Meyer et al.:

Detection of Low Levels of Genotoxic Compounds in Food Contact Materials Using an Alternative HPTLC-SOS-Umu-C Assay

Supplementary Data

Tab. S1: Data of extracted/migrated amount
Data on extraction (IDs 35-37, 39 and 65) and migration (ID 64) samples; tin cans were weighed before and after extraction/migration

Tin can sample	Mass of extracted or migrated material (mg)	Amount (mg/dm^2)
ID 35	7.80	3.73
ID 36	12.16	5.82
ID 37	34.90	16.70
ID 39	34.90	16.70
ID 65	17.80	8.52
ID 64	8.00	3.83

Tab. S2: Data of the dose-response study (extract samples)
Data for the dose-response curves obtained by the new RP-HPTLC-UV/Vis/FLD-SOS-Umu-C assay for the five different *n*-hexane – acetone extracts

Tin can sample	Correlation coefficient	Relative standard deviation [%]
ID 35	0.987	9
ID 36	0.995	6
ID 37	0.997	5
ID 39	0.988	8
ID 65	0.993	7
Mean	**0.992**	**7**

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Tab. S3: Data of the dose-response study
Data for the repeated dose-response curves (n = 5) obtained by the new RP-HPTLC-UV/Vis/FLD-SOS-Umu-C assay for the 95%-ethanol migration sample ID 64

Plates for ID 64	Correlation coefficient	Relative standard deviation [%]
1	0.990	8
2	0.998	5
3	0.995	7
4	0.989	11
5	0.990	9
Mean	**0.992**	**8**

Tab. S4: Data of LOBD/LOBQ study
Data on determination of the LOBD and LOBQ of 4-NQO in six spiked tin can coatings via the new RP-HPTLC-UV/Vis/FLD-SOS-Umu-C assay

Tin can ID	LOBD [pg/band]	LOBD [ng/L]	LOBD [nmol/L]	Mean LOBD [pg/band] Precision [*%RSD*]
64	13	67	0.35	**17** **16%**
35	16	32	0.17	
36	18	59	0.31	
37	21	71	0.37	
39	16	54	0.28	
65	15	50	0.26	
Tin can ID	**LOBQ [pg/band]**	**LOBQ [ng/L]**	**LOBQ [nmol/L]**	**Mean LOBD [pg/band] Precision [*%RSD*]**
64	40	202	1.06	**50** **16%**
35	49	98	0.51	
36	53	177	0.93	
37	65	215	1.13	
39	49	163	0.86	
65	45	150	0.79	

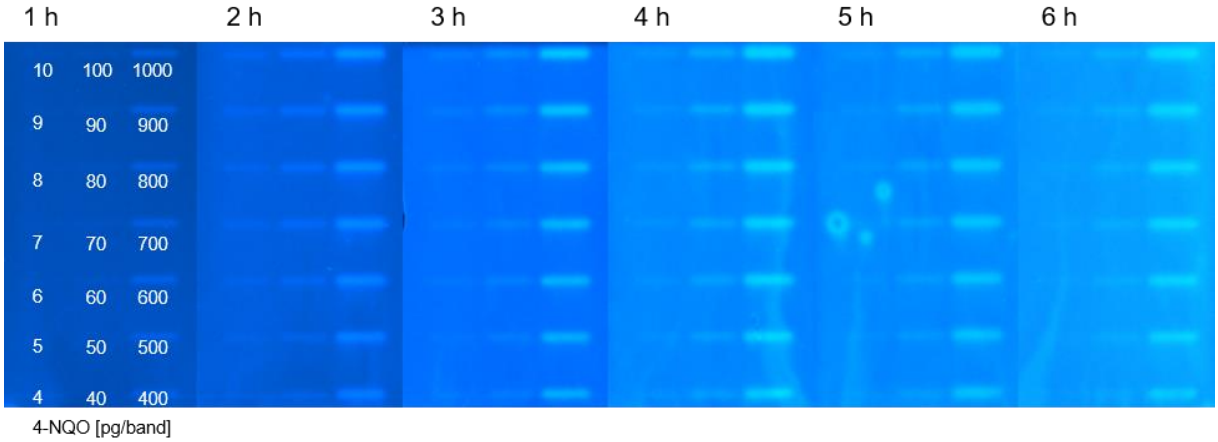

Fig. S1: Influence of incubation times of 1-6 h on response (images)
Planar SOS-Umu-C assay image at FLD 366 nm for different *Salmonella* incubation times of 1 h to 6 h on the adsorbent, all at OD_{660} of 0.2.

