



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1588

Organics in Cod Liver Oil

This Standard Reference Material (SRM), a cod liver oil, is intended for use in developing and validating analytical methods for the determination of chlorinated biphenyls, chlorinated pesticides, and alpha-tocopherol in cod liver oil or in other similar complex lipophilic matrices. SRM 1588 consists of five sealed ampoules per unit, each ampoule containing approximately 1.2 mL of the cod liver oil.

Certified Concentrations

The certified concentrations and uncertainties for ten chlorinated pesticides, five individual polychlorinated biphenyl (PCB) congeners, and alpha-tocopherol, which are all naturally present in the cod liver oil, are given in Table 1. These values are based on results obtained from the analyses of this material using two independent sample preparation procedures and two measurement methods based on gas chromatography with electron capture and mass spectrometric detection. A summary of the analytical results by the various methods is shown in Table 2. Alternate names, Chemical Abstracts Service Nomenclature and Registry Numbers of the certified components are listed in Table 3.

Noncertified Concentrations

Noncertified concentrations of six added polychlorodibenzo-p-dioxins and octachlorodibenzofuran, which are given for information only, are listed in Table 4.

NOTICE AND WARNING TO USERS

Handling: This material contains added polychlorodibenzo-p-dioxins and octachlorodibenzofuran. Each is present in this material at a concentration of 1 ng/g or less. Although some of these compounds are reported to be toxic and/or carcinogenic, their concentrations are too low to be considered hazardous under OSHA regulations. However, the material should be handled with care. Use proper disposal methods.

Expiration of Certification: This certification is valid within the specified uncertainty limits for one year from the date of shipment. In the event that the certification should become invalid before then, users will be notified by NIST. Please return the attached registration form to facilitate notification.

Storage: Sealed ampoules, as received, should be stored in the dark at temperatures less than 30 °C.

Use: Samples of this SRM for analysis should be withdrawn from ampoules immediately after opening and used without delay for the certified values listed in Table 1 to be valid within the stated uncertainties. Certified values are not applicable to material in ampoules stored after opening, even if they are resealed.

Preparation and analytical determinations were performed in the NIST Organic Analytical Research Division by J. M. Brown-Thomas, F. R. Guenther, and R. M. Parris.

Consultation on the statistical design of the experimental work and evaluation of the data were provided by R. C. Paule of the National Measurement Laboratory.

The coordination of the technical measurements for certification was under the direction of S. N. Chesler, R. M. Parris and W. E. May of the Organic Analytical Research Division.

Gaithersburg, MD 20899
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Stanley D. Rasberry, Chief
Office of Standard Reference Materials

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The technical and support aspects involved in the preparation, certification and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

PREPARATION AND ANALYSIS

This SRM is a cod liver oil that has been fortified with selected chlorinated dibenzodioxins and dibenzofurans. It was donated to NIST by the University of Ulm, Federal Republic of Germany.

Weighed aliquots of gravimetrically prepared 2,2,4-trimethylpentane solutions of six polychlorinated dibenzo-p-dioxins and octachlorodibenzofuran were added to a known mass of the cod liver oil. The concentrations of these components, which are included here for information only and are not certified values, were calculated from these gravimetric measurements and are listed in Table 4. The oil was homogenized by stirring for 10 h in a 9-L glass bottle. Each 2-mL amber ampoule was purged with argon just prior to the addition of approximately 1.2 mL of oil to the ampoule which was then flame sealed.

PCB's and Chlorinated Pesticides The SRM was analyzed for selected PCB's and chlorinated pesticides using gas chromatography with electron capture detection (GC-ECD) and gas chromatography with mass spectrometric detection (GC-MS). Two independent sample "cleanup" methods were also used. For the GC-ECD analyses, an N-N-dimethylformamide (DMF)-water/hexane liquid-liquid partitioning procedure was used to remove the majority of the lipid and the very polar components of the oil. Additional analytical interferences were removed from this extract on a semi-preparative aminosilane (NH₂) liquid chromatographic column from which analytes of interest were collected in two fractions according to previously calibrated retention zones. GC-ECD was employed for the final quantitative analysis of each fraction. Aliquots from randomly selected ampoules were analyzed using a 60-m immobilized-phase, non-polar capillary column. A representative chromatogram of each of the two fractions from these analyses is shown in Figure 1. These chromatograms are provided for information only.

