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ESTIMATING DIOXIN-LIKE POLYCHLORINATED BIPHENYL TOXIC EQUIVALENTS FROM TOTAL POLYCHLORINATED BIPHENYL MEASUREMENTS IN FISH

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Abstract—Polychlorinated biphenyls (PCBs) are 209 related compounds, a dozen of which are known as dioxin-like PCBs (dl-PCBs) and are among the most toxic PCBs. Polychlorinated biphenyls contribute to many adverse effects to human health, including cancer, and are a major cause of fish advisories in North America. It is a common perception that individual PCB compounds, especially dl-PCBs, rather than total PCB need to be quantified to predict the environmental hazard because of differences in their toxicity potential and distribution among various environmental matrices, including aquatic food webs. Because the current analytical methods for quantifying dl-PCBs are complex and four- to fivefold more expensive, limited fish samples are analyzed for dl-PCBs. Using what likely is the largest dl-PCB fish data set (n = 912) with a wide distribution of fish species (n = 22), size (19–112 cm), weight (100–14,300 g), sex (male:female, 51:49), and PCB contamination level (20–7,300 ng/g wet wt), we show that the comparatively less expensive and rapid measurements of total PCB in fish can be utilized to assess dl-PCB–related toxicological hazard, measured as 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalents (TEQ). A regression equation of dl-PCB–related TEQ (i.e., $TEQ_{dl\text{-}PCB}$) to total PCB in fish is presented ($TEQ_{dl\text{-}PCB} = [2.56 \times 10^{-5}]C_{total\ PCB}$, $r = 0.89$, $p < 0.001$). The regression was evaluated by applying it to three independent data sets of substantial sizes (n = 55, 141, and 176). The $TEQ_{dl\text{-}PCB}$ estimated using the regression and total PCB measurements were within a reasonable factor of two to three of the $TEQ_{dl\text{-}PCB}$ calculated from the dl-PCB measurements. The successful evaluation indicates versatility of the regression.

Keywords—Toxic equivalents Polychlorinated biphenyls Dioxin-like polychlorinated biphenyls Fish advisory Analytical method

INTRODUCTION

Fish are an important part of a healthy diet [1]; however, fish may contain contaminant levels that could pose health risks to humans and wildlife [2–6]. Various levels of government protect humans from possible risks of eating contaminated fish by issuing fish consumption advisories [4,6]. In North America, high levels of polychlorinated biphenyls (PCBs) in fish are a major concern [4,6]. Polychlorinated biphenyls are 209 related compounds, known as congeners, that contribute to many adverse effects to human health, including cancer [2]. Twelve PCB congeners are defined as dioxin-like PCBs (dl-PCBs), because they interact with organisms by the same mechanism as the most toxic dioxin compound, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) [7]. The toxicological properties of dl-PCBs differ from those of the non-dl-PCBs, and toxicological hazard associated with these two groups of PCBs generally is assessed separately. Dioxin-like PCBs are among the most toxic PCBs and drive the risk assessment of PCBs in the environment [7,8]. When analyzed, dl-PCBs often are found at levels considered to be detrimental to ecosystem health and are a major cause of restrictions concerning fish consumption [4].

Toxicity potential of dl-PCBs varies over five orders of magnitude [7]. After careful review of available scientific data, the toxic equivalency factors (TEFs), defined as the toxicity relative to that of 2,3,7,8-TCDD, have been assigned to each dioxin-like compound, including dl-PCBs [7]. The toxicological hazard associated with a dl-PCB mixture in fish is assessed using a 2,3,7,8-TCDD toxic equivalent (TEQ) concentration of the mixture, as shown in Equation 1:

$$TEQ_{dl\text{-}PCB} = \sum_{i=1}^{12} (TEF_i C_{dl\text{-}PCB,i}) \quad (1)$$

where $C_{dl\text{-}PCB,i}$ is the concentration of the dl-PCB congener i.

An overall TEQ for all dioxin-like contaminants that interact with organisms by the same mechanism as 2,3,7,8-TCDD is calculated by summing their individual TEQs. Among these contaminants, dl-PCBs generally are the greatest contributors to overall TEQs in fish. For example, Hites et al. [3] globally assessed organic contaminants in farmed and wild salmon and found that $TEQ_{dl\text{-}PCB}$ typically contributed 75% to the overall TEQ.

