

# EVALUATION OF POSSIBLE PCDD/F, DIOXIN LIKE PCB AND PAH CONTAMINATION IN OLIVE AND OLIVE OIL SAMPLES FROM AREAS AFFECTED BY THE FIRES IN SUMMER 2007 IN GREECE

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## Introduction

During the summer of 2007, a series of massive forest fires broke out in several areas across Greece<sup>1</sup>. The fires mainly affected western and southern Peloponnese as well as southern Evia.

Serious concern arose about possible polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) contamination of the affected areas and especially about the danger of contaminated animal and agricultural food products from the local production, since food intake is the main route of human exposure to PCDD/Fs and dioxin-like PCBs<sup>2</sup>. In Greece, the dominant source of PCDD/Fs and PCBs is considered to be the uncontrolled combustion of municipal solid waste in open landfills and accidental fires in forest, rural and industrial areas<sup>3</sup>. Our previous case study of food and soil contamination after a fire in the public landfill of Tagarades, a sanitary landfill in northern Greece, has shown that PCDD/Fs levels were higher in all lipid containing food samples than those normally found in respective products randomly collected from the Greek production and market. Among vegetable samples, contamination was detected only in olive samples<sup>4</sup>. Because of our previous findings from Tagarades, olive oil, which is a very significant agricultural product of Greece, was the major concern in this case.

Other possible contaminants of concern in the fire-affected areas were polycyclic aromatic hydrocarbons (PAHs). In contrast to the results for PCDD/Fs, vegetable samples from the same landfill area of Tagarades did not indicate any contamination with PAHs, that shows a different potential of the two groups of substances to contaminate food (unpublished data). The most studied PAH is benzo[a]pyrene (BAP), a possible human carcinogen, according to International Agency for Research on Cancer (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7), which is used as a marker of the occurrence of carcinogenic PAHs in food according to the opinion of the Scientific Committee on Food ([http://ec.europa.eu/food/fs/sc/scf/out154\\_en.pdf](http://ec.europa.eu/food/fs/sc/scf/out154_en.pdf)). Fifteen PAHs are believed to be mutagenic/genotoxic in vivo: cyclopenta[c,d]pyrene (CPP), benzo[a]anthracene (BAA), chrysene (CHR), 5-methylchrysene (5MCHR), benzo[j]fluoranthene (BJF), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF), benzo[a]pyrene (BAP), dibenzo[a,l]pyrene (DaIP), dibenzo[a,h]pyrene (DahA), benzo[g,h,i]perylene (BghiP), indeno[1,2,3-cd]pyrene (IND), dibenzo[a,e]pyrene (DaeP), dibenzo[a,i]pyrene (DaiP) and dibenzo[a,h]pyrene (DahP). These PAHs, except BghiP, have also shown carcinogenic properties in vivo. According to the European Committee Recommendation 2005/108/EC these 15 PAHs + 1 (benzo[c]fluorine), evaluated by Joint FAO/WHO Expert Committee on Food Additives (64<sup>th</sup> meeting, 2005, Summary and Conclusions) should be measured and the results transmitted to EU.

The existing legislation for PCDD/Fs, dioxin-like PCBs and PAHs in food is EC Regulation 1881/2006 as amended, where maximum levels are set in various foodstuffs. Especially for fats and oils intended for direct human consumption or as food ingredients, the maximum level for PCDD/Fs is 0.75 pg TEQ/g, for the sum of PCDD/Fs and dioxin-like PCBs 1.25 pg TEQ/g and for BAP 2 mg/kg (ppm).

For these reasons, in order to ensure the quality of the Greek olive oil, the Ministry of Rural Development and Food decided to study PCDD/F, dioxin-like PCB and PAH contamination levels in olive samples collected from oil producing areas that were affected by the massive forest fires.

## Methods and materials

### *Materials*

All solvents used were residue analysis picograde and were purchased from Promochem (Germany). The isomers for the preparation of the  $^{13}\text{C}_{12}$  internal standard solutions were purchased from Wellington Laboratories (Canada) and Cambridge Isotope Laboratories (USA). Carbosphere 80/100 mesh was purchased from Alltech (USA). Alumina was Basic activity Super 1 for dioxin analysis, MP Biochemicals GmbH (Germany). The sulfuric acid impregnated silica gel was prepared as follows: Silica gel (100 g, 60-200 mesh, Merck) was activated in an oven at 200 °C for at least 2 days and then mixed with concentrated sulfuric acid (44 g). Solid phase extraction columns used were WATERS C18 2g, capacity 15 mL and ISOLUTE Florisil 500 mg, capacity 3 mL.

