Residue Analysis of Organophosphorus and Organochlorine Pesticides in Fatty Matrices by Gas Chromatography Coupled with Electron-Capture Detection

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A multiresidue method for the simultaneous determination of 22 organochlorine (OCs) and organophosphorus (Ops) pesticides (including isomers and metabolites), representing a wide range of physicochemical properties, was developed in fatty matrices extracted from meat. Pesticides were extracted from samples with acetonitrile/*n*-hexane (v:v, 1:1). The analytical screening was performed by gas chromatography coupled with electron-capture detection (ECD). The identification of compounds was based on their retention time and on comparison of the primary and secondary ions. The optimized method was validated by determining accuracy (recovery percentages), precision (repeatability and reproducibility), and sensitivity (detection and quantitation limits) from analyses of samples fortified at 38 to 300 ng/g levels. Correlation coefficients for the 22 extracted pesticide standard curves (linear regression analysis, $n = 3$) ranged from 0.998 to 1.000. Recovery studies from 2 g samples fortified at 3 levels demonstrated that the GC-ECD method provides 64.4–96.0% recovery for all pesticides except 2,4′-DDE (44.6–50.4%), 4,4′-DDE (51.1–57.5%) and 2,4′-DDT (50.0–51.2%). Both repeatability and reproducibility relative standard deviation values were $< 20\%$ for all residues. Detection limits ranged from 0.31 to 1.27 ng/g and quantification limits were between 1.04 and 4.25 ng/g. The proposed analytical method may be used as a simple procedure in routine determinations of OCs and Ops in meat. It can also be applied to the determination of pesticide multi-residues in other animal products such as butter and milk.

Key words: Pesticides, Multiresidues, Fatty Matrices

Introduction

Pesticides are often applied to crops to control pests that may reduce yields (Pico *et al*., 2000). The hazardous nature of organochlorine pesticides (OCs) is the result of their high toxicities, chemical and biological stabilities, and lipophilicities. The characteristics make OCs prone to bioaccumulation along the food chain (Biziuk *et al*., 1996). As a consequence, although the use of most OCs has been restricted or even banned in many countries, they are still widespread not only in the environment but also in biotic matrices. These compounds can generate certain harmful effects on humans as well as on animals (Daston *et al*., 1997; Smith and Gangolli, 2002). In agriculture practices, they have been replaced by organophosphorus pesticides (Ops), which are considered less persistent. The insecticides of this type typically act through inhibition of the enzyme acetylcholinesterase (Sultatos, 1994), and display large variation in physicochemical properties such as polarity and water solubility.

Due to their chemical stability and persistence, pesticides residues were accumulated in adipose tissue of meat and fat-rich dairy products and exposing consumers of dairy products to significant levels of contamination (Bentabol and Jodral, 1995). This fact has caused concern since meat and dairy products play a central role in human nutrition. In order to minimize the human health risk, attempts have to be made to ensure that the organochlorines and the organophosphorus residues in food commodities are kept well under the recommended tolerance levels. Therefore, the present

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ment of a stock solution with the same concentration, which was directly injected into the GC system.

Detection sensitivity was determined by calculating the limit of detection (LOD) and the limit of quantification (LOQ) in the fortified samples. The LOD of the smallest amount of pesticides was obtained on the basis of a signal-to-noise (S/N) ratio of 3. The LOQ of the lowest content of pesticides in samples was calculated on the basis of S/N ratio 10. Where appropriate, values are expressed as means ± standard deviation (SD).

Results

Linearity and detection limit

Several standard solutions were injected in the GC-ECD system to obtain the linearity of detector response and the detection limits of the 22 compounds studied. The ECD response for all pesticides was linear in the concentration assayed with determination coefficient > 0.998 for all pesticides. Table I summarizes the limits of detection (LOD; obtained at the signal-to-noise ratio 3) and the limits of quantification (LOQ, obtained at the signal-to-noise ratio 10) obtained for the individual pesticides in meat by GC-ECD.

A typical gas chromatogram obtained from blank beef fat using microwave-assisted extraction and solvent partitioning is shown in Fig. 1. No significant interferences were observed in the gas chromatogram comparing the standards with the blank chromatogram (Fig. 2). The 22 pesticides were successfully detected with excellent sensitivity (Fig. 3). The developed method provides clean blank extracts without interferences during GC.

