Analysis and Quality Control of WHO- and UNEP-Coordinated Human Milk Studies 2000–2019: Organochlorine Pesticides and Industrial Contaminants

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Abstract

The analytical method for determination of nonpolar organochlorine pesticides and industrial contaminants in human milk comprises extraction of lipids, the use of three internal standards, two chromatographic separation steps for clean-up and gas chromatography on various columns of different polarity and different detectors (GC-ECD, GC-MS/MS). The more polar analytes were determined applying the QuEChERS method and HPLC-MS/MS measurement.

As accredited laboratory since 1998, a comprehensive quality control programme was applied to prove the long-term reliability of results of the WHO/UNEP-coordinated surveys on human milk performed between 2000 and 2019. The concept comprised numerous quality control samples (spiked samples for quality control charts and left-over samples from proficiency tests as in-house reference material) and participation in 53 proficiency tests covering test samples of food of animal origin, plant oils and standard solutions. Trueness calculated from the spiked quality control samples and expressed as median recovery for 29 analytes was 99% (range 86%–110%), and the precision expressed as the median of the coefficient of variation (CV) was 12% (range 0.7%–31%). Trueness calculated from in-house reference material (375 results for 41 consensus values) was on average 96% with a precision expressed as CV of 7.6. Finally, the results of 53 proficiency tests were used to derive trueness for 23 analytes as deviation from the assigned value; this deviation was on average 14% (range 6%–27%). As conclusion of various approaches of analytical quality control, for the 2000–2019 period the long-term trueness was on average in the range 85%–95% with a precision on average better than 15%.

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The general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025, the analytical criteria for analyses of organochlorine pesticides and industrial contaminants in feed and food required by EU legislation and the criteria for monitoring information for the Parties to the Stockholm Convention were met.

Keywords

Analysis · Organochlorine pesticides · Organochlorine industrial contaminants · Long-term quality control · Trueness · Precision · Human milk · Global WHO/UNEP studies

1   Introduction

In a series of articles in this compendium, an overview of the World Health Organization (WHO) and United Nations Environment Programme (UNEP)-coordinated exposure studies on persistent organic pollutants (POPs) in human milk and their link to the Stockholm Convention on POPs is given in the introduction (Part I) (Malisch et al. 2023a). Part II presents the analytical methods, Part III the results and discussion, including for organochlorine pesticides and industrial contaminants (Malisch et al. 2023b), Part IV the assessments, including time trends for selected organochlorine pesticides (Malisch et al. 2022) and Part V the summary and conclusions.

The references used for the review on findings of POPs in human milk in Part I (Fürst 2023) show a wide range of analytical methods used for the determination of organochlorine pesticides and industrial contaminants. For official food control in Germany, a multi-method for determination of lipophilic pesticide residues in food of animal origin was of particular interest, which was established in the 1980s (DFG S19; Specht et al. 1995). Over time and after successful interlaboratory studies this method was adopted as a European Norm (European Norm (EN) 1528) and included in the collection of official methods in Germany (German Standard § 64 LFBG a). The method for determination of nonpolar pesticide residues in cow's milk can be applied for human milk, as well. For more polar analytes, the QuEChERS-multimethod was established (Anastassiades et al. 2003), which was further enhanced to cover a broad range of matrices (Anastassiades 2006) and later included in the collection of official methods in Germany for determination in food of animal origin, as well (German Standard § 64 LFBG b).

In this chapter, the analytical methods for the determination of organochlorine pesticides (and their relevant metabolites) aldrin, chlordane, chlordecone, dicofol, DDT, dieldrin, endosulfan, endrin, heptachlor, hexachlorobenzene, hexachlorocyclohexanes, mirex, pentachlorophenol/pentachloroanisole and toxaphene and of the industrial chemicals hexachlorobutadiene and pentachlorobenzene are presented, including the internal and external quality control.

2   Materials and Methods

The protocols for collection of samples and a general overview on participating countries with regional distribution and temporal differentiation are given in Part I (Malisch et al. 2023a). After collection of a large number of individual samples fulfilling protocol criteria, pooled samples were prepared considered to be representative for a country or subpopulation at the time of the sampling. A total of 82 countries submitted pooled samples during one or more of the five studies conducted from 2000 to 2019. 163 pooled samples were analysed for organochlorine pesticides and industrial chemicals (Malisch et al. 2023b), applying analytical methods described in this article.

2.1   Determination of Nonpolar Substances According to EN1528 (DIN EN 1528-1:1997-1-4)

For the determination of residues of nonpolar organochlorine pesticides and contaminants, a method based on the European Norm 1528 was used, which is also part of the official collection of test methods in Germany (European Norm (EN) 1528; German Standard § 64 LFGB a). It is based on a modular structure and covers a wide range of analyte-matrix combinations (suitable for food of animal origin and human milk).

After centrifugation at 2000 g for 10 min and separation of the cream, lipids were extracted with nonpolar solvents. The supernatant cream layer was transferred into a glass beaker. By stirring, sodium sulfate was added to the cream until the mixed material was of powdery consistence. This powder was then extracted 3–4 times with *n*-hexane by thorough stirring with a glass rod and filtering the extract. The solvent was evaporated to give the extracted lipids.

As internal standards, PCB 28 (2,4,4′-trichlorobiphenyl), triphenylphosphate and PCB 209 (decachlorobiphenyl) were used. After addition of the internal standards, up to 0.5 g lipids were separated by gel chromatography using polystyrene gel (Bio-Beads S-X3; column length 740 mm, 20 mm i.d., filling level 500–500 mm; cyclohexane/ethyl acetate mixture [1:1, v/v] used as eluent). After concentration to give a volume of about 2–3 mL, the eluate was further concentrated with iso-octane added and evaporated to about 1 mL. In order to completely remove the ethyl acetate additional isooctane was added and the mixture re-concentrated.

Chromatography on a small column of partially deactivated silica gel was performed as the final clean-up step. Silica gel (70–230 mesh) was heated overnight at 130 °C and allowed to cool in a desiccator. After adding 1.5% of water, it was shaken for 30 min and then stored in a tightly sealed container. The glass chromatographic column (length 365 mm, 10 mm i.d.) was packed with 1 g of deactivated silica gel. After pre-washing with 2x5 ml hexane, the isooctane solution was loaded onto the silica column and the analytes of interest were eluted by 10 ml toluene.

The toluene fraction was evaporated to a small volume (avoiding complete dryness) and immediately taken up with cyclohexane to the intended final volume for determination by various gas chromatographic (GC) methods using capillary GC with Electron Capture Detector (ECD) and tandem mass spectrometry (MS/MS). GC-ECD measurements were performed on various instruments, including, e.g., Trace Ultra Thermo and Trace 1310 GC Thermo, with injection on two capillaries of different polarity in parallel (PS088 and DB1701). GC-MS/MS triple quadrupole mass spectrometers were used for confirmation (e.g. Chromtech Evolution GC-MS/MS, Agilent 7000 TQ GC-MS/MS) with chromatography on HP-5MS (Ultra inert 30 m, 0.25 mm i.d., 0.25 μm film thickness).

2.2 Analysis of Chlordecone and Pentachlorophenol According to the QuEChERS Method

The more polar analytes, chlordecone and pentachlorophenol *(pentachloroanisole as recommended analyte in human milk was determined by the above presented method for nonpolar substances),* were determined by the QuEChERS (**Qu**ick **E**asy **Ch**eap **E**fficient **R**obust and **S**afe) method (Anastassiades et al. 2003; Anastassiades 2006). Initially the method was developed for food of plant origin. Later the method was expanded to food matrices of animal origin like meat, egg and milk, as well (German Standard §64 LFGB b).

10 g of sample were weighed into a 40 mL centrifuge tube, fortified with internal standard (^{13}C-labelled chlordecone and ^{13}C-labelled pentachlorophenol (PCP)) and 10 mL of acetonitrile was added. By vigorous shaking, the analytes were extracted in a single-phase extraction. After addition of salts (4 g magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate) for separation of water and buffering, the mixture was shaken vigorously again and centrifuged. The acetonitrile phase was separated.

Removal of residual water and clean-up were performed simultaneously by solid phase extraction (SPE) using 25 mg PSA (*P*rimary *S*econdary *A*min) per mL acetonitrile extract and 150 mg magnesium sulfate per mL acetonitrile extract ("dispersive SPE"). After shaking and centrifugation, 1 mL of the extract was concentrated by blowing down with nitrogen to 0.25 mL final volume.

The pesticide residues contained in this solution were determined by high performance liquid chromatography coupled with tandem mass selective spectrometry detector (HPLC-MS/MS in ESI negative mode) (Agilent TQ 6490) using a C18 column (length 2.1x50 mm, 1.8 μm particle size) and a gradient of water/acetonitrile for chlordecone and of 0.005% acetic acid/acetonitrile for PCP.

3   Analytical Criteria

Regulation (EC) No 396/2005 sets Maximum Residue Levels (MRLs) for residues of pesticides in feed and food, including a number of banned active substances, e.g. certain organochlorine pesticides (Regulation (EC) No 396/2005). The methods of analysis for determination of pesticide residues shall comply with the criteria set in provisions of Community law for official controls of food and feed. Technical guidelines describe the method validation and analytical quality control (AQC) requirements to support the validity of data (DG-SANTE 2021). This document is updated on a biennial basis. The procedures and criteria of these "Pesticide Guidelines" were applied also for analysis of the human milk samples of the WHO/UNEP-coordinated exposure studies. In addition, the recommendations of the guidance on the Global Monitoring Plan for POPs on analytical methodology and quality control were met (UNEP 2019).

4   Quality Control

Accuracy is a function of systematic errors and random components. A comprehensive quality control programme was applied to prove the long-time reliability of results over many years, for most parameters between 2000 and 2019. With each series of samples, a chemical blank value and at least one quality control sample (fortified "blank sample" of an uncontaminated pork fat matrix) were included. Possible systematic errors were checked by participation in proficiency tests of any kind of matrix and by analysis of surplus material of plant and animal origin oil remaining from proficiency tests. This validation should guarantee maximum accuracy and is part of the general quality control programme applied in the daily routine for analysis of all kinds of samples, as required for an accredited laboratory for the official food control. Results of the quality control samples, proficiency tests and remainder material samples are presented in the following.

4.1   Quality Control Samples

The comprehensive quality control programme included fortified samples of an uncontaminated pork fat matrix at various concentration levels, which were monitored in quality control charts. Furthermore, remainder material from proficiency test material of various oils of plant and animal origin with consensus values for organochlorine pesticides was used for quality assurance in routine analyses. Analyses were performed by different operators using different chemicals over a long time and data collected in separate runs—therefore, these quality control data collected under intermediate conditions are much more robust than data from a single validation when one technician performs repeated analyses under the same conditions using the same chemicals in one sequence.

