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Health Effects of Trimellitic Anhydride Occupational Exposure: Insights from Animal Models and Immunosurveillance Programs

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Abstract
Acid anhydrides are used by chemical industries as plasticizers. Trimellitic acid (TMA) is an acid anhydride widely utilized in factories to produce paints, varnishes, and plastics. In addition to causing direct irritant effects, TMA can augment antibody responses in exposed factory workers leading to occupational asthma. Therefore, industries producing TMA have implemented occupational immunosurveillance programs (OISPs) to ensure early diagnosis and medical management, involving exposure reduction/ complete removal of sensitized workers from exposure areas. Multiple animal models (mice strains, rat stains, guinea pig, swine) with different exposure patterns (dermal, nasal, vapor inhalation exposures for different time frames) have been described to elucidate the pathophysiology of TMA exposure. In TMA factories, in spite of implementing advanced environmental controls and personal protective measures to limit exposure, workers become TMA-sensitized. Animal models revealed sIgG, sIgE, sIgA, and sIgM along with pulmonary lesions, cellular infiltrates, alveolar hemorrhage, and pneumonitis associated with TMA exposure. Molecular studies showed involvement of specific functional gene clusters related to cytokine and chemokine responses, lung remodeling, and arginase function. However, thus far, there is no evidence supporting fetotoxic or carcinogenic effects of TMA. OISP data showed IgG and IgE responses in exposed factory workers. Interestingly, timelines for detectable sIgG response, in conjunction with its magnitude, have been shown to be a predictor for future sIgE response. OISPs have been very successful so far at creating a healthy and safe working environment for TMA-exposed factory workers.

Keywords Occupational health · Haptens · Trimellitic anhydride · Industrial asthma · IgE · Low molecular weight allergens

Background

An estimated 15% of chronic obstructive pulmonary disease and asthma cases are work-related, costing $7 billion in work productivity loss in the USA. [1] A significant portion of this is caused by workplace exposure to low molecular weight chemicals (LMW) that are irritating and potentially sensitizing. Acid anhydrides, such as trimellitic anhydride (TMA), are LMW agents used for the production of plastics, paints, varnishes, and adhesives. TMA (IUPAC name 1,3-dioxo-2-benzofuran-5-carboxylic acid) is an acid anhydride with a molecular weight of 192.126 g/mol. It is produced as powder and remains solid at normal temperature and pressure. It is instantly hydrolyzed in water and produces trimellitic acid though an exothermic reaction. [2] Approximately 100,000 metric tons/year TMA are produced worldwide, the majority of which (65,000 metric tons/year) are produced in the USA. TMA is widely used as a hardening agent by the plastic and paint industry (Fig. 1). However, it is known to cause a spectrum of work-related immunologic and non-immunologic respiratory health problems requiring special precautions in its manufacturing and handling. [3–5] In ambient moisture, TMA can be converted to trimellitic acid which acts as a respiratory irritant. But once systemically absorbed, TMA can also bind to human proteins capable of eliciting specific serum IgG- and/or IgE-mediated immune disorders including asthma [4, 6–8]. According to OSHA (Occupational Safety and Health Administration, USA) standards, the permissible exposure limit (PEL) for TMA is 0.04 mg/ml. However, In TMA plants, personal exposure ranges from <0.6 to 1900 μg/ml. [7, 8] Exposure to TMA may occur as dust and/or as a fume in the production phase where it is formed by catalytic oxidation of 1,2,4-trimethyl benzene or in packaging and logistic units of TMA factories. An estimated 20,000 factory workers in the USA are annually exposed to TMA in their occupational

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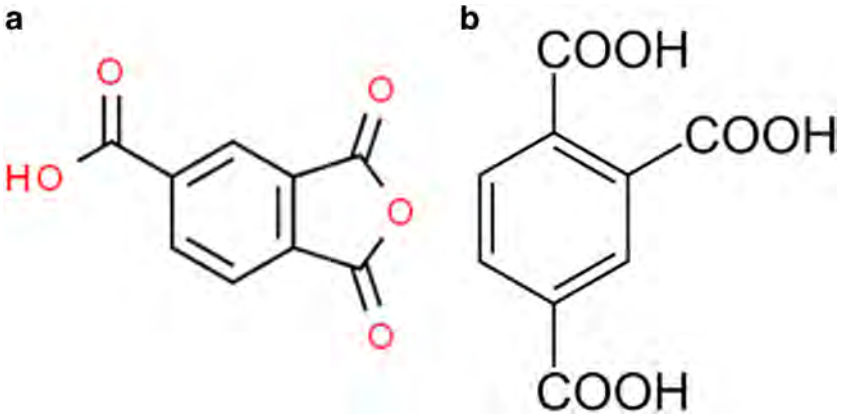

Fig. 1 The chemical structures of **a** trimellitic anhydride (TMA) and **b** trimellitic acid (TMLA). Free TMA can be converted to TMLA that can cause an irritant response. Furthermore, numerous TMA molecules can irreversibly bind to a large carrier protein (such as human serum albumin, HSA) to produce the complete antigen capable of augmenting immune responses that can lead to occupational asthma. TMA is therefore a unique molecule responsible for causing both irritant and allergenic responses in TMA-exposed factory workers

environments. [9] There have been no reports of TMA exposure from domestic sources. TMA, once utilized as a plasticizer, becomes converted to very stable ester derivatives which do not off-gas or migrate. [10] Airborne TMA can be sampled on adsorbents such as cellulose ester membrane filter, glass fiber, and PVC membrane. Absorbed TMA can be determined by high-performance liquid chromatography (HPLC), gas chromatography, or polarography. [11] More recently, a sensor has been developed which uses resorcinol dye on perfluorosulfonic acid (PSA) membrane to react with airborne TMA and generates a highly sensitive and selective optical signal suitable for continuous, on-site monitoring of personal exposure levels. [12] OSHA (Occupational Safety and Health Administration, USA), the ACGIH (American Conference of Governmental Industrial Hygienists), and local or regional occupational environmental health departments are the resources for identifying reputable TMA air sampling experts.

TMA workers in high-exposure areas can remain tolerant (no TMA-specific antibodies) or become sensitized to TMA (producing TMA-specific serum IgG and/or IgE). Previous investigators have demonstrated that TMA-specific serum IgG and IgE (henceforth referred as TMA sIgG and sIgE) are predictive of developing respiratory diseases among workers, while the absence of antibodies is a negative predictor [12]. Inhaled TMA reacts to lysin/arginine residues of endogenous carrier proteins (e.g., human serum albumin, HSA) to form stable antigenic trimellityl-protein complexes eliciting specific antibody responses in exposed workers which can lead to occupational asthma [6, 13]. Occupational immunosurveillance programs (OISPs) are therefore implemented to screen out sensitized workers and remove them from high-exposure areas to prevent the development of clinical symptoms. [8, 14] We have recently described the outcomes of a prototypic OISP conducted in a TMA factory. [14] The objective of this systematic review is to capture the spectrum of health effects of TMA exposure from available immunologic, biochemical/genomic, and clinical studies performed using animal models and in TMA-exposed factory workers enrolled in OISPs, and to assess whether current prototypic OISPs are effective to create safe working environment for TMA factory workers.

From NCBI PubMed, we retrieved all studies that aim at analyzing the immunologic, molecular, and clinical outcomes of TMA exposure using animal models and in humans. In vitro studies using cell lines were not included in the current analysis. To further interpret results from toxicogenomic data, we extracted the gene symbols of transcripts differentially regulated upon TMA exposure. Differentially regulated pathways and networks were identified using Metascape gene annotation and analysis suite and the networks were visualized using Cytoscape package. [15, 16]

Health Effects Related to TMA Exposure

The most common health effect of TMA is as an air toxic since it is rapidly converted to trimellitic acid, a respiratory irritant. Exposure to TMA dust and fumes can cause irritation to the eyes and respiratory system eliciting transient upper airway irritation, lacrimation, and rhinorrhea. The symptoms can occur either following a single high-level exposure to TMA in powder and/or fume forms or over time with persistent low levels of exposure [17]. In addition, pulmonary hemorrhage due to inhalation of fumes or powders containing trimellitic anhydride (TMA) has been described [18].

