Preparation, Stability and Analysis of Corn Oil Formulations containing 2,3,4,7,8-Pentachlorodibenzofuran (4-PeCDF), 3,3'4,4',5-Pentachlorobiphenyl (PCB-126) and 2,2'4,4',5,5'-Hexachlorobiphenyl (PCB-153) for Use in the National Toxicology Program Toxicity Equivalence Factor Dioxin Initiative.

Brian L. Burback[1], Cynthia S. Smith[2], Jon White [1], Ed Psurny[1], Barb Harritos[1] and Steve Graves[1]. [1]Battelle Memorial Institute, 505 King Avenue, Columbus Ohio 43201, [2] National Institute of Environmental Health Sciences, National Institutes of Health, 111 T. W. Alexander Drive, Research Triangle Park, NC 27709.

Abstract

The National Toxicology Program's (NTP) Toxicity Equivalence Factor Dioxin Initiative[1] requires the preparation, analysis and determination of stability for formulations of various polychlorinated aromatic hydrocarbons in corn oil containing 1% acetone as a cosolvent. The formulations were prepared by diluting the acetone solutions of the chemicals with corn oil. Analysis of PCB-153 formulations at concentrations ranging from 0.2 to 10 mg/mL was accomplished by diluting the formulations with acetone and analyzing by gas chromatography with electron capture detection (GC/ECD). Analysis of PCB-126 and 4-PeCDF formulations at concentrations of 1.2 ng/mL and above was done by saponification of the formulation, extraction with hexane[2]and analysis of the extract with gas chromatography/mass spectrometry detection (GC/MS). A stability study of these formulations was done at the lowest targeted dose concentration of each compound for at least 35 days and under simulated animal dosing conditions. All three formulations were determined to be stable and therefore suitable for use in studies planned by the NTP.

Introduction

The National Toxicology Program (NTP) is currently planning a series of toxicokinetic and chronic toxicology studies for 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), 3,3'4,4',5-pentachlorobiphenyl (PCB-126) and 2,2'4,4',5,5'-hexachlorobiphenyl (PCB-153) as part of their "Toxicity Equivalence Factor Dioxin Initiative". In order to carry out these studies it was necessary to develop methods to formulate doses, analyze these formulations and determine their stability.

Experimental Methods

Formulations of PCB-126, PCB-153 and 4-PeCDF were prepared by first diluting acetone solutions with corn oil to obtain the required concentration of chemical and ratio of

acetone to corn oil (1%). The mixtures were stirred for a minimum of 2 hours to ensure that the chemical was totally dissolved.

Validation of analytical methods was done using a minimum of six standards prepared from two independently prepared stock solutions. Triplicate standards were analyzed at the lowest and the highest standard concentrations. Acceptance criteria for the validations included precision, accuracy, adequate sensitivity, specificity, and linearity. The precision of the assay was acceptable if the relative standard deviation of the highest and lowest vehicle standards was less than 10%. The accuracy of the assay was acceptable if the relative error, or average relative errors, were less than 10% of the theoretical values. The sensitivity of the assay was acceptable if the limit of quantitation was at least 20% below the concentration of the lowest vehicle standard. The specificity of the assay was acceptable if the average response in the blanks was less than 10% of the lowest vehicle standard. The linearity of the assay was acceptable if the correlation coefficient (r) was greater than 0.99.

Analysis of PCB-153 formulations was accomplished by preparing standards of PCB-153 in corn oil containing 1% acetone at concentrations from 10 to 100 μg/mL. These standards were prepared for analysis by diluting 1 mL of standard and 1 mL of internal standard (hexachlorobenzene at 10 μg/mL in acetone) to 100 mL with acetone. Formulations with concentrations greater than the upper limit were diluted into the concentration range with acetone. One mL of the formulation (or diluted formulations) and 1 mL of internal standard were diluted to 100 mL with acetone. All standards and samples were analyzed using a Hewlett Packard 5890 series II GC with an electron capture detector using a PTE-5 15m x 0.53mm capillary column. Stability of these formulation was determined at 20 μg /mL when the formulation was stored in sealed amber glass containers with minimal headspace and under simulated dosing conditions.

Analysis of PCB-126 doses was accomplished by preparing standards of PCB-126 in corn oil containing 1% acetone at concentrations from 1 to 15 ng/mL. These standards were prepared for analysis by adding 0.5 mL of internal standard (^{13}C PCB126 at 40 μg/mL in acetone), 5 mL of potassium hydroxide and 2 mL of ethanol. The mixtures were then vortexed, sealed and mixed overnight. They were allowed to cool to room temperature. Five (5) mL of water and 5 mL of 95% ethanol were then added to each. They were vortexed and extracted with 5 mL of hexane (four times). The extracts were combined, evaporated, reconstituted in nonane and transferred to a GC vial. Formulations were diluted into the concentration range of the assay and treated identically. Standards and samples were analyzed by GC/MS using a VG Autospec Ultima with a DB5, 15 m X 0.25 mm (ID), 0.25 μm column. Stability of these formulations was determined at 1.2 ng /mL stored in sealed amber glass containers with minimal headspace and under simulated dosing conditions.

