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International workshop on biomarkers for isocyanates

Isocyanates, highly reactive compounds extensively used in industry, are the most commonly reported cause of occupational asthma in industrial populations. The most important isocyanate types include 2,4- and 2,6-toluene diisocyanate (TDI), hexamethylene 1,6-diisocyanate (HDI), and diphenylmethylene 4,4-diisocyanate (MDI).

Isocyanate-containing compounds are used in many products, including surface coatings, water-proofing coatings, paints, polyurethane foams, insulation, adhesives, resins, elastomers, binders, and sealants. For isocyanate-exposed workers, the estimated prevalence of developing asthma is 5—10%. People who are sensitized to isocyanates can develop immediate or late asthmatic response after isocyanate exposure. The mechanisms by which isocyanates cause asthma are not well defined. The limited information available suggests that isocyanate asthma is predominantly mediated via nonimmunoglobulin E mechanisms and that isocyanates act like foreign low-molecular-weight haptens, inducing antigen-specific T-cell responses and airway inflammation.

The purpose of the workshop [supported in part by the EU biomed programme (BMH4-CT95—0559)] convened in Helsinki on 14—15 December 1998 was to discuss the recent developments of the various biomarkers (exposure, effect, susceptibility) for the isocyanates and their use in occupational health.

The keynote lecture on the state of the art "Isocyanates and Occupational Asthma" was given by Professor Xaver Baur (Bochum, Germany). Different studies on isocyanate-challenged workers, as well as on animals, indicate that airway epithelium damage and cellular inflammation are common acute responses to long-term or high isocyanate exposure. Most subjects with isocyanate-induced respiratory disorders do not show immunoglobulin (Ig) E antibodies specific to isocyanate-HSA conjugates (HSA = human serum albumin). Professor Baur recommended that all isocyanate-exposed workers should be submitted to medical surveillance programs, including regular clinical check-ups, lung function tests, and suitable biological monitoring, to detect disorders in early reversible stages. Isocyanate-induced respiratory afflictions should be legally reported, recognized, and compensated as occupational diseases.

Dr Päivi Piirilä and her collaborators had followed the 245 workers diagnosed with isocyanate-induced occupational asthma in Finland during 1976—1992. Fifty-five percent of these people were no longer working, in the majority of cases (80%) because they had developed occupational asthma. Of those who were still working, 57% had changed profession. Patients who had had positive isocyanate-specific IgE used less medication and had fewer symptoms than those who had not shown isocyanate-specific IgE. Smoking was also associated with persistent respiratory symptoms.

When compared with the bronchial provocation test as the "gold" standard, IgE antibodies showed a sensitivity of about 20% and a specificity of 100%. Dr Henrik Nordman (Helsinki, Finland) discussed the reasons for the low sensitivity. He suggested that one factor may be the fairly short half-time of IgE (within 30 days the sensitivity seems to decrease by 50%).

Dr Jürgen Lewalter (Leverkusen, Germany) presented data on the possible modulating role of some enzyme polymorphisms in the clinical toxicology of isocyanates. Workers who metabolized aromatic isocyanates rapidly reported fewer symptoms than workers with a lower metabolism rate. He suggested that, at the same isocyanate exposure level, workers with low glutathione S-transferase and glucuronyl transferase activities, as well as high hydrolase and low N-acetyltransferase activities, generally report clinical symptoms of allergy depending mainly on the amount and half-time of HSA adducts.

According to Dr Lewalter's observations, in the routine occupational surveillance of isocyanate-exposed workers, individual recovery rates of free HSA lower than 50% during 16 hours after isocyanate exposure may be a sensitive and valid susceptibility marker for potentially IgE-induced sensitizations.

Professor Gabriele Sabbioni (Munich, Germany) and his collaborators had studied the work environment of German construction workers. For instance, during the surfacing of sports fields, MDI levels of 0.019—0.050 mg/m^3 and TDI levels of 0.016—0.033 mg/m^3 were measured. These levels correspond, at their highest, to 100% and 47% of the current German occupational exposure limit (MAK-werte; MDI 0.05 mg/m^3 and TDI 0.07 mg/m^3). MDA adducts were analyzed and calculated to correspond to an MDA exposure level of 66 ng/(kg · day). The estimated TD_{50} (defined as the dose that will halve the proportion of tumor-free animals at a specified point in time) for MDA is 12 500 000 ng/kg. According to these estimates, human exposure to MDA corresponds to 190 000-fold less than TD_{50}-level exposure in rodents.

