APPENDIX 6

BIOACCUMULATION PROGRAM

In 2005 the Bioaccumulation Program targeted, for selected priority pollutant analysis of tissues, two species common to the Palos Verdes Shelf. The white croaker (*Genyonemus lineatus*) is the most commonly taken fish in the local recreational fishery and has, in the past, been a target of a commercial gillnet fishery in the region. The Dover sole (*Microstomus pacificus*), while not taken in any Southern California fisheries, is a common member of the shelf-depth demersal fish community. However, Dover sole of the required target size were extremely rare on the Palos Verdes Shelf throughout 2005. Despite sampling efforts consistent with those required in the permit, the number collected was insufficient to allow analysis of any tissue sample for this species.

The tissues collected in this program were analyzed for the following priority pollutants as stipulated in the JWPCP NPDES monitoring program: total DDT, DDT derivatives, total PCBs, and PCB derivatives. Percent lipids and percent moisture were also determined.

CONTENTS

ANALYTICAL METHODS

EXTRACTION OF CHLORINATED PESTICIDES AND PCBS IN BIOLOGICAL SAMPLES

1. Scope and Application

This procedure is used to determine chlorinated pesticides (o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, and p,p'-DDT) and PCBs (Aroclors 1221, 1232, 1242, 1248, 1254, and 1260) in biological tissue samples. Other pesticides may also be determined by this procedure. Tissue types may include, but are not limited to, fish muscle tissue, sea urchin gonads, and fish livers.

2. Summary of Method

The biological tissue sample is mechanically extracted by homogenization with acetonitrile. The acetonitrile extract is extracted with diethyl ether in hexane in a separatory funnel. The diethyl ether in hexane extract is concentrated and cleaned-up using Florisil prior to gas chromatographic analysis.

3. Sample Handling and Preservation

3.1 Samples must be stored in glass containers with Teflon lined caps after dissection. An approved dissection protocol may be required or recommended.

3.2 Samples are frozen until extraction.

3.3 Sample extracts must be injected no later than 40 days after the extraction date.

4. Interferences

4.1 Common plastics contain varying amounts of easily extractable phthalates may cause interferences. Interferences by phthalate esters can pose a major problem when using the electron capture detector since they may obscure late eluting peaks in the chromatogram. Avoid contact of solvents and samples with plastics other than Teflon.

4.2 Other interferences attributed to unidentified organic compounds may arise. The affected sample extract may be colored and have either a rising baseline or many large unidentified peaks. This interference may be eliminated by treatment with sulfuric acid.

5. Apparatus

5.1 Separatory funnels, 500 mL with Teflon stopcocks and stoppers.

5.2 Beakers, 125 mL, 150 mL, 250 mL, and 400 mL Pyrex.

5.3 Chromatography columns, 400 mm long x 22 mm I.D., with Teflon stopcock, coarse frit (Kontes K-42054).

5.4 Chromatography columns, 300 mm x 22 mm I.D.

5.5 Centricones, 15 mL, graduated, with glass stoppers.

5.6 Tilter dispensers, 20 mL and50 mL.

5.7 J&L Scientific, solvent recovery and sample concentration apparatus, six position.

5.8 Brinkman Polytron blender (PT 10/35) with sawtooth blade (PTA 20 TS).

5.9 Kuderna-Danish concentrator apparatus:

5.9.1 Evaporative flask, Kuderna-Danish, 500 mL (Kontes K-570001-0500).
5.9.2 Concentrator tube, Kuderna-Danish, 10 mL (Kontes K-570050-1025).
5.9.3 Snyder column, Kuderna-Danish, three ball macro (Kontes K-503000-0121).

5.10 Oven, 130 EC.

5.11 Vials, glass, 7 mL with a Teflon lined cap.

6. Reagents

6.1 Hexane, pesticide grade.

6.2 Diethyl ether, pesticide grade.

6.3 Mixture of 6 % diethyl ether in hexane, volume/volume.

6.4 Mixture of 15 % diethyl ether in hexane, volume/volume.

6.5 Florisil, PR grade (Supelco) 60/100 mesh, stored at 130 EC until use. If Florisil other than P.R. grade is used, such as that from Baker, it is recommended that it be fired at 650 EC for 4 hrs and stored at 130 EC until use.

