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Accreditation of a Screening Method for Non–Dioxin-like Polychlorinated Biphenyl Detection in Fishery Products according to European Legislation

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ABSTRACT

European Commission Regulation 882/2004/EC requires that official control laboratories for foodstuffs in the member states are certified according to UNI EN ISO/IEC 17025:2005 (general requirement for the competence of calibration and testing laboratories). This mandatory requirement has resulted in a continuous adaptation and development of analytical procedures. The aim of this study was to develop a method for semiquantitative screening of polychlorinated biphenyls in fish for human consumption. According to the Commission Decision 657/2002/CE, the detection capability, the precision, the selectivity-specificity, and applicability-ruggedness-stability were determined to validate the method. Moreover, trueness was verified. This procedure resulted in rapid execution, which allowed immediate and effective intervention by the local health authorities to protect the health of consumers. Finally, the procedure has been recognized by the Italian accrediting body, ACCREDIA.

Polychlorinated biphenyls (PCBs) are environmentally persistent organic pollutants *(17, 19)* that include 209 congeners: 12 dioxin-like PCBs (DL-PCBs) and 197 non–dioxin-like PCBs (NDL-PCBs). These compounds have been widely produced and used by industry during the last century due to their dielectric and flame retardant properties; however, the use of PCBs as raw material or chemical intermediates has been banned in the European Union since 1985 *(3),* and Council Directive 96/59/EC *(5)* reports measurements for phasing out existing PCB-containing equipment and materials. Concentrations of PCBs in food may be high because of their chemical stability and their power to bioaccumulate, and they represent a risk to public health *(9, 13, 14)*. In particular, food of animal origin, such as fish, has been recognized as one of the main vehicles for human exposure to PCBs *(7–9)*. Note that study of dietary exposure of the general population to this group of persistent chemicals through consumption of fishery products has focused on DL-PCBs, although NDL-PCBs are analytically predominant in animal tissues *(10, 16)*. PCBs are associated with several adverse effects on humans, commonly attributed to DL-PCBs, although NDL-PCBs have also been shown to be toxic compounds *(1, 12, 15, 18)*. Moreover, analysis of the profiles of food contamination carried out by the European Food Safety Authority *(9),* reports that the six most common PCB congeners in the environment and food are PCBs 28, 52, 101, 138, 153, and 180 (according to International Union of Pure and Applied Chemistry nomenclature), belonging to NDL-PCBs and representing more than 50% of all congeners present in foodstuffs of animal origin and in human fat. The purpose of this study was to develop and validate a screening method for the quantitative determination of NDL-PCBs in fishery products (such as cod, bream, catfish, tuna, octopus, cuttlefish, squid, and mussels), following the reference text for analytical methods validation according to the European Commission Decision 657/2002/EC *(2)*.

According to prescriptions of the Commission Decision 657/2002/EC *(2)* for screening methods, the detection capability (CCβ) of the method, instead of the detection limit, was determined. CCβ is a parameter that tests the suitability of the method with regard to false negative responses; and the β-error, which is the probability of a false negative response, must be less than 5%. In addition to CCβ, the precision, the selectivity-specificity, and applicability-ruggedness-stability (as reported in table 9 of 657/2002/EC) were determined. The present method can also be easily converted to a confirmatory method by using a few additional parameters (CCα and veracity), according to the procedure described below.

MATERIALS AND METHODS

Reagents. Unless stated otherwise, all reagents were purchased from Carlo Erba (Milan, Italy). Diethyl ether, petroleum ether, and isooctane were used for pesticide analysis, Na_2SO_4 was ''anhydrous,'' and H_2SO_4 grade was 96%. PCBs and polybrominated diphenyl ether (PBDE) standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

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TABLE 1. *Recovery of blank samples spiked at 1.0 ng/g for each congener (6.0 ng/g in sum) of 20 replicates (nine mussel samples, five pangasius, and six cuttlefish) in four analytical sessions (repeatability between days)*

	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Σ (ng/g)
Mean recovery	0.85	0.93	0.85	0.88	0.90	0.91	5.30
SD	0.14	0.14	0.15	0.14	0.15	0.12	0.50
RSD% between days[a]	17	15	18	15	17	13	9
Minimum	0.67	0.73	0.69	0.72	0.69	0.70	4.70
Maximum	1.20	1.21	1.17	1.23	1.25	1.19	6.40
ML regulation 1259/2011/CE	—	—	—	—	—	—	75.00

[a] RSD%, relative standard deviation.

