Division of Science and Research

Dioxins in Tissues from Crabs from the Raritan/Newark Bay Systems

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TITLE: Dioxins in Tissues from Crabs from the Raritan/Newark Bay System

Submitted to

Dr. Robert Tucker
Division of Science and Research
New Jersey Department of Environmental Protection
401 East State Street
CN - 409
Trenton, New Jersey 08625

Prof. Angela Cristini
Biology Department
Ramapo College of New Jersey
505 Ramapo Valley Road
Mahwah, N.J. 07430

Prof. Michael Gross
Midwest Center for Mass Spectrometry
Department of Chemistry
University of Nebraska
Lincoln, NE 68588-0304

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MISSION STATEMENT

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For further information contact:

NJ Department of Environmental Protection and Energy
Division of Science and Research
CN 409
Trenton, NJ 08625-0409

(609) 984-6070

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INTRODUCTION

Many researchers have reported the presence of dioxins in environmental samples throughout the world. Data collected by the New Jersey Department of Environmental Protection has demonstrated that the tissues of the blue crab, Callinectes sapidus, and the american lobster, Homarus americanus, collected from Northern New Jersey waters contained elevated levels of several dioxins and dibenzofurans. The results of these studies suggest that additional data should be collected on the levels of these chemicals in the tissues of these species from the Raritan/Newark Bay system. These data will be used to help the DEP and the DOH set possible consumption guidelines for the crustacea from this ecosystem.

The specific goals of this project were to: (1) collect C.sapidus and H. americanus from 4 stations in the Raritan/Newark Bay system in the late Spring, Fall, and Winter of 1991 - 92 (2) dissect, archive, and ship the muscle and hepatopancreas tissues from these animals to MCMS (3) perform isomer specific analysis for PCDD/Fs in these samples. No lobsters were caught during any of the collecting trips at any of the stations, therefore the study was restricted to C. sapidus. The animals were collected using the resources of the New Jersey Marine Sciences Consortium. The tissues were analyzed by HRGC/HRMS using the Carlo-Erba GC/Kratos MS-50 system at MCMS.

This report describes analytical results of crab tissue samples collected from four stations in the Raritan/Newark Bay system during September 1991 and June 1992. The samples were analyzed for 17 selected 2,3,7,8-substituted polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans (PCDD/Fs). This report consists of five sections: 1) Sampling Methods, Analytical Results and Discussion; 2) Sample Preparation and Analysis; 3) Quality Assurance and Quality Control (QA/QC) for sample analysis; 4) Terminology, abbreviations and symbols employed in this report (in the appendix); 5) References.

SAMPLING METHODS, ANALYTICAL RESULTS AND DISCUSSION

1.1 Methods

Our research group executed an experimental design which maximized the efforts used for sampling and tissue preparation. We collected and saved tissue samples from more than twice the number of animals that were chemically analyzed. Therefore, if the results indicate that further samples will be needed to establish firm consumption guidelines no additional sampling must be carried out. In addition, this design allows for a seasonal "snap shot" of the contaminants in the tissues of this specie that has the possibility of being expanded using data from animals collected in the same time frame.

Discussions with Mr. J. Nickels from the NJMSC and Mr. P. Hauge from the NJDEPE resulted in the establishment of the following four stations: Station 1 - Sandy Hook Bay (40o26.38 min N, 74o00.90 min W); Station 2 - East Reach of Raritan Bay (40o28.99 min N, 74o04.61 min W); Station 3 - Newark Bay (40o42.08 min N, 74o07.56 min W); Station 4 - Wards Point (40o28.66 min N, 74o13.82 min W) (Map 1). Adult, legal size blue crabs were collected by Dr. Cristini and the NJMSC personnel using an otter trawl or a crab rake. Animals were collected on September, 1991; March, 1992; and June 1992.

As soon as the crabs were removed from the net they were placed in coolers containing crushed ice. They were stored on ice overnight; the next day at Ramapo College the crabs were sorted by size and sex, placed in labeled plastic bags while still alive and frozen in a large freezer at -80 0C. All of the crabs collected during the March sampling were archived and remain frozen at -80 0C at Ramapo college. Ten to fourteen crabs from each station were chosen for chemical analysis the remainder of the animals remain in the freezer at Ramapo college.

Before dissection the animals were measured, weighed, and staged for their position in the molt cycle. The muscle and hepatopancreas of 5 - 7 similar sized animals of the same sex were each dissected from the animal while still frozen and placed in new glass scintillation vials with foil lined caps that had been rinsed with methanol, acetone and methylene chloride. The ovaries of the female crabs were also removed and placed in scintillation vials for future analysis. The vials were kept in an ice bath during the dissection; after dissection the weight of the tissue contained in each vial was determined and the vials were stored at -80 C. The samples were sent to MCMS packed in dry ice.

1.2 Analytical Results and Discussion

Muscles and pancreas samples taken from the Newark Bay system were extracted and analyzed for selected 2,3,7,8-substituted PCDD/Fs. The method used was mass profile monitoring by gas chromatography/high resolution mass spectrometry (GC/HRMS). The notable results, the levels of 2,3,7,8-TCDD/Fs in all pancreas samples, are listed in Tables 1a and 1b. All other 2,3,7,8-PCDD/Fs were not detected or at levels less than 100 parts-per-trillion (ppt).

Tables 1a and 1b also give the levels of total TCDD/Fs. Three to six isomers of TCDF were detected, in addition to 2,3,7,8-TCDF, but their identities were not determined. One other isomer of TCDD, in addition to 2,3,7,8-TCDD, was also detected but not identified. The exact mass data and isotope ratio of the selected confirmation ions shown in mass profile are consistent with the assignments the compounds are TCDD/F isomers. The relative response factors of 2,3,7,8-TCDD and 2,3,7,8-TCDF were used for the quantification of the isomers.

Tables 2a through 5a and Tables 2b through 5b show the analytical results including concentration levels, detection limits, and internal standard recoveries for all selected 2,3,7,8-PCDD/Fs, respectively, in the crab tissue samples collected in September 1991 and in June 1992.

More detailed data for the analyses are presented in Table 8. These additional data include the criteria for the identification of the PCDD/Fs. Some typical QA/QC data are given in Table 10 and Table 11 as an indication of the reproducibility and integrity of the method.

The pancreas samples from station 3 have the highest concentration level, followed by station 4, station 2, and station 1. However, 2,3,7,8-TCDD/Fs were only detected in the muscle samples from station 3, and the levels are less than 50 ppt. The concentrations of the PCDD/Fs in sample composite 1 (male animal) are higher than those in composite 2 (female animal). The animals collected in September (1991) have higher dioxins contamination than those collected in June (1992).

Sulfur analogues of TCDF and P5CDF were also discovered in all samples containing 2,3,7,8-TCDD/Fs. The levels of the TCDF analogue are more than 5 times that of 2,3,7,8-TCDF if the response factor of 2,3,7,8-TCDF is used for the quantification.

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The results of the chemical analysis indicate that the levels of dioxin in both the hepatopancreas and the muscle tissues from crabs from Newark Bay are elevated well above the suggested FDA guidelines of 25ppt. The values are very similar to the levels found in crabs from this area in the 1982 DEPE study. These data strongly suggest that the ban on consumption of this species from these waters be continued. Personel observations indicate that there are a large number of people that catch blue crabs for consumption from Newark Bay and there are few signs to warn them of the consumption ban. This situation should be corrected in order to afford the maximum health protection to citizens of New Jersey.

The levels of dioxin in the hepatopancreas of all the animals from all of the stations in Raritan Bay are well above the suggested FDA guideline although the values for the muscle tissues were below 25ppt. These data indicate the necessity for a consumption advisory including directions for removal of the hepatopancreas before cooking. The advisory and cooking instructions should be well publicised because of the number of people that catch and consume crabs from Raritan Bay.

The data also indicate seasonal differences in the dioxin levels of the hepatopancreas; higher values appear to occur in September. This could possibly be the result of the "feeding state" of the animals; in September the crabs have been active and feeding for the 3-4 months before capture, in June the crabs have just begun to feed after inactivity because of the lower winter water temperatures. The present study collected crabs from all stations in March 1992; these animals are archived at Ramapo College. The tissues from these animals should be annalized for dioxin in order to complete the seasonal profile for this species. These values, from crabs that were not feeding when captured, would help to clarify the relationship between feeding and tissue levels of dioxin. These data are important, particularly since there is a commercial fishery for this species during the winter months.

In addition, the ovaries and egg masses from female crabs have been archived at Ramapo College. These tissues should also be analyzed for selected 2,3,7,8-substituted PCDD/F's in order to establish the levels of dioxin that are being passed to the next generation of C. sapidus that will be recruted as larvae into this and other estuaries along coastal New Jersey.