Recovery and reproducibility

A recovery test was performed by determining the concentrations of pesticides from fortified samples at three different levels. As indicated in Table II, the recoveries of the studied pesticides ranged from 64.4–96.0% recovery for all pesticides except DDT metabolites [2,4′-DDE (44.6–50.4%), 4,4′-DDE (51.1–57.5%) and 2,4′-DDT (50.0–51.2%)] and the relative standard deviations were $< 20\%$.

Discussion

Variety of methods such as Soxhlet extraction (Rho *et al.*, 1998), supercritical fluid extraction

Table I. Regression, limits of detection (LOD), limits of quantification (LOQ), and repeatability (RSD) of the studied pesticides by GC-ECD.

Compound	r^2	LOD [ng/g]	LOQ [ng/g]	Inter-assay RSD (%)	Intra-assay RSD (%)
γ-BHC (lindane)	1.000	0.31	1.04	11.433	1.143
Heptachlor	1.000	1.27	4.25	11.115	0.913
Chlorpyrifos-methyl	1.000	0.52	1.73	13.068	1.710
Dimetipin	1.000	0.38	1.25	18.860	2.156
Chlorpyrifos	1.000	0.40	1.34	10.732	0.897
Fenitrothion	0.999	0.40	1.33	16.358	2.475
Heptachlor epoxide	1.000	0.47	1.56	7.025	2.088
Chlordane/*trans*-chlordane	1.000	1.16	3.85	9.826	0.260
2,4′-DDE	1.000	0.84	2.81	7.614	1.175
cis-Chlordane	1.000	0.62	2.08	6.974	1.542
α-Endosulfan	1.000	0.62	2.05	6.823	1.221
Chinomethionate	1.000	0.58	1.94	9.031	1.221
4,4′-DDE	0.999	0.52	1.73	7.943	1.647
Dieldrin	1.000	0.52	1.73	6.875	1.390
2,4′-DDD	1.000	0.75	2.49	8.099	1.203
Endrin	1.000	0.66	2.19	7.288	1.541
2,4′-DDT	0.999	0.93	3.10	8.915	1.603
4,4′-DDD	0.999	1.06	3.53	8.097	0.938
β-Endosulfan	0.999	0.62	2.07	6.646	1.262
Ethion	0.998	0.72	2.39	8.869	0.791
4,4′-DDT	1.000	0.89	2.98	7.499	1.434
Endosulfan sulfate	0.999	1.05	3.50	7.395	1.540

