Supporting information for

Assessment of human internal exposure to rubber-derived chemicals

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Instrumental analyses

Determination of target analytes was conducted on an ultra-high performance liquid chromatography (UHPLC) equipped with a ZORBAX Extend-C18 column (3.5 μm, 2.1 × 100 mm, Agilent Technologies). The UHPLC was coupled to a 5500 triple quadrupole mass spectrometer (SCIEX, Redwood City, CA). The sample injection volume was 10 μL and the LC column was kept at 40°C. The mobile phases constituted 0.2 mM ammonium acetate (A) and methanol (B). The mobile phase flow rate was maintained at 0.5 mL/min. The following gradient program was employed: 10 % B (held for 0.5 min), linearly ramped to 50 % B in 1 min, followed by a linear increase to 99 % B in 6 min (held for 1.5 min), and then changed to 10%B in 0.1 min and equilibrated for 2 min. The MS was equipped with a TurboIonSpray® electrospray ionization (ESI) probe and operated in multiple reaction monitoring (MRM) mode (positive). The optimized MRM parameters are given in Table S2. The ESI source conditions were as follows: ion spray voltage, 4500 V; source temperature, 500 °C; ion source Gas 1, 30; ion source Gas 2, 40; curtain gas, 30; collision gas, medium. Data were collected and analyzed using the Analyst software (version 1.7.2; AB Sciex, Framingham, MA, USA). Quantification of target analytes was conducted based on calibration curves developed from a suite of calibration standards (prepared in methanol) at five to seven different concentrations.

Optimization of the extraction solvent

To achieve efficient extraction of target chemicals from plasma samples, we optimized the extraction solvent according to their structures based on our previously reported liquid-liquid extraction (LLE) method.[1] Briefly, an aliquot of 300 μL sheep serum was spiked with surrogate standards (5 ng each) and extracted with the extraction solvent under vortexing. After centrifugation at 3000 rpm, the supernatant was collected, and the extraction was repeated twice. The supernatants were combined, concentrated to 100 μL, frozen overnight at -80 °C, and then centrifuged to collect the final supernatant for instrumental analysis. Three different types of extraction solvents were tested, including ethyl acetate containing 0.1% ammonia, acetonitrile (ACN), and a mixture of

acetone and hexane (1:1, v/v). The recoveries of target chemicals were compared (Table S3) and the results showed that ACN exhibited the best recovery performance for most analytes.

Quality assurance and quality control (QA/QC) tests

Matrix effect. In brief, sheep serum (300 μL each) was extracted without the addition of any target analytes or surrogate standards and processed in six replicates. The final extract was reconstituted and then aliquoted into two sub-samples with equal volume (50 μL each). Sub-sample A was spiked with 30 μL of standards including a standard mixture of analytes (10 μL, 5 ng), 10 μL of surrogate standards (5 ng), and 10 μL of internal standards (5 ng). Sub-sample B was spiked with 20 μL of methanol and 10 μL of internal standards (5 ng). An external standard solution (S) was prepared by mixing the 30 μL of analyte mixtures (including target analytes, surrogate standards, and internal standards, 5 ng for each) with 50 μL methanol. The matrix effect was evaluated as the percentage of signal enhancement or suppression, using the following equation:

$$ME\% = \frac{A_i - B_i}{S_i} \times 100\%$$

where A_i, B_i, and S_i are the chromatographic peak areas of the analyte (i) in sub-samples A and B and external standard solution (S), respectively.

Precision. The precision was assessed by evaluation of the intra-batch and inter-batch variations. The intra-batch precision was calculated as the coefficients of variation (CVs) in the detected concentrations among six replicate sheep serum samples spiked with standards (2 ng each). The inter-batch precision was calculated as CVs in the detected concentrations between three batches (six samples per batch).

