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Consumption of organic meat does not diminish the carcinogenic potential associated with the intake of persistent organic pollutants (POPs)

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Abstract Numerous studies have shown an epidemiological link between meat consumption and the incidence of cancer, and it has been suggested that this relationship may be motivated by the presence of carcinogenic contaminants on it. Among the most frequently detected contaminants in meat are several types of persistent organic pollutants (POPs), and it is well known that many of them are carcinogenic. On the other hand, an increasing number of consumers choose to feed on what are perceived as healthier foods. Thus, the number of consumers of organic food is growing. However, environmental contamination by POPs is ubiquitous, and it is therefore unlikely that the practices of organic food production are able to prevent this contamination. To test this hypothesis, we acquired 76 samples of meat (beef, chicken, and lamb) of two modes of production (organic and conventional) and quantified their levels of 33 carcinogenic POPs. On this basis, we determined the human meat-related daily dietary exposure to these carcinogens using as a model a population with a high consumption of meat, such as the Spanish population. The maximum allowable meat consumption for this population and the carcinogenic risk quotients associated with the current pattern of consumption were calculated. As expected, no sample was completely free of carcinogenic contaminants, and the differences between organically and conventionally produced meats were minimal. According to these results, the current pattern of meat consumption exceeded the maximum limits, which are set according to the levels of contaminations, and this is associated with a relevant carcinogenic risk. Strikingly, the consumption of organically produced meat does not diminish this carcinogenic risk, but on the contrary, it seems to be even higher, especially that associated with lamb consumption.

Keywords Meat · Organic meat · Carcinogens · Persistent organic pollutants · Carcinogenic risk · PCBs · PAHs · Organochlorine pesticides

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Introduction

It is well known that the food is the primary route of exposure to pollutants from numerous chemical classes (Vogt et al. 2012). Among the multitude of different chemical compounds that food may contain, persistent organic pollutants (POPs) are especially worrisome, and during the last decades, many POPs have been highlighted as a cause of concern and have been the subject of extensive study and international regulation in part because of their carcinogenic potential (Boada et al. 2012; Casals-Casas and Desvergne 2011; Dickerson et al. 2011; Dorgan et al. 1999; Knerr and Schrenk 2006; Ribas-Fito et al. 2001; Valeron et al. 2009). Since these compounds are highly resistant to degradation and are highly distributed in the environment, their presence in food is very difficult to avoid (Li et al. 2006; Rychen et al. 2014). Thus, it has been established that the ingestion of food contributes more than 90 % to the total current exposure to these compounds, especially those food from animal origin such as fish, dairy products, or meat (Li et al. 2006). According to the literature, the content of POPs in meat is particularly relevant, and several studies have reported high levels of organochlorine pesticides (OCPs) (Letta and Attah 2013; Pardio et al. 2012; Schecter et al. 2010; Wang et al. 2011), polychlorinated biphenyls (PCBs) (Costabeber et al. 2006; Malisch and Kotz 2014; Schecter et al. 2010; Schwarz et al. 2014), and especially polycyclic aromatic hydrocarbons (PAHs), which although they are not strictly considered as POPs, they are usually included in this group due to their high environmental prevalence and lipophilicity (Helmus et al. 2013; Lammel et al. 2013; Martorell et al. 2012; Veyrand et al. 2013).

It is known that dietary practices may influence exposure to chemical contaminants such as POPs, through the food consumption pattern, the forms of cooking or processing the food, the production modes, packaging types, etc. (Luzardo et al. 2013a; Oates and Cohen 2011; Vogt et al. 2012). For example, a growing number of people choose organic food as a healthier choice, although the study of the profile of these consumers has indicated that they are also motivated by concern for environmental health, the animal welfare, or by the perception that organic food has a higher nutritional value than conventional products (Oates et al. 2012; Smith-Spangler et al. 2012; Vogt et al. 2012). In fact, in the USA, organic food production increased by 50 % during the last decade, and in Europe, this increase has been even higher (in some countries like Spain, the land surface devoted to organic production has tripled during this period) (FIBL-IFOAM 2012).

Numerous studies have compared organic and conventional food production, both in relation to their nutritional value and in relation to its content of chemical residues (Smith-Spangler et al. 2012). With respect to nutrient content, most studies indicate that organic food production does not have a higher nutritional value than conventional production (Smith-Spangler et al. 2012). However, studies have shown that organically produced foods have much less risk of being contaminated by residues of pesticides or other chemical pollutants than conventional foods (Smith-Spangler et al. 2012). Organic livestock are fed with organically produced feed that is free of pesticides and animal by-products (Beane 2013), and therefore it is supposed that there should be lower accumulation of chemical residues. However, practically, there are no studies on the chemical residues' content in organic meat, although some authors have studied the presence of residues of veterinary drugs, heavy metals, microorganisms, and antibiotic resistance in organically and conventionally produced pigs (Hoogenboom et al. 2008). Therefore, the comparison with conventional production meat has also been scarcely studied.

The study of chemical contamination of meat is relevant because the consumption of meat has been associated with the increased incidence of different types of cancer (Abid et al. 2014), and different studies have linked this increased risk of cancer with the presence of carcinogenic chemical substances in meat (Trafialek and Kolanowski 2014). Meat consumption in Europe is high (51.2 kg/year/person) (Chamorro et al. 2012), and according to the Integrated Risk Information System, a variety of the most common pollutants in meat, such as PCBs, hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT) and its metabolites, and some congeners of PAHs, have been classified in group B of carcinogenicity (probable human carcinogens) (WHO 2014). Although cancer slope factors (CSFs) have been calculated for all these probable carcinogens (EPA 2014) and this would allow an estimate of the risk of cancer associated with continuous exposure to them through foodstuff, very few studies have attempted to estimate the carcinogenic risks that are associated with the current pattern of consumption of meat and meat products (Trafialek and Kolanowski 2014). To our knowledge no study to date has considered studying whether organic meat production could be an option to reduce the carcinogenic potential of meat consumption in relation to their content of chemical carcinogens, especially POPs.

