News on Ethoxyquin – Overview of findings in fish

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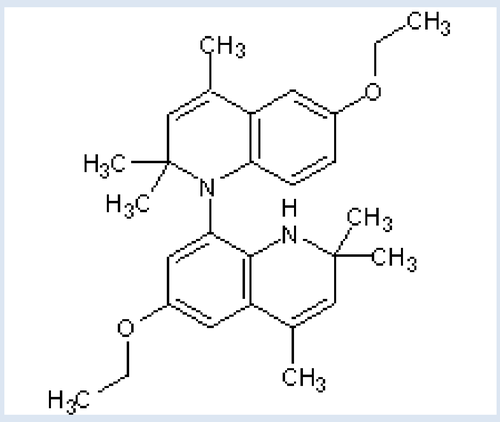
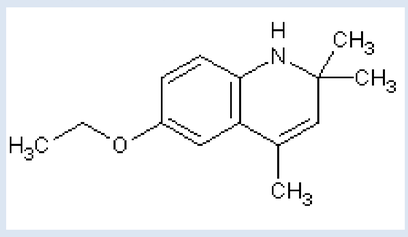
INTRODUCTION

Ethoxyquin is legally used as an antioxidant. Chemically, it belongs to the group of chinolene derivatives. As ethoxyquin has a fungicide effect, it was used as a pesticide. In 2011 the authorisation for the use as pesticide was revoked [1].

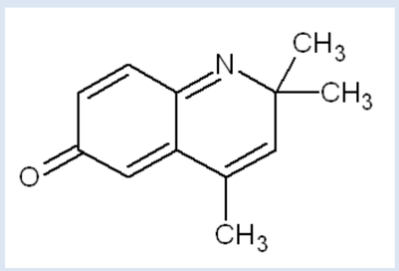
For the analysis of ethoxyquin it is important to avoid degradation of the analyte during sample preparation and extraction. EURL-SRM has evaluated, that using ascorbic acid prior to QuEChERS extraction can avoid significant losses of ethoxyquin during analysis [2]. The method developed by EURL-AO is based on the existing QuEChERS-AO extraction with the addition of ascorbic acid and clean-up followed by LC-MS/MS determination. It is possible to determine ethoxyquin and the metabolites ethoxyquin C-N-dimer as well as ethoxyquin quinolene imine (EQI).



ethoxyquin C-N-dimer



ethoxyquin



ethoxyquin quinolene imine (EQI)

METHOD INFORMATION

The method is based on QuEChERS-AO with an addition of ascorbic acid to the sample to avoid degradation during sample preparation and extraction.
The method was successfully validated according to DIN 32645 (calibration curve approach) and SANTE document 11813/2017. Therefore, the method is suitable for the determination of ethoxyquin and ethoxyquin C-N-dimer [6; 7]. The limit of quantification (LOQ) is 0.002 mg/kg for ethoxyquin and 0.005 mg/kg for the ethoxyquin C-N-dimer. Additionally Ethoxyquin quinone imine (EQI) was added to the method with an LOQ of 0.005 mg/kg.

HPLC-MS/MS CONDITIONS

System: Agilent LC (1200 series) coupled with Agilent MS Triple-Quad 6410

HPLC parameters		
Injection volume	3 µL	
Column	Phenomenex Luna C18(2) 100A, 50 x 2 mm, 5 µm @	
Temperature	25 °C	
Gradient LC elution	Solvent A: 0.01 % formic acid Solvent B: Methanol	
MS/MS parameters		
Scan type	Dynamic MRM, ESI positive	
Gas temperature	320 °C	
Gas Flow	10L/min	
Nebulizer	40 psi	
Capillary voltage	4000 V	**Transition**
Mass transitions	ethoxyquin	218.2 > 174.1 218.2 > 160.1 218.2 > 148.1
	ethoxyquin-dimer	433.3 > 375.3 433.3 > 216.2 433.3 > 188.2
	ethoxyquin-quinone imine (EQI)	188.1 > 173.1 188.1 > 145.1

Time (min)	Flow rate	B [%]
0	0.3	30
0.5	0.3	30
10	0.4	100
12	0.4	100
12.3	0.3	30
17	0.3	30

LEGAL ASSESSMENT OF THE RESULTS

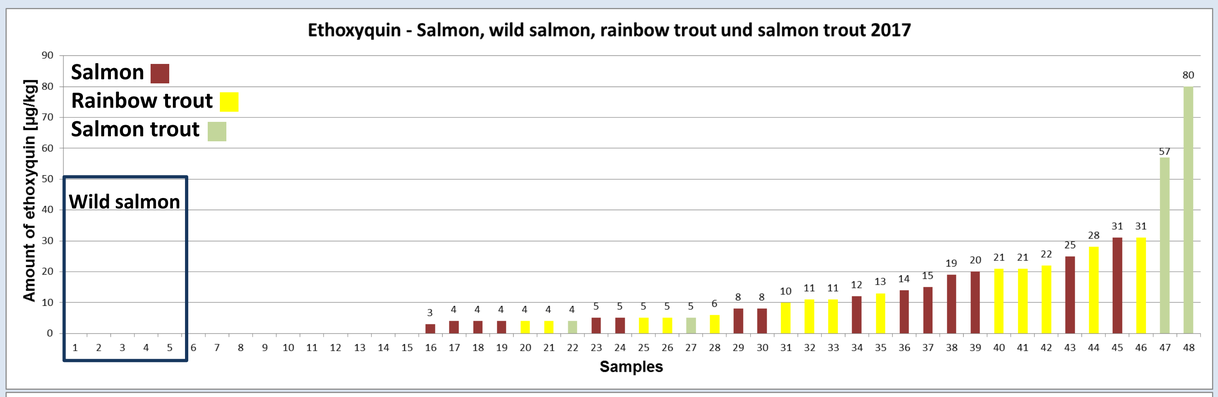
Ethoxyquin is approved as <u>feed</u> additive but not as <u>food</u> additive. In 2011 the authorisation for the use as pesticide was revoked. As pesticide, ethoxyquin is regulated in Regulation (EC) No 396/2005 [3]. However, the matrix fish is not part of this regulation. As a consequence there is no common European Union regulation for ethoxyquin in fish.

