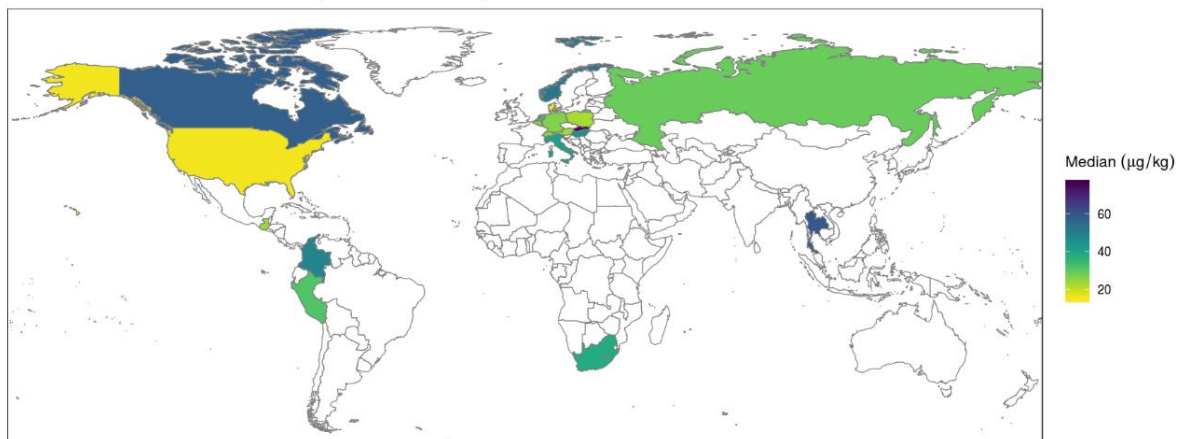


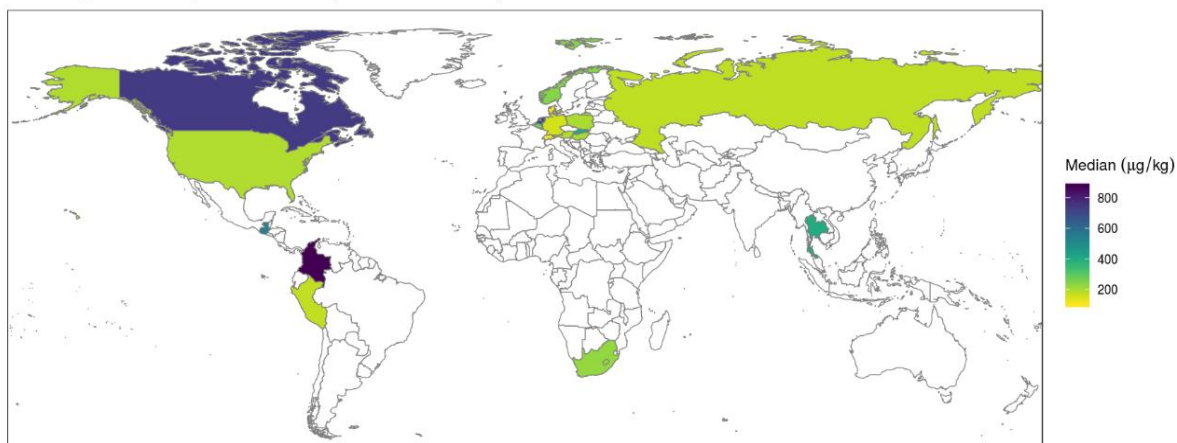
# Co-occurrence of DON and Emerging Mycotoxins in Worldwide Finished Pig Feed and Their Combined Toxicity in Intestinal Cells

Abdullah Khan Khoshal, Barbara Novak, Pascal G.P. Martin, Timothy Jenkins, Manon Neves, Gerd Schatzmayr, Isabelle P. Oswald and Philippe Pinton

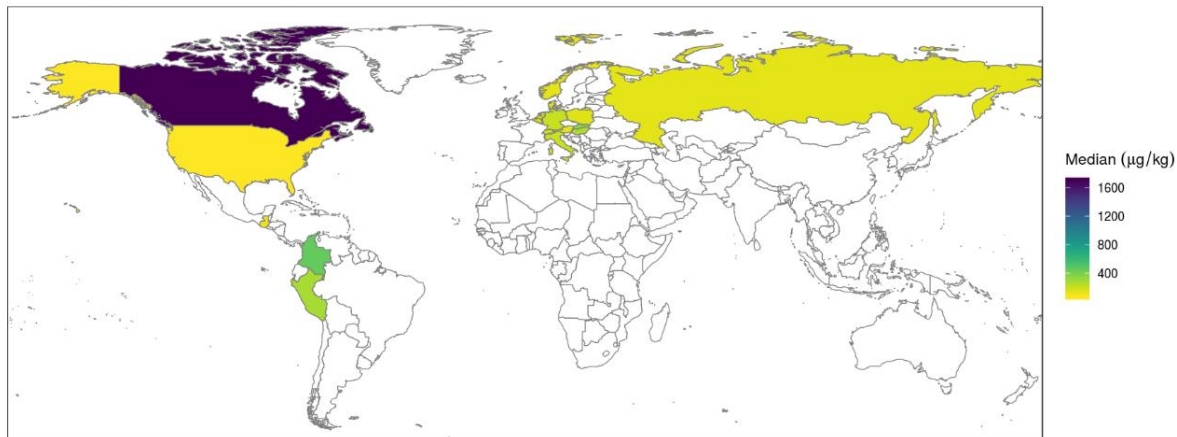
Median Brevianamid F contamination (Countries with  $n > 4$ )



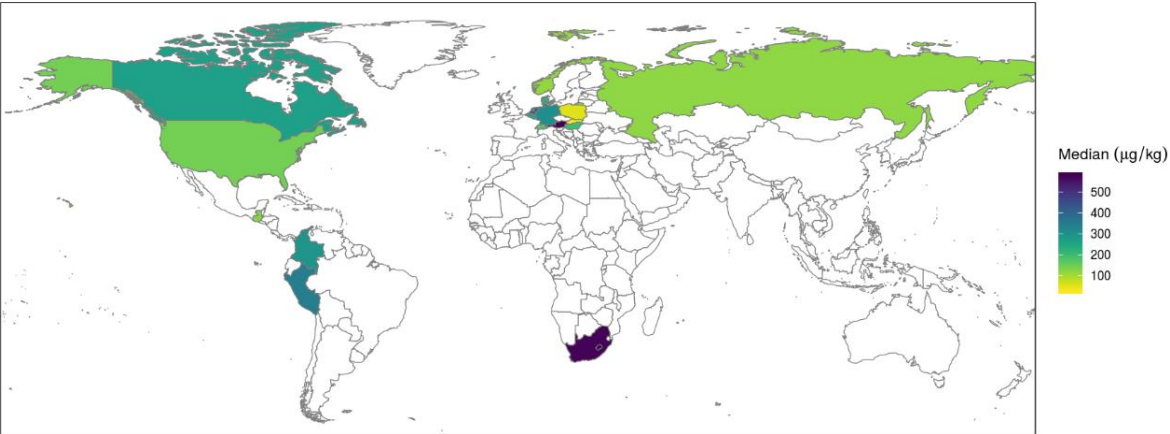
Median cyclo L-Pro-L-Tyr contamination (Countries with  $n > 4$ )



