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Analytical Procedures and Quality Assurance Plan for the Determination of PCDD/PCDF in Fish

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U.S. Environmental Protection Agency

National Dioxin Study - Phase II

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NOTICE

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FOREWORD

Directed by Congressional mandate, the U.S. Environmental Protection Agency during 1983 initiated the National Dioxin Study, a survey of environmental contamination by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the United States. Results of this study are published in the National Dioxin Study: Tiers 3,5,6, and 7, EPA 400/4-82-003. This laboratory, the Environmental Research Laboratory- Duluth, was responsible for one part of the Study, the analysis of fish samples. The most significant findings of these analyses was the observation that fish contamination was more widespread than previously thought, and that a primary source of TCDD was discharge from pulp and paper production using chlorine.

A second more detailed characterization of anthropogenic organic chemical contaminants in fish was conducted in subsequent analyses during what is now called Phase II of the National Dioxin Study. This document describes the analytical methods used for the determination of the level of contamination of fifteen biosignificant polychlorinated dibenzo-p-dioxins and dibenzofurans in fish. A companion document (EPA /600/3-90/023) describes the analytical methods used for the determination of levels of contamination of polychlorinated biphenyls, pesticides, and industrial compounds in those same fish.

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I. Introduction

This document, "Analytical Procedures and Quality Assurance Plan for the Determination of PCDD/PCDF in Fish" has been drafted in response to the need for the Environmental Research Laboratory of Duluth (ERL-D) to perform analysis for tetrachloro- to octachloro- congeners/isomers of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF), Table 1.

Table 1. Biosignificant PCDDs/PCDFs

Analyte	CASRN
2378-TCDF	51207-31-9
2367-TCDF	
3467-TCDF	
2378-TCDD	1746-01-6
12378-PeCDF	57117-41-6
23478-PeCDF	57117-31-6
23467-PeCDF	70648-29-9
12378-PeCDD	40321-76-4
123467-HxCDF	
123478-HxCDF	70648-26-9
123678-HxCDF	57117-44-9
234678-HxCDF	60851-34-5
123789-HxCDF	72918-21-9
123478-HxCDD	32598-13-3
123678-HxCDD	57753-85-7
123789-HxCDD	19408-74-3
1234678-HpCDF	67562-39-4
1234789-HpCDF	55673-89-7
1234678-HpCDD	37871-00-4

These analyses are limited by lack of analytical standards; however isomer specificity may be determined using specially developed standards. Analytical results will, therefore, be reported as concentration (pg/g) for each gas chromatography (GC) peak in a congener class by making the assumption that the response for the molecular ion of all isomers in that class is equal to the response observed for the isomer for which ERL-D does have a standard. The target minimum level of detection (MLD) for specific PCDD/PCDF isomers is given in Table 2 below. This document is meant to be only a guideline for analyses and may be modified as needed to satisfactorily analyze any sample.

Table 2. Minimum Level of Detection Values

PCDD/PCDF	Target Minimum Level of Detection
TCDD, TCDF	1 pg/g
PeCDD, PeCDF	2 pg/g
HxCDD, HxCDF	4 pg/g
HpCDD, HpCDF	10 pg/g

II. Sample Preparation

A. Grinding: Frozen fish wrapped in aluminum foil are sent to the ERL-Duluth laboratory. How the fish is ground, (whole body or fillet), is dependent on the species. Bottom feeders are ground whole and predators are filleted with the skin off. Fish tissue is ground frozen in a stainless steel power meat grinder. Each sample is processed through the grinder three times which homogenizes it thoroughly. The ground tissue is stored at -20° C in solvent rinsed glass jars with aluminum lined plastic lids.

B. Extraction: Tissue (20 g) is blended with enough anhydrous sodium sulfate to dry the tissue (100 g). Two-thirds of the sample is placed in a glass Soxhlet thimble, spiked with 100 ul of each Standard Solution A and B (Table 3) and then the remainder of the sample is added to the thimble. The sample is extracted at least twelve hours with a 1:1 mixture of hexane and methylene chloride in a Soxhlet extractor. The sample is quantitatively transferred to a 500 ml Kuderna-Danish apparatus and prewashed boiling chips are added.

C. Percent Lipid Determination: The sample extracted in section I.B. of sample preparation is used to determine percent lipid. After sample concentration, the KD lower tube is placed in a 60° C water bath under a gentle stream of dry carbon filtered air. After any remaining solvent has been evaporated, the lower

tube and contents are weighed. The lipid is then quantitatively transferred to the macro column as described in Section I.D. of sample preparation. After transfer, the empty lower tube and boiling chips are weighed. The percent lipid is calculated from the weight differences.

Table 3. Internal Standard Solutions.

Compound	Concentration in solution (pg/uL)	Concentration in tissue (pg/g*)
Internal Standard Solution A. (100 uL)		
$^{37}Cl_4$ 2,3,7,8-TCDD	2.0	10.0
$^{13}C_{12}$ 2,3,7,8-TCDD	5.0	25.0
$^{13}C_{12}$ 2,3,7,8-TCDF	5.0	25.0
$^{13}C_{12}$ 1,2,3,7,8-PeCDD	5.0	25.0
$^{13}C_{12}$ 1,2,3,7,8-PeCDF	5.0	25.0
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDD	12.5	62.5
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDF	12.5	62.5
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDD	12.5	62.5
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDF	12.5	62.5
$^{13}C_{12}$ OCDD	25.0	125.0
$^{37}Cl_4$ 2,3,7,8-TCDF	2.0	10.0
Internal Standard Solution B.		
1,2,3,4-TCDD	1.0	5.0
1,2,4,7,8-PeCDD	1.0	5.0
1,2,3,4-TCDF	1.0	5.0
1,2,3,6,7-PeCDF	1.0	5.0
Internal Standard Solution C.		
$^{13}C_{12}$ 1,2,3,4-TCDD	50.0	50.0

* Assumes a 20 g sample.