This study was designed to test this hypothesis, where the concentrations of 7 PAHs, 18 PCBs, and 8 OCPs for which the CSFs have been calculated were determined in samples of meats (chicken, beef, and lamb) from organic and conventional production. The samples were acquired in large suppliers who serve the entire European territory. The main objective of this study was to use these data to estimate the carcinogenic risk associated with the current level of meat consumption by the European population considering two possible scenarios: consumers that choose organic meats and consumers that choose conventional meats. The methodology that has been recently used to estimate the carcinogenic risk in other food groups, such as fish (Yu et al. 2014), was applied, using the data of food consumption of the Spanish population.

Materials and methods

Sampling

Two purchases of meat samples of the two modes of production (organic and conventional) were made in the last quarter of 2013 and the first of 2014. These purchases were made in supermarkets belonging to large European retail chains located in the Canary Islands (Spain), which have common suppliers, and can therefore be considered representative of the products available to consumers throughout the continent. A total of 76 samples of meat were acquired, which were distributed as follows: 16 samples of lamb (8 from conventional production and 8 from organic production), 32 samples of chicken (20 conventional and 12 organic), and 28 samples of beef (16 conventional and 12 organic). The samples were processed immediately after arrival at the laboratory. Each meat sample was finely chopped with a knife and milled using a stainless steel food processor. Then, all the samples were frozen at −18 °C until analysis.

Chemicals, reagents, and analytes of interest

Dichloromethane, hexane, ethyl acetate, and cyclohexane (purity >99.9 %) were purchased from Fisher Scientific (Leicestershire, UK). Ultrapure water was produced using a Milli-Q Gradient A10 (Millipore, Molsheim, France). Diatomaceous earth was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCB congeners, and the internal standards (ISs, PCB 202, p,p′-DDE-d8, phenanthene-d10, tetrachloro-m-xylene, and heptachloro epoxide cis) were purchased from DrEhrenstorfer, Reference Materials (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc. (CT, USA). Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20 °C. Solutions diluted from 0.05 to 100 ng/mL were used for calibration curves.

The analytes selected for this study were 8 OCPs (p,p′-DDT, p,p′-DDE, p,p′-DDD, hexachlorobencene, and the four isomers of hexachlorocyclohexane (α-, β-, γ-, δ-HCH)); 18 PCB congeners, including marker-PCBs (M-PCBs) and dioxin-like PCBs (DL-PCBs) (IUPAC numbers # 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189); and 7 PAHs listed as carcinogens in the Toxics Release Inventory Program of the USA and the EPA's Priority Chemical list (EPA 2001) (benzo(a)anthracene, benzo(a)phenanthrene (chrysene), benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene).

Extraction and cleanup procedure

All the contaminants included in this study are completely lipid-soluble and therefore are found in the lipid fraction of tissues. For this reason, the fat from the meat was firstly extracted. Thus, the samples (5 g) were homogenized in 5 mL of ultrapure water with a disperser (Ultra-turrax, IKA, China). This homogenate was spiked with the ISs mix in acetone (10 μg/mL) to yield a final concentration of 100 ng/mL and was mixed with 30 g of diatomaceous earth to absorb any moisture. The extraction and cleanup method followed the procedures recommended by the European Standard for the determination of pesticides and PCBs in fatty foods (EN 1996a, b), which had been previously validated in our laboratory for different fatty samples of animal origin (Almeida-Gonzalez et al. 2012; Garcia-Alvarez et al. 2014; Luzardo et al. 2014). This method achieves acceptable recoveries that ranged between 71.5 and 103.2 %. Briefly, the fat was extracted using a Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed, Denmark) apparatus, which consisted of an extraction unit, a control unit, and a drive unit. The samples were placed into the extraction unit, and 20 mL of dichloromethane was added to each of the extraction cups in a closed system, and the cups were heated using an electric heating plate. The three-step extraction consisted of boiling, rinsing, and solvent recovery. The solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40 °C to prevent analyte losses. Using a precision balance, the fat obtained was carefully weighted into a zeroed glass tube to determine the fat content of each meat sample (percentage). The weighted fat was dissolved in 2 mL of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (BioBeads SX-3) using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 mL/min as the eluent. The first 25-min elution volume, which contained the great majority of lipids (>98 %), was discarded. The 25–85-min elution volume (120 mL), which contained all the analytes that were co-extracted with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally, the solvent was evaporated to dryness under a gentle nitrogen stream. The residue was then reconstituted in 1 mL of cyclohexane, and the sample was transferred to a GC vial that was used for the chromatographic analysis. The amount of pollutants per gram of fat was obtained by multiplying by the corresponding correction factor. The amount of contaminants in fresh meat was obtained by correcting for the fat percentage of each sample.

Chemical analysis procedure

All the compounds, plus ISs, were analyzed by gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) (Quantum XL, Thermo Fisher Scientific Inc., Waltham, MA, USA) as previously described (Camacho et al. 2013, 2014; Luzardo et al. 2013b). Briefly, a 30 m×0.25 mm i.d., 0.25-μm film thickness column (BPX5, SGE Inc., Austin, TX, USA) was used as the stationary phase. Helium (99.999 %) was used as the carrier gas at a constant flow of 1 mL/min. The 61-min oven temperature program was as follows: 60 °C held for 1 min, ramped to 210 °C at 12 °C/min and then to 320 °C at 8 °C/min and held for 6 min. The injector temperature was set at 270 °C, and the transfer line was heated to 310 °C. The injection volume was 1 μl in the splitless mode. A timed selected reaction monitoring (SRM) method for the simultaneous analysis of all the compounds in a single run was constructed. The operation conditions of the mass spectrometer were as follows: electron impact ionization (70 eV) in SRM; emission current, 50 μA; ionization source temperature, 220 °C; electron multiplier voltage, 1500 V; scan width, 0.15; scan time, 0.05 s; and peak width, *m/z* 0.7, and Da. Argon (99.99 %) was used as the collision gas at 0.2 Pa.

