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Research Article

Detection of Pollutants in Organic and Non-Organic Food: Are PAHs Coming from Pesticides?

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Abstract

Thirty-five samples of organic and non-organic equivalent foods were assessed for pollutants, including 800 pesticides. Sausages and cheeses were tested because they come from animals that can have bioaccumulation capacity. Since formulants of pesticides have been shown to include petroleum residues, such as Polycyclic Aromatic Hydrocarbons (PAHs) and metals, in total 24 PAHs were also tested, using accredited methods. Surprisingly, only a few food products contained declared active substances of pesticides above the regulatory threshold of 10 µg/kg. This threshold is not adapted for a real risk assessment, since, for instance, blood testosterone in a man is usually below this level, at which most pesticides are endocrine and nervous disruptors. Metals were detected in all samples, with more Si in non-organic cheeses, but PAHs were found at a maximum of 6.5 times higher in non-organic sausages with 3 carcinogenic ones, in comparison to organic products; this makes a toxicity 6,606 times higher. Due to this large difference for PAHs, and their generally recognised carcinogenic, endocrine, and nervous toxicity, we propose that these could be considered as markers of chemical food pollution, possibly linked to the presence of numerous pesticides below the threshold.

Keywords: Cheeses; Heavy Metals; Pesticides; Polycyclic aromatic hydrocarbons; PAHs; Organic food; Sausages

Abbreviations: MRL: Maximum Residue Level; NO: Non-Organic; O: Organic; PAHs: Polycyclic Aromatic Hydrocarbons

Introduction

The presence of pollutants in food is a general matter of concern that is important to the understanding of the origin of long-term chronic diseases or malformations [1] that may be due to chemicals. Numerous mutagens, for instance, can be ingested either by inhalation, food, or drinks, and may present epigenetic [2] or more general health effects. Some have been authorized in agriculture and are monitored for acute or short-term adverse consequences, but less is studied on their long-term impacts on the ecosystem and health, even by regulatory agencies, and even less on their mixtures. This is particularly concerning due to the increases in biodiversity loss [3] and, for instance, endocrine or nervous chronic diseases [4] including some cancers [5].

In this context, we focused on pesticides that are intentionally toxic to one or several levels of the ecosystem and are used in non-organic agriculture. These can undergo a trophic magnification, in common with other persistent contaminants in the food chain [6], especially in lipophilic products. A debate is ongoing on the protective effects of the culture and consumption of organic food on chronic diseases [7,8] though their ecosystemic benefits are more widely recognized [9].

We recently discovered the presence of PAHs and other petroleum residues, together with heavy metals, in the formulations of various commercialized synthetic pesticides [10,11]. The presence of these non-declared chemicals, also called formulants (since these are products in formulations), could be caused by the manufacturing process of pesticides synthesized from petroleum, which can thus be rich in petroleum residues [12]. This phenomenon can greatly amplify the toxicity of pesticide formulations in comparison with the toxicity of the isolated pesticide molecules, declared as active ingredients, which are tested and assessed alone to determine their Acceptable Daily Intakes (ADI) and Maximum

Residue Levels (MRL) in food [13,14]. This toxicity amplification by pesticides in formulations, as sold and used, is estimated at around three orders of magnitude more than isolated declared active ingredients [15,16].

Considering the trophic magnification of contaminants in the food chain due to their bioaccumulation, as well as their toxicity amplification by formulants of pesticides, we measured the presence of pesticides, PAHs, and metals in organic and non-organic foods. We chose highly processed and lipophilic ones, namely sausages and cheeses from European countries. In the European Union the regulatory thresholds and controls are often believed to be more stringent than in other parts of the world.

Materials and Methods

Food: Equivalent organic and non-organic sausages and cheeses were collected in stores in France, Germany, and Spain, and freshly analysed. The full list appears in the raw data.

Pesticides: The extraction was performed by QuEChERS TS/EN method 15662. The screening for 800 pesticides included glyphosate, glufosinate and AMPA. It was performed either by gas or liquid chromatography, followed by mass spectrometry (using either one of these methods or both), according to regulatory methods adapted for food, especially through § 64 LFGB L 00.00-34: 2010-09, or 00.00-113: 2015-03.

