
Halibut Liver^	.666	-.565	.948
Scorpionfish Liver	.654	-.520	.826
Dover sole Liver	.687	-.535	.849
White croaker Liver	.632	-.403	.766
Crab Digestive Gland^	.703	-.600	.949
Shrimp Muscle	.557	-.502	.892
Whole Inverts	.467	-.333	.747

$p < .01$ if $r_s > .491$; $p < .05$ if $r_s > .382$.

^ Volatile Organics were not analyzed in this sample, therefore we used the average values for all the other species for the statistical analysis.

Table 4. Results of the analysis of scorpionfish from the Channel Islands (Anacapa and Catalina) and Dana Pt. caged at Palos Verdes to determine uptake rates of trace organic compounds.

Station		Whole Body Weight gm	Total Length mm	Liver Weight gm	Total DDT mg/ wet kg	Total PCB mg/ wet kg	Total Parent Compounds mg/wet kg
Channel Islands; Non-Treated; Collected 11 Mar 83							
	mean	540	310	14	1.6	0.30	1.9
Liver	±1SD	130	22	7	0.4	0.21	0.5
n=5	%CV	24	7	49	25	71	28
Dana Pt.; Non-Treated; Collected 5 Mar 82							
	mean	370	250	23	6.1	0.39	6.5
Liver	±1SD	140	27	10	6.6	0.28	6.9
n=10	%CV	37	11	46	110	74	110
Dana Pt.; Non-Treated; Collected 20 Oct 82							
	mean	350	260	8.2	6.7	1.2	7.9
Liver	±1SD	130	26	3.8	3.3	0.4	3.7
n=10	%CV	38	10	47	49	35	46
	mean	*	*	*	0.078	0.011	0.089
Muscle	±1SD	*	*	*	0.038	0.006	0.044
n=10	%CV	*	*	*	49	49	49
Dana Pt.; Caged at Palos Verdes for 12 days							
	mean	390	280	11	6.8	1.2	7.9
Liver	±1SD	79	19	4	3.3	0.6	3.8
n=10	%CV	20	7	33	49	53	50
Dana Pt.; Caged at Palos Verdes for 27 days							
	mean	340	270	10	11	2.2	13
Liver	±1SD	150	36	6	13	2.5	15
n=9	%CV	43	14	61	120	110	120
Dana Pt.; Caged at Palos Verdes for 45 days							
	mean	290	260	5.9	6.9	0.97	7.9
Liver	±1SD	87	26	3.7	4.2	0.58	4.7
n=9	%CV	30	10	63	60	60	60
	mean	*	*	*	0.073	0.009	0.082
Muscle	±1SD	*	*	*	0.038	0.004	0.042
n=5	%CV	*	*	*	52	40	40

Table 5. Results of the analysis of scorpionfish from Palos Verdes (PV7-3) non-treated and depurated in clean conditions in the laboratory.

Station		Whole Body Weight gm	Total Length mm	Liver Weight gm	Total DDT mg/ wet kg	Total PCB mg/ wet kg	Total Parent Compounds mg/wet kg
Palos Verdes; Non-Treated; Collected 29 Mar 82							
	mean	190	210	7.3	35	2.1	37
Liver	±1SD	59	20	5.2	21	1.3	22
n=10	%CV	30	9	72	60	61	60
	mean	*	*	*	0.45	0.021	0.47
Muscle	±1SD	*	*	*	0.35	0.013	0.36
n=10	%CV	*	*	*	77	61	76
Palos Verdes; Non-Treated; Collected 22 Oct 82							
	mean	280	250	7.5	0.31	0.024	0.34
Muscle	±1SD	51	16	2.9	0.22	0.013	0.23
n=10	%CV	18	6	38	69	52	68
Palos Verdes; Caged at Palos Verdes for 72 days							
	mean	330	260	18	37	2.2	39
Liver	±1SD	34	7	6	25	1.5	26
n=10	%CV	10	3	36	68	68	68
	mean	*	*	*	0.30	0.030	0.33
Muscle	±1SD	*	*	*	0.27	0.043	0.29
n=10	%CV	*	*	*	89	140	88
Palos Verdes; Depurated in the Lab for 28 days							
	mean	180	220	9.8	26	1.8	28
Liver	±1SD	31	14	7.8	13	0.9	13
n=5	%CV	18	6	79	49	50	49
	mean	*	*	*	0.25	0.019	0.27
Muscle	±1SD	*	*	*	0.15	0.008	0.16
n=5	%CV	*	*	*	59	43	58
Palos Verdes; Depurated in the Lab for 56 days							
	mean	180	220	8.1	31	3.7	34
Liver	±1SD	11	3	3.1	10	3.9	9
n=5	%CV	6	1	39	32	110	25
	mean	*	*	*	0.54	0.056	0.60
Muscle	±1SD	*	*	*	0.65	0.059	0.71
n=5	%CV	*	*	*	120	110	120
Palos Verdes; Depurated in the Lab for 126 days							
	mean	200	220	15	13	0.87	14
Liver	±1SD	20	8	3	7	0.31	7
n=5	%CV	10	4	22	52	36	50
	mean	*	*	*	0.21	0.026	0.24
Muscle	±1SD	*	*	*	0.10	0.008	0.11
n=5	%CV	*	*	*	48	31	45

