Appendix 7

Standard Operating Procedure

for the USGS Reston, Virginia Environmental Organic Geochemistry Laboratory

Instrumental Analysis for Chlorinated Hydrocarbons

Note: A version of this Standard Operating Procedure was accepted by the National Oceanic and Atmospheric Administration (NOAA) in August 1993 as part of the Analytical Chemistry Quality Assurance Plan for the Southern California Natural Resource Damage Assessment.

Contents

Instrumental Analysis for Chlorinated Hydrocarbons

1. **Application:** This document describes procedures to be used for quantitation of PCBs (polychlorinated biphenyls) and DDTs (dichlorodiphenyltrichloroethane-related compounds) in marine sediment samples. The procedures described here are for 84 individual PCB congeners + three surrogates (identified as RS_n, where n = 1,2,3) and eight DDT compounds. A description of the standards used in this procedure is given below (section 4.). It is assumed that the samples were previously extracted according to procedures described in Appendix 4 and fractionated according to procedures given in Appendix 2.

a. ***Tested concentration range.*** This procedure is only one part of a complete analytical method. Expected concentrations of the PCBs and DDTs in marine sediments are given in Appendix 4.

b. ***Sensitivity.*** Following is a tabulation of peak areas obtained near (but above) the detection limit based upon initial multipoint calibration experiments for the PCBs and DDTs (Table 1). All injections were of one microliter, and the data represent the average results obtained from three replicate injections.

Table 1. Tabulation of peak areas for PCB congeners and DDT compounds near the method detection limit.

Congener	Amount (pg)	Peak Area (μV-sec)
1	22.6	11,269
3	13.9	3,365
9	8.38	23,576
6	1.48	4036
8	3.89	9791
19	4.44	11,493
30 (RS1)	8.33	28,045
18	3.89	16,722
17	2.74	19,859
24	4.71	19,294
16	2.07	7032
29	4.75	17,194
26	6.70	20,561
25	4.73	20,563
31/28	7.88	30,222
33	4.80	14,904

Table 1 cont'd. Tabulation of peak areas for PCB congeners and DDT compounds near the method detection limit cont'd.

Congener	Amount (pg)	Peak Area (μV-sec)
22	5.29	17,449
46	5.74	18,893
69	8.26	32,557
52	3.91	13,388
49	3.91	14,243
47	8.05	35,270
35	6.84	19,738
44	3.93	16,223
42	3.99	17,831
41	6.77	24,037
40	7.41	26,851
100	5.04	20,396
74	3.91	12,920
70	3.91	15,375
66	3.91	14,971
121 (RS2)	4.66	19,773
91	5.94	25,185
60	3.93	9,763
92	5.21	24,929
84	3.91	16,354
101	3.93	18,115
99	3.91	16,048
119	3.78	13,829
83	4.84	18,036
97	3.91	13,673
87	3.93	13,779
136	5.00	19,023
110	3.93	16,151
151	4.09	19,624
107	4.29	14,299
123	3.89	15,422

Table 1 cont'd. Tabulation of peak areas for PCB congeners and DDT compounds near the method detection limit cont'd.

Congener	Amount (pg)	Peak Area (μV-sec)
118	3.96	18,039
134	3.52	12,977
114	3.91	14,114
153	3.93	15,911
105	3.91	15,574
141	5.12	24,088
179	3.74	12,846
137	5.58	16,141
130	4.42	23,798
138	3.89	14,037
158	3.93	15,334
129	4.87	19,804
187	3.93	15,410
183	3.89	14,645
128/167	11.9	44,343
185	5.55	24,803
174	3.96	17,415
177	3.96	15,437
171/156	7.69	28,718
157/201	7.88	30,416
172	3.75	15,574
180	3.93	13,787
193	4.15	17,361
200	4.26	17,291
169	3.85	12,173
170	3.93	15,064
198 (RS3)	3.04	11,538
196	3.89	15,940
189	3.95	13,613
195	3.95	15,198

Table 1. Tabulation of peak areas for PCB congeners and DDT compounds near the method detection limit cont'd.

Congener	Amount (pg)	Peak Area (μV-sec)
194	3.91	17,748
205	2.78	11,382
206	3.87	15,380
209	3.96	16,807
p,p'-DDNU[a]	--	--
p,p'-DDMU	3.76	15,083
o,p'-DDE	3.78	18,589
p,p'-DDE	3.78	17,318
o,p'-DDD	3.67	14,720
p,p'-DDD	3.76	12,114
o,p'-DDT	3.72	12,194
p,p'-DDT	3.72	18,732

[a] *p,p*'-DDNU is not included because it was not available when the MDL experiment was performed.

c. ***Detection limits.*** Method detection limits (MDLs) were determined for the 87 PCB congeners and seven DDT compounds in sediments according to the procedure described in 40 CFR Part 136 (USEPA, 1992). The results are based on analysis of eight replicates of precombusted sand. *P,p*'-DDNU is not included because this compound was not available at the time the MDL experiments were performed. Table 2 provides information on the measured MDLs, sources of the analytes used in preparing standard solutions ('source'), and the mixtures into which they were incorporated during standard solution preparation ('mix'). For more information on these mixtures, see discussion to follow (section 4.).

Table 2. Method detection limits for the PCBs and DDTs.

Congener	Source/mix[a]	MDL (ng/dry g)
1	Ultra Scientific (USGS-1)	10.0
3	Chem Service (USGS-3)	5.62
9	Ultra Scientific (USGS-1)	6.06
6	Ultra Scientific (USGS-5)	0.560
8	NIST (PCB92A-1)	0.192
19	Ultra Scientific (USGS-5)	--
30 (RS1)	Ultra Scientific	0.238

Table 2. Method detection limits for the PCBs and DDTs cont'd.

