# Determination of PCBs and Dioxins and Their Stabilities in Corn Oil Formulations

Hamid Shafiei, Alice Shan, Rachel Rose-Mansfield, Mark Davis, Robert E. Smith
Midwest Research Institute, 425 Volker Blvd., Kansas City, MO 64110
and Diane Overstreet
NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

## Introduction

Methods based on high resolution gas chromatography (HRGC) and HRGC coupled to high resolution mass spectrometry (HRGC/HRMS) and isotope dilution were developed to determine the concentrations of 2,3,7,8-tetrachlorobenzo-p-dioxin (2,3,7,8-TCDD), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,2'4,4'5,5'-hexachlorobiphenyl (PCB 153) in corn oil dose formulations. Methods for sample clean-up were developed, too. This *in vitro* study was conducted to provide data on the feasibility of conducting toxicology studies on these compounds in rats. One dose of 4 ng/mL PCB 126 plus 4 μg/mL PCB 153, and another dose of 1.32 ng/mL 2,3,7,8-TCDD, 2.64 ng/mL 2,3,4,7,8-PeCDF, plus 13.3 ng/mL PCB126 were prepared in corn oil. A mixture of acidified and neutral silica was sufficient to remove corn oil and permit determination of μg/mL levels of PCB 153 by high resolution gas chromatography with electron capture detection (HRGC/ECD). However, further clean-up with alumina columns was needed to remove the 1000-fold excess of PCB 153 before determining ng/mL levels of PCB 126. All other analytes were determined by HRGC/HRMS utilizing a Fison's Autospec Ultima, operating at a resolving power of 10,000. Both doses were found to be stable for 35-days

## Materials and Methods

Matrix standards containing 20-150 μg/mL PCB 153 and 20-150 ng/mL PCB 126 were prepared in corn oil. Then, PCB 138 and $^{13}$C-PCB 126 internal quantitation standards were added. Also, standards in nonane containing ~ 1-100 pg/μL 2,3,7,8-TCDD and ~ 2-200 pg/mL PeCDF, along with ~ 50 pg/μL each of $^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-PeCDF, and $^{13}C_{12}$-1,2,3,4-TCDD were prepared. Triplicate dose formulations of 1.32 ng/mL 2,3,7,8-TCDD, 2.64 ng/mL 2,3,4,7,8-PeCDF and 13.4 ng/mL PCB 126 in corn oil were prepared, along with triplicate dose formulations of 4 ng/mL PCB 126 and 4 μg/mL PCB 153. To clean up the 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and PCB 126 formulation, acidified (40% $H_2SO_4$; w/w) silica and FMS carbon were used. Silica columns were used to clean up the PCB 126 plus PCB 153 formulation, also. Because there was one thousand times more PCB 153, no more clean up was required for PCB 153 analysis by GC/ECD. However, excess PCB 153 had to be removed before PCB 126 could be quantified. This was accomplished by applying samples to columns packed with neutral silica, acidic

alumina, and ~4 g of $Na_2SO_4$. The samples were analyzed for PCB 126 by HRGC/HRMS using a Fisons Autospec Ultima ® mass spectrometer operating with an accelerating voltage of 8000 volts, trap current of 600 μAmps, electron energy of 35 eV, photomultiplier voltage of 400 volts, source temperature of 280 °C, mass resolution of > 10,000 (10% valley definition) and an overall selective ion monitoring (SIM) cycle time of 1 sec. was used. It was equipped with a 60 m by 0.25 mm i.d. gas chromatography column with a DB5 column coating with a film thickness of 0.25 μm. The helium head pressure was 30 psi, with a total flow of 40 mL/min and a purge flow of 2 mL/min. Splitless injection (1 min), with an injection temperature of 290 °C, injection volume of 1 μL and an interface temperature of 280 °C were used. The temperature program was 150 °C to 230 °C at 50°C/min with a second hold time of 2.0 min, then a second temperature ramp of 230 °C to 235 °C at 1°C/min, followed by a third hold time of 2.0 min and a final hold time of 3 min.

At the same time, there is only 1/1000 as much PCB 126 as PCB 153 in the matrix standards. Thus, PCB 153 was analyzed directly by GC with no interference from PCB 126 using a Hewlett-Packard 6890 GC with a 6890 autosampler, a 60 meters long Restek Rtx-5 column, 0.25 mm i.d., and 0.25 μm film thickness. The oven temperature was 60°C (2 min hold), then 50°C/min to 220°C (1 min hold), then 8°C/min to 310°C (5 min hold). The electron capture detector was set at 350° C, with an inlet temperature of 260° C. The carrier gas was helium with a flow rate of 0.8 mL/min. The make-up gas was nitrogen with a flow rate of 50 mL/min. Splitless injection (1 μL) was used with the purge turned on 1 min after injection. The data system was Turbo Chrom, Version 4.

