Summary

The third round of interlaboratory comparison on the determination of the 2,3,7,8-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) as well as dioxin-like non-ortho and mono-ortho chlorinated biphenyls (PCBs) in three food items was conducted in 2002. The objectives were to assess the in-between laboratory reproducibility, to offer a quality assurance instrument for the participating laboratories and to assess the readiness of expert laboratories world-wide to determine low levels of dioxins and dioxin-like PCBs in food.

The 2002 study was performed on samples of tuna filet, pork meat and egg yolk. In addition, three standard solutions were provided containing known concentrations of a) PCDDs/PCDFs, b) non-ortho PCBs and c) mono-ortho PCBs. Fifty-three (53) participating laboratories from 19 countries received the testing materials in February 2002 and results were returned from 46 laboratories by the deadline in May. Most laboratories participated in all three food items. This final report, made available as a pdf-file on the web in June, has been discussed among the participants at a consultation meeting during the Dioxin 2002 Symposium on August 13 in Barcelona, Spain.

This final report presents all the results reported from the participating laboratories for the 29 analytes assigned a toxic equivalency factor by the WHO, all seventeen 2,3,7,8-substituted PCDDs and PCDFs, the non-ortho substituted PCBs #77, 81, 126 and 169, and the eight mono-ortho substituted PCBs #105, 114, 118, 123, 156, 157, 167, 189, in the three food items on a fresh weight and lipid weight basis. Assigned values for the analytes were determined by the participant consensus technique using simple statistics. Non-detected congeners were assigned a concentration corresponding to the reported detection limit. The consensus values for each analyte in the three food samples were determined as follows: The median of all reported concentrations for each analyte was calculated. All values above two times of the median were then removed from the calculation. The consensus median and mean plus standard deviation were calculated from the remaining data. TEQs were calculated from the consensus values of individual congeners using the WHO-derived toxic equivalency factors. Z-scores were calculated for each laboratory's result of PCDD/PCDF TEQ using a deviation of ±20% of the consensus TEQs.

The consensus value for the standard solutions were calculated in the same manner except that values outside ± 50% of the median of all values were removed prior to final calculation of the consensus median and mean.

The consensus values for the lipid content were calculated by first excluding results deviating more than two standard deviations (± 2 SD1) from the mean of all values and then re-calculating the median, mean and standard deviation.

Two of the samples, tuna filet and pork meat, showed low levels of contamination with dioxins and dioxin-like PCBs. It was therefore a major challenge to detect many of the congeners. Consequently, only laboratories with very low detection limits and no contamination problems are assumed to have presented results with z-scores within ±2. For tuna filet, these were 38% and for pork meat 43% of the laboratories. For the egg yolk sample, 71% of the laboratories reach this goal.

Recent reports from several countries show decreasing levels of dioxins and dioxin-like PCBs in general foodstuffs. Further, when determining intake of dioxin-like compounds from various low contaminated but high consumption foods and performing cumulative exposure assessments, it is of course better to work with measurable, though low levels, than to handle the uncertainties associated with “less than” values. This implies that there is an increasing requirement to the laboratories to improve the sensitivity of their analytical methods in order to be able to report measured values in foods of decreasing contamination with dioxin-like compounds.

Introduction

In order to ensure consumer protection and reduce human exposure to dioxins through food consumption, the Commission of the European Communities recently issued maximum levels for dioxins in foodstuffs as well as feeding stuffs. In addition, action levels will be introduced as a tool for competent authorities and operators to highlight those cases were it is appropriate to identify a source of contamination and to take measures for its reduction or elimination, i.e., when significant levels of dioxins above background level are found in foodstuffs and feedingstuffs. So far, these limit levels are set for dioxins only, not including the dioxin-like PCBs, given the very limited data available on the prevalence of the latter. A need was therefore identified to generate reliable data not only on the presence of dioxins, but especially of dioxin-like PCBs in a wide range of foodstuffs and feeding stuffs in order to obtain a reliable database. Accordingly, Member States are requested to perform frequent monitoring of the presence of dioxins and dioxin-like PCBs in food and feed.

