**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/dm²) |
|----------------|---------------------------------------------|-----------------|
| 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          |                      |                  |
| 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          |                      |                  |
| Mean LOBD  | 17             | 50          | 0.89          | 16%                  |                  |

| Tin can ID | LOBD [pg/band] | LOBD [ng/L] | LOBD [nmol/L] | Mean LOBD [pg/band] | Precision [%RSD] |
|------------|----------------|-------------|---------------|----------------------|------------------|
| 64         | 40             | 202         | 1.06          |                      |                  |
| 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          |                      |                  |
| Mean LOBD  | 50             | 16%         |               |                      |                  |
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 hRF 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:

\[
\text{nM} = \frac{m(4\text{-NQO}[pg])}{M(4\text{-NQO}[pmol]) \times 200 \mu L} \times 5 \times 1000 \times \frac{1}{1000} = \frac{\text{nMol}}{L}
\]

(Eq.S-1)

with \( \frac{m(4\text{-NQO}[pg])}{M(4\text{-NQO}[pmol]) \times 200 \mu L} \) = 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.