EURL-SRM - Analytical Method Report

Analysis of Residues of Carbofuran (sum) Using QuEChERS Method

Reported by: EURL-SRM
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Brief description:

A QuEChERS-based method for the analysis of carbofuran (CF) (sum) in fruits and vegetables is presented. Carbofuran (sum) entails 5 components: carbofuran (CF), 3-hydroxy-carbofuran (3-OH-CF), carbosulfan (CS), benfuracarb (BF) and furathiocarb (FT). The method presented involves a conversion of CS, BF and FT to CF under acidic conditions directly from the QuEChERS extracts. Conversion to CF was considered crucial, as it reduces the number of compounds to be analytically determined from five to only two (CF and 3-OH-CF). Direct analysis of CS and BF would be highly challenging, as these two compounds tend to degrade at various stages of analysis. Validation was conducted following spiking of 0.001 mg/kg of BF, CS or FT (corresponds to 0.00052-0.00058 mg/kg CF). In addition 3-OH-CF, the only component included in the residue definition in food of animal origin, was successfully validated in milk at 0.001 mg/kg (corresponds to the MRL) using QuEChERS without hydrolysis.

Background:

Originally CF and its 3 pro-pesticides (BF, CS, FT) were regulated separately; however given the tendency of CS, BF and FT to degrade during analysis and food processing a new residue definition was introduced, which includes CF, its three pro-pesticides and the metabolite 3-OH-carbofuran (3-OH-CF) expressed as CF. For products of animal origin the residue definition refers to 3-OH-CF (free and conjugated). Given the strong acute neurotoxicity of CF, very low MRLs for CF (sum) were established for food products with substantial short-term consumption (e.g. 0.001 mg/kg for potatoes, apples and milk and 0.002 mg/kg for stone fruits, grapes, fruiting vegetables, brassica vegetables, salad plants and stem vegetables).

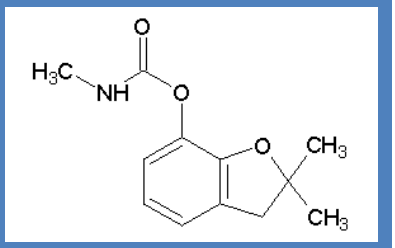
Compound profile:

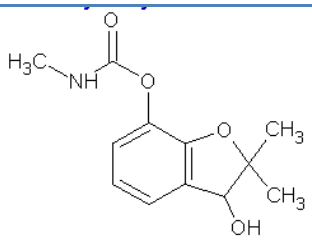
CF is an insecticide, nematicide and acaricide of the carbamate group. CS, FT and BF are pro-pesticides that degrade to CF, which is the active substance. None of the four compounds is approved in the EU, but there are still uses in other countries. Carbofuran acts against soil-dwelling and foliar-feeding insects (including wireworms, white grubs, millipedes, symphylids, fruit flies, aphids and thrips, etc.) as well against nematodes in a multitude of crop types including vegetables, cereals, citrus, vines and berries [1] . A recent import-

tolerance request for mushrooms from China (exposed through rice straw) was rejected following the toxicological re-evaluation of CF. The ARfD values of CF, CS, BF and FT were 0.00015, 0.005, 0.02 and 0.006 mg/kg bw/d, respectively. 3-OH-CF is considered to exhibit similar toxicity as the parent.

Compound facts at a glance:

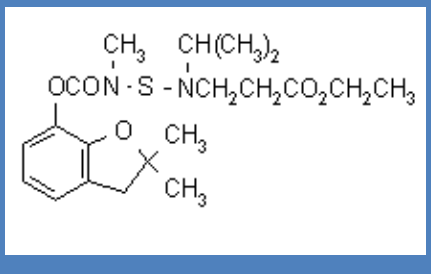
Carbofuran	
Mode of action	Systemic, with predominantly contact and stomach action [1]
LogP	1.8 at 20°C, no effect of pH [2]
Water solubility	315 mg/L at 19.5°C +/- 2.0 °C, no effect of pH [2]
Stability	Unstable in alkaline media. Stable in acidic and neutral media. Decomposes >150 °C [1]
Residue definition EU	**Food of plant origin and honey**: "*Carbofuran (sum of carbofuran (including any carbofuran generated from carbosulfan, benfuracarb or furathiocarb) and 3-hydroxycarbofuran expressed as carbofuran)*" **Food of animal origin:** "*3-hydroxycarbofuran (free and conjugated) expressed as carbofuran*"
Registration Status	Not approved within the EU
ADI	0.00015 mg/kg bw/day (2009) [2]
ARfD	0.00015 mg/kg bw (2009) [2]
Main metabolite	3-Hydroxycarbofuran

