SUPPORTING INFORMATION

Literature review and evaluation of biomarkers, matrices and analytical methods for chemicals selected in the research program Human Biomonitoring for the European Union (HBM4EU)

Gabriele Sabbioni[1,2,3*], Argelia Castaño[4], Marta Esteban López[4], Thomas Göen[5], Hans Mol[6], Margaux Riou[7], and Romuald Tagne-Fotso[7].

[1]Università della Svizzera Italiana (USI), Research and Transfer Service, Lugano, Switzerland

[2]Institute of Environmental and Occupational Toxicology, Airolo, Switzerland

[3]Walther-Straub-Institute for Pharmacology and Toxicology, Ludwig-Maximilians-University Munich, Munich, Germany

[4]National Centre for Environmental Health, Instituto de Salud Carlos III (ISCIII), Majadahonda, Spain

[5]Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander Universität Erlangen-Nürnberg (IPASUM), Erlangen, Germany

[6]Wageningen Food Safety Research, Part of Wageningen University & Research, Wageningen, Netherlands

[7]Department of Environmental and Occupational Health, Santé publique France, the national public health agency, Saint-Maurice, France

*Gabriele Sabbioni, gabriele.sabbioni@bluewin.ch; Argelia Castano, castano@isciii.es; Marta Estaban López, m.esteban@isciii.es; Thomas Göen, thomas.goeen@fau.de; Hans Mol, hans.mol@wur.nl; Margaux Riou, Margaux.RIOU@santepubliquefrance.fr; Romuald Tagne-Fotso, Romuald.TAGNE-FOTSO@santepubliquefrance.fr.

*Corresponding author: Prof. Dr. Gabriele Sabbioni, Institute of Environmental and Occupational Toxicology, CH-6780 Airolo, Switzerland

Tables

Table S1. Chemical structures of biomarkers found after exposure to acrylamide.

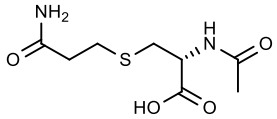
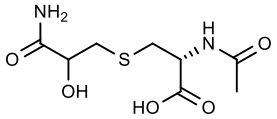
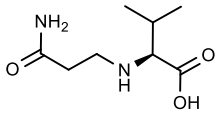
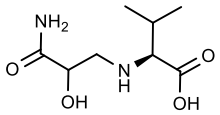
Acronym; compound name; CAS no.	Structure
AAMA N-Acetyl-S-(2-carbamoylethyl)-L-cysteine; N-Acetyl-S-(3-amino-3-oxopropyl)-L-cysteine CAS:81690-92-8	
GAMA N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine; N-Acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)-L-cysteine CAS:137698-08-9	
AAVal N-(2-Carbamoylethyl)valine; N-(3-Amino-3-oxopropyl)-L-valine CAS:51078-53-6	
GAVal N-(2-Carbamoyl-2-hydroxyethyl)valine; N-(3-Amino-2-hydroxy-3-oxopropyl)-L-valine CAS252663-74-4	

Table S2. Chemical structures of biomarkers found after exposure to mycotoxins.