According to the "pesticides guidelines" (DG-SANTE 2021), acceptable limits for individual recovery results should usually be within the range of the mean recovery +/− 2x RSD. Mean recoveries from initial validation should be within the range 70–120%. A practical default range of 60–140% (control level range) may be used for individual recoveries in routine analysis. In principle, recoveries outside this default range of 60–140% would require re-analysis of the batch, but the results may be acceptable in certain justified cases. For example, if the individual recovery is unacceptably high and no residues are detected, it is not necessary to re-analyse the samples to prove the absence of residues. However, consistently high recoveries or RSDs outside ±20% must be investigated. In practise, as requested by DIN EN ISO/IEC 17025 for accredited laboratories (European Norm (EN) 17025), root cause analysis and corrective measures were initiated and further quality assurance measures were taken (e.g. repeated analyses with standard addition), when the mean recovery +/− 1x RSD was outside the range 70–120% (warning level range). The results of the quality control charts for the analytes of interest are shown in the following to illustrate the extensive compliance with the acceptable limits and exceedances in few cases.

### 4.1.1 Nonpolar Substances (Method EN1528; Spiked Samples)

Results of the quality control charts obtained over many years from uncontaminated spiked pork fat matrix samples show the reliability of the determination of the nonpolar organochlorine analytes determined by the DIN EN 1528-1:1997-1-4 method (European Norm (EN) 1528) for the pesticides aldrin, chlordane, DDT, dicofol, dieldrin, endosulfan, endrin, heptachlorepoxideee, hexachlorobenzene, hexachlorocyclohexanes, mirex and toxaphene, furthermore for pentachloroanisole as main metabolite of pentachlorophenol and for the industrial chemical hexachlorobutadiene.

Aldrin

Aldrin is rapidly metabolized to dieldrin and is not normally found in humans; it was not detected in any sample (<0.5 μg/kg lipid) (Malisch et al. 2023b). The determination of aldrin in a total of 22 quality control samples between 2016 and 2019 proves the good recoveries of this analyte (Fig. 1). The mean recovery from all quality control samples covering concentrations of 4, 20, 40 and 100 μg/kg lipid was 98.9% with a coefficient of variation (CV) of 8.8%. The annual mean recovery including the CV was within the warning limits in the respective years, which reflects successful quality assurance of the analytical results.

Chlordane

According to the residue definition for pesticides in food, the sum parameter "chlordane complex" comprises *cis*-chlordane (= "α-chlordane") and *trans*-chlordane (= "γ -chlordane") (both more relevant for food of plant origin) and the metabolite oxychlordane (relevant for food of animal origin; also the relevant parameter for human milk). Between 2005 and 2019, a total of 139 quality control samples were analysed for oxychlordane as the most important analyte of the
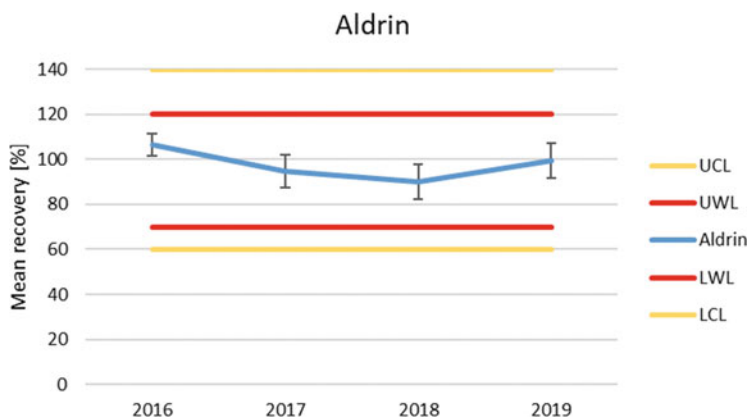
Fig. 1 Quality control chart for aldrin for the period 2016 to 2019. 22 quality control samples (uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg aldrin/kg lipid, respectively) were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level
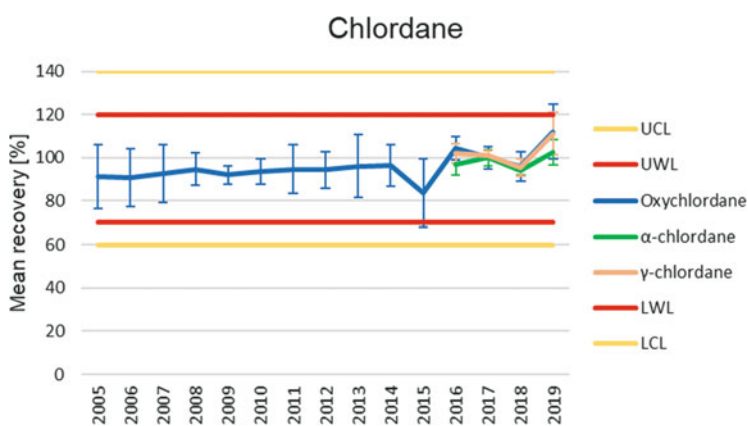
Fig. 2 Quality control chart for (i) oxychlordane based on 139 quality control samples for the period 2005 to 2019 and (ii) for α-chlordane and γ-chlordane based on 23 quality control samples for the period 2016 to 2019. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg chlordane/kg lipid, respectively, were analysed alternatingly. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

chlordane complex. Since 2016, additional 23 quality control samples were included to cover α-chlordane and γ-chlordane. The mean recovery from all quality control samples in these years was within the warning limits (Fig. 2). The mean recovery for all samples for oxychlordane was 94.5% with a CV of 12.9%. The mean recovery for

all samples for α-chlordane was 99.0% with a CV of 5.4%. The mean recovery for all samples for γ-chlordane was 103.0% with a CV of 8.2%.

DDT

Between 2000 and 2019, a total of 1192 quality control samples were analysed for p, p'-DDE as most important parameter for the sum parameter "DDT complex": In most of the human milk samples, p,p'-DDE contributed about 95%, while p,p'-DDT contributed about 5% to the sum total, and concentrations of o,p'-DDD, p,p'-DDD and o,p'-DDE were below the limit of quantification (0.5 µg/kg lipid) (Malisch et al. 2023b). In addition to p,p'-DDE, from 2015 to 2019, a total of 298 quality control samples were analysed also for o,p-DDT and p,p'-DDT, and from 2016 to 2019, a total of 31 quality control samples for p,p'-DDD. Figure 3 illustrates the mean recovery of all quality control samples in the respective year. The mean recoveries for p,p'-DDE, o,p'-DDT and p,p'-DDD were within the warning limits, but p, p'-DDT exceeded the UWL on few occasions.

In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when recoveries were outside the warning levels and further quality assurance measures were taken (e.g. repeated analyses with standard addition), to ensure that the mean recovery for the analytes was again successfully within the warning limits. The mean recovery for all samples for p,p'-DDE in the period 2000–2019 was 96.2% with a CV of 14.3%. The mean recovery for all samples for the sum parameter "DDT complex" in the period 2016–2019 was 108.1% with a CV of 15.3%.
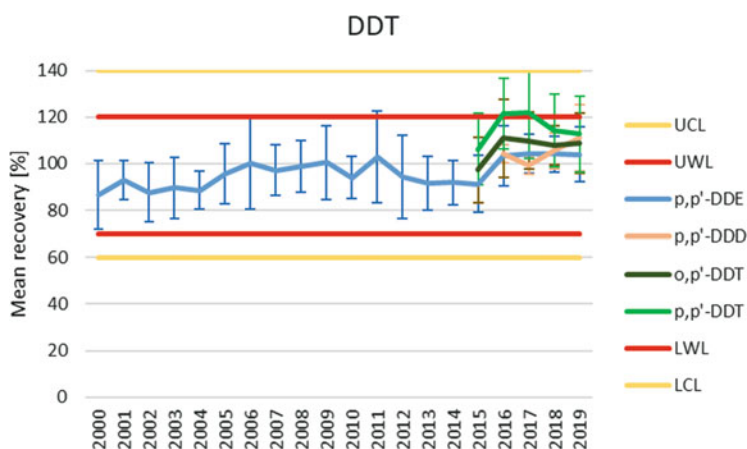
Fig. 3 Quality control chart for DDT used from 2000 to 2019 (1192 quality control samples for p, p'-DDE, 298 quality control samples for o,p'-DDT and p,p'-DDT and 31 quality control samples for p,p'-DDD). Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg DDT/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level
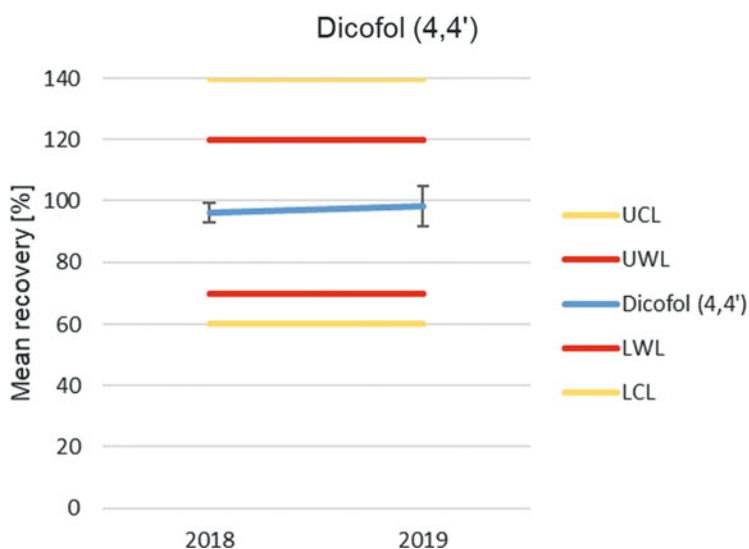
Dicofol (4,4′)

Dicofol was listed in the Stockholm Convention in 2019 and, in agreement with UNEP the analytical spectrum for the 2016–2019 survey included this compound in order to have a complete picture of all 30 POPs as covered by the Convention until 2019 (Malisch et al. 2023b). Between 2018 and 2019, a total of 13 quality control samples were analysed for the dicofol (4,4′) (Fig. 4). The mean recovery from all samples was 98.4% with a CV of 5.9% and within the warning limits.

Dieldrin

The determination of dieldrin in a total of 256 quality control samples between 2015 and 2019 proves the good recoveries of this analyte (Fig. 5). The mean recovery derived from quality control samples was 103.0% with a CV of 11.5% and was always within the warning limits in the respective years.

