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Risk assessment for children exposed to DDT residues in various milk types from the Greek market

Ioannis N. Tsakiris [a], Marina Goumenou [b], Manolis N. Tzatzarakis [c], Athanasios K. Alegakis [c], Christina Tsitsimpikou [d], Eren Ozcagli [e], Dionysios Vynias [c], Aristidis M. Tsatsakis [c,*]

[a] TEI of Western Macedonia, Florina Branch, Department of Agricultural Products Management and Quality Control, TermaKontopoulou, 53100 Florina, Greece
[b] European Food Safety Authority, Parma, Italy
[c] Laboratory of Toxicology, Department of Medicine, University of Crete, Voutes 71003, Heraklion, Greece
[d] General Chemical State Laboratory of Greece, 16, An. Tsocha Str., 11521, Ampelokipi, Athens, Greece
[e] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Beyazit–Istanbul 34116, Turkey

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ABSTRACT

The occurrence of residues of DDT and its metabolites was monitored in 196 cow milk samples of various pasteurized commercial types collected from the Greek market. Residue levels were determined by GC-MS analysis. In 97.4% of the samples at least one DDT isomer or one of the DDT metabolites was detected, in levels not exceeding the maximum permitted residue level by the EU. Hazard Index for both carcinogenic and non-carcinogenic effects was estimated under two assumptions: a) using DDT concentrations from positive samples and b) imputing LOD/2 as an arbitrary concentration for negative samples. No statistically significant differences in detected or summed residue ($p > 0.05$) concentrations between different milk types were observed, with the exception of specific metabolites of DDT in some milk types. Exposure assessment scenarios were developed for children aged 1, 3, 5, 7 and 12 years old based on estimated body weights and daily milk consumption. Hazard Indices for non-carcinogenic effects were below 0.109 covering also carcinogenic effects according to WHO approach. The cancer risk values for carcinogenic effects according to the US EPA Cancer Benchmark Concentration approach, ranged from 0.4 to 18. For both effects the highest values were calculated for the 1- to 3-year-old age groups.

1. Introduction

Organochlorine pesticides (OCPs) are typically characterized by high vapor pressures and lipophilicity. They are persistent and highly stable under most environmental conditions. Additionally, their fat solubility promotes bioaccumulation through the food chain (Pardio et al., 2003). OCP residues are regarded as severe environmental contaminants and they are toxic to both humans and animals. OCPs are also considered as endocrine-disrupting chemicals and carcinogenic compounds (Stoker et al., 2011). Dichlodiphenyl-trichloroethane (DDT), a previously widely used OCP, is officially classified according to EU regulation as suspected of causing cancer (Carc 2) and responsible for causing damage to organs through repeated and prolonged exposure (Annex VI of Regulation 1272/2008/EC), while the Integrated Risk Information System (IRIS) of United States Environmental Protection Agency (USEPA) has classified DDT and some of its major metabolites as probable human carcinogens (Group B2) (Luo et al., 2014). Despite the fact that the production and usage of the great majority of OCPs has been banned in Europe (Regulation 528/2012/EU) and substantially reduced worldwide, their residues may still be detected in food products from different regions (e.g. Heck et al., 2007; Tsatsakis et al., 2008).

Human exposure to OCPs occurs mainly through the food chain. OCPs, including DDT, are among the most common persistent organic

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Abbreviations: ADI, Acceptable Daily Intake; CBC, Cancer Benchmark Concentration; CI, confidence of interval; DDD, dichlorodiphenyldichloroethane (metabolite of DDT); DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (metabolite of DDT); DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; DDTs, sum of DDT and its metabolites; EDI, Estimated Daily Intake; GC-MS, gas chromatography–mass spectrometry; HI, Hazard Index; IRIS, Integrated Risk Information System; JMPR, Joint FAO-WHO Meeting of Pesticides Residues; MRLs, Maximum Residues Levels; OCPs, organochlorine pesticides; PTDI, Provisional Tolerable Daily Intake; RfD, Reference Dose; TCN, 1,2,3,4-tetrachloronaphthalene; UHT, Ultra High Temperature (milk treatment).