Congener	Source/mix[a]	MDL (ng/dry g)
18	NIST (PCB92A-1)	0.357
17	AccuStandard (USGS-4)	0.110
24	Ultra Scientific (USGS-5)	0.076
16	Ultra Scientific (USGS-3, USGS-5)	0.227
29	Ultra Scientific (USGS-5)	0.154
26	Ultra Scientific (USGS-3)	0.160
25	Ultra Scientific (USGS-2)	0.148
31/28	NIST (PCB92A-1)	0.186
33	Analabs (USGS-2)	0.212
22	Ultra Scientific (USGS-1)	0.712
46	AccuStandard (USGS-2)	0.173
69	Ultra Scientific (USGS-3)	0.558
52	NIST (PCB92A-1)	0.167
49	NIST (PCB92A-1)	0.236
47	ChemService (USGS-4)	1.87
35	Ultra Scientific (USGS-3)	0.594
44	NIST (PCB92A-1)	2.24
42	Ultra Scientific (USGS-1)	0.282
41	AccuStandard (USGS-5)	1.18
40	Ultra Scientific (USGS-4)	0.798
100	Ultra Scientific (USGS-4)	0.176
74	NIST (PCB92A-1)	0.094
70	NIST (PCB92A-1)	0.112
66	NIST (PCB92A-1)	0.166
121 (RS2)	Ultra Scientific	0.121
91	AccuStandard (USGS-2)	0.197
60	NIST (PCB92A-1)	0.310
92	AccuStandard (USGS-3)	--
84	NIST (PCB92A-1)	0.245
101	NIST (PCB92A-1)	0.275
99	NIST (PCB92A-1)	0.297
119	Ultra Scientific (USGS-1)	0.144
83	AccuStandard (USGS-5)	0.138
97	NIST (PCB92A-1)	0.136

Table 2. Method detection limits for the PCBs and DDTs cont'd.

Congener	Source/mix[a]	MDL (ng/dry g)
87	NIST (PCB92A-1)	0.127
136	Ultra Scientific (USGS-1)	0.106
110	NIST (PCB92A-1)	0.144
151	Ultra Scientific (USGS-4)	0.121
107	AccuStandard (USGS-4)	0.082
123	NIST (PCB92A-1)	0.153
118	NIST (PCB92A-1)	0.277
134	AccuStandard (USGS-5)	3.48
114	NIST (PCB92A-1)	--
153	NIST (PCB92A-1)	0.177
105	NIST (PCB92A-1)	2.74
141	Ultra Scientific (USGS-3)	0.162
179	AccuStandard (USGS-5)	0.015
137	Chem Service (USGS-1)	0.136
130	AccuStandard (USGS-4)	0.160
138	NIST (PCB92A-1)	0.111
158	NIST (PCB92A-1)	0.108
129	Ultra Scientific (USGS-3)	0.159
187	NIST (PCB92A-1)	0.072
183	NIST (PCB92A-1)	0.102
128/167	NIST (PCB92A-1), Ultra (USGS-2)/NIST (PCB92A-1)	0.291
185	Ultra Scientific (USGS-3)	1.22
174	NIST (PCB92A-1)	0.371
177	NIST (PCB92A-1)	0.689
171/156	Chem Service (USGS-1)/NIST (PCB92A-1)	0.432
157/201	NIST (PCB92A-1)/NIST (PCB92A-1)	0.255
172	AccuStandard (USGS-2)	0.251
180	NIST (PCB92A-1)	0.118
193	AccuStandard (USGS-5)	0.144
200	Ultra Scientific (USGS-2)	18.6
169	Ultra Scientific (USGS-5)	0.851
170	NIST (PCB92A-1)	0.416
198 (RS3)	Ultra Scientific	9.60

Table 2. Method detection limits for the PCBs and DDTs cont'd.

Congener	Source/mix[a]	MDL (ng/dry g)
196	NIST (PCB92A-1)	0.446
189	NIST (PCB92A-1)	0.120
195	NIST (PCB92A-1)	0.041
194	NIST (PCB92A-1)	0.443
205	Ultra Scientific (USGS-3)	0.219
206	NIST (PCB92A-1)	0.086
209	NIST (PCB92A-1)	0.128
Analyte	**Source/mix[a]**	**MDL (ng/dry g)**
p,p'-DDMU	NIST (PESTA-1)	0.220
o,p'-DDE	NIST (PESTA-1)	0.708
p,p'-DDE	NIST (PESTA-1)	0.210
o,p'-DDD	NIST (PESTA-1)	0.084
p,p'-DDD	NIST (PESTA-1)	0.236
o,p'-DDT	NIST (PESTA-1)	6.09
p,p'-DDT	NIST (PESTA-1)	0.252

[a]Source/mix indicates sources of chemicals and mixture (in parentheses) used in preparation of standard solutions (*cf.*, Figures 1,2).

d. ***Interferences.*** Interferences that could possibly be encountered during the determination of the seven DDT compounds (*p,p*'-DDNU not included) and the PCBs are listed in Appendix 4. In the case of the PCBs, this subject is discussed at length in the main body of this Open-File Report.

e. ***Analysis Rate.*** Chromatographic runs on the GC/ECD (gas chromatograph/electron capture detector) take approximately 90 minutes including cool down time for the gas chromatograph. Since there are approximately 15 runs for 10 field samples/compound class (2-3 single-point calibration runs, one SRM [standard reference material], one blank, one MS/MSD [matrix spike/matrix spike duplicate] equivalent + 10 samples), it takes roughly 22.5 hours to complete a run of 10 field samples (with the aid of an autosampler). This does not include final preparation of the samples (~20 minutes/sample) and other miscellaneous preliminary activities (instrument checkout, computer/data system checkout). Initial reduction of the data follows immediately (and automatically) after the run. However, each chromatographic run is inspected for quality of the chromatogram, obvious interference problems, peak identification, baseline, and integration. If necessary, reprocessing (or reanalysis) is carried out. Hence, 24 hours is a conservative estimate of the time required to complete the instrumental analyses for 10 samples for one compound class (either PCBs or DDTs). Please note also that at least 10% of all samples are analyzed by GC/MS (gas chromatography/mass spectrometry) for purposes of confirmation. These runs are of similar duration.

2. Chemistry: No chemical reactions are involved in these procedures.

3. Apparatus:

a. ***Instrumentation.*** All quantitative analyses of PCBs and DDTs are performed by gas chromatography/electron capture detection. Confirmation of the retention time-based identifications from GC/ECD analysis are established using mass spectral information produced with the GC/MS (gas chromatograph/mass spectrometer). Following are descriptions of these instruments.

