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Stress Proteins in Amphipods as Biomarkers of Sediment Pollution in San Francisco Bay

Ingeborg Werner, Kurt F. Kline, and James T. Hollibaugh

Technical Report 48

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Interagency Ecological Program for the San Francisco Bay/Delta Estuary

A Cooperative Program of:

California Department of Water Resources
State Water Resources Control Board
U.S. Bureau of Reclamation
U.S. Army Corps of Engineers
California Department of Fish and Game
U.S. Fish and Wildlife Service
U.S. Geological Survey
U.S. Environmental Protection Agency
National Marine Fisheries Service

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Project Title: Stress Proteins in Amphipods as Biomarkers of Sediment Pollution in San Francisco Bay

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SUMMARY

This study investigates the use of stress proteins hsp60 and hsp70 in two infaunal (*Ampelisca abdita*, estuarine; *Rhepoxynius abronius*, marine) and one epifaunal (*Hyalella azteca*, freshwater) amphipod species as sublethal biomarkers for contaminant exposure in sediments. All three species are routinely used in standard sediment bioassays. In a first phase, the test organisms were exposed for 24 hours to solutions of the heavy metal cadmium, the pesticides diazinon and dieldrin, and the PAH fluoranthene. In the second phase, the effects of natural factors (life stage, moulting, starvation, salinity, and temperature changes) were investigated. In a third phase, the estuarine species *A. abdita* was exposed in 10-day bioassays to field sediments from 23 locations in San Francisco Bay. Results of stress protein analyses were compared to percent survival and concentrations of xenobiotic compounds in the test sediments. Analysis of hsp60 and hsp70 was performed using Western blotting techniques with subsequent comparative quantification by densitometry.

Results demonstrated compound and species-specific induction or inhibition of stress protein expression. Whereas a member of the hps70 protein family showed the most sensitive response to xenobiotic compounds in *H. azteca*, several members of the hsp60 protein family were the main proteins detected in *A. abdita* and *R. abronius*. Sensitivity of the stress protein response was highest in *H. azteca*, with significant effects detected at concentrations 110-, 50-, >1000- and >500-fold lower than LC50 values for cadmium, diazinon, dieldrin and fluoranthene, respectively. The corresponding ratios were >5, 0.7, >10, and 2.9 for *A. abdita* and >2, 3.1, >100, and >2.9 for *R. abronius*. Other proteins detected by staining SDS-Page gels with Coomassie-blue may prove to be more sensitive indicators for exposure to xenobiotic compounds than the hsp60 and hsp70 proteins detected by Western blotting.

Potential natural stressors like salinity and temperature changes induced stress proteins when known tolerance limits of the organisms were exceeded. Exposure to the moulting hormone ecdyson for 6 days did not alter stress protein expression in *H. azteca*, whereas starvation induced stress proteins in *R. abronius* but not in *H. azteca* within 10 days. Differences in stress protein response and sensitivity for cadmium and diazinon were detected between adult and juvenile *A. abdita*. Juvenile animals were less sensitive to cadmium and more sensitive to diazinon than adults. The stress protein response followed this pattern: it was 5 times less sensitive to cadmium exposure and 10 times more sensitive to diazinon exposure in juvenile animals.

Comparison of stress protein induction to survival after 10-day sediment bioassays with *A. abdita* showed that significantly reduced survival was associated with elevated stress protein levels in 36% of cases. In 6 out of 10 tests where elevated stress protein levels were measured,

survival was significantly reduced. Generally, induction of stress proteins was highest in sediments with high concentrations of PAHs and organotin compounds. The sediment, which caused highest mortality, also induced the strongest hsp64 response and contained high concentrations of PAHs (39.8 mg/kg dw) and phthalate esters (1280 µg/kg dw). Total concentrations of heavy metals were not correlated with percent survival or with stress protein induction.

Results from the first part of this study indicate that the stress proteins investigated might not be suitable as integrative biomarkers for complex mixtures of xenobiotic compounds often present in sediments. Furthermore, the sensitivity of the stress protein response detected in *A. abdita* is not high compared to survival after 10 days of exposure. Our results suggest that measuring hsp60 and hsp70 expression alone is presently not a reliable means of assessing the integrated stress caused by environmental pollutants. More information is needed on the link between increased (or decreased) stress protein expression, uptake of xenobiotic compounds, species specific metabolism, and longer-term effects in organisms.

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INTRODUCTION

In recent years, the search for more sensitive methods of detecting toxicity in the environment has led to increasing scientific interest in the cellular heat shock protein response (Sanders 1990, 1993; Dyer *et al.* 1990; Köhler *et al.* 1992; Hightower & Schulz 1991; Oedberg-Ferragut *et al.* 1991). The available information indicates that stress proteins confer protection from environmentally induced cellular damage, and accumulation of stress proteins in the cell has been linked to the intensity of stress. Relative to the abundance of information on the response in cell cultures, the ecologically more relevant investigations of these processes on an organismal level are scarce (Sanders 1993).

Heat shock proteins are induced by a wide variety of stressors (Sanders 1993; Hightower 1991), which appear to share the common property of either damaging proteins directly or causing cells to synthesize aberrant proteins (Pinto *et al.* 1991; Hightower 1991). The more general term "stress proteins", which includes the heat shock proteins as a subset, has therefore been widely applied. The main groups are categorized by molecular weights and include the hsp70 (or stress70) and hsp60 (or cpn60) protein families. Whereas hsp70s are predominantly cytoplasmic proteins, the inducible form of hsp60 is localized in the mitochondrial matrix. Functions of both protein families include the stabilization or generation of unfolded protein precursors before assembly, translocation of proteins into organelles, rearrangement of protein oligomers, dissolution of protein aggregates, and degradation of denatured proteins (Gething & Sambrook 1992).

Sediments bind and accumulate a wide variety of metals and organic compounds (Senesi and Chen 1989). These range from compounds with demonstrated genotoxicity to compounds with developmental toxicity and acute toxicity. Direct or indirect transfer of these chemicals from sediment to organisms is considered to be a major route of exposure for many species. Estuaries in urban areas such as San Francisco Bay are particularly impacted by anthropogenic pollution.

Most effort to assess potential contaminant impacts has been devoted to measurements of amounts of contaminants in the environment and/or in organisms. It is however apparent that chemical approaches alone are insufficient to address sediment pollution (Iannuzzi *et al.* 1995). Many of the chemicals are not present in bioavailable forms (Hamelink *et al.* 1994), and we lack the full information on their ability to cause deleterious effects. Whereas sensitive bioassay endpoints have been developed and applied in effluent studies, the assessment of sediment toxicity still proves problematic with endpoints being survival and/or growth of the test organism, reproduction, or accumulation of xenobiotics in body tissues. Due to their sensitivity, amphipods are routinely used in standard 10-day sediment bioassays (ASTM 1993). Their survival is used as a criterion to assess toxicity. These assays are generally short-term tests, which do not address sublethal contaminant effects. They cannot tell us how and if contaminants affect the organisms over a longer period, nor can they be used as an early warning of potential damage to the ecosystem.

The present study attempts to evaluate the induction of two heat shock proteins, hsp60 and hsp70, as potential sublethal biomarkers of contaminant exposure in a freshwater *(Hyalella azteca)*, an estuarine *(Ampelisca abdita)*, and a marine/estuarine *(Rhepoxynius abronius)* amphipod species (suborder: Gammaridea). We proposed analysis of stress protein hsp60 in animals exposed to San Francisco Bay sediment in standard 10-day bioassays and comparison of stress protein levels with bioassay results. The availability of an antibody cross-reacting with amphipod hsp70 enabled us to include this important stress protein in our investigation.

In a first phase, a series of 24-hour experiments was conducted with known concentrations of four toxic compounds. We chose a heavy metal (cadmium), a PAH (fluoranthene), an organochlorine pesticide (dieldrin), and an organophosphate pesticide (diazinon) as test compounds. All of these are common environmental contaminants, but they differ in structure and mode of action. We measured the accumulation of hsp70 and hsp60 after exposure to a range of concentrations of each compound to evaluate the presence and the sensitivity of the response. In addition, results from Western blotting were compared to protein patterns on Coomassie stained SDS-PAGE gels.

In the second phase, we investigated the effects of natural factors such as age of the animals, influence of moulting hormone (ecdyson), and starvation on hsp inducibility. We also conducted experiments on the effects of stressors such as salinity and temperature changes, which are difficult to control in field experiments.

In a third phase, *A. abdita* were exposed in standard 10-day bioassays to field sediments from 23 locations in San Francisco Bay, and survival was measured. The chemical composition of sediments was analyzed. Surviving amphipods were analyzed for hsp60 and hsp70 and results were compared to survival rates and xenobiotic content of the sediments tested. The time course of induction was investigated over a 10-day period.

Stressor-Specific Response

Animals: Sources and Holding

Animals were collected in Yaquina Bay, Oregon (*Rhepoxynius abronius)* and San Francisco Bay, California (*Ampelisca abdita*) or cultured in the laboratory *(Hyalella* azteca). During and after transport, *R. abronius* were maintained in sediment from the collection site at 15°C. The overlying sea water (30-33‰), obtained from Bodega Bay, California, was aerated. Tubes of *A. abdita* were maintained in aerated San Francisco Bay water, and the animals were slowly acclimated to the desired experimental temperature and/or salinity (2°C and/or 2-3‰ per day). Minimum holding time in the laboratory was 48 hours before experiments. Cultures of *H. azteca* were maintained at 25°C in aerated fresh water of moderate hardness (160-180 mg/L $CaCO_3$; Borgmann *et al.* 1989).

Experimental Design: Xenobiotics

Tests were performed exposing the animals to 4 or 5 concentrations of fluoranthene, diazinon, dieldrin, and cadmium chloride. Stock solutions were prepared in Pyrex beakers by adding CdCl2 (J.T. Baker Inc.) and diazinon (ChemService) directly to distilled water for nominal concentrations of 10 g/L $CdCl_2$ and 3.04 mg/L diazinon. We dissolved 10 mg dieldrin (ChemService) in 60 µL of acetone and 10 mg fluoranthene (ChemService) in 150 µL of acetone. The acetone mixture was then added to 1 L of distilled water and stirred for 24 hours. Stock solutions of cadmium, dieldrin, and fluoranthene were analyzed by atomic absorption spectroscopy and gas chromatography/mass spectroscopy. Measured concentrations were 4.8 g/L cadmium, 5.9 mg/L dieldrin, 1 mg/L (*H. azteca* test*)* or 3.5 mg/L (*A. abdita, R. abronius* tests) fluoranthene. The stock solution of diazinon was analyzed using the EnviroGard test kit (Millipore), which contained 4.3 mg/L diazinon. All final experimental concentrations of diazinon for the exposure experiment with *H. azteca* were analyzed. Nominal concentrations were used in subsequent exposure experiments with *A. abdita* and *R. abronius* to diazinon. Experimental concentrations were prepared by serial dilution with 0.22 µm filtered Bodega Bay (*R. abronius*, 31‰), San Francisco Bay (*A. abdita*, 25‰), or fresh water (*H. azteca*, 160-180 mg/L $CaCO_3$) in Pyrex beakers. Three replicate test containers per concentration were set up containing 150 mL of the test solution. Control treatments contained 0.22 µm-filtered water (Cd and diazinon exposures) or 1 µL/L (dieldrin exposure) and 3 µL/L acetone (fluoranthene exposure) in 0.22 µm-filtered water.

To the replicates, 10 (*H. azteca*), 20 (*R. abronius)*, or 30 (*A. abdita*) adult animals were added at random. Oxygen content of the water, pH, temperature and salinity were measured at the beginning and end of the experiments. Temperatures were 20°C for experiments with *A. abdita* and *H. azteca*, and 15°C for *R. abronius*. Infaunal species (*A. abdita, R. abronius*) were exposed in the dark, the epifaunal species (*H. azteca*) at a 16:8-hour light: dark cycle. After 24 hours, mortality was

recorded and part of the surviving animals were rinsed and stored at –70°C until stress protein analysis. To examine delayed mortality, 10 *R. abronius* and 10 *A. abdita* were taken from each test beaker and transferred into sediment from their respective collection sites, where they were maintained under standard protocol conditions (ASTM 1993) for 10 days.

The LC50s (24 hours) were determined for Cd and diazinon by exposing animals to 2, 1, 0.1, 0.01, and 0.001 mg/L Cd and 3, 0.3, 0.03, and 0.003 mg/L diazinon. Point estimates were calculated using probit analysis (US EPA 1991). Experimental concentrations were chosen such that the highest concentration was close to the LC50. No acutely toxic effect was observed within the 24-hour period for dieldrin and fluoranthene at concentrations up to 60 µg/L dieldrin and 500 µg/L fluoranthene. The 24-hour LC50s could, therefore, not be determined experimentally, and available values from experiments conducted for longer periods were taken from the literature (US EPA 1993; Swartz *et al.* 1990) for comparison. Except for the dieldrin experiment with *H. azteca*, experimental concentrations of dieldrin and fluoranthene were chosen such that the test concentrations bracketed available LC50 values.

Natural Stress Factors

Additional experiments were carried out to investigate the effect of environmental variables (variations of salinity and temperature, moulting hormone ecdyson, starvation) on stress protein expression. Animals were exposed for 24 hours to 0.22-µm-filtered water of different salinities bracketing the salinity tolerance of each species. The effect of environmentally relevant temperature changes was tested by exposing animals to temperatures 5-10°C above their adapted temperature for 24 hours. To investigate the effects of moulting on hsp60 and hsp70 expression, *H. azteca* were exposed to a 1-µM solution of the moulting hormone ecdyson (E. Chang, Bodega Marine Laboratory) for 6 days. This species was chosen due to its relatively short moulting cycle (7-8 days at 26-28°C; Bovee 1950). The effects of starvation were tested in 10-day experiments with *H. azteca* and *R. abronius*. *Hyalella azteca* were kept in aerated 0.22-µm-filtered water. Control animals were fed a mixture of Tetramin, a common aquarium fish food, trout chow, and alfalfa leaves every second day. *Rhepoxynius abronius* were allowed to burrow in pure fine granular silica (0.3-0.4 mm; Fisher) and maintained under ASTM protocol conditions for 10 days. Control animals were kept in sediment from their collection site.

Influence of Life Stage

To investigate the effect of age on the sensitivity and stress response of the animals, juvenile *A. abdita* were exposed to the same xenobiotic treatments. These animals were collected in July 1993 and were 2-3 mm long. *A. abdita* of this size are immature (Mills 1967; Kinnetic Laboratories Inc. 1980).

10-Day Sediment Bioassays with *Ampelisca abdita*

Animals: Source and Holding

Animals were collected in San Francisco Bay, California. Tubes of *A. abdita* were maintained in aerated San Francisco Bay water, and the animals were slowly acclimated to the desired experimental temperature and/or salinity (2°C and/or 2-3‰ per day). Minimum holding time in the laboratory was 48 hours before experiments.

Sediments: Collection and Treatment

Control Sediments: Sediment from northern San Francisco Bay where large, permanent populations of *A. abdita* are found was collected by means of a Van Veen grab sampler and used as control sediment in *Ampelisca* bioassays. Chemical analysis of the sediments revealed no or very low levels of contamination.

Test Sediments: Sediment samples were taken from 23 sites in San Francisco Bay. (Table 1, Figure 1). Three to five stations per site were sampled using a gravity corer or a vibra-corer, depending on water depth at the selected site.

Table 1
SEDIMENT SAMPLING SITES IN SAN FRANCISCO BAY

1	Benicia	9	Unocal
2	Carquinez	10	Alcatraz
3	Exxon-1 Exxon-2	11	Treasure Island
4	San Pablo Bay	12	Port of Oakland
5	Bel Marin Keyes	13	San Francisco Dry Dock-A San Francisco Dry Dock-B San Francisco Dry Dock-D
6	Paradise Cay	14	San Mateo, Oyster Point
7	San Rafael-A San Rafael-B		
8	NFISC 1-1 NFISC 1-2 NFISC 1-3 NFISC 1-5 NFISC 1-6 NFISC 1-9		

Sediment Handling: Samples were packed in insulated containers, cooled with ice and non-toxic ice-packs to 4°C, and transported to the Bioassay Laboratory of ABT Inc. in Tiburon, California. First, the samples from each station were thoroughly mixed in stainless steel bowls until they were a consistent color and texture. Then, each station was thoroughly mixed with the other four stations from the respective sampling site. All sediments were then placed in cold storage and maintained at 4°C prior to testing.

Sediment Physical Characteristics: Subsamples for grain-size analysis were collected and analyzed by MEC Analytical Systems, Inc., Carlsbad, California. The method for grain-size analysis follows Plumb (1981).

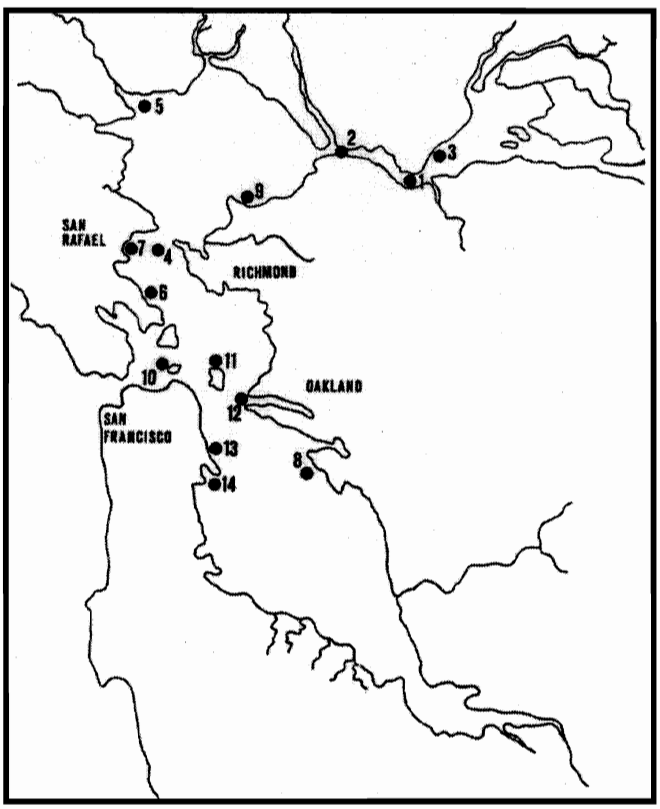

Figure 1
SEDIMENT SAMPLING SITES IN SAN FRANCISCO BAY

Cadmium-Spiked Sediment

Sediment Spiking: Control sediment was sieved (1-mm mesh), placed in Pyrex containers, and weighed. We added 15 mg cadmium to 1 kg of sediment (wet weight) as a solution of $CdCl_2$, then stirred the mixture by hand with a plastic rod for 1 minute. The sediment was then shaken for 24 hours and allowed to settle for 24 hours in the dark. The resulting Cd concentration was 10.3 mg/kg sediment (wet weight).

Bioassay: The 10-day bioassay was performed as described above. After 1, 2, 4, 7, and 10 days, the animals of 3 replicates each of Cd-spiked and control sediment were removed, treated as above, and stored until further analysis.

Stress Protein Analysis

Sample Processing for Stress Proteins

Between 4 and 6 animals (*R. abronius, H. azteca*) or 10-15 animals (*A. abdita*) were pooled for one sample and homogenized by hand on ice in a glass homogenizer for 2 minutes with 2.5 volumes of a hypotonic solution containing 66 mM tris buffer (Trizma Base, Sigma, pH 7.5), 1% Nonidet P-40 (Sigma), and 0.2 mM phenylmethylsulfonylfluoride (Sigma). The homogenized sample was placed in microcentrifuge tubes and centrifuged at 7000 g for 30 minutes at 4°C. The pellets were resuspended in about 50 µL 1% SDS-homogenization buffer, vortexed for 30 seconds, and centrifuged at 7000 g for 30 minutes at 4°C. The total protein concentration in each supernatant was determined by the Biorad DC Protein Assay, which is based on the method of Lowry (1951). Sample buffer (Lämmli 1970) was immediately added to each sample at a 5:1 dilution, and samples were boiled for 2 minutes.

1-D Electrophoresis

Samples containing 50 µg total protein were loaded on a 10% polyacrylamide gel with a 5% stacking gel (Blattler *et al.* 1972) using the buffer system described by Lämmli (1970). The gels were run at 25 mA per gel for about 1.5 hours (Biorad Mini-Protean). A parallel gel was prepared using 25 µg total protein per sample and stained with Coomassie blue for comparison.

Western Blotting

The gels were equilibrated in transfer buffer for 30 minutes, and proteins were electroblotted onto Immobilon-P membrane (Millipore) (Towbin *et al.* 1979) at constant current (200 mA) for 1 hour and 12 minutes. A standard (Sigma, see below) was used, and proteins were stained with Ponceau S solution to check for transfer efficiency. Membranes were blocked with 5% skim milk in 20 mM tris buffer and 0.4 M NaCl (pH 7.5) with 0.05% Tween-20 for 30 minutes. The following primary antibodies were used as probes:

- Polyclonal antibody, directed against hsp60 from *Synechococcus* sp. (cyanobacteria; Stressgen), dilution 1:1,500
- Polyclonal anti-hsp60 directed against hsp60 from moth sperm (dilution 1:1,500; Stressgen)
- Monoclonal, anti-hsp 70 (MA3-001) antibody (dilution 1:500; Affinity Bioreagents)

Sediment Chemistry: Sediments were analyzed for heavy metals, pesticides and polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), phthalate esters and organotin compounds. The following compounds were measured:

- Heavy metals: As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Zn
- Pesticides and PCBs: alpha-BHC, gamma-BHC, beta-BHC, heptachlor, delta-BHC, aldrin, heptachlor epoxide, endosulfan I, 4,4'-DDE, dieldrin, endrin, 4,4'-DDD, endosulfan II, 4,4'-DDT, endrin aldehyde, endosulfan sulfate, methoxychlor, toxaphene, chlorodane, PCB 1016, 1221, 1232, 1242, 1248, 1254, 1260
- PAHs: naphthalene, 2-methylnaphthalene, acenaphthylene, dibenzofuran, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)-anthracene, chrysene, benzo(b+k)fluoranthene, benzo(a)pyrene, ideno(1,2,3-cd)-pyrene, dibenz(ah)anthracene, benzo-(g,h,i)perylene
- Phthalate esters: dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, butylbenzyl phthalate, bis(2-ethylhexyl) phthalate, di-n-octyl phthalate
- Organotin compounds: tributyltin, dibutyltin, monobutyltin

The analysis of total organic carbon was performed using a persulfate wet oxidation method (Menzel and Vaccaro 1964).

Sediment Bioassays

The complete procedure for the infaunal amphipod bioassay is found in Swartz *et al.* (1985) and ASTM (1993). Sediments were sieved through a 1.0-mm stainless steel mesh to remove large materials and organisms. Bioassays were performed as 10-day static tests with solid phase sediment and overlying water in aerated 1-L glass test chambers (five replicates). Twenty amphipods per replicate were exposed to about 200 mL of sediment for 10 days at a temperature of 20°C. Salinity of the overlying water was 25-28‰. The animals were adapted to test conditions over an appropriate period prior to the tests. During the test period, NH_3/NH_4, O_2, pH, and temperature were recorded daily. Mortality and sublethal effects such as emergence from sediment were recorded. Surviving animals were collected and immediately frozen and stored at –70°C for subsequent stress protein analysis.

Time Course of Stress Protein Induction in Field-Collected Sediment and Cadmium-Spiked Sediment

Sediment from Site NFISC 1-9

A 10-day sediment bioassay with 3 replicate test containers and 50 animals per container was performed according to the standard test protocol (ASTM 1993) at 20°C and a salinity of 25‰. After 1, 3, 5, 7, and 10 days, the animals of three replicates containing NFISC 1-9 sediment and three replicates containing control sediment were sieved, counted, rinsed with distilled water, and stored at –70°C until further analysis.

After the cross-reactivity of the primary antibodies with amphipod heat shock proteins was examined, each blot (membrane) was probed with both anti-hsp60 and anti-hsp70.

Blots (membranes) were incubated for 1.5 hour with primary anti-hsp60 antibody and then washed three times for 30 minutes in tris-buffered saline solution containing 0.05% Tween-20. Alkaline phosphatase-conjugated, secondary antibodies, anti-rabbit (Biorad; 1:1,000; incubation time: 1.5 hour) was used to detect the hsp60 antibody probes. Membranes were washed again, incubated for 1.5 hour with primary anti-hsp70 antibody, and washed three times. Anti-rat IgG (Sigma; 1:10,000; incubation time: 1.5 hour) was used as the secondary antibody to detect anti-hsp70. After washing the substrates, p-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate were used to visualize the bound antibodies. The developed blots (membranes) were scanned on a densitometer (Biorad GS-670).

One of the proteins contained in Sigma HMW protein standard cross-reacted with anti-hsp60. We used this as our internal standard to check for transfer efficiency and freshness of reagents. Additionally, gels were stained with Coomassie blue after transfer to ensure that complete protein transfer had occurred. Being aware of the semi-quantitative nature of Western blotting, 2-3 lanes contained samples from control treatments on each of the two blots (supernatant and pellet) per experiment. Each xenobiotic treatment was usually represented by 3 replicate lanes per blot. Wherever possible, all samples to be compared were analyzed on one blot. Densitometer readings of samples from xenobiotic treatments were compared to readings of control samples on the same blot. Membranes from several experiments were incubated and developed simultaneously to ensure that blot development conditions were identical. Fresh antibody solutions were used each time. We examined variation in bands of equal amounts of protein standard within one blot. The standard error of densitometer readings within one blot was 8.7%. Initially, blots were produced in duplicate. Subsequent nested analysis of variance showed that variation between blots accounted for about 7% of total variance.

Statistical Analysis

Treatment effects on stress protein levels were determined using one-way analysis of variance (ANOVA) (Sokal & Rohlf 1981). Differences between treatment data and control data were analyzed by T-test (Sokal & Rohlf 1981). If $p < 0.05$, stress protein levels were considered significantly different from controls. Nested ANOVA was performed to examine error due to variation between Western blots, test beakers, and treatments. Differences between treatment data and control data from 10-day sediment bioassays with *A. abdita* were analyzed by t-test. Regression analysis was used to determine correlation coefficients for the relationship between survival data and stress protein levels. All statistical results were calculated in SAS (SAS Inst. Inc. 1988).

Stressor-Specific Response

Water Quality

Oxygen content in test containers varied between 6.9-7.2 mg/L (*R. abronius*), 7.5-8.4 mg/L (*A. abdita*), and 8.5-9.0 mg/L (*H. azteca*). The pH ranged from 7.7 to 8.4.

LC50s and Delayed Mortality

LC50s from this study and other sources are listed in Table 2. Survival after 24 hours of cadmium exposure was above 98% for *R. abronius* exposed to 1.25, 2.5, 5, and 10 mg Cd/L, but all animals died

Table 2
TEST CONCENTRATIONS IN 24-HOUR EXPERIMENTS AND LC50s

Species	Compound Tested	Test Concentrations	LC50
Hyalella azteca	Cadmium	50, 25, 5, 0.5 µg/L	55 µg/L (24h; 0‰)*
	Diazinon	32, 5.8, 0.6, 0.03 µg/L	30 µg/L (24h)*; 19 µg/L (48h)*
	Dieldrin	6, 0.6, 0.06, 0.006 µg/L	>6 µg/L (24h)*; 7.6 µg/L (96h) — USEPA (1993) >53 mg/kg sediment (10d) — Albrecht** (pers. comm.)
	Fluoranthene	500, 100, 10, 1 µg/L	>500 µg/L (24h)*; 15.4 mg/kg sediment (10d) (about 72 µg/L) — DeWitt *et al.*, (1989)
Ampelisca abdita	Cadmium	2.5, 0.5,0.05, 0.005 mg/L	>2.5 mg/L(24h; 25‰)*; 0.33 mg/L (96h) — ASTM (1993)
	Diazinon	30, 3, 0.3, 0.03 µg/L (nom.)	21 µg/L (24h)*; 10 µg/L (48h)*
	Dieldrin	60, 6, 0.6, 0.06, 0.006 µg/L	>60 µg/L (24h)*; 9.95 mg/kg sediment (10d) — Albrecht** (pers. comm.)
	Fluoranthene	100, 35, 3.5, 0.35, 0.035 µg/L	>100 µg/L (24h)* 3.3-9.9 µg/L (96h) — ASTM (1993)
Rhepoxynius abronius	Cadmium	10, 5, 2.5, 1.25 mg/L	>10 mg/L(24h; 31‰)* 0.92 mg/L (96h) — ASTM (1993)
	Diazinon	30, 3, 0.3, 0.03 µg/L (nom.)	9.2 µg/L (24h)*
	Dieldrin	60, 6, 0.6, 0.06 µg/L	>60 µg/L (24h)*
	Fluoranthene	100, 70, 35, 3.5, 0.35 µg/L	>100 µg/L (24h)*; 23.8 µg/L (10d) — Swartz *et al.* (1990)

* This study.
**B. Albrecht, TRACK Labs, Pensacola, Florida.

during a 10-day recovery period in home sediment. No *R. abronius* survived a 10-day recovery period after 24-hour exposure to 30 µg/L diazinon, but 100% of animals exposed to 3, 0.3, and 0.03 µg/L diazinon survived. All *A. abdita* transferred after exposure to 0.5, 0.05, and 0.005 mg/L Cd survived the 10-day recovery period. All animals exposed to 2.5 mg/L Cd died during this time. Animals showed delayed mortality (within 10 days) after 24-hour exposure to 30 µg/L (68% of transferred animals died) and 3 µg/L (48% of transferred animals died) of diazinon. Survival was above 98% after 24 hours in fluoranthene and dieldrin treatments for all species, and no mortality was recorded after 10-day recovery in *A. abdita* and *R. Abronius*.

Antibody Cross-Reactivity

Amphipod heat shock proteins that cross-reacted with the antibody probes used are shown in Figure 2. A distinction is made between supernatant and pellet fractions. Arrows show protein bands quantified by densitometry. Since we started our analysis of *H. azteca* samples from exposure experiments using anti-hsp60 from moth, we continued to use this antibody for *H. azteca*. Due to limited availability of the original moth antibody, anti-hsp60 from cyanobacteria was used as probe for hsp60 in the analyses of *R. abronius* and *A. abdita*. Cross-reactivity of both hsp60-antibodies was tested. Dominant proteins detected after exposure experiments are listed in Table 3.