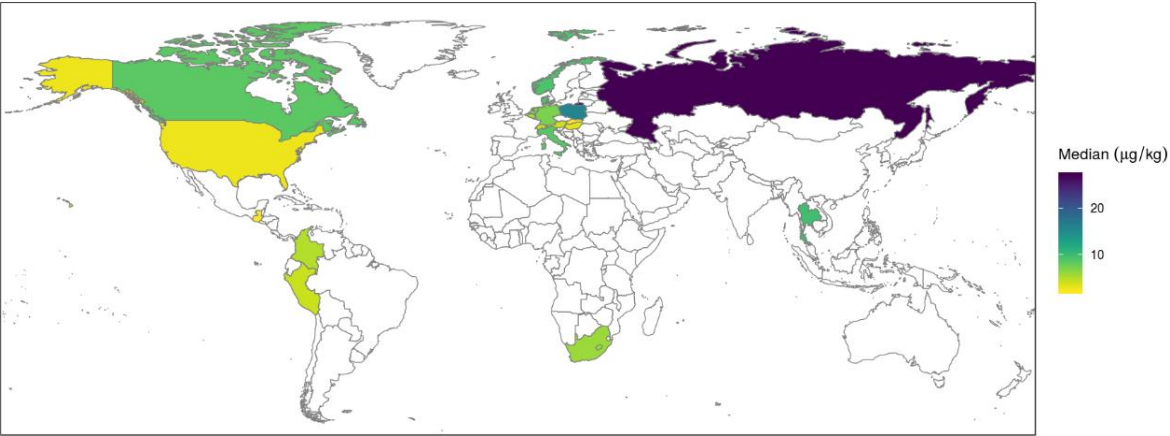
Median Tryptophol contamination (Countries with  $n > 4$ )



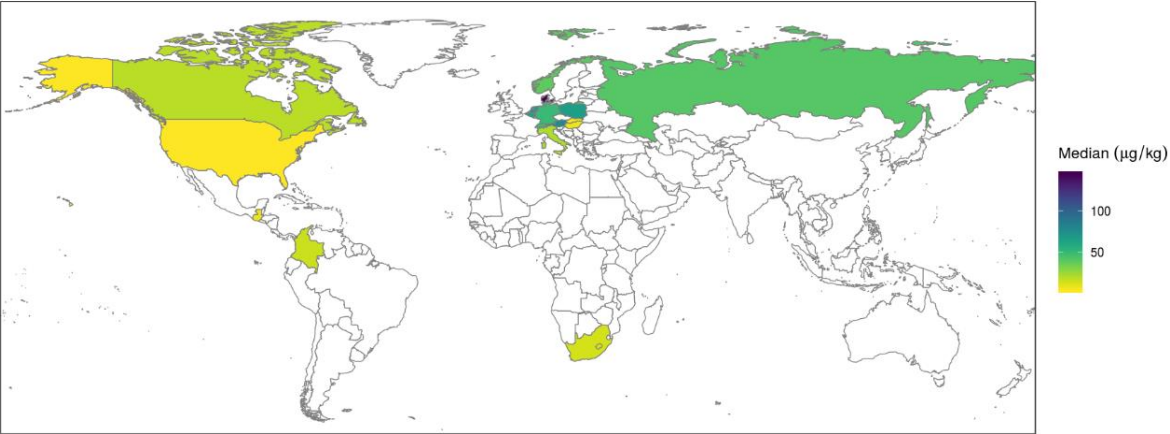
Median Aurofusarin contamination (Countries with n > 4)



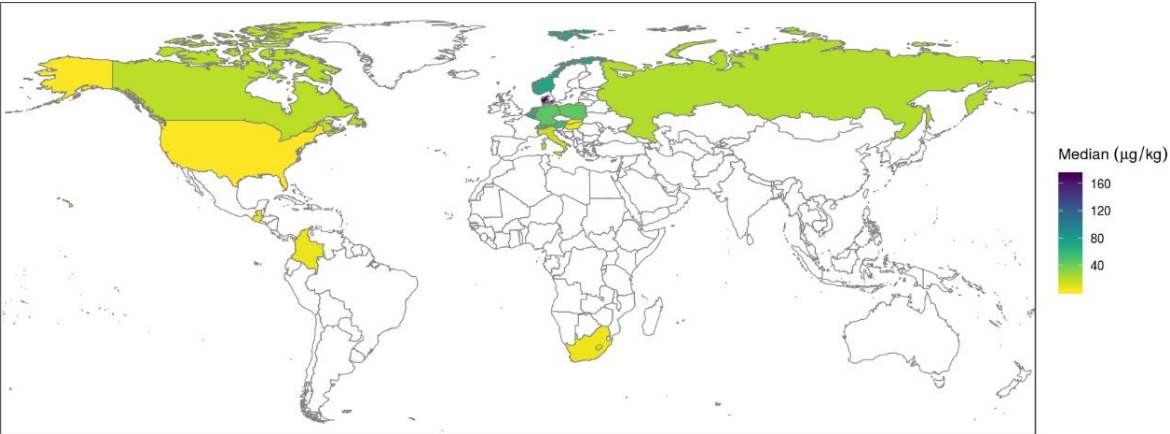
Median Emodin contamination (Countries with n > 4)



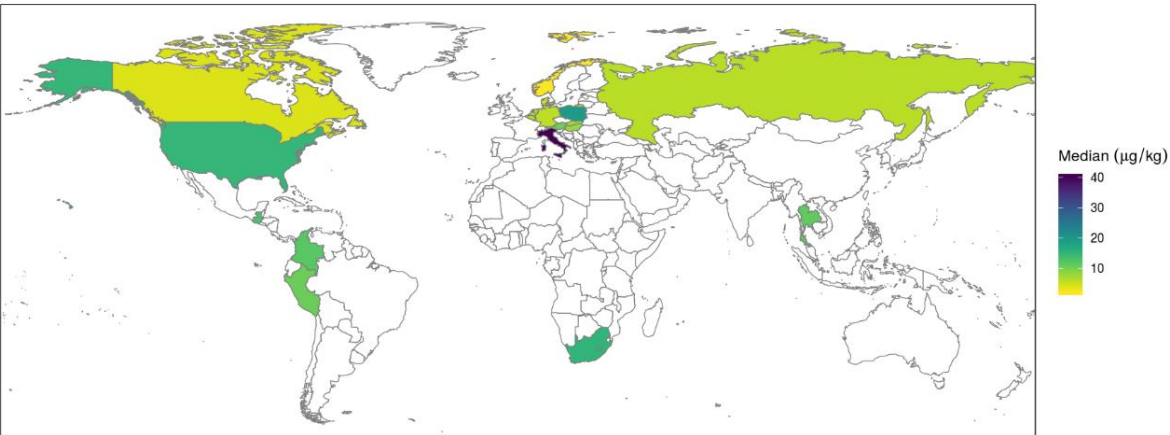
Median Enniatin B1 contamination (Countries with n > 4)



