LEVELS OF DECHLORANE PLUS AND RELATED COMPOUNDS (DECHLORANE 602, 603 AND 604) IN VEGETABLE AND MARINE OILS

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Introduction

Dechlorane Plus (DP) is a flame retardant additive used in polymeric systems such as electrical hard plastic connectors in televisions and computer monitors, wire coatings and furniture[1]. This product, currently classified as a high production volume chemical by the U.S. Environmental Protection Agency, was created by OxyChem in the 1970s when Mirex, another flame retardant from the same company, was banned.

The existence of DP in the environment was first detected in air, fish and sediment samples from the Great Lakes in 2006[2]. Since then, many studies have been performed in order to determine the environmental behavior and presence of this compound and its main relatives: Dechlorane 602 (Dec 602), Dechlorane 603 (Dec 603) and Dechlorane 604 (Dec 604). These compounds have shown similar properties to other persistent organic pollutants, such as long-range transport potential, bioaccumulation, biomagnification and low degradation rate. There are still few studies on the toxicity of DP and related compounds, but it has been demonstrated their influence in oxidative stress and neurotoxicity in terrestrial animals as well as toxicity to aquatic animals[3,4].

DP and its analogues have been mainly studied in environmental matrices such as water, air, soil and biota with monitoring purposes, but the dietary exposure to these chemicals has been hardly investigated[5,6]. Thus, there is a need for the development of analytical methods for the determination of these compounds on food and feed matrices in order to generate data that allow us to evaluate their intake by human beings.

Materials and methods

Chemicals

*N*on-labeled *syn*- and *anti*-DP standards were purchased from Wellington Laboratories (Ontario, Canada). ^{13}C *anti*-DP and ^{13}C PBDE 183 standards were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dec 602, Dec 603 and Dec 604 standards were purchased from Toronto Research Chemicals (Ontario, Canada). Silica 60 (70-230 mesh) and sodium sulfate were supplied by Merck (Darmstadt, Germany), hexane and toluene were supplied by LGC-Promochem (Wesel, Germany) and Sigma Aldrich (St. Louis, MO, USA), respectively, sulfuric acid was supplied by Scharlau (Barcelona, Spain) and sodium hydroxide and silver nitrate were supplied by Panreac (Barcelona, Spain).

Samples analyzed

Oil samples from different origin and used for different purposes were analyzed. Most of them were produced from marine oils as health supplements for human consumption, but also some fish oils for feed were included in the study. Additionally, two samples of health supplements, which were mainly constituted by vegetable oils, and two of mixture of fish and vegetable oil were analyzed (Table 1).

Sample preparation

1 g of sample was weighed and dissolved in approximately 1 ml of hexane. Then, internal standard (^{13}C *anti*-DP) was added. Clean-up was performed using multi-layer silica columns packed with alternated layers of activated silica and silica impregnated with sulfuric acid, sodium hydroxide and silver nitrate. Columns were eluted with hexane.

Further purification based on preparative HPLC chromatography was studied. For that purpose, a 1050 HPLC (Hewlett Packard, Palo Alto, CA, USA) equipped with a 2-(1-pyrenyl)ethyl column (4.6 x 250 mm, 5 µm) (Nacalai Tesque Inc., Kyoto, Japan) was used and hexane as mobile phase at 1.0 ml/min. About 250 µl were manually injected and fractions were collected at different times in order to study the retention times of the analytes and to determine the volume of eluate to be discarded.

Analyte-containing fractions were concentrated to 15 µl and recovery standard (^{13}C PBDE 183) was added.

Table 1. Oil samples analyzed in this study

Sample code	*Purpose*	*Main constituent*
F1	Food	Salmon oil
F2	Food	Cod liver oil
F3	Food	Fish (unspecified) oil
F4	Food	Fish (unspecified) oil
F5	Food	Fish (unspecified) oil
F6	Food	Anchovy oil
F7	Food	Fish (unspecified) oil
F8	Food	Krill oil
F9	Food	Cold-water fish oil
F10	Food	High seas fish oil
F11	Food	Fish (unspecified) oil
F12	Feed	Salmon oil, crude
F13	Feed	Tuna oil, refined
F14	Feed	Salmon oil
F15	Feed	Fish (unspecified) oil
V1	Food	Primula oil
V2	Food	Vegetables (unspecified) oil
M1	Food	Salmon and borage oil
M2	Food	Soy oil, marine coral, borage oil, rice bran and grapes

