DESIGN AND PREPARATION OF A CUSTOM 58-CONGENER PCB MIXTURE DOSING SOLUTION FOR AVIAN EGG INJECTION STUDIES

HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT

HUDSON RIVER NATURAL RESOURCE TRUSTEES

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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. As described in the Trustees' Avian Egg Injection Study Plan, one of the contaminants of concern being tested in the study is a PCB mixture. This report provides the U.S. Geological Survey (USGS) report on the preparation of the PCB mixture used in the Trustees' avian egg injection studies. The USGS report also describes the rationale for the selection of PCB congeners used in the mixture and the preparation of dosing solutions used in the avian egg injection studies.

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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). 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.

In 2006, the Trustees released the "Study Plan for Avian Egg Injection Study" (Avian Egg Injection Study Plan), dated May 12, 2006 (Hudson River Natural Resource Trustees, 2006, 2007).

2.0 INTRODUCTION

The Avian Egg Injection Study Plan noted that the objective of the investigation is to evaluate the toxicity and adverse effects of embryonic exposure of multiple avian species to dose ranges of PCB 126 or a PCB mixture. That PCB mixture, made up of individual PCB congeners, fits a profile similar to the mixture of PCBs occurring in the eggs of birds nesting in the Upper Hudson River. Appendix A of this report provides the U.S. Geological Survey (USGS) report on the preparation of the PCB mixture used in the Trustees' avian egg injection studies. The USGS report also describes the rationale for the selection of PCB congeners used in the mixture and the preparation of dosing solutions used in the avian egg injection studies.

3.0 LITERATURE CITED

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Appendix A

Design and Preparation of a Custom 58-Congener PCB Mixture Dosing Solution for Avian Egg Injection Studies (U.S. Geological Survey report)

Design and preparation of a custom 58-congener PCB mixture dosing solutions for Japanese quail egg injection studies.

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Attachment 1. AccuStandard custom PCB 58-congener mixture certificate of analysis.

Attachment 2. AccuStandard isooctane procedural blank certificate of analysis.

Introduction

The Hudson River Natural Resource Trustees are conducting studies to evaluate whether avian reproduction and/or development is injured as a result of exposure to PCBs from the Hudson River. As part of the NRDA, Japanese quail egg injection studies have been conducted in order to establish the effective dosage and reliable measurement end points that will then be applied to tests in other avian species. This report describes the rationale for selection of the PCB congeners selected for testing in avian species and the preparation of the mixture used in the avian egg injection studies with Japanese quail.

The mixture of PCBs, and possibly dioxins and furans, present in biota from the Hudson River are largely the result of releases of Aroclors into the river (see NRDA websites for details: http://www.fws.gov/contaminants/restorationplans/hudsonriver/hudsonriver.cfm or http://www.dec.state.ny.us/website/dfwmr/habitat/nrd/). Aroclors are the commercial names for heat transfer fluids used in electrical components such as capacitors and transformers. Aroclors were chiefly comprised of polychlorinated biphenyls, but also contained polychlorinated dibenzofurans (PCDFs) as a by-product of manufacture and use (Erickson et al. 1989). Based on our current knowledge of toxicity in avian species, PCBs have a subset of congeners which can bind the Ah-receptor and also cause dioxin-like toxicity. PCBs with chlorine atoms at one (mono-) or no (non-) ortho positions of the biphenyl rings and chlorine atoms on the lateral positions (meta and para) of the biphenyl rings fall into this dioxin-like category of toxicity. These non-ortho-chloro PCBs and mono-ortho-chloro PCBs are referred to as “dioxin-like PCBs.” Other PCB congeners have been shown to work through different modes of action and can cause neurotoxicity, malignant transformations of cells and tissues, as well as other untoward effects in exposed species. However, knowledge of the exact mechanisms by which the non-dioxin-like PCB congeners cause these effects is limited.

The amounts of PCDFs in Aroclors although small on a mass basis, are significant on the basis of toxicological potency. Thus, any mixture that is prepared to evaluate the toxicological potency of chemicals associated with the use and release of Aroclors into the Hudson River, must consider the toxic potency of PCDFs as well as PCBs. The relative toxicological importance of PCDFs in fish and wildlife species in the Hudson River is a direct function of the exposure experienced by the particular species. Fundamental principles of toxicokinetics (uptake, distribution, metabolism, and elimination) in the organism are important variables for the assessment of PCDFs, as well as PCBs and other chemicals which bioaccumulate and contribute to dioxin-like toxic equivalents (TEQs). The environmental fate of the PCDFs and PCBs present in Aroclors is dependent, in part, on physical chemical properties of the individual congeners (persistence, volatility, sorption, etc). Thus, the chemical exposure experienced by a species in the Hudson River ecosystem is a function of its trophic level and placement in the food web.

Rationale and Design of PCB Congener Mixture

The rationale for selection of chemicals to include in a mixture representing avian species found in the Hudson River basin was based on the toxicological concerns for these chemicals (ie. exposure and toxicity). Polychlorinated dibenzodioxins (PCDDs) and PCDFs are both referred to as “dioxin-like” because they exert their toxicity through the same cytosolic receptor (Ah-receptor) and cause the same suite of toxicological symptoms in avian species. Certainly, no model of toxicity comparable to that of dioxin-like toxicity (van den Berg et al. 1998) is available for the non-dioxin-like PCB congeners. Therefore, toxicological evaluation and assessment of PCBs is logically divided into two categories, dioxin-like PCBs and the non-dioxin-like PCBs. The lack of a detailed model of toxicity for the non-dioxin-like PCB congeners forces us to evaluate these congeners on the basis of mass or simple concentrations. The dioxin-like PCBs, on the other hand, can be evaluated based on concentration and their individual potency (toxic equivalency factor, TEF). Thus, evaluation and selection of PCB congeners to include in a mixture for toxicological testing in Japanese quail was based on concentrations of the non-dioxin-like PCB congeners and the toxic potencies (concentrations X TEF) for each of the dioxin-like PCB congeners. The evaluation of PCDD and PCDF congeners was based on their potency to cause dioxin-like effects and the relative amounts present in avian wildlife from the Hudson River.

Chemical Data on Belted Kingfisher, Spotted Sandpiper, and Tree Swallows from the Hudson River

The exposure data on belted kingfisher, spotted sandpiper, and tree swallows collected from the Hudson River in 2004 (Hudson River Natural Resource Trustees 2005) was the main source of information for the selection of chemicals that comprised the mixture for toxicity testing. A summary of the chemical exposure data used for the selection of congeners that were added to the synthetic mixture are provided for this report. The data summary for the selection process is provided in two manners: 1) toxic potencies or TEQs for the dioxin-like PCB, PCDD, and PCDF congeners (Table 1); and 2) mean concentrations and their ranks for the non-dioxin-like PCBs (Table 2).

The TEQs present in the avian species collected from the Hudson River in 2004 were remarkable with respect to the concentrations of PCB-related TEQs as compared to PCDF- and PCDD-related TEQs (Table 1). In all three of these species the PCB-related TEQs were 99% or greater of the total TEQs observed in each species. This is remarkable in the sense that even though we know that Aroclors released into the Hudson River were first and foremost PCBs, they also contained PCDFs as manufacturing by-products. Some of these PCDFs would be subject to metabolism (eg. 2,3,7,8-TCDF) and might not be observed at significant concentrations for this reason. The overall conclusion that can be drawn from these data is that any dioxin-like toxicity observed or expected in developing embryos of birds from the Hudson River study area appears to be almost exclusively related to PCBs. Consequently, our toxicity testing in Japanese quail is focused on PCB congeners that are present. Testing of the contributions of PCDFs and PCDDs to the developmental toxicity of the dioxin-like chemicals in these avian species would necessarily have to take a much smaller research or assessment priority.