Collection of samples. Samples were collected from an Italian market. After sampling, fish samples were stored at −20°C until analysis. Cod, bream, catfish, tuna, octopus, cuttlefish, and squid samples were thawed and gutted, and the edible parts were homogenized; mussel samples were thawed and peeled, and whole mussels (edible part) were homogenized.

Extraction method for fish and shellfish. Each sample (5.00 ± 0.01 g) was homogenized and subsequently extracted by agitation for 12 h with 20 ml of diethyl ether in a 25-ml Pyrex glass tube with a ground glass stopper. The extract was filtered over Na_2SO_4, dried under nitrogen flow, and reconstituted with 2 ml of petroleum ether.

Hydrolysis of extracts. A diatomaceous earth solid support (Extrelut NT3, Merck, Darmstadt, Germany) was conditioned with 3 ml of H_2SO_4. Extract was loaded on the solid support, and mineralization was carried out for approximately 5 min (to a maximum 20 min) at room temperature. Compounds were eluted with 20 ml of petroleum ether, passed through a 6-ml solid-phase clean-up Florisil cartridge (Isolute, Uppsala, Sweden), dried in the Rotavapor (Büchi, Assago, Milan) set at 40°C, and reconstituted with 1,000 ± 0.002 ml of isooctane.

Instrumental determination. Sample was filtered on a 0.45-μm nylon syringe filter (Millipore, Bedford, MA) and injected into the gas chromatograph (Autosystem XL, Perkin-Elmer, Waltham, MA), which was equipped with an electron capture detector and a capillary column (30 m by 0.25 mm by 0.25 μm; 35% phenyl–65% dimethylpolysiloxane fused silica; Zorbax, Phenomenex, Torrance, CA). Instrumental parameters were injection volume of 0.5 μl, injector at 250°C, and detector at 380°C; the oven temperature was programmed as follows: 100 to 250°C at a rate of 15°C/min, 250 to 300°C at a rate of 5°C/min, hold for 1 min. Chromatographic conditions were tested also for possible interference of PBDE 47 (stock solution at 10 ng/ml), which is the most predominant congener in the environment, at retention time (R_t) of PCB 180. The retention time window for the identification of analytes was R_t ± 0.5%, and the amount of each compound was determined by external standardization with a three-point curve (1.0, 10.0, and 20.0 ng/g of PCB mixture in isooctane).

Spiking of the sample. A mixture (50 μl) of the six PCB congeners of interest (28, 52, 101, 138, 153, and 180) at a concentration of 0.100 mg/liter was added to eight samples of PCB-free mussels, six samples of pangasius, and six samples of cuttlefish. The mixture was prepared by dilution with isooctane of the stock solution at 10 ng/ml of each PCB congener. These samples were subjected to extraction, purification, and instrumental determination on three different days (Table 1), as required by Commission Decision 657/2002/EC *(2)*.

Validation procedure. The validation study was conducted in accordance with Directive 93/99/EEC *(4)*, thus according to the criteria set out in Decision 657/2002/EC *(2)*. The CCβ was determined for the edible part; the CCβ is the lower analyte concentration at which the β error is less than 5%, similar to the limit of detection. To calculate the β error, 20 PCB-free fish samples were spiked at 1.0 ng/g (wet weight) with a mix of six NDL-PCBs of interest (Σ = 6.0 ng/g), and the presence of the peak (signal-to-noise ratio greater than 3) was checked at R_t of each PCB congener. The false-negative rate is considered higher than 5% if only one sample reports a signal-to-noise ratio less than 3 (1 [5%] of 20) for each PCB congener. Precision was evaluated on

