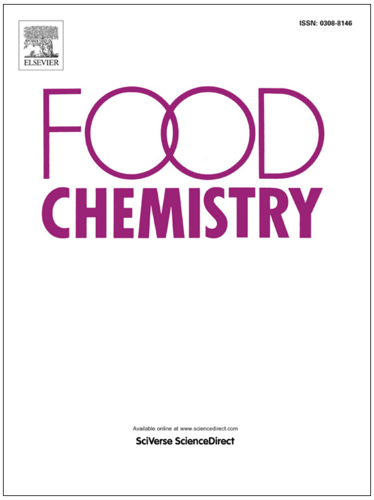

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Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production

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Abstract

The purpose of the present work was the experimental evaluation of pesticides transfer to virgin olive oil during the production step and prediction of their processing factors, which could be eventually used for the calculation of maximum residue limits (MRLs) in olive oil from the MRLs set in olives. A laboratory-scale Abencor system was used for the production of olive oil from olives spiked with the 104 pesticides studied, three different chromatographic methods being used for the analysis of raw olives and the obtained olive oil: (i) gas chromatography-tandem mass spectrometry (GC-MS/MS) for GC-amenable pesticides; (ii) hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) for polar pesticides, and; (iii) reversed-phase liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for low to medium polarity pesticides. Processing factors experimentally calculated were correlated to their octanol-water partitioning coefficient (log K_{ow}), enabling the calculation of the equivalent MRLs in olive oil from the MRLs in olives, considering the percentage of oil extracted (oil yield) and the log K_{ow} of each pesticide.

Keywords: olive oil; olives; pesticide; processing factors; GC-MS; LC-MS.

1. Introduction

Nowadays, the use of pesticides in crops is widely extended in order to counteract the adverse effects of pests, while, at the same time, increasing harvest yields to meet the food demands of a growing global population. However, even trace amounts of pesticides in food can cause health problems. To avoid an inadequate use of pesticides and an unreasonable transfer of them to the processed foodstuffs, several institutions worldwide have established guidelines for good agricultural practices and stringent regulations establishing the maximum concentration of pesticides allowed in foodstuffs. The maximum residue limits (MRLs) set by the European Union (EU), (European Commission, 2005) or the Codex Alimentarius Commission (REP15/CAC, 2015) are a couple of examples. These MRLs have been calculated individually for each foodstuff, depending on the physicochemical properties of the active substances, and on their toxicity. For selected compounds, a default value of 10 $\mu g \cdot kg^{-1}$ (European Commission, 2008) has been established.

MRLs have not been set for some processed vegetables such as olive oil. In the particular case of virgin olive oils, a default conversion factor of 5 (*viz*. assuming a standard production yield of 20% olive oil from raw olives) was proposed for the first time in 2014 to convert the MRLs set in olives to an indicative maximum concentration level of pesticide residues authorized in virgin olive oil during official control (European Commission, 2014). However, the actual processing factor (PF) of each pesticide strongly depends on its relative solubility and affinity towards the aqueous phase or to the oil phase during olive oil production. In 2015, the EU differentiated between fat-soluble and fat insoluble compounds, setting processing factors of 5 and 1, respectively (European Commission, 2015). Thus, the MRL in olive oils should be estimated taking into account the concentration or dilution performed during olive oil production. In fact, annex VI of Regulation EC N^{o} 396/2005 is expected to deliver PFs for each compound in several raw commodities. Unfortunately, to the best our knowledge this annex has not been yet established, (European Commission, 2017). Meanwhile, there is a particular concern from the European vegetable oil industry and its suppliers (Fediol & Coceral, 2012)

Given the industrial two-phase extraction used in olive mills and the partitioning undergone by all the olive paste components during malaxation and centrifugation, the polarity of the compounds (e.g., the octanol-water partition coefficient (log K_{ow})) may be a useful parameter to estimate the degree of transfer and, thus, to predict the PFs during olive oil production. Previous studies have calculated PFs in other vegetables crops including tomatoes (Abou-Arab, 1999), (Liu, et al., 2014), cucumbers (Ramezani & Shahriari, 2015) and spinach (Bonnechère et al., 2012); apples (Li, M. et al., 2015), (Martin et al., 2013), raisins (Shabeer, et al., 2015), grapes (Pazzirota, T. et al., 2013) and oranges (Li, Y. et al., 2012); in cereals, such as soybeans (Zhao, Ge, Liu, & Jiang, 2014) and sorghum (Han, Y. et al., 2016). However, all these studies have been undertaken for a limited number of pesticides, and with the goal of studying the effect of different steps carried out in industrial processing, such as drying, washing, peeling, hulling, milling, water addition and other processes (Keikotlhaile, Spanoghe, & Steurbaut, 2010).

Nevertheless, scarce literature is available on the study of PFs in olive oil. To the best our knowledge, only one previous study has estimated the PFs for thirteen pesticides in olives and olive oils (Amvrazi & Albanis, 2008). In this study, the objective was to examine the effect of the amount of water added during olive oil production for pesticide reduction, revealing that an increase of water content yielded a decrease of the pesticides transferred. With this scenario in mind, the aim of this work is to examine and tentatively predict levels of pesticides during olive oil production. For this task, 104 commonly used pesticides was selected and the PF were calculated using a laboratory-scale olive mill (Abencor system). Different analytical methods based on gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were used for the determination of pesticides in the initial spiked olives and in the olive oil produced by Abencor.