Table 6. Mean (± 1SD) concentrations (mg/wet kg) of total DDT parent compounds and their oxygenated metabolites comparing samples extracted with and without hydrolysis (n=5).

Station	Parent DDT Compounds^	Heat-Catalyzed Base-Hydrolysis	DDTols*	DDTols/Total Contaminants X 100
SHRIMP MUSCLE				
@ SMB	0.031±0.014	Without	<0.01	0%
		With	10 ± 7	99%
# PV	0.85 ± 0.38	Without	<0.01	0%
		With	4.8±1.7	85%
SCORPIONFISH LIVER				
SMB	14 ± 11	Without	<0.01	0%
		With	13 ± 7	48%
PV	28 ± 10	Without	<0.01	0%
		With	15 ± 9	35%
SEDIMENT				
SMB	0.067±0.011	Without	<0.01	0%
		With	0.48±0.92	88%
PV	6.3 ± 2.7	Without	<0.01	0%
		With	26 ± 14	80%

^ o,p' + p,p'-(DDT+DDD+DDE).
* p,p'-DDA + p,p'-DDOH.
@ Santa Monica Bay station 2-3 (Figure 1).
# Palos Verdes station 7-3 (Figure 1).

Table 7. Mean (± 1SD) concentrations (mg/wet kg) of Aroclor 1254 and its oxygenated metabolites comparing samples extracted with and without hydrolysis (n=5).

Station	Parent PCB Compounds^	Heat-Catalyzed Base-Hydrolysis	PCBols *	PCBols/Total Contaminants X 100
SHRIMP MUSCLE				
@ SMB	0.016±0.009	Without	<0.1	0%
		With	1.3 ± 0.6	99%
# PV	0.039±0.027	Without	<0.1	0%
		With	0.38±0.86	91%
SCORPIONFISH LIVER				
SMB	2.0 ± 1.3	Without	<0.1	0%
		With	18 ± 20	90%
PV	1.9 ± 0.5	Without	<0.1	0%
		With	16 ± 10	89%
SEDIMENT				
SMB	0.011±0.003	Without	<0.1	0%
		With	4.0±1.8	99%
PV	0.22 ± 0.09	Without	<0.1	0%
		With	12 ± 11	98%

^ Aroclor 1254.
* 2',3',4',5'-tetrachloro-3-biphenylol + 2',3',4',5'-tetrachloro-4-biphenylol + 3,3',4,4'-tetrachloro-4,4'-biphenyldiol + 2',3,3',4',5'-pentachloro-2-biphenylol + 2',3',4',5,5'-pentachloro-2-biphenylol.
@ Santa Monica Bay station 2-3 (Figure 1).
# Palos Verdes station 7-3 (Figure 1).

Table 8. Concentrations (mg/wet kg) of total DDT and total PCB parent compounds and their oxygenated metabolites in mussels (*Mytilus californianus*) soft parts collected from the coastal waters of southern California in December 1981 (n=10).

Station	Total Tissue Parent Compounds (DDT+DDE+DDD)	Total Cytosolic Oxy-Metabolites (DDTols=DDA+DDOH)	Oxy-Metabolites/ Total Contaminants X 100
Point Dume	0.039	62	99%
Redondo Beach	0.11	75	99%
Palos Verdes	0.22	21	99%

Station	Total Tissue Parent Compounds (PCB 1242+1254)	Total Cytosolic Oxy-Metabolites (PCBols)^	Oxy-Metabolites/ Total Contaminants X 100
Point Dume	0.005	0.85	99%
Redondo Beach	0.057	1.5	96%
Palos Verdes	0.028	1.6	98%

^ 3,4',5-trichloro-3-biphenylol + 2',3,3',4',5'-pentachloro-2-biphenylol + 2',3',4',5,5'-pentachloro-2-biphenylol.

