

# Tobacco Eco-toxic Fallout: Organochlorine pesticides and polychlorinated biphenyls residues in hookah wastewater

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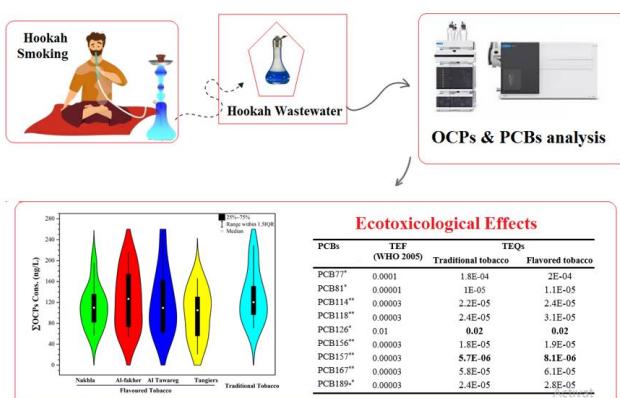
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## Graphical abstract



## Abstract

Concerns about both flavored and traditional waterpipe smoking continue to grow because of their considerable environmental and health impacts. This study aims to conduct a comprehensive evaluation of the levels of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) present in wastewater generated from waterpipe use. Samples from four major brands of flavored tobacco (Nakhla, Al-fakher, Al-tawareq, Tangiers) and traditional tobacco were analyzed. The mean concentration levels of  $\Sigma$ OCPs in Nakhla, Al-fakher, Al-tawareq and Tangiers brands were 111.25, 125.51, 122.70, and 98.13 ng/L, respectively, and in traditional tobacco samples was 132.23 ng/L. The PCBs concentrations in flavored tobacco samples were 10.36 -

11.94  $\mu$ g/L, which exceeds the concentration in traditional tobacco at 10.26  $\mu$ g/L.  $\beta$ -HCH was the most prevalent OCP, representing up to 51% in flavored tobacco wastewater and 45% in traditional tobacco wastewater. PCB194 was the most common PCB congener, consistently making up 24-25% of the entire PCB concentration. The risk quotient (RQ) for values traditional and flavored hookah wastewater were ranged from 0.00 ( $\delta$ -HCH) to 2.46 (endosulfan) and 0.00 ( $\delta$ -HCH) to 3.50 (endosulfan), respectively. The environmental and health impacts of hookah wastewater, particularly concerning PCBs and OCPs and other toxic compounds, are becoming increasingly concerning. Despite the comparatively low toxic equivalent (TEQ) values associated with hookah wastewater, the continuous discharge of such waste into aquatic environments present considerable threats to the wellbeing of both natural habitats and people. Addressing this contamination requires concerted efforts from policymakers, researchers, and communities worldwide to develop effective solutions.

**Keywords:** Waterpipe, Wastewater, Tobacco, Organochlorine pesticide, Polychlorinated biphenyls

## 1. Introduction

Tobacco smoking represents a significant global health challenge, as a major preventable cause of mortality and morbidity (Danaei *et al.* 2017; De Silva *et al.* 2024; Masjedi *et al.* 2023d). Tobacco consumption patterns have shifted in recent years, with a decline in cigarette smoking but an

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increase in waterpipe tobacco smoking, which is one of the most traditional and widespread methods worldwide (Dehvari and Babaei 2022; Khodadost *et al.* 2020). This increased prevalence of waterpipe tobacco smoking causes concern about both the health risks to smokers and the potential adverse environmental impacts associated with the frequent disposal of concentrated hazardous wastes from waterpipe smoking (Heydari *et al.* 2024a; Kassem *et al.* 2020a; Masjedi *et al.* 2023a; Masjedi *et al.* 2023d; Maziak *et al.* 2015; Rashidi *et al.* 2024a; Soleimani *et al.* 2024a; Soleimani *et al.* 2025b; Termeh-Zonoozi *et al.* 2023).

A waterpipe is made up of a water container with a bowl at the top filled with waterpipe tobacco. The tobacco is ignited by burning charcoal placed on top, and the generated smoke flows through the water before it is breathed in (Edwards *et al.* 2021; Termeh-Zonoozi *et al.* 2023). After each use, the charcoal residues, aluminum foil used to cover the waterpipe tobacco during smoking, and burnt or partially burnt tobacco residues are discarded as waste. Additionally, approximately 94% of the smoked water from waterpipe is disposed of as wastewater in bathroom or kitchen sinks, where it flows into onsite septic systems or municipal sewer systems, with some also being discarded in toilet, backyard soil, or street storm drain (Edwards *et al.* 2021; Hsieh *et al.* 2021; Kassem *et al.* 2020a).

Previous scientific reports confirmed high concentrations of various pollutants, including BTEX (Heydari *et al.* 2020; Jafari *et al.* 2020; Masjedi *et al.* 2023e), PAHs (Masjedi *et al.* 2023c; Rashidi *et al.* 2024b; Soleimani *et al.* 2025a), heavy metals (Masjedi *et al.* 2023b; Masjedi *et al.* 2020), and aromatic amines (Heydari *et al.* 2024b; Soleimani *et al.* 2024b) in fresh tobacco and tobacco smoke. However, these toxic chemicals may be present in the waterpipe wastes and wastewater. As a result, releasing waterpipe wastewater into the environment can add these pollutants to soil and water systems, leading to their buildup and contributing to overall environmental contamination (Edwards *et al.* 2021). Few researches evaluated the pollutants, such as toxic elements (Al-Kazwini *et al.* 2015; Jafari *et al.* 2020; Qamar *et al.* 2015), BTEX (Masjedi *et al.* 2023d), aldehydes, nicotine (Edwards *et al.* 2021), furanic compounds (Schubert *et al.* 2012), and primary aromatic amines (Heydari *et al.* 2024a; Schubert *et al.* 2011) in waterpipe wastewater. Despite the detection of various chemicals in waterpipe wastewater, other toxic compounds that may also be present, such as biphenyls, polychlorinated biphenyls (PCBs), pesticides, and herbicides, have not been thoroughly investigated.