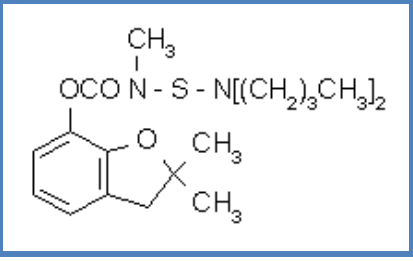


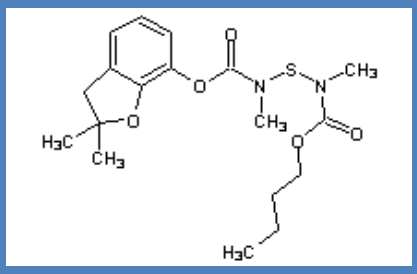


The following pro-pesticides metabolize to carbofuran (the active component):

Benfuracarb	
Mode of action	Systemic and contact insecticide with stomach and contact action [1]
LogP	4.22 (25°C) [2]
Water solubility	8 (20°C, pH 4) [2]
Stability	Stable in neutral and weakly basic media, but unstable in acidic and strongly basic media. Stable up to 190 °C [1]
Residue definition EU	**See carbofuran**
Registration Status	Not approved within the EU
ADI	0.01 mg/kg bw/day (2009) [2]
ARfD	0.02 mg/kg bw (2009) [2]



Carbosulfan		
Mode of action	Systemic insecticide with contact and stomach action [1]	
LogP	7.42 (25°C, pH-independent) [2]	
Water solubility	0.11mg/L (25°C, pH 9) [2]	
Stability	Hydrolysed in aqueous media [1]	
Residue definition EU	**See carbofuran**	
Registration Status	Not approved within the EU [3]	
ADI	0.005 mg/kg bw/day (2009) [2]	
ARfD	0.005 mg/kg bw (2009) [2]	

Furathiocarb		
Mode of action	Systemic insecticide with contact and stomach action. [1]	
LogP	4.6 (25°C) [1]	
Water solubility	11 (25°C) [1]	
Stability	Stable up to 400 °C [1]	
Residue definition EU	**See carbofuran**	
Registration Status	Not approved within the EU	
ADI	0.0035 mg/kg bw/day (RMS 1999) [3]	
ARfD	0.006 mg/kg bw (RMS 1999) [3]	

Standard Materials used (exemplary[1]):

Carbofuran (purity 99,5%), purchased from Dr. Ehrenstorfer (Cat #: C11010000)
Benfuracarb (purity 92%), purchased from Dr. Ehrenstorfer (Cat #: CA10475000)
Furathiocarb (purity 99%), purchased from Dr. Ehrenstorfer (Cat #: C13970000)
Carbosulfan (purity 98%), purchased from Dr. Ehrenstorfer (Cat #: CA11030000)
3-Hydroxycarbofuran (purity 98%), purchased from Dr. Ehrenstorfer (Cat #: C 11011000)

[1] Disclaimer: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Apparatus and Consumables:
Use materials described in the QuEChERS standard procedure (EN15662). As a mechanical shaker you can use a horizontally or vertically reciprocating shaker or a rotatory shaker (e.g. HS260 by IKA or GenoGrinder by Spex or SSL1 Labscale Orbital Shaker by Stuart). To filter the extract use e.g. polyester disposable syringe filters of 0.45 µm pore size.

Extraction: Apply the **citrate buffered QuEChERS (EN 15662)**.
Weigh 10 g of frozen fruit or vegetable homogenate or 5 g of cereals; adjust water content to 10 mL where necessary, add 10 mL acetonitrile and internal standard (e.g. 100 µL of an appropriately concentrated solution of Carbofuran-D3[2]). Shake 15 min using a mechanical shaker. Add a mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate, shake 1 min and centrifuge.

Cleanup: Cleanup via dispersive SPE is optional for fruits and vegetables.

Hydrolysis: Transfer 1 mL of raw extract into vial and add 10 µL 5N H_2SO_4. Nearly quantitative transformation of BF, CS and FT into CF is achieved by heating the vials for 3h at 80 °C.

LC-MS/MS analysis: For screening purposes CF, 3-OH-CF as well as BF, FT and CS may be analyzed by LC-MS/MS directly in QuEChERS raw extracts or cleaned-up extracts. In case of positive findings the hydrolysis step can be conducted as described above and LC-MS/MS analysis of CF repeated.
See exemplary measurement conditions in Table 1 and Table 2.

1mL final extract will represent approximately 1 g matrix.