Table 9. Concentrations (mg/wet kg) of total DDT and its oxygenated metabolites in white croaker (*Genyonemus lineatus*) from Dana Pt. and Palos Verdes collected in September 1981 (Composite of n=10).

Station	Total Tissue Parent Compounds (DDT+DDE+DDD)	Total Cytosolic Oxy-Metabolites (DDTols=DDA+DDOH)	DDTols/Total Contaminants X 100
Dana Pt.	0.55	9.7	95%
Palos Verdes	36	25	61%

Table 10. Caged scorpionfish liver pathology ratings (n=10).

Condition Rated^	Sample and Exposure Period: Dana Pt. 0 Days	Dana Pt. 12 Days	Dana Pt. 27 Days	Dana Pt. 45 Days	PV7-3 0 Days	Statistics@ F	PR>F
Hepatic Cord Structure	19	10	13	16	22	2.25	.08
Degenerative Foci	0	2	8	8	2	2.58	.05
Focal Hypertrophy	10	10	15	5	11	2.39	.06
Nuclear Pleomorphism	0	0	1	0	0	*	*
Megalocytes	0	0	1	0	0	*	*
Adenomatus Foci	0	0	0	0	0	*	*
Vacuolation	16	15	17	12	20	2.06	.10
Hepatic Sclerotic Foci	0	0	5	2	1	3.87	.01
Focal Hyperplasia	1	0	3	6	2	2.17	.09
Hepatocellular Necrosis	1	1	5	3	1	1.08	.38
Pycnotic	1	1	0	0	0	*	*
Cytoplasmic Inclusions	8	4	6	0	4	4.81	<.01
Sinusoidal Compression	17	16	15	10	16	1.10	.37
Sinusoidal Congestion	3	5	1	8	6	1.53	.21
Melanin	10	7	8	5	7	1.44	.24
Melanin Macrophage Centers	8	5	9	7	8	0.81	.52
Cholangiole Fibrosis	4	3	3	2	5	0.55	.82

^ Each fish was blind rated 0,1,2 or 3 (0 being best) for each condition then totalled for statistical analysis.
* Not Analyzed Statistically.
@ ANOVA; PR>F = Probability of Rejection Greater Than F.