PCBs and organochlorine pesticides (OCPs) belong to the group of persistent pollutants that can persist for long durations in the environment and build up in adipose tissue (Moon *et al.* 2017). Exposure to PCBs and OCPs is associated with numerous harmful health outcomes, including damage to immune, nervous and reproductive systems, an heightened risk of various cancers—including breast cancer—and adverse effects on

neurodevelopment (Dickerson *et al.* 2019; Koual *et al.* 2019; Mouly and Toms 2016; Rezek *et al.* 2008; Zhang *et al.* 2010). Despite restrictions on their use, PCBs and several OCPs, including hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), and dichlorodiphenyltrichloroethane (DDT) have remained as environmental concerns in some regions (Wang *et al.* 2016c). PCBs are widespread contaminants found throughout the environment owing to their persistent chemical properties and affinity for accumulating in fats (Dickerson *et al.* 2019). Some researchers have reported the negative impacts of smoking on PCBs concentrations in human body, exhibiting higher levels in smokers than nonsmokers (D'Errico *et al.* 2012; Dickerson *et al.* 2019; Moon *et al.* 2017; Oltramare *et al.* 2025). OCPs were widely used for control of insect-borne epidemics, such as malaria, and agricultural cultivation (Karimi *et al.* 2020; Qi *et al.* 2014; Taufeeq *et al.* 2021a), and may be applied during the cultivation of tobacco products (Arfaeinia *et al.* 2024b). Previous studies detected residues of OCP in present in tobacco and its derivatives (Arfaeinia *et al.* 2024b; Qi *et al.* 2014; Taufeeq *et al.* 2021a). In a study, PCBs and OCPs were detected in leachate from smoked cigarette butts, indicating that these compounds are released from discarded cigarette butts into the aquatic ecosystem (Arfaeinia *et al.* 2024b). Exposure to these hazardous substances in humans may occur through consuming contaminated fatty foods (Arisawa *et al.* 2011), inhalation and/or dermal contact (Schettgen *et al.* 2012).

This study introduces novel insights by comprehensively measuring concentrations of PCBs and OCPs—persistent organic pollutants—in waterpipe wastewater from both flavored and traditional tobacco, an area previously underexplored compared to prior research on general chemical toxicity, heavy metals, or solid waste. It tests hypotheses of potentially harmful levels, quantifies ecological risks, and differentiates wastewater impacts, thereby highlighting new contaminants from this emerging tobacco waste source and advancing environmental toxicology beyond existing aquatic toxicity assessments. The primary objective of this work was to comprehensively assess the concentration levels of PCBs and OCPs compounds in waterpipe wastewater, with a particular focus on both flavored and traditional tobacco. We hypothesized that wastewater from waterpipe may contain OCPs and PCBs at concentrations capable of posing environmental harm. In addition, we evaluated the ecological risks associated with the detected OCP and PCB compounds. This study seeks to provide meaningful insight into the levels of these hazardous chemicals in waterpipe wastewater, thereby enhancing understanding of their contribution to environmental pollution and supporting the development of improved waste management practices.

## 2. Material and Methods

### 2.1. Study protocol and sample collection

In this study, the presence and concentrations of 11 PCBs and 12 OCPs were measured in wastewater collected from hookah bowls using both flavored and traditional

tobaccos. Four commonly available flavored tobacco brands—Nakhla, Al-Fakher, Al Tawareg, and Tangiers—were selected for analysis. After a typical smoking session at local cafés, the wastewater remaining in the hookah bowls was immediately collected to preserve analyte integrity. For each flavored tobacco brand, 10 wastewater samples were obtained. Additionally, 10 wastewater samples from bowls using freshly smoked traditional (non-flavored) tobacco were collected for comparison and analysis of the target contaminants. A total of 50 wastewater samples were collected immediately following waterpipe use. Of these, 40 samples were derived from four distinct flavored tobacco brands, while the remaining 10 originated from traditional (unflavored) tobacco. All samples were placed in opaque, foil-wrapped glass bottles, transported to the laboratory within approximately 2 hours of collection in a chilled container containing ice packs, and subsequently stored at  $-4^{\circ}\text{C}$  in the dark conditions until analysis. To preserve analyte stability and prevent volatilization, samples were kept at this temperature without exposure to light during storage. Sample extraction was carried out within a maximum holding time of 48 hours after collection for all samples.

### 2.2. Sample preparation and extraction

The target pollutants were extracted from waterpipe wastewater samples with using the dispersive liquid-liquid microextraction (DLLME) method based on our previous study (Jorfi *et al.* 2022; Soleimani *et al.* 2023). The extraction solvent was dichloromethane (DCM) and dispersion solvent was acetone. The extraction process involved placing 10 ml of the waterpipe wastewater sample in a 15 ml polypropylene tube and then adding 50  $\mu\text{l}$  of triphenyl phosphate (TPP) solution. A cloudy solution was formed through rapid injection of 1 ml of DLLME solution composed of acetone and dichloromethane (9:1). Following one minute of agitation, the mixture was centrifuged at 6500 rpm for five minutes, and the lower dichloromethane layer containing PCBs and OCPs was extracted for further analysis and quantification.

To minimize contamination and guarantee methodological accuracy, all glassware and polypropylene tubes were meticulously rinsed with methanol and DCM, and blank samples (containing only solvents and internal standard, without wastewater) were processed together with the real wastewater samples. The trace levels quantified in blank samples were applied to adjust the samples results accordingly, ensuring that any background contamination was subtracted from the sample measurements.

### 2.3. OCPs and PCBs analysis

The gas chromatograph-mass spectrometer (GC-MS) instrument comprising the Agilent 7890 GC and Agilent 5975 MS (Agilent Technologies, Palo Alto, CA) was utilized for OCPs and PCBs analysis. This setup featured a split/splitless injection port and an ultra-inert column measuring 30 meters long, millimeters in diameter, and 25 micrometers thick. The oven temperature program was initiated at  $55^{\circ}\text{C}$ , held for one minute, subsequently rose at a rate of  $1^{\circ}\text{C}$  per minute to  $150^{\circ}\text{C}$  for two min, and

finished with a  $20^{\circ}\text{C}/\text{min}$  increase to  $280^{\circ}\text{C}$  for 15 min. For OCPs, the oven was first heated to  $80^{\circ}\text{C}$  for 1 min, then ramped at  $30^{\circ}\text{C}/\text{min}$  to  $175^{\circ}\text{C}$  (hold for 4 min), and finally gradually increased at  $3^{\circ}\text{C}/\text{min}$  to  $225^{\circ}\text{C}$ , holding this temperature for 10 min to ensure complete elution of analytes. The system was injected with one microliter of every extract, and its pulse was maintained at 40 psi for 0.2 min at  $300^{\circ}\text{C}$ . To identify and quantify the analytes, a selected ion monitoring (SIM) mode was employed, consisting of one quantitative ion and two qualifier ions for each compound. Quality control and quality assurance (QA/QC) were carried out according to our previous study, and information such as retention time, spiked concentration (ppb), average of obtained concentration (ppb), average of spike recovery (%), acceptable range for spike recovery (%), intraday RSD% of spike recovery, acceptable range for intraday RSD (%), interday RSD% of spike recovery, acceptable range for interday RSD (%), LOD (ppb) and LOQ (ppb) are provided in that study as well as in **Table S1** (Jorfi *et al.* 2022; Soleimani *et al.* 2023). For PCBs compounds, LODs were between 0.03 and 0.07  $\mu\text{g}/\text{L}$  and LOQs were between 0.10 and 0.23  $\mu\text{g}/\text{L}$ , For OCPs compounds, LODs were between 0.06 and 0.08  $\mu\text{g}/\text{L}$  and LOQs were between 0.18 and 0.26  $\mu\text{g}/\text{L}$ . This indicated that the analytical method is capable of detecting and reliably quantifying the target compounds at trace levels.

