

ENDOCRINE DISRUPTORS AND ONE HEALTH: LABORATORY-BASED EVIDENCE FROM TURKIYE

Begum YURDAKOK-DIKMEN¹, Ozgur KUZUKIRAN², Ilker SIMSEK²,
Recep UYAR³, Ayhan FILAZI¹

¹Ankara University, Faculty of Veterinary Medicine, Ankara, Turkiye

²Çankırı Karatekin University, Sabanozu Health Services Vocational School, Cankiri, Turkiye

³Ankara University, Institute of Stem Cell, Ankara, Turkiye

Corresponding author email: byurdakok@yahoo.com

Abstract

Endocrine-disrupting compounds (EDCs) are of great concern, which interfere with hormonal systems, posing risks to human, animal, and environmental health. This study synthesizes findings from multidisciplinary investigations focused on the detection, distribution, and biological effects of EDCs across various matrices. Using gas chromatography–mass spectrometry (GC-MS) techniques, EDCs such as PCBs, OCPs, PAHs, PBDEs, and phthalates were quantified in dairy products, eggs, propolis, water bodies, and animal tissues. High levels of lipophilic EDCs were found in first-lactation bovine milk, fruit-added yogurt, organic eggs, and propolis, with contamination patterns influenced by fat content and production type. In vitro assays revealed cytotoxic and endocrine-disrupting effects of phthalates and BPA in cell lines from humans, cattle, dogs, and crayfish. Wildlife biomonitoring confirmed bioaccumulation of POPs in sea turtles and bats, while placental analyses demonstrated species-specific contaminant profiles and transcriptomic alterations affecting fetal development. These findings highlight the persistence and prevalence of EDCs and underscore the need for integrated monitoring and mitigation strategies. Adopting a One Health approach is essential to safeguard ecosystem integrity and public health from EDC-related risks.

Key words: animal origin food, endocrine disrupters, one health, residues, toxicity.

INTRODUCTION

Endocrine-disrupting compounds (EDCs) are exogenous chemicals that interfere with the endocrine systems of humans and animals, disrupting physiological homeostasis, or altering hormone synthesis and metabolism; which potentially causes developmental, reproductive, neurological, and immune dysfunctions.

Recent studies have identified estrogenic, androgenic, and progestogenic activities of EDCs in environmental samples, particularly in wastewater, even at extremely low concentrations. Furthermore, many samples have also demonstrated significant antagonistic hormonal effects, highlighting the complexity and potency of these substances in disrupting endocrine functions.

A substantial proportion of endocrine-disrupting chemicals (EDCs) originate from human and veterinary pharmaceutical products, as well as from personal care items, which often enter aquatic systems through direct discharge into

wastewater. Additional sources of EDCs include effluents from the food industry (e.g., phytoestrogens) and various industrial processes. These industrial sources may release a broad array of chemicals such as bisphenols, alkylphenols, polychlorinated biphenyls (PCBs), surfactants, pesticides, detergents, anti-corrosive agents, dyes, and flame retardants. Some of these compounds are semi-volatile and lipophilic, enabling them to travel long distances in the atmosphere either as free molecules or adsorbed onto particulates. As a result, they can reach surface waters and sediments far from their original emission sources. While certain EDCs-such as dichlorodiphenyltrichloroethane (DDT) and PCBs-are persistent in the environment, others degrade more rapidly (e.g., sex steroids, alkylphenols, and phthalates). Nevertheless, some of these substances can bioaccumulate in animal tissues, remain in ecosystems over extended periods, and ultimately enter the food chain. In addition to organic EDCs, certain inorganic substances-such as cadmium, mercury, arsenic, lead,

manganese, and zinc-as well as boric acid, have also been shown to interact with hormone pathways, further broadening the scope of concern regarding endocrine disruption.

This study summarises the results of multidisciplinary research projects investigating the detection, distribution and biological effects of endocrine-disrupting chemicals (EDCs) in various matrixes.

MATERIALS AND METHODS

This study synthesizes data from a series of interdisciplinary investigations conducted in our laboratory, focusing on the detection, biological impact, and environmental distribution of EDCs. Analytical evaluations were primarily performed using gas chromatography-mass spectrometry (GC-MS; Thermo Finnigan, USA) to quantify key EDCs including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and phthalates (Table 1).