D. Anthropogenic Chemical Isolation: The sample extract is quantitatively transferred to a 30 cm x 2.5 cm glass chromatography column (MACRO-columns) fitted with a 300 mL reservoir on top. The column has been packed with a plug of glass wool (bottom to top), 2 g silica gel, 2 g potassium silicate, 2 g sodium sulfate 10 g celite/sulfuric acid and 2 g sodium sulfate, and previously washed with 100 mL hexane. The column is eluted with 100 mL benzene/hexane (5%) and the eluent is collected in a Kuderna-Danish (KD) apparatus (Caution: benzene is a known carcinogen). Isooctane (1.0 mL) is added, the volume is reduced and then transferred to the florisil column.

E. Florisil Chromatography: A 1.0 cm x 20.0 cm glass chromatography column fitted with a 100 mL reservoir is packed with a plug of glass wool (bottom to top), 5.0 cm (1.5 g) activated florisil and 1.0 cm sodium sulfate. The florisil is activated at 120° C for 24 hours. The column is washed with 20 mL methylene chloride followed by 10 ml hexane. Sample and two 1 ml hexane rinses are quantitatively applied in small "plugs". The column is eluted with 20 mL 2% methylene chloride/hexane and the eluate discarded. This wash is followed by 50 mL methylene chloride which flows directly onto the micro carbon/silca gel column for PCDD/PCDF isolation.

F. PCDD/PCDF Isolation: Effluent from the florisil column is passed onto a 4 mm x 200 mm column (micro-column) containing 300 mg silica gel/carbon (see sec. III.A.6) which was previously rinsed with 10 ml toluene followed by 10 ml methylene chloride. The column is fitted with a solvent reservoir. After the sample has almost completely eluted from the micro-column, the reservoir is washed twice with 2 mL 25% benzene/methylene chloride and the

column is finally eluted with an additional 11 mL 25% benzene/ methylene chloride. The column is inverted on the reservoir and the PCDD/PCDF are eluted with toluene (25 mL). The toluene fraction is collected in a pear shaped flask (25 ml) and reduced in volume to 0.1 mL in a 60° C water bath under a gentle stream of dry carbon filtered air. The sample is transferred to a microvial using toluene to rinse the flask. Prior to GC/MS analysis, the sample is allowed to evaporate to dryness and is spiked with 20 ul of Standard Solution C (Table 3).

III. Reagents and Standards:

A. Reagents:

1. Solvents: Only pesticide grade distilled in glass solvents are used. They are: hexane, isooctane, methylene chloride, benzene, toluene, acetone, and methanol (Burdick and Jackson, Fischer Scientific).

2. Sodium Sulfate: Sodium sulfate (Baker Chemical Company reagent grade anhydrous) is baked at 650°C in a furnace for 24 hours, cooled, and stored in an empty hexane solvent bottle.

3. Silica Gel: Silica-Gel-60 (Merck-Darmstadt), is Soxhlet extracted eight hours with methanol, placed on solvent rinsed foil, air dried for 12 hours, and vacuum oven dried (125°C) for 24 hours. It is stored in an empty hexane solvent bottle. Prior to use it is activated at 105° C for 24 hours.

4. Sulfuric Acid/Celite: Sulfuric acid (Baker Chemical Company, Ultrex) (5 mL) is blended in a 250 mL beaker with Celite 545 (Baker) (10 g).

5. Potassium Silicate: High purity potassium hydroxide (Aldridge Chemical Company) (56 g) is dissolved in methanol (300 mL). Silica-gel (100 g) is added to the mixture and stirred (1 hour, 60° C). The mixture is cooled and the solvent is removed using a Buchner funnel. The potassium silicate is rinsed twice with 100 ml of methanol and once with 100 ml of methylene chloride. The solids are placed on aluminum foil in a fume hood and allowed to dry for approximately 2 hours. The solids are placed in a vacuum oven and dried overnight at 105°C. The reagent is placed in a rinsed beaker and stored (activated) at 120°C until use.

6. Silica Gel/Carbon: Silica Gel-60 (100 g) (Merck-Darmstadt) is Soxhlet extracted with methanol (200 mL) for 24 hours, air dried in a hood, and further dried in vacuum oven for 24 hours. AMOCO PX-21 Carbon (5 g) is added and then blended until uniform in color. The Silica Gel/Carbon is stored in a closed jar at room temperature until use.

7. Florisil: Florisil 60-100 mesh (Baker Analyzed) is soxhlet extracted with methanol for 24 hours, placed on solvent rinsed foil, air dried and stored in an empty hexane bottle. Prior to use it is activated at 120°C for 24 hours.

B. Standards:

1. Analytical Standard Spiking Solution

Table 3 provides details of the spiking solutions. The surrogate analytes are used by the data reviewer to insure that calculated MLD values are reasonable.

2. Quantification Standards: Quantification standards were prepared by Wright State University. The concentration of 2,3,7,8-TCDD was

checked against a primary standard obtained from the U.S. National Bureau of Standards. A table of the concentrations of each isomer in each standard is given in Table 4.

3. Qualitative Standards: ERL-D has developed two qualitative analytical standards, one containing all 75 PCDD's and all 138 PCDF's was developed from an extraction of municipal incinerator fly ash (Tables 5 and 6) and the other containing only the biosignificant isomers was developed by exposure of fish to an extract of municipal incinerator fly ash and processing the exposed fish for PCDD/PCDF. These standards will be used to assign structures for isomer specific analyses.

   Standard solutions are sonicated for 5 to 10 minutes before use.

4. Mass Spectrometer Mass Calibration Compounds: Perfluorokerosene (PFK) is used for the initial mass calibration of the mass spectrometer. Perfluorodecalin (PFD) is used daily for determining mass resolution on m/z 392.9761.