Quality control

All the recoveries were above 71 %, and it was thus considered acceptable to use this method for all the pollutants. All the individual measurements were corrected by the recovery efficiency for each analyte. All the samples were injected three times, and the values used for the calculations were the mean of the three values. In each batch of samples, three controls were included for every nine vials (three samples): a reagent blank consisting of a vial containing only cyclohexane, a vial containing 2 ng/mL of each of the pollutants in cyclohexane, and an internal laboratory quality control (QC) consisting of melted meat fat spiked with a mixture of all the pesticides (20 μg/kg), and processed using the same method that was used for the samples. The results were considered to be acceptable when the quantification of the analytes in the QC was within 15 % of the deviation of the theoretical value, which occurred in all the injections. The limit of quantification (LOQ) was set to 0.1 ng/g for all the analytes. A zero value was assigned to all the compounds below the limit of detection (LOD), and those compounds below the LOQ were assigned half of the LOQ.

Dietary intake estimates and calculations

To estimate the daily intake of pollutants through the consumption of a certain type of meat, it is necessary to know the concentration of pollutants in that meat (median and mean values expressed in ng/g fresh product) and multiply that value by the daily average consumption of that meat in a given population. Data on food consumption in Europe are published by the European Food Safety Authority (EFSA) from data provided by the Member States of the EU (EFSA 2011). However, the data for the whole EU are available by food groups (meat) rather than for individual foods (pork, beef, and chicken). Given that Spain is one of the EU countries with the highest meat consumption per capita (the third after the Czech Republic and Hungary in the adult population, and the first in child and adolescent population), the values of consumption of individual foods by the Spanish population were used in this study, which have been published by the Spanish Agency for Consumer Food Safety and Nutrition (AECOSAN 2006, 2011). While it has been established that regular organic food consumers tend to consume less amount of meat (up to 33 %) than non-consumers (Kesse-Guyot et al. 2013), in this paper, we have assumed that consumption is identical, for comparison purposes. For the risk assessment, two groups were considered: adults (18 years old and above, average weight 70.1 kg) and children (6 to 10 years old, average weight 30.4 kg).

For calculations of this paper, analytical values have been considered separately and grouped as follows: the total value of OCP residues (∑OCPs) as the sum of the 8 OCPs and metabolites measured; the total value of DDTs (∑DDT) as the sum of the measured values of p,p′-DDT, p,p′-DDE, and p,p′-DDD; and the total value of HCH residues (∑HCH) as the sum of the 4 HCH isomers measured (α-, β-, δ-, and γ-HCH); the total value of PCB residues (∑PCBs) as the sum of the 18 PCB congeners measured; the total value of the marker PCB residues (∑M-PCBs) as the sum of the 7 congeners considered as markers of environmental contamination by PCBs (#28, 52, 101, 118, 138, 153, and 180); the total value of dioxin-like PCBs (∑DL-PCBs) considered as the sum of the measurements of the 12 individual congeners (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189); and the total content of carcinogenic PAHs (∑c-PAHs) as the sum of the values of the 7 US-EPA compounds following the EFSA recommendations (EFSA 2008). Additionally, for the risk estimation, we calculated the potential toxicity (in terms of toxic equivalence to dioxins, TEQs) for the DL-PCBs using the toxic equivalency factors (TEFs) as revised by the World Health Organization (WHO) in 2005 (Van den Berg et al. 2006), and the potential toxicity in terms of benzo[a]pyrene toxic equivalents ($B[a]P_{eq}$) using the TEFs, which are established for the carcinogenic PAHs (Nisbet and LaGoy 1992).

The CFSs of the carcinogens included in this study were taken from the EPA's IRIS (EPA 2014) and were as follows: 1 per mg of substance/kg body weight-day (mg/kg-day) for marker PCBs (based on Aroclors 1260, 1254, 1242, and 1061), 1.1×10^5 per mg/kg-day for dioxin-like PCBs (based on 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8,-TCDD)), 0.34 per mg/kg-day for DDTs, 1.8 per mg/kg-day for HCHs (as there are not CFS values listed for total HCHs, the values listed for β- and γ-HCH were used), 1.6 per mg/kg-day for hexachlorobencene, and 7.3 per mg/kg-day for PAHs (based on benzo[a]pyrene).

Carcinogenic risk calculation

To estimate whether chemical contamination by carcinogens of meat endangers the consumers, we applied the risk assessment index, known as the risk quotient (RQ), using the methodology that has been used for other food groups, such as fish (Yu et al. 2014). RQ is defined as the ratio between the current consumption of meat (R_{meat}) and the maximum tolerable consumption of these products, which is calculated taking into account the concentrations of carcinogens in these foods (CR_{lim}) as follows:

$$CR_{\mathrm{lim}} = \frac{ARL \cdot BW}{\sum_{m=1}^{X} C_{\mathrm{m}} \cdot CSF_{\mathrm{m}}}$$

where CR_{lim} is the maximum allowable consumption rate (kg/day) for a particular meat; ARL is the maximum acceptable individual lifetime risk level (dimensionless), and a value of 10^{-5} was used in this study (Yu et al. 2014); BW is the body weight (kg); C_m is the median concentration of contaminant m in a particular meat (mg/kg) as determined in this study; and CSF_m is the cancer slope factor of a contaminant m (mg/kg/day) with carcinogenic potential. In the case of multiple contaminants with the same CSF, their concentrations in a particular type of meat were summed (from m=1 to m=x).