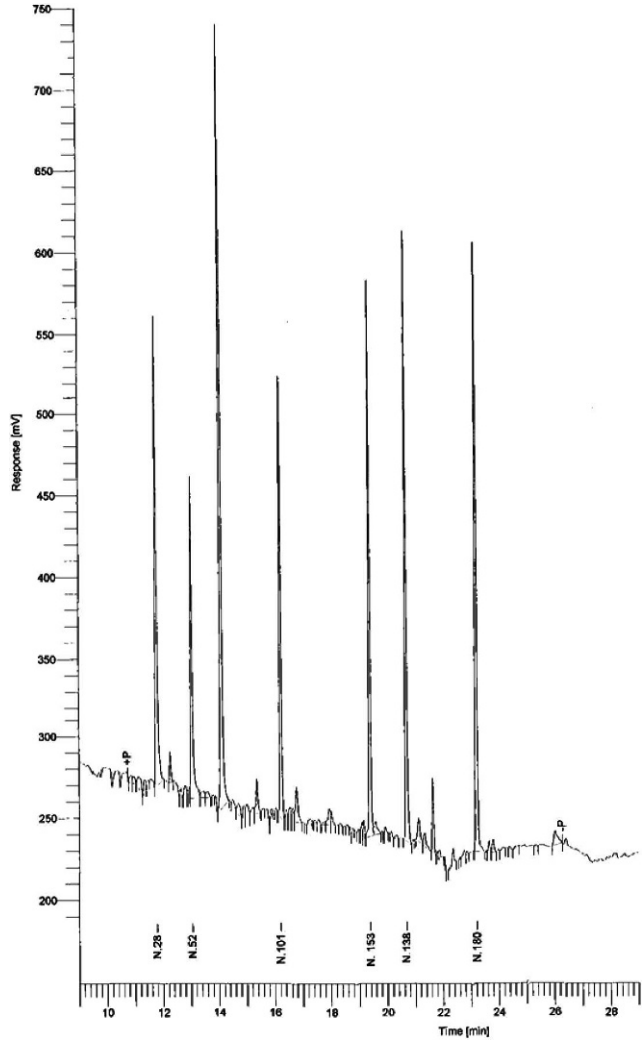

FIGURE 1. *PCB-free mussel sample spiked at 1.0 ng/g with mix of the six congeners of interest.*

TABLE 2. *PCB levels occurring in fishery products received by the laboratory during routine control activity in the period 2010 to 2012, expressed as the sum of the six PCBs of interest*

No.	Yr	Region	City	Commercial type	Sample (edible parts)	Σ PCBs (ng/g)[a]
1	2010	Campania	Napoli	Breeding plant	*Mytilus galloprovincialis*	3.2
2	2010	Campania	Napoli	Sample import	*Mugil cephalus*	7.7
3	2010	Campania	Napoli	Retail	Canned fish	5.2
4	2010	Lazio	Latina	Wholesale	Cephalopods	2.0
5	2010	Campania	Salerno	Wholesale	*Fistularia commersonii*	0.6
6	2010	Campania	Napoli	Wholesale	Cephalopods	1.8
7	2010	Lazio	Roma	Wholesale	Mix fish fillets	1.7
8	2011	Campania	Mugnano di Napoli	Wholesale	Seawater fish	ND
9	2011	Campania	Battipaglia	Wholesale	Seawater fish	0.1
10	2012	Calabria	Mangone	Wholesale	Seawater fish	0.1
11	2012	Calabria	Mangone	Wholesale	Canned fish	3.9
12	2012	Calabria	Cosenza	Retail	Seawater fish	0.1
13	2012	Calabria	Mangone	Retail	Processed fish	0.4
14	2012	Calabria	Cosenza	Wholesale	Seawater fish	ND
15	2012	Calabria	Rogliano	Retail	Canned fish	0.3
16	2012	Campania	Pastorano	Wholesale	*Oncorhynchus gorbuscha*	2.0
17	2012	Calabria	Rende	Wholesale	Processed fish	0.4
18	2012	Campania	Gricignano di Aversa	Wholesale	Frozen fish	0.6

[a] PCBs 28, 52, 101, 138, 153, and 180. ND, not detected.

the same 20 PCB-free samples spiked at 1.0 ng/g (wet weight), with the mix of the six NDL-PCBs of interest (Σ = 6.0 ng/g), applying the criteria from Decision EC 657/2002 stating that, for a value less than 100 ng/g, relative standard deviation shall be as low as possible (table 3 of Decision 657/2002/EC). To determine selectivity-specificity experimentally, 20 PCB-free samples were used (mussels [11], pangasius [3], cuttlefish [1], codfish [1], octopus [1], tuna [1], squid [1], sea bream [1]) (Table 1), and the absence of peaks was verified (signal-to-noise ratio less than 3) in R_t of each congener of PCB. To determine applicability-ruggedness-stability of the method, the same 20 PCB-free samples spiked at 1.0 ng/g (wet weight), with the mix of the six indicator congeners of NDL-PCBs (Σ = 6.0 ng/g), were used with minor changes (section 3.1.1.3 of Decision 657/2002/EC), in which the selected variables were different production batches of sulfuric acid and different production batches of solid-phase extraction Florisil columns. Trueness, an additional parameter required by Decision 657/2002/EC *(2)* only for confirmatory methods, was verified using the standard reference material 2977 (mussel tissue provided by National Institute of Standards and Technology [NIST], Gaithersburg, MD), complying with the ranges indicated by NIST whereby dry and wet samples were compared considering 80% (wt/wt) as the average water content of mussels. Finally, to comply with the Italian national accrediting body ACCREDIA, the method was also tested by circuit interlaboratory proficiency testing provided by the European Union Reference Laboratory in 2011, which consisted of searching for the six PCB congeners of interest in salmon and fish oil.