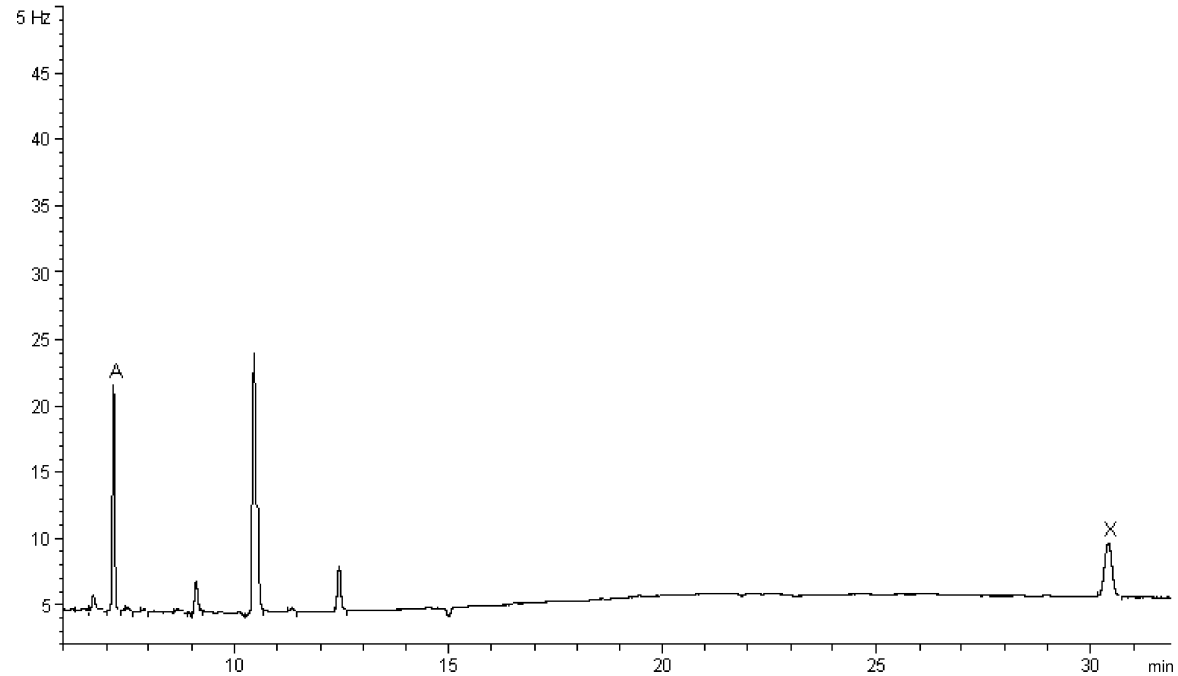

Fig. 1. Gas chromatogram of blank beef fat sample. A, PCNB (7.172); X, dibromo-DDE (30.416).

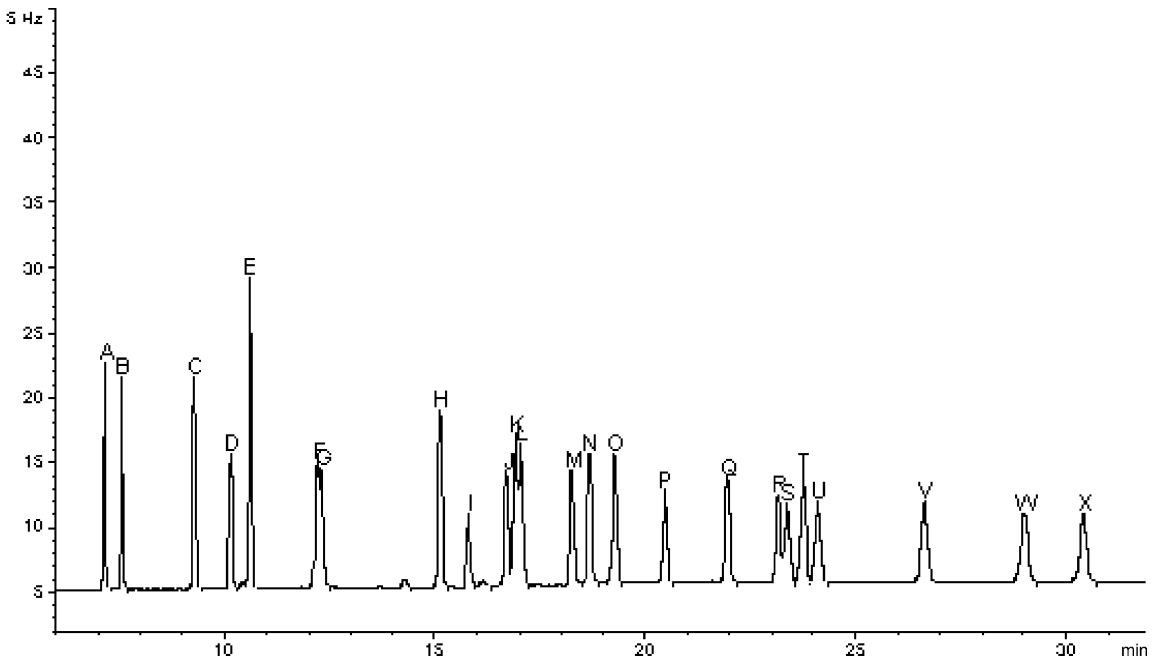

Fig. 2. Gas chromatogram of the standard pesticides mixture. A, PCNB (7.165); B, γ-BHC (7.568); C, heptachlor (9.281); D, chlorpyrifos-methyl (10.156); E, dimetipin (10.609); F, chlorpyrifos (12.216); G, fenitrothion (12.302); H, heptachlor epoxide (15.134); I, chlordane/*trans*-chlordane (15.80); J, 2,4′-DDE (16.705); K, *cis*-chlordane (16.92); L, α-endosulfan (17.042); M, chinomethionate (18.258); N, 4,4′-DDE (18.677); O, dieldrin (19.262); P, 2,4′-DDD (20.470); Q, endrin (21.958); R, 2,4′-DDT (23.166); S, 4,4′-DDD (23.378); T, β-endosulfan (23.749); U, ethion (24.104); V, 4,4′-DDT (26.632); W, endosulfan sulfate (29.011); X, dibromo-DDE (30.40).