GC/ECD: The instrumentation includes a Hewlett-Packard 5890 Series II high resolution gas chromatograph equipped with a ^{63}Ni electron capture detector and a split/splitless capillary injector. A Hewlett-Packard 7673A autosampler is used to perform splitless injections into the split/splitless capillary injector which is equipped with a Merlin Microseal septum. The analytical column is a 30 m x 0.25 mm (id) DB-5 fused silica capillary with a 0.25 μm film thickness (J& W Scientific). Chromatographic conditions are given in section 5.b. (Table 5). The instrument (both autosampler and GC) is controlled by a Perkin-Elmer Series 900 Link interface with data acquisition and reprocessing through the PerkinElmer TotalChrom Workstation (v 6.2.1) software which runs on a Dell Optiplex GX300 computer. The chromatographic data are processed automatically following the run and reprocessed at a later time. Acquired runs and subsequent quantitation files are backed up on a separate hard disk.

GC/MS: The instrumentation includes an Agilent 6890 high resolution gas chromatograph equipped with cool on-column and split/splitless injectors with electronic pressure control. The analytical column is identical to that used for the GC/ECD analyses of the F2 fractions. The capillary column is directly interfaced to an Agilent 5973 mass selective detector by way of the standard heated transfer line. The mass spectrometer interface is held isothermal at 285 °C, and the manifold and ion source regions are held isothermal at 150 °C and 250 °C, respectively. Analyte molecules are ionized by electron impact at 70 eV. The instrument is operated in full scan mode, scanning from 50 to 500 amu at 1.68 scans/second. The mass spectrometer is tuned prior to calibration using PFTBA (perfluorotributylamine; Autotune) and is controlled by Agilent ChemStation software, D01.00 Build 75, running under Windows XP Professional (SP1). Data are acquired by the Agilent ChemStation data system and stored on the hard disk of a Dell GX270 Optiplex desktop computer. Confirmations are based on mass spectral and retention time data for authentic standards as well as spectra in the NIST (National Institute of Standards and Technology) mass spectral library. Acquired runs are backed up on a separate hard disk.

b. ***Parameters.*** Section 5.b. provides information on the parameters used for instrumental analysis.

c. ***Hardware/glassware***. The following are used to prepare samples for analysis and for carrying out the instrumental analysis on the GC/ECD.

-microsyringes: 10, 25, 50, 100, 250 and 500 μL
-Target amber DP vials (National Scientific, #C4000-2W)
-Target 250-μL conical glass inserts (National Scientific, #C4010-629L)
-autosampler syringe (HP# 5181-1267)
-nitrogen blowdown system (custom made)
-11-mm LB-2 injector septa (Supelco # 2-0654)
-DB-5 column (J&W Scientific # 122-5032)
-graphite ferrules (Agilent #5080-8853)
-graphite ferrules (Agilent #5062-3506)

d. ***Chemicals***. For chemicals used in preparation of standard solutions, see section 4 of this document. The only other chemicals used in this procedure are glass-distilled hexane (Burdick & Jackson, High Purity grade) and glass-distilled dichloromethane (Burdick & Jackson, High Purity grade).

4. Standards:

a. ***Calibration standards.*** There are five calibration standards being used in this work (all made up in hexane): three single-point calibration standards, one for the PCBs and two for the DDTs (used primarily as continuing calibration verification, CCV, standards), and a set of multipoint calibration standards for each of the two compound classes (seven levels each). Individual component concentrations for the single-point calibration standards (designated with a 'CS' in the name) are approximately 50 pg/μL, whereas the nominal component concentrations in the multipoint calibration standard solutions (designated with an 'MP' in the name) range from ~5-250 pg/μL. All compounds were obtained either from NIST as a solution in isooctane or from a reputable supplier as neat material (Analabs, Ultra Scientific, Chem Service, AccuStandard) with guaranteed purity for every compound of 99+ %. All solutions were tested for purity by GC/ECD shortly following preparation and prior to serial dilution. They are stored in sealed ampoules within secure boxes in a locked freezer at -20 °C.

As shown in the PCB and DDT standard solution preparation flow charts below (Figures 1,2), the NIST solution of PCBs (41components: **PCB92A-1**) and DDTs (eight components: **PESTA-1**) were supplemented with additional analytes, surrogates and internal quantitation standards obtained from commercial sources. In the case of the PCBs, 42 additional congeners (Table 2) + three surrogates (*cf*., section 4.b.) + three internal (quantitation) standards (*cf*., section 4.c.) were added to make the complete calibration standard solutions. In the case of the DDTs, one surrogate (DBOFB-dibromoocatafluorobiphenyl) and three internal (quantitation) standards (tetrachloro-*meta*-xylene + PCB congeners 11 and 207) were added to make the complete calibration solutions. For the **Palos Verdes Remediation Project** an additional single-point calibration standard solution, DDT-CS-02-1/1, was prepared. This standard solution includes all of the DDT analytes, surrogate (DBOFB) and internal standards found in DDT-CS-01-1/1 plus the DDT degradation product, *p,p*'-DDNU.

b. ***Surrogates (recovery).*** Because the PCBs and DDTs are isolated in the same chromatographic fraction (*cf*. Appendix 2), three surrogates, PCB congeners 30, 121 and 198 (IUPAC; International Union of Pure and Applied Chemistry), were used to track recovery of both the PCBs and DDTs. These congeners approximate the range of physical-chemical properties, chromatographic properties (adsorption and gas chromatography) and degree of chlorination of the PCB congeners and DDT analytes expected to be found in the samples to be tested. In the original NOAA-sponsored work (1992-94) and during the **Palos Verdes Remediation Project** these surrogates were used to assess method performance, not to adjust measured analyte concentrations.

Several dilutions of the surrogate solutions were prepared in anticipation of the need to spike samples of widely varying concentration. Following are the concentrations of surrogates in the PCB and DDT spiking solutions.

Table 3. Tabulation of surrogate solution concentrations (all solutions in hexane).