Instrumental determination

Determination of Dechloranes was performed with a 6890N Gas Chromatograph (Agilent, Santa Clara, CA, USA) coupled to an Autospec Ultima High Resolution Mass Spectrometer (Waters, Manchester, UK) using electron impact (EI). A capillary RTX-5MS column (15 m x 0.25 mm i.d., 0.25 μm) (Restek Corporation, Bellefonte, PA, USA) was used, with helium at 1.0 ml/min as the carrier gas. The injector was programmed in splitless mode (2 min), set at a temperature of 280°C and the injection volume was 2 μl. The oven temperature program started at 120°C (held for 2.10 min), ramped at 35°C/min to 280°C (held for 1.00 min), then 5°C/min to 300°C (held for 3.00 min) and finally 50°C to 320°C (held for 1.00 min).

The HRMS operated in the SIR mode at 35eV, with 270°C as ion source and transfer line temperatures. The monitored and quantitative ions were as follows: *m/z* 271.8102 and 273.8072 for Dec 602, *anti*- and *syn*-DP, *m/z* 260.8599 and 262.8570 for Dec 603, *m/z* 417.7025 and 419.7005 for Dec 604, *m/z* 276.8297 and 278.8240 for ^{13}C *anti*-DP and *m/z* 413.8116 and 415.8096 for ^{13}C PBDE 183. An initial acquisition method of one single function was performed, but the possibility of using a three function method was also studied, with a first window acquisition from 6.00 to 9.86 min for Dec 602 and Dec 603, 9.86 to 11.00 min for Dec 604 and ^{13}C PBDE 183, and finally from 11.00 to 20.00 min for *syn*-DP, *anti*-DP and 13C *anti*-DP.

Study of method performance

Four aliquots of one gram each of a sample of fish oil from health supplements were dissolved in hexane and three of them were spiked with approximately 175 pg of Dec-602, 140 pg of Dec-603, 175 pg of Dec-604, 130 pg of *syn*-DP and 120 pg of *anti*-DP. Clean-up procedure and instrumental determination were preformed, and accuracy and precision were evaluated. Limits of detection were calculated as the concentration giving a signal equal to three times the baseline noise. Procedure blanks were performed in each batch of samples and the concentration of every compound was found to be below the detection limit.

Results and discussion

Analytical method

After studying fractions obtained with the preparative HPLC, results showed that dechlorane compounds were eluted between 3.5 min and 10 min in the conditions mentioned above, while residual interferences from the matrix were discarded in the first fraction (0 to 3.5 min). This improvement in the clean-up, monitored by

perfluorokerosene traces in the HRMS, implied chromatograms with less noise and better recoveries of the analytes.
Regarding the acquisition method, the use of a three function method in the instrumental determination, allowed an increase of sensitivity between 15 and 35%, depending on the compound.

The study of precision and trueness of the method, revealed that coefficient of variation values were below 12 % and trueness were below 30%. Despite these values, caused by the low concentrations of analytes and the use of only one internal standard for the quantification, the concentration range of the samples can be evaluated by this method. Limits of detection were between 0.9 and 1.7 pg in the validation sample for all the analytes except for Dec-604, which had a slightly higher value of 3.4 pg. The difference between limits of detection can be attributed to the difference on the chemical structure of Dec604 (partially brominated) compared to the other dechloranes (chlorinated).

Samples
The concentrations of Dec 602, Dec 603, Dec 604, *syn*- and *anti*-DP were determined for each sample and values between below the limit of detection and 384 pg/g were obtained (Table 2).These concentrations are in the same order as those reported by other authors in food and feed matrices[6,7,8].
Dec-602 and Dec-603 were not detected in vegetable oil samples but were found in most of the fish oil samples (Figure 1), while Dec 604 was below the limit of detection in all the studied samples. Generally, concentrations were higher in feed oils than in food oils. This could be explained by the fact that most of the food oils studied were health supplements and they are usually exhaustively purified to avoid the presence of pollutants and bad odors or taste[9].
The relative abundance of the *anti*- isomer (f_{anti}) was calculated by dividing the concentration of *anti*-DP by the sum of the *syn*- and *anti*-DP concentrations in the cases both compounds were detected. This value was below the industrial rate (0.75) in all cases, suggesting that some enrichment of the *syn*- isomer is produced. The relative isomer composition of DP in the environment is reported to vary for many reasons such as stereospecific photodegradation, biodegradation and biota isomer-specific uptake or elimination[10].

