SENSITIVE INSTRUMENTAL METHOD FOR QUANTITATIVE DETERMINATION OF HIGH-BROMINATED FLAME RETARDANTS IN HUMAN SERUM SAMPLES

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SENSITIVE INSTRUMENTAL METHOD FOR QUANTITATIVE DETERMINATION OF HIGH-BROMINATED FLAME RETARDANTS IN HUMAN SERUM SAMPLES.

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Abstract

Background

High-brominated flame retardants can be released into the environment from consumer products, such as electric and electronic equipment, and enter the human body by different pathways. Due to their toxicity and the regulations, is very relevant to know their levels and trends in human samples. However, chromatographic serum analysis of some of these compounds represents nowadays a challenge in general population.

Objective

To optimise and validate an instrumental method based on gas chromatography coupled to mass spectrometry, which, together with, a simple sample preparation procedure allows the analysis of decabromodiphenyl ether (BDE-209), decabromodiphenyl ethane (DBDPE) and tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (TBBPA-DBPE) in human serum samples from the general population.

Methods

In order to minimise the high degradation during instrumental analysis, GC parameters such as injection volumes, carrier flow rates and column lengths, were assessed and optimized. This instrumental approach in combination with solid-phase extraction (SPE) followed by multi-layer silica gel column purification allowed satisfactory analysis using only 1 mL of serum.

Results

The performance of the complete method was evaluated at three spiking levels, 0.01, 0.05 and 0.2 ng/mL Recoveries in the range 87-108 % were obtained whereas the relative standard deviation in interday measurements, were in general lower than 19%. Limits of detection were in the range of 0.0045–0.0070 ng/mL. The optimized procedure was successfully applied to the determination of the investigated pollutants in real human samples of general population.

Conclusion

The proposed method could contribute to the inclusion of these environmental pollutants in human biomonitoring studies, increasing the knowledge of levels and trends in the general population.

Highlights

GC-MS parameters optimization to minimise instrumental analytes degradation.

Successful application to human serum samples from the general population.

Tetrabromobisphenol A bis(2,3-dibromopropyl ether) human serum levels are reported first time.

Introduction

Brominated flame retardants (BFRs) are synthetic organobromine compounds that have been widely used in many consumer and industrial products since 1970´s, including electric and electronic equipment, buildings and construction materials, furniture, textiles and plastics, in order to delay fire starting and progressing. BFRs represents about 20% by volume of the global flame retardants production, being one of the most relevant the commercial mixture decaBDE, where BDE-209 is the predominant congener (around 94%). Recently, the global in-use and waste stocks of decaBDE were estimated in 400 and 100 kt, respectively. In addition, recycling process can contribute to the reintroduction of BDE-209 in the environment (1, 2).

Due to their ubiquity, long-distance transport, persistence and human health affects as endocrine disruption and neurotoxic, PBDEs, have been a subject of global concern (3, 4). Regarding DecaBDE, their restrictions started in the first decade of the 21st century in the European Union and were focus on electrical and electronic equipment (5). Finally, in 2017, was globally regulated within the framework of Stockholm Convention with some use exceptions. Therefore, in recent years novel BFRs have been introduced in the market as alternatives to DecaBDE, such as decabromodiphenyl ethane (DBDPE) and TBBPA derivates like TBBPA-bis(2,3-dibromopropylether) (TBBPA-DBPE) (6, 7). Although human toxicity studies for these compounds are scarce, they are also suspected of being endocrine disruptors and toxic effects on model organisms such as hepatotoxicity or neurotoxicity have been described (7, 8). Furthermore, TBBPA-DBPE contains 2,3-dibromo-1-propanol in its substructure that is considered as possibly carcinogenic to humans (9).

Human Biomonitoring (HBM) is a powerful approach for assessing human exposure to environmental pollutants since biomonitoring data directly reflect the total body burden as allow integrating all routes and exposure source. Nowadays, HBM studies are used to support the implementation and effectiveness assessment of health policies (10).

Although high brominated flame retardants have been analyzed in recent years in environmental and food samples by GC-MS and GC-MS/MS, and to lesser extent by LC-MS/MS (11-13), limited studies on human samples have reported levels for BDE-209 and DBDPE and none for TBBPA-DBPE (2, 14). As of today, sensitive quantification of high brominated compounds in human serum is not easy to achieve since, together with the low levels expected and typical high background levels, relevant degradation process take place during GC analysis, in particular in sample injection and chromatography separation (2, 15). Therefore a carefully instrument optimization are required in order to achieve low enough quantification limits to be applied in human samples from the general non-exposed population.