In *H. azteca*, both hsp60-antibodies (moth, cyanobacterium) recognized primarily three proteins (hsp58, hsp61, hsp64; Figure 2a). We quantified hsp58 and hsp64 bands because these were consistently observed after exposure to xenobiotic compounds. Anti-hsp70 recognized a protein of about 71 kDa in both sample

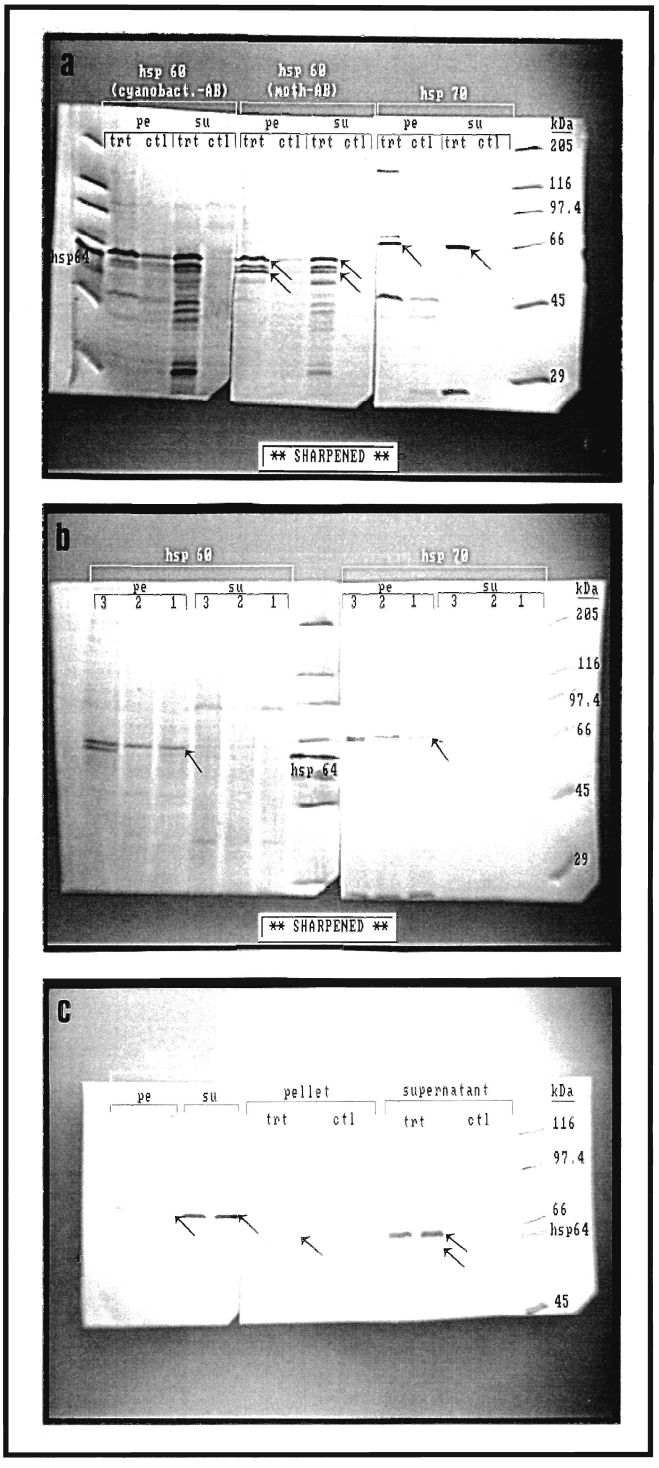

Figure 2
CROSS-REACTIVITY OF PRIMARY ANTIBODIES WITH AMPHIPOD PROTEINS
Western blots of (a) *H. azteca*, (b) *A. abdita*, (c) *R. abronius*.

su = supernatant, pe = pellet, ctl = control sample,
trt = diazinon treated,
1 = heat-shocked, 2,3 = diazinon treated
Arrows indicate bands quantified on densitometer.

Table 3
CROSS-REACTIVITY OF PRIMARY ANTIBODIES

	Approximate Molecular Weight of Cross-Reacting Proteins (kDa)					
	H. azteca		*A. abdita*		*R. abronius*	
Antibody	Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet
anti-hsp60	**58**	**58**	64	62	60	60
(SPA-805; moth;	61	61		64	64	64
rabbit polyclonal)	**64**	**64**				
anti-hsp60	58	58	**54**	**54**	**54**	**54**
(SPA-804;	61	61	**64**	62	**60**	**60**
cyano-bacterium;	64	64		**64**	**64**	**64**
rabbit polyclonal)						
anti-hsp70	28	**71**	—	27	**71**	**71**
(MA3-001;	**71**	73		**71**	76	140
human, rat monoclonal)		140		140		

— Non-detectable
Molecular weight of proteins quantified by densitometry printed in bold.

fractions. Additionally, it bound to a 28-kDa protein in supernatants and to proteins of about 73 and 140 kDa in the pellet fraction. Only the most consistent band at 71-kDa protein was quantified. The secondary antibody (anti-rat) was shown to bind to a protein of about 47 kDa (Werner 1995).

In *A. abdita,* heat shock proteins were detected primarily in the pellet fraction (Figure 2b). Only after strong insults were hsp60-proteins observed in the supernatant fraction (Table 3). Anti-hsp60 from cyanobacterium recognized proteins of 54, 62, and 64 kDa. Hsp54 is not shown in Figure 2b. Anti-hsp70 recognized a 71-kDa protein and additionally bound to proteins of about 27 and 140 kDa. Only consistently occurring protein bands of 54, 64, and 71 kDa were quantified by densitometry.

In *R. abronius*, anti-hsp60 from cyanobacterium detected proteins of 54, 60, and 64 kDa in both sample fractions (54 kDa and 60 kDa proteins not shown in Figure 2c). Anti-hsp70 recognized proteins of 71 and 76 kDa in supernatants (76 kDa protein not shown), and proteins of 71 and 140 kDa in pellets (Figure 2c).

Hsp60 and Hsp70 Response in *Hyalella azteca*

Supernatants: Hsp71 was measured predominantly in the pellet fraction and was detected in supernatants only after exposure to 32 µg/L diazinon (Figure 3). At relatively low concentrations of xenobiotic compounds, hsp58 and hsp64 seemed to shift from the supernatant to the pellet fraction. Whereas these two members of the hsp60 protein family were detected in supernatants of control animals, they were not detectable in supernatants of animals exposed to 5-25 µg/L cadmium, and 6 and 60 ng/L dieldrin but were significantly elevated in pellets. After exposure to 0.03 µg/L and 0.6 µg/L diazinon, a significant decrease in hsp58 and hsp64 levels was measured in the supernatant fraction. After comparison with control values from other tests, this decrease was considered negligible. A diazinon concentration of 32 µg/L significantly increased hsp58, hsp64, and hsp71 in supernatants.

Pellets: Stress protein levels in pellets were elevated following exposure of the animals to cadmium, diazinon and dieldrin (Figure 3a-c). Induction of hsp71

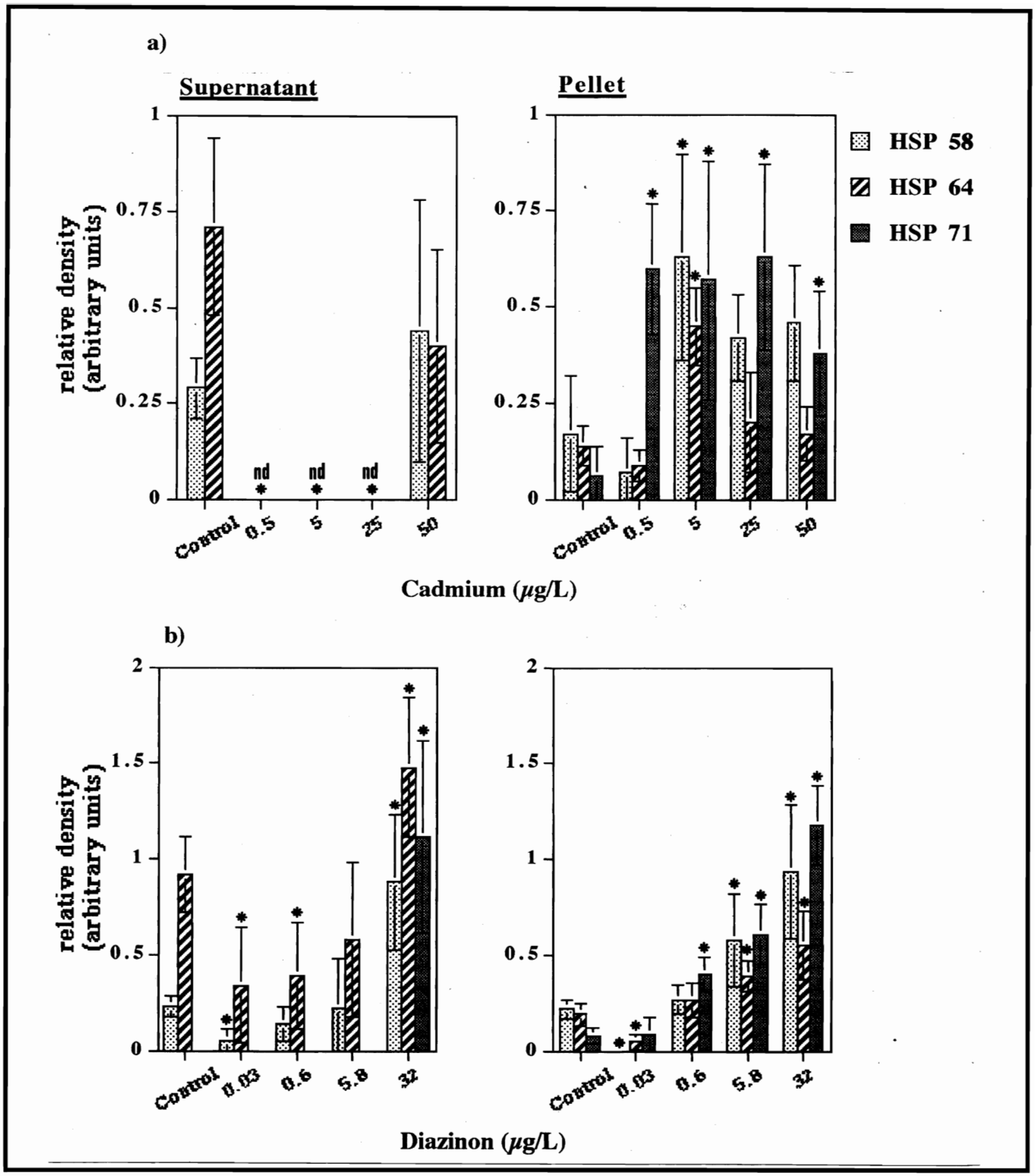

Figure 3
HSP EXPRESSION FOLLOWING 24-HOUR EXPOSURE OF *HYALELLA AZTECA* TO CADMIUM, DIAZINON, DIELDRIN, AND FLUORANTHENE

Mean optical density of bands detected by Western blot analysis and 95% confidence limits; n = 6;
*Significantly different from control, significance level $p<0.05$;
nd = not detected.

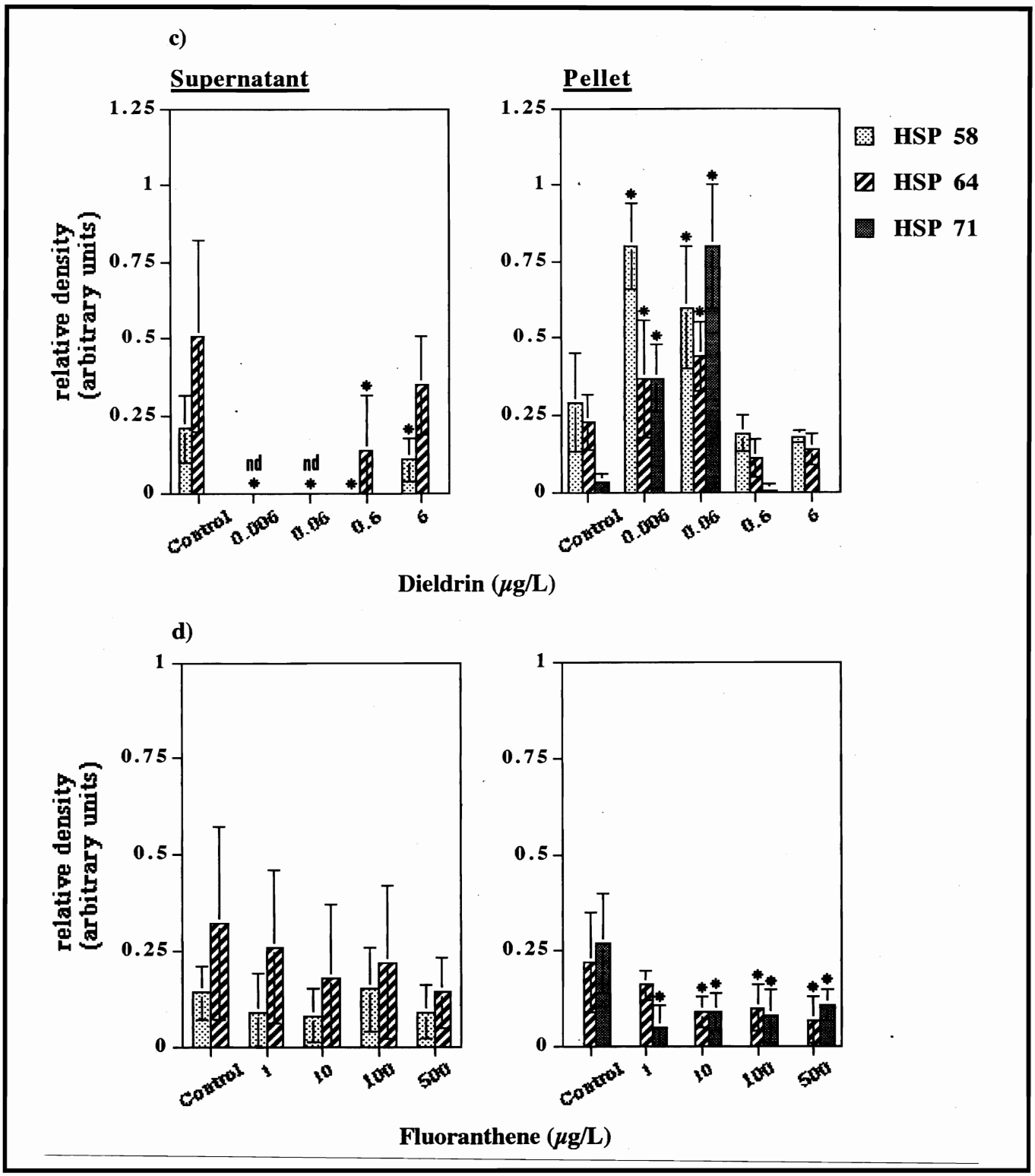

Figure 3 (continued)
HSP EXPRESSION FOLLOWING 24-HOUR EXPOSURE OF *HYALELLA AZTECA* TO CADMIUM, DIAZINON, DIELDRIN, AND FLUORANTHENE

Mean optical density of bands detected by Western blot analysis and 95% confidence limits; n = 6;
*Significantly different from control, significance level $p<0.05$;
nd = not detected.

generally occurred at lower xenobiotic concentrations than induction of hsp64 or hsp58. Hsp71 levels in pellets were increased about tenfold after exposure to 0.5, 5, and 25 µg/L cadmium, but only fivefold after exposure to 50 µg/L cadmium. Hsp64 and hsp58 were significantly elevated at 5 µg/L cadmium (Figure 3a). After exposure to diazinon, hsp71 was significantly elevated at concentrations of 0.6 µg/L, 5.8 µg/L, and 32 µg/L. Hsp58 and hsp64 were significantly induced in animals exposed to 5.8 and 32 µg/L diazinon (Figure 3b). Hsp58 and hsp64 seemed to be decreased at 0.03 µg/L diazinon, but considering the variability of low values, we considered this decrease as inconclusive. Exposure to dieldrin led to a significant increase of hsp58, hsp64, and hsp71 at dieldrin concentrations of 6 and 60 ng/L (Figure 3c). No induction was detected after exposure to 0.6 µg/L and 6 µg/L dieldrin. Fluoranthene appeared to have an inhibiting effect on stress protein production (Figure 3d). Levels of hsp64 and hsp71 in pellets were significantly reduced relative to controls at a concentration as low as 1 µg/L fluoranthene, although no effect on survival was observed even at 500 µg/L fluoranthene, where animals were observed to clump together and were clearly lethargic.

Hsp60 and Hsp70 Response in *Ampelisca abdita*

Heat shock proteins 54, 64, and 71 were detected predominantly in the pellet fraction. Hsp54 and hsp64 were detectable in the supernatant fraction only after exposure to 30 µg/L diazinon (data not shown). Hsp64 and hsp54 levels in pellets were elevated 5- to 15-fold in animals exposed to cadmium, diazinon, and fluoranthene (Figure 4a, b, d), whereas hsp71 showed no response. Significant hsp64 increases were measured following exposure to 0.5 mg/L and 2.5 mg/L cadmium, 30 µg/L diazinon, and 35 µg/L (but not 100 µg/L) fluoranthene. Hsp54 was significantly elevated in animals exposed to 2.5 mg/L cadmium, 30 µg/L diazinon, and 35 µg/L (but not 100 µg/L) fluoranthene. Expression of hsp54 was significantly decreased at dieldrin concentrations of 6 and 60 µg/L, and expression of hsp64 was significantly decreased at dieldrin concentration of 60 µg/L (Figure 4c).

Life Stage Dependence of Stress Protein Induction in *A. abdita*

Stress proteins hsp54 and 64 were detected only in the pellet fraction (Figure 5a-d). Hsp71 was not detected. The stress protein response to cadmium was less sensitive in juvenile *A. abdita* than in adults (about fivefold). Both proteins were induced in juveniles at a concentration of 2.5 mg/L cadmium, whereas induction of hsp64 in adults occurred at 0.5 mg/L cadmium. All of the juvenile animals exposed to 2.5 mg/L cadmium survived the 24-hour test period but died within the following 10 days in "clean" sediment. Diazinon caused induction of hsp64 in juveniles at concentrations of 3 and 30 µg/L; *i.e.*, 10 times lower than in adults. Hsp64 levels were about twice as high at 3 µg/L diazinon than at 30 µg/L. Dieldrin and fluoranthene neither caused mortality nor increased levels of stress proteins within the 24-hour test period.

Hsp60 and Hsp70 Response in *Rhepoxynius abronius*

As opposed to both *H. azteca* and *A. abdita*, a stress protein response to xenobiotic compounds was primarily detected in the supernatant fraction of the samples (Figure 6). Only under severe stress (10 mg/L Cd, 30 µg/L diazinon) were stress proteins detected in the pellet fraction. Hsp60 was induced by all com-

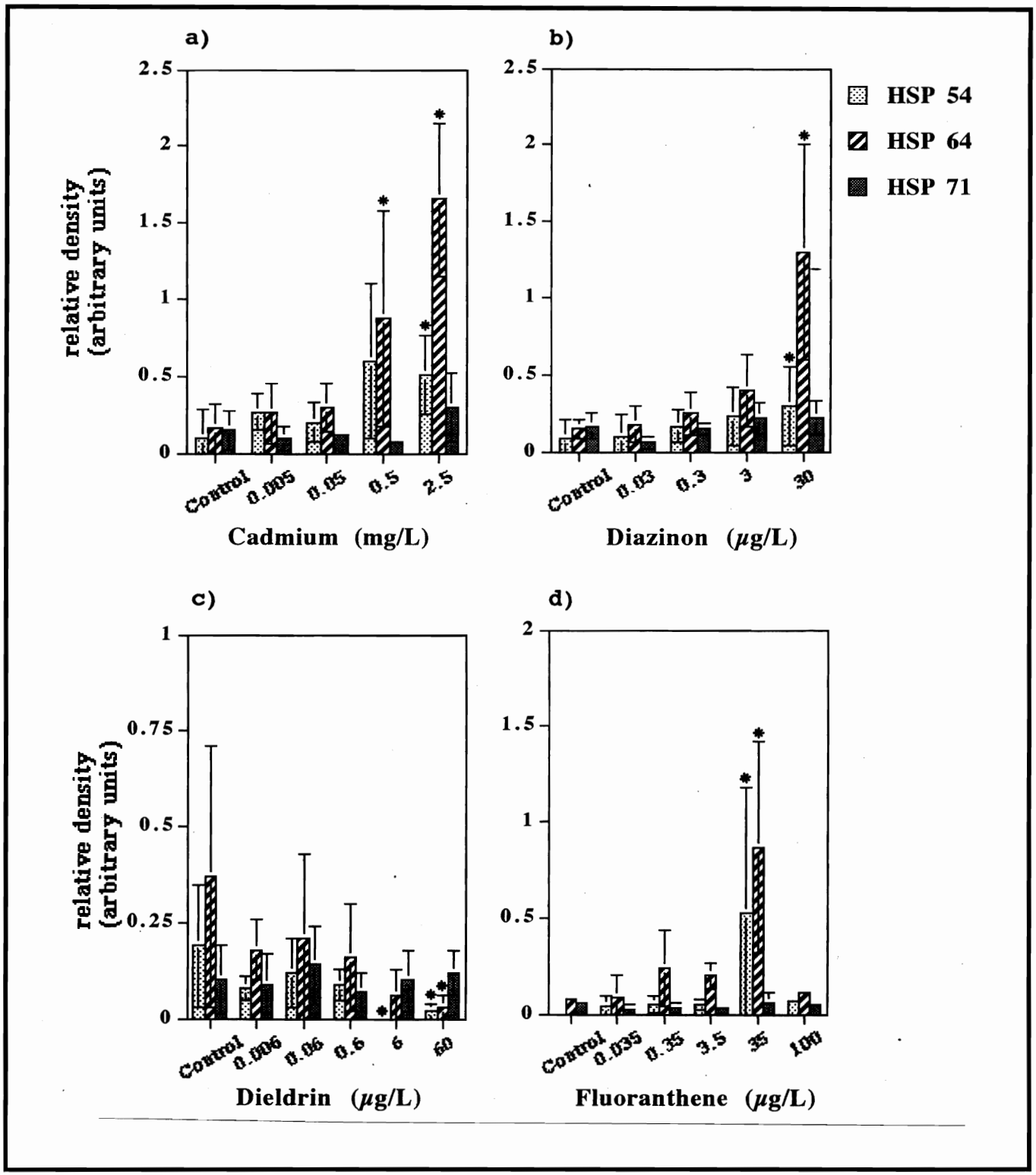

Figure 4
HSP IN PELLET FRACTION FOLLOWING 24-HOUR EXPOSURE OF ADULT *AMPELISCA ABDITA* TO CADMIUM, DIAZINON, DIELDRIN, AND FLUORANTHENE

Mean optical density of bands detected by Western blot analysis and 95% confidence limits; n = 6;
*Significantly different from control, significance level $p<0.05$;
nd = not detected

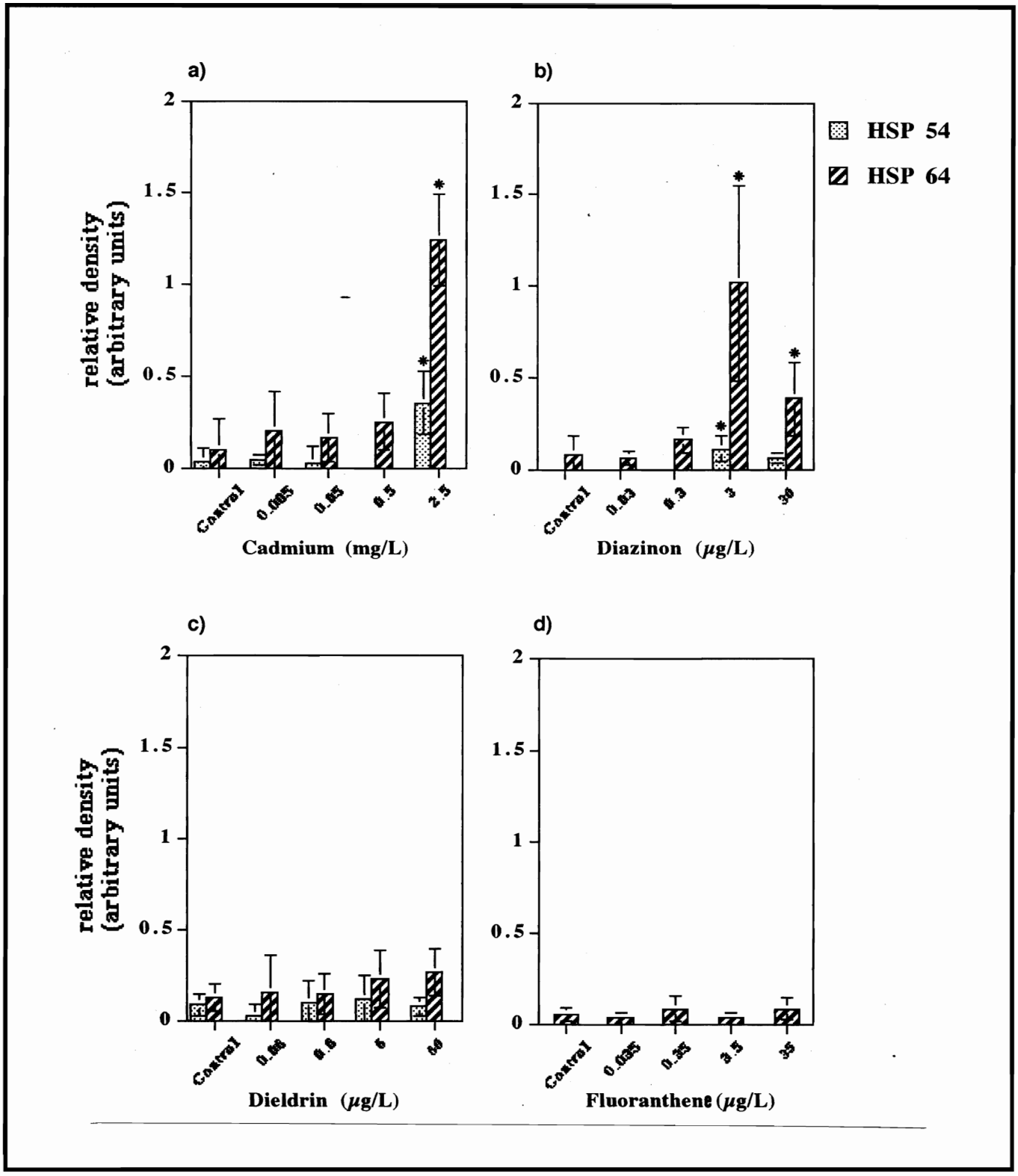

Figure 5
HSP60 PROTEINS IN JUVENILES OF *AMPELISCA ABDITA* (PELLET) AFTER 24 HOURS IN CONCENTRATIONS OF CADMIUM, DIAZINON, DIELDRIN, AND FLUORANTHENE

Means of densitometer readings and 95% confidence limits of proteins detected by Western blotting using hsp60 and hsp70 antibodies; n = 6;
*Significantly different from controls; $p < 0.05$; nd = not detected

pounds tested, whereas only diazinon caused a significant increase in hsp54, hsp64, and hsp71.

Hsp60 levels in the supernatant fraction were elevated about twofold in response to exposure to 5 and 10 mg/L cadmium (Figure 6a). Ten mg/L cadmium caused hsp54, hsp60, and hsp64 levels to rise significantly in pellets. Although no animal died within the 24-hour test period, we found that all of the exposed animals died within a 10-day recovery period in clean sediment (Werner 1995). Following exposure to 30 µg/L diazinon (Figure 6b), hsp54, hsp60, hsp64, and hsp71 were induced. Significant induction of hsp54 was measured at nominal diazinon concentrations of 3 and 30 µg/L, and 30 µg/L diazinon caused an increase in hsp64 and hsp71 pellets. Dieldrin (Figure 6c) caused significant induction of hsp60 at a concentration of 0.6 µg/L only. Fluoranthene concentrations of 35 and 70 µg/L (but not 100 µg/L; Figure 6d) induced hsp60. Elevated levels of hsp54 and hsp60 were detected in pellets following exposure to 35 µg/L fluoranthene; they were, however, highly variable and not significantly different from controls.

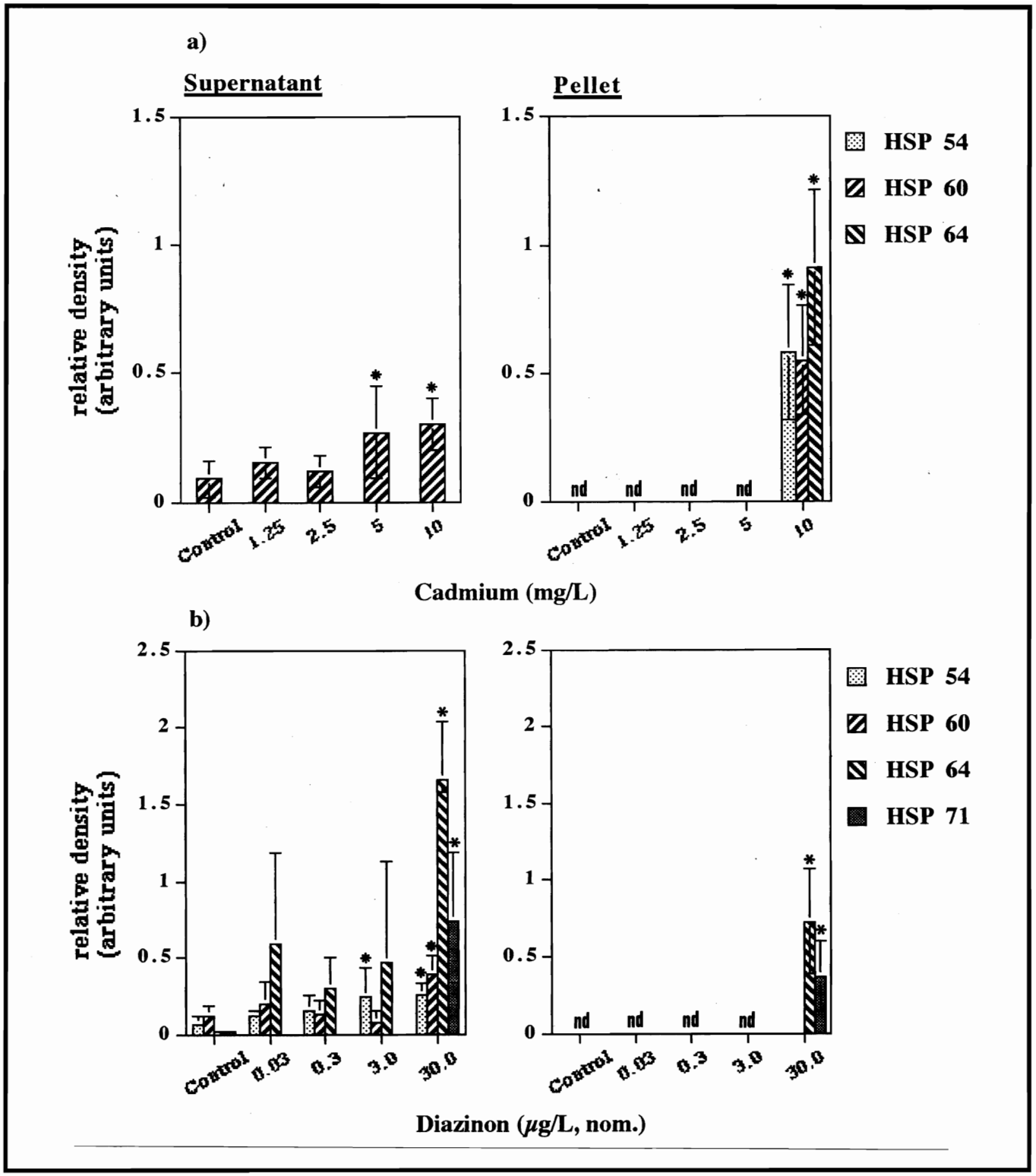

Figure 6
HSP EXPRESSION FOLLOWING 24-HOUR EXPOSURE OF *RHEPOXYNIUS ABRONIUS* T CADMIUM, DIAZINON, DIELDRIN, AND FLUORANTHENE

Mean optical density of bands detected by Western blot analysis and 95% confidence limits; n = 6.
*Significantly different from control, significance level $p<0.05$; nd = not detected.