References

1. Huang W, Pan X-F, Tang S, et al. 2023. Target Exposome for Characterizing Early Gestational Exposure to Contaminants of Emerging Concern and Association with Gestational Diabetes Mellitus. **Environ Sci Technol** 57(36):13408-13418. https://pubmed.ncbi.nlm.nih.gov/37651547/, doi:10.1021/acs.est.3c04492..

Table S1. Detailed information on the rubber-derived chemicals included in this study.

Chemicals	Full Name	CAS	Supplier[a]
Rubber-derived chemicals			
6PPD	N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine	793-24-8	AccuStandard
6PPD-Q	N-(1,3dimethylbutyl)-N′-phenyl-p-phenylenediamine quinone	2754428-18-5	TRC
IPPD	N-isopropyl-N′-phenyl-1,4-phenylenediamine	101-72-4	AccuStandard
IPPD-Q	N-isopropyl-N′-phenyl-1,4-phenylenediamine quinone	68054-73-9	TRC
DPPD	N, N′-diphenyl-p-phenylenediamine	74-31-7	AccuStandard
CPPD	N-phenyl-N′-cyclohexyl-p-phenylenediamine	101-87-1	AccuStandard
DTPD	N, N′-di(o-tolyl)-p-phenylenediamine	27417-40-9	AccuStandard
44PD	N, N′-di-2-butyl-p-phenylenediamine	101-96-2	AccuStandard
7PPD	N-(1,4-dimethylpentyl)-N'-phenyl-1,4-benzenediame	3081-01-4	AccuStandard
DAPPD	N, N'-diacetyl-p-phenylenediamine	140-50-1	TCI
4s DPA	4-Nitrosodiphenylamine	156-10-5	TCI
DPA	Diphenylamine	122-39-4	AccuStandard
4-NO$_2$-DPA	4-Nitrodiphenylamine	836-30-6	TCI
2-NO$_2$-DPA	2-Nitrodiphenylamine	119-75-5	TCI
2,4-NO$_2$-DPA	2,4-Dinitrodiphenylamine	961-68-2	TRC
4,4'-NO$_2$-DPA	Bis (4-nitrophenyl) amine	1821-27-8	TCI
PANA	N-Phenylnaphthyl-1-amine	90-30-2	AccuStandard
DPG	1,3-Diphenylguanidine	102-06-7	AccuStandard
DTG	1,3-Di-o-tolylguanidine	97-39-2	AccuStandard
DPB	N, N′-Diphenylbenzidine	531-91-9	TRC
HMMM	Hexa(methoxymethyl) melamine	3089-11-0	TCI
Surrogate standards			

6PPD-Q-d$_5$	6PPD-quinone-d$_5$	NA[b]	TRC
DPG-d$_{10}$	N, N′-Diphenylguanidine-d$_{10}$	NA	TRC
DPA-d$_{10}$	Diphenylamine-d$_{10}$	37055-51-9	Aladdin
Internal standard			
CMP-d$_{10}$	Coumaphos-d$_{10}$	287397-86-8	AccuStandard

[a]Suppliers: AccuStandard, AccuStandard Inc. (New Haven, Connecticut, USA); TRC, Toronto Research Chemicals (Toronto, Canada); TCI, TCI America (Seekonk, Massachusetts, USA); Aladdin, Aladdin Chemical Co. Ltd (Shanghai, China). [b]NA, not available.

Table S2. Optimized MRM parameters of the rubber-derived chemicals in this study.