Table 2. Concentrations (pg/g) of Dec 602, Dec 603, *syn*-DP, *anti*-DP, ΣDPs, and f_{anti} in vegetable and fish oil samples

Sample code	Dec 602	Dec 603	syn-DP	anti-DP	ΣDPs	f_{anti}
F1	36.4	<5.5	<9.7	<7.6	-	-
F2	7.6	<1.6	17.3	35.7	53.0	0.67
F3	4.3	4.3	<3.6	<2.9	-	-
F4	<3.5	11	8.9	7.5	16.4	0.46
F5	10.9	9.8	<5.8	<4.6	-	-
F6	1.7	1.5	<3.7	4.4	-	-
F7	61.7	<1.7	<4.4	<3.5	-	-
F8	17.2	<2.4	<6.2	9.3	-	-
F9	6.1	9.3	12	9.8	21.8	0.45
F10	5.1	4.9	9	10.5	19.5	0.54
F11	13.6	9.7	9.6	12.8	22.4	0.57
F12	384	81.7	143.1	52.7	196	0.27
F13	192	12.9	<11.5	52.5	-	-
F14	7.7	3.1	6.1	8.4	14.5	0.58
F15	114	33.3	30.9	55.3	86.2	0.64
M1	32.6	29.1	14.5	19.1	33.6	0.57
M2	<3.4	<1.9	70.8	48.8	120	0.41
V1	<3.4	<3.2	14.7	34.4	49.1	0.70
V2	<3.1	<2.7	21.8	27.4	49.2	0.56

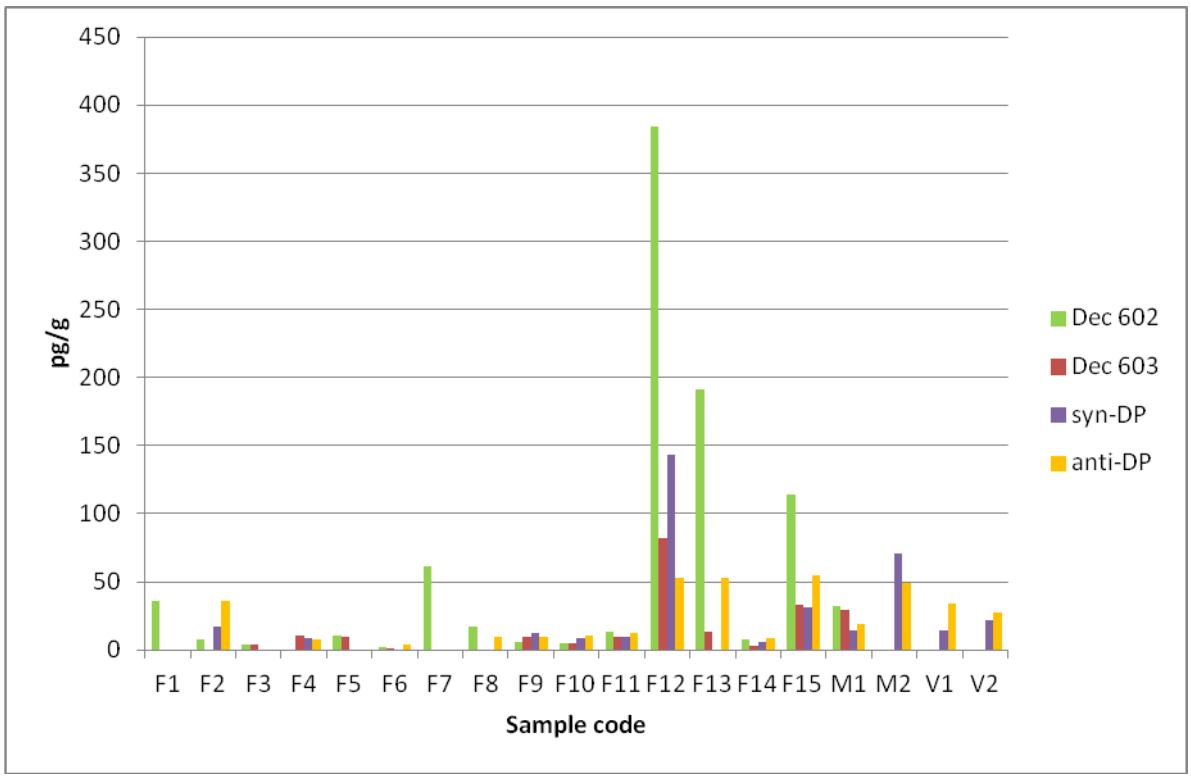

Figure 1 Distribution of Dec 602, Dec 603, *syn*- and *anti*-DP in oil samples

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