A. Forster, F. Schüll, A. Gahr, R. Schmidt, A. Faltermeier, R. Kugel and S. Laupheimer

Transfer of Hop Pesticide Residues into Beer during Brewing Process

The use of pesticides in hop cultivation raises concerns about the transfer of pesticide residues (PRs) into beer during brewing. This study investigates the transfer rates (TR) of PRs from hops to beer throughout the brewing process. A spiking technique was employed to simulate elevated pesticide concentrations of relevant active ingredients on hops, with its accuracy assessed against originally contaminated samples. A key finding was the significant variability in TR, ranging from 0 % to over 70 %, largely dependent on the solubility of individual actives. Special aspects of a brewing process were evaluated. The TRs were similar compared hopping in the brew house and dry hopping. Steps like fermentation, agitation during dry hopping, centrifugation and filtration have a minimal impact on TRs. These results provide valuable insights into the behaviour of PRs during brewing and contribute to a more comprehensive understanding of their transfer, which is crucial for ensuring food safety in beer production. While not OECD-compliant, the spiking method offers a practical approach for studying transfer behaviour of PRs, particularly for compounds that are challenging to detect in originally contaminated samples.

Descriptors: Pesticide residues, hops, transfer behaviour, spiking, food safety

1 Introduction

In conventional hop production, hops are treated with plant protection products (PPPs) throughout the growing season. General information on the use of PPPs can be found in [1] and annually updated application recommendations including a list of approved PPPs in Germany are published by the Bavarian State Research Center for Agriculture in the so-called “Grünes Heft" [2]. Changes result from new authorisations of a PPP or a regular expiration of authorisation. National authorities may also withdraw the authorisation of PPPs. The most important active ingredients for hop cultivation (excluding herbicides) for the years 2013, 2020 and 2024 are listed in table 1 (see page 162) with the respective maximum residue levels (MRLs) set by the EU commission in accordance with EU Regulation (EC) 396/2005 [3].

In an annual joint monitoring programme, the hop industry tests several hundred hop lots per harvest for marketability to a specified extent. In addition to providing a neutral quality assessment, this monitoring serves to ensure the correct use of authorised PPPs and to check compliance with the respective MRLs [4].

Only a few studies focused on the transfer of PRs of hops during beer production. In [5], for example, the fate of six active ingredients was determined via the dosage of pellets. Despite a high dosage of 600 g/hL in form of pellets conventionally treated with PPPs, no residues were found in the beer. Only the technique of spiking (enriching hop samples with active ingredients of PPPs) allowed the detection of four active ingredients in the beer. Another study [6] monitored the transfer behaviour of four active substances during hot hopping. The transfer rates (TRs) were estimated and were found to be 90 % for Dimethomorph, 62 % for Azoxystrobin and approximately 20 % for Myclobutanil. Quinoxyfen could not be found in the final beer. Additionally, [6] analysed the transfer of PRs during dry hopping at 0 °C. This resulted in TRs of 15 to 25 % for Azoxystrobin and Dimethomorph. Myclobutanil and, again, Quinoxyfen, could not be found anymore. The authors concluded that at high dosages in the hot wort, a subsequent dry hopping could already lead to saturation effect.

Walsh et al. [7] investigated conventionally grown hops at various dosages in a small-scale approach and found only two PRs, Boscalid and Bifenazate, in the final beer. More detailed studies of transfer behaviour can be found in Dušek et al. [8]. Beers were experimentally produced on a very small-scale with 400 ml batches and they tracked the transfer of 58 active ingredients into wort and beer with remarkable ranges from a few to 100 %.

The transfer behaviour of PPPs from hops into beers can be estimated from their solubility in water. Data are available on partition

https://doi.org/10.23763/BrSc24-15forster

Authors

Adrian Forster, Florian Schüll (ORCID ID: 0009-0009-9194-6486), HVG Hopfenverwertungsgenossenschaft e.G., Wolnzach; Andreas Gahr (ORCID ID: 0000-0003-0386-3226), Roland Schmidt, Alois Faltermeier, Hopfenveredlung St. Johann GmbH, Train-St. Johann; Reinhold Kugel, Silvana Laupheimer (ORCID ID: 0009-0009-2620-1541), BarthHaas GmbH & Co. KG, Nürnberg; corresponding authors: f.schuell@hvg-germany.de, Silvana.laupheimer@barthhaas.de

Abbreviations

PR: pesticide residue
PPP: plant protection product
TR: transfer rate
PF: processing factor
MRL: maximum residue level

Table 1 **Active ingredients (in alphabetical order) of plant protection products (PPPs) authorised in Germany in 2013, 2020 and 2024 and their respective MRLs in the EU given in mg/kg. [1)]yes = No MRL required. [2)]Change in residue definition between 2013 and 2020/2024. The MRL describes the sum of Flonicamid including the metabolites TFNA, TFNA-AM and TFNG. [3)]Regular authorisation in 2024 after publication of the "Grünes Heft". [4)]PPP in grace period**

Active Ingredients	Approved in 2013	EU MRL 2013	Approved in 2020	EU MRL 2020	Approved in 2024	EU MRL 2024
Abamectin	+	0,05				
Acequinocyl			+	15	+	20
Ametoctradin			+	100	+	90
Azoxystrobin	+	30	+	30	+	30
Boscalid	+	60	+	80	+	80
Cymoxanil	+	2	+	0,1	+	0,1
Dimethomorph	+	50	+	80	+	80
Dithianon	+	100	+	100	+	100
Fatty Acid Potassium salts					+	yes[1)]
Fosetyl-Al	+	1500	+	2000	+	2000
Flonicamid (sum[2)])	+	2	+	3[2)]	+	3[2)]
Fluopicolide			+	0,7	+	0,15
Fluopyram[3)]					+	60
Folpet (sum)					+	400
Hexythiazox	+	20	+	20	+	3
Imidacloprid	+	10				
Potassium hydrogen carbonate			+	yes	+	yes
Copper	+	1000	+	1000	+	yes
Lambda-Cyhalothrin	+	10	+	10	+	10
Maltodextrin			+	yes	+	yes
Mandipropamid	+	50	+	90	+	90
Metalaxyl-M	+	10			+	15
Metrafenone			+	80	+	80
Milbemectin	+	0,2	+	0,2	+	0,1
Myclobutanil	+	2	+	5		
Pymetrozin	+	15				
Pyraclostrobin	+	15	+	15	+	15
Quinoxyfen	+	2				
Sulfur	+	100	+	yes	+	yes
Spirodiclofen	+	30	+	40		
Spirotetramat (sum)					+	15
Triadimenol	+	10	+	15[4)]		
Trifloxystrobin	+	30	+	40	+	40

coefficients in *n*-octanol-water (K_{ow} or logP values), which include the solubility in octanol and water [9]. Based on this, [10] refers to the low solubility of PRs in water considering the K_{ow} values. However, the assessment of the transfer behaviour of PRs requires extensive preliminary investigations, such as those carried out in [8]. An exclusive consideration of the K_{ow} value may lead to incorrect conclusions.