### 2.4. Eco-toxicological risk assessments for OCPs and PCBs

The toxic equivalent (TEQ) was calculated based on approach described by Van *et al.* (2006b). This involved applying the detected concentrations of PCBs in waterpipe wastewater samples by the World Health Organization's (WHO 2005) toxic equivalency factors (TEFs) for humans and other mammals, as indicated in Equation (1):

$$\text{TEQ} = \sum [C_i \times \text{TEF}_i] \quad (1)$$

The toxic equivalency of a mixture is computed by aggregating the concentrations of its individual components ( $C_i$ ) after each concentration has been weighted by a corresponding TEF.

The environmental hazard associated with the identified OCP compounds in waterpipe wastewaters was assessed by employing the risk quotient (RQ) approach. The specific formula for calculation of the RQ is as follow:

$$\text{RQ} = \frac{\text{MEC}}{\text{PNEC}} \quad (2)$$

Where MEC is the quantified level of OCPs ( $\text{ng}/\text{l}$ ) in waterpipe wastewater samples, while PNEC is the predicted no-effect concentration values derived from the Guo *et al.* (2023) research. The PNEC values obtained through division of the  $\text{LC}_{50}$  or  $\text{EC}_{50}$  by Assessment Factor (AF) set at a constant value of 1000, utilizing a consistent Assessment Factor (AF) of 1000 for all substances.

The application of an  $\text{AF} = 1000$  is in accordance with widely accepted ecological risk assessment frameworks, including the European Union Technical Guidance

Document (EU TGD), Organization for Economic Co-operation and Development (OECD) guidelines (ECB-JRC 2003), and the European Chemicals Agency (ECHA) guidance under the European Union Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) (ECHA 2008). This conservative assessment factor is recommended when available toxicity data are limited to acute laboratory tests and the lack of chronic toxicity data (ECHA 2008; Arfaeinia *et al.* 2024a; ECB-JRC, 2003). The use of a consistent AF across all compounds guarantees methodological consistency and delivers a conservative estimate of ecological risk in complex matrices such as wastewater (Arfaeinia *et al.*, 2024a). Environmental risk is considered moderate when RQ values fall between 0.1 and 1 and values less than 0.1 correspond to a low risk (Nie *et al.*, 2015).

### 2.5. Statistical analyzes

Data analysis was conducted using SPSS (Version 27). Specifically, Independent Samples Test was employed to identify significant differences in PCBs/OCPs concentrations across the two fruit-flavored and traditional waterpipe wastewater samples. The p-value less than 0.05 was considered the cutoff for statistical significance.

## 3. Results and discussion

### 3.1. The OCPs concentration levels in waterpipe wastewater

**Table 1.** The individual concentrations (mean  $\pm$  sd, ng/L) of OCPs in waterpipe wastewater from flavored and traditional tobacco.

Pesticide	Flavored				Traditional
	Nakhla	Al-fakher	Al Tawareg	Tangiers	
Aldrin	1.01 $\pm$ 0.73	1.77 $\pm$ 1.03	1.74 $\pm$ 1.40	1.7 $\pm$ 1.21	2.39 $\pm$ 1.29
Dieldrin	4.38 $\pm$ 1.78	3.93 $\pm$ 2.60	3.96 $\pm$ 1.82	4.5 $\pm$ 1.82	5.12 $\pm$ 3.01
Heptachlor	9.12 $\pm$ 4.61	10.55 $\pm$ 6.883	11.21 $\pm$ 2.35	7.51 $\pm$ 4.18	13.06 $\pm$ 7.83
Chlordane	2.14 $\pm$ 1.46	3.60 $\pm$ 2.74	1.74 $\pm$ 1.59	2.35 $\pm$ 2.20	1.05 $\pm$ 0.59
Endosulfan	8.39 $\pm$ 6.32	10.49 $\pm$ 7.54	7.10 $\pm$ 7.26	7.02 $\pm$ 8.32	4.91 $\pm$ 3.67
$\alpha$ -HCH	38.8 $\pm$ 17.41	41.94 $\pm$ 31.04	54.07 $\pm$ 35.57	42.42 $\pm$ 17.76	44.07 $\pm$ 15.14
$\beta$ -HCH	53.65 $\pm$ 29.07	65.62 $\pm$ 37.23	84.50 $\pm$ 51.70	55.80 $\pm$ 30.61	66.02 $\pm$ 34.26
$\gamma$ -HCH	0.357 $\pm$ 0.31	0.42 $\pm$ 0.26	0.33 $\pm$ 0.31	0.84 $\pm$ 0.78	0.66 $\pm$ 0.63
$\delta$ -HCH	0.83 $\pm$ 0.59	1.13 $\pm$ 0.78	1.77 $\pm$ 1.06	1.27 $\pm$ 1.01	1.37 $\pm$ 1.49
$\Sigma$ HCH	89.36 $\pm$ 33.85	102.2 $\pm$ 53.42	103.92 $\pm$ 60.43	79.98 $\pm$ 40.47	102.91 $\pm$ 43.77
p,p'-DDE	ND	ND	ND	ND	0.52 $\pm$ 0.24
p,p'-DDD	ND	ND	ND	ND	4.18 $\pm$ 2.41
p,p'-DDT	ND	ND	ND	ND	2.06 $\pm$ 0.35
$\Sigma$ DDT	ND	ND	ND	ND	5.83 $\pm$ 2.53

Due to its susceptibility to a wide range of diseases, tobacco cultivation often needs the utilize of various chemical treatments, including organic pesticides (López Dávila *et al.* 2020). OCPs are used as pesticides in agriculture, including tobacco farming. Residues from these pesticides can remain on the crops and be present in final products (Kartalović *et al.* 2020a). As well as, OCP residues can accumulate in soil and water over time and be absorbed by plants during growth (Guo *et al.* 2019). In addition to these, there is a possibility that manufacturing equipment or materials could introduce small amounts of OCP residues into tobacco products. Therefore, both environmental factors (such as contamination during