However, as mentioned, a unique characteristic of this low molecular weight irritant is its ability to form an immunogenic antigen after forming a conjugate with endogenous proteins that it is capable of eliciting TMA sIgG and sIgE-mediated immune responses leading to asthma and other immune

respiratory disorders. [4, 7, 19, 20] The most common immunologic clinical conditions described for TMA-sIgE-sensitized workers has been occupational rhinitis (OR) and occupational asthma (OA). [21] Once a worker becomes sensitized, symptoms can occur within minutes after TMA re-exposure; however, typically exposed workers experience a latency period (time between initial exposure and development of symptoms which includes sensitization).of weeks to years after exposure prior to the development of sensitization and onset of symptoms. [4, 6] Commonly, TMA sensitized workers initially report symptoms of rhinitis and/or conjunctivitis prior to the occurrence of asthma symptoms. [22] Thus, exposed TMA workers can experience a spectrum of non-immunologic (transient irritant-induced) as well as immunologic (antibody-mediated) mediated symptoms. For example, in one cross-sectional study conducted at a TMA-manufacturing plant with 474 employees, 6.75% ($n = 32$) developed TMA-associated immune disorders consisting of asthma/rhinitis ($n = 12$), 2% developed active late respiratory systemic syndrome (LRSS) ($n = 10$) and 1% had LRSS in remission ($n = 5$), less than 1% developed late onset asthma ($n = 4$), and one worker experienced late onset arthralgia ($n = 1$). This study illustrates the spectrum of clinical responses that can occur in exposed TMA workers. [21]

Most immunosurveillance studies have used TMA sIgE and sIgG antibody assays as a positive and negative predictor for developing occupational respiratory disorders. [4, 20, 21, 23, 24] A limited number of studies have also used an acid anhydride-HSA conjugated skin test reagent to correlate worker sensitization to clinical disease and exposure. [25, 26] Barker et.al. demonstrated that a positive TMA skin test response was an independent risk factor for developing work-related bronchial hyperresponsiveness. [25] In this study, using multivariate analysis, the investigators found that the number of smoking pack years, sensitization to common inhalant allergens (i.e., atopy), and age were also independent risk factors for TMA-induced OA. However, after adjusting data for FEV1, these factors were no longer significant. [25] Interestingly, although TMA skin testing was found to correlate with the development of serum-specific IgE antibody responses and subsequent clinical disease, it did not conclusively correlate to ambient TMA exposure concentrations indicating that the magnitude of exposure was not the critical factor for developing sensitization. [25, 27]

There are only two case series ($n = 4$ sensitized workers per report) describing the correlation of acid anhydride skin testing to sIgE responses. [6, 28] Both case series found good correlation between the acid anhydride skin test reagent and serum sIgE assays. [6, 28] However, the study by Baur et.al., which evaluated workers exposed to a number of different acid anhydrides including TMA, found that the TMA serum sIgE assay was more sensitive than skin prick testing. [28]

Currently, industries that either manufacture TMA or use TMA in their work process have implemented rigid environmental control measures to reduce workers' exposure. In some instances, comprehensive immunosurveillance programs have been established to monitor TMA-exposed workers for development of TMA sensitization and clinical disease. Once detected, TMA-exposed workers who are IgE-sensitized can be removed from exposure areas to prevent the progression to clinical disease.

It is certain from human epidemiological studies and animal models that TMA is a potent respiratory sensitizer but is less clear at what exposure levels clinical symptoms manifest. Although questions about whether TMA has the potential for being carcinogenic, genotoxic, or fetotoxic have been previously raised, there is no evidence supporting these concerns. [29] According to a NIOSH (National Institute of Occupational Safety and Health) report, TMA-exposed employees reported eye and nose irritation, shortness of breath, coughing, nausea, headache, skin irritation, and throat irritation at levels averaging 1.5 and 2.8 mg/m^3 (NIOSH 1974c/Ex.1–1181. [30] Pulmonary hemorrhage and hemolytic anemia were reported at unspecified levels (Ahmad, Morgan, Patterson et al. 1979/Ex. 1–460). Rat models showed intraalveolar hemorrhage following a 0.01 ppm TMA exposure (Amoco Chemical Corporation 1978, ACGIH 1986/Ex. 1–3, p. 606). Based on these reports, the PEL for trimellitic anhydride was reviewed by OSHA and set at 0.005 ppm 8-h TWA (time-weighted average) level [30]. The Agency suggested that setting this exposure limit will protect workers from observed pulmonary effects, sensitization, and skin and upper respiratory tract irritation. An identical PEL was also set by NIOSH (0.005 ppm/10 h) and by ACGIH (American Conference of Governmental Industrial Hygienists; 0.005 ppm, where 1 ppm = 7.86 mg/m^3 at normal temperature and pressure, NTP). The next several sections will review in more detail work conduction in animal models and humans that assessed the health effects of TMA.

Animal Models

Multiple exposure models have been proposed by investigators using different animals (monkey, dog, rat, mice, and guinea pig models), methods of TMA exposure, and outcome assessments (Table 1). Animal studies were retrieved by querying the PubMed database using search terms "trimellitic anhydride and animal model." Route of exposure varied from nasal to dermal/intradermal with or without intraperitoneal pre-sensitization. Antibody responses were assessed using standard ELISA or RIA techniques. Functionality of antibodies were demonstrated using hemagglutination, passive sensitization, and histamine release assay. Cell-mediated responses were demonstrated using lymphocyte proliferation, and clinical/pathological manifestations were demonstrated using

Table 1 Summary of animal models demonstrating that TMA is a potent irritant and can be inducive of specific antibody responses leading to airway inflammation

Author	Model animal	Methods	Results	Reference
Turner et al	Rhesus monkeys, Human cells and human sera.	Direct and indirect hemagglutination and hemolysis	Functional antibody isotypes in workers; Anti-hapten antibody in TMA_exposed Rhesus monkey	Clin Exp Immunol. 1980 Feb;39(2):470–6.
Liu et al	12-week-old (BALB/c x A/J)F1 hybrids (CAF1) mice	Intraperitoneal injection of TMA-keyhole limpet hemocyanin (KLH) and TMA-(D)Glu-(D)Lys polymer	TMA-(D)Glu-(D)Lys polymer can suppress TMA-specific IgG and IgE antibody response in sensitized mice	J Allergy Clin Immunol. 1980 Oct;66(4):322–6.
Sale et al	Dogs ($n = 8$) and Rabbits ($n = 7$)	Intrabronchial application	Detectable antibody responses (predominantly specific IgG), lymphocyte reactivity and pathogenic signs at autopsy	Int Arch Allergy Appl Immunol. 1982;67(4):329–34
Zeiss et al	Sprague-Dawley rats	Trimellitic anhydride inhalation-model development, lung and serology	Serum antibody measurements, lung pathology	J Allergy Clin Immunol. 1987 Jan;79(1):59–63.
Chandler et al	Rat model	ELISA-based determination of Ig isotypes	Hapten-specific IgG, IgA and IgM in bronchoalveolar lavage and serum	J Allergy Clin Immunol. 1987 Aug;80(2):223–9.
Ziess et al	Sprague-Dawley rats	Inhaled TMA—short-term exposure mimicking industrial exposure; blood and lung pathology	Kinetics and magnitude of specific IgG, IgA, and IgM responses; lung foci	J Allergy Clin Immunol. 1989 Aug;84(2):219–23.
Tao et al	Guinea pigs	Passive sensitization, active immunization, IgG1, IgG2 and airway injury evaluation	Antibody-mediated lung injury	Int Arch Allergy Appl Immunol. 1991;96(2):119–27.
Dearman et al	BALB/c mice	Inhalation exposure to 5 mg/m^3 TMA	Serum IgG and IgE anti-hapten antibody	Int Arch Allergy Appl Immunol. 1991;95(1):70–6.
Dearman et al	BALB/c mice	Dermal exposure, passive immunization	Topical exposure can cause delayed (24 h) and immediate (1 h) dermal reaction, unlike DNCB which caused only delayed reaction.	Int Arch Allergy Immunol. 1992;97(4):315–21.
Hayes et al	Guinea pigs	Intradermal injection of varying concentrations and doses	IgG1 humoral response by ELISA and ELISA-inhibition; bronchial reactivity and passive cutaneous anaphylaxis.	Clin Exp Allergy. 1992 Jul;22(7):694–700.
Hayes et al	Guinea pigs	Intradermal injection	IgG1, IgE, lung resistance, airway microvascular leakage detected	Am Rev. Respir Dis. 1992 Nov;146(5 Pt 1):1306–10.
Hayes et al	Guinea pigs	Intradermal sensitization followed by Inhaled trimellitic anhydride (TMA) dust	Pulmonary inflation pressure, IgG1, bronchial reactivity accompanied by an eosinophilic inflammation	Am Rev. Respir Dis. 1992 Nov;146(5 Pt 1):1311–4
Potter et al	BALB/c mice	TMA dermal exposure in acetone:olive oil	Elevated total IgE by ELISA	Fundam Appl Toxicol. 1995 Jun;26(1):127–35
Arakawa	Guinea pigs	intradermal injection	IgG1 by ELISA as well as airway response	Int Arch Allergy Immunol. 1995 Nov;108(3):274–80.
Lauerma et al	BALB/c mice	Topical application	ear swelling response	J Appl Toxicol. 1997 Nov-Dec; 17(6):357–60.