2. Experimental section

2.1 Reagents and pesticide standards

HPLC-grade solvents: methanol (MeOH), acetonitrile (MeCN), ethyl acetate (EtOAc) and *n*-hexane were purchased from Sigma-Aldrich (Madrid, Spain) as well as sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$) and formic acid (HCOOH). Acetic acid (HOAc) was purchased from J.T. Baker (Center Valley, PA). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA) was used throughout the study to obtain the HPLC-grade water used during the analyses. EMR-Lipid sorbent employed to extract the pesticides was acquired from Agilent Technologies (Santa Clara, CA). A suite of 104 pesticides were selected (Table SD-1, *Supplementary Data*), including representative compounds from different families typically used in olive groves, including insecticides, herbicides, fungicides and acaricides. All the analytes are included in the latest Annex 1 (last Annex 1/2016) of European Regulation 396/2005, (European Commission, 2017). Analytical standards (>99% purity) of each pesticide were supplied by Sigma-Aldrich or Dr. Ehrenstorfer (Augsburg, Germany). Standard solutions of each pesticide (500 $\mu g \cdot mL^{-1}$) were prepared in MeOH or MeCN for the analysis by LC-MS/MS and in EtOAc or *n*-hexane for the compounds analyzed by GC-MS/MS. Solutions containing mixtures of the studied compounds (5 $\mu g\ mL^{-1}$) were prepared in MeCN or *n*-hexane and stored in amber glass vials with caps at –20 °C until use.

2.2. Sample treatment and laboratory-scale olive oil extraction from crushed olives

Olive samples were collected from an olive grove (Jaén, Spain), and stored at –20 °C. Before the sample treatment, olives were crushed using a mill (Talleres Lopera, Priego de Córdoba, Spain). To obtain virgin olive oil samples for the analyses, portions of olive samples were selected and processed by means of a lab-scale Abencor system (MC2 Ingeniería y Sistemas, S.L., Sevilla, Spain) to extract olive oil. Milled samples were weighed (ca. 150 g) and transferred to the malaxer, equipped with thermometer (MC2 model TB-100), where the crushed olive paste was shaken for 30 min at temperature less than 20.6 °C. Subsequently, the paste obtained was centrifuged for 1 min at 3500 rpm (Abencor centrifuge MC2 model CF-100). In this final step, the virgin olive oil was separated from pomace juice and water that constitute the olive, and frozen until the moment of analysis.

2.3. Procedures for pesticide determination in olive oil and olives

2.3.1. Sample treatment for pesticides with low or medium polarity. The “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) method for pesticides in fatty matrices (AOAC Official Method 2007.01, 2011), was used with the modification of using a novel sorbent (EMR-Lipid), which provides some advantages in terms of method precision and matrix effects (López-Blanco et al., 2016). In brief, the modification consisted of a two-step cleanup procedure of the acetonitrile extract, using (1) EMR-Lipid sorbent and (2) $MgSO_4$

and NaCl. The description of the procedure is detailed in the supplementary data section. For the compounds studied by LC-MS/MS, the method performance in terms of recovery rates was already assessed (López-Blanco et al., 2016), being the recovery rates used to correct and compensate analyte losses during analysis. In the case of pesticides analyzed by GC-MS/MS, the recovery rates obtained with this sample treatment procedure are included in Table SD-2 (*Supplementary Data*).

2.3.2 Sample treatment for polar pesticides. A method for polar pesticides, the so-called QuPPe (Quick Polar Pesticides), was adapted for pesticide analyses of highly polar compounds in both olive oil and olives (EU Reference Laboratories for Residues of Pesticides (EURLs), 2016). The detailed description is addressed in the *Supplementary Data* section. Briefly, an extraction with methanol (1% formic acid)/water (1:1 v/v) was performed, aided by a heating step to extract some compounds. The method performance in terms of recovery rates was already assessed (Nortes-Méndez et al., 2016), the recovery rates obtained being used to correct and compensate analyte losses during analysis.

2.4. Determination of pesticides in olive oil and olives using LC-MS/MS and GC-MS/MS

From the 104 pesticides selected, 60 pesticides (with low or medium polarity) were analyzed using QuEChERS and UHPLC-MS/MS (Thermo Scientific Quantiva, San José, CA) with a C18 column and a method described elsewhere and detailed in *Supplementary Data* (López-Blanco et al., 2016). Seven polar pesticides were analyzed with QuPPe and HPLC-MS/MS with a HILIC column (Nortes-Méndez et al., 2016), and the remaining 37 compounds were analyzed by GC-MS/MS using an ion trap instrument (Polaris Q-Ion Trap; Thermo Scientific, USA) with the method described in *Supplementary Data*.

Briefly, the reversed-phase UHPLC-MS/MS method consisted of a 20-min run on a short C18 column (2.1 mm i.d. × 50 mm, 1.8 µm), using a gradient of a mixture of water (0.1% formic acid) and acetonitrile (0.1% formic acid). The HILIC-MS/MS method employed a longer HILIC column (2.1 mm i.d. x 100 mm, 1,8 µm) for a total analysis time of 16 min. Mobile phase **A** was water (ammonium formate 100 mM, adjusted to pH 2.85 with formic acid)/acetonitrile (1:2 *v/v*), and mobile phase **B** was acetonitrile. Finally, the GC-MS/MS method was based on a temperature ramp from 70 to 300 °C (with different steps) in 43 min.