This study describes the optimization and validation of a new instrumental approach based on (GC-MS) for the sensitive quantification of BDE-209, DBDPE and, for the first time to our knowledge, TBBPA-DBPE, in small volume of human serum. The method proposed was successfully applied to real samples of general population from a national HBM study carried out in Spain (16). Therefore, this approach can be a valid tool to evaluate the recent Deca-BDE replacement efficiency and human exposure level of the alternatives.

Experimental

Standards, reagents and samples

Standards of BDE-209 and $^{13}C_{12}$-labelled BDE-209, and DBDPE were obtained from Wellington Laboratories (Ontario, Canada) whereas TBBPA-DBPE was supplied by

Merck (Darmstadt, Germany). $^{13}C_{12}$-labelled BDE-209 at 0.1 ng/mL was used as injection standard to compensate instrumental fluctuations.

All solvents used were of organic trace analysis quality. n-hexane, methanol and isooctane were purchased by Scharlab (Barcelona, Spain), dichloromethane (DCM) by J.T. Baker (Deventer, the Netherlands) and acetonitrile by Lab-Scan Ltd (Gliwice, Poland). Milli-Q® ultrapure water quality was used (Merck Millipore). Granular anhydrous sodium sulphate was obtained from Scharlab. Sulphuric acid (98%), pro analysis quality, and silica gel 60 were purchased from Merck and SPE OASIS^{TM}HLB cartridges (3 mL/60 mg) were obtained from Waters (Mildford, MA, USA).

Human serum samples, used for method validation, were obtained from Arctic Monitoring and Assessment Program, AMAP 2016-2017 (Ring Test for Persistent Organic Pollutants in Human Serum, National Institute of Public Health, Quebec, Canada). Rabbit serum was assessed for calibration curves.

In order to assess method feasibility for determination of these pollutants in the general population, the proposed method was applied to a random subsample of human serum samples from Madrid and Castilla La Mancha collected in the framework HBM study Bioambient.es (16).

The samples were stored at -80 ºC in glass vials until chemical analysis.

The retardants concentrations were individually lipid-adjusted. Both total cholesterol and triglycerides were quantified by enzymatic method and further determination using spectrophotometry at 500 nm (17).

Sample treatment

The sample preparation procedure was adapted from others previously described to achieve rapid and simple extraction and purification of these analytes in a small volume human serum sample (18, 19). Briefly, 1 mL of human serum, 1 mL of formic acid and 100 µL of acetonitrile is added to a 10 mL glass tube to serum proteins denaturation. Then, the mixture is loaded into a pre-conditioned SPE cartridge, i.e. methanol and Milli-Q water, rinsed with 2 mL of Milli-Q water and vacuum dried. The analytes are eluted with 2 mL of n-hexane and 2 mL of DCM. Once concentrated up to 1 mL, the eluent is cleaned up in a multilayer silica gel column, i.e. 0.17 g activated silica gel, 0.8 g silica modified with sulphuric acid (44%, w/w) and anhydrous sodium sulphate, packed in an 3 mL empty glass column and eluted with 3 mL of n-hexane:DCM (1:1, v/v). The extract is evaporated to nearly dryness using Reacti-Therm III Heating Module from Thermo, at 40 °C, with a gentle flow of nitrogen and vials inserts of 300 µL. Finally, it is reconstituted with 100 µl of $^{13}C_{12}$-labelled BDE-209 solution in isooctane at 1 ng/mL prior to instrumental analysis.

Procedure blanks and sample controls were prepared following the same procedure as for serum samples but using Milli-Q water instead of serum. No significant background interferences were found by the methodology proposed.

Gas chromatographic analysis

The identification and quantification of the BFRs selected was performed by GC (HP 7890A Series, Hewlett-Packard, Palo Alto, CA) equipped with a multimode inlet (MMI) and coupled with a MS operating in negative chemical ionization (NCI, 5975C Agilent). Thus, 10 µL were injected in large volume injection (LVI) mode at 160 µL/min. An ultra inert, dimpled liner with an internal diameter of 2 mm was used (Agilent 5190-2297). It was changed every 30-40 injections. The inlet temperature program was set at 80 ºC for 0.1 min, then increased to 350 ºC at 750 ºC/min and held for 5 min. A vent pressure of 5 psi until 0.1 min, purge flow of 60 mL/min (2.6 min) and vent flow at 150 mL/min were set.