study was undertaken to present a simple and rapid extraction method to analyze 22 organochlorines and organophosphorus pesticides in fatty matrices extracted from meat samples by gas chromatography combined with electron-capture detection. Selected pesticides were chosen on the basis of their lipophilicity and possibility of detection by gas chromatography.

Materials and Methods

Chemicals and reagents

Pesticide standards of heptachlor (98.5%), heptachlor epoxide (99.5%), dieldrin (98.0%), endrin (99.9%), 2,4′-DDE (99.0%), 4,4′-DDE (99.0%), 2,4′-DDD (99.5%), 2,4′-DDT (98.0%), 4,4′-DDD (99.0%), 4,4′-DDT (99.0%), α-endosulfan (99.3%), β-endosulfan (99.0%), endosulfan sulfate (98.8%) and ethion (95.0%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). γ-BHC (lindane) (98.9%), chlorpyrifos-methyl (98.3%), dimetipin (99.0%), chlorpyrifos (99.5%), fenitrothion (98.7%), chlordane/*trans*-chlordane (98.0%), *cis*-chlordane (99.5%) and chinomethionate (98.0%) were supplied from ChemService (West Chester, Pennsylvania, USA). All solvents used were of HPLC grade.

Instruments

An Agilent 6890N gas chromatography system (Palo Alto, CA, USA) coupled with an electron-capture detector (ECD) and a HP 7673A automatic injector system was used for the analysis of pesticides. A SPB™-608 fused silica capillary column (30.0 m × 0.25 mm ID and 0.25 μm film thickness) supplied by Supelco (Sigma-Aldrich Korea, Seoul, Korea) was employed, with nitrogen as makeup gas at 0.7 ml/min. The operating conditions were as follows: The detector and injector were operated at 300 and 250 °C, respectively; the oven temperature was maintained at 210 °C for 10 min and then increased to 230 °C at a rate of 2 °C/min.

Samples and extraction

Fats were extracted according to the procedure described by the Canadian official method with some modification. Briefly, 25 g of meat were cut into pieces of 1 cm^3 and heated at microwave for 10 min with full power (Martz, 1999). For extraction, (2 ± 0.1) g of fats were drenched with 25 ml of *n*-hexane in a 50 ml centrifuge tube. The mixture was then transferred to a 250 ml separatory funnel and extracted twice with 25 ml acetonitrile. The organic phase was collected and concentrated to approx. 3 ml using rotatory vacuum evaporation (Büchi Rotavapor R-114, Germany). The remained extract was transferred to a 15 ml centrifuge tube and maintained at −70 °C for 20 min (for precipitation), where after the mixture was centrifuged for 10 min at 3,000 × *g*. After centrifugation, the retention time and volume correction (RTVC) standards [pentachloronitrobenzene (PCNB) and dibromo-DDE] were added to the decanted supernatant and evaporated to dryness under a stream of nitrogen. Then, the residue was reconstituted with 1 ml of isooctane for analysis.

Stock solutions

Solutions of 1000 μg/ml of each pesticide standard were prepared by dissolving 0.025 g of a pesticide in 25 ml methanol. A pesticide intermediate standard solution was prepared by transferring 1 ml from each pesticide solution to a 10 ml volumetric flask and diluting to volume with methanol to obtain a concentration of 100 μg/ml. The solutions were stable for 12 months when stored at 4 °C or lower. Several standard solutions, with concentrations of 0.15–1.2 μg/ml, were injected three times to obtain the linearity of detector response.

Reproducibility

Reproducibility was investigated by inter-assay and intra-assay coefficient variations. Inter-assay coefficients of variation (day-to-day variability) were calculated using data generated from 10 consecutive assays whereas single fortified samples were run in sets of five to determine the intra-assay coefficients of variation (within-day variability). Retention times of pesticides were determined by injecting a standards mixture into the gas chromatograph for 10 times.