### *Collection of samples*

Olive samples were collected from the fire-affected areas. After collection, olive samples were ground and pressed. Resulting olive oil was collected in appropriate containers. Subsequently, in order to check the ordinary olive oil production, samples were collected in one fire-affected area from the first conventional olive oil produced after the fire and the oil produced one year later.

### *Dioxin and PCBs analysis*

Sample preparation was performed as previously described <sup>5</sup>. Quantification standards ( $^{13}\text{C}$ -labelled solutions of PCDD/Fs and PCBs in toluene) were added to each sample prior to extraction.

10 g of each sample were dissolved in dichloromethane and brought onto the top of a Carbosphere column, which was washed with dichloromethane to remove residual fat, refluxed with toluene to elute dioxin-like PCBs, inverted and refluxed overnight to elute PCDD/F fraction.

The obtained fractions were further cleaned up using a column containing 0.5 g of 44 %  $\text{H}_2\text{SO}_4$ -silicagel, and 5 g of alumina. The column used for the PCB fraction was eluted with hexane/dichloromethane mixture (1:1 v/v). The eluate was evaporated to dryness and re-dissolved in n-nonane containing injection standard  $^{13}\text{C}_{12}$  PCB-80.

The alumina column used for the PCDD/F fraction was washed with a hexane/dichloromethane mixture (93:7 v/v) and eluted with a hexane/dichloromethane mixture (60:40 v/v). Finally, the eluate was evaporated to dryness and re-dissolved in n-nonane containing injection standard  $^{13}\text{C}_{12}$  1,2,3,4-TCDD.

For mono-ortho PCBs 0.5 g of each sample was dissolved in 5 mL of hexane and brought onto a column containing 44%  $\text{H}_2\text{SO}_4$ -silica gel. The column was eluted with hexane. The fraction obtained was further cleaned up using an alumina column as described above and eluted with hexane/dichloromethane mixture (1:1 v/v). Finally, the eluate was evaporated to dryness and re-dissolved in n-nonane containing injection standard  $^{13}\text{C}_{12}$  PCB 80.

**Instrumental analysis:** The quantification of PCDD/Fs, non-ortho and mono-ortho PCBs was performed by HRGC-HRMS (EI) in MID mode on a Trace GC gas chromatograph (ThermoFinnigan) coupled to a MAT-95 XP mass spectrometer (ThermoFinnigan) equipped with a CTC A 200S autosampler at 10.000 resolving power (10% valley definition). Instrumental conditions and purity control criteria were according to the EPA 1613 and EPA 1668A methods (US Environmental Protection Agency, 1994, 1999). The quantification of concentration and recovery was carried out by the isotopic dilution method. The limit of detection (LOD) for each congener was determined as the concentration in the extract that produced an instrumental response at two different ions to be monitored with a signal to noise ratio of 3:1 for the less sensitive signal (EC Directive 2004/44/EC). For toxicity equivalent (TEQ) calculations the WHO-98 toxicity equivalent factors (TEF) were used <sup>6</sup>.

### *PAHs analysis*

The method performed is based on the method described in ISO 15753: 2006 modified "in house". It is an internal standard method using as internal standard the PAH Benzo(b)Chrysene (B(b)Chr 250 ppb). The method involves the gradual enrichment in PAHs of the oil phase by using liquid-liquid partition followed by a clean up stage of two solid phase extractions (SPE) in order to isolate PAHs. Eighteen PAHs were measured by HPLC, using photodiode array UV-VIS detector (DAD) and fluorescence detector (FLD). The PAHs measured are: anthracene (AN), fluoranthene (FAN), pyrene (PYR), cyclopenta[c,d]pyrene (CPP), benzo[a]anthracene (BAA), chrysene (CHR), 5-methylchrysene (5MCHR), benzo[j]fluoranthene (BJF), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF),

benzo[a]pyrene (BAP), dibenzo[a,l]pyrene (DalP), dibenzo[a,h]pyrene (DahA), benzo-[g,h,i]perylene (BghiP), indeno[1,2,3-cd]pyrene (IND), dibenzo[a,e]pyrene (DaeP), dibenzo[a,i]pyrene (DaiP) and dibenzo[a,h]pyrene (DahP). The results were corrected by the recovery of each PAH.

## Results and discussion

The control of possible contamination of the local food products after a fire is very significant for the protection of public health, since food is responsible for over 90% of human exposure to dioxin and dioxin-like compounds <sup>2</sup>. Due to their high lipid content, olives have the ability to absorb dioxins, dioxin-like compounds and PAHs, which are transferred by contaminated fly ash in cases of fire. Therefore, olives could be considered as an indicator of dioxin and PAH contamination. In Regulation 1881/2006/EC, the European Union has set maximum levels of dioxins and sum of dioxins and dioxin-like PCBs in olive oil (0.75 and 1.25 pg TEQ/g respectively) and maximum level in edible fats and oils for BAP (2 mg/kg). No maximum level has been set for either PCDD/Fs and PCBs or PAHs for olives or other vegetables. For that reason, the olive samples collected were sent to a model olive oil press for the production of olive oil following a process that excludes contamination during production. The olive oil was analysed for PCDD/F, dioxin-like PCB and PAH content in order to obtain results that can be compared with the EU maximum levels.