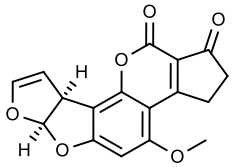
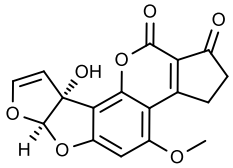
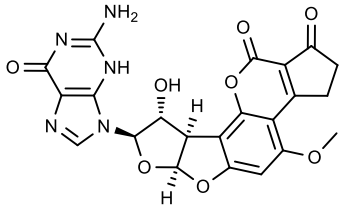
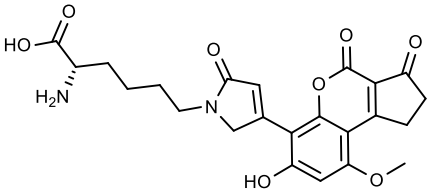
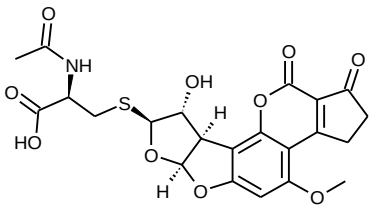
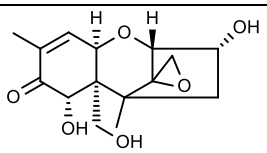
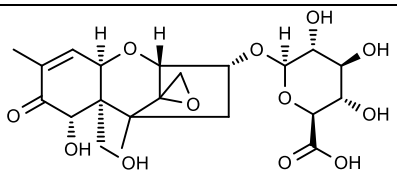
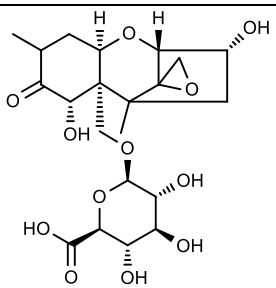
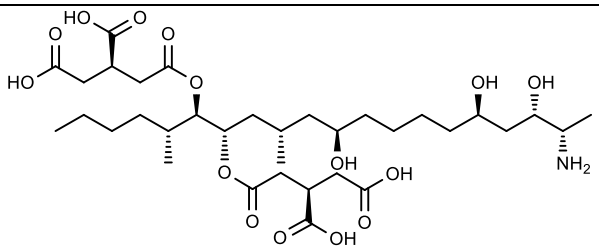
Acronym; compound name; CAS no.	Structure
AFB Aflatoxin B1 CAS:1162-65-8	
AFM1 Aflatoxin M1 CAS:6795-23-9	
AFB-N7-guanine CAS:79982-94-8	
AFB-Lys 2-Amino-6-(4-(7-hydroxy-9-methoxy-3,4-dioxo-1,2,3,4-tetrahydrocyclopenta[*c*]chromen-6-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid CAS:131919-04-5	
AFB-Cys-NAc N-Acetyl-S-(1,2,3,6a,8,9,9a,11-octahydro-9-hydroxy-4-methoxy-1,11-dioxocyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-8-yl)-L-cysteine CAS: 90358-63-7	
DON Deoxynivalenol CAS:51481-10-8	
DON-3GlcA Deoxynivalenol 3-glucuronide CAS:1000000-13-4	
DON-15-GlcA Deoxynivalenol 15-glucuronide CAS:1372859-16-9	
Fumonisin B1 CAS: 116355-83-0	

Table S3. Chemical structures of biomarkers found after exposure to diisocyanates.