Analysis of 4-PeCDF doses was accomplished by preparing standards of 4-PeCDF in corn oil containing 1% acetone at concentrations of 1.6 to 15 ng/mL. These standards were prepared for analysis by adding 0.5 mL of internal standard (^{13}C 4-PeCDF at 40 μg/mL in acetone), 5 mL of potassium hydroxide and 2 mL of ethanol. The mixtures were then vortexed, sealed and mixed overnight. They were allowed to cool to room temperature. Five (5) mL of water and 5 mL of 95% ethanol were then added to each. They were vortexed and extracted with 5 mL of hexane (four times). The extracts were combined, evaporated, reconstituted in nonane and transferred to a GC vial. Formulations were diluted into the concentration range of the assay and treated identically. Standards and samples were analyzed by GC/MS using a VG Autospec Ultima with a DB5, 15 m X 0.25 mm (ID), 0.25 μm column. Stability of these

formulations was determined at 4 ng /mL stored in sealed amber glass containers with minimal headspace and under simulated dosing conditions.

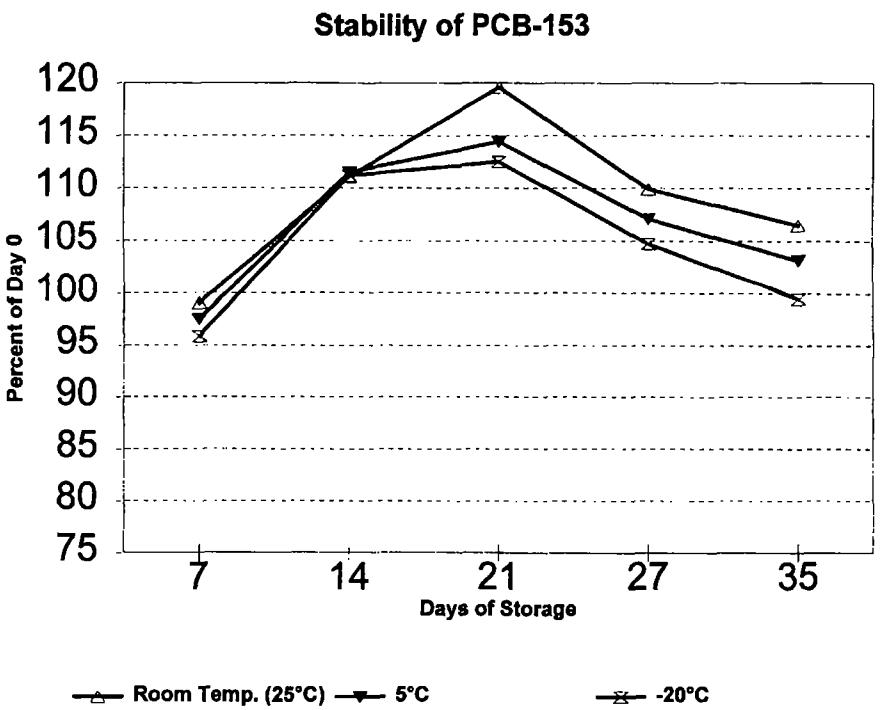
Results and Discussions

The preparation of formulations containing PCB-126, PCB-153 and 4-PeCDF in corn oil containing acetone requires that the doses be stirred for at least two hours to ensure that a solution is obtained.

Methods have been developed and validated for the analysis of formulations in corn oil containing 1% acetone at concentrations greater than 10 µg/mL for PCB-153, 1.0 ng/mL for PCB-126 and 1.6 ng/mL for 4-PeCDF. These methods are suitable for use in the toxicokinetic and toxicity studies planned by NTP