Endosulfan

Technical-grade endosulfan, which contains a number of related isomers, was listed in the Stockholm Convention in 2011. The sum parameter endosulfan complex comprises the analytes α-endosulfan, β-endosulfan and endosulfan-sulfate. Between 2005 and 2019, a total of 139 quality control samples were analysed for endosulfan-sulfate. From 2016 to 2019, a total of 24 quality control samples were analysed for β-endosulfan, and from 2017 till 2019, an additional 19 quality control samples for

Fig. 4 Quality control chart for dicofol (4,4′) used from 2018 to 2019. 13 quality control samples (uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg dicofol (4,4′)/kg lipid, respectively) were analysed alternately over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level
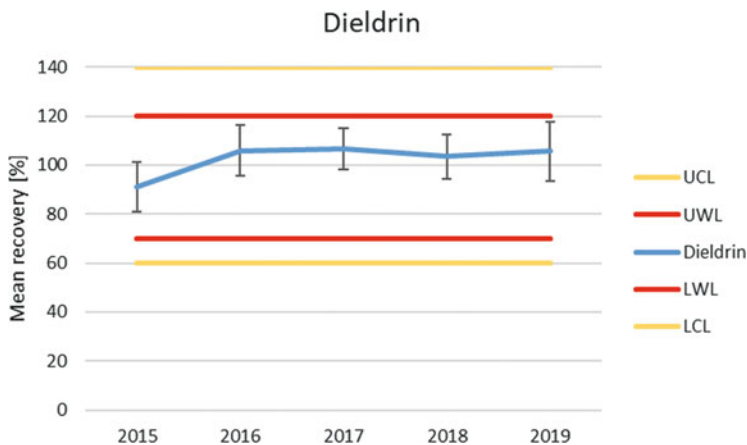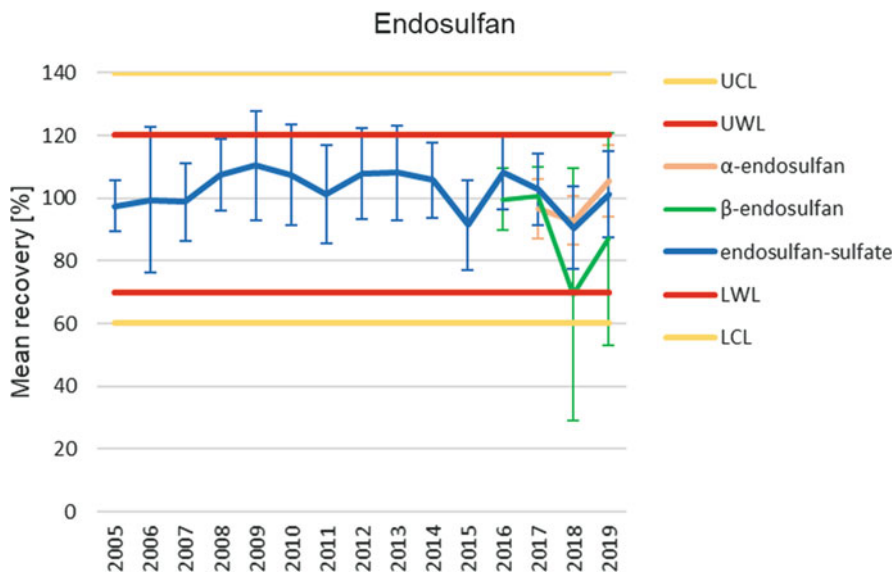
Fig. 5 Quality control chart for dieldrin for the period 2015 to 2019. 256 quality control samples (uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg dieldrin/kg lipid, respectively) were analysed alternately over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

α-endosulfan were analysed. Figure 6 illustrates the mean recovery from the quality control samples in the respective year, which were within the warning limits. The mean recovery for all samples for endosulfan-sulfate for the period 2005–2019 was 102.2% with a CV of 15.6%. The mean recovery for all samples for the sum parameter endosulfan complex for the period 2017–2019 was 95.4% with a CV of 19.5%.

Endrin
Between 2016 and 2019, a total of 22 quality control samples were analysed for endrin. Figure 7 illustrates the mean recovery from all quality control samples in the respective years. The mean recovery from all quality control samples was 100.5% with a CV of 13.4% and therefore within the warning limits.

Heptachlorepoxide
Heptachlor is rapidly metabolized to heptachlorepoxideee. In humans only cis-heptachlorepoxideee is normally found. Between 2003 and 2019, a total of 365 quality control samples were analysed for cis-heptachlorepoxideee. Between 2017 and 2019, 19 quality control samples were analysed for trans-heptachlorepoxideee, as well. Figure 8 illustrates the mean recovery from all quality control samples in the respective years. The mean recoveries for cis- and trans-heptachlorepoxideee were nearly identical and within the warning limits. The mean recovery for all samples for cis-heptachlorepoxideee in the period 2003–2019 was 98.9% with a CV of 13.1%. The mean recovery for all samples for trans-heptachlorepoxideee in the period 2017–2019 was 101.7% with a CV of 10.0%.

Fig. 6 Quality control chart for (i) endosulfan-sulfate for the period 2005 to 2019 with 139 quality control samples, (ii) for β-endosulfan for the period 2016 to 2019 with 24 quality control samples and (iii) for α-endosulfan for the period 2017 to 2019 with 19 quality control samples. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg endosulfan/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Hexachlorobenzene

Between 2000 and 2019, a total of 1116 quality control samples were analysed for hexachlorobenzene (HCB). Figure 9 illustrates the mean recovery from all quality control samples in the respective year, which were all in the warning limits. The mean recovery for all samples for HCB in the period 2000–2019 was 85.0%, with a CV of 18.0%.

Hexachlorobutadiene

Production and use of hexachlorobutadiene (HCBD) were prohibited by listing HCBD in 2015 in Annex A (Elimination) of the Stockholm Convention in 2015. HCBD can also be formed as unintentional by-product and was therefore listed in 2017 also in Annex C (Unintentional production). Therefore, analyses were performed for samples of the 2016–2019 period after validation of the analytical method. In 2018 and 2019, a total of 12 quality control samples were analysed for HCBD. Figure 10 illustrates the mean recovery from all quality control samples in the respective year. The mean recovery was 107.8% with a CV of 7.6% and within the warning limits.
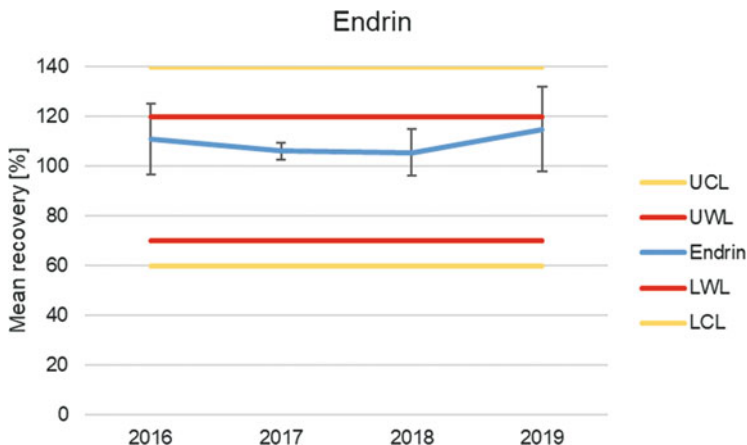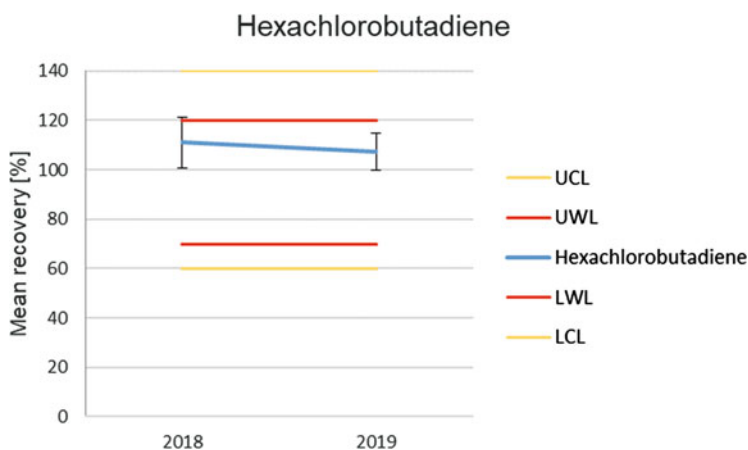
Fig. 7 Quality control chart for endrin for the period 2016 to 2019 (22 quality control samples). Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg endrin/kg lipid, respectively, were alternatingly analysed over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level
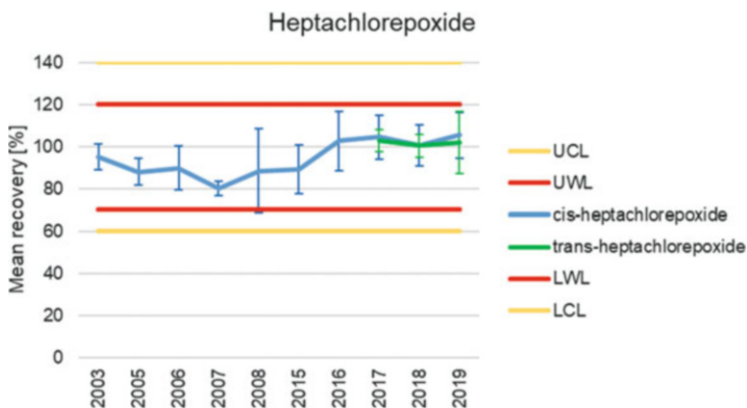
Fig. 8 Quality control chart for cis-heptachlorepoxidee for the period 2003 to 2019 (365 quality control samples) and for trans-heptachlorepoxidee for the period 2017 to 2019 (19 quality control samples). Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg cis-heptachlorepoxidee/kg lipid and in the period 2017–2019 in addition spiked with 2, 10, 25 and 50 µg trans-heptachlorepoxidee/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level
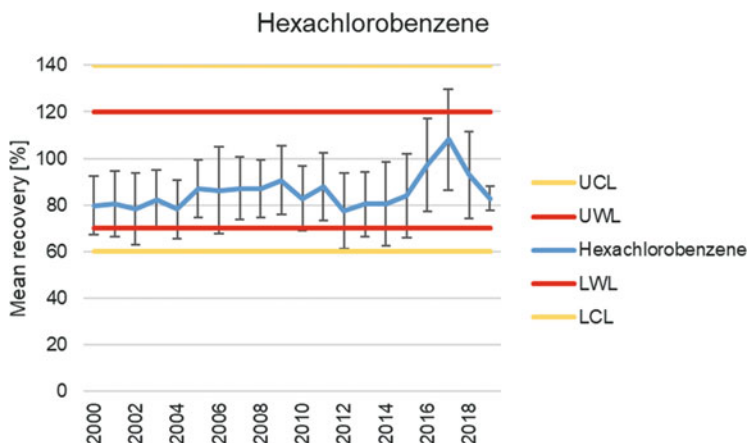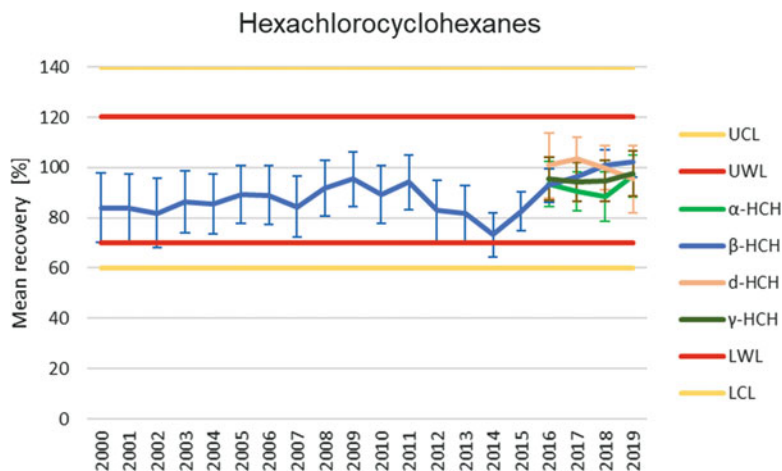
Fig. 9 Quality control chart for hexachlorobenzene for the period 2000 to 2019. 1116 lipid quality control samples were analysed during this period. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg HCB/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Fig. 10 Quality control chart for hexachlorobutadiene for the period 2018 to 2019. 12 lipid quality control samples were analysed during this period. Uncontaminated pork fat matrix samples spiked with 4 µg HCBD/kg lipid, respectively, were analysed over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH)

Technical-grade hexachlorocyclohexane (HCH) is a mixture mainly of three isomers comprising about 65–70% α-HCH, 7–20% β-HCH and 14–15% γ-HCH. These

Fig. 11 Quality control chart for hexachlorocyclohexanes. In the period 2000 to 2019, 1163 quality control samples were analysed for β-HCH, in the period 2016 to 2019, 185 samples for α–HCH, 218 samples for δ-HCH and 55 samples for γ-HCH. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg HCH/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

isomers were listed in 2009 in Annex A (for elimination) by the Stockholm Convention. However, the minor component δ-HCH; 6–10% was not listed. Metabolism results in the accumulation of mainly β-HCH in humans, whereas the concentrations of α-HCH and γ-HCH were below the limit of quantification (< 0.5 μg/kg lipid) in most human milk samples (Malisch et al. 2023b). Therefore, the main focus of quality control in the period 2000–2019 was on β-HCH; α-, γ- and δ-HCH were added for particular control during the 2016–2019 study.

