

Simultaneous Detection of Multiple DNA Adducts in Human Lung Samples by Isotope-Dilution

UPLC-MS/MS

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Table S-1. Information about human tissue samples.

sample id	sex	age	reason of surgical intervention	smoking status^a
P1	m	80	lung tumor	cigarette smoker
P2	m	72	lung tumor	-
P3	m	77	lung tumor	-
P4	m	74	lung tumor	cigarette smoker
P5	f	74	lung tumor	non-smoker
P6	f	64	lung tumor	cigarette smoker
P7	f	72	lung tumor	-
P8	f	55	lung tumor	-
P9	f	50	lung tumor	-
P10	f	72	lung tumor	-

^a The smoking habits were not always reported and pack-years are not known for smokers.

Table S-2. Mass spectrometric parameters for the detection of DNA adducts by multiple reaction monitoring. Traces used as quantifier signals are highlighted if multiple transitions were available.

adduct	transitions		collision energy (eV)	Cone voltage (V)
	parent ion	daughter ion		
1,N ⁶ -εdA	276.1	160	13	15
[¹⁵ N ₅]1,N ⁶ -εdA	281.1	165	13	15
3,N ⁴ -εdC	252.1	136	13	15
[¹⁵ N ₃]3,N ⁴ -εdC	255.1	139	13	15
M1dG	304.1	188	10	13
[¹⁵ N ₃]M1dG	307.1	191	10	13
N ² -FFM-dG	376.1	109	15	32
	376.1	260.1	15	12
[¹⁵ N ₅ , ¹³ C ₁₀]N ² -FFM-dG	391.1	109	15	32
	391.1	270.1	15	12
N ⁶ -FFM-dA	360.1	109	25	35
	360.1	148	25	25
	360.1	244.1	25	15
[¹⁵ N ₅]N ⁶ -FFM-dA	365.1	109	25	35
	365.1	153	25	25
	365.1	249.1	25	15
N ² -MF-dG	348.1	81.1	17	37
	348.1	164	17	25
	348.1	232	17	12
[¹⁵ N ₅ , ¹³ C ₁₀]N ² -MF-dG	363.1	81.1	17	37
	363.1	174	17	25
	363.1	242	17	12
N ⁶ -MF-dA	332.1	81.1	27	35
	332.1	148.1	27	25
	332.1	216.1	27	15
[¹⁵ N ₅]N ⁶ -MF-dA	337.1	81.1	27	35
	337.1	153.1	27	25
	337.1	221.1	27	15
N ² -MIE-dG	444.1	164.1	22	27
	444.1	177.1	22	27
	444.1	328.1	22	11
[¹⁵ N ₅]N ² -MIE-dG	449.1	169.1	22	27
	449.1	177.1	22	27
	449.1	333.1	22	11
N ⁶ -MIE-dA	428.1	146.1	33	44
	428.1	177.2	33	28
	428.1	312.1	33	18
[¹⁵ N ₅]N ⁶ -MIE-dA	433.1	146.1	33	44

	433.1	177.2	33	28
	433.1	317.1	33	18
N^2 -1MIM-dG	427.1	145.1	20	43
	427.1	160.1	20	23
$[^{15}N_5]N^2$ -1MIM-dG	427.1	311.1	20	11
	432.1	145.1	20	43
	432.1	160.1	20	23
	432.1	316.1	20	11
N^6 -1MIM-dA	411.1	145.1	29	40
	411.1	160.1	29	23
$[^{15}N_5]N^6$ -1MIM-dA	411.1	295.1	29	15
	416.1	145.1	29	40
	416.1	160.1	29	23
	416.1	300.1	29	15
C8-PhIP-dG	490.1	250.1	35	55
	490.1	374.1	35	25
$[^{15}N_5, ^{13}C_{10}]$ C8-PhIP-dG	505.1	252.1	35	55
	505.1	384.1	35	25
C8-ABP-dG	435.2	210	23	50
	435.2	319.1	23	18
$[^{13}C_{10}]$ C8-ABP-dG	445.2	210	23	50
	445.2	324.1	23	18
N^2 -BPDE-dG	570.3	152	25	35
	570.3	257.1	25	35
$[^2H_7]N^2$ -BPDE-dG	570.3	454.1	25	10
	577.3	152	25	35
	577.3	264.1	25	35
	577.3	461.1	25	10
N^2 -MP-dG	482.1	215.1	20	45
	482.1	366.1	20	10
$[^{15}N_5, ^{13}C_{10}]N^2$ -MP-dG	497.1	215.1	20	45
	497.1	376.1	20	10
N^6 -MP-dA	466.2	215.1	30	35
	466.2	350.1	30	20
$[^{15}N_5]N^6$ -MP-dA	471.2	215.1	30	35
	471.2	355.1	30	20