When assessing the extent to which a PR from a raw material is transferred into a final food product, it is customary to determine and specify a processing factor (PF), which is calculated according to equation 1 [11].

However, the determination of a PF does not consider a very important variable such as the amount of raw material used. A PF is usually determined in the context of safety and risk assessment studies for PRs, but there is a lack of a general regulation and each company submitting a PPP for approval is acting individually. The German Federal Institute for Risk Assessment (BfR) published PFs for some active ingredients, also for hops [12]. However, only guidelines for the application of PFs are available [13] but there are currently no adopted rules for the determination of PF within the EU. PFs only give an indication of the PR to be expected in the beer from the addition of hop. For an accurate calculation of PRs in beer, the dosage rate of hops is crucial. Without this information, a PR in the used raw material cannot be deduced from a detected PR in the beer. Hop dosages can range from 30-50 g hops/pellets per hectolitre (hL) for normal international lager beers to 100 – 300 g/hL for standard pilseners. For craft beers, IPAs or NEIPAs, common hop dosages are up to

3000 g/hL. The wide range of hop quantities in a beer from 30 to 3000 g/hL clearly shows that a PF does not allow a sufficient estimation of PRs in brewing processes.

Alternatively, the determination of a transfer rate (TR) of PRs by considering the hop dosages in beer might be a more appropriate method (Eq. 2). TR expresses the percentage of a PR that passed into the beer from the used raw material divided by the used amount of PR. The latter value is calculated from the hop dosage used [g/hL] multiplied with the residue found in the raw hops or pellets used [mg/kg]. All results of this study are given as the calculated TR based on equation 2. Measured residue levels can be provided upon reasonable request.

$$\text{Processing factor (PF)} = \frac{\text{Residue in food [mg/kg]}}{\text{Residue of the used raw material [mg/kg]}} \quad \text{(Eq. 1)}$$

$$\text{Transfer rate (TR) of a pesticide residue (PR)}[\%] = \frac{\text{PR in beer } [\frac{\text{mg}}{\text{kg}}]}{\text{used amount of PR } [\frac{\text{mg}}{\text{kg}}]} \; x\,100 \quad \text{(Eq. 2)}$$

A major problem in tracking PRs in brewing trials is finding hop samples that contain a sufficient number of active ingredients and a relevant amount of PRs. Former experiments show that usually only low and inhomogeneous PRs on randomly chosen hop lots are available, which are representing the realistic situation of PRs on hop lots, but insufficient for comparable test brews and TR development. Many repetitions would be required to analyse the relevant range of PRs. This may also be a reason for the low data density. Residues in hops are often too small to follow their fate in beer accurately. For this reason, it is being investigated whether the method of 'spiking', i.e. artificially contaminating hops with PRs, leads to reliable TRs that are comparable to those of originally contaminated batches. If it can be shown that spiked batches behave similarly to original batches, all important PRs could be analysed at once. The analytical accuracy also increases with increasing levels of PM residues in the beer. However, spiking of food products or additives with PRs to track their fate during food production is not allowed in the OECD rules [14]. The justification for this prohibition can be illustrated by an example of fruit and berries. Those commodities are washed before being processed into juice or jam; hence, spiked PRs would be at least partially washed off and do not contribute to a residue in the food. This would simulate a low TR or PF. However, spiked hop samples do not have the same effect as they go through the whole brewing process without any prior cleaning or washing.

The aim of this work is the investigation of TRs of relevant PRs. The method of spiking is tested for its suitability to track PRs from hops to beer throughout the brewing process. In addition, filtration and timing of hop addition during beer production, where a reduction of PRs could be expected, will be analysed. The essential parameters of hopping, including dry hopping will be conducted at a 2-hL research brewery with proven good reproducibility [15]. These results may contribute to a more comprehensive understanding of their transfer behaviour, which is crucial for ensuring food safety in beer production.

2 Methods

2.1 Selection of PRs

Based on an evaluation of more than 3000 spray records from the hop trading companies BarthHaas GmbH & Co. KG and HVG e. G., the relevant PPPs for hop cultivation were identified. In addition, it was considered whether PRs are detectable in raw hops or pellets and whether degradation processes of the active ingredient during the brewing process are known. Referring to table 1, it is known that Folpet and the closely related compound Captan are hydrolysed during brewing and therefore not detectable in beer [16]. Furthermore, seven active ingredients were not found in any of the approximately 500 hop samples analysed in 2012. Hence, those were not included in the evaluation of the experiments shown in this study and we end up with 16 active ingredients that we classify as "relevant" (Table 2).

2.2 Original and spiked hop samples

For all brewing trials we used original hop samples and spiked hop samples. The technique of spiking has already been presented in a poster [17] and is briefly described here. The hop dosage for each trial was mixed with an ethanolic solution containing the calculated amounts of the 16 active ingredients. The active ingredients Quinoxyfen and Flonicamid were weighed separately into an ethanolic solution and added to the spiking solution in a dilution step. This was necessary because the very small quantities of these two active ingredients required could not be weighed in directly. Metalaxyl-M was first pre-dissolved in ethyl acetate due to its poor solubility in ethanol. The correspondingly diluted ethanolic solution was added to the calculated hop dosage in a ½ or 1 kg can, usually used for hop extract. To ensure complete evaporation of the solvent, the cans were left open for 24 hours before sealing.

Table 2 **Analysed residue levels of 16 active ingredients (in alphabetical order) from PPPs classified as relevant. The mean and maximum values from 500 monitored batches from 2012 are given in mg/kg**

Active ingredient	mean	max
Azoxystrobin	0.7	5.8
Boscalid	7.6	29.0
Dimethomorph	2.0	23.0
Flonicamid	0.1	0.3
Fluopicolide	–	–
Hexythiazox	0.4	3.9
Imidacloprid	0.1	0.1
Mandipropamid	3.7	18.0
Metalaxyl-M	3.1	5.1
Myclobutanil	0.3	2.3
Pymetrozin	0.1	0.1
Pyraclostrobin	1.8	5.6
Quinoxyfen	0.2	0.6
Spirodiclofen	2.0	8.1
Triadimenol	0.2	1.8
Trifloxystrobin	1.1	9.1

Dušek et al. [8] also used the method of spiking with a standardised amount of 15 mg/kg per active ingredient without taking into account the permitted MRLs. However, as these vary over a wide range, e.g. from 1 to 100 mg/kg, it seemed advisable to use the legally defined MRLs. Our spiked quantities varied between 0.5 and 1.5 times of the MRL, corresponding to the measurement uncertainty of 50 % around the measured value, which is taken into account in the EU by [18]. A quantity of 1.5 times of the MRL simulates a high contamination and could lead to higher values in beers with lower standard deviation of the analysis values. In the brewing trials described in section 3.6, a spiking quantity of 1.0 times of the MRL was used. In a first step, two pellets with detectable background contamination were selected. Table 3 shows the PRs in the original pellets (O) and the resulting amounts in the spiked pellets (O+0.5*MRL and O+1.5*MRL). Six of the 16 selected active ingredients were not detected in any of the original pellets.