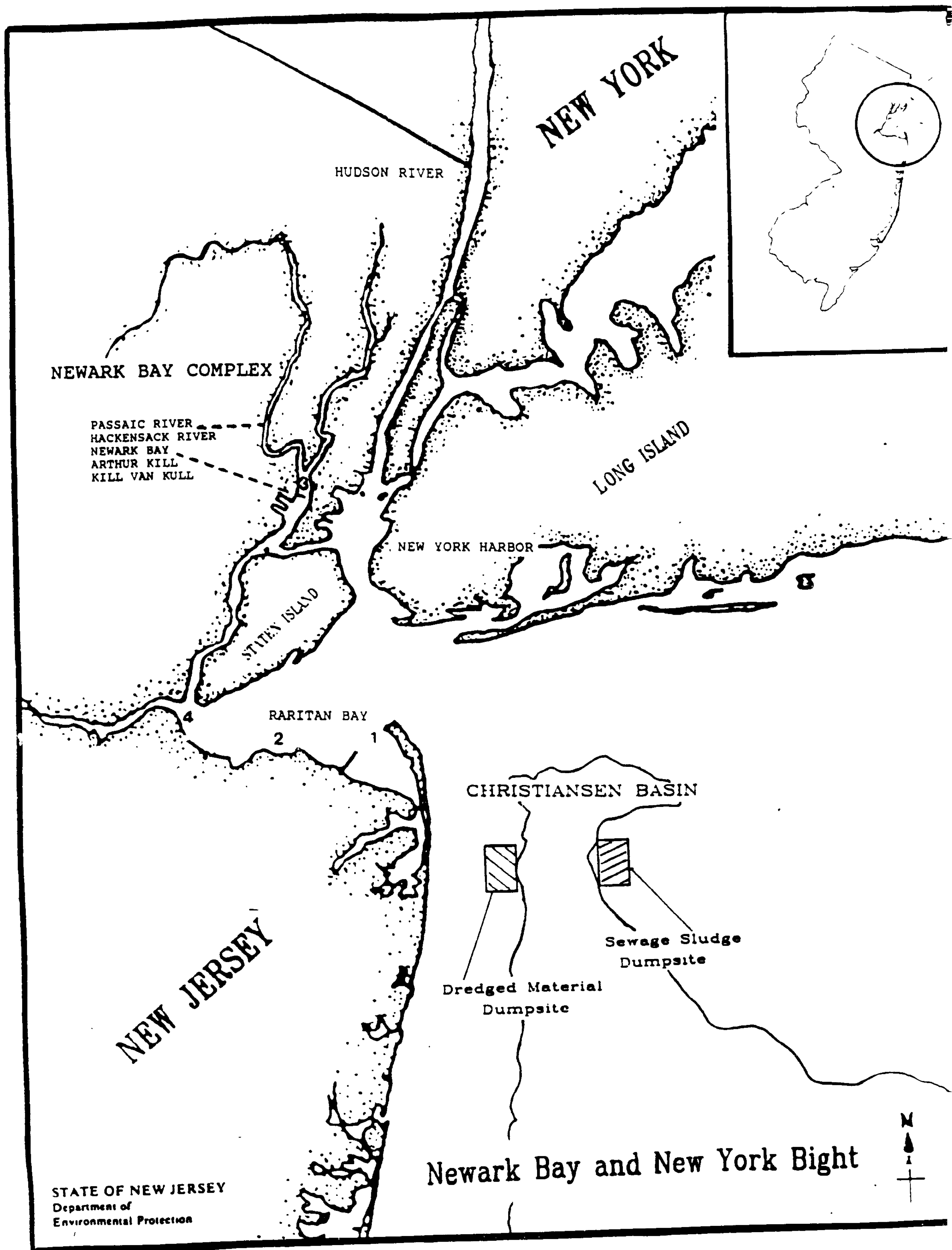

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Table 1a: Concentration of 2,3,7,8-TCDD/Fs and Total TCDD/Fs in the Crab Tissue Samples.

Samples Collected Date: September 1991
Concentration: ppt

Sample ID	Station	Composite	tissue	2378-TCDD	Total TCDD	2378-TCDF	Total TCDF
NJCL9E	1	1	m	ND (6)	ND	ND (8)	ND
NJCL9D	1	2	m	ND (5)	ND	ND (7)	ND
NJCL7A	2	1	m	ND (5)	ND	ND (14)	ND
NJCL7B	2	2	m	ND (10)	ND	ND (15)	ND
NJCL8E	3	1	m	45	45	15	65
NJCL8D	3	2	m	40	40	ND (8)	35
NJCL8F	4	1	m	ND (12)	ND	ND (10)	ND
*NJCL8G**	4	*1*	*m*	*ND (16)*	*ND*	*ND (14)*	*ND*
NJCL9F	4	2	m	ND (7)	ND	ND (5)	ND
*NJCL9G**	4	*2*	*m*	*ND (8)*	*ND*	*ND (7)*	*ND*
NJCL9A	1	1	p	50	90	100	360
NJCL8A	1	2	p	40	40	75	215
*NJCL12A**	1	*2*	*p*	*35*	*35*	*90*	*260*
NJCL7C	2	1	p	90	130	150	510
NJCL7D	2	2	p	90	150	140	440
NJCL8C	3	1	p	940	1120	200	1170
NJCL8B	3	2	p	690	850	160	680
*NJCL12B**	3	*2*	*p*	*600*	*810*	*185*	*790*
NJCL9B	4	1	p	210	320	220	800
NJCL9C	4	2	p	60	110	125	400
*NJCL12C**	4	*2*	*p*	*65*	*125*	*115*	*410*

* = results for analysis of a second portion of the sample; data given in italics.
m = muscle; p = pancreas.
ND = not detected with the detection limit in parenthesis.

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Table 1b: Concentration of 2,3,7,8-TCDD/Fs and Total TCDD/Fs in the Crab Tissue Samples.

Samples Collected Date: June 1992
Concentration: ppt

Sample ID	Station	Composite	tissue	2378-TCDD	Total TCDD	2378-TCDF	Total TCDF
NJCL11A	1	1	m	ND (5)	ND	ND (5)	ND
NJCL11B	2	1	m	ND (10)	ND	ND (5)	ND
NJCL11C	2	2	m	ND (10)	ND	ND (5)	ND
NJCL11D	3	1	m	30	30	ND (6)	ND
*NJCL12D**	3	*1*	*m*	*40*	*40*	*ND (10)*	*35*
NJCL11F	3	2	m	20	ND	ND (6)	ND
NJCL11G	4	1	m	ND (10)	ND	ND (5)	ND
NJCL11H	4	2	m	ND (10)	ND	ND (15)	ND
NJCL10A	1	1	p	80	80	100	340
NJCL10B	2	1	p	70	100	110	370
NJCL10G	2	2	p	45	45	70	120
*NJCL10H**	2	*2*	*p*	*65*	*65*	*90*	*150*
NJCL10C	3	1	p	475	590	130	670
*NJCL12E**	3	*1*	*p*	*425*	*520*	*140*	*665*
NJCL10E	3	2	p	580	710	150	800
*NJCL12F**	3	*2*	*p*	*480*	*580*	*150*	*630*
NJCL10D	4	1	p	80	80	115	400
NJCL10F	4	2	p	60	60	110	300

* = results analysis of a second portion of the sample; data given in italics.
m = muscle; p = pancreas.
ND = not detected with the detection limit in parenthesis.

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Table 2a: Results of Dioxins Analysis for Crab Tissue Samples From Station #1.

Samples Collected Date: September 1991
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL9E			NJCL9D			NJCL9A			NJCL8A(12A)		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	ND	80	6	ND	70	5	50	70	6	40	45	10
12378-P5CDD*	ND	80	7	ND	75	7	ND	65	9	ND	50	15
123478-H6CDD	ND	--	6	ND	--	10	ND	--	10	ND	--	13
123678-H6CDD	ND	--	10	ND	--	10	ND	--	15	ND	--	15
123789-H6CDD*	ND	80	6	ND	60	7	ND	110	10	ND	65	13
1234678-H7CDD*	ND	70	10	ND	60	8	ND	95	8	ND	60	20
12346789-OCDD*	ND	60	31	ND	45	90	ND	50	53	ND	45	41
2378-TCDF*	ND	85	8	ND	70	7	100	80	7	75	60	10
12378-P5CDF*	ND	80	6	ND	70	8	ND	80	29	ND	55	10
23478-P5CDF	ND	--	10	ND	--	10	ND	--	10	ND	--	10
123478-H6CDF*	ND	70	9	ND	50	12	ND	60	18	ND	50	25
123678-H6CDF	ND	--	15	ND	--	20	ND	--	20	ND	--	20
123789-H6CDF	ND	--	10	ND	--	15	ND	--	20	ND	--	25
234678-H6CDF	ND	--	9	ND	--	12	ND	--	18	ND	--	25
1234678-H7CDF*	ND	70	20	ND	40	50	ND	50	21	ND	45	30
1234789-H7CDF	ND	--	20	ND	--	50	ND	--	21	ND	--	30
12346789-OCDF	ND	60	55	ND	--	52	ND	--	28	ND	--	48
Lipid Content	< 1%			< 1%			9%			8%		

MCMS ID = sample ID given at MCMS, ID in parenthesis is the ID of duplicate sample.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

Table 2b: Results of Dioxins Analysis for Crab Tissue Samples From Station #1.

Samples Collected Date: June 1992
Concentration: ppt

Samples	Muscle Composite #1						Pancreas Composite #1					
MCMS ID	NJCL11A						NJCL10A					
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	ND	80	5				80	75	7			
12378-P5CDD*	ND	75	6				ND	65	15			
123478-H6CDD	ND	--	10				ND	--	10			
123678-H6CDD	ND	--	10				ND	--	10			
123789-H6CDD*	ND	70	10				ND	75	10			
1234678-H7CDD*	ND	60	15				ND	65	15			
12346789-OCDD*	ND	60	25				ND	60	30			
2378-TCDF*	ND	80	5				100	80	5			
12378-P5CDF*	ND	80	5				ND	65	20			
23478-P5CDF	ND	--	10				ND	--	20			
123478-H6CDF*	ND	70	10				ND	70	15			
123678-H6CDF	ND	--	15				ND	--	15			
123789-H6CDF	ND	--	15				ND	--	15			
234678-H6CDF	ND	--	15				ND	--	15			
1234678-H7CDF*	ND	65	10				ND	60	20			
1234789-H7CDF	ND	--	20				ND	--	20			
12346789-OCDF	ND	60	25				ND	--	20			
Lipid Content	< 1%						8%					

MCMS ID = sample ID given at MCMS.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

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Table 3a: Results of Dioxins Analysis for Crab Tissue Samples From Station #2.

