

**PREPARATION OF INDIVIDUAL AND CUSTOM PCB MIXTURE  
DOSING SOLUTIONS FOR AVIAN EGG INJECTION STUDIES  
ASSOCIATED WITH INJURY DETERMINATIONS  
UNDER THE HUDSON RIVER NRDA**

**USCG BIOCHEMISTRY & PHYSIOLOGY BRANCH  
FINAL LABORATORY REPORT FY 2011  
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**HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT**

**HUDSON RIVER NATURAL RESOURCE TRUSTEES**

STATE OF NEW YORK

U.S. DEPARTMENT OF COMMERCE

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*\* Certain information has been redacted to maintain confidentiality.*



## **EXECUTIVE SUMMARY**

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

As part of the Hudson River NRDA, the Trustees are conducting an avian egg injection study. This report provides the U.S. Geological Survey (USGS) report on the preparation of dosing solutions used in the Trustees' avian egg injection studies. Specifically this report addresses the preparation of dosing solutions used in the Trustees' avian egg injection studies in years subsequent to 2006, including the dosing solutions of PCB 77, PCB 126, the spotted sandpiper PCB mixture and the tree swallow PCB mixture.

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**PREPARATION OF INDIVIDUAL AND CUSTOM PCB MIXTURE DOSING SOLUTIONS FOR AVIAN EGG INJECTION  
STUDIES ASSOCIATED WITH INJURY DETERMINATIONS UNDER THE HUDSON RIVER NRDA**

## 1.0 BACKGROUND

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

In 2002, the Hudson River Natural Resource Trustees released an Assessment Plan for the Hudson River (Hudson River Natural Resource Trustees, 2002). That Assessment Plan documented that natural resources of the Hudson River had been, and continued to be, exposed to contamination by PCBs, and provided information regarding three major steps in the assessment: pathway and injury determination, injury quantification, and damage determination and restoration. That Assessment Plan further noted that the Trustees were considering conducting injury determination and quantification for a number of Hudson River resources, including birds, and that the Trustees' currently proposed approach to injury determination and quantification entailed several specific investigations focused on birds, including a preliminary avian evaluation, a breeding bird survey, a bird egg study, an evaluation of avian exposure from feeding on floodplain organisms, and bald eagle monitoring.

In 2004, the Trustees released the “Study Plan for Year 2004 Avian Investigations for the Hudson River – Final, Public Release Version” (Avian Injury Study Plan), dated June 15, 2004 (Hudson River Natural Resource Trustees, 2004), following up on the Assessment Plan and avian work that had been conducted by the Trustees in the interim. That Avian Injury Study Plan described the activities that constituted the Trustees' planned approach to conducting investigations of injury to avian species as part of the Hudson River NRDA, including an avian egg injection study.

## 2.0 INTRODUCTION

In 2006, the Trustees released the “Study Plan for Avian Egg Injection Study” (Avian Egg Injection Study Plan), dated May 12, 2006, revised January 31, 2007 (Hudson River Natural Resource Trustees, 2007a). Year 1 (2006) avian egg injection work focused on injection of test PCBs and development of injection and incubation protocols for eggs from tree swallow (*Tachycineta bicolor*), American kestrel (*Falco sparverius*) and chicken (*Gallus domesticus*) (Hudson River Natural Resource Trustees 2007a). The PCB mixture used in the 2006 work is described in Appendix B of the 2006 Avian Egg Injection Study Plan, in the report, “Design and Preparation of a Custom 58-Congener PCB Mixture Dosing Solution for Avian Egg Injection Studies.” This 58-congener mixture is also referred to as the “spotted sandpiper PCB mixture” and is described in Hudson River Natural Resource Trustees 2007b. The Trustees subsequently conducted additional avian egg injection studies in 2007-2010.

Year 2 (2007) work included an evaluation of the effects of a PCB mixture relevant to tree swallows from the Upper Hudson River (a 66-congener mixture) in an egg injection study, and a pilot study of injection of that PCB mixture into eggs of Eastern bluebirds (*Sialia sialis*) (Hudson River Natural Resource Trustees 2007b). This 66-congener mixture is also referred to as the “tree swallow PCB mixture.”

Year 3 (2008) work included further egg injection studies on tree swallows, American kestrels and Eastern bluebirds, along with a pilot study of injection of a PCB mixture into eggs of Eastern screech owl (*Otus asio*) (Hudson River Natural Resource Trustees 2008). In 2008 tree swallow eggs were injected with PCB 77, and eggs of American kestrel, Eastern bluebird and Eastern screech owl were injected with the 58-congener mixture.

Egg injection work on Japanese quail was done in Years 4 and 5 (2009-2010) to inform the results of work done under previously approved study plans.

The objective of these investigations is to evaluate the toxicity and adverse effects of embryonic exposure of multiple avian species to individual PCB congeners or PCB mixtures.

This report provides the U.S. Geological Survey report on the preparation of dosing solutions used in the Trustees' avian egg injection studies in years subsequent to 2006, including the dosing solutions of PCB 77, PCB 126, the spotted sandpiper PCB mixture and the tree swallow PCB mixture.

### 3.0 LITREATURE CITED

- Hudson River Natural Resource Trustees. 2002. Hudson River Natural Resource Damage Assessment Plan. September 2002. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2004. Study Plan for Year 2004 Avian Investigations for the Hudson River. Final. Public Release Version. June 15, 2004. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2007a. Study Plan for Avian Egg Injection Study. Hudson River Natural Resource Damage Assessment. Final. Public Release Version. May 12, 2006; revised January 31, 2007. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2007b. Avian Injury Study. Avian Egg Injection Study Plan. Amendment for Year 2 (2007). Hudson River Natural Resource Damage Assessment. Final. Public Release Version. June 1, 2007. U.S. Department of Commerce, Silver Spring, MD.
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**Preparation of individual and custom PCB mixture dosing solutions for avian egg injection studies associated with injury determinations under the Hudson River NRDA.**

Biochemistry & Physiology Branch Final Laboratory Report FY 2011

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## **Attachments**

Attachment 1. AccuStandard Certificate of Analysis: Custom, spotted sandpiper, PCB mixture, S-13907-250mL.

Attachment 2. AccuStandard Certificate of Analysis: Isooctane, procedural blank, S-13907-BLK-250mL.

Attachment 3. AccuStandard Certificate of Analysis: Custom, tree swallow, PCB mixture, S-15880-250 mL

Attachment 4. AccuStandard Certificate of Analysis: isooctane, procedural blank S-15880-BLK-250 mL.

Attachment 5. AccuStandard Certificate of Analysis:PCB 77, C-077N, received 4-25-08.

Attachment 6. AccuStandard Certificate of Analysis:PCB 126, C-126N, received 12-22-08.

Attachment 7. AccuStandard Certificate of Analysis:PCB 77, C-077N, received 1-20-09.

## **1.0 Introduction**

The Hudson River Natural Resource Trustees are conducting studies to evaluate whether exposure to PCBs has resulted in injury to avian reproduction and/or development along the Hudson River. As part of the NRDA injury assessment, avian egg injection studies have been conducted to establish toxicity thresholds. This report describes the preparation of injection solutions for individual congeners and PCB mixtures used in the avian egg injection studies conducted by Dr. Mary Ann Ottinger and colleagues, University of Maryland.

Several dosing solutions of individual PCB congeners and PCB mixtures for avian toxicity testing were prepared for the avian injury studies. A PCB mixture designed to mimic the composition of PCBs found in spotted sandpiper eggs collected along the Hudson River was previously formulated and is referred to as the “spotted sandpiper PCB mixture” throughout the rest of this report. The rationale and preparation methods for the dosing solutions described in this report were the same as those described previously for the spotted sandpiper PCB mixture. This report contains the preparation details for a PCB mixture designed to represent PCB congeners found in tree swallow eggs found along the Hudson River. The tree swallow data are listed in the previous report and this mixture is referred to as the “tree swallow PCB mixture.” Individual PCB solutions are referenced by their congener numbers, the targeted species to be studied, and the injection carrier solution used in the preparation.

### ***1.1 Spotted sandpiper PCB mixture order & receipt***

The custom, spotted sandpiper, PCB mixture dissolved in 250-mL of isooctane and corresponding 250-mL of blank isooctane were purchased from AccuStandard, Inc. (New Haven, CT; Invoice # 270876; S-13907-250mL; Lot B5110052). The custom, spotted sandpiper, PCB mixture solution and isooctane blank were received in separate brown glass bottles with Teflon-lined screw caps. AccuStandard personnel used their exact measured weights of each congener and GC/MS –based congener purities to generate a list of “prepared concentrations” and “certified analyte concentrations” provided in the certificate of analysis for the custom, PCB congener, and standard solution (Attachment 1). A similar certificate of analysis was provided for the isooctane blank (Attachment 2).

This custom PCB congener package was opened by Diane Nicks and James L. Zajicek upon receipt at CERC and the bottles were inspected for concurrence with attached documentation. Both bottles were received in good condition and the documentation was verified. To verify these solution concentrations, triplicate 100- $\mu$ L sub-samples of each were taken with a 100- $\mu$ L Hamilton syringe and placed in cleaned, amber, 1.5-mL, autosampler vials with yellow, Teflon-faced, septum-lined screw caps. The autosampler vials were weighed before and after sample addition using both a Mettler AE260 and a Mettler-Toledo A6245 so that mass of the transferred volume could be determined. The six samples were given to Dr. Kathy Echols of the CERC Organic Chemistry section for analysis. The spotted sandpiper PCB mixture and the isooctane blank were secured and stored in a locked box placed in the CERC Biochemistry Section hazardous compound storage area.

### ***1.2 Tree swallow PCB mixture order and receipt***

The custom tree swallow PCB mixture, dissolved in 250 mL of isooctane, and the corresponding 250 mL of blank isooctane were purchased from AccuStandard, Inc. (New Haven, CT; Invoice # 297036; S-15880-250 mL; Lot B7040178 ). The tree swallow PCB mixture solution and isooctane blank were received in separate brown glass bottles with Teflon-lined screw caps. AccuStandard personnel used their exact measured weights of each congener and GC/MS –based congener purities to generate a list of “prepared concentrations” and “certified analyte concentrations” provided in the certificate of analysis for the custom PCB congener standard solution (Attachment 3). A similar certificate of analysis was provided for the isooctane blank (Attachment 4).

The custom PCB congener package was opened by Diane Nicks and James L. Zajicek upon receipt at CERC and the bottles were inspected for concurrence with attached documentation. Both bottles were received in good condition and the documentation was verified. The Tree Swallow PCB mixture and the isooctane blank were secured and stored in a locked box placed in the CERC Biochemistry Section hazardous compound storage area.

### ***1.3 PCB congener order & receipt***

Neat PCB standards, congener 126 and 77 (two separate shipments), were purchased from AccuStandard, Inc. (New Haven, CT) in exact weight aliquots, prepared by AccuStandard personnel in small amber vials with PTFE screw caps. Each shipment was accompanied by a certificate of analysis indicating purity (Attachments 5, 6 & 7).

The PCB congener packages were opened by Diane Nicks and James L. Zajicek upon receipt at CERC and the vials were inspected for concurrence with attached documentation. All vials were received in good condition and documentation was verified.

### ***1.4 Glassware preparation***

**Transfer tubes** – Glass culture tubes (25 or 30 mL) with Teflon<sup>®</sup>-faced, rubber lined screw caps were used for transfer and sub-sampling of the original Spotted Sandpiper and Tree Swallow, PCB mixtures from AccuStandard. These transfer tubes were prepared by solvent rinsing with three consecutive washes of acetone, securing the Teflon-lined screw cap during each rinse to eliminate any residual manufacture contaminants in both the tube and cap. These transfer tubes were uniquely labeled by etching and any residual acetone was allowed to evaporate. Two, tube, heating blocks with accompanying nitrogen purge apparatuses were prepared in separate fume hoods, one designated for the PCB mixture(s) and one for the blank(s). Heating blocks were set to maintain heat at approximately 35°C (range 32 – 38°C).

**Dosing solution vials** - New 3-mL or 5-mL v-vials (Sigma, Z115096, Z115118) and corresponding black Teflon-lined screw caps (Sigma, Z164313) were etched with an individual number or identification. Using ultra pure, ultra filtered 0.2 µm ASTM Type I water (Synergy UV, Millipore Corp., Bedford, MA),

vials were marked for volume at the required volumes for each dosing solution to be made. The water was then decanted and each vial was solvent rinsed with three consecutive washes of acetone, securing the Teflon-lined screw cap during each rinse to eliminate any residual water or manufacture contaminants. Rinsed vials and caps were air dried in the fume hood. All vials & caps were uniquely labeled by etching, autoclaved at 120° C, 20 psi, 10 min sterilization and then dried at 35° C for 2 hours.

## **2.0 Preparations of spotted sandpiper PCB mixture dosing solutions**

### ***2.1 Spotted sandpiper PCB mixture and procedural blank for kestrel egg injection in corn oil solvent study 4-24-06***

#### **Sub-sampling**

Calculations indicated that 50 mL of the 250 mL original spotted sandpiper PCB mixture would be required to make the dosing solutions for the Kestrel egg injection studies (Table 1). Using a new, sterile, 10.0-mL, serological pipette 12.5 mL of the spotted sandpiper PCB isooctane mixture received from AccuStandard was transferred to one of the pre-washed, pre-weighted, 30-mL culture tubes (labeled JZ2). A second new, sterile, 10.0-mL, serological pipette was used to transfer 12.5-mL of the isooctane blank received from AccuStandard into another pre-washed, pre-weighted, 30 mL culture tube (labeled JZ1). The two tubes were placed into separate, pre-designated heating blocks, and a gentle stream of nitrogen was used to evaporate the solvent (isooctane) from each tube. The volume of each tube was concentrated to approximately 3 mL and then another 12.5 mL of the original spotted sandpiper PCB mixture from AccuStandard (for tube JZ2) or the isooctane blank received from AccuStandard (for tube JZ1) were carefully added to the respective transfer tubes. The cycle of transfer and evaporation (from the spotted sandpiper PCB mixture solution to transfer tube JZ2 or the isooctane blank to transfer tube JZ1) was continued until a total of 50 mL of the original solutions (PCB or blank) from AccuStandard had been added to each tube. After the addition of the final 12.5 mL aliquot, the volume in each transfer tube was allowed to concentrate to approximately 1 mL.

The concentrated, spotted sandpiper PCB solution (transfer tube JZ2) was quantitatively transferred using a baked (450 °C) Pasteur pipette to a previously prepared 5-mL dosing solution vial, labeled JLZ2-SD. Vial JLZ2-SD was placed in the PCB designated heating block and secured with gently purging nitrogen to facilitate evaporation of the isooctane washes. Transfer tube JZ2, containing the PCB mixture, was rinsed 12 times with 1 to 1.5-mL isooctane and each rinse was transferred to vial JLZ2-SD as space was made available due to evaporation. The isooctane blank was treated in exactly the same manner, transferring the contents of transfer tube JZ1 to 5-mL vial labeled JLZ1-IB. The isooctane blank solution in transfer tube JZ1 was rinsed 12 times and rinses transferred to vial JLZ1-IB, which was placed in the isooctane heating block. After the final rinse was transferred, the heating block temperatures were reduced to approximately 25 °C and both vials (vial JLZ1-IB and vial JLZ2-SD) were concentrated to the pre-etched mark at 0.8 mL (etching described above).