Analyte	Q1 (Da)	Q3 (Da)	DP (V)	EP (V)	CE (V)	CXP (V)
6PPD	269.0	184.1	125	10	30	13
	269.0	212.4	89	10	28	13
6PPD-Q	299.2	241.1	175	10	41	13
	299.2	215.1	205	10	26	13
IPPD	227.2	184.1	95	10	27	13
	227.2	107.1	95	10	50	13
IPPD-Q	257.1	187.1	165	10	34	13
	257.1	215.1	118	10	19	13
DPPD	261.2	184.1	180	10	34	13
	261.2	107.1	247	10	51	13
CPPD	267.2	185.1	96	10	22	13
	267.2	93.1	95	10	47	13
DTPD	289.2	198.1	178	10	34	13
	289.2	183.0	189	10	51	13
44PD	221.2	164.1	296	10	21	13
	221.2	135.1	295	10	37	13
7PPD	283.2	184.1	95	10	34	13
	283.2	93.1	95	10	47	13
DAPPD	193.0	109.0	125	10	30	13
	193.0	151.0	270	10	26	13
4s DPA	199.0	128.0	162	10	55	13
	199.0	181.1	162	10	38	13
4-NO$_2$-DPA	215.1	167.1	160	10	46	13
	215.1	139.1	189	10	85	13
2-NO$_2$-DPA	215.2	77.0	183	10	55	13
	215.2	180.1	183	10	27	13
4,4'-NO$_2$-DPA	260.1	243.0	172	10	24	13
	260.1	213.1	116	10	31	13
2,4-NO$_2$-DPA	260.3	123.0	174	10	22	13
	260.3	115.0	170	10	35	13
DPA	170.1	93.0	268	10	36	13
	170.1	65.1	282	10	43	13
DPG	212.0	119.0	130	10	43	13
	212.0	94.1	131	10	38	13
DTG	240.2	133.1	130	10	44	13
	240.2	108.1	112	10	48	13
PANA	220.1	115.1	189	10	63	13
	220.1	92.1	261	10	32	13
DPB	337.2	156.1	220	10	78	13
	337.2	183.1	220	10	68	13
HMMM	391.0	177.0	54	10	34	13

	391.0	207.1	45	10	33	13
6PPD-Q-d$_5$	304.0	246.0	77	10	33	13
	304.0	192.0	87	10	34	13
DPG-d$_{10}$	222.0	99.0	60	10	44	13
	222.0	123.0	79	10	34	13
DPA-d$_{10}$	180.0	98.0	64	10	29	13
	180.0	96.0	66	10	26	13
CMP-d$_{10}$	373.0	228.0	100	10	34	13

Abbreviations. Q1, precursor ion; Q3, product ion; DP, decluttering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

Table S3. Optimization of the extraction solvent in liquid-liquid extraction-based sample preparation method.

Analyte	Recovery (%)		
	Solvent 1	Solvent 2	Solvent 3
6PPD	18.4	85.5	0.1
6PPD-Q	60.3	88.0	103.3
IPPD	18.7	79.1	0.0
IPPD-Q	69.4	80.1	72.1
DPPD	13.9	98.5	14.1
CPPD	19.1	72.2	0.1
DTPD	5.9	57.3	12.1
44PD	50.3	48.2	48.1
7PPD	11.7	63.5	0.1
DNPD	5.9	22.4	3.2
4s DPA	65.8	51.7	1.7
DAPPD	87.1	66.5	49.6
OPPD	6.8	24.0	0.0
4-NO$_2$-DPA	97.8	89.0	99.3
2-NO$_2$-DPA	63.9	66.4	51.6
4,4'-NO$_2$-DPA	57.8	63.3	50.0
2,4-NO$_2$-DPA	116.7	76.8	38.3
DPA	41.8	60.3	9.8
DPG	70.7	71.9	58.7
DTG	57.8	84.3	54.6
PANA	48.0	77.2	1.1
DPB	39.5	43.6	2.8
HMMM	101.7	83.5	157.0

Note: Solvent 1, ethyl acetate containing 0.1% ammonia; Solvent 2, acetonitrile ACN; Solvent 3, a mixture of acetone and hexane (1:1, v/v).

Table S4. Procedural blank, matrix effects, and precision of the rubber-derived chemicals.