Dr Christina Rosenberg presented data on exposure to isocyanates in Finnish polyurethane foam plants. The

measured levels of TDI varied, depending on the technology used, between 10% and 80% of the Finnish occupational exposure limit (OEL) (0.035 mg/m^3, expressed as isocyanate groups). The measured MDI levels were considerably lower. The highest concentration measured from a breathing-zone sample was 3% of the Finnish OEL. She emphasized the importance of the sampling method and derivatization reagent used in obtaining reliable results. Solvent-free sampling using reagent [1-(2-methoxyphenyl)piperazine] impregnated glass fiber filters was a reliable method for monitoring breathing-zone concentrations of monomeric isocyanates. Di-butylamine and impinger sampling was recommended for sampling complex mixtures (eg, polymeric isocyanates in thermal degradation processes of polyurethane).

Isocyanate-derived urinary metabolites are good biomarkers of recent exposure to isocyanates. Dr Rosenberg showed that the urinary excretion of 2,6-toluenediamine accurately reflects the exposure condition in flexible foam plants. However, this relationship was not established between urinary methylenedianiline and airborne concentrations of MDI, although the urinary levels clearly verified exposure to MDI.

Professor Peter Farmer (Leicester, United Kingdom) presented data on mass spectral assays for detecting aryl isocyanate adducts in DNA (deoxyribonucleic acid). DNA, spiked with synthetic standards of 4-methylphenyl isocyanate adducts with 2-deoxyadenosine (dA), 2'-deoxycytidine (dC) and 2'-deoxyguanosine (dG) and subsequently digested with enzymes to deoxynucleosides, was studied with liquid chromatography-mass spectrometry with electrospray ionization. Enhanced selectivity was achieved by mass spectrometry-mass spectrometry, with multiple reaction monitoring. The detection limit was 10—20 fmol/injection. Evidence for some adducts was seen for 4-methylphenyl isocyanate-treated and digested calf thymus DNA. Further improvements in the purification procedures and mass spectral sensitivity are needed for detecting nucleoside and nucleotide adducts of DNA exposed in vivo to isocyanates.

Dr Elizabeth Martin (Leicester, United Kingdom) discussed the potential to use ^{32}P-postlabeling to detect isocyanate adducts. This technique is currently the most sensitive method for detecting DNA adducts. Reference standards, dGp-AcMDA and dGp-MDA, were synthesized by the reaction of 4,4'-methylenedianiline (MDA) with deoxyguanosine 3'-monophosphate. After postlabeling, DNA modified in vitro with N-hydroxy-N'-acetyl-MDA showed 1 main component which corresponded to postlabeled dGp-AcMDA. No adducts were detected in lymphocyte DNA from workers exposed to MDI.

Although diisocyanates are highly reactive, information on their genotoxicity is scarce. Dr Hannu Norppa (Helsinki, Finland) showed results of studies on the genotoxic effects of diisocyanate exposure among Finnish polyurethane foam workers. Cytogenetic biomarkers in peripheral lymphocytes were examined in 56 rigid polyurethane foam workers exposed to MDI, 17 flexible polyurethane foam workers exposed to TDI, and 70 unexposed referents. TDI exposure was found to affect the frequency of cells with chromatid-type aberrations in nonsmokers, while MDI exposure resulted in a slightly increased frequency of sister chromatid exchanges (SCE). The subjects were also genotyped for genetic polymorphisms of 6 enzymes putatively involved in isocyanate metabolism. The NAT2 slow genotype (expected to result in slow *N*-acetyltransferase 2 activity) was associated with an increased baseline rate of cells with chromatid breaks, and subjects with the GSTT1 null genotype (no glutathione *S*-transferase T1 activity) showed an increased base-line SCE frequency.

Dr Claudia Bolognesi (Genoa, Italy) presented findings on the analysis of micronuclei (MN) in peripheral blood lymphocytes and buccal mucosa cells of the same Finnish polyurethane foam workers. A total of 41 MDI- and 17 TDI-exposed subjects and 56 referents were analyzed for MN frequency in lymphocytes; buccal mucosa samples were examined from 54, 17, and 64 subjects, respectively. TDI exposure was associated with an increased MN rate in both lymphocytes and buccal cells. In the MDI-exposed subjects, an increased MN rate was evident in buccal cells in nonsmoking subjects. The NAT1 slow genotype (slow *N*-acetyltransferase 1 activity) was associated with a decreased lymphocyte MN frequency in the exposed subjects and in smokers. The GSTM1 null genotype (no glutathione *S*-transferase M1 activity) was associated with an increased lymphocyte MN frequency in diisocyanate-exposed workers; a similar association was observed for the NAT2 slow genotype in MDI-exposed and reference subjects. Taken together, the results presented by Drs Norppa and Bolognesi showed that occupational exposure to TDI and MDI increases cytogenetic damage, indicating a possible genotoxic hazard of diisocyanate exposure.