According to Equation 1, a reliable measurement of the 12 dl-PCBs is required to calculate $TEQ_{dl\text{-}PCB}$. Polychlorinated biphenyl levels generally are monitored as Aroclor-equivalent total PCB based on a limited set of chromatographic peaks or by congener-specific analysis in which individual congeners can be quantified in the high pg/g (i.e., parts per trillion [ppt]) range. Most congener-specific analyses therefore fail to quantify lower but toxicologically important levels of dl-PCBs in fish. A detailed, congener-specific dl-PCB analysis can quantify dl-PCBs at low-ppt levels; however, high cost ($800 to $2,000 per sample) and time requirement because of analytical complexity limit the number of fish samples that can be analyzed for dl-PCBs [9–11]. Consequently, the risk of these

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toxic chemicals to humans and ecosystem health may not be fully recognized.

Attempts have been made to reduce the analytical cost by measuring only selected compounds that can predict TEQ. Although some of the dl-PCB congeners (e.g., PCBs 126 and 118) can be very good predictors of $TEQ_{dl\text{-}PCB}$, such an approach is of little use because of minimal difference in the analytical cost of selected versus full dl-PCB congener analysis [10]. Alternatively, use of PCB congeners (e.g., PCBs 138, 153, and 118), which do not require a very low detection limit (e.g., 0.1 ppt) and can be correlated well with TEQs, has been proposed to estimate $TEQ_{dl\text{-}PCB}$ [10]. This method, however, also requires a relatively costly congener-specific PCB analysis. In contrast, monitoring of total PCB levels is four- to fivefold faster and less expensive ($200 to $500 per sample) and is routinely performed by most agencies [9]. Therefore, at the very least, the assessment of toxicological hazard associated with dl-PCBs in fish can be simplified, and deficiencies in fish advisories and risk assessment because of a lack of dl-PCB measurements can be alleviated if total PCB measurements can be used to estimate $TEQ_{dl\text{-}PCB}$.

Limited studies [10,12] conducted to correlate $TEQ_{dl\text{-}PCB}$ and total PCB measurements were inconclusive about generalizing the relationship because of their small sample size, narrow geographic representation, and/or limited number of fish species considered. In the present study, we analyze what likely is the largest data set of total PCB and dl-PCB fish concentrations with a wide distribution of fish species, size, weight, sex, and PCB contamination level. We first present a regression relating total PCB and $TEQ_{dl\text{-}PCB}$ and then evaluate performance of the regression by applying it to three independent data sets of substantial sample sizes. Finally, the reasons behind the relationship and applicability of the regression are discussed.

MATERIALS AND METHODS

Sampling, extraction, and analysis

Fish samples were collected from various locations in Ontario (Canada) as part of the ongoing Sport Fish Contaminant Monitoring Program coordinated by the Ontario Ministry of the Environment (MOE) in collaboration with the Ontario Ministry of Natural Resources and Health Canada. The samples generally were collected using gill nets during late summer or early fall. Fish were stored frozen as whole fish and shipped to MOE laboratories for chemical analysis. Fish were measured for length and weight, sexed, and filleted before analysis.

Total PCB

The total PCB level was quantified in fish fillet samples using MOE method E3136 [13]. Tissue homogenates were weighed (5.0 g) and transferred to centrifuge tubes. A 0.5-ml aliquot of a 5:2 mixture of decachlorobiphenyl and 1,3,5-tribromobenzene (100 μg/ml) were added to each sample as a surrogate spike. Concentrated, reagent-grade hydrochloric acid (18 ml) was added to each tube and digested overnight. A 20-ml aliquot of a 25% (v/v) dichloromethane (DCM) in hexane solvent was added. Solutions were mixed for 45 min on a bench-top rotator, then racked for 24 to 48 h to permit emulsion separation. Upper solvent layers were quantitatively withdrawn and transferred to 100-ml volumetric flasks, and volumes were made up to 100 ml with DCM/hexane. Evaporated sample extracts (1 ml) were added to dry Florisil® columns (Caledon Laboratories, Georgetown, ON, Canada) and allowed to drain to the top of the packing. Pure hexane was then added in 1-ml portions until the columns were completely wet. This was followed by 25 ml of hexane to elute PCBs. Column effluents, containing PCBs and mirex (fraction 1), were collected in 40-ml, graduated tubes, and any hexane remaining at the top of the tubes was drained off. Columns were then eluted with a 25% (v/v) DCM/hexane solution (25 ml), with effluents containing mostly organochlorine pesticides (fraction 2) collected in 40-ml tubes. Pure iso-octane (1 ml) was added to fractions 1 and 2, and the sample extracts were evaporated to 1 ml. Gas–liquid chromatography was used to determine total PCB concentrations using a HP 5890 Series-II gas chromatograph and Ni^{63} electron-capture detector (Agilent Technologies, Santa Clara, CA, USA). The column (DB-17; length, 30 m; inner diameter, 0.53 mm; film thickness, 0.1 μm; J&W Scientific, Folsom, CA, USA) head pressure was 3.5 psi, and the temperature program for fraction 1 was as follows: 80°C for 1 min, a ramp from 80 to 180°C at 10°C/min, a ramp from 180 to 260°C at 5°C/min, and then a hold at 260°C for 6 min. Total PCB were determined using a 4:1 mixture of Aroclors 1254 and 1260 for quantification. This ratio of Aroclors best resembled the congener patterns detected for most fish samples. Quantification was carried out using the 23 largest peaks. Peak areas were summed to determine the total PCB in the standards and samples. A five-point calibration procedure with continuing single-point calibration was used. A minimum of 11 peaks was required for a positive identification of samples. A method blank and spiked sample (4:1 mixture of Aroclors 1254 and 1260) was analyzed with each set of 20–25 samples.