GC separation was carried out by DB-5MS column (0.25 mm × 0.10 µm × 10 m in length, Agilent) and the GC oven temperature was programmed from 80 ºC (held for 2.6 min) to 200 ºC at 30 ºC/min, then up to 275 ºC at 5 ºC/min and finally to 315 ºC (2 min) at 40 ºC/min. Helium was used as the carrier gas at a constant flow of 4.0 mL/min. Mass spectrometer, in NCI mode, used methane as reagent gas and 70 eV of electron energy. Transfer line, quadrupole and ion source temperatures were set at 300, 150 and 150 ºC, respectively.

Quantification was performed in selected ion monitoring (SIM). The m/z for quantitation/confirmation were selected after injection of standards in SCAN mode and they were in line with other similar studies (20) (see Supplementary Table 1). Calibration curves levels were 0.005, 0.010, 0.025, 0.050, 0.100, 0.250 and 0.500 ng/mL of serum, whereas the confirmation criteria established were: i) detection of the target compound peak at the same retention time that the corresponding standard, ii) the maintenance of the two selected ions ratio within ± 15% of the standard value.

Results and discussion

Optimization of the GC-MS procedure

Some instrumental parameters have been assessed previously mainly for BDE-209, however, the added value of the present study is the inclusion of other high brominated compounds (21, 22).

Different injection volume of standard mixtures at 5 ng/mL were tested, i.e. 2µL in pulsed splitless injection mode and 5 µL and 10 µL multimode inlet (MMI) for large injection volumes (LVI). Whereas, TBBPA-DBPE only was detected by LVI, BDE-209 and DBDPE abundances were increased about 10.5 times for LVI (10 µL) with respect to splitless injection (Table 1). In addition, BDE-209 thermal degradation, that may occur during sample injection, as evidenced by the appearance of signals corresponding to debrominated products such as BDEs 206, 207 and 208 (23). Whereas nona-BDEs abundance was reduced about 30% by increasing volume injected from 5 to 10 µL, degradation of BDE-209 seems constant with both injection mode, probably due to the formation of other thermal degradation compounds other than nona-BDE. For these reasons, the injection of 10 µL with a MMI was selected.

Injection speed was the next parameter assessed. Based on the value calculated by the Agilent Solvent Elimination Calculator software and related bibliography (24): 100, 160, 200 and 300 µL/min were evaluated. Analysis of 5 ng/mL standard mixtures showed how TBBPA-DBPE was sensitive to changes in the injection speed. Whereas higher speed i.e. 300 µL/min led to a slight decrease in sensitivity of TBBPA-DBPE, at 100 µL/min it could not be detected. Regarding BDE-209 and DBDPE, no relevant differences were observed, although an injection speed of 160 µl/min provided a decrease of BDE-209 degradation products. Therefore, injection speed of 160 µL/min was selected for further analysis.

Column length and flow rate were assessed using standard solution (1 ng/mL), because of their known influence on higher brominated compounds degradation. Fast temperature gradient was not considered since the aim was to get a sufficiently generic method that would allow the simultaneous analysis of other high-brominated compounds and could be applied to different types of human samples, such as fat. A 15 meters DB-5MS column (0.25 mm id, 0.10 µm film thickness) was firstly considered, however, it was observed a noticeable degradation mainly for DBDPE and TBBPA-DBPE when the number of standard injections increases (see Supplementary Figure 1). This loss of sensitivity over time could endanger the method reproducibility and/or increase the cost per analysis and could be explained by changes in the column film caused by the time the column is kept at high temperature.

For these reasons, a non-conventional shorter column (10 m), obtained from longer ones, was tested. With this column, BDE-209, DBDPE and TBBPA-DBPE abundance were higher than those found with a new 15 m column, increasing method sensitivity between 3 and 7 times (Fig. 1A).

In the same way, i.e. reduce the interaction time of analytes with stationary, flow rates higher than traditional ones were evaluated (Fig 1B). A flow of 4.0 mL/min provides an abundance increase about 25% for the three analytes selected, without compromise MS detection.