Analyte	Procedural blank (ng/mL)[a]	Precision		Matrix effect (%, n = 6)
		Intra-batch (%, n = 6)	Inter-batch (%, n = 3)	
6PPD		19.6	15.1	103.1
6PPD-Q		19.2	8.9	98.2
IPPD		7.4	7.4	155.2
IPPD-Q		20.7	17.1	80.4
DPPD		20.8	10.8	106.1
CPPD		23.9	8.6	128.3
DTPD		16.1	18.1	123.5
44PD		20.3	8.8	90.2
7PPD		19.6	18.2	114.9
DAPPD		17.0	15.1	68.5
4s DPA	0.041	17.9	12.5	98.6
4-NO$_2$-DPA	0.27	13.3	6.7	102.5
2-NO$_2$-DPA		19.6	14.0	114.0
4,4'-NO$_2$-DPA		17.3	19.5	85.5
2,4'-NO2-DPA	0.11	14.9	16.5	94.0
DPA		19.9	12.3	133.0
DPG		11.5	3.9	34.8
DTG		18.4	18.5	109.4
PANA		16.7	20.7	145.8
DPB		18.6	17.8	68.6
HMMM	0.035	15.1	13.3	105.3

[a]No fill indicates undetectable in blank samples.

Table S5. Gender and age distribution of the rubber manufacturer workers.

