MINI-REVIEW

Methyl Isocyanate and Carcinogenesis: Bridgeable Gaps in Scientific Knowledge

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Abstract

Methyl isocyanate may have a role in cancer etiology, although the link is unclear. There is evidence in the literature that it can induce cancer in animals but the carcinogenic potency is weak. Pheochromocytoma of adrenal medulla and acinar cell tumors of pancreas have been observed in methyl isocyanate exposed animals. Conversely, emerging data from population-based epidemiological studies are contradictory since there is no evidence of such cancers in methyl isocyanate exposed humans. Recently, we reported a high prevalence of breast and lung cancers in such a population in Bhopal. In vitro findings appearing in the latest scientific literature suggest that genomic instability is caused by methyl isocyanate analogs in lung, colon, kidney, ovary epithelial cells, and that hepatocytes may undergo oncogenic transformation, have obvious implications. The conflicting information prompted us to present this update over the last three decades on methyl isocyanate-induced cancers after an extensive literature search using PubMed. While the pertinent literature remains limited, with a scarcity of strong laboratory analyses and field-epidemiological investigations, our succinct review of animal and human epidemiological data including in vitro evidences, should hopefully provide more insight to researchers, toxicologists, and public health professionals concerned with validation of the carcinogenicity of methyl isocyanate in humans.

Keywords: Methyl isocyanate - carcinogenicity - need for validation - review

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Introduction

Isocyanates (IC) (N=C=O) are low molecular weight organic, aromatic and aliphatic compounds consist of two double bonds with strong chemical reactivity. Methyl isocyanate (MIC), Toluene di-isocyanate (TDI), Diphenyl methane di-isocyanate (MDI), Hexamethylene di-isocyanate (HDI), and Naphthalene di-isocyanate (NDI) are the most commonly used IC's for industrial purpose (CCC, 2008; Nakashima et al., 2002). MIC is a well-known toxic industrial chemical (TIC) that raises serious concern to human health as well as environment. Twenty seven years after the accident (3rd December, 1984), Bhopal MIC gas leak is still deemed as a prototype of world's worst chemical catastrophe. The accidental exposure killed 3600 individuals and an estimated 2,00,000 was lethally exposed. 50,000 were exposed and expected to survive with long-term effects (Dhara and Kriebel, 1993). The perilous potential of MIC is known to cause short-term and long-term disabilities and chronic health effects (Mehta et al., 1990). At the time of the catastrophe, toxicological profile and complex mechanisms of MIC was not understandable in animals and humans. Immediate and later studies were done only after the MIC disaster. However, very little work has recognized the toxicity of MIC in humans. This scarce situation incited the Indian Council of Medical Research (ICMR) now to call for a research proposal in the context of biomonitoring for studying the long-term effects in the MIC exposed population after 27 years of MIC gas leak in Bhopal (ICMR, 2010).

MIC ($CH_3N=C=O$) is an intermediate used in the last stages of carbamate pesticides such as carbaryl, sevin, aldicarb, methomyl, and carbofuran (Bucher, 1987; ATSDR, 2002; New Jersey HSFS, 2002). MIC is a direct pulmonary irritant that targets the lungs on inhalation at very low levels (Gupta et al., 1991; Jones et al., 1992). Occupational or accidental exposure to MIC is known to cause chronic respiratory symptoms like asthma, rhinitis and hypersensitivity pneumonitis (Vandenplas et al., 1993; Baur et al., 1994). Immunotoxicity (Luster, 1986; Karol et al., 1986), genotoxicity (Tice et al., 1986; Conner et al., 1987; Shelby et al., 1987), reproductive and developmental toxicity (Schwetz et al., 1987) of MIC are well documented in the literature. But, very little is known about the carcinogenic potential of MIC in animals and the accidental or occupational hazard of MIC in humans with respect to cancer is not exactly known.

As far as the carcinogenic potential of MIC is concerned, information cited in the literature is divergent in the current

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phase and has not received considerable interest or serious attention in relevance to humans. Material data sheets of toxic chemical substances and analyzing agencies are stating that MIC is a non-carcinogenic substance. But, there is a growing recognition of *in vitro* evidences are contradictory to this hypothesis and illustrating the oncogenic transformations in human cells treated with a surrogate chemical of MIC (Mishra et al., 2009abd; Raghuram et al., 2010; Hariom and Mishra, 2011; Hariom et al., 2011). Perhaps, the clinical-epidemiological evidences are questioning the MIC exposure in lung and breast cancer etiology (Senthilkumar et al., 2011). Hence, the link of MIC in cancer etiology is really puzzled in the current scientific knowledge.

