

ASSESSMENT OF THE POTENTIAL FOR ISOCYANIC ACID AND OTHER MONOISOCYANATES TO CAUSE RESPIRATORY IRRITATION AND SENSITISATION

Uses, occurrence, and occupational exposure to monoisocyanates

The primary use of methyl isocyanate (MIC, CAS no. 624-83-9) is as a chemical intermediate in the production of carbamate pesticides. MIC is also used to produce polyurethane foam and plastics (ATSDR, 2002). Isocyanic acid (ICA, Cas no. 75-13-8) does not have commercial uses because of its instability. The potential for occupational exposure to ICA largely arises when it is generated as a thermal degradation product of other industrial processes. Ethyl isocyanate is a liquid used commercially to make pharmaceuticals and pesticides (NJDHSS, 2000). Phenyl isocyanate (PIC, CAS no. 103-71-9) is a trace constituent in commercial diphenyl methane diisocyanate products.

There are a number of reports of workplace exposure to monoisocyanates. This information is relatively new because it has only recently become possible to analyse these substances in mixed chemical exposures such as those resulting from thermal breakdown. For example, heating of materials containing polyurethane (PUR) or phenol-formaldehyde-urea (PFU) has been found to give rise to monomeric diisocyanates and a number of other monoisocyanates, including ICA, MIC, and ethyl, propyl or butyl isocyanates in air (Karlsson *et al* 2000; 2001). These polymers are widely used in industry. Insulation materials (e.g. mineral wool) that contain PFU as a binder are used for insulation of ovens and pipes, whereas thermal degradation of PUR lacquers often occurs in car repair shops during finishing and machining processes that generate heat (Karlsson *et al*, 2001). Thermal degradation of PUR foams also occurs during flame bonding in the lamination and other industries (HSL, 2007). A number of factors have been reported to affect the emission of isocyanates to air during the thermal degradation of PFU and PUR-coatings; these are the applied temperature, the duration of heating and the type and quality of coatings that have been used (Karlsson *et al*, 2001).

Some of the reports of exposure to monoisocyanates relate to car body repair shops, and are associated with certain tasks that generate high temperatures such as grinding, welding or turning (Henriks-Eckerman *et al*, 2002; Sennbro *et al*, 2004 and White, 2003). In one study many different species of isocyanates were formed during thermal decomposition of polyurethane coatings; MIC, ethyl-, propyl-, butyl-, and phenyl isocyanate were found in air at concentrations of up to 290, 60, 20, 9 and 27 $\mu\text{g}\cdot\text{m}^{-3}$, respectively, in addition to various diisocyanates (Karlsson and Spanne, 2000). In two body repair training schools and a body repair shop the generation of aliphatic, alkenyl and aromatic di- and mono- isocyanates during the thermal degradation of polyurethane-based car paints was detected (Boutin *et al*, 2006). The most abundant isocyanates were monomeric diisocyanates and MIC. During cutting and grinding processes, the airborne concentration of MIC was 0.64-76.8 $\mu\text{g}\cdot\text{m}^{-3}$ close to the source of emission and 0.61-2.83 $\mu\text{g}\cdot\text{m}^{-3}$ in workers' breathing zone. A number of other monoisocyanates (including ethyl-, propyl-, butyl-, pentyl-, hexyl-, propylene-,

butylenes-, pentylene- hexylene- and phenyl isocyanates) were detected close to the emission source at concentrations of 0.11-3.45 $\mu\text{g.m}^{-3}$, but only ethyl isocyanate was detected in the breathing zone.

The Norwegian Labour Inspection detected low molecular weight isocyanates in air when materials containing some combination of phenol-formaldehyde-urea were heated (Karlsson *et al*, 1998). MIC and ICA were detected in air in significant concentrations.

Concentrations of ICA in the range 50-700 $\mu\text{g.m}^{-3}$ have been reported in air samples collected in an iron foundry during casting in sand moulds with furan resins (Karlsson *et al*, 2001). Relatively high airborne concentrations of MIC and ICA were also reported in a study of emissions from a chemical core binder system (Hot Box) based on formaldehyde-carbamide resin used in some Swedish die-casting foundries. Air concentrations in short-term samples (n=298) from workers ranged from <4 to 68 $\mu\text{g.m}^{-3}$ and from <4 and 280 $\mu\text{g.m}^{-3}$ for MIC and ICA, respectively. Based on these findings, the authors indicated that air concentrations of ICA may exceed local occupational exposure limits at a large number of workplaces associated with these industrial processes (Westberg *et al*, 2005).