Table 1 (continued)

Author	Model animal	Methods	Results	Reference
Fraser	Guinea pigs	Passive sensitization with TMA-specific IgG2, IgG1, or a combination of both	Significantly greater bronchoconstrictor with a combination of the IgG2 and IgG1 compared to either of the antibody isotypes	Toxicol Appl Pharmacol. 1998 Jun;150(2):218–27
Regal	BALB/c mice	Intradermal sensitization followed by intranasal TMA-mouse serum albumin	Higher serum total IgE and lung eosinophilia	Toxicol Appl Pharmacol. 2001 Sep 15;175(3):234–42.
Larsen	Guinea pigs (immature and mature—male and female)	Intradermal	TMA-induced lung cellular infiltrationvaries with age	Int Arch Allergy Immunol. 2002 Jan;127(1):63–72.
Zhang	Brown Norway rats	Topical application of dry TMA powder; IgG and IgE determination by ELISA	Dermal exposure of dry TMA powder can elicit specific IgG and IgE response	Allergy. 2002 Jul;57(7):620–6.
Larsen et al	Guinea pigs	Intradermal injection with TMA or vehicle, followed by intratracheal application of TMA powder.	TMA dust elicits airway obstruction and eosinophilia in non-sensitized and sensitized (greater obstruction) animals.	Toxicology. 2002 Sep 2;178(2):89–99
Zhang et al	Brown Norway rats	Sensitization: dry TMA powder to dorsal skin; Challenge: aerosolized TMA inhalation	IgE response, respiratory response by whole-body plethysmography—early- and late-phase reactions	J Allergy Clin Immunol. 2004 Feb;113(2):320–6.
Valster et al	Brown Norway rats	Dermal exposure on alveolar macrophage-depleted animals	Alveolar macrophages potentiate TMA-mediated immediate lung function decrease, dampen 24 h inflammatory reaction.	Toxicol Appl Pharmacol. 2006 Feb 15;211(1):20–9.
Vanoirbeek et al	BALB/c mice	Dermal (two exposures), followed by intranasal exposure of TMA and DNCB	Both compounds induce a mixed T(H)1-T(H)2 response, but only TMA induced ventilatory changes.	J Allergy Clin Immunol. 2006 May;117(5):1090–7
Mirshahpanah et al	BALB/c mice	Dermal exposure	Ear swelling response, inflammation/ lymphocyte infiltration; Blocking CCR4 and CCR10 can be useful in controlling reactions.	Exp Dermatol. 2008 Jan;17(1):30–4.
Fukuyama et al	BALB/c mice	Low-level exposure (dermal contact/ ear dorsal surface) on an long model (32 days).	Elicited hapten-specific serum IgE, Th2 cytokines (IL-4, IL-5, IL-10, and IL-13) responses.	Toxicol Lett. 2008 Jul 30;180(1):1–8
Schneider C et al	BALB/c mice	Chronic and acute models of TMA dermal exposure	Chronic dermal exposure can induce stronger skin inflammation, infiltration of T cells, eosinophils, mast cells and Th2 cytokine profile with higher serum IgE levels, compared to acute exposure.	J Invest Dermatol. 2009
Tarkowski M	BALB/c mice			

Table 1 (continued)

Author	Model animal	Methods	Results	Reference
et al		Dermal applications on days 1 and 7 followed by intranasal instillation on day 14.	Dose-dependent increase of CD44 expression on T cells	Int J Occup Med Environ Health. 2008;21(3):253–62
OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts
De Jong WH	BALB/c mice	Inhalation	Lymph node IL-4, IL-10 and proliferative response	Toxicology. 2009 Jul 10;261(3):103–11
Zhang XD	Brown Norway rats	Dermal exposure followed by inhalation challenge	Dose-dependent specific IgE, early- and late-phase airway responses	Clin Exp Allergy. 2009 Nov;39(11):1746–53
OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts	OSHA, the ACGIH and local or regional occupational environmental health departments are resources for identifying reputable TMA air sampling experts
Li M, Fan X, Ji L, Fan Y, Xu L	BALB/c mice	Sensitization by intraperitoneal injection and challenged by inhalation	Exacerbating effects of trimellitic anhydride in ovalbumin-induced asthmatic mice and the gene and protein expressions of TRPA1, TRPV1, TRPV2 in lung tissue	Int Immunopharmacol 69:159–168. doi:https://doi.org/10.1016/j.intimp.2019.01.038

plethysmography, bronchoalveolar lavage cytology, and lung anatomy/immunohistochemistry. In summary, the results from these studies all confirm that TMA is a potent irritant, but can bind to carrier proteins to induce humoral immune responses eliciting sIgG, sIgE, sIgA, and sIgM antibodies. Studies also confirmed the potential roles of hapten-specific IgG (IgG1 and IgG2) and IgE in producing clinical effects including increased airway resistance and inflammation. Humoral and cell-mediated immune responses, irritant responses, and other toxic outcomes of TMA exposure have been summarized below:

Assessment of Antibody Responses

Animal studies have confirmed that the respiratory and immune system are the principal targets of TMA-IgE-mediated reactions, resulting in activation of a network of immunologic pathways that clinically lead to occupational respiratory disorders. Animal models of TMA-exposure used mice, rats, guinea pigs, and monkeys. Oral, inhalation, and dermal LD50 values have been determined using rat models (oral 2030 to 3340 mg/kg; inhalation >2330 mg/m^3; dermal 5600 mg/kg). [31, 32] In vitro assays have not shown significant genotoxicity of TMA. [32] Reproductive tissue damage also has not been observed following sub-chronic exposures to TMA. [32] Furthermore, no teratogenic or fetotoxic effects were found in developmental toxicity studies. [31, 32]

The principal effect of TMA exposure is immunogenic. Leach et al. (1988) showed that rats pre-treated with the immunosuppressant, cyclophosphamide, before exposing them to TMA did not develop lung lesions or TMA-specific serum antibodies. Similar results were reported by other investigators, which clearly indicated that the health effect of TMA exposure is predominantly immune-mediated. [33, 34] Interestingly, Dykewicz et al. (1988) passively sensitized rhesus monkeys using serum from a worker with documented TMA-induced asthma and high TMA-HSA sIgE, sIgG, and sIgA antibody titers and were able to elicit bronchospasm in the monkeys when they were challenged with aerosolized TMA-HSA. [35]

Rat models were extensively used for periodic exposure studies to partially mimic human exposure. In this investigation, rats were exposed to 0, 10, 30, 100, or 300 μg/m^3 of TMA dust, 3 h/day for 5 days. Hemorrhagic lung foci were identified at the 30–300 μg/m^3 TMA exposure concentration. These lesions healed 12 days after stopping the exposure, but reoccurred after repeated exposure. [36] Histologic assessment of TMA-induced lung lesions revealed extensive cellular infiltrates (including macrophages), alveolar hemorrhage, and pneumonitis. Of note, in this model the lungs were the only organ affected and the extent of lung damage increased proportionally with increasing TMA concentration. [37]