Many methods such as gas chromatography, liquid chromatography, ELISA can be used to analyse EDCs. Since we have a GC-MS device in our laboratory, the analyses were carried out with this device. However, in order to guarantee the accuracy of the measurements, the method must first be validated and quality control parameters must be made. Chromatographic methods are more quantitative and the results obtained with them are more reliable. Our most important limitations are the scarcity of placentas, especially from dogs, budgetary constraints and lack of time. Using different matrices in each study and re-validation to test the reliability of the method as the matrix changes requires both intensive labour and high budget. However, the number of samples used in the studies was selected by statistical methods to represent the general population and was considered sufficient to show the importance of the subject.

Animal-Origin Food Analysis

Milk and Yogurt: Bovine milk and traditional strained yogurt samples were collected during different lactation periods (Simsek et al., 2024). Comparative analysis was conducted between plain and fruit yogurt formulations (Sireli et al., 2017).

Hen Eggs: Eggs sourced from battery-cage, free-range, and organic production systems were analyzed for PAEs, OCPs, PCBs, and PBDEs (Kuzukiran et al., 2018).

Bovine Fat Tissues: PCB distributions were analyzed across various fat depots (subcutaneous, perirenal, mesenteric) in cattle, with stratification by age and gender (Kuzukiran et al., 2020).

Propolis: Solid-phase extraction (SPE) techniques were used to prepare propolis samples for GC-MS-based EDC quantification (Simsek et al., 2020).

In Vitro and Cell-Based Assays

Colorectal Cell Lines: Phthalate mixtures were evaluated for their effects on DLD1 and HT29 human colorectal adenocarcinoma cell lines (Yurdakok-Dikmen et al., 2015).

Bovine Spermatozoa: The effects of PCBs 28, 30, and 118 on bovine sperm viability and morphology were assessed in vitro (Yurdakok et al., 2015).

Prostate Cancer Cells: Cytotoxicity of phthalates and the protective effects of alpha-lipoic acid were studied in prostate cancer cell lines (Kismali et al., 2017).

Bovine Testicular Cells: Testicular cells derived from cattle were exposed to phthalates to assess endocrine disruption at the gonadal level (Yurdakok-Dikmen et al., 2019).

Canine Testicular Cells: Primary testicular cells from dogs were cultured and treated with phthalates and BPA to assess toxicity and endocrine function interference (Tekin et al., 2020).

Crayfish Primary Cells: Narrow-clawed crayfish (*Astacus leptodactylus*) cells from hepatopancreas, gill, muscle, intestine, and gonads were exposed to DEHP, PCB118, and BPA. Cytotoxicity was measured using MTT assays (Yurdakok-Dikmen et al., 2020).

Mammary Cancer: MCF7 cell line treated with vincristine and tamoxifen in combination with BPA and DEHP to investigate EDC-induced chemoresistance (Uyar et al, 2022).

Ecotoxicological Field Studies

Aquatic Environments: Tap water, sea water, river water, and marine sediment samples were collected from various ecosystems and screened for multi-class EDCs, including legacy POPs and emerging pollutants (Yurdakok Dikmen et

al., 2016; Kuzukiran et al., 2016a, Kuzukiran et al., 2016b).

Sea Turtles: Loggerhead sea turtles (*Caretta caretta*) were examined post-mortem. EDC concentrations in fat, liver, kidney, and muscle tissues were quantified to evaluate bioaccumulation patterns (Kuzukiran et al., 2024).

Wild Bats: Liver and adipose tissue from free-living bats in Türkiye were analyzed for 20 organochlorine compounds. Data were stratified by species, sex, diet type, and fat content to

assess exposure variability and ecological risk (Kuzukiran et al., 2021).

Epidemiological and Transcriptomic Analysis
Human and Canine Placentas: Placental tissues from dogs and humans were screened for EDC residues. RNA-seq analyses were performed to investigate differential gene expression, with a focus on cholinergic receptors, estrogen receptors, and membrane transporters related to endocrine and placental function (Yurdakok-Dikmen et al., 2022; Kuzukiran et al., 2024).