Between 2000 and 2019, a total of 1163 quality control samples were analysed for β-HCH. In the period 2016–2019, additional 185 quality control samples were included for the determination of α-HCH, 55 samples for γ-HCH and 218 samples for δ-HCH. Figure 11 illustrates the mean recovery from all quality control samples in the respective year. The mean recoveries from all quality control samples were within the warning limits. The mean recovery for all samples for β-HCH in the period 2000–2019 was 89.0% with a CV of 16.5%. The mean recovery for all samples for α-, β-, γ-, δ-HCH in the period 2016–2019 was 96.4% with a CV of 11.3%.

Mirex

Between 2000 and 2019, a total of 1437 quality control samples were analysed for mirex. Figure 12 illustrates the mean recovery in the respective year, which were within the warning limits. The mean recovery for all samples for mirex in the period 2000–2019 was 93.3% with a CV of 13.9%.
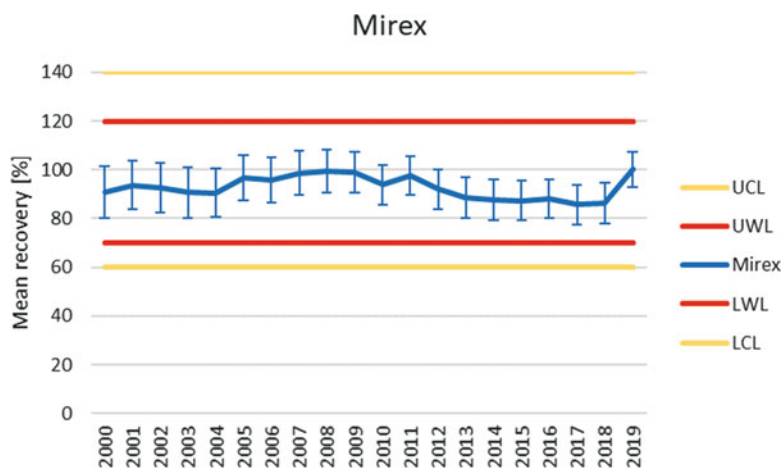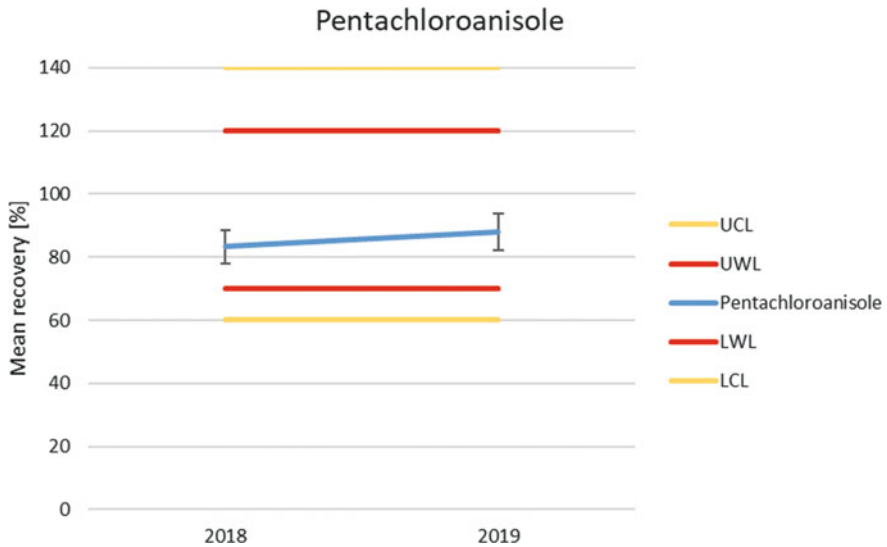
Fig. 12 Quality control chart for mirex for the period 2000 to 2019. 1437 lipid quality control samples were analysed for mirex as representative analyte and internal standard. Uncontaminated pork fat matrix samples spiked with concentration level of 4 μg mirex/kg lipid and 40 μg mirex as internal standard/kg lipid were determined over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Pentachloroanisole

Between 2018 and 2019, a total of 11 quality control samples were analysed for pentachloroanisole. Figure 13 illustrates the mean recovery from all quality control samples in the respective year. The mean recovery from all quality control samples was 87.9% with a CV of 5.9% and in these years within the warning limits.

Toxaphene

Between 2000 and 2019, a total of 160 quality control samples were analysed for the toxaphene marker compounds Parlar 26, Parlar 50 and Parlar 62. Figure 14 illustrates the mean recovery from all quality control samples in the respective year. Except some outliers in the period 2001–2002 for Parlar 62, the mean recoveries from these quality control samples were within the warning limits. In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when recoveries were outside the warning levels and further quality assurance action was taken (e.g. repeated analyses with standard addition), to ensure that the mean recovery for all toxaphene analytes was within the warning limits. The mean recovery for the sum parameter of Parlar 26, Parlar 50 and Parlar 62 was 97.2% with a CV of 23.7%.

Fig. 13 Quality control chart for pentachloroanisole for the period 2018 to 2019. 11 uncontaminated pork fat matrix quality control samples spiked at 20 μg pentachloroanisole/kg lipid were analysed. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level
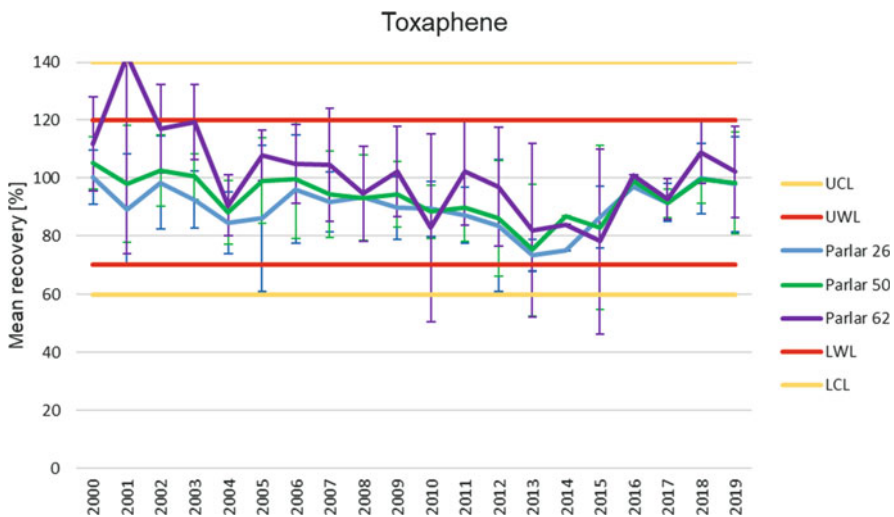
Fig. 14 Quality control chart for toxaphene for the period 2000 to 2019. 160 uncontaminated pork fat matrix quality control samples spiked at 10 μg toxaphene/kg lipid with each of the three marker compounds Parlar 26, Parlar 50 and Parlar 62 were analysed over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

4.1.2 Chlordecone and Pentachlorophenol (QuEChERS Method; Spiked Samples)

The validation of the more polar organochlorine pesticides chlordecone and pentachlorophenol in samples of the 2016–2019 period is based on a LC-MS/MS method.

Chlordecone

For validation of chlordecone, raw cow's milk with a fat content of 4% was spiked at 5 concentrations and the recovery determined by 6 replicates. The mean recovery (%) and the CV % fulfilled the requirements for analytical quality control for pesticide residue analysis (mean recovery 70–120%, CV < 20%) (DG SANTE, 2021) (Table 1).

Two human milk matrix quality control samples were fortified with 10 µg/kg fresh weight and analysed for chlordecone. The mean recovery was 92.0%.

Pentachlorophenol (PCP)

In a minimal validation of pentachlorophenol by LC-MS/MS determination, a pork muscle tissue matrix was used. The pork matrix was spiked at 2 levels and determined in 6 replicates, respectively. The requirements for analytical quality control for pesticide residue analysis (mean recovery 70–120%, CV < 20%) (DG SANTE 2021) were met (Table 2).

Two human milk matrix quality control samples were spiked at 10 µg/kg fresh weight and analysed for pentachlorophenol. The mean recovery was 96.6%.

4.1.3 Summary: Trueness and Precision Derived from Spiked Quality Control Samples

Table 3 summarizes the results of the quality control data for spiked samples with regard to trueness (expressed as mean recoveries in %) and precision (expressed as CV in %). The trueness for all analytes was on average 99% (range 86%–110%) and the precision on average 11.5% (range 0.7%–31.4%). In conclusion, based on quality control samples, the applied analytical methods achieved a very good long-term performance for trueness and precision over the 2000–2019 period.