Finally, transfer line temperatures (from 275 to 300 ºC), ion source and quadrupole temperatures (from 150 to 250 ºC) were tested without significant sensitivity improvements, therefore, in consonance with other studies transfer line temperature was set at 300 ºC and ion source and quadrupole at 150 ºC (12, 25).

Method validation

The method did not provide interferences that could compromise quantification since reagent black samples after method application presented low and reproducible signals of target compounds as usual for high brominated BFRs. External standard calibration curve was used since rabbit serum provided higher levels for the analytes selected than those observed in reactive blanks therefore, reducing the method sensitivity (Supplementary Figure 2).

To evaluate matrix effect, interlaboratory human serum samples, with assigned values for BDE-209, were quantified with two calibration approaches (standard calibration curve and matrixmatched calibration curve), after applying the proposed analytical method. As shows Supplementary Table 2, there was no difference between both methods in terms of accuracy.

Linearity study was carried out taking into account the expected levels in serum from general population. Good linearity was obtained with an eight-level standard calibration curve built with the mean of eight inter-day curves from 0.005-0.500 ng/mL for all analytes (Table 2).

For recovery and reproducibility study eight inter-day experiments (n=3), of 1 mL of Milli-Q water samples spiked at 0.01 ng/mL, 0.05 ng/mL and 0.2 ng/mL were carried out. Recoveries ranged from 87 to 108 % with RSDs, in terms of reproducibility, lower than 19 %, except for TBBPA-DBPE only at the lowest level studied.

The method detection and quantification limits were calculated as 3 x SD and 10 x SD, respectively, plus average blank values. SD was determinate by blanks quantification in eight different calibration curves over two months. LOD and LOQ was obtained interpolating the value for each compound in the mean calibration curve (n=8). The methodology provided LODs lower than 0.0070 ng/mL and LOQs lower than 0.0091 ng/mL (Table 2). These limits were lower than those obtained in previous studies with GC-NCI-MS (2, 26).

Currently, no certified reference materials for serum, or similar, are available for these analytes and on the other hand, interlaboratory exercises of serum only include BDE-209. Therefore, the external validation was carried out for BDE-209 using twelve human serum samples human from AMAP Program. Results obtained showed satisfactory degree of agreement, between the concentration reported by the method proposed and assignment values, which is indicative of the robustness and accuracy of the method developed (see Table 3).

Application to real human samples

Finally, this method was successfully applied to a random human serum subsample (n=40) from general adult population within the framework of Bioambient.es project, demographic details are showed in Supplementary material, Table 3. As an example, Supplementary Figure 3 shows the typical chromatogram obtained for 1 mL of serum sample from general population. The method did not provide interferences that compromise the sensitive and accurate quantification of the target compounds.

Although the samples were collected in 2009 and 2010, i.e. when DecaBDE restrictions were not generalized, DBDPE and TBBPA-DBPE were quantified in 40 % and 23 % of the volunteers respectively (Table 4). Regarding levels found, median concentration of BDE-209, i.e. GM: 1.89 ng/g lipids, were in the range of those reported in Europe, but with a percentage of samples above the LOD, in general, higher than other studies. Whereas studies in Germany and Denmark found BDE-209 levels very similar to those found in this study, i.e. 1.76 and 1.71 ng/g lip respectively (27, 28), levels reported in Sweden or Greece were almost half of those found in this work (GM: 0.90 and 1.18 ng/g lip respectively (29, 30) Among the non-European countries also South Korea reported lower BDE-209 levels (GM: 0.91 ng/g lip) (31), although the levels found in Canada were up to 5 times higher than those reported in this work (32). Very few studies included DBDPE in the general population and the results showed low detection frequencies (below 10%) and slightly higher concentrations. These differences could be due to the significant lower LOD obtained with the method proposed which were three to ten times (33, 34). To our knowledge, this is the first time TBBPA-DBPE levels have been reported in human serum samples. These results and the detection frequency of these environmental contaminants which are present in products of daily use, confirm the importance of their inclusion in HBM programs.