Then, the RQ for each food item and contaminant was calculated as follows:

$$RQ = \frac{R_{\text{meat}}}{CR_{\text{lim}}}\ (\text{for a single contaminant})$$

$$RQ = R_{\text{meat}} \cdot \sum_{m=1}^{x} \frac{1}{CR_{\text{lim}}}\ (\text{for multiple contaminants})$$

Thus, if the value of RQ is equal to or less than 1, there is no carcinogenic risk associated with the ingestion of contaminants through the consumption of a particular type of meat. Otherwise, the population is considered to be at carcinogenic risk when RQ is greater than 1, indicating that the current consumption of that foodstuff is greater than its CR_{lim} value.

Statistical analysis

The PASW Statistics v 19.0 software package (SPSS Inc., Chicago, IL, USA) was used to manage the database of the study and to perform the statistical analyses. Normality was examined using the Kolmogorov-Smirnov test. The POP distributions in the meat samples lacked normality and homoscedasticity; therefore, we used non-parametric tests (the Mann-Whitney and Kruskal-Wallis tests). The results are reported as the medians and percentiles 25th–75th ranges. Probability levels of less than 0.05 (two-tailed) were considered statistically significant.

Results and discussion

Distribution of persistent organic pollutants with carcinogenic potential in organically and conventionally produced beef, chicken, and lamb

The main objective of this paper is to provide an estimate of the level of exposure to carcinogenic POPs through consumption of beef, chicken, or lamb, depending on their mode of production (conventional production or organic production). While the levels of many of these substances have been identified in previous studies carried out in different parts of the world, all these works have been performed in conventionally produced meat. As far as we know, no work has been done specifically on organic meats. Also, as one might expect, the levels of carcinogenic POPs in meats published to date are highly variable (sometimes very significantly) (Costabeber et al. 2006; Letta and Attah 2013; Malisch and Kotz 2014; Pardio et al. 2012; Polder et al. 2010; Schecter et al. 2010; Tornkvist et al. 2011; Wang et al. 2011), which is logical because it is very common to find regional variations in contaminant levels. Since the objective of this paper is not to compare our results with previous works but to make a comparison of exposure depending on the product chosen by consumers, to make a realistic estimate, we preferred to directly quantify the contaminants in a representative sample of the main types of meat that any European consumer can find in supermarkets of the continent, and directly determine over them carcinogenic contaminant levels. Table 1 presents a summary of the data obtained directly from these samples, expressed as median and percentiles 25th and 75th.

As it would be expected in foods of animal origin, none of the samples was free of all the contaminants investigated. Both, meat samples from organic production and from conventional production, presented an average of 19 residues (ranging from 11 to 24 residues out of 33). In any case, the levels found in all samples were below the levels legally established in Europe (maximum residue levels, MRLs) (EC 2006a, b). The highest levels of contaminants found in this study were those of the M-PCBs in lamb, both organically and conventionally produced, and those of DDTs, also in organic and conventional lamb. In fact, these two sets of pollutants, the M-PCBs and DDTs, were the most abundant in all types of meat, as shown in Table 1. With regard to the PCB content of meats, it is remarkable that these were completely dominated by congeners 118, 138, 153, and 180 in all the cases, and thus, the ∑M-PCBs contributed with 94.3–99.8 % to the ∑PCBs. In fact, DL-PCBs were the contaminants that reached the lowest levels in all meat types, and therefore the toxic equivalent quantity (TEQ) levels for dioxin-like PCBs in the meat samples analyzed had low median values (range from 0.01 to 0.41 pg/g w.w.) (Table 1).

Two facts attracted attention of our results. First, the fact that pollution levels are quite different between distinct types of meats. Thus, lamb is by far the one with the highest levels of all pollutants studied. At the other extreme, we find the chicken (skinless) having the lowest levels in all cases. The beef meat shows intermediate values (Table 1). These differences are probably attributable to the very different percentages of fat of each type of meat, because when we compare the data expressed as nanograms of carcinogen per gram of fat rather than per gram of fresh product, the differences are much smaller (data not shown).Second, it is interesting to note that the differences between the two modes of production, organic and conventional, can be considered minimal, generally speaking. Table 1 shows the values of statistical significance found for each of the pollutants and meats. As seen above, the highest differences were found between organic and

Table 1 Concentrations of contaminants with carcinogenic potential (ng g^{-1} w.w.) in samples of organic and conventional meat available in the European market

	∑DDTs		∑HCHs		HCB		∑M-PCBs		∑DL-PCBs		$\sum TEQ_{DL\text{-}PCBs}$ [a]		$\sum B[a]P_{eq}$	
	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)
Lamb														
Conventional	196.0	247.4 (34.6–345.2)	9.3	9.8 (2.7–15.2)	314.4	325.1 (99.3–452.1)	51.7	50.6 (23.9–70.4)	3.5	2.7 (0.0–7.9)	0.29	0.27 (0.19–0.41)	3.7	3.3 (1.4–6.3)
Organic	436.9	300.5 (34.1–661.2)	179.9	164.4 (5.4–369.9)	294.0	302.3 (96.2–472.6)	61.3	64.8 (37.3–81.7)	1.1	1.1 (0.0–2.2)	0.07	0.06 (0.01–0.13)	3.4	2.5 (0.9–6.7)
Ratio[b]	0.82		0.06		1.01		0.78		2.45		4.5		1.32	
P value[c]	*0.8918*		*0.0123*		*0.9842*		*0.2709*		*0.1324*		*0.0004* ***		*0.7590*	
Chicken														
Conventional	8.51	7.5 (4.6–10.7)	2.9	2.6 (0.8–4.5)	2.3	3.0 (0.3–3.3)	13.9	14.0 (5.6–17.2)	0.3	0.0 (0.0–0.7)	0.02	0.01 (0.01–0.03)	0.3	0.2 (0.1–0.3)
Organic	7.2	7.6 (3.8–12.4)	1.3	0.9 (0.3–2.1)	5.5	4.3 (1.3–9.2)	14.3	16.8 (7.9–18.2)	0.2	0.2 (0.0–0.4)	0.02	0.02 (0.01–0.03)	0.2	0.2 (0.2–0.3)
Ratio[b]	0.99		2.88		0.70		0.83		0.5		0.5		1	
p value	*0.9796*		*0.0275**		*0.0105* *		*0.8676*		*0.6927*		*0.7386*		*0.9527*	
Beef														
Conventional	25.6	20.4 (10.6–37.1)	1.1	1.1 (0.1–1.4)	2.1	2.4 (1.1–2.7)	28.2	25.6 (17.2–37.6)	2.4	2.1 (1.4–3.3)	0.15	0.12 (0.08–0.21)	1.2	0.9 (0.6–1.5)
Organic	14.9	14.2 (8.7–20.8)	9.0	11.4 (0.1–15.4)	6.8	7.9 (2.1–10.4)	23.6	17.7 (13.6–34.5)	1.2	1.1 (0.7–1.4)	0.07	0.06 (0.04–0.11)	0.6	0.6 (0.4–0.7)
Ratio[b]	1.43		0.09		0.30		1.44		1.91		2		1.5	
p value	*0.3626*		*0.0604*		*0.0131**		*0.3482*		*0.0014* **		*0.0009* ***		*0.0054* **	

