

Fig. 1. Synthesis process to adduct MDA to the lysine side chain on a peptide.

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### Quantitation of 4,4'-methylene diphenyl diisocyanate human serum albumin adducts

Toxicology Reports, Volume 1, 2014, 743–751

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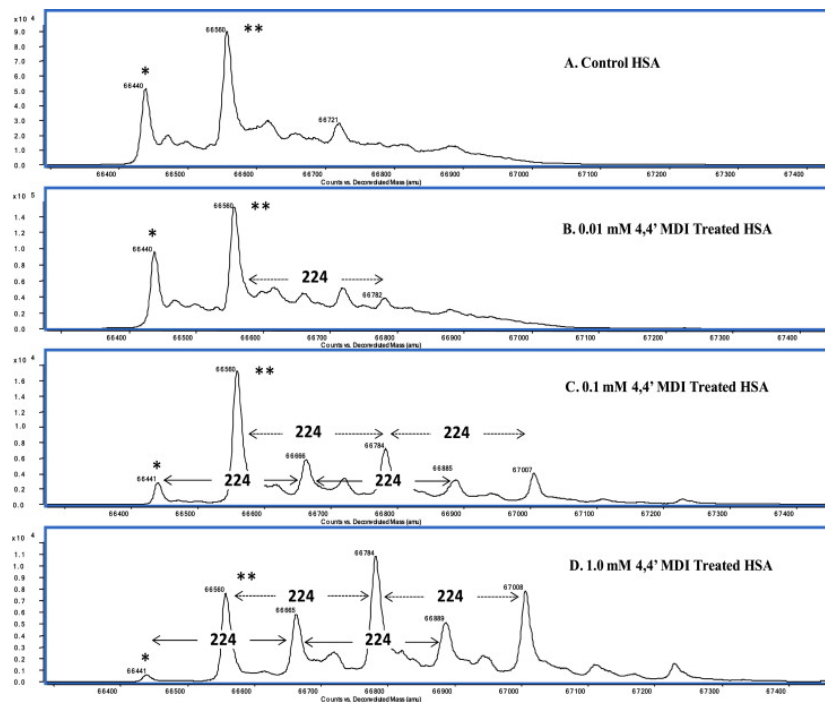


Fig. 2. Deconvoluted full scan mass spectra taken in positive ion electrospray ionization mode of (A) control HSA; (B) 0.01 mM 4,4'-MDI adducted HSA; (C) 0.1 mM 4,4'-MDI adducted HSA; and (D) 1.0 mM 4,4'-MDI adducted HSA. The single asterisk indicates the first...

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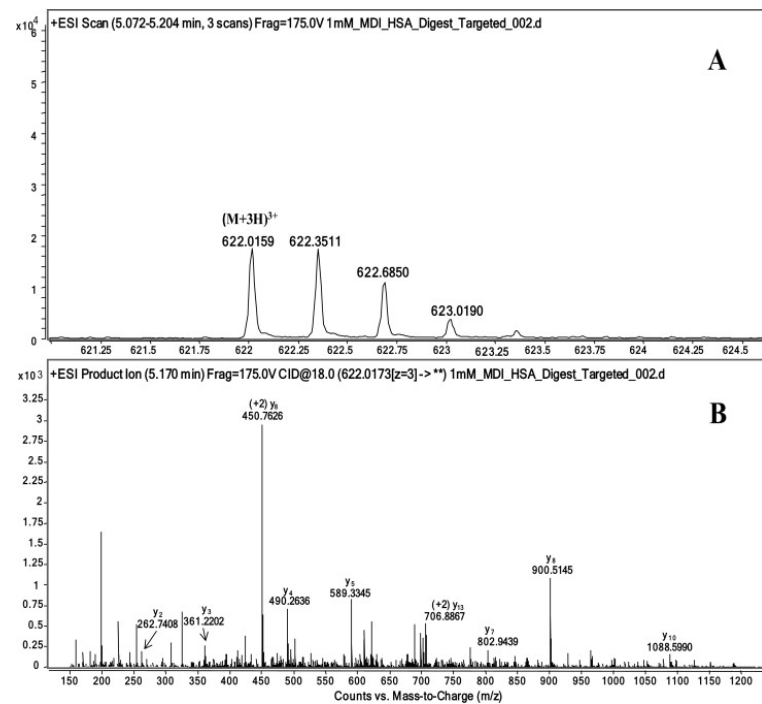


Fig. 3. Adducted peptide KVPQVSTPTLVEVSR (A) full scan and (B) product ion scan.

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Table 2.

Multiple reaction monitoring transitions and corresponding collision energies for labeled and unlabeled adducted HSA peptides. Underlined lysine (K) indicates the site of MDI adduction.

Adducted  $\uparrow$  Monoisoto Optimized collision energies

	Precursor	Product	
AFKAWAV	<u>622.3</u> (+2)	673.1	16
KQTALAEL	<u>662.9</u> (+2)	844.5	16
YTK	635.3 (+1)	371.2	16
KVPQVSTP	<u>622.0</u> (+3)	900.5	18
		589.3	18
		450.8	18
KVPQVSTP	<u>624.3</u> (+3)	906.5	18
		595.3	18

Table 3.