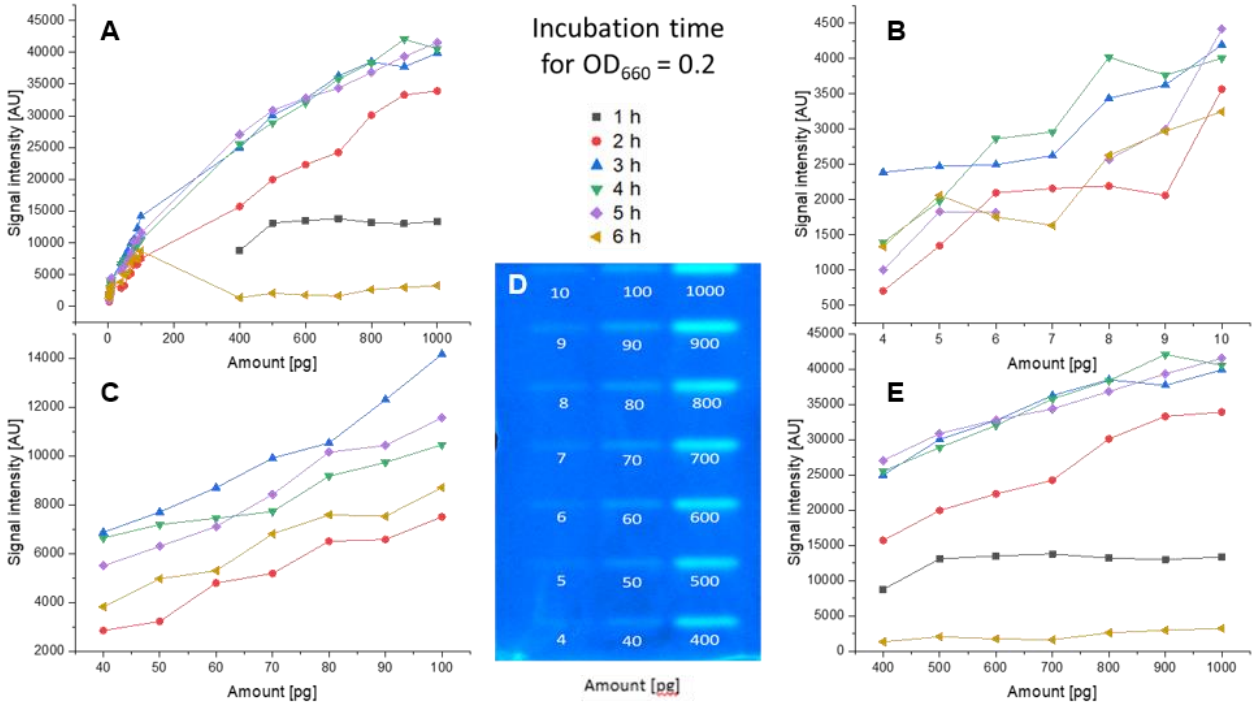

Fig. S2: Influence of incubation times on response for different amount ranges
Plots of signal intensities in densitograms at FLD 366/>400 nm of the planar SOS-Umu-C assay (image at FLD 366 nm after a 3-h incubation, D) against the applied 4-NQO amount for incubation times of 1-6 h (different colors): (A) 4-1000 pg/band region; zoom to the (B) 4-10 pg/band, (C) 40-100 pg/band and (E) upper 400-1000 pg/band region.

Fig. S3: Investigated tin cans with different coatings
FCM model studied consisting of five different tin can coatings (ID 35-37, 39 and 65), kindly provided for research purposes by the packaging supplier to Nestlé Research, Switzerland.