[a] $\sum TEQ_{DL\text{-}PCBs}$ are expressed in pg g^{-1} w.w.

[b] Ratio of the median levels (conventional to organic)

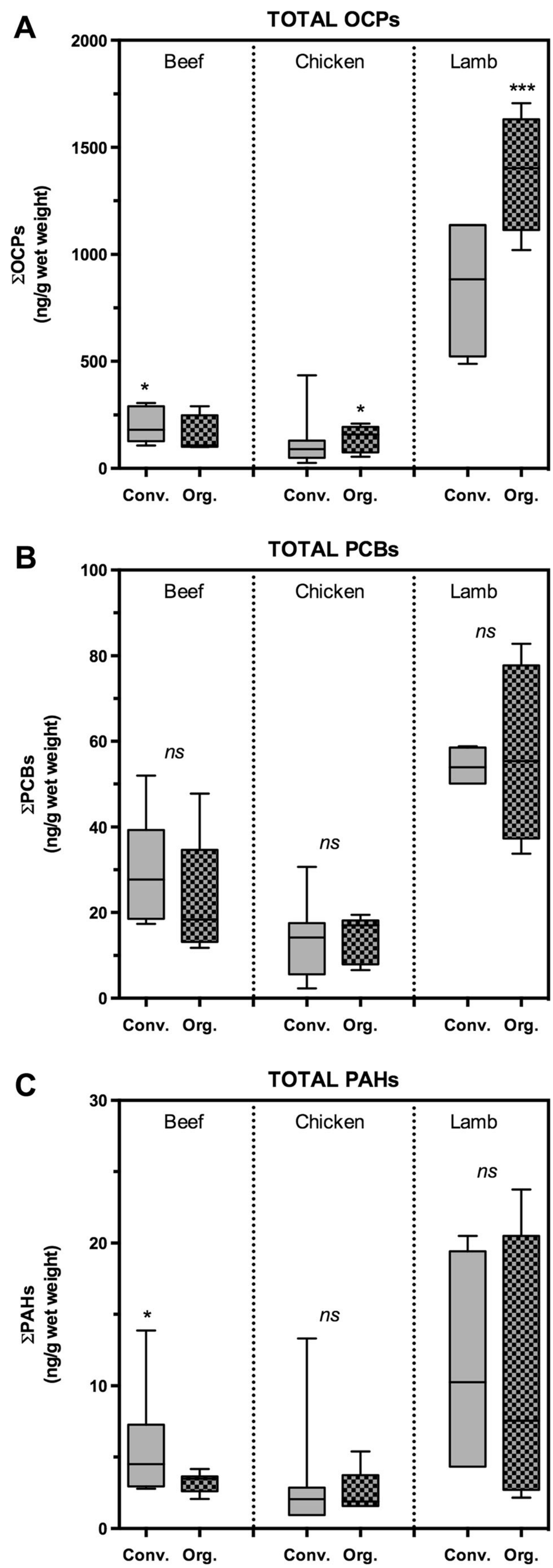
[c] *P* value results from the comparison between the medians (Mann-Whitney test), where:

*=p<0.05

**=p<0.01

***=p<0.001

Fig. 1 Box plots of the levels of ∑OCPs (**a**), ∑PCBs (**b**), and ∑c-PAHs (**c**) in the three types of meat studied, and comparison between the two modes of production of these meats (conventional vs. organic). The *line* inside the *boxes* represents the median, the *bottom* and *top* of the *boxes* are the first and third quartiles of the distribution, and the *lines* extending vertically from the *boxes* indicate the variability outside the upper and lower quartiles



conventional beef. However, contrary to what one might think, not in all cases the values were lower in meat from organic production. Thus, as it can be observed in Table 1, in the case of hexachlorobenzene (HCB), levels found in samples of organic beef and organic chicken were significantly higher than those found in the same meats from conventional production. Also noteworthy is the case of HCH isomers (∑HCH) since, although not statistically significant, a trend is observed that the levels are higher in lamb and beef from organic production. However, in relation to these contaminants, the trend was reversed in chicken meat (in this case with statistical significance, $p>0.05$, Table 1).