Conclusions

An instrumental method based on GC-MS analysis has been developed and its suitability demonstrated for the determination of three high-brominated flame retardants (BDE-209, DBDPE and TBBPA-DBPE) in human serum once applied simple sample preparation procedure. Instrumental parameters optimization, as injection mode, length column or unusual high gas flow, allowed minimize compounds degradation in the chromatographic system and provided successfully recoveries (between 87 and 108%) and reproducibility (in general, RSD interday < 20%). The levels and number of samples above LOD observed in the present studied population, proved that the proposed procedure is suitable for the accurate determination of the target analytes at levels in expected general population and including for the first time TBBPA-DBPE analysis in humans. Therefore, the results demonstrated the need to have sensitive methods for the inclusion of these contaminants in HBM studies. In addition, this approach would allow evaluating time trends by HBM studies, the control of the presence of BDE-209, as well as the alternatives used for their replacement in general population and therefore the assessment of restrictive measures effectiveness implemented in recent years.

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References

1. Abbasi, G., Li L., & Breivik K. (2019) *Environ. Sci. Technol.* **53**, 6330–6340. doi: 10.1021/acs.est.8b07032
2. Gao, L., Li, J., Wu, Y., Yu, M., Chen, T., Shi, Z., Zhou, X., & Sun, Z. (2016) *Anal. Bioanal. Chem.* **408**, 7835–7844. doi: 10.1007/s00216-016-9887-z
3. Zota, A.R., Park, J.S., Wang, Y., Petreas, M., Zoeller, R.T., & T.J. Woodruff. (2011) *Environ. Sci. Technol.* **45,** 7896–7905. doi: 10.1021/es200422b
4. Gascon, M., Fort, M., Martinez, D., Carsin, A.E, Forns, J., Grimalt, J.O., Santa Marina, L., Lertxundi, N., Sunyer, J., & Vrijheid, M. (2012) *Environ. Health Perspect.* **120**, 1760–1765. doi: 10.1289/ehp.1205266
5. Directive 2002/95/EC of the European Parliament and of the Council of 27 January 2003 on the restriction of the use of certain hazardous substances in electrical and electronic equipment. (2003) *Official Journal of the European Union* **L37**, 19–23
6. Stockholm Convention, (2022) http://chm.pops.int/ (accesses 2 sep 2022)
7. Okeke, E.S., Huang B., Mao G., Chen, Y., Zhengjia, Z., Qian, X., Wu, X., & Feng, W. (2022) *Environ. Res*. **206**, 112594 doi: 10.1016/j.envres.2021.112594
8. Dong, L., Wang, S., Qu, J., You, H., & Liu, D. (2021) *Ecotoxicol. Environ. Saf.* **208**, 111570. doi: 10.1016/j.ecoenv.2020.111570
9. International Agency for Research on Cancer, IARC Monographs on the evaluation of carcinogenic risks to humans. (2000) *IARC Publications Website* **77**, 439–458. https://publications.iarc.fr/95 (accesses 2 sep 2022)
10. Global Monitoring Plan Stockholm Convention (2022) http://chm.pops.int/Implementation/GlobalMonitoringPlan/Overview/tabid/83/Default.aspx (accesses 2 sep 2022)

11. Covaci, A., Voorspoels, S., & de Boer, J. (2003) *Environ. Int.* **29,** 735–756. doi: 10.1016/s0160-4120(03)00114-4

12. Kalachova, K., Cajka, T., Sandy, C., Hajslova, J., & Pulkrabova, J. (2013) *Talanta* **105,** 109–116. doi: 10.1016/j.talanta.2012.11.073

13. Fromme, H., Hilger, B., Kopp, E., Miserok, M., & Voelkel, W. (2014) *Environ. Int.* **64** 61–68. doi: 10.1016/j.envint.2013.11.017

14. Lu, D., Jin, Y., Feng, C., Wang, D., Lin, Y., Qiu, X., Xu, Q., Wen, Y., She, J., Wang, G., & Zhou, Z. (2017) *Anal. Bioanal. Chem.* **409**, 5307–5317. doi: 10.1007/s00216-017-0476-6

15. Covaci, A., & Voorspoels, S. (2005) *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **827**, 216–223. doi: 10.1016/j.chromb.2005.09.020

16. Perez-Gomez, B., Pastor-Barriuso, R., Cervantes-Amat, M., Esteban, M., Ruiz-Moraga, M., Aragones, N., Pollan, M., Navarro, C., Calvo, E., Roman, J., Lopez-Abente, G., & Castano, A. (2013) *Environ. Sci. Pollut. Res.* **20,** 1193–1202. doi: 10.1007/s11356-012-1320-3