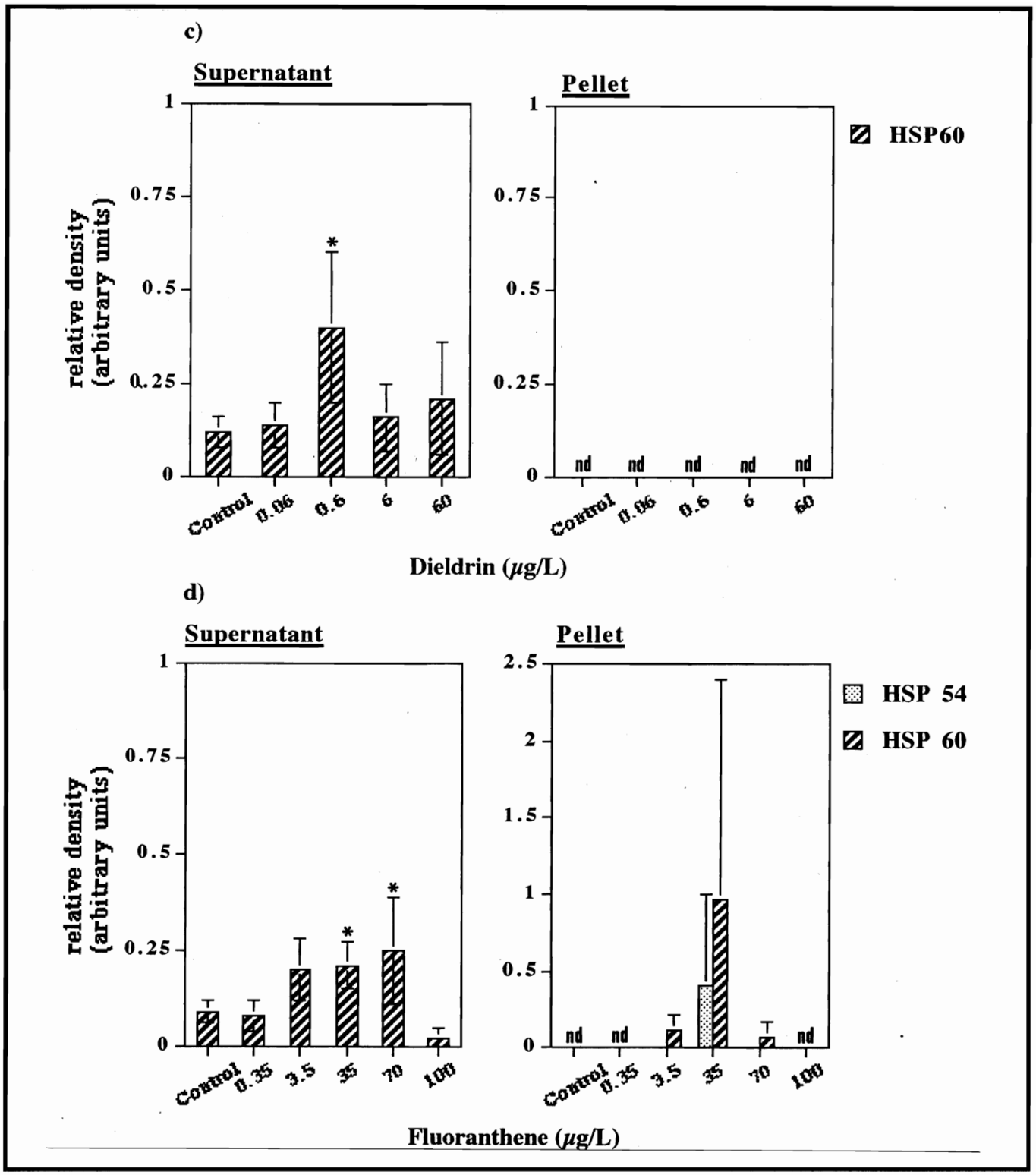

Figure 6 (continued)
HSP EXPRESSION FOLLOWING 24-HOUR EXPOSURE OF *RHEPOXYNIUS ABRONIUS* TO CADMIUM, DIAZINON, DIELDRIN, AND FLUORANTHENE

Mean optical density of bands detected by Western blot analysis and 95% confidence limits; n = 6.
*Significantly different from control, significance level $p<0.05$; nd = not detected.

Effect of Natural Stressors

Table 4 summarizes results of experiments carried out on the effects of potential natural inducers of stress proteins.

Salinity: Levels of hsp61 (pellet) in *H. azteca* maintained for 24 hours at 0, 10, and 15‰ (adapted salinity: 0‰) showed no significant increase, but at 20‰, hsp61 increased significantly (Figure 7) and about 40% of the animals died. Levels of hsp71 (pellet) appeared to decrease at higher salinity, but differences were not statistically significant. *A. abdita* exposed for 24 hours to salinities of 15, 20, 26, and 32‰ (adapted salinity: 26‰) showed no significant change in hsp64 or hsp71 levels. Two stress proteins (hsp64, hsp71; supernatant) were elevated in *R. abronius* (adapted salinity: 30‰) exposed to 20‰ compared to hsp levels measured in animals from the 25‰ treatment. Survival was above 95% in all treatments with the exception of *H. azteca* maintained at 20‰.

Temperature: Levels of hsp58, hsp64, hsp71, and hsp140 (pellet fraction), a possible dimer of hsp71, were elevated in *Hyalella azteca* exposed for 24 hours to 35°C (from 25°C) (Figure 8). No induction was measured in *A. abdita* exposed for 24 hours to 25°C (from 15°C). *R. abronius* showed elevated levels of hsp54 and hsp64 (supernatant fraction) after 24-hour exposure to 25°C (from 15°C).

Moulting hormone: Ecdyson did not cause measured hsp levels to rise in *H. azteca* (Figure 9). Beùserve increased moulting in exposed animals, it is uncertain whether the animals took up the ecdyson from solution. However, uptake of ecdyson from solution has been observed in lobsters, and it results in a shorter moulting cycle (E. Chang, University of California, Bodega Marine Laboratory, personal communication).

Starvation: Maintaining *R. abronius* for 10 days in fine-granuled silica significantly raised stress protein levels (Figure 10a) and caused 55% mortality compared to controls. Relative to fed controls, stress protein levels (hsp64, hsp71) decreased in starved *H. azteca* at the end of a 10-day experiment (Figure 10b). Survival of *H. azteca* was 100%.

Table 4
INDUCTION OF HSP60 AND HSP70 PROTEINS BY NATURAL STRESSORS

Stressor	*H. azteca*	*A. abdita*	*R. abronius*
Salinity Change (24h)	hsp64 [20‰]	No Induction [15 - 30‰]	hsp64, hsp71 [20‰]
Temperature Increase (24h, 10°C)	[35°C] hsp58, hsp64, hsp71	[25°C] No Induction	[25°C] hsp54, hsp64
1 µM Ecdyson (6 days)	No Induction	NT	NT
Starvation (10 days)	No Induction	NT	hsp54, hsp64, hsp71

NT = not tested

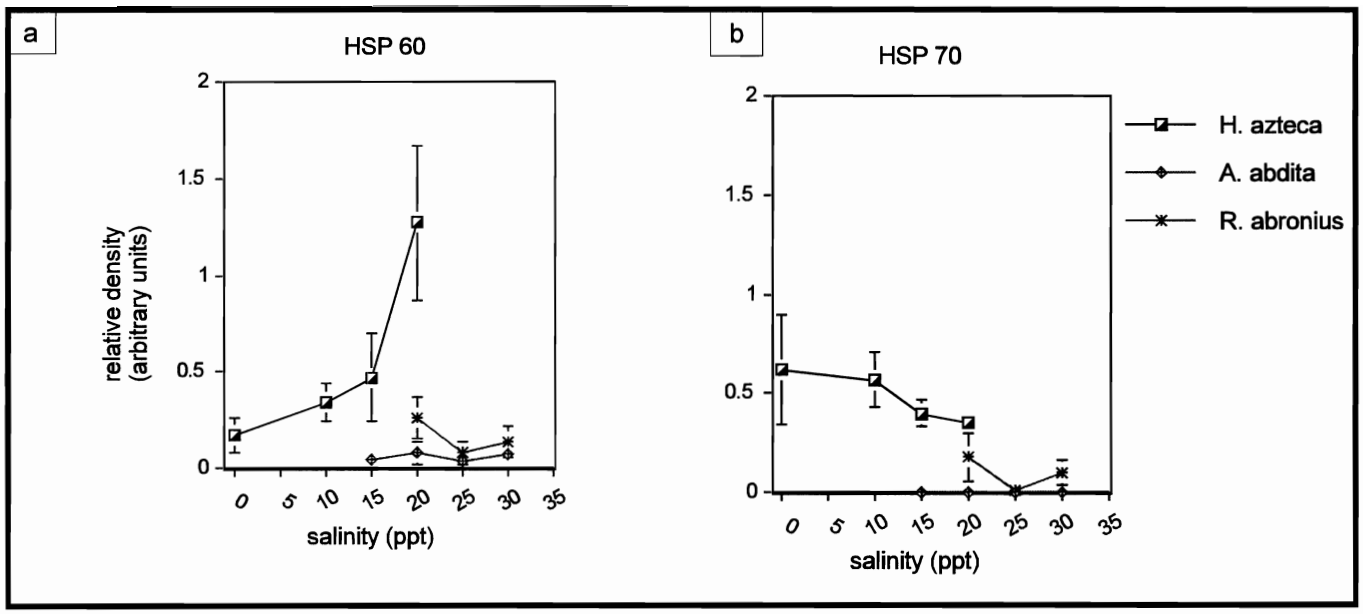

Figure 7
EFFECT OF SALINITY CHANGES ON HSP EXPRESSION

Mean optical density of bands detected by Western blot analysis and 95% confidence limits. Animals were exposed to a range of salinities for 24 hours. Proteins detected by anti-hsp60 were hsp61 (*H. azteca*) and hsp64 (*A. abdita* and *R. abronius*); the protein detected by anti-hsp70 was hsp71 in all three species; n = 6.

*Significantly different from hsp values in adapted salinities (see text); $p<0.05$.

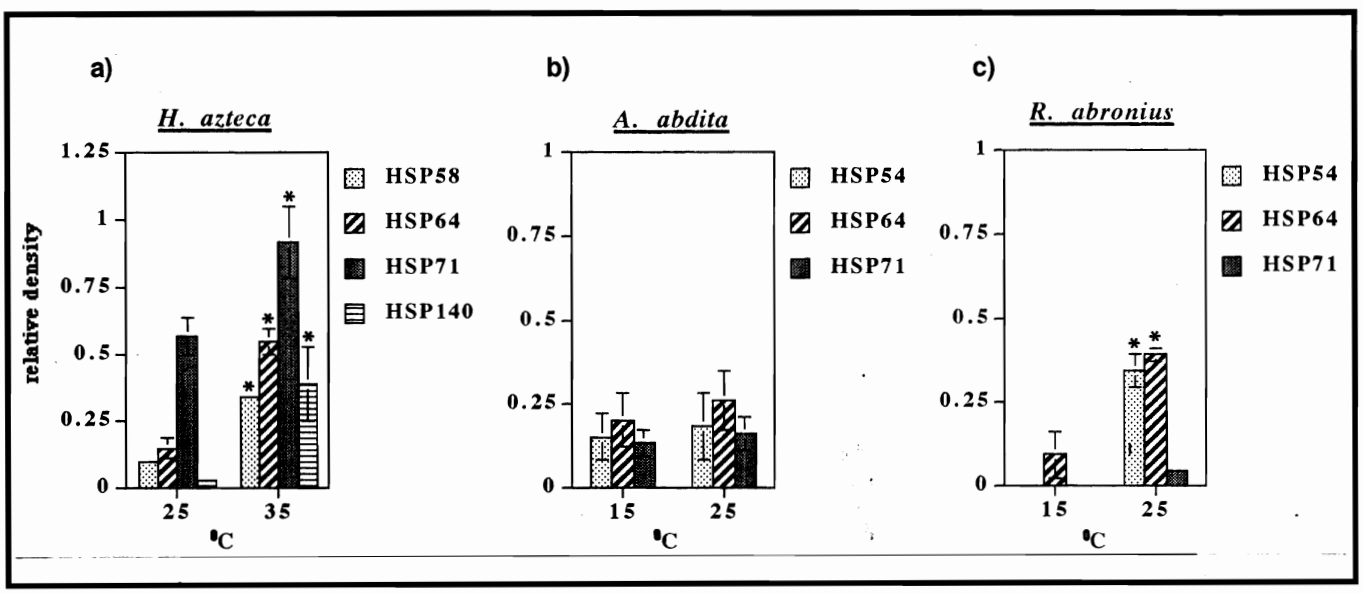

Figure 8
EFFECT OF TEMPERATURE INCREASE

Expression of stress proteins in *H. azteca, A. abdita,* and *R. abronius* after 24 hours at normal temperatures and 10°C above normal temperature. Graphs show mean and standard error (n=3) of densitometer readings for proteins detected by Western blotting using hsp60 and hsp70 antibodies.

*Significantly different from controls; significance level $p<0.05$; nd = not detected.

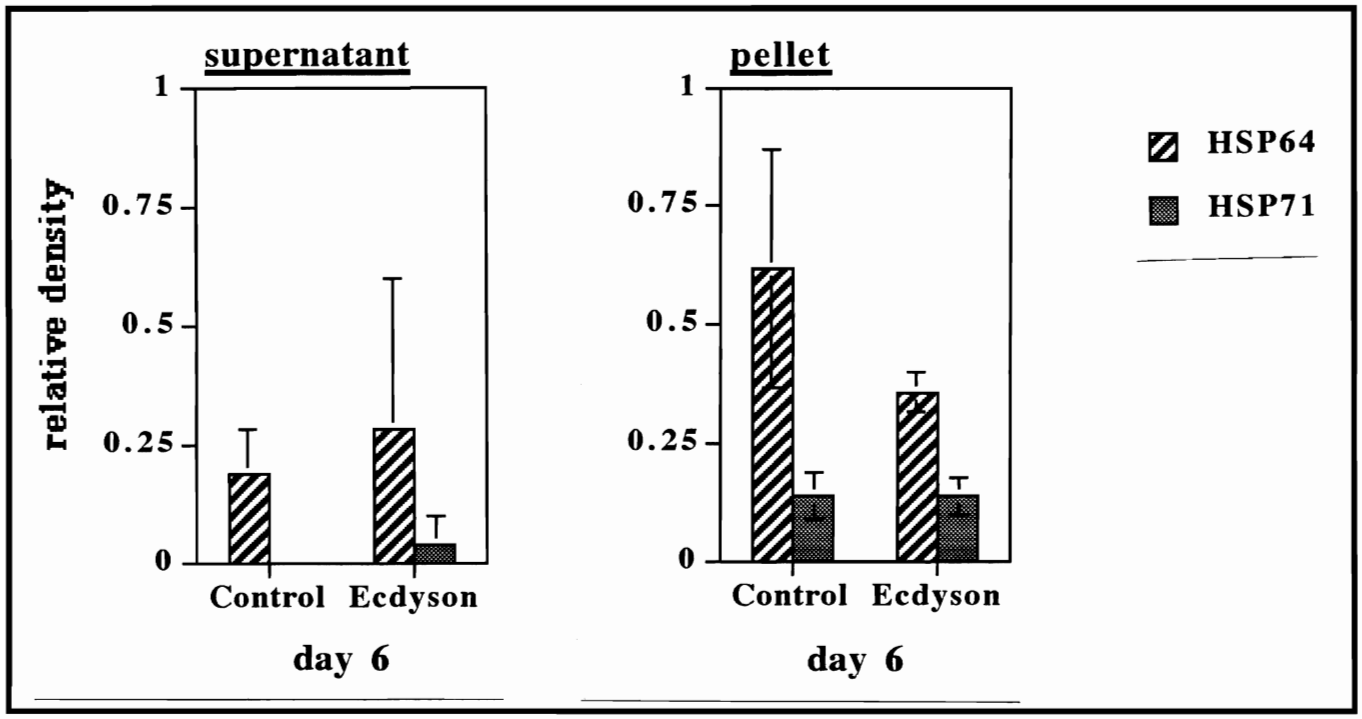

Figure 9
EFFECT OF ECDYSON ON HSP EXPRESSION

Expression of hsp60 and hsp70 proteins in *H. azteca* after 6 days in a solution of 1 µM ecdyson. Graphs show mean and 95% confidence limits of densitometer readings for proteins detected by Western blotting in controls and *H. azteca* exposed to ecdyson (n=6).

*Significantly different from controls; $p<0.05$; nd = not detected.

Observed Induction of Coomassie-Stained Proteins

Various Coomassie-stained proteins in SDS-PAGE gels from animals exposed to xenobiotic compounds appeared as stronger bands than proteins of the same molecular weight from control animals (Table 5; Figures A1-A19 in appendix). This increased expression was considered to be a consequence of exposure to xenobiotic compounds. For some of these proteins, induction was observed at lower concentrations of chemicals than induction of hsp60 and/or hsp70 proteins detected by Western blotting.

H. azteca: Induction of proteins was observed in supernatant and pellet fractions (Table 5). In the pellet, proteins of 36 kDa and 45 kDa were a particularly sensitive measure for the influence of xenobiotics. They were induced by all cadmium and diazinon concentrations tested (0.5-50 µg/L cadmium, 0.03-32 µg/L diazinon) and by 6 and 60 ng/L dieldrin. Strong bands from these proteins were present in gels from control animals of the experiment using fluoranthene as test compound as well as in fluoranthene-exposed animals, suggesting that all animals were stressed independently from fluoranthene exposure.

A. abdita (adult)*:* Increased expression of proteins was observed only in pellets (Table 5). After exposure to 2.5 mg/L cadmium and 30 µg/L diazinon, proteins of 34, 36, 45, and 50 kDa as well as numerous high molecular weight proteins (HMW; 97-200 kDa) were induced. A 34-kDa protein indicated influence of

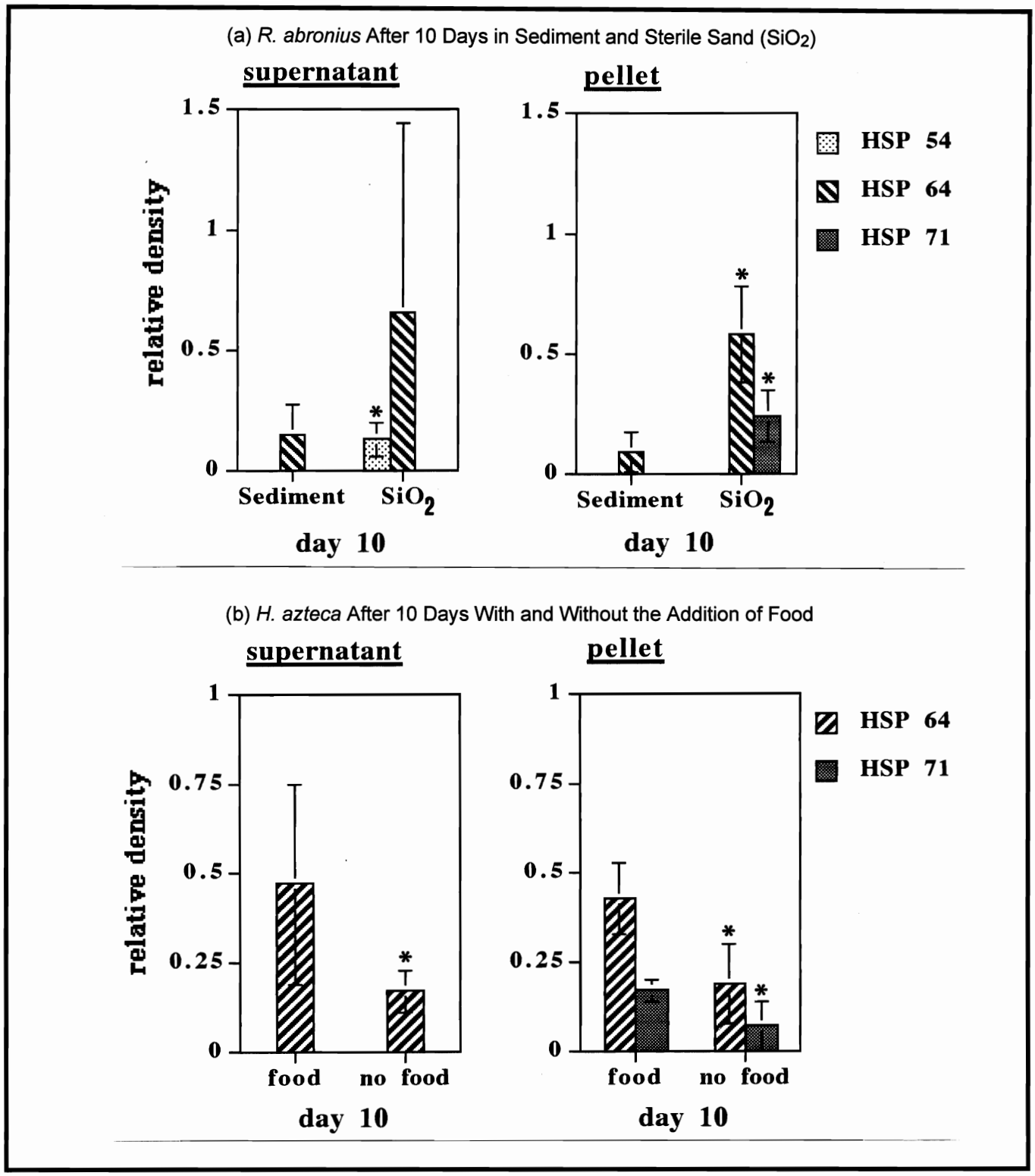

Figure 10
EFFECT OF STARVATION ON HSP EXPRESSION

Expression of hsp60 and hsp70 proteins in *R. abronius* after 10 days in sediment and sterile sand (SiO2) and in *H. azteca* after 10 days with and without the addition of food. Graphs show mean densitometer readings and 95% confidence limits of proteins detected by Western blotting; n=6.

*Significantly different from controls; $p<0.05$; nd = not detected.

dieldrin and fluoranthene at much lower concentrations than hsp60 and/or hsp70 proteins detected by Western blotting. Increased expression of this protein was observed after exposure of the animals to 0.06 and 0.6 µg/L dieldrin and all fluoranthene concentrations tested (0.035-100 µg/L).

A. abdita (juvenile): A 78-kDa protein was detected in the supernatant fraction, which was induced by 2.5 mg/L cadmium, 3 and 30 µg/L diazinon, and all test concentrations of dieldrin (0.06-60 µg/L) and fluoranthene (0.35-35 µg/L). In pellets, induction of 34 kDa, 36 kDa, 45 kDa, and HMW-proteins was observed after exposure to xenobiotics (Table 5).

R. abronius: Induction of a number of proteins was observed in both fractions only after exposure to 30 µg/L diazinon (Table 5).

Table 5
COOMASSIE-STAINED PROTEINS (SDS-PAGE) INDUCED AFTER EXPOSURE OF *HYALELLA AZTECA, AMPELISCA ABDITA,* AND *RHEPOXYNIUS ABRONIUS* TO XENOBIOTICS

	Induced Proteins (kDa)							
	H. azteca		*A. abdita* (Adult)		*A. abdita* (Juvenile)		*R. Abronius*	
Xenobiotic Compound	Super-natant	Pellet	Super-natant	Pellet	Super-natant	Pellet	Super-natant	Pellet
Cadmium	42+	45+++ 36+++ HMW++	—	50++ 45+++ 36++ 34+++ HMW+	78++	45++ 36++ 34+++ HMW+	—	—
Diazinon	42+ 40+	45+++ 36+++ HMW+	—	50++ 45+++ 36++ 34+++ HMW+	78+++	45+++ 36++ 34+++ HMW++	45+ 38+ 36+ 34++ HMW+	68+ 66+ 54+ 45++ 42+ 38+ 36+ HMW+
Dieldrin	—	45+++ 36+++ HMW+	—	34++	78+++	36++ 34++ HMW++		
Fluoranthene	—	45* 36* HMW+	—	34++ 50++	78+++	50++	—	—

+ Only visible after strong insult
++ Moderate band
+++ Strong band
* Detectable in controls
— Non-detectable

Sediment Physical and Chemical Characteristics

Grain size distribution, total organic carbon content and results from chemical analysis of each test sediment are listed in Tables A1 - A15 (in Appendix).

Comparison of Results from Bioassays and HSP Analyses with Xenobiotic Concentrations

Results of 10-day bioassays and stress protein analysis are listed in Table 6 and shown graphically in Figure 11 for comparison with Figure 12. Survival of *A. abdita* ranged from 32±3.7% (NFISC 1-3)

Table 6
RESULTS OF 10-DAY SEDIMENT BIOASSAYS WITH *AMPELISCA ABDITA*

Percent Survival and Stress Proteins HSP71 and HSP64

Sample Site		Survival [% ± SE; n=5]	hsp71 [rd ± SE; (n)]	hsp64 [rd ± SE; (n)]
1	Benicia	98 ± 1.8	0.08 ± 0.03* (6)	—
2	Carquinez	90 ± 1.9	—	—
3	Exxon-1	71 ± 3.7*	—	—
	Exxon-2	77 ± 1.5*	—	—
4	San Pablo Bay	88 ± 1.7	—	0.16 ± 0.07* (6)
5	Bel Marin Keyes	85 ± 1.0*	—	—
6	Paradise Cay	87 ± 2.1	—	—
7	San Rafael-A	79 ± 0.8*	—	—
	San Rafael-B	70 ± 2.3*	—	—
8	NFISC 1-1	64 ± 2.3*	—	0.09 ± 0.04* (6)
	NFISC 1-2	32 ± 3.7*	0.14 ± 0.07* (5)	0.55 ± 0.26* (5)
	NFISC 1-3	70 ± 3.2*	0.12 ± 0.08* (5)	—
	NFISC 1-5	90 ± 1.2	—	—
	NFISC 1-6	81 ± 2.2*	—	—
	NFISC 1-9	47 ± 0.9*	—	0.09 ± 0.04* (5)
9	Unocal	84 ± 7.6	—	—
10	Alcatraz	71 ± 7.0	0.03 ± 0.01* (6)	0.04 ± 0.01* (6)
11	Treasure Island	75 ± 2.7*	—	—
12	Port of Oakland	70 ± 6.5*	—	—
13	San Francisco Dry Dock - A	67 ± 4.1*	0.12 ± 0.03 (5)	0.2 ± 0.09* (5)
	San Francisco Dry Dock - B	81 ± 5.4	0.26 ± 0.03 (5)	—
	San Francisco Dry Dock - D	87 ± 2.6	—	—
14	San Mateo, Oyster Point	77 ± 4.9*	0.12 ± 0.03 (6)	—
15	Control	95 ± 0.4	—	—

— Non-detectable
* Significantly different from control ($p<0.05$)
rd Relative density of bands on Western blots
SE Standard Error

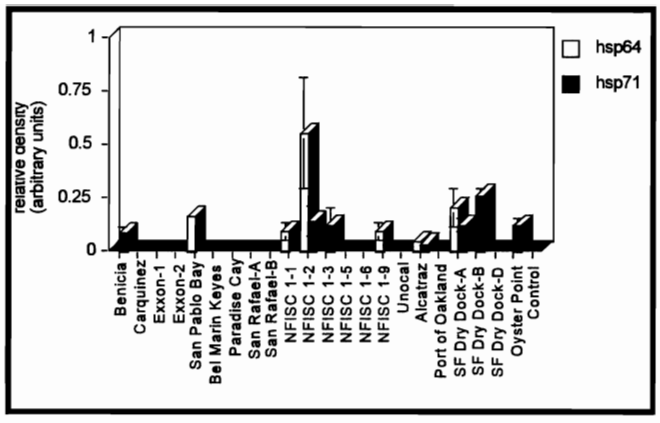

Figure 11
RESULTS OF WESTERN BLOT ANALYSIS OF A. ABDITA AFTER 10-DAY BIOASSAYS WITH SEDIMENTS FROM SAN FRANCISCO BAY

The graph shows mean densitometer readings (n=5) ± standard deviation of protein bands detected in replicate samples after incubation with hsp60 and hsp70 antibodies.

to 98±1.8% (Benicia). Only three tests resulted in elevated levels of both hsp71 and hsp64, three others showed elevated levels of hsp64 and in four hsp71 was increased.