For the GC-MS analyses of the cod liver oil, preparative scale size exclusion chromatography (SEC) with an organic mobile phase was used to isolate the PCB's and chlorinated pesticides from most of the lipid matrix. Five aliquots of the SRM material from randomly selected sets of three adjacent ampoules were each injected onto a divinylbenzene-styrene copolymer column and the fractions containing the PCB and pesticide analytes were collected. The fractions for each set of ampoules were combined and refractionated on the size exclusion column prior to analysis using capillary GC-MS in the selective ion monitoring mode.

In both methods, two PCB congeners and deuterated (d₈) 4,4'-DDT not significantly present in the native cod liver oil were added as internal standards for quantification. Calibration response factors for the analytes relative to the internal standards were determined by fractionating and analyzing fractions of gravimetrically prepared calibration solutions of the analytes of interest and internal standards.

Additionally, one aliquot of cod liver oil was fractionated using two LC isolation techniques (size exclusion and normal-phase [aminosilane] chromatography) and was analyzed using GC-ECD equipped with the 60-m capillary column. The internal standard method of quantification was used and aliquots of SRM 1492 "Chlorinated Pesticides in Hexane" and SRM 1493 "Polychlorinated Biphenyls in 2,2,4-Trimethylpentane" were fractionated and chromatographed to obtain analyte response factors. The results are shown as column 3 of Table 2.

alpha-Tocopherol The concentration of alpha-tocopherol was determined using independent methods based on normal-phase (NP) and reversed-phase (RP) HPLC. UV detection was used for both methods. The NP-HPLC analyses utilized a semi-preparative aminosilane column with quantification by the external standard method. For the RP-HPLC analyses, aliquots of the oil, which were diluted with hexane and spiked with tocol as the internal standard, were first fractionated on a gel permeation column and the resulting fraction was then analyzed using a polymerically-bonded C₁₈ column.

Table 1. Certified Concentrations of Chlorinated Pesticides, Chlorinated Biphenyls and alpha-Tocopherol in SRM 1588

Compound ^a	Concentration, ng/g
Hexachlorobenzene	148 ± 21 ^b
alpha-HCH	86 ± 19
trans-Chlordane	50 ± 13
cis-Chlordane	158 ± 8
trans-Nonachlor	209 ± 11
Dieldrin	150 ± 12
4,4'-DDD	277 ± 15
4,4'-DDE	641 ± 62
2,4'-DDT	156 ± 5
4,4'-DDT	529 ± 45
PCB 101	129 ± 5
PCB 138	261 ± 29
PCB 153	276 ± 40
PCB 170	45 ± 5
PCB 180	107 ± 4
	Concentration, µg/g
alpha-Tocopherol	112 ± 5 ^b

^aPCB numbering scheme taken from Ballschmiter, K. and M. Zell, Fresenius Z. Anal. Chem. 302, 20 (1980). Complete Chemical Abstracts Service (CAS) Nomenclature and Registry Numbers are given in Table 3.

^bUncertainty expressed as two standard deviations of the certified values. Statistical analyses of variance based on the within- and between-method variances were made. In general, the largest source of variability was that among the three methods of analysis from which the certified values were derived (see Table 2). The sample homogeneity, in terms of ampoule-to-ampoule variability, was satisfactory and did not contribute to the above uncertainties.

Table 2. Summary of Analytical Results^a

Analytical Method Preparative Technique(s)	-----Concentration, ng/g-----		
	GC-MS (30 m) SEC	GC-ECD (60 m) DMF NH ₂ Fract.	GC-ECD (60 m) SEC NH ₂ Fract.
Compound			
Hexachlorobenzene	163 ± 6	130 ± 17	143
alpha-HCH	94 ± 6	74 ± 20	---
trans-Chlordane	57 ± 1	44 ± 3	---
cis-Chlordane	160 ± 8	158 ± 11	158
trans-Nonachlor	218 ± 18	208 ± 13	209
Dieldrin	158 ± 3	140 ± 9	151
4,4'-DDD	269 ± 6	292 ± 23	286
4,4'-DDE	615 ± 7	678 ± 50	---
2,4'-DDT	153 ± 3	162 ± 10	160
4,4'-DDT	552 ± 3	506 ± 4	---
PCB 101	132 ± 2	124 ± 3	131
PCB 138	240 ± 5	286 ± 20	260
PCB 153	245 ± 7	312 ± 18	274
PCB 170	47 ± 1	40 ± 2	48
PCB 180	104 ± 1	106 ± 3	111