Respiratory effects associated with methyl isocyanate

The toxicity of MIC has been extensively reviewed (ATSDR 2002, NRC 2004, SCOEL 2005) and a consensus is apparent, so the primary literature has not been consulted again for the purposes of this paper. It is firmly established that MIC is a potent respiratory irritant and is very acutely toxic by the inhalation route. MIC vapour has been shown to be a sensory and pulmonary irritant in mice and guinea pigs, causing delays in the respiratory cycle and producing cough. Nasal wetness has been reported as a common sign in exposed animals. Also, histological investigations in animal inhalation experiments have provided evidence of direct irritation to all areas of the respiratory tract. A very low 4 h LC_{50} of 26 mg.m^{-3} has been determined in the rat. Humans exposed to MIC during the industrial gas leak at Bhopal showed similar signs of coughing and respiratory distress and many of the 5000 deaths were attributed to pulmonary damage. Adverse effects at Bhopal occurred immediately or after a latent period of hours or days. Additionally, respiratory tract and eye irritation has been reported in human volunteers exposed to MIC under experimental conditions; a NOAEL of 0.9 mg.m^{-3} (for a 1-5 min exposure has been identified in these studies (SCOEL 2005).

WATCH is already aware of the absence of an internationally accepted experimental test system to predict the asthmagenic potential of a low molecular weight substance. Hence there is little or no useful experimental data in relation to the asthma endpoint. No cases of respiratory sensitisation (or skin sensitisation) due to MIC have been reported in published literature. No cases of respiratory disease associated with occupational exposure to MIC have been reported as part of the GB Surveillance of Work-related and Occupational Respiratory Disease (SWORD) scheme (HSE, personal communication). It should be noted that absence of cases of sensitisation/respiratory disease could be due to limited occupational exposure to MIC and does not necessarily indicate the absence of hazardous properties to the respiratory tract. There is evidence that MIC can cause an immune response in

animals and humans, which is of interest because respiratory sensitisation is generally thought to represent an allergic reaction (although the mechanisms are not definitely proven). MIC-specific antibodies were detected in the sera of guinea pigs injected with reactive MIC. In the same study, sera from 11/99 subjects exposed to MIC in the Bhopal incident was found to contain MIC-specific antibodies to IgE, IgG and IgM, although the titres were generally low (Karol *et al*, 1987).

Respiratory effects of Isocyanic Acid

As there are no published reviews of the toxicity of ICA, a full literature search was conducted using PubMed. No data from either animal or human studies regarding the potential for ICA to irritate or sensitise the respiratory system were found. Furthermore, no studies were identified on other aspects of the toxicity of ICA. As is the case with MIC, no cases of respiratory disease associated with occupational exposure to ICA have been reported under the SWORD scheme (HSE, personal communication), which could be a function of limited occupational exposure to this substance. The lack of data on the toxicological effects of ICA has been noted elsewhere. For example, the Swedish Criteria Group on Occupational Standards of the National Institute for Working Life conducted a review of literature on the ICA as part of establishing the scientific basis for proposing an occupational exposure limit; they found no data regarding the toxic effects of ICA in animals or humans (Montelius, 2002).

In the absence of studies providing direct information on the toxicity of ICA, the physicochemical properties and biological reactivity of ICA have been considered as these may inform on the potential to cause respiratory tract irritancy and sensitisation.

Physical and chemical characteristics of isocyanic acid

ICA is a strong organic acid with a K_a of 3.5. ICA is a very reactive compound that can readily transform into other substances. It can lose a proton in an aqueous environment under certain conditions, particularly if a strong base is present, forming an isocyanate. ICA is a tautomer of the less stable, cyanic acid (CAS no. 420-05-3). These forms interchange by a tautomerisation reaction, involving the migration of a hydrogen atom or proton accompanied by a switch of a double bond. ICA is an unstable liquid above 0°C with a tendency to polymerise. The primary polymerisation product, which is also generated in the gas form, is cyanuric acid (CAS no. 108-80-5), a cyclic trimer. ICA is soluble in water, but disintegrates both via ionisation and by formation of ammonia and carbon dioxide.

Biological reactivity

Various studies in the literature discuss the biological effects of ICA formed by the spontaneous non-enzymic transformation of urea, which occurs at elevated levels in kidney disease. The reactive group of ICA can modify a variety of proteins by carbamylation. ICA carbamylated low-density lipoproteins were found to induce endothelial cell injury; expression of adhesion molecules; vascular smooth muscle cell proliferation in patients with kidney disease, leading to atherosclerosis (Shah *et al*, 2008). ICA generated from plasma urea in impaired renal function can carbamylate

haemoglobin. Carbamylated haemoglobin and other carbamylated proteins are markers for uraemia in kidney disease (Wynckel *et al*, 2000).