2.5. Study of processing factors: transfer from spiked olives to olive oil produced by a laboratory-scale Abencor system

Different batches of crushed olive samples were spiked with different concentrations of each pesticide in the range from 0.5 to 5 mg·kg^{-1} depending on method sensitivity and experiment. The mixture of pesticides was drop-spread over the paste in an open container. Then, the olive samples were stirred at room temperature for 60 min helped with a N_2 current to remove excess solvent. Afterwards, the olive paste was stored at 4 °C overnight. Before the olive oil extraction step, different fractions (n = 6) of the spiked and incubated olive paste were analyzed, to assess the homogenization of the paste and also to accurately determine the amount of pesticides present in each sample fraction, using the different methodologies described, according to the pesticide class and properties. Thereafter, olive oil was extracted from spiked olives using the Abencor system, following the procedure described in *Section 2.2*. Similarly, the olive oil obtained was analyzed to determine the pesticides transferred. The concentration levels detected were calculated for each analyte using matrix-matched calibration curves, and the recovery rate from the extraction procedure for pesticide analysis was used to correct the actual amount of pesticide transferred to the olive oil.

3. Results and discussion

3.1. Calculation of processing factor through determination of pesticide residue levels during olive oil production

Usually, PFs are applied to account for the effect of food processing on the decrease in pesticide residues. The aim of the present article is to establish the relationship between residue levels initially present in raw commodities (olives) and the levels remaining in the processed foodstuff after the transformation process (olive oil). The PF is calculated from the concentration levels found in the analyzed olives and olive oil samples from the same production batch. The olive oil yield (weight fraction of olive oil produced from a batch of olives) and the analyte losses during the measurement of pesticides (sample treatment recovery rate) must be also taken into account, so that any pesticide loss that cannot be attributed to the industrial process itself is compensated in the final PF calculated.

The PF(%) is calculated from pesticide mass balance during olive oil production. It is expressed as the percentage of the residue levels present in the raw commodity (olives) that are detected in the processed product (oil extracted from this one). The calculation of PF is shown in equation 1:

$$Processing\ Factor\ (\%) = \frac{[Pesticide]_{olive\ oil}}{[Pesticide]_{olives}} * \frac{Recovery_{\ olives}}{Recovery_{\ olive\ oil}} * oil\ yield\ (\%) \qquad [1]$$

where,

[Pesticide]$_{\text{olive oil}}$ is the concentration found in the olive oil extracted

[Pesticide]$_{\text{olives}}$ is the concentration found in the spiked olive paste

Recovery $_{\text{olives}}$ refers to the percentage of compound that is recovered (100% – Recovery(%)$_{\text{analyte}}$ is the analyte loss) during pesticide extraction and determination step

Recovery $_{\text{olives}}$ refers to the percentage of compound that is recovered (100 – Recovery(%)$_{\text{analyte}}$ is the analyte loss) during pesticide extraction and determination step

Oil yield (%) refers to the percentage of fat content (olive oil) obtained from the studied olives. It is calculated by weighing the olive oil obtained after Abencor extraction and the initial olives used to produce the olive oil.

The results obtained for the studied pesticides are summarized in **Table 1.** In addition, the plot of the PFs obtained for each pesticide against their octanol-water coefficient (log K_{ow}) (EURLs 2017) is shown in **Figure 1.** It has to be highlighted that, a linear trend between both parameters was obtained, with a correlation coefficient (r^2) of 0.5905. The following equation was obtained: *PF: 12.162 logK_{OW} + 4.98 (see Figure 1).* The correlation is moderate when considering the wide range of chemical classes studied.

<Table 1 and Figure 1>

As could be expected, highly polar pesticides (with log K_{ow} < 0) displayed negligible transfer rates. This fact is due to their low solubility in non-polar solvents or environments such as oil. They are mainly distributed in the aqueous phase derived from the water content of olives (olive pomace), which is separated from the oil phase during the centrifugation step.

For the pesticides with log K_{ow} > 0, there is a clear trend to be increasingly transferred to the oil phase as the polarity decreases. A breakdown of the graphic obtained according to pesticide class is shown in **Figure 2**. Up to five different chemical classes, including triazoles, triazines, neonicotinoids, carbamates and organophosphorus pesticides were studied. In the case of organophosphorus compounds, a significant number of them were evaluated across a wide range of polarity (log K_{ow} from −0.85 to 4.84). The slope of transfer correlation with log K_{ow} (13.33) was similar to that obtained in the overall equation. Similar results were obtained with triazines and carbamates, which exhibited a slightly higher slope. On the other hand, triazoles and neonicotinoids displayed different behavior, so that an increase of hydrophobicity did not cause such an increase of pesticide transfer efficiency for these two classes. This information is interesting as they might receive different consideration when specific MRLs in olive oil are to be set. Other classes, such as organochlorines and pyrethroids, were also studied. However, a limited number of these pesticide classes was studied, and they had similar octanol-water coefficients (in the first case around log K_{ow} ≈ 4.5, and log K_{ow} ≈ 6.0 respectively). Therefore, it was not possible to establish a pattern in these cases. Similar PF values were obtained for all these analytes with analogous polarities. Table 1 includes not only the experimental PFs but also those calculated from the regression equation (using the log K_{ow} values of each individual pesticide), and also those calculated from the class-specific regression equation when available. Taking into account these results, it can be concluded that PFs can be roughly modeled using log K_{ow} as a variable and might enable the correction of PFs. Compared with previous work where PFs were estimated for eight common pesticides (Amvrazi & Albanis, 2008), the PFs obtained in the current work were slightly higher. This can be partly attributed to the addition of water and the different production process used.