Chandler et al. using TMA conjugated to rat serum albumin (RSA) to quantitate TMA sIgG, sIgA, and sIgM antibodies in TMA-RSA-exposed rats, found higher total antibody concentrations in the bronchoalveolar lavage (BAL) compared to serum levels [33]. In other rat models, the humoral immune response to inhaled TMA was demonstrated to occur simultaneously with the development of lung lesions which was highly correlated with increased bronchoalveolar lavage (BAL) and serum anti-TMA antibody levels.[[38]

In an antibody kinetic study, rats were sensitized by inhalation to 500 μg/m^3 (high exposure group) or 330 μg/m^3 (low exposure group) TMA powder on days 1, 5, and 10 for 6 h/day and then challenged with TMA 540 μg/m^3 or 300 μg/m^3 on day 29 or 22 respectively. In the high exposure group, TMA-RSA-specific IgM and IgA antibodies began to increase at day 5 and peaked at day 20. TMA-RSA-specific IgG antibodies were detectable by day 7 and peaked at day 20. The investigators found a significant correlation between all of the TMA-RSA-specific antibody responses and lung injury [39]. However, TMA-RSA-specific IgE levels were not measured in this study.

Subsequently, numerous studies have demonstrated TMA-induced increased methacholine airway hyperresponsiveness, the presence of allergic inflammatory mediators and cellular infiltrates in the BAL of animals sensitized with TMA serum sIgE and with definitive lung pathology. [40, 41] Using whole-body plethysmography to measure respiratory function, it was found that TMA challenge of TMA-sensitized rats can clearly distinguished between irritant-induced changes and non-specific airway hyperresponsiveness 24 h post-challenge. [42] Furthermore, sensitization-dependent functional changes of the airways were clearly accompanied by characteristic asthmatic inflammatory changes and airway damage. [40] In a guinea pig model, histamine and thromboxane were found to be the principal mediators elevated in TMA-induced early- and late-phase bronchoconstriction responses. Activation of inducible nitric oxide synthase has also been demonstrated in bronchial tissue after challenge with TMA-guinea pig serum albumin conjugate in TMA-sensitized guinea pigs [43].

A more recent study using a Brown Norway Rat model where TMA sensitization was established by dermal exposure to 50% and 25% TMA w/v followed by an inhalational TMA challenge of 15 mg/m^3, demonstrated TMA-induced laryngeal inflammation, increased numbers of eosinophils, neutrophils, and macrophages in bronchoalveolar lavage (BAL), and increased sIgE levels in serum and lung tissue homogenate. Transcriptome analysis of lung tissue collected 24 h post-challenge showed altered expression levels of a number of Gene Ontology groups including upregulation of genes involved in inflammation and lung remodeling [44] (Fig. 2).

Dearman et al. (1991) also sensitized mice topically, by applying TMA dissolved in 4:1 acetone: olive oil mixture, to

a shaved flank under an occluded patch for 48 h. After 5 days, the mice ear thickness was measured, and then challenged with 25 μl of the TMA test material on the dorsal surface of both ears. It was found that TMA induced the production of TMA-specific IgE and IgG2b. This finding was in contrast to applying 2,4-dinitrochlorobenzene (DNCB), a potent contact sensitizer, which resulted in an increased TMA-specific IgG2a antibody response. [45] The results of this study indicated that dermal exposure to TMA can exhibit a sIgE-mediated immune response similar to inhalational studies which is distinct from other chemical contact sensitizers.

In another novel animal model experiment, Vanoirbeek et.al. exposed mice with TMA dermally and then challenged them with intranasal TMA. Mice that were TMA sensitized in this manner demonstrated a change in airway respiratory resistance (demonstrated by an increase in enhanced pause or Penh as measured by whole-body plethysmography) immediately after TMA intranasal challenge as well as an increase in methacholine airway hyperreactivity. In contrast, mice similarly sensitized to DNCB did not exhibit any ventilatory changes. Both TMA and DNCB demonstrated a mixed T_H1-T_H2 response based on serum antibody and lymph node cytokine assays; however, only TMA was found to induce changes in respiratory function by plethysmography [42].

Subsequently, a number of other studies using guinea pig models intracutaneously sensitized to TMA, followed by either intranasal or intratracheal TMA challenge protocols, revealed TMA-specific antibody responses associated with increased lung resistance and airway microvascular leakage compared to control animals [46–49]. The time course of these immune and airway responses in sensitized guinea pigs

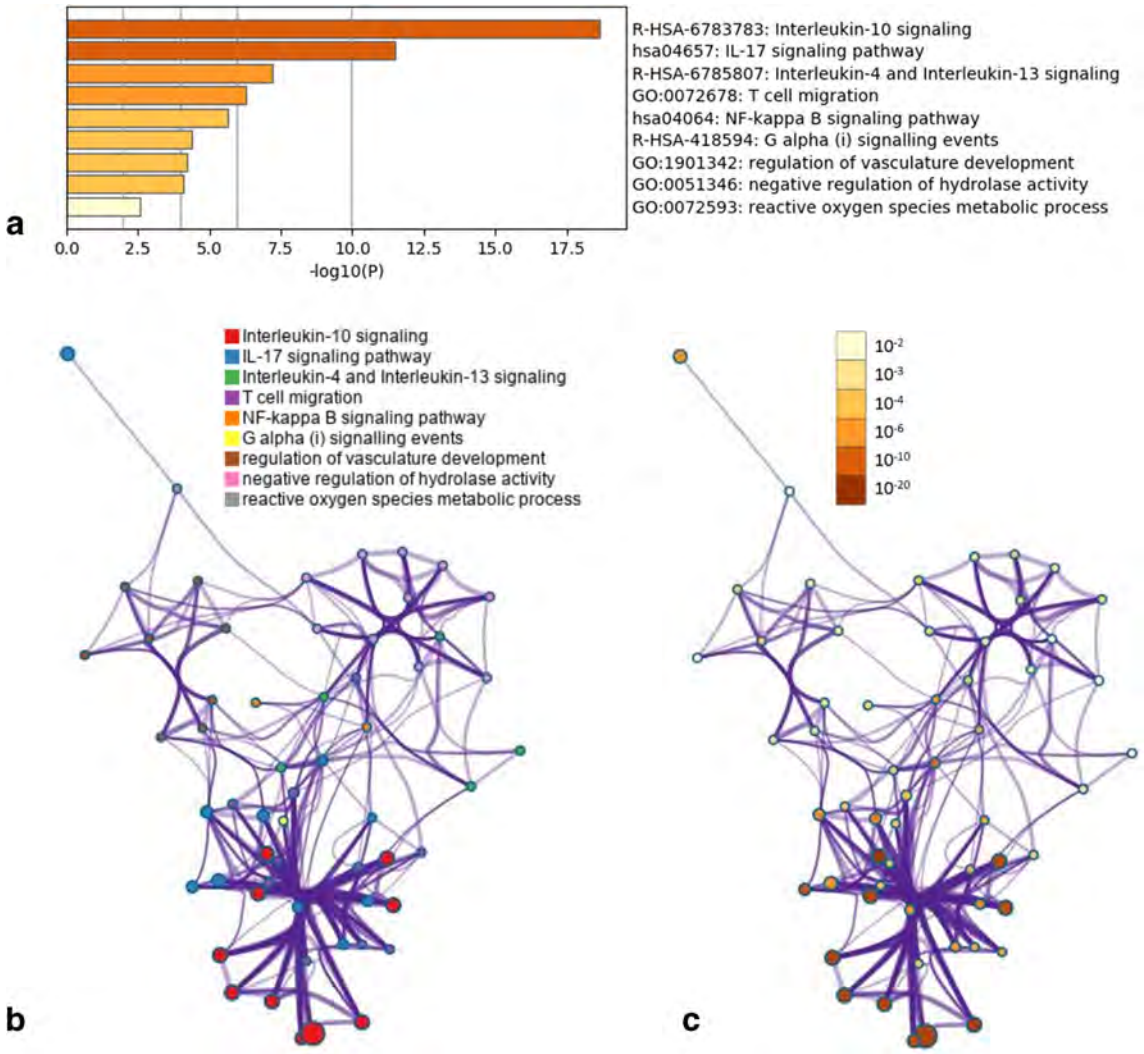

Fig. 2 Analysis of pulmonary gene expression in Brown Norway rats upon dermal sensitization with TMA followed by TMA inhalation challenge. The differentially regulated genes were obtained from Cooper et al. 2008 (Toxicol Pathol. 36(7):985–98). Whole-genome analysis of the lung, sampled 24 h after challenge, showed expression changes in genes involved in inflammation and remodeling. **a** Bar graph of enriched terms across input gene lists, colored by *p* values. **b** and **c** Network of enriched terms: (**b**) colored by cluster ID, where nodes that share the same cluster ID are typically close to each other; (**c**) colored by *p* value, where terms containing more genes tend to have a more significant *p* value

