Respiratory symptoms, sensitization and exposure-response associations in HDI exposed spray painters

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Online data supplement

Material and methods

Personal exposure estimates

Detailed assessment of exposure to a range of different isocyanate compounds in the car body repair shops and industrial painting companies specialized in ships and harbor equipment has recently been published (1). Briefly, personal samples were taken using midget impingers for sampling, di-n-butylamine as a reagent and LC-MS/MS for analysis. Diisocyanates, several monoisocyanates, aminoisocyanates and oligomers of HDI and MDI were quantified. Exposure is expressed in μg reactive isocyanate group (NCO) to be able to add up exposure to different isocyanate compounds. Since a large proportion of samples was below the limit of detection (LOD) this was incorporated in the formula. Widespread exposure to especially HDI oligomers was found with highest exposures during spray painting. Additional data from the airplane painting company indicated a similar exposure pattern to the previously reported exposures with higher exposures during especially spray painting. Separate task-based airborne exposure measurements were used for car body repair shops, industrial painting companies specializing in ships and harbor equipment and in airplanes. Estimates from car body repair shops were used for workers from companies specialized in furniture since walk-through surveys indicated that the spray-painting environment was very similar in these industries.

Cut-off values ImmunoCAP assay

For IgE antibodies a cut-off level of 0.35 kU/l was used as described by Phadia. For IgG antibodies a cut-off level of 5 mg/l was used based on the mean + 3 *standard deviation of specific IgG levels in 20 healthy non-exposed controls.

Preparation of HDI-HSA conjugates using fluid phase reaction

HDI-HSA conjugates (HDI_L-HSA) were prepared in a liquid phase reaction essentially as described earlier (2). HDI (Sigma-Aldrich, Steinheim, Germany) was dissolved in dioxane (0.15 ml in 10 ml), and the 10 mL solution was added dropwise to 100 ml of a 1% solution of HSA (Sigma, low endotoxin, ≤0.1 ng/mg solid, St. Louis, MO, USA) in a 0.05 mol/l KCl, 0.05 mol/l Na borate buffer while stirring (3h, room temperature). Reactions were terminated by adding 2 ml of ethanolamine and after centrifugation (2100g, 1 hour), the supernatant was dialyzed against PBS (3 times, 3 hours, 4°C), dialyzed (3500 Da Spectrapor, Spectrum Laboratories Inc.,Rancho Dominiquez, Ca, USA) against deionized water (overnight, 4°C) and frozen. The HDI/HSA ratio of the conjugate (25-30) was based on free NH_2 groups, measured with TNBS (3).

Preparation of HDI-HSA conjugates using vapor phase reaction

Vapor-induced HDI-HSA (HDI_V-HSA) conjugates were prepared as previously described (3). HDI (Sigma; St. Luois, MO) vapor in the concentration range of 20-200 ppb was passively generated in a closed circuit system and monitored with an Autostep monitor (GMD, Pittsburgh, Pa, USA). A 0.5% solution of low

endotoxin HSA (Sigma) in tissue culture grade PBS (GibcoBRL; Grand Island, NY) was exposed overnight in open 60-mm petri dishes (Becton Dickinson, Franklin lakes, NJ, USA). The vapor exposure unit was sterilized with 70% ethanol and HSA solutions were sterile (0.2 μm) filtered (Corning; Corning, NY) before and after exposure, aliquoted and stored at –20°C. The HDI/HSA ratio of the conjugate (9-10) was based on free NH_2 groups, measured with TNBS (3).

Preparation of oligomeric HDI-HSA conjugates

HSA-conjugates were also prepared with Desmodur N-100 and N-3300 (Bayer; Pittsburgh, PA), commercially used biuret and isocyanurate oligomers of HDI. HDI oligomers were mixed 1:1 with acetone (J.T.Baker; Phillipsburg, NJ) then with 0.5 % HSA in tissue culture-grade PBS to achieve a final concentration of 0.1% (v/v) HDI oligomer. Reactants were mixed end-over-end for 2 hr at 37°C, 0.2 μm filtered, dialyzed against tissue-culture grade PBS 4X, using Spectra/Por cellulose membranes sterilized by irradiation (Spectrum Labs, Inc.; Los Angeles, CA), and refiltered (0.2 μm). HDI/HSA ratios of the conjugates (8-8.5 for N100-HSA; 5.5-6 for N3300-HSA) were based on free NH_2 groups, measured with TNBS (3).

EIA

Microtiter plates (Greiner, Frickenhausen, Germany) were coated overnight at 4 °C with conjugates (HDI_L-HSA, HDI_V-HSA, N100-HSA, N3300-HSA) and unmodified, non-treated HSA in PBS-azide, at 10 μg/ml (IgE) and 20 μg/ml (IgG).

Volumes of 0.1 ml/well were used in this step and all subsequent steps. Serum samples diluted 1/10 (IgE) or 1/100 (IgG) in PBS-Tween with 0.2% gelatin (PBTG) were added and incubated for 2 h at 37°C. In IgE analyses antibody binding was detected with mouse monoclonal anti-human IgE (CLB, Sanquin Reagents, Amsterdam, The Netherlands) 1:16000 (1h 37°C) followed by rabbit anti-mouse/biotin (DAKO, DakoCytomation Denmark, Glostrup, Denmark) 1:5000, and avidin/HrP (DAKO) 1:2000 (4). In IgG analyses antibodies were detected with peroxidase-labelled rabbit anti-human IgG/PO (DAKO) 1:2000 (1h 37°C). OPD was used as the substrate (30 min 20°C), the reaction was stopped with 2M HCl and the optical density (OD) was read at 492 nm. For each serum, the OD measured in the HSA-coated well was subtracted from the OD values measured in each of the conjugate-coated wells.

In each EIA plate a complete row of microwells was included as 'reagent blanks' or 'no serum controls'. The OD values for test sera were not further adjusted for non-specific binding of the detection reagent since these no serum control OD values showed no significant differences between wells coated with HSA alone or with any of the isocyanate-HSA conjugates.

Cut-off values EIA

All sera with a HSA-corrected OD value for an IgE reaction of > 0.05 for any of the conjugates were retested on the whole panel. A cut-off value for HSA corrected OD values of 0.1 was chosen because of optimal concordance (analyzed by kappa values [κ]) for repeated measurements (κ ≥ 0.8). When using

an OD ratio (OD(conjugate)/OD(HSA)) > 2 as a cut-off level similar positive samples and associations were found (data not shown).

For IgG analyses a random sample of 145 samples was reanalyzed to determine cut-off levels. A cut-off value for HSA corrected OD values of 0.3 was chosen because of optimal concordance for repeated measurements. At this cut-off level κ was around 0.7 for HDI_L-HSA and HDI_V-HSA and around 0.9 for N3300-HSA and N100-HSA. Sera with strong reactions with HSA (OD>0.3) were retested on the whole panel, and if in the second test the OD on HSA was <0.3, the results of the second test were used. Statistical analyses were performed with and without samples with a repeatedly strong reaction (OD >0.3) in the HSA-coated well (n=22).

Physiological testing

At the time of selection of the subset of the population in which physiological testing was carried out results for specific IgE and IgG against diisocyanates measured by ImmunoCAP assay (Phadia, Uppsala, Sweden) were available. All workers from companies with at least one worker with detectable specific antibodies were invited to participate. The company participation rate was 90% and worker participation rate was 66%. Companies were visited between January and June 2006. All tests were carried out on a working day in a secluded room at the company or in an especially equipped van at the company premises.

References

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