Recovery and detection sensitivity

The recovery test was performed by determining the concentrations of pesticides from fortified samples at levels ranging from 38–300 ng/g. We measured the recovery of the extraction procedure by comparing the peak height, which was acquired from measurement of a spiked sample with known concentration, with the peak height from measure-

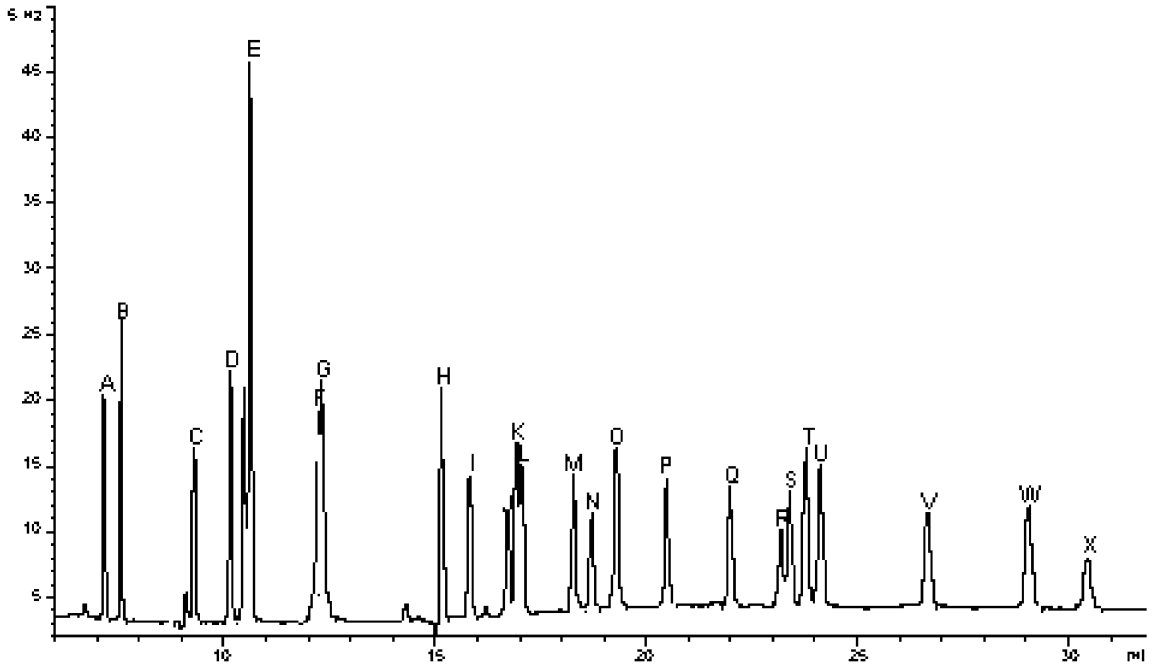

Fig. 3. Gas chromatogram of fatty matrices fortified with pesticides. A, PCNB (7.174); B, γ-BHC (7.577); C, heptachlor (9.289); D, chlorpyrifos-methyl (10.164); E, dimetipin (10.616); F, chlorpyrifos (12.236); G, fenitrothion (12.326); H, heptachlor epoxide (15.143); I, chlordane/*trans*-chlordane (15.814); J, 2,4′-DDE (16.717); K, *cis*-chlordane (16.932); L, α-endosulfan (17.052); M, chinomethionate (18.266); N, 4,4′-DDE (18.689); O, dieldrin (19.257); P, 2,4′-DDD (20.481); Q, endrin (21.971); R, 2,4′-DDT (23.180); S, 4,4′-DDD (23.397); T, β-endosulfan (23.769); U, ethion (24.120); V, 4,4′-DDT (26.652); W, endosulfan sulfate (29.034); X, dibromo-DDE (30.432).

Table II. Recovery of pesticides from spiked fat samples.