Dioxin-like PCBs

The 12 dl-PCBs (i.e., four non-*ortho* or coplanar PCBs [PCBs 77, 81, 126, and 169] and eight mono-*ortho* PCBs [PCBs 105, 114, 118, 123, 156, 157, 167, and 189]) and seventeen 2,3,7,8-substituted congeners of polychlorinated dioxins and furans (PCDD/Fs) were analyzed using MOE method DFPCB-E3418 [14]. This method entails hydrochloric acid digestion of 5 g of homogenized fish tissue, followed by extraction with hexane and a three-stage column cleanup procedure. The first column contained sulfuric acid/silica covered with anhydrous sodium sulfate. This column was eluted with hexane. The concentrated extract was transferred to a column containing 5.0 g of activated alumina and 2.0 g of anhydrous sodium sulfate. The alumina column was eluted with hexane followed by 10% (v/v) carbon tetrachloride/hexane to collect most mono-*ortho* PCBs (fraction A). The PCDD/Fs, coplanar PCBs, and remaining mono-*ortho* PCBs were eluted with 10% (v/v) methanol/DCM (fraction B). Fraction B was loaded onto a column containing 0.35 g of 5% (w/w) Amoco PX21-activated silica, eluted with 40 ml of 25% DCM/hexane, and added to fraction A. The column was inverted and eluted with 160 ml of toluene to isolate a PCDD/F and coplanar PCB fraction. The gas chromatography–high-resolution mass spectrometry (GC-HRMS) system was tuned to a resolving power of greater than 10,000 (10% valley definition). Fractions A and B were analyzed in separate GC-HRMS runs. Analyses for dl-PCBs and PCDD/Fs were performed on a Micromass Autospec HRMS at a resolving power of 10,000 with a HP6890 gas chromatograph (Agilent Technologies) using a DB-5 column (length, 40 m; inner diameter, 0.18 mm; film thickness, 0.18 μm; J&W Scientific).

The dl-PCB extracts were analyzed in splitless mode with He carrier gas at a linear velocity of 1.5 cm/s, and injector temperature and transfer-line temperature were maintained at 280 and 300°C, respectively. The temperature program for fraction A was as follows: 150°C for 1 min, a ramp from 150 to 200°C at 5°C/min, a ramp from 200 to 235°C at 3°C/min, a hold at 235°C for 10 min, a ramp from 235 to 300°C at 12°C/min, and then a hold at 300°C for 1 min. The temperature program for fraction B was as follows: 100°C for 1 min, a ramp from 100 to 200°C at 30°C/min, a ramp from 200 to 235°C at 3°C/min, a hold at 235°C for 10 min, a ramp from 235 to 300°C at 6°C/min, and then a hold at 300°C for 12 min. All dl-PCB data were corrected for surrogate recoveries. Method blanks and spiked blank matrix samples were processed with each set of 10 field samples. Actual detection limits were calculated from each chromatogram using a signal to noise ratio of 5:1.

Data set

The data set generated by the Sport Fish Contaminant Monitoring Program of MOE include dl-PCB measurements in 912 skinless fillet samples of 22 different fish species (Appendix 1 [http://dx.doi.org/10.1897/06-321.S1]). The samples were collected between 1996 and 2004 from 80 locations across the province of Ontario (Canada) and varied in size (range, 19–112 cm; median, 60 cm; 25th to 75th quartiles, 53–70 cm) and weight (range, 100–14,300 g; median, 2,353 g; 25th to 75th quartiles, 1,457–3,834 g). The male to female ratio of the collected samples was almost equal (51:49). Total PCB ranged from 20 to 7,300 ng/g wet weight (median, 280 ng/g; 25th to 75th quartiles, 100–720 ng/g).

Statistical analysis

The $TEQ_{dl\text{-}PCB}$ values were calculated using Equation 1, measured dl-PCB concentrations, and TEFs for mammalian species published by the World Health Organization in 2005 [7]. Congener concentrations below the detection levels were treated as half the detection limit. The correlation between $TEQ_{dl\text{-}PCB}$ and total PCB was determined with the Pearson correlation coefficient using SPSS® (Ver 12.0.1, 2003; SPSS, Chicago, IL, USA).