elicited through intracutaneous injections (administered as two injections, each of 0.1 ml of 0.3% TMA in corn oil) and intratracheal challenge have been characterized by Arakawa et al. [50]. The investigators used TMA conjugated to guinea pig serum albumin to measure hapten-sIgG1 at 3-, 5-, and 8-week intervals and found that maximal response occurred 5 weeks after sensitization.

Assessment of Irritant Effects of TMA Exposure

Recent studies have focused on investigating the differential gene expression responses that can potentially distinguish contact sensitizers (e.g., 1-chloro-2,4-dinitrobenzene, i.e., DNCB), from respiratory sensitizer (TMA) and non-sensitizing irritants (e.g., methyl salicylate and nonanoic acid) using a local lymph node assay [51, 52]. It was found using this in vitro model that immune-specific gene functions, such as dendritic cell activation, were unique to the sensitizers (DNCB and TMA) which may allow distinguishing between sensitizing and irritant-inducing chemical agents. In addition, genes for mast cell proteases were strongly upregulated by respiratory sensitizers compared to contact sensitizers which may represent potential biomarkers for further discriminating between contact and respiratory sensitizers [53].

Assessment of Toxicant/ Toxicogenomic Effects

Ryan et al. (1988) used a guinea pig model which demonstrated that inhalation of TMA 0.5 mg/m^3 for 6 h/day on days 6–15 of gestation did not produce any signs of fetotoxicity or teratogenicity in offspring [54]. However, they observed inflammatory lung foci and increased TMA-specific antibody responses in pregnant rats and increased TMA-specific antibody levels also in the neonatal pups. Inflammatory lung foci were only observed in the neonatal offspring following TMA challenge exposure, where the mothers had not completely recovered from the original TMA exposure. However, these inflammatory lung foci did not persist in adult offspring. Furthermore, there were no observed signs of fetotoxicity or teratogenicity in any offspring from TMA-sensitized guinea pigs in this study.

When tested in an ovalbumin model of asthma, TMA exacerbated symptoms in BALB/c mice exhibiting severe airway inflammation, airway hyperresponsiveness, and lung injury. OVA-sensitized mice when challenged using ovalbumin plus TMA showed higher level of Th2 cytokines in BALF and pulmonary homogenate indicating that TMA might augment symptoms in established asthma. Increased gene and protein level expressions of TRPA1, V1, and V2 in lung tissue suggested that exacerbating effects of TMA in OVA-induced asthma might be related to the regulation of TRPA1, V1, V2, and relevant neurokinins. [55]

Further transcriptomic studies identified the following four functional clusters of differentially regulated genes in the lungs of a mouse model of TMA-induced asthma: (a) chemokines and cytokines (CCL8, CCL9, CCL6, CCR5, CCR1, CCL13), (b) secretion and remodeling (Gob5, Tff2, MMP12, Muc5AC), (c) cell adhesion and lectins (Chi3l3, Clec4a2, CD83, CD53, VCAM-1), complement system (complement component 1 and components 3), and (d) arginase metabolism genes (glycine amidinotransferase, arginase type II). [56] The gene showing highest fold-change is Gob5 (gene alias CLCA3), which encodes a putative calcium-activated chloride channel involved in the regulation of mucus production and secretion, known in other forms of airway dysfunction including asthma, COPD, cystic fibrosis, and asbestos-induced lung fibrosis. [57–60] The arginase activity in the lung was higher in mRNA and protein/enzyme levels in case of ovalbumin exposure compared to TMA exposure.

In an attempt to discriminate between irritant versus allergenic response, human plasmacytoid dendritic cells (pDC) expressing FcER1$^+$ were sensitized with factory workers' sera ($n = 2$; 1:20 dilution) containing TMA-specific IgE and exposed to either (a) HSA (unconjugated non-immunogenic carrier protein); (b) free TMA (irritant hapten), or (c) TMA-HSA conjugate (complete antigen). DEGs associated with free TMA exposure involved innate immune and cell migration pathways including IL23A, IL15, epiregulin, and endothelin. In contrast, TMA-HSA exposure was associated with DEGs involved in humoral immune responses, cytokine, and adhesion molecules including CD300LB, SORBS1, TNFSF13B, and LYN tyrosin kinase. Pathway analyses revealed over-representation of Granzyme B pathway genes and inflammatory immune genes for the free TMA and TMA-HSA groups, respectively. Specific cytokines (IL1, IL6) and chemokines (CCL4, CCL20) were upregulated in both groups indicating that gene expression of pDCs exposed to free TMA is distinctly different from pDCs exposed to TMA-HSA in sensitized subjects. [61]

Health Effects of TMA Exposure: Human Studies

Extensive PubMed and Scopus database searches using "Trimellitic Anhydride and human exposure" search words (in Title, abstract, keyword) identified ten case series studies reported between years 1977–1982.

In an early report, Fewcett at al. (1977) described case studies involving seven workers directly exposed to epoxy resin/anhydride powders (trimellitic and phthalic anhydrides) at their workplaces. The workers reportedly developed TMA-flu and/or asthma. TMA sensitivity was confirmed by inhalation challenge. In the same year, Zeiss et al. described the spectrum of TMA-induced respiratory symptoms (asthma and rhinitis of the immediate type, late onset asthma with systemic symptoms, and airway irritation) in a group of 14

TMA-exposed factory workers. [6] The authors reported the relevance of hapten-specific IgE and IgG antibodies in predicting clinical outcomes. Antibody isotypes specific to TMA-HSA were determined using radioimmunoassay. Positive lymphocyte reactivity of TMA-HSA (in three representative workers) and leukocyte histamine release (in one worker with high specific IgE and severe acute rhinitis and asthma) were also demonstrated in symptomatic workers.

In 1978, Patterson and colleagues described in great detail the preparation and use of a TMA-HSA conjugate for quantitating TMA-specific antibodies in the serum samples of TMA-exposed factory workers. [62] The investigators classified workers as having TMA-induced asthma, LRSS (late respiratory systemic syndrome), or both or as workers exposed to TMA without symptoms ($n = 5$) in relation to TMA exposure (except for an occasional irritant exposure episode). The authors used a TMA-HSA conjugate for skin testing and solid-phase radioimmunoassay to detect TMA-HSA-specific IgG, sIgM, and sIgE antibody isotypes. Specific IgE antibody was detected in 3 out of 5 workers with physician-diagnosed asthma. In addition, 8 out of 10 symptomatic (with asthma/LRSS or both) workers showed detectable serum hapten-TMA-specific serum IgA. Interestingly, higher antibody responses were found in symptomatic workers compared to asymptomatic workers even after long periods of removal from TMA exposure. Although TMA-HSA-specific IgM antibody was also reported, it was not found to be significantly different between symptomatic and asymptomatic workers. [62]