Dr Ari Hirvonen reported findings on the potential role of metabolic genotypes in individual susceptibility to isocyanate-induced asthma. His group had analyzed the GSTM1, GSTM3, GSTP1, GSTT1, NAT1, and NAT2 genotypes from the aforementioned study population (ie, the 105 Finnish workers with isocyanate-induced asthma who had been exposed to MDI or TDI) and the 74 similarly exposed healthy workers. The GSTM1 null genotype, GSTP1 slow activity genotype, and NAT1 slow acetylator genotype conferred about a 2-fold risk of this disorder compared with the GSTM1-positive genotype, GSTP1 fast activity genotype, and NAT1 fast acetylator genotype, respectively. A combination of these at-risk genotypes showed no significant concurrent effects in individual susceptibility to isocyanate-induced asthma, and other studied genotypes had no significant

role in this context. These preliminary results suggest that impaired metabolic capacity may indeed increase the individual risk of isocyanate-induced asthma due to a decreased elimination of isocyanates from the body.

Dr Jürgen Pauluhn (Wuppertal, Germany) presented results on the acute inhalation toxicity of isocyanates in rats. His data indicated that different isocyanates show a wide range of acute toxicity and that the acute pathomechanisms appear to depend upon both specific chemical reactivity and physicochemical properties. According to Dr Pauluhn, the use of acute inhalation toxicity data on rats for regulatory classification purposes is not a simple process but requires judicious expert judgment.

In summary our main observations of the meeting are as follows:

1. Ambient air monitoring of isocyanates is both feasible and recommendable. A convenient procedure for the personal monitoring of exposure to monomeric isocyanates is sampling with reagent [eg, 1-(2-methoxyphenyl)piperazine] impregnated glass fiber filters.

2. Biological monitoring of exposure is practical and also to be recommended for use in occupational health surveillance. Urinary concentrations of toluenediamine, TDI-derived amine, reflect accurately the level of TDI exposure. Urinary methylenedianiline, MDI-derived amine, clearly indicates exposure to MDI, although a correlation between urinary amine and air concentrations has not been established.

3. Exposure to TDI and MDI in polyurethane foam production leads to an increased occurrence of cytogenetic changes both in circulating peripheral lymphocytes and in exfoliated cells from buccal mucosa and therefore suggests that occupational exposure to diisocyanates is a genotoxic hazard.

4. Preliminary results suggest that certain genotypes of drug-metabolizing enzyme genes (such as GSTM1 null genotype, NAT1 slow-acetylator genotype, and GSTP1 slow-activity genotype) may confer an increased risk of isocyanate-induced asthma.

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Which way epidemiology

"Take me wherever you wish. I am needed everywhere", Herbert von Karajan, in his typical modesty, is told to have replied to a taxi driver's question about destination. This is how Hans-Olov Adami opened his keynote address at the First Panum Seminar: The Future of Epidemiology in Copenhagen, on 25 January 1999, arguing that epidemiology, like von Karajan, is needed everywhere.

The seminar, chaired by Jorn Olsen, drew some 300 participants. The discussions concerned questions such as whether the future is for macrosocial public health epidemiology, for molecular epidemiology, or for something in between; whether epidemiology needs substantive theories; or whether it is a general toolbox rather than a science, as Stig Wall formulated it in his keynote; and whether methodological or substance-matter limits should be set for epidemiology. It was generally agreed that all levels are important. As Mervyn Susser put it, we need a multilevel conceptual framework of disease causation and prevention. A problem remains, Neil Pearce noted, for resource allocation seems to favor molecular epidemiology, drawing from public health epidemiology. Many of the discoveries on the causes of cancer, for example, have their origins in systematic international comparisons of cancer incidence. On the other hand, soon we will know the human genome, as David Hunter reminded, pointing towards the vast potential for molecular studies. But just as epidemiologists need to look inside the black box, so should biologists look outside it. In this spirit, Walter W Holland called for a broad focus for epidemiology and disease control. Many speakers were also worried about the paucity of preventive intervention studies, including those done in the Third World.

The invited speeches will be published in the *International Journal of Epidemiology*. Follow also the *Epidemiology Monitor*. A continuation is expected as a satellite seminar at the International Epidemiological Association meeting in Kaunas, Lithuania, in August 2000.

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