



Congener Profile and Estimated Dietary Intake of Dioxins in Egyptian Food

Thesis Submitted

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ABSTRACT



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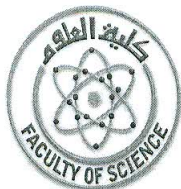
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AIM OF STUDY

- ✓ Survey of Dioxins and DL-PCBs contamination levels in some foodstuffs samples collected from different markets in Egypt.
- ✓ Studying the contribution of Dioxins, DL-PCBs to the toxic equivalent quantities (WHO-TEQ) in the studied foodstuffs.
- ✓ Assessment the risk on Egyptian customers as dietary exposure to such chemical contaminants through estimated their dietary intake relative to the acceptable daily intakes stated by WHO and FAO.

List of Abbreviations

Abbreviations	Synonyms
µg	Micro-gram, 10 ⁻⁶ g.
mg	Milli-gram, 10 ⁻³ g.
Kg	Kilo-gram, 10 ³ g.
b.w	Body weight.
ng	Nano-gram, 10 ⁻⁹ g
pg	Pico-gram, 10 ⁻¹² g.
FAO	Food and Agriculture Organization.
WHO	World Health Organization.
JECFA	Joint FAO/WHO Expert Committee on Food Additives.
WHO/GEMS	WHO/Global Environment Monitoring System.
EFSA	European Food Safety Authority.
EU	European Union.
EGY	Egyptian Standards.
HRGC-HRMS	High Resolution Gas Chromatography - High Resolution Mass Spectrometer.
PCDDs	Polychlorodibenzo-p-dioxins.
PCDFs	Polychlorodibenzofurans.
DL-PCBs	Dioxin-like polychlorinated biphenyls.
TEF	Toxicity equivalent factor.
n	Number of analysed samples
EPTDI	Estimated provisional tolerable daily intake.
EPTWI	Estimated provisional tolerable weekly intake.
APTDI	Accepted provisional tolerable daily intake.
APTWI	Accepted provisional tolerable weekly intake.
TEQ	Toxic equivalency quantity.
SCF	Scientific Committee on Food.
WHO-TEQ ₂₀₀₅	Toxic equivalency quantity determined by World Health Organization in 2005.



كلية معتمدة



مكتب وكيل الكلية لشئون الدراسات العليا والبحوث



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* عنوان البحث:

Patterns of Polychlorodibenzo-p-Dioxins and Furans and study the dietary intake in commercialized orange in Egypt

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العليا والبحوث



Patterns of Polychlorodibenzo-p-Dioxins and Furans and study the dietary intake in commercialized orange in Egypt

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ABSTRACT

A total of 18 orange samples were collected from 9 different governorates in Egypt and analyzed for dioxins, consisting of polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs), using High Resolution Gas Chromatography - High Resolution Mass Spectrometer (HRGC-HRMS). The mean concentration of Σ PCDD/Fs in orange samples was 1.8596 pg/g whole weight (w.w.). 1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (OCDD) was the dominant congener of PCDD/Fs in orange samples. The Toxic Equivalency (TEQ) concentrations of PCDD/Fs were calculated using the Toxicity Equivalent Factors (TEF) established by the World Health Organization (WHO) in 2005. The mean TEQ concentration of Σ PCDD/Fs in orange samples was 0.1616 pgWHO-TEQ₂₀₀₅/g w.w. This study showed that the average TEQ concentration of Σ PCDDs (0.1061 pgWHO-TEQ₂₀₀₅/g w.w.) is higher than the average TEQ concentration of Σ PCDFs (0.0555pgWHO-TEQ₂₀₀₅/g w.w.) which contributed about 65.66% from the total TEQ concentration of Σ PCDD/Fs in commercialized orange in Egypt. This study showed that the PCDD/Fs levels in orange were lower than the maximum permissible limits of Egyptian Standardization and European Community (0.30 pgWHO-TEQ₂₀₀₅/g w.w.). The consumption of Egyptian people for orange attained according to GEMS/Food World Health Organization (WHO) consumption rate of orange for the Middle Eastern people is 38g/person/day, which showed that the Estimated Daily Intake (EDI) of Σ PCDD/Fs of the Egyptian consumer is 0.1023 pg WHO-TEQ/kg body weight/day lower than the WHO acceptable daily intake which is 4 pg WHO-TEQ/ kg b.w/day.

Keywords: PCDD/Fs; HRGC/MS; Dietary intake; Orange and Egypt.

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Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) are two groups of chemicals within the persistent organic pollutants (POPs) found in the environment because of their persistent, toxic, and bioaccumulative properties. Furthermore, they are the most toxic POPs ^[1]. The two families of POPs consist of 75 PCDDs and 135 PCDFs theoretical individual congeners, based on the number and position of chlorine atoms in the chemical structure ^[2]. Although they are 210 PCDD/Fs congeners, only 17 (those with a 2,3,7,8 substitution) have so far been found to be toxic. Among them, the most toxic compound is 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD. The toxicity of other PCDD/Fs is measured in relation to TCDD. PCDD/Fs have well described toxicities at extremely low concentration, a highly competent system, which allows inter-comparison of toxicities between different isomers on the basis of toxic equivalents (TEQ), has been developed for humans and wildlife ^[3]. In general, PCDD/Fs compounds are of unintentional anthropogenic nature linked to several industrial processes that include thermal and combustion processes, waste incineration, industrial reservoir source, metal smelting and refining and production of pesticides ^[4, 5].