FIGURE 1
LOCATION OF SAMPLING STATIONS

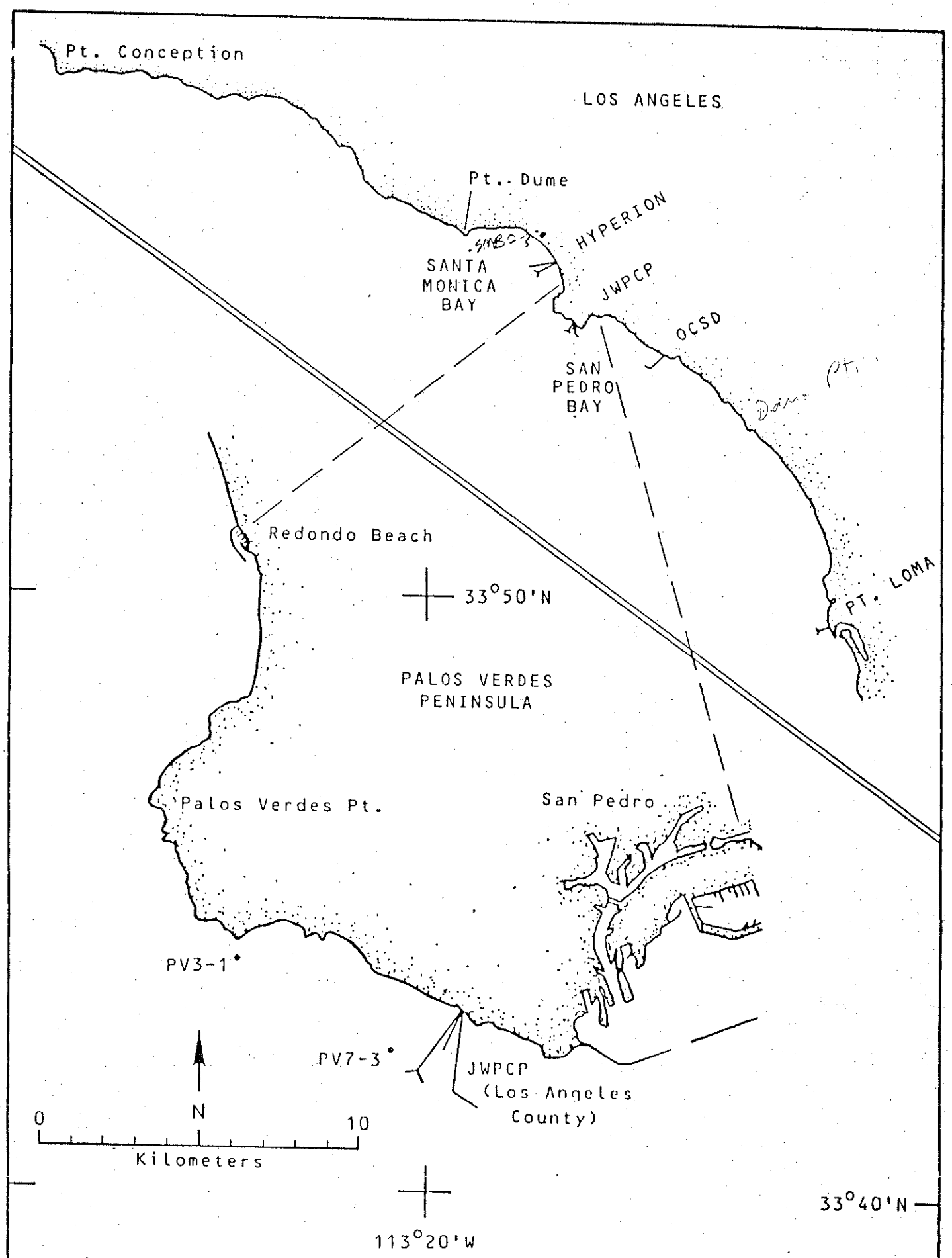


Figure 1. Location of the sediment and organisms sampling stations.

FIGURE 2
CYTOSOLIC DISRTIBUTION OF OXYGENATED METABOLITES IN THE WHOLE SOFT PARTS OF MUSSELS COLLECTED FROM THREE STATIONS DURING SEPTEMBER AND DECEMBER