In these circumstances, we analyzed the carcinogenic data of MIC through an extensive literature search using the term "methyl isocyanate and cancers" in PubMed for the articles published up to April 2012. In light to our knowledge, literature pertinent is too limited and not much review or literature has been reported the carcinogenic potential of MIC. Hence, we made an attempt to review the available literature, those shown the carcinogenic evidence of MIC in animals, humans and *in vitro* experiments. This article could be useful to researchers, toxicologists, and public health professionals to assess the carcinogenic potential of MIC in future.

Numerous testing approaches are used to assess the carcinogenic risk of chemicals. Systematic carcinogenicity testing mainly depends on animal bioassays, human epidemiological evidence and laboratory experiments with human cells *in vitro*. Hence, this review will focus on up-to-date carcinogenic data of MIC in particular, animal studies done *in vivo*, data derived from clinical and epidemiological studies in humans, *in vitro* reports on oncogenic transformation in cell lines with special regard to genomic instability and a discussion on future needs.

Animal studies

It is of paramount importance to note that the animal studies are the validating evidence in carcinogenicity testing. The base-line evidence was provided by Gassert et al. (1986), observed the peribronchial lymphoid hyperplasia in male lister hooded rats of 14 months after 2-hour exposures to 11, 21, or 31 ppm of MIC. After this preliminary work, to date, only two pivotal studies have made great contribution to our understanding on MIC-induced carcinogenicity.

First effort was made by Ennever and Rosenkranz, (1987), reported the genotoxic carcinogenicity potential of MIC using prediction and battery selection method. This study finding stated that MIC has a significant potential for inducing cancer in rodents. In conclusion, this study revealed MIC has low and weak carcinogenic potency and the risk may be identified upon the level, duration and mode of exposure. However, this study assumed that the chemicals carcinogenic to rodents are also potentially carcinogenic to humans.

Subsequent *in vivo* study of Bucher and Uraih (1989) reported carcinogenicity and pulmonary pathology associated with a single 2-hour inhalation exposure of laboratory rodents to MIC. This study observed an increase of pheochromocytoma of adrenal medulla in male rats and less frequent in female groups exposed to MIC (3- and 10-ppm concentration). These findings suggested the occurrence of adrenal gland tumors in MIC inhaled rats. Adenomas of pancreatic acinar cells were also found to be increased in male rats of the exposure group. However, the evidence in support of this association is weak.

Research findings originated from these animal studies at the interface suggested that MIC has low carcinogenic potential. After these two studies, the predictive risk assessment of MIC has not received much attention in animals and humans. If animal experiments provide direct link of a TIC in cancer etiology, there is a substantial need to validate the cause-effect relationships through clinical-epidemiological investigations relevance to populations.

Human population-based epidemiological studies

Further evidence appears from IC exposed population-based epidemiological studies from the accidental and occupational settings as well as cancer registries. Population groups that may be exposed to IC accidentally or from the occupational setup includes the workers of polyurethane foam industries (PFI) and pesticide handling or production or using IC as an intermediate, and habitants living near the vicinity of the production plant. Scarce in epidemiological evidence of MIC- induced cancer morbidity data in the literature imposed us to address the cancer morbidity status of general IC exposure evidences along in the context.

The first survey of cancer morbidity in IC exposure was conducted by Sorahan and Pope (1993) demonstrated the site-specific cancer mortality in TDI exposed PFI workers of United Kingdom (UK). Larynx, lung, kidney and pancreatic cancers were significantly observed in the female workers. Increased cancer incidence in women occurred at sites of cancer known to be related to cigarette smoking and other factors. However, the study concluded that cancer rates in TDI exposed workers were lower than the general population.

Hagmar et al. (1993a) investigated the cancer risk in Swedish polyurethane foam manufacturing industry (PFMI) workers exposed to TDI and MDI. Rectal cancer and non-Hodgkin's lymphoma (NHL) was observed among the workers. However, the occurrence of these cancers was non-significant and the overall cancer incidence was low in the cohorts. Hagmar et al. (1993b) subsequently studied the association of occupational exposure to TDI and MDI for cancer risk in the PFMI. The cohort based case-referent study revealed a weaker association of colon cancers was reported. However, no association of rectal cancer and NHL was observed. Schnorr et al. (1996) investigated the cancer mortality in TDI exposed PFMI workers. This study results stated the occurrence of rectal cancer and NHL, however non-significant association was found. No increase of breast cancer reported in women workers. These findings were in concordance to the conclusion of Hagmar et al. (1993b). A study conducted by Sorahan and Nichols (2002),

updated the Mortality and morbidity status of TDI and MDI exposed UK flexible PFI workers for the time period 1958-1998. No significant risk or association found in lung cancer. However, the study was unable to link the work related exposure to IC in lung cancer or non-malignant diseases of the respiratory system. Mikoczy et al. (2004) updated the cancer incidence for the time period 1959–98, in Swedish PFI workers exposed to TDI and MDI. Increased lung cancer risk was found among the female workers. As similar to the findings of Sorahan and Nichols (2002), this study also unable to link isocyanate exposure with lung cancer risk.