Table 1 Validation data for chlordecone in raw cow's milk

µg/kg milk	µg/kg milk fat	Recovery [%]	(CV) [%]
0.01	0.25	98.5	11.8
0.02	0.5	100.1	12.8
0.05	1.25	101.9	8.1
0.1	2.5	99.3	6.0
0.2	5.0	103.1	4.8

Table 2 Validation data of pentachlorophenol (PCP) in pork muscle matrix milk

µg PCP/kg pork muscle	Average [µg PCP /kg pork muscle]	Recovery [%]	CV [%]
10	9.7	96.7	2.8
50	51.3	102.6	1.6

Table 3 Mean recoveries (%) and CVs (%) for 16 parameters with 29 analytes

Analyte	Mean recoveries (%)	CV (%)
Aldrin	98.9	8.8
Chlordecone	92.9	3.4
Chlordane		
Oxychlordane	94.5	12.9
α-chlordane	99	5.4
γ-chlordane	103.8	8.2
DDT		
p,p'-DDE	96.2	14.3
p,p'-DDT	107.3	13.8
o,p'-DDT	107.3	13.8
p,p'-DDD	105.7	9.4
Dicofol	98.4	5.9
Dieldrin	103	11.5
Endosulfan		
Endosulfan-sulfate	102.2	15.6
α-endosulfan	99.8	11.3
β-endosulfan	90	26.4
Endrin	110.5	13.4
Heptachlorepoxide		
cis-heptachlorepoxidee	98.9	13.1
trans-heptachlorepoxidee	101.7	10
Hexachlorobenzene	85.7	18
Hexachlorobutadiene	107.8	7.6
Hexachlorocyclohexanes		
α–HCH	91.1	9.2
β-HCH	89	16.5
γ-HCH	95.6	8.5
δ-HCH	99.6	11.7
Mirex	100.7	6.9
Pentachloroanisole	87.9	5.9
Pentachlorophenol	96.6	0.7
Toxaphene		
Parlar 26	91.6	15.4
Parlar 50	95	15.8
Parlar 62	104.9	31.4
Min	85.7	0.7
Median	98.9	11.5
95% Percentile	107.6	23.0
Max	110.5	31.4
N_1 = number of analytes	29	
N_2 = number of samples	6465	

4.2   Remainders of Samples from Proficiency Tests as in-house Reference Material

10 samples (plant oil and animal origin oil spiked with several organochlorine pesticides) from excess materials provided for proficiency tests with defined consensus values were used as in-house quality control samples. Trueness calculated from 375 results for 41 consensus values was on average 96% and the precision expressed as CV 7.6% (Table 4).

4.3   Participation in Proficiency Tests for Pesticide Residues

Between 2000 and 2019, CVUA Freiburg participated in 53 proficiency tests (PTs) for determination of pesticide residues in test samples mainly of food of animal origin, of plant oils and in two cases of human milk (Table 5). The analytical methods applied for these different matrices use the same modular elements. In certain PTs, standard solutions also had to be analysed. Due to the limited availability of toxaphene proficiency tests, the results of the 1999 proficiency test on toxaphenes in a fish (halibut) matrix were also included as additional information.

In these proficiency tests, around 150 different analytes were determined resulting in a total of around 800 data. 26 of these analytes were organochlorine pesticides providing a total of around 170 results.

In order to summarize the evaluation of all 53 samples of these proficiency tests performed between 1999 and 2019, the results were calculated on a lipid basis, fresh weight basis or solution basis (according to the "pesticides guidelines" (DG-SANTE 2021)), regardless of the requirements for reporting data for individual proficiency tests. The general idea of this summarizing evaluation was to check how the results reported by CVUA Freiburg deviated from the respective consensus value.

Figures 15, 17, 19, 20, 22, 24, 26, 28, 30, 31 show the deviation (%) from the consensus values for all results obtained in the 53 PTs for the analytes chlordane (cis-chlordane; trans-chlordane; oxychlordane), DDT (o,p'-DDD; p,p'-DDD, o,p'-DDE; p,p'-DDE, o,p'-DDT, p,p'-DDT), dieldrin, endosulfan (α-endosulfan; β-endosulfan; endosulfan-sulfate), endrin, hexachlorobenzene, hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), heptachlorepoxide (cis-heptachlorepoxide; trans-heptachlorepoxide), mirex and toxaphene (Parlar 26, Parlar 50 and Parlar 62). In all figures, the deviations for results calculated on a lipid, fresh weight and solution basis are marked as dots, triangles and squares, respectively, as described in the table caption.

Figures 16, 18, 21, 23, 25, 27, 29, 32 illustrate the z-score values obtained in 51 of the 53 PTs. The evaluation of these 51 PTs is based on the same principle and therefore gives comparable results: The consensus values were calculated as robust mean or median from all results without obvious outliers and the z-score calculations were performed by applying a robust standard deviation or the standard deviation according to Horwitz. The results of the two PTs following the Cofino-model were not included in the comparison. In accordance with the ISO 17043, z-scores of

Table 4 Mean recoveries (%) and CVs (%) for 10 left-over oil samples of plant and animal origin

Matrix	Analyte	Consensus µg/kg	Number of values	Mean µg/kg	Recovery (%)	CV (%)
Oil	Chlordane-oxy	20.0	8	17.4	87.2	**3.0**
Oil	Chlordane-oxy	31.2	6	29.8	95.4	**7.6**
Oil	Chlordane-oxy	55.6	17	53.1	95.5	**6.8**
Oil	Chlordane-trans	43.9	6	39.8	90.6	**7.8**
Oil	Chlordane-trans	84.8	6	73.4	86.5	**1.3**
Oil	DDE-pp'	21.6	9	21.0	97.2	**11.8**
Oil	DDE-pp'	24.3	8	23.3	95.8	**7.9**
Oil	DDE-pp'	76.8	6	78.1	101.7	**5.0**
Oil	DDE-pp'	93.7	17	89.2	95.2	**6.8**
Oil	DDE-pp'	524.1	6	568.5	108.5	**18.4**
Oil	DDT-pp'	33.0	8	33.2	100.7	**3.9**
Oil	DDT-pp'	90.6	6	91.7	101.2	**10.9**
Oil	DDT-pp'	93.4	17	90.5	96.9	**8.7**
Oil	Dieldrin	10.7	6	9.57	89.4	**14.8**
Oil	Dieldrin	13.3	8	11.9	89.3	**5.1**
Oil	Dieldrin	56.5	17	53.4	94.5	**6.8**
Oil	Dieldrin	173.3	9	182.8	105.5	**6.3**
Oil	Endosulfane-α	3.80	6	3.18	83.8	**13.4**
Oil	Endosulfane-α	71.4	6	70.0	98.0	**3.4**
Oil	Endosulfane-β	6.70	6	6.00	89.6	**30.3**
Oil	Endosulfane-β	37.8	6	36.2	95.8	**5.4**
Oil	Endosulfane sulfate	26.6	6	23.5	88.3	**14.0**
Oil	Endrin	19.5	6	15.2	77.8	**5.4**
Oil	Endrin	21.5	8	15.6	72.4	**5.4**
Oil	Endrin	39.2	17	33.2	84.7	**14.9**
Oil	Endrin	44.8	6	43.9	97.9	**4.2**
Oil	Endrin	46.3	9	46.0	99.4	**11.4**
Oil	HCB	21.9	8	22.6	103.0	**7.3**
Oil	HCB	43.0	9	46.3	107.6	**12.5**
Oil	HCB	65.0	6	71.1	109.4	**7.3**
Oil	HCB	76.6	17	76.4	99.8	**8.7**
Oil	HCB	362.2	6	355.1	98.0	**16.9**
Oil	HCH-β	12.4	8	13.4	108.0	**8.5**
Oil	HCH-β	20.7	6	18.9	91.1	**8.5**
Oil	HCH-β	48.5	17	44.1	91.0	**13.6**
Oil	HCH-γ	33.7	8	32.4	96.2	**6.5**
Oil	HCH-γ	63.4	6	68.7	108.3	**3.0**
Oil	HCH-γ	67.9	6	86.0	126.6	**13.2**
Oil	HCH-γ	82.1	17	77.8	94.7	**5.7**
Oil	Heptachlorepoxide-cis	22.8	8	18.0	78.8	**5.4**
Oil	Heptachlorepoxide-cis	44.7	17	35.9	80.2	**10.1**

(continued)

Table 4 (continued)

Matrix	Analyte	Consensus µg/kg	Number of values	Mean µg/kg	Recovery (%)	CV (%)
	Min	3.8	6.0	3.2	**72.4**	**1.3**
	Median	43.9	8.0	39.8	**95.8**	**7.6**
	95% percentile	173.3	17.0	182.8	**108.5**	**16.9**
	97.5% percentile	362.2	17.0	355.1	**109.4**	**18.4**
	Max	524.1	17.0	568.5	**126.6**	**30.3**
	N	**41**	**375**			

Table 5 Overview of proficiency tests between 1999 and 2019

No	Year	Organization	Matrix
1	2000	FAPAS	Plant oil
2	2000	LVU Herbolzheim	Plant oil
3	2001	FAPAS/BgVV	Meat (poultry)
4	2002	LVU Herbolzheim	Plant oil
5	2003	FAPAS	Fish
6	2003	FAPAS	Plant oil
7	2003	FAPAS	Meat (poultry)
8	2003	LVU Herbolzheim	Solution
9	2004	FAPAS	Milk powder
10	2004	LVU Herbolzheim	Plant oil
11	2004	BVL	Meat (boiled sausage)
12	2004	FAPAS	Plant oil
13	2004	FAPAS	Milk powder
14	2004	FAPAS	Meat (poultry)
15	2005	FAPAS	Milk powder
16	2006	FAPAS	Fish meal
17	2006	LVU Herbolzheim	Plant oil
18	2007	BVL	Egg
19	2007	FAPAS	Fish
20	2008	FAPAS	Milk powder
21	2009	FAPAS	Fish
22	2009	FAPAS	Milk powder
23	2010	FAPAS	Meat (poultry)
24	2010	FAPAS	Milk powder
25	2011	FAPAS	Minced Oily Fish
26	2011	FAPAS	Plant oil
27	2012	FAPAS	Milchpulver
28	2013	FAPAS	Minced Oily Fish
29	2013	FAPAS	Pork, muscle
30	2013	BIPEA	Honey
31	2014	FAPAS	Milk powder

Table 5 (continued)

No	Year	Organization	Matrix
32	2014	FAPAS	Animal Fat
33	2014	FAPAS	Rapeseed oil
34	2015	FAPAS	Animal fat (Pork)
35	2015	FAPAS	Fish oil
36	2015	FAPAS	Milk powder
37	2016	FAPAS	Animal fat (pork)
38	2016	FAPAS	Milk powder
39	2016	FAPAS	Infant formula
40	2016–2017	UNEP	Fish
41	2016–2017	UNEP	Human milk
42	2017	BIPEA	Egg powder
43	2017	FAPAS	Chicken egg
44	2017	FAPAS	Oily fish
45	2017	FAPAS	Olive Oil
46	2017	FAPAS	Infant formula
47	2017	FAPAS	Offal (Liver)
48	2018	ANSES	Fish
49	2018–2019	UNEP	Fish
50	2018–2019	UNEP	Human milk
51	2018	FAPAS	Infant Formula
52	2019	FAPAS	Infant Formula
53*	1999	BgVV	Halibut

* Proficiency test results for additional information for the quality measures for toxaphene