Percent survival was compared to elevated hsp64 and hsp71 levels. Significantly reduced survival was associated with elevated stress protein levels in 36% of cases, and 6 out of 10 tests where elevated stress protein levels were measured showed significantly decreased survival. Correlation between percent survival and hsp64 was $y=0.67‰10^{-0.012x}$ with $r^2=0.37$ (Figure 13a). There was no correlation between percent survival and hsp71 values (Figure 13b).

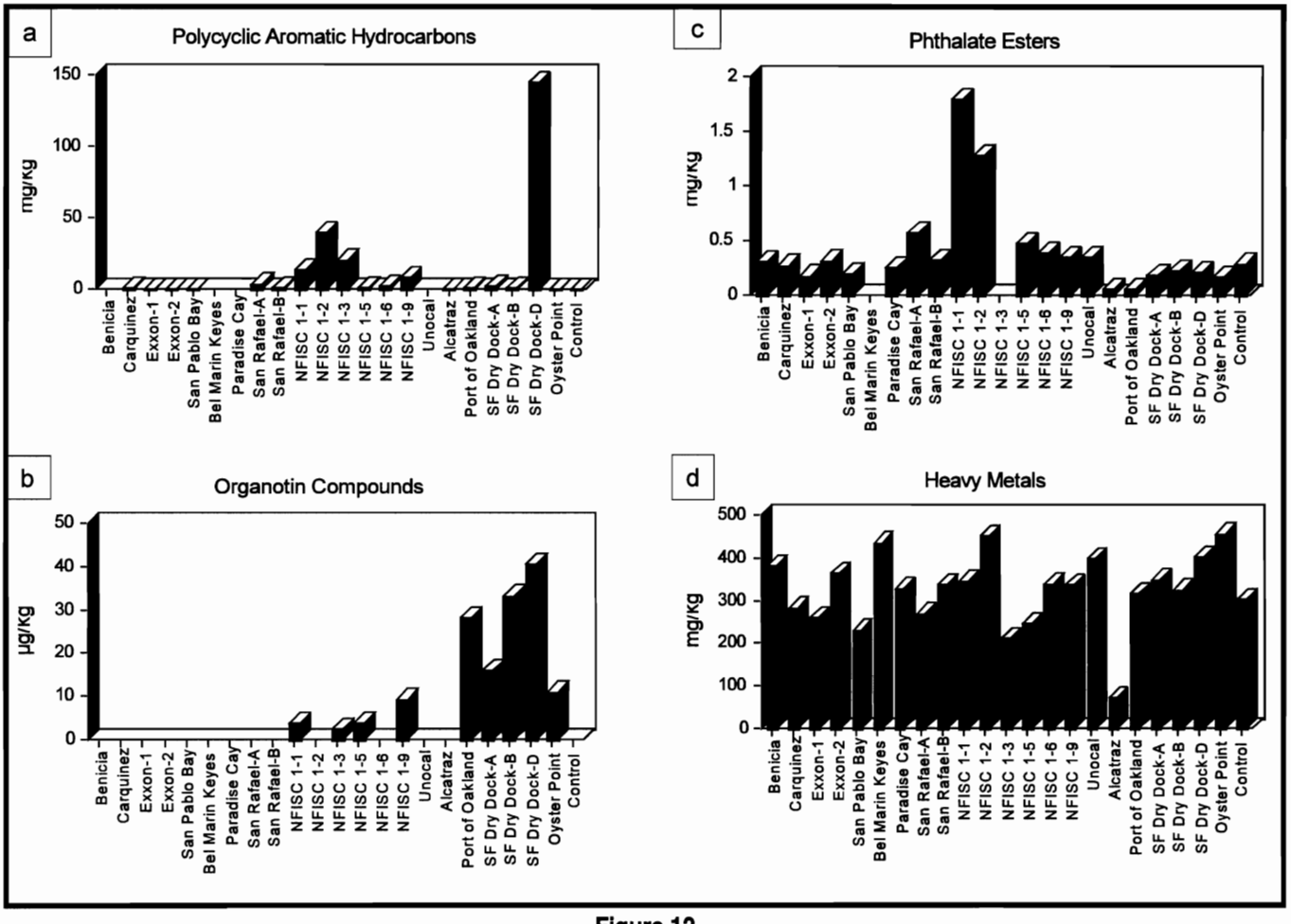

Figure 12
RESULTS OF CHEMICAL ANALYSES OF SEDIMENT SAMPLES FROM SAN FRANCISCO BAY

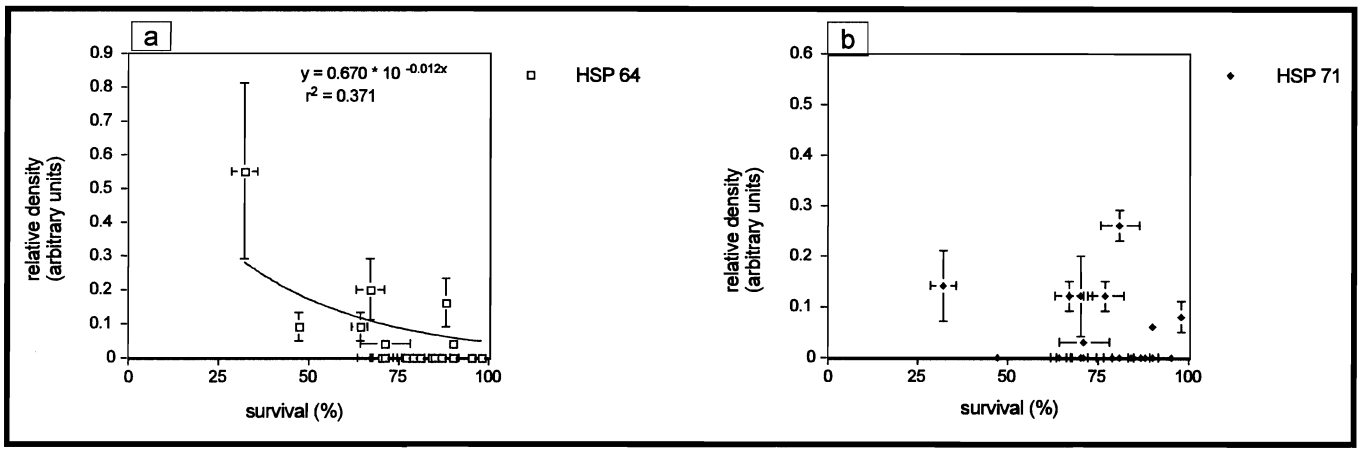

Figure 13
RESULTS OF 10-DAY SEDIMENT BIOASSAYS WITH A. ABDITA AS THE TEST ORGANISM

Correlation of percent survival with hsp64 and hsp71 densitometer readings. Hsp64 and hsp71 were detected by Western blotting. Mean relative density ± standard error (n=5 or 6; see Table 6) are shown.

Figure 12 shows total amounts of PAHs, organotin compounds, heavy metals and phthalate esters of each test sediment. Chlorinated hydrocarbons were detected in only five locations, with highest concentrations at sites NFISC 1-3 (58 µg/kg dry sediment) and Unocal (5.27 µg/kg dry sediment). Amounts detected in sediments from Port of Oakland and San Francisco Dry Dock-B and -D were below 0.1 µg/kg, and results of analyses for these compounds are not shown in Figure 12. Comparison of total concentrations of different groups of xenobiotics in test sediments with percent survival and measured stress proteins showed that induction of stress proteins was highest in sediments from locations where high concentrations of PAHs and organotin compounds were found. These were NFISC 1-1, 1-2, 1-3, and 1-9. One exception is site 13-D (San Francisco Dry Dock), where concentrations of PAHs (145.1 mg/kg dry weight) and organotin compounds (40.5 µg/kg dry weight) were very high. This was not reflected in percent survival (87±2.6) or in stress protein levels. Organotin concentrations were highest in sediment samples from Port of Oakland, San Francisco Dry Dock, and Oyster Point.

Hsp71 was elevated after exposure of the animals to sediment from San Francisco Dry Dock-A and -B and from Oyster Point. Elevated hsp64 levels were measured for San Francisco Dry Dock-A only. The sediment, which caused highest mortality (NFISC 1-2; percent survival: 32 ± 3.7%), also induced the strongest hsp64 response and contained high concentrations of PAHs (39.84 mg/kg dw) and phthalate esters (1280 µg/kg dw). Total concentrations of heavy metals were not correlated with percent survival or with hsp71 or hsp64 levels.

Time Course of Stress Protein Induction under Continuous Exposure to Contaminated Sediments

NFISC 1-9 Sediment: Temperature of the overlying water was 23.0-24.5°C for the first 2 days of the test and was subsequently maintained at 19-20.6°C for the remaining 8 days. Oxygen content and pH of the water was 7.3-7.6 mg/L and 7.9-8.0, respectively. Salinity was 25‰. Percent survival after 10 days was 96± 2.6% (SE; n=3) in control sediment and 68±5% in NFISC 1-9 sediment (Figure 14).

No stress protein was detected in control animals. Hsp62 and hsp64 levels were elevated in animals exposed to NFISC 1-9 sediment only on days 3 and 5, whereas hsp71 was not detected. Increased expression of Coomassie-stained proteins of 34 and 36 kDa was observed on days 3, 5, 7, and 10 (Figure A18).

Cadmium-Spiked Sediment: Chemical analysis of the sediment after spiking resulted in a Cd concentration of 10.3 mg/kg wet weight (10 day LC50: 10 mg/kg ww). Temperature in the overlying water was 18.2-19.6°C. Oxygen content was 7.4-7.6 mg/L, pH was 8.0-8.1, and salinity was 25‰. Percent survival after 10 days was 83±7% (SE; n=3) in control sediment, and 49±13% in Cd-spiked sediment (Figure 15). No stress proteins were detected in control animals. Hsp64 and hsp54 were detected on days 4 and 7 in animals from Cd-spiked sediments; hsp71 was not detected. Increased expression of a Coomassie-stained protein of 45 kDa was detected on day 10.

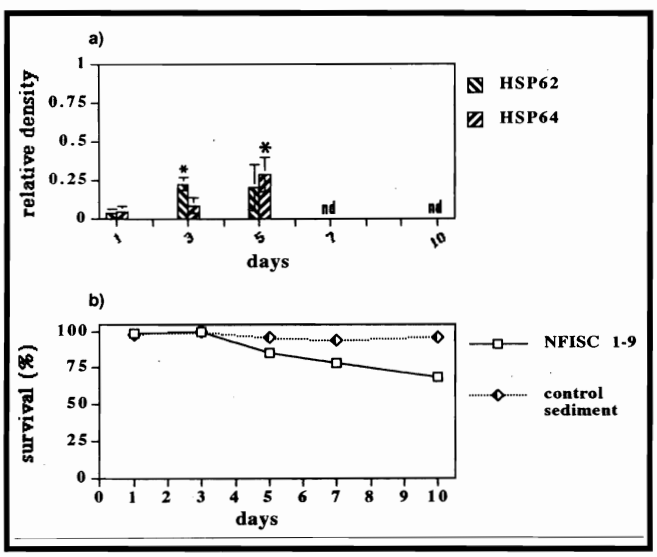

Figure 14
TIME COURSE OF STRESS PROTEIN EXPRESSION IN *A. ABDITA* EXPOSED TO SEDIMENT FROM NFISC 1-9

(a) Mean densitometer readings ± standard deviation (n=6) of hsp62 and hsp64 bands detected by Western blotting (anti-hsp60). No hsp71 was detected. Stress protein concentrations in control animals were below the detection limit.

(b) Mean percent survival ± standard error (n=3) of *A. abdita* in control sediment and NFISC 1-9 sediment.

*Significantly different from controls; p<0.05; nd = not detected.

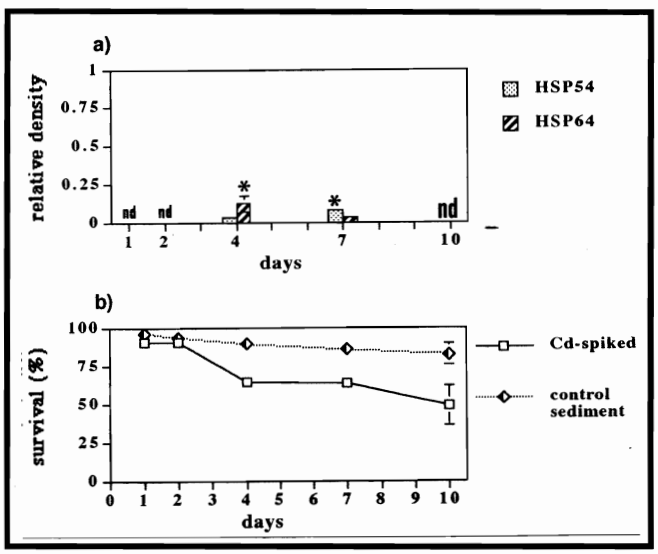

Figure 15
TIME COURSE OF STRESS PROTEIN EXPRESSION IN *A. ABDITA* EXPOSED TO CADMIUM-SPIKED SEDIMENT (10.3 mg/kg dry weight)

(a) Mean densitometer readings ± standard deviation (n=6) of hsp54 and hsp64 bands detected by Western blotting (anti-hsp60). No hsp71 was detected. Stress protein concentrations in control animals were below the detection limit.

(b) Mean percent survival ± standard error (n=3) of *A. abdita* in control sediment and Cd-spiked sediment.

*Significantly different from controls; p<0.05; nd = not detected.

Stressor-Specific Response

Xenobiotic Compounds

Results of our study demonstrate the complex nature of the stress protein response when animals are exposed to xenobiotic compounds. With the exception of diazinon, where stress protein induction in all three species was similar, the intensity and sensitivity of this response was dependent on both the species and the compound tested. We found both induction and inhibition of stress protein expression following exposure to xenobiotic compounds. Natural stressors like temperature and salinity changes did not induce hsp60 or hsp70 unless they exceeded the tolerance range of the organism, whereas the effects of starvation could potentially complicate interpretation of field data.

In *H. azteca*, the most sensitive response was seen in the expression of a member of the hsp70 protein family, hsp71, whereas members of the hsp60 protein family were the dominant proteins detected in *A. abdita* (hsp54, hsp64) and *R. abronius* (hsp54, hsp60, hsp64). An hsp60 protein also appears to be the major inducible stress protein in two species of *Hydra* and the rotifer *Brachionus plicatilus*, where synthesis of an hsp70 protein is constitutive but not inducible (Cochrane *et al.* 1991; Bosch *et al.* 1988).

Biomarkers should be indicators of sublethal, potentially harmful concentrations of xenobiotic compounds. For cadmium, the Lowest Observed Effective Concentration (LOEC) for altered stress protein expression in *H. azteca* ($LOEC_{hsp}$; see Table 7) was 0.5 µg/L, half of the 42 day-LC50 of 1 µg/L reported by Borgmann *et al.* (1989). Cadmium concentrations in the environment have been measured to be as high as 0.4 µg/L in rivers (Streit 1994) and 10 µg/L or 140 mg/kg sediment (dry weight) in marine habitats (Sundelin 1983). Cadmium LC50 and $LOEC_{hsp}$ for *A. abdita* and *R. abronius* from our study were well above reported concentrations in natural environments

Table 7
COMPARISON OF $LOEC_{hsp}$ TO CONCENTRATIONS OF XENOBIOTIC COMPOUNDS DETECTED IN THE ENVIRONMENT

Xenobiotic Compounds	$LOEC_{hsp}$ *H. azteca*	*A. abdita*	*R. abronius*	Environmental Concentrations
Cadmium	**0.5 µg/L** (0‰)	0.5 mg/L (25-30‰)	5 mg/L (30-33‰)	0.4 µg/L (fresh water) 10 µg/L (marine)
Diazinon (µg/L)	**0.6**	30.0	**3.0**	3.8
Dieldrin (µg/L)	**0.006 - 0.06**	6.0	0.6	0.09
Fluoranthene (µg/L)	**1.0**	35.0	35.0	16

LOEChsp = Lowest Observed Effective Concentration that significantly altered hsp60 or hsp70 expression. Environmentally relevant $LOEC_{hsp}$ values are printed in bold.

(Table 7). Cadmium concentrations in interstitial water of sediments are, however, likely to be higher than in the overlying water body, but the relatively high Cd concentrations needed to induce the stress protein response in *A. abdita* and *R. abronius* make it seem unlikely that the hsp60 or hsp70 response of these species could act as a sensitive indicator of cadmium contamination in the environment. For *H. azteca*, the $LOEC_{hsp}$ could be a biomarker of adverse effects caused by cadmium in freshwater environments.

The $LOEC_{hsp}$ for diazinon was 0.6 µg/L in *H. azteca*, 30 µg/L in *A. abdita*, and 3 µg/L in *R. abronius*. The 36 h-LC50 for *Ceriodaphnia dubia* (Cladocera) is reported to be 0.6 µg/L (Katznelson 1994), and reproduction of *C. dubia* is reduced at 0.08 µg/L. Concentrations of diazinon measured in Orestimba Creek, a tributary of the San Joaquin River (California) were as high as 3.8 µg/L (Anonymous 1994). Diazinon also accumulates in sediments. Up to 40 µg/kg diazinon (dry weight) were detected in sediments of an urban creek in Alameda County, California (R. Wetzig, Alameda County Flood Control District, personal communication). These numbers show that detected environmental concentrations of diazinon are well within concentrations able to cause a stress protein response in *H. azteca* and mortality in *C. dubia*. Interstitial water concentrations of diazinon in sediments containing 40 µg/kg diazinon (see above) need to be determined before potential deleterious effect on infaunal organisms can be assessed.

Dieldrin caused a strong stress protein response in *H. azteca* at low concentrations (6 and 60 ng/L; Figure 3c) without increasing mortality. These concentrations are within the range of concentrations measured in the environment. Mean concentrations measured in surface waters of the United States were 1-90 ng/L (WHO 1989). $LOEC_{hsp}$ values of the saltwater species *A. abdita* and *R. abronius* are likely to be above concentrations present in the aquatic environment. Measurements of ocean water samples in the western North Atlantic showed maximum dieldrin concentrations of 19.4 ng/L. However as much as 1 mg/kg (dry sediment) dieldrin has been detected in sediments (WHO 1989). Known acute 96 h-LC50s range from 5 to 740 µg/L for freshwater crustaceans and 0.7 to 50 µg/L for saltwater crustaceans (US EPA 1993; WHO 1989).

The "biphasic" stress protein response to dieldrin in *H. azteca* confirmed results of an earlier experiment, where animals of this species were exposed to dieldrin-spiked sediment for 10 days (Werner and B. Albrecht, unpublished). Elevated hsp64 values were measured after exposure to 2.4-18.0 mg/kg dry sediment (interstitial water concentrations: about 0.4-3 µg/L dieldrin; US EPA 1993) but not at 53 mg/kg dieldrin (interstitial water concentrations: about 10 µg/L dieldrin; US EPA 1993); these dieldrin concentrations did not affect survival. The response pattern suggests a possible link with hormesis, often observed in toxicity studies (Laughlin & Guard 1981). Hormesis is the induction of beneficial effects by low doses of otherwise harmful physical or chemical agents. This effect has been linked to increased stress protein expression (Smith-Sonneborn 1993). No induction of stress proteins was measured in *A. abdita*, which is more sensitive to dieldrin-spiked sediment than *H. azteca* (B. Albrecht, TRACK Labs, Pensacola FL, personal communication), and only one test concentration caused a significant increase in hsp60 in *R. abronius*. An alternative explanation for the observed effects may be derived from the potential influence of organochlorine compounds such as dieldrin in the steroid hormone system. These compounds can bind to steroid hormone receptors and have

been linked to "feminization" or "masculinization" effects in several species. Hormones and "pseudohormones" are active in extremely small concentrations, but receptors are inactivated if the organism is exposed to high concentrations (Raloff 1994, 1995). It has recently been shown that a member of the hsp70 protein family is involved in the activation of steroid hormone receptors (Bohen *et al.* 1995).

Organisms readily accumulate dieldrin (bioconcentration factors of aquatic animals: 400-68,000; US EPA 1993), and sublethal effects like reduction of growth rates and reproductive success have been demonstrated in fish at a concentration of 0.75 µg/L dieldrin (WHO 1989). An investigation of the correlation of the stress protein response with such longer-term effects was, however, beyond the scope of this study.

Results from stress protein measurements following exposure to fluoranthene are difficult to interpret. Expression of hsp71 and hsp64 in pellets of *H. azteca* was significantly less than in controls after exposure to fluoranthene. However, measured hsp71 values were elevated in controls compared to control samples from other experiments, and hsp64 levels were within the range seen in samples, which were not significantly different from controls (*e.g.* Figure 3a). Significant induction of hsp60 proteins was measured in the more sensitive species *A. abdita* and *R. abronius* (ASTM 1993; Swartz *et al.* 1990). Inducing concentrations were 10 times the reported 96h-LC50 (ASTM 1993) for *A. abdita* and slightly above the 10d-LC50 for *R. abronius* (Swartz *et al.* 1990), whereas the highest concentration to which the animals were exposed (100 µg/L) did not cause an increase in hsp60 proteins. Inhibition of stress protein expression by fluoranthene was, however, observed in *A. abdita*, where hsp71 but not hsp64 was reduced in heat-shocked animals subsequently exposed to 10 and 100 µg/L fluoranthene (Werner 1995). Fluoranthene concentrations in waste water have been measured to be as high as 16 µg/L (US EPA 1980). Sediments accumulate fluoranthene, and reported concentrations are as high as 1791 mg/kg wet sediment (Kennish 1991).

The inhibitory effect of other xenobiotic compounds or concentrations of chemicals on the stress protein response has previously been reported. In sea urchin embryos, copper inhibited the heat-shock response (Sanders, personal communication), and Edington *et al.* (1989) found that deuterium oxide and glycerol inhibited stress protein induction in cell cultures. Whether inhibition of stress protein expression can be used as an indicator of xenobiotic effects still needs to be determined. It certainly complicates interpretation of data from field studies, where generally mixtures of xenobiotic compounds are present.

Analysis of two different cell fractions (supernatant = soluble fraction, pellet = insoluble fraction) revealed that in the three species of amphipods used in our experiments, the stress proteins accumulated in different fractions. Tissue homogenization was the same for all samples. Under normal conditions, the heat shock proteins are soluble. After stress, heat shock proteins are the predominant insoluble proteins detected in cell homogenates (Pinto *et al.* 1991; Bensaude et al. 1990). The changes might represent structural modification of these proteins, or they might be a consequence of the overall structural changes in stressed cells. In this study, the redistribution of hsps into the soluble fraction was observed in *H. azteca* (Figure 3), whereas in *A. abdita* hsps were predominantly detected in the pellet ("insoluble" or SDS-soluble fraction; Figures 4 and 5) and in *R. abronius* the

majority were found in the supernatant ("soluble" fraction; Figure 6). It is possible that the composition of membrane phospholipids can influence the homogenization process. Membrane phospholipids of cold- and warm-adapted organisms have different ratios of saturated to unsaturated fatty acids (Hochachka and Somero 1984). The percentage of unsaturated fatty acids is higher in cold-adapted organisms, such as *R. abronius*, to secure fluidity of the membranes. This higher fluidity may cause membranes to "break up" more easily in the homogenization process, which could explain the higher "solubility" of stress proteins in *R. abronius*.

Sensitivity of the Stress Protein Response

The most sensitive stress protein response was measured in the freshwater species *H. azteca* (Figure 3). For better comparison of the sensitivity of the detected stress protein response between species, the ratio LC50:$LOEC_{hsp}$ was calculated (Table 8), where LC50 is the 24-hour value listed in Table 2, and $LOEC_{hsp}$ is the lowest xenobiotic concentration where significant hsp60 or hsp70 induction was detected.

High variation of measured hsp levels between individual samples is thought to be partially responsible for the relatively low sensitivity of the hsp60 and hsp70 response in *A. abdita* and *R. abronius*. In a study by Stringham and Candido (1994), only 13-43% of nematodes exposed to various xenobiotic compounds expressed ß-galactosidase as a stress protein (resulting from a hsp16-*lacZ* reporter fusion in transgenic animals). A weaker stress protein response may also be a consequence of evolutionary adaptation to relatively constant environmental conditions (Sanders 1993; Bosch *et al.* 1988). Sanders *et al.* (1991b) found that two closely related mollusc species (*Collisella scabra, Collisella pelta*) showed different patterns of stress protein induction dependent on their respective habitats. The species that lived under relatively constant environmental conditions showed no or a weaker stress protein response than the species adapted to fluctuating conditions. This could explain the weak stress protein response in *R. abronius*. However, the estuarine species *A. abdita* lives in an environment characterized by a great deal of physical variability, and the sensitivity of the stress protein response was almost equal to that of *R. abronius*, which naturally occurs in a less variable marine environment. Nevertheless, *H. azteca* appears to be the most adaptable and tolerant of the three species investigated (US EPA 1994).

Physical variables such as water hardness, salinity, and temperature can alter solubility, chemical form, and uptake of chemical compounds (McLusky *et al.* 1986; Stephenson 1983). Increased salinity considerably decreases the toxic effects of cadmium due to complexation with Cl- (McLusky *et al.* 1986), which explains the much higher LC50s of *A. abdita* and *R. abronius* for this compound. Increased temperature increases the toxicity of cadmium. Acute toxicity of diazinon increased from freshwater to marine species (Table 7). It is not known whether this was due to salinity effects or solely to species specificity. Dieldrin toxicity does not appear to vary with salinity (US EPA 1993), and fluoranthene toxicity was not affected by salinity in experiments with the euryhaline amphipod species *Eohaustorius estuarius* (DeWitt *et al.* 1989).

Differences in the intensity of the response to exposure of different xenobiotic compounds may also be due to tissue-specific induction of different stress proteins (Sanders *et al.* 1993; Dyer *et al.* 1990). Due to the size of our test animals, we were unable to investigate this aspect of the stress protein response by Western blotting. Stringham and Candido (1994) have demonstrated stressor-

Table 8
SENSITIVITY OF THE STRESS PROTEIN RESPONSE: COMPARISON OF 24h-LOEChsp AND 24h-LC50

Species	Xenobiotic Compound	LOEChsp	LC50	LC50:LOEChsp
H. azteca	cadmium	0.5 µg/L	55 µg/L	110
	diazinon	0.6 µg/L	30 µg/L	50
	dieldrin	6·10-3 - 6·10-2 µg/L	>6 µg/L	>1000
	fluoranthene	1 µg/L	>500 µg/L	>500 (inhibition)
A. Abdita (adult)	cadmium	0.5 mg/L	>2.5 mg/L	>5
	diazinon	30 µg/L	21 µg/L	0.7
	dieldrin	6 µg/L	>60 µg/L	>10 (inhibition)
	fluoranthene	35 µg/L	>100 µg/L	>2.9*
A. abdita (juvenile)	cadmium	2.5 mg/L	>2.5 mg/L	>1
	diazinon	3 µg/L	5 µg/L	1.7
	dieldrin	—	>60 µg/L	—
	fluoranthene	—	>35 µg/L	—
R. abronius	cadmium	5 mg/L	>10 mg/L	>2
	diazinon	3 µg/L	9.2 µg/L	3.1
	dieldrin	0.6 µg/L	>60 µg/L	>100**
	fluoranthene	35 µg/L	>100 µg/L	>2.9

LOEChsp = Lowest concentration that significantly altered hsp60 or hsp70 expression (lowest observed effect concentration).
* Only elevated at 35 µg/L
** Only elevated at 0.6 µg/L
— Non-detectable

specific sites of stress protein induction in nematodes. Whereas arsenic elicited a "whole body" response, Pb, Cd, Hg and Cu had very specific sites of action. Cadmium affected muscle, nerve, and epidermal cells of the pharynx. Differences in expression at the tissue level reflected the mode of action of various chemicals in fathead minnows (*Pimephales promelas)*, and induction was greatest in their respective target tissues (Sanders 1993). Diazinon, an acetylcholinesterase inhibitor, increased all major stress proteins in muscle and brain tissue, whereas only hsp90, hsp70, and hsp32 were induced in gill tissue. When whole animals are homogenized and analyzed as in this study, a tissue-specific stress response is masked, which would compromise the sensitivity of the assay. Diazinon exerts its strongest effect in nerve and muscle cells; this could explain the strong and sensitive response in all three amphipod species. The effect of cadmium is organ specific and may be altered by the chemical form of the metal (Cochrane *et al.* 1991), which could help explain the weak response in *R. abronius*.

Stress Protein Response in Different Life Stages

To assess the environmental risk of xenobiotic compounds, knowledge of the life stage dependence of toxic effects is important. Certain life stages may not be able to express stress proteins (Roccheri *et al.* 1986; Lindquist 1986). Sanders (1993) reported a link between thermotolerance and the ability of sea urchin embryos to express stress proteins. A comparative investigation of hsp60 and hsp70 in medaka (*Oryzias latipes*, Pisces) embryos and larvae showed that

hsp60 was constitutively expressed in higher amounts in embryos than in larvae (Soimasuo *et al.* in prep.). Embryos of *Artemia salina* expressed hsp89 and hsp68 in higher quantities than larvae of this species (McLennan & Miller 1990).

In this study, the stress protein response to various xenobiotic compounds in juvenile and adult *A. abdita* was compared. LC50 values for cadmium were similar in both life stages, but the stress protein response was about 5 times more sensitive in adult animals. The LC50 for diazinon was 4 times lower for juveniles than for adult *A. abdita*, and significant induction of hsp60 proteins occurred at diazinon concentrations 10 times lower than in adult animals. Juvenile animals were less sensitive to cadmium and more sensitive to diazinon than adult *A. abdita*. In both cases, the stress protein response was clearly a more sensitive indicator of xenobiotic influence than LC50 values.

The most pronounced difference observed between juvenile and adult *A. abdita* was the induction of a 78 kDa protein detected after staining of SDS-PAGE gels with Coomassie blue. This protein band was not detectable in control samples, but was visible after exposure of juveniles to cadmium, diazinon, all dieldrin concentrations down to 0.06 µg/L, and fluoranthene concentrations as low as 0.35 µg/L. Neither hsp60 nor hsp70 proteins were detectable in Western blots of juveniles after exposure to dieldrin and fluoranthene.

Natural Stressors

For biomarkers to be useful in field studies, stress proteins should not be induced by changes in environmental variables such as salinity and temperature. Moulting and starvation also constitute potential stress factors that may complicate interpretation of data from field studies.