As the differences were not very relevant when analyzed by pollutants, we also performed the comparison by grouping them by chemical classes: OCPs, PCBs, and PAHs. The results are shown in Fig. 1. The only significant differences in the level of pollutants according to the mode of production were found for OCPs (in the three types of meat) and PAHs (in beef). The most striking results were that organic lamb meat contained much higher levels of OCPs than conventionally produced lamb ($p<0.001$, Fig. 1). These higher levels were also found in chicken meat, but the differences were much smaller ($p<0.05$). However, for beef meat, the situation was reverse: conventional production beef showed higher levels of OCPs than beef from organic production, and the same was obtained for the levels of PAHs ($p<0.05$ in both cases).

Dietary intake of carcinogenic POPs through the consumption of organic and conventional meats by adults and children

Dietary exposure calculations are performed by combining data on consumption habits with the concentrations of contaminants found in food samples. The estimation of food consumption, nutrient intake, and contaminant exposure is a topic of growing interest in the field of public health as a means to inform and guide the actions on food security and nutrition, and as a predictive method for determining the state of health of populations. For this reason, the European Food Safety Authority (EFSA) performs a collection of food consumption data from the different Member States (MS), which must develop nutritional surveys in their territories. On this basis, the Authority prepares an European database of Food Consumption (http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm). For the estimates in this paper, the data provided by the authorities on food security of our country, Spain (AECOSAN 2006, 2011), were used. The reason for this is that Spain overall meat consumption has steadily increased over the last few decades (Kanerva 2013; Leon-Munoz et al. 2012), and currently, the meat industry is ranked in fifth position in the industrial sector of the Spanish economy and is ranked first among the agro-food industries (Chamorro et al. 2012). Thus, meat consumption in Spain increased from one of the lowest ones in the EU reaching an average per capita consumption of 52.7 kg/year/person, which is even higher than the European average (51.2 kg/year/person) (Chamorro et al. 2012). In any case, using food consumption data from any EU country, and the concentrations of pollutants reported in this paper, daily intake levels of these pollutants for the different European populations can be easily obtained. It should be noted that in our estimates, we have assumed that all consumers (strict consumers of organic products, occasional consumers of organic products, and non-consumers of organic products) equally fit the pattern of consumption defined in nutrition surveys. This assumption has been made for comparison purposes, although it has been recently described that those strict consumers of organic products tend to consume up to 30 % less meat than consumers of conventional products (Kesse-Guyot et al. 2013), and therefore their actual exposure would be lower than the estimates presented here.

Table 2 summarizes the dietary intake of all the contaminants included in this study arranged by groups (on the basis of their similar carcinogenic potential), for children (6–10 years) and adults (>18 years).

First, with regards to the OCPs, our results show that the estimated daily intake (EDI) of ∑DDTs through the consumption of these three types of meat for adults living in Spain is 55.71 ng/kg body weight (b.w.)/day if they choose to consume conventional products, and 86.75 ng/kg b.w./day if they choose to consume organic meats, mainly due to the contribution of lamb meat in both cases. In children, the exposure to ∑DDTs through meat consumption is even higher, being 91.02 ng/kg b.w./day for consumers of conventionally produced meat, and up to 119.86 ng/kg b.w./day for consumers of organic meats. In any case, it should be noted that these consumptions represent only between 0.5 and 1.2 % (depending on the consumer and their choice) of the provisional tolerable daily intake (TDI) for humans established by the World Health Organization for these contaminants (0.01 mg/kg b.w./day)(JECFA 2000). With regard to the exposure to ∑HCHs through meat consumption, the EDIs were much higher in adults and children consuming organic products (36.8 and 51.59 ng/kg b.w./day, respectively) than in consumers of conventionally produced meats (4.08 and 7.67 ng/kg b.w./day, respectively) ($p<0.005$). However, it is remarkable that, again, in this case, the exposure to ∑HCHs is also far from the established TDIs (5000 ng/kg b.w./day) (JECFA 2000), representing less than 1 % of this value even in the worst scenario. The main contributors of this exposure to ∑HCHs were organic lamb and organic beef. Finally, within the group of OCPs, with regard to the intake of HCB, we found that once again the major contributor was by far lamb meat. However, in this case, organically and conventionally produced lamb contributed almost equally to the exposure to this contaminant. Thus, the HCB EDIs for adults and children who consume organic meat were 56.49 and 76.57 ng/kg b.w./day, respectively, and were 54.49 and 68.62 ng/kg b.w./day for

Table 2 Median values of dietary intakes of carcinogenic POPs (ng/kg b.w./day) by means of the consumption of organic and conventional meats for Spanish adults and children

	Food consumption (g/day)	∑DDT	∑HCH	HCB	∑M-PCB	∑DL-PCB	∑TEQ[a]	$\sum B[a]P_{eq}$
Adults								
Lamb (conventional)	11.5	32.16	1.52	51.59	8.49	0.58	0.05	0.61
Lamb (organic)	11.5	71.68	29.52	48.24	10.05	0.18	0.01	0.55
Chicken (conventional)	42.7	5.18	1.75	1.41	8.46	0.16	0.01	0.17
Chicken (organic)	42.7	4.36	0.80	3.35	8.71	0.13	0.01	0.13
Beef (conventional)	50.4	18.37	0.81	1.49	20.24	1.74	0.11	0.86
Beef (organic)	50.4	10.71	6.48	4.90	16.94	0.83	0.05	0.40
Children								
Lamb (conventional)	6.0	38.69	1.83	62.06	10.21	0.70	0.06	0.73
Lamb (organic)	6.0	86.24	35.52	58.03	12.09	0.21	0.01	0.66
Chicken (conventional)	43.1	12.07	4.07	3.28	19.70	0.37	0.03	0.41
Chicken (organic)	43.1	10.14	1.87	7.80	20.27	0.31	0.03	0.31
Beef (conventional)	47.9	40.26	1.77	3.28	44.36	3.82	0.23	1.89
Beef (organic)	47.9	23.48	14.20	10.74	37.13	1.82	0.10	0.88