Evaluation of the non-dioxin-like PCB congeners present in these avian species from the Hudson River basin was based on the concentrations of the chemicals present, the rank order of their concentrations within the eggs of a species, and the cumulative percent of total PCBs attributable to individual congeners (Table 2). The PCB congener concentrations (ng/g, ranked from greatest to least) are followed by a column of the relative percentage of each congener as compared to the total PCB concentration for the species, and the last column under each species is a cumulative percentage as compared to the total PCB content in that species.

The composition of the PCB congeners with the greatest concentrations in each species was fairly consistent. As one evaluates the cumulative percentage of the congeners, starting from the congener with the greatest concentration, there are a large number of congeners (or congener pairs) which are the same in each of the three species. If one looks at the first 36 congeners for each species, 29 of 36 congeners are the same when comparing the congeners found in greatest concentrations in the tree swallow to that of the belted kingfisher, while 31 of the first 36 congeners are the same between the spotted sandpiper and the belted kingfisher. The cumulative percentages of individual PCB congener contributions to total PCBs up until the 36th ranked congener (or congener set) are approximately 95%, 90% and 88% for spotted sandpiper, belted kingfisher, and tree swallow, respectively. That is to say, the first 36 PCB congeners (or congener sets) account for approximately 88% of the total PCB content in tree swallows; 90% of the total PCB content in belted kingfisher; and 95% of the total PCB content in spotted sandpiper.

Design of a PCB mixture similar to Hudson River spotted sandpipers

The congener composition developed for the laboratory toxicity testing was based on the spotted sandpiper eggs collected from the Hudson River in 2004. The rationale for the selection was based on: 1) the greater concentrations of total PCBs and TEQs in the eggs of the spotted sandpiper; 2) the large degree of similarity between the congener patterns in tree swallows, spotted sandpipers and belted kingfisher eggs from the Hudson River samples; and 3) the high concentrations of PCBs observed in spotted sandpiper eggs in 2004, suggesting that this species might be a candidate for an egg injection study. The first 36 PCB congeners (or congener sets) in the spotted sandpiper were selected from the list (Table 2). This represented approximately 95% of the total PCB content measured in spotted sandpiper eggs on a mass basis. Additionally, the dioxin-like PCBs were added to the mixture based upon their importance from a toxicological standpoint. Thus, the dioxin-like PCB congeners that were not among in the first 36 congeners or congener sets were added to the PCB mixture in direct proportion to their concentrations found in spotted sandpiper eggs (Table 2), even when their concentrations were small and the ranks of the dioxin-like PCBs were below the first 36 congeners. Accordingly, another 9 congeners were included in the synthetic PCB mixture on this basis. The exact concentrations of the dioxin-like and non-dioxin-like PCB congeners in the mixture reflect the concentrations found in the spotted sandpiper egg samples.

The final selection of congeners to be included into the PCB mixture was based on the composition of PCBs in the spotted sandpiper eggs taken in the initial 11 samples collected from the Hudson River in 2004, the presence of these congeners in Aroclor mixtures, and the relative amounts of some congeners in Aroclors. The field identification and species of these 11 samples were as follows: 1) BK04-105-E, Belted Kingfisher; 2) BK04-150-E, Belted Kingfisher; 3) BK04-171-E, Belted Kingfisher; 4) BK04-216-E, Belted Kingfisher; 5) SS04-22-E1, Spotted Sandpiper; 6) SS04-140-A-E, Spotted Sandpiper; 7) SS04-237-A-E1, Spotted Sandpiper; 8) TS04-JV-40-EA, Tree Swallow; 9) TS04-JV-42-EA, Tree Swallow; 10) TS04-REM-83-EA, Tree Swallow; and 11) TS04-RI-128-EA, Tree Swallow. The analysis of PCB congeners included co-elution of some congeners (Table 2). The first 36 ranked congeners or congener sets included 18 pairs of congeners that co-eluted and 5 congener sets that potentially included three co-eluting congeners (Table 3). Construction of the congener mix for toxicity testing required us to evaluate the possible composition of each individual congener within a set of co-eluting congeners. The composition of individual congeners within a set was estimated from the composition of these congeners within a 1:1:1:1 Aroclor 1242:1248:1254:1260 mixture. Of the 18 pairs of co-eluting congeners, 13 of the pairs contained a congener that was present in only very small amounts (<1%) relative to the amount of the other co-eluting congener. The PCB congeners with relative proportions of <0.01 (ie. <1%) as compared to the other co-eluting congener were omitted from the PCB mixture (Table 3). Thus, PCB congeners 80, 61, 106, 73, 76, 127, 120, 160, 86, 93, 182, 111, and 107 were not added to the PCB mixture, due to negligible concentrations assumed to be present. The individual congeners of the 5 remaining co-eluting pairs of congeners were added into the PCB mixture at concentrations proportional to their relative amounts in the Aroclor 1:1:1:1 mixture (Table 3).

The same selection criteria and rules for proportions were used for the 5 sets of co-eluting congeners that appeared in the top 36 ranked congeners or congener sets from the original data set on spotted sandpiper eggs (Table 2). Congeners 90 and 116 were omitted from the mixture, due to assumed negligible amounts. The remainder of the individual congeners in the 5 sets of triplet peaks were added into the PCB mixture at the amounts given in Table 3. The composition of the PCB mixture resulted in 58 individual PCB congeners; the 49 congeners selected based on their importance to the total mass of PCBs and the 9 congeners selected based on their dioxin-like toxicological properties (Table 3). The 58-congener PCB mixture, with proportions equivalent to that of the congener composition of spotted sandpiper eggs from the Hudson River, was special ordered from AccuStandard, Inc. (New Haven, CT; Table 4).

The objective of this work was to prepare the mixture of PCB congeners and develop a serial dilution set of dosing solutions to be used to study PCB-related toxicity in avian embryos and chicks. The remainder of this report describes the preparation of six concentrations (doses) of the custom 58-congener PCB mixture emulsified in 0.75% (v/v) corn oil:1,2-propanediol and an emulsified 0.75% corn oil:1,2-propanediol blank under sterile conditions. These solutions were prepared for use as egg injection dosing solutions in a Japanese quail egg dosing study conducted at the University of Maryland. The solutions may potentially be used in subsequent egg injection studies on other species.

Preparation of custom 58-congener PCB mixture dosing solutions

PCB mixture order and receipt

The custom 58-congener PCB standard dissolved in 250-mL of isooctane and corresponding 250-mL of blank isooctane were purchased from AccuStandard, Inc. (New Haven, CT; Invoice # 270876). The custom PCB congener 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 1). A similar certificate of Analysis was provided for the isooctane blank (Attachment 2).

Upon receipt at CERC, the custom PCB congener package was opened by CERC staff and the bottles were inspected for concurrence with attached documentation. Both bottles were received in good condition and documentation was verified. To verify these solution concentrations triplicate 100-µL sub-samples of each were taken with a 100-µL Hamilton syringe and placed in cleaned amber 1.5-mL autosample vials with yellow Teflon-faced septa screw caps. The autosample 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 the CERC Organic Chemistry section for analysis. The 58-congener PCB mixture and the isooctane blank were secured and stored in a locked box placed in the CERC Biochemistry Section hazardous compound storage area.

Glassware preparation

Transfer tubes - Two 30-mL glass culture tubes with Teflon-faced rubber lined screw caps (Fisher # 149 3010G, 20 x 125 mm) were used for transfer and sub-sampling of the original 58-congener PCB mixture from AccuStandard. We prepared these transfer tubes 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 numbered (8 & 9) by etching and any residual acetone was allowed to evaporate. Two tube heating blocks with accompanying nitrogen purge apparatuses were prepared in separate hoods, one designated for the PCB mixture (tube #8) and one for the blank (tube #9). Heating blocks were set to maintain heat at approximately 35^{o}C (range 32 – 38^{o}C).