$|z| \leq 2.0$ indicate a "satisfactory" performance and generate no signal, z-scores between the range $2.0 < |z| < 3.0$ indicate "questionable" performance and generate warning signals and z-scores above $|z| \geq 3.0$ indicate "unsatisfactory" performance and generate action signals. In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when z-scores were above $|z| \geq 2.0$.
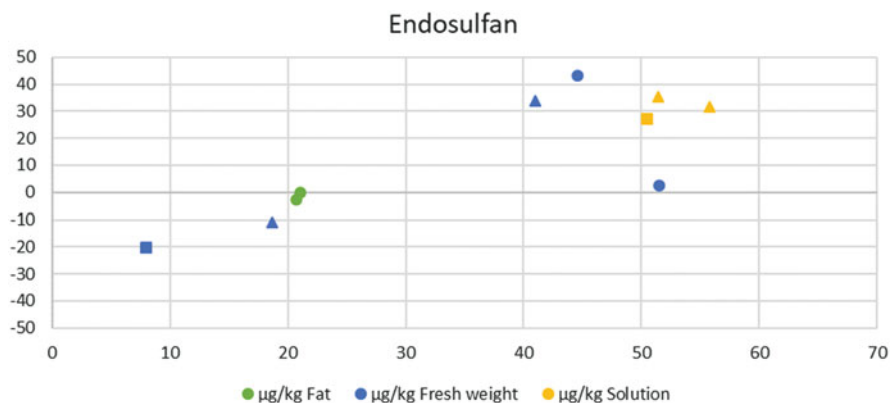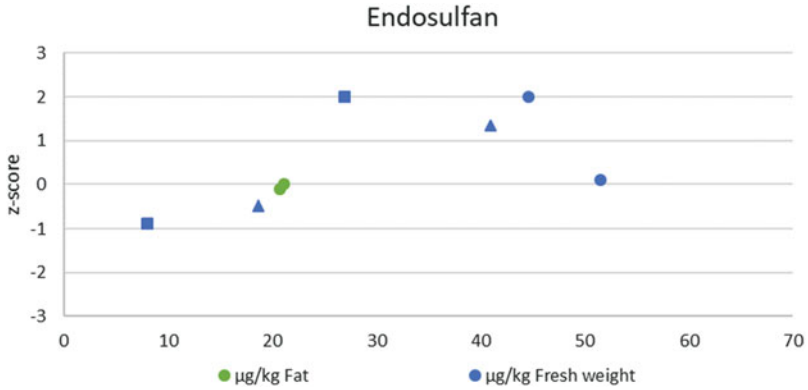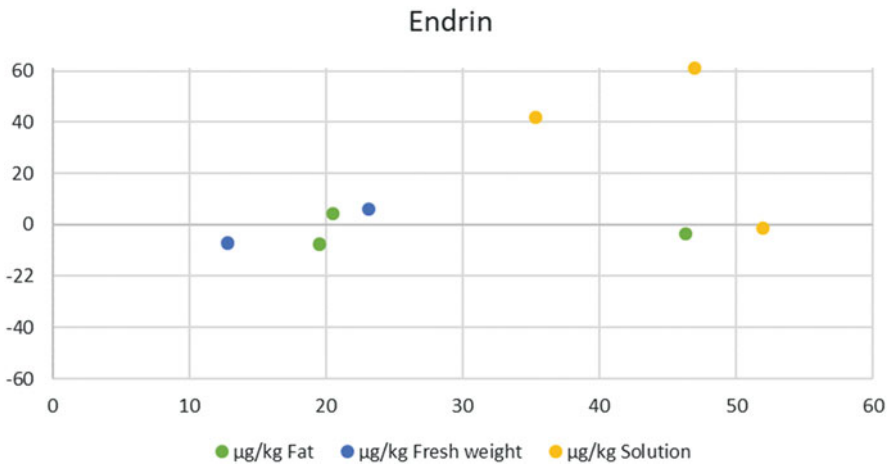
4.3.1 Chlordane
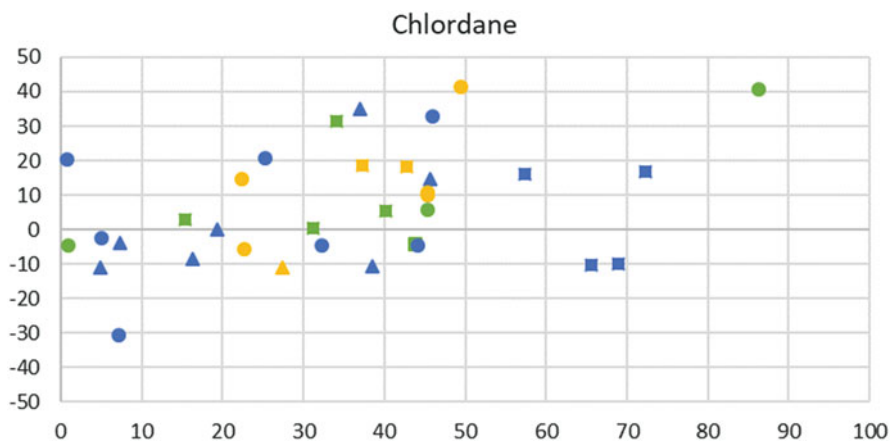
Fig. 15 Deviation (%) from the assigned values of 34 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for chlordane in proficiency tests performed between 2000 and 2019 (cis-chlordane visualized as dots, trans-chlordane as triangles and oxy-chlordane as squares)
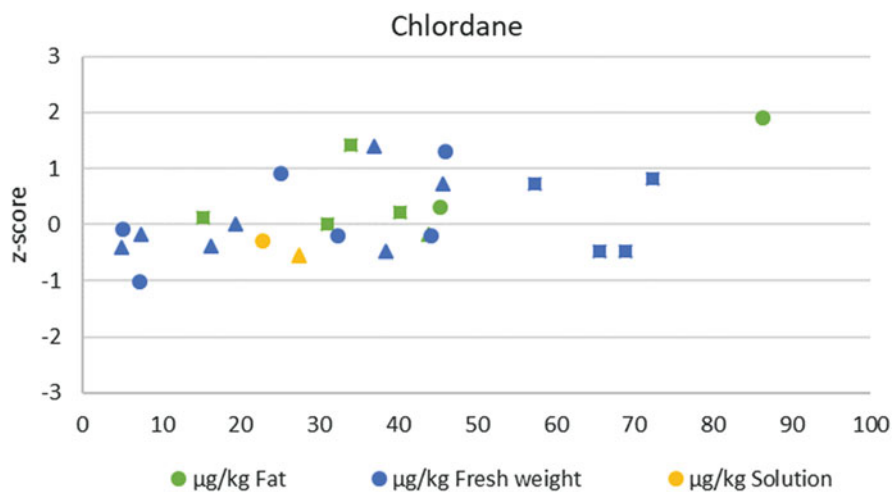
Fig. 16 Z-score of 26 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for cis-chlordane, trans-chlordane, oxy-chlordane for deviation from the assigned values of proficiency tests performed between 2000 and 2019 (cis-chlordane visualized as dots, trans-chlordane as triangles and oxy-chlordane as squares)

4.3.2   DDT
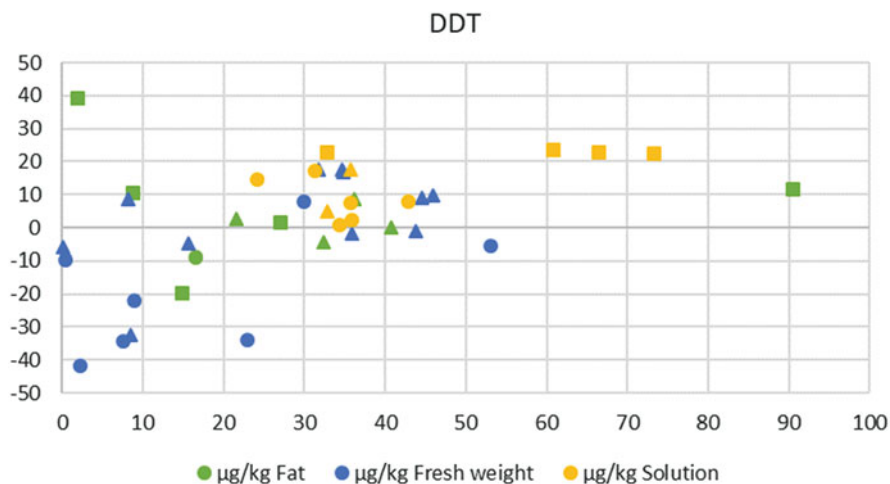
Fig. 17 Deviation (%) from the assigned values of 40 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for DDT in proficiency tests performed between 2000 and 2019 (o,p'-DDD and p, p'-DDD visualized as dots, o,p'-DDE and p,p'-DDE as triangles, o,p'-DDT and p,p'-DDT as squares)
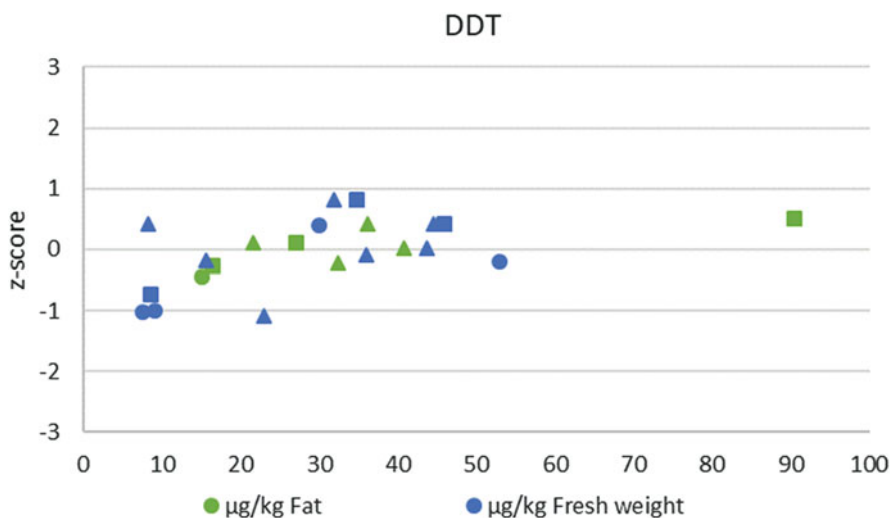
Fig. 18 Z-score of 23 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for o,p'-DDD, p, p'-DDE, o,p'-DDT and p,p'-DDT for deviation from the assigned values of proficiency tests performed between 2000 and 2019 (o,p'-DDD and p,p'-DDD visualized as dots, o,p'-DDE and p,p'-DDE as triangles, o,p'-DDT and p,p'-DDT as squares)

4.3.3   Dieldrin
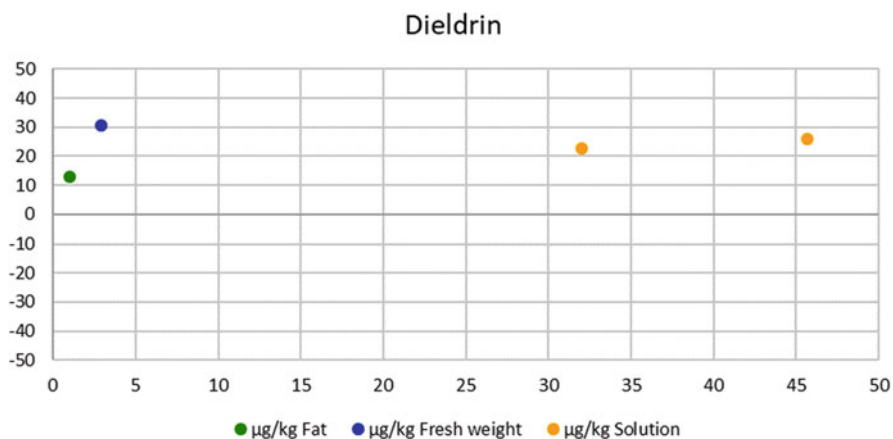
Fig. 19 Deviation (%) from the assigned values of 4 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for dieldrin in proficiency tests performed between 2016 and 2019 (no calculation of z-scores in these PTs)