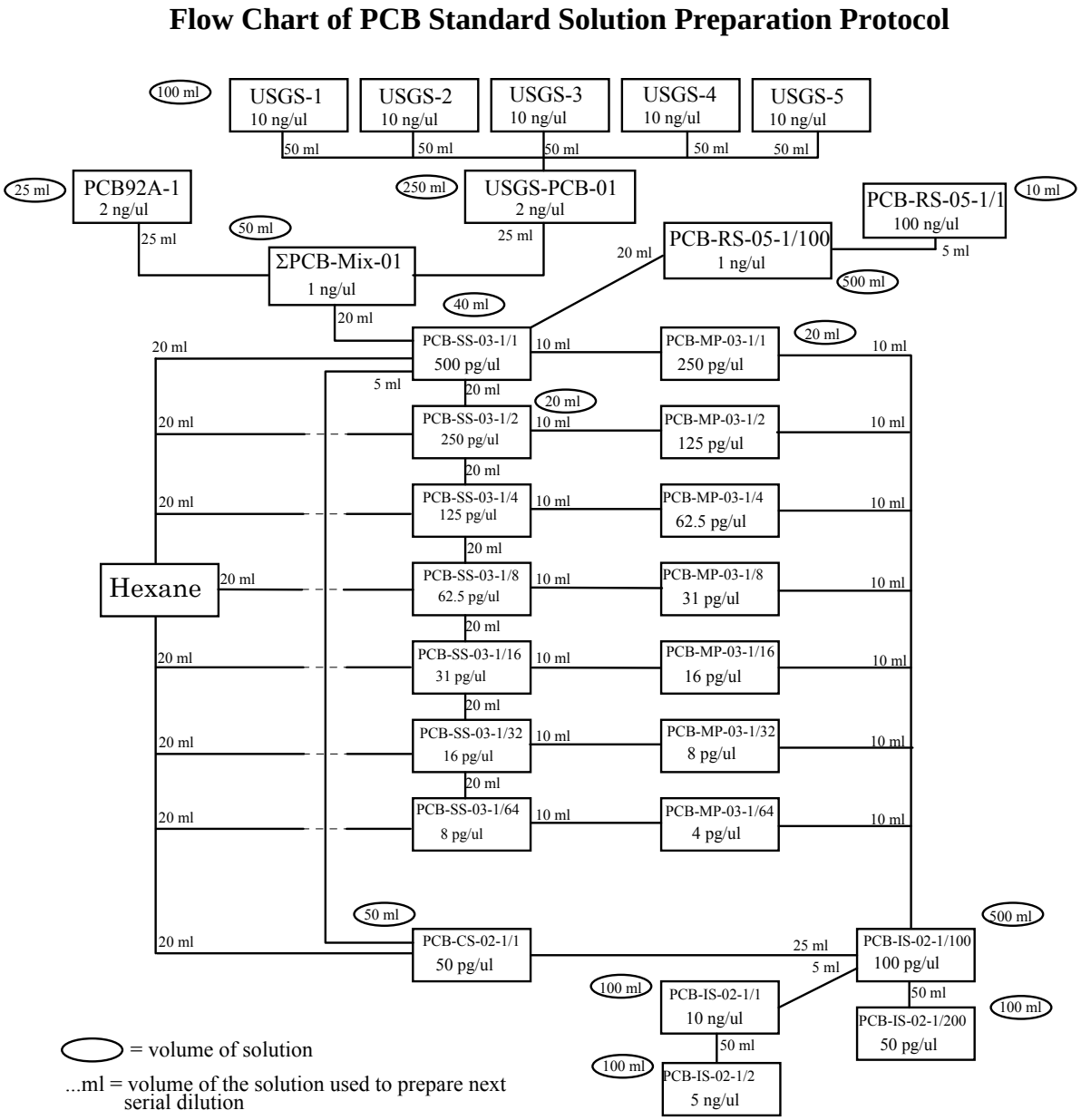
Compound	PCB-RS-05-1/100 (ng/μL)	PCB-RS-05-1/1000 (pg/μL)
PCB 30	2.13	213.5
PCB 121	1.19	119.3
PCB 198	0.78	77.9

c. ***Internal (quantitation) standards.*** Three compounds were included as potential internal quantitation standards, tetrachloro-*meta*-xylene (TCMX), PCB 11 and PCB 207. All of these compounds elute at times in the gas chromatograms different from the expected PCB or DDT analytes (Note: PCB 207 has been found in trace amounts only in Aroclor 1260; Schulz *et al*., 1989). The internal (quantitation) standards are incorporated into the calibration standard solutions (*cf*. Figures 1, 2) and are added to the samples just prior to instrumental analysis. The specific solution to be used with a given F2 fraction split depends upon whether PCBs or DDTs are being measured (see section 5.a. below). Following is a tabulation of the internal standard solutions that have been prepared.

Table 4. Tabulation of internal standard solution concentrations for quantitation of PCBs and DDTs (all solutions in hexane).