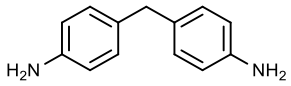
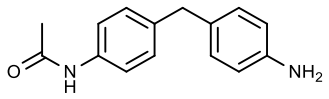
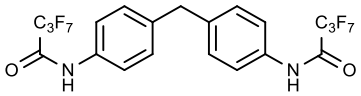
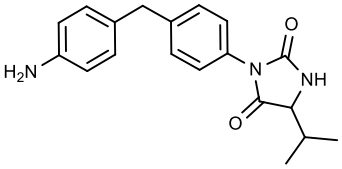
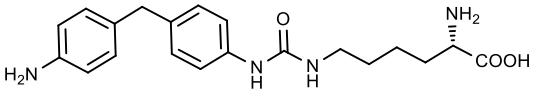
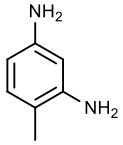
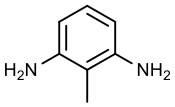
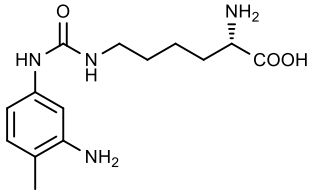
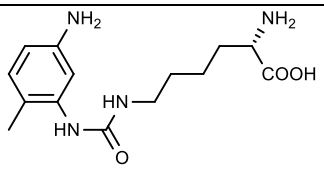
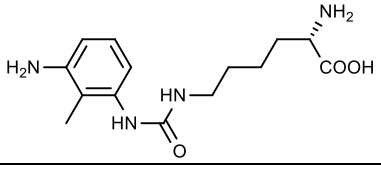
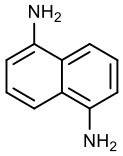
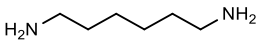
Acronym; compound name; CAS no.	Structure
MDA 4,4'-Methylenedianiline CAS:101-77-9	
AcMDA N-Acetyl-4,4‘-methylenedianiline CAS: 24367-94-0	
MDA-diHFBA N,N'-(methylenedi-4,1-phenylene)bis[2,2,3,3,4,4,4-heptafluorobutanamide]; CAS: 124232-73-1	
MDI-Val-Hyd 3-[4-[(4-Aminophenyl)methyl]phenyl]-5-(1-methylethyl)-2,4-imidazolidinedione CAS:264285-90-7	
MDI-Lys N6-[[[4-[(4-Aminophenyl)methyl]phenyl]amino]carbonyl]-L-lysine ; CAS:1200446-89-4	
24TDA 2,4-toluenediamine CAS: 95-80-7	
26TDA 2,6-toluenediamine CAS: 823-40-5	
3A4MP-Lys N6-[[(3-Amino-4-methylphenyl)amino]carbonyl]-L-lysine CAS:1416719-26-0	
5A2MP-Lys N6-[[(5-amino-2-methylphenyl)amino]carbonyl]-L-lysine CAS:1416719-28-2	
3A2MP-Lys N6-[[(3-Amino-2-methylphenyl)amino]carbonyl]-L-lysine CAS:1416719-29-3	
NDA 1,5-Naphthalenediamine CAS:2243-62-1	
HDA 1,6-Hexanediamine CAS:124-09-4	

Table S4. Conditions of the methods listed in table S2 used to hydrolyze urine of workers exposed to diisocyanates.

Urine from workers exposed to diisocyanates: hydrolysis conditions	Analyzed compound with/without derivatization	Analytical Method
0.6M HCl, 80°C, 4h[1]	24TDA, 26TDA, MDA, NDA	LC-MS/MS
0.6M HCl, 80°C, 4h[2]	HDA	LC-MS/MS
1.5M H_2SO_4, 100°C, 1.5h[3]	24TDA-, 26TDA-, MDA-, HDA-diHFBA	GC-MS(NCI)
1.6M H_2SO_4, 100°C, 1h[4]	MDA	LC-MS/MS
1.6M H_2SO_4, 80°C, 16h[5]	24TDA-, 26TDA-, MDA-, HDA-diAc	LC-MS/MS
1.6M H_2SO_4, 100°C, 4h[6]	HDA-diHFBA	GC-MS(NCI)
1.6M H_2SO_4, 100°C, 16h[7]	24TDA-, 26TDA-, MDA-, HDA-diHFBA	GC-MS(NCI)
1.8M H_2SO_4, 100°C, 4,8,16,24,48h[8]	24TDA-, 26TDA-, MDA-diPFPA	GC-MS(NCI)
1.8M H_2SO_4, 100°C, 16h[9]	24TDA-,26TDA-,MDA-,NDA-,HDA-diHFBA	LC-MS/MS
2.4M HCl, 100°C, 2h[10]	HDA-diHFBA	GC-MS(NCI)
3.6M HCl, 100°C, 16h[11]	24TDA-, 26TDA-diPFPA	GC-MS(NCI)
3.6M HCl, 100°C, 4,8,16,24,48h[8]	24TDA-, 26TDA-diPFPA	GC-MS(NCI)
4M HCl, 100°C, 0.5h[12]	MDA-diHFBA	GC-MS(NCI)
0.2M NaOH, 100°C, 24h[13]	24TDA-, 26TDA, MDA-, NDA-diPFPA	GC-MS(NCI)
5M NaOH, 100°C, 4,8,16,24,48h[8]	24TDA-, 26TDA-diPFPA	GC-MS(NCI)