[*] Corresponding author. Toxicology Laboratory, Medical School, University of Crete, Heraklion, 71409, P.O. Box 1393, Crete, Greece. Tel.: +30 (2810) 542098 or 394679; fax: +30 (2810) 394688 or 542098.

E-mail address: aris@med.uoc.gr (A.M. Tsatsakis).

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pollutants detected in dietary milk (e.g. Losada et al., 1996; Tsakiris et al., 2013a); they accumulate in fat-rich food products including dairy products, such as cheese, butter and milk (e.g. Salem et al., 2009).

Quantitative exposure assessment is now widely used to estimate human exposure to xenobiotics through the consumption of food and therefore to provide a quantitative estimate of possible risks to human health. Risk assessment outputs are the scientific basis for risk management decisions and option analysis (FAO/WHO, Food Standards Programme, Codex Alimentarius Commission, 2008).

The objectives of the present study were to evaluate children's exposure to DDTs (DDT and its metabolites) via dietary milk consumption in Greece, and to assess the respective potential risks to children's health in terms of cancer and non-cancer effects.

2. Materials and methods

2.1. Sampling

A total of 196 samples of dietary cow milk from different market outlet points were collected. The characteristics of the milk samples are presented in Fig. 1. Of the 196 samples, 154 (79%) were conventionally produced while 42 (21%) were organic (certified by Greek certification bodies) and 30 (15%) were milk formula products specifically for children older than 12 months. Pasteurized milk samples represented 51% of the total samples while the rest were Ultra High Temperature (UHT) processed. The majority of samples (89%) were bottled in Tetrapack® aseptic packages.

Sampling was conducted according to European Commission Directive 2002/63/EC. All samples were transported at 2–4 °C to the laboratory and were analyzed at the same day of sampling; additional portions for replicate and confirmatory analysis were stored deep-frozen.

2.2. Analytical method

2.2.1. Reagents and materials

Analytical grade standards (opDDE, ppDDE, opDDD, ppDDD, opDDT, ppDDT) were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). All solutions were stored at −20 °C.

Hexane (Riedel-deHaen, Sigma-Aldrich, Laborchemikalien, D-30926) and dichloromethane (Fluka, Riedel-deHaen, Laborchemikalien, D-30926) were of pro analysis grade. Hydrochloric acid 37% and concentrated sulfuric acid 95–97%, were analytical grade reagents (Merck).

2.2.2. Sample extraction procedure

Milk treatment was based on a slightly modified previously published method (Campoy et al., 2001). Briefly, 5 ml of milk were placed in a glass vial. 2.5 ml methanol and 0.1 g of sodium oxalate were added. The solution was shaken for 5 min and 5 ml of ethyl ether/hexane (1:1 v/v) were added for extraction. The extracted samples were centrifuged for 5 min at 3000 rpm and the organic phase was separated. The extraction procedure was repeated twice. The collected organic phases were concentrated under a gentle stream of N_2 to a final volume of 1 ml. A volume of 0.5 ml of concentrated sulfuric acid was added and centrifugation was performed for 5 min at 3000 rpm. The acid residue was extracted twice with 1 ml of hexane. The organic phases were collected and hexane was evaporated to dryness.

2.2.3. Clean up step

The dry residue was re-dissolved in 1 ml of hexane cleaned up on solid phase extraction (SPE) columns, packed with 500 mg of acidified silica and 250 mg anhydrous Na_2SO_4 (analytical grade). The modified silica gel was prepared as follows: 27 ml of concentrated sulfuric acid were added drop wise into 50 g of anhydrous silica gel (60–200 Mesh; Merck), while the mixture was stirred to ensure good homogeneity. The acidified silica was stirred for another 30 minutes. Both sodium sulfate and acidified silica gel were heated at 120 °C for 3 hours before use.

The SPE cartridges were activated by addition of 2 ml of hexane:dichloromethane (4:1 v/v) and 2 ml of hexane. The elution solvent was hexane:dichloromethane (1:1 v/v). The final eluate was concentrated to dryness under a gentle nitrogen stream and 100 µl of a solution (0.1 ppm) of 1,2,3,4-tetrachloronaphthalene (TCN) internal standard was added and transferred to a GC-MS vial.