Table 1. Endocrine disrupting compounds mentioned in this study, their sources, health effects, and legal thresholds

Compounds	Sources	Health Effects	Thresholds
Organochlorine Pesticides (OCPs)	Agricultural pesticides (DDT, chlordane, dieldrin) Industrial chemicals Contaminated soils and sediments Atmospheric long-range transport Bioaccumulation in food chain	Endocrine disruption Reproductive toxicity Neurodevelopmental effects Carcinogenic potential (Group 1-2B IARC) Immunotoxicity Liver dysfunction	EU DDT: 1.0 mg/kg (fat) Dieldrin: 0.2 mg/kg WHO ADI: DDT: 0.02 mg/kg bw/day US EPA: Various limits 0.1-5 mg/kg
Polychlorinated Biphenyls (PCBs)	Electrical equipment (transformers, capacitors) Industrial coolants and lubricants Paint additives and flame retardants Contaminated sediments Incineration and thermal processes	Cancer (Group 1 IARC carcinogen) Immunosuppression Neurological effects Reproductive disorders Endocrine disruption Chloracne (skin effects)	EU: Sum PCBs: 0.04 mg/kg WHO TDI: 1-4 pg TEQ/kg bw/day US EPA: 2.0 mg/kg (fish) 0.5 mg/kg (poultry)
Polybrominated Diphenyl Ethers (PBDEs)	Flame retardants in electronics Furniture and textile treatment Construction materials Automotive industry Waste incineration Indoor dust and air	Thyroid hormone disruption Neurodevelopmental toxicity Reproductive effects Behavioral changes Potential carcinogenicity Oxidative stress	EU RoHS: 1000 mg/kg (electronics) US EPA: No federal limits Health Canada: TDI: 0.1 µg/kg bw/day (BDE-209)
Polycyclic Aromatic Hydrocarbons (PAHs)	Incomplete combustion processes Vehicle emissions Industrial activities Coal and wood burning Cigarette smoke Grilled and smoked foods	Carcinogenicity (Group 1-2A IARC) Mutagenic effects Respiratory irritation Skin sensitization Immunosuppression Developmental toxicity	EU: B[a]P: 0.002 mg/kg Sum PAH4: 0.012 mg/kg US EPA: B[a]P: 0.005 mg/L (water) WHO: Air quality: 1 ng/m ³
Phthalates	Plastic manufacturing (PVC plasticizers) Personal care products Medical devices Food packaging materials Cosmetics and fragrances Building materials	Endocrine disruption Reproductive toxicity Developmental effects Anti-androgenic effects Metabolic disorders Asthma and allergies	EU: DEHP: 1.5 mg/kg DBP: 0.3 mg/kg US EPA TDI: DEHP: 20 µg/kg bw/day Toys Directive: 0.1% by weight limit

Note: Thresholds vary by matrix (food, water, air, soil) and regulatory authority. Values shown represent commonly referenced limits from major international organizations. ADI = Acceptable Daily Intake; TDI = Tolerable Daily Intake; TEQ = Toxic Equivalency; RoHS = Restriction of Hazardous Substances; IARC = International Agency for Research on Cancer.