### **Preparation of spotted sandpiper PCB mixture stock and procedural blank in corn oil solutions**

The dosing solutions of this PCB mixture were prepared in corn oil to attain concentrations great enough for toxicity testing. A PCB stock solution (in vial labeled JLZ2-SD) and procedural blank solution (in vial labeled JLZ1-IB) were prepared from the concentrated sub-samples described above. The targeted concentration of the PCB stock dosing solution was 246 µg PCB/µL in a final volume of 1.6-mL. A procedural blank solution was like-wise prepared from the isooctane sub-sample concentrate. These solutions were prepared as follows. Small volumes of sterile (0.2-µm filtered) corn oil (Sigma, Cat. # C8267, batch 103K0107, density 0.918 g/mL) were added to each of the 5-mL vials; 49.1 µL to vial JLZ1-IB (blank) and 47.2 µL to vial JLZ2-SD (PCB mixture); volumes calculated based on density. Each vial was gently mixed by slow vortex mixing. This was done to allow the corn oil to act as a co-solvent for the PCB congeners, and the rest of the isooctane was allowed to evaporate. Any residual isooctane was allowed to evaporate under gentle nitrogen purge with the vials maintained at approximately 25° C. The vials were periodically mixed by slow vortex mixing. The evaporation was continued until vials reached a constant weight and the masses of the contents (PCB congeners) in each of the two vials were determined by difference.

Sterile corn oil was added to both vials to bring them to the previously marked volume of 1.6 mL. Calculated volumes based on the masses of the corn oil added confirmed the volume added to each vial. The contents of each 5-mL vial were vigorously vortex mixed until they appeared homogeneous. The resulting corn oil solution of the spotted sandpiper, PCB standard, stock dosing solution (vial JLZ2-SD) was at the target concentration of 246 µg PCB/µL, while the procedural blank corn oil solution (vial JLZ1-IB) was the negative control for the toxicity testing; the procedural blank, stock dosing solution.

### **Serial dilution of the spotted sandpiper PCB mixture stock in corn oil for kestrel studies**

The various dosing solutions were prepared by serial dilution of the stock, PCB mixture, corn oil solution. Five 3-mL vials previously prepared, weighed and marked at 0.5 and 1.0-mL were arranged so that they were consecutively labeled; 3-2F, 3-4F, 3-8F, 3-16F and 3-32F. A 500-µL aliquant of the spotted sandpiper, PCB standard, stock dosing solution (vial JLZ2-SD) was transferred to an empty 1-mL vial labeled 1-JLZ2-SD under sterile conditions. In the same manner, a 500-µL aliquant of the procedural blank, stock dosing solution (vial JLZ1-IB) was transferred to an empty 1-mL vial labeled 1-JLZ1-IB. Under sterile conditions, a 500-µL aliquant of corn oil was transferred to each of the vials 3-2F, 3-4F, 3-8F, 3-16F and 3-32F. The spotted sandpiper, PCB mixture, stock dosing solution (vial JLZ2-SD) was freshly vortex-mixed and using a calibrated Rainin P-1000, 500-µL was transferred to vial 3-2F. This resulted in a 2-fold dilution and vial 3-2F was vigorously vortex-mixed. In the same manner, 500-µL was transferred from vial 3-2F to vial 3-4F resulting in a 4-fold dilution of the original PCB emulsion. This process was repeated to produce the entire dosing solution dilution series (Table 2). Finally, a 500-µL aliquot was transferred from vial 3-32F to an empty 1-mL vial labeled 1-32F (Table 2).

### **Sub-sampling of the spotted sandpiper PCB mixture in corn oil dosing solutions prepared for kestrel studies for chemical analysis**

Aliquots (40- $\mu$ L) of each dosing solution (Table 2) were taken and placed in previously prepared, amber, 1.5-mL, autosampler vials with Teflon-faced septa screw caps. The autosampler vials were weighed before and after sample addition using a Mettler AE260 analytical balance so that the mass of the transferred volume could be determined. The seven sub-samples were archived in the locked box which was held in the CERC Biochemistry Section, hazardous compound storage area.

### **Shipment of the spotted sandpiper PCB mixture in corn oil dosing solutions for kestrel studies**

Each vial listed in Table 2 was opened under sterile conditions, the air space was purged with argon to remove oxygen, recapped, then individually bagged in a 4 x 4 "ziplock plastic bag, placed in a 3-mL vial shipping box and all were stored in an upright position at -80 °C in preparation for shipment. All other vials or bottles containing solutions prepared in this procedure were returned and secured to a locked box after all air spaces had been argon purged. The 3-mL vial shipping box containing the dosing solutions was packed in a Styrofoam shipping box with approximately 10 lbs. dry ice and shipped via FedEx with the appropriate documentation to Dr. Emma Lavoie, University of Maryland.

### **Nominal concentrations of PCBs and TEQs in the dosing solutions and eggs**

The certified concentrations provided by AccuStandard (Attachment 1) were used to calculate the nominal concentrations of individual PCB congeners in each of the corn oil-based egg dosing solutions (Table 3). Briefly, for each congener we calculated the mass in 50.0-mL of the original 250-mL spotted sandpiper PCB standard stock solution from AccuStandard (Table 1). This mass was converted to concentration units ( $\mu$ g/ $\mu$ L) using the final volume of the PCB stock dosing solution (1.60-mL in vial JL22-SD). Subsequently, dosing solution concentrations for the individual congeners were calculated based on dilution factors (Table 2). Finally, individual PCB congener egg dose concentrations were calculated by multiplying the dosing solution concentration of each congener times the injection volume (6  $\mu$ L/egg) and then dividing by the average mass of a Kestrel egg (15 g/egg). Expected doses of each of the PCB congeners were calculated in this fashion, for each of the six corn oil-based dosing solutions (Table 3).

For example: The AccuStandard certified concentration for PCB congener 28 (2, 4, 4'-trichlorobiphenyl) of 817.6  $\mu$ g/mL (Attachment 1), was multiplied by 50.0 mL, resulting in 40,880  $\mu$ g of PCB congener 28. Next, we divided by 1.60 mL and then by 1000  $\mu$ L/mL to give 25.55  $\mu$ g/ $\mu$ L; the concentration of PCB congener 28 in the corn oil, stock, dosing solution (98  $\mu$ g total PCB/g egg, vial 1-JL22-SD, see solution naming convention below). The PCB congener 28 concentration/g egg (expected nominal dose) was calculated by multiplying 25.55  $\mu$ g/ $\mu$ L by 0.4  $\mu$ L/g/egg and finally multiplying by 1000 ng/ $\mu$ g to obtain 10,220 ng/g (Table 3). The names for each of the corn oil dosing solutions (column headers) were given as the nominal total PCB dose expected to be delivered to an individual Kestrel egg from that solution (Table 3). Thus, the six corn oil dosing solutions were referred to as 98, 49, 25, 12, 6, and 3  $\mu$ g PCB/g egg doses (column headers in Table 3).

The dioxin toxic equivalents (TEQs) expected to be delivered to a quail egg at each dose were also estimated (Table 4). The dose from the 12 PCB congeners with dioxin-like potency (from Table 3) was multiplied times the potency of the congener (toxic equivalency factor, TEF) as determined for avian wildlife (van den Berg et al. 1998). The sum of the individual congener TEQs was estimated for each of the dosing solutions. The six dosing solutions, referred to as 98, 49, 25, 12, 6, and 3 µg PCB/g egg (Table 3), had total TEQs of approximately 12,600 pg/g, 6,300 pg/g, 3,100 pg/g, 1,600 pg/g, 790 pg/g, and 390 pg/g, respectively (Table 4).

## ***2.2 Spotted sandpiper PCB mixture and procedural blank for chicken egg injections in corn oil solvent study 10-17-06***

The previously prepared, stock, spotted sandpiper, PCB solution (in vial JLZ2-SD, at a nominal concentration of 244.8 µg/µL) and sterile corn oil were used to prepare targeted dilutions for chicken, egg injection studies. Using the techniques previously described, 1,500 µL of a 60.0 µg spotted sandpiper PCB mix/µL stock was made by adding 370 µL from vial JLZ2-SD to 1130 µL corn oil in vial 3-COS. The resulting solution was further diluted according to a 3-fold series into vials 3-3F, 3-9F, 3-27F, 3-81F, & 3-243F. This resulted in a solution series range of 60, 20, 6.67, 2.22, 0.74, and 0.25 µg spotted sandpiper PCB mix/µL corn oil (Table 5). Vial 3-COB was used for the sterile corn oil blank solution. A 50 µL subsample of each solution was taken and stored as previously described. Solutions were prepared for shipment and sent as stated above.

## ***2.3 Spotted sandpiper PCB mixture and procedural blank for eastern screech owl egg injections in fatty-acid-mix solvent study 4-1-08***

2.0 mL of a fatty acid mix was prepared according to the recipe received from Karen Dean (University of Maryland).

**Redacted**

This was placed in a sterile 5 mL vial and topped w/ argon.

Using the previously described techniques, 9.4 mL of the custom spotted sandpiper PCB mix prepared in isooctane (S-13907, AccuStandard) was used to make 250 µL of 295 µg total PCB mix/µL fatty acid mix (25.2 µg total PCB/g egg). A 1 mL portion of the custom spotted sandpiper PCB mix stock solution (9.4 mL total) was transferred into a sterile 1.0 mL vial along with 100 µL of the above-described fatty acid mix. The vial was placed in a heating block at 37° C and the isooctane was evaporated under a gentle stream of nitrogen. The rest of the 9.4 mL of the stock solution was added to the vial in 1.0-mL aliquots as the isooctane evaporated. The gentle stream of nitrogen was continued until all isooctane had been removed as evidenced by the constant vial weight. A fatty acid blank solution (250 µL) was prepared in the same manner using the isooctane blank (S-13907-BLK, AccuStandard). A ten-fold dilution of the 295 µg spotted sandpiper PCB mix/µL fatty acid was made by adding 20 µL of the 295 µg/µL solution to 180 µL of the fatty acid mix in a clean sterile vial (Table 6). A 20 µL sample of the 295

$\mu\text{g}/\mu\text{L}$  (25.2  $\mu\text{g}$  total PCBs/g egg) solution was transferred to a clean auto-sampler vial and stored (described above) for possible later concentration confirmation. The three dosing solution vials were prepared & shipped as described above.

#### ***2.4 Spotted sandpiper PCB mixture and procedural blank for Japanese quail egg injections in charcoal-stripped corn oil solvent study 1-7-09***

Two 25 mL clean, baked test tubes were marked # 5 & # 6. The tube # 5 was used to concentrate the sandpiper PCB mix and # 6 was used for the blank. Measured aliquots (20.0 mL) of the stock PCB or isooctane solutions were carefully pipetted into their respective tubes. The solvent was evaporated under a slow nitrogen stream as the tubes were held at  $\sim 40^\circ\text{C}$ . As the solution evaporated more was added in 10.0 mL aliquots and a final 205  $\mu\text{L}$  aliquot until a total volume of 128.205 mL was transferred to the tube. The solution was allowed to evaporate to  $\sim 2$  mL. The solutions were then transferred to clean, baked, weighed 3-mL vials labeled SS 1.2 mg/ $\mu\text{L}$  & Blank. Vials SS 1.2 mg/ $\mu\text{L}$  & Blank were marked for volume of 833.3 & 416.65  $\mu\text{L}$ , respectively. Each tube was rinsed 6 times with clean isooctane and each rinse was transferred to the corresponding vial with evaporation between rinses to allow space for subsequent rinses.

Vial SS 1.2 mg/ $\mu\text{L}$  was evaporated and weighed indicating that 1.1477 g of solution was in the vial. The decision was made to make the highest volume solution at a concentration of 1.2 mg total PCBs/ $\mu\text{L}$  and to make 2-fold dilutions of the resulting solution. Since 128.205 mL of the stock contains  $\sim 1000$  mg total PCBs, then:  $1000 \text{ mg}/1.2 \text{ mg}/\mu\text{L} = 833.3 \mu\text{L}$ .

As the vial weight approached the weight expected when all solvent was removed, 10  $\mu\text{L}$  of the charcoal-stripped corn oil was added. As the vial weight reduced with solvent removal an additional volume of corn oil was added to bring the solution to the 833.3  $\mu\text{L}$  mark. Vial weight changes indicated that a total of 80.5  $\mu\text{L}$  of the charcoal-stripped corn oil was added to vial TS 1.2 mg/ $\mu\text{L}$ .

Five other clean, baked, 3-mL vials had been prepared by labeling SS 0.6 mg/ $\mu\text{L}$ , SS 0.3 mg/ $\mu\text{L}$ , SS 0.15 mg/ $\mu\text{L}$ , SS 0.075 mg/ $\mu\text{L}$ , & SS 0.0375 mg/ $\mu\text{L}$ . These 3-mL vials were marked for volume at 833.3 & 416.65  $\mu\text{L}$  then filled with the charcoal-stripped corn oil to the 416.65 line. Then a serial dilution was made by removing 416  $\mu\text{L}$  from the TS 1.2 mg/ $\mu\text{L}$  vial and then adding it to the TS 0.6 mg/ $\mu\text{L}$  vial. The resulting dilution was mixed by repeated pipetting, vortex mixed, and then a 416- $\mu\text{L}$  aliquot was transferred to the next vial, etc. All vials were kept at  $40^\circ\text{C}$  to facilitate pipetting & mixing. An extra 416  $\mu\text{L}$  of the charcoal-stripped corn oil was added to the blank vial. All vials were then clearly labeled to indicate total spotted sandpiper PCB mix concentration (Table 7).

Seven clean 1-mL vials were labeled and weighed. A 20.0  $\mu\text{L}$  sample of each solution was removed to the corresponding 1-mL vial. The vials were weighed again and weights recorded. These subsamples were held in reserve in the CERC locked box. Dosing vials were prepared and shipped as previously described.

### **3.0 Preparations of tree swallow PCB mixture dosing solutions**

#### ***3.1 Tree swallow PCB mixture and procedural blank solutions for tree swallow egg injections in propylene glycol/corn oil emulsion study 4-30-07***

##### **Sub-sampling**

A portion (37.5 mL of the 250 mL) of the original Tree Swallow PCB mixture (S-15880) was used to make the dosing solutions for the tree swallow egg injection studies (Table 8). Using new, sterile, 10.0-mL, serological pipette 12.5 mL of the Tree Swallow PCB isooctane mixture received from AccuStandard was transferred to one of the pre-washed, pre-weighted, 30 mL culture tubes (labeled PCB). A second new, sterile, 10.0-mL, serological pipette was used to transfer 12.5 mL of the isooctane blank received from AccuStandard into another pre-washed, pre-weighted, 30-mL culture tubes (labeled iso). The tubes were placed into separate, pre-designated heating blocks and a gentle stream of nitrogen was used to evaporate the solvent (isooctane) in each tube. The volume of each tube was concentrated to approximately 3 mL and then another 12.5 mL aliquot of the original Tree Swallow PCB mixture from AccuStandard (tube PCB) or the isooctane blank received from AccuStandard (tube iso) were carefully added to the respective transfer tubes. The cycle of transfer and evaporation (from the Tree Swallow PCB mixture solution to transfer tube PCB or the isooctane blank to transfer tube iso) was continued until a total of 37.5 mL of the original solutions (PCB or blank) from AccuStandard had been added to each tube. After the addition of the final 12.5 mL aliquot, the volume in each transfer tube was allowed to concentrate to approximately 1 mL.