Compound	[ng/g]	Av. rec (%) (n = 3)	[ng/g]	Av. rec (%) (n = 3)	[ng/g]	Av. rec (%) (n = 3)
γ-BHC (lindane)	38	73.1 ± 2.3	75	78.1 ± 5.4	150	79.5 ± 9.1
Heptachlor	38	68.0 ± 7.2	75	67.9 ± 9.0	150	65.4 ± 5.0
Chlorpyrifos-methyl	50	89.2 ± 10.6	100	88.2 ± 8.5	200	85.6 ± 10.9
Dimetipin	75	96.0 ± 9.5	150	90.1 ± 9.3	300	95.4 ± 18.0
Chlorpyrifos	38	80.0 ± 11.8	75	79.0 ± 8.2	150	80.2 ± 7.5
Fenitrothion	38	94.8 ± 17.8	75	92.0 ± 16.7	150	90.0 ± 16.4
Heptachlor epoxide	38	76.2 ± 5.4	75	74.6 ± 6.6	150	79.7 ± 4.9
Chlordane/*trans*-chlordane	38	81.8 ± 5.7	75	81.1 ± 6.1	150	85.0 ± 8.4
2,4′-DDE	38	49.5 ± 6.8	75	50.4 ± 7.9	150	44.6 ± 3.4
cis-Chlordane	38	84.7 ± 11.3	75	85.6 ± 12.3	150	89.5 ± 4.2
α-Endosulfan	38	73.3 ± 9.4	75	73.8 ± 11.0	150	75.2 ± 3.8
Chinomethionate	38	64.4 ± 3.1	75	65.7 ± 5.3	150	66.4 ± 5.1
4,4′-DDE	38	51.1 ± 6.6	75	51.8 ± 9.1	150	57.5 ± 3.0
Dieldrin	38	84.2 ± 10.6	75	83.3 ± 11.5	150	80.4 ± 4.2
2,4′-DDD	38	71.6 ± 9.4	75	70.0 ± 8.8	150	76.5 ± 5.4
Endrin	38	67.8 ± 8.0	75	67.5 ± 9.3	150	65.6 ± 4.1
2,4′-DDT	38	50.0 ± 5.9	75	50.1 ± 6.7	150	51.2 ± 3.7
4,4′-DDD	38	78.3 ± 7.1	75	76.8 ± 8.8	150	75.6 ± 5.7
β-Endosulfan	38	77.9 ± 10.5	75	75.7 ± 2.2	150	72.9 ± 4.8
Ethion	38	74.3 ± 8.8	75	79.2 ± 8.0	150	79.3 ± 7.0
4,4′-DDT	38	73.4 ± 7.7	75	72.8 ± 8.4	150	76 ± 4.2
Endosulfan sulfate	38	87.9 ± 8.8	75	86.6 ± 9.9	150	83.2 ± 5.7

(Janda *et al.,* 1993), and sonication extraction (Castro *et al*., 2001; Sánchez-Brunete *et al*., 2002) have been widely used for extraction of pesticides from soil. Additionally, some of organochlorine and organophosphorus pesticides were extracted using microwave-assisted extraction (Eskilsson and Björklund, 2000). In this study, we used the microwave-assisted extraction technique for extraction of lipids from meat tissue as previously described by van Zoonen (1996).

The relatively low recovery of DDT metabolites might be related to its persistence at *n*-hexane layer during solvent partitioning. Further, it was difficult to pretreat the sample extracts individually to separate the interesting pesticide as well as to remove lipid interferences from the extracts. Therefore, DDT metabolites showed low recovery compared to the other pesticides analyzed by the same method. However, the instrumental limit of detection of these pesticides ranged from 0.52 to 0.93 ppb for 2,4′-DDE, 4,4′-DDE, and 2,4′-DDT. These results showed a satisfactory level for detecting the maximum residual limit of these pesticides (total sum 5.0 ppm for DDT metabolites). In accordance to recoveries and removal of interference, microwave-assisted extraction of fat from meat and solvent partitioning and cleanup is simple and effective for the reliable screening analysis of lipophilic pesticides.

Organochlorine pesticides could persist in the environment for several years and subsequently accumulate in aquatic organisms. Despite the use of DDT had been restricted for decades and its usage has been diminished, this would suggest that residue problem will decreases but the persistence of this compound in the environment will be encountered for many years. DDT may contaminate soils, plants, grazed cattle, dairy cow, and human in the way of the food chain. Therefore, for food safety and public health, laboratory examination of food products should be more enforced to reduce the public health risk of hazardous pesticides in livestock. This work shows the need to carry out further monitoring studies in order to improve food safety since these pesticides represent a potential risk to human health.

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