ICA carbamylation of the amino terminus of proteins can block N-terminal sequencing and further protein characterisation. ICA can also attack the side chains of lysine and arginine residues rendering a protein unsuitable for many enzymatic digests.

Whilst the relevance of these studies to the potential for ICA to induce respiratory effects following inhalation exposure is unclear, these findings nevertheless indicate that ICA is biologically reactive, can interact with a number of different molecules and can cause damage to biological systems.

Structure-activity relationships

Quantitative structure-activity relationship (QSAR) analysis is a process that can be used to quantitatively correlate chemical structure with a well defined process, such as biological activity or chemical reactivity. QSAR techniques have been used to investigate the relationship between chemical structure and the occupational asthma hazard of low molecular weight organic compounds and a software model¹ having the objective to predict the asthmagenic potential of chemicals from their structure has been developed (Jarvis *et al*, 2005). The Occupational Asthma Hazard Prediction model uses logistic regression to predict the hazard index of chemicals in the range 0 to 1 where a prediction > 0.5 signifies asthmagenic potential. The model predicts that ICA has an asthma hazard of 0.11, commensurate with having a low probability of being an asthmagen (Centre for Occupational and Environmental Health, personal communication). The QSAR analysis of isocyanate species with two –NCO reactive groups per molecule indicated that these had a hazard index >0.5. However, the difference in asthma hazard index predicted for di- and mono isocyanates by the model is largely a reflection on there being published positive respiratory sensitisation data for some diisocyanates and no comparable data for monoisocyanates. Hence, the model is simply reflecting back the existing pattern of data, rather than making a prediction based on further insight.

It has recently been hypothesised that diisocyanates may be more likely to induce immunological reactions than monoisocyanates because of the presence of two rather than one reactive –NCO group. Diisocyanates with two reactive –NCO groups may be able to directly crosslink cellular proteins in a way that alters their conformation, thus making them immunologic (Wisnewski *et al*, 2000). The mechanism of diisocyanate induced asthma is still, however, largely unknown.

Respiratory effects associated with other monoisocyanates in commercial use

A literature review identified no reports of respiratory sensitisation or information on respiratory tract irritancy in humans exposed to other monoisocyanates. As is the case

¹ The asthma hazard prediction model is freely accessible through the internet at <http://www.coeh.man.ac.uk/research/asthma>

with MIC and ICA, no cases of respiratory disease associated with occupational exposure to other monoisocyanates have been reported under the SWORD scheme (HSE, personal communication), although this could be a function of limited occupational exposure to these substances. However, there is some evidence from animal models, provided by three studies, that some monoisocyanates can elicit responses that are comparable to certain changes seen in human cases of respiratory sensitisation. Also, in humans the presence of antibodies against monoisocyanates has been demonstrated in TDI-exposed workers, providing evidence of immunoreactivity of monoisocyanates.

The respiratory effects of PIC were investigated in rats following 2 weeks (6 hr.day⁻¹, 5 days.week⁻¹) inhalation exposure to PIC at concentrations between 0 and 10 mg.m⁻³ (Pauluhn *et al*, 1995). Intraluminal inflammation of airways, hypertrophy of bronchial smooth muscle, epithelial desquamation and eosinophilia of the airways was observed in rats exposed to PIC at and above 7 mg.m⁻³. The authors proposed that effects observed were fully consistent with a persistent inflammatory response involving tissue of direct contact. In addition, they suggested that the experimental animal model for exposure to PIC had been able to reproduce the major pathological features of the asthmatic airway including: airway hyper-responsiveness, infiltration of airway walls with eosinophils and neutrophils, hypertrophy of smooth muscle, mucus secretion, airway plugging and partial occlusion of the airway lumen with mucus and cellular debris. Although the authors propose that 'asthma-like' effects were observed based on a number of biological features that have been associated with asthma, other classical features of allergic response (i.e. IgE-mediated hypersensitivity) were not investigated. It is not therefore possible to determine the sensitisation potential of PIC from the study. Nevertheless, the findings indicate that PIC can induce direct chronic inflammation and irritation in rats following inhalation exposures at and above 7 mg.m⁻³.