There is a large demand for chemical laboratories able to monitor these contaminants at low levels in food and feed. Such analyses require high quality standards using highly selective and sensitive analytical techniques and validated procedures, accreditation by recognised bodies and successful participation in interlaboratory comparisons or proficiency testing. So far, only a few interlaboratory comparisons on the measurement of dioxins and dioxin-like PCBs in foods are available.

This study is the third round of a world-wide interlaboratory comparison study on dioxin-like compounds in food and has been organised by the Department of Analytical Chemistry, Division of Environmental Medicine, Norwegian Institute of Public Health in Oslo, Norway.

The exercise took place from February 2002, when the samples were shipped to the laboratories for analysis, to beginning of May 2002, when the last reports on the results were received. This final report is available to the participants on the web (http://www.fhi.no) in July and has been discussed during a consultation meeting at the Dioxin 2002 Symposium on August 13 in Barcelona, Spain.

The main objective of this exercise was to assess the in-between laboratory reproducibility of dioxin-like compounds analyses in frequently consumed foods with background contamination. It also serves as a QA/QC instrument for each participating laboratory to contribute to its proficiency. A further objective has been to assess the world-wide readiness and capacity of dioxin analyses of food. All of the participants from the two previous rounds of "Interlaboratory Comparisons on Dioxin in Foods" were invited to participate. In addition, several other laboratories announced their participation. There was no limit to the total number of participating laboratories. The 46 laboratories that submitted results, and thereby contributed to the actual study results, are presented in Table 1. Complete affiliations and addresses of all participants in Dioxins in Food 2002 are given in Appendix A.

Table 1. Participants that reported results in the Third Round of Interlaboratory Comparison on Dioxins in Food 2002.

AgriQuality Environmental Lower Hutt, New Zealand	GfA Gesellschaft für Arbeitsplatz- und Umweltanalytik Münster, Germany	Norwegian Institute of Public Health Oslo, Norway
Analytical Solutions Rio De Janeiro, Brazil	Health Canada Bureau of Chemical Safety, Ottawa, Canada	Oekometric GmbH Bayreuth, Germany
Australian Government Analytical Laboratories Pymble, NSW, Australia	Health Canada Health Products and Food Branch Food Directorate Scarborough, Ontario, Canada	RIKILT Wageningen, The Netherlands
AXYS Analytical Services Ltd. Sidney, B. C., Canada	Institut Quimic de Sarria Environmental Laboratory Barcelona, Spain	RIVM/LOC Bilthoven, The Netherlands
Canadian Food Inspection Agency Calgary, Alberta, Canada	Istituto Superiore di Sanità Lab TCE Roma, Italy	Scientific Analysis Laboratories Ltd. Manchester, United Kingdom
CARSO Lyon, France	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" Teramo, Italy	Scientific Institute of Public Health Brussels, Belgium
CART-ULG Liège, Belgium	Japan Food Research Laboratories Tama-City, Japan	SGS Controll-GmbH Wismar, Germany
Central Science Laboratory York, United Kingdom	Laboratori de Dioxines Dept. de Ecotechnologies, IIQAB-CSIC Barcelona, Spain	The District of Public Health Department- Chemical Laboratory Frydek Mistek, Czech Republic
Chemisches und Veterinärunter-suchungsamt Freiburg, Germany	Micropollutants Technologie Thionville, France	TNO Environment Energy and Process Innovation Apeldoorn, The Netherlands
Consorzio Interuniversitario Nazionale la Chimica per l'Ambiente Marghera (Venezia), Italy	MPU Mess- und Prüfstelle Technischer Umweltschutz Berlin, Germany	Triangle Laboratories, Inc. Durham, NC, USA
Department of Chemistry National Tsing Hua University Taiwan, Republic of China	National Institute of Environmental Analysis and Environmental Protection Chung Li City, Taiwan, Republic of China	TÜV Süddeutschland Donzdorf, Germany
Department of Environmental and Occupational Health Tainan, Taiwan, Republic of China	National Public Health Institute Laboratory of Chemistry Kuopio, Finland	U.S. Environmental Protection Agency Stennis Space Center MS, USA
Dr. Wessling Laboratorien GmbH Altenberge, Germany	National Veterinary School of Nantes LABERCA Nantes, France	Umeå University Umeå, Sweden
Eco-Center Bolzano, Italy	Norwegian Institute for Air Research (NILU) Kjeller, Norway	Unilever R&D Colworth Sharnbrook, Bedfordshire, United Kingdom
ERGO-Forschungsgesellschaft mbH Hamburg, Germany		VITO Mol, Belgium
Federal Environment Agency Vienna, Austria		Wellington Laboratories Guelph, Ontario, Canada