Salinity changes caused stress protein induction when natural tolerance limits of organisms were exceeded. Ingersoll *et al.* (1992) report that *H. azteca* can tolerate salinity up to 29‰, but does not reproduce above 15‰. In this study, stress protein hsp64 was induced significantly by a 24-hour salinity change from 0 to 20‰, but not to 15‰. Hsp71 levels decreased with increasing salinity. Stress proteins were not induced in *A. abdita* at 15-30‰ salinity. Bousfield (1973) reported that *A. abdita* inhabits areas with 10-35‰ salinity. *R. abronius* is the least tolerant of variations in environmental conditions, and survival was reduced significantly in 10-day tests at 18.8‰ salinity (Swartz *et al.* 1985). Stress proteins hsp64 and hsp71 were elevated after *R. abronius* was exposed to 20‰ for only 24 hours.

A 24-hour temperature increase of 10°C induced stress proteins when known tolerance limits of the organisms were exceeded. *H. azteca* tolerates temperatures up to 33°C (Bovee 1949). Stress proteins were significantly elevated at 35°C. *A. abdita* tolerates temperatures up to 25°C (ASTM 1993). A 24-hour exposure at 25°C did not induce stress proteins. The same treatment caused increased expression of hsp60 proteins in *R. abronius*. *R. abronius* is a stenothermal species, and we know that it tolerates temperatures above 15°C for short periods only (ASTM 1993). McLennan and Miller (1990) obtained comparable results in their study on cysts and larvae of *Artemia salina*. Whereas larvae and cysts increased expression of hsp89 and hsp68 after a heat shock at 40°C, normal larval protein synthesis was repressed at 40°C, but cysts did not show an effect until 47°C. Cysts are known to be more thermotolerant than larvae.

Our study showed that hsp60 and hsp70 proteins in *H. azteca* were not affected by a 6-day exposure to ecdyson. These findings confirmed results from an investigation of Lindquist (1986) on the effect of ecdyson in *Drosophila*. No hsp70 or hsp68 were induced, but in certain life stages, ecdyson caused an increased expression of hsp22, hsp23, and hsp28.

Starvation of *R. abronius* and *H. azteca* for 10 days caused an increased level of stress proteins and decreased survival of *R. abronius* compared to control animals and lower levels of stress proteins but no effect on survival of *H. azteca* (Figure 10). Several factors complicate interpretation of our results. *R. abronius* were maintained for 10 days in sterile SiO_2; it is possible that the artificial substrate with its relatively large grain size (0.3-0.4 mm) caused an increased energy demand for the animals, which normally move through the sediment feeding on small benthic organisms. This may have enhanced the effect caused by starvation alone. *H. azteca* feeds on bacteria and algae in the water. Although the water where they were kept was filter-sterilized (0.22 µm), the animals themselves could have introduced microorganisms. Increased levels of stress protein in control animals may be due to the introduction of excessive amounts of food. The results are difficult to interpret, but we cannot exclude starvation as a potential inducer of stress proteins.

Sediment Bioassays

We measured stress proteins hsp64 and hsp71 in *A. abdita* exposed to sediments from 23 sampling sites in San Francisco Bay to determine whether they are suitable indicators of deleterious sublethal effects of the complex mixtures of xenobiotic compounds in sediments. Comparison of hsp induction to survival after 10-day bioassays showed that stress protein values were significantly elevated in only 36% of tests where survival was significantly reduced (Figure 13). Whereas there was a correlation between percent survival, level of contamination, and hsp64 expression in sediments from NFISC sites, site 13-D (San Francisco Dry Dock) did not cause stress protein induction or reduced survival despite very high concentrations of PAHs and organotin compounds (Figure 12, Table 6). The fluoranthene concentration at site 13-D was measured to be 19.4 mg/kg dry sediment, which is above the 10-day LC50 of *H. azteca*, which is less sensitive to this compound than *A. abdita*. The results from site 13-D are difficult to interpret and should be verified using fluoranthene-spiked sediment.

Results from the first part of this study indicated that stress proteins might not be suitable as an integrative biomarker of complex mixtures of xenobiotic compounds present in sediments. Furthermore, it became clear that the sensitivity of the stress protein response in *A. abdita* is not high compared to survival. This is reflected in results from the 10-day sediment bioassays. Other investigations on stress proteins in field-exposed animals confirm the difficulties associated with complex mixtures of chemicals. Pedersen & Lundebye (1995) did not find a correlation between hsp70 in crabs (*Carcinus maenas*) exposed to sediments containing high concentrations of heavy metals (Cd, Cu, Zn) and heavy metal concentrations accumulated by the animals. Sanders & Steinert (1995) concluded from results of a field study with molluscs in San Diego Bay that induction of hsp60 and hsp70 proteins depended on the type of contaminants present at different locations and did not reflect total contaminant burden of the sediments. Results from an investigation of biomarkers in *Leponus macrochirus* (Pisces; Theodorakis *et al.* 1992) showed that hsp70 concentrations were elevated

during the first and second week of exposure in sediments contaminated with PCBs, PAHs, and heavy metals, but that after 16 weeks, hsp70 expression in exposed animals was lower than in control animals. Veldhuizen-Tsoerkan *et al.* (1991a) found no induction of stress proteins in *Mytilus edulis* (Mollusca) exposed for 5 months in highly contaminated sediments from the Scheldt estuary, but an additional heat shock resulted in induction of different stress proteins in molluscs exposed to contaminated sediment compared to those found in control animals held in clean sediment. They observed both induction and inhibition of various stress proteins. Whereas hsp36 was strongly induced by heat shock in molluscs exposed to contaminated sediment, hsp43 and hsp70 were clearly inhibited by the same treatment. Sediments from the Scheldt estuary contain high levels of cadmium, zinc, chromium, lead, PCBs, and pesticides (dieldrin, DDT).

The data show how difficult it is to determine the toxicity of contaminated sediments. Sediments usually contain complex mixtures of chemicals that are bound to sediment particles or organic material or dissolved in interstitial water (Ozretich *et al.* 1995). This latter fraction of dissolved xenobiotic compounds in interstitial water is mostly responsible for toxic effects on infaunal organisms (Borgmann *et al.* 1991; Landrum & Robbins 1990; Swartz *et al.* 1990; Black & McCarthy 1988; Landrum *et al.* 1985).

In addition to the questions associated with bioavailability of xenobiotic compounds in sediments (Iannuzzi *et al.* 1995), the treatment and storage of sediment samples for laboratory investigations can influence results of toxicity tests (Dillon *et al.* 1994). The toxicity of interstitial water can change even within a 10-day test period (Carr & Chapman 1995). The nature and extent of these changes are not predictable.

Time Course of Stress Protein Induction

To be suitable biomarkers, stress protein concentrations should increase rapidly under the influence of toxic substances and stay elevated for the duration of exposure. Lindquist (1986) observed a transient expression of stress proteins under continuous temperature stress in bacteria and yeast, where stress proteins play an important role in temperature adaptation. In cells of higher organisms, however, stress proteins are generally expressed as long as the stress continues or until cell death occurs (Welch 1990). Sanders *et al.* (1992) found that in *Mytilus edulis* (Mollusca) maintained at 10°C above their normal temperature, concentrations of hsp70 and hsp60 were elevated over an experimental period of 8 weeks. Concentrations of stress proteins in a fish cell culture remained elevated during a 24-hour exposure to cadmium and copper (Ryan & Hightower 1994). In an investigation by Stringham & Candido (1994), expression of stress proteins in nematodes exposed to arsenic increased within 24 hours and remained elevated for the entire 96-hour test period. Contrary to these findings, Hiranuma *et al.* (1993) observed a transient induction of hsp70-RNA by cadmium in human hepatoma cells, whereas concentrations of metallothionein- and hsp60-RNA increased with time.

We performed 10-day tests to investigate the time course of stress protein induction in *A. abdita* exposed to contaminated sediments, the toxicity of which had been determined previously. Despite significant reduction of percent survival both in sediment from site NFISC 1-9 and cadmium-spiked sediment, increased

expression of hsp60 proteins was transient in both tests. Toxicity of the NFISC 1-9 sediment measured as percent mortality after 10 days was reduced compared to the first bioassay. This could be due to additional storage time of about 2 weeks prior to the time course experiment (Carr & Chapman 1995; ASTM 1993). Increased expression of hsp60 proteins was measured on days 3 and 5 in animals exposed to NFISC 1-9 sediment. No stress proteins were detected in control animals. This increase could have been triggered by elevated temperature on days 1 and 2 (23-24.5°C). An additive effect of stressors such as xenobiotic compounds and elevated temperature was also found by Müller *et al.* (1995) in a study on *Ephydatia fluviatilis* (Porifera). Stress caused by elevated temperature increased induction of hsp70 by organic xenobiotics. Veldhuizen-Tsoerkan *et al.* (1991b) demonstrated that *Mytilus edulis* exposed to sublethal cadmium concentrations (16.5 µg/L) for 9 and 11 months did not have elevated stress protein concentrations, but an additional heat shock caused significantly stronger expression of stress proteins in cadmium-exposed animals than in control animals.

Longer-Term Consequences of Elevated Stress Protein Concentrations

Information on the link between increased stress protein expression and long-term effects in organisms is scarce. Sanders *et al.* (1991a) correlated hsp70 levels to scope-for-growth in *Mytilus edulis*. Stress protein induction was followed by apoptosis in vertebrate cells (Buchman *et al.* 1993). Incorporation of 35S into stress proteins of gill tissue in *Pimephales promelas* (Pisces) was positively correlated with mortality after 96 hours of exposure to xenobiotic compounds (Dyer *et al.* 1993). Boon-Niermeijer (1991) linked induction of heat shock proteins in molluscs to teratogenic effects. As severe heat-shock or other adverse influences inhibit the splicing of RNA (Yost & Lindquist 1986) and consequently normal cellular protein synthesis (Ciavarra & Simeone 1990). Suppressed gene expression in a specific developmental stage has been proposed as the likely cause of malformations in *Drosophila melanogaster* (Boon-Niermeijer 1991). This example shows that even brief and transient stress may have longer-term consequences for an organism. Knowledge of $LOEC_{hsp}$ values for a wide variety of species and xenobiotic compounds may, therefore, be of importance for the determination of adequate threshold concentrations.

Much more research is needed to evaluate the ecological implications of elevated stress protein levels in organisms. The species specificity of the response is linked to uptake and metabolism of xenobiotic compounds and does not allow an extrapolation of observed effects to other species of the same habitat. Moreover, inhibition as well as induction of the stress protein response has been measured as a consequence of uptake of xenobiotic compounds. More information is needed on the effects of complex mixtures of chemical compounds as found in most sediments and their interaction with naturally occurring physical stressors (Müller *et al.* 1995). It is possible that stress proteins other than the ones detected by Western blotting in this study represent more sensitive indicators of pollutant stress. The present study suggests that measuring hsp60 and hsp70 expression alone is not a reliable means of assessing the integrated stress caused by environmental pollutants.

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Appendix A

CHEMICAL AND BIOCHEMICAL DATA

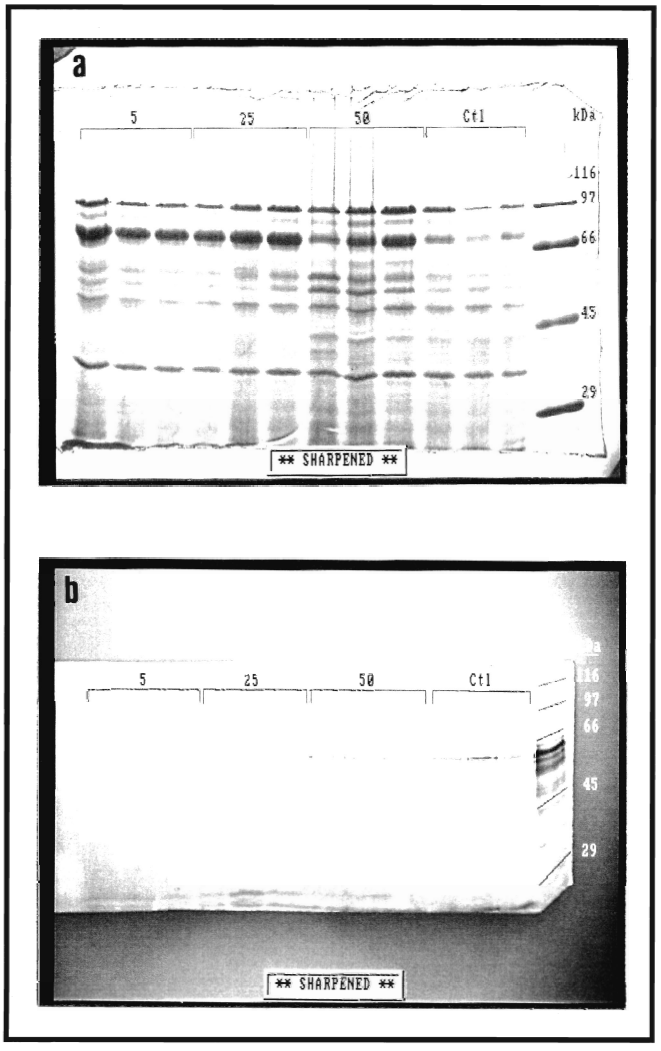

Figure A1-1
***H. AZTECA* - SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF CADMIUM CONCENTRATIONS**
a: Coomassie-stained SDS-PAGE gel
b: Western blot

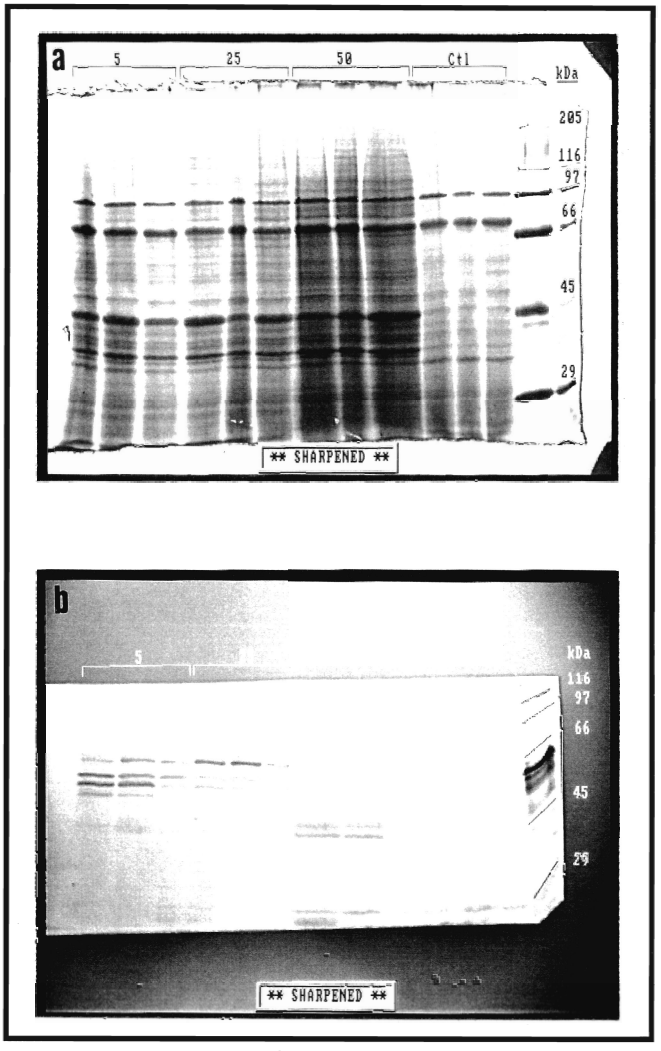

Figure A1-2
***H. AZTECA* - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF CADMIUM CONCENTRATIONS**
a: Coomassie-stained SDS-PAGE gel
b: Western blot

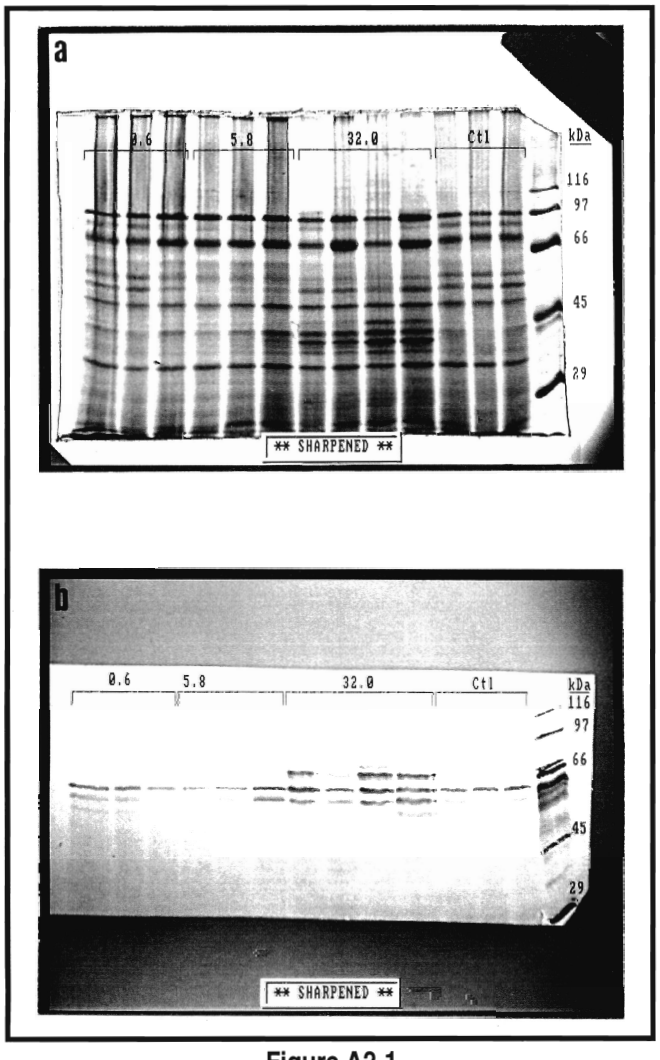

Figure A2-1
***H. AZTECA* - SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIAZINON CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

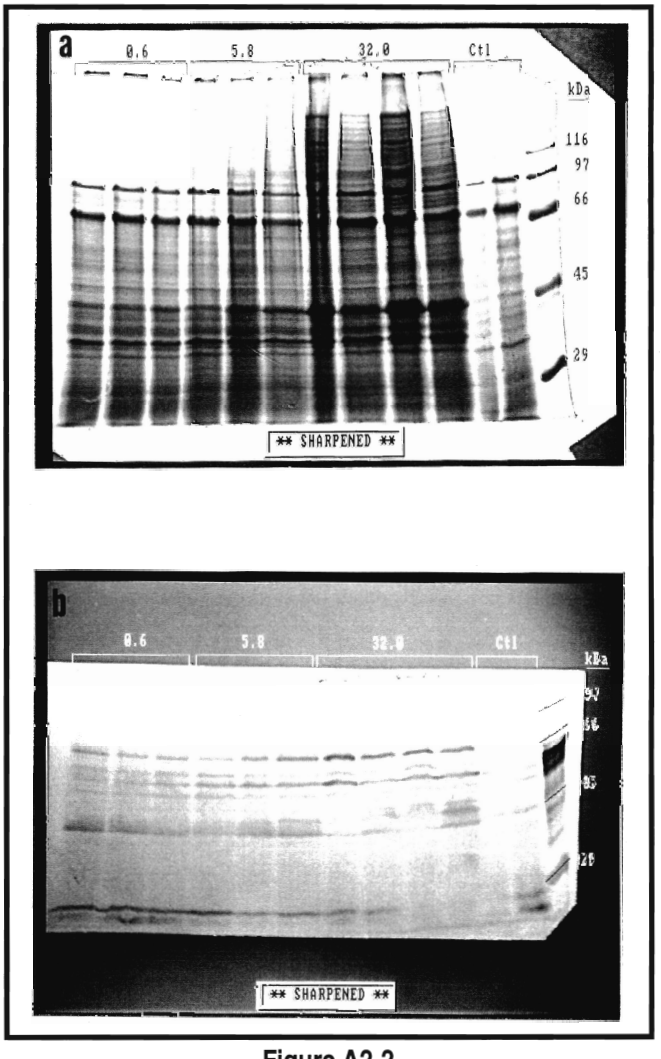

Figure A2-2
***H. AZTECA* - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIAZINON CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

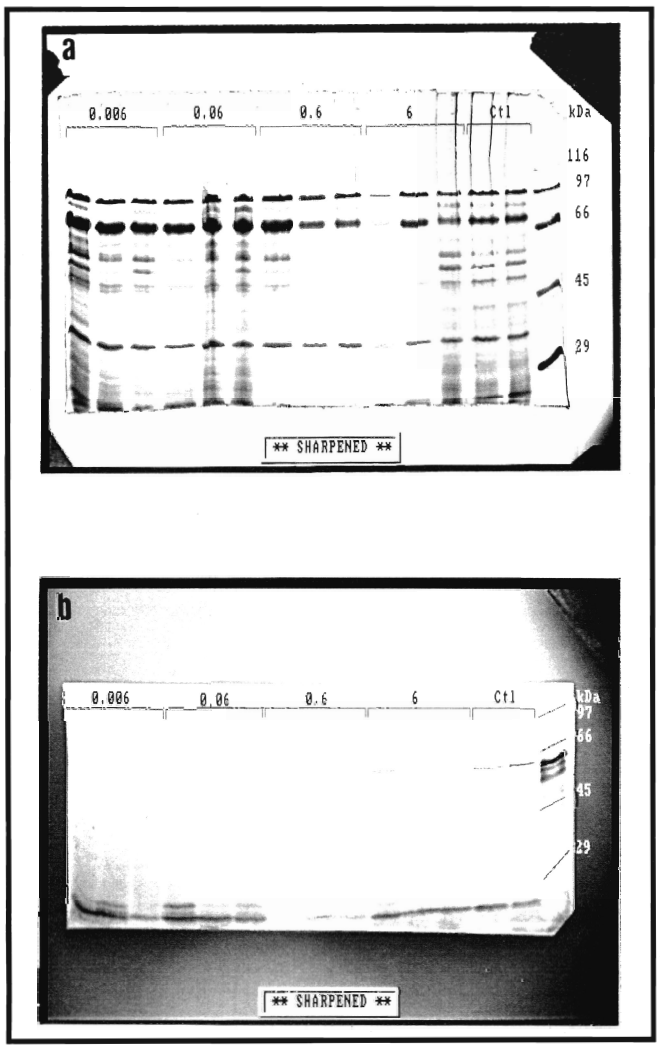

Figure A3-1
***H. AZTECA* - SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIELDRIN CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

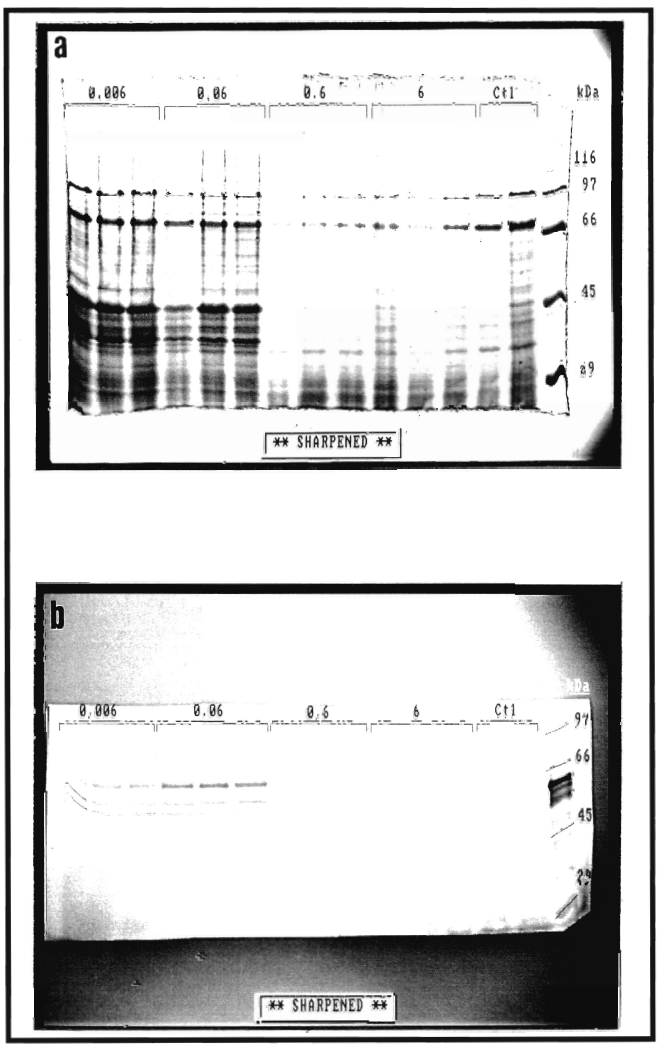

Figure A3-2
***H. AZTECA* - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIELDRIN CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

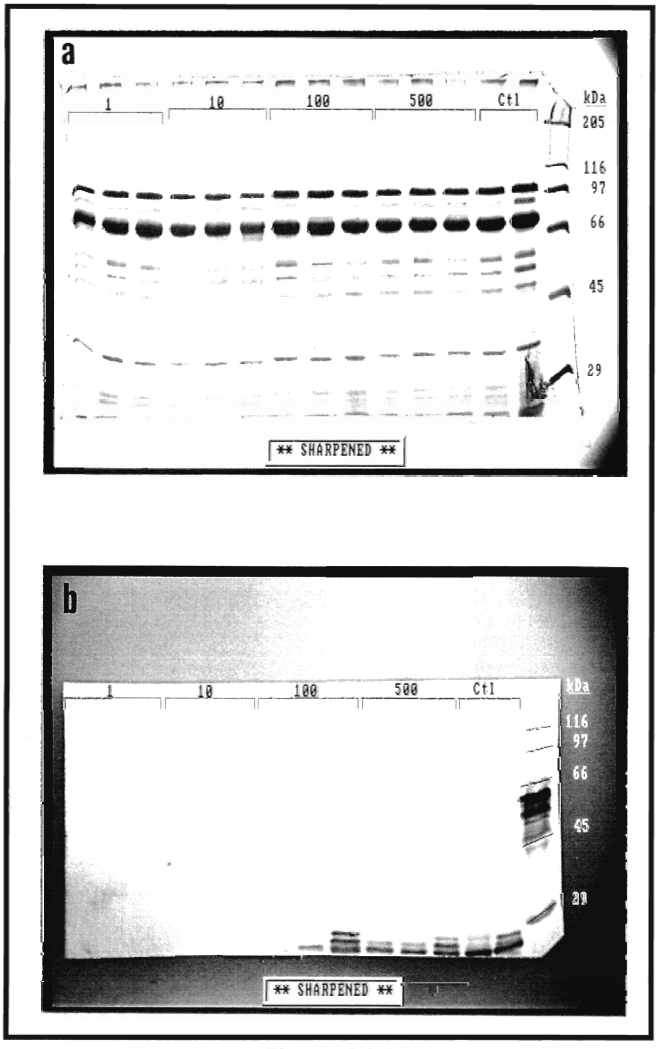

Figure A4-1
***H. AZTECA* - SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF FLUORANTHENE CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

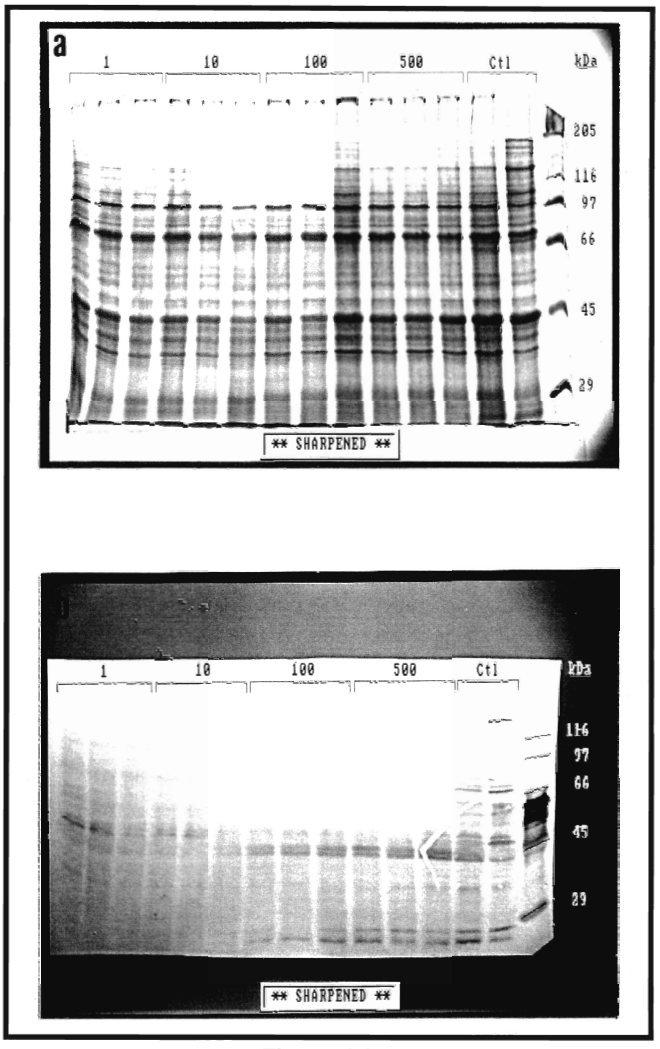

Figure A4-2
***H. AZTECA* - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF FLUORANTHENE CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