Dose solution vials - Six new 3-mL reaction-vials (Sigma, Z115096) and corresponding black Teflon-lined screw caps (Sigma, Z164313) were etched with a number and marked for volume at 0.80 and 1.6 mL using ultra pure water, ultra filtered 0.2 μm ASTM Type I water (Hydro Service & Supplies, Inc. Research Triangle Park , NC). Each was then 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. Six additional new 3-mL reaction vials were etched with a number and marked for volume at 0.50 and 1.0-mL and processed as described above. Each vial and cap was etched with the same number and vial and cap weights were recorded separately and as a pair so they could be used together.

Sub-sampling of the PCB mixture and procedural blank from AccuStandard

We calculated that 50-mL of the 250-mL original 58-congener PCB mixture (20%) would be required to make the dosing solutions for the Japanese quail egg injection studies (Table 4). Using new, sterile, 10 mL, serological pipette 12.5-mL of the 58-congener isooctane mixture received from AccuStandard was transferred to one of the pre-washed, pre-weighted, 30-mL culture tubes (labeled #8). A second new, sterile, 10-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 #9). 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 of the original 58-congener PCB mixture from AccuStandard (for tube #8) or the isooctane blank received from AccuStandard (for tube #9) were carefully added to the respective transfer tubes. The cycle of transfer and evaporation (from the 58-congener PCB mixture solution to transfer tube #8 or the isooctane blank to transfer tube #9) 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 58-congener PCB solution (transfer tube #8) was quantitatively transferred using a baked Pasteur pipette to a previously prepared 3-mL dosing solution vial, labeled 3-2. Vial 3-2 was placed in the PCB designated heating block and secured with gently purging nitrogen to facilitate evaporation of the isooctane washes. Transfer tube # 8, containing the PCB mixture, was rinsed 12 times with 1 to 1.5-mL isooctane and each rinsate was transferred to vial 3-2 as space was made available due to evaporation. The isooctane blank was treated in exactly the same manner, transferring the contents of transfer tube # 9 to 3-mL vial labeled 3-1. The isooctane blank solution in transfer tube #9 was rinsed 12 times and rinses transferred to vial 3-1, which was placed in the isooctane heating block. After the final rinse was transferred, the heating block temperatures were reduced to approximately 25 ^{o}C and both vials (vial 3-1 and vial 3-2) were concentrated to the pre-etched mark at 0.8 mL (described above).

Preparation of 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 3-2) and procedural blank emulsion (in vial labeled 3-1) were prepared from the sub-samples described above. The targeted concentration of the PCB stock emulsion dosing solution was 246 µg PCB/ µL in a final volume of 1.6-mL. A procedural blank emulsion was also prepared. These emulsions were prepared as follows. A small amount of sterile corn oil (Sigma, Cat. # C8267, batch 103K0107, density 0.9 g/mL) was added to each of the 3-mL vials; 12.2-µL to vial 3-1 (blank) and 12.7-µL to vial 3-2 (PCB mixture), calculated based density. Each vial was gently mixed by slow vortexing. 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 ^{o}C. The vials were periodically mixed by slow vortexing. 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.

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 1.6-mL. Calculated volumes based on the masses of the 1,2-propanediol added confirmed the volume added to each vial. The contents of each 3-mL vial were vigorously vortex-mixed until they became thoroughly emulsified. The resulting emulsion of the 58-congener PCB standard dosing stock solution (vial 3-2) was at a target concentration of 246 µg PCB/uL, while the procedural blank emulsion (vial 3-1) was the negative control for the toxicity testing blank dosing solution.

Serial dilution of the PCB mixture stock emulsion

The various 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 101.2-µL of sterile corn oil with 13.4710-mL of sterile 1,2-propanediol in a sterile 15-mL glass culture tube with a Teflon-faced rubber-lined screw cap (volumes confirmed by weight). This 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, 3-8 to 3-12. A 500-µL aliquant of the 58-congener PCB standard dosing solution stock emulsion (vial 3-2) was transferred to the empty vial 3-7 under sterile conditions. In the same manner, a 500-µL aliquant of the procedural blank dosing solution emulsion (vial 3-1) was transferred to vial 3-4. Under sterile conditions, a 500-µL aliquant of the freshly vortex-mixed sterile stock emulsion of 0.75% (v/v) corn oil:1,2-propanediol from the 15-mL culture tube was transferred to each of the vials 3-8, 3-9, 3-10, 3-11, and 3-12. The 58-congener PCB mixture emulsion dosing solution stock (vial 3-2) was freshly vortex-mixed and using a calibrated Rainin P-1000, 500-µL was transferred to vial 3-8. This resulted in a 2-fold dilution and vial 3-8 was vigorously vortex-mixed. In the same manner, 500-µL was transferred from vial 3-8 to vial 3-9 resulting in a 4-fold dilution of the original PCB emulsion. This process was repeated to produce the entire dosing solution dilution series (Table 5).

Sub-sampling of dosing solution emulsions for chemical analysis

A 20-µL sample of each dosing solution (Table 5) was taken and placed in previously prepared amber 1.5-mL autosample vial with Teflon-faced septa screw caps. The autosample 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 given to the CERC Environmental Chemistry Branch for analysis. The results of these analyses will be reported in a separate report from the Environmental Chemistry Branch, CERC.

Shipment of the dosing solution emulsions

Each vial listed in Table 5 was individually bagged in a 4 x 4 "Zip-loc" type plastic bag, placed in a 3 mL vial shipping box and all were stored at -80 ^{o}C in preparation for shipment. All other vials or bottles containing solutions prepared in this procedure were returned and secured to a locked box. 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 the laboratory conducting the avian egg injection studies with Japanese quail.

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 emulsified egg dosing solutions (Table 6). Briefly, for each congener we calculated the mass in 50.0-mL of the original 250-mL 58-congener PCB standard stock solution from AccuStandard (Table 4). This mass was converted to concentration units (µg/µL) using the final volume of the PCB stock dosing solution emulsion (1.60-mL in vial 3-2). Subsequently, dosing solution concentrations for the individual congeners were calculated based on dilution factors (Table 5). Finally, individual PCB congener egg dose concentrations were calculated by multiplying the dosing solution concentration of each congener times the injection volume (4 µL/egg) and then dividing by the average mass of a Japanese quail egg (10 g/egg). Expected doses of each of the 58 PCB congeners were calculated in this fashion, for each of the six dosing solutions (Table 6).

For example: The AccuStandard certified concentration for PCB congener 28 (2, 4, 4'-trichlorobiphenyl) of 817.6 µg/mL, was multiplied by 50.0 ml, resulting in 40,880 µg of PCB congener 28. Next, we divided by 1.60 mL and then by 1000 µL/mL to give 25.55 µg/µL; the concentration of PCB congener 28 in the emulsified stock dosing solution (98 µg total PCB/g egg, vial 3-7). The PCB congener 28 concentration/g egg (expected nominal dose) was calculated by multiplying 25.55 µg/µL by 4 µL, dividing by 10 g/egg and finally multiplying by 1000 ng/µg to obtain 10,220 ng/g (Table 6). The names for each of the dosing solution emulsions (column headers) were given as the nominal total PCB dose expected to be delivered to an individual Japanese quail egg from that solution (Table 6). Thus, the six dosing solution emulsions were referred to as 98, 49, 25, 12, 6, and 3 µg PCB/g egg doses (column headers in Table 6).