Multiple studies have demonstrated the utility of monitoring hapten-specific serum antibodies in exposed workers for potential clinical correlation. [63] McGrath et al. evaluated 20 workers (in the year 1979) exposed to TMA powder and documented that out of 6 workers with TMA-HSA serum sIgE, 3 had LRSS and 2 had TMA-induced allergic rhinitis /allergic rhinitis and asthma. [64] One asymptomatic worker had antibody against TMA-HSA. Finally, the investigators monitored a total of 32 workers over 4 years (from 1979 through 1983) including 11 TMA-naïve workers hired in 1982 after environmental control measures were implemented to reduce exposure, and concluded that reducing environmental exposure was helpful in reducing or preventing sensitization. [64]

Another prospective study by the same group between 1979 and 1985 (6-years) in workers chronically exposed to TMA found that five of 17 workers exhibited respiratory symptoms (LRSS, TMA-induced allergic rhinitis, with or without asthma). [65] Additionally, one asymptomatic worker had detectable antibody. The authors pointed out the following measures implemented in the factory were the most effective at reducing exposure: (a) workers' education program on safe handling, (b) increased employee contact with a medical surveillance team, (c) improved respiratory protection with proper fitting techniques, (d) improvement in the local exhaust system and clean up with vacuum cleaners rather than dry sweeping, (e) reduced use of TMA-powder with increased use of TMA flakes, and (f) improved handling procedures. In addition, their study indicated that removal of symptomatic workers from further TMA exposure resulted in decreased clinical symptoms with concomitant decrease in serum TMA-specific IgG and IgE antibody titers, emphasizing the importance of monitoring TMA-hapten serum-specific antibody responses in exposed worker's sera over time.

Table 2 summarizes the clinical immunologic studies on TMA factory workers retrieved from PubMed using search terms "trimellitic anhydride and worker." Published data indicate that exposure leading to sensitization occurs primarily through inhalation of TMA dust and/or powder, although dermal contact also a likely sensitizing route and the primary adverse health outcomes include LRSS, early/late-onset asthma, and rhinitis (Table 2). Most of the early studies used radioimmunoassay to measure TMA-specific antibody isotypes (especially TMA-sIgE) which have now been supplanted with enzyme-linked immunoassays (ELISAs) as they have equivalent sensitivities and specificities against TMA-conjugated proteins but do not require the safety precautions used for radioisotopes and are overall more cost-effective. A more recent, longitudinal monitoring study found that incorporating TMA-sIgG and sIgE serum antibody responses in exposed workers was useful as a long-term surveillance strategy to prevent adverse respiratory health effects and improve workers' safety. [14] In general, studies overwhelmingly support immunosurveillance as being very effective for detecting early TMA sensitization so workers can be removed from further exposure before the development of clinically overt rhinitis and/or asthma symptoms.

OIS Programs: Clinical Insights and Recommendations

Early Findings

From these early TMA exposure studies (mostly observational), it has been well recognized that workplace exposure to TMA chemicals can lead to occupational asthma if clinical symptoms are not recognized early by removing TMA sIgE-sensitized workers from further exposure. In response to these early studies, TMA manufacturers have proactively established occupational immune-surveillance programs (OISP) in an attempt to mitigate or prevent work-related respiratory conditions secondary to respiratory sensitizing and/or irritating chemical agents such as TMA. [66–68] Occupational immune-surveillance programs can be applicable to prevent occupational respiratory diseases in a wide range of industries where factory workers are exposed to various low molecular weight such as diisocyanates (used to produce polyurethane products such as rigid and flexible foams,

Table 2 Summary of clinical studies' routes of exposure, sensitization, and health effects of TMA

Investigator	Mode of exposure	Clinical outcomes/ findings	Reference
Fawcett DW et al	Fume	Asthma, chronic bronchitis	Clinical Allergy 7(l): −14, Jan 1977
Zeiss CR et al.	TMA synthesis unit—dust/fume	Asthma and rhinitis of the immediate type, late onset asthma with systemic symptoms, and airway irritation. TMA-specific serum IgE	J Allergy Clin Immunol. 1977 Aug;60(2):96–103.
Sale SR et al	TMA powder	Late respiratory systemic syndrome (LRSS), immediate rhinitis and asthma, irritant reaction	J Allergy Clin Immunol. 1981 Sep;68(3):188–93.
Bernstein DI et al	TMA powder and phthalic anhydride fumes	Rhinitis, asthma, LRSS, and irritant responses; specific serum antibodies (IgG and IgE isotypes) demonstrated	J Allergy Clin Immunol. 1982 Mar;69(3):311–8
Zeiss CR et al	Dust/fume inhalation	Asthma-rhinitis, LRSS, and irritant response; positive skin reaction to TMA-HSA.	Ann Intern Med. 1983 Jan;98(1):8–12
Bernstein DI et al	TMA powder	Rhinitis and LRSS; specific IgE measured in the serum using RIA. Specific IgE level went down with decrease in TMA exposure.	J Allergy Clin Immunol. 1983 Dec;72(6):709–13.
McGrath et al	TMA powder	LRSS, allergic rhinitis, asthma; environmental control of TMA exposure can be helpful in reducing sensitization	J Occup Med. 1984 Sep;26(9):671–5.
Dykewicz MS et al	Dust/fume in TMA factory	Asthma with high IgE, IgG, IgM, and IgA to trimellityl-human serum albumin; serum was able to cause passive sensitization in Monkeys	J Lab Clin Med. 1988 Apr;111(4):459–65.
Zeiss CR et al	Dust/fume in TMA factory	LRSS, asthma, late onset asthma, arthralgia and myalgia; IgE sensitization demonstrated by RIA and skin test. Environmental controls and workers' education was helpful in controlling adverse health outcomes.	Allergy Proc. 1990 Mar-Apr;11(2):71–7.
Bernstein JA, Ghosh D et al	Occupational exposure	TMA-HSA conjugate characterization. Conjugate showed a sensitivity of 73% in SPT, which could be improved to 93% using subsequent intracutaneous testing.	J Occup Environ Med 53 (10):1122–1127
Ghosh D et al	Occupational exposure	Early onset of sIgG along with a higher magnitude of response might be useful for identifying TMA-exposed workers more likely to develop TMA-specific serum IgE.	Allergy 73 (5):1075–1083

coatings, adhesives, sealants and elastomers, insulation, spray paint, etc.) and phthalic anhydrides (used to make plasticizers, dyes, pharmaceuticals, varnishes, etc.) and high molecular weight sensitizing agents such as enzymes used in the manufacturing of detergents [69–72] In general, these immunosurveillance safety programs are designed to promote the health, safety, and improved quality of life for exposed workers by collecting, analyzing, and interpreting clinical and immunologic data to prevent occupational induced respiratory disease.

Several studies have now confirmed a strong association between TMA-sIgG and sIgE serum antibodies and subsequent development of occupational respiratory disease [4, 20, 21, 23, 24]. However, other factors such as smoking history, atopic status (i.e., sensitization to common inhalant allergens), and age have not been consistently found to be significant risk factors for TMA-induced OA after controlling for FEV1 (forced expiratory volume in 1 s) [25]. Furthermore, while TMA sIgE has been found to be highly associated with the development of TMA-induced occupational rhinitis (OR) and OA in TMA-exposed workers [7, 21], the role of sIgG in OA has been less clear even though it has been usually regarded as a marker of TMA exposure [29, 73]. In the study by Grammer et al. previously discussed above, they have found a relationship between TMA sIgE and respiratory disease with continued exposure [23]. In this study, of the 16 individuals with specific IgE against TMA-HSA, three developed asthma while working and another six developed asthma during the 5-year follow-up, but none of the 165 workers without TMA-HSA sIgE developed clinical asthma and only one out of 102 developed asthma within the 5-year follow-up period. In addition, among the 44 subjects with TMA-HSA sIgG, six developed an immunologic respiratory disease (LRSS or LA) and two others developed an immunologic respiratory disease within the 5-year follow-up period. In contrast, none of the 137 subjects without TMA-HSA sIgG developed TMA sIgE sensitization or developed immunologic respiratory disease while working directly with TMA, nor did any of the 80 subjects followed for 5 years develop TMA sensitization or clinical symptoms. Thus, the authors concluded that development of TMA-HSA, sIgE, and sIgG is predictive of immune-mediated respiratory disease due to TMA exposure.