Table 4: Calibration Standards

Concentrations in Calibration Solutions in pg/ul Tridecane

Calibration Standard	W1	W2	W3	W4	W5	W6	W7	W8
2,3,7,8-TCDD	200	100	50	25	10	5	2.5	1
2,3,7,8-TCDF	200	100	50	25	10	5	2.5	1
1,2,3,7,8-PeCDD	200	100	50	25	10	5	2.5	1
1,2,3,7,8-PeCDF	200	100	50	25	10	5	2.5	1
2,3,4,7,8-PeCDF	200	100	50	25	10	5	2.5	1
1,2,3,4,7,8-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,6,7,8-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,7,8,9-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,6,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,7,8,9-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
2,3,4,6,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,6,7,8-HpCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,6,7,8-HpCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,7,8,9-HpCDF	500	250	125	62.5	25	12.5	6.25	2.5
OCDD	1000	500	250	125	50	25	12.5	5
OCDF	1000	500	250	125	50	25	12.5	5
$^{13}C_{12}$ 2,3,7,8-TCDD	50	50	50	50	50	50	50	50
$^{13}C_{12}$ 2,3,7,8-TCDF	50	50	50	50	50	50	50	50
$^{13}C_{12}$ 1,2,3,7,8-PeCDD	50	50	50	50	50	50	50	50
$^{13}C_{12}$ 1,2,3,7,8-PeCDF	50	50	50	50	50	50	50	50
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDD	125	125	125	125	125	125	125	125
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDF	125	125	125	125	125	125	125	125
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDD	125	125	125	125	125	125	125	125
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDF	125	125	125	125	125	125	125	125
$^{13}C_{12}$ OCDD	250	250	250	250	250	250	250	250
$^{37}Cl_4$ 2,3,7,8-TCDD	20	20	20	20	20	20	20	20
$^{37}Cl_4$ 2,3,7,8-TCDF	20	20	20	20	20	20	20	20
$^{13}C_{12}$ 1,2,3,4-TCDD	50	50	50	50	50	50	50	50

Table 5: Relative Retention Times for 4-8 PCDD Isomers

Compound	RRT DB5	RRT SP2330
1368	0.814	0.826
1379	0.838	0.871
1369	0.861	0.948
1378	0.912	0.916
1469	0.912	1.072
1247	0.912	0.948
1248	0.912	0.948
1246	0.921	1.014
1249	0.921	1.014
1268	0.934	0.972
1478	0.940	0.990
1279	0.960	1.027
1234	0.985	1.014
1236	0.985	1.027
1269	0.985	1.105
1237	0.993	1.014
1238	0.993	1.014
2378	1.000	1.000
1239	1.009	1.088
1278	1.028	1.072
1267	1.048	1.130
1289	1.079	1.216
12468	1.224	1.111
12479	1.224	1.111
12469	1.265	1.268
12368	1.293	1.148
12478	1.308	1.188
12379	1.320	1.209
12369	1.348	1.307
12467	1.348	1.321
12489	1.348	1.321
12347	1.368	1.268
12346	1.368	1.352
12378	1.400	1.288
12367	1.415	1.363
12389	1.443	1.463
124679	1.620	1.473
124689	1.620	1.473
123468	1.673	1.473
123679	1.700	1.546
123689	1.700	1.546
123469	1.700	1.681
123478	1.764	1.604
123678	1.775	1.618
123467	1.802	1.789
123789	1.802	1.721
1234679	1.976	2.135
1234678	2.023	2.297
12346789	2.234	3.225

Table 6: Relative Retention Times for 4-8 PCDF Isomers

Compound	RRT DB5	RRT SP2330	Compound	RRT DB5	RRT SP2330
1368	0.730	0.777	13478	1.202	1.083
1468	0.752	0.875	13479	1.217	1.103
2468	0.763	0.989	23469	1.217	1.173
1247	0.782	0.885	12479	1.233	1.142
1347	0.782	0.865	13469	1.253	1.204
1378	0.782	0.853	23468	1.253	1.278
1346	0.782	0.919	12469	1.253	1.278
2368	0.782	1.071	12347	1.253	1.173
1367	0.801	0.881	12346	1.253	1.231
1348	0.801	0.900	12348	1.280	1.216
1379	0.801	0.853	12378	1.280	1.216
1268	0.835	0.943	12367	1.295	1.252
1248	0.835	0.919	23489	1.309	1.388
1467	0.853	0.989	12379	1.309	1.237
1478	0.853	0.943	23478	1.359	1.557
1369	0.863	0.943	12489	1.359	1.446
1237	0.863	0.943	13489	1.359	1.350
2467	0.863	1.109	12369	1.359	1.373
1234	0.880	0.977	23467	1.371	1.612
2349	0.880	0.977	12349	1.392	1.420
1236	0.880	0.989	12389	1.446	1.590
1469	0.880	1.061			
1238	0.880	0.989	123468	1.556	1.336
1278	0.902	1.017	134678	1.570	1.370
1349	0.920	1.013	124678	1.570	1.348
1267	0.920	1.049	134679	1.570	1.348
2378	0.939	1.169	124679	1.602	1.428
2348	0.939	1.175	124689	1.621	1.521
2347	0.939	1.140	123467	1.663	1.533
2346	0.939	1.193	123478	1.663	1.489
1246	0.939	0.940	123678	1.676	1.502
1249	0.939	1.071	123479	1.676	1.489
1279	0.939	1.049	123469	1.712	1.668
2367	0.973	1.206	123679	1.730	1.562
1239	0.988	1.140	123689	1.744	1.668
1269	0.988	1.162	234678	1.744	2.012
3467	0.988	1.264	123789	1.827	1.871
1289	1.071	1.341	123489	1.827	1.940
13468	1.120	1.008	1234678	1.954	1.936
12468	1.120	1.028	1234679	1.979	2.001
23479	1.190	1.065	1234689	2.024	2.161
12368	1.202	1.103	1234789	2.043	2.463
12478	1.202	1.121			
13467	1.202	1.142	12346789	2.240	3.165
12467	1.202	1.160			

IV. Instrumental Parameters:

All gas chromatography/mass spectrometry analyses (GC/MS) will be done on a Finnigan-MAT 8230 high resolution GC/high resolution MS (HRGC/HRMS) system. Instrumental parameters are given in Table 7.

Table 7: HRGC/HRMS Operating Parameters

Data Acquisition: Multiple Ion Selection Electric Sector Scan.