The dioxin toxic equivalents (TEQs) expected to be delivered to a quail egg at each dose were also estimated (Table 7). The dose from the 12 PCB congeners with dioxin-like potency (from Table 6) 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 solution emulsions, referred to as 98, 49, 25, 12, 6, and 3 µg PCB/g egg (Table 6), had total TEQs of approximately12,600 pg/g, 6,300 pg/g, 3,100 pg/g, 1,600 pg/g, 790 pg/g, and 390 pg/g, respectively (Table 7).

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Tables

Table 1. Concentrations (pg/g) of dioxin-like toxic equivalents (TEQs) derived from PCBs, PCDDs, and PCDFs measured in spotted sandpiper, belted kingfisher, and tree swallow eggs collected from the Hudson River basin in 2004*.

	Tree swallow TEQ (pg/g)	Belted Kingfisher TEQ (pg/g)	Spotted Sandpiper TEQ (pg/g)
PCB-81	600	600	1700
PCB-77	2000	650	1500
PCB-123	0	0	0
PCB-114	1	2	6
PCB-126	200	900	500
PCB-167	0	0	0
PCB-156	4	5	16
PCB-157	1	1	3
PCB-169	0	0	0
PCB-189	0	0	0
PCB 118/106	4	5	16
PCB 105/127	17	23	76
PCB Sub-total	2828	2187	3818
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PECDD	3	2	1
1,2,3,4,7,8-HXCDD	0	0	0
1,2,3,6,7,8-HXCDD	0	0	0
1,2,3,7,8,9-HXCDD	0	0	0
1,2,3,4,6,7,8-HPCDD	0	0	0
OCDD	0	0	0
PCDD Sub-total	4	3	2
2,3,7,8-TCDF	1	1	1
1,2,3,7,8-PECDF	0	0	0
2,3,4,7,8-PECDF	5	2	2
1,2,3,4,7,8-HXCDF	0	0	0
1,2,3,6,7,8-HXCDF	0	0	0
2,3,4,6,7,8-HXCDF	0	0	0
1,2,3,7,8,9-HXCDF	0	0	0
1,2,3,4,6,7,8-HPCDF	0	0	0
1,2,3,4,7,8,9-HPCDF	0	0	0
OCDF	0	0	0
PCDF Sub-total	6	3	3
Total TEQs	2837	2194	3823

TEQs based on concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) measured in eggs collected in 2004 and avian TEF values (van den Berg et al. 1998). Concentrations of co-eluting congeners PCB-118/PCB-106 and PCB-105/PCB-127, were assumed to be all PCB-118 and PCB-105, respectively, for TEQ estimation purposes. Concentrations for the PCB congeners are based on GC-LR/MS analysis and concentrations of PCDDs and PCDFs are based on GC-HR/MS analysis of the initial 11 avian egg samples taken in 2004 (see text for sample identification numbers).

Table 2. Concentrations (ng/g egg), relative amount (% of total), and cummulative percentage of rank ordered polychlorinated biphenyl (PCB) congeners measured in the eggs of birds collected along the Hudson River in 2004.

	Tree swallow averages (ng/g)	Rel. %	Cumm.%		Belted Kingfisher averages (ng/g)	Rel. %	Cumm.%		Spotted Sandpiper averages (ng/g)	Rel. %	Cumm.%
TOTAL PCB	8203	100		TOTAL PCB	9198	100		TOTAL PCB	25579	100	
PCB 52/73	506	6	6.2	PCB 138/163/164	642	7	7.0	PCB 28	2548	10	10.0
PCB 28	459	6	11.8	PCB 153	580	6	13.3	PCB 66/80	2290	9	18.9
PCB 66/80	426	5	17.0	PCB 52/73	566	6	19.4	PCB 74/61	1679	7	25.5
PCB 70/76	409	5	21.9	PCB 118/106	548	6	25.4	PCB 118/106	1604	6	31.7
PCB 31	382	5	26.6	PCB 66/80	523	6	31.1	PCB 47/48/75	1538	6	37.8
PCB 49/43	370	5	31.1	PCB 47/48/75	492	5	36.4	PCB 138/163/164	1410	6	43.3
PCB 118/106	368	4	35.6	PCB 74/61	428	5	41.1	PCB 90/101/89	1289	5	48.3
PCB 138/163/164	347	4	39.8	PCB 90/101/89	410	4	45.5	PCB 52/73	1250	5	53.2
PCB 90/101/89	318	4	43.7	PCB 99	395	4	49.8	PCB 49/43	1099	4	57.5
PCB 41/71/64/68	316	4	47.5	PCB 49/43	386	4	54.0	PCB 153	1016	4	61.5
PCB 74/61	305	4	51.3	PCB 110	304	3	57.3	PCB 99	998	4	65.4
PCB 47/48/75	283	3	54.7	PCB 28	282	3	60.4	PCB 70/76	913	4	68.9
PCB 153	266	3	58.0	PCB 105/127	227	2	62.9	PCB 105/127	764	3	71.9
PCB 110	259	3	61.1	PCB 149/139	218	2	65.2	PCB 31	743	3	74.8
PCB 44	231	3	63.9	PCB 41/71/64/68	181	2	67.2	PCB 56/60	720	3	77.6
PCB 56/60	214	3	66.5	PCB 187/182	164	2	69.0	PCB 41/71/64/68	645	3	80.2
PCB 99	178	2	68.7	PCB 180	160	2	70.7	PCB 110	453	2	81.9
PCB 105/127	172	2	70.8	PCB 56/60	144	2	72.3	PCB 85/120	437	2	83.6
PCB 95/93	146	2	72.6	PCB 31	139	2	73.8	PCB 87/115/116	330	1	84.9
PCB 87/115/116	138	2	74.3	PCB 70/76	136	1	75.3	PCB 128	250	1	85.9
PCB 42/59	137	2	76.0	PCB 85/120	128	1	76.7	PCB 149/139	217	1	86.8
PCB 149/139	125	2	77.5	PCB 95/93	123	1	78.0	PCB 92	213	1	87.6
PCB 37	119	1	78.9	PCB 87/115/116	120	1	79.3	PCB 180	180	1	88.3
PCB 97/86	99	1	80.1	PCB 92	115	1	80.6	PCB 158/160	178	1	89.0
PCB 85/120	83	1	81.1	PCB 146	108	1	81.7	PCB 146	165	1	89.6
PCB 180	70	1	82.0	PCB 170/190	99	1	82.8	PCB 97/86	159	1	90.3
PCB 128	61	1	82.7	PCB 128	87	1	83.8	PCB 156	155	1	90.9
PCB 26	52	1	83.4	PCB 111/117	86	1	84.7	PCB 95/93	155	1	91.5
PCB 92	50	1	84.0	PCB 63	74	1	85.5	PCB 187/182	141	1	92.0
PCB 132/168	48	1	84.6	PCB 91	62	1	86.2	PCB 170/190	132	1	92.5
PCB 16/32	47	1	85.1	PCB 158/160	54	1	86.8	PCB 111/117	130	1	93.0
PCB 170/190	47	1	85.7	PCB 156	53	1	87.3	PCB 141	118	0.5	93.5
PCB 22	46	1	86.3	PCB 199	52	1	87.9	PCB 130	84	0.3	93.8
PCB 158/160	44	1	86.8	PCB 151	51	1	88.4	PCB 107/109	81	0.3	94.1
PCB 91	44	1	87.3	PCB 44	49	1	89.0	PCB 137	78	0.3	94.5
PCB 146	42	1	87.9	PCB 107/109	47	1	89.5	PCB 42/59	71	0.3	94.7
PCB 156	42	1	88.4	PCB 196/203	46	0.5	90.0	PCB 144/135	65	0.3	95.0
PCB 77	40	0.5	88.8	PCB 132/168	42	0.5	90.4	PCB 91	65	0.3	95.2
PCB 33/20/21	38	0.5	89.3	PCB 72	41	0.4	90.9	PCB 132/168	60	0.2	95.5
PCB 17	38	0.5	89.8	PCB 97/86	38	0.4	91.3	PCB 63	60	0.2	95.7
PCB 18	36	0.4	90.2	PCB 183	38	0.4	91.7	PCB 114	57	0.2	95.9
PCB 141	35	0.4	90.6	PCB 177	37	0.4	92.1	PCB 119	50	0.2	96.1
PCB 63	34	0.4	91.0	PCB 141	36	0.4	92.5	PCB 167	49	0.2	96.3
PCB 15	33	0.4	91.4	PCB 130	31	0.3	92.8	PCB 183	44	0.2	96.5
PCB 187/182	33	0.4	91.8	PCB 144/135	30	0.3	93.2	PCB 177	40	0.2	96.6
PCB 67	31	0.4	92.2	PCB 194	30	0.3	93.5	PCB 123	39	0.2	96.8
PCB 53	29	0.4	92.6	PCB 178	29	0.3	93.8	PCB 174/181	39	0.2	96.9
PCB 107/109	28	0.3	92.9	PCB 42/59	28	0.3	94.1	PCB 82	38	0.1	97.1
PCB 82	27	0.3	93.3	PCB 137	28	0.3	94.4	PCB 151	37	0.1	97.2
PCB 151	25	0.3	93.6	PCB 174/181	27	0.3	94.7	PCB 157	33	0.1	97.4
PCB 111/117	24	0.3	93.9	PCB 119	26	0.3	95.0	PCB 147	32	0.1	97.5
PCB 25	23	0.3	94.1	PCB 133	25	0.3	95.3	PCB 72	31	0.1	97.6
PCB 144/135	23	0.3	94.4	PCB 167	22	0.2	95.5	PCB 77	30	0.1	97.7
PCB 84	22	0.3	94.7	PCB 114	22	0.2	95.8	PCB 22	30	0.1	97.8
PCB 40	20	0.2	94.9	PCB 26	22	0.2	96.0	PCB 129	29	0.1	98.0
PCB 130	20	0.2	95.2	PCB 147	21	0.2	96.2	PCB 199	26	0.1	98.1
PCB 137	20	0.2	95.4	PCB 123	18	0.2	96.4	PCB 196/203	25	0.1	98.2
PCB 167	16	0.2	95.6	PCB 206	17	0.2	96.6	PCB 171	23	0.1	98.2
PCB 183	16	0.2	95.8	PCB 154	17	0.2	96.8	PCB 133	21	0.1	98.3
PCB 174/181	14	0.2	96.0	PCB 193	15	0.2	97.0	PCB 33/20/21	20	0.1	98.4