17. Bernert, J.T., Turner, W.E., Patterson, D.G., & Needham, L.L. (2007) *Chemosphere* **68**, 824–831. doi: 10.1016/j.chemosphere.2007.02.043

18. Ramos, J.J., Gomara, B., Fernandez, M.A., & Gonzalez, M.J. (2007) *J. Chromatgr. A* **1152**, 124–129. doi: 10.1016/j.chroma.2006.12.050

19. Roosens, L., D'Hollander, W., Bervoets, L., Reynders, H., Van Campenhout, K., Cornelis, C., Van Den Heuvel, R., Koppen, G., & Covaci, A. (2010) *Environ. Poll.* **158**, 2546–2552. doi: 10.1016/j.envpol.2010.05.022

20. Dodson, R.E., Perovich, L.J., Covaci, A., Van den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., & Rudel, R.A. (2012) *Environ. Sci. Technol.* **46**, 13056–13066. doi: 10.1021/es303879n

21. Björklund, J., Tollbäck, P., Hiärne, C., Dyremark, E., & Ostman, C. (2004) *J Chromatogr A.* **1041**, 201–210. doi: 10.1016/j.chroma.2004.04.025

22. Tollbäck, P., Björklund, J., & Ostman, C. (2003) *J Chromatogr A.* **991**, 241–253. doi: 10.1016/s0021-9673(03)00174-2

23. Cariou, R., Antignac, J.P., Debrauwer, L., Maume, D., Monteau, F., Zalko, D., le Bizec, B., & Andre, F. (2006) *J. Chromatogr. Sci*. **44**, 489–497. doi: 10.1093/chromsci/44.8.489

24. Stapleton, H.M. (2006) *Anal. Bioanal. Chem.* **386**, 807–817. doi: 10.1007/s00216-006-0400-y

25. Gómara B., Herrero, L., Pacepavicius, G., Ohta, S., Alaee, M., Gonzalez, M. J., (2011), Chemosphere **83(6)**, 799-805. doi: 10.1016/j.chemosphere.2011.02.080

26. Pirard, C., & Charlier, C. (2018) *Chemosphere* **211,** 918–925. doi: 10.1016/j.chemosphere.2018.08.012

27. Fromme, H., Becher, G., Hilger, B., Albrecht, M., Gries, W., Leng, G., & Völkel, W. (2016) *Int. J. Hyg. Environ. Health* **219**, 380–388. doi: 10.1016/j.ijheh.2016.03.003.

28. Frederiksen, M., Thomsen, C., Frøshaug, M., Vorkamp, K., Thomsen, M., Becher, G., & Knudsen, L.E. (2010) *Int. J. Hyg. Environ. Health* **213**, 233–242. doi: 10.1016/j.ijheh.2010.04.008

29. Darnerud, P.O., Lignell, S., Aune, M., Isaksson, M., Cantillana, T., Redeby, J., & Glynn, A. (2015) *Environ. Res.* **138**, 352–360. doi: 10.1016/j.envres.2015.02.031

30. Kalantzi, O.I., Geens, T., Covaci, A., & Siskos, P.A., (2011). *Environ. Int.* **37**, 349–353. doi: 10.1016/j.envint.2010.10.005

31. Kim, J., Kang, J. H., Park, H., Baek, S. Y., Kim, Y. H., & Chang, Y.S., *Environ. Poll.* **164**, 46–52. doi: 10.1016/j.envpol.2012.01.016

32. Health Canada, Report on Human Biomonitoring of Environmental Chemicals in Pooled Samples. (2020). https://www.canada.ca/content/dam/hc-sc/images/services/environmental-workplace-health/reports-publications/environmental-contaminants/report-human-biomonitoring-environmental-chemicals-pooled-samples/27-20-2746-01-human-biomonitoring-final-en.pdf (accesses 5 feb 2023)

33. Zhou, S.N., Buchar, A., Siddique, S., Takser, L., Abdelouahab, N., & Zhu, J., (2014) *Environ. Sci. Technol.* **48**, 8873–8880. doi: 10.1021/es5016839

34. Tay, J.H., Sellström, U., Papadopoulou, E., Padilla-Sánchez, J.A., Haug, L.S., & de Wit, C.A. (2019) *Environ. Res*. **178,** 108731. doi: 10.1016/j.envres.2019.108731

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Table 1. Volume injection optimization. Standard solution 10 ng/mL.