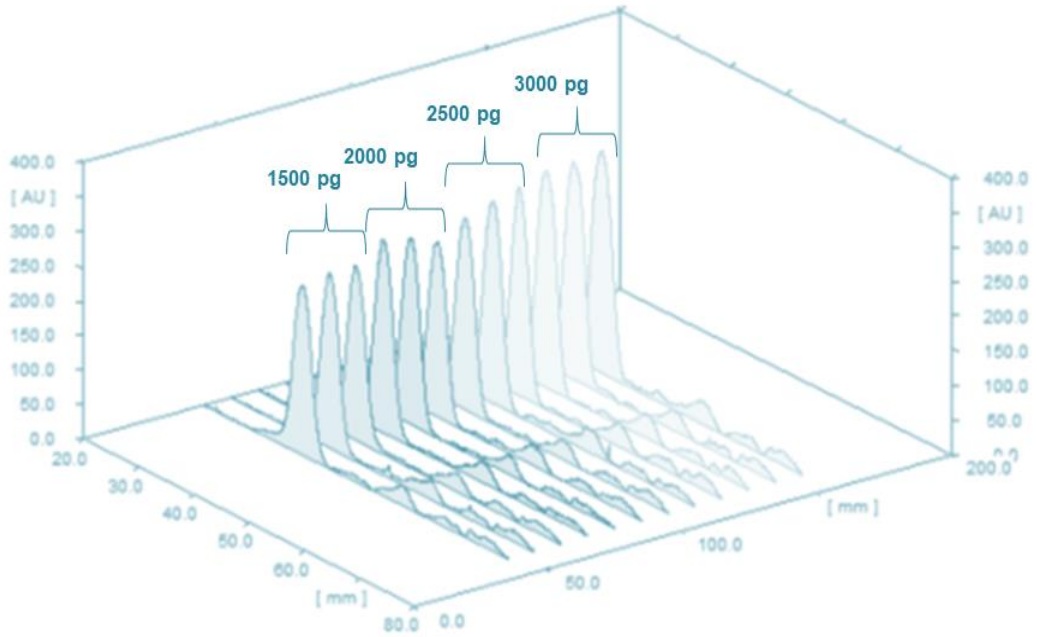

Fig. S4: Determination of the upper working range
3D densitogram (366/>400 nm) of the RP-HPTLC-SOS-Umu-C bioautogram for determination of the upper working range showing no substantial increase in signal intensity (peak area) above 1500 pg/band.

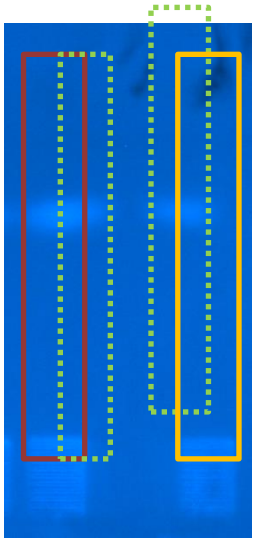

Fig. S5: Proof of absence of any matrix influence on separation
RP-HPTLC-SOS-Umu-C bioautogram at FLD 366 nm of a spiked (200 µL/area each, red box) *versus* original migrate sample ID 64 (orange box), partially oversprayed with 100 pg/area 4-NQO (green dotted box) showing no impact of the can matrix on the hR_F of 4-NQO at 48.