	----Concentration, µg/g----	
	RP-HPLC	NP-HPLC
alpha-tocopherol	115 ± 5	109 ± 5

^aThe summary of results given above is presented as background information. The listed uncertainties represent one standard deviation for a single measurement and recognize only the within-method variability.

Table 3. Names(s), Chemical Abstract Services (CAS) Nomenclature and Registry Number^a

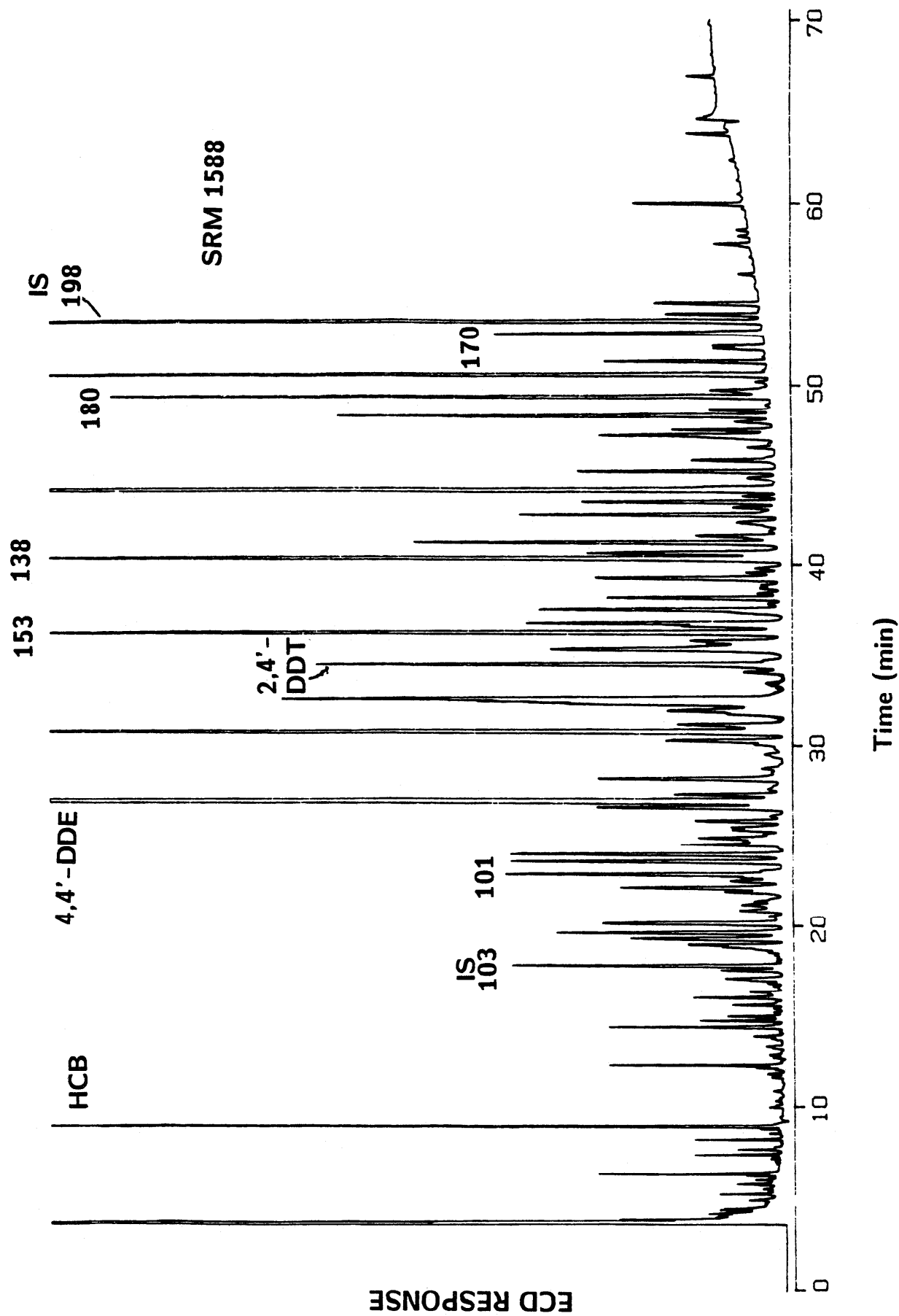
Compound (Alternate Name)	CAS Registry Number	CAS Nomenclature
PCB 101	37680-73-2	2,2',4,5,5'-pentachlorobiphenyl
PCB 138	35065-28-2	2,2',3,4,4',5'-hexachlorobiphenyl
PCB 153	35065-27-1	2,2',4,4',5,5'-hexachlorobiphenyl
PCB 170	35065-30-6	2,2',3,3',4,4',5-heptachlorobiphenyl
PCB 180	35065-29-3	2,2',3,4,4',5,5'-heptachlorobiphenyl
Hexachlorobenzene (HCB)	118-74-1	hexachlorobenzene
alpha-HCH (alpha-BHC)	319-84-6	(1 α ,2 α ,3 β ,4 α ,5 β ,6 β)-1,2,3,4,5,6- hexachlorocyclohexane
trans-Chlordane (gamma-Chlordane)	5103-74-2	(1 α ,2 β ,3 $\alpha\alpha$,4 β ,7 β ,7 $\alpha\alpha$)-1,2,4,5,6,7,8,8- octachloro-2,3,3a,4,7,7a-hexahydro-4,7- methano-1H-indene