Metals: A total of 11 metals and elements (Ag, As, Cd, Co, Cu, Fe, Ni, Pb, Si, Ti, Zn) were analysed by adapted regulatory methods for food, mostly by high frequency induced plasma emission spectrometry and mass spectrometry (ICP-MS) after pressurized digestion, called norms AFNOR, ME48, DIN EN ISO 15763, EN ISO 17294-2-E29.

Polycyclic Aromatic Hydrocarbons (PAHs): In total, 24 main PAHs were assessed by gas and/or high-performance liquid chromatography with reverse phase polarity and if necessary double mass spectrometry (GC/MS/MS), according to norm EU 208/2005 or an adapted version of NF EN ISO 15302 or GC/MS ISO N842 CD21035 modified.

Results

All food samples contained some detected substances. Metals were detected in all cases and PAHs in some instances. Pesticides were detected at levels over 10 µg/kg in rare cases.

Pesticides: The major pesticide detected, 2-phenylphenol (E231), is a food additive used as a fungicide and a preservative in non-organic products. We detected 2-phenylphenol in one sausage at 150 µg/kg and two cheeses at levels below 10 and 37. It can be added during the manufacturing process. Dodine was also detected in one organic cheese; it is a fungicide used on wood, on which organic cheese may have been stored together with non-organic ones, after the food processing. All other pesticides were below 10 µg/kg (Table 1).

Pollutants	Non-organic sausages	Organic sausages	Non-organic cheeses	Organic cheeses
Max pesticides µg/kg Min <10 (800 molec.)	150* (1/11)	<10 (7/7)	37 (1/12)	110* (1/5)
Mean metals mg/kg (Pb, Ni, Ti)	1.91 (9/11)	1.58 (6/7)	3,37 (11/12)	4.27 (5/5)
Si mg/kg	19.42 (8/11)	22.61 (2/7)	15.7 (2/12)	<10 (5/5)
Total max PAHs µg/kg	409.6 (6/11)	63.4 (3/7)	<10 (12/12)	<10 (5/5)

Table 1: Pollutants measured in non-organic and corresponding organic food. Maximal (max) levels of pesticides detected and minimum (min) for 800 molecules, compared to mean levels of metals and elements, with a focus on Pb, Ni, and Ti in food. Si is added as an additive, often in nanoparticles. In parenthesis is the number of cases testing positive out of the total.

*The pesticide found at the highest level (150) in non-organic sausages is 2-phenylphenol (E231), which is used intentionally as a food preservative. The maximal level of pesticide found in one case in one organic cheese (110) is entirely due to dodine, a fungicide and bactericide that can be used as a wood preservative on cheese wood aging boards. This could be an unintentional contamination.

The maximal difference between non-organic and organic foods is for carcinogenic or possibly carcinogenic PAHs in sausages, found at 6.5 times higher levels in non-organic foods than in organic ones.

Metals: Metals or other elements were found in every product in approximately the same range in sausages (total from 27 to 187 mg/kg), with a peak at 148 due to silicon in one organic sausage. Pb, Ni or Ti were detected (Table 1). For cheeses, there was a big difference between non-organic products only for silicon (15.7), and organic ones (<10).

Polycyclic Aromatic Hydrocarbons (PAHs): The only undeclared and non-authorized pollutants in all these foods are PAHs, which are generally petroleum residues. These are 2.9 (acenaphthene) and 5.8 µg/kg (anthracene, fluorene, and pyrene) in two organic sausages (German) out of six. In an additional chorizo the highest content in PAHs in organic products was discovered (59.5). By contrast, PAHs were detected at levels from 0.4 to 405.4 µg/kg due to 17 molecules in 6 non-organic sausages (3 German products, 2 French and 1 Spanish chorizo, the most contaminated) (Figure 1). The maximum peaks were 191.1 µg/kg for phenanthrene, 58.2 for pyrene and 45.2 for fluoranthene. Some PAHs are carcinogens, while others are probable or possible ones. These are anyway carcinogens in mixtures. Their relative toxicity is described in Table 2. Surprisingly, non-organic sausages were found to be 81,920 times more toxic than organic ones (12.4) due to only one organic chorizo. PAHs were found at a maximum of 6.5 times higher in non-organic sausages with 3 known carcinogenic ones, in comparison to organic products; this makes a toxicity 6,606 times higher, which is very elevated. In all cheeses, these PAHs were not found at levels over 10 µg/kg.