6.6 Sodium sulfate, anhydrous, granular, fired at 550 EC for 4 hrs and stored at 130 EC.

6.7 Deionized water, passed through a charcoal column (Fisher).

6.8 Saturated solution of sodium sulfate in deionized water.

6.9 Standards in acetonitrile:

6.9.1 Standard 8088 - containing alpha-BHC, beta-BHC, gamma-BHC (lindane), delta-BHC, heptachlor, aldrin, heptachlor epoxide B, heptachlor epoxide A, trans-chlordane, o,p'-DDE, endosulfan I, cis-chlordane, trans-nonachlor, oxychlordane, p,p'-DDE, dieldrin, o,p'-DDD, endrin, endosulfan II, p,p'-DDD, o,p'-DDT, endrin aldehyde, endrin ketone, endosulfan sulfate, p,p'-DDT, and methoxychlor in acetonitrile, all with nominal concentrations of 10 µg/L of each pesticide.

6.9.2 Standard 42/54 - containing Aroclors 1242 and 1254 in acetonitrile, at concentrations of 200 and 120 µg/L, respectively.

6.9.3 Fish spiking solution - containing o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, Aroclor 1242, Aroclor 1254, and Aroclor 1260 at the concentrations listed in the Table I. The relative concentrations of the standard components are intended to mirror the relative concentrations found in biological samples from the JWPCP ocean outfall.

6.10 Acetonitrile, pesticide grade.

7. Procedures

7.1. All glassware must be rinsed three times with 15 % diethyl ether in hexane prior to use.

7.2 The tissue sample is homogenized by stirring or chopping with a blade, where appropriate.

7.3 Weigh about 5 grams of the tissue sample into a 150 mL beaker and record the weight to the nearest 0.01 g. Lesser sample weights may also be used but may result in poor analytical precision.

7.4 Add 40 mL of acetonitrile to the beaker with the tilter dispenser just prior to homogenization. The tissue sample will harden if allowed to remain under the acetonitrile for too long, making the blending step difficult.

7.5 Homogenize the sample in acetonitrile with the Brinkman Polytron blender for about 1 min, completely grinding all of the tissue.

7.6 Rinse the blade of the Brinkman Polytron blender with another 40 mL portion of acetonitrile and add it to the sample beaker.

7.7 Prepare a blank by adding 80 mL of acetonitrile to a 150 mL beaker.

7.8 Prepare process standards by adding the standards of the components of interest in acetonitrile to 80 mL of acetonitrile in a 150 mL beaker.

7.9 Prepare a duplicate analysis and a spiked sample at a 10 % frequency or one for every batch. The spiking level should ideally be at least double the highest expected analyte concentration.

7.10 Transfer the sample extract to a 500 mL separatory funnel. Rinse the beaker with 50 mL of 15 % diethyl ether in hexane and add the rinse to the separatory funnel. Repeat this step three more times for a total of 200 mL of 15 % diethyl ether in hexane.

7.11 Add 50 mL of charcoal filtered water to each separatory funnel. Add 5 mL of saturated sodium sulfate solution to each separatory funnel to prevent emulsions from forming.

7.12 Shake each separatory funnel for 2 min and allow the phases to separate.

7.13 Drain and discard the lower layer in the separatory funnel. Add 25 mL of water to each separatory funnel and shake briefly, about 3 or 4 times, and allow for complete phase separation. Drain and discard the lower layer of each separatory funnel.

7.14 Transfer the extract in each separatory funnel to a Kuderna-Danish concentrator apparatus. Rinse the separatory funnel with 15 % diethyl ether in hexane and add the rinse to the Kuderna-Danish concentrator apparatus.