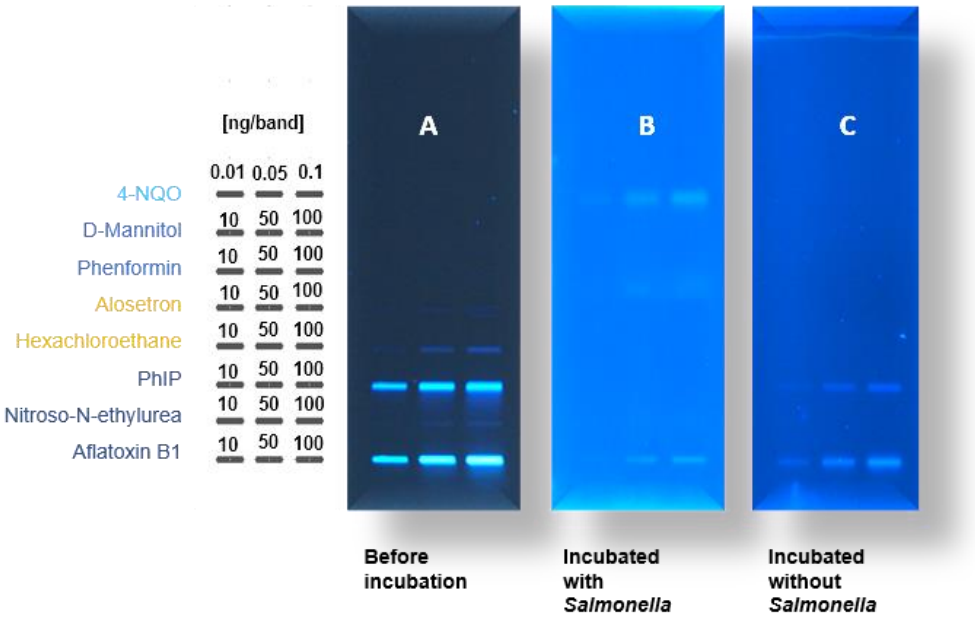

Fig. S6: Investigation of false positive responses
Images at FLD 366 nm showing fluorescent bands of the different substances, each applied on the HPTLC plate RP-18 W in three different amounts: (A) native fluorescence, (B) after the planar SOS-Umu-C assay and (C) same procedure as B but without *Salmonella* clearly identifies aflatoxin B1 as a false positive caused by its native fluorescence.

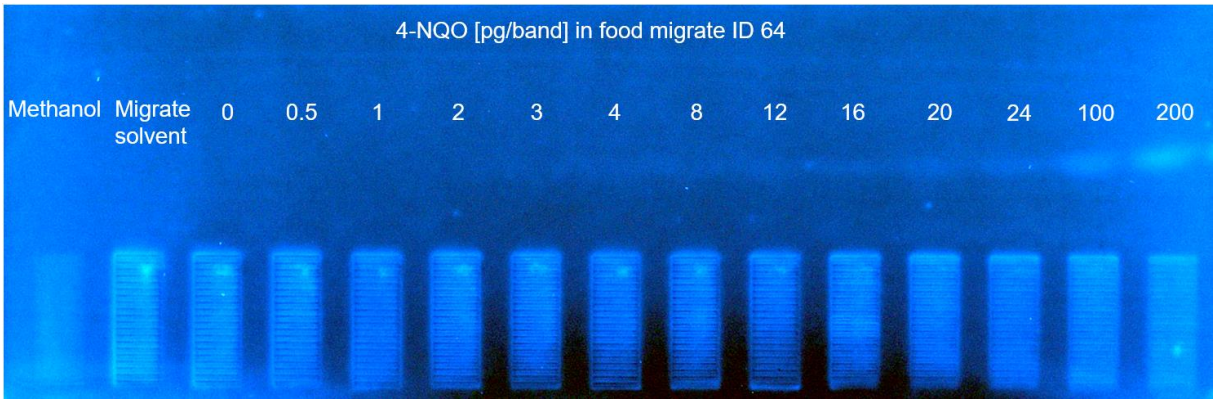

Fig. S7: Confirmation of the LEC in matrix
RP-HPTLC-SOS-Umu-C bioautogram at FLD 366 nm in matrix, showing food migrate ID 64 (200 µL each) spiked with 0 to 200 pg 4-NQO and applied as 7 mm x 20-mm area on the pretreated HPTLC RP-18 W plate (comparatively more cells settled down in the rills of the start area caused during spray-on application); it confirmed the LEC of 4-NQO in matrix (3 pg/band, 0.08 nM; experiment was performed twice).