	Abundance		
Compound	**2 µL (splitless)**	**5 µL (LVI)**	**10 µL (LVI)**
BDE 209	76201	373226	802739
DBDPE	3624	24561	58702
TBBPA-DBPE	ND	1901	13827
ΣBDEs 206/207/208	7468	40713	58492
% degradation BDE-209	9.80	10.91	7.29

Table 2**.** Average recoveries and reproducibility (relative standard deviations, RSDs) of the NBFRs (n = 8, inter-day). LODs and LOQs (ng/mL) and coefficients of determination (r^2) for the BFRs analyzed.

Spiked level (ng/ml)	**BDE 209**	**DBDPE**	**TBBPA-DBPE**
0.01	97 (8.4)	93 (13.2)	102 (31.3)
0.05	92 (6.1)	87 (10.8)	105 (13.7)
0.2	91 (17.2)	87 (15.9)	108 (18.9)
LOD	0.0045	0.0069	0.0070
LOQ	0.0091	0.0131	0.0231
linearity r^2	0.998	0.996	0.993

Table 3~~Supplementary material, Table 2~~**:** Assigned values (ng/mL) and method values from the analysis of BDE-209 in twelve certified serum samples (4 rounds) from AMAP (2016 - 2017) using the optimized method.

Sample	**Method value**	**Assigned value (Acceptable range)**	**Recovery (%)**
		2016	
Sample 1	1.07	1.42 (0.834 – 2.01)	75
Sample 2	0.840	1.15 (0.639 – 1.66)	73
Sample 3	0.230	0.276 (0.135 – 0.417)	83
Sample 4	0.370	0.432 (0.234 – 0.630)	86
Sample 5	0.750	0.904 (0.528 – 1.28)	83
Sample 6	1.28	1.67 (0.930 – 2.41)	77
		2017	
Sample 7	1.08	1.06 (0.673 – 1.45)	102
Sample 8	0.137	0.172 (0.0787 – 0.265)	80
Sample 9	0.500	0.605 (0.358 – 0.852)	83
Sample 10	0.315	0.367 (0.201 - 0.533)	86
Sample 11	1.318	1.01 (0.598 – 1.42)	130
Sample 12	1.155	1.73 (1.05 – 2.41)	67

Table 4. Concentrations of BFRs selected (ng/g lipids) found in human serum samples (n=40) after applying the proposed method. Only samples above LOD were considered for arithmetical mean (AM) and geometrical mean (GM)

Sample	**BDE-209**	**DBDPE**	**TBBPA-DBPE**
M1	<LOD	<LOD	<LOD
M2	<LOD	<LOD	<LOD
M3	2.54	1.50	1.20
M4	1.08	<LOD	<LOD
M5	<LOD	<LOD	<LOD
M6	17.32	1.07	<LOD
M7	<LOD	1.48	<LOD
M8	<LOD	3.93	<LOD
M9	1.47	4.13	<LOD
M10	<LOD	<LOD	<LOD
M11	3.35	<LOD	0.89
M12	<LOD	<LOD	<LOD
M13	1.21	<LOD	<LOD
M14	0.70	2.78	<LOD
M15	<LOD	<LOD	1.57
M16	0.89	<LOD	7.32
M17	<LOD	3.85	0.77
M18	<LOD	1.47	<LOD
M19	<LOD	1.31	1.31
M20	1.86	5.76	<LOD
M21	3.82	9.86	<LOD
M22	<LOD	<LOD	<LOD
M23	<LOD	1.70	<LOD
M24	<LOD	<LOD	<LOD
M25	2.91	<LOD	<LOD
M26	1.42	<LOD	<LOD
M27	1.82	<LOD	<LOD
M28	1.89	<LOD	<LOD
M29	<LOD	<LOD	<LOD
M30	<LOD	<LOD	3.96
M31	< LOD	1,53	< LOD
M32	1,83	< LOD	< LOD
M33	1,17	< LOD	< LOD
M34	1,26	3,90	< LOD
M35	0,80	< LOD	0,91
M36	<LOD	<LOD	<LOD
M37	1,49	1,67	< LOD
M38	4,34	< LOD	< LOD

M39	<LOD	<LOD	<LOD
M40	2,62	1,54	< LOD
<LOD	47%	60%	80%
AM	2,66	2,97	2,24
GM	1,89	2,40	1,61
Max	17.32	9.86	7.32