Compound	Mass Window	m/z value Quant.	Confir.*
TCDF	1	305.8986	303.9016
$^{37}Cl_4$-TCDF	1	311.8898	
$^{13}C_{12}$-TCDF	1	317.9389	315.9419
TCDD	1	321.8936	319.8965
$^{37}Cl_4$-TCDD	1	327.8847	
$^{13}C_{12}$-TCDD	1	333.9338	331.9368
PeCDF	2	339.8597	341.8567
$^{13}C_{12}$-PeCDF	2	351.9000	349.9029
PeCDD	2	355.8546	353.8576
$^{13}C_{12}$-PeCDD	2	367.8949	369.8919
HxCDF	3	373.8207	375.8178
$^{13}C_{12}$-HxCDF	3	385.8610	387.8580
HxCDD	3	389.8156	391.8127
$^{13}C_{12}$-HxCDD	3	401.8559	403.8530
HpCDF	4	407.7817	409.7788
$^{13}C_{12}$-HpCDF	4	419.8220	421.8191
HpCDD	4	423.7766	425.7737
$^{13}C_{12}$-HpCDD	4	435.8169	437.8140
OCDF	5	443.7498	445.7369
$^{13}C_{12}$-OCDF	5	455.7801	453.7831
OCDD	5	459.7348	457.7377
$^{13}C_{12}$-OCDD	5	471.7750	473.7721

Sample Introduction:	Capillary Column, Splitless Injection.
Ionization:	Electron Impact, 70eV, 1mA Emission Current.
Source Pressure:	1×10^{-5} torr.
Ionizer Temperature:	250° C.
Mass Resolution:	5000, 10% valley.
Scan Rate:	1 MIS cycle per second.
GC Column:	30 m DB-5, 60 m SP2330
Linear Velocity:	35 cm/sec Helium.
Temperature Program:	180° C (hold 1 min); 13°/min to 200°; 3°/min to 270°; 270° hold 4 min.

Mass windows are monitored sequentially during the temperature programs with the windows defined by the elution of standards.

* Quant. = Quantifcation ion; Confir. = Confirmation ion.

V. Quality Assurance/Quality Control (QA/QC)

A. General Procedures of Operation

1. Analysis of Samples: Samples are analyzed in sets of twelve consisting of:

   a. Blank: Method Blank (extraction apparatus) is prepared in the laboratory and subjected to the same sample preparation procedures as environmental samples. The Method Blank is used in every sample set.

   b. Fortified Matrix: Native analytes (100 uL) (Table 8) are added to a blank sample matrix. The levels of fortification of native analytes in the matrix spike will be above the target detection limit to provide an estimate of the method's sensitivity, and for determination of percent accuracy of quantification. This sample may be substituted with a reference sample that has been analyzed at least three times and a mean value of contamination has been established.

   c. Detection Limit Verification Sample: An environmental sample with nondetectable amounts of native analyte (determined from a previous analysis) will be spiked with native analytes (Table 8) and analyzed with the next sample set. The addition of the QA/QC sample will be done for only the first three sample sets of any matrix type to establish that the calculated MLD is achievable. If analytical results show difficulty in obtaining the MLD, then this QA/QC sample must be in each set. If no problem is experienced, then this QA/QC sample may be dropped.

Table 8: Native PCDD/PCDF spiking solution (100 uL)

Compound	Concentration (pg/uL Tridecane)		
	Solution A	Solution B	Solution C
2,3,7,8-TCDD	0.50	1.00	1.50
2,3,7,8-TCDF	0.50	1.00	1.50
1,2,3,7,8-PeCDD	0.50	1.00	1.50
1,2,3,7,8-PeCDF	0.50	1.00	1.50
2,3,4,7,8-PeCDF	0.50	1.00	1.50
1,2,3,4,7,8-HxCDD	1.25	2.50	3.75
1,2,3,6,7,8-HxCDD	1.25	2.50	3.75
1,2,3,7,8,9-HxCDD	1.25	2.50	3.75
1,2,3,4,7,8-HxCDF	1.25	2.50	3.75
1,2,3,6,7,8-HxCDF	1.25	2.50	3.75
2,3,4,6,7,8-HxCDF	1.25	2.50	3.75
1,2,3,7,8,9-HxCDF	1.25	2.50	3.75
1,2,3,4,6,7,8-HpCDD	1.25	2.50	3.75
1,2,3,4,6,7,8-HpCDF	1.25	2.50	3.75
OCDD	2.50	5.00	7.50
OCDF	2.50	5.00	7.50

d. Duplicate Sample: Two separate portions of the same environmental sample are processed and analyzed.

e. Environmental Samples: The total number of environmental samples analyzed is eight if the Detection Limit Verification sample is used; otherwise nine samples are analyzed.

2. Sample Tracking and Labeling of Samples:

a. Logging Incoming Samples: ERL-D completes the chain of custody forms and informs the Sample Control Center (SCC) that samples arrived safely or informs SCC of any problems with the samples. Each sample received by ERL-D had previously been assigned two numbers by the Sample Control Center, the Sample Control Center number (SCC#) and an Episode number. The SCC# number is unique for each sample and provides

a means for tracking a given sample throughout its analysis and its permanent storage at the locker plant. The samples are placed into freezer A upon arrival at ERL-Duluth, homogenized, (see II.A.), and an aliquot (100-500 g) is placed into freezer B. After the samples are extracted they are put into freezer C. If all the data meets QA requirements after mass spectral analysis and quantification, the samples are transferred to a locker plant for permanent storage (-20° C).

b. Logging and Labeling Samples During Preparation: A laboratory identification code (lab ID) is randomly assigned to each sample in a set of twelve at the start of sample preparation. The code consists of a letter, A through L, date of extraction, and two initials of the sample preparation chemist, (e.g. A091587ML). This code is used to identify the sample throughout the analysis period. The SCC#, lab ID, sample description, weight of sample, and amount of analytical standards added to each sample are recorded in the sample preparation log book at the start of extraction. The lab ID is written on labeling tape which is transferred from beaker to flask during sample preparation. The lab ID is written into the MS log book along with the mass spectra analysis number.

3. Data System Sample Tracking: ERL-D has developed the National Dioxin Study (NDS) Phase II, Bioaccumulative Pollutants in Fish: Sample Tracking Database to facilitate record keeping and summary report generation for each sample on the DEC-VAX 11/785 (Digital Equipment Corporation). For each sample, including QA samples, information pertinent to each sample is entered into the

database. Quantification data (final concentration, ion ratios, percent recovery, MLDs, and signal to noise) are automatically uploaded to the database once all QA criteria have been met. Figure 1 is an example of the NDS database.