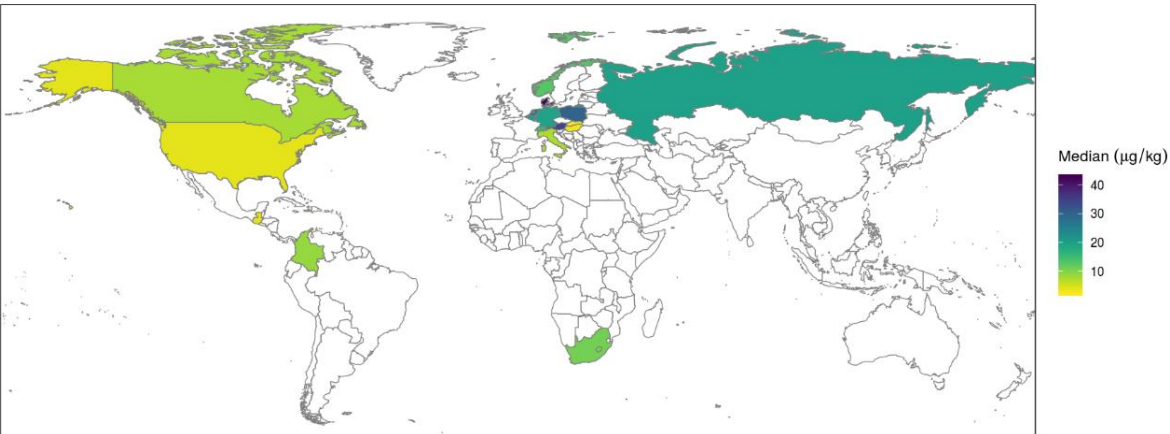
Median Enniatin B contamination (Countries with n > 4)

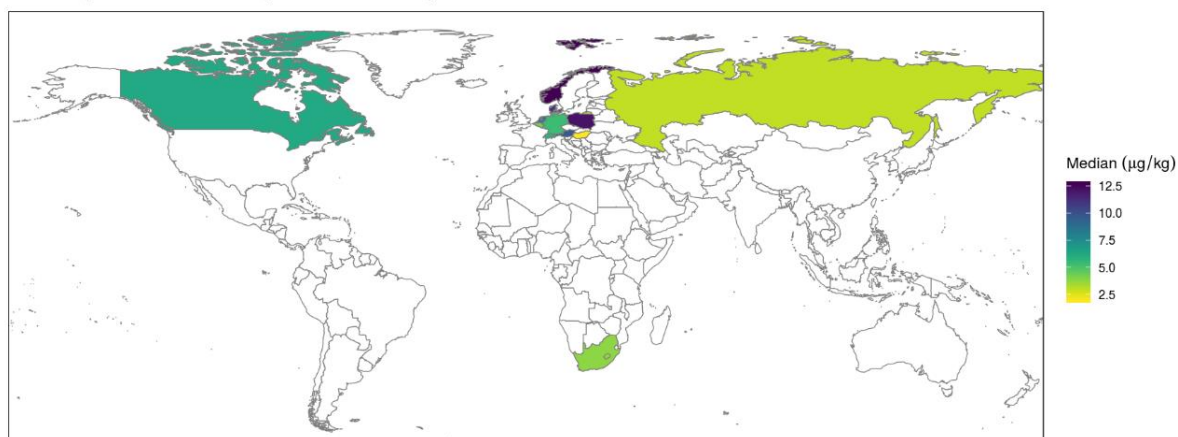


Median Beauvericin contamination (Countries with n > 4)