Once PFs were experimentally calculated and a preliminary mathematical model based on the use of log K_{OW} adopted, they can be incorporated to correct experimental MRLs in olive oil. Nowadays, there are only MRLs set in olives (for both table olives and olives intended for olive oil production), whereas olive oil as a derivative product does not have specific MRLs. The Regulation (EC) 400/2014 (European Commission 2014) established for the first time a default preconcentration factor of 5 to calculate MRLs in fat-soluble pesticides. This factor comes from the assumption of an estimated oil yield of 20% and assuming a completely effective pesticide transfer (100% of PFs). By contrast, a factor of 1 is applied for those pesticides that are not transferred (European Commission 2015). As demonstrated during this work, all the compounds do not have the same PF, which is correlated with the log K_{ow} of the studied compounds. Thus, it would be more reasonable to introduce this variable in the calculation of MRLs for olive oil, considering the substantial differences of physicochemical properties across the pesticides. A new equation is proposed including the impact of PFs, and expressed using log K_{OW} according to the linear regression obtained for the experimental PFs calculated. The proposed data and the calculated MRLs are included in **Table 2**. The data reveal that the estimated MRLs are higher than the current default MRLs using the oil yield factor of 20% and assuming the complete transfer of pesticides during olive oil production (European Commission 2014, European Commission 2015). Neither assumption is met as was demonstrated in the present study. Thus, the proposed work clearly shows the need to compile data from individual processing factors and incorporate them into Annex VI of the EU regulation 396/2005.

<Table 2>

$$MRL_{olive\ oil} = MRL_{olives} * \frac{100}{oil\ yield} * \frac{100}{[Processing\ factor\ ]}$$

$$MRL_{olive\ oil} = MRL_{olives} * \frac{10^4}{oil\ yield * [(12.162 * \log K_{ow} + 4.98)\ \pm 5.2]}$$

4. Conclusions

Currently, the general procedure for the establishment of MRLs for pesticides in olive oil is the application of a processing factor (PF) to the MRL existing for the raw commodity (Regulation (EC) 396/2005), considering only a default oil yield (of 20%) for fat-soluble compounds (Regulation (EC) 400/2014, Regulation (EU) 2015/595). A factor of 1 is applied to fat-insoluble compounds. Pesticide PFs not only depend on the industrial process and their yields, but also on the singular physicochemical properties of each pesticide. In the present work, the PFs of 104 pesticides covering a wide polarity range have been calculated during olive oil production at laboratory scale. As expected, lipophilic pesticides were transferred to the oil phase to a higher extent than the hydrophilic ones, whose concentrations are negligible, as they are mostly transferred to the water phase to a higher extent (olive pomace). A linear dependence between log K_{ow} and the PF (%) has been established. The proposed model based on log K_{OW} has shown differences on the behavior of pesticides that may be considered in the calculation of MRLs.

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Figure captions

Figure 1. Plot of processing factors (PF) (%) obtained for each pesticide *versus* log $K_{OW.}$

Figure 2. Class-specific plots of processing factors (PF) (%) obtained for each class *versus* log K_{OW}.