The first two letters of the SCC number indicate whether the sample is an Environmental, Method or Matrix Blank, Duplicate Sample or a mass spectral confirmation analysis of an environmental sample. All environmental samples begin with the letter D, or S if it is a mass spectral confirmation analysis of a previously analyzed environmental sample. The Blank and Duplicate samples begin with the letter Q followed by a D or an R for duplicate or reference fish sample, respectively. Table 9 lists the possible codes for the SCC number, and matrix type. Episode numbers for Blanks and Fortified Matrix samples are entered as 0000.

Figure 1: Database Format for Sample Information.

NDS Phase II: Bioaccumulative Pollutants in Fish:
Sample Tracking System ERL-D loc:25

EPISODE #: 0000 SCC #: QR071486
Sampling Information:
Sampling Office:
State & City:
Sampling Contact:
Date Sampled: 0/ 0/ 0
Site Location:
Latitude: N 0 0' 0" Longitude: W 0 0' 0"
Analysis Lab: D Date Received: 0/ 0/ 0
Matrix Type: R Rerun: 0

Analytical:	PCDD/PCDF	Pesticide & Industrial Chemicals
Extraction Date:	7/14/86	0/ 0/ 0
GC/MS ID:	MAT86824	
LAB ID:	K071486LH	
Weight:	20.00	0.00
% Lipid:	5.2	0.0

Mass Lipid on GPC: 0.00

Comments: Reference fish 86

NDS Phase II: Bioaccumulative Pollutants in Fish

EPISODE #: 0000 SCC #: QR071486 ERL-D Loc: 25

DATA FOR BIOSIGNIFICANT POLYCHLORINATED DIBENZODIOXINS AND FURANS:

Analyte	CAS NO.	I/R	S/N	%REC	DL	Amount(pg/g)
2,3,7,8-TCDF	51207-31-9	0.74	55.75	62	0.0000	5.26
2,3,6,7-TCDF		1.00	8.28	62	0.9726	ND
3,4,6,7-TCDF		1.71	16.56	62	0.4863	ND
2,3,7,8-TCDD	1746-01-6	0.78	40.75	73	0.0000	15.63
1,2,3,7,8-PeCDF	57117-41-6	1.33	16.72	54	1.0892	ND
2,3,4,7,8-PeCDF	57117-31-6	1.10	11.15	54	1.6357	ND
2,3,4,6,7-PeCDF	70648-29-9	0.00	8.36	54	2.1784	ND
1,2,3,7,8-PeCDD	40321-76-4	0.25	4.24	57	4.0729	ND
1,2,3,4,6,7-HxCDF *						
1,2,3,4,7,8-HxCDF	70648-26-9	0.00	57.03	47	0.7327	ND
1,2,3,6,7,8-HxCDF	57117-44-9	0.67	28.52	47	1.4654	ND
2,3,4,6,7,8-HxCDF	60851-34-5	1.25	57.03	47	0.7327	ND
1,2,3,7,8,9-HxCDF	72918-21-9	0.00	57.03	47	0.7327	ND
1,2,3,4,7,8-HxCDD	32598-13-3	0.00	29.08	49	1.3863	ND
1,2,3,6,7,8-HxCDD	57753-85-7	1.31	4.67	49	0.0000	3.23
1,2,3,7,8,9-HxCDD	19408-74-3	0.00	29.08	49	1.3863	ND
1,2,3,4,6,7,8-HpCDF	67562-39-4	0.62	18.97	39	0.0000	ND
1,2,3,4,7,8,9-HpCDF	55673-89-7	0.00	37.94	39	0.0000	ND
1,2,3,4,6,7,8-HpCDD	37871-00-4	1.13	10.50	39	0.0000	5.93

* Coelutes with 1,2,3,4,6,7-HxCDF on a DB5.

I/R = Ion Ratio; S/N = Signal to Noise; DL = Detection Limit

Table 9: Codes for the SCC Number and Matrix Type

SCC number first letter options:

D -- Environmental samples
Q -- QA samples
S -- MS confirmation analysis

Second letter options for Environmental Samples

A - Region 1	G - Region 7
B - Region 2	H - Region 8
C - Region 3	Y - Region 9
D - Region 4	J - Region 10
E - Region 5	T - All regional data
F - Region 6	

Second letter options for QA samples:

B - Method or matrix blank
D - Labrotory duplicate
R - Reference fish or fortified matrix

Matrix Type:

PF - Predator Fillet
WB - Whole Bottom
WP - Whole Predator
BF - Bottom Fillet
R - Reference
Y - Blank
L - Laboratory Duplicate

B. Instrumental Quality Control

1. Gas Chromatograph

a. Operation and Maintenance: Operation and maintenance of the gas chromatograph will be done according to manufacturer's recommendations.

b. Column Performance: GC column performance will be evaluated by:

i. Resolution of 1,2,3,4-TCDD from 2,3,7,8-TCDD (Table 10).

ii. The R^2 value of the regression of the sample relative retention time of all biosignificant PCDD/PCDF, to the library relative retention should not be less than 0.995.

iii. Elution of all PCDD/PCDF during analysis from a GC window defining solution of select PCDD/PCDF (Table 11).

Table 10: GC Column Performance Quality Control

Resolution of 1,2,3,4-TCDD from 2,3,7,8-TCDD will be used to evaluate general column performance. Resolution (R) must be 0.75 or greater.

$$R = \frac{2d}{W_1 + W_2}$$

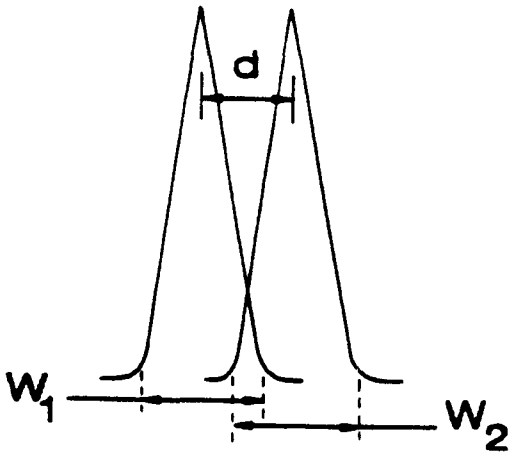


Table 11: GC Elution Window Defining Solutions for DB-5 Column

Congener Group	First Eluting	Last Eluting
TCDD	1,3,6,8	1,2,8,9
TCDF	1,3,6,8	1,2,8,9
PeCDD	1,2,4,7,9 /1,2,4,6,8	1,2,3,8,9
PeCDF	1,3,4,6,8	1,2,7,8,9
HxCDD	1,2,4,6,7,9 / 1,2,4,6,8,9	1,2,3,4,6,7
HxCDF	1,2,3,4,6,8	1,2,3,4,8,9
HpCDD	1,2,3,4,6,7,9	1,2,3,4,6,7,8
HpCDF	1,2,3,4,6,7,8	1,2,3,4,7,8,9