The concentrated Tree Swallow PCB solution (transfer tube PCB) was quantitatively transferred using a baked Pasteur pipette to a previously prepared 3-mL dosing solution vial, labeled X2. Vial X2 was placed in the PCB designated heating block and secured with gently purging nitrogen to facilitate evaporation of the isooctane washes. Transfer tube PCB, containing the PCB mixture, was rinsed 12 times with 1 to 1.5 mL isooctane and each rinse was transferred to vial X2 as space was made available due to evaporation. The isooctane blank was treated in exactly the same manner, transferring the contents of transfer tube iso to the 3-mL vial labeled X1. Transfer tube iso was rinsed 12 times; the rinses were transferred to vial X1, and vial X1 was placed in the isooctane heating block. After the final rinse of each transfer vial was transferred, each heating block temperature was reduced to approximately 25 °C and the contents of both vials (vial X2 and vial X1) were concentrated to the pre-etched marked at 0.5 mL (described above).

##### **Preparation of tree swallow PCB mixture stock emulsion and procedural blank emulsion**

The dosing solutions of the PCB mixture were prepared as emulsions of 0.75% (v/v) corn oil in 1,2-propanediol (propylene glycol) to attain concentrations great enough for toxicity testing. A PCB stock emulsion (in vial labeled X2) and procedural blank emulsion (in vial labeled X1) were prepared from the sub-samples described above. The targeted concentration of the PCB stock emulsion dosing solution was 250 µg PCB/µL (or 250 mg/mL) in a final volume of 1.37 mL. A procedural blank emulsion was also prepared. These emulsions were prepared as follows. Approximately, 11 µL of sterile corn oil (Sigma, Cat. # C8267, batch 103K0107, density 0.9 g/mL) was added to each of the 3-mL vials; 10.6-µL

to vial X1 (blank) and 12.2- $\mu$ L to vial X2 (PCB mixture), exact volumes calculated based density. Each vial was gently mixed by slow vortex mixing. This was done to allow the corn oil to act as a co-solvent for the PCB congeners and the rest of the isooctane was allowed to evaporate. Any residual isooctane was allowed to evaporate under gentle nitrogen purge with the vials maintained at approximately 25 °C. The vials were periodically mixed by slow vortex mixing. The evaporation was continued until vials reached a constant weight and the masses of the contents (PCB congeners or non-volatile material in the procedural blank) in each of the two vials were determined by difference.

Filter sterilized 1,2-propanediol (Fisher # P355-1, density 1.036 g/mL) was added to both vials to bring them to the previously marked volume of 1.37-mL. Calculated volumes based on the masses of the 1,2-propanediol added confirmed the volumes added to the respective vials. The contents of each 3-mL vial were vigorously vortex-mixed until they became thoroughly emulsified. The resulting emulsion of the Tree Swallow, PCB standard, stock, dosing solution (vial X2) was at the target concentration of 250  $\mu$ g PCB/ $\mu$ L, while the procedural blank emulsion (vial X1) was the negative control or the toxicity testing, blank, stock dosing solution.

### **Serial dilution of the PCB mixture stock emulsion**

The target dosing solutions were prepared by serial dilution of the stock PCB mixture emulsion. A sterile emulsion of corn oil and 1,2-propanediol (propylene glycol) was first prepared to make all of the subsequent dilutions. The sterile stock emulsion of 0.75% (v/v) corn oil:1,2-propanediol was prepared by combining 37.5- $\mu$ L of sterile corn oil (Sigma, C8267) with 4.963-mL of sterile 1,2-propanediol (Fisher, P-355) in a sterile 15-mL glass culture tube with a Teflon-faced, rubber-lined, screw cap (volumes were confirmed by their weights). The resulting mixture was vigorously vortex mixed to produce a stable emulsion. The six 3-mL vials previously prepared, weighed, and marked at 0.5 and 1.0 mL were arranged so that they were consecutively numbered, X3 to X8. A 500- $\mu$ L aliquant of the tree swallow PCB standard stock dosing solution emulsion (vial X2) was transferred to the empty vial X3 under sterile conditions. Also using sterile conditions, 500- $\mu$ L aliquots of the freshly vortex mixed, sterile, stock emulsion of 0.75% (v/v) corn oil:1,2-propanediol from the 15-mL culture tube were transferred to vials X4, X5, X6, X7, and X8. The tree swallow, PCB mixture, stock, dosing solution, emulsion (vial X2) was freshly vortex mixed, and a 500- $\mu$ L aliquot was transferred to vial X4 using a calibrated Rainin P-1000. Vial X4 was vigorously vortex mixed to provide the targeted 2-fold dilution. In the same manner, a 500- $\mu$ L aliquot was transferred from vial X4 to vial X5 resulting in a 4-fold dilution of the original PCB emulsion. This process was repeated to produce the entire targeted, dosing solution, dilution series. Vial X1 remained as prepared to serve as blank. (Table 9).

### **Sub-sampling of dosing solution emulsions for chemical analysis**

A 20- $\mu$ L sample of each dosing solution (Table TS PCB emulsion sol concs) was taken and placed in previously prepared amber 1.5-mL autosampler vials with Teflon<sup>®</sup>-faced, septum-lined screw caps. The autosampler vials were weighed before and after sample addition using a Mettler AE260 so that mass of the transferred volume could be determined. The seven samples were held in reserve, secured, and stored in a locked box placed in the CERC Biochemistry Section hazardous compound storage area.

### **Shipment of the dosing solutions**

Each vial shipped was opened under sterile conditions, the air space was purged with argon to remove oxygen, recapped, then individually bagged in a 4 x 4 "Zip-loc" type plastic bag, placed in a vial shipping box, and all were stored in an up-right position at -80 °C in preparation for shipment. All other vials or bottles containing solutions prepared in this procedure were returned and secured in a locked box after all air spaces had been argon purged. The vial shipping box containing the dosing solutions was packed in a Styrofoam shipping box with approximately 10 lbs. dry ice and shipped via FedEx on 30 April, 2007 with the appropriate documentation to Emma Lavoie or Mary Ann Ottinger, University of Maryland.

### **Nominal concentrations of PCBs in the dosing solutions and eggs**

The certified concentrations provided by AccuStandard (Attachment 3) were used to calculate the nominal concentrations of individual PCB congeners in each of the emulsified egg dosing solutions (Table 10). Briefly, for each congener we calculated the mass in 37.5-mL of the original 250-mL Tree Swallow PCB standard stock solution from AccuStandard (Table 8). This mass was converted to concentration units ( $\mu\text{g}/\mu\text{L}$ ) using the final volume of the PCB stock dosing solution emulsion (1.37-mL in vial X2). Subsequently, dosing solution concentrations for the individual congeners were calculated based on dilution factors (Table 9). Finally, individual PCB congener egg dose concentrations were calculated by multiplying the dosing solution concentration of each congener times the injection volume ( $0.8 \mu\text{L}/\text{egg}$ ) and then dividing by the average mass of a tree swallow egg (2 g/egg). Expected doses of each of the Tree Swallow PCB congeners were calculated in this fashion, for each of the six dosing solutions (Table 10).

### ***3.2 Tree swallow PCB mixture and procedural blank for Tree Swallow egg injections in corn oil study 5-3-07***

This series of solutions was prepared as described above with the following variations. A measured aliquot (27.28 mL of the original 250 mL) of the Tree Swallow, PCB mixture was used to make the dosing solutions for the corn oil vehicle, tree swallow egg, injection studies (Table 8). Using a new, sterile, 10.0-mL, serological pipette a 12.5-mL aliquot of the tree swallow, PCB isooctane mixture, received from AccuStandard, was transferred to one of the pre-washed, pre-weighted, 30 mL culture tubes (labeled PCB). A second new, sterile, 10.0 mL, serological pipette was used to transfer a 12.5-mL aliquot of the isooctane blank (AccuStandard) into another pre-washed, pre-weighted, 30-mL culture tube (labeled iso). The tubes were placed into separate, pre-designated heating blocks and a gentle stream of nitrogen was used to evaporate the solvent (isooctane) in each tube. The volume of each tube was concentrated to approximately 3 mL, then another 12.5 mL aliquot of the original, tree swallow, PCB mixture (AccuStandard, tube PCB) or the isooctane blank (AccuStandard, tube iso) were carefully added to the respective transfer tubes. The cycle of transfer and evaporation (from the tree swallow PCB mixture solution to transfer tube PCB or the isooctane blank to transfer tube iso) was continued until a total of 27.28 mL of the original solutions (PCB or blank) from AccuStandard had been added to each tube. After the addition of the final 2.28 mL aliquot, the volume in each transfer tube was allowed to concentrate to approximately 1 mL.

The concentrated, tree swallow, PCB solution (transfer tube PCB) was quantitatively transferred using a baked Pasteur pipette to a previously prepared 3-mL dosing solution vial, labeled X10. Vial X10 was placed in the PCB designated heating block and secured with gently purging nitrogen to facilitate evaporation of the isooctane washes. Transfer tube PCB, containing the PCB mixture, was rinsed 12 times with 1 to 1.5 mL isooctane and each rinse was transferred to vial X10 as space was made available due to evaporation. The isooctane blank was treated in exactly the same manner, transferring the contents of transfer tube iso to 3-mL vial labeled X9. The isooctane blank solution in transfer tube iso was rinsed 12 times and rinses transferred to vial X9, which was placed in the isooctane heating block. After the final rinses were transferred, the heating block temperatures were reduced to approximately 25 °C and both vials (vial X10 and vial X9) were concentrated to the pre-etched marked at 0.50 mL (described above).

The dosing solutions of the PCB mixture in corn oil were prepared to provide targeted concentrations for toxicity testing. A PCB stock solution (in vial labeled X10) and procedural blank solution (in vial labeled X9) were prepared from the sub-samples described above. The targeted concentration of the PCB mixture, stock, corn oil, dosing solution was 250 µg PCB/µL (250 mg/mL) in a final volume of 1.0-mL. A procedural blank stock corn oil solution was similarly prepared. These stock solutions in corn oil were prepared as follows. Approximately 11 µL of sterile corn oil (Sigma, Cat. # C8267, batch 103K0107, density 0.9 g/mL) was added to each of the 3-mL vials. Each vial was gently mixed by slow vortex mixing. This was done to allow the corn oil to act as a co-solvent for the PCB congeners and the rest of the isooctane was allowed to evaporate. Any residual isooctane was allowed to evaporate under gentle nitrogen purge with the vials maintained at approximately 25 °C. The vials were periodically mixed by slow vortex mixing. The evaporation was continued until vials reached a constant weight and the masses of the contents (PCB congeners or non-volatile material in the isooctane procedural blanks) in each of the two vials were determined by difference.

Filter sterilized corn oil (Sigma, Cat. # C8267, batch 103K0107, density 0.9 g/mL) was added to both vials to bring them to the previously marked volume of 1.0-mL. Calculated volumes, based on the masses of the corn oil added, confirmed the volumes added to the two vials. The contents of each 3-mL vial were vigorously vortex mixed until they appeared homogenous. The resulting solution of the tree swallow, PCB standard mixture, stock, dosing solution (vial X10) was at the target concentration of 250 µg PCB/µL, while the procedural blank solution (vial X9) was the negative control or the toxicity testing, stock, blank, dosing solution.

The targeted dosing solutions were prepared by serial dilution of the stock, corn oil, PCB mixture. Sterile corn oil (Sigma, C8267) was used to make all of the subsequent dilutions. Six 3-mL vials (previously prepared, weighed, and marked at 0.5 and 1.0-mL) were arranged so that they were consecutively numbered, X11 to X15. Using sterile techniques, a 500-µL aliquant of sterile corn oil was transferred to vials X11, X12, X13, X14, and X15. The tree swallow, PCB mixture, stock, dosing solution, in corn oil (vial X10) was freshly vortex mixed and using a calibrated Rainin P-1000, a 500-µL aliquot was transferred to vial X11. Vial X11 was vigorously vortex mixed to provide the desired 2-fold dilution. In the same manner, 500-µL was transferred from vial X11 to vial X12 resulting in a 4-fold

dilution of the original stock PCB mixture in corn oil. This process was repeated to produce the entire dosing solution dilution series. Vial X9 remained as prepared to serve as blank. (Table 11).

A 20- $\mu\text{L}$  sample of each dosing solution (Table TS PCB corn oil sol concs) was taken and placed in previously prepared amber 1.5-mL autosampler vial with Teflon-faced septa screw caps. The autosampler vials were weighed before and after sample addition using a Mettler AE260 so that mass of the transferred volume could be determined. These seven samples were held in reserve, secured, and stored in a locked box held in the CERC Biochemistry Section hazardous compound storage area.

Each vial was prepared & shipped as described above. Nominal concentrations were identical to those of the tree swallow PCB mix/corn oil-propylene glycol emulsion described above (Table 11).

### ***3.3 Tree swallow PCB mixture and procedural blank for Japanese quail egg injections in charcoal stripped corn oil solvent study 1-7-09***

Two 25-mL clean baked screw cap culture tubes were marked # 3 & # 4. The tube # 3 was used to concentrate the tree swallow PCB mix and # 4 was used for the isooctane blank. A measured aliquot (20.0) mL of the stock tree swallow PCB mix or iso-octane was carefully pipetted into their respective tubes. The solvent was evaporated under a slow nitrogen stream as the tubes were held at  $\sim 50^{\circ}\text{C}$ . As the solution evaporated more was added in 10.0- mL aliquots. The last aliquot added was 9.0 mL plus 0.123 mL so that 109.123 mLs (1000  $\mu\text{g}$  total PCB's) were placed in the tube. The temperature was reduced to  $\sim 40^{\circ}\text{C}$  and each solution was allowed to evaporate to  $\sim 2$  mL. The solutions were then transferred to weighed 5-mL vials II 1 & II 2, tree swallow PCB mix solution & blank respectively. Vials II 1 & II 2 were marked at 300 & 500  $\mu\text{L}$ . Each culture tube was rinsed 6 times with clean iso-octane, and each rinse was transferred to the corresponding vial with evaporation between rinses to allow space for subsequent, consecutive rinses.

The contents of Vial II 1 were evaporated to approximately 1 mL. The measured weight difference from the empty vial weight indicated of 1.0869 g of PCB mix plus isooctane remained in the vial. The decision was made to maximize the volume of solution that would have a concentration of 1.2 mg/ $\mu\text{L}$  and subsequently, make 2 fold dilutions.

109.123 mL of the stock contains  $\sim 1000$  mg total PCBs.