A

B

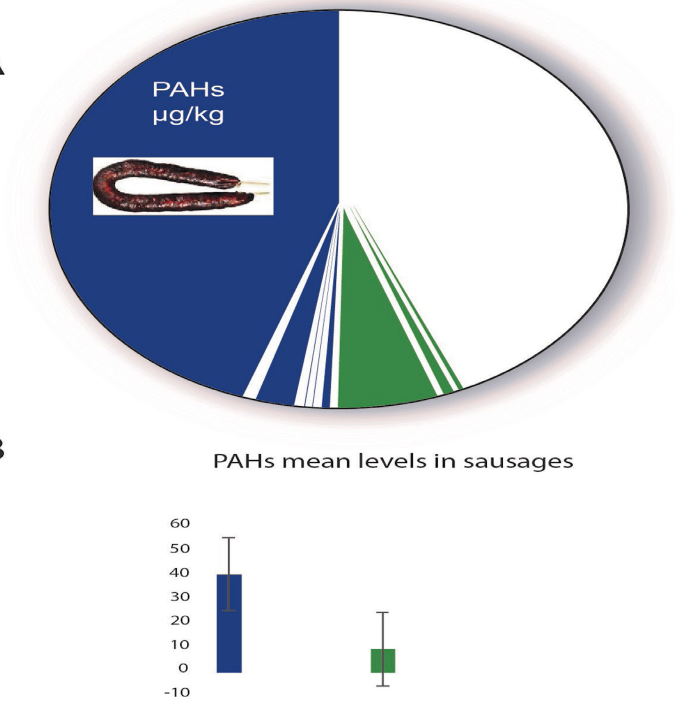

Figure 1: Levels of PAHs detected in sausages. A. Relative levels per sausage: maximum 405.4 µg/kg in one non-organic chorizo and less in 5 others (blue), the organic sausages (green) reach 59.5 at the maximum. **B.** Mean levels in the same products.

PAHs µg/kg	max NO	Max O	tox	max/tox No	maxtox O
Acenaphthene	3.2	2.9	60		
Acenaphthene	20.3	2.4	1	20.5	2.4
Anthracene	38.8	3.2	10	3.9	
Benzo(a)anthracene	12.9	1	0.1	129	10
Benzo(a)pyrene	2.1		0.3	7	
Benzo(b)fluoranthene	2.5		0.005	500	
Benzo(c)fluorene	1.2		K	yes	
Benzo(g,h,i)perylene	0.6		30		
Benzo(j)fluoranthene	0.6		K	yes	
Benzo(k)fluoranthene	1		5		
Chrysene	5.2	1.1	10		
Cyclopenta(cd)pyrene	1.8		K	yes	
Dibenzo(a,e)pyrene	<1		K		
Dibenzo(a,h)anthracene	<0.5		0.5		
Dibenzo(a,h)pyrene	<1		K		
Dibenzo(a,i)pyrene	<1		K		
Dibenzo(a,l)pyrene	<,1		K		
Fluoranthene	45.2	6.2	10	4.52	
Fluorene	15	3.6	10	1.5	
Indeni(1,2,3-c,d)pyrene	<0.8		5		
5-Methylchrysene	<1		K		
Naphtalene	9.7	12	20		
Phenanthrene	191.1	25	40	4;8	
Pyrene	58.2	6	30	1.9	
Total	409.6	63.4	0.005;K	81,920	12.4
	17 PAH	10 PAH			

Table 2: Relative toxicity of PAHs in non-organic (NO) versus organic (O) sausages.

17 PAHs have been detected in NO sausages, versus 10 in O ones. Maximal (max) levels are presented over their toxicity levels (tox) recognized by international agencies, for oral chronic consumption. Above 11 detected, the toxicity norm is considered to be 0.005 µg/kg. For the total NO measurement, it is 81,920 times over the norm (with 3 highly carcinogenic, K), and only 12.4 times for organic products.