2. Mass Spectral Performance: The performance of the mass spectrometer is evaluated for resolution, sensitivity and linearity. The mass resolution used for these analyses is set at a minimum of 5000 (10% valley definition). The mass spectrometer is tuned each day to the required resolution according to the procedures established by the instrument manufacturer. Sensitivity and linearity is evaluated by the use of calibration standards varying in concentration (Table 4). A calibration curve is established for each standard. The curve must be linear over the range of concentrations used in the calibration standards. The percent relative standard deviations for the mean response factors must be less than 20 percent.

C. Evaluation of Data:

1. Accuracy: Accuracy, the degree to which the analytical measurement reflects the true level present, will be evaluated in two ways for each sample set. These are: the difference of measurement of a PCDD/PCDF isomer added to a blank matrix, or difference of measurement of a PCDD/PCDF from the level in an established reference material; and the efficiency for recovery

of the internal standard added for each congener group. The QA requirements for accuracy and method efficiency are provided in Table 12. Percent Accuracy and Percent Method Efficiency are defined as follows:

$$\% \text{ accuracy} = \frac{\text{measured value}}{\text{amount native isomer added to blank matrix}} \times 100$$

$$\% \text{ Method efficiency} = \frac{\text{measured value}}{\text{amount internal standard added to each sample}} \times 100$$

Table 12: Quality Assurance Parameters

	Ion Ratio	Method* Efficiency	Accuracy* at 10 pg/g	Precision** at 10 pg/g	S/N Minimum
TCDD	0.76± 15%	>40%, <120%	±50%	±50%	3.0
PCDD	0.61± 15%	>40%, <120%	±50%	±50%	3.0
HxCDD	1.23± 15%	>40%, <120%	±100%	±100%	3.0
HpCDD	1.02± 15%	>40%, <120%	±100%	±100%	3.0
OCDD	0.88± 15%	>40%, <120%	±200%	±100%	3.0
TCDF	0.76± 15%	>40%, <120%	±50%	±50%	3.0
PCDF	1.53± 15%	>40%, <120%	±50%	±50%	3.0
HxCDF	1.23± 15%	>40%, <120%	±100%	±100%	3.0
HpCDF	1.02± 15%	>40%, <120%	±200%	±200%	3.0
OCDF	1.53± 15%	>40%, <120%	±200%	±200%	3.0

* Variance of measured value from actual.
** Variance of difference of duplicates from mean.

2. Precision: Precision, a measure of mutual agreement among individual measurements of the same pollutant in replicate samples, is evaluated for each sample set by the ratio of the difference of duplicate values to their mean value. Table 12 provides QA requirements for precision. Precision is determined only when both values are above the detection limit.

Precision is defined as follows:

$$\text{Precision} = \frac{\text{difference between duplicate samples}}{\text{mean value for the duplicates}} \times 100$$

3. Signal Quality: The quality of the mass spectral signals used for qualitative and quantitative analysis is evaluated using two parameters: the ion intensity ratio for the two ions monitored in each congener group, and the signal to noise (S/N) ratio. Table 12 provides QA requirements for signal quality. In addition, qualitative identification will be based on coelution with the stable isotope labeled compound, or relative retention time correlation (Tables 5 and 6).

4. Polar Gas Chromatographic Confirmation Analysis: Ten percent of the sample extracts analyzed are seleceted for GC/MS confirmation analysis on the more polar SP2330 column, (Supelco, Belafonte, PA). Samples which were positive for 2,3,7,8-TCDD were selected for analysis.

D. Quality Assurance Problems and Corrective Actions:

Problem	Corrective Action
MS performance outside QA	Adjust MS parameters for resolution, rerun initial curve and reanalyze sample(s).
GC column performance outside QA.	Reanalyze standards and samples on modified or alternate column.
Method efficiency outside of QA.	If 2378-TCDD method efficiency <40%, reanalyze sample set. If method efficiency <40% for analytes other than 2378-TCDD, flag and report data.
Accuracy outside of QA for spiked matrix. Precision of duplicates outside QA.	If more than 20% of the analytes are outside of QA for accuracy and precision, reanalyze the sample set.
Detection of analyte in blank for 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PCDD	Reextract and reanalyze all samples for which the level of contamination, or MLD, is < 2.5 x blank level.
For other analytes in blank	Record blank concentration in comment field of samples.
Analyte exceeds calibration standard range.	Measure method efficiency. Dilute sample 100:1 respike with each standard solution (A and B), adjust volume and reanalyze.
Method efficiency for blank outside of QA or blank lost	Reextract and reanalyze all positives in set.

Because of the complexity of these analyses types, it is not expected that all analytes will meet all QA criteria. Therefore, a complete review of the data by a chemist is essential. Responsibility for the evaluation of data is that of the sample preparation chemist and the mass spectrometer operator. Review of the data, including QA, and resolution of data quality problems is the responsibility of the Principal Investigator/Program Manager Resolution of data questions may require reanalysis of samples to include the addition of confirmatory ions or analysis on different types of GC columns.