Samples Collected Date: September 1991
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL7A			NJCL7B			NJCL7C			NJCL7D		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	ND	75	5	ND	80	10	90	90	7	90	85	6
12378-P5CDD*	ND	70	8	ND	68	15	ND	70	8	ND	65	8
123478-H6CDD	ND	--	12	ND	--	18	ND	--	8	ND	--	15
123678-H6CDD	ND	--	15	ND	--	20	ND	--	10	ND	--	20
123789-H6CDD*	ND	65	12	ND	58	18	ND	56	8	ND	60	15
1234678-H7CDD*	ND	75	8	ND	75	10	ND	60	6	ND	65	10
12346789-OCDD*	ND	65	20	ND	50	35	ND	55	18	ND	50	26
2378-TCDF*	ND	85	14	ND	80	15	150	90	8	140	90	6
12378-P5CDF*	ND	75	6	ND	65	14	ND	65	15	30	70	17
23478-P5CDF	ND	--	10	ND	--	15	25	--	15	30	--	20
123478-H6CDF*	ND	65	10	ND	65	15	ND	60	10	ND	55	16
123678-H6CDF	ND	--	15	ND	--	20	ND	--	15	ND	--	20
123789-H6CDF	ND	--	20	ND	--	15	ND	--	20	ND	--	20
234678-H6CDF	ND	--	10	ND	--	15	ND	--	10	ND	--	13
1234678-H7CDF*	ND	70	10	ND	70	14	ND	60	10	ND	60	14
1234789-H7CDF	ND	--	10	ND	--	14	ND	--	12	ND	--	14
12346789-OCDF	ND	--	15	ND	--	50	ND	--	20	ND	--	27
Lipid Content	< 1%			< 1%			7%			8%		

MCMS ID = sample ID given at MCMS.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

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Table 3b: Results of Dioxins Analysis for Crab Tissue Samples From Station #2.

Samples Collected Date: June 1992
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL11B			NJCL11C			NJCL10B			NJCL10G(10H)		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	ND	70	10	ND	70	10	70	70	8	55	70	9
12378-P5CDD*	ND	75	8	ND	85	5	ND	70	12	ND	65	10
123478-H6CDD	ND	--	10	ND	--	10	ND	--	10	ND	--	15
123678-H6CDD	ND	--	10	ND	--	10	ND	--	10	ND	--	15
123789-H6CDD*	ND	70	10	ND	65	10	ND	70	10	ND	75	15
1234678-H7CDD*	ND	60	20	ND	60	15	ND	75	10	ND	65	20
12346789-OCDD*	ND	65	15	ND	60	20	ND	50	35	ND	55	40
2378-TCDF*	ND	80	5	ND	75	5	110	75	5	80	70	7
12378-P5CDF*	ND	85	5	ND	90	5	ND	65	20	ND	70	15
23478-P5CDF	ND	--	5	ND	--	15	ND	--	15	ND	--	15
123478-H6CDF*	ND	65	15	ND	70	10	ND	65	10	ND	60	20
123678-H6CDF	ND	--	15	ND	--	20	ND	--	15	ND	--	20
123789-H6CDF	ND	--	20	ND	--	10	ND	--	20	ND	--	20
234678-H6CDF	ND	--	15	ND	--	10	ND	--	10	ND	--	20
1234678-H7CDF*	ND	60	25	ND	60	15	ND	60	20	ND	55	25
1234789-H7CDF	ND	--	20	ND	--	15	ND	--	20	ND	--	25
12346789-OCDF	ND	--	40	ND	--	15	ND	--	25	ND	--	40
Lipid Content	< 1%			< 1%			6%			6%		

MCMS ID = sample ID given at MCMS, ID in parenthesis is the ID of duplicate sample.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

Table 4a: Results of Dioxins Analysis for Crab Tissue Samples From Station #3.

Samples Collected Date: September 1991
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL8E			NJCL8D			NJCL8C			NJCL8B(12B)		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	**45**	60	10	**40**	65	10	**940**	60	3	**690**	65	4
12378-P5CDD*	ND	70	7	ND	60	20	ND	55	3	ND	60	4
123478-H6CDD	ND	--	9	ND	--	25	ND	--	5	ND	--	7
123678-H6CDD	ND	--	15	ND	--	15	ND	--	10	ND	--	10
123789-H6CDD*	ND	70	10	ND	55	25	ND	60	2	ND	45	5
1234678-H7CDD*	ND	55	25	ND	60	20	ND	50	8	ND	45	7
12346789-OCDD*	ND	55	32	ND	50	40	ND	50	20	ND	50	9
2378-TCDF*	**15**	70	7	ND	70	8	**200**	80	3	**160**	70	2
12378-P5CDF*	ND	75	6	ND	65	12	**70**	85	10	**25**	62	5
23478-P5CDF	ND	--	10	ND	--	10	**90**	--	15	**15**	--	10
123478-H6CDF*	ND	65	10	ND	60	20	**95**	65	10	**45**	45	7
123678-H6CDF	ND	--	20	ND	--	20	ND	--	20	ND	--	10
123789-H6CDF	ND	--	20	ND	--	20	ND	--	20	ND	--	10
234678-H6CDF	ND	--	12	ND	--	20	ND	--	8	ND	--	9
1234678-H7CDF*	ND	60	17	ND	55	26	ND	50	20	ND	50	15
1234789-H7CDF	ND	--	17	ND	--	26	ND	--	20	ND	50	15
12346789-OCDF	ND	--	40	ND	--	45	ND	50	35	ND	--	30
Lipid Content	<1 %			< 1%			5%			6%		

MCMS ID = sample ID given at MCMS, ID in parenthesis is the ID of duplicate sample.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

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Table 4b: Results of Dioxins Analysis for Crab Tissue Samples From Station #3.

Samples Collected Date: September 1991
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL11D(12D)			NJCL11F			NJCL10C(12E)			NJCL10E(12F)		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	**35**	75	6	**20**	75	7	**450**	80	3	**530**	85	3
12378-P5CDD*	ND	80	5	ND	65	10	ND	70	5	ND	80	5
123478-H6CDD	ND	--	5	ND	--	10	ND	--	5	ND	--	10
123678-H6CDD	ND	--	5	ND	--	10	ND	--	10	ND	--	10
123789-H6CDD*	ND	70	10	ND	65	10	ND	60	10	ND	80	5
1234678-H7CDD*	ND	75	10	ND	70	15	ND	55	10	ND	70	10
12346789-OCDD*	ND	60	20	ND	70	20	ND	55	20	ND	60	10
2378-TCDF*	ND	80	6	ND	80	6	**135**	75	3	**150**	60	10
12378-P5CDF*	ND	75	5	ND	80	5	**45**	70	10	**40**	80	5
23478-P5CDF	ND	--	10	ND	--	10	**70**	--	15	**50**	--	10
123478-H6CDF*	ND	65	5	ND	80	10	**50**	60	10	**75**	45	10
123678-H6CDF	ND	--	10	ND	--	10	ND	--	10	ND	--	10
123789-H6CDF	ND	--	10	ND	--	20	ND	--	10	ND	--	10
234678-H6CDF	ND	--	10	ND	--	20	ND	--	10	ND	--	10
1234678-H7CDF*	ND	45	30	ND	65	20	ND	55	10	ND	50	15
1234789-H7CDF	ND	--	15	ND	--	20	ND	--	10	ND	50	15
12346789-OCDF	ND	--	20	ND	--	25	ND	60	15	ND	--	25
Lipid Content	< 1 %			< 1%			5%			6%		

MCMS ID = sample ID given at MCMS, ID in parenthesis is the ID of duplicate sample.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

Table 5a: Results of Dioxins Analysis for Crab Tissue Samples From Station #4.

Samples Collected Date: September 1991
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL8F(8G)			NJCL9F(9G)			NJCL9B			NJCL9C(12C)		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	ND	70	12	ND	75	7	**210**	80	6	**60**	80	6
12378-P5CDD*	ND	75	15	ND	70	5	ND	75	7	ND	70	9
123478-H6CDD	ND	--	18	ND	--	9	ND	--	10	ND	--	5
123678-H6CDD	ND	--	20	ND	--	15	ND	--	10	ND	--	10
123789-H6CDD*	ND	55	20	ND	65	9	ND	65	10	ND	75	5
1234678-H7CDD*	ND	70	11	ND	70	20	ND	60	15	ND	75	7
12346789-OCDD*	ND	65	20	ND	50	50	ND	60	30	ND	70	22
2378-TCDF*	ND	70	10	ND	75	5	**220**	85	6	**125**	85	4
12378-P5CDF*	ND	75	11	ND	75	5	**45**	80	17	**20**	95	7
23478-P5CDF	ND	--	15	ND	--	15	**50**	--	15	**25**	--	10
123478-H6CDF*	ND	60	17	ND	60	15	**20**	70	15	ND	80	8
123678-H6CDF	ND	--	20	ND	--	20	ND	--	15	ND	--	10
123789-H6CDF	ND	--	20	ND	--	20	ND	--	15	ND	--	10
234678-H6CDF	ND	--	15	ND	--	15	ND	--	10	ND	--	8
1234678-H7CDF*	ND	65	12	ND	50	25	ND	65	21	ND	80	11
1234789-H7CDF	ND	--	12	ND	--	25	ND	--	21	ND	--	11
12346789-OCDF	ND	--	30	ND	--	46	ND	--	20	ND	--	22
Lipid Content	< 1%			< 1%			8%			7%		

MCMS ID = sample ID given at MCMS, ID in parenthesis is the ID of duplicate sample.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

Table 5b: Results of Dioxins Analysis for Crab Tissue Samples From Station #4.