Summary demographic and classification information for all sera samples.

Parameter Exposed to diisocyanates non-asthmatic n = 7 Exposed to diisocyanates related asthma n = 7 Not exposed to diisocyanates non-asthmatic n = 7 Not exposed to diisocyanates related asthma n = 7

Parameter	Exposed to diisocyanates non-asthmatic n = 7	Exposed to diisocyanates related asthma n = 7	Not exposed to diisocyanates non-asthmatic n = 7	Not exposed to diisocyanates related asthma n = 7
Gender				
Male	13	8	8	1
Female	2	2	1	6
Age (years)	38.5 ± 9.9	36.5 ± 9.9	44.9 ± 7.5	37.9 ± 11.0
Mean (±SD)	66.8 ± 83.0	60.5 ± 54.3	152.5 ± 10.0	Not applicable
Last exposure (years)	94.8 ± 136.0	140.9 ± 111.0	170.9 ± 25.0	Not applicable
Challenge (Yes/No)	No	No	No	No
Detect or not detect	4 detect	3 detect	1 detect	
Sabbioni	4 NQ	1 NQ	2 NQ	0 NQ
Modified	7 ND	5 ND	4 ND	6 ND

Table S1. MDI exposed worker demographics and classification criteria positive for occupational asthma.

Code	Diagnosis	Age at time of Sampling (years)	Gender	Smoking Status	Exposures in the workplace	Challenge agent	Challenge reaction to MDI	Days between sample collection and last occupational exposure	Duration of Occupational Exposure to Diisocyanates (months)	fmol adduct/mg albumin ND=Not Detectable NQ= Detectable, Non-quantifiable
<b>DRA* -1</b>	Diisocyanate related asthma	27.5	Male	Non-smoker	MDI	MDI	Prolonged immediate	1	81.7	NQ
<b>DRA -2</b>	Diisocyanate related asthma	24.0	Male	Non-smoker	MDI	MDI	Prolonged immediate	4	65.9	NQ
<b>DRA -3</b>	Diisocyanate related asthma	36.4	Male	Ex-smoker	MDI	MDI	Immediate	137	168.8	ND
<b>DRA -4</b>	Diisocyanate related asthma	53.0	Male	Ex-smoker	MDI	MDI	Progressive	238	9.0	ND
<b>DRA -5</b>	Diisocyanate related asthma	33.6	Male	Non-smoker	MDI	MDI	Isolated late	1	58.4	7.55
<b>DRA -6</b>	Diisocyanate related asthma	29.4	Male	Non-smoker	MDI	MDI	Isolated late	130	3.1	15.7
<b>DRA -7</b>	Diisocyanate related asthma	42.3	Male	Ex-smoker	MDI	MDI	Isolated late	3	16.6	8.97
<b>DRA -8</b>	Diisocyanate related asthma	38.3	Male	Ex-smoker	MDI	MDI	Early late	4	35.1	ND
<b>DRA -9</b>	Diisocyanate related asthma	35.4	Male	Ex-smoker	MDI	MDI	Isolated late	25	80.1	NQ
<b>DRA -10</b>	Diisocyanate related asthma	36.1	Female	Non-smoker	MDI	MDI	Dual	185	6.0	ND
<b>DRA -11</b>	Diisocyanate related asthma	43.4	Male	Ex-smoker	MDI	MDI	Early late	64	25.2	ND
<b>DRA -12</b>	Diisocyanate related asthma	54.1	Male	Ex-smoker	MDI	MDI	Late	126	39.9	15.7
<b>DRA -13</b>	Diisocyanate related asthma	25.9	Male	Non-smoker	MDI	MDI	Isolated change in PC20	500	46.7	ND
<b>DRA -14</b>	Diisocyanate related asthma	46.3	Male	Non-smoker	MDI	MDI	Late	3	326.3	NQ
<b>DRA -15</b>	Diisocyanate related asthma	52.6	Female	Non-smoker	MDI	MDI	Isolated change in PC20	1	39.0	ND
<b>*Diisocyanate related asthmatic</b>	Mean	38.5						94.8	66.8	
	SD	9.9						136.9	83.0	

Table S2. MDI exposed worker demographics and classification criteria negative for asthma (non-asthma of any kind).