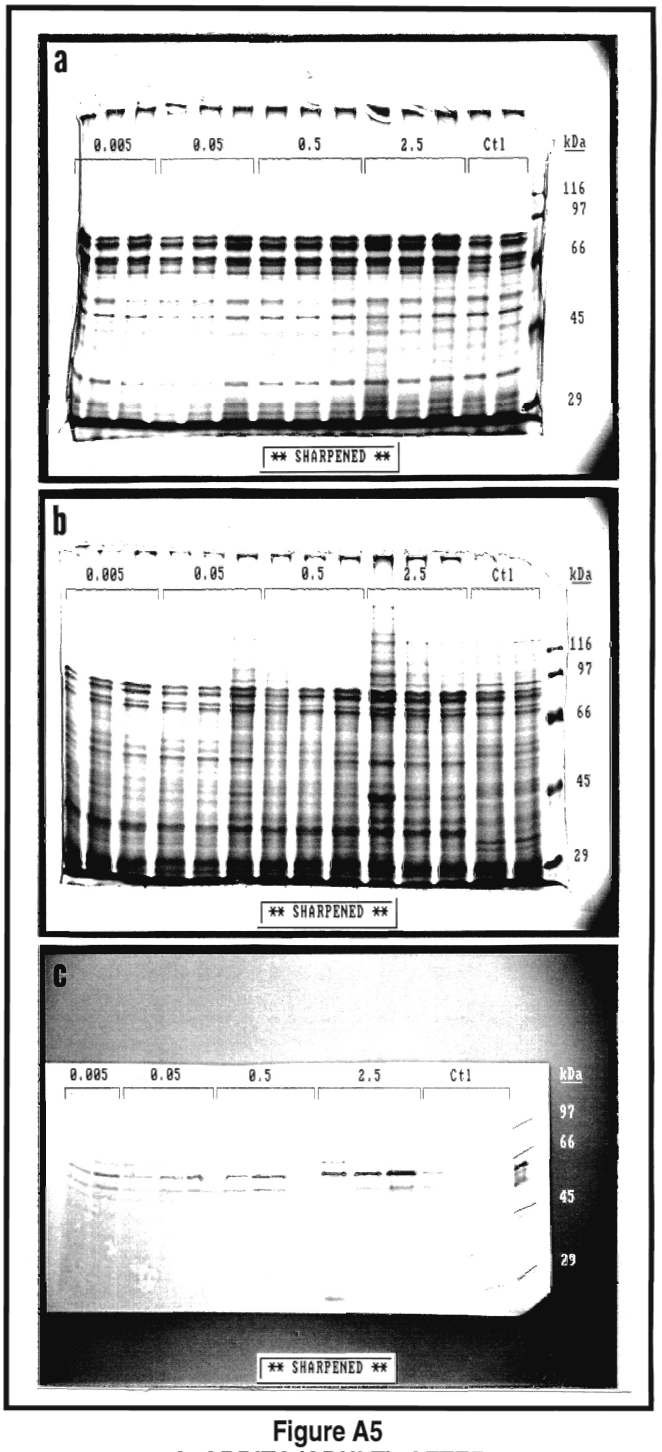

Figure A5
***A. ABDITA* (ADULT) AFTER**
24-HOUR EXPOSURE TO A RANGE OF
CADMIUM CONCENTRATIONS

a: Coomassie-stained SDS-PAGE gel, supernatant
b: Coomassie-stained SDS-PAGE gel, pellets
c: Western blot

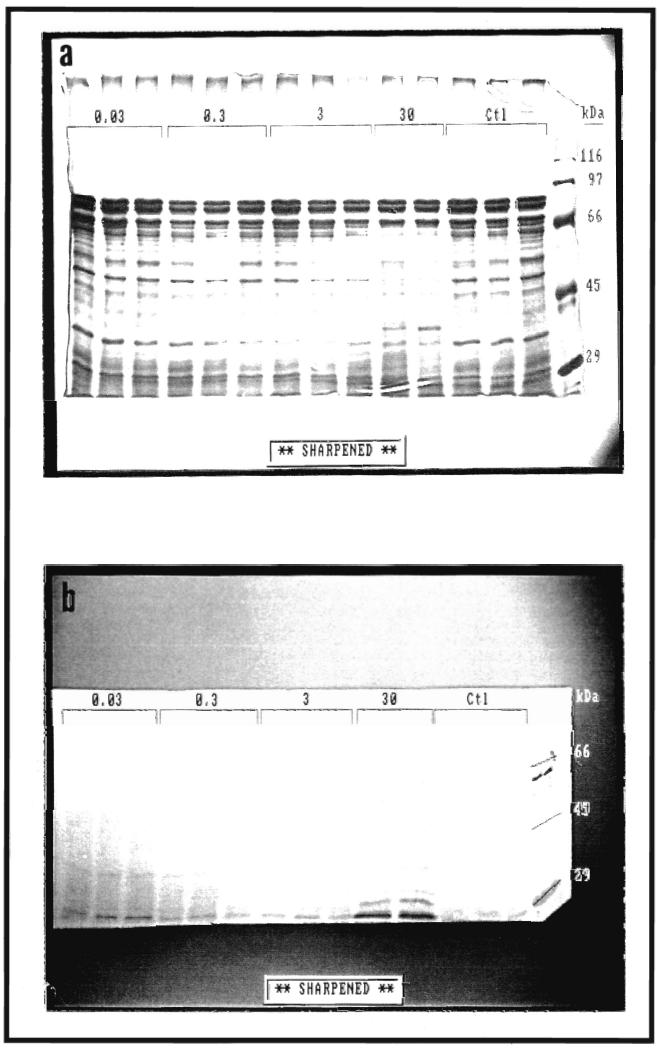

Figure A6-1
***A. ABDITA* (ADULT) - SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIAZINON CONCENTRATIONS**
a: Coomassie-stained SDS-PAGE gel
b: Western blot

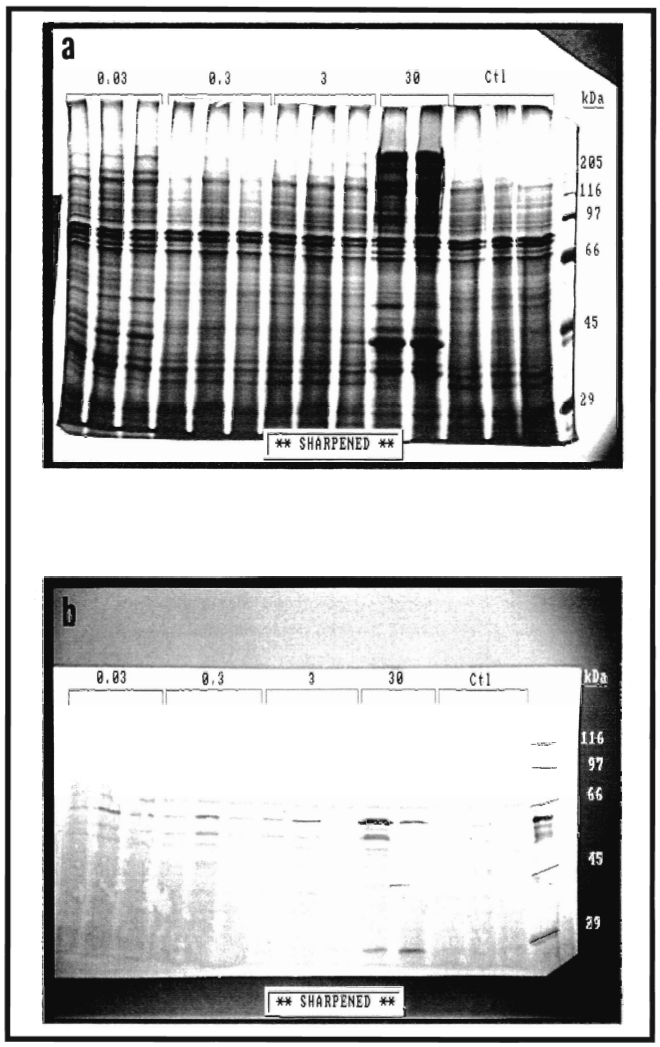

Figure A6-2
***A. ABDITA* (ADULT) - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIAZINON CONCENTRATIONS**
a: Coomassie-stained SDS-PAGE gel
b: Western blot

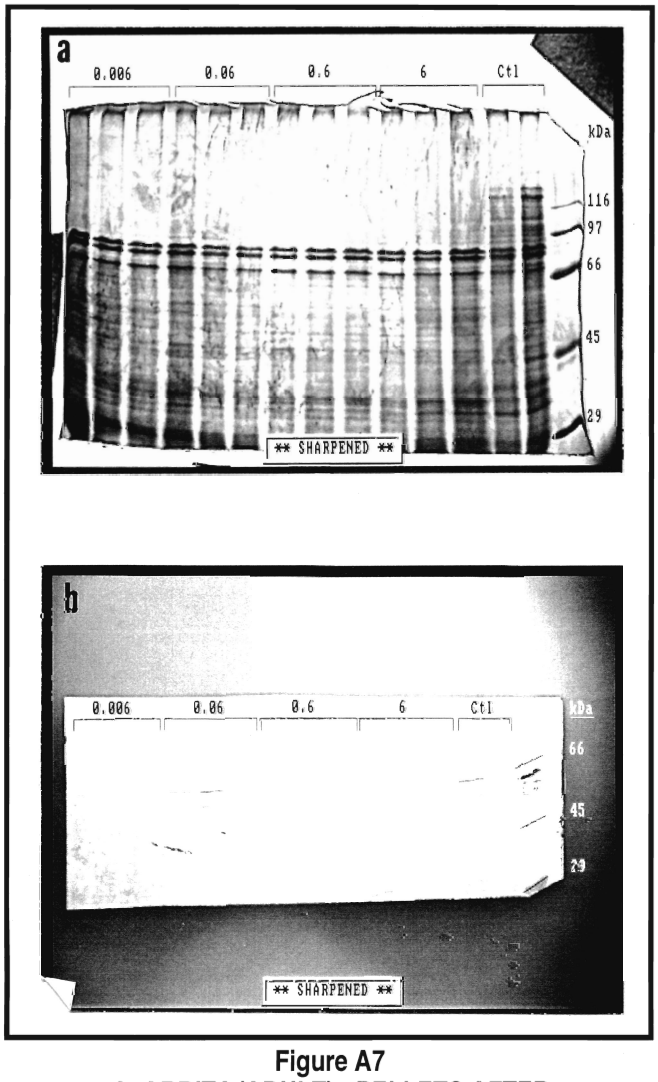

Figure A7
***A. ABDITA* (ADULT) - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIELDRIN CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

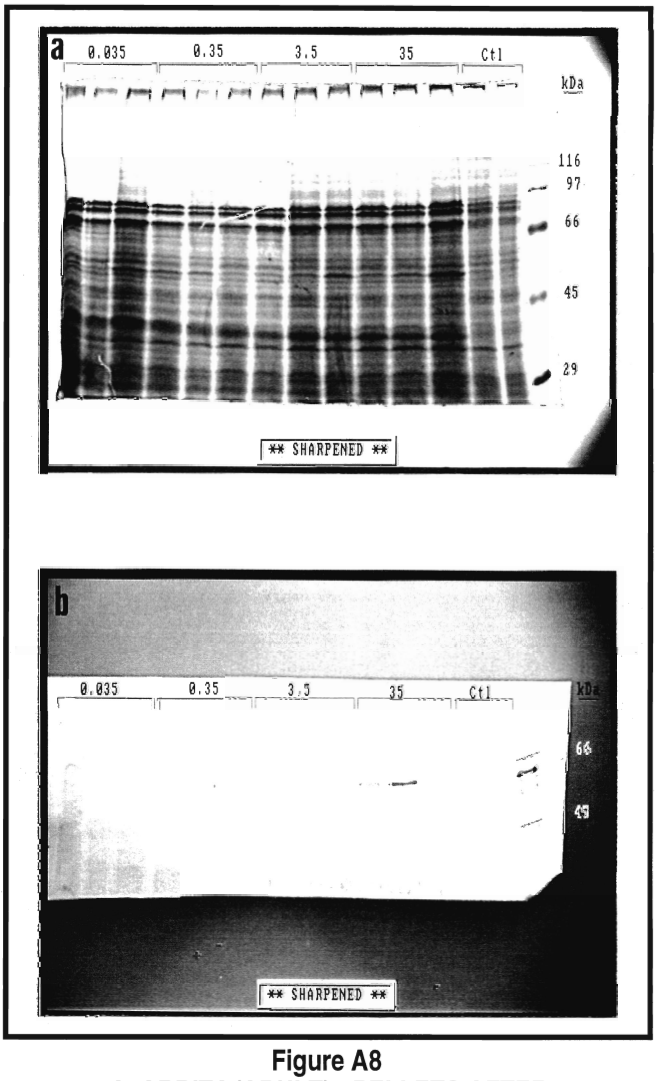

Figure A8
***A. ABDITA* (ADULT) - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF FLUORANTHENE CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

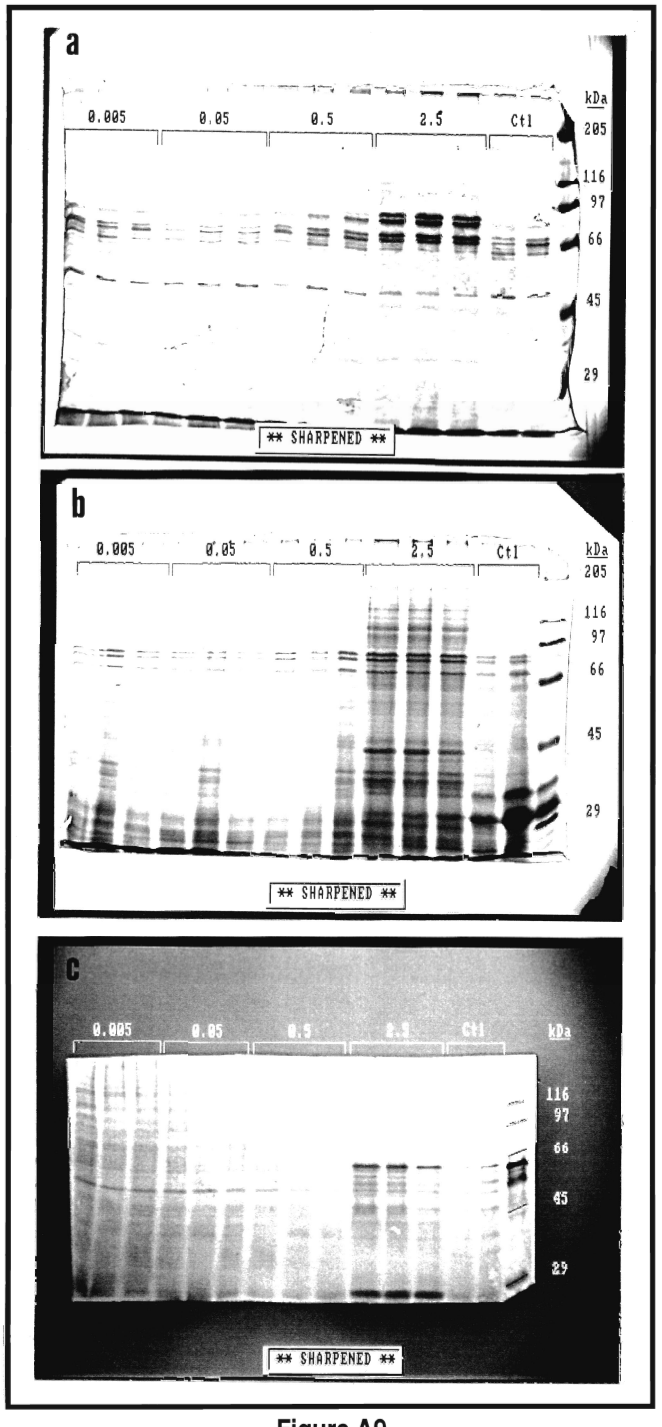

Figure A9
***A. ABDITA* (JUVENILE) AFTER**
24-HOUR EXPOSURE TO A RANGE OF
CADMIUM CONCENTRATIONS

a: Coomassie-stained SDS-PAGE gel, supernatant
b: Coomassie-stained SDS-PAGE gel, pellets
c: Western blot

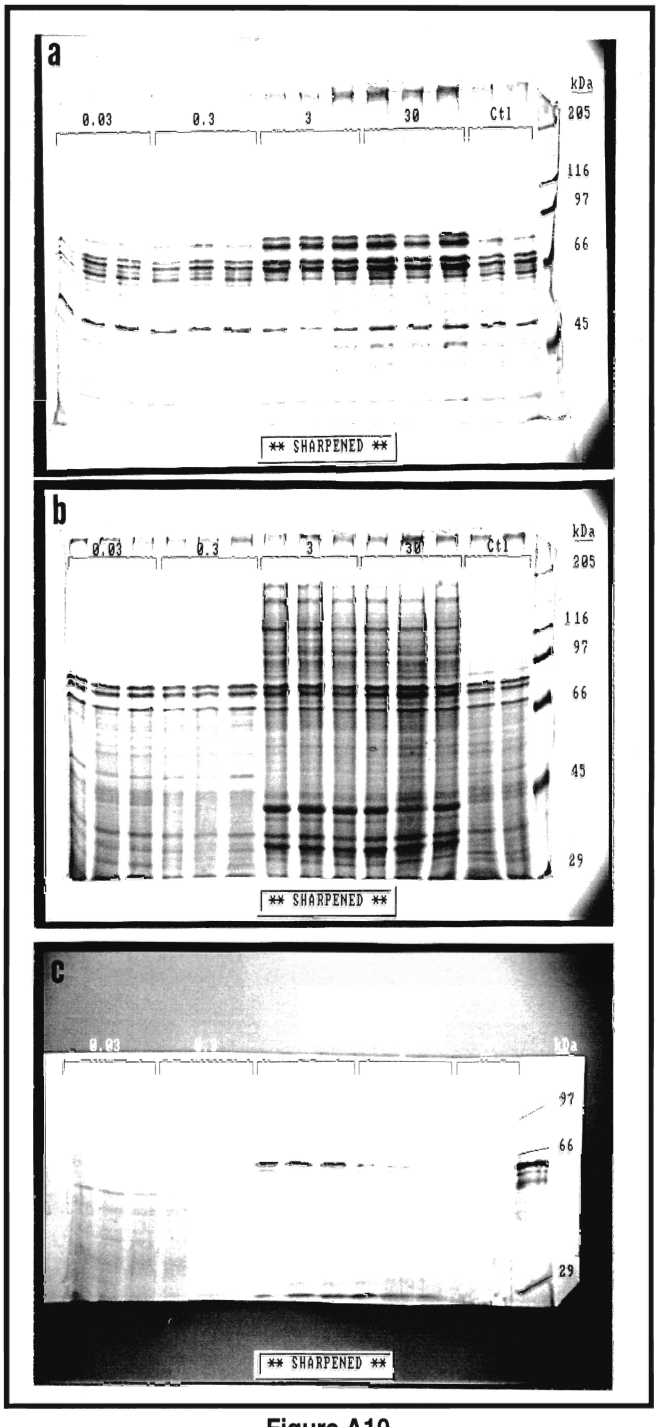

Figure A10
***A. ABDITA* (JUVENILE) AFTER**
24-HOUR EXPOSURE TO A RANGE OF
DIAZINON CONCENTRATIONS

a: Coomassie-stained SDS-PAGE gel, supernatant
b: Coomassie-stained SDS-PAGE gel, pellets
c: Western blot

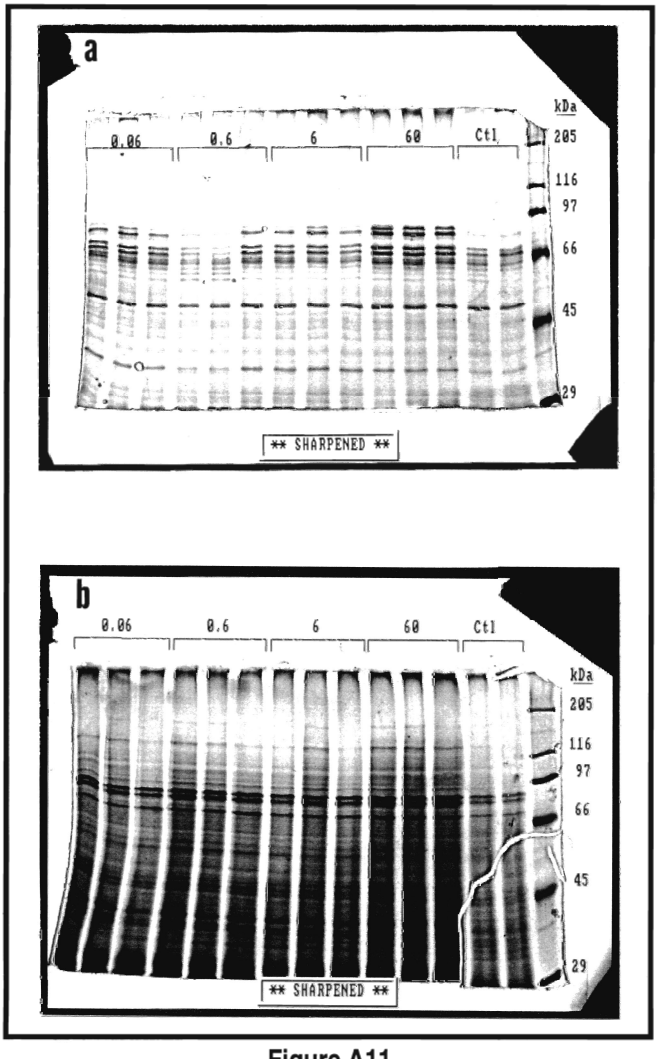

Figure A11
***A. ABDITA* (JUVENILE) - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIELDRIN CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Coomassie-stained SDS-PAGE pellets

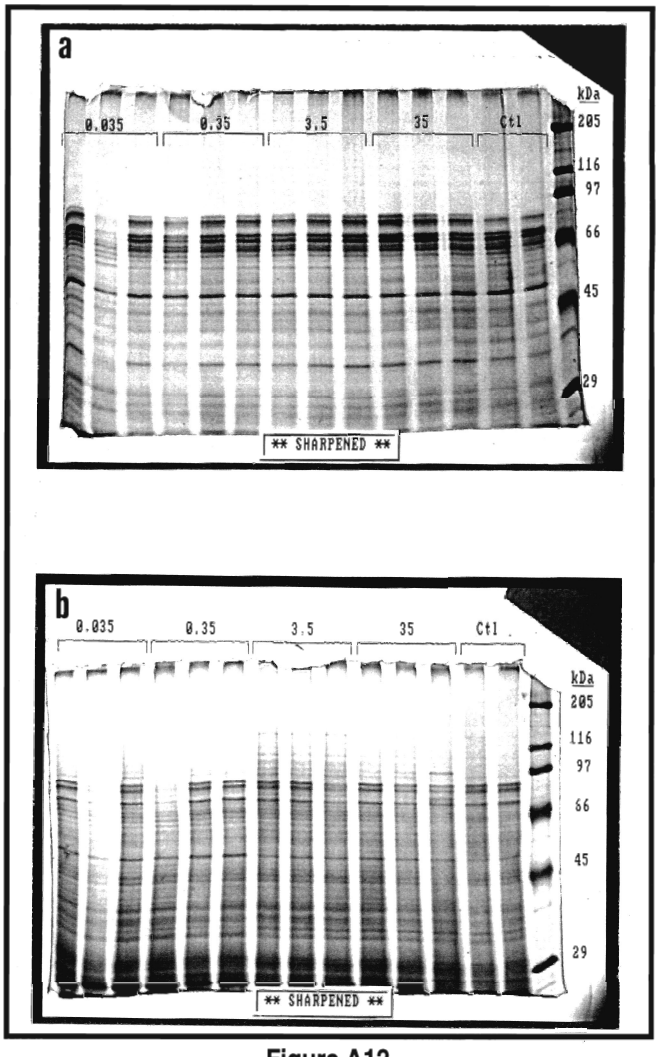

Figure A12
***A. ABDITA* (JUVENILE) - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF FLUORANTHENE CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Coomassie-stained SDS-PAGE pellets

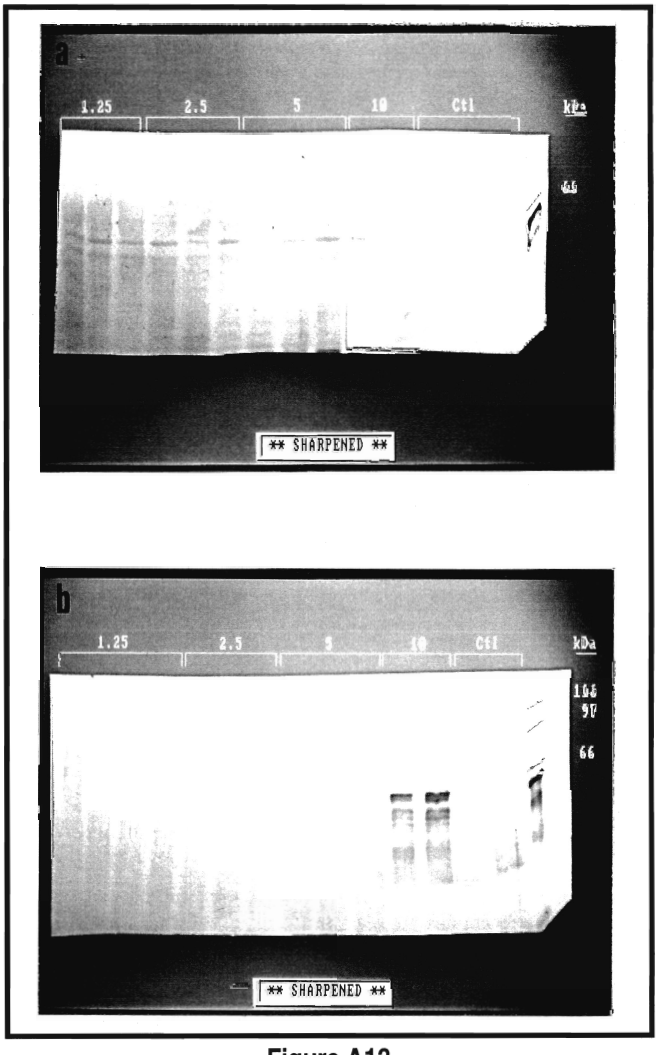

Figure A13
***R. ABRONIUS* - WESTERN BLOTS AFTER 24-HOUR EXPOSURE TO A RANGE OF CADMIUM CONCENTRATIONS**

a: Supernatants
b: Pellets

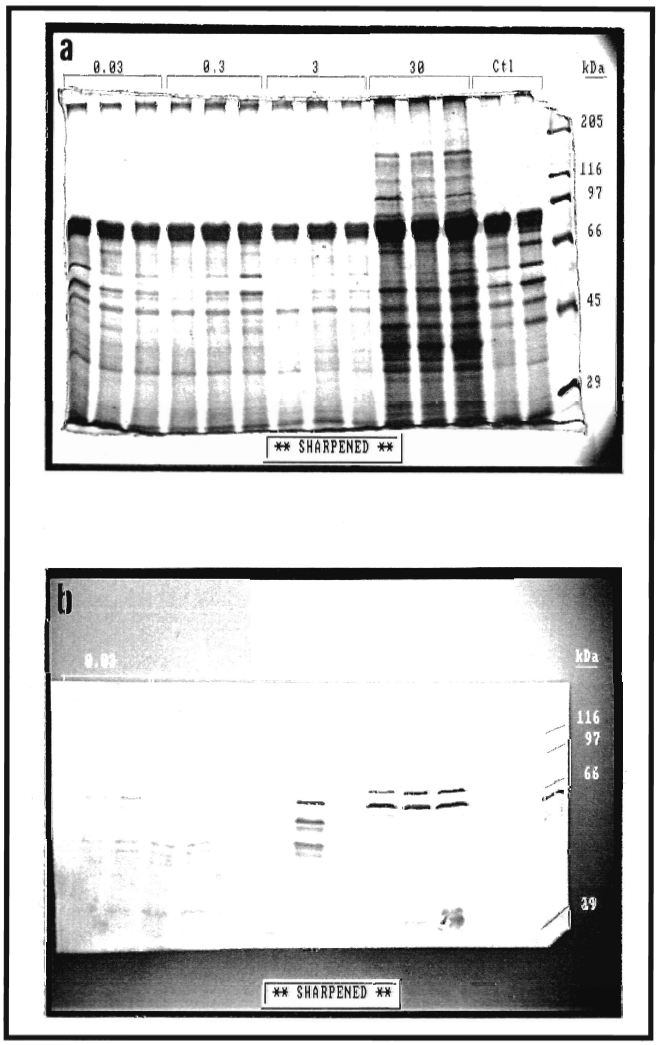

Figure A14-1
***R. ABRONIUS* - SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIAZINON CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