Table S-3. Recovery values of different extraction methods for DNA adduct enrichment.

adduct	recovery $R_{M,Ad}$ (%) ^a			
	extraction with methanol	extraction with 1-butanol	SPE with C18 columns	SPE with Oasis columns
1, <i>N</i> ⁶ - ϵ dA	86.3	49.1	84.2	95.8
3, <i>N</i> ⁴ - ϵ dC ^b	-	-	-	-
M1dG	88.4	73.1	101.1	33.0
<i>N</i> ² -FFM-dG	105.3	66.4	88.3	93.8
<i>N</i> ⁶ -FFM-dA	95.2	91.4	101.8	79.4
<i>N</i> ² -MF-dG	99.7	71.5	96.4	91.8
<i>N</i> ⁶ -MF-dA	50.4	49.4	60.4	53.9
<i>N</i> ² -MIE-dG	87.4	72.9	89.6	88.9
<i>N</i> ⁶ -MIE-dA	76.8	59.5	87.6	91.3
<i>N</i> ² -1MIM-dG	84.9	56.2	74.1	78.4
<i>N</i> ⁶ -1MIM-dA	44.1	65.1	77.8	70.8
C8-PhIP-dG	89.0	44.6	2.1	18.2
C8-ABP-dG	57.1	35.3	53.4	60.6
<i>N</i> ² -BPDE-dG	79.1	84.0	71.7	58.2
<i>N</i> ² -MP-dG	86.9	69.2	54.1	77.2
<i>N</i> ⁶ -MP-dA	12.2	10.0	8.2	25.5

a The $R_{M,Ad}$ values were determined as follows. Five samples of 350 μ g commercially available DNA were spiked with a mix of isotope-labeled reference substances of the DNA adducts, subjected to enzymatic hydrolysis and processed by one of the work-up methods. The internal standards were analyzed by UPLC-MS/MS and the mean peak areas \bar{A}_1 of the quantifier signals were determined. In a second set of five samples, we analyzed the internal standards mixed with the residuals of DNA digests, which were subjected to one of the extraction methods (\bar{A}_2). The relative signal loss of a specific analyte due to the extraction procedure was calculated ($R_{M,Ad} = \bar{A}_1/\bar{A}_2 \cdot 100$ %). Maximum performance values are highlighted in dark grey whereas second best methods are shaded in light grey.

b A sizeable signal in unmodified herring sperm DNA used for this assay co-eluted with the internal standard and prohibited the calculation of $R_{M,Ad}$ for 3,*N*⁴- ϵ dC.

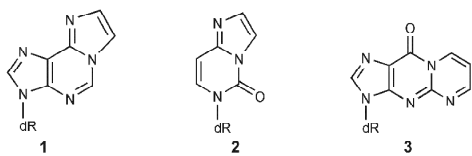
Table S-4. Effects of DNA residuals from different extraction methods on the UPLC-MS/MS analytical results of DNA adducts.

adduct	$IS_{M,Ad} (\%)^a$			
	extraction with methanol	extraction with 1-butanol	SPE with C18 columns	SPE with OASIS columns
1, <i>N</i> ⁶ - ϵ dA	9.9	49.6	18.0	94.6
3, <i>N</i> ⁴ - ϵ dC ^b	-	-	-	-
M1dG	35.3	59.5	62.5	53.2
<i>N</i> ² -FFM-dG	31.4	59.5	52.3	65.0
<i>N</i> ⁶ -FFM-dA	64.7	101.3	71.1	76.9
<i>N</i> ² -MF-dG	22.0	55.0	43.0	60.2
<i>N</i> ⁶ -MF-dA	31.1	39.4	36.6	43.2
<i>N</i> ² -MIE-dG	59.0	87.5	62.8	85.2
<i>N</i> ⁶ -MIE-dA	57.1	81.6	63.3	43.1
<i>N</i> ² -1MIM-dG	51.5	66.5	40.8	89.7
<i>N</i> ⁶ -1MIM-dA	56.7	71.3	52.6	52.3
C8-PhIP-dG	99.7	86.3	95.1	77.2
C8-ABP-dG	57.1	90.0	55.7	76.3
<i>N</i> ² -BPDE-dG	57.6	94.0	63.6	91.3
<i>N</i> ² -MP-dG	58.8	78.9	63.7	70.9
<i>N</i> ⁶ -MP-dA	51.2	78.9	53.0	53.8