Dikshit and Kanhere (1999) studied the MIC exposed population of Bhopal to reveal the effect of gas exposure for cancer risk. Three most common cancer sites (lung, oropharynx and oral activity) studied for the incidence rates from 1987 to 1992 in males. Relative risks (RR) were estimated using the cases from the cancer registry and controls from a tobacco survey for the gas-affected regions. This descriptive study estimated the RR of lung (1.4), oropharynx (1.3), and oral cavity cancer (0.7) for gas-affected regions in the year 1992 in comparison to gas-unaffected regions and the year 1987-1990. This case-control investigation estimated the RR of lung (0.9), oropharynx (1.4) (adjusted for smoking) and oral cavity cancer (1.2) (adjusted for tobacco chewing) as the effect of the gas exposure. The study concluded that full potential of excess risk, if any, may not manifest for 15-20 years after the gas exposure. However, the findings suggested that Bhopal population should be closely monitored in the future to observe the effect of gas exposure on cancer risk and highly recommended that the effect of gas exposure on cancer must be studied in other settings in the future. Next to Dikshit and Kanhere (1999), Ganesh et al. (2005) studied eight years (1994-2002) cancer pattern in MIC gas survivor and their offspring. The incidence rate was higher in male survivors and male offsrings as compare to female survivors and female offsprings. Cancer of breast (26.16%) was highly reported in the MIC exposed population. However, the breast cancer incidence was low when compared with the non-exposed cancer population (32.71%). This study had many limitations and failed to report the age-specific gender-wise cancer pattern in this population.

It is noteworthy to that our recent population-based epidemiological study reported age-specific gender-wise cancer morbidity in MIC exposed long-term survivors and their offspring (Senthilkumar et al., 2011). We updated the five year morbidity status of 1261 cancer cases those are surviving after exposure to MIC on December 3rd, 1984, in Bhopal. For the first time, high prevalence of breast (n=231) (18.31%) and lung (n=103) (8.16%) cancers were reported as compared to any other site. Besides, the relative percentage of cancer morbidity was higher in females (n=639) (50.67%) as compare to males (n=622) (49.32%). However, there are no significant differences in morbidity status between males and females. Our study also observed some of the cancers such as chronic lymphoid leukemia (CLL), cancer of stomach, gall bladder, anal canal, glandular tissue's, sarcoma's which were not previously reported in this population by Ganesh et al. (2005). Moreover, our findings suggested the increased incidence of cancers in MIC affected population of Bhopal and recommended the need of large population-based case-control investigations in future. Unfortunately, as similar to the findings of Mikoczy et al. (2004), our study also failed to link the MIC exposure in lung cancer risk. However, vulnerability of this population to cancer is coherent. This circumstantial hypothesis is supported by many studies stated the association of environmental contaminants, organic solvents and chemicals especially organochlorines and pesticides in breast (Blair et al., 1998; Ibarluzea et al., 2004; Engel et al., 2005; Newby and Howard, 2006) and lung cancer etiology (Burns, 2005). After 27 years of exposure, in support to the cancer morbidity study (Senthilkumar et al., 2011), our continuous biomonitoring efforts reported a wide-spread of MIC exposure-induced genetic alterations in Bhopal gas tragedy survivors (Malla et al., 2011). An elevated level of chromosomal aberrations suggested the genetic instability among the MIC exposed population. Our study findings suggested that this population is more vulnerable to cancers. These findings are validated in concordance to the pivotal studies reported previously by Hagmar et al. (1994: 1998) and Bonassi et al. (2000).

