Diisocyanate-lysine conjugates in urine samples of diisocyanate exposed workers.

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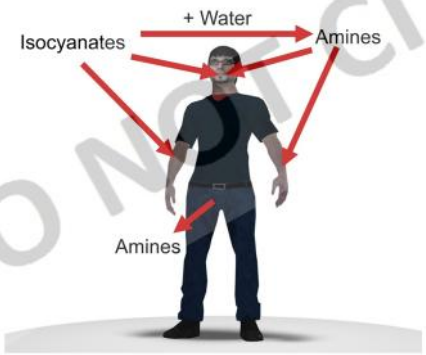
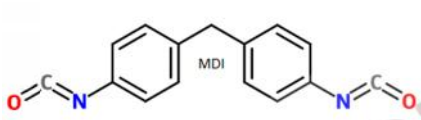
Issues

BM methods cannot distinguish between diisocyanate and diamine exposure in urine.



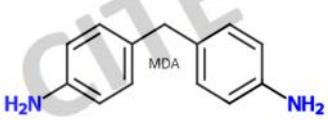
MDI and MDA

Methylene diphenyl diisocyanate (MDI)



- Adhesives, polyurethane foam
- Sensitiser
- UK BMGV = 1 µmol/mol creatinine

Methylene dianinilne (MDA)



- Manufacture of MDI, azo dyes
- Suspect Carcinogen (IARC 2B)
- UK BMGV = 50 µmol/mol creatinine

Co-exposure

- Both diisocyanates and diamines used in the same workplace
- Generation of diamines during diisocyanate use
  - Diamines in air samples during polyurethane production (Jones *et al*, 2011)
  - Diamines found on finished polyurethane products (Lewandowski *et al*, 2005)

Specific biomarkers are needed

JONES, K., JOHNSON, P. D., BALDWIN, P. E. J., COLDWELL, M., COOKE, J., KEEN, C., HARDING, A. H., SMITH, D. & COCKER, J. 2017. Exposure to Diisocyanates and Their Corresponding Diamines in Seven Different Workplaces. Ann Work Expo Health, 61, 383-393.
LEWANDOWSKI, T. A., HAYES, A. W. & BECK, B. D. 2005. Risk evaluation of occupational exposure to methylene dianiline and toluene diamine in polyurethane foam. *Hum Exp Toxicol*, 24, 655-62.

Previous Research

- Diisocyanates react readily with protein groups
  - Bind to amino acids on albumin (Wisnewski *et al*, 2011)
  - MDI-Lysine (MDI-Lys) and acetylMDI-Lys (acMDI-Lys) reported in HSA samples of workers (Sabbioni *et al*, 2010)
  - MDI-Lys in sera samples after specific inhalation challenge (Luna *et al*, 2014)
- No reports on specific urinary metabolites of MDI
  - Less invasive than blood sampling

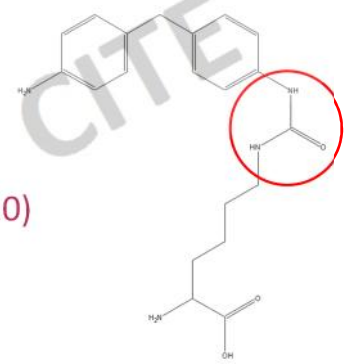
WISNEWSKI, A. V., HETTICK, J. M. & SIEGEL, P. D. 2011. Toluene Diisocyanate Reactivity with Glutathione Across a Vapor/Liquid Interface and Subsequent Transcarbamoylation of Human Albumin. *Chemical Research in Toxicology*, 24, 1686-1693.
SABBIONI, G., DONGARI, N. & KUMAR, A. 2010. Determination of a new biomarker in subjects exposed to 4,4'-methylenediphenyl diisocyanate. Biomarkers, 15, 508-15.
LUNA, L. G., GREEN, B. J., ZHANG, F., ARNOLD, S. M., SIEGEL, P. D. & BARTELS, M. J. 2014. Quantitation of 4,4'-methylene diphenyl diisocyanate human serum albumin adducts. *Toxicology Reports*, 1, 743-751.