VI. Quantification Procedures

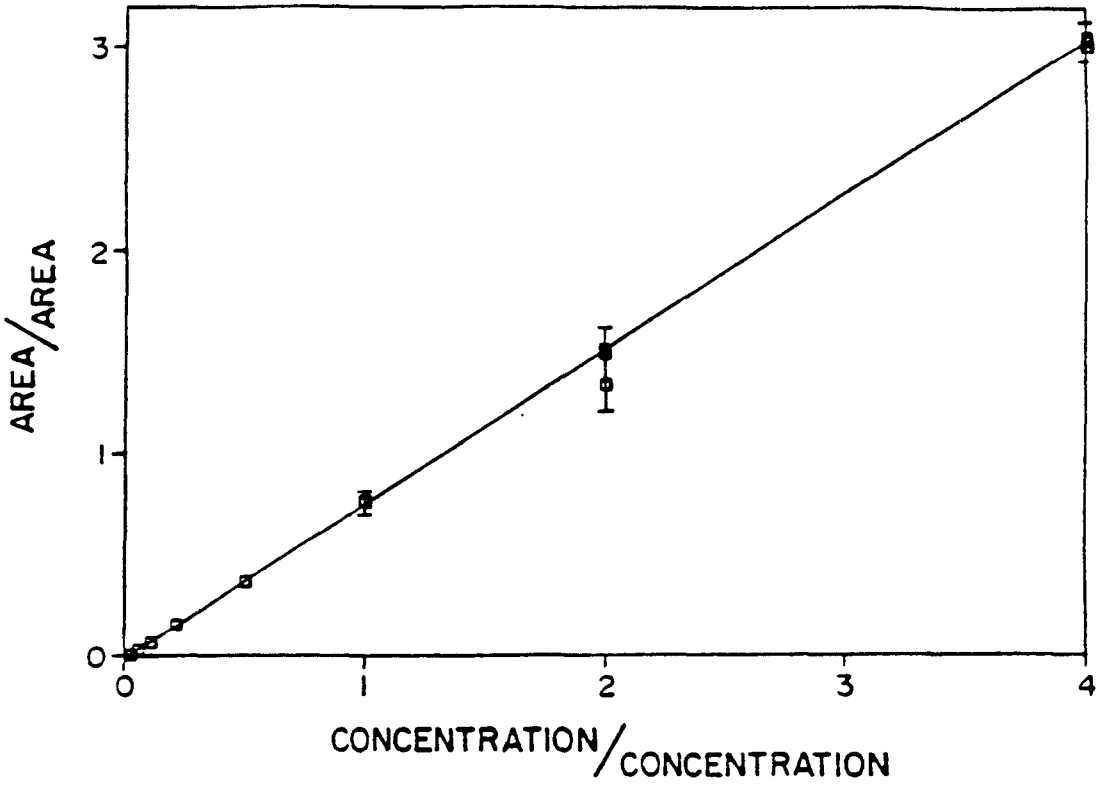
Quantification of analytes is accomplished by assigning isomer identification, integrating the area of mass specific GC peaks, and calculating an analyte concentration based upon an ion relative response factor between the analyte and standard.

A. Initial and Daily Calibration of the HRMS: An initial calibration of the instrument will be performed as needed. This will include making three replicate injections of each calibration standard (Table 4). Weighted least-squares linear regression is used to generate a calibration curve for each analyte. The weighting factor is inversely proportional to the variance among the replicate injections of each calibration standard. The slope of the regression line is the response factor used to quantify the analyte. At least two calibration standards are injected daily to insure that any response factors used for quantification and recovery calculations do not deviate from the initial calibration by more than 20 percent. If the daily calibration generates values outside this margin, and less drastic corrective action does not solve the problem, a new set of initial calibration curves is generated and the old response factor libraries discarded. An example of a typical calibration curve, using 2,3,7,8-TCDD as an example, is shown in Figure 2.

Figure 2

2,3,7,8-TCDD

WEIGHTED CALIBRATION CURVE



SLOPE = RESPONSE FACTOR

B. Signal Quality

1. Minimum Level of Detection (MLD): Minimum Level of Detection is defined as the concentration predicted from the ratio of baseline noise area to labeled standard area, plus three times the standard error of the estimate derived from the initial calibration curve for the analyte of interest.

Initial Calibration Based Method of MLD: MLD is estimated from the ratio of the noise area to the isotopically labeled internal standard area, plus three times the standard error of the estimate (SE) for the area ratio, or Y-axis, of the initial calibration curve. The Y-intercept (INT) is subtracted from this quantity, in keeping with the normal formalism for "inverse prediction" of a point on the X, or concentration ratio axis, from a point on the Y, or signal ratio axis. The SE term is derived from an analysis of variance (ANOVA) performed during the weighted least squares fit of the initial calibration curve. This term represents the random error in the replicate injections used to generate the calibration curve, the error not accounted for by the linear model. The weighting is necessary because of the relation often observed in instrumental analysis, of increasing variance with increasing concentration. MLD, according to this scheme, is defined below:

$$MLD = \frac{[(N_A/I334) + (3 \times SE) - INT] \times C334}{RF(N/I334) \times K}$$

where: N_A = noise area in the window for the major ion of the native analyte,

I334 = labeled internal standard peak area in the sample,

INT = the Y-axis intercept on the initial calibration curve,

C334 = labeled internal standard concentration,

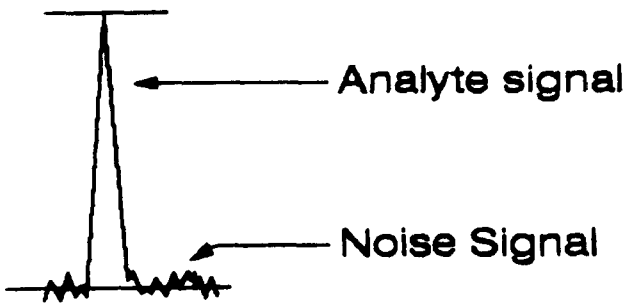
K = constant to adjust for sample size and final volume,

RF(N/I334) = response factor for major native ion to $^{13}C_{12}$ 1,2,3,4-TCDD ion, the slope of the initial calibration curve,

SE = standard error of the estimate of the initial calibration curve.

In addition, fish tissue is spiked with surrogate analytes (see Internal Standard Solution B, Table 3) prior to extraction. The surrogate analytes serve as an added check to insure that MLD values calculated from the initial calibration curve, as discussed above, are reasonable.

2. Signal to Noise (S/N): The method of determining the signal to noise ratio is shown below.



$$S/N = \frac{\text{Analyte Signal Peak Area}}{\text{Noise Signal Peak Area}}$$

$$S/N = \frac{\text{Analyte Signal Peak Area}}{\text{Noise Signal Peak Area}}$$

The noise area is calculated by integrating over a peak width equivalent to the analyte signal, typically about 10 seconds.