RESULTS AND DISCUSSION

The method of sample preparation for multiresidual analyses in complex matrices that was used in the past was particularly laborious, especially as regards acid hydrolysis of the lipid extracts. The use of diatomaceous earth solid supports for single-layer acid chromatography, which takes a few minutes, allowed the process to be done in a single day; this replaced the slower (overnight) typical sulfuric acid hydrolysis and was simpler to execute than the multilayer reactive columns *(20)*. As required by Decision 657/2002/EC *(2),* CCβ, selectivity-specificity, and applicability-ruggedness-stability were determined. The results of the validation study showed that all 20 samples, fortified with the PCB mixture, were positive (Table 1), with an accuracy in terms of recovery between 67 and 125%. The β error was less than 5%, according to the calculation method described above, and the CCβ was fixed at 1.0 ng/g (wet weight) for each of the congeners. This method was selective-specific for each indicator PCB because there was not any matrix interference (Fig. 1) in all 20 replicates of PCB-free samples. Moreover, as to possible interference by PBDEs, the absence of coelution of environmentally predominant PBDE 47 with PCB 180 was verified. As for precision, relative standard deviation was between 9 and 18, as low as possible (Table 1). The method was found to be applicable-rugged-stable because no variables significantly influenced the results between the two groups of 10 samples ($0.12 < P < 0.27$, including the sum of six congeners) in the same 20 spiked samples used to calculate the CCβ. Regarding trueness, the additional parameter, all concentrations obtained were the average of three replicates within these ranges from NIST standard reference material 2977: 5.17 ± 0.36 ng/g for PCB 28, 8.02 ± 0.56 ng/g for PCB 52, 10.6 ± 0.9 ng/g for PCB 101, 7.94 ± 0.63 ng/g for PCB 138, 14.1 ± 1.3 ng/g for PCB 153 g, and 2.74 ± 0.25 ng/g for PCB 180. Finally, participation in circuit interlaboratory proficiency testing provided by the European Union Reference Laboratory gave satisfactory results for both salmon (z-score = −1.8) and fish oil (z-score = −1.1).

The method for determination of the six indicator NDL-PCBs has proved suitable for the execution of screening assays on fishery products, as it is able to detect the analytes specifically studied with a β error less than 5% at levels greater than or equal to 1.0 ng/g (wet weight) for each

compound, in accordance with Commission Decision 657/2002/EC *(2)*. The main benefit of the present method is its agreement with Decision 657/2002/EC *(2),* the European reference text; therefore, the performance of these methods can be unequivocally demonstrated and they can be standardized for use in food control. In 2010 to 2012, this method was tested in the routine control of 18 fish samples received in the laboratory (Table 2). During the analytical sessions, compliance with ISO/IEC 17025:2005 *(11)* and the quality of the results were further verified by inserting one or more spiked samples within each test run, whose recovery levels were compatible with the range available in Shewhart-like control charts (data not shown). Finally, the method has been accredited by the Italian national accreditation body, ACCREDIA.

In conclusion, nowadays the search for NDL-PCBs is no longer a matter of basic research but strictly of food safety, given their undesirable presence in foods (Commission Regulation 1259/2011/CE *(6)*). It is necessary to develop and validate control methods; however, previously published methods for detection of NDL-PCBs in food are not in accordance with European standards. The procedure outlined here has already been recognized by ACCREDIA, and compared with similar methods reported in the literature, it is simpler to execute and requires less time, with the benefit of allowing immediate and effective intervention by the local health authorities to protect the health of consumers. This is a precaution-based, screening method of food security; the analytical data should be uniquely confirmed by a confirmatory method in order to establish whether the legal limits set by Regulations 1259/2011/CE *(6)* are exceeded for certain types of food, including those covered by the validation study. As to application, fishery products were considered of great interest to food safety because PCBs are predominant in fish, representing a risk to human health, and because this food, an important economic resource for the countries producing fish, is widely consumed as one of the main foods of the ''Mediterranean diet.''