Discussion

The first observation regarding French, Spanish and German sausages is that only one pesticide was detected in one case at 150 µg/kg, in a non-organic sample from Spain, and in two other ones to a lesser extent. It is not only used as a general biocide but also as an additive or disinfectant. It is otherwise known as E231 and is also authorized as a food conservative in non-organic production. Considering all these data, one could conclude that it has been used intentionally. Some experiments evidence its irritant properties; it is potentially a carcinogenic endocrine and nervous disruptor over the long-term [17]. By contrast, no pesticides were detected in organic sausages. Organochlorine pesticide residues have been detected in other studies in various Spanish meat products, including in sausages at levels between 10 and 100 µg/kg at the beginning of the 1990s [18].

The pesticide 2-phenylphenol was also found at 37 µg/kg and below 10 µg/kg respectively in a French and a German non-organic cheese; this could have been added intentionally in the manufacturing process, as indicated. This pesticide was also detected in Spanish dairy products and cheeses by others, below the regulatory threshold [19]. Dodine was detected in one organic cheese; it is a fungicide applied to wood, on which organic cheese may have been stored together with non-organic ones, after food processing. The presence in this case is most probably not intentional but due to contamination.

However, in general, the regulatory threshold for accredited methods to assess pesticides is nowadays 10 µg/kg, which is often considered as the Maximum Residue Level (MRL), although technically it is possible to measure pesticides well below. This high threshold of 10 µg/kg or ppb does not appear to be adequately protective for health. For chronic oral consumption, since many of the measured pesticides are known as endocrine and nervous system disruptors [4], this level is not sufficiently low, and overall, it is not logical. To explain that assertion, testosterone itself acts in the organism at levels below this threshold in the entire life of a man. This hormone is generally found at levels between 3-9 µg/L in an adult man [20]. The detection threshold in water is 0.1 µg/L for a pesticide; this threshold for pesticide detections is in fact not a technical but a regulatory decision [21] to facilitate many measurements at once; or issued from political decisions to avoid detecting too many pesticides. To lower the threshold is feasible since lindane, another pesticide, together with one of its metabolites, has been found below or just around 10 µg/kg recently (2020) in meat sausages from Bosnia and Herzegovina [22]. Pesticides have again been detected very recently in Egyptian meat sausages and burgers at levels around 0.1 µg/kg or higher and the authors conclude that these should be monitored with precise methods because of public health concerns [23]. The presence of associated PAHs may lower the toxicity level of concern to 1,000 times less (0.1 ng/kg), as explained in the introduction.

Metals and trace elements were measured and found at physiological levels (see raw data); we focused on Pb, Ni and Ti, since these can be toxic and are used as nanoparticle additives, as well as silicon (Table 1). These molecules can cross the blood, brain or testis barriers once ingested [24-26]. The use of some of these nanoparticles as whitening agents, for instance, may explain their relatively high presence in cheeses for Pb, Ni and Ti (1-2 mg more per kg than in sausages, Table 1). They were, however, found at comparable levels in organic and non-organic products, except for Si which was not detected in organic samples, but in non-organic ones. In fact, silicon dioxide (E551) is a common anti-aggregant in food, used mostly in non-organic products.

The most important concern is about PAHs and other petroleum residues that have been linked to the presence of pesticides, whether glyphosate-based [27,28] or not [11]. Due to the pesticide measurement threshold, even if very low levels of PAHs are present together with each of the 800 declared active substances measured below 10 µg/kg (in addition to possible anthropogenic environmental contaminants), their impact could be serious.