Table 3 **Analysed PRs in mg/kg in hop samples for each used pellet. O = Original pellets; O+0,5*MRL and O+1,5*MRL = Original pellets spiked with 0.5x or 1.5x of MRL, n.d. = not detectable**

Active ingredient	Pellet 1			Pellet 2		
	O	O+0.5*MRL	O+1.5* MRL	O	O+0.5*MRL	O+1.5*MRL
Azoxystrobin	0.1	15.1	45.1	0.4	15.4	45.4
Boscalid	9.1	39.1	99.1	10.6	40.6	100.6
Dimethomorph	5.1	30.1	80.1	8.6	33.6	83.6
Flonicamid	n.d.	1.0	3.0	0.09	1.1	3.1
Fluopicolide	n.d.	0.0	0.0	n.d.	0.0	0.0
Hexythiazox	0.1	10.1	30.1	3.9	13.9	33.9
Imidacloprid	n.d.	5.0	15.0	n.d.	5.0	15.0
Mandipropamid	5.8	30.8	80.8	0.4	25.4	75.4
Metalaxyl-M	n.d.	5.0	15.0	n.d.	5.0	15.0
Myclobutanil	n.d.	1.0	3.0	n.d.	1.04	3.0
Pymetrozin	n.d.	7.5	22.5	n.d.	7.5	22.5
Pyraclostrobin	1.6	6.6	16.6	2.2	7.2	17.2
Quinoxyfen	0.1	0.3	0.8	n.d.	0.3	0.8
Spirodiclofen	n.d.	20.0	60.0	8.1	28.1	68.1
Triadimenol	n.d.	5.0	15.0	n.d.	5.0	15.0
Trifloxystrobin	0.1	15.1	45.1	0.2	15.2	45.2

2.3 Beer production

All brewing trials were carried out at the 2-hL research brewery in St. Johann, Germany. This facility has proven in earlier studies comparability between repetitions and provide much more reliable results than laboratory approaches on a millilitre or litre scale. A standard brewing process for bottom-fermented beers of 11.0 °Plato and Pilsner malt as the raw material were used. Infusion mashing was followed by solid-liquid separation in the lauter tun. Wort boiling with an internal boiler lasted 70 minutes. For hot hopping, the appropriate pellets were dosed at the beginning (BB), in the mid (MB) and at the end (EB) of the wort boiling, as well as in combinations of those. Dry hopped beers were firstly hopped with 8 g/hL of alpha acids in form of CO_2 extract from hops without any detectable PRs, to prevent wort foaming and infection with other microbial contaminants of beer. To avoid losses during hopping, the solution and the can were dosed directly to the brew kettle. For dry hopping the pellets were prepared in a permeable bag made of a neutral mesh fabric and were applied directly together with the bag in the maturation tank.

2.3.1 Dry hopping

To determine the TR of PR from spiked hop pellets in beer, many test batches were required. To optimise the process and produce several test series from a single brew, a method has been developed in which the green beer is divided into 10-litre kegs after the main fermentation has been completed. This allows precise and reproducible hop addition. This approach creates a consistent starting point for each small 10-litre batches, ensuring reliable analysis of PR transfer. After wort boiling, the brew was divided as follows:

Portion 1:

I. Fermentation at 8 °C for 8 days

II. Transfer to 120 L tank and mature at 14 °C for 8 days; hop pellet dosage (450 – 500 g/hL). The warmer fermentation temperature and the alcohol content favour the transfer of PR.

III. Storage at 0 °C for 3 weeks

IV. Kieselguhr filtration and bottling

Portion 2:

Proceed as for the portion 1 until primary fermentation and then divided into 8 kegs of 10 litres volume each. A coarse filtration was performed beforehand in order to achieve a good homogeneity of the hops in the cask. This prevented the absorption of pesticide residues on trub and yeast and ensured undisturbed transfer to the beer. To test the suitability of spiking PRs on hop samples, the following treatments were obtained:

I. Mock keg: no further hop addition

II. 2 kegs with hop dosage of 50 g per keg (= 500 g/hL) original pellets (O)

III 2 kegs with hop dosage of 50 g pellets, each spiked with 0.5*MRL

IV. 2 kegs of hop dosage of 50 g pellets spiked with 1.5*MRL

All were followed by a run-through as for the first part of the brew with maturation at 14 °C for 1 week, a cold storage for 2 weeks, and bottling.

The respective pellet samples were weighed into permeable bags made of a neutral mesh fabric and placed in both the tanks and the kegs. One variable affecting dissolution behaviour is movement in a tank or keg. It can be assumed that mixing or movement in a tank promotes mass transfer. The aim of dry hopping is to extract hop aroma. In addition to beer-soluble compounds such as esters, ketones and alcohols, hops contain a large number of poorly soluble, non-polar compounds such as mono- and sesquiterpenes. Various techniques are used in breweries to facilitate the transfer of these substances [1]. However, increased extraction of desirable hop compounds should also have an effect on the transfer behaviour of PRs.

The trials in the tank were only hopped with the original pellets spiked with 0.5*MRL (O+0.5*MRL). The keg trials were hopped with all 3 pellet types (O, O+0.5*MRL, and O+1.5*MRL). To simulate better mixing, CO_2 was blown into the tanks via the cone once a day to mix the green beer. Kegs were rotated once a day to agitate and circulate the beers. The influence of beer clarification on the fate of PRs was also of interest. To this end, beers were analysed for PRs before and after centrifugation. In addition, tank samples were compared before and after standard filtration with kieselgur. A total of 58 samples were analysed in the dry hopping tests, comparing the original pellets and the spiked pellets. The focus was on whether spiking was acceptable, whether there was a difference between keg and tank, and the effects of movement, centrifugation and filtration.

2.3.2 Wort hopping

Pellet 2 with O+0.5* MRL was used with the following hop dosages. The different time points of hopping ranged from zero (EB) to 70 minutes (BB) and provide an indication of possible thermal decomposition. Higher TR in the late additions would be an indication of this. The wort and beers were analysed to determine the possible depletion of PRs during beer production. Three beers were subjected to kieselguhr filtration and subsequently membrane filtered.