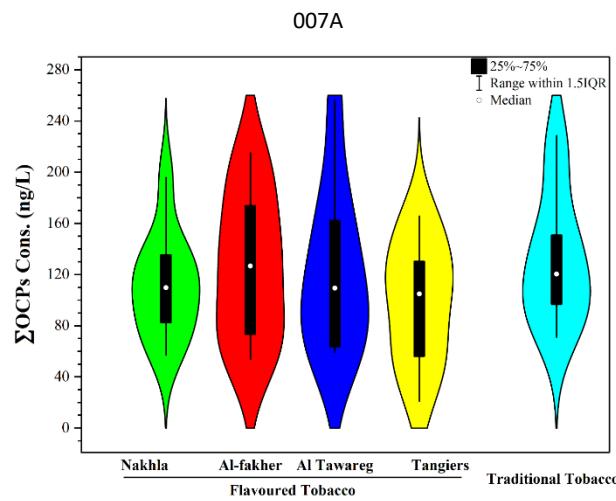
cultivation) and industrial processes (like pesticide use or combustion) contribute to the presence of PCBs and OCPs in tobacco products. The extensive application of pesticides in tobacco fields leads to their accumulation in the final product, and when used with hookah, some of it is also trapped in the water in the hookah bowl, creating a toxic wastewater (Quadroni and Bettinetti 2019). The increased pesticide levels found in waterpipe wastewaters of traditional tobaccos may be because this type of tobaccos are comprised entirely of tobacco, whereas flavored tobaccos are only 30% tobacco, with the rest consisting of sweeteners and flavorings added during processing (Kassem *et al.* 2020b; Kassem *et al.* 2018).

Thus, during waterpipe use OCPs residues leach into wastewater, with higher concentrations in traditional tobacco—composed entirely of tobacco—compared to flavored varieties (only ~30% tobacco, diluted by additives), resulting in elevated pesticide-to-mass ratios and greater trampot in hookah water.

Hexachlorocyclohexane (HCH) isomers had the maximum concentration level in both fruit-flavored and traditional tobacco wastewaters samples. The concentration of  $\Sigma$ HCH in Nakhla, Al-Fakher, Al Tawareg and Tangiers brands were 89.36, 102.19, 103.91, and 79.98 ng/L, respectively, and in traditional tobacco samples was 102.91 ng/L. The elevated concentrations of HCH compounds in the waterpipe wastewater samples could result from their persistent and non-volatile characteristics nature, combined with careless pesticide application in tobacco farming and the considerable microbial breakdown resistance of HCHs (Abbas 2021; Wang *et al.* 2016a). The predominance of  $\beta$ -HCH comes from its higher chemical stability and lower biodegradability in comparison with other HCH isomers. Unlike  $\alpha$ - and  $\gamma$ -HCH,  $\beta$ -HCH shows greater resistance to microbial decomposition and volatilization, leading to its accumulation preferentially in environmental matrices such as wastewater (Mehboob *et al.* 2013; Qian *et al.* 2019). The presence of HCHs, a type of insecticide, is frequently observed in agricultural areas, including rivers in India (Chakraborty *et al.* 2016), Pakistan (Taufeeq *et al.* 2021b), Iran (Kafaei *et al.* 2020), Egypt (Barakat *et al.* 2013), and China (Wang *et al.* 2016b), which are affected by activities such as tobacco farming (Taufeeq *et al.* 2021b). Significant amounts of  $\alpha$ - and  $\beta$ -HCH, by-product substances in the making of lindane, were improperly discarded, posing environmental and health risks (Ma *et al.* 2020; Sineli *et al.* 2016), particularly because of the elevated toxicity of HCHs that are related to endocrine, neurological, renal, and gastrointestinal concerns, along with immune system damage (Ali *et al.* 2020; Kong *et al.* 2014), with  $\beta$ -HCH being the most hazardous to mammals (Jackovitz and Hebert 2015; Kafaei *et al.* 2020). Subsequent to HCHs, heptachlor exhibited the next highest concentration among the waterpipe wastewater samples analyzed. The concentration of this pollutant in the samples from fruit-flavored tobacco in Nakhla, Al-Fakher, Al Tawareg and Tangiers brands were 9.12, 10.55, 11.21, and 7.51 ng/L, respectively, and in traditional tobacco samples was 13.05 ng/L. The International Agency for Research on Cancer (IARC) has classified heptachlor as a substance that may cause cancer in humans (Vainio *et al.* 1991). Because of their long-lasting nature and tendency to lipids, heptachlor residues persist in the environment, affecting life forms in both land and water ecosystems (McManus *et al.* 2013). Interestingly, DDT isomers were not found in waterpipe wastewater samples of fruit-flavored tobacco, but were present in waterpipe wastewater samples of local tobacco samples at a  $\Sigma$ DDT levels of 5.82  $\mu$ g/L. In a 2019 research by Quadroni and Bettinetti (Bettinetti *et al.* 2012), DDT pesticide was detected to be the most prevalent in 78.57% of in tobacco products samples, with concentration levels of 9 ng/g of pp'-DDT and 13 ng/g of

pp'-DDE. A research by Rahman *et al.*, (Rahman *et al.* 2012) reported that the average DDT residue in tobacco leaf samples collected from different areas in Kushtia, Bangladesh was 4000 ng/g. DDT and its by-products such as DDD and DDE can adversely affect the liver as well as normal and cancerous tissues that respond to estrogen (Liu *et al.* 2022; Wang *et al.* 2022).

These results underscore varietal differences driven by tobacco content and combustion dynamics, with traditional types posing higher risks from undiluted pesticide legacies, while HCH/heptachlor ubiquity reflects global agricultural bans' incomplete mitigation. Elevated  $\beta$ -HCH toxicity to mammals further amplifies concerns for endocrine/neurological damage in exposed ecosystems. This interpretation extends literature by mechanistically linking cultivation practices to waterpipe-specific waste pollution, advocating refined monitoring in high-use regions.



**Figure 1.** Violin plot of  $\Sigma$ OCPs concentrations levels in waterpipe wastewater from fruit-flavored and traditional tobacco.

### 3.2. The PCBs concentration levels in waterpipe wastewater

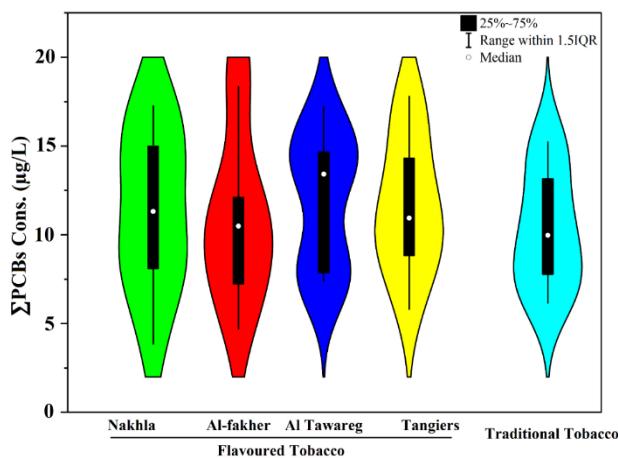
The measured concentrations of  $\Sigma$ PCBs in wastewater samples generated from both flavored and traditional waterpipe tobacco are presented in **Table 2**. All tested waterpipe wastewater samples contained PCBs, indicating that waterpipe tobacco wastewater represents a significant source of these environmental pollutants. As shown in **Table 2**, the mean concentrations of  $\Sigma$ PCBs in wastewater from fruit-flavored tobacco brands—Nakhla, Al-Fakher, Al-Tawareg, and Tangiers—were 11.41, 11.05, 11.94, and 11.65  $\mu$ g/L, respectively. The mean concentration in traditional tobacco wastewater samples was 10.26  $\mu$ g/L. According to our findings, the average  $\Sigma$ PCB concentrations in tobacco brand wastewaters, ranked from highest to lowest, are: Al-Tawareg, Tangiers, Nakhla, Al-Fakher, followed by traditional brands. The violin plot of  $\Sigma$ PCBs concentrations levels in waterpipe wastewater from fruit-flavored and traditional tobacco are shown in **Figure 2**. The results of Independent Samples Test are presented in **Table S2**. The detection of  $\Sigma$ PCBs in all waterpipe wastewater samples indicates that waterpipe tobacco—regardless of flavor type—serves as a consistent source of these persistent organic pollutants.