cis-Chlordane (alpha-Chlordane)	5103-71-9	(1 α ,2 α ,3 $\alpha\alpha$,4 β ,7 β ,7 $\alpha\alpha$)-1,2,4,5,6,7,8,8- octachloro-2,3,3a,4,7,7a-hexahydro-4,7- methano-1H-indene
trans-Nonachlor	39765-80-5	(1 α ,2 β ,3 α ,3 $\alpha\alpha$,4 β ,7 β ,7 $\alpha\alpha$)- 1,2,3,4,5,6,7,8,8-nonachloro- 2,3,3a,4,7,7a-hexahydro- 4,7-methano-1H-indene
Dieldrin (HEOD)	60-57-1	(1 α ,2 β ,2 $\alpha\alpha$,3 β ,6 β ,6 $\alpha\alpha$,7 β ,7 $\alpha\alpha$)- 3,4,5,6,9,9-hexachloro- 1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6- dimethanonaphth[2,3-b]oxirene
4,4'-DDE (<i>p,p'</i> -DDE)	72-55-9	1,1'-(dichloroethenylidene)bis[4- chlorobenzene]
4,4'-DDD (<i>p,p'</i> -DDD) (<i>p,p'</i> -TDE)	72-54-8	1,1'-(2,2-dichloroethylidene)bis[4- chlorobenzene]
2,4'-DDT (<i>o,p'</i> -DDT)	789-02-6	1-chloro-2-[2,2,2-trichloro-1-(4- chlorophenyl)ethyl]benzene
4,4'-DDT (<i>p,p'</i> -DDT)	50-29-3	1,1'-(2,2,2-trichloroethylidene)bis[4- chlorobenzene]
alpha-Tocopherol	59-02-9	alpha-tocopherol

^aChemical Abstracts, Eleventh Collective Index, Index Guide, American Chemical Society, Columbus, Ohio, 1986.