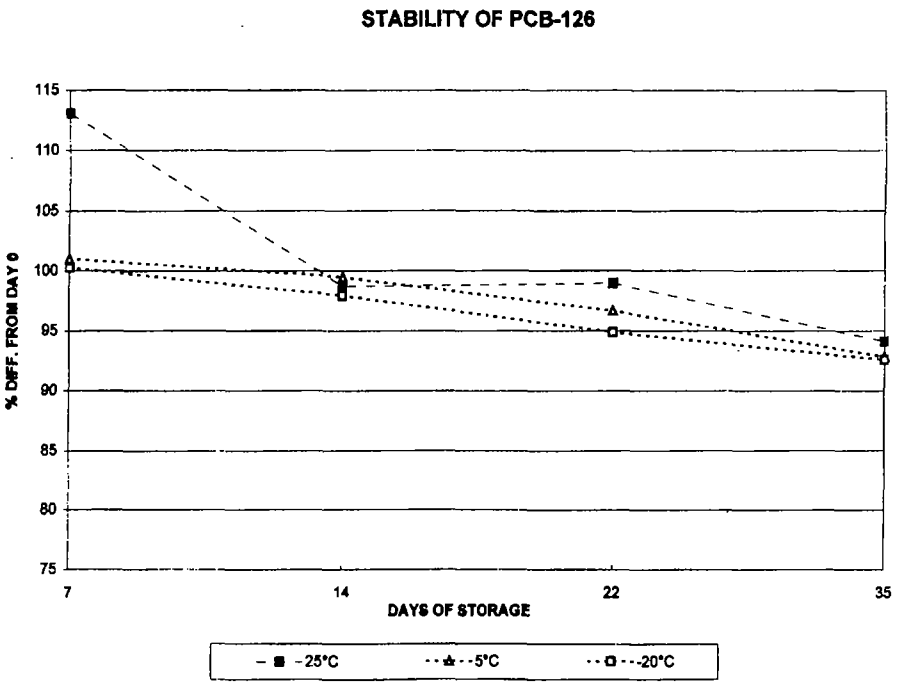
Formulations of PCB-153 at 20 µg/mL were determined to be stable (Figure 1) for at least 35 days at room temperature (25°C), 5°C, and –20°C. The method used in preparing standards on day 21 probably caused lower than nominal standard concentrations, resulting in high values for this day.

Figure 1.



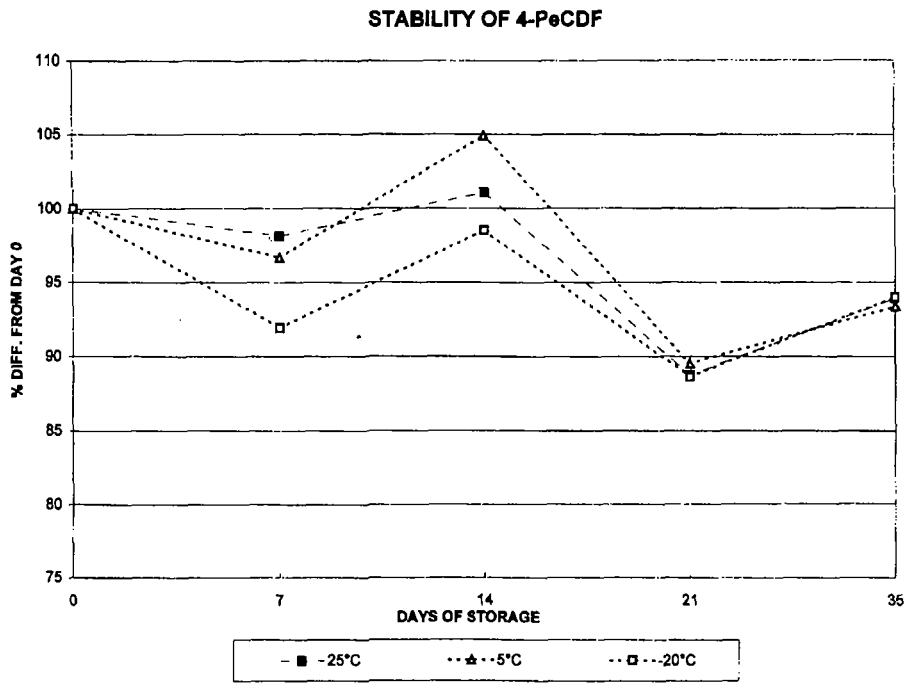
Doses of PCB-126 at 1.2 ng/mL were determined to be stable (Figure 2) for at least 35 days at room temperature (25°C), 5°C, and –20°C.

Figure 2.



Doses of 4-PeCDF at 4 ng/mL were determined to be stable (Figure 3) for at least 35 days at room temperature (25°C), 5°C, and –20°C.

Figure 3.



The formulations are stable and are suitable for use in the toxicokinetic and the toxicology studies.

Acknowledgments

This work was done in support of the Chemistry Support Services for the Environmental Toxicology Program and was funded by the National Institute of Environmental Health Sciences.

Literature Cited

(1) National Toxicology Program "Studies to Evaluate Dioxin Equivalency Factors Initiative" Current Study Funded by the National Institute of Environmental Health Sciences.
(2) Collins, B. Private Communication from the National Institute of Environmental Health Sciences. *1996.*