Brew	Hop dosage
1	50 g/hL pellets at the begin of boiling (BB) plus 200 g/hL at the end of boiling (EB), giving a total of 250 g/hL
2	200 g/hL pellets at BB
3	200 g/hL pellets at mid of boiling (MB)
4	200 g/hL pellets at EB
5	50 g/hL pellets at BB, 200 g/hL each at MB and EB, giving a total of 450 g/hL

2.3.3 Combined hopping

To test for a saturation effect, a series of combined hot and dry hopping were carried out. Pellet 2 was used in both concentrations, O+0.5*MRL and O+1*MRL. The amounts were set at 100 g/hL for BB and 200 g/hL each for MB and EB, giving a total of 500 g/hL. The two brews were divided into 6 kegs after primary fermentation. In addition to the batch without dry hopping, both brews were hopped at 500 g/hL with original pellets, O+0.5*MRL and O+1.0*MRL. Additionally, we were also interested in the filtration behaviour of four beers. In total, we analysed ten beers. As the previous trials shown in this study provided a good and reliable reproducibility, the beers from the combined hopping in the kegs could be analysed in one sample for analysis.

2.3.4 Additional tests in 2020

The list of currently authorised PPPs changed between 2013 and 2020 and 2024 (Table 1). Ametoctradin, Fluopyram, Metrafenone, Flonicamid(sum), and Spirotetramat(sum) were assessed as newly approved active ingredients. On the other hand, Abamectin, Imidacloprid, Myclobutanil, Pymetrozine, Quinoxyfen, Spirodiclofen, and Triadimenol were no longer authorised in 2020. However, it is useful to investigate PPPs that are no longer authorised, as pellets and extracts from previous years may still be in use. In addition to the new active ingredients, 6 previously analysed moderately to highly soluble actives were retested to verify the 2013 results. Due to the extensive series from 2013, it was possible to streamline the analytical system considerably. All active ingredients were spiked to their MRL value in one pellet. Two brews were sufficient to determine the TRs. Hopping was carried out at the end of boiling with 250 g/hL or 500 g/hL of the spiked pellets. Afterwards, the brews were split into kegs and again hopped with 250 or 500 g/hL followed by kieselguhr filtration at the end of maturation.

2.4 Analysis of active substances

The performed analytical methods are based on the methods S (special analyses) of the German Research Foundation (DFG). The methods used refer to the appendix in ASU § 64 LFGB, L00.00-113 for the liquid/liquid method [19] and L00.00-34:20019-09 [20] for gas chromatography. Quantification was performed by the addition of commercially available standards (= active ingredients). All analyses in hops and beers were carried out in the laboratory of Eurofins Sofia GmbH, Berlin, according to [20]. The limits of quantification of the active ingredients in hops were mostly 0.1 mg/kg, in some cases even lower at 0.05 mg/kg, and in beer 0.001 mg/kg. The standard deviations of the analytical values were between 0.2 and 0.6 mg/kg for hops and between 0.003 and 0.008 mg/kg for beer [20], depending on the absolute values. The TR is calculated from the ratio of the analysed values of pesticides in beer to the dosed amounts in hops. Particularly for low values in beer, there are inevitably large variations in the order of < 20 % to > 40 %. Two examples illustrate this:

Low level in beer

1. Determination: $$\frac{0.002\ \frac{mg}{kg}(measured\ value)}{0.009\ \frac{mg}{kg}(dosage\ rate)} x\ 100 = 22\ \%\ TR$$

2. Determination: $$\frac{0.004\ \frac{mg}{kg}(measured\ value)}{0.009\ \frac{mg}{kg}(dosage\ rate)}\ x\ 100 = 44\ \%\ TR$$

Table 4 **Comparison of TRs in % from 16 relevant active ingredients from two independent repetitions in kegs. Active ingredients could be classified by their solubility according to the calculated TR: not soluble – TR = 0%; poorly soluble – TR ≤ 10 %; medium soluble – TR ≤ 50 % and good soluble – TR > 50 %. The four categories are differently coloured in blue (or grey) scales**

Active Ingredient	TR in Kegs		Solubility
	Serie 1	Serie 2	
Fluopicolide	0	0	Not soluble TR = 0 %
Pyraclostrobin	0	0	
Quinoxyfen	0	0	
Hexythiazox	8	1	Poorly soluble TR ≤ 10 %
Spirodiclofen	6	1	
Trifloxystrobin	7	4	
Pymetrozin	5	10	
Dimethomorph	28	24	Medium soluble TR ≤ 50 %
Mandipropamid	26	17	
Azoxystrobin	36	23	
Boscalid	35	23	
Myclobutanil	33	18	
Triadimenol	45	44	
Flonicamid	92	72	Good soluble TR > 50 %
Imidacloprid	70	62	
Metalaxyl-M	75	71	
Ø n = 13	**35.8**	**26.7**	

Table 5 **TRs of poorly, medium and good soluble active ingredients (Table 4) in %. Beers were hopped in kegs with original hop samples (O), O+0,5*MRL or O+1,5*MRL. Results are given as the average from four independent repetitions. x = PR was not detectable in the original pellet and a TR could not be calculated. # = Active ingredients that were detectable in the original hop samples without spiking and their corresponding average. Those allowed a comparison with spiked hop samples**

Active ingredient	O	O+0,5*MRL	O+1,5*MRL
Hexythiazox	x	11	7
Spirodiclofen#	4	9	5
Trifloxystrobin	x	8	6
Pymetrozin	x	5	6
Dimethomorph#	25	32	29
Mandipropamid#	19	27	29
Azoxystrobin#	50	41	37
Boscalid#	36	39	32
Myclobutanil	x	35	32
Triadimenol	x	46	46
Flonicamid	x	92	93
Imidacloprid	–	66	76
Metalaxyl-M	–	74	77
Ø n = 5#	**26.8**	**29.6**	**26.4**
Ø n = 13		**37.3**	**36.5**

High level in beer

1. Determination: $$\frac{0.13\,\frac{mg}{kg}\,(measured\ value)}{0.61\,\frac{mg}{kg}\,(dosage\ rate)}\ x\ 100 = 21\,\%\,TR$$

2. Determination: $$\frac{0.19\,\frac{mg}{kg}\,(measured\ value)}{0.61\,\frac{mg}{kg}\,(dosage\ rate)}\ x\ 100 = 31\,\%\,TR$$

The analysis error of the dosage rate is not taken into account here. The errors of the TR must therefore be considered when making comparisons. In our study, all tests were performed in duplicates or in some cases in quadruplicate.