4.3.4   Endosulfan

Fig. 20 Deviation (%) from the assigned values of 13 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for endosulfan in proficiency tests performed between 2000 and 2019 (endosulfan-sulfate visualized as dots, β-endosulfan as triangles, α-endosulfan as squares)

Fig. 21 Z-score of 8 results (μg/kg fresh weight and μg/kg lipid) for α-endosulfan, β-endosulfan and endosulfan-sulfate for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (endosulfan-sulfate visualized as dots, β-endosulfan as triangles, α-endosulfan as squares)

4.3.5   Endrin

Fig. 22 Deviation (%) from the assigned values of 8 results (μg/kg fresh weight, μg/kg lipid or μg/kg solution) for endrin in proficiency tests performed between 2000 and 2019
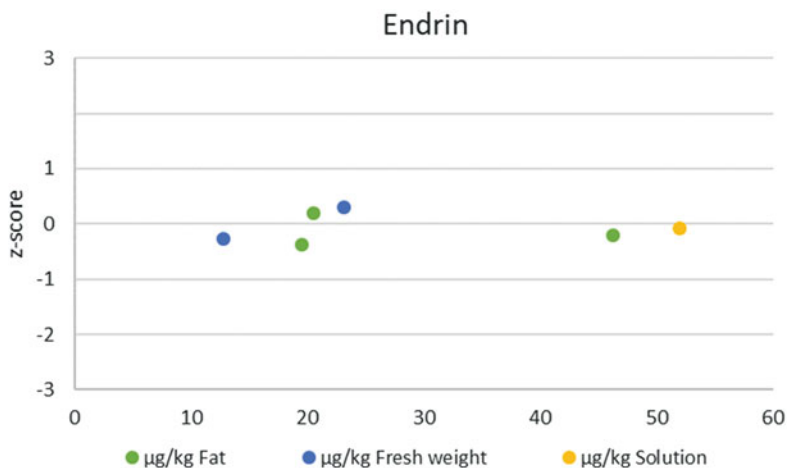
Fig. 23 Z-score of 6 results (μg/kg fresh weight, μg/kg lipid or μg/kg solution) for endrin for deviation from the assigned values of proficiency tests performed between 2000 and 2015
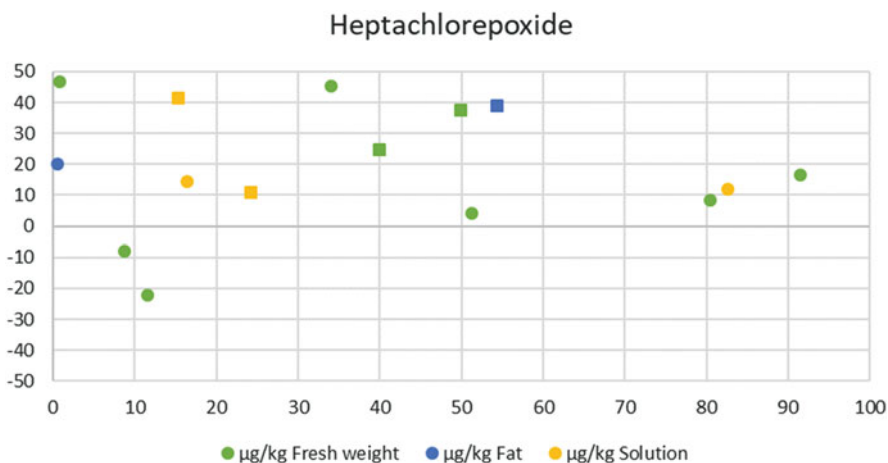
4.3.6 Hexachlorobenzene (HCB)
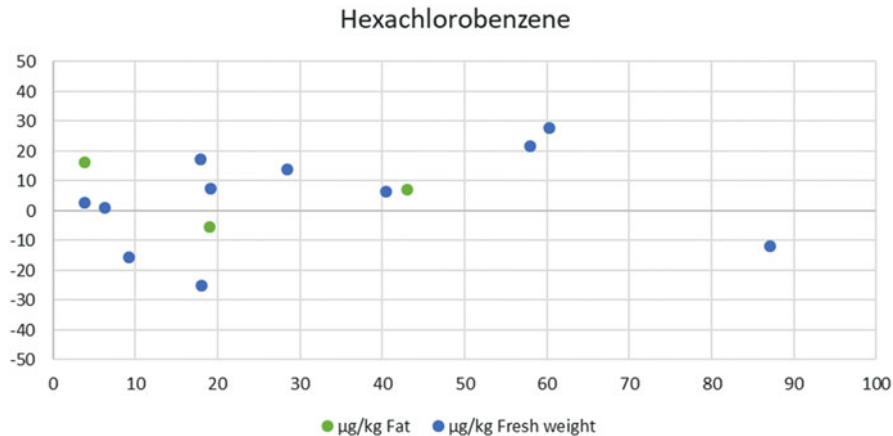
Fig. 24 Deviation (%) from the assigned values of 15 results (μg/kg fresh weight or μg/kg lipid) for hexachlorobenzene in proficiency tests performed between 2000 and 2019

Hexachlorobenzene
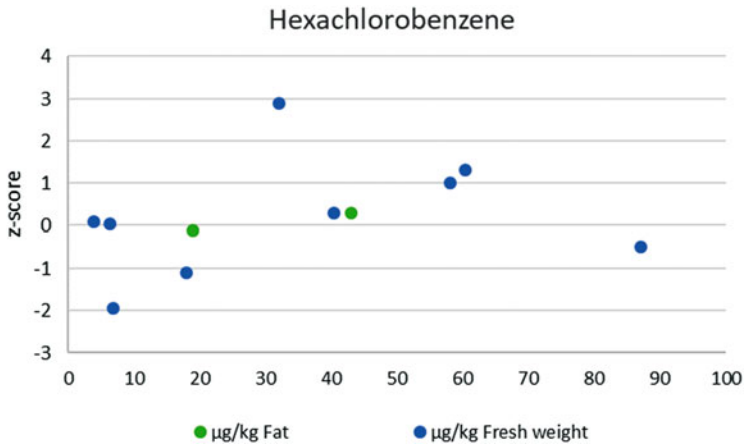
Fig. 25 Z-score of 11 results (µg/kg fresh weight or µg/kg lipid) for hexachlorobenzene for deviation from the assigned values of proficiency tests performed between 2000 and 2017

4.3.7 Hexachlorocyclohexanes (HCH)

Hexachlorocyclohexanes
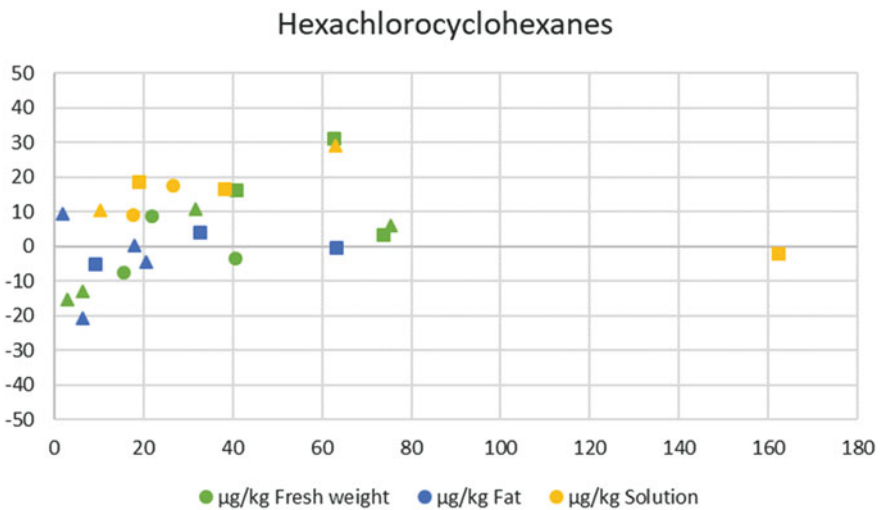
Fig. 26 Deviation (%) from the assigned values of 24 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for hexachlorocyclohexanes in proficiency tests performed between 2000 and 2019 (α-HCH results visualized as dots, β-HCH results as triangles, γ-HCH results as squares)
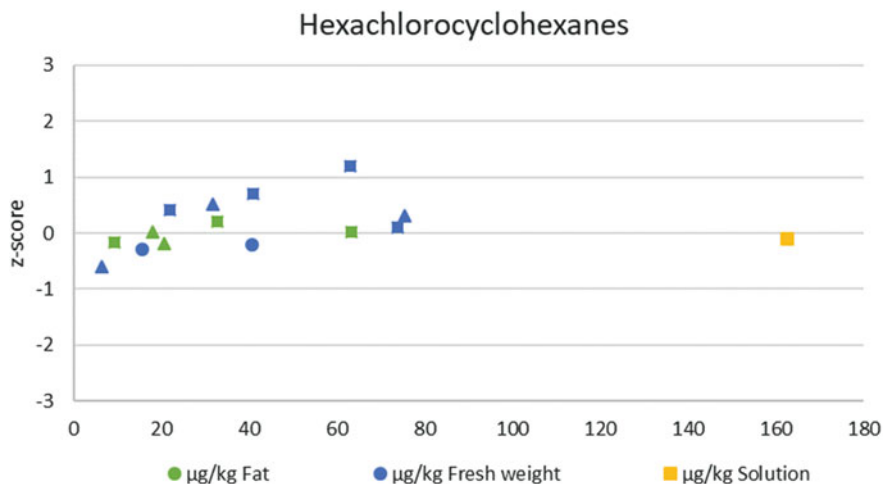
Fig. 27 Z-score of 15 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH) for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (α-HCH results visualized as dots, β-HCH results as triangles, γ-HCH results as squares)
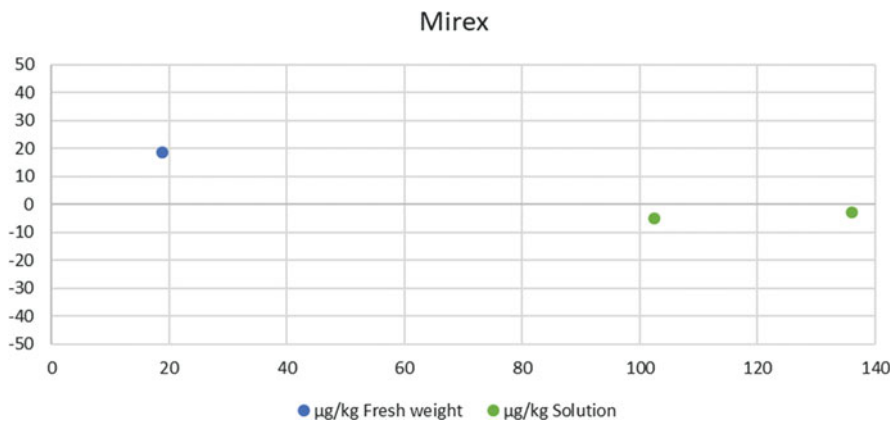
4.3.8 Heptachlorepoxide