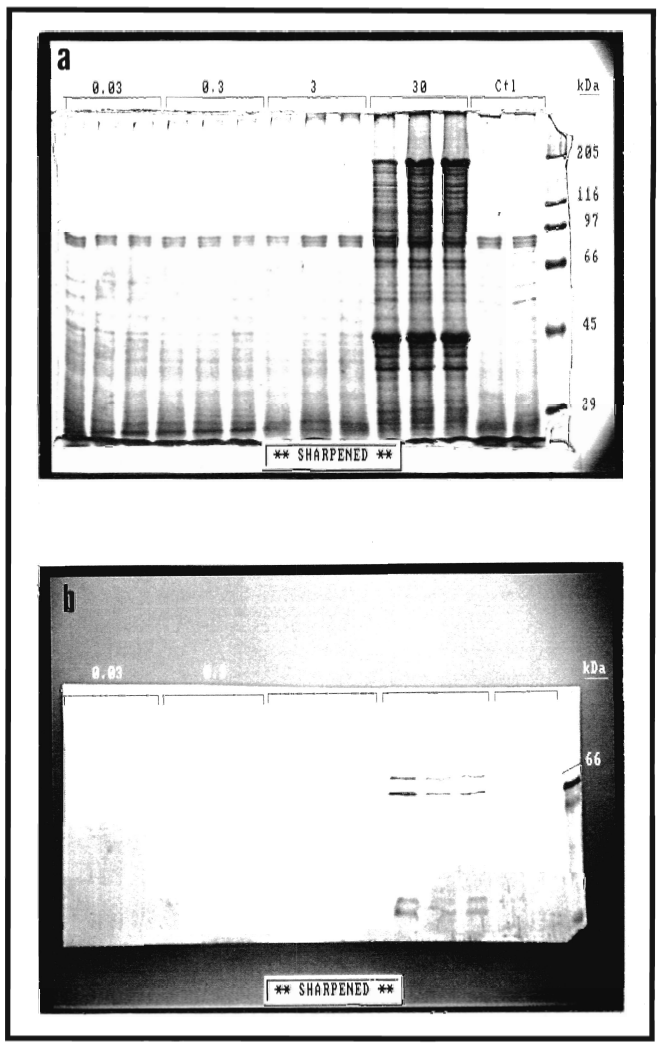

Figure A14-2
***R. ABRONIUS* - PELLETS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIAZINON CONCENTRATIONS**

a: Coomassie-stained SDS-PAGE gel
b: Western blot

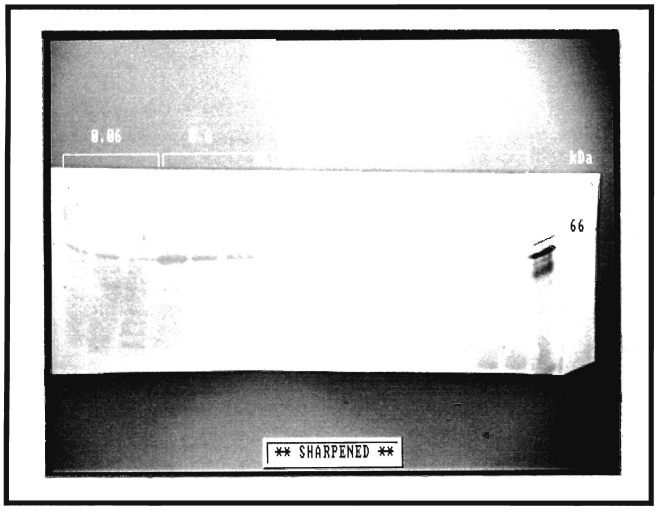

Figure A15
***R. ABRONIUS* - WESTERN BLOT OF SUPERNATANTS AFTER 24-HOUR EXPOSURE TO A RANGE OF DIELDRIN CONCENTRATIONS**

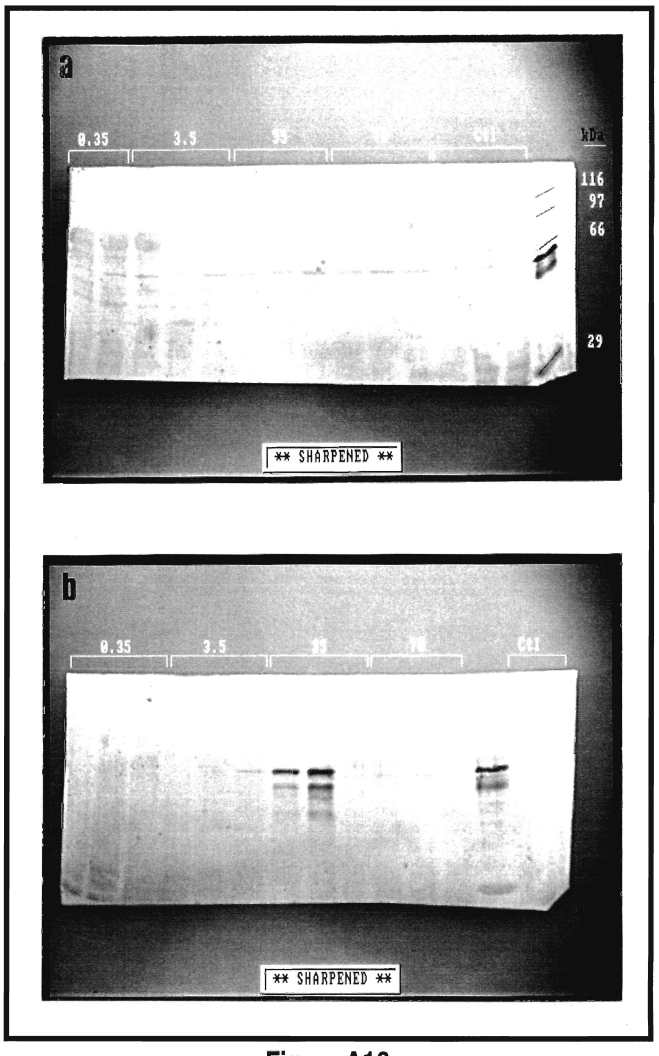

Figure A16
***R. ABRONIUS* - WESTERN BLOTS AFTER 24-HOUR EXPOSURE TO A RANGE OF FLUORANTHENE CONCENTRATIONS**

a: Supernatants
b: Pellets

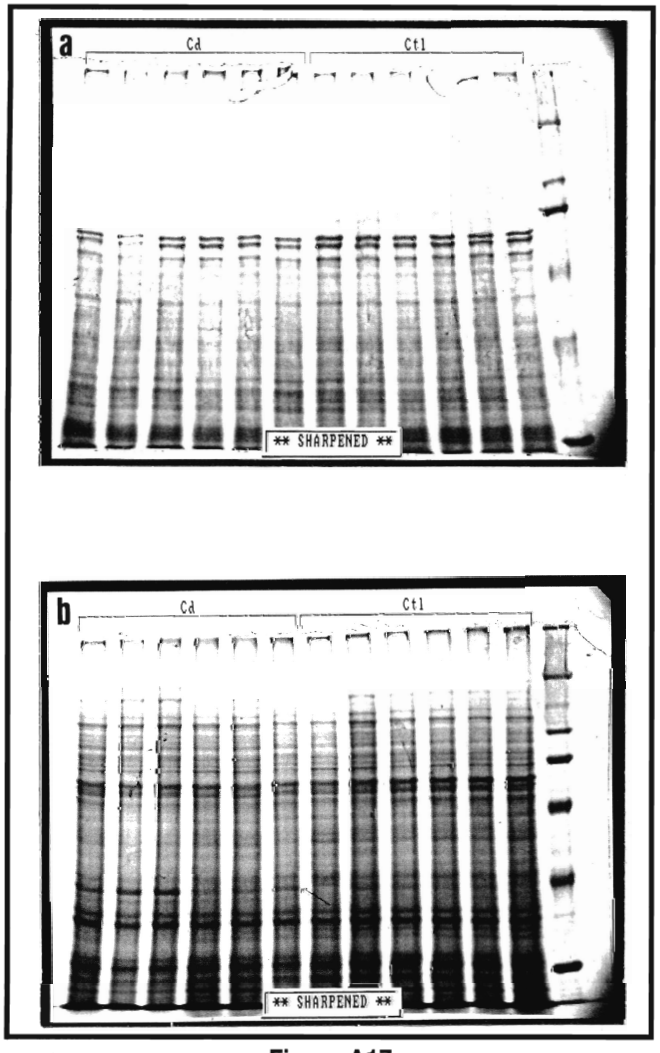

Figure A17
***A. ABDITA* (ADULT) - PELLETS:**
COOMASSIE-STAINED SDS-PAGE GELS AFTER EXPOSURE TO CADMIUM-SPIKED SEDIMENT

(10.3 mg/kg wet weight)

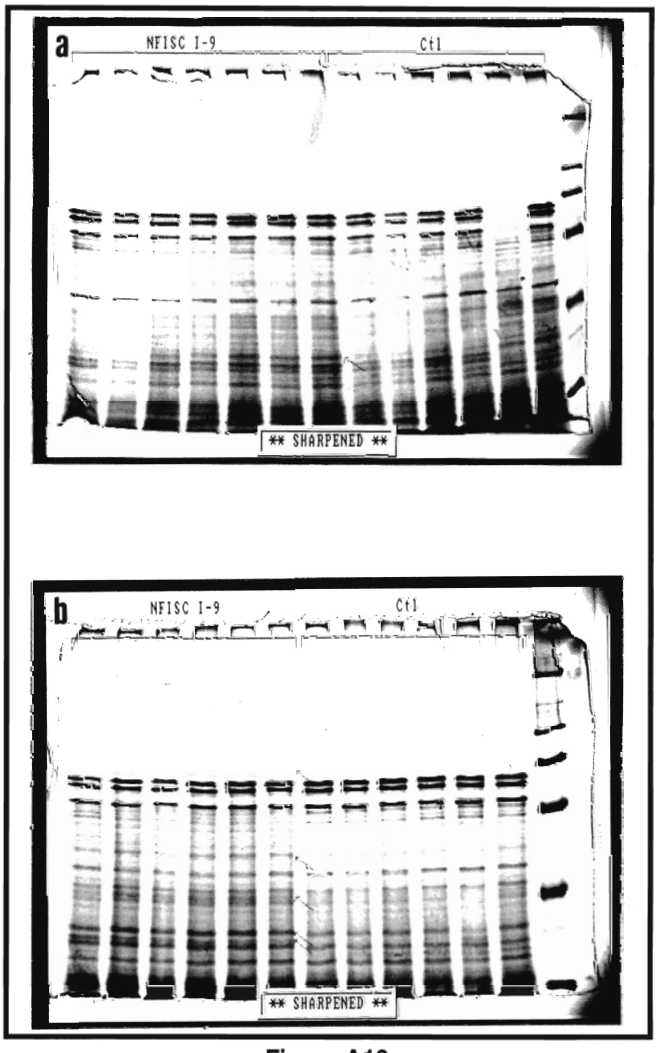

Figure A18
***A. ABDITA* (ADULT) - PELLETS:**
COOMASSIE-STAINED SDS-PAGE GEL AFTER EXPOSURE TO SEDIMENT FROM SITE 8 - NFISC 1-9

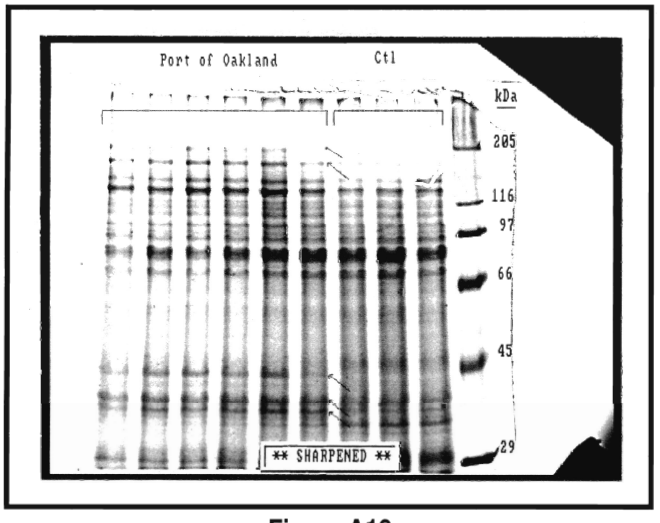

Figure A19
***A. ABDITA* (ADULT) - COOMASSIE-STAINED SDS-PAGE GEL AFTER 10-DAY EXPOSURE TO SEDIMENT FROM SITE 12 - PORT OF OAKLAND**

Table A-1
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 1/BENICIA

All chemical analyses are given as dry weight basis unless noted.

	Benicia	Detection Limit
Grain size (%)		
Gravel	0.0	
Sand	4.9	
Silt	40.5	
Clay	54.6	
Solids (%)	42.0	
Sulfides (mg/kg)		
Total	138	
Water Soluble	0.048	0.25
Total Organic Carbon (mg/kg)	15 300	
TRPH (mg/Kg)	26.7	10
Organotins (µg/Kg)		
Tributyltin	<2.38	
Dibutyltin	<2.38	
Monobutyltin	<2.38	
Metals (mg/Kg)		
Arsenic (As)	11.1	
Cadmium (Cd)	0.450	
Chromium (Cr)	75.7	
Copper (Cu)	62.6	
Lead (Pb)	28.6	
Mercury (Hg)	0.238	
Nickel (Ni)	85.0	
Selenium (Se)	<0.236	0.099
Silver (Ag)	<0.190	0.080
Zinc (Zn)	119	
Pesticides and PCBs (µg/kg)		
4,4' - DDD	ND	2
4,4' - DDE	ND	2
4,4' - DDT	ND	2
Aldrin	ND	2
alpha-BHC	ND	2
beta BHC	ND	2
Chlordane	ND	25
delta BHC	ND	2
Dieldrin	ND	2
Endosulfan I	ND	10
Endosulfan II	ND	2
Endosulfan Sulfate	ND	25

	Benicia	Detection Limit
Endrin	ND	2
Endrin Aldehyde	ND	10
Heptachlor	ND	2
Heptachlor Epoxide	ND	10
gamma-BHC	ND	2
Toxaphene	ND	25
PCB 1016	ND	20
PCB 1221	ND	20
PCB 1232	ND	20
PCB 1242	ND	20
PCB 1248	ND	20
PCB 1254	ND	20
PCB 1260	ND	20
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	ND	20
Pyrene	ND	20
Benzo (A) Anthracene	ND	20
Chrysene	ND	20
Benzo (B) Fluoranthene	ND	20
Benzo (K) Fluoranthene	ND	20
Benzo (A) Pyrene	ND	20
Dibenzo (A,H) Anthracene	ND	
Ideno (1,2,3-CD) Pyrene	ND	
Benzo (G,H,I) Perylene	ND	20
Total	0	
Phthalate Esters (µg/kg)		
Bis(2-Ethylhexyl)Phthalate	110	
Butyl Benzyl Phthalate	ND	8.5
Di-N-Butyl Phthalate	197	9.0
Diethyl Phthalate	ND	12.0
Dimethyl Phthalate	ND	7.3
Di—N-Octyl Phthalate	ND	75.0
Total	307	

Table A-2
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 2/CARQUINEZ

All chemical analyses are given as dry weight basis unless noted.

	Carquinez	Detection Limit
Grain size (%)		
Gravel	5.4	
Sand	30.7	
Silt	32.4	
Clay	31.5	
Solids (%)	57.7	
Sulfides (mg/kg)		
Total	255	
Water Soluble	0.052	0.25
Total Organic Carbon (mg/kg)	8 890	
TRPH (mg/Kg)	31.2	10
Organotins (µg/Kg)		
Tributyltin	<1.73	
Dibutyltin	<1.73	
Monobutyltin	<1.73	
Metals (mg/Kg)		
Arsenic (As)	17.9	
Cadmium (Cd)	0.364	
Chromium (Cr)	51.8	
Copper (Cu)	39.7	
Lead (Pb)	25.1	
Mercury (Hg)	0.094	
Nickel (Ni)	54.9	
Selenium (Se)	<0.172	0.099
Silver (Ag)	<0.139	0.080
Zinc (Zn)	92.4	
Pesticides and PCBs (µg/kg)		
4,4' - DDD	ND	2
4,4' - DDE	ND	2
4,4' - DDT	ND	2
Aldrin	ND	2
alpha-BHC	ND	2
beta BHC	ND	2
Chlordane	ND	25
delta BHC	ND	2
Dieldrin	ND	2
Endosulfan I	ND	10
Endosulfan II	ND	2
Endosulfan Sulfate	ND	25

	Carquinez	Detection Limit
Endrin	ND	2
Endrin Aldehyde	ND	10
Heptachlor	ND	2
Heptachlor Epoxide	ND	10
gamma-BHC	ND	2
Toxaphene	ND	25
PCB 1016	ND	20
PCB 1221	ND	20
PCB 1232	ND	20
PCB 1242	ND	20
PCB 1248	ND	20
PCB 1254	ND	20
PCB 1260	ND	20
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	151	20
Pyrene	225	20
Benzo (A) Anthracene	58.6	20
Chrysene	80.2	20
Benzo (B) Fluoranthene	ND	20
Benzo (K) Fluoranthene	ND	20
Benzo (A) Pyrene	168	20
Dibenzo (A,H) Anthracene	ND	20
Ideno (1,2,3-CD) Pyrene	ND	20
Benzo (G,H,I) Perylene	ND	20
Total	682.8	
Phthalate Esters (µg/kg)		
Bis(2-Ethylhexyl)Phthalate	73.7	
Butyl Benzyl Phthalate	ND	8.5
Di-N-Butyl Phthalate	196	9.0
Diethyl Phthalate	ND	12.0
Dimethyl Phthalate	ND	7.3
Di—N-Octyl Phthalate	ND	75.0
Total	269.7	

Table A-3
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 3/EXXON

All chemical analyses are given as dry weight basis unless noted.

	Exxon-1	Exxon-2	Detection Limit
Grain size (%)			
Gravel	0.1	0.1	
Sand	33.4	13.3	
Silt	26.4	32.3	
Clay	40.0	54.4	
Solids (%)	56.5	41.6	
Sulfides (mg/kg)			
Total	23.2	<2.40	
Water Soluble	<1.8	<2.4	
Total Organic Carbon (%)	1.21	1.42	
TRPH (mg/kg)	8.0	7.2	
Organotins (µg/kg)			
Tributyltin	<1.8	<2.4	1.0
Dibutyltin	<1.8	<2.4	1.0
Monobutyltin	<1.8	<2.4	1.0
Metals (mg/kg)			
Arsenic (As)	6.94	8.85	
Cadmium (Cd)	<0.035	<0.048	0.020
Chromium (Cr)	53.6	77.2	
Copper (Cu)	41.1	62.0	
Lead (Pb)	14.9	22.6	
Mercury (Hg)	0.241	0.313	
Nickel (Ni)	65.8	81.5	
Selenium (Se)	<0.708	<0.962	0.400
Silver (Ag)	<0.142	<0.192	0.080
Zinc (Zn)	77.2	113	
Pesticides and PCBs (µg/kg)			
4,4' - DDD	ND	ND	2
4,4' - DDE	ND	ND	2
4,4' - DDT	ND	ND	2
Aldrin	ND	ND	2
alpha-BHC	ND	ND	2
beta BHC	ND	ND	2
Chlordane	ND	ND	25
delta BHC	ND	ND	2
Dieldrin	ND	ND	2
Endosulfan I	ND	ND	10
Endosulfan II	ND	ND	2
Endosulfan Sulfate	ND	ND	25

	Exxon-1	Exxon-2	Detection Limit
Endrin	ND	ND	2
Endrin Aldehyde	ND	ND	10
Heptachlor	ND	ND	2
Heptachlor Epoxide	ND	ND	10
Lindane	ND	ND	2
Toxaphene	ND	ND	25
PCB 1016	ND	ND	20
PCB 1221	ND	ND	20
PCB 1232	ND	ND	20
PCB 1242	ND	ND	20
PCB 1248	ND	ND	20
PCB 1254	ND	ND	20
PCB 1260	ND	ND	20
PAHs (µg/kg)			
Naphthalene	ND	ND	20
Acenaphthylene	ND	ND	20
Acenaphthene	ND	ND	20
Fluorene	ND	ND	20
Phenanthrene	ND	ND	20
Anthracene	ND	ND	20
Fluoranthene	53.6	63.2	20
Pyrene	65.7	64.2	20
Benzo (A) Anthracene	ND	ND	20
Chrysene	ND	ND	20
Benzo (B) Fluoranthene	ND	ND	20
Benzo (K) Fluoranthene	ND	ND	20
Benzo (A) Pyrene	ND	ND	20
Dibenzo (AH) Anthracene	ND	ND	20
Ideno (1,2,3-CD) Pyrene	ND	ND	20
Benzo (G,H,I) Perylene	ND	ND	20
Total	119.3	127.4	
Phthalate Esters (µg/kg)			
Bis (2-ethylhexyl) Phthalate	171	310	50
Butylbenzyl Phthalate	ND	ND	8.5
Di-n-butyl Phthalate	ND	ND	9
Diethyl Phthalate	ND	ND	12
Dimethyl Phthalate	ND	ND	7.3
Di-n-octyl Phthalate	ND	ND	75
Total	171	310	

Table A-4
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 4/SAN PABLO BAY

All chemical analyses are given as dry weight basis unless noted.

	San Pablo Bay	Detection Limit
Grain size (%)		
Gravel	4.5	
Sand	57.8	
Silt	14.3	
Clay	23.4	
Solids (%)	64.6	
Sulfides (mg/kg)		
Total	113	
Water Soluble	<0.2	0.1
Total Organic Carbon (%)	0.673	
TRPH (mg/kg)	4.5	
Organotins (µg/kg)		
Tributyltin	<1.5	1.0
Dibutyltin	<1.5	1.0
Monobutyltin	<1.5	1.0
Metals (mg/kg)		
Arsenic (As)	7.12	
Cadmium (Cd)	0.150	
Chromium (Cr)	58.8	
Copper (Cu)	26.3	
Lead (Pb)	13.8	
Mercury (Hg)	0.183	
Nickel (Ni)	56.3	
Selenium (Se)	0.443	
Silver (Ag)	<0.124	0.08
Zinc (Zn)	67.2	
Pesticides and PCBs (mg/kg)		
4,4' - DDD	ND	2
4,4' - DDE	ND	2
4,4' - DDT	ND	2
Aldrin	ND	2
alpha-BHC	ND	2
beta BHC	ND	2
Chlordane	ND	25
Delta-BHC	ND	2
Dieldrin	ND	2
Endosulfan I	ND	2
Endosulfan II	ND	2
Endosulfan Sulfate	ND	25

	San Pablo Bay	Detection Limit
Endrin	ND	2
Endrin Aldehyde	ND	10
Heptachlor	ND	2
Heptachlor Epoxide	ND	10
Lindane	ND	2
Toxaphene	ND	25
PCB 1016	ND	20
PCB 1221	ND	20
PCB 1232	ND	20
PCB 1242	ND	20
PCB 1248	ND	20
PCB 1254	ND	20
PCB 1260	ND	20
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	44.0	20
Pyrene	59.9	20
Benzo (A) Anthracene	ND	20
Chrysene	ND	20
Benzo (B) Fluoranthene	ND	20
Benzo (K) Fluoranthene	ND	20
Benzo (A) Pyrene	ND	20
Dibenzo (A,H) Anthracene	ND	20
Ideno (1,2,3-CD) Pyrene	ND	20
Benzo (G,H,I) Perylene	ND	20
Total	103.9	
Phthalate Esters (µg/kg)		
Bis (2-ethylhexyl) Phthalate	105	50
Butylbenzyl Phthalate	ND	8.5
Di-n-butyl Phthalate	83.9	9
Diethyl Phthalate	ND	12
Dimethyl Phthalate	ND	7.3
Di-n-octyl Phthalate	ND	75
Total	188.9	

Table A-5
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 5/BEL MARIN KEYES

All chemical analyses are given as dry weight basis unless noted.

	Bel Marin Keyes	Detection Limit
Grain size (%)		
Gravel	0.0	
Sand	0.7	
Silt	33.7	
Clay	65.6	
Solids (%)	43.4	
Metals (mg/kg)		
Antimony	5.25	
Arsenic	12.4	
Barium	56.7	
Beryllium	0.797	
Cadmium	<0.369	0.16
Chromium	89.9	
Cobalt	22.9	
Copper	54.4	
Lead	47.0	
Mercury	0.415	
Molybdenum	<0.230	0.1
Nickel	98.2	
Selenium	<1.15	0.5
Silver	<0.184	0.08
Thallium	<0.230	0.1
Vanadium	77.0	
Zinc	133	

	Bel Marin Keyes	Detection Limit
Organics (µg/kg)		
Aldrin	<4.4	1.9
Chlordane	<23.0	10.0
4,4' - DDT	<10.8	4.7
4,4' - DDE	<12.9	5.6
4,4' - DDD	<6.5	2.8
2,4-D	<2.5	1.1
Dieldrin	<5.8	2.5
Endrin	<23.0	10.0
Heptachlor	<4.4	1.9
Kepone	<11.5	5.0
Lindane	<7.1	3.1
Methoxychlor	<23.0	10.0
Mirex	<11.5	5.0
Pentachlorophenol	<8.3	3.6
PCBs	<23.0	10.0
Toxaphene	<23.0	10.0
Trichloroethylene	<11.5	5.0
2,4,5-TP	<2.8	1.2
Organic Lead (mg/kg)	<1.2	0.5
Dioxins		
2,3,7,8-TCDD	ND	0.027
Asbestos	ND	
Nitrate-N	<4.61	
Nitrite-N	<23.0	
Sulfate	2440	
Fluoride	10.1	

Table A-6
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 6/PARADISE CAY

All chemical analyses are given as dry weight basis unless noted.

	Paradise Cay	Detection Limit
Grain size (%)		
Gravel	0.0	
Sand	0.8	
Silt	45.7	
Clay	53.5	
Solids (%) (Dry Wt.)	44.6	
Sulfides (mg/kg)		
Total	159	
Water Soluble	<0.2	0.1
Total Organic Carbon (%)	1.09	
TRPH (mg/kg)	6.5	
Organotins (µg/kg)		
Tributyltin	<2.2	1.0
Dibutyltin	<2.2	1.0
Monobutyltin	<2.2	1.0
Metals (mg/kg)		
Arsenic (As)	8.39	
Cadmium (Cd)	0.182	
Chromium (Cr)	74.0	
Copper (Cu)	48.0	
Lead (Pb)	22.2	
Mercury (Hg)	0.341	
Nickel (Ni)	70.0	
Selenium (Se)	0.767	
Silver (Ag)	0.704	0.08
Zinc (Zn)	103	
Pesticides and PCBs (mg/kg)		
4,4' - DDD	ND	2
4,4' - DDE	ND	2
4,4' - DDT	ND	2
Aldrin	ND	2
alpha-BHC	ND	2
beta BHC	ND	2
Chlordane	ND	25
Delta-BHC	ND	2
Dieldrin	ND	2
Endosulfan I	ND	2
Endosulfan II	ND	2
Endosulfan Sulfate	ND	25

	Paradise Cay	Detection Limit
Endrin	ND	2
Endrin Aldehyde	ND	10
Heptachlor	ND	2
Heptachlor Epoxide	ND	10
Lindane	ND	2
Toxaphene	ND	25
PCB 1016	ND	20
PCB 1221	ND	20
PCB 1232	ND	20
PCB 1242	ND	20
PCB 1248	ND	20
PCB 1254	ND	20
PCB 1260	ND	20
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	ND	20
Pyrene	ND	20
Benzo (A) Anthracene	ND	20
Chrysene	ND	20
Benzo (B) Fluoranthene	ND	20
Benzo (K) Fluoranthene	ND	20
Benzo (A) Pyrene	ND	20
Dibenzo (A,H) Anthracene	ND	20
Ideno (1,2,3-CD) Pyrene	ND	20
Benzo (G,H,I) Perylene	ND	20
Total	ND	
Phthalate Esters (µg/kg)		
Bis (2-ethylhexyl) Phthalate	161	50
Butylbenzyl Phthalate	ND	8.5
Di-n-butyl Phthalate	93.3	9
Diethyl Phthalate	ND	12
Dimethyl Phthalate	ND	7.3
Di-n-octyl Phthalate	ND	75
Total	254.3	

Table A-7
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 7/SAN RAFAEL

All chemical analyses are given as dry weight basis unless noted.

	San Rafael A	San Rafael B	Detection Limit
Grain size (%)			
Gravel	0.3	0.0	
Sand	14.1	17.6	
Silt	41.5	37.2	
Clay	44.2	45.1	
Solids (%)	45.7	39.6	
Sulfides (mg/kg)			
Total	202	321	
Water Soluble	<0.2	<0.3	0.1
Total Organic Carbon (%)		0.996	1.64
TRPH (mg/kg)	12.9	3.5	
Organotins (µg/kg)			
Tributyltin	<2.2	<2.5	1.0
Dibutyltin	<2.2	<2.5	1.0
Monobutyltin	<2.2	<2.5	1.0
Metals (mg/kg)			
Arsenic (As)	10.3	11.2	
Cadmium (Cd)	0.208	0.258	
Chromium (Cr)	62.6	75.8	
Copper (Cu)	41.6	53.5	
Lead (Pb)	15.9	20.5	
Mercury (Hg)	0.324	0.369	
Nickel (Ni)	59.5	74.2	
Selenium (Se)	0.691	0.662	
Silver (Ag)	0.278	0.429	0.08
Zinc (Zn)	77.5	98.5	
Pesticides and PCBs (mg/kg)			
4,4' - DDD	ND	ND	2
4,4' - DDE	ND	ND	2
4,4' - DDT	ND	ND	2
Aldrin	ND	ND	2
alpha-BHC	ND	ND	2
beta BHC	ND	ND	2
Chlordane	ND	ND	25
Delta-BHC	ND	ND	2
Dieldrin	ND	ND	2
Endosulfan I	ND	ND	2
Endosulfan II	ND	ND	2
Endosulfan Sulfate	ND	ND	25
Endrin	ND	ND	2
Endrin Aldehyde	ND	ND	10
Heptachlor	ND	ND	2
Heptachlor Epoxide	ND	ND	10
Lindane	ND	ND	2
Toxaphene	ND	ND	25
PCB 1016	ND	ND	20
PCB 1221	ND	ND	20
PCB 1232	ND	ND	20
PCB 1242	ND	ND	20
PCB 1248	ND	ND	20
PCB 1254	ND	ND	20
PCB 1260	ND	ND	20
PAHs (µg/kg)			
Naphthalene	ND	ND	20
Acenaphthylene	81.0	ND	20
Acenaphthene	ND	ND	20
Fluorene	ND	ND	20
Phenanthrene	359	ND	20
Anthracene	149	ND	20
Fluoranthene	689	422	20
Pyrene	930	601	20
Benzo (A) Anthracene	387	222	20
Chrysene	438	ND	20
Benzo (B) Fluoranthene	ND	ND	20
Benzo (K) Fluoranthene	ND	ND	20
Benzo (A) Pyrene	597	ND	20
Dibenzo (A,H) Anthracene	ND	ND	20
Ideno (1,2,3-CD) Pyrene	ND	ND	20
Benzo (G,H,I) Perylene	ND	ND	20
Total	3630	1245	
Phthalate Esters (µg/kg)			
Bis (2-ethylhexyl) Phthalate	289	198	50
Butylbenzyl Phthalate	ND	ND	8.5
Di-n-butyl Phthalate	219	118	9
Diethyl Phthalate	ND	ND	12
Dimethyl Phthalate	ND	ND	7.3
Di-n-octyl Phthalate	56.5	ND	75
Total	564.5	316	

Table A-8
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 8/NAVAL FACILITIES INDUSTRIAL SUPPLY CENTER

All chemical analyses are given as dry weight basis unless noted.