$1000 \text{ mg}/1.2\text{mg}/\mu\text{L} = 833.3 \mu\text{L}$

Seven clean baked 3-mL vials were marked for volume at 833.3 & 416.65  $\mu\text{L}$ . Vials were labeled (TS 1.2 mg/ $\mu\text{L}$ , TS 0.6 mg/ $\mu\text{L}$ , TS 0.3 mg/ $\mu\text{L}$ , TS 0.15 mg/ $\mu\text{L}$ , TS 0.075 mg/ $\mu\text{L}$ , TS 0.0375 mg/ $\mu\text{L}$ , & Blank) weighed and weights recorded. The PCB mix solution was transferred from vial II 1 to the TS 1.2 mg/ $\mu\text{L}$  vial then the II 1 vial was rinsed 6 times with clean isooctane and each rinse was transferred to the TS 1.2 mg/ $\mu\text{L}$  vial with evaporation at  $40^{\circ}\text{C}$  between rinses to allow space for consecutive rinses. The blank solution was transferred in the same manner. As the vial weight approached the weight expected when all solvent was removed 10  $\mu\text{L}$  of the charcoal stripped corn oil was added. As the vial

weight reduced with solvent removal an additional volume of corn oil was added to bring the solution to the 833.3  $\mu\text{L}$  mark. Vial weight changes indicated that a total of 83  $\mu\text{L}$  of the charcoal stripped corn oil was added to vial TS 1.2 mg/ $\mu\text{L}$ .

All other 3-mL vials were filled with the charcoal-stripped corn oil to the 416.65 line. Then a serial dilution was made by removing 416  $\mu\text{L}$  from the TS 1.2 mg/ $\mu\text{L}$  vial and then adding it to the TS 0.6 mg/ $\mu\text{L}$  vial. This solution was vortex mixed and a similar 416  $\mu\text{L}$  was removed/transferred to the next vial, etc. All vials were kept at 40°C to facilitate pipetting & mixing. An extra 416  $\mu\text{L}$  of the charcoal stripped corn oil was added to the blank vial. All vials were then clearly labeled (Table 12).

Seven clean 1-mL autosampler vials were labeled and weighed. A 20  $\mu\text{L}$  sample of each solution was removed to the corresponding 1-mL vial. The vials were weighed again and weights recorded in excel spreadsheet "analysis sample vial wt info 011209."

Solutions were prepared & shipped as described above.

#### **4.0 Preparations of individual PCB dosing solutions**

##### ***4.1 Preparation of PCB 77 dosing solution in fatty acid mix for tree swallow egg injection studies 4-29-08***

Neat PCB 77 (C-077N) was purchased from AccuStandard with exact weight recorded. Two vials, each containing ~25 mg were received on 4/25/08 by Mandy Annis (CERC) & transferred to Diane Nicks. The catalog # was C-077N, lot # 981009LB-AC. A measured volume (2.565 mL) of methylene chloride ( $\text{CH}_2\text{CL}_2$ ) was added (using 500 & 100  $\mu\text{L}$  Hamilton syringes) to the vial containing 0.02565 g of PCB 77 to result in a 10 mg/mL solution. The vial was marked at the volume and alternately vortex mixed & sonicated until all PCB 77 crystals were in solution.

Three clean 3-mL vials & caps were marked at 1-mL volume; solvent rinsed and allowed to dry. 3 mL of a fatty acid mix was prepared in a clean 5-mL vial according to the recipe received from Karen Dean 4/23/08 **Redacted**

**Redacted**

The fatty acid mix was vortex mixed and placed in a 45° C heating block to ensure melting & mixing of fatty acids. After all fatty acids had melted the mix was kept at 37° C to allow for pipette transfer.

A 1-mL aliquot of  $\text{CH}_2\text{CL}_2$  was placed in one of the previously marked vials and labeled B. A 100- $\mu\text{L}$  aliquot of the 10 mg PCB 77/mL  $\text{CH}_2\text{CL}_2$  stock solution was placed in a second vial (with a Hamilton syringe), then 900  $\mu\text{L}$   $\text{CH}_2\text{CL}_2$  was added to result in a 1 mg/mL solution. This vial was labeled 77 1. Likewise, a 1-mL aliquot of the 10 mg PCB 77/mL  $\text{CH}_2\text{CL}_2$  was placed in a third vial with a Hamilton syringe. This vial was labeled 77 10.

All three vials were placed under a gentle stream of nitrogen in a 37° C heating block and evaporated to approximately 500 µL. To each was added 900 µL of the fatty acid mix, vortex mixed, sonicated and again placed under a gentle stream of nitrogen in a 37° C heating block. All vials were periodically vortex mixed, sonicated and again placed under a gentle stream of nitrogen in a 37° C heating block until all of the CH<sub>2</sub>CL<sub>2</sub> had been removed; as evidenced by the constant weight. All vials were brought up to the 1-mL mark by adding additional amounts of the fatty acid mix (Table 13). A 20 µL subsample was taken from each vial, stored at CERC for possible analysis, and then all vials were topped with argon, capped, prepared for shipment and shipped as described above.

#### **4.2 Preparation of PCB 126 dosing solution in carbon stripped corn oil for Japanese quail egg studies 12-30-08**

A total of ~15 mg (3 by 5 mg) of neat PCB 126 was purchased from AccuStandard (C-125N). The neat PCB 126 material was received in three AccuStandard vials, accurate weights in 5 mg range, were those listed below:

vial # 1:	0.00569 g
vial #2:	0.00553 g
vial #3:	<u>0.00532 g</u>
Total:	0.01654g

Sample calculations:

The target PCB 126 stock dosing solution concentration was 25 µg/µL so 0.01654 g = 16540 µg

16540 µg/25 µg/µL = 661.6 µL

A clean, baked, 5-mL vial was marked at a volume of 661.6 µL.

To each of the three received vials about 1 mL of CH<sub>2</sub>CL<sub>2</sub> was added to dissolve the PCB 126 powdered material. Then resulting solution was transferred to the marked 5-mL vial and some of the CH<sub>2</sub>CL<sub>2</sub> was evaporated at ~ 40°C under a slow stream of nitrogen. Each vial was rinsed 6 times with clean CH<sub>2</sub>CL<sub>2</sub> and the rinses transferred to the 5-mL vial. The 5-mL vial marked at 661.6 µL was brought up to volume with CH<sub>2</sub>CL<sub>2</sub>.

Using a 500-µL Hamilton syringe seven, clean, baked, 1-mL vials were marked at 300 µL and at 500 µL. These were then etched I 1 thru I 7. A 300 µL aliquot of CH<sub>2</sub>CL<sub>2</sub> was added to vials I 2 thru I 6 and 500 µL CH<sub>2</sub>CL<sub>2</sub> to vial I 7. Using a 250-µL Hamilton syringe 500 µL of the PCB 126 CH<sub>2</sub>CL<sub>2</sub> stock was transferred from the 5-mL vial to vial I 1. Then using the same syringe 200 µL of the PCB 126 CH<sub>2</sub>CL<sub>2</sub> stock was transferred from vial I 1 to I 2, mixed and then 200 µL was transferred to I 3, etc. resulting in a 2.5 fold serial dilution (25, 10, 4, 1.6, 0.64, 0.256 µg/µL). A measured aliquot (100 µL) of sterile, charcoal-stripped, corn oil was added to each vial, then all vials were placed under a slow nitrogen stream at ~ 40°C. The CH<sub>2</sub>CL<sub>2</sub> was evaporated by a gentle purge of nitrogen. To ensure all of the CH<sub>2</sub>CL<sub>2</sub> was removed vials were periodically sonicated, heated & vortex mixed. As the CH<sub>2</sub>CL<sub>2</sub> was

removed a second 100  $\mu\text{L}$  aliquot of sterile, charcoal-stripped, corn oil was added. The 25  $\mu\text{g}/\mu\text{L}$  solution required heat and sonication to get the PCB 126 into solution. All other concentrations remained in solution during the solvent transfer from  $\text{CH}_2\text{Cl}_2$  to corn oil. Vials I 1 thru I 5 were brought up to the 300- $\mu\text{L}$  mark with the sterile, charcoal-stripped, corn oil, while vials I 6 & I 7 were brought up to the 500  $\mu\text{L}$  mark, I 6, the final dilution and I 7 the blank (Table 14).

A 20  $\mu\text{L}$  sample of each dosing solution was removed to clean, labeled vials and held at CERC for later analysis, if required. Vials I 1 through I 7 were labeled, topped with nitrogen, capped and sealed with Teflon tape. The vials were then frozen ( $-80^\circ\text{C}$ ), packed, and prepared for shipment as stated above.

#### **4.3 Preparation of PCB 77 dosing solution in carbon stripped corn oil for Japanese quail egg studies**

Neat PCB 77 (C-077N, 600 mg) was purchased at accurate weight from AccuStandard.

The vial containing C-077N) was received by Diane Nicks (CERC) and the accurate weight recorded at 0.60060 g. The requested concentration was 1.2  $\text{mg}/\mu\text{L}$  so the result of the equation below indicates how this concentration could be achieved using all of the neat PCB 77 in the vial.

$$600.6 \text{ mg} / 1.2 \text{ mg}/\mu\text{L} = 500.5 \mu\text{L}$$

A clean baked 3-mL vial labeled 3-5 was marked at the volume 500.5  $\mu\text{L}$ . Six other clean baked 1 mL vials were marked for volume at 250 & 500  $\mu\text{L}$ . To the received vial containing the neat PCB 77, about 2 mL of  $\text{CH}_2\text{Cl}_2$  was added to dissolve the PCB 77. The neat PCB 77 did not dissolve completely and resulted in a white colored suspension. This suspension was transferred to vial 3-5, the  $\text{CH}_2\text{Cl}_2$  was evaporated under a gentle flow of nitrogen and the original vial was rinsed 8 times with  $\text{CH}_2\text{Cl}_2$  and each rinse was transferred to vial 3-5 with evaporation. About 3 mL of  $\text{CH}_2\text{Cl}_2$  was added to vial 3-5 to attempt to get the PCB 77 into solution. The PCB 77 still did not go into solution with heat & sonication. To allow for more head volume the solution was transferred with 8 rinses to a clean baked 5 mL vial labeled II-4. To help get the PCB 77 into solution  $\sim 250 \mu\text{L}$  (0.2027 g) of sterile, charcoal stripped corn oil was added to the vial. (The idea was that the PCB 77 would dissolve into the corn oil more easily.) The vial was sonicated & heated to about  $56^\circ\text{C}$  and the  $\text{CH}_2\text{Cl}_2$  was evaporated. A second  $\sim 250 \mu\text{L}$  (0.2132 g) of sterile, charcoal stripped corn oil was added to the vial. With sonication, heat and evaporation the  $\text{CH}_2\text{Cl}_2$  was removed but the PCB 77 did not go into solution. More corn oil was added to try to get the PCB 77 into solution, up to  $\sim 1000 \mu\text{L}$  (1.0659 g which calculates to 949  $\mu\text{L}$  so  $\sim 633 \mu\text{g}/\mu\text{L}$ ). With heat ( $56^\circ\text{C}$ ), sonication & vortexing the PCB 77 would not go into solution. But it did appear to hold in a suspension for a short period of time.

To test for solubility, 100  $\mu\text{L}$  of the PCB 77/corn oil suspension was transferred to a clean, baked, 1-mL vial. After several additions of corn oil with little success it was decided to make the highest concentration at 20  $\mu\text{g}/\mu\text{L}$ . So a 32-fold dilution of the suspension in vial II-4 was made to result in two 1-mL vials of a solution at 20  $\mu\text{g}$  PCB 77/ $\mu\text{L}$  corn oil. These vials were sonicated, heated ( $56^\circ\text{C}$ ), and vortex mixed to get the PCB 77 completely into solution. Due to the extended time the vial II-4 was open for evaporations and corn oil additions, the 2 mL of the warm 20  $\mu\text{g}$  PCB 77/ $\mu\text{L}$  corn oil was passed through a 0.2  $\mu\text{m}$  syringe filter to collect 1 mL of the sterilized solution into a clean, sterile, baked, marked, 1-mL vial. A one to one serial dilution of this filtered nominal concentration 20  $\mu\text{g}/\mu\text{L}$  solution was made into clean, baked, marked-for-volume vials to result in 500  $\mu\text{L}$  each of 20, 10, 5, 2.5,

1.25 & 0.625 µg/µL solutions. An additional 1 mL of the sterile, charcoal-stripped, corn oil was placed into another vial to serve as vehicle control (Table 15).

A 20 µL sub-sample of each dosing solution and the unfiltered 20 µg/µL solution were removed to clean, labeled vials and held at CERC for later analysis, if required. These 20 µL subsample vials were weighed before and after the sample additions, and the measured weights were recorded in “analysis sample vial wt info.xls.” The PCB 77 dosing solution vials were labeled, topped with argon, capped, and sealed with Teflon tape. The vials were then frozen (-80°C), packed, and prepared for shipment as previously described.

## 5.0 References

CERC SOP P.561. 1998. Accurate formulation of dosing solutions for injection studies.

Material Safety Data Sheet, Corn Oil C8267. Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103.

Material Safety Data Sheet, propylene glycol. Fisher Scientific, 1 Reagent Lane, Fairlawn, NJ 07410.

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**Table 1. Final selected PCB congeners, total mass, and percentage of total PCB in the stock, 50-mL aliquant of the original, Spotted Sandpiper, PCB mixture solution.**

PCB Congener	PCB congener mass ( $\mu\text{g}$ ) in the 50-mL aliquant of the original stock solution received from AccuStandard.	Relative amount (%)
PCB 28	40,880	10.46%
PCB 66	36,660	9.38%
PCB 74	26,840	6.87%
PCB 118	25,530	6.53%
PCB 47	14,420	3.69%
PCB 48	9,405	2.41%
PCB 75	802	0.21%
PCB 138	17,000	4.35%
PCB 163	3,980	1.02%
PCB 164	1,607	0.41%
PCB 101	19,980	5.11%
PCB 89	600	0.15%
PCB 52	20,100	5.14%
PCB 49	15,820	4.05%
PCB 43	1,816	0.46%
PCB 153	16,215	4.15%
PCB 99	15,935	4.08%
PCB 70	14,515	3.72%
PCB 105	12,280	3.14%
PCB 31	11,800	3.02%
PCB 56	5,780	1.48%
PCB 60	5,760	1.47%
PCB 41	1,998	0.51%
PCB 71	1,614	0.41%
PCB 64	6,780	1.74%
PCB 110	7,220	1.85%
PCB 85	6,940	1.78%
PCB 87	4,818	1.23%
PCB 115	398.4	0.10%
PCB 128	4,026	1.03%
PCB 149	3,380	0.87%
PCB 139	68	0.02%
PCB 92	3,413.5	0.87%
PCB 180	2,802	0.72%
PCB 158	2,822	0.72%
PCB 146	2,583.5	0.66%
PCB 97	2,584	0.66%
PCB 156	2,412	0.62%
PCB 95	2,398	0.61%
PCB 187	2,196.5	0.56%
PCB 170	1,814	0.46%
PCB 190	400.8	0.10%
PCB 117	1,992	0.51%
PCB 141	1,788	0.46%
PCB 130	1,390	0.36%

PCB 109	1,206	0.31%
PCB 137	1,198.5	0.31%
PCB 42	795	0.20%
PCB 59	197.2	0.05%
PCB 114	1,008	0.26%
PCB 167	796	0.20%
PCB 123	599	0.15%
PCB 157	594	0.15%
PCB 77	400	0.10%
PCB 81	200.4	0.05%
PCB 126	84.35	0.02%
PCB 189	70.2	0.02%
PCB 169	0.72	0.0002%
Total	390,713	100.0%

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**Table 2. Target and nominal concentrations of total PCB in each kestrel corn oil dosing solution.**

Dose ID	Vial & Cap #	Solution Description	Target Concentration ( $\mu\text{g}/\mu\text{L}$ ) <sup>1</sup>	Nominal Concentration ( $\mu\text{g}/\mu\text{L}$ ) <sup>2</sup>
7	1-JLZ2-SD	Stock spotted sandpiper PCB mixture	246	244
6	3-2F	2-fold dilution	123	122
5	3-4F	4-fold dilution	62	61
4	3-8F	8-fold dilution	31	30.5
3	3-16F	16-fold dilution	15	15
2	1-32F	32-fold dilution	8	7.5
1	1-JLZ-IB	Isooctane blank	0	0

<sup>1</sup> Target concentrations for the dosing solutions of the custom spotted sandpiper PCB mixture.