RESULTS AND DISCUSSIONS

Quantitative analysis of dairy products revealed that both milk and yogurt can serve as reservoirs of lipophilic endocrine disruptors. In the first-lactation period bovine milk samples consistently exhibited higher concentrations of OCPs, PCBs, and PBDEs, correlating with elevated fat content, followed by the third and second periods. The same ranking is valid for the fat content in milk. Yogurt samples-particularly fruit-added types-showed greater phthalate contamination, likely due to processing and packaging materials. Eggs from free-range and organic systems displayed elevated POPs compared to battery-produced eggs, likely reflecting environmental exposure pathways. Propolis samples were also confirmed to harbor a diverse spectrum of persistent organic pollutants (POPs), including PAHs and PCBs, suggesting that even bee-derived products reflect ambient contamination levels. Age and gender influence PCB accumulation in cattle, with older animals-particularly females-showing higher levels. Among various tissues, perihepatic fat, muscle, and kidney contained the highest concentrations, especially of PCB101, PCB153, and PCB138. This highlights the breakdown of traditional safety assumptions that organic systems inherently mitigate chemical risks, instead indicating the influence of environmental context over production type. Ecotoxicological monitoring in aquatic environments revealed a multi-class contamination profile in tap water, river water, sea water, and marine sediments. Advanced GC-MS methodologies, including ultrasound-assisted microextraction (USAEME) and dispersive liquid-liquid microextraction (DLLME), enabled sensitive detection of trace-level PCBs and phthalates. Wildlife biomonitoring studies illustrated bioaccumulation of OCPs and PCBs in the liver and adipose tissues of free-living bats, with significant interspecies, sex, and fat content-related differences. Similarly, necropsy findings from loggerhead sea turtles (*Caretta caretta*) confirmed organ-specific contamination, with hepatic and adipose tissues carrying the heaviest pollutant loads. Phthalates promoted cytotoxic effects in prostate cancer cell lines, while α -lipoic acid

provided partial protective effects by restoring mitochondrial function. In mammary cancer models (MCF7), co-treatment with BPA and DEHP enhanced resistance to tamoxifen and vincristine via upregulation of multidrug resistance genes and apoptotic suppression.

Narrow-clawed crayfish tissues demonstrated dose- and tissue-specific sensitivity to BPA, DEHP, and PCB118. MTT assays revealed highest cytotoxicity in gonadal and intestinal cell cultures, underscoring the relevance of aquatic invertebrates as EDC biomarkers. Phthalates disrupted bovine sperm viability and acrosomal integrity, with PCB 118 having the most detrimental effects among the congeners tested. Canine testicular cells also showed significant sensitivity to BPA and DEHP, confirming cross-species relevance.

Species-specific differences in the placental accumulation of PCBs, OCPs, PBDEs, and PAHs between dogs and humans, with dogs showing distinct bioaccumulation patterns and potentially higher excretion rates. In humans, contaminant levels in placental tissues were influenced by factors such as BMI, smoking status, and educational background, highlighting the role of lifestyle and environmental exposure. Given the placenta's vital role in fetal development, minimizing maternal exposure to environmental pollutants-particularly prior to conception-is essential for promoting healthier outcomes in future generations. Transcriptomic analyses (RNA-seq) from dog and human placental tissues confirmed differential expression of cholinergic receptors, estrogen receptors, and membrane transporters. These molecular alterations may compromise placental barrier function and hormone signaling, affecting fetal development from the first trimester onward.

The One Health framework emphasizes the interconnectedness of human, animal, and environmental health. Endocrine-disrupting chemicals (EDCs), such as polychlorinated biphenyls (PCBs), phthalates, and bisphenol A (BPA), epitomize this nexus by exerting parallel and often synergistic effects across biological systems and ecosystems. Although individual EDCs exert weak estrogenic effects on their own, in mixtures the effects add up. For example, phthalates antagonise androgen receptors, BPA activates estrogen receptors and

triclosan disrupts thyroid hormone synthesis. Exposure to them together causes simultaneous disruption of multiple hormone systems. In the agricultural sector, dairy and egg products-often consumed daily-serve as vectors for lipophilic EDCs due to bioaccumulation in animal fat. The environmental interface is equally concerning. EDCs discharged into aquatic ecosystems bioaccumulate through trophic levels, disrupting endocrine functions in aquatic fauna and predatory species, including humans.

CONCLUSIONS

The body of evidence in our laboratories indicate the presence and biological impact of EDCs across diverse environmental matrices and species. Dairy products, eggs, and bee-derived substances such as propolis are confirmed vectors of lipophilic EDCs, reflecting both direct exposure and environmental contamination. Organic and free-range production systems, traditionally perceived as safer, do not necessarily confer reduced chemical risk, highlighting the dominant role of environmental exposure over farming method. Aquatic and wildlife monitoring reveals multi-compartmental contamination with persistent organic pollutants (POPs), results showed bioaccumulation patterns influenced by species, tissue type, age, sex, and environmental context. Molecular and cellular toxicity studies further demonstrate that EDCs compromise reproductive health, mitochondrial function, and treatment efficacy in cancer models, with observed cross-species effects.