2.2.4. GC-MS analysis

Electron ionization mass spectrometric analysis of milk extracts was performed on a GC-2010 Shimadzu system equipped with a BPX5 capillary (SGE), 30 m × 0.25 mm i.d. × 0.25 µm (Supelco). Pure helium with a column flow of 1 ml/min was used as a carrier gas. Two microliters of each analyte solution were injected into the system in splitless mode and analyzed under the following conditions: The column temperature was initially held at 60 °C for 1 min, raised to 180 °C at 15 °C/min, held for 1 min, raised to 250 °C at 4 °C/min, held for 1 min and finally raised to 300 °C at 25 °C/min, where it remained stable for 2 min. The injector, interface and ion source temperatures were set at 270 °C, 310 °C and 230 °C. The mass spectrometer was operated in the selected ion-monitoring mode (SIM).

2.2.5. Assay validation

For the recovery estimation of the extraction method, blank milk samples were spiked with each analyte at concentration levels of 0.5, 1.0, 2.5 and 5.0 ng/ml. The mean recoveries for opDDE, ppDDE, opDDD, ppDDD, opDDT and ppDDT were 63.5%, 62.3%, 60.3%, 70.2%, 80.2% and 81.7% respectively.

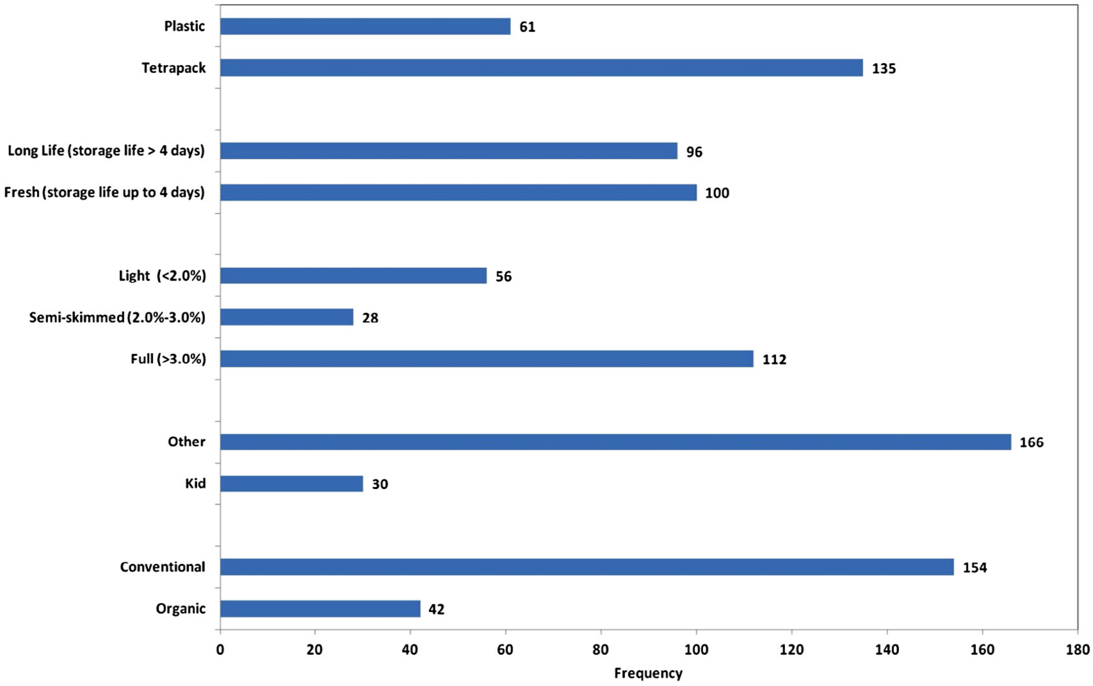

Fig. 1. Characteristics of milk samples collected from Greek market during 2009–2010 and analyzed for DDTs.