Samples Collected Date: June 1992
Concentration: ppt

Samples	Muscle Composite #1			Muscle Composite #2			Pancreas Composite #1			Pancreas Composite #2		
MCMS ID	NJCL11G			NJCL11H			NJCL10C			NJCL10F		
Analyte	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL	Conc	%Rec	DL
2378-TCDD*	ND	65	10	ND	65	10	80	85	6	60	75	5
12378-P5CDD*	ND	80	5	ND	75	5	ND	70	10	ND	70	15
123478-H6CDD	ND	--	10	ND	--	10	ND	--	10	ND	--	15
123678-H6CDD	ND	--	10	ND	--	10	ND	--	10	ND	--	15
123789-H6CDD*	ND	70	10	ND	70	10	ND	75	10	ND	65	15
1234678-H7CDD*	ND	70	10	ND	65	20	ND	65	15	ND	70	15
12346789-OCDD*	ND	70	20	ND	60	20	ND	60	35	ND	60	30
2378-TCDF*	ND	80	5	ND	65	15	115	90	5	110	85	5
12378-P5CDF*	ND	80	5	ND	80	5	25	80	10	ND	70	15
23478-P5CDF	ND	--	5	ND	--	5	ND	--	15	ND	--	15
123478-H6CDF*	ND	70	10	ND	60	10	ND	65	15	ND	70	15
123678-H6CDF	ND	--	10	ND	--	10	ND	--	15	NI	--	15
123789-H6CDF	ND	--	10	ND	--	10	ND	--	15	ND	--	15
234678-H6CDF	ND	--	10	ND	--	10	ND	--	15	ND	--	15
1234678-H7CDF*	ND	75	10	ND	60	20	ND	65	20	ND	65	20
1234789-H7CDF	ND	--	10	ND	--	20	ND	--	20	ND	--	20
12346789-OCDF	ND	--	20	ND	--	20	ND	--	20	ND	--	30
Lipid Content	< 1%			< 1%			6%			7%		

MCMS ID = sample ID given at MCMS.
Conc = results on tissue weight basis (ppt).
%Rec = recovery for each $^{13}C_{12}$-labeled internal standard (identified by *).
DL = detection limit for each congener (ppt).
ND = not detected.

2. SAMPLE PREPARATION AND ANALYSIS.

The analytical protocol that is used is based on both updated versions of EPA procedures[1,2], specifically Method 1613 (1989), and over 15 years of accumulated experience. In brief, the protocol consists of basic digestion, liquid/liquid extraction, acid wash, multiple column chromatographic clean-up, and GC/HRMS analysis[3-8]. Strict criteria are required in GC/HRMS for positive identification of the PCDD/Fs in a sample, including correct mass measurement, correct retention time, and proper isotope and signal-to-noise ratios. The data were acquired in the mass profile mode[8], and internal standard method was used for quantification of the PCDD/Fs[1-7]. Quality assurance/quality control samples were run in parallel with the unknown samples under the same conditions, and the results met strict requirements for the analysis to be valid.

2.1. Sample Composite.

The crab tissue samples were received from Prof. Cristini in good condition. The samples contained crab muscles and pancreas from four stations in the Raritan/Newark Bay system. Two composites of the muscle and two of the pancreas (each from 5-7 animals) were made for each station. The samples were homogenized and stored in a freezer kept below -20 ^{o}C until dioxin analysis was performed. An MCMS sample ID was given to each composite.

2.2. Sample Preparation.

2.2.1. Crab Tissue Samples.

Ten grams crab tissue was accurately weighted and fortified with the following $^{13}C_{12}$-labeled PCDD/Fs as internal standards:

5 ng $^{13}C_{12}$-2,3,7,8-TCDD
5 ng $^{13}C_{12}$-1,2,3,7,8-P5CDD
5 ng $^{13}C_{12}$-1,2,3,7,8,9-H6CDD
5 ng $^{13}C_{12}$-1,2,3,4,6,7,8-H7CDD
10 ng $^{13}C_{12}$-OCDD

5 ng $^{13}C_{12}$-2,3,7,8-TCDF
5 ng $^{13}C_{12}$-1,2,3,7,8-P5CDF
5 ng $^{13}C_{12}$-1,2,3,4,7,8-H6CDF
5 ng $^{13}C_{12}$-1,2,3,4,6,7,8-H7CDF

The fortified sample was digested with 40% KOH (50 mL for muscle and 70 mL for pancreas) and 20 mL ethanol at room temperature until all tissue was visibly saponified. The analytes were extracted three times with 60 mL hexane. The combined hexane extracts were evaporated to 50 mL and washed with 30 mL water to remove some highly polar interferences, washed with successive 20 mL portions of concentrated sulfuric acid until both layers became clear, and finally washed with 30 mL portions of water until the pH of the aqueous phase was 6 - 7. The hexane solution was concentrated to approximately 1 mL under a stream of dry nitrogen for the subsequent column chromatographic clean up.

2.2.2. Method Blank Sample.

A mixture of 20 mL ethanol and 50 mL 40% KOH solution was fortified with all the labeled internal standards mentioned above and served as a method blank. The method blank was subjected to the complete procedure of sample preparation and GC/HRMS analysis.

2.2.3. Matrix Blank Sample.

Catfish tissue (500 grams) was obtained commercially at a Lincoln supermarket and was diced into reasonably small pieces and carefully mixed. Ten grams of the homogenized catfish tissue was fortified with all ^{13}C-labeled internal standards, and the complete analysis procedure was performed.

2.2.4. Fortified Matrix Blank Sample.

Ten grams catfish tissue that had been previously analyzed and proven to contain no dioxin contamination was fortified with some or all of the native 2,3,7,8-PCDD/Fs. The fortified native standards were allowed to become absorbed into the sample for at least 1 hour. The sample was then fortified with the related internal standards and was subjected to the complete analysis procedure.

2.2.5. Recovery Test Sample.

A sample was prepared as in Section 2.2.4, except that the internal standards were added into sample extract prior to GC/HRMS analysis.

2.3. Column Chromatographic Clean Up.

2.3.1. Silica Chromatography.

Silica gel for chromatography was used without any activation. A 4-cm column was prepared in a disposable Pasteur pipet, and the silica gel was capped with a 0.25-cm layer of anhydrous sodium sulfate. The column was washed with ca. 6 mL of hexane prior to transferring the sample extract. The extract was quantitatively transferred to the column and eluted with 6 mL of hexane and then with 5 mL of 20% benzene in hexane. The eluates were combined and concentrated to approximately 1 mL, while benzene was being replaced with hexane, in preparation for alumina chromatography.

2.3.2. Alumina Chromatography.

Alumina was washed by saturating with methylene chloride, removing excess solvent, then activating at 200 °C for at least 24 hours. A 4-cm column was prepared in a disposable Pasteur pipet and capped with a 0.25-cm layer of anhydrous sodium sulfate. The column was cooled to room temperature in a desiccator before use. Six mL of hexane was used to wet the column before the sample was added. The sample eluent from the silica chromatography step was quantitatively transferred to the column. The column was eluted with 6 mL hexane, then with 6 mL 25% methylene chloride in hexane. The methylene chloride/hexane fraction was collected and concentrated under nitrogen to 1 mL in preparation for the carbon chromatography. All other fractions were discarded.

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2.3.3. Carbon Chromatography.

Carbon (Amoco PX-21) was mixed with previously extracted (in a Soxhlet with methanol) silica gel (2 g of silica and 50 mg of carbon). A disposable Pasteur pipet was cut at the narrow end (end A), and glass wool was used as a plug at the other end (end B). The pipet was then filled with a 0.5-cm layer of silica and 2-cm layer of carbon/silica mixture. The column was then capped with a 0.25-cm layer of anhydrous sodium sulfate and plugged with glass wool. The column was washed in the direction B to A with 5 mL methylene chloride and 5 mL toluene. Subsequently, it was washed in A to B direction with 7 mL hexane, and the sample eluent from the alumina chromatography was transferred onto the column. It was then eluted with methylene chloride (5 mL), 3:1 methylene chloride/benzene (5 mL) in succession, and finally the PCDD/Fs were eluted in the reverse flow (B to A) with 8 mL toluene. The toluene eluent was collected in a centrifuge tube and evaporated to dryness under a gentle stream of nitrogen. The PCDD/Fs were then redissolved in 10 μL of toluene for GC/HRMS analysis.

2.4. GC/HRMS Analysis.

Analysis of the sample extracts were performed on a Kratos MS-50 high resolution mass spectrometer directly coupled to a Carlo-Erba gas chromatograph.

2.4.1. Operating Conditions of the Instrument.

A fused silica capillary column, DB-5 (60 m x 0.25 mm i.d., 0.25 μ film thickness) was used for the gas chromatographic separation. The GC conditions were: on column injection (injection volume 1 or 2 μL); helium carrier gas at a head pressure of 1.5 kg/cm^2; column temperature initially held at 80 °C for 1 min, then programmed to 300 °C at a rate of 25 °C/min (Program A). A second temperature program (Program B) was used, if necessary, to get better GC separation (see Section 2.4.2.2): column temperature initially held at 80 °C for 2 min, programmed to 230 °C at a rate of 35 °C/min and held for 5 min, then heated from 230 °C to 300 °C at 5 °C/min.

The entire GC column effluent was passed directly into the mass spectrometer ion source via an interface operated at 250 °C. The mass spectrometer was operated in the EI mode (70 eV, source temperature 250 °C) at 10,000 resolving power (10% valley definition). Data acquisition and processing for the mass spectrometer were controlled by a Kratos MACH-3 computer system. Selected ion monitoring (SIM) of five groups of selected ions (see Table 6) was used for the analysis. Two ions were selected for each analyte, one as the confirmation ion and the other, more abundant one to provide for quantification. A quantification ion was also selected for each internal standard. Mass profiles of the selected ions were acquired at an amplifier bandwidth of 1000 Hz, a sweep width of 300 ppm, and a scan time of 0.8 sec/cycle. The mass chromatograms and mass profiles for the selected ions of the target compounds were obtained during GC elution over the retention time windows. The mass profile data were used for qualitative and quantitative analysis of the PCDD/Fs.

Table 6: Selected ions for the analysis of the PCDD/Fs by using the SIM technique.