3 Results

3.1 PRs identification after dry hopping in kegs

In two independent series with a double approach, three beers each were brewed, hopped with 500 g/hL of O, O+0.5*MRL, and O+1.5*MRL, respectively, and initially analysed only in hazy state. In the first step, the average value of each series in kegs (Table 4) are shown. No significant differences were observed between the two pellet types. The average TRs from both series were 35.8 % and 26.7 %, respectively. Based on the calculated TRs, the detected active ingredients were grouped into four solubility categories. Fluopicolide, Pyraclostrobin and Quinoxyfen cause no residues above the detection limit in kegs at a dosage of 500 g/hL. They appear to be not soluble (TR = 0 %). Another four active ingredients showed poor solubility with a TR of < 10 %. With a TR between 10 % and 50 %, six active ingredients could be classified as moderately soluble. Three other active substances had TRs significantly above 50 % and were classified as good soluble. The large differences in TRs, ranging from 0 to 75 %, are noteworthy. The insoluble active ingredients will be not considered in further experiments.

3.2 Comparison of original and spiked hops by dry hopping in kegs

The next step was to investigate potential differences in the transfer behaviour of PRs of original hop samples compared to spiked pellets. For this purpose, again kegs were used with the three different spiked concentrations O, O+0.5*MRL, and O+1.5*MRL. Table 5 shows the TRs of four independently brewed beers. Five active ingredients were also detectable in the original hop samples, namely Spirodiclofen, Dimethomorph, Mandipropamid, Azoxystrobin and Boscalid. The overall average of those five was 26.8 % (O), 29.6 % (O+0.5*MRL) and 26.4 % (O+1.5*MRL). These results lead to the conclusion that the model of spiking hop samples may lead to results in beer that are comparable to original hops samples. All 13 compounds also showed a well-matched TR at O+0.5*MRL of 37.3 % and O+1.5*MRL of 36.5 %. Spiking at half or even one and a half times the maximum dose did not indicate a deviation from a linear trend. Spiking hops with active ingredients should be considered as an useful method for determining the transfer behaviour of PRs into beer during brewing.

3.3 Comparison of TRs in kegs and tanks with dry hopping

Table 6 Comparison of TRs in % of the relevant active ingredients (Table 4) from beers brewed in kegs or tanks

Active ingredient	Keg	Tank
Hexythiazox	4	2
Spirodiclofen	3	3
Trifloxystrobin	5	2
Pymetrozin	8	5
Dimethomorph	24	11
Mandipropamid	21	10
Azoxystrobin	28	15
Boscalid	28	13
Myclobutanil	25	15
Triadimenol	44	25
Flonicamid	81	55
Imidacloprid	66	35
Metalaxyl-M	71	30
Ø n = 13	**31**	**17**

Table 7 TRs in % of relevant active ingredients from beers in kegs hopped with original hop samples (O), O+0,5*MRL or O+1,5*MRL that were subsequently rotated for mixing the content or not

Active ingredient	O		O+0,5*MRL		O+1,5*MRL	
	not mixed	mixed	not mixed	mixed	not mixed	mixed
Hexythiazox	–	–	–	–	1	1
Spirodiclofen	–	–	–	–	–	0,3
Trifloxystrobin	–	–	3	4	3	4
Pymetrozin	–	–	20	17	14	18
Dimethomorph	27	31	19	17	21	27
Mandipropamid	22	22	13	10	14	18
Azoxystrobin	–	–	23	19	22	28
Boscalid	25	32	18	15	20	26
Myclobutanil	–	–	-	27	16	20
Triadimenol	–	–	44	35	42	51
Flonicamid	–	–	74	83	74	56
Imidacloprid	–	–	68	53	64	61
Metalaxyl-M			76	53	79	75
Ø n = 3	**25**	**28**	**17**	**14**	**18**	**24**
Ø n = 10			**35**	**27**	**38**	**38**

The mean values of the TRs in kegs are compared with the TRs in tanks in table 6. The TRs in kegs are almost twice as high as in tanks. We assume that (I) the brewing volume can have an effect on the TRs as they are higher in smaller units. (II) The TRs measured in kegs might reflect a worst-case scenario and (III) dry hopping in the kegs may lead to reliable results with a tolerable effort. The TRs on a small scale are likely to be higher than those of a production operation.

3.4 Influence of technical factors during brewing on TRs

To elucidate the effect of different standard techniques during the brewing process, we conducted further trials in kegs and tanks to clarify the following factors:

(I) What is the influence of the mixing of the tank contents?

(II) What is the influence of kieselguhr filtration?

(III) What is the effect of centrifuging the beer?

Firstly, we analysed the TRs of beers that were mixed or not mixed. We used again all three concentrations O, O+0.5*MRL, and O+1.5*MRL. Table 7 shows the TRs in kegs, divided into three differently hopped keg contents subsequently mixed daily or not mixed. The three active ingredients present in the original hop samples have comparable TRs compared to the spiked hop samples. The average of those three actives was 25 and 28 % in the original pellet, 17 and 14 % in the O+0.5*MRL and 18 and 24 % in the O+1.5*MRL. This leads once more to the conclusion that spiking hop samples with active ingredients is a reasonable model. However, mixing the casks did not result in an increase in TR. Overall, the TRs of these beers are comparable to the observations discussed in 3.2 (see Table 5).

Table 8 TRs in % of beers in tanks and hopped with O+0,5*MRL that were moved with CO_2 or not and either subsequently filtered using kieselgur or not filtered

Active ingredient	no movement		movement	
	before filtration	after filtration	before filtration	after filtration
Trifloxystrobin	3	1	1	0
Pymetrozin	0	35	23	20
Dimethomorph	13	10	9	5
Mandipropamid	7	6	5	4
Azoxystrobin	14	11	9	5
Boscalid	12	10	7	4
Triadimenol	27	23	19	8
Flomicamid	74	56	74	56
Imidacloprid	66	53	41	33
Metalaxyl-M	70	53	33	25
Ø n = 10	**29**	**26**	**22**	**16**

Secondly, we tested the influence of kieselguhr filtration in combination with mixing as a further technological factor. The results are presented in table 8 and show the TRs of beers in tanks hopped with O+0.5*MRL. Only 10 of the 13 active ingredients from table 5 were found in the tank samples. Hexythiazox, Spirodiclofen and Myclobutanil were not found in any of the tank samples. Only Trifloxystrobin and Pymetrozine from the poorly soluble group were detected in the tank samples. The overall average of the 10 detectable active ingredients showed little difference and allowed two conclusions to be drawn: (I) Agitation by CO_2 injection does not lead to higher active ingredient transfer and (II) filtration tended to have a lower TR in the number of active ingredients. The remaining compounds tended to have a lower TR.