As a result, EDCs bind to hormone receptors by mimicking (agonist effect) or inhibiting (antagonist effect) natural hormones. For example, bisphenol A binds to estrogen receptors and exerts estrogenic effects, while phthalates antagonise androgen receptors. They have also been reported to have epigenetic effects.

In the European Union, according to the International Stockholm Convention on Persistent Organic Pollutants (Regulation EC 850/2004), organochlorine pesticides DDT, aldrin, dieldrin, endrin are classified as POPs and their use is prohibited. Regulation EC 396/2005 sets the maximum residue limits for pesticide residues in food as 1.0 mg/kg for DDT

on oil basis. Directive 96/59/EC regulates the disposal of PCBs, while Regulation EC 1881/2006 sets PCB limits in food (0.04 mg/kg for total PCBs). REACH Regulation (EC 1907/2006) prohibits the production and marketing of PCBs. RoHS Directive (2011/65/EU) limits the use of certain PBDEs in electronic devices to 1000 mg/kg. Tetra-, penta-, hexa- and heptaBDEs are banned under the Stockholm Convention. For PAHs, Regulation EC 1881/2006 sets a limit of 0.002 mg/kg for benzo(a)pyrene and 0.012 mg/kg for the sum of PAH4 in food. REACH Regulation Annex XVII restricts the use of some PAHs in consumer products. Toys Safety Directive (2009/48/EC) sets a limit of 0.1% for DEHP, DBP, BBP in toys for phthalates. REACH Regulation has included DEHP, DBP, DIBP and DIBP in the list of substances of high concern. Regulation EC 1881/2006 sets a maximum limit of 1.5 mg/kg for DEHP in infant formula. The detection of pollutants in placental tissues of both humans and animals, along with associated transcriptomic alterations, raises concerns about developmental toxicity and transgenerational effects. These findings reinforce the critical need to address environmental pollution through a One Health approach, recognizing the intertwined health of humans, animals, and ecosystems. Coordinated strategies to monitor, regulate, and mitigate EDC exposure are essential to safeguard biological integrity and public health. Therefore, continuous monitoring of EDC residues, especially in foods, is recommended.

REFERENCES

- Kismali, G., Yurdakok-Dikmen, B., Kuzukiran, O., Arslan, P., & Filazi, A. (2017). Phthalate induced toxicity in prostate cancer cell lines and effects of alpha lipoic acid. *Bratislava Medical Journal*, 118(8), 460–466.
- Kuzukiran, O., Filazi, A., Sevin, S., Yurdakok-Dikmen, B., Yikilmaz, Y., Erdoğan, E., Şen, F., Totan, F. E., Celik, C., & Kirmizibayrak, O. (2020). Determination of the polychlorinated biphenyls distribution in different fat tissues of cattle by age and gender. *Archives of Environmental Contamination and Toxicology*, 78(2), 294–302.
- Kuzukiran, O., Simsek, I., Kara, E., Yurdakok-Dikmen, B., Boztepe, U. G., Toprak, M., & Filazi, A. (2024). An investigation of some persistent organic pollutants in loggerhead sea turtles (*Caretta caretta*). *Marine Pollution Bulletin*, 205, 116670.