REFERENCES

1. Al-Anati, L., J. Högberg, and U. Stenius. 2008. Non-dioxin-like PCBs interact with benzo[a]pyrene-induced p53-responses and inhibit apoptosis. *Chemosphere* 73:278–283.
2. Commission of the European Communities. 2002. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Union* L 221:8–36.
3. Council of the European Communities. 1985. Council Directive of 1 October 1985 amending for the sixth time (PCBs/PCTs) Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. *Off. J. Eur. Union* L 269:56–58.
4. Council of the European Communities. 1993. Council Directive 93/99/EEC of 29 October 1993 on the subject of additional measures concerning the official control of foodstuffs. *Off. J. Eur. Union* L 290:14–17.
5. Council of the European Union. 1996. Council Directive 96/59/EC of 16 September 1996 on the disposal of polychlorinated biphenyls and polychlorinated terphenyls (PCB/PCT). *Off. J. Eur. Union* L 243:31–35.
6. European Commission. 2011. Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. *Off. J. Eur. Union* L 320:18–23.
7. European Commission, Scientific Committee on Food. 2000. Opinion of the scientific committee on food on the risk assessment of dioxins and dioxin-like PCBs in food. Scientific Committee on Food, Health and Consumer Protection Directorate-General, European Commission (Brussels). Available at: http://europa.eu.int/comm/food/fs/sc/scf/out78_en.pdf. Accessed 10 January 2011.
8. European Food Safety Authority. 2005. Scientific Panel on Contaminants in the Food Chain of EFSA (CONTAM Panel). *EFSA J.* 284:1–137.
9. European Food Safety Authority. 2010. Scientific report of EFSA. Results of the monitoring of non dioxin-like PCBs in food and feed. *EFSA J.* 8(7):1701. doi:10.2903/j.efsa.2010.1701.
10. Fattore, E., R. Fanelli, E. Dellatte, A. Turrini, and A. Di Domenico. 2008. Assessment of the dietary exposure to non-dioxin-like PCBs of the Italian general population. *Chemosphere* 73:278–283.
11. International Organization for Standardization. 2005. ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories, 2nd ed. International Organization for Standardization, Geneva.
12. Machala, M., P. Simeckova, L. Al-Anati, U. Stenius, K. Pencikova, J. Prochazkova, P. Krcmar, J. Slavik, and J. Vondracek. 2009. Effects of non dioxin-like PCBs (NDL-PCBs) on signalling pathways contributing to liver tumor promotion and carcinogenesis. *Toxicol. Lett.* 189S:S137.
13. Muir, D. C. G., R. J. Norstrom, and M. Simon. 1988. Organochlorine contaminants in arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordane-related compounds. *Environ. Sci. Technol.* 22:1071–1079.
14. Olsson, A., K. Valters, and S. Burreau. 2000. Concentrations of organochlorine substances in relation to fish size and trophic position: a study on perch (*Perca fluviatidis*). *Environ. Sci. Technol.* 34:4878–4886.
15. Seegal, R. F. 1998. Neurochemical effects of co-planar and non-coplanar polychlorinated biphenyls. *Neurotoxicol. Teratol.* 20:349–350.
16. Serpe, F. P., M. Esposito, P. Gallo, M. Salini, P. Maglio, T. Hauber, and L. Serpe. 2010. Determination of heavy metals, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in *Mytilus galloprovincialis* from Campania coasts, Italy. *Fresenius Environ. Bull.* 19:2292–2296.
17. Stockholm Convention on Persistent Organic Pollutants. 22 May 2001. Stockholm.
18. Strathmann, J., M. Schwarz, J. C. Tharappel, H. P. Glauert, B. T. Spear, L. W. Robertson, K. E. Appel, and A. Buchmann. 2006. PCB 153, a non-dioxin-like tumor promoter, selects for beta-catenin (Catnb)-mutated mouse liver tumors. *Toxicol. Sci.* 93:34–40.
19. World Health Organization. 1993. Polychlorinated biphenyls and terphenyls. Environmental Health Criteria 140. International Programme on Chemical Safety, World Health Organization, Geneva.
20. Zajicek, J. L., D. E. Tillitt, J. N. Huckins, J. D. Petty, M. E. Potts, and D. A. Nardone. 1996. Application of enzyme-linked immunosorbent assay for measurement of polychlorinated biphenyls from hydrophobic solutions, chap. 26, p. 307–325. *In* J. M. Van Emon, C. L. Gerlach, and J. C. Johnson (ed.), Environmental immunochemical methods. ACS Symposium Series 646. American Chemical Society, Washington, DC.