PCDDs and PCDFs may be transported over long distances from their source and travel via water, air and ground. Because of their high stability, low volatility and high resistance to degradation, they can remain long periods with half-lives between 7 and 10 years ^[6]. These characteristics render dioxins to be highly persistent pollutants that experience bioaccumulation and bio magnification phenomena, so they can access into the feed and food chain ^[7-10].

Oranges represent around 30 percent of the total Egyptian fruit production and 65 percent of citrus production. Egypt is one of the world's leading orange producers and exporters. Egyptian orange production is high yielding and competitive due to the availability of irrigation water (the Nile), suitable climatic conditions, good soil, low labor costs, an early harvest compared to other major producers in the region, and Egypt's proximity to major importing countries. Egypt is the sixth orange producer in the world after Brazil, China, US, EU, and Mexico. Several orange varieties are produced in Egypt but the dominant varieties are six (*Baladi Orange*, *Valencia Orange*, *Blood Orange*, *Navel Orange*, *Khalily Orange* and *Sweet Orange*). Valencia and navel are the main exported varieties while the others are used more for domestic consumption ^[11].

A wide variety of extreme health effects have been related to high exposure to dioxin derivatives, such as growth retardation of the fetus and infants, developmental defects, reproductive effects, chloracne, hormonal dysfunctions, mood alterations, reduced mental performance, endometriosis, changes in white blood cells, dental defects and diabetes ^[12-17].

Since 1990s food has been identified as a pathway of human exposure to dioxin compounds. Dietary intake contributes about 90-98% of the total daily dioxin intake of the general population ^[18, 19]. The World Health Organization (WHO) has established a tolerable daily intake (TDI) range of 1–4 pg TEQ (Toxic Equivalency)/kg b.w. (body weight) for dioxins ^[20]. Likewise, a tolerable weekly intake (TWI) of 14 pg WHO-TEQ/kg b.w. (body weight) has been set up by the European Union through the Scientific Committee on Food ^[21].

Therefore, the aim of this study is the assessment of the risk on the human as dietary exposure to dioxins in consumed orange in Egypt through estimating their

dietary intake relative to the acceptable daily intakes stated by the World Health Organization (WHO).

Materials and methods

Sampling

The procedures of sampling were performed following the Codex Alimentarius Commission regulation ^[22]. A total of 18 orange samples were purchased from 9 different governorates (Qaliubiya, Giza, Alexandria, Ismailia, Sharkia, Fayoum, Menoufiya, Beni Suef and Gharbiya) in Egypt to determine levels of Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). Samples were collected during the years 2015–2016 and were homogenized using a blender and stored at -20°C until analysis.

Chemicals and reagents

All solvents used were analytical grade and purity not less than 99%. Silica gel and basic alumina were purchased from Aldrich (Brockmann I, Standard grade, Milwaukee, USA). Calibration standard solutions, labeled standard and injection solutions specified in Environmental Protection Agency (EPA) Method 1613B were obtained from Cambridge Isotopes Laboratories (Andover, USA).

Apparatus and instrument

Soxhlet (50mm ID, 200 ml capacity with 500 ml flask), Top bench balance" Mettler Toledo" ranged from 0.1 mg to 210, Electrical apparatus for sample homogenization (e.g Bamix), Thimble (43mm ID*123mm H) to fit Soxhlet, Rotary evaporator (Heidolph VV2000), Calibrated Micro-liter pipettes and Heating Electromantle (Cat. No EM0500/C to fit 500 ml round-bottom flask).

High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS).The High Resolution Mass Spectrometry (Micromass Auto-Spec Ultima) attached to GC chromatography (Agilent 6890 N).The GC equipped with Auto injector (Agilent 7683 series), Split/Splitless injection port for capillary column and a DB-5ms fused silica column (60 m × 0.25 mm × 0.25 µm).

Method of analysis

Extraction

Orange samples were grinded well. A known weight of samples (25 g) was spiked with known amounts of mixture of labeled PCDD/Fs. Next, samples were extracted by soxhlet for at least 18-24 h with Dichloromethane /Hexane 1/1 v/v. The extract was rotary evaporated and concentrated to near dryness. Complete removal of the solvent was performed using a stream of nitrogen. The extract residue was dissolved in 5 ml of n-hexane for cleanup.

Cleanup and purification process

These steps were conducted according to U.S EPA 1613(B) Method ^[23], using Anthropogenic, Multilayer silica gel, Alumina and active Carbon column.

HRGC/HRMS analysis

The qualitative and quantitative analyses of PCDD/Fs congeners and dl-PCBs congeners were performed using GC/HRMS in the selected ion monitoring mode at a resolution $\geq 10,000$. Separation of congeners was carried out using a capillary column (60m length, 0.25 mm ID, and 0.25 µm thickness) coated with a DB-5 stationary phase. Helium was used as a carrier gas at a flow rate of 1ml/min. The temperature of the injector and the interface were 280 °C, respectively. The column temperature program was as follows: initial temperature was 90-220 °C, at 15 °C/min, then kept at 220 °C for 15min then raised again from 220-290 °C at a rate of 8 °C/min and kept at 290 °C for 17.6 min ^[23].