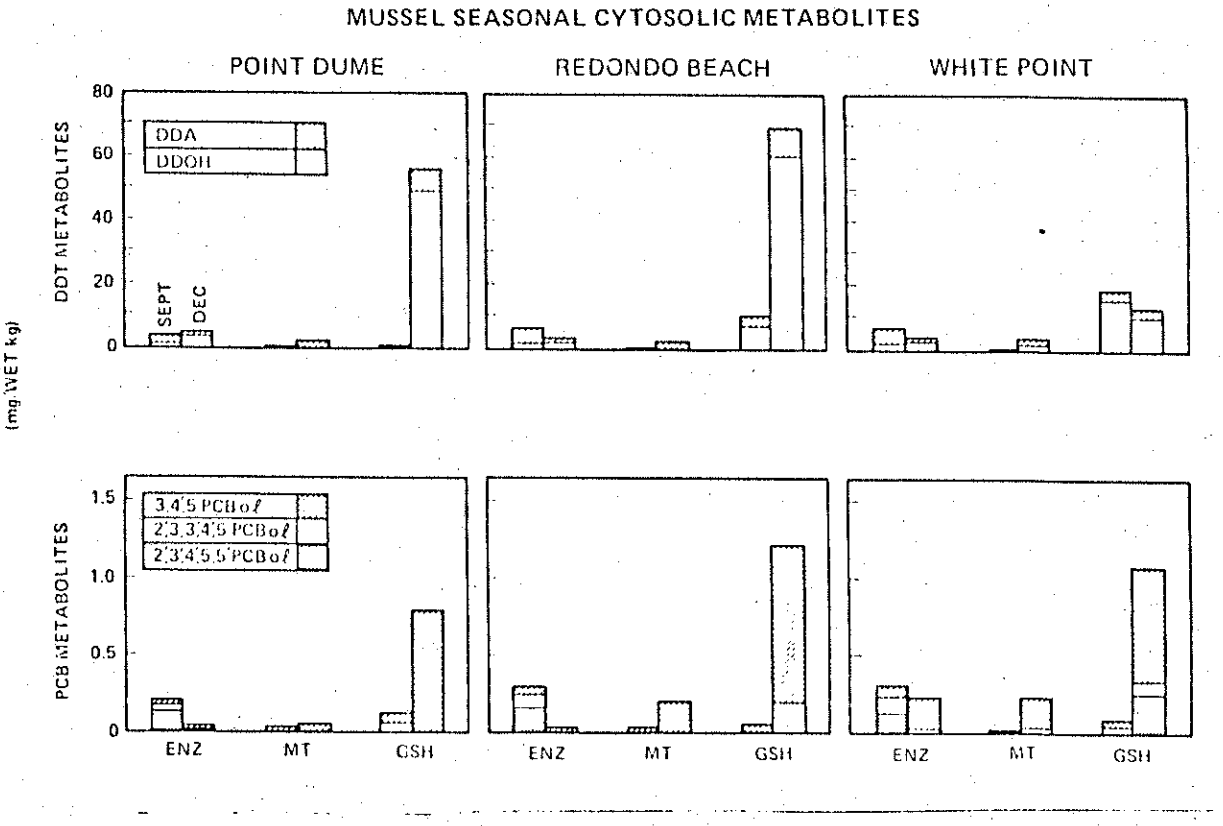

MUSSEL SEASONAL CYTOSOLIC METABOLITES
POINT DUME
REDONDO BEACH
WHITE POINT
DDT METABOLITES
(mg WET kg)
DDA
DDOH
SEPT
DEC
80
60
40
20
0
PCB METABOLITES
3,4,5 PCB ol
2,3,3',4,5 PCB ol
2,3,4',5,5' PCB ol
1.5
1.0
0.5
0
ENZ
MT
GSH

FIGURE 3
CYTOSOLIC DISTRIBUTION OF THE OXYGENATED METABOLITES OF DDT IN LIVER TISSUE OF WHITE CROAKER COLLECTED FROM DANA PT. AND PALOS VERDES