Fig. 28 Deviation (%) from the assigned values of 15 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for heptachlorepoxidee in proficiency tests performed between 2000 and 2019 (cis-heptachlorepoxide results visualized as dots, trans-heptachlorepoxide results as squares)
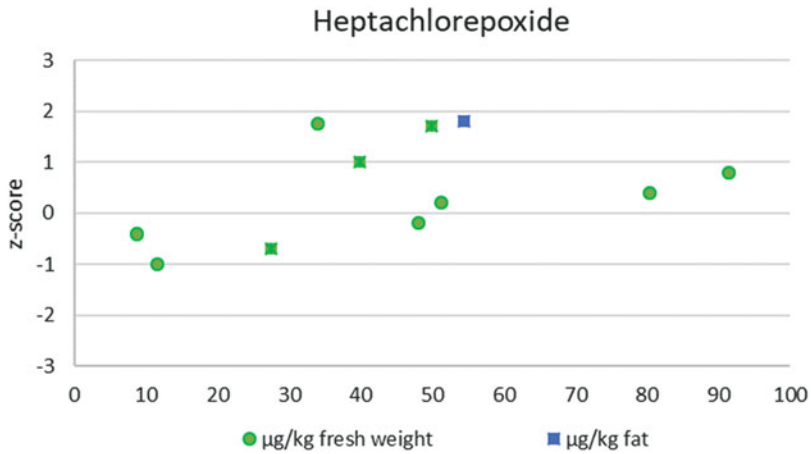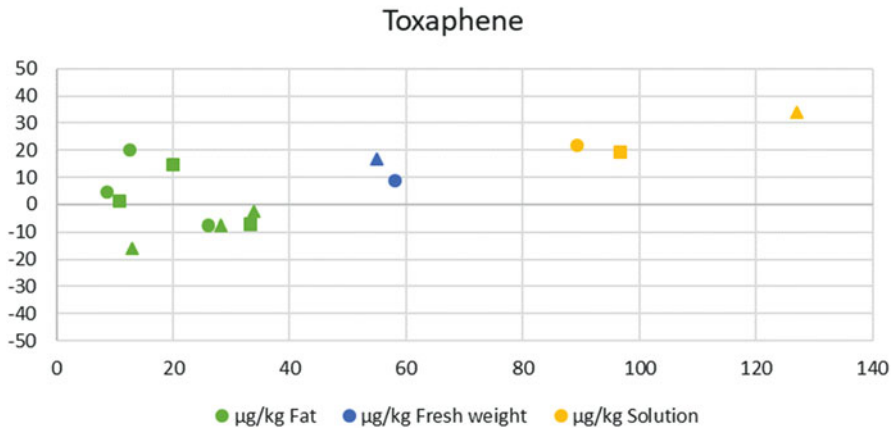
Fig. 29 Z-score of 11 results for heptachlorepoxidee for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (cis-heptachlorepoxide results visualized as dots, trans-heptachlorepoxide results as squares)

4.3.9 Mirex

Fig. 30 Deviation (%) from the assigned values of 3 results (µg/kg fresh weight or µg/kg solution) for mirex in proficiency tests performed between 2016 and 2019 (no z-scores for these PTs)

4.3.10 Toxaphene

Fig. 31 Deviation (%) from the assigned values of 14 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for toxaphene in proficiency tests performed between 1994 and 2019 (Parlar 26 results visualized as dots, Parlar 50 results as triangles and Parlar 60 results as squares)
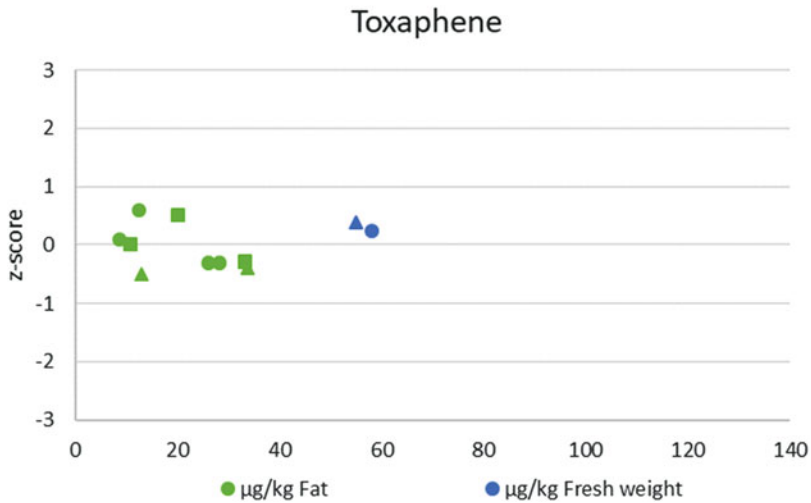
Fig. 32 Z-score of 11 results for toxaphene (Parlar 26, Parlar 50 and Parlar 60) for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (Parlar 26 results visualized as dots, Parlar 50 results as triangles and Parlar 60 results as squares)

4.3.11 Average Deviation

A summary of the average deviation CVs (%) of all results from assigned values of proficiency tests performed between 1999 and 2019 regardless of the required reporting data (on lipid, fresh weight or volume basis) is shown in Table 6. No evaluation was possible for many individual parameters (n.a.) because the provider did not provide an assigned value or there was no offer. As result, based on the assigned values of proficiency tests, the methods achieved on average a long-term trueness over the 2000–2019 period calculated as deviation from the assigned value of 17.5%; 90% of the results differed by less than 27% from the assigned value.

The z-score performance in 51 proficiency test samples (see Sect. 4.3 above) for the analytes chlordane (cis-chlordane, trans-chlordane, oxychlordane), DDT (p,p'-DDT, p,p'-DDE, p,p'DDD), endosulfan (alpha-endosulfan, beta-endosulfan, endosulfan sulfate), endrin, hexachlorobenzene, hexachlorocyclohexanes (alpha-HCH, beta-HCH, gamma-HCH), heptachlor (heptachlor, cis-heptachlorepoxidee, trans-heptachlorepoxidee), toxaphene (Parlar 26, Parlar 50 and Parlar 62) and chlordecone is illustrated in Fig. 33. In accordance with the ISO 17043, z-scores of $|z| \leq 2.0$ indicates a "satisfactory" performance and generates no signal, z-scores between the range $2.0 < |z| < 3.0$ indicates a "questionable" performance and generates a warning signal and z-scores above $|z| \geq 3.0$ indicates a "unsatisfactory" performance and generates an action signal. In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when z-scores were above $|z| \geq 2.0$. As conclusion, 92% of the z-score values were satisfactory.

4.4   Accreditation

In 1993, quality standards were introduced for laboratories entrusted by the Member States of the European Economic Community with the official control of foodstuffs: Laboratories had to comply with the general criteria for the operation of testing laboratories laid down in European Standard EN 45001 supplemented by standard operating procedures and the random audit of their compliance by quality assurance personnel not later than November 1998 (Council Directive 93/99/EEC). In a revision of the regulations on official controls in 2004, it was stipulated that laboratories that were designated for official control should operate and be assessed and accredited in accordance with the European Standard EN ISO/IEC 17025 (European Norm (EN) 17025) on "General requirements for the competence of testing and calibration laboratories" (EU Regulation 882/2004, succeeded by Regulation (EU) 2017/625). Therefore, the CVUA Freiburg was accredited in 1998 and has since been re-accredited continuously.

As a result, all analyses performed by CVUA Freiburg for the WHO/UNEP-coordinated exposure studies for the period 2000–2019 followed the strict rules of the accreditation system and the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025.

Table 6 Average absolute deviation (%) and median absolute deviation (%) for 14 parameters with 23 analytes

Analyte	Average absolute deviation (%)	Median absolute deviation (%)
Aldrin	n.a.	n.a.
Chlordane		
• Oxychlordane	11.8	10.5
• α-chlordane	18.1	10.0
• γ-chlordane	12.4	11.3
DDT		
• p,p'-DDE	12.2	8.4
• p,p'-DDT	18.7	18.3
• o,p'-DDT	22.4	22.4
• p,p'-DDD	21.2	9.5
Dicofol	n.a.	n.a.
Dieldrin	23.1	24.3
Endosulfan		
• Endosulfan-sulfate	25.8	23.1
• α-endosulfan	33.2	26.8
• β-endosulfan	27.8	32.5
Endrin	16.6	6.5
Heptachlorepoxide		
• cis-heptachlorepoxidee	19.8	15.6
• trans-heptachlorepoxidee	30.5	37.2
Hexachlorobenzene	16.7	13.9
Hexachlorobutadiene	n.a.	n.a.
Hexachlorocyclohexanes		
• α–HCH	9.4	8.4
• β-HCH	11.9	10.5
• γ-HCH	10.5	7.0
• δ-HCH	n.a.	n.a.
Mirex	8.9	5.1
Pentachlorophenol	12.5	12.5
Toxaphene		
• Parlar 26	12.5	8.6
• Parlar 50	15.3	16.0
• Parlar 62	10.4	10.8
Summary		
Min	8.9	5.1
Median	16.6	11.3
Average	17.5	15.2
90% Percentile	27.4	26.3
95% Percentile	30.1	31.7
Max	33.2	37.2
n.a.	No evaluation possible	
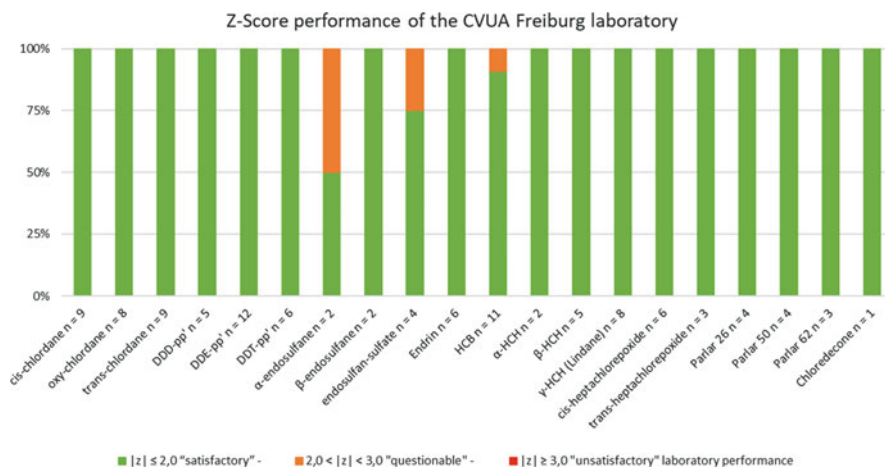
Fig. 33 Z-Score performance

5 Conclusion

The data of a comprehensive quality control gathered over two decades prove that the analytical methods applied for all samples of the WHO/UNEP-coordinated exposure studies performed between 2000 and 2019 met the requirements for acceptance of results as requested by the analytical criteria for analyses of organochlorine pesticides in food by EU legislation.

As a result, it was made sure that any differences in levels in this wide span of altogether 20 years (when the samples of the studies reported here were collected), e.g. lower levels found in later years, were not the result of an analytical variation but the result of decreasing temporal trends. As conclusion of various approaches of analytical quality control, for the 2000–2019 period the long-term trueness was on average in the range 85%–95% with a precision on average below 15%.

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