	NFISC 1-1	NFISC 1-2	NFISC 1-3	NFISC 1-5	NFISC 1-6	NFISC 1-9	Detection Limit
Grain size (%)Grain size (%)							
Gravel	13.4	1.6	2.0	0.3	0.0	5.3	
Sand	41.1	28.1	66.3	45.5	35.2	30.2	
Silt	13.4	22.7	9.5	17.3	16.7	22.3	
Clay	32.1	47.7	22.2	36.8	48.1	42.2	
Solids (%)	50.4	46.7	63.1	50.8	46.8	51.1	0.1
Volatile Solids (%)	2.76	3.09	2.74	2.69	3.00	3.46	
Sulfides (mg/kg)							
Total	212	266	190	238	271	243	0.1
Water Soluble	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.1
Total Organic Carbon (%)	0.998	1.34	0.682	0.87	1.16	1.13	
TRPH (mg/kg)	81.7	255	149	39.6	35.5	123	1.0
Organotins (µg/kg)							
Tributyltin	<2.0	<2.1	<1.6	<2.0	<2.1	5.5	1.0
Dibutyltin	3.8	<2.1	2.4	3.7	<2.1	3.7	1.0
Monobutyltin	<2.0	<2.1	<1.6	<2.0	<2.1	<2.0	1.0
Tetrabutyltin	<2.0	<2.1	<1.6	<2.0	<2.1	<2.0	1.0
Metals (mg/kg)							
Arsenic (As)	6.79	9.08	4.03	7.17	7.82	0.689	0.1
Cadmium (Cd)	0.361	0.724	0.320	0.191	0.387	0.354	0.1
Chromium (Cr)	52.4	66.8	41.5	53.1	68.8	64.6	0.1
Copper (Cu)	62.3	69.0	33.3	39.4	55.6	59.5	0.1
Lead (Pb)	26.4	39.6	28.7	21.5	34.6	33.7	0.1
Mercury (Hg)	0.345	0.702	0.228	0.366	0.444	0.325	0.2
Nickel (Ni)	75.0	62.1	35.2	47.6	61.1	58.3	0.1
Selenium (Se)	<1.59	<0.857	<0.634	<1.57	<1.71	<1.57	0.1
Silver (Ag)	0.333	0.857	0.716	0.260	0.526	0.552	0.1
Zinc (Zn)	121	202	67.2	76.0	109	119	0.1
Pesticides and PCBs (µg/kg)							
4,4' - DDD	ND	ND	13.6	ND	ND	ND	2
4,4' - DDE	ND	ND	ND	ND	ND	ND	2
4,4' - DDT	ND	ND	ND	ND	ND	ND	2
Aldrin	ND	ND	ND	ND	ND	ND	2
alpha-BHC	ND	ND	ND	ND	ND	ND	2
beta BHC	ND	ND	ND	ND	ND	ND	2
Chlordane	ND	ND	ND	ND	ND	ND	25
Delta-BHC	ND	ND	ND	ND	ND	ND	2
Dieldrin	ND	ND	ND	ND	ND	ND	2
Endosulfan I	ND	ND	ND	ND	ND	ND	2
Endosulfan II	ND	ND	ND	ND	ND	ND	2
Endosulfan Sulfate	ND	ND	ND	ND	ND	ND	25
Endrin	ND	ND	ND	ND	ND	ND	2
Endrin Aldehyde	ND	ND	ND	ND	ND	ND	10
Heptachlor	ND	ND	ND	ND	ND	ND	2
Heptachlor Epoxide	ND	ND	ND	ND	ND	ND	10
Lindane	ND	ND	ND	ND	ND	ND	2

Table A-8 (continued)
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 8/NAVAL FACILITIES INDUSTRIAL SUPPLY CENTER

All chemical analyses are given as dry weight basis unless noted.

	NFISC 1-1	NFISC 1-2	NFISC 1-3	NFISC 1-5	NFISC 1-6	NFISC 1-9	Detection Limit
Methoxychlor	ND	ND	ND	ND	ND	ND	25
Toxaphene	ND	ND	ND	ND	ND	ND	20
PCB Arochlor 1016	ND	ND	ND	ND	ND	ND	20
PCB Arochlor 1221	ND	ND	ND	ND	ND	ND	20
PCB Arochlor 1232	ND	ND	ND	ND	ND	ND	20
PCB Arochlor 1242	ND	ND	ND	ND	ND	ND	20
PCB Arochlor 1248	ND	ND	ND	ND	ND	ND	20
PCB Arochlor 1254	ND	ND	44.4	ND	ND	ND	20
PCB Arochlor 1260	ND	ND	ND	ND	ND	ND	20
Phenols (µg/kg)							
4-chloro-3-methylphenol	ND	ND	ND	ND	ND	ND	
2-chlorophenol	ND	ND	ND	ND	ND	ND	
2,4-dichlorophenol	ND	ND	ND	ND	ND	ND	
2,4-dimethylphenol	ND	ND	ND	ND	ND	ND	
2,4-dinitrophenol	ND	ND	ND	ND	ND	ND	
2-methyl-4,6-dinitrophenol	ND	ND	ND	ND	ND	ND	
2-nitrophenol	ND	ND	ND	ND	ND	ND	
4-nitrophenol	ND	ND	ND	ND	ND	ND	
Pentachlorophenol	ND	ND	ND	ND	ND	ND	
Phenol	ND	ND	ND	ND	ND	ND	
2,4,6-trichlorophenol	ND	ND	ND	ND	ND	ND	
PAHs (µg/kg)							
Naphthalene	ND	ND	ND	ND	ND	ND	
Acenaphthylene	182	ND	ND	ND	ND	86.5	20
Acenaphthene	159	2420	ND	ND	ND	185	20
Fluorene	128	2240	ND	ND	ND	121	20
Phenanthrene	449	11 800	1 980	39.6	76.7	568	20
Anthracene	321	1 500	515	ND	64.5	247	20
Fluoranthene	2 670	9 190	6 590	98.2	141	1630	20
Pyrene	3 110	7 750	6 700	173	246	1810	20
Benzo (A) Anthracene	901	1 510	903	ND	104	507	20
Chrysene	1 090	2 330	2 440	94.9	363	642	20
Benzo (B) Fluoranthene	1 590	ND	ND	ND	276	738	20
Benzo (K) Fluoranthene	2 260	ND	ND	ND	ND	1290	20
Benzo (A) Pyrene	798	1 100	1 090	156	188	423	20
Dibenzo (A,H) Anthracene	ND	ND	ND	ND	ND	69.1	20
Ideno (1,2,3-CD) Pyrene	ND	ND	ND	92.5	ND	ND	20
Benzo (G,H,I) Perylene	ND	ND	ND	132	141	ND	20
Total (PAH)	13 658	39 840	20 218	786.2	1 600.2	8 316.6	
Phthalate Esters (µg/kg)							
Bis (2-ethylhexyl) Phthalate	1 760	1 280	ND	470	389	348	50.0
Butylbenzyl Phthalate	35.1	ND	ND	ND	ND	ND	8.5
Di-n-butyl Phthalate	ND	ND	ND	ND	ND	ND	9.0
Diethyl Phthalate	ND	ND	ND	ND	ND	ND	12.0
Dimethyl Phthalate	ND	ND	ND	ND	ND	ND	7.3
Di-n-octyl Phthalate	ND	ND	ND	ND	ND	ND	75.0
Total	1 795.1	1 280	0	470	389	348	

Table A-9
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 9/UNOCAL (RICHMOND)

All chemical analyses are given as dry weight basis unless noted.

	UNOCAL	Detection Limit
Grain size (%)		
Gravel	0.4	
Sand	8.8	
Silt	31.8	
Clay	59.0	
Solids (%)	44.4	
Sulfides (mg/kg)		
Total	313	0.1
Water Soluble	<0.2	0.1
Total Organic Carbon (%)	0.14	
TRPH (mg/kg)	6.5	1.0
Organotins (µg/kg)		
Tributyltin	<2.3	1.0
Dibutyltin	<2.3	1.0
Monobutyltin	<2.3	1.0
Metals (mg/kg)		
Arsenic (As)	10.5	
Cadmium (Cd)	<0.090	0.04
Chromium (Cr)	78.8	
Copper (Cu)	57.7	
Lead (Pb)	39.2	
Mercury (Hg)	0.455	
Nickel (Ni)	74.3	
Selenium (Se)	<1.13	0.5
Silver (Ag)	0.270	
Zinc (Zn)	137	
Pesticides and PCBs (µg/kg)		
4,4' - DDD	5.27	2
4,4' - DDE	ND	2
4,4' - DDT	ND	2
Aldrin	ND	2
alpha-BHC	ND	2
beta BHC	ND	2
Chlordane	ND	25
Delta-BHC	ND	2
Dieldrin	ND	2
Endosulfan I	ND	2
Endosulfan II	ND	2
Endosulfan Sulfate	ND	25

	UNOCAL	Detection Limit
Endrin	ND	2
Endrin Aldehyde	ND	10
Heptachlor	ND	2
Heptachlor Epoxide	ND	10
Lindane	ND	2
Toxaphene	ND	25
PCB 1016	ND	20
PCB 1221	ND	20
PCB 1232	ND	20
PCB 1242	ND	20
PCB 1248	ND	20
PCB 1254	ND	20
PCB 1260	ND	20
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	ND	20
Pyrene	ND	20
Benzo (A) Anthracene	ND	20
Chrysene	ND	20
Benzo (B) Fluoranthene	ND	20
Benzo (K) Fluoranthene	ND	20
Benzo (A) Pyrene	ND	20
Dibenzo (A,H) Anthracene	ND	20
Ideno (1,2,3-CD) Pyrene	ND	20
Benzo (G,H,I) Perylene	ND	20
Total		
Phthalate Esters (µg/kg)		
Bis (2-ethylhexyl) Phthalate	345	50
Butylbenzyl Phthalate	ND	8.5
Di-n-butyl Phthalate	ND	9
Diethyl Phthalate	ND	12
Dimethyl Phthalate	ND	7.3
Di-n-octyl Phthalate	ND	75
Total	345	

Table A-10
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 10/ALCATRAZ

All chemical analyses are given as dry weight basis unless noted.

	Alcatraz	Detection Limit
Grain size (%)		
Gravel	5.5	
Sand	91.1	
Silt	0.7	
Clay	2.7	
Solids (%)	84.3	
Total Organic Carbon (mg/kg)	0.19	
Sulfides (mg/kg)		
Total	1.0	
Water Soluble	ND	
Organotins (µg/kg)		
Tributyltin	ND	1.0
Dibutyltin	ND	1.0
Monobutyltin	ND	1.0
TRPH (mg/kg)	ND	
Metals (mg/kg)		
Arsenic (As)	5.3	0.2
Cadmium (Cd)	0.03	0.01
Chromium (Cr)	15.9	2
Copper (Cu)	3.8	1
Lead (Pb)	6.4	0.01
Mercury (Hg)	0.02	0.02
Nickel (Ni)	22.6	5
Selenium (Se)	ND	0.5
Silver (Ag)	ND	0.01
Zinc (Zn)	18.7	2
Pesticides and PCBs (mg/kg)		
alpha-BHC	ND	0.002
gamma-BHC	ND	0.002
beta BHC	ND	0.006
Heptachlor	ND	0.002
delta BHC	ND	0.002
Aldrin	ND	0.002
Heptachlor Epoxide	ND	0.002
Endosulfan I	ND	0.002
4,4' - DDE	ND	0.002
Dieldrin	ND	0.002
Endrin	ND	0.002

	Alcatraz	Detection Limit
4,4' - DDD	ND	0.002
Endosulfan II	ND	0.002
4,4' - DDT	ND	0.002
Endrin Aldehyde	ND	0.002
Endosulfan Sulfate	ND	0.002
Methoxychlor	ND	0.004
Toxaphene	ND	0.03
Chlorodane	ND	0.01
PCB 1016	ND	0.01
PCB 1221	ND	0.01
PCB 1232	ND	0.01
PCB 1242	ND	0.01
PCB 1248	ND	0.01
PCB 1254	ND	0.01
PCB 1260	ND	0.01
PAHs (µg/kg)		
Naphthalene	29	10
2-Methylnaphthalene	ND	10
Acenaphthylene	ND	10
Dibenzofuran	ND	10
Acenaphthene	ND	10
Fluorene	ND	10
Phenanthrene	18	10
Anthracene	ND	10
Fluoranthene	21	10
Pyrene	32	10
Benz (A) Anthracene	ND	10
Chrysene	ND	10
Benzo (B+K) Fluoranthene	15	10
Benzo (A) Pyrene	10	10
Ideno (1,2,3-CD) Pyrene	ND	10
Dibenz (AH) Anthracene	ND	10
Benzo (G,H,I) Perylene	52	10
Total	177	
Phthalate Esters (µg/kg)		
Dimethyl Phthalate	ND	20
Diethyl Phthalate	ND	20
Di-n-butyl Phthalate	ND	20
Butylbenzyl Phthalate	ND	20
Bis (2-ethylhexyl) Phthalate	52	20
Di-n-octyl Phthalate	ND	20

Table A-11
RESULTS FROM GRAIN SIZE ANALYSIS, SAMPLE SITE 11/TREASURE ISLAND

Site	Percent Sand	Percent Silt	Percent Clay
Control	12.211	38.699	49.090
Treasure Island	8.757	47.272	43.971

Table A-12
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 12/PORT OF OAKLAND

All chemical analyses are given as dry weight basis unless noted.

	Port of Oakland	Detection Limit
Grain size (%)		
Gravel	0.0	
Sand	8.0	
Silt	40.2	
Clay	51.7	
Solids (%)	44	
Total Organic Carbon (mg/kg)	0.19	
Sulfides (mg/kg)		
Total	310	
Water Soluble	18	
Organotins (µg/kg)		
Tributyltin	12	1.0
Dibutyltin	ND	1.0
Monobutyltin	16	1.0
TRPH (mg/kg)	139	
Metals (mg/kg)		
Arsenic (As)	9.6	0.2
Cadmium (Cd)	0.18	0.01
Chromium (Cr)	64.5	2
Copper (Cu)	41	1
Lead (Pb)	33.6	0.01
Mercury (Hg)	0.35	0.02
Nickel (Ni)	66.8	5
Selenium (Se)	1.9	0.5
Silver (Ag)	0.29	0.01
Zinc (Zn)	98.7	2
Pesticides and PCBs (mg/kg)		
alpha-BHC	ND	0.002
gamma-BHC	ND	0.002
beta BHC	ND	0.006
Heptachlor	ND	0.002
delta BHC	ND	0.002
Aldrin	ND	0.002
Heptachlor Epoxide	ND	0.002
Endosulfan I	ND	0.002
4,4' - DDE	ND	0.002
Dieldrin	ND	0.002
Endrin	ND	0.002
4,4' - DDD	0.002	0.002

	Port of Oakland	Detection Limit
Endosulfan II	ND	0.002
4,4' - DDT	<0.003	0.002
Endrin Aldehyde	ND	0.002
Endosulfan Sulfate	ND	0.002
Methoxychlor	ND	0.004
Toxaphene	<0.09	0.03
Chlorodane	ND	0.01
PCB 1016	ND	0.01
PCB 1221	ND	0.01
PCB 1232	ND	0.01
PCB 1242	ND	0.01
PCB 1248	ND	0.01
PCB 1254	ND	0.01
PCB 1260	0.01	0.01
PAHs (µg/kg)		
Naphthalene	24	10
2-Methylnaphthalene	ND	10
Acenaphthylene	13	10
Dibenzofuran	ND	10
Acenaphthene	ND	10
Fluorene	ND	10
Phenanthrene	81	10
Anthracene	25	10
Fluoranthene	180	10
Pyrene	240	10
Benz (A) Anthracene	68	10
Chrysene	72	10
Benzo (B+K) Fluoranthene	15	10
Benzo (A) Pyrene	130	10
Ideno (1,2,3-CD) Pyrene	ND	10
Dibenz (AH) Anthracene	ND	10
Benzo (G,H,I) Perylene	110	10
Total	1234	
Phthalate Esters (µg/kg)		
Dimethyl Phthalate	ND	20
Diethyl Phthalate	ND	20
Di-n-butyl Phthalate	ND	20
Butylbenzyl Phthalate	ND	20
Bis (2-ethylhexyl) Phthalate	52	20
Di-n-octyl Phthalate	ND	20

Table A-13
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 13/SAN FRANCISCO DRY DOCKS A, B, D, AND CONTROL SEDIMENT

All chemical analyses are given as dry weight basis unless noted.

	Dry Dock Site A	Dry Dock Site B	Dry Dock Site D	Control Sediment	Detection Limit
Grain size (%)					
Gravel	0.0	0.2	1.0	0.0	
Sand	4.1	5.3	7.5	7.9	
Silt	42.7	40.7	39.9	43.6	
Clay	53.2	53.9	51.6	48.5	
Solids (%)		43.7	42.3	46.9	40.9
Total Organic Carbon (%)		1.44	1.46	1.42	1.38
Sulfides (mg/kg)					
Total	270	250	320	1.5	
Water Soluble	ND	ND	ND	ND	
Organotins (µg/kg)					
Tributyltin	16.0	27.1	31.8	ND	1.0
Dibutyltin	<2.2	6.01	8.65	ND	1.0
Monobutyltin	<2.2	<2.4	<2.1	12	1.0
TRPH (mg/kg)	225	195	210	ND	
Metals (mg/kg)					
Arsenic (As)	9.1	8.2	10.0	9.2	0.2
Cadmium (Cd)	0.20	0.18	0.27	0.19	0.01
Chromium (Cr)	77.5	76.1	89.0	91	2
Copper (Cu)	50.0	45.0	68.7	58	1
Lead (Pb)	31.7	29.0	37.6	26.9	0.01
Mercury (Hg)	0.32	0.38	0.39	0.37	0.02
Nickel (Ni)	74.4	69.3	81.3	93	5
Selenium (Se)	ND	ND	ND	ND	0.5
Silver (Ag)	0.37	0.37	0.44	0.26	0.01
Zinc (Zn)	105	95.9	114	126	2
Pesticides and PCBs (µg/kg)					
alpha-BHC	ND	ND	<0.004	ND	0.002
gamma-BHC	ND	ND	<0.004	ND	0.002
beta BHC	ND	ND	ND	ND	0.006
Heptachlor	ND	ND	<0.004	ND	0.002
delta BHC	ND	ND	<0.004	ND	0.002
Aldrin	ND	ND	<0.004	ND	0.002
Heptachlor Epoxide	ND	ND	<0.004	ND	0.002
Endosulfan I	ND	ND	<0.004	ND	0.002
4,4' - DDE	ND	ND	0.004	ND	0.002
Dieldrin	ND	ND	<0.004	ND	0.002
Endrin	ND	ND	<0.004	ND	0.002
4,4' - DDD	ND	0.002	0.008	ND	0.002
Endosulfan II	ND	ND	<0.006	ND	0.002
4,4' - DDT	ND	ND	<0.02	ND	0.002

Table A-13 (continued)
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 13/SAN FRANCISCO DRY DOCKS A, B, D, AND CONTROL SEDIMENT

All chemical analyses are given as dry weight basis unless noted.

	Dry Dock Site A	Dry Dock Site B	Dry Dock Site D	Control Sediment	Detection Limit
Endrin Aldehyde	ND	ND	<0.004	ND	0.002
Endosulfan Sulfate	ND	ND	<0.004	ND	0.002
Methoxychlor	ND	ND	<0.04	ND	0.004
Toxaphene	<0.05	<0.05	<0.05	<0.1	0.03
Chlorodane	<0.02	<0.02	<0.02	<0.4	0.01
PCB 1016	<0.02	<0.02	ND	<0.2	0.01
PCB 1221	<0.02	<0.02	ND	<0.2	0.01
PCB 1232	<0.02	<0.02	ND	<0.2	0.01
PCB 1242	<0.02	<0.02	ND	<0.2	0.01
PCB 1248	<0.02	<0.02	ND	<0.2	0.01
PCB 1254	<0.02	<0.02	0.06	<0.2	0.01
PCB 1260	<0.02	<0.02	ND	<0.2	0.01
PAHs (µg/kg)					
Naphthalene	ND	ND	554	ND	20
Acenaphthylene	ND	ND	4160	ND	20
Acenaphthene	ND	ND	3690	ND	20
Fluorene	ND	ND	3070	ND	20
Phenanthrene	99.8	64.1	30300	92	20
Anthracene	77.6	ND	12900	ND	20
Fluoranthene	288	188	19400	270	20
Pyrene	310	224	21900	350	20
Benz (A) Anthracene	ND	ND	10600	140	20
Chrysene	201	141	12000	110	20
Benzo (B) Fluoranthene	149	102	5580	250	20
Benzo (K) Fluoranthene	106	77.9	4120	ND	20
Benzo (A) Pyrene	192	128	8800	180	20
Dibenz (AH) Anthracene	ND	ND	1440	ND	20
Ideno (1,2,3-CD) Pyrene	104	ND	3160	140	20
Benzo (G,H,I) Perylene	142	ND	3430	120	20
Total	1,669	925	145,104	1,652	
Phthalate Esters (µg/kg)					
Dimethyl Phthalate	ND	ND	ND	ND	20
Diethyl Phthalate	ND	ND	ND	ND	20
Di-n-butyl Phthalate	ND	ND	ND	ND	20
Butylbenzyl Phthalate	85	91	84	43	20
Bis (2-ethylhexyl) Phthalate	97	130	120	210	20
Di-n-octyl Phthalate	ND	ND	ND	ND	20

Table A-14
RESULTS FROM SEDIMENT ANALYSIS, SAMPLE SITE 14/OYSTER POINT (SAN MATEO)

All chemical analyses are given as dry weight basis unless noted.

	Oyster Point	Detection Limit
Grain size (%)		
Gravel	0	
Sand	0.684	
Silt	36.936	
Clay	62.379	
Solids (%)	42.2	
Total Organic Carbon (%)	0.206	
Sulfides (mg/kg)		
Total	450	
Water Soluble	<0.2	
Organotins (µg/kg)		
Tributyltin	<2.4	1.0
Dibutyltin	10.9	1.0
Monobutyltin	<2.4	1.0
TRPH (mg/kg)	<23.7	
Metals (mg/kg)		
Arsenic (As)	10.8	
Cadmium (Cd)	<0.38	<0.3
Chromium (Cr)	102	
Copper (Cu)	67.1	
Lead (Pb)	48.8	
Mercury (Hg)	0.308	<0.02
Nickel (Ni)	86.7	
Selenium (Se)	0.379	<0.1
Silver (Ag)	<0.19	
Zinc (Zn)	140	
Pesticides and PCBs (µg/kg)		
alpha-BHC	ND	2
beta BHC	ND	2
Heptachlor	ND	2
delta BHC	ND	2
Aldrin	ND	2
Heptachlor Epoxide	ND	10
Endosulfan I	ND	10
4,4' - DDE	ND	2
Dieldrin	ND	2
Endrin	ND	2
4,4' - DDD	ND	2
Endosulfan II	ND	2
4,4' - DDT	ND	2
Endrin Aldehyde	ND	10
Endosulfan Sulfate	ND	25
Lindane	ND	2
Toxaphene	ND	25
Chlordane	ND	25
PCB 1016	ND	20
PCB 1221	ND	20
PCB 1232	ND	20
PCB 1242	ND	20
PCB 1248	ND	20
PCB 1254	ND	20
PCB 1260	ND	20
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	93.1	20
Pyrene	144	20
Benzo Anthracene	ND	20
Chrysene	74.2	20
Benzo (B) Fluoranthene	84.4	20
Benzo (K) Fluoranthene	52.9	20
Benzo (A) Pyrene	ND	20
Ideno (1,2,3-CD) Pyrene	55.4	20
Dibenzo (AH) Anthracene	ND	20
Benzo (G,H,I) Perylene	ND	20
Total	311.3	
Phthalate Esters (µg/kg)		
Dimethyl Phthalate	ND	7.3
Diethyl Phthalate	ND	12
Di-n-butyl Phthalate	75.8	9
Butylbenzyl Phthalate	ND	8.5
Bis (2-ethylhexyl) Phthalate	84.1	50
Di-n-octyl Phthalate	ND	75.0

Table A-15
RESULTS FROM SEDIMENT ANALYSIS, CONTROL SEDIMENT
(From sampling site of *A. abdita*)

All chemical analyses are given as dry weight basis unless noted.

	Control	Detection Limit
Grain size (%)		
Gravel	2.1	
Sand	8.8	
Silt	27.5	
Clay	35.1	
Solids (%)	56.8	
Total Organic Carbon (%)	0.88	
Sulfides (mg/kg)		
Total	185	0.1
Water Soluble	<0.2	0.1
Organotins (µg/kg)		
Tributyltin	<1.8	1.0
Dibutyltin	<1.8	1.0
Monobutyltin	<1.8	1.0
TRPH (mg/kg)	10.4	
Metals (mg/kg)		
Arsenic (As)	10.4	0.2
Cadmium (Cd)	<0.07	0.01
Chromium (Cr)	61.3	2
Copper (Cu)	39.8	1
Lead (Pb)	25.4	0.01
Mercury (Hg)	0.349	0.02
Nickel (Ni)	66.9	5
Selenium (Se)	<0.88	0.5
Silver (Ag)	0.25	0.01
Zinc (Zn)	96.8	2
Pesticides and PCBs (µg/kg)		
alpha-BHC	ND	0.002
gamma-BHC	ND	0.002
beta BHC	ND	0.006
Heptachlor	ND	0.002
delta BHC	ND	0.002
Aldrin	ND	0.002
Heptachlor Epoxide	ND	0.002
Endosulfan I	ND	0.002
4,4' - DDE	ND	0.002
Dieldrin	ND	0.002
Endrin	ND	0.002
4,4' - DDD	ND	0.002

	Control	Detection Limit
Endosulfan II	ND	0.002
4,4' - DDT	ND	0.002
Endrin Aldehyde	ND	0.002
Endosulfan Sulfate	ND	0.002
Methoxychlor	ND	0.004
Toxaphene	ND	0.03
Chlorodane	ND	0.01
PCB 1016	ND	0.01
PCB 1221	ND	0.01
PCB 1232	ND	0.01
PCB 1242	ND	0.01
PCB 1248	ND	0.01
PCB 1254	ND	0.01
PCB 1260	ND	0.01
PAHs (µg/kg)		
Naphthalene	ND	20
Acenaphthylene	ND	20
Acenaphthene	ND	20
Fluorene	ND	20
Phenanthrene	ND	20
Anthracene	ND	20
Fluoranthene	82.7	20
Pyrene	118	20
Benz (A) Anthracene	ND	20
Chrysene	ND	20
Benzo (B) Fluoranthene	ND	20
Benzo (K) Fluoranthene	ND	20
Benzo (A) Pyrene	ND	20
Dibenz (AH) Anthracene	ND	20
Ideno (1,2,3-CD) Pyrene	ND	20
Benzo (G,H,I) Perylene	ND	20
Total	200.7	
Phthalate Esters (µg/kg)		
Dimethyl Phthalate	282	20
Diethyl Phthalate	ND	20
Di-n-butyl Phthalate	ND	20
Butylbenzyl Phthalate	ND	20
Bis (2-ethylhexyl) Phthalate	ND	20
Di-n-octyl Phthalate	ND	20

Table A-16
ANOVA (a = 0.05) RESULTS FROM 24-HOUR EXPERIMENTS

Species	Stressor	Stress Protein	ANOVA result
H. azteca	cadmium	hsp 58 (1)	$F_{4,23} = 10.7$; $p<0.0002$
		hsp 64 (1)	$F_{4,23} = 27.33$; $p<0.0001$
		hsp 58 (2)	$F_{4,23} = 4.95$; $p<0.01$
		hsp 64 (2)	$F_{4,23} = 5.45$; $p<0.0066$
		hsp 71 (2)	$F_{4,23} = 5.45$; $p<0.0066$
	diazinon	hsp 58 (1)	$F_{4,23} = 15.54$; $p<0.0001$
		hsp 64 (1)	$F_{4,23} = 15.09$; $p<0.0001$
		hsp 58 (2)	$F_{4,23} = 15.57$; $p<0.0001$
		hsp 64 (2)	$F_{4,23} = 12.8$; $p<0.0001$
		hsp 71 (2)	$F_{4,23} = 68.2$; $p<0.0001$
	dieldrin	hsp 58 (1)	$F_{4,23} = 22.25$; $p<0.0001$
		hsp 64 (1)	$F_{4,23} = 11.20$; $p<0.0002$
		hsp 58 (2)	$F_{4,23} = 29.03$; $p<0.0001$
		hsp 64 (2)	$F_{4,23} = 10.88$; $p<0.0001$
		hsp 71 (2)	$F_{4,23} = 23.35$; $p<0.0001$
	fluoranthen	hsp 64 (2)	$F_{4,25} = 5.13$; $p<0.0037$
		hsp 71 (2)	$F_{4,25} = 11.23$; $p<0.0001$
	starvation	hsp 64 (1)	$F_{1,10} = 12.52$; $p<0.0054$
		hsp 64 (2)	$F_{1,10} = 15.25$; $p<0.0029$
		hsp 71 (2)	$F_{1,10} = 9.97$; $p<0.01$
	salinity	hsp 64 (2)	$F_{3,8} = 12.25$; $p<0.0023$
	35°C (24h)	hsp 64 (2)	$F_{1,4} = 12.01$; $p<0.0257$
A. abdita (adult)	cadmium	hsp 54 (2)	$F_{4,25} = 5.32$; $p<0.003$
		hsp 64 (2)	$F_{4,25} = 12.08$; $p<0.0001$
	diazinon	hsp 54 (2)	$F_{4,15} = 4.49$; $p<0.0139$
		hsp 64 (2)	$F_{4,15} = 11.08$; $p<0.0002$
	dieldrin	hsp 54 (2)	$F_{4,25} = 3.38$; $p<0.0242$
		hsp 64 (2)	$F_{4,25} = 1.97$; $p<0.1300$
	fluoranthen	hsp 54 (2)	$F_{4,25} = 3.61$; $p<0.0187$
		hsp 64 (2)	$F_{4,25} = 10.64$; $p<0.0001$
A. abdita (juvenile)	cadmium	hsp 54	$F_{4,25} = 22.39$; $p<0.0001$
		hsp 64	$F_{4,25} = 120.24$; $p<0.0001$
	diazinon	hsp 64	$F_{4,25} = 14.44$; $p<0.0001$
R. abronius	cadmium	hsp 60 (1)	$F_{4,25} = 4.78$; $p<0.0053$
	diazinon	hsp 54 (1)	$F_{4,25} = 2.84$; $p<0.045$
		hsp 60 (1)	$F_{4,25} = 9.14$; $p<0.0001$
		hsp 64 (1)	$F_{4,25} = 13.28$; $p<0.0001$
		hsp 71 (1)	$F_{4,25} = 18.58$; $p<0.0001$
		hsp 64 (2)	$F_{4,25} = 15.00$; $p<0.0001$
		hsp 71 (2)	$F_{4,25} = 35.64$; $p<0.0001$
	dieldrin	hsp 60 (1)	$F_{4,25} = 5.74$; $p<0.002$
	fluoranthen	hsp 60 (1)	$F_{4,25} = 2.73$; $p<0.05$
	starvation	hsp 54 (1)	$F_{1,10} = 18.92$; $p<0.0014$
		hsp 64 (2)	$F_{1,10} = 48.94$; $p<0.0001$
		hsp 71 (2)	$F_{1,10} = 30.42$; $p<0.0003$
	salinity	hsp 71 (2)	$F_{2,15} = 6.69$; $p<0.0084$

$F_{x,y} = z$; x = degrees of freedom (treatments);
y = degrees of freedom (samples);
z = F-value
(1) supernatant fraction
(2) pellet fraction