The chemical names and structures of 24TDA, 26TDA, MDA, NDA, and HDA are presented in Table S1. The acronyms are listed in Table S19. Most of the methods listed in this table used the same conditions to hydrolyze whole plasma. [1] (Bhandari et al. 2016), [2] (Bhandari et al. 2018), [3] (Cocker and Jones 2017), [4] (Lépine et al. 2019), [3] (Cocker and Jones 2017), [4] (Lépine et al. 2019), [5] (Lepine et al. 2020), [6] (Gaines et al. 2010), [7] (Rosenberg et al. 2002), [8] (Lind et al. 1996), [9] (Marand et al. 2004), [10] (Maitre et al. 1996), [11] (Skarping et al. 1994), [12] (Schutze et al. 1995; Sepai et al. 1995), [13] (Sennbro et al. 2003; Sennbro et al. 2005).

Table S5. Chemical structures of biomarkers found after exposure to pyrethroids.

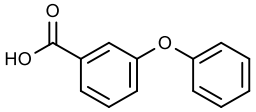
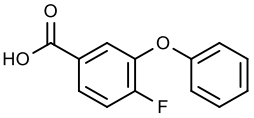
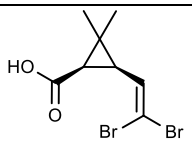
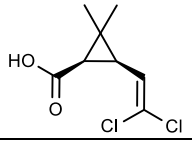
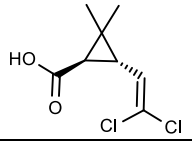
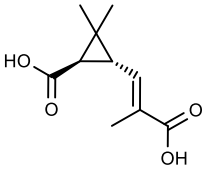
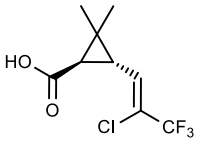
Acronym; compound name; CAS no.	Structure
3PBA 3-phenoxybenzoic acid CAS:3739-38-6	
4F3PBA 4-fluoro-3-phenoxybenzoic acid CAS:77279-89-1	
cis-DBCA cis-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid CAS:63597-73-9	
cis-DCCA cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid CAS:59042-49-8	
trans-DCCA trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid CAS:59042-50-1	
trans-CDCA trans-Chrysanthemumdicarboxylic acid CAS:72120-98-0	
ClF3CA cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid CAS:72748-35-7	