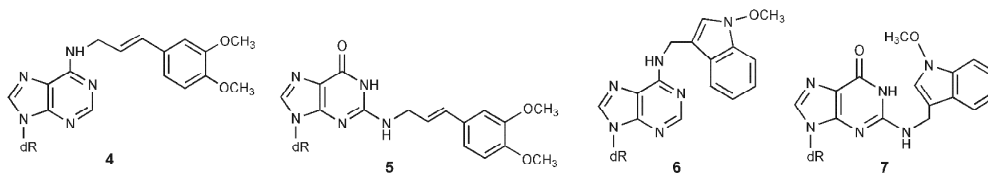
a The $IS_{M,Ad}$ values were determined as follows. Digests of 350 μ g commercially available DNA were processed with the work-up methods. The residuals were spiked with the internal reference compounds, which were then analyzed by UPLC-MS/MS. The resulting mean peak areas (\bar{A}_1) were compared to those resulting from direct injection of the internal standards (\bar{A}_2). The relative signal intensity was calculated ($IS_{M,Ad} = \bar{A}_1/\bar{A}_2 \cdot 100 \%$). Maximum remaining peak areas are highlighted in dark grey whereas second best methods are shaded in light grey.

b A sizeable signal in unmodified herring sperm DNA used for this assay co-eluted with the internal standard and prohibited the calculation of $IS_{M,Ad}$ for 3,*N*⁴- ϵ dC.

DNA adducts from lipid peroxidation products



DNA adducts from plant metabolites



DNA adducts from food contaminants

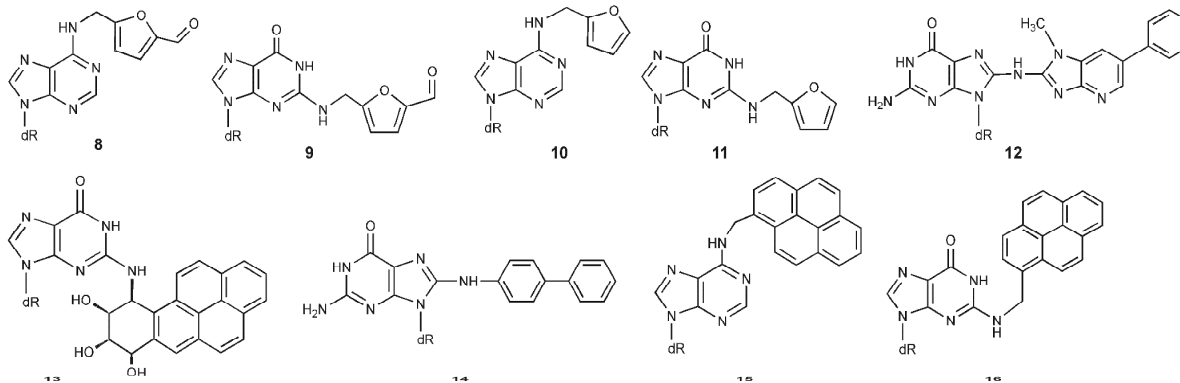


Figure S-1. Structural representations of DNA adducts available as isotope-labeled internal reference compounds. The adducts are formed from 1) lipid peroxidation products (N^6 - ϵ -dA **1**, $3,N^4$ - ϵ -dC **2** and M1dG **3**), 2) secondary plant metabolites methyleugenol (N^6 -MIE-dA **4**, N^2 -MIE-dG **5**) and neoglucobrassicin (N^6 -1MIM-dA **6**, N^2 -1MIM-dG **7**), or 3) food contaminants 5-hydroxymethylfurfural (N^6 -FFM-dA **8**, N^2 -FFM-dG **9**), furfuryl alcohol (N^6 -MF-dA **10**, N^2 -MF-dG **11**) the heterocyclic aromatic amine PhIP ($C8$ -PhIP-dG **12**), benzo[*a*]pyrene (N^2 -BPDE-dG **13**), 4-aminobiphenyl ($C8$ -ABP-dG **14**) and 1-methylpyrene (N^6 -MP-dA **15**, N^2 -MP-dG **16**).