RESULTS

A strong positive relationship was observed between total PCB and $TEQ_{dl\text{-}PCB}$ (Fig. 1). A regression analysis performed on the measured values provided a correlation coefficient (r) of 0.89 ($p < 0.001$, $n = 912$) for the equation shown in Figure 1a and Equation 2:

$$TEQ_{dl\text{-}PCB} = (2.56 \times 10^{-5}) C_{total\ PCB} \quad (2)$$

where $C_{total\ PCB}$ is the concentration of total PCB. The concentration units (e.g., pg/g) of $TEQ_{dl\text{-}PCB}$ and total PCB are the same. This regression equation was calculated with the relationship passing through the origin given that TEQ should be zero when the total PCB value is zero. When we did not pass the regression line through origin, it intercepted on the positive y-axis. This would be interpreted as a certain amount of dl-PCBs being present when the total PCB value is zero, which is theoretically erroneous.

Linearity of the relationship between dependent and independent variables and normal distribution of the error in data are key assumptions in linear-regression models. Therefore, the use of a nonlinear (e.g., log) transformation to the dependent and/or independent variables may be required to linearize relationships and stabilize variance [15]. A regression analysis on log-transformed $TEQ_{dl\text{-}PCB}$ and $C_{total\ PCB}$ is shown in Figure 1b and Equation 3:

$$\log_{10}(TEQ_{dl\text{-}PCB}) = 1.0544 \cdot \log_{10}(C_{total\ PCB}) - 4.9094 \quad (3)$$

Again, the concentration units (e.g., pg/g) of $TEQ_{dl\text{-}PCB}$ and total PCB are same. The ratio of $TEQ_{dl\text{-}PCB}$ from Equation 2 to $TEQ_{dl\text{-}PCB}$ from Equation 3 ranged from 0.88 to 1.21, with the values from Equation 2 being, on average, 5% higher. In the subsequent discussion, we focus on the $TEQ_{dl\text{-}PCB}$ obtained using Equation 2. Figure 1 suggests a larger variation in observed $TEQ_{dl\text{-}PCB}$ at lower levels of total PCB (<110 ng/g wet wt). We attribute this scatter to proportionally higher analytical error at lower levels of PCBs and influence of the concentrations below the detection limits. To examine the impact of adopting half the detection limits for nondetected values on the regression (Eqn. 2), we performed separate regressions on the data points that included the detection limits and zero value for the nondetects. We found negligible impact of variation in the treatment of nondetects in the statistical analysis on Equation 2 (results not shown).

The linear relationship between total PCB and $TEQ_{dl\text{-}PCB}$ (Eqn. 2) is a result of the relatively constant fractions of dl-PCBs (especially PCB-126: median, 0.024%; 25th to 75th

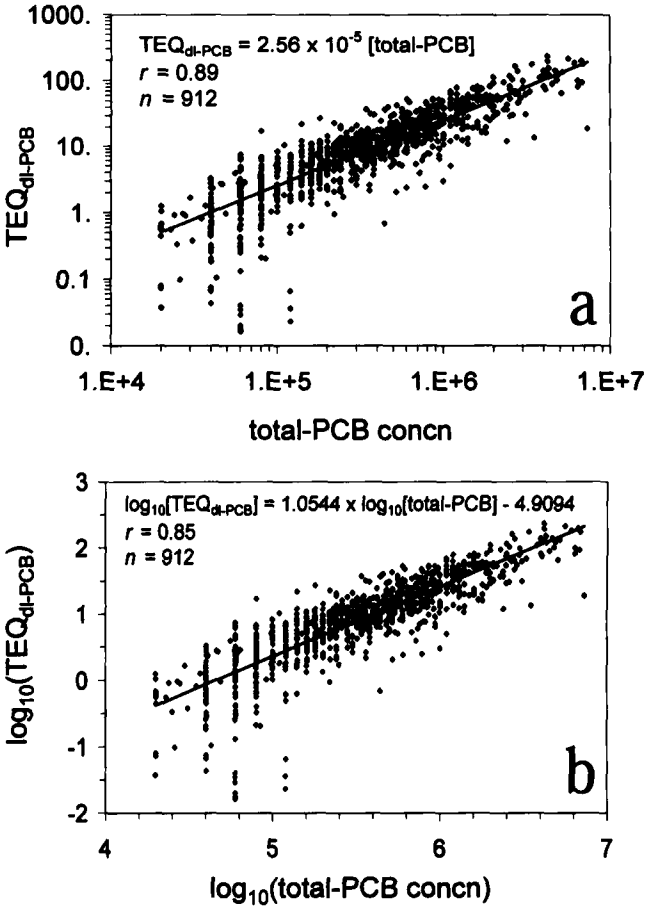

Fig. 1. Toxic equivalent concentrations of dioxin-like polychlorinated biphenyls ($TEQ_{dl\text{-}PCB}$) as a function of total PCB concentrations for (a) simple and (b) log-transformed data. The concentration units of $TEQ_{dl\text{-}PCB}$ and total PCB in the correlation are the same. Data are plotted in terms of pg/g wet weight, r is the Pearson correlation coefficient, and n is the sample size. For (a), the regression equation was prepared for the relationship passing through the origin.