Design and practical implementation

Study design

As in the previous rounds of this interlaboratory comparison study on dioxin-like compounds in food, the test material chosen represented naturally contaminated food samples. The analytes to be determined by each participating laboratory were all seventeen 2,3,7,8-substituted PCDDs and PCDFs, the four non-ortho substituted PCBs #77, 81, 126 and 169, and the eight mono-ortho substituted PCBs #105, 114, 118, 123, 156, 157, 167, 189. Analysis should be performed using the laboratory's own methods for sample preparation and instrumental analysis, their own standards and quantification procedures, and their own method for lipid determination.

It was recommended that laboratories report as many as possible of all 2,3,7,8-substituted PCDDs/ PCDFs and dioxin-like PCBs. The report should include the determined lipid percent for the test samples. Also the actual sample and lipid intake (g) for each determination should be reported. For each sample, laboratories should report one concentration on fresh and lipid weight basis for each congener which is detected (S/N ≥3) as well as the level of determination (LOD, S/N =3). Non-detected congeners (S/N <3) should be marked "N.D." in the Comments column of the Report form.

In addition, three standard solutions containing known concentrations of a) seventeen 2,3,7,8-substituted PCDDs/PCDFs, b) four non-ortho PCBs and c) eight mono-ortho PCBs were to be analysed using the laboratory's own quantification standards and methods, and the results had to be reported.

The test material consisted of tuna filet, pork meat, and egg yolk, representing samples with background contamination. The laboratory could choose to participate in analysing one, two or all three of the food samples.

Collection, preparation, and distribution of samples

The food items were chosen to represent background contamination levels in regular foods. The test materials consisted of three non-fortified natural products. Filet from Mediterranean tuna was purchased at a store in Oslo, ground pork meat was obtained from a butcher in Northern Jutland, Denmark and egg yolk was obtained from the Norwegian egg and poultry supplier co-operation Prior Norway, Oslo.

Homogeneity of the tuna filet and ground pork meat was obtained by repeatedly grinding portions of the food item in a meat grinder and homogenizing this portions in a mixer. Egg yolk was homogenised using the mixer only. Sub-samples of at least 150 g tuna filet (T), 150 g pork meat (P) and 50 g egg yolk (E) were placed into carefully cleaned screw-cap glass bottles and stored at –20 °C until shipment. The frozen samples were shipped to the participating laboratories marked as test material T, P and E.

Reporting and handling of data

Detailed instructions for participants and Excel report forms were sent out to the participants together with the samples in February 2002. For each analyte in each sample, participants were requested to report a single value for the concentration or indicate non-detected congeners by "N.D.". In addition, detection limits had to be given for each analyte. Concentrations were to be reported both on lipid and on wet weight basis including the lipid content of the sample. Additionally, the concentrations of each analyte in the three standard solutions, determined by the laboratories' own quantification standards and methods, had to be reported.

Each participating laboratory was given a code number by the co-ordinators. Participants had access to their own code only and laboratory codes were not revealed to third parties.