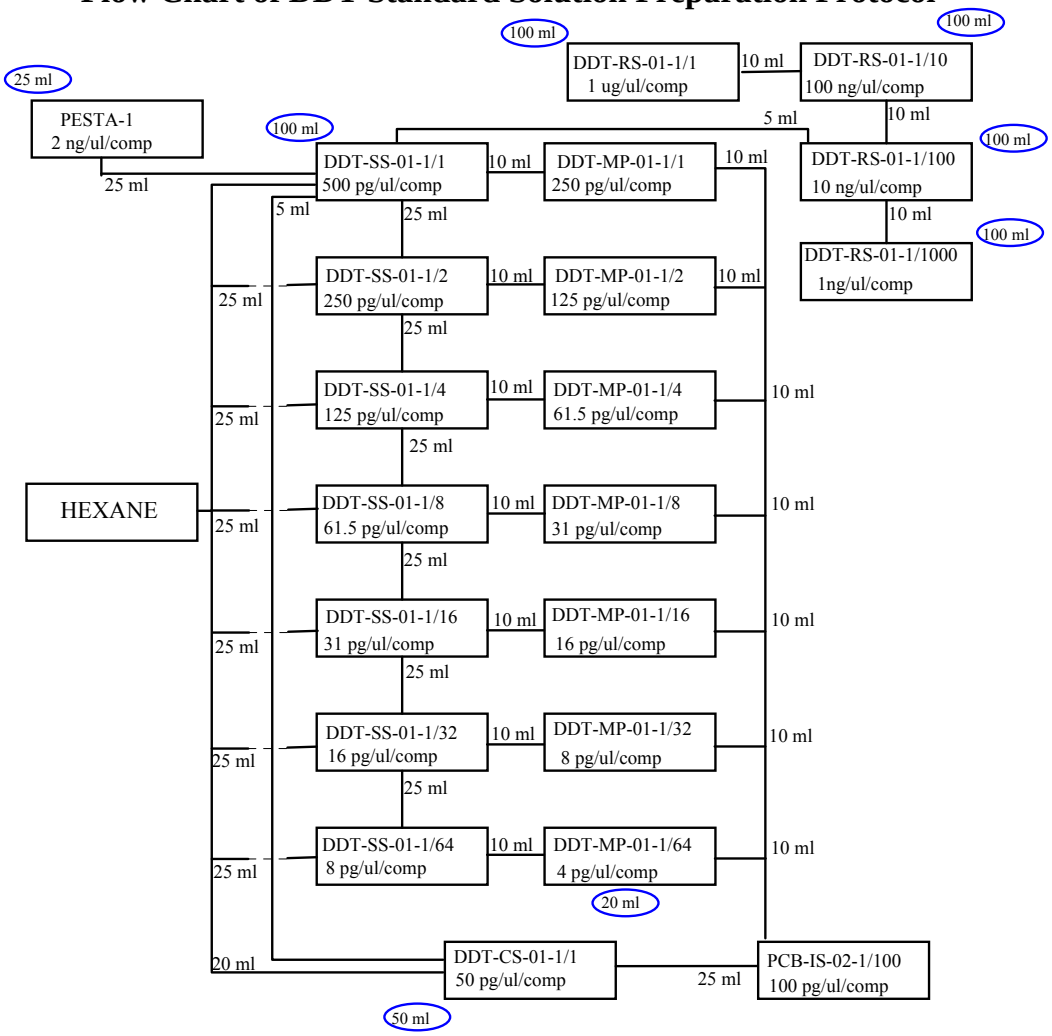
Compound	PCB-IS-02-1/1 (ng/μL)	PCB-IS-02-1/2 (ng/μL)	PCB-IS-02-1/100 (pg/μL)	PCB-IS-02-1/200 (pg/μL)
TCMX	16.1	8.03	161.	80.3
PCB 11	35.3	17.7	153.	177.
PCB 207	10.4	5.18	104.	51.8

Figure 1. Flow chart showing the PCB standard solution preparation scheme (all solutions in hexane).



Note: SS=spike solution, MP=multipoint calibration solution, CS=single point calibration solution, IS=internal standard solution, RS=surrogate solution. See Table 2 for components included in **PCB92A-1** (from NIST) and **USGS-1,2,3,4,5** (prepared from commercially available materials).

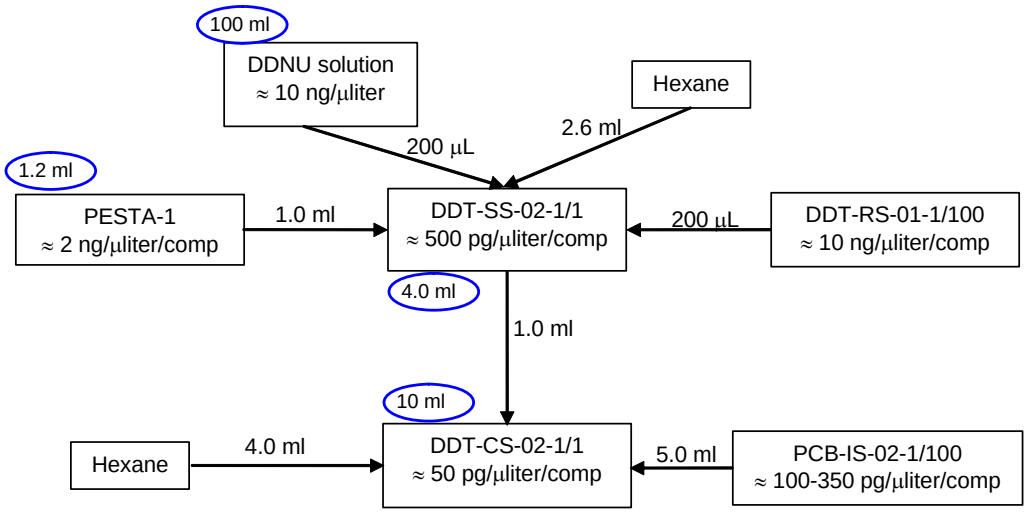
Figure 2. Flow chart showing the DDT standard solution preparation scheme (all solutions in hexane).



Note: SS=spike solution, MP=multipoint calibration solution, CS=single point calibration solution, IS=internal standard solution, RS=surrogate solution. See Table 2 for components included in **PESTA-1** (from NIST).

Figure 3. Flow chart showing the DDT-CS-02-1/1 standard solution preparation scheme (all solutions in hexane).

Flow Chart of DDT-CS-02-1/1 Standard Solution Preparation Protocol



Note: SS=spike solution, MP=multipoint calibration solution, CS=single point calibration solution, IS=internal standard solution, RS=surrogate solution.

5. Procedure:

a. ***Preparation of F2 fractions for instrumental analysis.*** Because the PCBs are expected to be found at much lower concentrations than the most abundant DDT analyte (*p,p'*-DDE) and because both compound classes are recovered in a single chromatographic fraction, it is beneficial to split the F2 fraction and analyze PCBs and DDTs in separate aliquots of the same F2 fraction. Moreover, because the long-chain alkylbenzenes are being determined in the F2 fraction by GC/MS on the same sediment samples (*cf.*, Appendix 5), a third split is necessary for them. Following is a description of the general approach to be taken, recognizing that the exact procedure may vary slightly on a sample-by-sample basis based on professional judgment and prior experience.

The F2 fractions are stored in the freezer in colorless borosilicate ½-dram vials (*cf*., Appendix 2). The volume is adjusted to 1 mL with dichloromethane. This is done by bringing the liquid level in the sample vial equivalent to the mark on an identical colorless ½-dram vial previously calibrated to exactly 1.0 mL. An aliquot of the F2 fraction corresponding to 5 % (*i.e.* 50 µL) is removed using a 100-µL syringe and transferred to a screw cap autosampler (A/S) vial. This is the DDT F2 split. The DDT F2 splits are gently evaporated to just dryness under a stream of dry nitrogen gas and immediately taken up in 1,000 µL of dichloromethane. An aliquot (25-300 µL depending on the sample) is then removed with a microsyringe and transferred to a second screw cap A/S vial. This is gently evaporated to dryness under a stream of dry nitrogen gas and immediately taken up in 500 µL of PCB-IS-02-1/200 (see Table 4, Figure 1; V_{final}). Both A/S vials can then be stored in the freezer until analysis is imminent.