<sup>2</sup> Nominal total PCB concentrations are based on the sum of the certified analyte concentrations from AccuStandard (Attachment 1), a volume of 50 mL of the 250 mL of original custom 58-congener PCB mixture, a stock PCB volume of 1.6 mL, and the appropriate serial dilution for each dose (corn oil).

**Table 3. Nominal individual PCB congener doses (ng/g egg) expected in the kestrel eggs injected with each of the dosing solutions**

PCB congener	Nominal egg dose (ng/g egg)					
	98 µg PCB/g dose (vial 1-JLZ2-SD)	49 µg PCB/g dose (vial 3-2F)	24 µg PCB/g dose (vial 3-4F)	12 µg PCB/g dose (vial 3-8F)	6 µg PCB/g dose (vial 3-16F)	3 µg PCB/g dose (vial 1-32F)
PCB 28	10,220	5,110	2,555	1,278	639	319
PCB 66	9,165	4,583	2,291	1,146	573	286
PCB 74	6,710	3,355	1,678	839	419	210
PCB 118	6,383	3,191	1,596	798	399	199
PCB 47	3,605	1,803	901	451	225	113
PCB 48	2,351	1,176	588	294	147	73
PCB 75	201	100	50	25	13	6
PCB 138	4,250	2,125	1,063	531	266	133
PCB 163	995	498	249	124	62	31
PCB 164	402	201	100	50	25	13
PCB 101	4,995	2,498	1,249	624	312	156
PCB 89	150	75	38	19	9	5
PCB 52	5,025	2,513	1,256	628	314	157
PCB 49	3,955	1,978	989	494	247	124
PCB 43	454	227	114	57	28	14
PCB 153	4,054	2,027	1,013	507	253	127
PCB 99	3,984	1,992	996	498	249	124
PCB 70	3,629	1,814	907	454	227	113
PCB 105	3,070	1,535	768	384	192	96
PCB 31	2,950	1,475	738	369	184	92
PCB 56	1,445	723	361	181	90	45
PCB 60	1,440	720	360	180	90	45
PCB 41	500	250	125	62	31	16
PCB 71	404	202	101	50	25	13
PCB 64	1,695	848	424	212	106	53
PCB 110	1,805	903	451	226	113	56
PCB 85	1,735	868	434	217	108	54
PCB 87	1,205	602	301	151	75	38
PCB 115	100	50	25	12	6	3
PCB 128	1,007	503	252	126	63	31
PCB 149	845	423	211	106	53	26
PCB 139	17	9	4	2	1	1
PCB 92	853	427	213	107	53	27

PCB 180	701	350	175	88	44	22
PCB 158	706	353	176	88	44	22
PCB 146	646	323	161	81	40	20
PCB 97	646	323	162	81	40	20
PCB 156	603	302	151	75	38	19
PCB 95	600	300	150	75	37	19
PCB 187	549	275	137	69	34	17
PCB 170	454	227	113	57	28	14
PCB 190	100	50	25	13	6	3
PCB 117	498	249	125	62	31	16
PCB 141	447	224	112	56	28	14
PCB 130	348	174	87	43	22	11
PCB 109	302	151	75	38	19	9
PCB 137	300	150	75	37	19	9
PCB 42	199	99	50	25	12	6
PCB 59	49	25	12	6	3	2
PCB 114	252	126	63	32	16	8
PCB 167	199	100	50	25	12	6
PCB 123	150	75	37	19	9	5
PCB 157	149	74	37	19	9	5
PCB 77	100	50	25	13	6	3
PCB 81	50	25	13	6	3	2
PCB 126	21	11	5	3	1	1
PCB 189	18	9	4	2	1	1
PCB 169	0.18	0.09	0.05	0.02	0.01	0.01
Total PCB	97,678	48,839	24,420	12,210	6,105	3,052

Estimation based on the following:

Highest Dose = 98 µg/g egg

Injection volume/g egg = 0.4 µL/g egg

**Table 4. Calculated doses and contributions to TEQs (pg/g egg) of individual dioxin-like PCB congeners for each dose of the spotted sandpiper PCB mixture used for injection of kestrel eggs.**

PCB congener	TEF value (WHO)	TEQ (pg/g)					
		98 ug PCB/g egg dose	49 ug PCB/g egg dose	25 ug PCB/g egg dose	12 ug PCB/g egg dose	6 ug PCB/g egg dose	3 ug PCB/g egg dose
PCB 118	0.00001	64	32	16	8	4	2
PCB 105	0.0001	307	154	77	38	19	10
PCB 156	0.0001	60	30	15	8	4	2
PCB 114	0.0001	25	13	6	3	2	1
PCB 167	0.00001	2	1	0.50	0.25	0.12	0.06
PCB 123	0.00001	1	1	0.37	0.19	0.09	0.05
PCB 157	0.0001	15	7	4	2	1	0.46
PCB 77	0.05	5000	2500	1250	625	313	156
PCB 81	0.1	5010	2505	1253	626	313	157
PCB 126	0.1	2109	1054	527	264	132	66
PCB 189	0.00001	0.18	0.09	0.04	0.02	0.01	0.01
PCB 169	0.001	0.18	0.09	0.05	0.02	0.01	0.01
Total TEQ		12594	6297	3148	1574	787	394

**Table 5. Nominal concentrations, expected egg dose and TEQ dose of the spotted sandpiper PCB mixture dosing solutions (corn oil) used for injection into chicken egg.**

Vial and cap id (etched)	Dosing sol'n nominal Concentration ( $\mu\text{g PCB}/\mu\text{L}$ )	Injection volume ( $\mu\text{L}/\text{g-egg}$ )	Egg Dose ( $\mu\text{g PCB}/\text{g-egg}$ )	Egg Dose ( $\text{pg TEQ}/\text{g-egg}$ )
3-COS	60.8	0.1	6.08	810
3-3F	20.6	0.1	2.06	270
3-9F	7.0	0.1	0.70	90
3-27F	2.4	0.1	0.24	30
3-81F	0.78	0.1	0.078	10
3-243F	0.27	0.1	0.027	3
3-COB	0.00	0.1	0.00	0

**Table 6. Nominal concentrations and estimated total spotted sandpiper PCB mixture dosing solutions (in fatty acids) used for injection into eastern screech owl eggs.**

Dosing sol'n nominal Concentration ( $\mu\text{g PCB}/\mu\text{L}$ )	Estimated egg dose ( $\mu\text{g PCB}/\text{g-egg}$ )
295.0	25.20
29.5	2.52
0.00	0.00

**Table 7. Nominal concentrations of spotted sandpiper PCB mixture dosing solution (in charcoal-stripped corn oil) used for injections of Japanese quail eggs.**

Vial & Cap #	Solution Description	Nominal Concentration (mg/ $\mu$ L)
SS 1.2 mg/ $\mu$ L	Stock spotted sandpiper PCB mixture	1.2
SS 0.6 mg/ $\mu$ L	2-fold dilution	0.6
SS 0.3 mg/ $\mu$ L	4-fold dilution	0.3
SS 0.15 mg/ $\mu$ L	8-fold dilution	0.15
SS 0.075 mg/ $\mu$ L	16-fold dilution	0.075
SS 0.0375 mg/ $\mu$ L	32-fold dilution	0.0375
Blank	Isooctane blank	0

Table 8. Target PCB congeners observed in Hudson River Tree Swallow, *Tachycineta bicolor*, eggs in rank order along with estimated concentrations and masses required for preparation of an egg dosing stock solution (calculations based on injecting 0.4 uL/g into a 2-g egg; 0.8 uL injected)

PCB Congener	HR Tree Swallow Mean PCB Conc. (ng/g)	Concentrations (ng/g) for a 100 ug/g dose	Mass (ng) in 2 g egg at 100 ug/g dose	Dosing stock solution (ug/uL)	Ordered from AccuStandard		Received from AccuStandard			
					total Mass (mg) in 250 mL of dosing stock solution	total Mass (mg) in 250 mL of dosing stock solution	Cert. Conc. received (mg/mL)	Cert. total cong. Mass (ug)	Cert. mass (% of nominal)	% of total
28	533.35	7,467	14,934	19	169,371	169.4	680.100	170,025	100	7.42
52	471.08	6,595	13,190	16	149,596	149.6	596.800	149,200	100	6.51
66	452.10	6,329	12,659	16	143,570	143.6	576.300	144,075	100	6.29
138	331.56	4,642	9,284	12	139,651	139.7	560.700	140,175	100	6.12
163	76.26	1,068	2,135	3	107,808	107.8	432.400	108,100	100	4.72
164	33.16	464	928	1	105,290	105.3	424.500	106,125	101	4.63
118	439.76	6,157	12,313	15	104,194	104.2	420.400	105,100	101	4.59
153	339.49	4,753	9,506	12	95,201	95.2	380.400	95,100	100	4.15
74	328.11	4,593	9,187	11	94,618	94.6	378.000	94,500	100	4.12
47	188.77	2,643	5,286	7	90,329	90.3	363.700	90,925	101	3.97
48	122.70	1,718	3,436	4	84,429	84.4	340.100	85,025	101	3.71
75	9.44	132	264	0	67,446	67.4	272.000	68,000	101	2.97
43	284.45	3,982	7,965	10	63,693	63.7	256.200	64,050	101	2.80
49	34.13	478	956	1	63,654	63.7	255.200	63,800	100	2.78
70	299.79	4,197	8,394	10	59,946	59.9	240.100	60,025	100	2.62
101	297.95	4,171	8,343	10	51,136	51.1	208.100	52,025	102	2.27
31	265.87	3,722	7,444	9	38,965	39.0	156.100	39,025	100	1.70
41	45.09	631	1,262	2	36,777	36.8	148.300	37,075	101	1.62
71	35.43	496	992	1	34,494	34.5	138.900	34,725	101	1.52
64	161.03	2,254	4,509	6	30,857	30.9	123.700	30,925	100	1.35
110	212.39	2,973	5,947	7	30,054	30.1	119.900	29,975	100	1.31

105	200.57	2,808	5,616	7	30,054	30.1	119.000	29,750	99	1.30
99	200.45	2,806	5,613	7	29,547	29.5	118.900	29,725	101	1.30
56	94.64	1,325	2,650	3	29,376	29.4	119.100	29,775	101	1.30
60	94.64	1,325	2,650	3	26,725	26.7	107.600	26,900	101	1.17
149	115.81	1,621	3,243	4	24,217	24.2	100.300	25,075	104	1.09
139	2.32	32	65	0	21,814	21.8	87.900	21,975	101	0.96
95	108.62	1,521	3,041	4	18,768	18.8	76.090	19,023	101	0.83
37	97.17	1,360	2,721	3	18,161	18.2	75.550	18,888	104	0.82
85	93.04	1,303	2,605	3	17,190	17.2	71.930	17,983	105	0.78
87	84.16	1,178	2,356	3	17,159	17.2	72.200	18,050	105	0.79
115	8.42	118	236	0	16,602	16.6	68.080	17,020	103	0.74
180	92.50	1,295	2,590	3	15,595	15.6	64.320	16,080	103	0.70
128	68.69	962	1,923	2	15,524	15.5	63.640	15,910	102	0.69
170	49.11	688	1,375	2	14,318	14.3	59.860	14,965	105	0.65
190	10.80	151	303	0	13,312	13.3	55.960	13,990	105	0.61
92	59.10	827	1,655	2	13,116	13.1	55.560	13,890	106	0.61
146	57.19	801	1,601	2	12,764	12.8	51.480	12,870	101	0.56
187	54.13	758	1,516	2	12,544	12.5	51.520	12,880	103	0.56
63	54.03	756	1,513	2	11,645	11.6	48.240	12,060	104	0.53
158	52.28	732	1,464	2	11,313	11.3	48.280	12,070	107	0.53
44	48.89	684	1,369	2	11,250	11.2	48.240	12,060	107	0.53
156	41.92	587	1,174	1	11,001	11.0	44.190	11,048	100	0.48
117	41.30	578	1,156	1	10,839	10.8	44.200	11,050	102	0.48
91	40.19	563	1,125	1	10,529	10.5	43.910	10,978	104	0.48
97	39.50	553	1,106	1	9,910	9.9	39.680	9,920	100	0.43
42	29.74	416	833	1	9,734	9.7	39.320	9,830	101	0.43
59	8.92	125	250	0	9,443	9.4	38.080	9,520	101	0.42
109	36.67	513	1,027	1	8,593	8.6	34.400	8,600	100	0.38
77	35.63	499	998	1	8,304	8.3	33.900	8,475	102	0.37
132	34.64	485	970	1	7,805	7.8	30.890	7,723	99	0.34
141	31.21	437	874	1	6,695	6.7	26.800	6,700	100	0.29
15	30.65	429	858	1	6,392	6.4	25.500	6,375	100	0.28

151	27.06	379	758	1	5,587	5.6	22.340	5,585	100	0.24
130	26.15	366	732	1	5,182	5.2	21.000	5,250	101	0.23
72	24.58	344	688	1	3,431	3.4	14.030	3,508	102	0.15
137	21.08	295	590	1	3,412	3.4	13.870	3,468	102	0.15
183	20.13	282	564	1	3,268	3.3	13.180	3,295	101	0.14
167	17.59	246	493	1	2,997	3.0	11.950	2,988	100	0.13
114	16.32	228	457	1	2,833	2.8	11.440	2,860	101	0.12
157	10.75	150	301	0	2,673	2.7	10.760	2,690	101	0.12
123	10.29	144	288	0	736	0.7	2.951	738	100	0.03
189	2.06	29	58	0	653	0.7	2.647	662	101	0.03
81	1.87	26	52	0	595	0.6	2.405	601	101	0.03
126	0.84	12	24	0	268	0.3	1.081	270	101	0.01
169	0.02	0	1	0	7	0.0	0.028	7	102	0.0003
Total PCBs	7,157.57	100,206	200,412	251	2,272,958	2,272.96	9,164	2,291,051		100.00

**Table 9. Target and nominal concentrations of total PCB in each tree swallow PCB mixture dosing solution (corn oil-propylene glycol emulsion).**

Vial & Cap #	Solution Description	Target Concentration ( $\mu\text{g}/\mu\text{L}$ ) <sup>1</sup>	Nominal Concentration ( $\mu\text{g}/\mu\text{L}$ ) <sup>2</sup>
	Stock tree swallow PCB		
X3	mixture	250	251
X4	2-fold dilution	125	125.5
X5	4-fold dilution	62.5	62.75
X6	8-fold dilution	31.25	31.38
X7	16-fold dilution	15.6	15.69
X8	32-fold dilution	7.8	7.84
X1	Isooctane blank	0	0

<sup>1</sup> Target concentrations for the dosing solutions of the custom tree swallow PCB mixture.