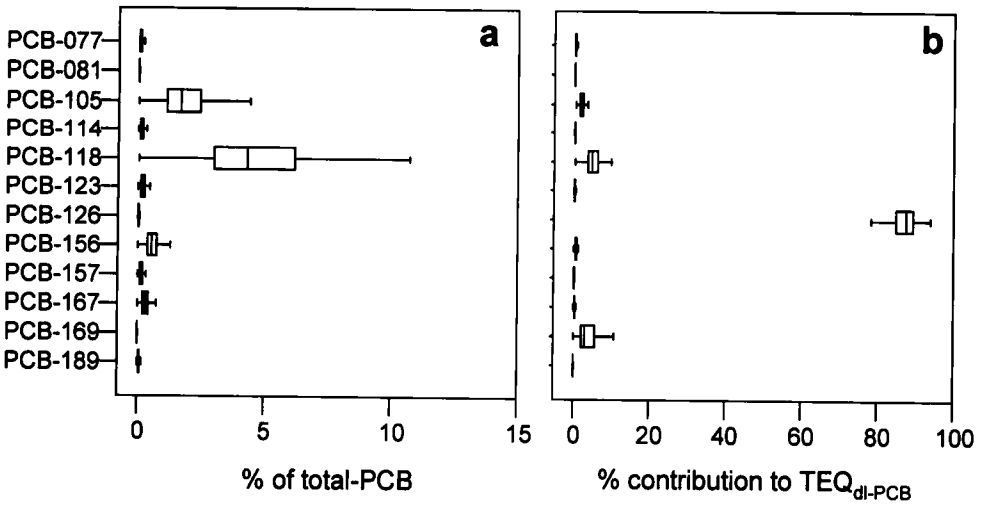

Fig. 2. (**a**) Percentage composition of total polychlorinated biphenyls (total PCB) attributed to dioxin-like PCBs (dl-PCBs) and (**b**) percentage contribution of dl-PCBs to the dl-PCB–related toxic equivalent ($TEQ_{dl\text{-}PCB}$). These results are presented as box plots prepared using SPSS® (Ver 12.0.1, 2003; SPSS, Chicago, IL, USA) in which the line within the box indicates the median, the box indicates the 25% and 75% quartile values, and the whiskers indicate the upper and lower values not classified as statistical outliers or extremes. The outliers and extremes were values more than 1.5- and 3.0-fold, respectively, the 25th to 75th interquartile range away from the closest end of the box.

quartiles, 0.015–0.036%) in total PCB (Fig. 2a), because TEFs do not vary with total PCB levels. Similar fractions have been reported for Lake Ontario lake trout (LOLT; 1977–1993; total PCB, ~2,000–9,500 ng/g [16]), Great Lakes trout and walleye (1991–1998; total PCB, 334–3,720 ng/g [17]), and four horn sculpin (*Myoxocephalus quadricornis*) and short horn sculpin (*Myoxocephalus scorpeus*) from the Canadian Arctic [18]. The analysis shows that although PCB 126 is a minor component (median, 0.024%; 25th to 75th quartiles, 0.015–0.036%) of total PCB (Fig. 2a), PCB 126 is the major contributor (median, 88%; 25th to 75th quartiles, 85–89%) to $TEQ_{dl\text{-}PCB}$ (Fig. 2b). This is a result of the relatively high toxicity potential (TEF = 0.1) of PCB 126 compared to other dl-PCBs (TEFs = 0.00003–0.03). The dl-PCBs 169 and 118 are the other important contributors to $TEQ_{dl\text{-}PCB}$ (Fig. 2b), largely on account of the high toxicity potential of PCB 169 (TEF = 0.03) and greater abundance of PCB 118 (Fig. 2a). The present analysis also is consistent with a uniform composition of the most abundant PCBs found at the higher trophic levels of the Great Lake food webs [19–21].