Table S6. List of acronyms used in this article

Acronym	Full name
AAMA	N-Acetyl-S-(2-carbamoyl-ethyl)cysteine
AAVal	N-(2-Carbamoylethyl)valine
AcMDA	N-Acetyl-4,4‘-methylenedianiline
AFB	Aflatoxin B1
AFB-Lys	2-amino-6-(4-(7-hydroxy-9-methoxy-3,4-dioxo-1,2,3,4-tetrahydrocyclopenta[*c*]chromen-6-yl)-2-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid
AFB-N7-guanine	8-(2-amino-6-oxo-1H-purin-9(6H)-yl)-9-hydroxy-4-methoxy-2,3,9,9a-tetrahydrocyclopenta[c]furo[3',2':4,5]furo[2,3-h]chromene-1,11(6aH,8H)-dione
AFM1	Aflatoxin M1
AFB-Cys-NAc	AFB- mercapturate
Alb	Albumin
3A4MP-Lys	N6-[[(3-Amino-4-methylphenyl)amino]carbonyl]-L-lysine
5A2MP-Lys	N6-[[(5-amino-2-methylphenyl)amino]carbonyl]-L-lysine
3A2MP-Lys	N6-[[(3-Amino-2-methylphenyl)amino]carbonyl]-L-lysine
AOP	Adverse outcome pathway
APCI	Atmospheric-pressure chemical ionization
bw	Body weight
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention
ClF3CA	cis-3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-Dimethylcyclopropanecarboxylic acid
COPHES	Consortium to Perform Human Biomonitoring on a European Scale
trans-CDCA	trans-Chrysanthemumdicarboxylic acid
cis-DBCA	cis-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid
cis-DCCA	cis-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
trans-DCCA	trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DMF	N,N-Dimethylformamide
DON	Deoxynivalenol
ECHA	European Chemicals Agency
EI	Electron ionization
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assays
EQUAS	External quality assurance programs
ESI	Electrospray ionization
4F3PBA	4-Fluoro-3-phenoxybenzoic acid
FB1	Fumonisin B1
FITC	Fluorescein isothiocyanate
FLD	Fluorescence detector
FLESH	Flemish Environmental and Human Health Studies
GAMA	N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine
GAVal	N-(2-Carbamoyl-2-hydroxyethyl)valine
GC	Gas chromatography
G-EQUAS	German external quality assurance programs
GC-MS	Gas chromatography-mass spectrometry
GC-MS/MS	Gas chromatography-tandem mass spectrometry
GC-NPD	Gas chromatography with nitrogen-phosphorus detector
GerES V	German Environmental Survey V
GlcA	Glucuronide
GSH	Glutathione
Hb	Hemoglobin
HBM	Human Biomonitoring
HBM4EU	Human Biomonitoring for the European Union
HDA	1,6-Hexamethylenediamine
HDI	1,6-Hexamethylene diisocyanate
HDA-diAc	N,N'-Diacetylhexamethylenediamine, N,N'-1,6-hexanediylbis-acetamide
HDA-diHFBA	N,N'-1,6-hexanediylbis[2,2,3,3,4,4,4-heptafluorobutaneamide]
HFBA	Heptafluorobutyric acid anhydride
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry

IAC	Immunoaffinity column
IARC	International Agency for Research on Cancer
ICC	Intraclass correlation coefficient
ICI	Interlaboratory comparison investigation
Iso-GAMA	N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine
IVIVE	*In vitro* to *in vivo* extrapolation
LC	Liquid chromatography
LC-MS/MS	High performance liquid chromatography-tandem mass spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MDA	4,4‘-Methylenedianiline, 4,4'-diaminodiphenylmethane
MDA-diAc	N,N'-Diacetyl-4,4'-diaminodiphenylmethane, N,N'-(methylenedi-4,1-phenylene)bis-acetamide
MDA-diHFBA	N,N'-(methylenedi-4,1-phenylene)bis[2,2,3,3,4,4,4-heptafluorobutanamide]
MDI	4,4‘-Methylenediphenyl diisocyanate
MDI-Lys	N6-[[[4-[(4-Aminophenyl)methyl]phenyl]amino]carbonyl]-L-lysine
MDI-Val-Hyd	3-[4-[(4-Aminophenyl)methyl]phenyl]-5-(1-methylethyl)-2,4-imidazolidinedione
Multi-method	Refers to the possibility to analyze different class of compounds with one method
NCI	Negative chemical ionization
NDI	1,5-Naphthylene diisocyanate
NDA	1,5-Naphthylenediamine
NDA-diAc	N,N'-(1,5-naphthylene)bis-acetamide
NDA-diHFBA	N,N'-1,5-Naphthalenediylbis[2,2,3,3,4,4,4-heptafluorobutanamide]
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NRHEEC	National Report on Human Exposure to Environmental Chemicals
NTP	National Toxicology Program
14PDA	1,4-Phenylenediamine
3PBA	3-Phenoxybenzoic acid
PBPK	Physiologically based pharmacokinetic
PFPA	Pentafluoropropionic acidanhydride
PFPITC	Pentafluorophenylisothiocyanate
PITC	Phenylisothiocyanate
QA/QC	Quality Assurance/Quality Control
SPE	Solid phase extraction
TCPy	3,5,6-Trichloro-2-pyridinol
24TDA	2,4-Toluenediamine
24TDA-diHFBA	N,N'-(4-methyl-1,3-phenylene)bis[2,2,3,3,4,4,4-heptafluorobutanamide]
24TDA-diAc	N,N‘-Diacetyl-toluenediamine, 2,4-Diacetylaminotoluene, N,N'-(4-methyl-1,3-phenylene)bis-acetamide
24TDI	2,4-Toluene diisocyanate
26TDA	2,6-Toluenediamine
26TDA-diHFBA	N,N'-(2-methyl-1,3-phenylene)bis[2,2,3,3,4,4,4-heptafluorobutanamide]
26TDA-diAc	N,N‘-Diacetyl-toluenediamine, 2,6-Diacetylaminotoluene, N,N'-(4-methyl-1,3-phenylene)bis-acetamide
26TDI	2,6-Toluene diisocyanate
UHPLC	Ultra High Performance Liquid Chromatography
US EPA	United States Environmental Protection Agency