Although the concentrations did not differ significantly between flavored and traditional tobacco ( $p$ -value $>0.05$ ), the slightly elevated  $\Sigma$ PCB levels observed in flavored brands (11.05–11.94  $\mu\text{g/L}$ ) compared to traditional tobacco (10.26  $\mu\text{g/L}$ ) may reflect compositional and processing differences between the two product types. Elevated concentrations of PCBs in wastewater samples of fruit-flavored can arise from the use of flavoring agents, substantial quantities of organic chemicals, sweeteners, scents, and essential oils introduced during processing of these type of tobacco (Farley *et al.* 2018; Masjedi *et al.* 2023b). Moreover, the slight variations in PCBs concentrations across wastewaters from various brands may also be linked to environmental factors affecting leaf contamination pathways: tobacco grown in soils impacted by historical PCB emissions, near industrial areas, or irrigated with contaminated water can accumulate PCB residues that later transfer into tobacco smoke and

ultimately into the waterpipe bowl (Verma *et al.* 2010; Zhu *et al.* 2014). The similar magnitude of PCB concentrations between flavored and traditional varieties suggests that agricultural contamination pathways remain dominant; however, the slightly higher levels in flavored products may reflect an additive effect of processing-related inputs. These findings reinforce the hydrophobic and combustion-stable nature of PCBs, their ability to partition into the water phase during smoking, and the potential role of both agricultural environments and manufacturing practices in shaping PCB burdens in waterpipe wastewater. This mechanistic understanding highlights why PCB pollution persists across tobacco types and supports the need for greater monitoring of additive-rich flavored products, whose complex composition may enhance the mobilization or introduction of persistent pollutants.

**Table 2.** The individual concentrations (mean  $\pm$  sd,  $\mu\text{g/L}$ ) of PCBs in waterpipe wastewater from flavored and traditional tobacco.

PCBs	Flavored				Traditional
	Nakhla	Al-fakher	Al Tawareg	Tangiers	
PCB77	2.14 $\pm$ 1.15	2.17 $\pm$ 1.21	1.84 $\pm$ 1.10	2.03 $\pm$ 1.29	1.84 $\pm$ 1.06
PCB81	1.16 $\pm$ 0.67	0.98 $\pm$ 0.68	1.09 $\pm$ 0.59	1.17 $\pm$ 0.65	1.19 $\pm$ 0.62
PCB114	0.78 $\pm$ 0.46	0.74 $\pm$ 0.30	0.82 $\pm$ 0.46	0.86 $\pm$ 0.46	0.74 $\pm$ 0.4
PCB118	0.91 $\pm$ 0.65	0.87 $\pm$ 0.80	1.17 $\pm$ 0.67	1.17 $\pm$ 0.82	0.80 $\pm$ 0.64
PCB123	0.41 $\pm$ 0.27	0.34 $\pm$ 0.25	0.42 $\pm$ 0.27	0.43 $\pm$ 0.27	0.34 $\pm$ 0.18
PCB126	0.20 $\pm$ 0.19	0.21 $\pm$ 0.18	0.22 $\pm$ 0.19	0.22 $\pm$ 0.18	0.21 $\pm$ 0.20
PCB156	0.59 $\pm$ 0.23	0.56 $\pm$ 0.19	0.58 $\pm$ 0.21	0.69 $\pm$ 0.24	0.60 $\pm$ 0.25
PCB157	0.17 $\pm$ 0.14	0.16 $\pm$ 0.12	0.19 $\pm$ 0.14	0.19 $\pm$ 0.14	0.19 $\pm$ 0.11
PCB167	2.01 $\pm$ 1.12	1.76 $\pm$ 1.23	2.28 $\pm$ 1.31	2.05 $\pm$ 1.22	1.79 $\pm$ 1.00
PCB189	0.93 $\pm$ 0.31	0.86 $\pm$ 0.27	0.91 $\pm$ 0.28	0.86 $\pm$ 0.44	0.92 $\pm$ 0.17
PCB194	3.08 $\pm$ 1.65	2.72 $\pm$ 1.60	3.20 $\pm$ 1.72	3.06 $\pm$ 1.55	2.74 $\pm$ 1.47



**Figure 2.** Violin plot of  $\Sigma$ PCBs concentrations levels in waterpipe wastewater from fruit-flavored and traditional tobacco.

The individual concentrations of PCBs in wastewaters samples from different fruit-flavored tobaccos as well as traditional tobacco are also detailed in **Table 2**. As indicated, average concentration of PCB77, PCB81, PCB114, PCB118, PCB123, PCB126, PCB156, PCB157, PCB167, PCB189 and PCB194 in wastewater samples of waterpipe with fruit-flavored tobacco were  $2.05 \pm 1.19$ ,  $1.10 \pm 0.65$ ,  $0.81 \pm 0.43$ ,  $1.03 \pm 0.75$ ,  $0.43 \pm 0.29$ ,  $0.22 \pm$