	Tree swallow averages (ng/g)	Rel. %	Cumm.%		Belted Kingfisher averages (ng/g)	Rel. %	Cumm.%		Spotted Sandpiper averages (ng/g)	Rel. %	Cumm.%
PCB 136	13	0.2	96.1	PCB 171	14	0.2	97.1	PCB 124	20	0.1	98.5
PCB 114	13	0.2	96.3	PCB 100	14	0.1	97.3	PCB 178	19	0.1	98.6
PCB 196/203	12	0.1	96.4	PCB 172/192	14	0.1	97.4	PCB 16/32	18	0.1	98.6
PCB 124	12	0.1	96.6	PCB 77	13	0.1	97.6	PCB 194	18	0.1	98.7
PCB 129	12	0.1	96.7	PCB 202	13	0.1	97.7	PCB 81	17	0.1	98.8
PCB 177	12	0.1	96.9	PCB 157	12	0.1	97.8	PCB 100	15	0.1	98.8
PCB 51	12	0.1	97.0	PCB 136	11	0.1	98.0	PCB 103	14	0.1	98.9
PCB 72	11	0.1	97.2	PCB 195	10	0.1	98.1	PCB 172/192	14	0.1	98.9
PCB 98/102	11	0.1	97.3	PCB 126	9	0.1	98.2	PCB 154	14	0.1	99.0
PCB 24/27	11	0.1	97.4	PCB 208	8	0.1	98.2	PCB 53	14	0.1	99.1
PCB 199	11	0.1	97.6	PCB 98/102	8	0.1	98.3	PCB 26	14	0.1	99.1
PCB 123	10	0.1	97.7	PCB 103	7	0.1	98.4	PCB 193	12	0.05	99.2
PCB 157	9	0.1	97.8	PCB 16/32	7	0.1	98.5	PCB 8/5	11	0.04	99.2
PCB 119	9	0.1	97.9	PCB 84	6	0.1	98.5	PCB 44	11	0.04	99.2
PCB 194	9	0.1	98.0	PCB 159	6	0.1	98.6	PCB 67	11	0.04	99.3
PCB 83/108	8	0.1	98.1	PCB 81	6	0.1	98.7	PCB 37	11	0.04	99.3
PCB 171	7	0.1	98.2	PCB 113	5	0.1	98.7	PCB 25	10	0.04	99.4
PCB 147	7	0.1	98.3	PCB 129	5	0.1	98.8	PCB 166	8	0.03	99.4
PCB 46	7	0.1	98.4	PCB 82	5	0.1	98.8	PCB 98/102	7	0.03	99.4
PCB 81	6	0.1	98.4	PCB 53	4	0.05	98.9	PCB 206	7	0.03	99.5
PCB 45	6	0.1	98.5	PCB 67	4	0.05	98.9	PCB 24/27	7	0.03	99.5
PCB 178	6	0.1	98.6	PCB 209	4	0.04	99.0	PCB 162	7	0.03	99.5
PCB 19	6	0.1	98.6	PCB 162	4	0.04	99.0	PCB 202	7	0.03	99.5
PCB 55	5	0.1	98.7	PCB 201	4	0.04	99.1	PCB 113	6	0.02	99.6
PCB 38	5	0.1	98.8	PCB 165	4	0.04	99.1	PCB 136	6	0.02	99.6
PCB 133	5	0.1	98.8	PCB 166	4	0.04	99.1	PCB 195	6	0.02	99.6
PCB 172/192	5	0.1	98.9	PCB 25	4	0.04	99.2	PCB 69	5	0.02	99.6
PCB 8/5	5	0.1	99.0	PCB 69	3	0.04	99.2	PCB 159	5	0.02	99.6
PCB 206	5	0.1	99.0	PCB 124	3	0.04	99.3	PCB 126	5	0.02	99.7
PCB 4/10	5	0.1	99.1	PCB 179	3	0.04	99.3	PCB 140	5	0.02	99.7
PCB 193	4	0.05	99.1	PCB 189	3	0.03	99.3	PCB 185	5	0.02	99.7
PCB 57	4	0.04	99.2	PCB 176	3	0.03	99.4	PCB 189	4	0.02	99.7
PCB 154	3	0.04	99.2	PCB 112	3	0.03	99.4	PCB 17	4	0.02	99.7
PCB 103	3	0.04	99.3	PCB 185	3	0.03	99.4	PCB 191	4	0.01	99.8
PCB 202	3	0.03	99.3	PCB 24/27	3	0.03	99.5	PCB 83/108	4	0.01	99.8
PCB 134/143	3	0.03	99.3	PCB 140	3	0.03	99.5	PCB 84	3	0.01	99.8
PCB 179	3	0.03	99.4	PCB 191	3	0.03	99.5	PCB 131/142	3	0.01	99.8
PCB 131/142	3	0.03	99.4	PCB 57	3	0.03	99.5	PCB 208	3	0.01	99.8
PCB 195	3	0.03	99.4	PCB 125	2	0.03	99.6	PCB 175	3	0.01	99.8
PCB 122	2	0.03	99.5	PCB 4/10	2	0.03	99.6	PCB 40	3	0.01	99.8
PCB 100	2	0.03	99.5	PCB 148	2	0.02	99.6	PCB 179	3	0.01	99.8
PCB 166	2	0.03	99.5	PCB 83/108	2	0.02	99.6	PCB 55	3	0.01	99.8
PCB 162	2	0.03	99.5	PCB 18	2	0.02	99.7	PCB 18	2	0.01	99.9
PCB 208	2	0.03	99.6	PCB 175	2	0.02	99.7	PCB 4/10	2	0.01	99.9
PCB 126	2	0.03	99.6	PCB 207	2	0.02	99.7	PCB 122	2	0.01	99.9
PCB 96	2	0.02	99.6	PCB 88/121	2	0.02	99.7	PCB 165	2	0.01	99.9
PCB 189	2	0.02	99.6	PCB 198	2	0.02	99.7	PCB 201	2	0.01	99.9
PCB 69	2	0.02	99.7	PCB 19	2	0.02	99.8	PCB 94	2	0.01	99.9
PCB 29	2	0.02	99.7	PCB 51	2	0.02	99.8	PCB 176	2	0.01	99.9
PCB 113	2	0.02	99.7	PCB 40	2	0.02	99.8	PCB 15	2	0.01	99.9
PCB 185	1	0.02	99.7	PCB 22	2	0.02	99.8	PCB 148	2	0.01	99.9
PCB 191	1	0.02	99.7	PCB 200	1	0.02	99.8	PCB 125	2	0.01	99.9
PCB 176	1	0.02	99.8	PCB 94	1	0.01	99.8	PCB 57	2	0.01	99.9
PCB 159	1	0.02	99.8	PCB 205	1	0.01	99.9	PCB 88/121	1	0.01	99.9
PCB 209	1	0.02	99.8	PCB 17	1	0.01	99.9	PCB 134/143	1	0.01	99.9
PCB 125	1	0.01	99.8	PCB 197	1	0.01	99.9	PCB 209	1	0.004	99.9
PCB 94	1	0.01	99.8	PCB 37	1	0.01	99.9	PCB 198	1	0.004	99.9
PCB 175	1	0.01	99.8	PCB 150	1	0.01	99.9	PCB 51	1	0.004	99.95
PCB 58	1	0.01	99.8	PCB 131/142	1	0.01	99.9	PCB 205	1	0.003	99.96
PCB 140	1	0.01	99.9	PCB 45	1	0.01	99.9	PCB 200	1	0.003	99.96
PCB 88/121	1	0.01	99.9	PCB 33/20/21	1	0.01	99.9	PCB 45	1	0.003	99.96
PCB 201	1	0.01	99.9	PCB 58	1	0.01	99.9	PCB 19	1	0.003	99.97
PCB 50	1	0.01	99.9	PCB 122	1	0.01	99.9	PCB 207	1	0.003	99.97
PCB 6	1	0.01	99.9	PCB 34/23	1	0.01	99.95	PCB 173	1	0.003	99.97
PCB 7/9	1	0.01	99.9	PCB 96	1	0.01	99.96	PCB 197	1	0.002	99.97
PCB 112	1	0.01	99.9	PCB 188	0.4	0.005	99.96	PCB 29	1	0.002	99.98