<sup>2</sup> Nominal total PCB concentrations are based on the sum of the certified analyte concentrations from AccuStandard (Attachment 3), a volume of 37.5 mL of the 250-mL of original custom tree swallow PCB mixture, a stock PCB volume of 1.37 mL, and the appropriate serial dilution for each dose (corn oil).

**Table 10. Target concentrations of PCB congeners in tree swallow PCB mixture dosing solutions (in corn oil-propylene glycol emulsion) used for tree swallow egg injection studies.**

PCB Congener	HR Tree swallow	Congener	Congener	Congener	Congener	Congener	Congener
	Mean PCB Conc. (ng/g)	concentrations (ng/g) for a 100 ug/g dose	concentrations (ng/g) for a 50 ug/g dose	concentrations (ng/g) for a 25 ug/g dose	concentrations (ng/g) for a 12.5 ug/g dose	concentrations (ng/g) for a 6.25 ug/g dose	concentrations (ng/g) for a 3.12 ug/g dose
28	533.35	7,467	3,733	1,867	933	467	233
52	471.08	6,595	3,298	1,649	824	412	206
66	452.10	6,329	3,165	1,582	791	396	198
138	331.56	4,642	2,321	1,160	580	290	145
163	76.26	1,068	534	267	133	67	33
164	33.16	464	232	116	58	29	15
118	439.76	6,157	3,078	1,539	770	385	192
153	339.49	4,753	2,376	1,188	594	297	149
74	328.11	4,593	2,297	1,148	574	287	144
47	188.77	2,643	1,321	661	330	165	83
48	122.70	1,718	859	429	215	107	54
75	9.44	132	66	33	17	8	4
43	284.45	3,982	1,991	996	498	249	124
49	34.13	478	239	119	60	30	15
70	299.79	4,197	2,099	1,049	525	262	131
101	297.95	4,171	2,086	1,043	521	261	130
31	265.87	3,722	1,861	931	465	233	116
41	45.09	631	316	158	79	39	20
71	35.43	496	248	124	62	31	15
64	161.03	2,254	1,127	564	282	141	70
110	212.39	2,973	1,487	743	372	186	93
105	200.57	2,808	1,404	702	351	175	88
99	200.45	2,806	1,403	702	351	175	88
56	94.64	1,325	662	331	166	83	41

60	94.64	1,325	662	331	166	83	41
149	115.81	1,621	811	405	203	101	51
139	2.32	32	16	8	4	2	1
95	108.62	1,521	760	380	190	95	48
37	97.17	1,360	680	340	170	85	43
85	93.04	1,303	651	326	163	81	41
87	84.16	1,178	589	295	147	74	37
115	8.42	118	59	29	15	7	4
180	92.50	1,295	648	324	162	81	40
128	68.69	962	481	240	120	60	30
170	49.11	688	344	172	86	43	21
190	10.80	151	76	38	19	9	5
92	59.10	827	414	207	103	52	26
146	57.19	801	400	200	100	50	25
187	54.13	758	379	189	95	47	24
63	54.03	756	378	189	95	47	24
158	52.28	732	366	183	91	46	23
44	48.89	684	342	171	86	43	21
156	41.92	587	293	147	73	37	18
117	41.30	578	289	145	72	36	18
91	40.19	563	281	141	70	35	18
97	39.50	553	277	138	69	35	17
42	29.74	416	208	104	52	26	13
59	8.92	125	62	31	16	8	4
109	36.67	513	257	128	64	32	16
77	35.63	499	249	125	62	31	16
132	34.64	485	242	121	61	30	15
141	31.21	437	218	109	55	27	14
15	30.65	429	215	107	54	27	13
151	27.06	379	189	95	47	24	12
130	26.15	366	183	92	46	23	11
72	24.58	344	172	86	43	22	11

137	21.08	295	148	74	37	18	9
183	20.13	282	141	70	35	18	9
167	17.59	246	123	62	31	15	8
114	16.32	228	114	57	29	14	7
157	10.75	150	75	38	19	9	5
123	10.29	144	72	36	18	9	5
189	2.06	29	14	7	4	2	1
81	1.87	26	13	7	3	2	1
126	0.84	12	6	3	1.5	0.7	0.4
169	0.022	0.302	0.151	0.075	0.038	0.019	0.009
<hr/>							
Total PCBs	7,158	100,206	50,103	25,051	12,526	6,263	3,131

**Table 11. Target concentrations of PCB congeners in tree swallow PCB mixture dosing solutions (in corn oil-propylene glycol emulsion) used for tree swallow egg injection studies.**

Vial & Cap #	Solution Description	Target Concentration ( $\mu\text{g}/\mu\text{L}$ ) <sup>1</sup>	Nominal Concentration ( $\mu\text{g}/\mu\text{L}$ ) <sup>2</sup>
	Stock tree swallow PCB mixture	250	251
X10		250	251
X11	2-fold dilution	125	125.5
X12	4-fold dilution	62.5	62.75
X13	8-fold dilution	31.25	31.38
X14	16-fold dilution	15.6	15.69
X15	32-fold dilution	7.8	7.84
X9	Isooctane blank	0	0

<sup>1</sup> Target concentrations for the dosing solutions of the custom spotted sandpiper PCB mixture.

<sup>2</sup> Nominal total PCB concentrations are based on the sum of the certified analyte concentrations from AccuStandard (Attachment 3), a volume of 27.28 mL of the 250 mL of original custom tree swallow PCB mixture, a stock PCB volume of 1.0 mL, and the appropriate serial dilution for each dose (corn oil).

**Table 12. Nominal concentrations of tree swallow PCB mixture dosing solutions (in each charcoal stripped corn oil) used for Japanese quail egg injection studies.**

Vial & Cap #	Solution Description	Nominal Concentration (mg/ $\mu$ L)
TS 1.2 mg/ $\mu$ L	Stock spotted sandpiper PCB mixture	1.2
TS 0.6 mg/ $\mu$ L	2-fold dilution	0.6
TS 0.3 mg/ $\mu$ L	4-fold dilution	0.3
TS 0.15 mg/ $\mu$ L	8-fold dilution	0.15
TS 0.075 mg/ $\mu$ L	16-fold dilution	0.075
TS 0.0375 mg/ $\mu$ L	32-fold dilution	0.0375
Blank	Isooctane blank	0

**Table 13. Nominal concentrations of PCB 77 dosing solutions (in fatty acid) used for tree swallow egg injections.**

Vial designation	Dosing sol'n nominal concentration ( $\mu\text{g}/\mu\text{L}$ )
77 10	10.00
77 1	1.00
B	0.00

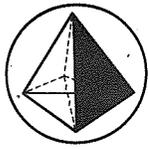
**Table 14. Nominal concentrations of PCB 126 dosing solutions (in charcoal-stripped corn oil) used for Japanese quail egg injection studies.**

Vial designation	Solution Description	Dosing sol'n nominal concentration ( $\mu\text{g}/\mu\text{L}$ )
I 1	stock PCB 126	25.00
I 2	2.5-fold dilution	10.00
I 3	6.25-fold dilution	4.00
I 4	15.6-fold dilution	1.60
I 5	39.1-fold dilution	0.64
I 6	97.7-fold dilution	0.26
I 7	Isooctane blank	0.00

**Table 15. Nominal concentrations of PCB 77 dosing solutions (in charcoal-stripped corn oil) used for Japanese quail egg injection studies.**

Vial designation	Solution Description	Dosing sol'n nominal concentration (µg/µL)
20	Stock PCB 77	20
10	2-fold dilution	10
5	4-fold dilution	5
2.5	8-fold dilution	2.5
1.25	16-fold dilution	1.25
0.625	32-fold dilution	0.625
0	Isooctane blank	0.0

Attachment 1. AccuStandard Certificate of Analysis: Custom, spotted sandpiper,  
PCB mixture, S-13907-250mL.



# AccuStandard<sup>®</sup>, Inc.

Chemical Reference Standards • The Standard for Excellence

## WARRANTIES:

Manufacturer (AccuStandard<sup>®</sup>, Inc.) warrants that its products shall conform to the description of such products as provided in its catalog or on the specific products' label. This warranty is exclusive, and AccuStandard, Inc. makes no other Warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose.

## PRODUCT STABILITY:

AccuStandard's products are monitored regularly to ensure they meet Catalog Specifications (on-going stability studies). The integrity of these products is dependent upon proper handling and storage by the end-user.

AccuStandard recommends the following storage conditions:

Volatiles	-10 to -20 °C
Semi-Volatiles	4 °C

Exceptions: Highly concentrated solutions (e.g. Z-014J) should be stored at room temperature.

Note: Allow ampules to equilibrate to 20 °C prior to opening.

## LIABILITY:

AccuStandard, Inc. products are for research use only. Due to their hazardous nature, they should be handled by trained personnel. AccuStandard's liability will be limited to replacement of products or refund of purchase price. Failure to give notice of claim within thirty (30) days from date of delivery will constitute a waiver by buyer of any and all claims.





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## CERTIFICATE OF ANALYSIS

CATALOG NO: S-13907-250ML

EXPIRATION: Nov 9, 2015

DESCRIPTION: Custom PCB Congener Standard

LOT: B5110052

See reverse for additional certification information.

SOLVENT: Isooctane

This product is guaranteed accurate to + 0.5% of the Certified Analyte concentration through the Expiration Date on the Label.

Component	CAS #	Purity % (GC/MS)	Prepared Concentration <sup>1</sup> (µg/mL)	Certified Analyte Concentration <sup>2</sup> (µg/mL)
2,4,4'-Trichlorobiphenyl	7012-37-5	100	817.6	±32.70 817.6
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	100	733.2	±29.33 733.2
2,4,4',5-Tetrachlorobiphenyl	32690-93-0	100	536.8	±21.47 536.8
2,3',4,4',5-Pentachlorobiphenyl 118	31508-00-6	99.5	513.2	±20.53 510.6
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	100	288.4	±11.54 288.4
2,2',4,5-Tetrachlorobiphenyl 48	70362-47-9	99.9	188.3	±7.53 188.1
2,4,4',6-Tetrachlorobiphenyl	32598-12-2	99.5	16.12	±0.64 16.04
2,2',3,4,4',5'-Hexachlorobiphenyl 138	35065-28-2	100	340.0	±13.60 340.0
2,3,3',4',5,6-Hexachlorobiphenyl 167	74472-44-9	99.0	80.40	±3.22 79.60
2,3,3',4',5',6-Hexachlorobiphenyl 164	74472-45-0	99.7	32.24	±1.29 32.14
2,2',4,5,5'-Pentachlorobiphenyl 101	37680-73-2	99.4	402.0	±16.08 399.6
2,2',3,4,6'-Pentachlorobiphenyl 89	73575-57-2	99.9	12.01	±0.48 12.00
2,2',5,5'-Tetrachlorobiphenyl 52	35693-99-3	100	402.0	±16.08 402.0
2,2',4,5'-Tetrachlorobiphenyl 49	41464-40-8	100	316.4	±12.66 316.4
2,2',3,5-Tetrachlorobiphenyl 43	70362-46-8	99.9	36.36	±1.45 36.32
2,2',4,4',5,5'-Hexachlorobiphenyl 153	35065-27-1	99.6	325.6	±13.02 324.3
2,2',4,4',5-Pentachlorobiphenyl 99	38380-01-7	99.6	320.0	±12.80 318.7
2,3',4',5-Tetrachlorobiphenyl 70	32598-11-1	99.0	293.2	±11.73 290.3
2,3,3',4,4'-Pentachlorobiphenyl 105	32598-14-4	100	245.6	±9.82 245.6
2,4',5-Trichlorobiphenyl 31	16606-02-3	100	236.0	±9.44 236.0
2,3,3',4'-Tetrachlorobiphenyl 56	41464-43-1	99.6	116.1	±4.64 115.6
2,3,4,4'-Tetrachlorobiphenyl 60	33025-41-1	99.0	116.4	±4.66 115.2
2,2',3,4-Tetrachlorobiphenyl 41	52663-59-9	99.3	40.24	±1.61 39.96
2,3',4',6-Tetrachlorobiphenyl 71	41464-46-4	100	32.28	±1.29 32.28
2,3,4,6-Tetrachlorobiphenyl 64	52663-58-8	99.0	137.0	±5.48 135.6
2,3,3',4',6-Pentachlorobiphenyl 110	38380-03-9	99.7	144.8	±5.79 144.4
2,2',3,4,4'-Pentachlorobiphenyl 85	65510-45-4	99.0	140.2	±5.61 138.8
2,2',3,4,5'-Pentachlorobiphenyl 87	38380-02-8	99.5	96.84	±3.87 96.36
2,3,4,4',6-Pentachlorobiphenyl 115	74472-38-1	99.5	8.008	±0.32 7.968
2,2',3,3',4,4'-Hexachlorobiphenyl 128	38380-07-3	99.7	80.76	±3.23 80.52
2,2',3,4',5',6-Hexachlorobiphenyl 149	38380-04-0	99.0	68.28	±2.73 67.60
2,2',3,4,4',6-Hexachlorobiphenyl 139	56030-56-9	99.4	1.368	±0.05 1.360
2,2',3,5,5'-Pentachlorobiphenyl 92	52663-61-3	99.7	68.48	±2.74 68.27
2,2',3,4,4',5,5'-Heptachlorobiphenyl 180	35065-29-3	100	56.04	±2.24 56.04
2,3,3',4,4',6-Hexachlorobiphenyl 158	74472-42-7	100	56.44	±2.26 56.44
2,2',3,4',5,5'-Hexachlorobiphenyl 146	51908-16-8	98.9	52.24	±2.09 51.67
2,2',3',4,5-Pentachlorobiphenyl 97	41464-51-1	99.0	52.20	±2.09 51.68

1. All weights are traceable through NIST, Test No. 822/270236-04  
 2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for this product is the Combined Uncertainty  $u_c(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$  where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). Values reported above are Expanded Combined Uncertainty.  
 3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

Page 1 of 2 Certified by: R. Cooper

# CERTIFICATION REPORT

- 1. Intended Use:** The product covered by this Certificate is designed for Calibration or for use in Quality Control procedures for the specified chemical compounds listed on the reverse side. This product can be used for Identification and/or Quantification. This product can also be used as a Reference Material to validate analytical procedures, subject to the conditions under Section 8.
- 2. Raw Materials:** Reference Standards are prepared from the highest quality starting materials with defined purities. All analytes and solvents are obtained from pre-qualified vendors and then analyzed or evaluated prior to use according to ISO9001 requirements.
- 3. Manufacturing:** AccuStandard, Inc. manufactures its products under an ISO 9001 certified quality system. Balances used in the manufacturing process are calibrated regularly. All weights are traceable through the National Institute of Standards and Technology (NIST), Test No. 822/254480.
- 4. Homogeneity Assessment:** Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the ISO 9001 Quality System.
- 5. Stability Assessment:** AccuStandard, Inc. guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
- 6. Analytical Quality Control:** Products are tested by validated analytical methods covered under the company's ISO 9001 Quality System.
- 7. Uncertainty Statistics and Confidence Limits:** The maximum Uncertainty stated on the face of this certificate has been calculated in accordance with the EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement - Second Edition. The Uncertainty given is the Expanded Combined Uncertainty and represents an estimated Standard Deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$ , where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). The Expanded Uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of the product.
- 8. Legal Notice and Limit of Liability:** This product is for research use only. No warranty for any particular application is expressed or implied. Due to their hazardous nature, they should be handled by trained personnel. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.