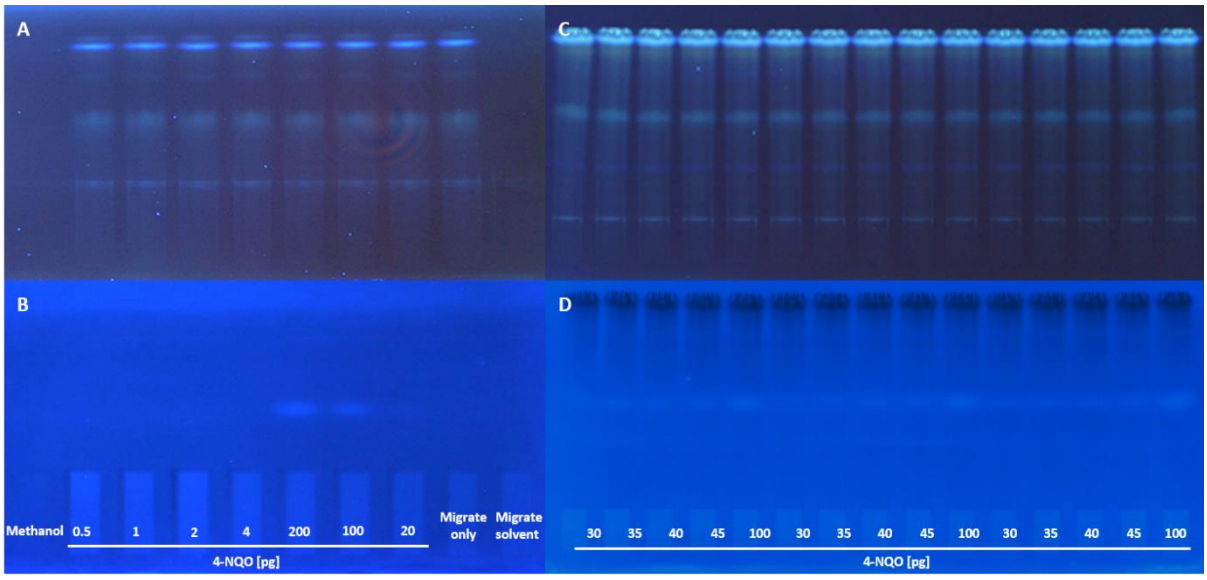

Fig. S8: Comparison of high and low level of migrated/extracted compounds
RP-HPTLC-chromatograms (A,C) and RP-HPTLC-SOS-Umu-C bioautograms (B,D) at FLD 366 nm, showing food migrate ID 64 (A,B; 200 µL each area) spiked with 0 to 200 pg 4-NQO and applied as 7 mm x 20 mm area (A,B), and food extract ID 39 (C,D; 500 µL each area) spiked with 30 to 100 pg 4-NQO and applied as 7 mm x 10 mm area on the pretreated HPTLC RP-18 W plate, together with respective negative controls (A,B). The migrate ID 64 is an example for the lowest level of migrated compounds (8 mg/can) and the extract ID 39 for higher levels of extracted compounds (35 mg/can, Tab. S1).

Eq. S1: Conversion of the units for the LEC determination.
Conversion of the amount on the HPTLC plate (pg/band) into nM, as used for the LEC determination experiment: 4-NQO was solved in methanol or migration sample. Twelve concentrations were prepared ranging from 0.5 to 200 pg/200 µL, whereby 200 µL of methanol or migration sample were applied, respectively. The following formula was used to calculate the nM concentration for each amount:

$$nM = \frac{m(4-NQO[pg])}{M(4-NQO[pMol])*200\ \mu L} * 5 * 1000 * \frac{1}{1000} = \frac{nMol}{L} \qquad \text{(Eq.S-1)}$$

with $\frac{m(4-NQO[pg])}{M(4-NQO[pMol]*200\ \mu L}$ = mass of 4-NQO in pg solved in 200 µL divided by the mol mass of 4-NQO in pMol, a factor of 5 taking into account the conversion from 200 µL to 1 mL, a factor of 1000 for the conversion of 1 mL to 1 L and a factor of 1/1000 to convert pmol into nmol.