- Kuzukiran, O., Simsek, I., Yorulmaz, T., Yurdakok-Dikmen, B., Ozkan, O., & Filazi, A. (2021). Multiresidues of environmental contaminants in bats from Turkey. *Chemosphere*, 282, 131022.
- Kuzukiran, O., Yurdakok-Dikmen, B., Filazi, A., Sevin, S., Aydin, F. G., & Tutun, H. (2016). Determination of polychlorinated biphenyls in marine sediments by ultrasound-assisted isolation and dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry. *Analytical Letters*, 49(15), 2525–2536.
- Kuzukiran, O., Yurdakok-Dikmen, B., Sevin, S., Şireli, U. T., İplikcioglu-Cil, G., & Filazi, A. (2018). Determination of selected endocrine disruptors in organic, free-range and battery-produced hen eggs and risk assessment. *Environmental Science and Pollution Research*, 25(35), 35376–35386.
- Kuzukiran, O., Yurdakok-Dikmen, B., Totan, F. E., Celik, C., Orhan, E. C., Bilir, E. K., Kara, E., & Filazi, A. (2016). Analytical method development and validation for some persistent organic pollutants in water and sediments by gas chromatography mass spectrometry. *International Journal of Environmental Research*, 10(3), 401–410.
- Kuzukiran, O., Yurdakok-Dikmen, B., Uyar, R., Turgut-Birer, Y., Çelik, H. T., Simsek, I., Karakas-Alkan, K., Boztepe, U. G., Ozyuncu, O., Kanca, H., Ozdag, H., & Filazi, A. (2024). Transcriptomic evaluation of metals detected in placenta. *Chemosphere*, 142929.
- Sevin, S., Kuzukiran, O., Yurdakok-Dikmen, B., Tutun, H., Aydin, F. G., & Filazi, A. (2018). Selected persistent organic pollutants levels in the Ankara River by months. *Environmental Monitoring and Assessment*, 190(12), 705.
- Simsek, I., Kuzukiran, O., Yurdakok-Dikmen, B., Snoj, T., & Filazi, A. (2020). Determination of persistent organic pollutants (POPs) in propolis by solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). *Analytical Letters*, 54(10), 1666–1682.
- Simsek, I., Kuzukiran, O., Boztepe, U. G., Toprak, M., Harbi, M. A., Hariry, R. E., ... & Filazi, A. (2024). The concentrations of selective endocrine disruptors in milk from different lactation periods of cows. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 30(4), 507–516.
- Şireli, U. T., Filazi, A., Yurdakok-Dikmen, B., İplikcioglu-Cil, G., Kuzukiran, O., & Orhan, C. (2017). Determination of phthalate residues in different types of yogurt by gas chromatography-mass spectrometry and estimation of yogurt-related intake of phthalates. *Food Analytical Methods*, 10(9), 3052–3062.
- Tekin, K., Arslan, P., Cil, B., Filazi, A., Akcay, E., & Yurdakok-Dikmen, B. (2020). Companion animals get close to the toxic aspects of anthropogenic world: Cytotoxicity of phthalates and bisphenol A on dog testicular primary cells. *Cytotechnology*, 72(5), 629–638.
- Uyar, R., Yurdakok-Dikmen, B., Turgut, Y., & Filazi, A. (2022). Diethylhexyl phthalate and bisphenol A promote vincristine and tamoxifen resistance in vitro. *Chemical Research in Toxicology*, 35(3), 538–546.
- Yurdakok, B., Tekin, K., Daskin, A., & Filazi, A. (2015). Effects of polychlorinated biphenyls 28, 30 and 118 on bovine spermatozoa in vitro. *Reproduction in Domestic Animals*, 50(1), 41–47.
- Yurdakok-Dikmen, B., Alpay, M., Kışmalı, G., Filazi, A., Kuzukiran, Ö., & Şireli, U. T. (2015). In vitro effects of phthalate mixtures on colorectal adenocarcinoma cell lines DLD1 and HT29. *Journal of Environmental Pathology, Toxicology and Oncology*, 34(2), 115–123.
- Yurdakok-Dikmen, B., Kuzukiran, O., Filazi, A., & Kara, E. (2016). Measurement of selected polychlorinated biphenyls (PCBs) in water via ultrasound assisted emulsification-microextraction (USAEME) using low-density organic solvents. *Journal of Water and Health*, 14(2), 214–222.
- Yurdakok-Dikmen, B., Stelletta, C., Tekin, K., Kuzukiran, O., Daskin, A., & Filazi, A. (2019). Effects of phthalates on bovine primary testicular culture and spermatozoa. *Cytotechnology*, 71(5), 935–947. <https://doi.org/10.1007/s10616-019-00336-z>
- Yurdakok-Dikmen, B., Turgut, Y., Gunal, A. Ç., Uyar, R., Filazi, A., & Erkoc, F. (2020). In vitro effects of selected endocrine disruptors (DEHP, PCB118, BPA) on narrow-clawed crayfish (*Astacus leptodactylus*) primary cells. *In Vitro Cellular & Developmental Biology-Animal*, 56, 783–791.