C. Quantification of PCDD/PCDF: The concentration of a natural PCDD/PCDF is determined by calculating a response factor between PCDD/PCDF and the stable isotope labeled PCDD/PCDF for the congener group. Calculations are performed as follows:

Standard:

$$RF(N/L) = \frac{A_N \times C_L}{A_L \times C_N}$$

Sample:

$$V_N = \frac{A_N \times S_L}{A_L \times RF(N/L)}$$

where: $RF(N/L)$ = response factor native to labeled,

A_N = peak area native,

A_L = peak area labeled,

C_N = concentration of native standard,

C_L = concentration of labeled standard,

S_L = labeled spiking level in sample,

V_N = level of native analyte in sample.

D. Method Efficiency: The method efficiency for the recovery of stable isotope labeled compounds is determined by calculating the amount of stable isotope labeled compound in the final extract and dividing by the amount spiked into the sample at the start of the cleanup procedure. This is done by determining the relative response factor between the Internal Standard Solution C, $^{13}C_{12}$ 1,2,3,4-TCDD and the stable isotope labeled internal standard (Solution A).

Determine Response Factor:

$$RF = \frac{A_L \times C_{IS}}{A_{IS} \times C_L}$$

where: RF = response factor,

A_L = area of stable isotope labeled internal standard, (solution A),

A_{IS} = area of 13C12 1,2,3,4-TCDD,

C_L = concentration of stable isotope labeled internal standard, (solution A),

C_{IS} = concentration of 13C12 1,2,3,4-TCDD.

The response factor is then used in calculating the concentration of the internal standard in the final solution,

$$C_L = \frac{A_L \times C_{IS}}{A_{IS} \times RF}$$

where: C_L = concentration of stable isotope labeled internal standard, (solution A).

The concentration in the final solution times the final volume equals the total amount present. The method efficiency is then calculated by:

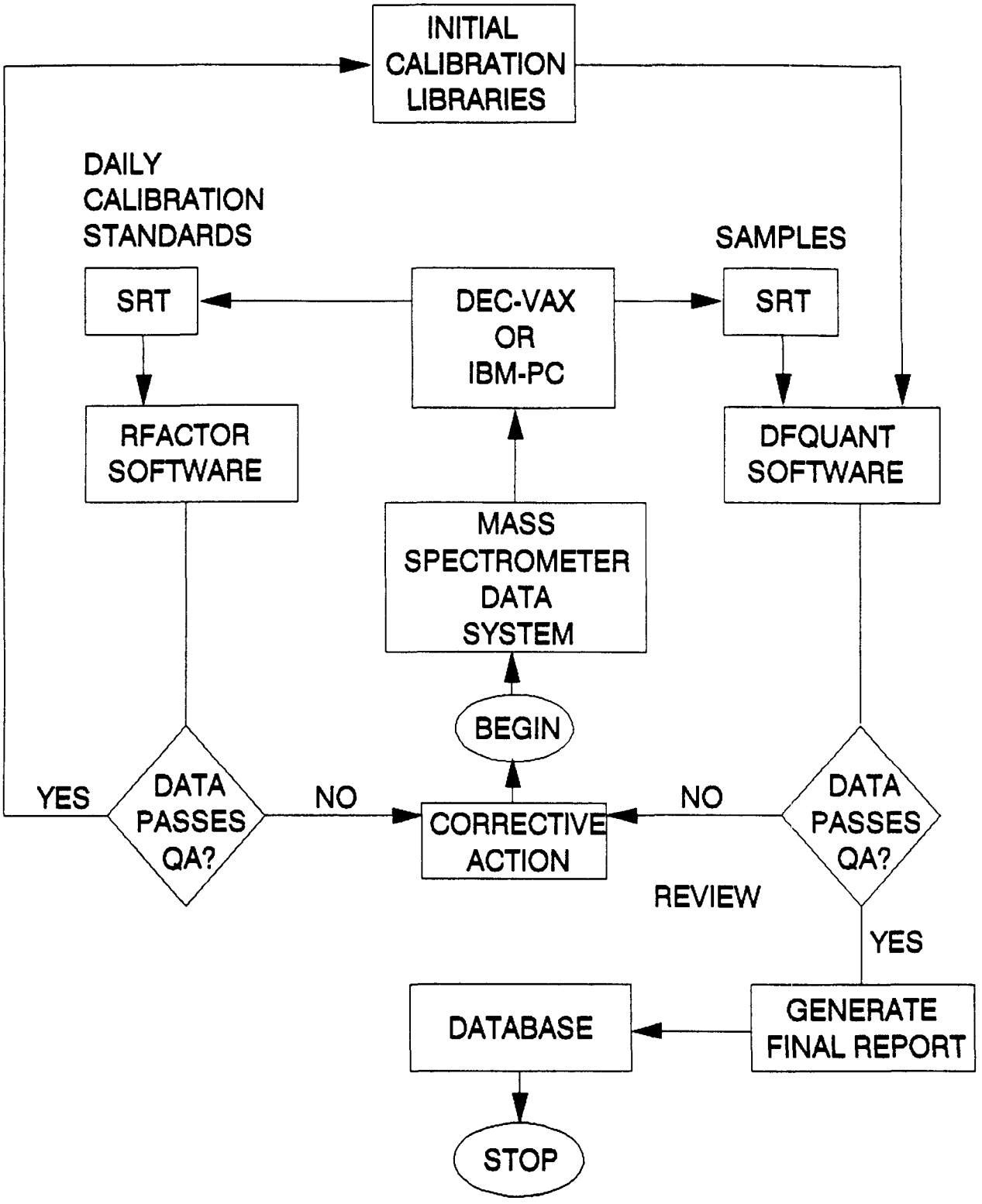
$$\% \text{ Recovery} = \frac{\text{CL found}}{\text{CL spiked}} \times 100$$

E. Integration of Automated Data Processing and Quality Assurance:

QA parameters for method efficiency, ion ratios, retention time correlations, signal/noise ratio, accuracy and precision are monitored with the aid of software either developed in-house, or modified from existing programs included with the HRMS data system. Raw data is sorted and edited using the mass spectrometer's dedicated data system, transferred to the DEC-VAX system and processed using software programs RFACTOR and DFQUANT (Figure 3.). Data is reviewed by the Project Director before entering into the NDS data base.

Figure 3

DATA REDUCTION FOR PCDD/PCDF
NATIONAL DIOXIN STUDY



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