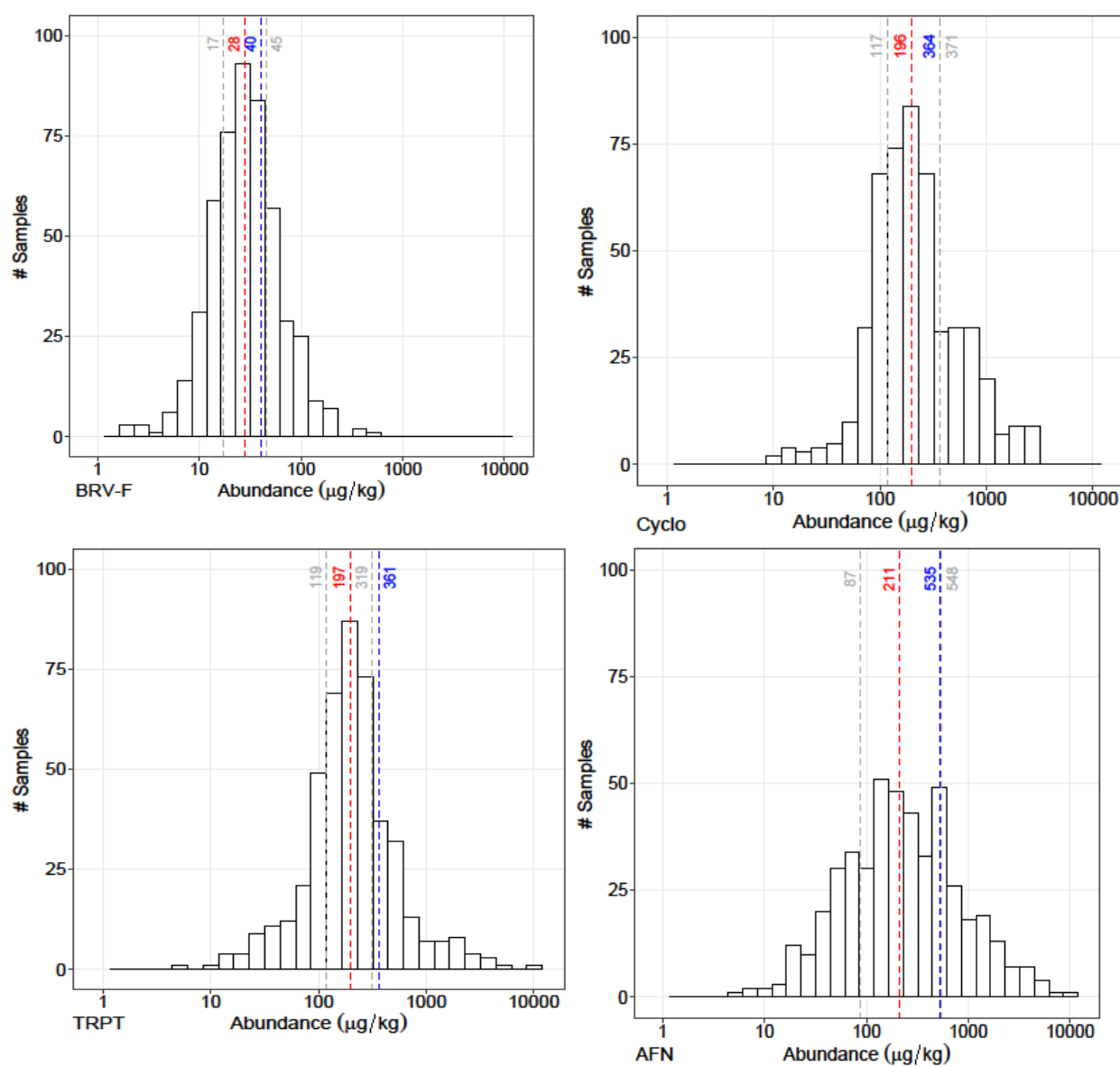


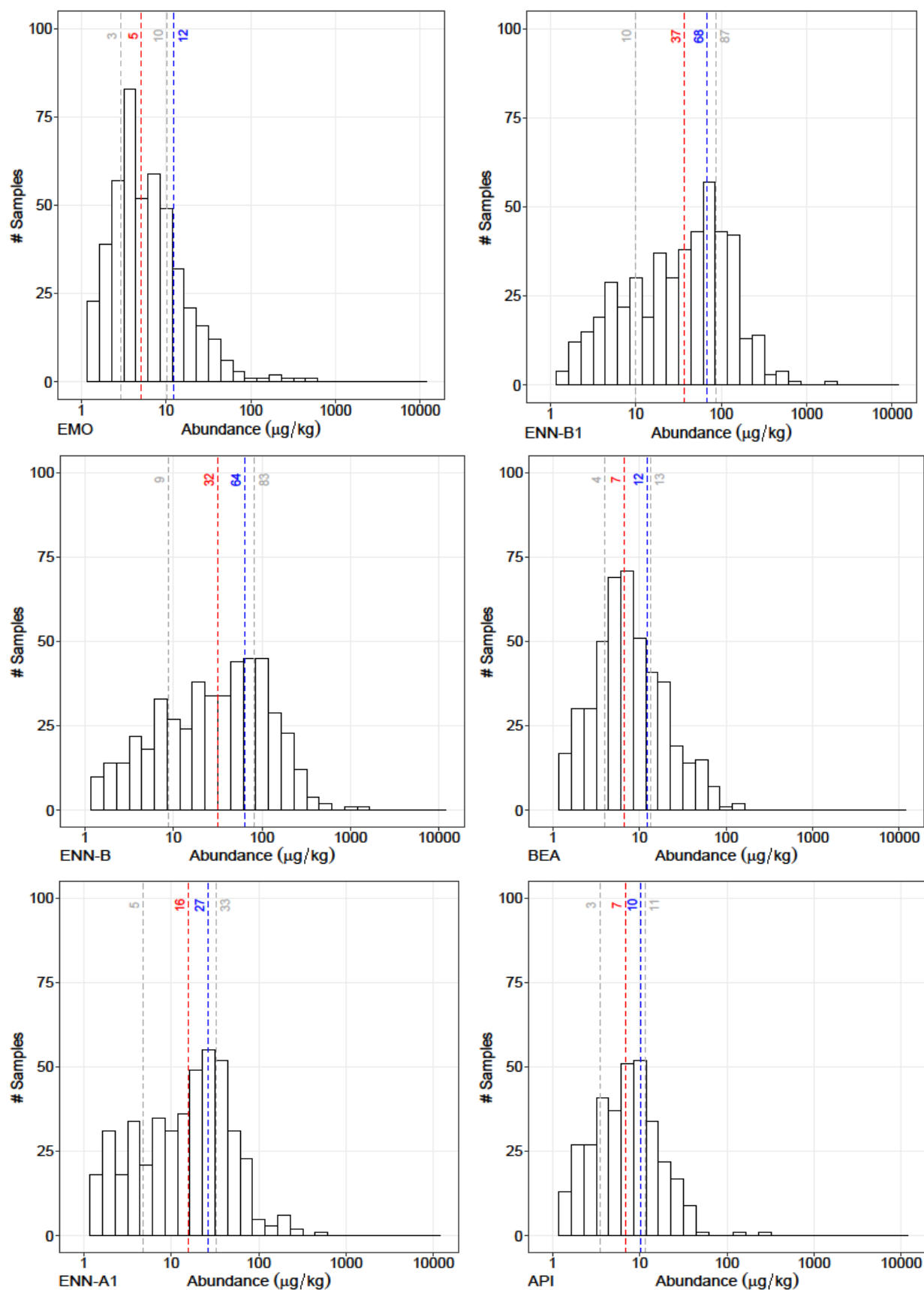
Median Enniatin A1 contamination (Countries with n > 4)



Median Apicidin contamination (Countries with  $n > 4$ )

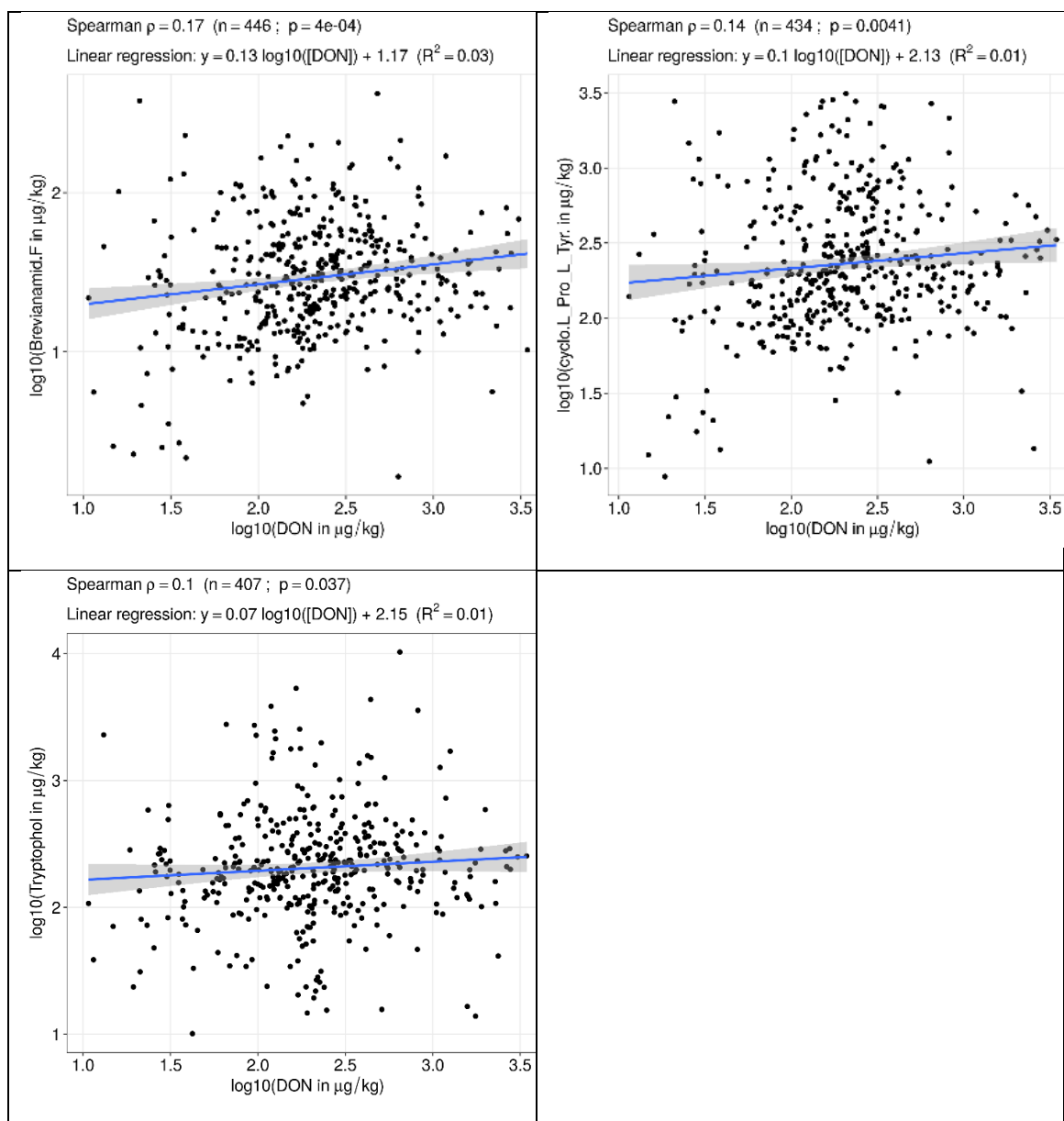
**Figure S1.** Worldwide contamination of BRV-F, Cyclo, TRPT, EMO, ENN-B1, AFN, ENN-A1, ENN-B, BEA and API.