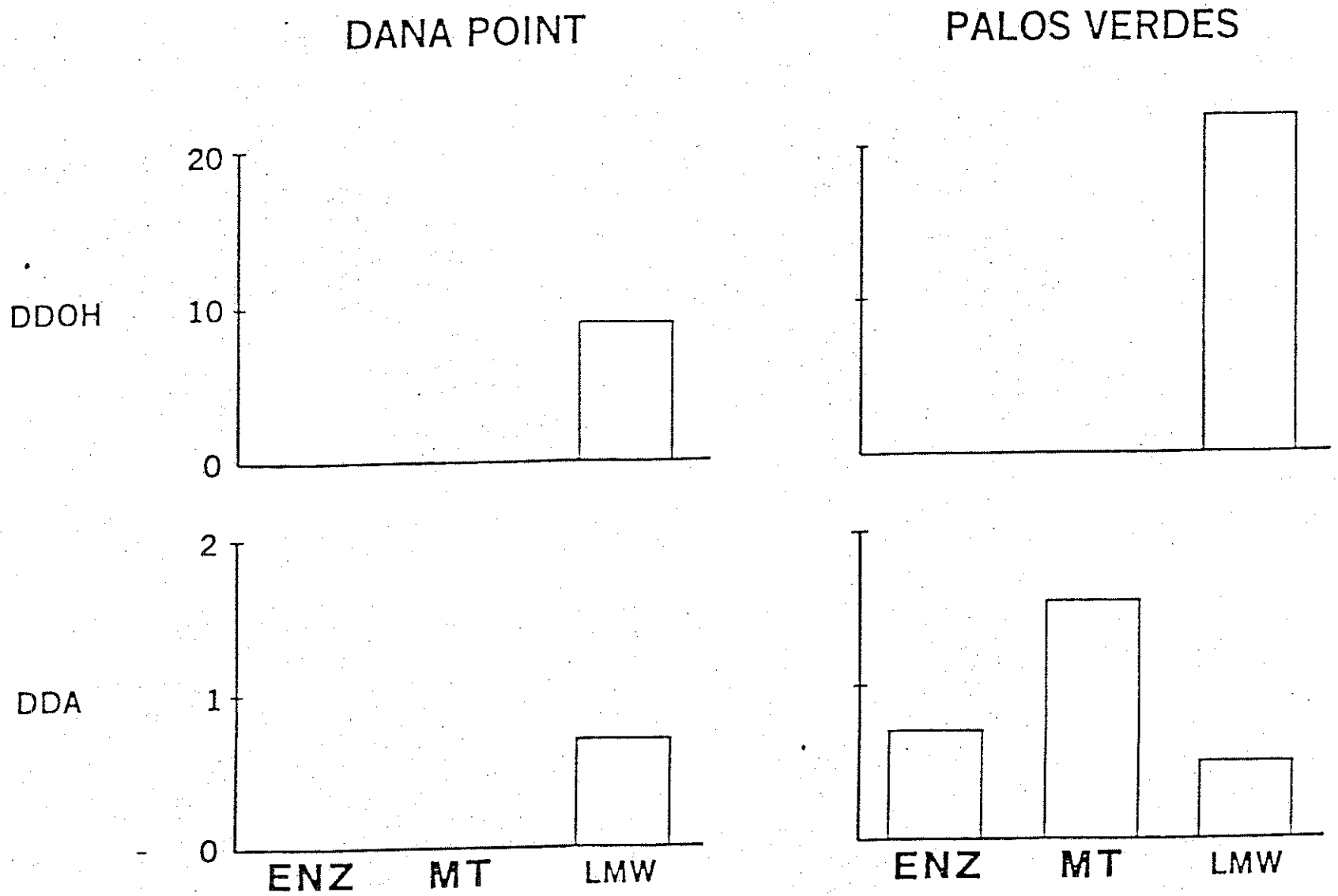

DANA POINT
PALOS VERDES
DDOH
DDA
20
10
0
2
1
0
ENZ
MT
LMW
ENZ
MT
LMW

FIGURE 4
CYTOSOLIC OXYGENATED DDT METABOLITES, GLUTATHIONE AND CATALASE IN LIVER TISSUE OF SCORPIONFISH (COMPOSITE OF 10 FISH) COLLECTED FROM DANA PT. AND CAGED AT PALOS VERDES

Cytosolic Oxygenated DDT Metabolites, Glutathione, and Catalase in Liver Tissue of Scorpion Fish (composite of 10) Captured at Dana Point and Caged at Palos Verdes (PV) for 0 (T_0) and 45 (T_{45}) Days.