Age (years)	Female	Male	Total
20-39	17	34	51
40-59	37	74	111
Total	54	108	162

6PPD **IPPD** **DPPD**

6PPD-Q **IPPD-Q** **CPPD**

DTPD **7PPD** **44PD**

DAPPD **4s DPA** **4-NO$_2$-DPA**

2-NO$_2$-DPA **2,4-NO$_2$-DPA** **4,4'-NO$_2$-DPA**

DPA **DPG** **DTG**

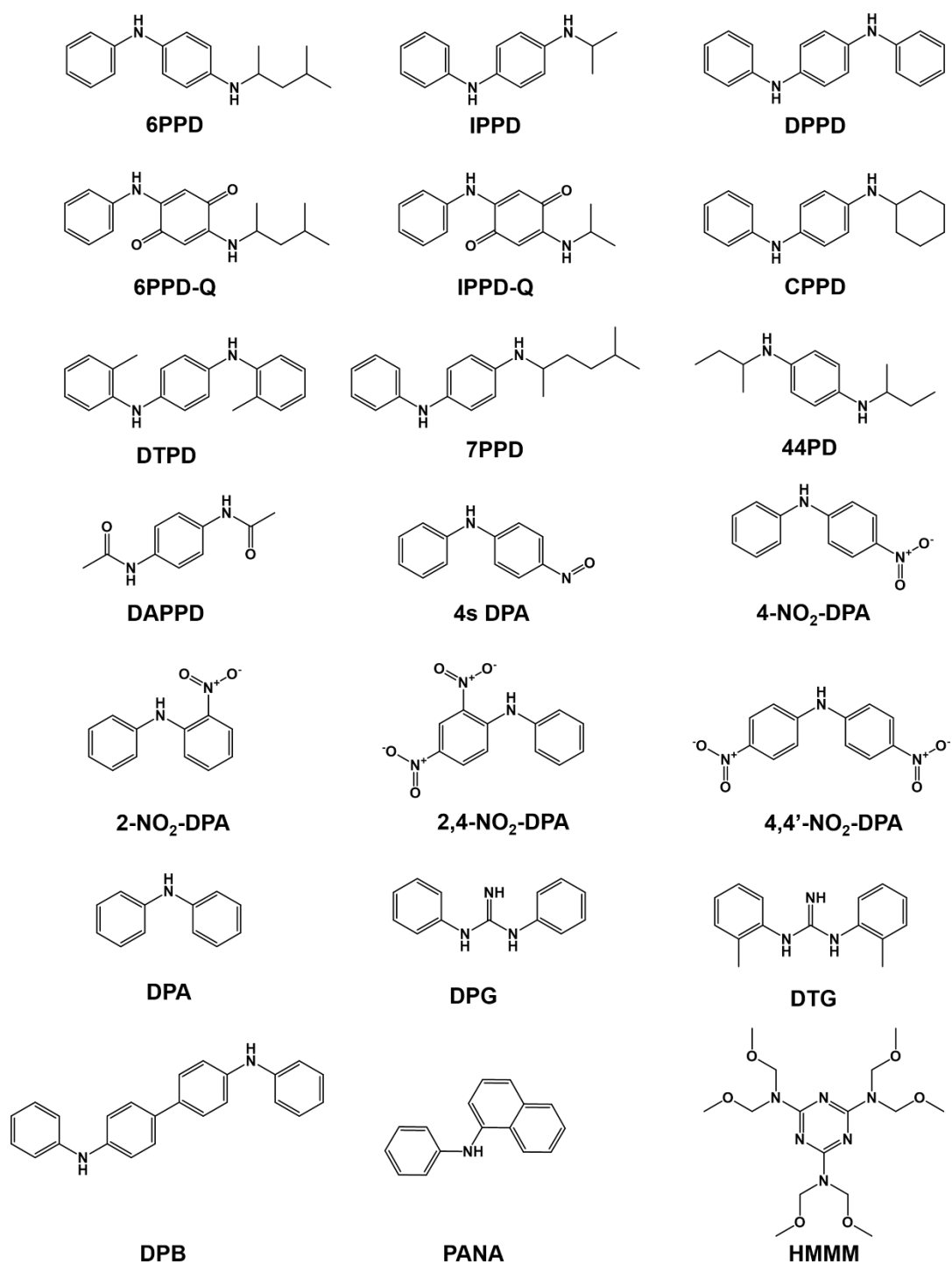**DPB** **PANA** **HMMM**

Figure S1. Structures of the rubber-derived chemicals included in this study.
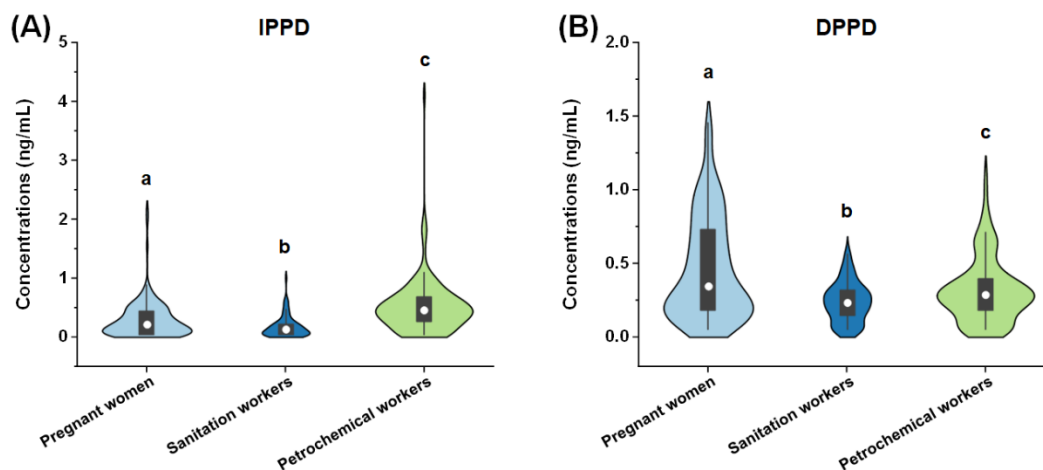
Figure S2. Distribution and comparison of IPPD and DPPD in pregnant women, sanitation workers, and rubber manufacturer workers. IPPD, *N*-isopropyl-*N'*-phenyl-1,4-phenylenediamine; DPPD, *N, N'*-diphenyl-p-phenylenediamine. Different letters above the violins indicate statistically significant differences at *P* < 0.05 across the populations tested using one-way ANOVA followed by Dunnett's post hoc test.