In vitro studies

There has been a great progress in the last few years in understanding the carcinogenic mechanisms of MIC. Laboratory experiments with human cells *in vitro* is the hallmark testing approach used to validate the oncogenic transformation ability of a specific chemical. Two decades later to animal studies (Ennever and Rosenkranz, 1987; Bucher and Uraih, 1989), initiative efforts of Mishra et al. (2009) on seminal *in vitro* studies emphasized that N-succinimidyl N-methylcarbamate, a carbamate esters and surrogate chemical of MIC may induce inflammation, DNA damage, cell cycle arrest and apoptosis in IMR-90 human lung fibroblasts may leads to genomic instability. This is the first *in vitro* study to conclude MIC-induced genomic alteration's involvement in the development of carcinogenicity. Mishra et al. (2009b) subsequently studied MIC - induced genomic instability in human colon epithelial cells. The results revealed that MIC exposure leads to genomic instability with DNA double-strand breaks and impairs DNA damage-response pathways in cultured human colon epithelial-FHC cells. Significantly, the findings evidenced IC can cause genomic instability in colonocytes. In another study, Mishra et al. (2009c: 2011) studied p53, cyclin-E, and Rad50 expression in surgically resected gallbladder cancer specimens of MIC affected population. This study finding concluded an altered expression of p53, Rad50, and cyclin-E in the malignant transformation of gallbladder carcinoma in MIC exposed cohort. Mishra et al. (2009d) investigated IC-mediated mitochondrial oxidative stress in human kidney epithelial HEK-293 cells. This study reported a wide range of chromosomal instability through mitochondrial-mediated oxidative stress, deregulated cell cycle progression and pro-tumoural instigation of senescence in the human kidney epithelial cells.

Raghuram et al. (2010) analyzed the IC exposure induced oncogenic transformations in B/CMBA.Ov mouse ovarian epithelial cells. The study observed stress-induced premature senescence and plausible association of chromosomal and microsatellite instability on IC exposure. The findings suggested that stress-induced premature senescence in ovarian epithelial cell proliferation may lead to ovarian dysfunction and carcinogenesis. Hariom et al. (2011) investigated MIC-induced cell cycle deregulation in cultured NCTC clone 1469 mouse liver epithelial cells. The study concluded MIC-induced alterations in cell cycle proteins that lead to genomic instability may involve in liver carcinogenesis. Consequently (2011) reported the IC - induced toxicity in HPAE-26 pulmonary arterial endothelial cells. The study observed MIC substitute induced tumorigenicity with decreased expression of different tumor suppressor and repair proteins.

Collective findings of *in vitro* studies (Mishra et al., 2009; Raghuram et al., 2010; Hariom and Mishra, 2011; Hariom et al., 2011) concluded that MIC analog-induced genomic instability in lung, colon, kidney, ovary epithelial cells, and liver may undergo oncogenic transformation and have implications in carcinogenesis.

Future needs validation

For the first time, this article projected the carcinogenicity data of MIC with its clinical-epidemiological and *in vitro* studies relevance to cancer. From this milieu, we discuss some of the confrontations to be resolved and need to fill the gaps in future.

Human epidemiological studies and *in vitro* evidences are confounding to animal data. While MIC has low carcinogenic effect in animals showed only two types of cancer (Pheochromocytoma of adrenal medulla and acinar cell tumors of pancreas) in the *in vivo* study, where as epidemiological data suggesting the possibility of lung and breast cancers in MIC exposure. No evidence of pheochromocytoma and acinar cell tumors found in the human data. However, a very less percentage of other pancreatic malignancies were observed in MIC exposed males (n=622) (1.12%) and females (n=639) (0.46%) (Senthilkumar et al., 2011). A spectrum of cancers explored frequently in human epidemiological studies was not observed in animals, whereas the same trend is parallel to *in vitro* studies. These comparisons reflect the limitation of extrapolative analysis done in animal studies till now. Inadequate approach of animal experiments reflects the uncertainties of available data. However, the major progress was made only after the *in vitro* experiments and clinical-epidemiological studies stating the profound role of MIC in cancers.

As similar to MIC, pheochromocytoma was often reported in animals exposed to hazardous substances like Methyl Isothiocyanate (MITC) (HSDB, 1997; RCD, 2003), Metam Sodium (Sodium N-Methyldithiocarbamate) (HSDB, 1997; RCD, 2004), Carbon tetrachloride (JPRC, 1998; Nagano et al., 1998; ATSDR, 2005), 4,4'-methylenedianiline dihydrochloride (Lamb et al., 1986), Dichlorobenzene (NTP, 1987; Elovaara, 1998), 4-Chloroaniline (CICAD, 2003), Ethoprop (RCD, 1995), Propylene Oxide and TDI (NTP, 2011ab) and 2-Butoxyethanol (IARC, 2006). Acinar cell tumors also frequently observed in Metam Sodium (Sodium N-Methyldithiocarbamate) (HSDB, 1997; RCD, 2004), Methyl Isothiocyanate (MITC) (HSDB, 1997; RCD, 2004), Butyl Benzyl Phthalate (IARC, 1999), and Perfluorooctanoic acid (Lau et al., 2007; Peters and Gonzalez, 2011).