Thirty-two olive samples were collected from locations up to 250 m away from the affected area, during a time period beginning immediately after the fire, and ending 2 months later. Twenty of them were analysed for the 17 PCDD/Fs and 12 dioxin-like PCBs, expressed as WHO98 PCDD/F TEQ values <sup>6</sup> and are listed in Table 1. The levels of PCDD/Fs and dioxin-like PCBs in all samples studied were below maximum dioxin levels accepted according to the EU requirements (Regulation 1881/2006/EC) and at levels usually detected in Greek olive oil <sup>5</sup>. Moreover, PCDD/Fs and dioxin-like PCBs levels of the olive oil samples collected in Evia from the first conventional olive oil production after the fire and one year later are presented in Table 2. These samples were also found below maximum EU levels.

PCDD/Fs concentration differences between the sets of samples were compared by t-test. Significance probabilities ( $p^*$  values) were calculated for the respective number of samples analyzed, based on the formula for comparison of two groups of normally distributed values for a confidence level of 95%. The results show that the PCDD/F concentration decreased with time. This is due to the increase of lipid content which takes place during the maturation process of oil-producing olives. The PCDD/F concentration measured immediately after the fires in August was higher than some months later, in November, and the conventional olive oil production collected one year later in Evia was even lower, equal to the detection limit, indicating that there was no long-term exposure to PCDD/Fs of the olives due to soil contamination.

The PCDD/F congener profile was uniform, since all congeners were detected at low levels.

In the present study, in contrast to a previous report <sup>4</sup>, where PCDD/F contamination was found in olive samples collected at locations near an accidental fire in a municipal landfill, no clear evidence of PCDD/F contamination was found. It is possible that the strong winds during the fire immediately dispersed pollutants to the wider area, therefore they were not significantly absorbed by the olives close to the burning sites. Moreover, in the previously reported case, probably massive pyrolysis of PVC products during waste burning caused dioxin contamination.

Dioxin-like PCBs levels were normal in all analyzed samples as PCBs are generally not connected to combustion. All thirty-two samples were also examined for PAH content. Our results show that all samples examined had BAP content below maximum levels set by Regulation 1881/2006/EC (values measured range from 0.020 mg/kg to 0.927 mg/kg). The levels of other 17 PAHs measured in the samples examined are considerably low compared to the results from other samples of extra virgin olive oil coming either from olive oil mills or from retail market.

**Table 1.** Dioxin and dioxin like PCBs levels of olive samples from fire-affected areas.

Sample code	Distance from the affected area	PCDDs/Fs pg WHO TEQ 1998 /g fat	PCBs pg WHO TEQ 1998 /g fat
Helia: H2.10.07	50 m	0.41	0.02
H3.10.07	10 – 50 m	0.41	0.03
H4.10.07	50 m	0.51	0.05

	H5.10.07	200 – 250 m	0.44	0.07
	H7.10.07	20 – 50 m	0.45	0.06
	H8.10.07	10 – 50 m	0.40	0.03
Evia:	E1.10.07	200 m	0.44	0.03
	E2.10.07	150 m	0.36	0.02
	E3.10.07	50 m	0.46	0.03
	E4.10.07	50 m	0.49	0.04
	E5.10.07	100 m	0.49	0.02
Arcadia:	AR1.10.07	200 m	0.34	0.02
Korinthia:	K2.10.07	10 – 50 m	0.45	0.02
Achaia:	AX1.10.07	Ø (inside the affected area)	0.49	0.03
	AX2.10.07	200 m	0.37	0.04
	AX3.10.07	600 m	0.43	0.02
Messinia:	M2.10.07	100 m	0.46	0.03
	M4.10.07	350 m	0.38	0.03
	M5.10.07	100 m	0.41	0.03
Lakonia:	L4.10.07	80 m	0.45	0.03

**Table 2.** PCDD/Fs and dioxin-like PCBs levels of the olive oil samples from the conventional production.

Sample code	PCDDs/Fs pg WHO TEQ 1998 /g fat	PCBs pg WHO TEQ 1998 /g fat
First olive oil production 3 months after the fire		
1872	0.42	0.02
1873	0.50	0.02
1874	0.42	0.03
1875	0.44	0.04
Olive oil production one year later		
2393	0.36	0.13
2394	0.34	0.04
2395	0.34	0.02
2396	0.36	0.03
2397	0.36	0.04

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