[a] Expressed in pg/kg b.w./day

Table 3 Values of maximum allowable consumption rate of conventional or organic meat (CR_{lim}), expressed in g/day

	Food consumption (g/day)	∑DDT	∑HCH	HCB	∑M-PCB	∑DL-PCB	∑B[a]P$_{eq}$
CR_{lim} for adults (g/day)							
Lamb (conventional)	11.5	10.2	40.8	1.4	13.1	210.5	25.1
Lamb (organic)	11.5	4.6	2.1	1.4	11.1	936.6	27.8
Chicken (conventional)	42.5	234.9	131.6	184.0	48.9	2512.9	325.8
Chicken (organic)	42.5	279.7	286.2	77.3	47.6	2853.1	427.4
Beef (conventional)	50.4	78.3	335.5	204.4	24.2	418.3	77.6
Beef (organic)	50.4	134.2	41.9	62.4	28.9	929.6	167.0
CR_{lim} for children (g/day)							
Lamb (conventional)	6.0	5.3	21.0	0.7	6.8	108.3	12.9
Lamb (organic)	6.0	2.4	1.1	0.7	5.7	482.1	14.3
Chicken (conventional)	43.1	120.9	67.7	94.7	25.2	1293.4	167.7
Chicken (organic)	43.1	144.0	147.3	39.8	24.5	1468.5	220.0
Beef (conventional)	47.9	40.3	172.7	105.2	12.4	215.3	39.9
Beef (organic)	47.9	69.1	21.6	32.1	14.9	478.5	85.9

The current values of consumption of these products by the Spanish population (g/day) are included for reference

those adults and children consuming conventionally produced meats. In the case of this contaminant, these EDIs represent between 6.8 and 9.57 % of the TDI set by WHO for HCB (JECFA 2000).

With regard to the group of M-PCBs, the exposure through meat consumption was very similar between consumers of organic and conventional meats, as seen in Table 2. The main dietary intake of these contaminants is from beef. Thus, adult population would be exposed to 37.19 ng/kg b.w./day if they consume conventionally produced meat, and to 35.71 ng/kg b.w./day if they consume organic meats. In children, the PCB EDIs are almost double than in adults (74.27 and 69.49 ng/kg b.w./day for conventional and organic meat consumers, respectively). As, for the total PCBs, a TDI value has not been established, it is necessary to consider the intake of the most toxic compounds, the DL-PCBs, expressed in terms of the equivalency to dioxin toxicity, to assess the level and exposure. Using this approach, our estimates of $\sum TEQ_{DL\text{-}PCBs}$ would represent 3.5 to 16 % of the TDI of 2 pg/kg b.w./day (SCF 2000). In this case, the highest levels of exposure were found for children who consume conventionally produced meats. Our results are in accordance to other studies where authors have estimated that meat is an important source of dioxins and dioxin-like compounds in a high percentage of the samples analyzed (Costabeber et al. 2006; Malisch and Kotz 2014; Schecter et al. 2010; Schwarz et al. 2014; Tornkvist et al. 2011). These results are worrisome because the possibility exists that certain consumers may be subject to high dietary exposures to dioxins, even though they choose to consume organic food.

Finally, with regard to the last chemical group studied, the c-PAHs, we found that conventionally produced beef meat was the major contributor. As shown in Table 2, the EDIs (expressed as equivalents of B[a]P) for the adult population is 1.6 ng/kg b.w./day if they consume conventionally produced meat, and a little bit lower if they choose to consume organic meats (1.08 ng/kg b.w./day). In children, the EDIs are 3.03 and 1.85 ng/kg b.w./day for consumers of conventional and organic meats, respectively. To date, the WHO has not yet established TDI values for c-PAHs or benzo[a]pyrene (JECFA 2000). However, other references may be used. Thus, using the Contaminated Land Exposure Assessment (CLEA) model of the UK, which has established a TDI for B[a]P$_{eq}$ of 20 ng/kg b.w./day (CLEA-UK 2008), the current meat consumption in Spain would represent up to 15 % of these values (in children consuming conventionally produced meats).

POP-associated carcinogenic potential of the current consumption of organically or conventionally produced meats

As shown in the previous section, in all cases, the intake of contaminants through the consumption of meat represents a relatively discrete percentage of the TDI established for each of them. However, the main objective of our research is focused on the study of the carcinogenic potential associated with the consumption of carcinogenic POPs with meat. It has been established that the RQ evaluation is a good method to estimate the risk of a population (in this case the carcinogenic risk), and to establish exposure limits to chemicals (USEPA