In animal study, Bucher et al. (1987) reported pheochromocytoma and acinar cell tumors only in male rats groups exposed to MIC. Based on cancer incidence in animal experiments, we may assume that the male group is more vulnerable to MIC as compared to females. But, this gender-wise trend is totally contradictory to human epidemiological data. We have also observed an increased breast cancer incidence in females and lung cancers in males of MIC exposed population. On the basis of overall gender-wise morbidity and high incidence of cancers, our study revealed that females are more vulnerable to cancers on exposure to MIC (Senthilkumar et al., 2011). Mikoczy et al. (2004) also observed an increased lung cancer risk among Swedish polyurethane foam industry female workers exposed to IC.

OEHHA (2001) guidelines indexed MIC as a hazardous substance that targets the respiratory and reproductive system on exposure. If lung is a target organ to MIC, it may possibly exhibits toxic effects accordingly to the exposure criteria. While there is no evidence of lung cancer etiology in MIC exposed animals, the human data arising from population based-epidemiological studies shown increased lung cancer incidence in MIC exposed population (Senthilkumar et al., 2011).

Material data information stating that MIC is primarily used in the last stages of carbamate pesticides, polyurethane foams, plastics and polymers (Bucher, 1987; Hrhyhorczuk et al, 1992; New Jersey HSFS, 2002; ATSDR, 2002). Up-to-date investigations are supporting the link of carbamate pesticide in the etiology of lung cancer. A study conducted by Pesatori et al. (1992) observed twofold excess lung cancer risk in Florida pesticide applicators exposed to carbamate insecticides, organophosphate and phenoxyacetic acid herbicides. Case-control studies conducted by McDuffie et al. (2001) and Zheng et al. (2001) reported the risk of NHL in carbamate insecticide exposure. As an intermediate in the production of carbamate pesticides MIC cannot be underestimated. The mechanism of action or indirect plausibility on carcinogenic effect may be masked to some extent.

On the other hand, directly tested *in vitro* studies are more clear and supportive to the human data obtained in the population-based epidemiological studies (Mikoczy et al., 2004; Senthilkumar et al., 2011). *In vitro* evidences are suggesting the positive interaction of MIC with lung and pulmonary arterial endothelial cells (Mishra et al., 2009a; Hariom and Mishra, 2011), colon (Mishra et al., 2009b), kidney (Mishra et al., 2009d), ovary epithelial cells (Raghuram et al., 2010) and liver (Hariom et al., 2011) may leads to oncogenic transformation and also assumed the possibility of site-specific cancers. However,

except lung the other sites assumed for cancers in the *in vitro* studies are less reported in the exposed population. Lung (8.16%), Colon (1.58%), GB (2.29%), Kidney (0.95%), Ovary (3.40%), and Liver (2.14%) were reported among 1261 cancer cases of MIC exposed population of Bhopal (Senthilkumar et al., 2011). In support, many literatures are linking the chromosomal abnormalities in MIC exposed population (Ghosh et al., 1990; Malla et al., 2011), obviously the genomic instability or chromosomal abnormalities are known to involve in the pathway of carcinogenesis (Bonassi et al., 2000: 2008).

Presently, in our opinion, *in vitro* human cell line experiments and large case-control investigations on population groups exposed to MIC extended to all types of cancers, in particular to lung and breast carcinoma's deserves full priority in validation. Finally, with respect to cancer prevention, *in vitro* experimental research data and clinical-epidemiological evidence must be correlated more in detail to unmask the cancer risk in MIC affected population. To achieve this goal, clinical-experimental toxicologists and epidemiologist should work together to understand the association of MIC in cancer risk.

To sum up, based on limited knowledge with direct and indirect plausibility on MIC exposure-induced cancers, there is an utmost need to fill this incongruence gap by studying the toxicological science of MIC *in vitro* as well as to include well-powered clinical-epidemiological data more comprehensively. Constant laboratory analysis and field-epidemiological evidence-based investigations can help to fill the gap between animals, humans and *in vitro* evidences. In-depth carcinogenic evidence of MIC must be explored through *in vitro* strategies with relevant to human cancers previously reported in the population-based epidemiological studies or we have to wait for the future ICMR findings to understand the MIC-induced carcinogenesis and toxic effect in humans.

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