Figures

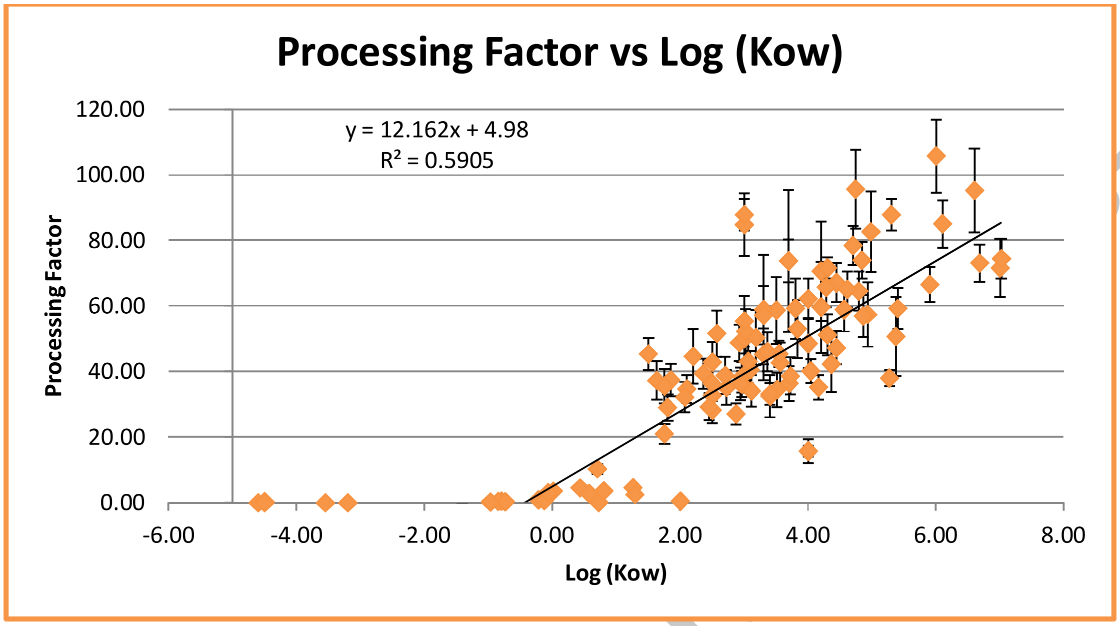

Figure 1

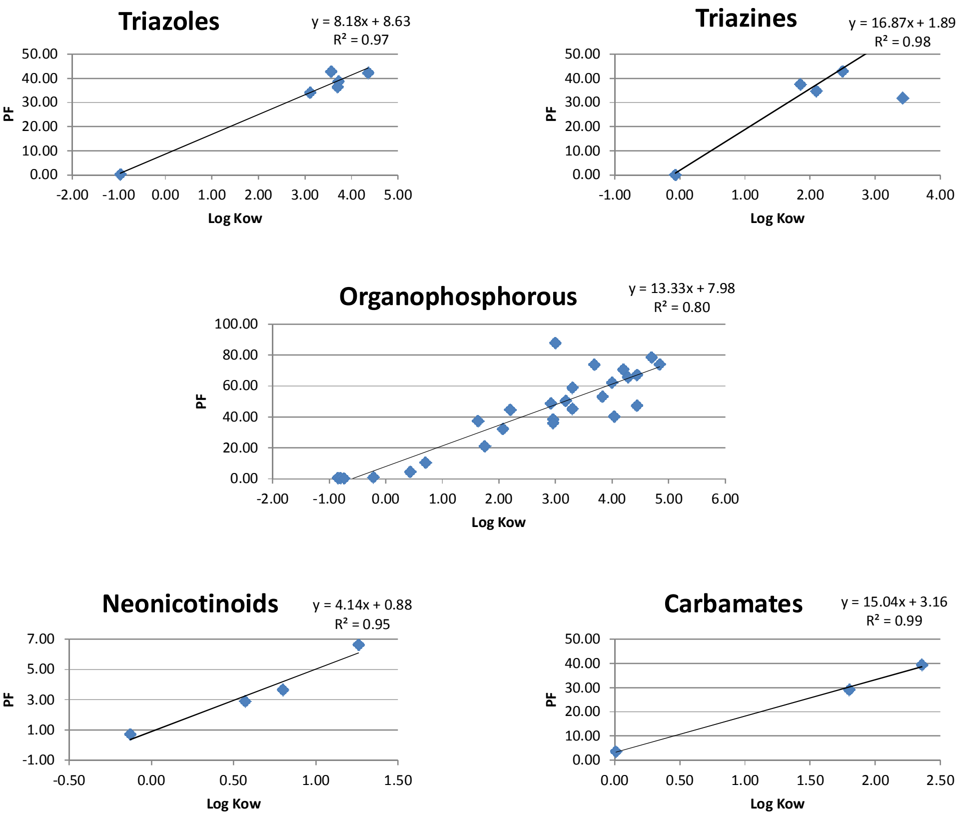
Triazoles
y = 8.18x + 8.63
R² = 0.97
PF
50.00
40.00
30.00
20.00
10.00
0.00
-2.00
-1.00
0.00
1.00
2.00
3.00
4.00
5.00
Log Kow
Triazines
y = 16.87x + 1.89
R² = 0.98
PF
50.00
40.00
30.00
20.00
10.00
0.00
-1.00
0.00
1.00
2.00
3.00
4.00
Log Kow
Organophosphorous
y = 13.33x + 7.98
R² = 0.80
PF
100.00
80.00
60.00
40.00
20.00
0.00
-2.00
-1.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Log Kow
Neonicotinoids
y = 4.14x + 0.88
R² = 0.95
PF
7.00
5.00
3.00
1.00
-1.00
-0.50
0.00
0.50
1.00
1.50
Log Kow
Carbamates
y = 15.04x + 3.16
R² = 0.99
PF
50.00
40.00
30.00
20.00
10.00
0.00
0.00
0.50
1.00
1.50
2.00
2.50
Log Kow

Figure 2

Table 1. Calculation of experimental processing factors (PF (%)) (*n* = 6)), calculated PF (%) using the log K_{ow} overall model, and the PF using the class-specific regression equation.