7.15 Concentrate the extract to about 10 mL using the J&L Scientific sample concentration apparatus.

7.16 Prepare a Florisil column. Add the predetermined amount of Florisil (reference 11.2) to the column, topped with 2 cm of anhydrous sodium sulfate. Rinse the column with 60 mL of hexane. Attach a clean Kuderna-Danish concentrator to the Florisil column. Close the stopcock on the Florisil column when the solvent reaches the sodium sulfate layer. Place a Kuderna-Danish flask with a concentrator tube beneath the Florisil column.

7.17 Load the sample extract on the Florisil column. Rinse the concentrator tube and add the rinse to the column. Elute the column with 100 mL of 6 % diethyl ether in hexane followed by 100 mL of 15 % diethyl ether in hexane, followed by 100 mL of diethyl ether.

7.18 Concentrate the combined Florisil column eluates to 7-10 mL in the Kuderna-Danish concentrator using the J&L Scientific sample concentration apparatus. Remove the Kuderna-Danish flask from the concentrator tube. Rinse the ground glass joint of the flask into the concentrator tube with a small amount of hexane.

7.19 Quantitatively transfer the concentrated extract, with successive rinses, to a 10 mL centricone. The final volume of the extract should be 10 mL.

7.20 The concentrated extract is treated with concentrated sulfuric acid to remove interfering substances:

7.20.1 In a small vial (7 mL), add up to 3 mL of sample extract and 3 mL of concentrated sulfuric acid. Shake vigorously for 30 seconds. Allow the layers to separate.

7.20.2 If the color of the acid phase is light yellow or light green, proceed with the water rinse (section 7.21.3). If the color is dark yellow or orange, perform additional sulfuric acid treatments (section 7.21.1) until a light yellow or light green color is observed.

7.20.3 Transfer the hexane layer to another vial; add 3 mL of water and shake vigorously. Let the layers separate, then pipette the hexane layer into another vial for gas chromatographic analysis.

8. Calculations

The sample extraction personnel provide the gas chromatographic analyst with sample weights and the final volumes of the extracts to be entered into the data system.

9. Quality Assurance Guidelines

9.1 Each sample set of ten samples or less contains one process blank.

9.2 Each sample set of ten samples or less contains two process standards (made from the 8088 and 42/54 standard spiking solutions).

9.3 Each sample set of ten samples or less contains a duplicate sample analysis. Additionally, a third replicate sample is spiked with the fish spiking solution.

9.4 Each sample set of ten samples or less contains a sample which is spiked with the fish spiking solution.

10. Method Performance

Precision and accuracy data for process standard recovery, duplicate percent deviation, and spike percent recovery are tracked by control charting. Control limits are revised periodically.

11. References

11.1 USEPA Method 608, "Organochlorine Pesticides and PCBs," in the Federal Register (40 CFR Part 136) Vol. 49, No. 209, Oct. 26, 1984, Rules and Regulations, pp. 43321 - 43336.

11.2 Mills, P.A. “Variation of Florisil Activity: Simple Method for Measuring Absorbent Capacity and Its Use in Standardizing Florisil Columns,” Journal of the Association of Official Analytical Chemists, 51, 29, (1968).

TABLE 1
Fish Spiking Solution, in acetonitrile.

Component	Concentration (ug/L)
o,p’-DDE	550
p,p’-DDE	10,000
o,p’-DDD	100
p,p’-DDD	250
o,p’-DDT	100
p,p’-DDT	100
Aroclor 1242	750
Aroclor 1254	1500
Aroclor 1260	1000

GRAVIMETRIC DETERMINATION OF LIPIDS IN BIOLOGICAL TISSUE SAMPLES

1. Scope and Application

    1.1 This procedure is applicable to the extraction of lipids in biological samples under CSDLAC test code 411.

2. Summary of Procedure

    2.1 The samples are weighed out and covered with a mixture of water, methanol, and chloroform.

    2.2 The mixture is blended and the lipids are partitioned into the chloroform layer.

    2.3 A portion of the chloroform layer is removed and evaporated to dryness.

    2.4 The residue is desiccated, weighed to determine the lipid content and reported as total lipids.

3. Sample Handling and Preservation

    3.1 The samples should be stored at 4EC until analyzed or kept frozen if they can not be analyzed immediately.

4. Interferences

    4.1 Contaminants/residues introduced to glassware after initial tares may cause false high results.

5. Apparatus

    5.1 Homogenizer, Brinkman Polytron, Model PT-20, with sawtooth head PT-20ST or Brinkman Polytron, Model PT-10/35 with PTA2OTS generator.