	Tree swallow averages (ng/g)	Rel. %	Cumm.%		Belted Kingfisher averages (ng/g)	Rel. %	Cumm.%		Spotted Sandpiper averages (ng/g)	Rel. %	Cumm.%
PCB 54	1	0.01	99.9	PCB 134/143	0.4	0.004	99.97	PCB 150	0.5	0.002	99.98
PCB 12/13	1	0.01	99.9	PCB 54	0.4	0.004	99.97	PCB 6	0.4	0.002	99.98
PCB 207	1	0.01	99.9	PCB 8/5	0.3	0.004	99.98	PCB 96	0.4	0.002	99.98
PCB 198	1	0.01	99.9	PCB 155	0.3	0.003	99.98	PCB 58	0.4	0.002	99.98
PCB 173	1	0.01	99.9	PCB 50	0.2	0.003	99.98	PCB 155	0.4	0.001	99.98
PCB 34/23	0.5	0.01	99.9	PCB 55	0.2	0.002	99.98	PCB 184	0.4	0.001	99.99
PCB 35	0.4	0.01	99.95	PCB 173	0.2	0.002	99.99	PCB 65/62	0.3	0.001	99.99
PCB 165	0.4	0.01	99.96	PCB 46	0.2	0.002	99.99	PCB 112	0.3	0.001	99.99
PCB 205	0.4	0.01	99.97	PCB 152	0.2	0.002	99.99	PCB 161	0.3	0.001	99.99
PCB 148	0.4	0.005	99.97	PCB 15	0.2	0.002	99.99	PCB 54	0.3	0.001	99.99
PCB 200	0.3	0.004	99.97	PCB 78	0.1	0.002	99.99	PCB 7/9	0.3	0.001	99.99
PCB 39	0.3	0.004	99.98	PCB 39	0.1	0.002	99.99	PCB 38	0.2	0.001	99.99
PCB 197	0.2	0.003	99.98	PCB 161	0.1	0.001	99.996	PCB 46	0.2	0.001	99.99
PCB 150	0.2	0.002	99.98	PCB 184	0.1	0.001	99.998	PCB 188	0.2	0.001	99.99
PCB 30	0.2	0.002	99.98	PCB 65/62	0.1	0.001	99.999	PCB 34/23	0.2	0.001	99.99
PCB 65/62	0.1	0.002	99.99	PCB 104	0.1	0.001	100	PCB 50	0.2	0.001	99.99
PCB 152	0.1	0.001	99.99	PCB 7/9	0.1	0.001	100	PCB 39	0.1	0.001	99.99
PCB 78	0.1	0.001	99.99	PCB 6	0.1	0.001	100	PCB 12/13	0.1	0.0005	99.995
PCB 184	0.1	0.001	99.99	PCB 35	0.04	0.0005	100	PCB 78	0.1	0.0005	99.996
PCB 161	0.1	0.001	99.99	PCB 169	0.04	0.0004	100	PCB 152	0.1	0.0004	99.996
PCB 145	0.1	0.001	99.99	PCB 38	0.03	0.0004	100	PCB 79	0.1	0.0004	99.997
PCB 155	0.1	0.001	99.99	PCB 29	0.03	0.0003	100	PCB 35	0.1	0.0003	99.997
PCB 104	0.1	0.001	99.99	PCB 145	0.03	0.0003	100	PCB 36	0.1	0.0002	99.997
PCB 11	0.1	0.001	99.995	PCB 1	0	0	100	PCB 30	0.1	0.0002	99.998
PCB 79	0.1	0.001	99.996	PCB 2	0	0	100	PCB 145	0.05	0.0002	99.998
PCB 188	0.03	0.0004	99.997	PCB 3	0	0	100	PCB 186	0.05	0.0002	99.998
PCB 36	0.03	0.0004	100	PCB 14	0	0	100	PCB 169	0.05	0.0002	99.998
PCB 1	0	0	100	PCB 11	0	0	100	PCB 14	0.04	0.0002	99.998
PCB 2	0	0	100	PCB 12/13	0	0	100	PCB 204	0.04	0.0001	100
PCB 3	0	0	100	PCB 30	0	0	100	PCB 1	0	0	100
PCB 14	0	0	100	PCB 36	0	0	100	PCB 2	0	0	100
PCB 169	0	0	100	PCB 79	0	0	100	PCB 3	0	0	100
PCB 186	0	0	100	PCB 186	0	0	100	PCB 11	0	0	100
PCB 204	0	0	100	PCB 204	0	0	100	PCB 104	0	0	100

The concentrations presented in this table are means of the initial 11 avian egg samples taken from the Hudson River by the trustees in 2004, with analysis by GC-LR/MS.