Furthermore, keg beers were analysed to determine the influence

Table 9 TRs in % of beers in kegs in two independent series comparing before and after centrifugation (1st Series) and an additional kieselgur filtration (2nd Series)

Active ingredient	1st Series		2nd Series		
	before centrifugation	after centrifugation	before centrifugation	after centrifugation	after filtration
Spirodiclofen	6	0	4	0	0
Trifloxystrobin	9	6	6	2	0
Pymetrozin	4	4	5	6	6
Dimethomorph	29	30	28	27	25
Mandipropamid	28	22	21	17	15
Azoxystrobin	46	41	34	29	26
Boscalid	38	34	32	27	96
Myclobutanil	34	36	32	29	29
Triadimenol	47	51	45	44	44
Flonicamid	100	100	89	99	99
Imidacloprid	64	75	73	71	71
Metalaxyl-M	76	74	76	70	65
Ø n = 12	**40**	**39**	**37**	**35**	**34**

Table 10 TRs in % of 13 active ingredients detected in the wort after different timings of hot hopping solo or in combination: BB = Begin of boiling; MB = Mid of boiling and EB = End of boiling. Data represent five independent repetitions and the average (Ø) of 10 (#) active ingredients that were soluble (≥ 10 %) in the wort as well as the average per active ingredient

Active ingredient	BB 200 g/hL	MB 200 g/hL	EB 200 g/hL	BB/EB 250 g/hL	BB/MB/EB 450 g/hL	Ø
Hexythiazox	0	0	0	3	0	1
Spirodiclofen	0	0	0	1	0	< 1
Trifloxystrobin	5	4	5	8	3	5
Pymetrozin#	30	42	69	43	44	46
Dimethomorph#	76	56	59	60	35	57
Mandipropamid#	49	37	37	43	18	37
Azoxystrobin#	77	54	60	51	35	55
Boscalid#	77	60	71	46	37	58
Myclobutanil#	50	40	40	54	18	40
Triadimenol#	76	60	66	77	48	64
Flonicamid#	100	100	100	62	85	89
Imidacloprid#	89	82	96	77	78	84
Metalaxyl-M#	76	59	69	77	59	68
Ø n = 10#	**70**	**59**	**67**	**59**	**46**	

of centrifugation and kieselguhr filtration. Table 9 shows the TRs before and after centrifugation and the TRs after filtration.

From the group of poorly soluble active ingredients, centrifugation resulted in a reduction of residues of Spirodiclofen. Similar to the results in table 8, a reduction in TR was only observed for the poorly soluble PRs. The moderately to highly soluble PRs remained in solution after centrifugation and filtration.

3.5 Determinations of TRs during wort hopping

Beside dry hopping also wort hopping was tested to elucidate potential PR transfers. Wort hopping was carried out only with pellets 2 (see Table 3), spiked with O+0.5*MRL. The hop dosage was done at three different times or in combination of those, at the beginning of boiling (BB), mid of boiling (MB) and at the end of boiling (EB). Table 10 summarises the TRs of the different treatments and leads to the conclusion that the boiling time has no effect on the TR of moderately to highly soluble PRs and Pymetrozine. The TRs do not differ by considering the large analytical variations mentioned in 2.4. We hypothesised, that (I) the solubility of the active ingredients is not influenced by short boiling times and (II) there is no evidence of thermal decomposition of the active ingredients, as there was no difference in TRs of different boiling times. These 10 PR were also classified as relatively stable in [8].

Only in one wort trial, the combination BB/EB, small amounts of Hexythiazox and Spirodiclofen were detected. The TR of Trifloxystrobin is between 3 % and 8 % in the 5 wort treatments and thus in a comparable range to that of dry hopping. The 7 moderately soluble PRs tend to be more soluble in wort with 52 % on average (see Table 10) than with dry hopping (30 %, see Table 4). The three good soluble PRs show TRs of 80 % on average in hot hopped beers, while 74 % on average in dry hopped beers. The most intensively hopped wort (BB/MB/EB with 450 g/hL) has a lower TR compared to the other four wort types, which may indicate that the solubility of PRs decreases slightly at very high amounts. The TRs of the beers made from the wort in table 10 were almost identical to those of the wort, so they are not listed. There was no decrease due to fermentation and storage. Some beers were additionally treated with membranes (1.2 µm and 0.45 µm) after kieselguhr filtration. None of those additional techniques resulted in a decrease on the TRs.

3.6 Influence on TRs by combining wort and dry hopping

To understand the effect of combined hopping (wort and dry hopping), we prepared two brews that firstly were hopped with 100 g/hL at the beginning of boiling and secondly hopped with 200 g/hL at the mid and end of the boiling. One brew was subsequently hopped with O+0.5*MRL and the other brew with O+1*MRL. Afterwards the wort was separated in two batches for further dry hopping. One tank was dry hopped with 500g/hL, and the other tank was main fermented, coarsely filtered and divided into 6 kegs each. Here, for dry hopping the three different concentrations of spiked pellets (O, O+0.5*MRL and O+1*MRL) were used. Again, the two repetitions of each treatment show comparable results and therefore are presented in table 11 as averages of both. This table summarises the mean values for the 8 moderately and poorly soluble compounds. Some of the not soluble or poorly soluble active ingredients were not detectable, namely Fluopicolide, Pyraclostrobin, Quinoxyfen, Hexythiazox, Pymetrozin and Spirodiclofen. The three good soluble actives Flonicamid, Imidacloprid and Metalaxyl-M were present in all treatments with comparably high TRs of over 60 %. Table 11 summarised the mean values for the 8 moderately and poorly soluble compounds, namely Trifloxystrobin, Pymetrozin, Dimethomorph, Mandipropamid, Azoxystrobin, Boscalid, Myclobutanil, and Triadi-

menol. We conclude that the values in the wort correspond to those of the not dry hopped beers. Also, fermentation, type of storage and filtration caused only slight decreases as already observed in the other brews. Again, keg beers hopped with O+0.5*MRL seem to have slightly higher TRs compared to tank beers. The TRs of the keg beers hopped with O+1*MRL are slightly lower than those kegs with O+0.5*MRL, indicating a moderate saturation effect.

Table 11 **Mean of TRs in % (n=2) of the wort and the 5 beers differently hopped with 500 g/hL of O+0,5*MRL or O+1*MRL. The mean given here shows the 8 active ingredients that were moderately or poorly soluble, namely Trifloxystrobin, Pymetrozin, Dimethomorph, Mandipropamid, Azoxystrobin, Boscalid, Myclobutanil, and Triadimenol**

	Wort only	Tank not dry hopped	Tank + 0.5*MRL	Keg + O	Keg + 0.5*MRL	Keg + 1*MRL
O+0.5*MRL	51	53	34	43	51	40
O+1*MRL	56	54	37	46	50	32

Additionally, the TRs of spiked beers decrease with the amount of added PRs, indicating a non-linear solubility, at least in the case of combined wort and dry hopping. A high level of TRs in the wort may therefore make it more difficult to dissolve the active ingredients by dry hopping.