Compound	Ions for Native PCDD/Fs					Labeled PCDD/Fs	
	m/z	type	*m/z*	type	isotope ratio	*m/z*	type
TCDF	303.9016	M	305.8987	M+2	0.77	317.9390	M+2
TCDD	319.8965	M	321.8936	M+2	0.77	333.9339	M+2
P5CDF	339.8598	M+2	341.8567	M+4	0.65	351.9000	M+2
P5CDD	355.8547	M+2	357.8518	M+4	0.65	367.8949	M+2
H6CDF	373.8209	M+2	375.8179	M+4	0.82	385.8612	M+2
H6CDD	389.8158	M+2	391.8128	M+4	0.82	401.8561	M+2
H7CDF	407.7820	M+2	409.7790	M+4	0.97	419.8222	M+2
H7CDD	423.7769	M+2	425.7739	M+4	0.97	435.8172	M+2
OCDF	441.7431	M+2	443.7401	M+4	0.88	455.7804	M+4
OCDD	457.7380	M+2	459.7350	M+4	0.88	471.7753	M+4

M = peak of the molecular ion on mass spectrum.
M + 2 = chlorine isotope peak of the molecular ion shifted two mass units higher.
M + 4 = chlorine isotope peak of the molecular ion shifted four mass units higher.

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2.4.2. Gas Chromatographic Separation of PCDD/Fs.

2.4.2.1. Test of GC Column Performance.

One mL of a TCDD standard mixture of 1,4,7,8-, 2,3,7,8-, 1,2,3,4-, 1,2,3,7-/1,2,3,8-, 1,2,7,8-, and 1,2,6,7-TCDD (200 pg/µl) was periodically injected to check GC column performance.

2.4.2.2. Retention Time of the 2,3,7,8-PCDD/Fs and SIM Retention Time Windows.

A standard solution containing all 17 selected 2,3,7,8-PCDD/Fs was routinely analyzed to obtain their GC retention times. The assignments of PCDD/F isomers in a sample are based on the comparison with GC/HRMS runs of native and labeled standard compounds containing the 2,3,7,8-PCDD/Fs analyzed under the same conditions. Assignments of the isomers for which no internal standards were available were based on chromatographic data in the literature[5] or in application notes. Two GC temperature programs were applied for the separation of PCDD/Fs (see Section 2.4.1). Program A was used for the routine analysis of the samples. Program B was designed to separate better the 1,2,3,4,7,8-H6CDD and 1,2,3,6,7,8-H6CDD as well as the 2,3,4,6,7,8-H6CDF and 1,2,3,7,8,9-H6CDF. However, it takes 10 min more for a GC/HRMS analysis by using program A than by using program B. Thus, program B was used only if any signals had been found at the retention time of the target compounds.

The SIM retention time windows were defined according to the GC retention time obtained. The windows were set for the temperature program A for 5 mass groups: (1) TCDD/F, (2) P5CDD/F, (3) H6CDD/F, (4) H7CDD, and (5) OCDD/F. The retention time range of each window covered the GC retention time of all analytes in the group. The windows and their time ranges, as well as the retention time of the analytes are given in Table 7.

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Table 7: SIM Windows and GC Retention Time Windows for Analysis of the PCDD/Fs.

Window	Time Range [1] (min)	Analyte	GC-Retention Time (min) Program A	Program B
1	13:00 - 16:30	2378-TCDF*	14:51 (1.000)[2]	21:26
		2378-TCDD*	15:07 (1.000)	21:56
2	16:00 - 18:30	12378-P5CDF*	16:36 (1.000)	24:33
		23478-P5CDF	16:59 (1.023)	25:11
		12378-P5CDD*	17:12 (1.000)	25:28
3	18:30 - 22:00	123478-H6CDF*	19:10 (1.000)	28:05
		123678-H6CDF	19:20 (1.008)	28:18
		234678-H6CDF	19:46 (1.031)	28:53
		123789-H6CDF	20:41 (1.079)	29:51
		123478-H6CDD	19:50 (0.977)	29:00
		123678-H6CDD	19:57 (0.983)	29:11
		123789-H6CDD*	20:18 (1.000)	29:33
4	22:00 - 26:30	1234678-H7CDF*	22:34 (1.000)	32:12
		1234789-H7CDF	24:42 (0.914)	34:35
		1234678-H7CDD*	23:59 (1.000)	33:49
5	26:30 - 33:00	OCDF	30:00 (0.991)	40:18
		OCDD*	29:43 (1.000)	40:03

1. This time range was designed for the temperature program A.
2. Relative retention time (in parenthesis): retention time of native PCDD/F related to its $^{13}C_{12}$-labeled internal standard (specified by *).

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2.4.3. Identification of PCDD/Fs.

A positive identification of PCDD/F satisfied all of the following criteria:

1. Signal to noise (S/N) of the peak determined either from mass chromatogram or from mass profile was greater than 3:1.
2. The relative retention time was equal to within ± 0.3% of that shown in Table 7.
3. The accurate masses of the selected ions were equal to the correct masses equal to within 8 ppm (measured with respect to the ions of internal standards).
4. The isotope ratio of the proper ions was equal to the theoretical value to within ± 0.10 (see Table 6).

The 2,3,7,8-PCDD/Fs identified in the crab tissue samples as well as the criteria information are shown in the Table 8.

For further identification, GC/low resolution MS was applied to the samples containing sufficient 2,3,7,8-TCDD/F so that a full mass spectrum could be obtained. The full-scan mass spectra (EI) of the analytes in the samples were compared with the EI mass spectra obtained from the analysis of authentic standards. Figure 1 demonstrates the identification of 2,3,7,8-TCDD and 2,3,7,8-TCDF in the sample NJCL8B by using GC/low resolution MS.

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Table 8: Identification of the Selected 2,3,7,8-PCDD/Fs in the Crab Tissue Samples.

Sample ID	Analyte	Relative RT	Isotope ratio	ΔM (ppm)
For the Samples Collected in September 1991				
NJCL7C	2378-TCDD	1.000	0.68	-3.4
	2378-TCDF	1.000	0.78	-2.2
	23478-P5CDF	1.020	0.63	5.9
NJCL7D	2378-TCDD	1.000	0.80	-0.8
	2378-TCDF	1.000	0.79	-2.7
	12378-P5CDF	1.000	0.68	-2.9
	23478-P5CDF	1.020	0.74	3.6
NJCL8A	2378-TCDF	1.000	0.82	-4.8
	2378-TCDD	1.000	0.79	6.2
NJCL8B	2378-TCDD	1.000	0.79	4.6
	2378-TCDF	1.000	0.70	3.3
	12378-P5CDF	1.000	0.65	-4.1
	23478-P5CDF	1.022	0.66	-3.6
	123478-H6CDF	1.000	0.77	-2.7
NJCL8C	2378-TCDD	1.000	0.70	0.8
	2378-TCDF	1.000	0.71	3.8
	12378-P5CDF	1.000	0.63	0
	23478-P5CDF	1.020	0.61	4.6
	123478-H6CDF	1.000	0.78	-2.3
NJCL8D	2378-TCDD	1.000	0.86	2.3
NJCL8E	2378-TCDD	1.000	0.70	-1.0
	2378-TCDF	1.000	0.79	-2.7
NLCL9A	2378-TCDD	1.000	0.74	3.2
	2378-TCDF	1.000	0.80	4.7

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Table 8: (continued)

NJCL9B	2378-TCDD	1.000	0.79	-2.6
	2378-TCDF	1.000	0.77	-1.0
	12378-P5CDF	1.000	0.55	-2.6
	23478-P5CDF	1.021	0.62	0.2
	123478-H6CDF	1.000	0.95	0.2
NJCL9C	2378-TCDD	1.000	0.75	2.5
	2378-TCDF	1.000	0.80	-5.9
	12378-P5CDF	1.000	0.68	-1.1
	23478-P5CDF	1.025	0.67	5.0
For the Samples Collected in June 1992				
NJCL10A	2378-TCDD	1.000	0.76	-1.6
	2378-TCDF	1.000	0.74	-5.9
NJCL10B	2378-TCDD	1.000	0.84	-8.0
	2378-TCDF	1.000	0.82	-0.1
NJCL10C	2378-TCDD	1.000	0.79	4.8
	2378-TCDF	1.000	0.78	4.8
	12378-P5CDF	1.000	0.66	-3.8
	23478-P5CDF	1.020	0.65	-2.7
	123478-H6CDF	1.000	0.81	-7.0
NJCL10D	2378-TCDD	1.000	0.81	-5.0
	2378-TCDF	1.000	0.81	-7.6
	12378-P5CDF	1.000	0.61	-4.0
NJCL10E	2378-TCDD	1.000	0.81	-0.4
	2378-TCDF	1.000	0.79	3.7
	12378-P5CDF	1.000	0.64	-2.4
	23478-P5CDF	1.022	0.63	-2.9
	123478-H6CDF	1.000	0.87	-1.3

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Table 8: (continued)

NJCL10F	2378-TCDD	1.000	0.70	0.9
	2378-TCDF	1.000	0.75	-3.8
	12378-P5CDF	1.000	0.66	-4.2
	23478-P5CDF	1.024	0.62	0.4
NJCL10G	2378-TCDD	1.000	0.70	1.2
	2378-TCDF	1.000	0.78	-4.9
NJCL11D	2378-TCDD	1.000	0.69	-2.7
NJCL11F	2378-TCDD	1.000	0.75	-2.2

See Tables 1 - 4 for the detailed information for the samples.
Relative RT = relative retention time (see Table 7).
Isotope ratio was the ratio of abundances of the two selected ions of the target PCDD/F. The theoretical values are 0.77 for TCDD/F and 0.65 for P5CDD/F isomers.
ΔM = relative *m/z* deviation between the measured *m/z* and the theoretical *m/z* of the quantification ion (measured with respect to the ions of internal standards).