Note on food of animal origin: *The residue definition for food of animal origin is as follows: "3-hydroxycarbofuran (free and conjugated) expressed as carbofuran". As the conjugates are not expected to be fully extractable into acetonitrile the hydrolysis of conjugated 3-OH-CF is recommended to be performed prior or during the extraction step. Lacking samples with incurred residues no deconjugation approach was developed for food of animal origin. The validation data on milk below concern free 3-OH-CF analyzed directly from QuEChERS extracts following dSPE-cleanup with ODS/PSA/$MgSO_4$.*

[2] Before using an internal standard, check if it is stable under the hydrolysis conditions

Measurement Conditions:

Table 1: Exemplary LC conditions for ABSciex 5500 Q-Trap

LC	WATERS Acquity UPLC		
MS/MS	ABSCIEX 5500 Q-Trap, run in ESI positive mode		
Column temperature	40°C		
Column	Acquity BEH C18, 2.1x100 mm, 1.7 µm		
Pre-column	Acquity BEH C18, 2.1x5 mm, 1.7 µm		
Mobile Phase	A: 5 mmol NH_4formate in purified water + 5% methanol B: 5 mmol NH_4formate in methanol		
Gradient	**Time** (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)
	0	100	0
	0.5	60	40
	7.5	10	90
	11.0	10	90
	11.1	100	0
	14.0	100	0
Flow	0.4 mL min^{-1}		
Injection volume	2 µL, partial loop with needle overfill		

Table 2: Exemplary LC conditions for AB 4000 Q-Trap

LC	WATERS Acquity UPLC		
MS/MS	ABSCIEX 4000 Q-Trap, run in ESI positive mode		
Column temperature	40°C		
Column	Acquity BEH C18, 2.1x100 mm, 1.7 µm		
Pre-column	Acquity BEH C18, 2.1x5 mm, 1.7 µm		
Mobile Phase	A: 5 mmol NH_4formate in purified water + 5% methanol B: 5 mmol NH_4formate in methanol		
Gradient	**Time** (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)
	0	95	5
	0.5	60	40
	2	10	90
	5	10	90
	5.1	95	5
	10	95	5
Flow	0.35 mL min^{-1}		
Injection volume	2 µL, partial loop with needle overfill		

Table 2: Exemplary MS/MS details for carbofuran group (ESI-positive mode), for ABSciex 5500 Q-Trap

Substance	Intensity	Q 1	Q 3	DP	CE	CXP
Carbofuran	1	222	123	76	29	6
	2		165	76	17	14
Benfuracarb	1	411	190	71	17	10
	2		102	71	45	6
	3		74	71	67	2
Furathiocarb	1	383	195	96	27	10
	2		252	96	19	2
	3		167	96	35	8
Carbosulfan	1	381	118	86	29	20
	2		160	81	21	14
	3		76	76	53	6
3-Hydroxy-carbofuran	1	238	163	76	21	10
	2		181	76	17	10
	3		220	76	11	40

Note: ***After conversion of BF, FT, CS to CF it is not necessary to include these compounds in the measurement. The transitions for these compounds could be used to monitor their presence in a first routine screening or to optionally verify their absence following the hydrolysis step.***

Validation data

Table 3: Recovery figures for BF, FT, CS after acidic conversion to CF

Matrix	Spiking Level parent (mg/kg)	n	BF Spiked level corresponds to 0.00058 mg/kg CF		FT Spiked level corresponds to 0.00058 mg/kg CF		CS Spiked level corresponds to 0.00054 mg/kg CF		MS/MS Instrument employed
			Rec.(%)	RSD (%)	Rec.(%)	RSD (%)	Rec.(%)	RSD (%)	
Grapes	0.001	5	112	2	111	2	113	2	ABSCIEX 5500 Q Trap
	0.001	5	104	5	102	2	98	4	ABSCIEX 4000 Q Trap
Potatoes	0.001	5	114	2	109	3	113	4	ABSCIEX 5500 Q Trap
	0.001	5	Not quantified due to interferences						ABSCIEX 4000 Q Trap

Table 4: Recovery figures for 3-OH-CF (following procedure involving hydrolysis step)

Matrix	Spiking Level (mg/kg)	n	3-OH-CF		MS/MS Instrument employed
			Rec.(%)	RSD (%)	
Grapes	0.001	5	78	5	ABSCIEX 5500 Q Trap
Potatoes	0.001	5	117	14	ABSCIEX 5500 Q Trap

Table 5: Recovery figures for CF and 3-OH-CF using QuEChERS as described in EN 15662

Matrix	Spiking Level (mg/kg)	n	CF		3-OH-CF		MS/MS Instru-ment employed
			Rec.(%)	RSD (%)	Rec.(%)	RSD (%)	
Wheat Flour	0.005	5	105	3	99	8	API 3200
Wheat Flour	0.01	5	105	4	105	6	API 3200
Oranges	0.001	5	91	6	96	8	API 3200
Oranges	0.002	5	98	4	104	5	API 3200
Onions	0.001	3	102	4	91	4	ABSCIEX 4000 Q Trap
Milk	0.001	5	-	-	97	3	ABSCIEX 4000 Q Trap

Supplementary Information

Matrix effects in the above commodities were in all cases negligible.

References

[1] e-Pesticide Manual V5.0, Author: C. D. S. Tomlin; 15th Edition, Version 5.0, 2009 Publisher/Contact: The British Crop Protection Council
[2] EFSA
[3] EU Pesticides Database

Document History

Date	Action	Changes
Apr. 2016	Publication of V1	