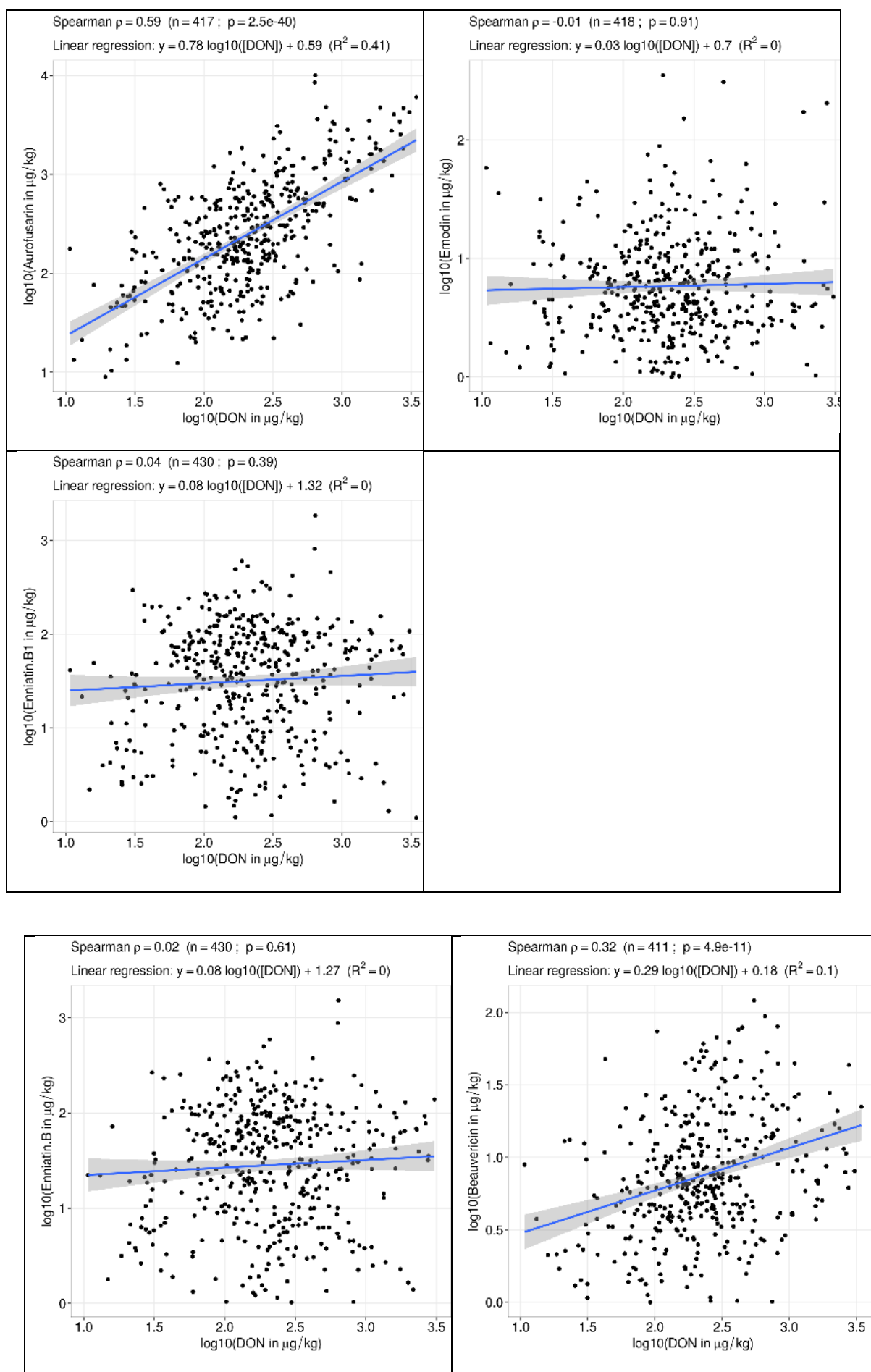


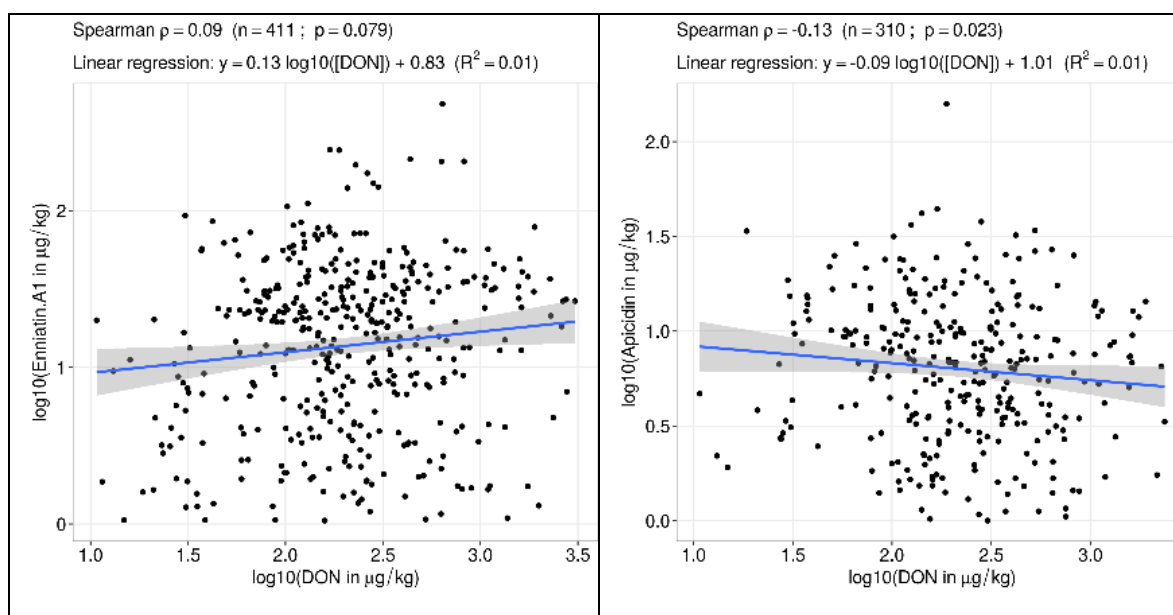
**Figure S2.** Abundance of emerging mycotoxins in pig feed (P25, median, mean, P75). X axis represents the distribution of the concentrations of fungal secondary metabolites (ppb). Y-axis describes the number of co-contaminated samples by DON and other emerging mycotoxins. Median concentrations