We evaluate the performance of the regression (Eqn. 2) developed here in three steps. First, we apply Equation 2 to predator fish fillet data recently collected by the U.S. Environmental Protection Agency (EPA) through a four-year (2000–2003) national study of freshwater fish contamination known as National Lake Fish Tissue Study (NLFTS) [22]. The NLFTS data set includes quantification of PCB congeners in fish samples collected from 500 randomly selected lakes and reservoirs in the lower 48 states. Each fillet sample was a composite of skin-on fillets of five adult fish of the same species and similar size from the same location. A subset of 55 predator fish fillet samples having measured values above the detection limit of the most toxic dl-PCB (PCB 126) was considered in this evaluation. The U.S. EPA method 1668 [23] was employed to measure PCB congeners. Note that the reported total PCB in the NLFTS data set are the sum-of-congener values and not the Aroclor equivalents, as often are measured. Estimates of total PCB using sum-of-congener values depend on the number of congeners and actual congeners selected for analysis and their detection limits. In addition, environmental parameters modify original Aroclor PCB congener patterns [8]. A close agreement (slope and intercept of 1.08 and 490, respectively; r^2 = 0.96) between the sum-of-congener value and the Aroclor equivalent concentration, however, has been reported for a variety of tissue and trophic-level samples collected between 1981 and 1997 from various locations, including the Arctic [24]. Also, the sum-of-congener values in the NLFTS data set are based on measurements for a large number of congeners (159 individual congeners plus remaining congeners as pairs). As such, the use of sum-of-congener values for an evaluation of the relationship that is based on Aroclor-equivalent measurements should not be a major concern.

The subset consisted of samples of 13 different fish species (Appendix 2 [http://dx.doi.org/10.1897/06-621.S1]) with the length, weight, and total PCB concentration ranging from approximately 18 to 68 cm (median, 40 cm; 25th to 75th quartiles, 35–48 cm), 70 to 3,115 g (median, 806 g; 25th to 75th quartiles, 614–1,076 g), and 10 to 700 ng/g wet weight (median, 43 ng/g; 25th to 75th quartiles, 28–81 ng/g), respectively. We first calculated the $TEQ_{dl\text{-}PCB}$ for the NLFTS subset, termed $TEQ_{observed}$, using TEFs and measured dl-PCBs. The $TEQ_{observed}$ was then compared with the estimated $TEQ_{dl\text{-}PCB}$ (i.e., $TEQ_{estimated}$) using Equation 2 and total PCB measurements from the NLFTS subset. The results suggest that the regression (Eqn. 2) provided very good estimates of $TEQ_{dl\text{-}PCB}$ with most (>95%) of the estimated values being either within a factor of two or conservative compared to the observed values (Fig. 3a). Although skin-on fillet PCB concentrations generally are greater than corresponding skinless fillet values [25], this evaluation indicates that the correlation is applicable to both types of measurements.

In the second step of the evaluation, we apply Equation 2 to whole-fish data rather than to fillet data from which the regression was derived. The validity of the regression for whole-fish measurements can enhance the utility of the relationship, because whole-fish measurements customarily are used to assess chemical dynamics in aquatic food webs. The regression was applied to previously published measurements of LOLT samples collected between 1977 and 1993 [16]. The

