## Supplementary Materials: Calcination Improves the in Vivo Efficacy of a Montmorillonite Clay to Bind Aflatoxin G1 in Broiler Chickens: A Toxicokinetic Approach

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Analyte	Precursor ( <i>m/z</i> ) <sup>a</sup>	ion	Product ( <i>m</i> / <i>z</i> )	ions	CE⁵ (eV)	Cone (V)	Retention (min)	time
AFG1	329.1		243.0 <sup>c</sup>		23	35	4.38	
			311.1		18	35		
<sup>13</sup> C <sub>17</sub> - AFG1	346.1		257.1°		25	40	4.38	
in or			328.1		20	40		

Table S1. MRM transitions and MS/MS parameters for AFG1 and the internal standard, <sup>13</sup>C<sub>17</sub>-AFG1.

Note: a m/z = mass-to-charge ratio, b CE = collision energy, c ion used for quantification.

**Table S2.** Results of the within-run and between-run precision and accuracy evaluation for the analysis of aflatoxin G1 in chicken plasma.

Theoretical concentration (ng/mL)	Mean concentration ± SD (ng/mL)	Precision, RSD (%)	Accuracy (%)
0.50 ª	$0.50 \pm 0.01$	2.1	-0.6
0.50 <sup>b</sup>	$0.53 \pm 0.07$	13.0	6.2
5.00 <sup>a</sup>	$4.88 \pm 0.14$	3.0	-2.4
5.00 ь	$5.20 \pm 0.68$	13.0	4.1
50.0 ª	$48.2 \pm 1.8$	3.7	-3.6
50.0 <sup>b</sup>	48.3 ± 2.2	4.5	-3.4

Note: <sup>a</sup> Within-run accuracy and precision (n=6); <sup>b</sup> Between-run accuracy and precision (n= 3 x 6); SD: standard deviation; RSD: relative standard deviation; Acceptance criteria: accuracy: < 1 ng/mL: -50% to +20%,  $\geq$  1 to < 10 ng/mL: -40% to +20%,  $\geq$  10 to < 100 ng/mL: -30% to +10%, within-run precision (RSD<sub>max</sub>): < 1 ng/mL: 30 %,  $\geq$ 1 to < 10 ng/mL: 25.0%,  $\geq$  10 ng/mL: 15.0%, between-run precision: < 1 ng/mL: 45%,  $\geq$ 1 to < 10 ng/mL: 32%,  $\geq$  10 to < 100 ng/mL: 16% [VICH GL49].

animistration and (b) so minimuter of a daministration of 2 mg / i G/kg b/r for possible phase i and phase if inclubontes.												
	Concentration	Retention	Calculated	Observed	Mass	Mass	Molecular					
	(ng/mL )	time	monoisotopic	mass	error	error	ion					
		(min)	mass	(m/z)	(mDa)	(ppm)						
			(m/z)									
Sample A : AFG1	89.2	9.40	329.0661	329.0676	1.5	4.5	[M-H]+					
Sample B : AFG1	11.7	9.40	329.0661	329.0659	-0.2	-0.6	[M-H]+					

**Table S3.** Results of the investigation of the UHPLC-HRMS extracted ion chromatograms (XIC) of a plasma sample taken at (A) 10 min after intravenous administration and (B) 30 min after oral administration of 2 mg AFG1/kg BW for possible phase I and phase II metabolites.



Figure S1. UHPLC-MS/MS chromatogram of (A) a blank plasma sample, (B) a blank plasma sample spiked at the LOQ level (AFG1 concentration: 0.50 ng/ml), a plasma sample taken at (C) 10 min after intravenous administration (AFG1 concentration : 101.7 ng/ml) and (D) 30 min after oral administration of 2 mg AFG1/kg BW (AFG1 concentration : 12.0 ng/ml).



**Figure S2.** UHPLC-HRMS extracted ion chromatogram (XIC) at m/z = 329.0661 of a plasma sample taken (A) before and (B) at 10 min after intravenous administration of 2 mg/kg BW, showing a peak of AFG1 at Tr = 9.42 min (concentration : 89.2 ng/ml); (C) low energy spectrum of the peak at Tr = 9.42 min, showing the [M-H]+ ion of AFG1 (observed accurate mass at m/z = 329.0699, mass error: 1.5 mDa or 4.5 ppm); (D) high energy spectrum of the same peak, showing the two major fragment ions of AFG1 at m/z = 243.0646 and 311.0546.