# AccuStandard Inc.

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## CERTIFICATE OF ANALYSIS

CATALOG NO: S-13907-250ML

EXPIRATION: Nov 9, 2015

DESCRIPTION: Custom PCB Congener Standard

LOT: B5110052

See reverse for additional certification information.

SOLVENT: Isooctane

This product is guaranteed accurate to +0.5% of the Certified Analyte concentration through the Expiration Date on the Label.

Component	CAS #	Purity % (GC/MS)	Prepared Concentration <sup>1</sup> (µg/mL)	Certified Analyte Concentration <sup>2</sup> (µg/mL)
2,3,3',4,4',5-Hexachlorobiphenyl 156	38380-08-4	100	48.24	± 1.93 48.24
2,2',3,5',6-Pentachlorobiphenyl 185	38379-99-6	99.1	48.40	± 1.94 47.96
2,2',3,4',5,5',6-Heptachlorobiphenyl 187	52663-68-0	99.4	44.20	± 1.77 43.93
2,2',3,3',4,4',5-Heptachlorobiphenyl 170	35065-30-6	100	36.28	± 1.45 36.28
2,3,3',4,4',5,6-Heptachlorobiphenyl 190	41411-64-7	100	8.016	± 0.32 8.016
2,3,4',5,6-Pentachlorobiphenyl 162	68194-11-6	99.0	40.24	± 1.61 39.84
2,2',3,4,5,5'-Hexachlorobiphenyl 141	52712-04-6	99.0	36.12	± 1.44 35.76
2,2',3,3',4,5'-Hexachlorobiphenyl 130	52663-66-8	99.3	28.00	± 1.12 27.80
2,3,3',4,6-Pentachlorobiphenyl 109	74472-35-8	100	24.12	± 0.96 24.12
2,2',3,4,4',5-Hexachlorobiphenyl 137	35694-06-5	99.7	24.04	± 0.96 23.97
2,2',3,4'-Tetrachlorobiphenyl 42	36559-22-5	99.4	16.00	± 0.64 15.90
2,3,3',6-Tetrachlorobiphenyl 59	74472-33-6	98.5	4.004	± 0.16 3.944
2,3,4,4',5-Pentachlorobiphenyl 114	74472-37-0	100	20.16	± 0.81 20.16
2,3',4,4',5,5'-Hexachlorobiphenyl 167	52663-72-6	99.0	16.08	± 0.64 15.92
2',3,4,4',5-Pentachlorobiphenyl 123	65510-44-3	99.8	12.00	± 0.48 11.98
2,3,3',4,4',5'-Hexachlorobiphenyl 177	69782-90-7	99.0	12.00	± 0.48 11.88
3,3',4,4'-Tetrachlorobiphenyl 77	32598-13-3	100	8.000	± 0.32 8.000
3,4,4',5-Tetrachlorobiphenyl 81	70362-50-4	100	4.008	± 0.16 4.008
3,3',4,4',5-Pentachlorobiphenyl 126	57465-28-8	100	1.687	± 0.07 1.687
2,3,3',4,4',5,5'-Heptachlorobiphenyl 189	39635-31-9	100	1.404	± 0.06 1.404
3,3',4,4',5,5'-Hexachlorobiphenyl 169	32774-16-6	100	0.0144	± 0.00 .0144

58 Components

1. All weights are traceable through NIST, Test No. 822/270236-04  
 2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for this product is the Combined Uncertainty  $u_c(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$  where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). Values reported above are Expanded Combined Uncertainty.  
 3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

Page 2 of 2 Certified by: R. Cooper

# CERTIFICATION REPORT

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- 3. Manufacturing:** AccuStandard, Inc. manufactures its products under an ISO 9001 certified quality system. Balances used in the manufacturing process are calibrated regularly. All weights are traceable through the National Institute of Standards and Technology (NIST), Test No. 822/254480.
- 4. Homogeneity Assessment:** Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the ISO 9001 Quality System.
- 5. Stability Assessment:** AccuStandard, Inc. guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
- 6. Analytical Quality Control:** Products are tested by validated analytical methods covered under the company's ISO 9001 Quality System.
- 7. Uncertainty Statistics and Confidence Limits:** The maximum Uncertainty stated on the face of this certificate has been calculated in accordance with the EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement - Second Edition. The Uncertainty given is the Expanded Combined Uncertainty and represents an estimated Standard Deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$ , where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). The Expanded Uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of the product.
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Attachment 2. AccuStandard Certificate of Analysis: Isooctane, procedural blank, S-13907-BLK-250mL.



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## CERTIFICATE OF ANALYSIS

CATALOG NO. S-13907-BLK-250ML

DESCRIPTION: Isooctane Control Blank

EXPIRATION: Nov 9, 2006

LOT: B5110053

SOLVENT: N/A

See reverse for additional certification information.

This product is guaranteed accurate to + 0.5% of the Certified Analyte concentration through the Expiration Date on the Label.

Component	CAS #	Purity % MFG	Prepared Concentration <sup>1</sup>	Certified Analyte Concentration <sup>2</sup>
Isooctane	540-84-1	99.9	N/A ±0	N/A

Please note: AccuStandard follows the U.S. conventions in reporting numerical values, on both certificates and labels.

A comma (,) is used to separate units of one-thousand or greater.  
A period (.) is used as a decimal place marker.

1. All weights are traceable through National Institute of Standards & Technology, Test No.
2. Certified Analyte Concentration = Purity x Prepared Concentration
3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

Certified by: R. Cooper

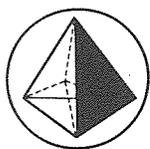
This product was manufactured to meet the quality system requirements of ISO 9001

QR-ORG/INO-001  
Rev. 11/02

# CERTIFICATION REPORT

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- 3. Manufacturing:** AccuStandard, Inc. manufactures its products under an ISO 9001 certified quality system. Balances used in the manufacturing process are calibrated regularly. All weights are traceable through the National Institute of Standards and Technology (NIST), Test No. 822/254480.
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- 5. Stability Assessment:** AccuStandard, Inc. guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
- 6. Analytical Quality Control:** Products are tested by validated analytical methods covered under the company's ISO 9001 Quality System.
- 7. Uncertainty Statistics and Confidence Limits:** The maximum Uncertainty stated on the face of this certificate has been calculated in accordance with the EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement - Second Edition. The Uncertainty given is the Expanded Combined Uncertainty and represents an estimated Standard Deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$ , where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). The Expanded Uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of the product.
- 8. Legal Notice and Limit of Liability:** This product is for research use only. No warranty for any particular application is expressed or implied. Due to their hazardous nature, they should be handled by trained personnel. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.

Attachment 3. AccuStandard Certificate of Analysis: Custom, tree swallow,  
PCB mixture, S-15880-250 mL



# AccuStandard<sup>®</sup>, Inc.

Chemical Reference Standards • The Standard for Excellence

## WARRANTIES:

Manufacturer (AccuStandard<sup>®</sup>, Inc.) warrants that its products shall conform to the description of such products as provided in its catalog or on the specific products' label. This warranty is exclusive, and AccuStandard, Inc. makes no other Warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose.

## PRODUCT STABILITY:

AccuStandard's products are monitored regularly to ensure they meet Catalog Specifications (on-going stability studies). The integrity of these products is dependent upon proper handling and storage by the end-user.

AccuStandard recommends the following storage conditions:

Volatiles	-10 to -20 °C
Semi-Volatiles	4 °C

Exceptions: Highly concentrated solutions (e.g. Z-014J) should be stored at room temperature.

Note: Allow ampules to equilibrate to 20 °C prior to opening.

## LIABILITY:

AccuStandard, Inc. products are for research use only. Due to their hazardous nature, they should be handled by trained personnel.

AccuStandard's liability will be limited to replacement of products or refund of purchase price. Failure to give notice of claim within thirty (30) days from date of delivery will constitute a waiver by buyer of any and all claims.





AccuStandard, Inc.



# CERTIFICATE OF ANALYSIS

CATALOG NO: S-15880-250ML

EXPIRATION: Apr 23, 2017

DESCRIPTION: Custom PCB Congener Standard

LOT #: B7040178

See reverse for additional certification information.

SOLVENT: Isooctane

This product is guaranteed accurate to  $\pm 0.5\%$  of the Certified Analyte concentration through the Expiration Date on the Label.

Component	CAS #	Purity % (GC/MS)	Prepared Concentration <sup>1</sup> ( $\mu\text{g/mL}$ )	Certified Analyte Concentration <sup>2</sup> ( $\mu\text{g/mL}$ )
4,4'-Dichlorobiphenyl	2050-68-2	100	39.32	$\pm 1.5728$ 39.32
2,4,4'-Trichlorobiphenyl	7012-37-5	100	680.1	$\pm 27.204$ 680.1
2,4',5'-Trichlorobiphenyl	16606-02-3	100	340.1	$\pm 13.604$ 340.1
3,4,4'-Trichlorobiphenyl	38444-90-5	99.7	124.1	$\pm 4.964$ 123.7
2,2',3,4-Tetrachlorobiphenyl	52663-59-9	99.3	60.28	$\pm 2.4112$ 59.86
2,2',3,4'-Tetrachlorobiphenyl	36559-22-5	100	38.08	$\pm 1.5232$ 38.08
2,2',3,5-Tetrachlorobiphenyl	70362-46-8	99.9	364.1	$\pm 14.564$ 363.7
2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	99.0	64.28	$\pm 2.5712$ 63.64
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	100	240.1	$\pm 9.604$ 240.1
2,2',4,5-Tetrachlorobiphenyl	70362-47-9	99.9	156.3	$\pm 6.252$ 156.1
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	100	44.20	$\pm 1.768$ 44.20
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	99.3	601.0	$\pm 24.04$ 596.8
2,3,3',4'-Tetrachlorobiphenyl	41464-43-1	99.6	120.4	$\pm 4.816$ 119.9
2,3,3',6-Tetrachlorobiphenyl	74472-33-6	98.5	11.61	$\pm 4.644$ 11.44
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1	99.0	120.2	$\pm 4.808$ 119.0
2,3,4',5-Tetrachlorobiphenyl	74472-34-7	100	72.20	$\pm 2.888$ 72.20
2,3,4',6-Tetrachlorobiphenyl	52663-58-8	100	208.1	$\pm 8.324$ 208.1
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	100	576.3	$\pm 23.052$ 576.3
2,3',4',5-Tetrachlorobiphenyl	32598-11-1	99.0	384.2	$\pm 15.368$ 380.4
2,3',4',6-Tetrachlorobiphenyl	41464-46-4	100	48.24	$\pm 1.9296$ 48.24
2,3',5,5'-Tetrachlorobiphenyl	41464-42-0	99.0	31.20	$\pm 1.248$ 30.89
2,4,4',5-Tetrachlorobiphenyl	32690-93-0	100	420.4	$\pm 16.816$ 420.4
2,4,4',6-Tetrachlorobiphenyl	32598-12-2	99.5	12.01	$\pm 4.804$ 11.95
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	100	48.28	$\pm 1.9312$ 48.28
3,4,4',5-Tetrachlorobiphenyl	70362-50-4	100	2.405	$\pm 0.962$ 2.405
2,2',3,4,4'-Pentachlorobiphenyl	65510-45-4	99.0	120.1	$\pm 4.804$ 118.9
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	99.5	108.1	$\pm 4.324$ 107.6
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8	99.0	52.00	$\pm 2.08$ 51.48
2,2',3,5,5'-Pentachlorobiphenyl	52663-61-3	99.7	76.32	$\pm 3.0528$ 76.09
2,2',3,5,6-Pentachlorobiphenyl	38379-99-6	99.1	140.2	$\pm 5.608$ 138.9
2,2',3',4,5-Pentachlorobiphenyl	41464-51-1	99.0	52.04	$\pm 2.0816$ 51.52
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	99.6	256.2	$\pm 10.248$ 255.2
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	99.4	380.3	$\pm 15.212$ 378.0
2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	100	256.2	$\pm 10.248$ 256.2
2,3,3',4,6-Pentachlorobiphenyl	74472-35-8	100	48.24	$\pm 1.9296$ 48.24
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	100	272.0	$\pm 10.88$ 272.0
2,3,4,4',5-Pentachlorobiphenyl	74472-37-0	100	21.00	$\pm 8.4$ 21.00
2,3,4,4',6-Pentachlorobiphenyl	74472-38-1	99.5	10.81	$\pm 4.324$ 10.76
2,3,4',5,6-Pentachlorobiphenyl	68194-11-6	99.0	56.12	$\pm 2.2448$ 55.56
2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	100	560.7	$\pm 22.428$ 560.7
2',3,4,4',5-Pentachlorobiphenyl	65510-44-3	99.8	13.21	$\pm 5.284$ 13.18
3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	100	1.081	$\pm 0.4324$ 1.081
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	99.7	88.16	$\pm 3.5264$ 87.90
2,2',3,3',4,5'-Hexachlorobiphenyl	52663-66-8	99.3	33.32	$\pm 1.3328$ 33.09
2,2',3,3',4,6'-Hexachlorobiphenyl	38380-05-1	99.8	44.28	$\pm 1.7712$ 44.19
2,2',3,4,4',5-Hexachlorobiphenyl	35694-06-5	99.7	26.88	$\pm 1.0752$ 26.80
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	100	424.5	$\pm 16.98$ 424.5
2,2',3,4,4',6-Hexachlorobiphenyl	56030-56-9	99.4	2.969	$\pm 1.1876$ 2.951
2,2',3,4,5,5'-Hexachlorobiphenyl	52712-04-6	99.0	40.08	$\pm 1.6032$ 39.68
2,2',3,4',5,5'-Hexachlorobiphenyl	51908-16-8	99.3	76.08	$\pm 3.0432$ 75.55
2,2',3,4',5,6-Hexachlorobiphenyl	38380-04-0	99.9	148.4	$\pm 5.936$ 148.3
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	100	34.40	$\pm 1.376$ 34.40
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	100	432.4	$\pm 17.296$ 432.4
2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4	99.5	56.24	$\pm 2.2496$ 55.96
2,3,3',4,4',5'-Hexachlorobiphenyl	69782-90-7	99.0	14.01	$\pm 5.604$ 13.87
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7	100	68.08	$\pm 2.7232$ 68.08
2,3,3',4',5,6-Hexachlorobiphenyl	74472-44-9	100	100.3	$\pm 4.012$ 100.3
2,3,3',4',5',6-Hexachlorobiphenyl	74472-45-0	99.7	44.04	$\pm 1.7616$ 43.91
2,3',4,4',5,5'-Hexachlorobiphenyl	52663-72-6	99.2	22.52	$\pm 9.008$ 22.34
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	100	0.028	$\pm 0.0112$ 0.028
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	100	64.32	$\pm 2.5728$ 64.32
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	99.0	120.3	$\pm 4.812$ 119.1
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	99.0	25.76	$\pm 1.0304$ 25.50
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	99.4	72.36	$\pm 2.8944$ 71.93
2,3,3',4,4',5,5'-Heptachlorobiphenyl	39635-31-9	99.9	2.650	$\pm 1.06$ 2.647
2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7	100	14.03	$\pm 5.612$ 14.03

received 4/26/07 *RRZ*

1. All weights are traceable through NIST, Test No. 822/272103-05  
 2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for this product is the Combined Uncertainty  $u_c(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$  where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). Values reported above are Expanded Combined Uncertainty.  
 3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

# *CERTIFICATION REPORT*

1. **Intended Use:** The product covered by this Certificate is designed for Calibration or for use in Quality Control procedures for the specified chemical compounds listed on the reverse side. This product can be used for Identification and/or Quantification. This product can also be used as a Reference Material to validate analytical procedures, subject to the conditions under Section 8.
2. **Raw Materials:** Reference Standards are prepared from the highest quality starting materials with defined purities. All analytes and solvents are obtained from pre-qualified vendors and then analyzed or evaluated according to ISO 9001 requirements prior to use.
3. **Manufacturing:** AccuStandard, Inc. manufactures its products under an ISO 9001 certified quality system. Balances used in the manufacturing process are calibrated regularly. All weights are traceable through the National Institute of Standards and Technology (NIST).
4. **Homogeneity Assessment:** Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the ISO 9001 Quality System.
5. **Stability Assessment:** AccuStandard, Inc. guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
6. **Analytical Quality Control:** Products are tested by validated analytical methods covered under the company's ISO 9001 Quality System.
7. **Uncertainty Statistics and Confidence Limits:** The maximum Uncertainty stated on the face of this certificate has been calculated in accordance with the EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement - Second Edition. The Uncertainty given is the Expanded Combined Uncertainty and represents an estimated Standard Deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$ , where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). The Expanded Uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of the product.
8. **Legal Notice and Limit of Liability:** This product is for research use only. No warranty for any particular application is expressed or implied. Due to their hazardous nature, they should be handled by trained personnel. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.