$0.18$ ,  $0.64 \pm 0.28$ ,  $0.27 \pm 0.39$ ,  $2.03 \pm 1.23$ ,  $0.92 \pm 0.40$ , and  $3.05 \pm 1.61 \mu\text{g/L}$ , respectively. The mean concentration levels of PCB77, PCB81, PCB114, PCB118, PCB123, PCB126, PCB156, PCB157, PCB167, PCB189 and PCB194 in wastewater samples of waterpipe with traditional tobacco were also  $1.84 \pm 1.06$ ,  $1.19 \pm 0.62$ ,  $0.74 \pm 0.40$ ,  $0.80 \pm 0.64$ ,  $0.33 \pm 0.18$ ,  $0.21 \pm 0.20$ ,  $0.60 \pm 0.25$ ,  $0.19 \pm 0.11$ ,  $1.79 \pm 1.00$ ,  $0.92 \pm 0.17$ , and  $2.74 \pm 1.47 \mu\text{g/L}$ , respectively. As can be seen, PCB194 exhibited the maximum concentration level among all quantified PCBs congeners. This observation could be explained by the durable chemical structure and resistance to degradation of PCB194, which tends to accumulate more easily in wastewater in relation to the other congeners (Beyer and Biziuk 2009). Moreover, its higher lipid affinity may contribute to stronger adsorption to particulate matter in the waterpipe residues, resulting in higher detected concentrations (Urbaniak *et al.* 2017). A study quantified dioxin-like PCB congeners (e.g., PCB 77, PCB 105, PCB 114, PCB 118) with toxicity equivalent values ranging from  $8.7 \times 10^{-6}$  to  $3.21 \times 10^{-4}$  ng WHO-TEQ per cigarette (Adesina *et al.* 2022). Inhalation of these pollutants was associated with non-carcinogenic health risks, as HQ values exceeded safe limits (Adesina *et al.* 2022). It should be noticed that industrial processes have caused the the emission of a significant amount of PCBs into the ecosystem, especially,

and the analogues readily build up in the environmental compartments (e.g., atmosphere, water, soil, and sediments), particularly in the nearby regions because of their persistence (Kim *et al.* 2017; Mao *et al.* 2021; Mila *et al.* 2022; Rivoira *et al.* 2022). Previous researches across many countries have consistently reported that the PCBs level in surface soil were closely associated with closeness to the source areas (Mao *et al.* 2021; Zhao *et al.* 2016). Numerous studies have shown that the increased PCB levels in the environmental matrices are a consequence of historical usage or improper disposal of PCBs-related goods (Meijer *et al.* 2003). PCBs can contaminate soil, air, and water and tobacco plants may absorb these chemicals from the environment during cultivation (Guo *et al.* 2019). The combustion of tobacco during smoking can release PCBs into the mainstream smoke. Studies have identified various PCB congeners in cigarette smoke, indicating that combustion processes contribute to their presence (Adesina *et al.* 2022). Although less documented, manufacturing processes might also introduce trace amounts of PCBs into tobacco products due to contamination or use of contaminated materials. Therefore, during waterpipe smoking, some of PCBs enters the smoke and is inhaled by the smoker, endangering his health. Additionally, some PCBs trapped in the waterpipe water, and the release of this water into the environment can have various eco-toxicological effects.

### 3.3. Environmental Toxicity of OCPs and PCBs

Although direct evidence connecting wastewater from waterpipe to substantial PCB or OCP contamination is limited compared with well-known sources such as industrial discharge or agricultural runoff, the release of these pollutants into aquatic environments still presents serious ecological and public-health risks due to their persistence, toxicity, and tendency to bio-accumulate in living organisms. The ecological risks related with OCPs in waterpipe wastewater for aquatic environment are provided in **Table 3**. The assessment of ecological risks posed by individual OCPs in traditional tobacco waterpipe wastewater showed that the RQ values were ranged from 0.00 ( $\delta$ -HCH) to 2.46 (endosulfan). The RQ value of endosulfan was greater than 1, with high ecological risk. As well as, the RQ values of  $\beta$ -HCH,  $\delta$ -HCH, chlordan, p,p'-DDE, p,p'-DDT and heptachlor were less than 0.1, with low ecological risks. Other compounds such as  $\alpha$ -HCH,  $\gamma$ -HCH, aldrin and dieldrin have medium ecological risks ( $0 < RQ < 1$ ). In the case of flavored tobacco, the RQ values were ranged from 0.00 ( $\delta$ -HCH) to 3.50 (endosulfan). The RQ value of endosulfan was generally greater than 1, with high ecological risk. As well as, the RQ values of  $\beta$ -HCH,  $\delta$ -HCH, chlordan, dieldrin and heptachlor were less than 0.1, with low ecological risks. Other compounds such as  $\alpha$ -HCH,  $\gamma$ -HCH and aldrin have medium ecological risks ( $0 < RQ < 1$ ).

**Table 3.** Ecological hazard metrics related to organochlorine pesticides (OCPs) in waterpipe wastewater for aquatic media

OCPs	Traditional Tobacco			Flavored Tobacco		
	MEC	PNEC	RQ	MEC	PNEC	RQ
$\alpha$ -HCH	44.07	370	0.12	38.51	370	0.11
$\beta$ -HCH	66.02	3140	0.02	54.13	3140	0.02
$\gamma$ -HCH	0.66	2.9	0.23	0.32	2.9	0.11
$\delta$ -HCH	1.37	1580	0.00	0.91	1580	0.00
Aldrin	2.39	10	0.24	1.03	10	0.11
Chlordan	1.05	90	0.01	1.87	90	0.02
Endosulfan	4.91	2	2.46	6.99	2	3.50
p,p'-DDE	0.52	15	0.03	<LOD	15	-
Dieldrin	5.12	50	0.11	3.05	50	0.06
p,p'-DDD	4.18	9	0.46	<LOD	9	-
p,p'-DDT	2.06	30	0.07	<LOD	30	-
Heptachlor	13.06	150	0.09	7.63	150	0.05

The TEQs of the PCBs in the flavored and traditional waterpipe wastewater for aquatic media ranged from  $8.1 \times 10^{-6}$  (PCB157) to 0.02 (PCB126) and  $5.7 \times 10^{-6}$  (PCB157) to 0.02 (PCB126) ng/L (**Table 4**). In a study, the TEQ values of dioxin-like PCBs in cigarette butts leachates were low, and it is reported that continuous discharge into water bodies could pose long-term environmental and health dangers (Arfaeinia *et al.* 2024a). The ecological impact of waterpipe wastewater is an increasing issue, particularly regarding its toxicological effects on aquatic ecosystems and human health. Despite the shallow TEQs observed in waterpipe wastewater, continuous discharge can lead to significant ecological and health risks. The TEF and TEQ approach is indeed a well-established method for

assessing the risk associated with exposure to PCBs and other dioxin-like substances (Van den Berg *et al.* 2006b), particularly in dietary contexts. However, its application in non-living environmental components (including soil, water, or air) presents several challenges (Arfaeinia *et al.* 2024b). Its application in abiotic environmental matrices requires careful consideration of bioavailability, transport mechanisms, and environmental fate. By addressing these challenges through targeted research and interdisciplinary collaboration, we can enhance our risk assessment frameworks and better protect human health and the environment. To accurately assess human risk for abiotic environments, it is suggested to utilize congener-targeted equations across the entire model rather than relying on

the TEQ model. This approach is crucial because the fate and transfer properties of different congeners vary significantly (Van den Berg *et al.* 2006a), which can lead to inaccuracies if a generalized TEQ framework is applied. While the concentrations of PCBs and OCPs from

waterpipe wastewater may be relatively low compared to other pollutants, their continuous release poses significant risks due to bioaccumulation effects on aquatic ecosystems.