Another comprehensive surveillance study evaluated 196 workers in a TMA factory between January 1976 and December 1987 [20] by clinical history, blood counts, chest

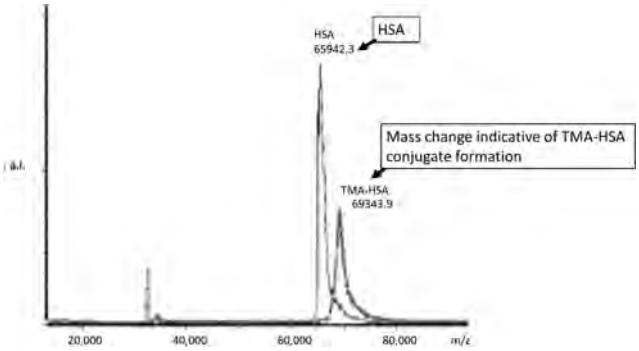

Fig. 3 Analysis of TMA-HSA conjugates by MALDI-TOF mass spectrometry (J Occup Environ Med, 2011. 53(10): p. 1122–7). The shift of the peak corresponding to an increase of (69,343.9 Da − 65,942.3 Da) = 4301.6 Da indicates conjugate formation. The number of TMA molecules bound to one molecule of human serum albumin (HSA) was estimated to be 18. This conjugate, when used in OISP, showed a SPT sensitivity of 73%, which could be improved to 93% using subsequent intracutaneous testing of SPT-negative workers at a 1:1000 w/v dilution.

X-ray, pulmonary function testing, and TMA-HSA IgE detected by skin testing and radioimmunoassay. Clinical outcomes included IgE-mediated asthma/rhinitis confirmed by a positive skin prick test, development of LRSS, or late onset asthma. The investigators confirmed a reduction in the number of workers with an immunologic respiratory syndrome during the 1982–1987 study period (7 of 85; 8%) compared to the 1976–1981 study period (26 of 111; 23%) despite an increase in TMA production, presumably due to improved environmental exposure control and worker education. However, in usual occupational settings, the true prevalence of sensitization is difficult to determine, since sensitized individuals with or without symptoms, once identified, are typically removed from exposure areas or change jobs, the latter which can potentially result in a lower reporting of sensitization prevalence, an effect referred to as the "healthy worker effect' (HWE) in occupational epidemiology. Similarly, the exclusion of physically unfit workers during the hiring process is another potential contributing factor to the HWE associated with OISPs. [74, 75]

Recent Findings and Recommendations

Most recent OIS studies identify sensitized workers and remove them from further exposure (therefore could be marked interventional). [14] TMA production factories have been divided into four TMA exposure areas: L1 (undetectable exposure, e.g., front office), L2 (very low exposure, e.g., research laboratory), L3 (high potential for exposure, e.g., TMA production area), and L4 (very high potential for TMA exposure, e.g., TMA packaging and warehouse). All L3 and L4 workers (production/ warehouse workers, packaging staff and quality control engineers who have frequent or consistent exposure to TMA dust or fumes) are required to wear personal protective exposure equipment (respirators and Tyvek suits). However, currently, in spite of implementing state of the art engineering controls and personal protective measures, susceptible workers continue to become TMA sensitized. [8, 14] Once a worker becomes IgE-sensitized, due to the increased risk of progressing to clinical disease, exposure reduction or complete removal from high TMA exposure areas is imperative. Thus immunosurveillance using TMA-specific serum antibodies as markers for exposure and sensitization has been

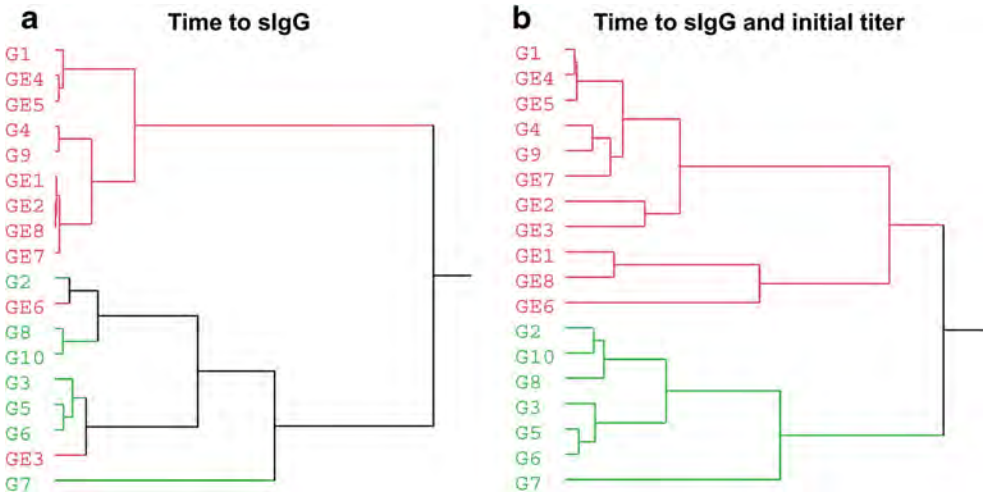

Fig. 4 A recent immunosurveillance study (Allergy 73(5):1075–1083) described clustering of TMA-exposed sensitized workers' serum samples by either (**a**) time to detectable sIgG alone or **b** in combination with initial sIgG titer value at the time of detection. Workers with sIgG (group 1, designated G1 through G10; shown in green) and who developed sIgE along with sIgG (group 2, designated GE1 through GE8; shown in red) were included in the analysis. Results showed that the time to detectable sIgG can discriminate between group 1 and group 2 with about 72% accuracy. However, this parameter when combined with the time of the initial sIgG titer can discriminate between these two groups with approximately 80% accuracy.

described to be very successful in preventing TMA-induced occupational lung disease [8].

A number of OISPs longitudinally monitoring factory workers exposed to trimellitic anhydride (TMA) have been in existence for several decades demonstrating that workers may remain resistant or tolerant (TMA serum-specific antibody negative) or become TMA sensitized (TMA serum specific IgG and/or IgE positive) over time. [3, 4, 20] TMA-specific antibodies can be measured using standard assays such as ImmunoCAP and ELISA. However, a major challenge of immunologically monitoring workers for TMA sensitization in these factories is the cost related to regularly obtaining serum from potentially hundreds of workers to assess whether they have increased TMA-specific IgG and/or IgE levels. Furthermore, delays in obtaining the results of these serologic assays can potentially prolong clinical decisions regarding the worker's disposition in the workplace. To address this concern, a recent study synthesized a TMA carrier protein conjugate and found that it was very useful for screening and identifying workers with TMA IgE sensitization [8]. In this study the epitope density of the conjugate was determined by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry prior to clinical applications (Fig. 3). TMA-exposed workers ($N = 40$) previously screened for the presence of TMA-specific serum antibodies (by ImmunoCAP) were skin tested using TMA–human serum albumin reagent (epitope density = 18 by MALDI-TOF) by nurses who were blinded to ImmunoCAP screening results (gold standard). The sensitivity of the test was 73%, which could be improved to 93% using intracutaneous testing of SPT-negative workers at a 1:1000 w/v dilution of this conjugate indicating that SPT could be used as an easy and relatively cost-effective method for longitudinally monitoring TMA-exposed workers for sensitization. Moreover, with a negative predictive value of 97% in SPT, it has a low chance to miss the early onset of TMA sensitization in exposed workers. [8]