of AFN, TRPT and Cyclo were  $\approx 200$  ppb, median concentrations of ENN-B1, ENN-B, BRV-F and ENN-A1 were 15–40 ppb, and median concentrations of API, BEA and EMO were 5–10 ppb.



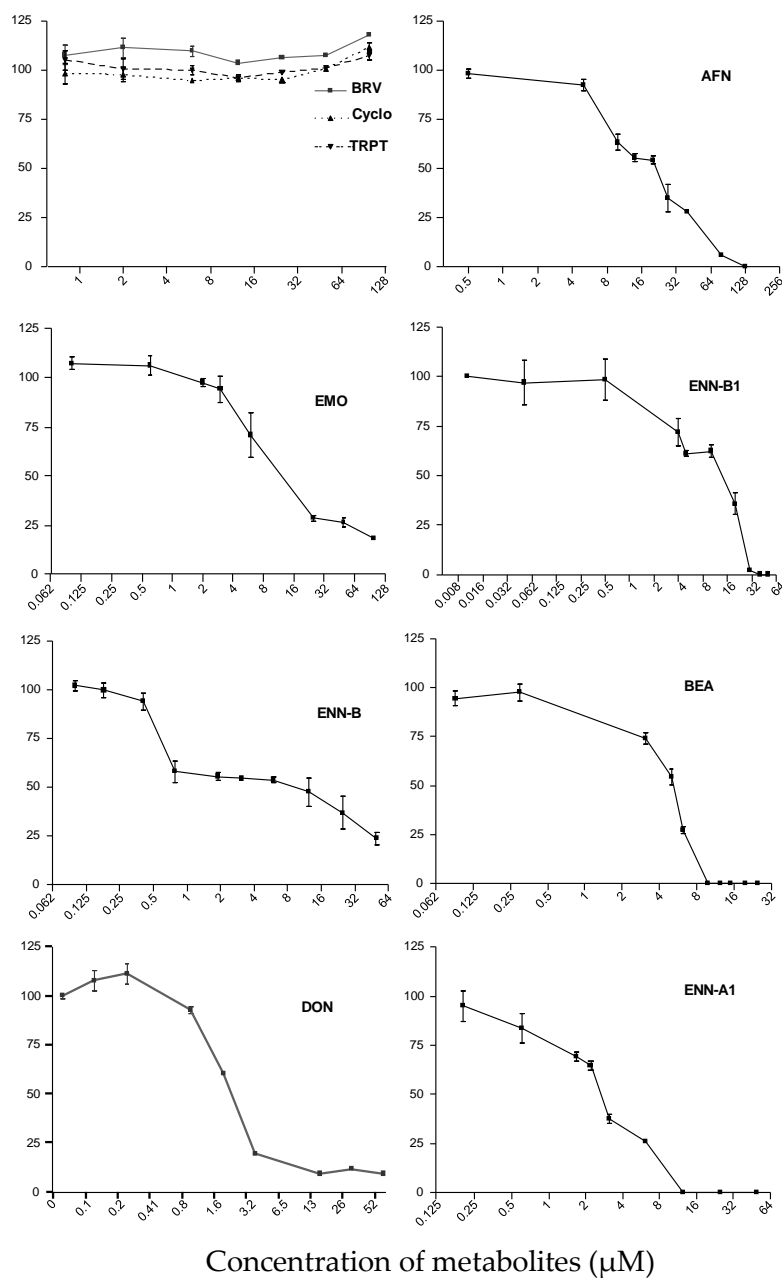




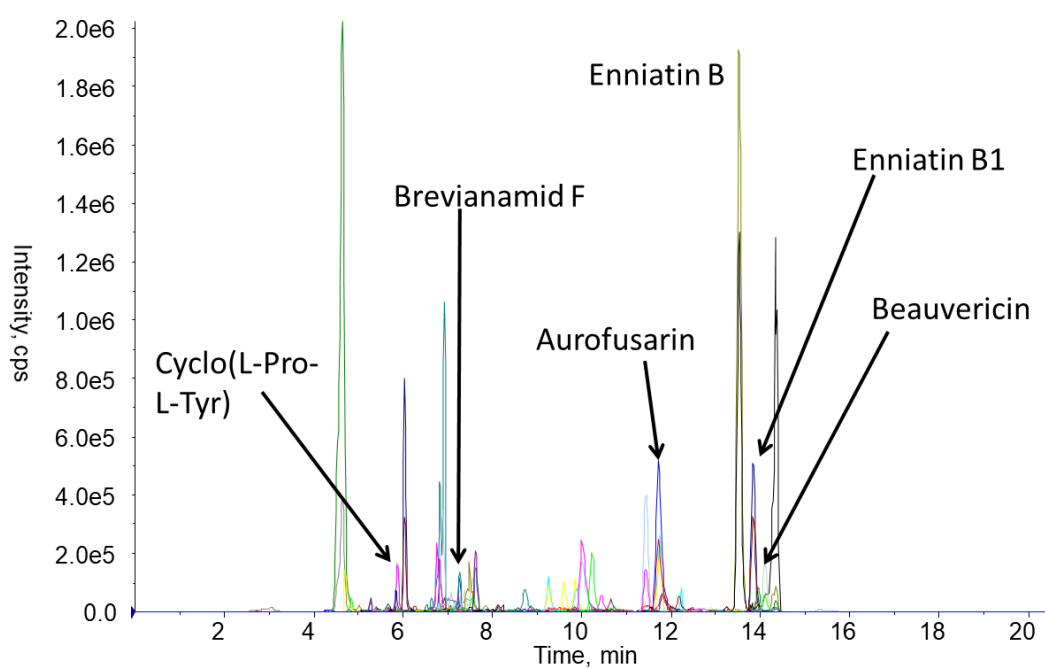


**Figure S3.** Scatter plot showing the correlation between the concentrations of BRV-F, Cyclo, TRPT, AFN, EMO, ENN-B1, ENN-B, BEA, DON, ENN-A1 and API in pig feed samples.