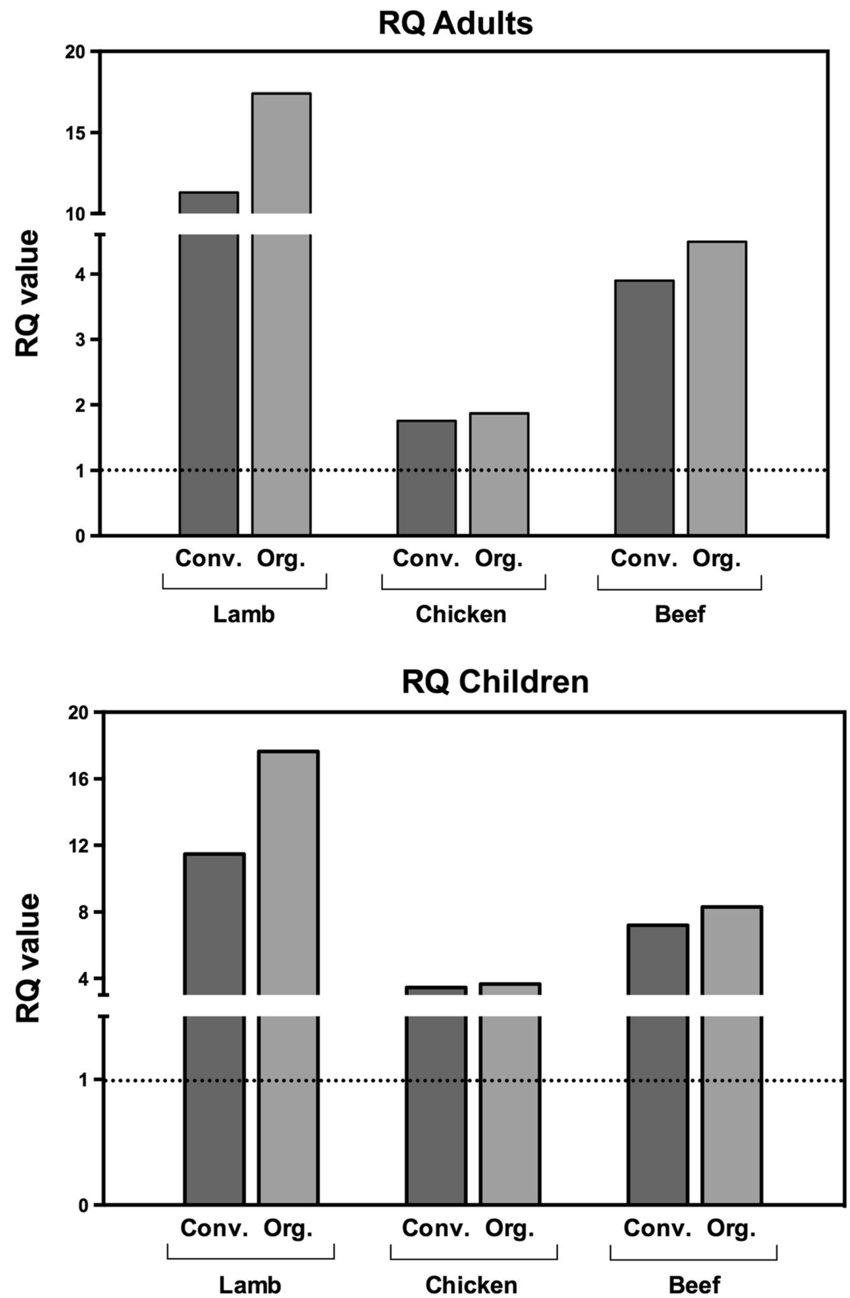


Fig. 2 Risk quotients of the contaminants for carcinogenic effects in adults (*upper panel*) and children (*lower panel*) via consumption of conventionally or organically produced meats. The *discontinuous horizontal line* indicates the threshold for carcinogenic risk (*RQ*=1)

2000). However, the application of this method to multiple chemical contaminants present in food has some weaknesses because assumptions must be made. Thus, it is well known that chemicals in foods are usually present in mixtures of various compounds belonging to different chemical classes, and thus they may exert their adverse effects on consumers interacting with each other in synergistic, additive, or even in antagonistic manners. However, the calculation model of the RQ implies the assumption that all pollutants cause similar toxicological effects and that the combined effect is the sum of the individual effects (USEPA 2000). Nevertheless, as recommended by the USEPA, we have considered that all carcinogens studied are similar and, therefore, we have used the additive model for calculating the RQs.

To do this, the CR_{lim} values (which represent the maximum allowable consumption of each meat type on the basis of their load of pollutants) were used for both adults and children (Table 3). According to these results, the current pattern of consumption of meat in the Spanish population implies that some of these limits are exceeded. Thus, the CR_{lim} of ∑M-PCBs is exceeded by the consumption of all three types of meat, either organic or conventional, both in adults and especially in children. The CR_{lim} of HCB and ∑DDTs are also exceeded by lamb consumption in both age groups, regardless the type of meat production and consumers' choice. Finally, current consumption of conventionally produced beef also implies that the CR_{lim} values of ∑DDTs and $B[a]P_{eq}$ are overpassed in children.

According to the CR_{lim}, we calculated *RQs* associated with the current consumption of each meat type for both adults and children (Fig. 2). From our results, two facts powerfully attract the attention. First, the current pattern of consumption of meat implies a carcinogenic risk ($RQ>1$) in all cases. The calculated carcinogenic risk ranges between 1.76 and 17.41 in adults, and between 3.45 and 17.65 in children. On the other hand, it is striking that the POP-associated carcinogenic risk tends to be higher in organic meats that in those which are conventionally produced. This is especially relevant in lamb meat, where the consumption of organic product implies a carcinogenic risk up to 1.5 times higher than the consumption of the conventionally produced option, both in adults and children.

Conclusions

In this research, the concentrations of 33 persistent organic pollutants with carcinogenic potential were determined in a large sample of meats available in the European supermarkets from two modes of production: conventional and organic. A mean of 19 of these 33 contaminants in all the samples tested (11–24) were found, but in no case the established MRLs were exceeded. Some significant differences in the levels of pollutants between organically and conventionally produced meats were found, but these differences can be considered of minor entity. As it is well known that continued exposure to carcinogens is not without risk (even at very low doses), the daily intake of these contaminants from the meat were estimated, taking a high meat consumer population such as the Spanish population as a model (adults and children). According to these estimates, exposure is similar in children than in adults, and also very similar if these consumers choose conventional or organic meats, generally speaking. The approximation of the risk ratio was used to evaluate the carcinogenic risk of the current pattern of consumption of these meats in the studied population, and a relevant risk was found in all the cases. Surprisingly, the risk seems to be even higher if the consumer chooses to consume organic meats (especially lamb). This work demonstrates once again that environmental contamination by POPs is ubiquitous and human exposure is very difficult to avoid. Even those consumers who choose to consume organic food, which is theoretically healthier, have exposure rates to these legacy pollutants that can become even higher than those of consumers who eat conventional food. This shows that efforts to minimize the environmental presence of these toxic pollutants should be continued and even strengthened.

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