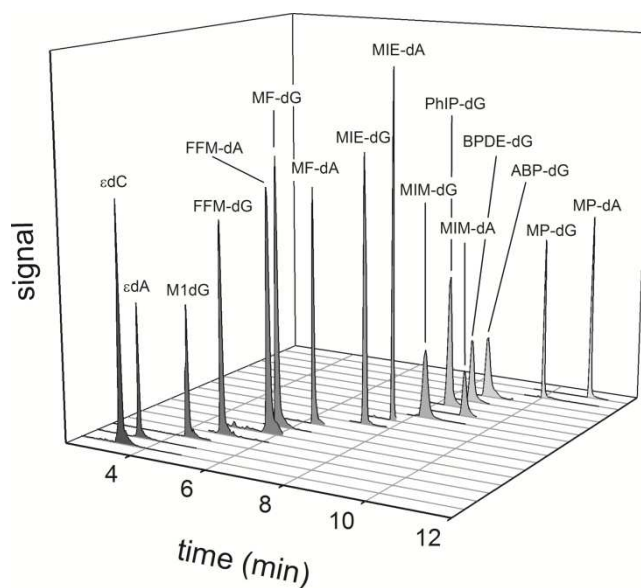


Figure S-2. Representative chromatograms of quantifier traces from isotope-labeled internal references of all DNA adducts included in this study. For the sake of clarity peaks are denoted with simplified acronyms omitting atomic positions and the sites of stable isotope labeling. Usually, the signals were recorded from injections of 200 fmol of the adducts on column. Amounts of 1000 fmol were required for comparable signal intensities of [$^{15}\text{N}_5, ^{13}\text{C}_{10}$]C8-PhIP-dG and [$^2\text{H}_7$] N^2 -BPDE-dG, whereas only 50 fmol were injected for the peaks of [$^{15}\text{N}_5$]1, N^6 - ϵ dA, [$^{15}\text{N}_5$] N^6 -MF-dA and [$^{15}\text{N}_5$] N^6 -MP-dA. The broadening of the peaks between 8 and 10 min is a technical artifact. The simultaneous recording of up to 38 fragmentations in this time interval reduced the resolutions, which in turn lead to peak broadening in the smoothing process. The analysis was conducted using water (solvent A) and acetonitrile (solvent B), both of which were acidified with 0.25 % acetic acid and 0.25 % formic acid, as eluents. Thus, retention times deviate from those chromatographic runs presented in the manuscript.