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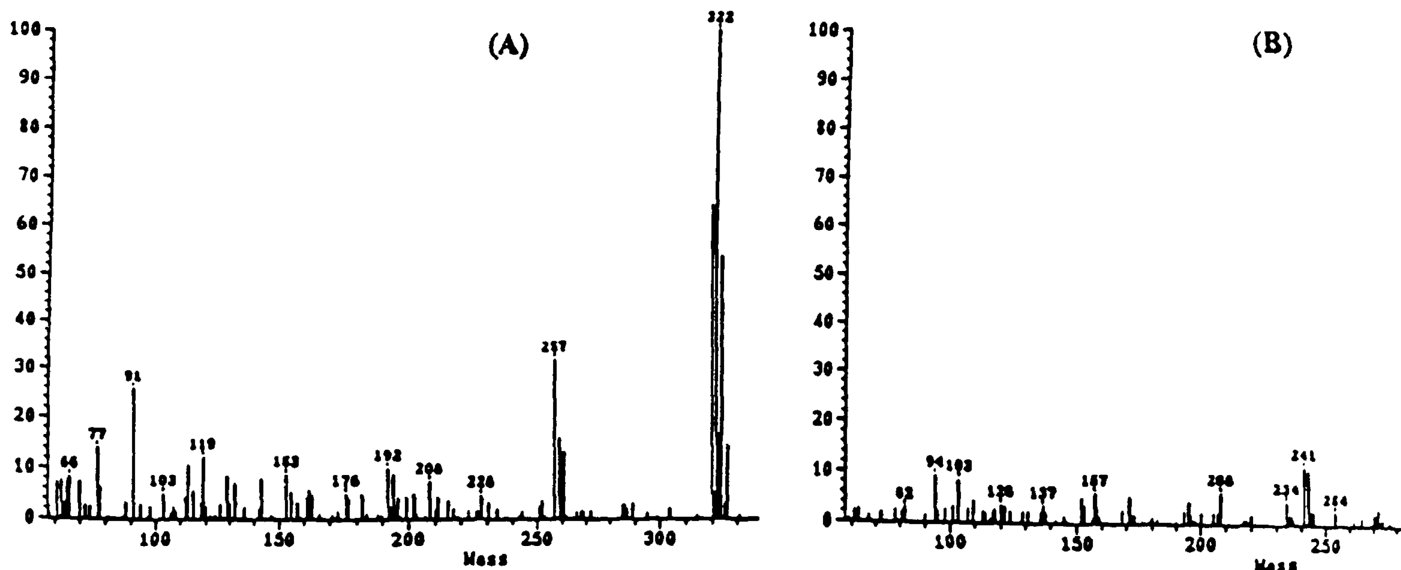
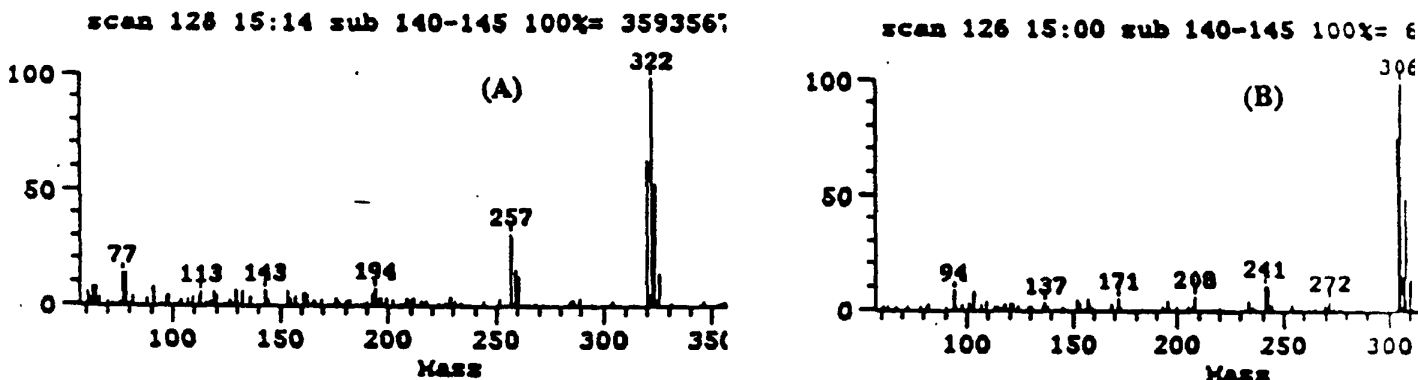
Figure 1. Identification of 2,3,7,8-TCDD and 2,3,7,8-TCDF in Sample NJCL8B (Pancreas, Station 3, Composite 2, Collected in September 1991) by Using GC/Low Resolution MS.



EI mass spectra of 2,3,7,8-TCDD (A) and 2,3,7,8-TCDF (B) obtained from a GC/MS analysis of the sample NJCL8B.



EI mass spectra obtained from a GC/MS analysis of authentic standards of 2,3,7,8-TCDD (A) and 2,3,7,8-TCDF (B). The injection amount was 500 pg for both compounds, respectively.

2.4.4. Quantification of PCDD/Fs.

The internal standard ratio method (also termed the isotope dilution method) was employed to quantify 2,3,7,8-PCDD/Fs in the samples[1-7]. The area of mass profile peaks of the quantification ions were used for the quantification.

2.4.4.1. Relative Response Factor.

The relative response factor (RRF), the ratio of the intensity of a native PCDD/F signal to that of the internal standard, was used for the quantitative analysis to improve the accuracy of the method. The RRF was obtained by the standard calibration (see Section 2.4.4.2) and was defined by the following equation:

$$RRF = (A_s \times C_{is})/(A_{is} \times C_s)$$

where A_s and A_{is} are the mass profile peak areas of the exact *m/z* for the native compound and the internal standard, respectively; C_s and C_{is} are the concentrations of the native compound and the internal standard in the GC/HRMS calibration standard, respectively.

2.4.4.2. Calibration

Calibration was done by using seven data points ranging from 10 pg to 1000 pg for 2,3,7,8-TCDD/F and three points for the other 2,3,7,8-PCDD/Fs (25 pg/50 pg/75 pg for P5CDD/F, H6CDD/F and H7CDD/F, 125 pg/250 pg/ 500 pg for OCDD/F). The amounts of the internal standards were constant for all calibration runs: 50 pg for each compound except OCDD (150 pg). Three GC/HRMS analyses were performed to obtain each point. The relative response factors were calculated and listed in Table 9. For 2,3,7,8-TCDD/F, the average relative response (native to labeled) vs concentration of native compound in standard solutions was plotted and a linear regression obtained (Figure 2). The data in Table 9 shows that each relative response factor was constant (less than 10% coefficient of variation) over the calibration points. Therefore, the average response factors (from > 9 determinations) were used for the quantification of the analytes.

Calibration standards were analyzed periodically. If the RRF value deviated by more than 20% of that determined from the slope of the calibration plot, a new calibration plot was determined over the entire concentration range.

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Table 9: Relative Response Factors Obtained From the Calibration Analysis.

	Calibration 1				Calibration 2				Calibration 3					
Compound	pg	RRF			pg	RRF			pg	RRF			$\overline{RRF}$	RSD
2378-4D*	25	0.68	0.72	0.89	50	0.86	0.84	0.74	100	0.79	0.69	0.86	0.84	9.6
12378-5D*	25	0.72	0.63	0.79	50	0.80	0.71	0.79	100	0.67	0.72	0.75	0.73	7.4
123478-6D	25	0.51	0.62	0.64	50	0.60	0.50	0.55	100	0.60	0.57	0.62	0.58	8.1
123678-6D	25	0.66	0.68	0.59	50	0.72	0.79	0.64	100	0.67	0.75	0.71	0.69	8.2
123789-6D*	25	0.75	0.82	0.78	50	0.84	0.88	0.79	100	0.89	0.77	0.84	0.82	5.6
1234678-7D*	50	0.65	0.62	0.53	100	0.55	0.55	0.56	200	0.56	0.51	0.48	0.56	8.8
OCDD*	125	0.95	0.93	1.06	250	0.96	0.86	1.0	500	1.0	0.96	0.93	0.96	5.5
2378-4F*	25	1.0	0.91	0.95	50	1.17	1.15	1.10	100	0.93	0.98	1.14	1.14	9.4
12378-5F*	25	0.58	0.56	0.59	50	0.64	0.67	0.65	100	0.62	0.60	0.60	0.61	5.8
23478-5F	25	0.67	0.62	0.61	50	0.75	0.71	0.68	100	0.62	0.56	0.63	0.65	8.2
123478-6F*	25	1.25	1.17	1.23	50	1.12	1.28	1.18	100	1.20	1.14	1.28	1.21	4.6
123678-6F	25	1.32	1.28	1.25	50	1.29	1.33	1.37	100	1.20	1.26	1.29	1.29	3.6
123789-6F	25	1.06	1.21	1.12	50	0.98	1.17	1.23	100	1.14	1.09	1.04	1.12	6.9
234678-6F	25	0.99	0.88	0.96	50	0.96	0.94	1.01	100	0.92	0.94	0.96	0.95	3.8
1234678-7F*	100	1.83	1.73	1.76	200	1.71	1.64	1.79	400	1.81	1.65	1.83	1.75	4.0
1234789-7F	50	0.96	0.91	0.89	100	0.77	0.71	0.86	200	0.92	0.76	0.86	0.85	9.3
OCDF	125	0.98	1.03	1.10	250	0.94	1.05	1.05	500	1.09	1.02	0.90	1.05	6.2

RRF = relative response factor of native PCDD/F to internal standard (specified by *).
4D/F = TCDD/F; 5D/F = P5CDD/F; 6D/F = H6CDD/F; 7D/F = H7CDD/F.
$\overline{RRF}$ = average RRF, $\overline{RRF}$ for 2378-TCDD/F were from 6 data points.
RSD = relative standard deviation (n = 9) in percentage.