Table 3. Calculations of the relative amounts for individual congeners added to the PCB mixture representing spotted sandpiper eggs from the Hudson River.

	Ratio of the components in an Aroclor mixture*	Major PCB component of combined peak	Congener concentration (ng/g)	Congener contribution (%)	Cummulative contribution (%)
TOTAL PCB			25579	100	
PCB 28			2548	10	10
PCB 66/80		66	2290	9	19
PCB 74/61		74	1679	7	25
PCB 118/106		118	1604	6	32
PCB 47/48/75	(1:0.65:0.05)		1538		
47	1	47	905	4	35
48	0.65	48	588	2	38
75	0.05	75	45	0.18	38
PCB 138/163/164	(1:0.23:0.1)		1410		
138	1	138	1060	4	42
163	0.23	163	244	1	43
164	0.1	164	106	0.41	43
PCB 90/101/89	(0:1:0.028)		1289		
90	0	90	NS		
101	1	101	1253	5	48
89	0.028	89	35	0.14	48
PCB 52/73		52	1250	5	53
PCB 49/43	(1:0.12)		1099		
49	1	49	981	4	57
43	0.12	43	118	0.46	57
PCB 153			1016	4	61
PCB 99			998	4	65
PCB 70/76		70	913	4	69
PCB 105/127		105	764	3	72
PCB 31			743	3	75
PCB 56/60	(1:1)		720		
56	1	56	360	1	76
60	1	60	360	1	78
PCB 41/71/64/68	(0.28:0.22:1:0)		645		
41	0.28	41	120	0.47	78
71	0.22	71	95	0.37	78
64	1	64	430	2	80
PCB 110			453	2	82
PCB 85/120		85	437	2	84
PCB 87/115/116	(1:0.1:0)		330		
87	1	87	300	1	85
115	0.1	115	30	0.12	85
116	0	116	NS		
PCB 128			250	1	86
PCB 149/139	(1:0.02)		217		
149	1	149	213	1	87
139	0.02	139	4	0.02	87

		Ratio of the components in an Aroclor mixture*	Major PCB component of combined peak	Congener concentration (ng/g)	Congener contribution (%)	Cummulative contribution (%)
PCB 92				213	1	88
PCB 180				180	1	88
PCB 158/160			158	178	1	89
PCB 146				165	1	90
PCB 97/86			97	159	1	90
PCB 156				155	1	91
PCB 95/93			95	155	1	91
PCB 187/182			187	141	1	92
PCB 170/190		(1:0.22)	170	132		
	170	1	190	108	0.42	92
	190	0.22		24	0.09	93
PCB 111/117			117	130	1	93
PCB 141				118	0.46	94
PCB 130				84	0.33	94
PCB 107/109			109	81	0.32	94
PCB 137				78	0.30	94
PCB 42/59		(1:0.3)		71		
	42	1	42	54	0.21	95
	59	0.3	59	16	0.06	95
PCB 114				57	0.22	95
PCB 167				49	0.19	95
PCB 123				39	0.15	95
PCB 157				33	0.13	95
PCB 77				30	0.12	96
PCB 81				17	0.07	96
PCB 126				5	0.02	96
PCB 189				4	0.02	96
PCB 169				0.05	0.0002	96

* The ratios of co-eluting congeners measured in an Aroclor 1:1:1:1 mixture of Aroclor 1242:1248:1254:1260. Congeners with relative concentrations <1% of the total for the co-eluting peaks were excluded. Congener concentrations for the co-eluting PCBs were based on the concentration of the entire co-eluting set of congeners in sandpiper eggs multiplied times the relative amount (%) of each individual congener in the set.

Table 4. Final selected PCB congeners, total mass and percentage of total PCB in the stock 50mL aliquant of the original PCB mixture solution.

PCB Congener	PCB congener mass (ug) in the 50 mL aliquant of the original stock solution received from AccuStandard.	Relative amount (%)
PCB 28	40880	10.46%
PCB 66	36660	9.38%
PCB 74	26840	6.87%
PCB 118	25530	6.53%
PCB 47	14420	3.69%
PCB 48	9405	2.41%
PCB 75	802	0.21%
PCB 138	17000	4.35%
PCB 163	3980	1.02%
PCB 164	1607	0.41%
PCB 101	19980	5.11%
PCB 89	600	0.15%
PCB 52	20100	5.14%
PCB 49	15820	4.05%
PCB 43	1816	0.46%
PCB 153	16215	4.15%
PCB 99	15935	4.08%
PCB 70	14515	3.72%
PCB 105	12280	3.14%
PCB 31	11800	3.02%
PCB 56	5780	1.48%
PCB 60	5760	1.47%
PCB 41	1998	0.51%
PCB 71	1614	0.41%
PCB 64	6780	1.74%
PCB 110	7220	1.85%
PCB 85	6940	1.78%
PCB 87	4818	1.23%
PCB 115	398.4	0.10%
PCB 128	4026	1.03%
PCB 149	3380	0.87%
PCB 139	68	0.02%
PCB 92	3413.5	0.87%
PCB 180	2802	0.72%
PCB 158	2822	0.72%
PCB 146	2583.5	0.66%

PCB Congener	PCB congener mass (ug) in the 50 mL aliquant of the original stock solution received from AccuStandard.	Relative amount (%)
PCB 97	2584	0.66%
PCB 156	2412	0.62%
PCB 95	2398	0.61%
PCB 187	2196.5	0.56%
PCB 170	1814	0.46%
PCB 190	400.8	0.10%
PCB 117	1992	0.51%
PCB 141	1788	0.46%
PCB 130	1390	0.36%
PCB 109	1206	0.31%
PCB 137	1198.5	0.31%
PCB 42	795	0.20%
PCB 59	197.2	0.05%
PCB 114	1008	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	390713	100.0%

Table 5. Target and nominal concentrations of total PCB in each dosing solution.

Dose ID	Vial & Cap #	Solution Description	Target Concentration (µg/µL)[1]	Nominal Concentration (µg/µL)[2]
7	3-7	Stock 58-congener PCB mixture	246	244
6	3-8	2-fold dilution	123	122
5	3-9	4-fold dilution	62	61
4	3-10	8-fold dilution	31	30.5
3	3-11	16-fold dilution	15	15
2	3-12	32-fold dilution	8	7.5
1	3-4	Isooctane blank	0	0

[1] Target concentrations for the dosing solutions of the custom 58-congener PCB mixture.

[2] 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 emulsion volume of 1.6 mL, and the appropriate serial dilution for each dose.

Table 6. Nominal individual PCB congener doses (ng/g egg) expected in the eggs of Japanese quail injected with each of the dosing solutions.