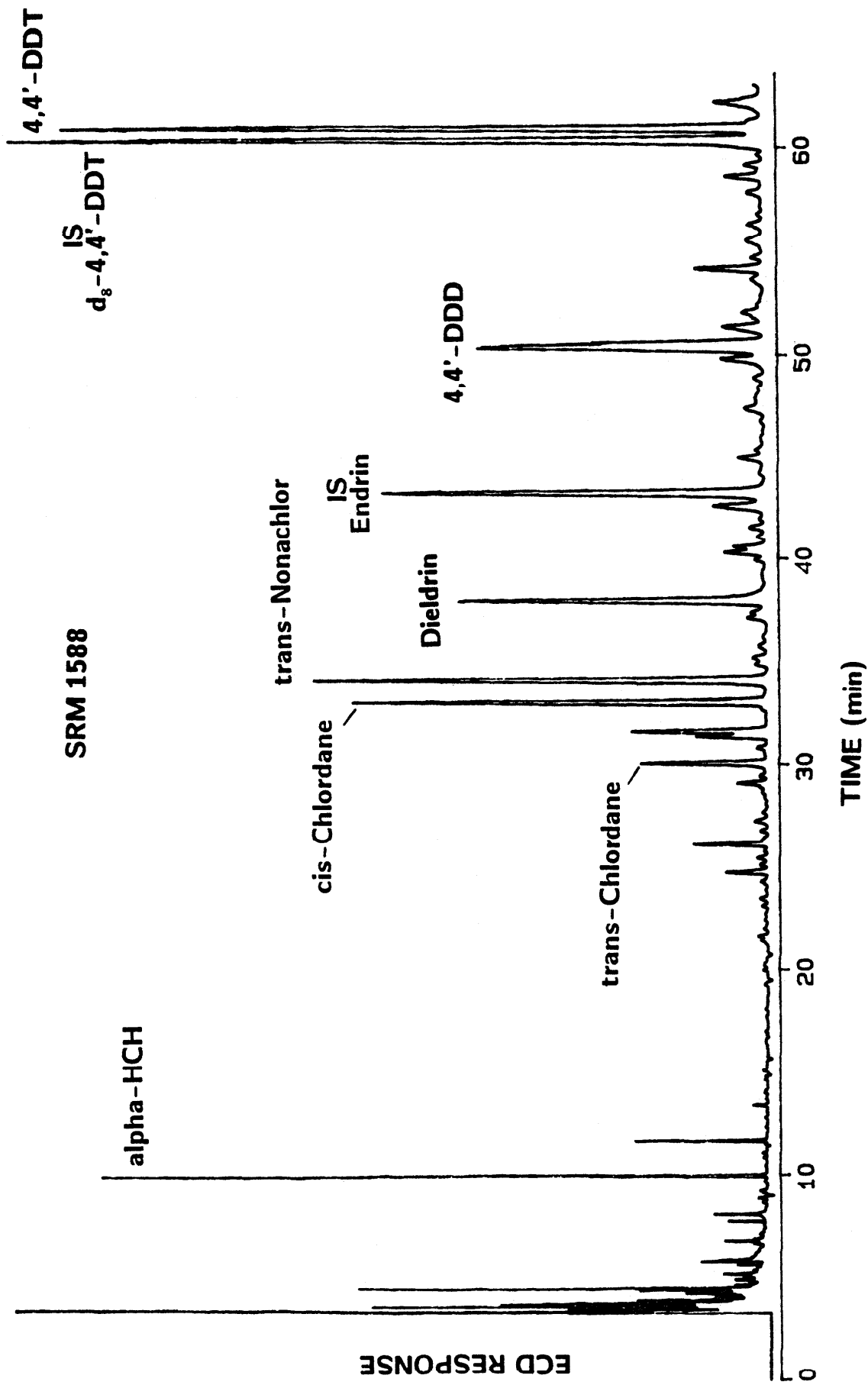
Table 4. Noncertified Concentrations of Organic Constituents in SRM 1588

NOTE: The values shown in this table are not certified because they are based only on gravimetry. They are provided for information only.

Compound	Concentration, ng/g
-dibenzo-<i>p</i>-dioxin	
1,2,7-trichloro	0.32
1,2,3,4-tetrachloro	0.38
2,3,7,8-tetrachloro	0.21
1,2,3,6,7,8-hexachloro	0.39
1,2,3,7,8,9-hexachloro	0.22
octachloro	1.01
octachlorodibenzofuran	1.00



Chromatogram of Aminosalane Fraction I of Cod Liver Oil Extract



Chromatogram of Aminosilane Fraction II of Cod Liver Oil Extract