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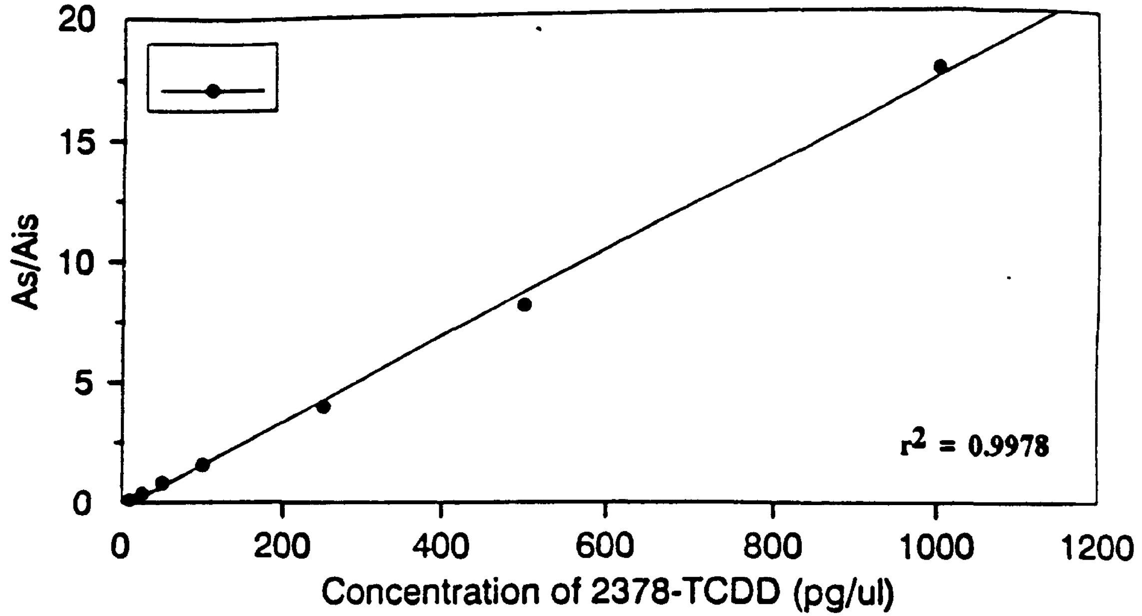
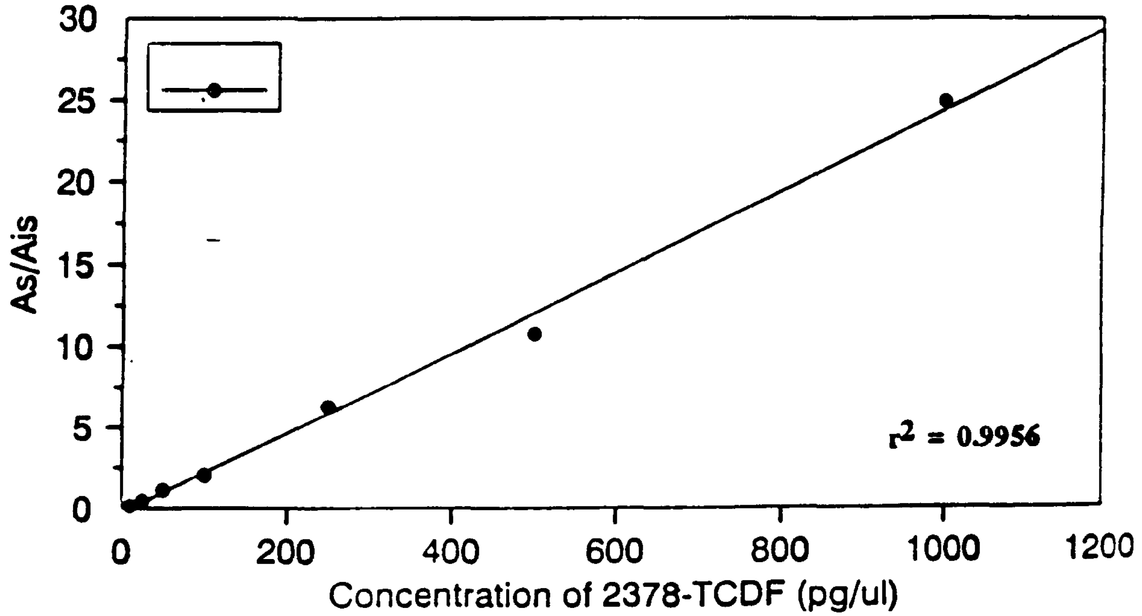
Figure 2: Calibration Curve of 2,3,7,8-TCDD and 2,3,7,8-TCDF.





2.4.4.3. Calculation of the Concentration of the 2,3,7,8-PCDD/Fs in Samples.

After identifying a PCDD/F from a GC/HRMS analysis, the concentration of the compound (C_x) in the sample was calculated according to the following formula:

$$C_x = [(Q_{is}/W)(A_x/A_{is})]/\overline{RRF}$$

where Q_{is} is the quantity of the internal standard fortified into the sample; W is the weight of the sample analyzed; A_x is the peak area of the exact *m/z* for the analyte; A_{is} and RRF are as defined as in Section 2.4.4.1.

2.4.4.4. Recovery of the 2,3,7,8-PCDD/Fs.

Recoveries of some selected 2,3,7,8-PCDD/Fs were determined for the column chromatographic clean-up procedures as well as for the complete procedure including sample preparation, clean-up, and analysis (see Section 2.2.5).

The recovery was calculated as follows:

$$\text{Recovery (\%)} = (100)\, C_x/C_f$$

where C_f and C_x are the fortified and determined concentration of a native PCDD/F, respectively.

The recovery of internal standard fortified in sample was based on the comparison of the peak areas for peaks centered at the exact *m/z* of the internal standard in a sample run and a standard run. Thus, this recovery not only depended on the sample preparation but also was affected by the instrument response, which changed from analysis to analysis.

The recoveries of internal standard were in the range of 40% - 110%, which is acceptable.

2.4.4.5. Detection Limits.

Detection limit (DL) is defined as the concentration of a PCDD/F required to produce a signal with an intensity of at least 3 times the baseline noise. The following formula is used for the calculation:

$$DL = (3)\,[(Q_{is}/W)(A_{no}/A_{is})]/\overline{RRF}$$

where A_{no} is integrated noise intensity of baseline nearest to and having the same width as the internal standard peak, measured at same *m/z* in mass profile. Other terms are defined in Sections 2.4.4.1 and 2.4.4.3.

For some samples, signals representing low levels of TCDD/Fs were detected but did not meet all the criteria for positive identification. In these cases, the detection limit was calculated on the basis of the actual signal and not on the basis of 3 times noise. For these samples, the detection limit was often slightly elevated.

2.4.4.6. Accuracy and Precision.

Accuracy data were obtained from the analysis of fortified matrix blank sample in which known amount native 2,3,7,8-PCDD/Fs were added. The accuracy was shown as a relative analysis error (RAE) and was calculated by the following formula:

$$RAE (\%) = (100) (C_x - C_f)/C_f$$

C_f and C_x are defined in Section 2.4.4.5.

The relative analysis error should be ± 50% for each analyte.

Precision was calculated from the analysis of a tissue sample and its duplicate samples as follows:

$$\text{precision} (\%) = (100) \mid (C_1 - C_2) \mid / [(C_1 + C_2)/2]$$

where C_1 and C_2 are the concentrations of a 2,3,7,8-PCDD/F determined for the sample and its duplicate sample.

The precision expected for duplicate analyses should be better than 40% for each analyte.

3. QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC).

MCMS follows the QA/QC and safety guidelines of the EPA method[2] and the QA/QC document specified by the Division of Science and Research of the New Jersey Department of Environmental Protection and Energy (NJDEPE)[9].

3.1. Sample Shipment and Receipt.

1. The samples were received from Prof. Cristini in good condition (less than -10 ^oC) and stored in a freezer at -20 ^oC until analyzed. Only the samples received and stored in good condition were analyzed.

2. A record of the shipment including the sample codes, date of receipt, shipment carrier, condition of the samples received and storage location was logged in the research logbook.

3. A chain-of-custody letter regarding the shipment was sent promptly to Prof. Cristini.

4. As suggested by Dr. Paul Hauge at the NJDEPE in his letter on March 17, 1992, the temperature of the samples was measured as soon as the samples were received. This was done for the samples collected on June 1992. The temperatures were less than - 10 ^oC and were recorded in-chain-of-custody letters.

5. A check solution containing native 2,3,7,8-TCDD/F and OCDD/F each at 500 pg/ul level was sent to Prof. Cristini, and then sent back to MCMS at ambient temperature. The standard solution was analyzed after it returned back to MCMS and showed no measurable changes for the compounds as well as for the concentrations.

6. Portions of samples that remained unused after analysis were kept in storage in the freezer at -20 ^oC.

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7. One batch of samples collected in June 1992 thawed during shipment, and many bottles were broken, causing samples to intermix. A replacement batch was sent by Prof. Cristini, and it was received in good condition (temperature on arrival was less than 10 ^{o}C) and was analyzed. The original batch was kept in the freezer and not analyzed.

3.2. Instrument System Performance Test.

1. The mass spectrometer was tuned to obtain acceptable resolution and sensitivity. The sample analysis was performed only when the instrument met the required conditions.

2. During the period of GC/HRMS analysis, accurate mass measurement and mass resolving power were frequently monitored and adjusted.

3. A GC column performance check solution was conducted to demonstrate adequate GC resolution to separate 2,3,7,8-TCDD from other TCDD isomers before samples were analyzed (see Section 2.4.2.1).

4. In addition to the calibration standard solution series, which was done at the beginning of each analysis period, one of the standard solutions was periodically "inserted" into sample runs.

5. A toluene blank was run on GC/HRMS, from time to time, particularly after a high concentration sample (e.g., the pancreas samples from station #3), to demonstrate the absence of any "memory effect".