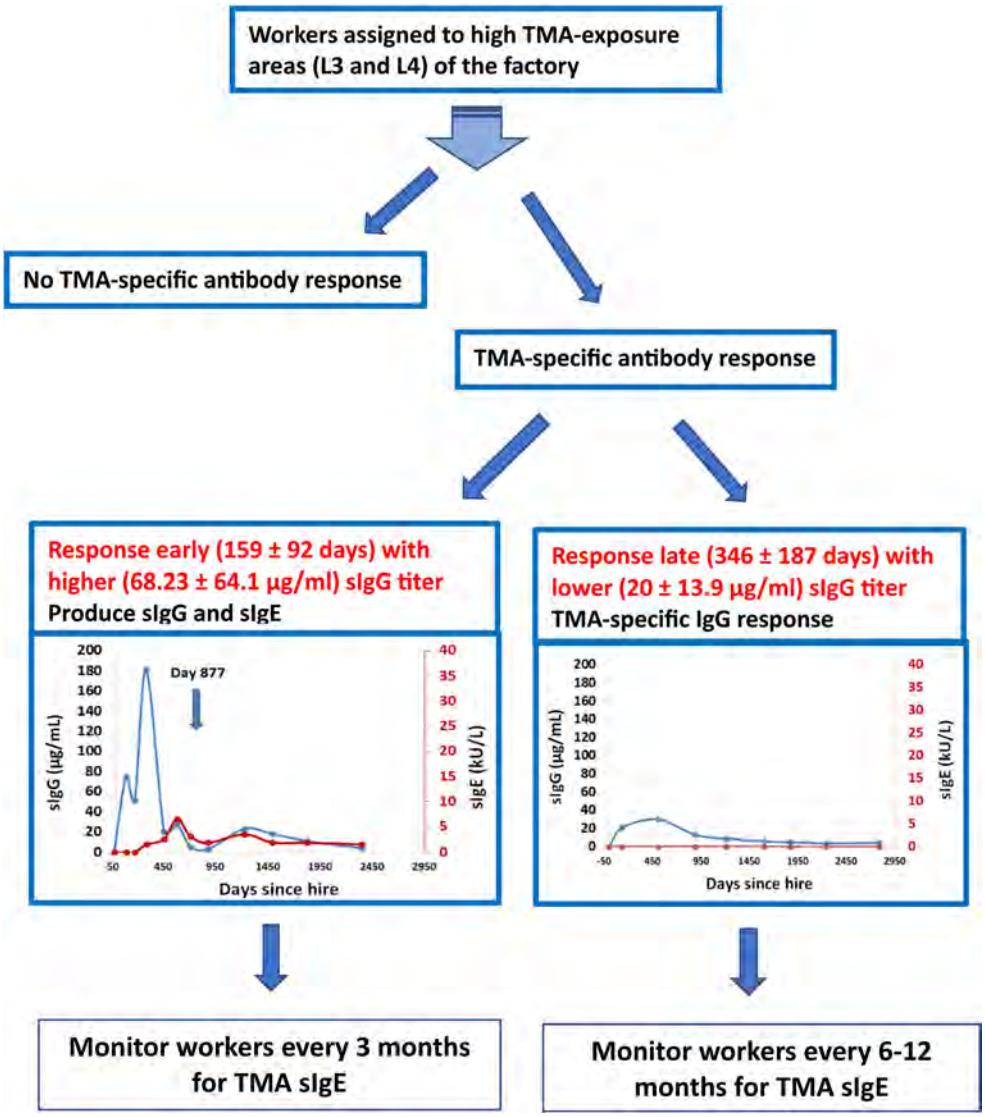

Fig. 5 Recommendations based on time course and magnitude of TMA-specific antibody responses (Allergy 73(5):1075–1083). Factory workers recruited to high-exposure areas (L3 and L4) can produce either no TMA-specific antibody, sIgG alone, or sIgG with sIgE. Exposed workers who produce sIgG early (after 159 ± 92 days of hire) with a higher magnitude of sIgG (68.23 ± 64.1 kU/L) are more likely to produce sIgG and sIgE (after 342 ± 186 days of hire) and required reassignment to lower exposure area. In contrast, those who produced sIgG later into their employment hire period (after 346 ± 187 days of exposure) with a lower magnitude of response (serum sIgG level 20.15 ± 13.9 kU/L) continued to produce only sIgG.

Although many studies have already established the relationship between specific antibody responses and health effects for TMA [76], there has been no clear elucidation of predisposing risk factors for developing TMA-specific serum IgE (sIgE) [77]. Current practice is therefore to remove workers from high TMA exposure areas only after developing sIgE, since present data is insufficient to predict early on whether a worker who develops TMA sIgG will go on to develop TMA sIgE or remain tolerant. Furthermore, limited data is available concerning long-term outcomes after sIgE-sensitized workers are removed from further exposure. Grammer and colleagues retrospectively investigated 29 TMA-exposed workers diagnosed with TMA-induced immunologic lung diseases who had been moved to low-exposure jobs for more than 1 year [78]. About half of the symptomatic workers showed significant improvement in their symptoms. For those workers who did not improve, their sIgE levels were higher leading to speculation that a higher sIgE could be a marker for poorer outcomes even after complete removal from further TMA exposure. This further indicates the need to identify workers early on who will develop specific IgE after being assigned to work in high-exposure areas and deploy them to areas with no or minimal TMA exposure.

A more recent study performed by our group monitored TMA-exposed workers in low, medium, and high TMA exposure areas by longitudinally measuring their serum-specific TMA sIgG, sIgG4, and sIgE levels. [14] Antibody kinetics for TMA sIgG and sIgE plotted against exposure duration were statistically compared between exposed TMA workers with sIgG only and those with sIgG who subsequently developed sIgE and were removed from further TMA exposure. In fact, TMA-exposed workers can be clustered based on the magnitude and time of onset of their IgG response (Fig. 4). It was found that early onset (usually within the first 6 months after exposure) of a high TMA-specific IgG antibody response after initial TMA exposure might serve as a useful biomarker for subsequent TMA sIgE sensitization (Fig. 5). Thus, this study supports an alternative role of TMA sIgG for identifying workers at increased risk of developing TMA sIgE sensitization versus immunotolerance.

Using this approach, it may now be possible to screen and remove TMA-exposed workers who produce high levels of TMA sIgG early on after initial exposure which will eventually lead to a workforce that is either tolerant or resistant to TMA IgE-mediated sensitization. However, it should be emphasized that although monitoring for specific antibody responses can prevent the development of immunologic mediated occupational lung disorders, it cannot prevent irritant-induced asthma (a.k.a. reactive airways dysfunction syndrome or RADS) which occurs without a latency period after a large irritant chemical exposure. Therefore, an OISP does not completely negate the need for continuous monitoring for TMA exposure and training of personnel to follow standard operating procedures and wear personal protective equipment to prevent TMA exposure as much as possible.

Interestingly, once workers developed TMA sIgE and were removed from further exposure, we observed a more rapid decline of sIgG4 compared to sIgG and sIgE over time. [14] In fact, sIgG and sIgE persisted much longer than anticipated after complete TMA avoidance. The sustained levels of TMA sIgG and sIgE may reflect the persistence of memory B lymphocytes which requires further investigation. The role of sIgG4 in development of sensitization and allergic diseases remains highly debated as to whether it represents a protective blocking antibody or a marker of high antigen exposure [79–81] [82, 83]. In addition, the finding that removed workers with high TMA sIgG and sIgE had a more rapid decline in TMA sIgG4, in contrast to actively exposed workers who had persistently higher IgG4 levels, supports the notion that sIgG4 could be a marker of persistent TMA exposure more so than TMA sIgG which requires further investigation. [80, 82–85]

Conclusions

In summary, TMA is a potential respiratory sensitizer and irritant. Extensive animal studies and epidemiological data suggest that workplace exposure to TMA can induce a spectrum of occupational respiratory disease. However, thus far, there is no evidence supporting toxic or carcinogenic effects of TMA other than irritant-induced or immunogenic respiratory symptoms. Primary prevention strategies such as the use of personal protective equipment and improved production and packaging engineering procedures have been effective at decreasing TMA exposure, although some TMA-exposed workers still develop TMA sIgE sensitization. Immunosurveillance which involves serial monitoring of TMA sIgG and sIgE has been highly effective at identifying these workers early on so they can be removed from further TMA exposure. Furthermore, specific timelines for TMA sIgG detection, in conjunction with its magnitude, can be used as a predictor for further stratifying workers at risk for developing TMA sIgE sensitization. Collectively, OISPs have been very successful at creating a healthy and safe working environment for TMA-exposed factory workers.

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Compliance with Ethical Standards

Conflict of Interest COI statement: Dr. Bernstein is a consultant to industry on matters related to work-related respiratory disease and immunosurveillance.

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