Figures

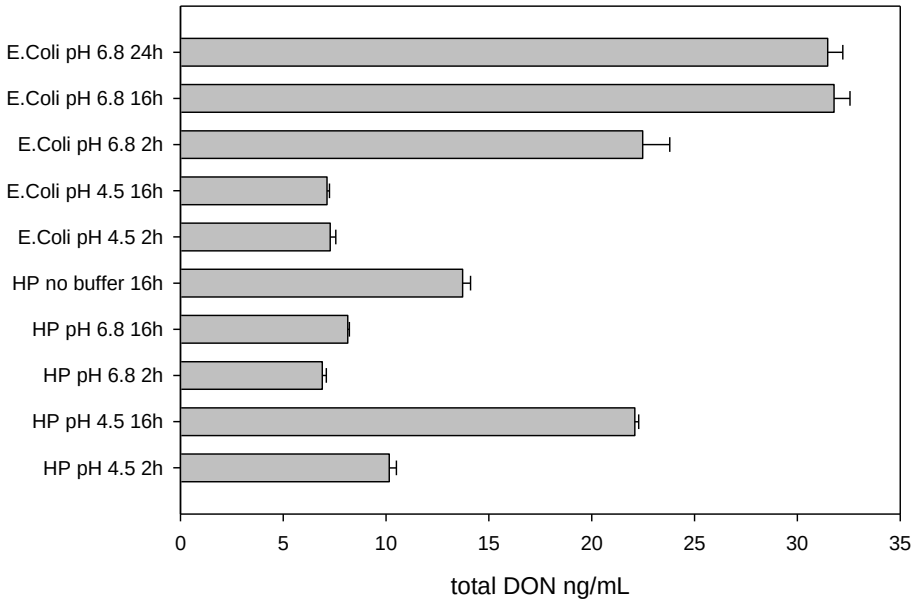


Fig. S1. Effect of deconjugation parameters (enzyme, pH and incubation time @37°C; triplicate experiments) on total DON found in a human urine sample. HP = *Helix Pomatia based* β-glucuronidase/sulfatase. E. coli = *E. coli-based* β-glucuronidase. Data were obtained from Hester van den Top, Wageningen Food Safety Research.

References

Bhandari, D.; Bowman, B.A.; Patel, A.B.; Chambers, D.M.; De Jesús, V.R.; Blount, B.C. UPLC-ESI-MS/MS method for the quantitative measurement of aliphatic diamines, trimethylamine N-oxide, and β-methylamino-l-alanine in human urine. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1083:86-92

Bhandari, D.; Ruhl, J.; Murphy, A.; McGahee, E.; Chambers, D.; Blount, B.C. Isotope Dilution UPLC-APCI-MS/MS Method for the Quantitative Measurement of Aromatic Diamines in Human Urine: Biomarkers of Diisocyanate Exposure. Anal Chem 2016;88:10687-10692