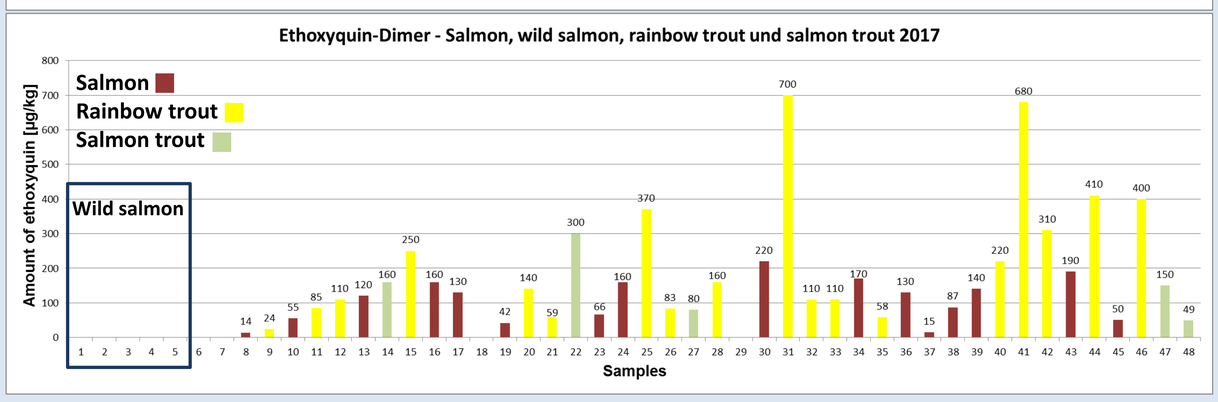
According to national regulation in Germany (“Rückstands-Höchstmengenverordnung”) there is a common maximum level of 0.01 mg/kg for the use as pesticide. As ethoxyquin may still be used legally as antioxidant so far no legally binding maximum level for ethoxyquin in fish exists. Also no residue definition, where the metabolites are included, has been defined.

EFSA stated in their opinion, that due to lack of information no safety of the additive ethoxyquin for any target animals, for consumers and for the environment can be concluded [4]. With Regulation (EU) 2017/962 the authorisation of ethoxyquin as feed additive is suspended. Until end of 2019 premixtures containing ethoxyquin may be used [5]. The Regulation shall be revised until end of 2020 at the latest. Maybe the lack of data EFSA reported is filled up and ethoxyquin might be used as antioxidant again or not.

RESULTS AND DISCUSSION

In total 48 samples of conventionally farmed salmon (20), wild salmon (5), rainbow trout (18) and salmon trout (5) were analysed. The samples were taken from the local market in supermarkets either freshly or frozen. The majority of the samples originated from Norwegian aquaculture. Additionally 5 samples of other species were analysed (e.g. wels catfish).





The highest ethoxyquin levels were detected in salmon trout in the range of nearly 0.08 mg/kg. The corresponding ethoxyquin C-N-dimer was found in a range of 0.05 mg/kg. The highest level of the ethoxyquin C-N-dimer was found in rainbow trout with concentrations up to 0.7 mg/kg. As expected, in the wild salmon sample neither ethoxyquin nor the C-N-Dimer were detected. One wels catfish contained 0.28 mg/kg ethoxyquin as well as 1 mg/kg ethoxyquin C-N-dimer. The metabolite EQI was not detected in the samples.

Almost all conventionally farmed fishes contained ethoxyquin and/or the C-N-dimer of ethoxyquin except two salmon samples. In this case alternative antioxidants like butylated hydroxytoluene (BHT) may have been used as feed preservatives.

LITERATURE

[1] COMMISSION DECISION of 3 March 2011 concerning the non-inclusion of ethoxyquin in Annex I to Council Directive 91/414/EEC and amending Commission Decision 2008/941/EC
[2] EURL-SRM - Analytical Observations Report - Analysis of Ethoxyquin and its Metabolites in Fish Using the QuEChERS Method Version 1 (last update: 10.05.2016)
[3] 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 and amending Council Directive 91/414/EEC
[4] Safety and efficacy of ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) for all animal species - EFSA Journal 2015;13(11):4272
[5] COMMISSION IMPLEMENTING REGULATION (EU) 2017/962 of 7 June 2017 suspending the authorisation of ethoxyquin as a feed additive for all animal species and categories
[6] DIN 32645/2008 - Chemical analysis - Decision limit, detection limit and determination limit under repeatability conditions - Terms, methods, evaluation
[7] SANTE/11813/2017 Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.

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