On receipt by the co-ordinators, the raw data from the laboratories were entered into a database. The draft final report was generated and made available to all participants on the web in June 2002. The draft of the final report was discussed at a consultation meeting at Dioxin2002 on August 13 in Barcelona, Spain

Statistical analysis

In the context of the last round of interlaboratory comparison "Dioxins in Food 2001" organised by the NIPH, the question arose of how to treat non-detected congeners (NDs) when determining the consensus from participant laboratories and calculating the toxic equivalent levels. It was clear that the relative standard deviation of the consensus mean could be unacceptably high and spoil the trueness of the assigned value when including data of insufficient quality. It could be reasonable to exclude all NDs as any value assigned the NDs is inevitably subjected to a higher uncertainty compared to the concentrations of the detected compounds. However, at low contamination levels, the resulting TEQ consensus value would most probably overestimate the true value, due to many low NDs. Furthermore, the consensus value may be based on a very small number of measured values. We therefore chose to include the NDs using the reported detection limits, because at low levels, the reported congeners are not proven to be present in the sample, they could actually be a result of sample contamination.

The resulting data set for each of the food samples appeared to be not normally distributed, with several laboratories reporting very high values either as a result of insufficient detection limits (high NDs) or a contamination problem (high blank). In order to avoid an unreasonable overestimation of the true value, medians were used and values above two times the median were not included in the further calculation.

Hence, congener-by-congener medians were calculated from food sample data of all reporting laboratories using the detection limit as concentration for non-detected congeners (upper bound concentration). Outliers were defined as those values above two times the median of all values and were removed from the data set. The consensus values were defined as the median of the remaining data for each congener. In addition, the consensus mean and standard deviation (SD) were calculated from this data set for each congener. Those congener data which had been removed prior to consensus calculation are marked in the tables presenting the individual results.

For the standard solutions, outliers were defined as those values outside ± 50% of the median of all reported values. Consensus median, mean and SD were calculated from the remaining data. The consensus of the lipid content was calculated as the mean after removal of values outside ± 2SD.

Not all laboratories determined all the 29 analytes and therefore the number of data used for the consensus between different congeners varies.

Toxic equivalents (TEQ) were calculated from the consensus values for three groups of analytes, PCDDs/PCDFs, non-ortho PCBs, and mono-ortho PCBs, using the toxic equivalency factors derived by WHO in 1998 (see Table below). As the detection limit was used for the concentration of non-detects, these TEQs represent upper bound concentrations.

Z-scores for PCDD/PCDF TEQs were calculated for each laboratory according to the following equation:

$$z = (x - X)/\sigma$$

where x = reported value; X = assigned value (consensus); σ = target value for standard deviation. A σ target value of 20% of the consensus was used, i.e. z-scores between +1 and –1 reflect a deviation of ± 20% from the consensus value.

The final report and certificate

The draft of the final report was prepared by members of the co-ordination group in May and June 2002. Opportunity was given to discuss the draft at the consultation meeting at the Dioxin 2002 Symposium on August 13 in Barcelona, Spain.

In the present report, the participants are presented in the tables and figures by their laboratory codes. Each laboratory has access to its own code only and the codes are not revealed to third parties. A certificate, stating the participant's code, is sent to each participant contributing to the results together with the printed report in the autumn 2002. Further copies of the report may then be ordered from the co-ordinators for a fee covering printing and mailing costs.

Co-ordination

The study was initiated and carried out by the Department of Analytical Chemistry, Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway. Members of the co-ordination committee were:

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Results

Results are presented in the chapters below. A participating laboratory will be able to compare its performance congener by congener with the other laboratories. Since variations in performances are based on several factors, it is recommended that each laboratory carefully evaluates the factors that, favourably or unfavourably, have contributed to its performance. A general reader of the report, who has no access to the laboratory codes, will be able to get a picture of the analytical performance of laboratories world-wide for determining dioxins in foods.

Presentation in the report

Fourty-six laboratories from 19 countries submitted results. A summary of the results is presented in the chapters below, including the consensus statistics on fresh and lipid weight basis for concentrations and TEQ values of individual congeners, total TEQ values for each laboratory, and the z-score plots based on a target deviation of ±20%. The results of the lipid determinations are presented as well as the sample weight used by each laboratory. Finally, individual results reported by the laboratories for concentrations for each congener are given for tuna filet, pork meat and egg yolk.