Pesticide	Log K_{ow}	RSD (*n* = 6)	PF (%)	PF theoretical	PF theoretical specific
Diquat	–4.60	–3.82*	NT	NT	NT
Paraquat	–4.50	–0.58*	NT	NT	NT
Fosetyl	–4.50	0.85*	NT	NT	NT
Mepiquat	–3.55	–2.00*	NT	NT	NT
Trimesium	–3.20	–1.74*	NT	NT	NT
Quinmerac	–1.41	–13.43	NT	NT	NT
Amitrole	–0.97	0.42*	NT	NT	NT
Acephate	–0.85	19.10	NT	NT	NT
Methamidophos	–0.80	15.32	NT	NT	NT
Omethoate	–0.74	20.05	NT	NT	NT
Monocrotophos	–0.22	17.47	NT	NT	NT
Thiamethoxam	–0.13	7.86	NT	NT	NT
Cyromazine	–0.07	3.41*	NT	NT	NT
Methomyl	0.01	13.57	NT	NT	NT
Trichlorfon	0.43	17.17	NT	NT	NT
Imidacloprid	0.57	16.54	NT	NT	NT
Dimethoate	0.70	13.98	10.37	13.49	17.29
Gibberellic Acid	0.72	26.33	NT	NT	NT
Acetamiprid	0.80	15.74	NT	NT	NT
Thiacloprid	1.26	23.20	NT	NT	NT
Dodine	1.29	13.86	NT	NT	NT
Thiophanate-methyl	1.45	14.41	NT	NT	NT
Carbendazim	1.50	10.86	45.42	23.22	NA
Fenthion-sulfoxide	1.63	13.92	37.37	24.80	29.66
Fenamiphos-sulfone	1.75	14.36	21.05	26.26	31.31
Metalaxyl	1.75	11.77	35.60	26.26	NA
Carbofuran	1.80	15.83	29.11	26.87	30.23
Terbuthylazine-desethyl	1.85	13.07	37.48	27.48	33.10
Fluroxypyr	2.00	14.54	NT	NT	NT
Malaoxon	2.07	13.56	32.27	30.16	35.51
Simazine	2.10	11.86	34.75	30.52	37.32
Methidathion	2.20	18.73	44.71	31.74	37.24
Carbaryl	2.36	14.55	39.50	33.68	38.65
Norflurazon	2.45	14.91	29.30	34.78	NA
Chlorotoluron	2.50	14.46	28.25	35.39	NA
Isoproturon	2.50	14.88	32.83	35.39	NA
Azoxystrobin	2.50	15.25	36.39	35.39	NA
Atrazine	2.50	11.71	42.95	35.39	44.07
Captan	2.57	13.69	51.68	36.24	NA
Dichlobenil	2.70	14.51	38.89	37.82	NA

Parathion-methyl	2.70	5.50	87.97	37.82	43.89
Dimethomorph	2.72	25.07	35.21	38.06	NA
Spinosyn A	2.80	19.75	15.81	39.03	NA
Diuron	2.87	13.97	27.12	39.88	NA
Malathion	2.92	10.61	48.88	40.49	46.82
Chlorothalonil	2.94	13.70	36.41	40.74	NA
Phosmet	2.96	14.71	36.26	40.98	47.35
Azinphos-methyl	2.96	17.43	38.41	40.98	47.35
Vinclozolin	3.00	12.79	50.07	41.47	NA
Pyrimethanil	3.00	11.32	84.91	41.47	NA
Folpet	3.02	12.87	52.28	41.71	NA
Quizalofop-p-ethyl	3.06	15.18	43.25	42.20	NA
Alachlor	3.09	11.46	40.63	42.56	NA
Iprodione	3.10	14.08	55.46	42.68	NA
Paclobutrazol	3.11	10.69	34.14	42.80	34.07
Azinphos-ethyl	3.18	16.76	50.45	43.66	50.27
Spinosyn D	3.20	12.11	15.83	43.90	NA
Fenamiphos	3.30	11.11	45.36	45.11	51.87
Procymidone	3.30	15.69	57.23	45.11	NA
Fenitrothion	3.30	28.26	58.96	45.11	51.87
Carfentrazone-ethyl	3.36	9.95	46.14	45.84	NA
Terbuthylazine	3.40	22.86	32.59	46.33	59.25
Kresoxim-methyl	3.40	29.24	33.27	46.33	NA
Fenhexamid	3.51	14.46	34.54	47.67	NA
Benalaxyl	3.54	21.50	45.50	48.03	NA
Tetraconazole	3.56	17.14	42.85	48.28	37.75
Diazinon	3.69	8.79	73.87	49.86	57.06
Tebuconazole	3.70	14.29	36.48	49.98	38.90
Penconazole	3.72	13.98	38.75	50.22	39.06
Lindane	3.80	17.29	58.76	51.20	NA
Pyrazophos	3.80	15.47	59.36	51.20	NA
Parathion	3.83	16.60	53.08	51.56	58.92
Fipronil	4.00	15.65	48.68	53.63	NA
Chlorpyrifos-methyl	4.00	9.90	62.27	53.63	61.18
Chlorfenvinphos	4.04	20.01	40.21	54.11	61.71
Rotenone	4.16	8.92	35.25	55.57	NA
Diflufenican	4.20	23.22	59.73	56.06	NA
Pirimiphos-methyl	4.20	10.80	70.69	56.06	63.84
Ethion	4.28	9.45	65.88	57.03	64.90
Endosulfan-sulfate	4.30	12.22	51.27	57.28	NA
Dicofol	4.30	4.53	71.71	57.28	NA
Difenoconazole	4.36	8.94	42.33	58.01	44.29
Quinalphos	4.44	12.99	47.32	58.98	67.03
Profenophos	4.44	8.89	67.16	58.98	67.03
Tolclofos-methyl	4.56	11.19	58.95	60.44	NA