## (A)

	l0min																	$\frown$			
	200307-030 Sm (Mn, 2x3)																	9.42			1: TOF MS ES+ 329.066 0.0500Da
а	100 8ª																	5928			6.81e4
	1.00 200307-030 Sm (Mn, 2x3)	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOF MS ES+
b	100		_~																		345.061 0.0500Da 549
~	0- <b>1</b>	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00
	200307-030 Sm (Mn, 2x3)																	9.42_			1: TOF MS ES+ 331.082 0.0500Da
С	»								<del></del>			<del></del>				+++++++++++++++++++++++++++++++++++++++		374		<del></del>	5.68e3
	1.00 200307-030 Sm (Mn, 2x3)	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOF MS ES+
d	100													7.7	7;113	8.19 133					327.051 0.0500Da 1.59e3
u	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00
	200307-030 Sm (Mn, 2x3)																				1: TOF MS ES+ 347.077 0.0500Da
e	0												····		<u>, , , , , , , , , , , , , , , , , , , </u>						779 Area
	1.00 200307-030 Sm (Mn, 2x3)	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOF MS ES+
f	100	~						_		_									~~~~		409.023 0.0500Da 256
	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00
~	200307-030 Sm (Min, 2X3)																				1: TOF MS ES+ 343.045 0.0500Da
g	* ************************************	1.50	2.00	2.50	2.00	2 50	4.00	4.50		5.50	6.00	6.50	7.00	7.50	·····				10.00	10.50	Δrea 11.00
	200307-030 Sm (Mn, 2x3)	1.00	2.00	2.00	3.00	3.00	4.00	4.00	5.00	5.50	0.00	0.00	7.00	7.50	0.00	0.00	9.00	9.00	10.00	10.50	1: TOF MS ES+
h	100					_										8.40;212		326			5.11e3
	1.00 200307_030_Sm (Mn_2x3)	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOE MS ES+
	100g											^									363.072 0.0500Da
1	0 <sup>4</sup>	1.50	2 00	2 50	3.00	3.50	4 00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00
	200307-030 Sm (Mn, 2x3)	1.00	2.00	2.00	0.00	0.00	4.00	4.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	5.00	5.00	10.00	10.00	1: TOF MS ES+ 505 098 0 0500Da
j	100	~~~					~~ <u>~</u>														323
-	1.00 200307-030 Sm (Mn, 2x3)	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOE MS ES+
1	100																				618.139 0.0500Da / 306
K	0°1	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00

**(B)** 

30	Imin																	$\frown$			
20	00307-103 Sm (Mn, 2x	3)																9.42_ 363			1: TOF MS ES+ 329.066 0.0500Da 6.01e3
a 20	1.00 0030*-103 Sm (Mn, 2x	1.50 3)	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOF MS ES+ 345.061 0.0500Da
b 20	0 1.00 00307-103 Sm (Mn, 2x	1.50 3)	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	623 Δrea 11.00 1: TOF MS ES+ 331.082.0.0500Da
C 20	100 0 1.00 00307-103 Sm (Mn, 2x	1.50 3)	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	316 Area 11.00 1: TOF MS ES+ 327.051.0.0500Da
d 20	100- 0 1.00 0307-103 Sm (Mn, 2x	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	617 4rea 11.00 1: TOF MS ES+
e		1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	347.077 0.0500Da 193 4rea 11.00
f	100 0 0 1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	409.023 0.0500Da 255 409.023 0.0500Da 255 4res
20 g	00307-103 Sm (Mn, 2x	3)	·		·····		~~~	~~~~~													1: TOF MS ES+ 343.045 0.0500Da 318 Area
20 h	1.00 00307-103 Sm (Mn, 2x	1.50 3)	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10:00	10.50	11.00 1: TOF MS ES+ 311.056 0.0500Da 333
20	0 1.00 0030*-103 Sm (Mn, 2x 100-	1.50 3)	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOF MS ES+ 363.072 0.0500D#
i 20	0 1.00 00307-103 Sm (Mn, 2x	1.50 3)	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00 1: TOF MS ES+ 505.098.0.05000
j 20	100 0 1.00 0307-103 Sm (Mn, 2x	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	145 145 11.00 1: TOF MS ES+
k		1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	618.139 0.0500Da 342 11 00

Figure S3. UHPLC-HRMS extracted ion chromatograms (XIC) of a plasma sample taken at (A) 10 min after intravenous administration (AFG1 concentration : 89.2 ng/ml) and (B) 30 min after oral administration of 2 mg AFG1/kg BW (AFG1 concentration : 11.7 ng/ml). The following mass-to-charge (m/z) values, corresponding with the theoretical exact mass of the protonated molecular ions [M-H]+, were extracted from the total ion chromatogram: (a) parent AFG1, C17H12O7 : 329.0661; (b) oxidation (+O), C17H12O8 : 345.0610; (c) reduction (+H2), C17H14O7 : 331.0818: (d) desaturation (-H2), C17H10O7 : 327.0505; (e) hydration (+H2O), C17H14O8 : 347.0767; (f) sulfation (+SO3), C17H12O10S : 409.0229; (g) oxidation + desaturation (+O-H2), C17H10O8 : 343.0454; (h) dehydration (-H2O), C17H10O6 : 311.0556; (i) dihydrodiol formation (+H2O2), C17H14O9 : 363.0716; (j) glucuronidation (+C6H8O6), C23H20O13 : 505.0982; (k) glutathione conjugation (+C10H15N3O5S), C27H27N3O12S ; 618.1394.



**Figure S4.** Correlation between the AFG1 plasma concentrations in 2 chickens that received an (A) oral and (B) intravenous administration of 2 mg/kg BW, after quantitative analysis using the UHPLC-MS/MS and UPLC-HRMS technique, respectively.