Code	Diagnosis	Age at time of Sampling (years)	Gender	Smoking Status	Exposures in the workplace	Challenge agent	Challenge reaction to MDI	Days between sample collection and last occupational exposure	Duration of Occupational Exposure to Diisocyanates (months)	fmol adduct/mg albumin ND=Not Detectable NQ= Detectable, Non-quantifiable
ENA ^ -1	No diisocyanate related asthma and Non asthmatic	30.1	Female	Smoker	MDI, HDI, TDI	MDI, HDI, TDI, cyanoacrylate	No Reaction	349	8.0	NQ
ENA -2	No diisocyanate related asthma and Non asthmatic	24.2	Male	Non-smoker	MDI	MDI	No Reaction	221	25.4	ND
ENA -3	No diisocyanate related asthma and Non asthmatic	25.3	Male	Non-smoker	MDI	MDI	No Reaction	133	53.9	ND
ENA -4	No diisocyanate related asthma and Non asthmatic	54.7	Male	Ex-smoker	MDI	MDI	No Reaction	3	28.5	ND
ENA -5	No diisocyanate related asthma and Non asthmatic	34.6	Male	Smoker	MDI	MDI	No Reaction	102	189.9	ND
ENA -6	No diisocyanate related asthma and Non asthmatic	47.4	Male	Smoker	HDI, MDI, metabisulfite, epoxy resin	HDI, MDI, metabisulfite, epoxy resin	No Reaction	118	27.2	60.8
ENA -7	No diisocyanate related asthma and Non asthmatic	29.8	Male	Non-smoker	HDI, MDI, TDI, IPDI, methacrylate	MDI, HDI, TDI, IPDI, methacrylate, recycle	No Reaction	145	55.1	11.3
ENA -8	No diisocyanate related asthma and Non asthmatic	38.9	Male	Non-smoker	MDI	MDI	No Reaction	61	54.5	7.39
ENA -9	No diisocyanate related asthma and Non asthmatic	43.6	Male	Non-smoker	MDI	MDI, latex, welding	No Reaction	276	117.7	57.3
ENA -10	No diisocyanate related asthma and Non asthmatic	36.8	Female	Non-smoker	MDI	MDI, fiberglass, glue	No Reaction	1	44.7	ND
^Exposed to Diisocyanates, and Non-Asthmatic	Mean	36.5						140.9	60.5	
	SD	9.9						113.07	54.3	

Table S3. MDI exposed workers demographics and classification criteria negative for occupational asthma.

Code	Diagnosis	Age at time of Sampling (years)	Gender	Smoking Status	Exposures in the workplace	Challenge agent	Challenge reaction to MDI	Days between sample collection and last occupational exposure	Duration of Occupational Exposure to Diisocyanates (months)	fmol adduct/mg albumin ND=Not Detectable NQ= Detectable, Non-quantifiable
EA†-1	No diisocyanate related asthma, asthmatic	46.5	Male	Smoker	MDI, TDI	MDI, TDI	No Reaction	1	61.5	7.65
EA3	No diisocyanate related asthma, asthmatic	56.0	Male	Ex-smoker	MDI	MDI	No Reaction	563	51.0	17.2
EA4	No diisocyanate related asthma, asthmatic	35.4	Male	Non-smoker	MDI	MDI	No Reaction	1	64.5	NQ
EA5	No diisocyanate related asthma, asthmatic	43.5	Male	Ex-smoker	MDI, TDI	MDI, TDI	No Reaction	170	256.7	NQ
EA6	No diisocyanate related asthma, asthmatic	42.1	Male	Smoker	MDI	MDI, aluminium welding	No Reaction	646	303.3	ND
EA7	No diisocyanate related asthma, asthmatic	36.1	Male	Smoker	MDI	MDI	No Reaction	215	9.8	ND
EA8	No diisocyanate related asthma, asthmatic	40.6	Male	Ex-smoker	MDI	MDI	No Reaction	1	174.9	10.4
EA9	No diisocyanate related asthma, asthmatic	48.1	Male	Smoker	MDI	MDI, MDF, formaldehyde	No Reaction	1	270.8	ND
EA10	No diisocyanate related asthma, asthmatic	56.0	Female	Ex-smoker	MDI	MDI, methacrylate, formaldehyde	No Reaction	3	180.3	ND
† Exposed to Diisocyanates, Asthmatic	Mean	44.9						177.9	152.5	
	SD	7.5						256.1	109.3	



Table S4. Non exposed worker control demographics and classification criteria.

Code	Diagnosis	Age at time of Sampling (years)	Gender	Smoking Status	Exposures in the workplace	Challenge agent	Challenge reaction to MDI	Days between sample collection and last occupational exposure	Duration of Occupational Exposure to Diisocyanates (months)	fmol adduct/mg albumin ND=Not Detectable NQ= Detectable, Non-quantifiable
<b>Control ‡ 1</b>	No asthma	25.3	Female	Non-smoker	None	pig, hen	No Reaction	4	NA	ND
<b>Control 2</b>	No asthma	40.2	Female	Smoker	None	pig	No Reaction	130	NA	ND
<b>Control 3</b>	No asthma	32.2	Male	Non-smoker	None	Flour	No Reaction	68	NA	ND
<b>Control 4</b>	No asthma	46.1	Female	Non-smoker	None	Flour	No Reaction	229	NA	151
<b>Control 5</b>	No asthma	24.5	Female	Non-smoker	None	silicone, latex,	No Reaction	128	NA	ND
<b>Control 6</b>	No asthma	43.0	Female	Ex-smoker	None	Latex gloves	No Reaction	57	NA	ND
<b>Control 11</b>	No asthma	53.9	Female	Non-smoker	None	Woodchips, MDF, melamine	No Reaction	2	NA	ND
<b>‡No Diisocyanate exposures, not asthmatic</b>	Mean	37.9						88.3	0.0	
	SD	11.0						80.7	0.0	