    5.2 Beakers, 150 mL.

    5.3 Bottles, centrifuge 250 mL glass.

    5.4 Tilter dispensers, 10 mL, 20 mL, 8 mL.

    5.5 Pasteur pipettes, 9 inches or Mohr-type pipets, 10 mL.

    5.6 Mill, analytical (Tekmar A-10).

5.7 Balance, analytical (weigh to 0.0001 g or 0.001 g).

5.8 Centricones with ground glass stoppers, graduated, 10 mL or 12 mL.

5.9 Balance, top loading.

5.10 Centrifuge, must be rated to spin 250 mL bottles at 10,000 rpm.

5.11 Infrared heat lamps (Fisher Infra Rediator).

5.12 Melting point capillary tubes, 150 mm x 1.1-1.2 mm.

5.13 Solvent evaporating manifold.

6. Reagents

6.1 Chloroform, reagent grade.

6.2 Methanol, reagent grade.

6.3 Water, Nanopure II or equivalent.

7. Procedure

7.1 Grind the fish tissue, if necessary, with a microanalytical mill to obtain a representative sample.

7.2 Weigh out approximately 1-2 grams into a 150 mL beaker using a top loading balance.

7.3 Add 10 mL chloroform, 20 mL methanol, and 8 mL water to the beaker with the sample. Homogenize the mixture for one minute with the Polytron blender.

7.6 Prepare a blank by adding the same volumes of solvent as that used for a sample.

7.5 Add an additional 10 ml chloroform to the mixture and blend again for 30 seconds at low speed.

7.6 Add an additional 10 mL water and blend briefly at low speed.

7.7 Load the mixture into a 250 mL centrifuge bottle and place in centrifuge head.

7.7.1 Do not rinse the sample beaker during the sample transfer.

7.8 Set the centrifuge speed at 10,000 rpm. Centrifuge for ten minutes.

7.8.1 Use refrigeration mode while centrifuging, if available.

7.9 Remove the bottle from the centrifuge head and remove half of the chloroform layer (10 mL).

7.9.1 The chloroform layer is the layer on the bottom. Be careful not to agitate the centrifuge bottle more than is necessary.

7.9.2 Place the 10 mL of chloroform in a 10 mL or 12 mL tared centricone. The centricone can be tared with the melting point tube inside if this is used in the evaporation step.

7.10 The chloroform solution is evaporated to dryness under a heat lamp with the aid of a stream of air. The centricone with the residue is placed in a desiccator overnight prior to weighing on an analytical balance.

8. Calculations:

% lipid = (A-B)/C X 200

Where A = net sample lipid weight
B = net blank weight
C = sample weight

9. Quality Assurance Guidelines

9.1 Duplicate samples should be run every 10 samples.

10. Precision and Accuracy

10.1 Insufficient data are available from the SJCWQL Shewhart program.

11. References

11.1 This procedure was developed by SJCWQL and JWPCPWQL analysts working from literature in the field.

11.2 Bligh, E. C., and W. J. Dyer. 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biochem. Physiol. 37:911-17.

FIELD SAMPLING AND TISSUE PREPARATION

DOVER SOLE (Microstomus pacificus)

FIELD SAMPLING: As was the case throughout the 1990s, Dover sole within the target size range (18-21 cm standard length size class) were extremely rare on the Palos Verdes Shelf in 2005. Despite sampling efforts exceeding those required in the permit, the number collected was insufficient to meet the sampling goals and no sample was submitted for analysis for 2005.