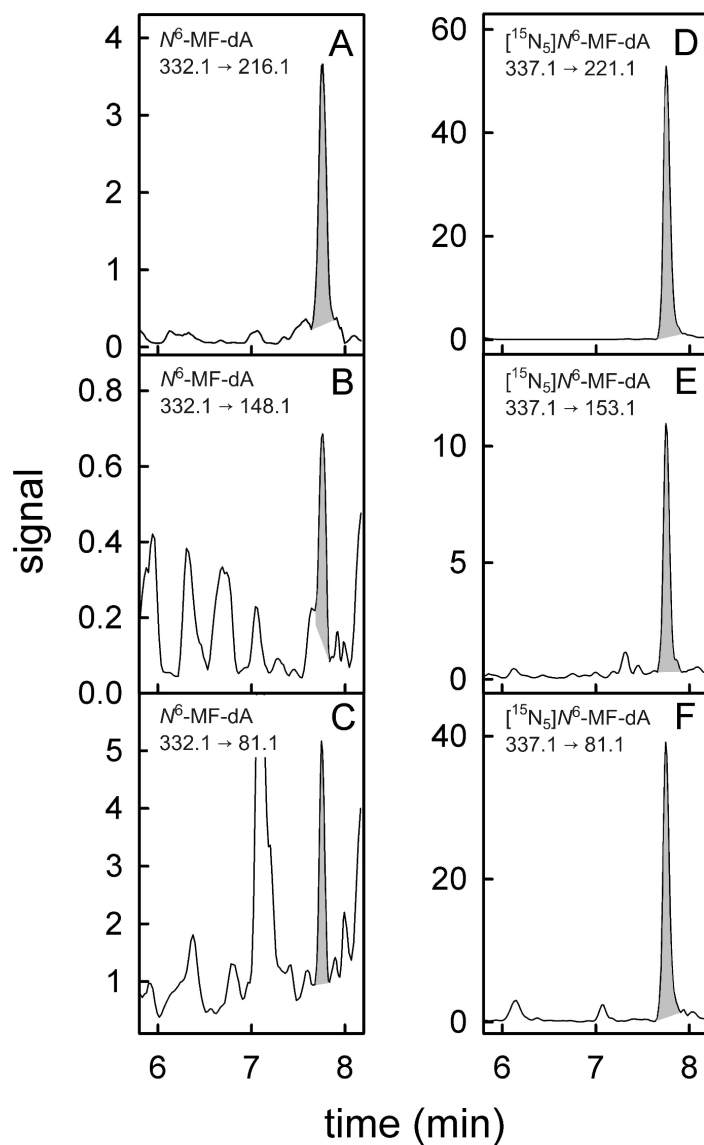


Figure S-3. UPLC-MS/MS chromatograms of a digested DNA sample from patient 8 containing N^6 -MF-dA. The left panels depict the chromatograms of N^6 -MF-dA with the fragmentations 332.1 \rightarrow 216.1 (A), 332.1 \rightarrow 148.1 (B) and 332.1 \rightarrow 81.1 (C) that were monitored together with the transitions 337.1 \rightarrow 221.1 (D), 337.1 \rightarrow 153.1 (E) and 337.1 \rightarrow 81.1 (F) of the internal isotope-labeled standard $[^{15}\text{N}_5]N^6$ -MF-dA. The most intensive signal (332.1 \rightarrow 216.1) was used as quantifier signal.