A second aliquot, corresponding to 25% of the F2 fraction (*i.e*. 250 µL), is transferred to a screw cap A/S vial for determination of the PCBs. This is the PCB F2 split. The PCB F2 split is gently evaporated to dryness under a stream of dry nitrogen gas. The sample is either immediately taken up in a volume (250 to 500 µL; V_{final}) of the internal standard solution (PCB-IS-02-1/200; see Table 4, Figure 1), in which case it is ready for analysis, or it is taken up in 1,000 µL of clean dichloromethane and an aliquot ranging from 250 to 500 µL is transferred to a second A/S vial. This second solution is gently evaporated to just dryness under a stream of dry nitrogen gas and immediately taken up in 400 µL PCB-IS-02-1/200 (see Table 4, Figure 1; V_{final}). The A/S vials can then be stored in the freezer until analysis is imminent. [Note: The remaining aliquot, corresponding to 70% of the F2 fraction (*i.e*. 700 µL), is used for determination of the long-chain alkylbenzenes (LCABs). This is the LCAB F2 split. It is stored in the freezer in the original borosilicate ½-dram vial from which the DDT and PCB F2 splits were taken (*cf.,* Appendix 5 for preparation procedures of LCAB F2 split).]

The actual volumes of the various DDT and PCB F2 dilutions are based on predictions using the LACSD (1992) data tabulations and results from the 1992 USGS core analyses (Bailey and Costa, 1994; Eganhouse et al., 2000).

b. ***Instrumental analysis.*** Following are general instructions for the analysis of the F2 fractions by GC/ECD and GC/MS.

GC/ECD: Sediment samples are analyzed for PCBs and DDTs by splitless injection on the split/splitless capillary injector of an HP5890 Series II gas chromatograph. Conditions of analysis are given in the following table (Table 5).

Table 5. Conditions used for GC/ECD analysis of F2 fractions for PCBs and DDTs.

Parameter	Setting
Column:	J&W Scientific, Inc.
Phase	DB-5
Length	30 meters
ID	0.25 mm
Film thickness	0.25 µm
Gas Chromatograph:	HP5890 Series II
Injector	split/splitless, Merlin Microseal septum
Injector temperature	275 °C
ECD temperature	285 °C
Program 1	40 °C to 100 °C @ 15 °C/min

Table 5. Conditions used for GC/ECD analysis of F2 fractions for PCBs and DDTs cont'd.

Program 2	100 °C to 285 °C 3 °C/min, 10 minute isothermal hold
Carrier, linear velocity	helium (upc), 30 cm/sec
Makeup gas, flow	nitrogen (upc), 60 mL/min
Data System:	PerkinElmer TurboChrom (v. 6.2.1)
Sampling rate	1.25 Hz
Interface	LINK 900 series

Following is a listing of the autosampler settings.

Table 6. Settings of HP7673A during quantitation of F2 fractions for PCBs and DDTs: autosampler injection.

Parameter	Setting
Autosampler:	HP7673A
No. sample pumps	6
No. pre-injection sample washes	0
No. post-injection solvent washes (bottle A)	3
No. post-injection solvent washes (bottle B)	3

Prior to initiating a series of runs, the inlet septum (Merlin Microseal) is inspected, a new liner (2 mm id, quartz; Agilent p/n 18740-80220) is installed in the split/splitless capillary injector, a new syringe is installed (if necessary) in the autosampler, and the solvent rinse bottles (A and B; *cf.* Table 5 above) are cleaned and refilled with fresh solvent (hexane). At this point, it is necessary to assure that the instrument is in good operating condition with Gaussian peak shapes for the analytes of interest, low bleed and acceptable response characteristics. This is accomplished by examining preliminary runs of the single-point calibration standard solutions (PCB-CS-02-1/1 and DDT-CS-01-1/1; *cf*., Figures 1 and 2) and comparing them with acceptable runs performed previously. If non-Gaussian peaks, high bleed and/or poor response is observed, remedial action must be taken. This could include replacement of the column, tightening or replacement of fittings, and so on. Following troubleshooting and problem correction, reanalysis of the single-point calibration standard solutions must be performed.

Once operating conditions are deemed acceptable, the column is conditioned (air injection), and the single-point calibration standard for the relevant compound class is analyzed (*i.e*. **DDTs**: DDT-CS-01-1/1; **PCBs**: PCB-CS-02-1/1). The conditioning run is performed in order to remove any material that may have accumulated in the injector during non-run periods, whereas the single-point calibration standard run serves as a final check of the system before proceeding

with the multipoint calibration. Assuming the conditioning run and single-point calibration standard run look acceptable, the multipoint calibration can be started.

Multipoint calibration is performed in the same fashion for DDTs and PCBs. However, these calibrations and their associated sample analyses are performed separately. In other words, the DDT F2 split samples are analyzed immediately after the DDT multipoint calibration and the PCB F2 split samples are analyzed immediately after the PCB multipoint calibration, but these calibrations and sample analyses are carried out at different times. Following is the sequence of runs that would typically occur.