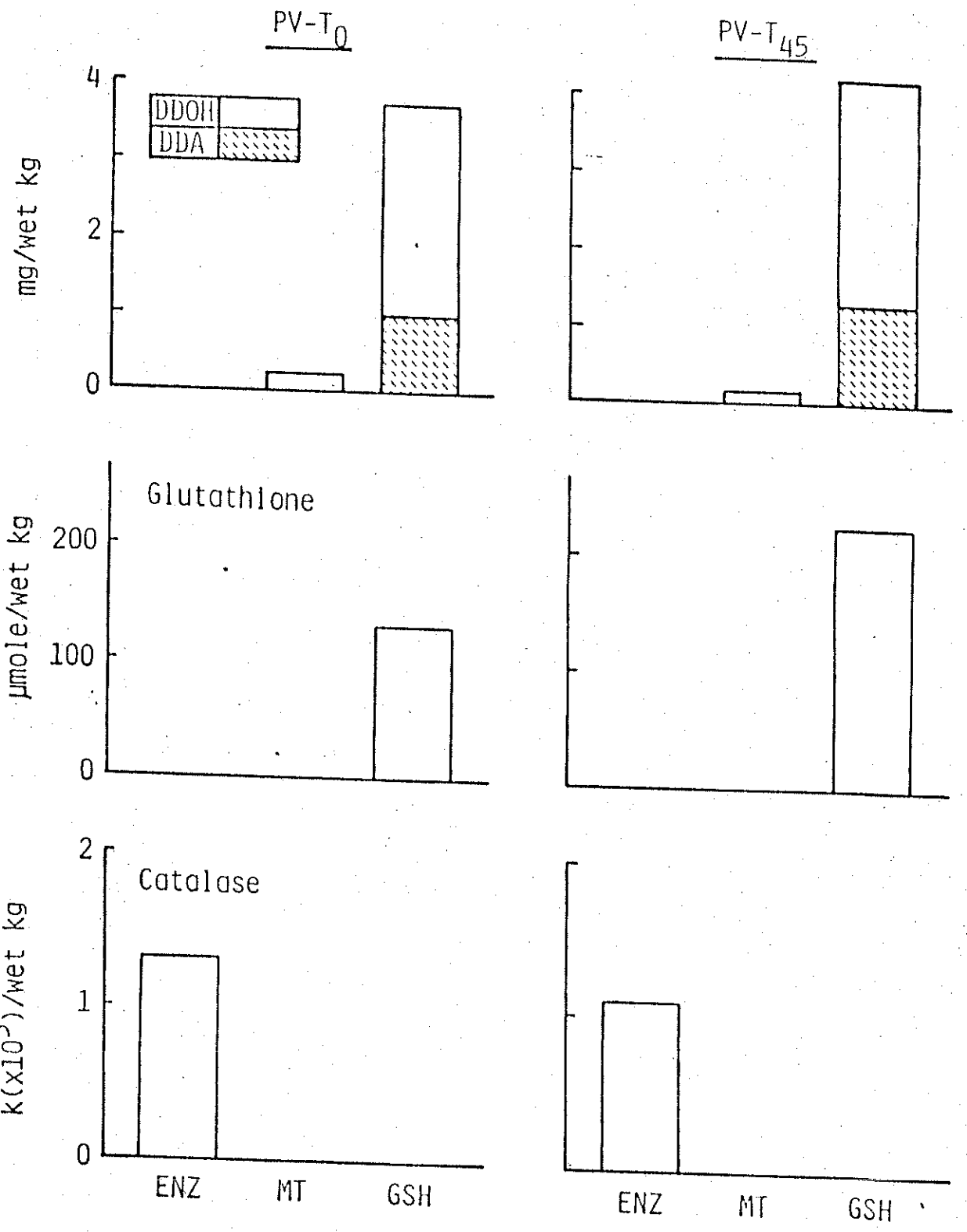
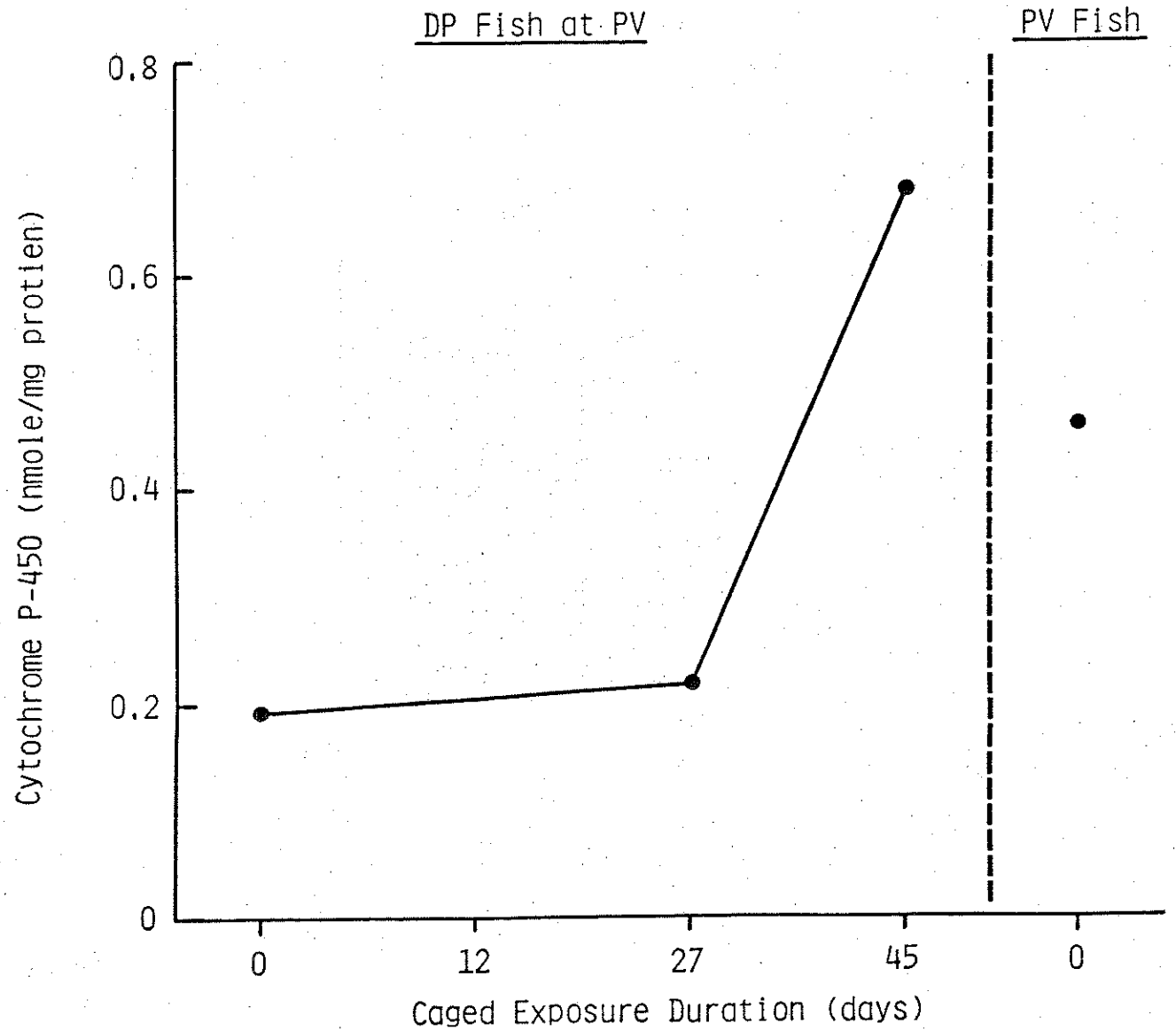