Attachment 4. AccuStandard Certificate of Analysis: isooctane, procedural blank S-15880-BLK-250 mL.



AccuStandard, Inc.



# CERTIFICATE OF ANALYSIS

CATALOG NO: S-15880-250ML

EXPIRATION: Apr 23, 2017

DESCRIPTION: Custom PCB Congener Standard

LOT #: B7040178

See reverse for additional certification information.

SOLVENT: Isooctane

This product is guaranteed accurate to  $\pm 0.5\%$  of the Certified Analyte concentration through the Expiration Date on the Label.

Component

CAS #

Purity %  
(GC/MS)

Prepared  
Concentration<sup>1</sup>  
( $\mu\text{g/mL}$ )

Certified Analyte  
Concentration<sup>2</sup>  
( $\mu\text{g/mL}$ )

1. All weights are traceable through NIST, Test No. 822/272103-05
2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for this product is the Combined Uncertainty  $u_c(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U_c(y)$  which is  $U_c(y) * K$  where K is the coverage factor at the 95% confidence level ( $K=2$ ). Values reported above are Expanded Combined Uncertainty.
3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

125 Market Street New Haven, CT 06513 USA

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Web [AccuStandard.com](http://AccuStandard.com)

OR-ORG/INO-001  
Rev. 10/06

Certified by: *R. Cooper*

# CERTIFICATION REPORT

1. **Intended Use:** The product covered by this Certificate is designed for Calibration or for use in Quality Control procedures for the specified chemical compounds listed on the reverse side. This product can be used for Identification and/or Quantification. This product can also be used as a Reference Material to validate analytical procedures, subject to the conditions under Section 8.
2. **Raw Materials:** Reference Standards are prepared from the highest quality starting materials with defined purities. All analytes and solvents are obtained from pre-qualified vendors and then analyzed or evaluated according to ISO 9001 requirements prior to use.
3. **Manufacturing:** AccuStandard, Inc. manufactures its products under an ISO 9001 certified quality system. Balances used in the manufacturing process are calibrated regularly. All weights are traceable through the National Institute of Standards and Technology (NIST).
4. **Homogeneity Assessment:** Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the ISO 9001 Quality System.
5. **Stability Assessment:** AccuStandard, Inc. guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
6. **Analytical Quality Control:** Products are tested by validated analytical methods covered under the company's ISO 9001 Quality System.
7. **Uncertainty Statistics and Confidence Limits:** The maximum Uncertainty stated on the face of this certificate has been calculated in accordance with the EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement - Second Edition. The Uncertainty given is the Expanded Combined Uncertainty and represents an estimated Standard Deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$ , where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). The Expanded Uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of the product.
8. **Legal Notice and Limit of Liability:** This product is for research use only. No warranty for any particular application is expressed or implied. Due to their hazardous nature, they should be handled by trained personnel. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.

Attachment 5. AccuStandard Certificate of Analysis:PCB 77, C-077N, received 4-25-08.

# CERTIFICATE OF ANALYSIS

CATALOG NO. C-077N

DESCRIPTION: Individual Congener

EXPIRATION: Jul 28, 2015

LOT: 981009LB-AC

SOLVENT: N/A

This product is guaranteed accurate to  $\pm 0.5\%$  of the Certified Analyte concentration through the Expiration Date on the Label.

See reverse for additional certification information.

Component	CAS #	Purity %	Prepared Concentration <sup>1</sup>	Certified Analyte Concentration <sup>2</sup>
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	100	N/A	N/A

*received  
4-25-08  
DLW*

Please note: AccuStandard follows the U.S. conventions in reporting numerical values, on both certificates and labels.

A comma (,) is used to separate units of one-thousand or greater.  
A period (.) is used as a decimal place marker.

<sup>1</sup> All weights are traceable through NIST, Test No. 822/254480  
<sup>2</sup> Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for is  $\pm 4\%$  which is the Combined Uncertainty  $uc(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $Uc(y) * K$  where K is the coverage factor at the 95% confidence level ( $K=2$ )  
<sup>3</sup> A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is certified the same lot# without the suffix.

Certified by

*R. Cooper*

# *CERTIFICATION REPORT*

1. **Intended Use:** The product covered by this Certificate is designed for calibration or for use in Quality Control procedures for the specified chemical compounds listed on the reverse side. This product can be used for identification and/or quantification. This product can also be used as a Reference Material to validate analytical procedures, subject to the conditions under Section 10.
2. **Quality Systems:** AccuStandard® is accredited to ISO/IEC 17025:2005
3. **Raw Materials:** Reference Standards are prepared from the highest quality starting materials with defined purities. All analytes and solvents are obtained from pre-qualified vendors and then analyzed or evaluated prior to use.
4. **Manufacturing:** Balances used in the manufacturing process are calibrated regularly. All weights are traceable through the National Institute of Standards and Technology (NIST).
5. **Homogeneity Assessment:** Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the appropriate Quality System requirements.
6. **Stability Assessment:** AccuStandard, Inc. guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
7. **Analytical Quality Control:** Products are tested by validated analytical methods covered under the company's Quality Systems.
8. **Uncertainty Statistics and Confidence Limits:** The maximum Uncertainty stated on the face of this certificate has been calculated in accordance with the EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement - Second Edition. The Uncertainty given is the Expanded Combined Uncertainty and represents an estimated Standard Deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $U_c(y) * K$ , where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ). The Expanded Uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of the product.
9. **Warranties:** AccuStandard, Inc. warrants that its products shall conform to the description of such products as provided in its catalog or on the specific product label. This warranty is exclusive, and AccuStandard, Inc. makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose.
10. **Legal Notice and Limit of Liability:** This product is for research use only. Due to the hazardous nature, it should be handled by trained personnel. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.

Attachment 6. AccuStandard Certificate of Analysis:PCB 126, C-126N, received 12-22-08.

# CERTIFICATE OF ANALYSIS

CATALOG NO. C-126N

DESCRIPTION: 3,3',4,4',5-Pentachlorobiphenyl

EXPIRATION: Jul 28, 2015

LOT: 081304MS-AC

SOLVENT: N/A

This product is guaranteed accurate to  $\pm 0.5\%$  of the Certified Analyte concentration through the Expiration Date on the Label.

See reverse for additional certification information.

Component	CAS #	Purity % (GC/MS)	Prepared Concentration <sup>1</sup>	Certified Analyte Concentration <sup>2</sup>
3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	99.4	N/A	N/A

*Received  
12/22/08  
OKW*

Please note: AccuStandard follows the U.S. conventions in reporting numerical values, on both certificates and labels.

A comma (,) is used to separate units of one-thousand or greater.  
A period (.) is used as a decimal place marker.

1. All weights are traceable through NIST, Test No. 822/254480
2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for this product is  $\pm 4\%$  which is the Combined Uncertainty  $uc(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is U which is  $Uc(y) * K$  where K is the coverage factor at the 95% confidence level ( $K=2$ ).
3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and identical to the same lot# without the suffix.

Certified by: *R. Cooper*

AccuStandard is accredited to ISO/IEC 17025:2005 and certified to ISO 9001:2000

# CERTIFICATION REPORT

1. Quality Documentation: This certificate is designed in accordance with ISO Guide 31 (Reference Materials – Contents of Certificates and Labels) and ISO Guide 35 (Reference Materials – General and Statistical Principles for Certification).

2. Quality Standards:

ISO 9001:2000 Quality Management System – Requirements,  
Eagle Registrations Certificate No. 3774



ISO 17025:2005 General Requirements for the Competence of  
Testing and Calibration Laboratories AClass Certificate No. AT-1339



3. Intended Use: The product covered by this certificate is designed for calibration or for use in quality control procedures for the specified chemical compounds listed on the reverse side. This product can be used for quantification and/or identification. This product can also be used as a reference material to validate analytical procedures, subject to the conditions under Section 11.
4. Raw Materials: Reference standards are prepared from the highest quality starting materials with defined purities. All analytes and solvents are obtained from pre-qualified vendors and then analyzed or evaluated prior to use.
5. Manufacturing: All balances are calibrated daily using an in-house procedure with weights that are compared annually to master weights and traceable to NIST. The balances are also calibrated annually by an ISO 17025 accredited calibration laboratory. Please refer to the NIST test number listed on the front of this certificate. Class A glassware is used in the manufacture and quality control of all standards and calibrated using an in-house procedure.
6. Homogeneity Assessment: Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the appropriate Quality System requirements.
7. Stability Assessment: The manufacturer guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
8. Analytical Quality Control: Products are tested by validated analytical methods specified in the manufacturer's quality system.
9. Uncertainty Statistics and Confidence Limits: The uncertainty values as stated on the face of this certificate have been determined using the EURACHEM/CITAC Guide (Quantifying Uncertainty in Analytical Measurement). We have evaluated both Type A (based on a series of observations) and Type B (manufacturers specifications and calibration data) factors and report a combined expanded uncertainty equal to the positive square root of the total variance of the uncertainty of the components using the following formula:  $u_m = \sqrt{(u(P))^2 + (u(m))^2 + (u(V))^2}$ . The expanded uncertainty, U, assumes a normal distribution and a coverage factor of k=2 is chosen using a 95% confidence level.
10. Warranties: The manufacturer warrants that its products shall conform to the description of such products as provided in its catalog or on the specific product label. This warranty is exclusive, and the manufacturer makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose.
11. Legal Notice and Limit of Liability: This product is for routine laboratory analysis and research purposes only. Due to the hazardous nature, only trained personnel should handle this product. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.

Attachment 7. AccuStandard Certificate of Analysis:PCB 77, C-077N, received 1-20-09.

# CERTIFICATE OF ANALYSIS

CATALOG NO. C-077N-600MG  
DESCRIPTION: 3,3',4,4'-Tetrachlorobiphenyl  
LOT: 010809KS  
SOLVENT: N/A  
See reverse for additional certification information.

EXPIRATION: Jan 8, 2019

This product is guaranteed accurate to  $\pm 0.5\%$  of the Certified Analyte concentration through the Expiration Date on the Label.

Component	CAS #	Purity % (GC/MS)	Prepared Concentration <sup>1</sup>	Certified Analyte Concentration <sup>2</sup>
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	100	N/A	N/A

*Received  
11/20/09  
OKW*

Please note: AccuStandard follows the U.S. conventions in reporting numerical values, on both certificates and labels.

A comma (,) is used to separate units of one-thousand or greater.  
A period (.) is used as a decimal place marker.

1. All weights are traceable through NIST, Test No. 822/272103-05
2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty calculated for this product is  $\pm 4\%$  which is the Combined Uncertainty  $uc(y)$ . It represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The Expanded Uncertainty is  $U$  which is  $Uc(y) * K$  where  $K$  is the coverage factor at the 95% confidence level ( $K=2$ ).
3. A product with a suffix (-1A, -2B, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

Certified by: *R. Cooper*

# CERTIFICATION REPORT

1. Quality Documentation: This certificate is designed in accordance with ISO Guide 31 (Reference Materials – Contents of Certificates and Labels) and ISO Guide 35 (Reference Materials – General and Statistical Principles for Certification).

2. Quality Standards:

ISO 9001:2000 Quality Management System – Requirements,  
Eagle Registrations Certificate No. 3774



ISO 17025:2005 General Requirements for the Competence of  
Testing and Calibration Laboratories AClass Certificate No. AT-1339



3. Intended Use: The product covered by this certificate is designed for calibration or for use in quality control procedures for the specified chemical compounds listed on the reverse side. This product can be used for quantification and/or identification. This product can also be used as a reference material to validate analytical procedures, subject to the conditions under Section 11.
4. Raw Materials: Reference standards are prepared from the highest quality starting materials with defined purities. All analytes and solvents are obtained from pre-qualified vendors and then analyzed or evaluated prior to use.
5. Manufacturing: All balances are calibrated daily using an in-house procedure with weights that are compared annually to master weights and traceable to NIST. The balances are also calibrated annually by an ISO 17025 accredited calibration laboratory. Please refer to the NIST test number listed on the front of this certificate. Class A glassware is used in the manufacture and quality control of all standards and calibrated using an in-house procedure.
6. Homogeneity Assessment: Homogeneity of the finished product is assessed by analyzing sample batches or by other methods consistent with the intended use of the product and by procedures that comply with the appropriate Quality System requirements.
7. Stability Assessment: The manufacturer guarantees the stability of this solution through the expiration date stated on the label, when handled and stored according to the conditions stated on the label. To ensure a uniform solution, mix the contents of the sealed container thoroughly prior to use. Care should be taken not to contaminate the contents of the original container.
8. Analytical Quality Control: Products are tested by validated analytical methods specified in the manufacturer's quality system.
9. Uncertainty Statistics and Confidence Limits: The uncertainty values as stated on the face of this certificate have been determined using the EURACHEM/CITAC Guide (Quantifying Uncertainty in Analytical Measurement). We have evaluated both Type A (based on a series of observations) and Type B (manufacturers specifications and calibration data) factors and report a combined expanded uncertainty equal to the positive square root of the total variance of the uncertainty of the components using the following formula:  $u_m = \sqrt{(u(P))^2 + (u(m))^2 + (u(V))^2}$ . The expanded uncertainty, U, assumes a normal distribution and a coverage factor of k=2 is chosen using a 95% confidence level.
10. Warranties: The manufacturer warrants that its products shall conform to the description of such products as provided in its catalog or on the specific product label. This warranty is exclusive, and the manufacturer makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose.
11. Legal Notice and Limit of Liability: This product is for routine laboratory analysis and research purposes only. Due to the hazardous nature, only trained personnel should handle this product. The company's liability will be limited to replacement of product or refund of purchase price. Notice of claims must be made within thirty (30) days from date of delivery.