Analysis – Sample Groups

- Samples
  - Post Shift urine samples
  - MDI exposed workers
    N = 64 (2 Companies)
  - MDA exposed workers
    N = 28 (1 company)
- All samples analysed by GC-MS
  - uMDA
  - 3rd group of NDs (None Detected)
    uMDA <LoQ (2 nmol/L) by GC-MS (N = 20)

Analysis - Method

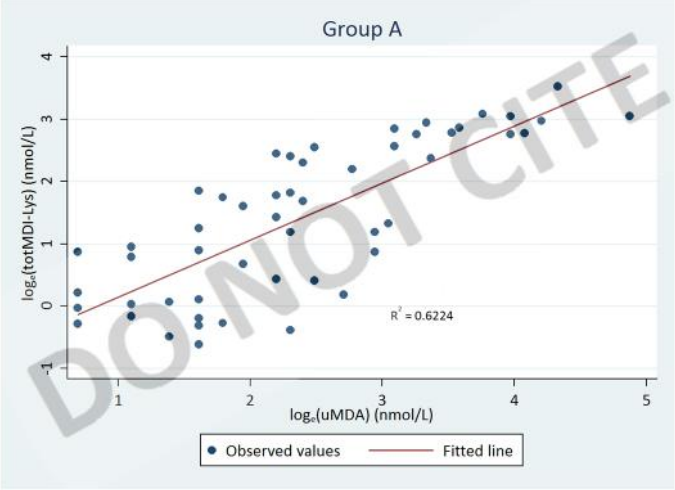
- Commissioned standards
  - MDI-lys
  - acMDI-lys
- Method (Sabbioni *et al*, 2010)
  - Pronase Hydrolysis
  - SPE clean up
  - LC-MS/MS analysis



Results

- Lysine conjugates detected
  - 88% of MDI exposed group
  - 57% of MDA exposed group
- acMDI-lys most abundant in all samples
  - Number of samples detected
  - Higher concentration when both present
- Not all positive for both uMDA and 1 lysine conjugate

Correlation – MDI exposed group



Results

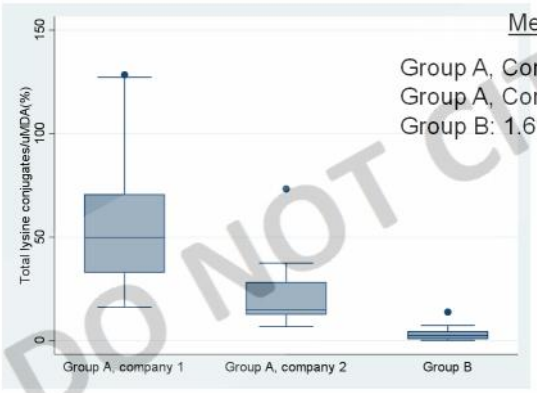
Analyte	Exposure Group		
	Group A, (Company 1, N = 38)	**Group A** (Company 2, N = 14)	**Group B** (N = 16)
uMDA	2 - 131	2 - 19	13 - 2557
MDI-lys	<LoQ - 3.6	<LoQ (All)	<LoQ - 7.6
acMDI-lys	1 - 30.3	0.5 - 2.4	0.8 - 44.1
Total Lysine (MDI-Lys + acMDI-Lys)	1 - 33.9	0.5 - 2.4	0.8 - 51.7

1 out of 20 NDs had acMDI-Lys (1.0 nmol/L), no MDI-Lys detected

Group A = MDI exposure
Group B = MDA exposure
LoQ uMDA = 2 nmol/L
LoQ MDI-Lys and acMDI-Lys = 0.5 nmol/L

Results

Ratio of total lysine/uMDA



Non-parametric test
$p < 0.001$

Discussion

- Group A, company 1
  - Adhesives
    - Low aerosol production
- Group A, Company 2
  - Injection moulding (foam)
    - Accessible curing ovens
    - Handling of finished products
      - Previous research has shown diamines present on cured diisocyanate products
- Group B
    - MDI used in the same workplace?

Summary

- MDI-Lys and acMDI-lys conjugates found in workers exposed to MDI
  - Moderate correlation with traditional amine biomarker
- Conjugates also found in MDA workers
  - Relatively low compared to uMDA
  - Possible co-exposure to MDI?
- MDI-Lys and acMDI-Lys may be a viable specific urinary metabolite for MDI exposure analysis
  - Combined with uMDA measurements by GCMS the major contribution to exposure can be determined

Further Work

- More Companies
  - Wider dataset
  - Greater contextual information
- Method improvements
  - Is hydrolysis needed?
    - Lysine conjugates or longer chain amino acid conjugates
- Other diisocyanates
  - HDI, TDI etc.

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