	Nominal egg dose (ng/g egg)					
PCB congener	98 µg PCB/g dose (vial 3-7)	49 µg PCB/g dose (vial 3-8)	24 µg PCB/g dose (vial 3-9)	12 µg PCB/g dose (vial 3-10)	6 µg PCB/g dose (vial 3-11)	3 µg PCB/g dose (vial 3-12)
PCB 28	10220	5110	2555	1278	639	319
PCB 66	9165	4583	2291	1146	573	286
PCB 74	6710	3355	1678	839	419	210
PCB 118	6383	3191	1596	798	399	199
PCB 47	3605	1803	901	451	225	113
PCB 48	2351	1176	588	294	147	73
PCB 75	201	100	50	25	13	6
PCB 138	4250	2125	1063	531	266	133
PCB 163	995	498	249	124	62	31
PCB 164	402	201	100	50	25	13
PCB 101	4995	2498	1249	624	312	156
PCB 89	150	75	38	19	9	5
PCB 52	5025	2513	1256	628	314	157
PCB 49	3955	1978	989	494	247	124
PCB 43	454	227	114	57	28	14
PCB 153	4054	2027	1013	507	253	127
PCB 99	3984	1992	996	498	249	124
PCB 70	3629	1814	907	454	227	113
PCB 105	3070	1535	768	384	192	96
PCB 31	2950	1475	738	369	184	92
PCB 56	1445	723	361	181	90	45
PCB 60	1440	720	360	180	90	45
PCB 41	500	250	125	62	31	16
PCB 71	404	202	101	50	25	13
PCB 64	1695	848	424	212	106	53
PCB 110	1805	903	451	226	113	56
PCB 85	1735	868	434	217	108	54
PCB 87	1205	602	301	151	75	38
PCB 115	100	50	25	12	6	3
PCB 128	1007	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 congener	98 µg PCB/g dose (vial 3-7)	49 µg PCB/g dose (vial 3-8)	24 µg PCB/g dose (vial 3-9)	12 µg PCB/g dose (vial 3-10)	6 µg PCB/g dose (vial 3-11)	3 µg PCB/g dose (vial 3-12)
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	97678	48839	24420	12210	6105	3052

Estimation based on the following:
Highest Dose = 98 µg/g egg
Injection volume = 0.4 µL/g egg
Egg weight = 10 g

Table 7. Calculated doses and contributions to TEQs (pg/g egg) of individual dioxin-like PCB congeners for each dose solution of the 58-congener PCB mixture prepared for the Japanese quail egg injection studies.

			TEQ (pg/g)					
PCB congener	WHO TEF· value	Rel. TEQ (% total)	98 µg PCB/g egg dose	49 µg PCB/g egg dose	24 µg PCB/g egg dose	12 µg PCB/g egg dose	6 µg PCB/g egg dose	3 µg PCB/g egg dose
PCB 118	0.00001	0.5	64	32	16	8	4	2
PCB 105	0.0001	2.4	307	154	77	38	19	10
PCB 156	0.0001	0.5	60	30	15	8	4	2
PCB 114	0.0001	0.2	25	13	6	3	2	1
PCB 167	0.00001	0.0	2	1	0.50	0.25	0.12	0.06
PCB 123	0.00001	0.0	1	1	0.37	0.19	0.09	0.05
PCB 157	0.0001	0.1	15	7	4	2	1	0.46
PCB 77	0.05	39.7	5000	2500	1250	625	313	156
PCB 81	0.1	39.8	5010	2505	1253	626	313	157
PCB 126	0.1	16.7	2109	1054	527	264	132	66
PCB 189	0.00001	0.0	0.18	0.09	0.04	0.02	0.01	0.01
PCB 169	0.001	0.0	0.18	0.09	0.05	0.02	0.01	0.01
Total TEQ		100.0	12594	6297	3148	1574	787	394

Estimated based on a 4 µL/egg injection volume and 10 g/egg assumed egg weight. Relative TEQ (%) was calculated based on the contribution of each congener to the total TEQ.

Attachments

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ISO 9001

CERTIFICATE OF ANALYSIS

CATALOG NO: S-13907-250ML

EXPIRATION: Nov 9,2015

DESCRIPTION: Custom PCB Congener Standard

LOT: B5110052

SOLVENT: Isooctane

See reverse for additional certification information.

This product is guaranteed accurate to + 0.5% of the Certified Analyte concen[illegible] Expiration Date on the Label.

Component	CAS #	Purity % (GC/MS)	Prepared Concentration[1] (µg/mL)		Certified Analyte Concentration[2] (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	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	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	35065-28-2	100	340.0	±13.60	340.0
2,3,3',4',5,6-Hexachlorobiphenyl	74472-44-9	99.0	80.40	±3.22	79.60
2,3,3',4',5',6-Hexachlorobiphenyl	74472-45-0	99.7	32.24	±1.29	32.14
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	99.4	402.0	±16.08	399.6
2,2',3,4,6'-Pentachlorobiphenyl	73555-57-2	99.9	12.01	±0.48	12.00
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	100	402.0	±16.08	402.0
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	100	316.4	±12.66	316.4
2,2',3,5-Tetrachlorobiphenyl	70362-46-8	99.9	36.36	1.45	36.32
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	99.6	325.6	±13.02	324.3
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	99.6	320.0	±12.80	318.7
2,3',4',5-Tetrachlorobiphenyl	32598-11-1	99.0	293.2	±11.73	290.3
2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	100	245.6	±9.82	245.6
2,4',5-Trichlorobiphenyl	16606-02-3	100	236.0	±9.44	236.0
2,3,3',4'-Tetrachlorobiphenyl	41464-43-1	99.6	116.1	±4.64	115.6
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1	99.0	116.4	±4.66	115.2
2,2',3,4-Tetrachlorobiphenyl	52663-59-9	W.3	40.24	±1.61	39.96
2,3',4',6-Tetrachlorobiphenyl	41464-46-4	100	32.28	±1.29	32.28
2,3,4',6-Tetrachlorobiphenyl	52663-58-8	99.0	137.0	a5.43	135.6
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	99.7	144 8	±5.79	144.4
2,2',3,4,4'-Pentachlorobiphenyl	65510-45-4	99.0	140.2	±5.61	138.8
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	W.5	96.84	±3.87	96.36
2,3,4,4',6-Pentachlorobiphenyl	74472-38-1	99.5	8.008	±0.32	7.968
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	99.7	80.76	±3.23	80.52
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	99.0	68.28	±2.73	67.60
2,2',3,4,4',6-Hexachlorobiphenyl	56030-56-9	99.4	1.368	±0.05	1.360
2,2',3,5,5'-Pentachlorobiphenyl	52663-61-3	99.7	68.48	±2.74	68.27
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	100	56.04	±2.24	56.04
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7	100	56.44	±2.26	56.44
2,2',3,4',5,5'-Hexachlorobiphenyl	51908-16-8	98.9	52.24	±2.09	51.67
2,2',3',4,5-Pentachlorobiphenyl	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

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. 8221254480.

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.

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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 Concentration¹ (µg/mL)		Certified Analyte Concentration² (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 95	38379-99-6	99.1	48.40	±1.94	47.96
2,2',3,4',5,5',6-Heptachlorobiphenyl	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	1 0	8.016	±0.32	8.016
2,3,4',5,6-Pentachlorobiphenyl [illegible]	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	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	[illegible]	12.00	±0.48	11 98
2,3,3',4,4',5'-Hexachlorobiphenyl 157	69782-90-7	[illegible]	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 1	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.

Certified by: R. Cooper

QR-OR-INO1002

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 IS09001 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 Wen 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 fhe 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.
Chemical Reference Standards • The Standard for Excellence

WARRANTIES:

Manufacture]: (AccuStandard®, 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 soludons (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-BLK-250ML

EXPIRATION: Nov 9, 2006

DESCRIPTION: Isooctane Control Blank

LOT: B5110053

SOLVENT: N/A

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

See reverse for additional certification information.

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

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.

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.

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

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 IS09001 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.

U.S. DEPARTMENT OF THE INTERIOR
MARCH 3, 1849

NOAA
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
U.S. DEPARTMENT OF COMMERCE

DEPARTMENT OF ENVIRONMENTAL CONSERVATION
NEW YORK STATE