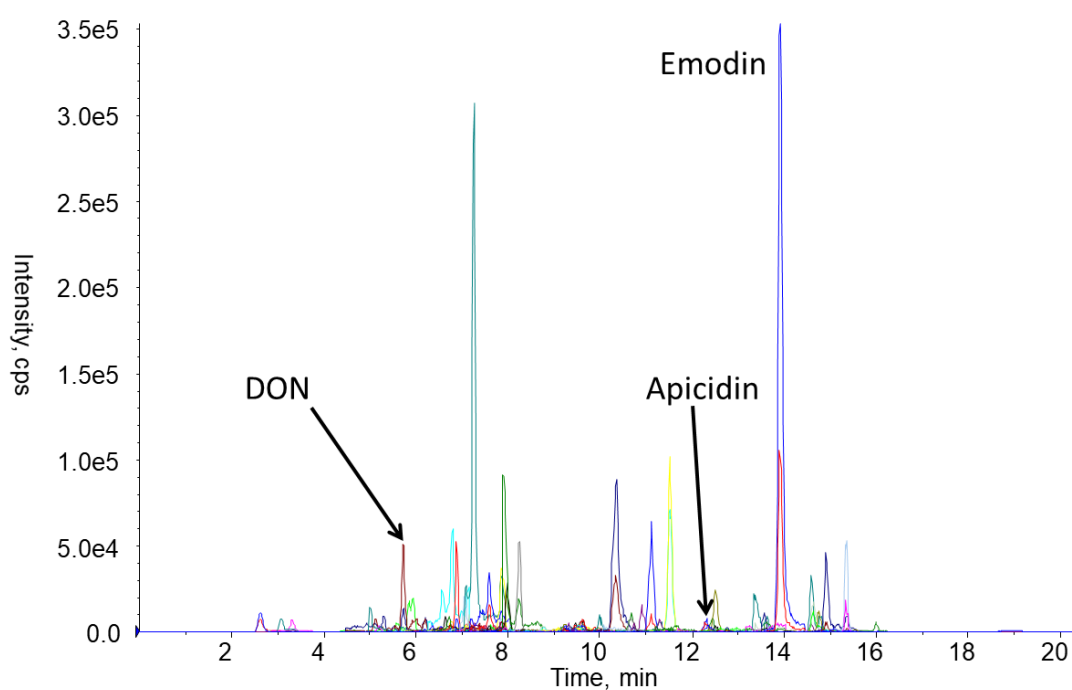




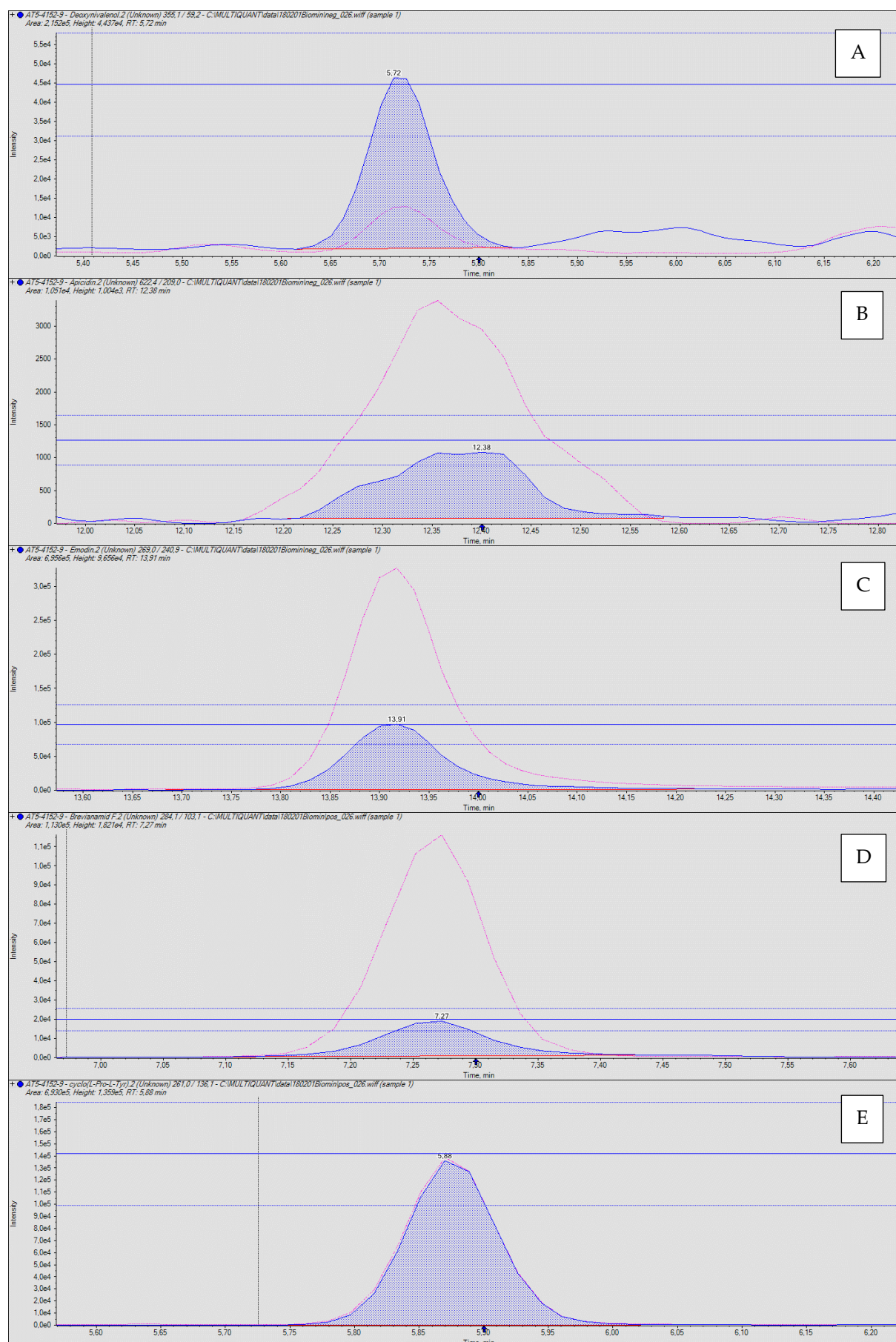
**Figure S4.** Dose effect curve of individual toxicity of 9 selected emerging mycotoxins (BRV-F, Cyclo, TRPT, AFN, EMO, ENN-B1, ENN-B, BEA, ENN-A1) and DON. Data are mean  $\pm$  SEM of three biological replicates.