Tetradifon	4.61	8.47	65.16	61.05	NA
Chlorpyrifos	4.70	7.52	78.55	62.14	70.49
Endosulfan-alpha	4.74	12.51	95.76	62.63	NA
Endosulfan-beta	4.79	9.26	64.59	63.24	NA
Fenthion	4.84	7.46	74.03	63.84	72.35
Oxyfluorfen	4.86	11.23	57.02	64.09	NA
Buprofezin	4.93	14.75	57.52	64.94	NA
Teflubenzuron	4.98	14.91	82.75	65.55	NA
Trifluralin	5.27	6.35	38.12	69.07	NA
Cypermethrin_(Sum)	5.30	5.50	87.97	69.44	NA
Pyriproxifen	5.37	23.67	50.79	70.29	NA
Bromopropylate	5.40	10.61	59.32	70.65	NA
Cyfluthrin-beta_(Sum)	5.90	8.11	66.65	76.74	NA
Fenpropathrin	6.00	10.51	105.88	77.95	NA
Permethrin (Sum)	6.10	8.48	85.16	79.17	NA
Bifenthrin	6.60	13.41	95.35	85.25	NA
Fenvalerate_(Sum)	6.68	7.80	73.21	86.22	NA
Cyhalothrin-lambda	7.00	12.34	71.74	90.11	NA
Fluvalinate-tau (Sum)	7.02	8.02	74.54	90.36	NA

*SD was obtained with three replicates for these compounds (n=3); NT: Not transferred; NA: Not Available

Table 2. PFs (%) and MRLs obtained for each pesticide in olive oil according to the conversion factor of 5 or with the proposed equation or the individual experimental PFs calculated.

Pesticide	PF (%)	MRL olives [a] (mg kg^{-1})	MRL olive oil (factor 5) [b] (mg kg^{-1})	Calculated MRL olive oil (using log K_{ow}) (mg kg^{-1})	Experimental MRL olive oil using individual PF (%) (mg kg^{-1})	Log K_{ow}
Paraquat	NT	0.02	0.1	NT	NT	−4.50
Diquat	NT	0.05	0.25	NT	NT	−4.60
Trimesium	NT	1.00	5	NT	NT	−3.20
Thiophanate-methyl	NT	0.10	0.5	NT	NT	1.45
Mepiquat	NT	0.05	0.25	NT	NT	−3.55
Quinmerac	NT	0.10	0.5	NT	NT	−1.41
Gibberellic Acid	NT	0.01	0.05	NT	NT	0.72
Amitrol	NT	0.05	0.25	NT	NT	−0.97
Omethoate	NT	2.00	10	NT	NT	−0.74
Fosetyl	NT	2.00	10	NT	NT	−4.50
Acephate	NT	0.01	0.05	NT	NT	−0.85
Fluroxypyr	NT	0.05	0.25	NT	NT	2.00
Methamidophos	NT	0.01	0.05	NT	NT	−0.80
Thiamethoxam	NT	0.05	0.25	NT	NT	−0.13
Monocrotophos	NT	0.01	0.05	NT	NT	−0.22
Dodine	NT	20.00	100	NT	NT	1.29
Imidacloprid	NT	0.50	2.5	NT	NT	0.57
Cyromazine	NT	0.05	0.25	NT	NT	−0.07
Methomyl	NT	0.02	0.1	NT	NT	0.01
Acetamiprid	NT	0.01	0.05	NT	NT	0.80
Triclorfon	NT	0.01	0.05	NT	NT	0.43
Thiacloprid	NT	4.00	20	NT	NT	1.26
Dimethoate	10.37	2.00	10	86.17	135.60	0.70
Spinosyn A	15.81	0.01	0.05	0.00	0.00	2.80
Spinosyn D	15.83	0.01	0.05	0.00	0.00	3.20
Fenamiphos-sulfone	21.05	0.02	0.1	0.44	0.67	1.75
Diuron	27.12	0.01	0.05	0.15	0.26	2.87
Chlorotoluron	28.25	0.01	0.05	0.16	0.25	2.50
Carbofuran	29.11	0.01	0.05	0.22	0.24	1.80
Norflurazon	29.30	0.01	0.05	167.18	239.97	2.45
Malaoxon	32.27	0.02	0.1	0.39	0.44	2.07
Terbuthylazine	32.59	0.05	0.25	0.63	1.08	3.40
Isoproturon	32.83	0.01	0.05	0.16	0.21	2.50
Kresoxim-methyl	33.27	0.20	1	2.51	4.23	3.40
Paclobutrazol	34.14	0.50	2.5	6.79	10.30	3.11
Fenhexamid	34.54	0.05	0.25	0.61	1.02	3.51
Simazine	34.75	0.01	0.05	0.19	0.20	2.10
Dimethomorph	35.21	0.01	0.05	0.15	0.20	2.72
Rotenone	35.25	0.01	0.05	0.10	0.20	4.16
Metalaxyl	35.60	0.05	0.25	1.11	0.99	1.75
Phosmet	36.26	3.00	15	42.56	58.18	2.96
Azoxystrobin	36.39	0.05	0.25	0.82	0.97	2.50
Chlorothalonil	36.41	0.01	0.05	0.14	0.19	2.94
Tebuconazole	36.48	0.05	0.25	0.58	0.96	3.70
Fenthion-sulfoxide	37.37	0.01	0.05	0.23	0.19	1.63
Terbuthylazine-desethyl	37.48	0.05	0.25	1.06	0.94	1.85
Trifluralin	38.12	0.05	0.25	0.42	0.92	5.27
Azinphos-methyl	38.41	0.05	0.25	0.71	0.92	2.96
Penconazole	38.75	0.05	0.25	0.58	0.91	3.72
Dichlobenil	38.89	0.01	0.05	0.15	0.18	2.70