1. Multipoint calibration. The multipoint calibration for the PCBs includes randomized analysis of calibration solutions at seven levels as depicted in the flow chart above (*i.e*. PCB-MP-03-1/n, n = 1,2,4,8,16,32,64; *cf*., Figure 1). Multipoint calibration for the DDTs includes randomized analysis of solutions at seven levels as shown in the flow chart above (*i.e*. DDT-MP-03-1/n, n = 1,2,4,8,16,32,64; *cf*., Figure 2). Following completion of the multipoint calibration analyses, the chromatograms are inspected for peak identification, peak shape, baseline and integration. If necessary, reprocessing is performed. Then calibration curves are plotted and statistics for the second order fit (with forcing through the origin) are computed for each analyte. The calibration curves are inspected for irregularities and the correlation coefficients (r) are compared with requirements of the **Southern California Damage Assessment Analytical Quality Assurance Plan** (Manen, 1994). Calibration curves for all analytes must achieve a correlation coefficient > 0.995 in order for analyses to proceed (*cf*., section 5.c., ***Instrumental QA/QC considerations***). The evaluation of the multipoint calibration is carried out as rapidly as possible.
2. First continuing calibration verification (CCV) run. For the PCBs this is an analysis of PCB-CS-02-1/1; for the DDTs this is an analysis of DDT-CS-01-1/1 **and** DDT-CS-02-1/1. [**Note**: The difference between the two DDT solutions is that the DDT-CS-02-1/1 single-point calibration standard solution contains *p,p*'-DDNU, but DDT-CS-01-1/1 does not. Thus, DDT-CS-01-1/1 is being used only for purposes of CCV, whereas DDT-CS-02-1/1 is used principally to quantify *p,p*'-DDNU in the samples but does supply additional CCV information.] The CCV runs are used to verify the multipoint calibration. The measured analyte concentrations in these runs are compared with the 'actual' analyte concentrations and must meet criteria described in the **Southern California Damage Assessment Analytical Quality Assurance Plan** (Manen, 1994; *cf*., ***QA/QC considerations*** below). If, and only if, these criteria are met, can sample analyses proceed. Otherwise, another multipoint calibration must be performed and evaluated with the appropriate CCV runs. The evaluation of the CCVs is carried out as rapidly as possible.
3. Pre-sample conditioning of column. Prior to the analysis of the F2 split sub-fraction analyses, a conditioning run is performed. This is

necessitated by the fact that the multipoint calibration results can take a considerable amount of time to review and evaluate, especially in the case of the PCBs. During this time, septum bleed may have accumulated on the column.

4. Sample runs. The F2 split sub-fractions of the samples are analyzed in succession with CCVs, blanks, SRM samples, and MS/MSD samples interposed at intervals as specified in the **Southern California Damage Assessment Analytical Quality Assurance Plan** (Manen, 1994). In the case of the CCV runs, the chromatograms are inspected immediately after analysis and automated data processing is complete. Then, reprocessing (if necessary) is performed, and the measured analyte concentrations are compared with the 'actual' analyte concentrations. Again, the CCV runs must meet criteria described in the **Southern California Damage Assessment Analytical Quality Assurance Plan** (Manen, 1994). If these criteria are met, sample analyses can continue. Otherwise, the multipoint calibration and evaluation must be repeated successfully before sample analyses can be resumed. Analyses continue until all samples, SRMs, blanks, and MS/MSDs have been completed. The last analysis is a CCV run, which must meet the criteria discussed above.

GC/MS confirmation: Confirmation of the identities of the DDT analytes is based on full scan electron impact GC/MS analysis of the LAB F2 split. The GC/MS analysis is conducted using analytical conditions similar to those used in the GC/ECD analyses (*cf*., Appendix 5). In the case of the PCBs, GC/electron capture negative chemical ionization mass spectrometry is used. This provides information on the molecular weight and degree of chlorination for congeners having from 5 to 10 chlorine substituents. Conditions of analysis have not been established for the **Palos Verdes Remediation Project** but will be similar to those described in Eganhouse *et al*. (2000).

c. ***Instrumental QA/QC considerations***. The principle test of proper instrument operation for the GC/ECD is multipoint calibration and maintenance of calibration during the period of sample analyses. Thus, considerable attention is given to the results obtained during the calibration exercises. The multipoint calibration for the PCBs involves 87 analytes and seven levels. It takes about 25 hours to complete the runs and 4-6 hours to review and reduce the data. As stated in the **Southern California Damage Assessment Analytical Quality Assurance Plan** (Manen, 1994), "...the correlation coefficient (r) for the curve will be greater than 0.9950. Failure to generate acceptable standard curves weekly (at a minimum) for each analyte will require recalibration...". In the case of the Continuing Calibration Verification (CCV) performed at the beginning of sample runs (immediately after the multipoint calibration) and at intervals between samples, we compare the concentrations of each analyte in the single-point calibration standard solutions (PCB-CS-02-1/1, DDT-CS-01-1/1, DDT-CS-02-1/1) as determined using the multipoint calibration with the "actual" concentrations. These results are used to construct control charts on which the measured concentrations of each analyte in the CCV are plotted over time along with the "actual" concentrations in the CCV. The criterion for acceptability of a

single-point calibration (CCV) run is that the "measured" concentrations of all analytes being monitored must fall within ± 25% of the 'actual' value. Temporal trends in the CCV control chart plots may indicate a general drift in the ECD, whereas abrupt changes that fall outside these criteria indicate a significant instrument problem that must be corrected.

d. ***Peak identification.*** Peaks are identified by the chromatography data system. The software first searches for "reference" peaks designated by the user for each analyte. The software then corrects the "expected" retention times of all detected peaks for any shift found for the reference peak. Absolute and relative retention time windows are also specified for each component. This serves to restrict the number of peaks that are identified by the data system as a specific targeted analyte. Because the peaks change in band width with respect to their elution time (band broadening) and because their separation from other nearby peaks is highly variable on an analyte-by-analyte basis, it is necessary to specify windows that will allow each peak to be found and consistently differentiated from other closely eluting peaks. In principle, if the method is set up optimally, even complex mixtures such as the PCBs can be analyzed with proper peak identification. In practice, however, peaks often exist as shoulders, the separations vary with time or other effects tend to cause misidentifications by the automated data processing software. For this reason, all chromatographic runs are inspected manually for correct peak identification. Until and unless GC/MS confirmation analyses are completed, identifications based on GC/ECD analysis must be viewed as tentative. When the GC/MS analyses are complete, the GC/ECD runs are again inspected for any misidentifications, and if need be, corrections are made, and the files are reprocessed.

At the time that the peak identifications are checked, the chromatograms are also inspected for proper peak integration and how the baseline has been drawn. Frequently, variations in sample composition will cause unexpected effects on the baseline and the integration of certain peaks. Thus, some reprocessing of the chromatogram is often necessary. This is done on a sample-by-sample basis (including the calibration standards), and final concentrations are not reported until all chromatograms have been examined and necessary changes to identifications and integrations (if any) have been made. [Note: Only concentrations above the MDL are reported. Data falling below the MDL are reported as less than the MDL (*e.g.* < 0.11 ng/dry g).] For complex mixtures such as the PCBs, it is difficult to prescribe specific procedures to be used. In general, the approach is as follows: 1) all chromatograms are inspected to identify recurrent problems, 2) a modified integration timed event is applied to a subset of the samples to determine if it will work acceptably, 3) successful modifications are applied systematically to all sample chromatograms so that peak integration and baselines will be consistent.