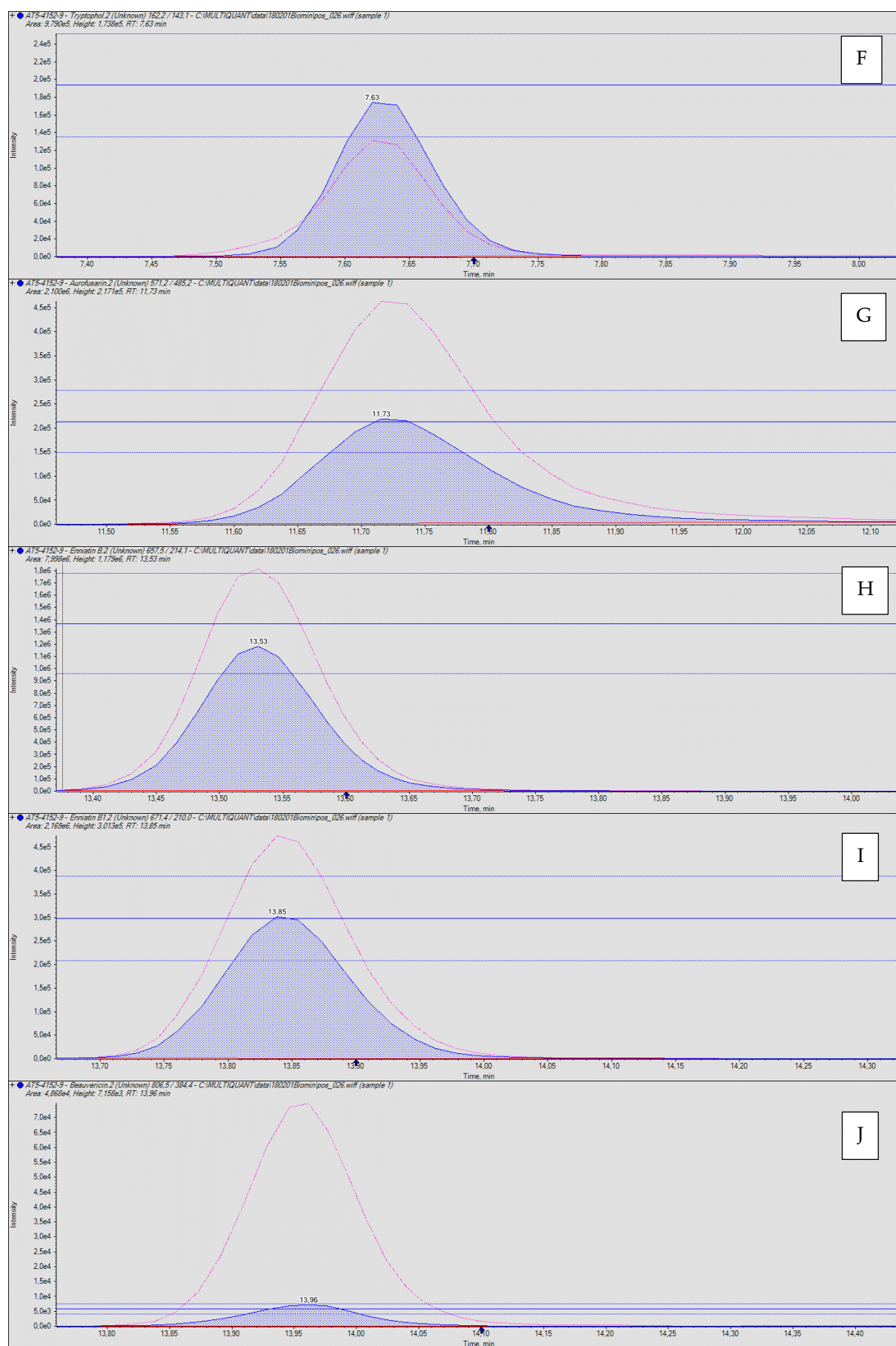


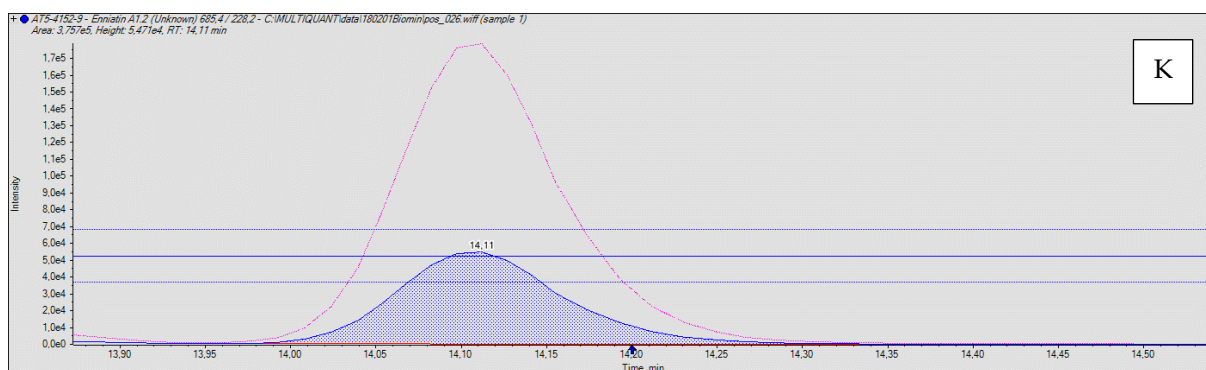
**Figure S5a.** LC-ESI(+)-MRM chromatogram (overlay of all acquired MRMs) of AT5-4153-009 (only compounds for toxicity tests are annotated).



**Figure S5b.** LC-ESI(-)-MRM chromatogram (overlay of all acquired MRMs) of AT5-4153-009 (only compounds for toxicity tests are annotated).







**Figure S6.** Overlay of the Extracted Ion Chromatograms of both quantifier (pink) as well as qualifier (blue; filled) of the 11 investigated compounds in sample AT5-4152-9; the horizontal lines denote the target height of the qualifier ( $\pm 30\%$  tolerance) calculated from the height of the quantifier and the target ion ratio calculated from the standards.

**Table S1.** Concentrations of DON and emerging mycotoxins converted in  $\mu\text{M}$  and the corresponding different ratios.

Metabolites	Concentrations at Selected Quartiles ( $\mu\text{M}$ )			Ratio DON/Emerging Mycotoxin		
	P25	P50	P75	Ratio 1	Ratio 2	Ratio 3
DON	0.38	0.70	1.31			
BRV-F	0.06	0.10	0.16	21.7	7.1	2.4
Cyclo	0.45	0.75	1.42	2.9	0.9	0.3
TRPT	0.74	1.22	1.98	1.8	0.6	0.2
AFN	0.15	0.37	0.96	8.7	1.9	0.4
EMO	0.01	0.02	0.04	122.7	36.5	9.9
ENN-B1	0.02	0.06	0.13	87.4	12.2	2.8
ENN-B	0.01	0.05	0.13	95.3	13.9	2.9
BEA	0.005	0.01	0.02	258.3	70.0	21.9
ENN-A1	0.01	0.02	0.05	184.0	29.4	7.7
API	0.006	0.01	0.02	236.2	63.4	20.5

The concentrations corresponding to the quartiles P25, median, P75 of emerging mycotoxins in  $\mu\text{g/kg}$  in pig feed were converted in  $\mu\text{M}$  assuming their dilution in 1 L of gastrointestinal fluid. Ratio 1 was calculated using the P25 (1<sup>st</sup> quartile) concentration of emerging mycotoxin and P75 (3<sup>rd</sup> quartile) concentration of DON. Ratio 2 was calculated using the median (2<sup>nd</sup> quartile) concentration of DON and each emerging mycotoxin. Ratio 3 was calculated using the P75 concentration of emerging mycotoxin and P25 concentration of DON.