Sample collection, sample preparation and laboratory analysis of DDT

The determination of DDT and other chlorinated pesticides was carried out based on the method described in the German Food and Feed Code (Lebensmittel- und Futtermittelgesetzbuch) § 64 LFGB L 00.00-34 (2010) Modul No. E8.

Materials and standards

Acetone and cyclohexane (for residue analysis, 99.0 %) were supplied by LGC Promochem (UK), n-hexane (for analysis of dioxins, furans and PCB) by Fluka (USA), ethyl-acetate (for GC) by Supleco (USA). Triphenyl-phosphate (≥ 99 %) and undecane (99 %) were sourced from Aldrich (USA). Merck (Germany) supplied laboratory-grade quartz sand and sodium sulphate (≥ 99 %). Helium and nitrogen (≥ 99.999) were supplied by Messer (Germany). The organochlorine Pesticide Mix (No. 32415; ≥ 96,0 %) was sourced from Restek (USA).

Equipment

For homogenisation of fish samples, a Retsch GM300 homogenizer was used. The gel permeation chromatography was carried out on an LC Tech GPC 1122 Solvent Delivery System (LCTech GmbH, Germany) with styrene-divinylbenzene copolymer gel (Bio-Beads S-X3 200-400 mesh) (Biorad, USA) and 50 g Bio-Beads S-X3 200-400, 32 cm long GPC column (Biorad, USA). For chromatographic separation and subsequent detection, a gas chromatograph coupled with mass spectrometry (Agilent 7890A GC+ Agilent 7000A QQQ in EI equipped with a 5UI MS column: 2*15 m x 250 μm x 0,25 μm (Agilent, USA) with mid-column backflush) was used.

Sample collection and homogenization

In order to obtain data from aquacultural sites in Hungary, 6 commercial sites were selected which varied in the geographical region and keeping technology. At each site, 15-15 individual 3-year-old carps (*Cyprinus carpio*) were collected, sacrificed and their filets (together with skin and scales) frozen. The homogenization required the whole filet to be ground in a Retsch GM300 homogenizer without thawing, in the presence of dry ice.

Sample preparation

Homogenised fish meat (approx. 25 g) was transferred to a mortar, then the internal standard was added (triphenyl-phosphate) and ground with 1:1 (w/w) sodium sulphate:quartz sand mixture. The mixture was extracted with 400 ml n-hexane:acetone (ratio 2:1 (v/v)) and the eluate was vacuum rotary evaporated to a final volume of 20 ml. The concentrated eluate was transferred to a 100 ml flask and set to 100 ml with n-hexane.

Determination of fat content was carried out by gravimetry: 10 ml of the extract was further concentrated in a water bath and left to dry at 105°C until constant weight, left to cool in a desiccator and then weighed again.

Preparation for analysis included 20 ml of the extract to be transferred to a 100 ml round-bottomed flask and vacuum rotary evaporated at 40°C to 2 ml, the evaporation completed under a weak nitrogen flow. The residue was resuspended in 10 ml cyclohexane:ethyl-acetate 1:1 (v/v) mixture.

Clean-up was carried out by gel permeation chromatography under the following conditions: GPC pump flow rate: 5 ml/min; sample loop: 5 ml; GPC phases: (GPC forerun: 1200 sec, GPC-tailing: 120 sec); moving phase: cyclohexane:ethyl-acetate 1:1 (v/v) mixture. 5 μl of undecane (Sigma-Aldrich,

USA) was added to the collected eluate, which was then vacuum rotary evaporated at 40°C until dry and resuspended in n-hexane so as the final concentration would be 1 g of the initial sample /ml.

Chromatographic conditions

The analysis of DDT and other chlorinated pesticides was carried out using a gas chromatograph coupled with MS/MS detector with the following method parameters: splitless inlet mode; carrier gas: helium, constant flow (approx. 1.2 ml/min); retention time locking: for chlorpyriphos-methyl; oven temp: 80°C (1 min), heat rate 32.7°C/min to 170°C (0 min), heat rate 10°C /min to 310°C(1+5 min); collision cell gas: N_2 1.5 ml/min and He 2.25 ml/min; transferline: 280°C; MS mode: MRM, retention times and quant transitions are given in Table 1. Retention time is given for reference only, identification is based on the ratio of the retention time of the internal standard and the analyte (RRT) during the measurement.

Table 1. Retention times and quant transitions used for analysis of DDT and metabolite compounds.

Compound name	Retention time	Precursor ion	Product ion
DDD, o,p'-	12.24	237.00	165.20
DDD, o,p'-	12.24	235.00	165.20
DDD, o,p'-	12.24	199.00	163.10
DDD, p,p'-/DDT, o,p'	12.90	236.90	165.20
DDD, p,p'-/DDT, o,p'	12.90	234.90	199.10
DDD, p,p'-/DDT, o,p'	12.90	234.90	165.10
DDE, o,p'-	11.53	317.80	284.00
DDE, o,p'-	11.53	248.00	176.20
DDE, o,p'-	11.53	246.00	176.20
DDE, p,p'-	12.15	317.80	246.00
DDE, p,p'-	12.15	315.80	246.00
DDE, p,p'-	12.15	246.10	176.20
DDT, p,p'-	13.58	237.00	165.20
DDT, p,p'-	13.58	235.00	199.10
DDT, p,p'-	13.58	235.00	165.20

Method verification and quality control

The method was carried out according to the method described in § 64 LFGB L 00.00 34 (2010) Modul No. E8. The accredited laboratory was operating under the guidance of the ISO 17025 standard. In order to ensure the validity of the results the analytical process included an internal control in each sample; a solvent blank, and a spiked sample in each batch, semiannually a repeat investigation and annually the investigation of certified reference material or participation in a ring trial.

The recovery in the spiked samples was expected to be within ± 2x RSD of the average recovery of the compound based on the SANCO/12495/2011 guidelines.

If the screening test was positive for any of the compounds, quantification was done using an at least three-point calibration curve.

The LOQ of the method for o,p-DDD, o,p-DDE, o,p-DDT, p,p-DDT, p,p-DDE, p,p-DDD was established to be 0.005 mg/kg.