Summarising comments on results

Analyte solution

For the three analyte solutions, 38 laboratories had reported concentrations for PCDDs/PCDFs, 33-36 laboratories for non-ortho PCBs, and 33-34 laboratories for mono-ortho PCBs. Even for the standard solutions with known concentrations, for each congener 1-3 reported values were outside two times the median of all values and were removed as outliers. The average relative standard deviation (RSD) for the 17 PCCD/PCDF congeners was 13% ranging from 8% for 2,3,4,6,7,8-HxCDF to 20% for TCDF. The average RSD for both non-ortho and mono-ortho PCBs was 15%. The calculation of z-scores for the TEQs (mean 13.8 pg/µl) of the PCDD/PCDF standard solution shows that 3 of the 38 laboratories have z-scores outside the range of a target ±20%, and as many as 10 are outside ±10%.

This may be regarded as a high number, as there are no interferences of sample matrix components in this determination. The results should stimulate several of the laboratories to carefully check their calibration standards.

Tuna filet

Forty-one (41) laboratories had determined PCDDs/PCDFs in tuna filet. The content of PCDDs/ PCDFs was unexpectedly low, and therefore the number of non-detects very high for many congeners, e.g., 32 laboratories were not able to detect 2,3,7,8-TCDD. The five major contributors to the PCDD/PCDF TEQ per gram fresh weight were (RSD given in parentheses): 1,2,3,7,8-PeCDD (58%); 2,3,4,7,8-PeCDF (40%); 2,3,7,8-TCDD (74%); 2,3,7,8-TCDF (40%); 1,2,3,6,7,8-HxCDD (60%) constituting 79% of the PCDD/PCDF TEQ.

PCBs were determined by 33 to 37 laboratories, and many more congeners were detected compared to the PCDDs/PCDFs. The four major contributors to the PCB TEQ on fresh weight basis were (RSD): PCB-169 (31%), 126 (41%), 118 (30%), 156 (36%) constituting 98% of the PCB-TEQ.

The total TEQ consensus value for tuna filet was 0.055 ppt on fresh weight and 2.5 on lipid weight basis. The contribution of the three groups of analytes to the total TEQ on fresh weight basis was 33%, 61% and 6% for PCDDs/PCDFs, non-ortho PCBs and mono-ortho PCBs, respectively. This confirms that dioxin-like PCBs make a dominating contribution to the total TEQ in marine fish.

The presentation of the z-scores for total PCDD/PCDF TEQ shows that many laboratories have reported too high values. Only 8 laboratories were within ±20% and 16 within ±40% of the consensus value for PCDD/PCDF TEQ on fresh weight basis, being as low as 0.018 ppt. In order to get an indication of the uncertainty in this PCDD/PCDD consensus TEQ, the relative standard deviation (RSD) of this consensus TEQ was calculated from the square root of the sum of squared products of standard deviations for individual congeners and the respective TEF. The RSD for the PCDD/PCDF TEQ of tuna filet on fresh weight basis was 29%. When including dioxin-like PCBs the RSD of total TEQ dropped to 18%.

The mean consensus of the reported lipid content was 2.1% with a relative standard deviation of 18.4%. Results from 6 laboratories were excluded from the consensus calculation.

Pork meat

For the pork meat sample, 40 laboratories determined PCDDs/PCDFs. Again, the dioxin content was low giving rise to many non-detected congeners. The five major contributors to PCDD/ PCDF TEQ on fresh weight basis were (RSD in parentheses): 1,2,3,7,8-PeCDD (53%); 2,3,4,7,8-PeCDF (53%); 2,3,7,8-TCDD (51%); 1,2,3,4,7,8-HxCDF (47%); 1,2,3,6,8,9-HxCDD (54 %) constituting 80% of the PCDD/PCDF TEQ. The PCDD/PCDF consensus TEQ on fresh weight basis was 0.035 with a RSD of 23%. The calculated RSD of total TEQ was 17%.