FIGURE 5
CONCENTRATION (nMOLE/mg PROTEIN) OF CYTOCHROME P-450 IN LIVER TISSUE OF SCORPIONFISH COLLECTED FROM DANA PT. AND CAGED AT PALOS VERDES

ENZYME ACTIVITY

Concentration (nmole/mg protien) of Cytochrome P-450 in Liver Tissue of Scorpion Fish Captured at Relatively Clean Dana Point (DP) and Caged at Highly Contaminated Palos Verdes (PV).



APPENDIX B

PUBLICATONS AND PRESENTATINS OF THE RESEARCH SUPPORTED BY THIS GRANT

1. Gossett, R.W., D.A. Brown and D.R. Young (1983). Predicting the Bioaccumulation of Organic Compounds in Marine Organisms using Octanol/Water Partition Coefficients. *Marine Pollution Bulletin*, Vol. 14, No.10, 387-392.

2. Brown, D.A., R.W. Gossett, P. Hershelman, H.A. Schafer, K.D.Jenkins and E.M.Perkins (1983). Bioaccumulation and Detoxification of Contaminants in Marine Organisms from Southern California Waters. In *Waste Disposal in the Oceans: Minimizing Impact, Maximizing Benefits* (D.F. Soule and D. Walsh, eds.), Procedings of the Southern California Academy of Sciences 1982, pp. 171-193. Boulder, Colorado.

3. Brown, D.A., R.W. Gossett, S.R. McHugh and K.D. Jenkins (1983). The Metabolism and Detoxification/Toxification of Xenobiotic Organics in the Marine Environment. The *Fourth International Ocean Disposal Symposium*, April 11-15, 1983. Plymouth, England. In Press.

4. Gossett, R.W., S.R. McHugh and D.A. Brown (1983). Relative Abundance of DDT and PCB Parent Compounds and Their Oxygenated Metabolites in Marine Sediments, Shrimp and Fish. In Preparation.

5. Gossett, R.W., S.R. McHugh and D.A.Brown (1983). Metabolism of Xenobiotic Organics in Scorpionfish Caged Near a Major California Municipal Outfall. The *Second International Symposium on Responses of Marine Organisms to Pollutants*. April 27-29,1983. Woods Hole, Massachusetts.

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7. Gossett, R.W., D.A.Brown and D.R. Young (1982). Predicting the Bioaccumulation and Toxicity of Organic Compounds. In *Southern California Coastal Water Reserach Project Bienial Report 1981-1982* (W.Bascom, ed.), pp. 149-156. Long Beach, California.

8. Brown, D.A., K.D.Jenkins, E.M. Perkins, R.W.Gossett and G.P.Hershelman (1982). Detoxification of Metals and Organic Compounds in White Croakers. In *Southern California Coastal Water Research Project Biennial Report 1981-1982* (W. Bascom, ed.), pp. 157-172. Long Beach, California.

9. Brown, D.A., E.M. Perkins, K.D. Jenkins, P.O. Oshida, S.M. Bay, J.F. Alfafara and V. Raco (1982). Seasonal Changes in Mussels. In *Southern California Coastal Water Research Project Biennial Report 1981-1982* (W. Bascom, ed.), pp. 179-192. Long Beach, California.

10. Brown, D.A., R.W. Gossett, V.E. Raco and K.D. Jenkins (1982). Bioaccumulation and Detoxification of Hydrocarbons in Marine Organisms. *Preceedings of the Biological Effects Workshop*, Asilomar, California. March

30-31, 1982. Lawrence Livermore Publications. In Press.

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