In another study, PIC was found to be a potent inducer of cellular and humoral responses in mice (Karol & Kramarik, 1996). The potency of PIC was assessed using the mouse ear swelling test. PIC was found to be the most potent isocyanate tested yielding an SD₅₀ (dose predicted to sensitise 50% of the mice) of 0.04 µmol.kg⁻¹ compared with SD₅₀ values of 0.5, 2.1, and 30.5 µmol.kg⁻¹ for the diisocyanates hexamethylene diisocyanate, methane diisocyanate and TDI, respectively. When tested for the ability to stimulate humoral immune responses, antibody titres to PIC were ten times greater than that of TDI. Hapten-specific IgE was not detected to either isocyanate. The clinical significance of these findings is uncertain, although the authors suggested that the demonstration of PIC as a potent inducer of both cellular and humoral immune response raises concerns that commercial products in which it occurs as a trace contaminant could have sensitisation potential.

In an earlier study, the ability of 5 monoisocyanate-ovalbumin (OA) conjugates including tolyl isocyanate (TIC) and hexyl monoisocyanates (HIC) to induce pulmonary hypersensitivity towards the hapten component was investigated in guinea pigs (De Ceaurriz *et al*, 1987). The animals were exposed daily to an aerosol of each monoisocyanate-ovalbumin conjugate for up to 15 days. Pulmonary responses were determined using a respiratory index (RI) calculation. The RI index was based on the observation in a previous study of an increased respiratory rate and decreased tidal volume followed by a slow gasping type respiratory pattern and collapse in sensitised

guinea pigs at challenge (Karol, 1978). Increases in respiratory rate and/or respiratory collapse occurred in guinea pigs exposed to TIC-OA and HIC-OA by days 9 and 15, with corresponding RI of 155 and 177, respectively. The authors proposed that these RI changes reflected sensitisation challenge responses to the TIC-OA and HIC-OA exposures during the latter part of the study, but antibody responses were not measured. Consequently, the study provides only limited insights into the potential for monoisocyanates to induce immunological effects.

The presence of serum IgE antibodies against two albumin-bound monoisocyanates, diphenylmethane 4-(mono) isocyanate (MMI) and TIC, has been demonstrated in TDI-exposed workers (Baur and Fruhmann, 1981). The study population was 195 TDI-processing workers, employed for between 2 weeks and 38 years, of which 55 had either TDI-related asthma or had developed COPD during employment at the plant. Among the workers with respiratory problems, 7 had IgE antibody reactivity to albumin-bound TIC and 5 had reactivity to albumin-bound MMI. Bronchial challenge testing to TDI conducted in some workers suggested that the TIC antibody activity might be correlated with the TDI challenge threshold concentration. The authors proposed that antibody reactivity to albumin-bound MMI and TIC in TDI workers suggested immunologic cross-reactivity of these different protein-bound isocyanates. They considered the findings to be clinically relevant suggesting that subjects who are sensitised to TDI could develop asthmatic reactions upon inhalation challenges to these monoisocyanates. Although serum IgE antibodies to certain monoisocyanates were observed in some workers with respiratory problems included in the study, no IgE mediated hypersensitivity to monoisocyanates was reported in these workers. On the basis of this study it is not therefore possible to determine whether individuals with prior exposures to diisocyanates are likely to develop asthmatic reactions when exposed by inhalation to monoisocyanates.

Summary

Monoisocyanates are not widely used in the workplace, but they can be process-generated, for example from the thermal breakdown of polyurethane coatings. The GB regulatory framework requires that the stringent workplace risk management measures applied to diisocyanates, appropriate because of the established respiratory sensitisation and irritant properties of this group of substances, are also applied to monoisocyanates.

With the exception of methyl isocyanate, information on the toxicity of the monoisocyanates is sparse. There is no direct evidence that any of the monoisocyanates can cause respiratory sensitisation. Considering indirect evidence, several studies in animal models have provided indications that certain monoisocyanates (phenyl- tolyl and hexyl- isocyanate) can elicit responses that could be due to allergenicity, but actual evidence of immunological involvement is absent or very weak. Two investigations have shown that some monoisocyanates (methyl- and tolyl- isocyanate) have the capacity to elicit an immunological response in the form of specific antibody production in humans. Also, the haptensation of proteins by monoisocyanates is biologically plausible because of their reactivity. However, this indirect information on the allergenicity and immunoreactivity of the monoisocyanates is very limited, and insufficient to reliably inform on their potential to cause respiratory sensitisation.

Concerning irritancy, it is known that methyl isocyanate is a potent respiratory tract irritant. For other monoisocyanates there is little direct information on their irritancy. However, isocyanic acid is a strong organic acid and it can therefore be predicted to possess irritant properties, as would other monoisocyanates.

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