PCB values were reported by 31to 36 laboratories. The major contributors to the PCB TEQ on fresh weight basis were (RSD in parentheses): PCB-126 (39%), 156 (29%), 118 (29%), 157 (36%) constituting 93% of the PCB TEQ.

The total TEQ consensus for pork meat was 0.054 ppt (fresh weight) and 0.24 ppt (lipid weight) to which the contributions were 65%, 19% and 16% from PCDD/PCDF, non-ortho and mono-ortho PCB, respectively. This pattern with a dominance of PCDDs/PCDFs contributing to the total TEQ seems to be typical for food produced from terrestrial animals.

As for the tuna filet sample, only a few laboratories (7) with very low detection limits and no blank problems had z-scores for fresh weight results ±1 and 17 had z-scores between +2 and –2.

The consensus for the lipid content was 19.9% with a relative standard deviation of 12%.

Egg yolk
For this sample, PCDDs/PCDFs were reported by 42 laboratories. The five major contributors to the PCDD/PCDF TEQ on fresh weight basis were (RSD in parentheses): 2,3,4,7,8-PeCDF (28%); 1,2,3,7,8-PeCDD (35%); 2,3,7,8-TCDF (25%); 2,3,7,8-TCDD (37%); 1,2,3,4,7,8-HxCDF (28%) constituting 86% of the PCDD/PCDF TEQ. The PCDD/PCDF consensus TEQ on fresh weight basis was 0.035 with a RSD of 14%. When including dioxin-like PCBs, the RSD of total TEQ was almost equal with 12%.

PCBs were reported by 34 to 38 laboratories. The congeners contributing most to the PCB TEQ on fresh weight basis were (RSD in parentheses): PCB-126 (25%), 118 (23%), 156 (26%), 105 (22%) constituting 94% of the PCB TEQ.

The total TEQ based on the consensus concentrations was 0.53 ppt on fresh weight and 2.0 ppt on lipid weight basis, and PCDDs/PCDFs, non-ortho and mono-ortho PCBs contributed 40%, 42%, 18% to this total TEQ, respectively.

For 25 laboratories (60%), the calculated z-scores for PCDD/PCDF TEQs were between ±1 at a consensus level of 0.21 ppt while for 30 laboratories (71%) the z-scores were within ±2.

Being typical for egg yolk, a high lipid content was determined with a consensus of 25.8% with a relative standard deviation 8.5%.

Conclusions

In this third round of interlaboratory comparison exercise two of the samples, tuna filet and pork meat, showed low contamination levels of dioxins and dioxin-like PCBs. It was therefore a major challenge to detect many of the congeners. Consequently, only laboratories with very low detection limits and no contamination problems are assumed to have presented results with z-scores within ±2. For tuna filet, these were 38% and for pork meat 43% of the laboratories. For the egg yolk sample, 71% of the laboratories reach this goal.

Using the median of all values and removing reported values above 2 times the median, seems to give a good estimate of the true value for low contaminated samples where a considerable number congeners are non detected.

Surprisingly, several laboratories do not show a sufficient analytical performance for the determination of the analytes in standard solutions with known concentrations.

In this third round, the majority of laboratories also determined dioxin-like non-ortho and mono-ortho PCBs. The importance to determine these compounds in food is demonstrated by their large contribution to the total TEQ especially in food from the marine environment.

Recent reports from several countries show decreasing levels of dioxins and dioxin-like PCBs in general foodstuffs. Further, when determining intake of dioxin-like compounds from various low contaminated but high consumption foods and performing cumulative exposure assessments, it is of course better to work with measurable, though low levels, than to handle the uncertainties associated with “less than” values. This implies that there is an increasing requirement to the laboratories to improve sensitivity of the analytical methods in order to be able to report measured values in foods of decreasing contamination with dioxin-like compounds.

Acknowledgements

The participating laboratories are acknowledged for their participation in this interlaboratory comparison and their interest in its overall objectives, thereby making it clear that they value good analytical performance. All the individual analysts are acknowledged for their contributions to the results.