It is known that PAHs can come from smoking of sausages, it is even considered as the major source [29]. Besides, most of smoked products, or chorizos-sausages containing smoked paprika (pimenton de la Vera), are positive for PAHs (Table 3). Similarly, non-smoked products do not contain PAHs over 0.2-10 µg/kg (threshold depending on the PAH) except 3 on 12 sausages (organic or not). Two non-organic chorizos, for instance, are smoked (only the paprika they contain, around 0.4-1.5%) and they provide 93.3% of the PAHs. The second most contaminated product is however the third chorizo, an organic one. Even without the three assayed chorizos, a mean of 2.3 times more PAHs was found in the non-organic smoked products in comparison to organic smoked ones. The smoking regulation for sausages is similar for both categories, according to written good practices. In general, mean levels of PAHs were however 4.2 times higher in non-organic than in organic samples. And for the maximum PAHs to which one can be exposed (Table 2), there is 6.5 times more in all non-organic samples, and they were essentially concentrated in sausages. A couple of similar sausages (organic and non-organic Landjäger and Wiener) were smoked naturally. They both presented 2-4 times more PAHs in products raised with pesticides. A non-organic smoked sausage (sample 15, raw data) did not contain detectable PAHs. All these results taken together evidence that PAHs cannot come only from smoking. They do not come also from the salt additives on the label. They could come from non-homogeneous environments, and this has to be further studied with more samples. However, considering the literature and the European regulated methods of productions, it is clear that other hypotheses have to be drawn. All these PAHs are not declared and not authorized. It therefore cannot be excluded that they come from several or many formulations of pesticides that were themselves present below the threshold of detection. The pesticide 2-phenylphenol (E231) was used as a conservative additive (sold as purified crystal) and does not contain other products of formulation like PAHs (sample 4, Table 3) by contrast to when it is sold as a pesticide for agriculture. It is the same case in cheeses.

NO sample	**2**	**4**	**12**	**14**	**22**	**24**	**5**	**31**	**15**	**25**	**32**
Sausage name	Botti-fara	Fuet	Tou-louse	Saucis-son sec	Wiener	Landjäg-ger	Chori-zo	Chori-zo	Knackies	Fleich wurst	Sau-cisse sèche
S/NS	NS	NS	NS	NS	S	S	S Papri-ka	S Papri-ka	S	NS	NS
PAH µg/kg	<10	<10	<10	<10	23.6	5.5	405.4	17	<10	0.4	0.5
O sample	**1**	**3**	**11**	**13**	**21**	**23**	**33**				
Name	Botti-fara	Fuet	Tou-louse	Saucis-son sec	Wiener	Landjäg-ger	Chori-zo				
S/NS	NS	NS	NS	NS	S	S	NS				
PAH µg/kg	<10	<10	<10	<10	5.8	2.9	59.5				
Total					**NO/O: 4.1**	**NO/O: 1.9**	**NO/O: 6.8**				

Table 3: Sausages smoked (S) or not (NS) and PAHs contents (µg/kg). Sausages studied here were smoked in 2/7 of the organic products, and 5/11 of the non-organic ones, according to the information on their labels, but also their declared processing and compositions, and their names or supplier websites or after phoning.

In this study we found lower levels or no detectable level of PAHs in all cheeses tested, like in the majority of organic sausages. In other recent studies, PAHs have been detected recently in dairy products like yogurt and butter, though below 10 µg/kg [30], but they appear to be found at higher levels in smoked cheeses [31]. All cheeses in this study were non-smoked. These could also contain lower levels of pesticides. In addition, it is known that various lactobacillus strains or yeasts necessary for cheese making may provoke degradation of PAHs; this has been recently demonstrated [32]. The mammary gland can also naturally filter partially some PAHs in comparison to meat and fat used for sausages, since the PAHs form adducts and bind to DNA in mammary glands [33], provoking cancers.

Non-organic sausages therefore have a considerably higher potential to act as endocrine and nervous system disruptors, and to be toxic and carcinogenic, compared with similar organic products. It is considered that for a mixture of 11 PAHs present or more in a product, which is the case for the 17 PAHs detected in non-organic products in this study, the carcinogenicity and toxicity potential is very high from 0.005 µg/kg at a regulatory level. Here for a maximum level of 409.6 (Table 2), the toxicity of non-organic sausages is thus extremely elevated, 409.6/0.005, i.e. 81,920. In comparison eating organic sausages brings only a risk of 12.4.

Other non-measured compounds may have similar toxic potential, such as PCBs, dioxins, nanoparticles, plasticizers, or radioactive chemicals. However, these PAHs are not declared, generally less considered for the risks of contaminants in mixtures, and overall, not considered for their association with pesticides, especially below the regulatory threshold for pesticides.

In conclusion, it is proposed that PAHs may be markers of food pollution. They present a toxicity far greater in non-organic than in organic meat products. PAHs are more present in non-organic products raised with pesticides, moreover they have already been demonstrated to be present in formulated pesticides [11]. Their presence associated to low levels of pesticides in non-organic food should be further investigated.

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