Cocker, J.; Jones, K.; Leng, G.; Gries, W.; Budnik, L.T.; Müller, J.; Göen, T.; Hartwig, A., MAK Commission, 2017. Hexamethylene diisocyanate, 2,4-toluene diisocyanate, 2,6-toluene diisocyanate, isophorone diisocyanate and 4,4′-methylene diphenyl diisocyanate – Determination of hexamethylenediamine, 2,4-toluenediamine, 2,6-toluenediamine, isophoronediamine and 4,4′-methylenedianiline in urine using gas chromatography-mass spectrometry [Biomonitoring Methods, 2017]. The MAK-Collection for Occupational Health and Safety 2017, Vol 2, No 3, 1436-1456. https://doi.org/10.1002/3527600418.bi82206e2217

Gaines, L.G.; Fent, K.W.; Flack, S.L.; Thomasen, J.M.; Ball, L.M.; Richardson, D.B.; Ding, K.; Whittaker, S.G.; Nylander-French, L.A. Urine 1,6-hexamethylene diamine (HDA) levels among workers exposed to 1,6-hexamethylene diisocyanate (HDI). Ann Occup Hyg 2010;54:678-691

Lepine, M.; Sleno, L.; Lesage, J.; Gagne, S. A validated UPLC-MS/MS method for the determination of aliphatic and aromatic isocyanate exposure in human urine. Anal Bioanal Chem 2020;412:753-762

Lépine, M.; Sleno, L.; Lesage, J.; Gagné, S. A validated liquid chromatography/tandem mass spectrometry method for 4,4′-methylenedianiline quantitation in human urine as a measure of 4,4′-methylene diphenyl diisocyanate exposure. Rapid Commun Mass Spectrom 2019;33:600-606

Lind, P.; Skarping, G.; Dalene, M. Biomarkers of toluene diisocyanate and thermal degradation products of Polyurethane, with special reference to the sample preparation. Anal Chim Acta 1996;333:277-283

Maitre, A.; Berode, M.; Perdrix, A.; Stoklov, M.; Mallion, J.M.; Savolainen, H. Urinary hexane diamine as an indicator of occupational exposure to hexamethylene diisocyanate. Int Arch Occup Environ Health 1996;69:65-68

Marand, A.; Karlsson, D.; Dalene, M.; Skarping, G. Determination of amines as pentafluoropropionic acid anhydride derivatives in biological samples using liquid chromatography and tandem mass spectrometry. Analyst 2004;129:522-528

Rosenberg, C.; Nikkila, K.; Henriks-Eckerman, M.L.; Peltonen, K.; Engstrorm, K. Biological monitoring of aromatic diisocyanates in workers exposed to thermal degradation products of polyurethanes. J Environ Monit 2002;4:711-716

Schutze, D.; Sepai, O.; Lewalter, J.; Miksche, L.; Henschler, D.; Sabbioni, G. Biomonitoring of workers exposed to 4,4'-methylenedianiline or 4,4'-methylenediphenyl diisocyanate. Carcinogenesis 1995;16:573-582

Sennbro, C.J.; Lindh, C.H.; Tinnerberg, H.; Gustavsson, C.; Littorin, M.; Welinder, H.; Joensson, B.A.G. Development, validation and characterization of an analytical method for the quantification of hydrolysable urinary metabolites and plasma protein adducts of 2,4- and 2,6-toluene diisocyanate, 1,5-naphthalene diisocyanate and 4,4'-methylenediphenyl diisocyanate. Biomarkers 2003;8:204-217

Sennbro, C.J.; Littorin, M.; Tinnerberg, H.; Jonsson, B.A. Upper reference limits for biomarkers of exposure to aromatic diisocyanates. Int Arch Occup Environ Health 2005;78:541-546

Sepai, O.; Henschler, D.; Sabbioni, G. Albumin adducts, hemoglobin adducts and urinary metabolites in workers exposed to 4,4'-methylenediphenyl diisocyanate. Carcinogenesis 1995;16:2583-2587

Skarping, G.; Dalene, M.; Lind, P. Determination of toluenediamine isomers by capillary gas chromatography and chemical ionization mass spectrometry with special reference to the biological monitoring of 2,4- and 2,6-toluene diisocyanate. J Chromatogr A 1994;663:199-210