**Results**

The concentrations of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and PCB 126 in the first formulation were determined accurately as shown in Table 1.

**Table 1. Analysis of First Spiked Corn Oil Formulation**

| 2,3,7,8-TCDD Expected (ng/g) | 2,3,7,8-TCDD Found (ng/g) | 2,3,4,7,8-PeCDF Expected (ng/g) | 2,3,4,7,8-PeCDF Found (ng/g) | PCB 126 Expected (ng/g) | PCB 26 Found (ng/g) |
|---|---|---|---|---|---|
| 1.00 | 0.90 | 2.00 | 1.90 | 10.0 | 10.0 |
| 1.00 | 0.83 | 2.00 | 1.85 | 10.0 | 9.49 |
| 1.00 | 0.92 | 2.00 | 1.84 | 10.0 | 9.35 |
| 1.50 | 1.30 | 3.00 | 2.81 | 15.0 | 14.1 |
| 2.50 | 2.39 | 5.00 | 4.67 | 25.0 | 23.1 |
| 5.00 | 4.69 | 10.0 | 9.23 | 50.0 | 44.1 |
| 10.0 | 9.28 | 20.0 | 19.6 | 100 | 90.0 |
| 20.0 | 18.5 | 40.0 | 40.0 | 200 | 189 |
| 20.0 | 16.1 | 40.0 | 39.8 | 200 | 193 |
| 20.0 | 18.3 | 40.0 | 40.1 | 200 | 182 |

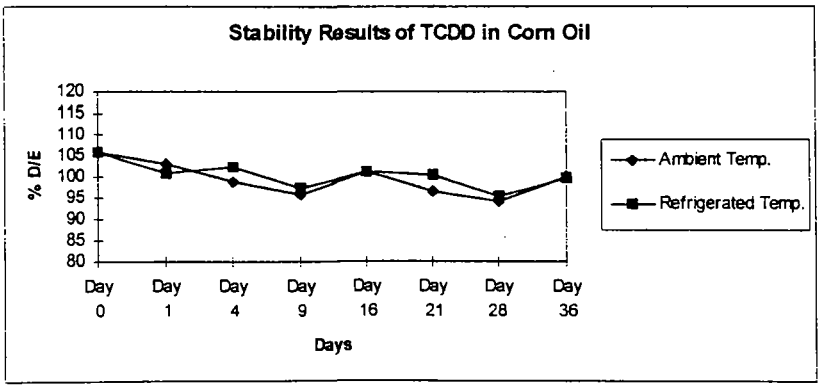
The results of the stability study in corn oil at ambient and refrigerated temperature are presented in Fig. 1a-c. The 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and PCB 126 were stable both at ambient and refrigerated storage conditions.

The concentrations of PCB 126 and PCB 153 in the second formulation were determined accurately as shown in Table 2. This formulation was stable for 35-days as shown in Fig. 2a-b.

**Table 2. Analysis of Second Spiked Corn Oil Formulations**

| PCB 126 Expected (ng/mL) | PCB 126 Found (ng/mL) | PCB 153 Expected (μg/mL) | PCB 153 Expected (μg/mL) |
|---|---|---|---|
| 3 | 2.94 | 3 | 2.85 |
| 3 | 2.95 | 3 | 3.15 |
| 3 | 3.03 | 3 | 2.95 |
| 20 | 20.5 | 20 | 18.0 |
| 20 | 20.9 | 20 | 18.8 |
| 20 | 20.6 | 20 | 18.4 |
| 30 | 30.9 | 30 | 29.9 |
| 50 | 51.2 | 50 | 53.0 |
| 75 | 75.4 | 75 | 78.5 |
| 100 | 99.0 | 100 | 102 |
| 150 | 154 | 150 | 148 |
| 150 | 152 | 150 | 154 |
| 150 | 150 | 150 | 146 |

**Fig. 1. Stabilities of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and PCB 126 in First Formulation in Corn Oil**



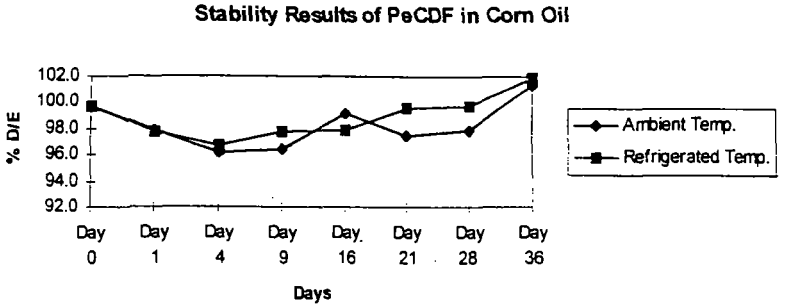



Fig. 2. Stability of PCB 126 and PCB 153 in Second Formulation in Corn Oil