6. **Calculations:** All calculations are carried out automatically by the PerkinElmer data system using the TotalChrom v. 6.2.1 software. The internal standard method of quantitation is used whereby all analyte concentrations (including those of the surrogates) are determined by comparing the ratio of the responses of the analytes to

internal standards in the sample with the ratio of responses of the analytes and internal standards in the calibration standards. In the NOAA-funded study (1992-94) and the **Palos Verdes Remediation Project** (2006), PCB 207 was the internal standard used for quantitation of all analytes (both PCBs and DDTs). Because the concentrations of the analytes and internal standards in the calibration standards and the internal standard in the sample are known, the equation(s) developed from the multipoint calibration curves can be applied to the sample to obtain concentrations (*cf*., equation 1 below). This calculation yields concentrations that are <u>not</u> corrected for analyte recovery. [<u>Note</u>: In multipoint calibrations performed in the Reston, VA laboratory, about ½ of the PCB analytes exhibit acceptable linear behavior ($r \geq 0.995$) over the entire calibration range (0-250 pg/μL). The remainder of the PCBs and all of the DDTs, however, exhibit calibration curves that are nonlinear and have to be modeled with second order equations. Thus, the calibration curve for each analyte is modeled with a second order equation.] Surrogate recovery is computed as the percent of each added surrogate that is measured in the sample (equation 2). These surrogate recoveries are <u>not</u> used to adjust analyte concentrations.

The equation used to compute concentrations of analytes in sediment samples is given below.

$$\left[C_i\right]_s = C_{IS}\left[\frac{-b-\sqrt{b^2-\left\{4a\times\left(\frac{A_i}{A_{IS}}\right)\right\}}}{2a}\right]\times\left[\frac{V_{final}\times DF}{M_S\times 1000}\right] \qquad (1)$$

<u>*where*</u>*:* $[C_i]_s$ = concentration of analyte *i* in the sediment sample (ng/dry g),

C_{IS} = concentration of the internal standard in the final sample solution (pg/μL),

a,*b* = dimensionless coefficients of x^2 and x, respectively, derived from quadratic fit to calibration data with forcing through origin,

A_i = area of analyte peak *i* in the final sample solution (μV-sec),

A_{IS} = area of internal standard in the final sample solution (μV-sec),

V_{final} = total volume of the sample solution at time of GC analysis (μL),

DF = dilution factor incorporating fraction of total extract fractionated, split taken of F2 fraction and subsequent dilutions, if any (dimensionless),

M_S = dry mass of sediments extracted (dry g).

The equation for determining surrogate recovery is given below.

$$R_i = \left[\frac{C_{RSi}\times M_S}{M_{RSi}}\right]\times 100 \qquad (2)$$

<u>*where*</u>*:* R_i = recovery of surrogate *i* in per cent,

C_{RSi} = concentration of surrogate *i* in the sample calculated using equation 1 (ng/dry g),

M_S = mass of dry sediments extracted (g),

M_{RSi} = mass of surrogate *i* added to the sample (ng).

7. **QA/QC Considerations:** Information on the number of blanks, SRMs, MS/MSDs, *etc*. that are processed along with a given number of samples or per batch can be found in the SOP entitled, "**Extraction of Sediments and Suspended Particles for Analysis of Trace Organics**" (Appendix 4). The quality control criteria that must be met with regard to the analysis of sediment samples for chlorinated hydrocarbons can be found in the **Southern California Damage Assessment Analytical Chemistry Quality Assurance Plan** (Manen, 1994) and will not be further elaborated here.

8. **Health, Safety, and Waste-Disposal Information:**

a. ***Personal protection*.** Safety glasses and protective gloves are recommended whenever reagents or samples are handled. For other precautions and safety procedures, consult the Material Safety Data Sheets (MSDS) for each chemical used. They are on file in the laboratory; *http://www.ilpi.com/msds/#Manufacturers* provides links to MSDSs of most chemical companies.

b. ***Electrical hazards.*** Electrical systems must conform to the National Electric Code, the National Fire Protection Association Code (NFPA 70-1971), and the American National Standards Institute (ANSI) Code (C1-1971). Consult the U.S. Geological Survey's Safety and Environmental Health Handbook (U.S. Geological Survey, 2002). Shock hazards exist inside the instruments. Only an authorized service representative or an individual with training in electronic repair should remove panels or circuit boards where voltages are greater than 20 V. The instruments require a third-wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.

c. ***Chemical hazards*.** Hexane and dichloromethane are solvents used in the preparation of samples for instrumental analysis. Gloves should be worn when handling organic solvents and, whenever possible, manipulations should be conducted in a fume hood. Waste solvents accumulated during rotary evaporation or other cleaning operations should be stored in a capped glass bottle (satellite accumulation point) and arrangements made for its disposal through the USGS Materials Management Office.

d. ***Gas cylinder handling*.** Compressed gas cylinders must be handled and stored according to the Safety and Environmental Health Handbook (U.S. Geological survey, 2002). Each cylinder must be 1) carefully inspected when received, 2) securely fastened at all times with an approved chain assembly or belt, 3) capped at all times when not in use, 4) capped when transported, 5) transported only by a properly designed vehicle (hand truck), and 6) stored separately with other full, empty, flammable, or oxidizing tanks of gas, as appropriate.

e. ***Sharps.*** Microsyringes with fixed or removable needles should be handled with care to avoid accidental skin punctures.

9. **References**: Following are citations from this SOP along with some additional sources (marked in bold) of information about the procedures that have been described here.

Bailey, A., and Costa, H.J. 1994. Chemical analyses of sediment cores data report: *in* U.S. Department of Justice, Southern California Bight Natural Resource Damage Assessment Expert Reports, 1994, chap. 2, appendix H, 138 p.

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Hendricks, T.J. and R.P. Eganhouse. 1992. Modification and Verification of Sediment Deposition Models. *Final Report to the California Water Resources Control Board*, September, 1992, 330pp.

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Shulz, D.E., G. Petrick and J.C. Duinker. 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environmental Science & Technology*, 23:852-859.

U.S. Geological Survey. 2002. USGS Handbook 445-3-H, Safety and Environmental Health Handbook, 435p.