The field sampling goal was determined by the specifications in the NPDES permit calling for the collection and analysis of three composites of ten individual Dover sole (30 fish) from each of three "zones" along the Palos Verdes peninsula. These zones, established in the 1988 revised permit, are defined as follows: Zone 1 (outfall area - Whites Pt to Bunker Pt), Zone 2 (intermediate area - Long Pt to Pt Vicente), Zone 3 (reference area - Palos Verdes Pt to Bluff Cove). Sampling is by otter trawl and is required to take place within the month of May. The permit stipulates that if sole within the target range (18 to 21 cm S.L.) are absent from the initial trawl (*i.e.*, sampling attempt) within a zone, no additional trawls need be attempted. If target-size sole are present in the initial trawl, one additional trawl shall be conducted in an attempt to collect the necessary number of individuals. Repeated attempts to collect Dover sole were made within all the zones during May 2005 and while these sampling efforts conform with the requirements of the permit, we were unable to collect sufficient material for any composite sample.

WHITE CROAKER (*Genyonemus lineatus*)

Muscle tissue of the white croaker (*Genyonemus lineatus*) was collected, dissected, and submitted to the JWPCPWQL for analysis of selected priority pollutants and lipids per the 2005 JWPCP NPDES monitoring program. The material was analyzed for total DDT, DDT derivatives, total PCB, PCB derivatives, % moisture, and % lipids.

FIELD SAMPLING: In 2005 a temporary redirection of our sampling effort for white croaker was made to support a regional survey of fish tissue contaminants conducted by the Montrose Settlements Restoration Program. This sampling took place in November and December, 2005. 30 individual white croaker were collected from two different depths (23 and 61m) along the Palos Verdes peninsula from one zone rather than three. Zone 1 is an area established in the 1988 revised permit, and defined as follows: Zone 1 (outfall area - Whites Pt to Bunker Pt). Collections were made by means of an otter trawl. Sampling in Zone 1 was conducted at night to increase the likelihood of capturing white croaker. All fish analyzed exceeded the 125 mm standard length (S.L.) minimum stipulated in the permit, ranging from 169 to 212 mm S.L. Upon collection each fish was placed in a foil bag with label, then placed in a labeled plastic bag and frozen. The fish were

transported to the MBL and held frozen until resectioning.

TISSUE PREPARATION: In the MBL each fish was weighed on a triple-beam balance to the nearest 0.1 gram and the standard length (mm) determined. The fish was then placed upon a methylene chloride rinsed glass surface for resection. Tools (Gerber fillet knife, forceps) were stainless steel and were cleaned in methylene chloride and then rinsed with deionized water. The work surface and tools were thoroughly cleaned with methylene chloride and deionized water between samples. Muscle tissue samples were from the dorso-lateral musculature and removed using a fisherman's style fillet technique where the muscle tissue and skin were removed together from the side of the fish. Then the fillet knife was used to horizontally slice the outer skin from the muscle tissue while holding the skin down with forceps. Several grams of muscle tissue was then placed in an acid-washed, methylene chloride-rinsed glass jar with a TFE-lined lid to make up the sample. The jar was labeled by means of an adhesive label and stored in a freezer. Following removal of the muscle sample, the body cavity of the fish was opened and the gonads removed. The gonads of each fish were weighed on an analytical balance to allow the calculation of the gonadal-somatic index (GSI = gonad wt/total body wt. X 100).

DESCRIPTION of DATA FILES

All data from the Bioaccumulation survey collected during 2005 are provided on the CD accompanying this report. The following descriptions explain the formatting of each of the data files covering the Bioaccumulation data. Data files are in comma separated value file (.CSV) format. Each file begins with a header row providing the field names for the data records. A brief definition of each field name is provided below. The term "sample" is a generic one, which, in the Bioaccumulation program, means resected tissue from organisms individually or as a composite from more than one individual.

Following the header row the data records begin. Data values can be either alpha, alpha-numeric characters or numeric. Any missing data values appear as ",," (*i.e.*, nothing between the commas). The last data value is not followed by a comma.