The tissue sample extracts were only analyzed when all the above criteria were met.

3.3. QA/QC Results for Sample Analysis.

1. Matrix blank (see Section 2.2.3): Analysis of matrix blank sample showed that no 2,3,7,8-substitued PCDD/Fs were found in the catfish. This result allowed the catfish to be used for the recovery test and for the samples of fortified matrix blank.

2. Recovery test (see Section 2.2.5): This was done before any samples were analyzed. The recovery of each compound was more than 50%.

Recoveries of the internal standards shown in the Tables 1 to 4 were better than 40% for each internal standard.

3. Blanks and Duplicates: For preparation and analysis of each batch of samples (6 - 8 samples), three QA/QC samples were included, as listed below:

a) Method blank (see Section 2.2.2): The method blanks (NJCL7G, NJCL8I, NJCL9J and NJCL12G) were analyzed and showed no positive response for native 2,3,7,8-PCDD/Fs.

b) Fortified matrix blank (see Section 2.2.4): Analysis of this sample provides an estimate of the sensitivity and accuracy of the analysis. The internal standard recovery was within a range of 40-120 percent. The accuracy, expressed as a relative analysis error was better than ± 40% (see Table 10).

c) Duplicate sample: The relative difference (precision) of the results for the samples and their duplicate samples were less than 40% for each analyte (see Table 11).

If the analytical results of any sample or any sample batch showed that any one of the above requirements was not met, the sample or the batch of the samples were re-analyzed. The following re-analysis were performed:

1. All tissue samples collected in September 1991 and in June 1992 were re-analyzed because the recoveries of the internal standards in previous analysis (NJCL3- to NJCL6-series) were < 10%.

2. Duplicate samples for pancreas of station 4 and composite 2 (NJCL9C, collected in September 1991) were re-analyzed because the precision (relative deviation) for 2,3,7,8-TCDD (55%) exceeded the precision limit (40%). The relative deviation for 2,3,7,8-TCDD from the re-analysis of the duplicate sample was 8% (see Table 11).

3. Duplicate samples NJCL12A and NJCL12B (for samples collected in September 1991), and NJCL12E and NJCL12F (for samples collected in June 1992) were analyzed again after the tissue samples had been stored for 5 months, in order to check whether any change of the results occurred during sample storage. Because extractions were done by three different persons (Z. Cai, V.M.S. Ramanujam, S. Monson), it was of interest to test agreement between different coworkers. The results were compared and shown in Table 11.

Table 10: QA/QC Data for Fortified Matrix Blank Samples.

Concentration: ppt

	NJCL8H			NJCL9I			NJCL10I		
Analyte	C_f	C_x	RAE	C_f	C_x	RAE	C_f	C_x	RAE
2378-TCDD	500	475	-5.0	500	495	-1.0	500	490	-2.0
2378-TCDF	500	470	-6.0	500	465	-7.0	500	495	-1.0
12378-P5CDD	NF	ND	--	500	475	-5.0	NF	ND	--
12378-P5CDF	NF	ND	--	500	550	+10	NF	ND	--
123478-H6CDD	NF	ND	--	500	540	+8.0	NF	ND	--
234678-H6CDF	NF	ND	--	500	675	+35	NF	ND	--
1234678-H7CDD	NF	ND	--	1000	980	-2.0	NF	ND	--
1234789-H7CDF	NF	ND	--	1000	675	-32	NF	ND	--
OCDD	1000	1190	+19	2500	2760	+10	1500	1680	+12
OCDF	NF	ND	--	NF	ND	--	NF	ND	--
	NJCL11I			NJCL12H			--		
2378-TCDD	500	495	-1.0	500	525	+5.0	--	--	--
2378-TCDF	500	465	-7.0	500	550	+10	--	--	--
12378-P5CDD	500	475	-5.0	500	520	+4.0	--	--	--
12378-P5CDF	500	550	+10	500	505	+1.0	--	--	--
123478-H6CDD	500	540	+8.0	500	575	+15	--	--	--
234678-H6CDF	500	675	+35	500	565	+13	--	--	--
1234678-H7CDD	1000	980	-2.0	1000	935	-6.5	--	--	--
1234789-H7CDF	1000	950	-5.0	1000	1020	+2.2	--	--	--
OCDD	2500	2760	+10	2500	2610	+4.4	--	--	--
OCDF	NF	ND	--	3500	3170	-9.4	--	--	--

C_f = fortified concentration of the PCDD/F in ppt.
C_x = determined concentration of the PCDD/F in ppt.
NF = not fortified, ND = not detected.
RAE = relative analytical error in percentage (see Section 2.4.4.6).

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Table 11: Analysis Results for 2,3,7,8-PCDD/Fs in Duplicate Samples.

Analyte	C_1 (ppt)	C_2 (ppt)	Rel. Dev. (%)	C_1 (ppt)	C_2 (ppt)	Rel. Dev. (%)
Sample collected	*September 1991*			*June 1992*		
	NJCL8F/8G (S4, m, Cp1)			NJCL11D/12D (S3, m, Cp1)		
2378-TCDD	ND	ND	--	30	40	28
2378-TCDF	ND	ND	--	ND	ND	--
	NJCL9F/NJCL9G (S4, m, Cp2)			--		
2378-PCDD/Fs	ND	ND	--	--	--	--
	NJCL8A/12A (S1, p, Cp2)			NJCL10G/10H (S2, p, Cp2)		
2378-TCDD	40	35	14	45	65	36
2378-TCDF	75	90	18	70	90	25
	NJCL8B/12B (S3, p, Cp2)			NJCL10C/12E (S3, p, Cp1)		
2378-TCDD	690	600	13	475	425	11
2378-TCDF	160	185	15	130	140	7
12378-P5CDF	25	35	33	45	45	0
23478-P5CDF	ND	15	--	75	60	22
123478-H6CDF	45	65	36	ND	50	--
1234678-H7CDF	ND	20	--	ND	ND	--
	NJCL9C/NJCL12C (S4, p, Cp2)			NJCL10E/12F (S3, p, Cp2)		
2378-TCDD	60	65	8.0	580	480	19
2378-TCDF	125	115	8.3	150	150	0
12378-P5CDF	20	20	0	45	40	12
23478-P5CDF	25	20	22	ND	50	--

C_1 and C_2 = concentration determined in the sample and in its duplicate sample.
Rel. Dev. = relative deviation (precision)
2378-PCDD/Fs = 2,3,7,8-substituted PCDD/Fs.
ND = not detected, p = pancreas, m = muscle, S = station, Cp = composite

3.4. Report and Storage of the Analytical Data.

1. Notes on the conditions of the samples receipt, storage, and sample preparation were recorded in research notebooks that are kept in the dioxin extraction laboratory. The operation parameters for GC/HRMS analysis were recorded in the trace analysis logbook routinely used at MCMS.

2. All original mass chromatogram and mass profile spectra acquired from the GC/HRMS analysis were printed out and stored at MCMS. They are also archived on magnetic tape for permanent storage.

3. The analytical data were processed by using the mathematical formulae described in Section 2 and directly recorded on the original spectra. The data were organized and reported by using computer software Lotus Manuscript.

4. This report was typed by using the Lotus Manuscript. The data and report can be transferred to NJDEPE via a computer diskette, if required.

APPENDIX

List of Terminology, Abbreviation and Symbols

EPA: Environmental Protection Agency

MCMS: Midwest Center for Mass Spectrometry

NJDEPE: New Jersey Department of Environmental Protection and Energy

PCDD/Fs: polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans

2378-PCDDs: 2,3,7,8-substituted PCDDs

2378-PCDFs: 2,3,7,8-substituted PCDFs

2378-PCDD/Fs: 2,3,7,8-substituted PCDD/Fs

TCDF: tetrachlorodibenzofuran

TCDD: tetrachlorodibenzo-*p*-dioxin

P5CDF: pentachlorodibenzofuran

P5CDD: pentachlorodibenzo-p-dioxin

H6CDF: Hexachlorodibenzofuran

H6CDD: Hexachlorodibenzo-p-dioxin

H7CDF: heptachlorodibenzofuran

H7CDD: heptachlorodibenzo-*p*-dioxin

OCDF: octachlorodibenzofuran

OCDD: octachlorodibenzo-*p*-dioxin

A_{is}: peak area at the exact m/z for an internal standard

A_{no}: area of noise baseline measured at the exact m/z

A_s: peak area at the exact m/z for a native PCDD/F standard

A_x: peak area at the exact m/z for an analyte

C_1 and C_2: concentrations of a PCDD/F determined in a sample and its duplicate sample

C_f: fortified concentration of a native PCDD/F

C_{is}: concentration of a ^{13}C-labeled internal standard

C_s: concentration of a native standard PCDD/F

C_x: determined concentration of an analyte

DL: detection limit

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GC/HRMS: capillary gas chromatography/high resolution mass spectrometry

GC/MS: capillary gas chromatography/mass spectrometry (low resolution)

ID: identification code

ΔM: deviation between the measured m/z and the theoretical m/z of a quantification ion

m: muscle of crab tissue

M: peak of the molecular ion

M + 2: chlorine isotope peak of the molecular ion shifted two mass units higher

M + 4: chlorine isotope peak of the molecular ion shifted four mass units higher

NF: not fortified

ND: not detected

P: pancreas of crab tissue

ppm: parts per million

ppt: parts per trillion

QA/QC: quality assurance/quality control

Q_{is}: quantity of the internal standard fortified into a sample

RAE: relative analysis error (for accuracy)

Rec: recovery of a $^{13}C_{12}$-labeled internal standard

Rel. Dev.: relative deviation (for precision)

RSD: relative standard deviation

RRF: relative response factor of a native PCDD/F to the internal standard

$\overline{RRF}$: average relative response factor

RT: gas chromatographic retention time

S/N: signal to noise ratio

W: the weight of a sample analyzed

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