3.7 Additional trials in 2020

The trials conducted so far were based on hop samples from 2013. Due to the changes of the PPPs registration situation, a second harvest from 2020 was chosen to include newly registered active ingredients in the study. On the other hand, it serves to validate the 2013 results using 6 active ingredients that were approved in both, 2013 and 2020 (Table 12). The actives Abamectin, Imidacloprid, Myclobutanil, Pymetrozine, Quinoxyfen, Spirodiclofen and Triadimenol were no longer authorised in 2020. Pellets from conventional cultivation were used and analysed for the relevant active ingredients and then spiked onto the 1*MRL (Table 12). Ametoctradin, Metrafenone and Spirotetramat were newly authorised. Spirotetramat is defined as the sum of Spirotetramat and Spirotetramat-enol, as listed in table 12. In contrast to 2013, the metabolites TFNG, TFNA and TFNA-AM are included in the residue definition of Flonicamid regulation (EC) 396/2005 of 2020 and are referred to as Flonicamid(sum). Fluoypyram was authorised as an emergency use in 2020 in accordance with Art. 53 of regulation (EC) 1107/2009.

Two batches were brewed and hopped with 250 g/hL and 500 g/hL just before boiling and split after primary fermentation. One part was additionally hopped with 250 g/hL and the other with 500 g/hL. The TR of the beers hopped at 250 and 500 g/hL were similar, hence only the average is given in table 13. The evaluation of the TRs from the pellets to the beer results in a categorisation for Spirotetramat and Flonicamid in “good” (TR over 50 %), for Fluopyram and Flonicamid(sum) in “medium” (TR between 20 and 50 %) and for Ametoctradin and Metrafenone in “poor” (TR below 10 %). Repetition of the analyses of six active ingredients from 2014 resulted in TRs as shown in table 14 (see page 170). The results are comparable with those from 2013. Flonicamid could not be analysed with the metabolites in 2013 and is therefore classified as having 'good' solubility. This is comparable to the observation of Flonicamid including metabolites by Dixius et al [21]. Only for Azoxystrobin is the solubility categorisation changed from medium to good.

The results of a study from the Federal Office of Consumer Protection and Food Safety (BVL) [22] are also worth mentioning in the context of our observations. The BVL analysed beers for pesticide residues. Boscalid was found in 5 and Mandipropamid in 8 of 120 samples in amounts up to max. 0.02 mg/l. The general conclusion is as follows: “Beer contains no or just very low levels of pesticide residues. One third of the samples contained mainly low levels of pesticide residues authorised for barley and hops. In terms of consumer risk, all findings were classified as harmless.”

Table 12 **Active ingredients (in alphabetical order) found in hop samples from harvest 2020 and their respective MRLs in the EU given in mg/kg. The analysed PR in original samples (O) and pellets spiked with O+1*MRL are given also in mg/kg. + = authorised in Germany in 2020. n.d. = not detected. Spirotetramat residues are defined as the sum of Spirotetramat and Spirotetramat-enol. * = Six active ingredients that were compared to results of 2013**

Active ingredient	Approved in 2020	EU MRL 2020	Pellet	
			O	O+1*MRL
Ametoctradin	+	100	7.9	92.1
Azoxystrobin*		30	0.5	29.5
Boscalid*		80	5.4	74.6
Dimethomorph*		80	3.4	76.6
Flonicamid*		3	n.d.	1
Fluopyram	+	50	n.d.	50
Mandipropamid*		90	18.3	71.7
Metrafenone	+	80	24.8	55.2
Myclobutanil*		2	0.3	1.7
Spirotetramat	+	15	0.04	3
Spirotetramat-enol			0.02	1.9

Table 13 **TRs in % of 2020 newly analysed active ingredients and their classification into groups based on TR and solubility**

Active ingredient	TR at late hopping	TR at late and dry hopping	Solubility
Ametoctradin	5	2	Poorly soluble TR ≤ 10 %
Metrafenone	4	2	
Fluopyram	50	43	Medium soluble TR ≤ 50 %
Flonicamid (sum)	36	35	
Flonicamid	100	80	Good soluble TR > 50 %
Spirotetramat (sum)	88	80	

Table 14 Comparison of TRs in % of six active ingredients found in 2013 and 2020 and their classification into groups based on TR and solubility

Active ingredient	2013 TR at late hopping	2013 TR only dry hopping	Solubility	2020 TR at late hopping	2020 TR at late + dry hopping	Solubility
Boscalid	42	29	Medium soluble TR ≤ 50 %	47	36	Medium soluble TR ≤ 50 %
Dimethomorph	44	26		65	43	
Mandipropamid	19	22		34	24	
Myclobutanil	37	26		–	44	
Azoxystrobin	42	30	**Medium soluble TR ≤ 50 %**	79	67	Good soluble TR > 50 %
Flonicamid solo	80	82	Good soluble TR > 50%	100	80	

4 Summary

Reliable data about the transfer behaviour of hop pesticides into beer during brewing are rarely available. They do not necessarily cover all relevant active ingredients, nor do they cover all aspects of beer production. The aim of this work is to gain a more systematic knowledge on the transfer of pesticide residues (PRs) from hops into beer.

An overview of the plant protection products (PPPs) currently allowed and used in hop cultivation in Germany led to the selection of 20 pesticides (16 from 2013, 4 from 2020) in total. A major problem by tracking PR transfers is the availability of raw hop material that contains residues of all relevant active ingredients for brewing trials. Some may not be present at all in some years, or only in very small quantities. Therefore, the technique of spiking hop samples with PRs has been extensively tested. Although spiking of samples is not permitted under OECD rules for registration procedures, it is desirable to obtain a better database using this technique for hops. The following results were obtained from a total of 78 tests on 98 wort and beer samples:

- Hop samples were spiked with 0.5 and 1.5 times of the approved EU MRL (Maximum residue level). Solubility was tested in kegs during dry hopping, as we speculate that this favours good transfer. There was no difference in the transfer rates (TRs) of PRs between the original samples and the spiked samples. This suggests that spiking hop samples with active ingredients is an effective method for obtaining comprehensive and reliable results. Spiking hop samples should be preferable because it eliminates the time and cost consuming search for suitably contaminated samples. Additionally, a higher amount of PR on the hop samples results in higher detectable residues in beer and therefore, leads to more reproducible results.
- The 21 relevant and analysed active ingredients can be divided into four groups according to their solubility or TR. Three are not soluble, six are poorly soluble, eight are medium soluble and four actives are good soluble. With dry hopping, the average TR of medium to good soluble PRs is around 20 % in the tank and around 40 % in the keg, probably due to the more intensive mass transfer in the 10-litre keg.
- Hopping in hot wort in the brewhouse resulted in only slightly higher TR than dry hopping. No correlation to the time of addition could be observed at a dose of 200 g/hL. The average TR of medium to highly soluble PRs was between 40 and 60 %.
- No effect of boiling time on the TR of moderately to good soluble substances was observed. A significant thermal decomposition of these compounds can therefore be excluded.
- A particularly intensive hop addition of 500 g/hL into the hot wort and a further 500 g/hL during dry hopping resulted in an average TR of 54 % in the wort, 36 % in the tank of the dry hopped beer and 42 % in the kegs. With very high dosages already in the wort, only about 27 % could be dissolved by dry hopping, indicating a moderate saturation effect.
- The pesticides dissolved in the wort did not decrease significantly during fermentation and storage.
- Only the three poorly soluble compounds can be reduced by centrifugation or filtration. The more soluble active ingredients do not or only slightly decrease when trub particles are removed.

The limit of quantification of the active ingredients in beer, which has now been lowered to 0.001 mg/kg, makes it likely that corresponding analysis values can also be detected in well hopped market beers. This follows the logic of residues in hops and their solubility or TR in the brewing process. The dynamic in PPP approvals or expirations requires follow-up monitoring, and newly approved active ingredients need to be also considered in future studies.

Conflict of interest

The authors declare there are no conflicts of interest.

5 References

1. Biendl, M.; Engelhard, B.; Forster, A.; Gahr, A.; Lutz, A.; Mitter, W.; Schmidt, R. and Schönberger, C.: Hops: Their Cultivation, Composition and Usage, Fachverlag Hans Carl, 2014.
2. Bavarian State Research Center for Agriculture (LfL): Grünes Heft, https://www.lfl.bayern.de/ipz/hopfen/022297/index.php, accessed: 04.10.2024, 2013, 2020, 2024.
3. European Commission: Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin, 2005.
4. Biendl, M.; Brunner, M.; Hörmannsberger, L. and Schmidt, R.: Three basic steps for best quality hops – made in Germany; Hopfen-Rundschau International, **13** (2012), pp. 8-18.
5. Schmidt, R.; Faltermeier, A.; Gehrig, M.; Fuchsbichler, G. and Gahr, A.: Fate of pesticide residues of hops during the brewing process,

Poster at the European Brewing Congress, 2007.
6. Kippenberger, M.; Hanke, S.; Biendl, M.; Stettner, G. and Lagemann, A.: Transfer of Nitrate and various Pesticides into Beer during Dry Hopping, BrewingScience – Monatsschrift für Brauwissenschaft, **67** (2014), no. 1/2, pp. 1-9.
7. Walsh, D.; O´Neal, S.; George, A.; Groenendale, D.; Henderson, R.; Groenendale, G. and Hengel, M.: Evaluation of Pesticide Residues from Conventional, Organic and Nontreated Hops on Conventionally Hopped, Late-hopped, and Wet-Hopped Beers, Journal of the American Chemical Society, **74** (2016), no. 1, pp. 53-56.
8. Dušek, M.; Jandovska, V. and Olsovska, J.: Tracking, Behavior and Fate of 58 Pesticides Originated from Hops during Beer Brewing, Journal of Agricultural and Food Chemistry, **66** (2018), no. 38, pp. 10113-10121.
9. Sangster, Q. J.: Octanol-Water Partition Coefficients. Fundamentals and Physical Chemistry, Vol. 2 of Wiley Series in Solution Chemistry, 1997.
10. Bavarian State Research Center for Agriculture (LfL): Reihe "Forschungsschwerpunkte am Hopfenforschungszentrum Hüll", 2022.
11. German Federal Institute for Risk Assessment (BfR): Mitteilung 003/2023 des BfR, https://www.bfr.bund.de/cm/343/eu-datenbank-zu-verarbeitungsfaktoren.pdf, accessed: 28.10.2024, 2023.
12. Zincke, F.; Fischer, A.; Kittelmann, A.; Kraus, C.; Scholz, R.; Michalski, B.: European database of processing factors for pesticides residues in food. https://zenodo.org/records/6827098, accessed: 29.11.2024, 2022.
13. European commission: SANTE/ 10704/2021: Information note on Article 20 of Regulation (EC) No 396/2005 as regards processing factors, processed and composite food and feed, 2022.
14. OECD: Test No. 508: Magnitude of the Pesticide Residues in Processed Commodities, OECD Guidelines for the Testing of Chemicals, Section 5, OECD Publishing, 2008.
15. Gahr, A.; Forster, A. and Van Opstaele, F.: Reproducibility Trials in a Research Brewery and Effects on the Evaluation of op Substances in Beer – Part 1: Reproducibility in fresh beers, BrewingScience, **69** (2016), no. 11/12, pp. 103-111.
16. Tomlin, C. D. S.: Pesticide Manual, 16. Edition, The British Crop Protection Council, 2012.
17. Forster, A.; Gahr, A.; Kugel, R. and Schmidt, R.: A Method to Enable Systematic Studies on the Transfer of Pesticides from Hops into Beer, Poster at the European Brewing Congress, 2015.
18. European commission: SANTE 11312/2021 v2: Analytical quality control and method validation procedures for pesticide residues analysis in food and feed, 2024.
19. Federal Office of Consumer Protection and Food Safety (BVL): L 00.00-113:2015-03, Untersuchung von Lebensmitteln – Bestimmung von Pestizidrückständen in pflanzlichen Lebensmitteln - LC-MS/MS-Verfahren mit Methanolextraktion und Reinigung an Diatomerde, 2010.
20. Federal Office of Consumer Protection and Food Safety (BVL): L 00.00-34:2010-09, Untersuchung von Lebensmitteln – Modulare Multimethode zur Bestimmung von Pflanzenschutzmittelrückständen in Lebensmitteln, 2009.
21. Dixius, D.; Hanke, S.; Stettner, G. and Lagemann, A.: Transfer of flonicamid and its metabolites (TFNA, TFNG and TFNA-AM) from hops into beer during wort boiling and during dry hopping, BrewingScience, **72** (2019), no. 5/6, pp. 109-117.
22. Federal Office of Consumer Protection and Food Safety (BVL): BVL-Report 13.4 Berichte zur Lebensmittelsicherheit – Monitoring 2017, 2017.

Received 4 November 2024, accepted 3 December 2024