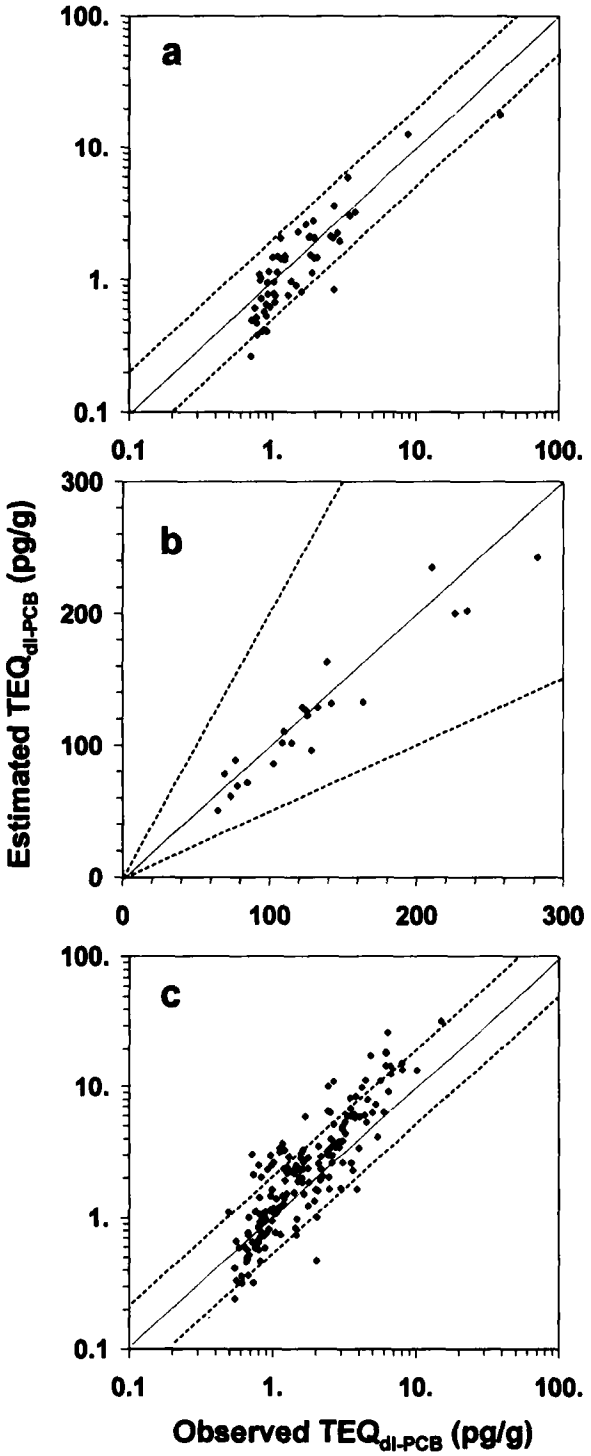

Fig. 3. Comparison of observed versus estimated dioxin-like polychlorinated biphenyl (dl-PCB)–related toxic equivalents ($TEQ_{dl\text{-}PCB}$, pg/g wet wt). The observed toxic equivalents ($TEQ_{observed}$) are based on toxic equivalency factors recommended by the World Health Organization in 2005 for mammalian species [7] and measured dl-PCB concentrations. The estimated TEQs ($TEQ_{estimated}$) are based on the regression between $TEQ_{dl\text{-}PCB}$ and total PCB developed in the present study (Eqn. 2) and total PCB measurements. The comparison is for (**a**) skin-on fillet samples (n = 56) collected by U.S. Environmental Protection Agency during the four-year National Lake Fish Tissue Study (NLFTS) [22], (**b**) annual (n = 22) values from 141 whole-fish samples of Lake Ontario (ON, Canada) lake trout (LOLT) collected yearly between 1977 and 1993 [16], and (**c**) bottom-dweller, whole-fish samples (n = 175) from the NLFTS data set [22]. The upper and lower dotted lines represent a factor-of-two prediction interval.

LOLT data include measurements for total PCB and all important dl-PCBs (i.e., PCBs 77, 81, 105, 118, 126, 156, and 169; total contribution of ~98% to $TEQ_{dl\text{-}PCB}$ in fish) (Fig. 2b). The LOLT data set contains 22 series of mean values reported from a total of 141 samples, with total PCB ranging from approximately 2,000 to 9,500 ng/g. The total PCB concentrations were measured using four different analytical methods. We selected the values reported for archived samples measured using the gas chromatography–electron-capture detection method, because it best resembled the method utilized in MOE measurements from which Equation 2 was derived.

The $TEQ_{dl\text{-}PCB}$ for the LOLT data set were calculated using measured dl-PCBs and TEFs. These $TEQ_{dl\text{-}PCB}$ were corrected by increasing the values by 2% to account for the contribution of missing dl-PCBs as mentioned above and were termed $TEQ_{observed}$. The $TEQ_{observed}$ were then compared with the $TEQ_{estimated}$ using Equation 2 and total PCB measurements from the LOLT data set. Again, the equation provided very good estimates of $TEQ_{dl\text{-}PCB}$. All the estimated $TEQ_{dl\text{-}PCB}$ values were within 25% of the observed values (Fig. 3b). These results confirm that the regression (Eqn. 2) is equally applicable to whole-fish total PCB measurements. Although fillet concentrations generally are lower than the corresponding whole-fish concentrations mainly because of low fat content [26], no significant difference in PCB congener pattern among fish tissues (e.g., liver and muscle) is observed [18,27,28]. Therefore, the difference between whole-fish and fillet fractions of dl-PCBs in total PCB is expected to be negligible. This characteristic of the dl-PCB distribution in fish and successful application of the regression to whole-fish data of the LOLT data set support the validity of Equation 2 for both fillet and whole-fish total PCB measurements.