FILE 1: BIOACCUMULATION CHEMISTRY

Number of Data Records (excluding header row) =510

Header Row Field Definitions:

Survey: Name of survey during which sample collected. Bioaccumulation survey is annual.

Zone: Identifies specific Bioaccumulation Zone from which sample collected

Replicate: Identifies position in sequence of replicate samples

Species: Name of species to which data record applies; scientific binomial

Common_Name: Name of species to which data record applies; local English name

Test_Material: Type of tissue composing the sample (e.g. muscle, liver, gonad)

Parameter Group: A grouping mechanism to categorize bioaccumulation chemistry parameters. There are 5 parameter groups:
PhysNCS– physical characteristics, Nitrogen, Carbon, Sulfide parameters
TICH- Total Identifiable Chlorinated Hydrocarbons
PAH- Polyaromatic Hydrocarbons
Metals- heavy metals
P Pollutants- priority pollutants (not covered in above categories)

Parameter: Name of chemistry parameter under which results are reported

#_in_Composite: Number of organisms contributing tissue to a bioaccumulation sample

Qualifier: Qualifies value reported in Results field

Result: Analytical result from sample analysis of the reported parameter

Result_Units: Units associated with the reported result

Sampling_Basis: Identifies whether reported result was analyzed as wet or dry weight

Data_Quality: Quality rating of the data. A= High quality data for use without qualification; B = Questionable data quality that may be used but see

metadata for qualifications; C= Highly questionable data, do not use, see metadata for explanation.; D= unknown data quality

Phylum: Taxonomic phylum to which taxon reported in record belongs

Class: Taxonomic class to which taxon reported in record belongs

Order: Taxonomic order to which taxon reported in record belongs

Family: Taxonomic family to which taxon reported in record belongs

Sample_Comments: Comments relevant to the sample

Sampling_ID: Unique sample identifier. A bioaccumulation sample may be made up of the same tissue from more than one organism.

FILE 2: BIOACCUMULATION ORGANISM INFO

Number of Data Records (excluding header row) =45

Header Row Field Definitions:

Survey: Name of survey during which sample collected. Bioaccumulation survey is annual.

Zone: Identifies specific Bioaccumulation Zone from which sample collected

Replicate: Identifies position in sequence of replicate samples

Species: Name of species to which data record applies; scientific binomial

Common_Name: Name of species to which data record applies; local English name

Standard_Length: Length of fish measured from anteriormost portion to end of hypural bone

Total_Length: Length of fish measured from anteriormost portion to tip of longest caudal fin ray

Length_Units: Units of measurement for length; CM=centimeter

Organism_Size: Size of a bioaccumulation organism other than a fish. For urchins size is the test diameter.

Sex: Gender of organism if applicable and determinable

Gonad_Weight: Weight of gonads (for use in calculating GSI)

Total_Weight: Total weight of the organism

GSI: GonadSomatic Index, a ratio of gonad weight to total body weight multiplied by 100

Tissue: Type of body tissue removed from an organism to make up a bioaccumulation sample (e.g. muscle, gonad, liver)

Tissue_Sample_Wt: Amount of wet weight of tissue removed from an organism to make up a bioaccumulation sample

Weight_Units: Units of measurement for total and tissue weights; G=gram

Phylum: Taxonomic phylum to which taxon reported in record belongs

Class: Taxonomic class to which taxon reported in record belongs

Order: Taxonomic order to which taxon reported in record belongs

Family: Taxonomic family to which taxon reported in record belongs

Sampling_Date: Date on which organism sampling occurred in the field in MM/DD/YY

Resectioning_Date: Date on which tissue was resected from organism in the record
Organism_Comments: Comments pertaining to the organism
Sampling_Depth: Depth at which sample was taken
Units_Depth: Unit of measurement for depth; M=meter:
Sampling_Latitude: Latitude in decimal degrees where sample taken (+ number =N)
Sampling_Longitude: Longitude in decimal degrees where sample taken (- number=W)
Sampling Gear: Gear used to collect sample
Sampling_ID: Unique sample identifier. A bioaccumulation sample may be made up of the same tissue from more than one organism.