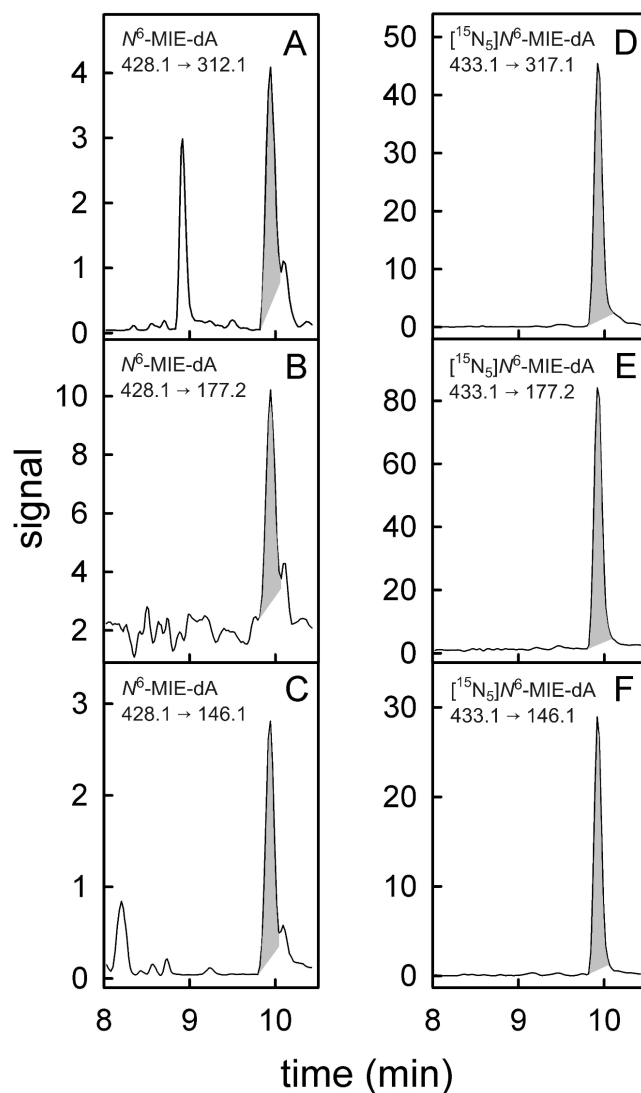


Figure S-4. UPLC-MS/MS chromatograms of a digested DNA sample from patient 6 containing the methyleugenol adduct N^6 -MIE-dA. Panels on the left hand side show the chromatograms of N^6 -MIE-dA with the trace 428.1 \rightarrow 312.1 (panel A), the quantifier trace 428.1 \rightarrow 177.2 (panel B) and the second qualifier trace 428.1 \rightarrow 146.1 (panel C). On the right hand side are the parallel recordings of the isotope-labeled standard $[^{15}\text{N}_5]N^6$ -MIE-dA with the transitions 433.1 \rightarrow 317.1 (panel D), 433.1 \rightarrow 177.2 (panel E) and 433.1 \rightarrow 146.1 (panel F).

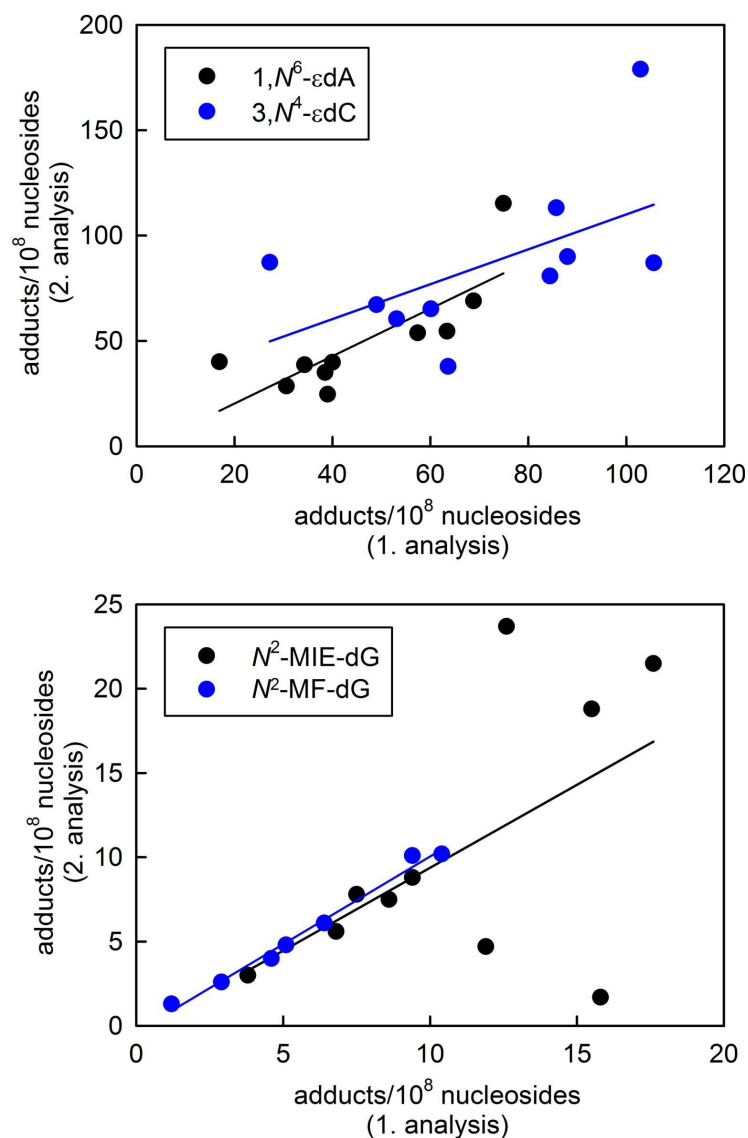


Figure S-5. Correlations between DNA adduct levels determined in two independent analyses in ten samples of human lung. Upper panel: 1,N⁶-εdA (black circles, $m = 1.13$, $r^2 = 0.632$) and 3,N⁴-εdC (blue circles, $m = 0.83$, $r^2 = 0.301$), lower panel: N²-MIE-dG (black circles, $m = 0.98$, $r^2 = 0.304$) and N²-MF-dG (blue circles, $m = 1.04$, $r^2 = 0.987$). Only seven N²-MF-dG levels were included, which exceeded the LOD in both analyses.