**Table 4.** The toxicity equivalent (TEQ) and Toxic Equivalency Factors (TEFs) values of PCBs in waterpipe wastewater for aquatic media.

PCBs	Concentrations		TEF (WHO 2005)	TEQs	
	Traditional tobacco	Flavored tobacco		Traditional tobacco	Flavored tobacco
PCB77*	1.84	2.05	0.0001	1.8E-04	2E-04
PCB81*	1.19	1.1	0.00001	1E-05	1.1E-05
PCB114**	0.74	0.8	0.00003	2.2E-05	2.4E-05
PCB118**	0.8	1.03	0.00003	2.4E-05	3.1E-05
PCB126*	0.21	0.22	0.01	0.02	0.02
PCB156**	0.6	0.64	0.00003	1.8E-05	1.9E-05
PCB157**	0.19	0.27	0.00003	5.7E-06	8.1E-06
PCB167**	1.79	2.03	0.00003	5.8E-05	6.1E-05
PCB189**	0.92	0.92	0.00003	2.4E-05	2.8E-05

\*: non-ortho substituted PCBs; \*\*: mono-ortho substituted PCBs

#### 4. Practical applications and future research prospects

Both PCBs and OCPs are classified as POPs, known for their carcinogenic, immunotoxic, and neurotoxic effects (Androutsopoulos *et al.* 2013; Lauby-Secretan *et al.* 2013). These compounds can bio accumulate in human tissues through inhalation or environmental exposure (Adesina *et al.* 2022; Kartalović *et al.* 2020b; Thakur and Pathania 2020; Zhu *et al.* 2022). This study showed that various hazardous pollutants including OCPs and PCBs can enter the environment through waterpipe wastewater and/or tobacco waste and negatively impact the terrestrial and aquatic ecosystems. To effectively diminish the ecological effect of waterpipe tobacco wastewaters, it is suggested that multi-pronged approach be taken. Countries with high rates of waterpipe use, particularly within the Middle East region, such as Iran, should prioritize thorough tobacco waste-specific waste management policies. Moreover, it is essential to highlight the duty of tobacco manufacturers in handling their products not just during production, but also after they are used and disposed. They must be forced to adopt Extended Producer Responsibility (EPR) programs to fulfill this. Moreover, to prevent these pollutants from entering the environment, specific actions are needed, such as public education and increasing smokers' awareness of the serious consequences of discharging these pollutants into the environment. In addition, while this study found that hookah wastewater can contain hazardous chemicals, its effects on terrestrial organisms/plants were not assessed. Additional scientific research is needed to understand the level of toxicity of this type of hazardous wastewater poses to both aquatic/terrestrial organisms. Simultaneously, researches of smokers' behavior could explore attitudes regarding the disposal of tobacco waste and highlights the significance of educational initiatives.

#### 5. Conclusion

This study evaluated the environmental health risks associated with wastewater generated from waterpipe tobacco use, with special emphasis on PCBs and OCPs. The

results showed that flavored and traditional waterpipe tobacco products contribute to the contamination of aquatic and terrestrial ecosystems, while flavored waterpipe tobacco products have higher concentrations of PCBs, because of the addition of flavoring material. The analysis showed that PCB194 was the most abundant PCBs in both flavored and traditional tobacco products, whereas  $\beta$ -HCH and  $\alpha$ -HCH were the most prevalent pesticides in all analyzed samples. In addition, waterpipe wastewater demonstrated considerable detection of OCP residues, which might underline the urgent need to control these toxic substances. The research put more focus on controlling the methods of disposal of waterpipe waste so as to suppress the negative consequences on the environment and public health. Proper management systems involving effective waste management strategies like wastewater treatment and proper disposal methods could reduce the adverse effects of both aquatic and terrestrial ecosystems. Such these insights help to improve the understanding of the toxic burden associated with waterpipe tobacco waste and set a foundation for further research and policy initiatives aimed at mitigating pollutants and protecting both the environment and human health. This would call for cooperation among policymakers and industry stakeholders in dealing with such challenges effectively, reducing the ecological impact of tobacco products and preserving human health.

#### 6. Credit author statement

The study was planned and overseen by **Hossein Arfaeinia** and **Gholamreza Heydari**. Samples were collected by **Farshid Soleimani** and **Mohammad Mahdi Hatami**. Experiments and data collection were carried out by **Hossein Arfaeinia**, **Farshid Soleimani**, and **Niloufar Borhani Yazdi**. **Ali Mouseli** was the advisor of the work. **Zohre Moeini** and **Masoumeh Tahmasbizadeh** performed the statistical analysis. **Farshid Soleimani** and **Zohre Moeini** prepared the initial draft of the manuscript. The completed manuscript was carefully reviewed and confirmed by all the authors.

## Declaration

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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## Supplementary Information

Table S1. Quality control and quality assurance of OCPs and PCBs quantitation analysis.

Chemicals	Retention Time	Spiked Concentration (ppb)	Average of Obtained Concentration (ppb)	Average of Spike Recovery (%)	Acceptable range for Spike recovery (%)	Intraday RSD% of Spike Recovery	Acceptable range for Intraday RSD (%)	Interday RSD% of Spike Recovery	Acceptable range for Interday RSD (%)	LOD (ppb)	LOQ (ppb)
a-Lindane	13.87		1.85	92.48		7.84		23.95		0.06	0.18
b-Lindane	15.01		1.45	72.58		7.36		15.35		0.06	0.18
c-Lindane	15.35		1.87	93.41		14.75		18.14		0.06	0.18
d-Lindane	16.78		1.89	94.55		14.65		23.99		0.06	0.18
Aldrin	20.74		1.53	76.54		10.37		15.78		0.08	0.26
Chlordan	24.11		1.88	94.18		12.07		21.70		0.07	0.24
Endosulfan	24.64		1.91	95.70		14.24		18.80		0.07	0.23
DDE	25.77		1.42	70.84		11.27		19.63		0.06	0.21
Dieldrin	25.81		1.98	99.07		16.50		15.36		0.07	0.25
DDD	27.29		1.78	88.80		7.79		18.27		0.07	0.22
DDT	28.48		1.49	74.73		13.67		20.95		0.06	0.21
Metoxychlor	30.21	2	1.52	76.10	60-115 (González and Herrador, 2007)	13.49	21 (González and Herrador, 2007)	20.14	32 (González and Herrador, 2007)	0.07	0.24
PCB18	9.23		1.97	98.35		7.91		21.24		0.04	0.12
PCB 28	11.89		1.99	99.51		13.43		16.65		0.04	0.12
PCB 52	12.55		1.91	95.53		16.38		22.63		0.03	0.11
PCB 44	13		1.97	98.55		7.07		15.19		0.03	0.10
PCB 77	15.84		1.97	98.63		7.35		20.91		0.04	0.14
PCB149	16.35		1.72	86.01		11.74		20.75		0.05	0.16
PCB 118	16.67		1.91	95.41		13.66		22.32		0.04	0.12
PCB 114	17.09		1.62	81.10		11.06		19.80		0.04	0.14
PCB 153	17.5		1.80	90.06		15.81		22.16		0.05	0.16
PCB 105	17.7		1.67	83.73		7.91		20.57		0.04	0.14
PCB 138	18.69		1.73	86.41		11.83		18.79		0.06	0.21
PCB 180	22.19		1.50	74.83		11.31		16.47		0.07	0.23