Finally, we evaluated the applicability of Equation 2 to total PCB measurements for bottom-dweller fish as opposed to predator fish, which dominated the MOE database from which Equation 2 was derived. We applied the regression to a NLFTS subset of 176 bottom-dweller, whole-fish samples having measured values above the detection limit of the most toxic dl-PCB (i.e., PCB 126). The evaluation also reexamined applicability of the regression to whole-fish measurements. The subset included samples of 17 different fish species (Appendix 3 [http://dx.doi.org/10.1897/06-621.S1]) dominated by common carp (*Cyprinus carpio*; 35%) and channel catfish (*Ictalurus punctatus*; 22%), with the length, weight, and total PCB concentrations ranging from approximately 22 to 85 cm (median, 46 cm; 25th to 75th quartiles, 39–54 cm), 120 to 10,000 g (median, 1,070 g; 25th to 75th quartiles, 620–2,155 g), and 9 to 1,266 ng/g wet wt (median, 86 ng/g; 25th to 75th quartiles, 39–147 ng/g), respectively. The $TEQ_{observed}$ and $TEQ_{estimated}$ were calculated and compared as mentioned earlier for the first part of the evaluation. Most (~80%) of the $TEQ_{estimated}$ values were within a factor of two compared to the $TEQ_{observed}$ values (Fig. 3c). Further, 94% of the $TEQ_{estimated}$ values were within a factor of three, and almost all (98%) were within a factor of two or conservative compared to the $TEQ_{observed}$ (Fig. 3c). Figure 3c shows a slope exceeding unity (slope and intercept of 2.04 and −0.9, respectively), which suggests that there may be some differences in the relationship for these benthic-feeding species [29]; this may be influenced by 35 and 22% of the samples being of common carp (*Cyprinus carpio*) and channel catfish (*Ictalurus punctatus*), respectively. The successful evaluation across these independent data sets, however, indicates the versatility of the regression.

DISCUSSION

The present study was prompted by growing interest in analyzing fish samples for congener-specific concentrations of PCBs, especially dl-PCBs. Our results suggest that relatively simple, cost-effective, and time-saving total PCB measurements can be readily utilized to assess toxicological hazard associated with dl-PCBs in fish. This simplified approach for $TEQ_{dl\text{-}PCB}$, in combination with a simple and inexpensive method (e.g., immunoassay analysis [30]) for quantification of $TEQ_{dioxin/furan}$, can substantially (four- to fivefold) reduce the monitoring cost of these contaminants in fish.

The regression equation (Eqn. 2) of total PCB and $TEQ_{dl\text{-}PCB}$ developed here is based on what likely is the largest data set of dl-PCBs and total PCB in fish. The data set consisted of many fish species of different trophic levels, which had wide ranges in size, weight, and PCB levels. Use of a different standard from the 4:1 mixture of Aroclors 1254 and 1260 utilized during the MOE total PCB analysis (see *Materials and Methods*) may affect total PCB measurement and, in turn, linearly affect the estimate of $TEQ_{dl\text{-}PCB}$. The $TEQ_{dl\text{-}PCB}$ estimated using the regression is expected to be within a factor of two to three of $TEQ_{dl\text{-}PCB}$ calculated using individual dl-PCB concentrations. We believe that such discrepancies should not be a major concern for the following three reasons: First, the expected up-to-45% variation in the congener-based $TEQ_{dl\text{-}PCB}$ because of 12 to 90% uncertainty in measured dl-PCB values [31]; second, the expected up-to-35% variation in total PCB–based $TEQ_{dl\text{-}PCB}$ because of 35% uncertainty in measured total PCB [31]; and finally, TEFs and, thereby, TEQs viewed as an order-of-magnitude estimate [32], because TEFs for a dl-PCB congener from individual studies (also known as relative potencies) vary by more than two to four orders of magnitude [7,33].

A number of studies have published dl-PCB patterns in fish from other geographical regions similar to those found in North America [34–37]. Therefore, the regression presented in the present paper likely is applicable to other parts of the world as well. At present, however, the uncertainty that will be embedded in such applications is unclear. A rigorous evaluation involving separate data sets of substantial sample sizes with low to high total PCB concentrations measured in both skin-on and skin-off fillet as well as whole-fish samples of many predator and bottom-dweller fish species, some of which were not a part of the original data set, strengthens the applicability of the correlation to fish from North America and, most likely, Europe. The deficiencies in, for example, exposure and risk assessment and fish advisories because of lack of expensive dl-PCB measurements can be alleviated using the regression and a simple, generally routine measurement of total PCB in fish. As a consequence, a true image of human and ecosystem health hazard of dl-PCBs in fish can be depicted at virtually no extra cost.

SUPPORTING INFORMATION

Appendix 1. Names, scientific names, and scientific family names of the fish species included in the Ontario Ministry of the Environment (MOE) data from which the regression between total PCB and $TEQ_{dl\text{-}PCB}$ (Eqn. 2) was derived.

Appendix 2. Names, scientific names, and scientific family names of the predator fish species included in the U.S. Environmental Protection Agency National Lake Fish Tissue Study (NLFTS) subset of 55 fillet samples utilized to evaluate the Equation 2.

Appendix 3. Names, scientific names, and scientific family names of the bottom-dweller fish species included in the U.S. Environmental Protection Agency National Lake Fish Tissue Study (NLFTS) subset of 176 whole fish samples utilized to evaluate the Equation 2.

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