Carbaryl	39.50	0.01	0.05	0.17	0.18	2.36
Chlorfenvinphos	40.21	0.01	0.05	0.11	0.17	4.04
Alachlor	40.63	0.01	0.05	0.14	0.17	3.09
Difenoconazole	42.33	2.00	10	20.05	33.23	4.36
Tetraconazole	42.85	0.02	0.1	0.24	0.33	3.56
Atrazine	42.95	0.05	0.25	0.82	0.82	2.50
Quizalofop-p-ethyl	43.25	0.05	0.25	0.69	0.81	3.06
Methidathion	44.71	0.02	0.1	0.37	0.31	2.20
Fenamiphos	45.36	0.02	0.1	0.26	0.31	3.30
Carbendazim	45.42	0.10	0.5	2.50	1.55	1.50
Benalaxyl	45.50	0.05	0.25	0.61	0.77	3.54
Carfentrazone-ethyl	46.14	0.01	0.05	0.13	0.15	3.36
Quinalphos	47.32	0.05	0.25	0.49	0.74	4.44
Fipronil	48.68	0.01	0.05	0.11	0.14	4.00
Malathion	48.88	0.02	0.1	0.29	0.29	2.92
Vinclozolin	50.07	0.05	0.25	0.70	0.70	3.00
Azinphos-ethyl	50.45	0.02	0.1	0.27	0.28	3.18
Pyriproxifen	50.79	0.05	0.25	0.41	0.69	5.37
Endosulfan-sulfate	51.27	0.05	0.25	0.51	0.69	4.30
Captan	51.68	0.02	0.1	0.32	0.27	2.57
Folpet	52.28	0.02	0.1	0.28	0.27	3.02
Parathion	53.08	0.05	0.25	0.56	0.66	3.83
Iprodione	55.46	0.02	0.1	0.27	0.25	3.10
Oxyfluorfen	57.02	1.00	5	9.07	12.33	4.86
Procymidone	57.23	0.01	0.05	0.13	0.12	3.30
Buprofezin	57.52	5.00	25	44.76	61.13	4.93
Lindane	58.76	0.01	0.05	0.11	0.12	3.80
Tolclofos-methyl	58.95	0.05	0.25	0.48	0.60	4.56
Fenitrothion	58.96	0.01	0.05	0.13	0.12	3.30
Bromopropylate	59.32	0.01	0.05	0.08	0.12	5.40
Pyrazophos	59.36	0.02	0.1	0.23	0.24	3.80
Diflufenican	59.73	0.05	0.25	0.52	0.59	4.20
Chlorpyrifos-methyl	62.27	0.05	0.25	0.54	0.56	4.00
Endosulfan-beta	64.59	0.05	0.25	0.46	0.54	4.79
Tetradifon	65.16	0.01	0.05	0.10	0.11	4.61
Ethion	65.88	0.01	0.05	0.10	0.11	4.28
Cyfluthrin-beta_(Sum)	66.65	0.02	0.1	0.15	0.21	5.90
Profenophos	67.16	0.02	0.1	0.20	0.21	4.44
Pirimiphos-methyl	70.69	0.05	0.25	0.52	0.50	4.20
Dicofol	71.71	0.05	0.25	0.51	0.49	4.30
Cyhalothrin-lambda	71.74	1.00	5	6.45	9.80	7.00
Fenvalerate_(Sum)	73.21	0.02	0.1	0.13	0.19	6.68
Diazinon	73.87	0.01	0.05	0.12	0.10	3.69
Fenthion	74.03	0.01	0.05	0.09	0.09	4.84
Fluvalinate-tau (Sum)	74.54	0.01	0.05	0.06	0.09	7.02
Chlorpyrifos	78.55	0.05	0.25	0.47	0.45	4.70
Teflubenzuron	82.75	0.05	0.25	0.44	0.42	4.98
Pyrimethanil	84.91	0.02	0.1	0.28	0.17	3.00
Permethrin (Sum)	85.16	0.05	0.25	0.37	0.41	6.10
Parathion-methyl	87.97	0.01	0.05	0.15	0.08	2.70
Cypermethrin_(Sum)	87.97	0.05	0.25	0.42	0.40	5.30
Bifenthrin	95.35	0.05	0.25	0.34	0.37	6.60
Endosulfan-alpha	95.76	0.05	0.25	0.46	0.37	4.74
Fenpropathrin	105.88	0.01	0.05	0.07	0.07	6.00

[a] MRLs for olives established to olive oil production

[b] Assuming 20% of oil yield (5 kg of olives yield 1 kg of olive oil, and all the pesticides are transferred (EC/400/2014).

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Highlights

Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production

- Need for individual processing factors to be addressed in Annex VI of 396/2005
- Processing factors (PF) calculated for 104 pesticides using laboratory-scale model.
- The PF obtained have been related with log K_{ow} values
- An equation to model and estimate PF for olive oil is proposed
- MRL values for pesticides in olive oil can be corrected with the PF obtained