Table S2. The comparison of OCPs and PCBs compounds levels between the two traditional and fruit-flavored waterpipe wastewater

	Levene's Test for Equality of Variances			t-test for Equality of Means					95% Confidence Interval of the Difference			
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference					
								Lower	Upper			
PCB77	Equal variances assumed		.141	.709	.911	47	.367	.39067	.42883	-.47204	1.25337	
	Equal variances not assumed											
PCB81	Equal variances assumed		.132	.718	.912	14.010	.377	.39067	.42833	-.52795	1.30928	

	Equal variances not assumed			.113	13.619	.911	.02881	.25401	-.51742	.57505
PCB114	Equal variances assumed	.116	.735	1.259	43	.215	.20107	.15969	-.12097	.52311
	Equal variances not assumed			1.174	13.286	.261	.20107	.17133	-.16825	.57039
PCB118	Equal variances assumed	1.144	.291	1.172	43	.248	.31424	.26811	-.22646	.85494
	Equal variances not assumed			1.239	15.835	.233	.31424	.25366	-.22394	.85243
PCB123	Equal variances assumed	1.534	.222	1.895	44	.065	.18972	.10011	-.01203	.39148
	Equal variances not assumed			2.154	17.681	.045	.18972	.08807	.00445	.37500
PCB126	Equal variances assumed	.028	.868	1.690	38	.099	.11423	.06758	-.02257	.25104
	Equal variances not assumed			1.695	15.543	.110	.11423	.06738	-.02895	.25742
PCB156	Equal variances assumed	.768	.387	1.418	37	.165	.15828	.11162	-.06788	.38444
	Equal variances not assumed			1.289	13.490	.219	.15828	.12276	-.10596	.42252
PCB157	Equal variances assumed	.574	.454	1.001	34	.324	.13028	.13009	-.13409	.39466
	Equal variances not assumed			1.467	33.662	.152	.13028	.08884	-.05033	.31089
PCB167	Equal variances assumed	1.075	.305	.577	47	.566	.24875	.43088	-.61808	1.11557
	Equal variances not assumed			.639	16.136	.532	.24875	.38944	-.57626	1.07375
PCB189	Equal variances assumed	.047	.830	1.240	40	.222	.18457	.14885	-.11627	.48541
	Equal variances not assumed			1.221	14.726	.241	.18457	.15114	-.13811	.50724
PCB194	Equal variances assumed	.258	.614	.547	48	.587	.31197	.57015	-.83440	1.45833
	Equal variances not assumed			.564	14.387	.582	.31197	.55355	-.87228	1.49621
TPCBs	Equal variances assumed	.962	.332	.893	48	.376	1.25080	1.40054	-.156518	4.06678
	Equal variances not assumed			1.010	16.486	.327	1.25080	1.23842	-.136826	3.86986
Aldrin	Equal variances assumed	2.016	.162	-1.412	47	.164	-.64833	.45904	-.157180	.27513
	Equal variances not assumed			-1.181	11.657	.261	-.64833	.54878	-.184794	.55128
Dieldrin	Equal variances assumed	.005	.942	-1.067	48	.292	-1.05075	.98522	-3.03167	.93017
	Equal variances not assumed			-.876	11.449	.399	-1.05075	1.19922	-3.67766	1.57616
Heptachlor	Equal variances assumed	2.182	.146	-2.376	48	.022	-5.42975	2.28529	-10.02463	-.83487
	Equal variances not assumed			-1.957	11.470	.075	-5.42975	2.77505	-11.50721	.64771
Chlordane	Equal variances assumed	9.746	.003	1.603	48	.116	1.14025	.71143	-.29017	2.57067
	Equal variances not assumed			2.729	44.065	.009	1.14025	.41789	.29808	1.98242
Endosulfan	Equal variances assumed	5.987	.018	1.220	48	.228	3.05937	2.50798	-.198326	8.10200
	Equal variances not assumed			1.743	27.244	.093	3.05937	1.75569	-.54150	6.66024
alphaHCH	Equal variances assumed	.240	.626	.321	48	.750	3.25825	10.14786	-.17.14540	23.66190
	Equal variances not assumed			.371	17.099	.715	3.25825	8.77935	-.15.25642	21.77292
betaHCH	Equal variances assumed	.800	.376	-.804	48	.425	-11.89675	14.80084	-41.65583	17.86233
	Equal variances not assumed			-.895	16.073	.384	-11.89675	13.29740	-40.07553	16.28203
gammaHCH	Equal variances assumed	1.664	.203	-1.185	48	.242	-.21325	.18001	-.57519	.14869
	Equal variances not assumed			-.963	11.358	.356	-.21325	.22143	-.69874	.27224
deltaHCH	Equal variances assumed	1.303	.259	-.490	48	.626	-.19175	.39112	-.97815	.59465
	Equal variances not assumed			-.380	10.976	.711	-.19175	.50470	-.1.30289	.91939

THCH	Equal variances assumed	.180	.673	-.521	48	.605	-9.04500	17.37225	-43.97424	25.88424
	Equal variances not assumed			-.546	14.718	.594	-9.04500	16.58032	-44.44427	26.35427
DDE	Equal variances assumed	90.717	.000	-8.685	48	.000	-.41600	.04790	-.51231	-.31969
	Equal variances not assumed			-4.205	9.000	.002	-.41600	.09894	-.63982	-.19218
DDD	Equal variances assumed	284.488	.000	-8.919	48	.000	-3.76000	.42155	-4.60758	-2.91242
	Equal variances not assumed			-4.318	9.000	.002	-3.76000	.87075	-5.72977	-1.79023
DDT	Equal variances assumed	76.347	.000	-11.578	48	.000	-1.65200	.14268	-1.93888	-1.36512
	Equal variances not assumed			-5.605	9.000	.000	-1.65200	.29472	-2.31870	-.98530
TDDT	Equal variances assumed	132.499	.000	-14.260	48	.000	-5.82800	.40870	-6.64974	-5.00626
	Equal variances not assumed			-6.904	9.000	.000	-5.82800	.84420	-7.73772	-3.91828
TOCPs	Equal variances assumed	.020	.889	-.973	48	.336	-17.82750	18.33090	-54.68424	19.02924
	Equal variances not assumed			-.981	14.012	.343	-17.82750	18.17147	-56.79836	21.14336